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Mass Spectrometric Identification of Catabolic Pathways in the Selected Amphibian Skin Peptides with the Saliva and the Major Salivary Protease, Kallikrein

By Xiaole Chen, He Wang, Lei Wang, Mei Zhou, Tianbao Chen & Chris Shaw
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Abstract- As nature has selected amphibian skin defensive peptides for inter-species delivery through the oral route in the recipient, structural stabilisation modifications may have occurred to facilitate this and such information would be most useful and could potentially provide new insights to the design of orally-active and selectively-targeted peptide therapeutics. The purposes of this study were to study catabolic pathways in saliva for selected but commonly occurring bioactive peptide types belonging to the protease inhibitor (PI) and bradykinin-related peptide (BRP) families, namely pLR (LVKGCWTKS-YPPKPCFVR), HV-BBI (SVIGCWTKSIPPRPC FVK) and I-11-R (IRRPPGFSPLR), and to extend this study by determining catabolic pathways with kallikrein – the major salivary protease. These data will aid in the establishment of a database of peptide stabilities that may be useful in the design of future orally-delivered peptide therapeutics.

Keywords: *amphibian; peptide; kallikrein; metabolism; catabolic; mass spectrometry.*

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Mass Spectrometric Identification of Catabolic Pathways in the Selected Amphibian Skin Peptides with the Saliva and the Major Salivary Protease, Kallikrein

Xiaole Chen ^α, He Wang ^σ, Lei Wang ^ρ, Mei Zhou ^ω, Tianbao Chen [¥] & Chris Shaw [§]

Abstract- As nature has selected amphibian skin defensive peptides for inter-species delivery through the oral route in the recipient, structural stabilisation modifications may have occurred to facilitate this and such information would be most useful and could potentially provide new insights to the design of orally-active and selectively-targeted peptide therapeutics. The purposes of this study were to study catabolic pathways in saliva for selected but commonly occurring bioactive peptide types belonging to the protease inhibitor (PI) and bradykinin-related peptide (BRP) families, namely pLR (LVGCGWTKS-YPPKPCFVR), HV-BBI (SVIGCWTKSIPPRPCFVK) and I-11-R (IRPPGFSPLR), and to extend this study by determining catabolic pathways with kallikrein – the major salivary protease. These data will aid in the establishment of a database of peptide stabilities that may be useful in the design of future orally-delivered peptide therapeutics.

Keywords: amphibian; peptide; kallikrein; metabolism; catabolic; mass spectrometry.

I. INTRODUCTION

The diverse compounds stored in the granular glands of amphibians have various pharmacological effects, such as cardiotoxic, myotoxic and neurotoxic activities. Even just one single species is able to produce a large number and variety of host-defence compounds reflecting the wide range of potentially pathogenic microorganisms in their living habitats as well as various species of predators including mammals, birds, snakes, etc. [1-3]. Under natural selective pressures, the amphibians have to develop and maintain effective anti-predator defence systems such that the compounds produced in the skin secretions not only conserve bioactive core sequence but also induce structural modifications for optimisation of chemical structures required for survival [4-6]. Once amphibians are attacked, the peptides secreted from skin glands are mostly delivered into the oral cavities of attacking predators where they play roles in several

bioactive processes ranging from the noxious to the toxic and in some instances, to the fatal. Noxious or toxic sequelae serve to make the predators uncomfortable therefore saving the amphibians lives and leaving the predator with a bad memory that may serve to save individual amphibians of that species in the future [5-6]. Killing ones assailant is of course then ultimate in anti-predator defence but aeons of natural wisdom renders this rare as such end-points may serve ultimately to create worse problems. This successful scheme of molecular evolution and adaptation of molecular structures for unusual purposes will without doubt supply the scientist with valuable insights into the stabilising/protease resistance modifications required for the peptide to access and activate/block its target.

It is quite difficult for the scientist to design therapeutic peptides to be given orally because they are so readily degraded by the plethora of proteases in the gut which results in a short duration of action if any action results at all. Also, even after injection for certain conditions of the nervous system, the peptides cannot readily access their targets due to the fact that they are not able to cross the blood-brain barrier. However, the defensive peptides secreted by frog skin, synthesised as parts of large inactive precursors and subsequently generated by selective proteolytic cleavages [7-8], are able to be delivered effectively via the oral route as they have been to predators for millions of years. Kallikrein, the major protease secreted by salivary gland, causes a trypsin-like cleavage in a wide range of proteins. It participates in the reactions that release lysyl-bradykinin from kininogen, which has the effect of increasing the permeability of blood vessels and capillaries of the salivary gland to generate some responses to the bioactive processes, such as vasodilatation and inflammatory [8-11]. Kallikrein is also important in cleavages of peptides and that recognises Lys-Lys, Lys-Arg, and Arg-Arg motifs as processing signals at either COOH-terminals or between pairs of basic amino acids in addition to cleavage at single basic residues [9-13]. Accordingly, kallikrein, a serine protease, is one of the most frequently-used enzymes in protein structure analysis [12, 14-15].

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Here, we describe the application of this method of studying the oral stability and catabolism of several widely-distributed amphibian skin peptides belonging to the protease inhibitor (PI) and bradykinin-related peptide (BRP) families, namely pLR and HV-BBI-A (PIs) and I-11-R (BRP). The two PIs have similar sequences with subtle differences but have been isolated from different species frogs. We have studied their biotransformation by whole saliva and then by the major salivary protease, kallikrein, in isolation, and have subsequently structurally-characterised metabolite oligopeptides and plotted the time courses of their generation/disappearance. The kallikrein experiments were performed to determine the overall contribution of this protease to the peptide catabolism observed in whole saliva and in addition, to determine if this protease could cleave the Arg residue at the C-terminus of I-11-R to supply additional evidence to support the previous observation by our previous study that kallikrein can not only cleave after the C-terminal side of Arg but can also cleave the bond at the N-terminal side of Arg when this residue occupied the C-terminus [4].

II. MATERIALS AND METHODS

a) Solid-phase peptide synthesis

Replicates of peptides were synthesised by solid-phase Fmoc chemistry using a PS3 automated peptide synthesiser (Protein Technologies, Tucson, AZ, USA). When the synthesis cycles were completed, the peptides were cleaved from the resins using 95/2.5/2.5 (v/v/v) TFA/TIPS/water for 6h, precipitated in ether over the next 24 h, washed exhaustively in six changes of ether and then allowed to completely dry over a further 24 h. The peptides were then dissolved in a minimal quantity of 0.05/99.5, v/v, TFA/water, snap frozen in liquid nitrogen and lyophilised. The degrees of purity and authentication of structures of the synthetic peptides were determined using MALDI-TOF MS as previously described.

b) Protease biotransformations

Kallikrein—synthetic peptides were dissolved in sodium phosphate buffer (BioReagent, pH 7.4, for molecular biology, Sigma) at a concentration of $1\mu\text{g}/\mu\text{l}$ and incubated with $1\mu\text{g}/\mu\text{l}$ bovine pancreatic kallikrein (Sigma) at an enzyme to substrate ratio 1:50 (w/w) at 37°C . Samples of $2\mu\text{l}$ were taken at 10min intervals in the first hour then subsequently at 60min intervals. The degradations were directly analyzed by MALDI-TOF-MS.

Human saliva--1 ml samples of human saliva (collected from author X.C and H.W) were incubated separately with $1\mu\text{g}/\mu\text{l}$ of respective synthetic peptides in a 37°C incubator for 48h. Samples were removed and treated as described above.

c) Mass spectrometric identification

Two microlitres of each sample from the kallikrein and saliva incubation experiments were placed

in separate wells of a MALDI-TOF sample plate, mixed with one microlitre of matrix solution (α -cyano-4-hydroxycinnamic acid in acetonitrile/0.1% TFA in water 30/70 (v/v)), air dried and subjected to analysis on a Perseptive Biosystems DE MALDI-TOF instrument (Perseptive Biosystems, Framingham, MA, USA).

III. RESULT

a) Synthesis and structural characterisation of replicates of natural peptides

pLR (LVKGCWTKSYPPKPCFVR), HV-BBI (SVIG-CWTKSIPPRPCFVK) and I-11-R (IRRPPGFSPRLR) were successfully solid-phase synthesised and their levels of purity were established by a combination of reverse phase HPLC followed by subjection to MALDI-TOF mass spectrometric analysis, where observed molecular ions consistent with predicted molecular masses of replicates of peptides indicated successful syntheses and confirmed high degrees (>95%) of purity of the expected products in each case.

b) Protease biotransformations

Incubation of pLR with saliva resulted in the rapid generation of a series of metabolites that are shown in Table1. The metabolites produced by kallikrein could be found in the series of metabolites produced by saliva (Table 2). Thus, it appeared that pLR, which is an antibacterial and trypsin-inhibitor peptide secreted by frog skin, could readily be cleaved by salivary proteases and that the major effective protease appeared to be kallikrein. A most unexpected finding was that a major catabolite, des-arginyl18-pLR (pLR 1-17) was produced. This observation was consistent with previous reports using bradykinins as substrates but this is the first demonstration of such a cleavage in peptide belonging to another structural family. Moreover, as the reaction time proceeded, this des-arginyl18-pLR (pLR 1-17) became the major metabolite (Figure1).

HV-BBI, which is likewise a trypsin inhibitor peptide from frog skin secretion and has a similar structure to pLR but with subtle differences, was also selected for study in these experiments. The results showed surprisingly that HV-BBI-A was not as readily degraded by saliva as its structural analogue, pLR, and no catabolism was detected in saliva under the same degradation conditions as pLR, although the peptide could be cleaved by kallikrein (Table 3), albeit rather slowly. The fragments (SVIGCWTKSIPPRPC, 1643 Da) and (KSIPPRPCFV, 1142 Da) were the major catabolic products (Figure 2). A further fragment, 5-17 (CWTKSIPPRPCFV, 1532 Da) was observed after 24h indicating that the X-Lys bond at the carboxyl terminus could be cleaved by kallikrein.

The BRP, I-11-R, which has an Arg residue as its C-terminus, was employed to assess yet again if this residue could be cleaved by kallikrein (Table 4), to confirm previous observations. As anticipated, this

residue was removed rapidly and the subsequent des-arginyl11-peptide generated represented a stable major catabolite (Figure 3).

IV. DISCUSSION

Amphibian skin gland secretions play key roles in everyday survival. As they contain a plethora of biologically-active molecules, they are a useful and unique source of material for scientific researchers, such as those involved in biophysical research, evolutionary studies and those searching for new pharmaceutical leads [1,16]. As many potent bioactive peptides continue to be isolated and identified from the skin secretions of amphibians, more and more attention is being paid to these special and amazing little animals. Caerulein first isolated from the Australian green tree frog, *Litoria caerulea*, is able to affect blood pressure at a very low concentration and epibatidine, a heterocyclic compound from the skin of *Epipedobates tricolor*, is non-addictive and 2000 times more active than morphine. Magainins 1 and 2 from the African clawed frog, *Xenopus laevis*, exhibit potent antibiotic activity against both Gram-negative and Gram-positive bacteria as well as fungi, and they possess anticancer activity at micromolar concentrations. Caerin 1, obtained from frogs of the genus *Litoria*, is not only a wide-spectrum antibiotic but also displays strong antiviral activity against enveloped viruses that include HIV and Herpes simplex 1 [1-3]. The bioactive peptides isolated from the secretions of amphibians where they have evolved as defensive bio-weapons, are considered to be new pharmacological agents that may serve to overcome serious clinical problems in humans as well as contribute to the study of aspects of delivery and stability of peptide therapeutics.

Over vast time periods of natural selection, amphibians have developed unique defence systems against the predators. These defensive secretions can induce some extraordinary behaviour, such as oral dyskinesias in predators involving small snakes, *Thamnophis ordinoides*, buying sufficient time for the amphibians to escape [1,16]. As the defensive peptides from the amphibian skin surface are firstly delivered into the oral cavities of attacking predators where they mix with saliva, it is quite logical to study the effects of such on the stability of these agents. Saliva has long been known to be one of the first lines of innate immune defence in both humans and animals. Histatins for example, are a class of peptides found in saliva that contain a very high proportion of histidine residues (18-29%) and are thus highly-cationic. Of no surprise, they also display potent antimicrobial activity [17-18]. Saliva is rich in proteins, enzymes and peptides that not only help animals lubricate and digest their food but can also reduce the effects of some toxic food components to protect the individual from damage [19-20]. Due to molecular wisdom accumulated over vast aeons that

leads to biochemical modifications, some amphibian skin peptides were found to retain their high-selectivity at cognate receptors after exposure to salivary proteases, with some catabolites even exhibiting enhanced bioactivity. Maximakinin, an N-terminally extended bradykinin obtained from the skin secretion of a Chinese toad, *Bombina maxima*, is a very good example of such a phenomenon [4]. The primary structures of amphibian peptides thus serve as good models to provide new insights into the study of peptide-stabilising and/or protease-resistance modifications to satisfy the requirements for oral therapeutic peptide delivery.

Kallikrein, as one of the most abundant proteolytic enzymes in the saliva, has been found in various organs including salivary glands, plasma and kidneys in mammals. In addition, kallikrein-like enzymes have been discovered in cells/tissues and biological fluids of other mammals and in other species and secretions such as some snake venoms [21]. To date, some conserved serine proteases which have sufficient chemical similarities to kallikrein, occur in multiple forms in human tissues and are described as the kallikrein-related peptidase family. Some of these kallikreins, such as 3 and 6, can act as potential biomarkers of disease and also have effects on various physiological processes through participating in the corresponding cleavages of propeptides and these include such as things as inflammation induction and maintenance and blood pressure control by releasing bradykinin from kininogens[7-10]. Kallikrein is predicted to recognize Lys-Lys, Lys-Arg, and Arg-Arg as processing signals either at the carboxyl-terminal or between pairs of basic amino acids of inactive propeptide substrates and cleave these peptide bonds to generate biologically-active peptides [7-9,12].

pLR, which was originally isolated from the skin of the Northern Leopard frog, and was identified as a histamine-releasing peptide with no haemolytic activity. pLR displays diverse biological functions involving antimicrobial activity, histamine release induction, mast cell activation and granulocyte macrophage colony formation inhibition without the induction of neutrophil apoptosis [22]. After pLR was incubated with salivary proteases and mammalian kallikrein, it was quite surprising to find that the degradation reactions observed, generated a spectrum of metabolites with the same discrete bioactivity as the parent compound. Kallikrein produced metabolites, as predicted, arising from cleavage of the Arg-Lys doublet, with cleavage at Arg-X predominating. An unusual observation however, was the removal of Arg18 generating des-arginyl18 pLR. This X-Arg cleavage has only been once reported for kallikrein as it is generally accepted that this enzyme cleaves at Arg-X bonds. Although kallikreins from different tissue sources may exhibit different site specificities, both bovine pancreatic and human/canine

salivary kallikreins were found to remove the C-terminal arginyl residues from pLR.

HV-BBI, a synthetic replicate of the natural mature peptide which is similar to the trypsin inhibitor (pLR), has only one difference in primary structure within their respective inhibitory loops that is the substitution of the Lys (K) residue in the presumed P5' position of ORB (a pLR/ranacyclin family member) by Arg (R). This substitution would make a large increase in potency highly unlikely. HV-BBI was not readily degraded by the saliva, in stark contrast to its related peptide, pLR, though it could be cleaved quite readily by kallikrein. While a series of catabolic cleavages were observed with kallikrein, these included the removal of the C-terminal Lys residue. I-11-R was a bradykinin-related peptide (BRP), isolated originally from the skin secretion of the North American frog, *Rana palustris*. A synthetic replicate of this endogenous frog skin secretion peptide, was rapidly attacked by kallikrein causing removal of its C-terminal Arg residue by the non-canonical kallikrein cleavage of an X-Arg bond. This cleavage was fully consistent with several previous observations with kallikrein using different peptide substrates.

Comprehensive analysis of all the catabolic data generated here, revealed that these were in agreement with previously reported data from our research group regarding the specificity of kallikrein, confirming that the peptides whose amino acid sequences contain Arg and/or Lys residues at the C-terminus can be cleaved specifically by kallikrein. The present data have also proved these original observations unequivocally for a broader range of peptide substrates. The catabolite generation time profiles demonstrated that different peptide bonds were cleaved with different time courses and preferences but the peptide bonds of Arg residues at the carboxyl terminus appeared to be generally cleaved by particular enzymes preferentially and 2x to 10x faster than Lys residues occupying the same position. Furthermore, after longer periods of incubation, there were numerous minor metabolites revealed with other cleavage sites like (Cys-Phe, Gly-Leu), which are not specific to kallikrein. This probably indicates the presence of minor contaminant proteases and it is probably biologically irrelevant, the rapid modifications being more likely to be relevant in this respect. The catabolic reactions observed for the BRP would undoubtedly produce a major shift in biological activity spectra as the des-arginyl forms of the kinins are highly-selective ligands for the B1 receptor subtype and do not activate the B2 receptor subtype at all [4]. The biotransformation observed in saliva for several amphibian skin-derived defensive peptides would suggest that the modified structures are highly selective for certain receptors and some have even enhanced bioactivity compared to original peptides. This may represent a most subtle and largely unexplored aspect of the efficacy of peptide

action in the survival strategy of amphibians and could readily supply the scientist with insights into the development of novel orally-active peptide drugs.

V. CONCLUSION

In this study, we present a new catabolic cleavage bond of an X-Arg/Lys by kallikrein, where the peptides containing Arg and/or Lys residues at the carboxyl terminus can be cleaved specifically by kallikrein. Moreover the peptide bond of Arg residues at the C-terminus was observed to be preferentially cleaved by particular enzymes and faster than Lys residues occupying the same position. The catabolic generation of several amphibian skin-derived defensive peptides are highly selective for certain receptors, even exhibiting enhanced bioactivities compared to original peptides, based on their modified structures. These data will aid in the establishment of a database of peptide stabilities that may be useful in the design of future orally-delivered peptide therapeutics.

VI. ACKNOWLEDGE

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Conflict of Interest statement

The authors declare that they have no conflict of interest.

Ethical statement

Secretions used in this study were obtained non-invasively from captive frogs.

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LEGENDS TO FIGURES

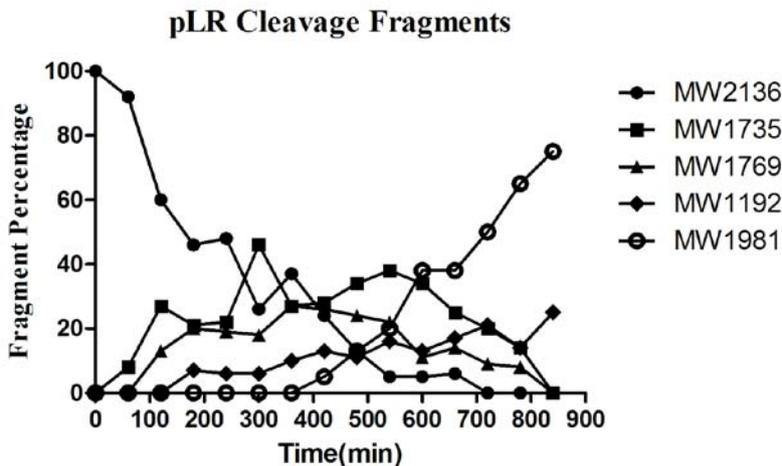


Figure 1 : The concentrations of pLR catabolism fragments with incubation time

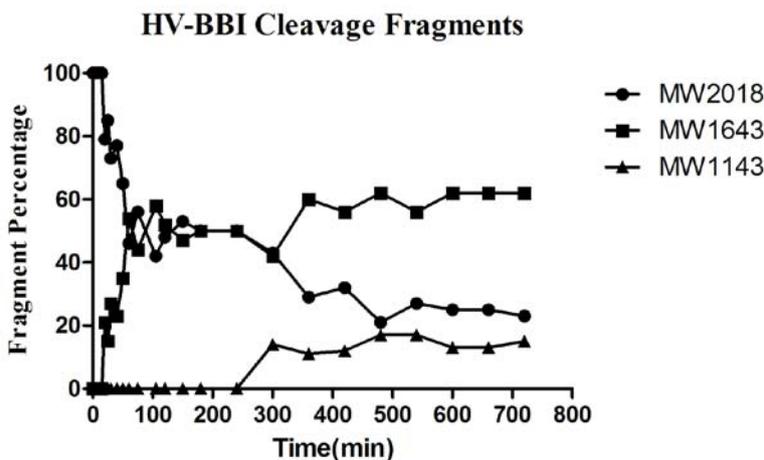


Figure 2 : The concentrations of HV-BBI catabolism fragments with incubation time

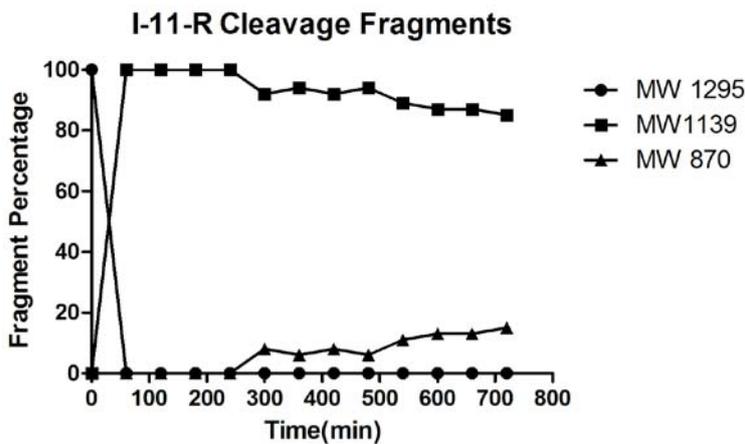


Figure 3 : The concentrations of I-11-R catabolism fragments with incubation time

Figures footnotes: The catabolite generation time figures generally show the change in relative concentration of each catabolic fragment with time. The percentage of each fragment compared with the total fragments represents their concentrations.

Table 1 : Mass spectrometric characterization of pLR fragments generated by saliva.

Cleavage time(min)	Calculated /Observed Mass (Da)	Sequence
0	2136.64/2136	LVRGCWTKSYPPKPCFVR
180	1769.14/1769	GCWTKSYPPKPCFVR
240	1192.46/1192	SYPPKPCFVR

LVR↓GCWTK↓SYPPKPCFVR
↓ = sites of saliva cleavage in pLR

Table 2 : Mass spectrometric characterization of pLR fragments generated by Kallikrein.

Cleavage time(min)	Calculated/Observed Mass	Sequence
0	2136.64/2136	LVRGCWTKSYPPKPCFVR
8	1734.14/1735	LVRGCWTKSYPPKPC
50	1769.14/1769	GCWTKSYPPKPCFVR
90	1192.46/1192	SYPPKPCFVR
120	1981.44/1981	LVRGCWTKSYPPKPCFV

LV↓R↓GCWTK↓SYPPKPC↓FV↓R
↓ = sites of kallikrein cleavage in pLR

Table 3 : Mass spectrometric characterization of HV-BBI fragments generated by Kallikrein.

Cleavage time(min)	Calculated /Observed Mass	Sequence
0	2018.53/2018	SVIGCWTKSIPPRPCFVK
10	1643.03/1643	SVIGCWTKSIPPRPC
420	1142.45/1143	KSIPPRPCFV
1620	1532.91/1533	CWTKSIPPRPCFV

SVIG↓CWT↓KSIPPRPC↓FV↓K
↓ = sites of kallikrein cleavage in HV-BBI-A

Table 4 : Mass spectrometric characterization of I-11-R fragments generated by Kallikrein.

Cleavage time(min)	Calculated /Observed Mass	Sequence
0	1295.57/1295	IRRPPGFSPLR
5	1139.38/1139	IRRPPGFSPL
300	870.03/870	RPPGFSPL

IR↓RPPGFSPL↓R
↓ = sites of kallikrein cleavage in I-11-R



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Miscarriage and Oxidative Stress of Iron Supplementation during Pregnancy

By Ahmed M. Issa

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Abstract- Background: Iron supplementations to the pregnant women in Iraq mostly considered as obligatory routine health care additive treatment without real assessing to the iron status in these people, therefore the acceptable range of iron must be estimated and the relationship between iron status and miscarriage should be explored.

Objective: In miscarriage and pregnant women that supplemented with iron tablets the levels of oxidative stress should be assessed and compared to the iron status to show the type and the strength of correlation between them.

Patients and methods: 96 pregnant women were participate in this study and categorized into. *First group:* Pregnant iron supplemented group (PIS group) includes 35 pregnant women (36.46%) during their 1st trimester or middle of 2nd trimester of pregnancy(18-36 year) and they were received an oral iron supplementation as 250 mg of ferrous gluconate tablet, three times a day.

Second group: Pregnant iron non– supplemented group (PINS group) includes 31 pregnant women (32.29%) during their 1st trimester or middle of 2nd trimester of pregnancy (19-37 year) and they were non supplemented with iron and they considered as control group.

Keywords: ROS, phenyl alanine mandelates, EC50, IC50, DPPH, ARP.

GJMR-B Classification : NLMC Code: WD 730



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Results: The study at first assess the iron status by measuring Hb, serum ferritin and serum iron in the three groups of women and shows that there is a significant differences $p < 0.005$ between oxidative stress parameters of the miscarriage women and the pregnant women that taken oral supplements of iron tablets. The MDA and PC were higher in miscarriage in comparison to pregnant. The decrement in GSH level in miscarriage was also significant (P value < 0.025). The correlations between serum iron concentration and oxidative stress parameters MDA, PC and GSH were plotted using the regression line analysis where the r^2 values were obtained respectively $r^2 = 0.63$, $r^2 = 0.55$ and $r^2 = 0.70$. It is obvious that there is a high correlation between the level of iron in the circulation and the oxidative stress of the reactive oxygen species that generated by the catalytic effect of this transition metal.

Conclusion: The high level of iron that obligatory supplemented to the pregnant women in Iraq should be restricted and early careful investigation should be performed to assess the status of iron before any iron supplements prescribed. The spontaneous abortion or miscarriage is highly

correlated to the oxidative stress that induced by the flood of iron in the circulation.

Keywords: iron; oxidative stress; miscarriage; free radicals.

I. INTRODUCTION

Iron is an essential metal for hemoglobin biosynthesis of erythrocytes, oxidation–reduction reactions, and cellular proliferation, whereas excess iron accumulation causes organ dysfunction through the production of reactive oxygen species (ROS) and other mechanisms. The total amount of body iron is approximately 3–4 g in subject with 70 kg, only 1–2 mg of iron is absorbed daily from the intestinal tract and circulated in the blood (1). Although the body loses iron e.g through menstruation, this way does not considered as a systematic excretion route of iron especially during pregnancy, and therefore the cumulative effect from supplementation can be dangerous; a continuous load exceeding 1-2 mg/day can eventually result in iron overload leading to organ failure and many other complications(2,3). The benefits of improved maternal iron status by iron supplementation could be offset by increased maternal and/or infant infection risk, due to increased availability of iron to host pathogens which cause prenatal infections and miscarriage (4).

Miscarriage, also known as spontaneous abortion and pregnancy loss, is the natural death of an embryo or fetus before it is able to survive independently. Some use the cutoff of 20 weeks of gestation after which fetal death is known as a stillbirth. The most common symptoms of a miscarriage is vaginal bleeding with or without pain. Sadness, anxiety, and guilt may occur (5). Tissue or clot like material may also come out of the vagina (6).

Ferrous iron is a principle pro-oxidant in human by being the most abundant transitional metal in human. It can initiate and potentiate the generation of free radicals through Fenton chemistry in which Fe^{+2} is oxidized to Fe^{+3} in existence of hydrogen peroxide and highly reactive hydroxyl free radical OH^* is produced (7).

It is well known that the absorption of dietary iron occurs in the intestinal duodenum (8). This amount of absorbed dietary iron is enough to compensate for the estimated 1 to 2 mg of unregulated lose of iron through sweat, dermal turnover, and incidental amounts excreted in urine (9,10).

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The need for iron varies markedly during each trimester of pregnancy. Iron requirements decrease during the first trimester because menstruation stops (11). During the second trimester, iron requirements begin to increase and continue to do so throughout the remainder of pregnancy (12). As pregnancy progresses, iron requirements for fetal growth rise steadily in proportion to the weight of the fetus, with most of the iron accumulating during the third trimester. The average iron content of a fetus weighing >3 kg is ~270 mg (13). Pregnancy, mostly because of the mitochondrial-rich (mitochondrial mass increase with gestational age), placenta is a conduction that favors oxidative stress transition metals, especially iron, which is particularly abundant in placenta, are important in the production of free radicals. At the same time placenta is highly vascular and exposed to high maternal oxygen partial pressure (14). When exposed to excess intake of iron, it is vulnerable to oxidative damage secondary to the continuous presence of a relatively some excess of iron intake (15).

This imbalance between pro-oxidants and antioxidants can lead to a number of reproductive diseases such as endometriosis, polycystic ovary syndrome (PCOS), and unexplained infertility. Pregnancy complications such as spontaneous abortion, recurrent pregnancy loss, and preeclampsia, can also develop in response to oxidative stress (16). Data from the United States National Health and Nutrition Examination Survey (NHANES) in 1999-2006 for 1171 pregnant women showed that pregnant women in the first trimester had the highest mean total body iron compared with that of pregnant women in the second or third trimesters (17,18). It has been reported by many investigators that the prevalence of fetal and maternal complications is higher in women with excess iron complications or β -thalassemia than in the general population (19-22).

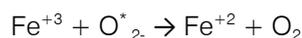
In the presence of excess iron the highly reactive OH^* (hydroxyl radical) species will be generated (23). In 1894, Fenton described the oxidizing potential of hydrogen peroxide when mixed with ferrous salts (24).



Forty years later, in 1934, Haber and Weiss identified the hydroxyl radical as the oxidizing species in this reactions: (25).



Overall summation of Fenton reaction in two steps gives the Haber-Weiss reaction



This reaction is often called the iron-catalyzed Haber-Weiss reaction, or sometimes the superoxide-driven Fenton reaction (25).

There are many different markers that can be used to demonstrate the presence of oxidative stress. In the current study reduced glutathione GSH, protein carbonyl group PC and malondialdehyde MDA all were used to monitor the oxidative stress because the free radicals are very unstable due to their high reactivity and cannot be practically determined at least by our humble laboratory (26). Protein carbonyl (PC) measurement is the most widely accepted possibility to evaluate the oxidative damage to the protein fraction as the content of carbonyl groups increases in free radical induced reactions (27,28).

The usage of (PC) groups as biomarkers of oxidative stress has some advantages in comparison with the measurement of other oxidation products because of the relative early formation and the relative stability of carbonylated proteins (29). Malondialdehyde is an aldehyde (3 carbon molecules with two aldehyde groups) (30). It is considered to be the terminal compound and the most important marker for monitoring lipid peroxidation and oxidative damage induced by ROS (31). About MDA Stewart AJ *et al* in 2005 mentioned that this naturally occurring end product of ROS is a marker of oxidative stress and used as a biomarker to determine oxidative stress level in organisms (32).

Glutathione is an essential nutrient for humans and, since it can be synthesized in the body from the amino acids L-cysteine, L-glutamic acid, and glycine, it does not have to be present as a supplement in the diet (33). The strong evidence that glutathione depletion causes cell death comes from the studies by Li and colleagues (34). They emphasized that a decrease in GSH triggers the activation of neuronal 12-lipoxygenase pathway which leads to the production of peroxides. However the direct depletion of cytoplasmic GSH resulted in increased generation of ROS (35).

Blood hemoglobin, serum iron and ferritin parameters were used to assess the iron status in the body. After considering many indicators, a World Health Organization (WHO) and Centers for Disease Control (CDC) and technical consultation on the assessment of iron status at the population level concluded that serum iron, Hb and ferritin were the most efficient combination of indicators for monitoring changes in the iron status of a population as a consequence of iron supplementation (36).

The iron as a routine administered treatment, given to most pregnant women in Iraq, need to be studied intensively to explore its effects on oxidation status in those subjects and which range would be acceptable and safe for iron administration during pregnancy to prevent more pregnancy complications or miscarriage problems.

II. PATIENTS AND METHODS

This was a 12 months cross-sectional study, conducted in Alzahra teaching hospital in Al-Najaf and some other private clinics, under supervision of specialist physicians, during 2014-2015. The study was designed in accordance with the Ethics Committee approval before the start of the practical part and blood collection. The 96 pregnant women were participate in this study and categorized into

First group: Pregnant iron supplemented group (PIS group) includes 35 pregnant women (36.46%) during their 1st trimester or middle of 2nd trimester of pregnancy (18-36 year) and they were received an oral iron supplementation as 250 mg of ferrous gluconate tablet, three times a day.

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Hemoglobin: Blood hemoglobin Concentration was determined by the Hb – Meter method. In the presence of alkaline potassium ferricyanide, hemoglobin is oxidized to methaemoglobin, which then react with potassium cyanide to form cyanmethaemoglobin complex which absorb the wave length of 540 nm .The absorbance is directly related to total hemoglobin concentration (37).

Serum Iron: After dissociation of iron-transferrin bound in acidic medium, ascorbic acid reduced Fe^{3+} iron into Fe^{2+} iron, Fe^{2+} iron then form a colored complex with 3-(2-pyridyl)-5,6-difuryl-1,2,4-triazine- disulfonate (ferene). The absorbance thus measured at 600nm (580-620) which is directly proportional to the amount of iron in the specimen. Thiourea is added in the reagent to prevent copper interference (38).

Serum ferritin: The quantitative determination of circulating ferritin concentration in human sera was done by the micro plate immunoenzymometric assay. The kit was purchased from Monobid inc, USA (39).

Serum malondialdehyde (MDA): level of serum malondialdehyde was determined spectrophotometrically by a modified procedure described by Guidet B. and Shah S.V. (40). The test is based on the reaction of MDA with thiobarbituric acid (TBA); forming MDA-TBA product that absorbs strongly at 532 nm.

Serum protein carbonyl (PC): Serum protein carbonyl (P.C) groups dimerizes with 2,4- dinitrophenylhydrazine (DNPH), which leads to the formation of a stable dinitrophenyl hydrazone (DNP) product, that can be detected by enzyme-linked immunosorbent assay (ELISA), We followed the method described by Levine et al in 1990(41).

Serum reduced glutathione (GSH): 5,5-Dithiobis(2-nitrobenzoic acid) (DTNB) is a disulfide chromogen that is readily reduced by sulfahydryl group of GSH to an intensely yellow compound. The absorbance of the reduced chromogen is measured spectrophotometrically at 412 nm, which is directly proportion to the GSH concentration (42).

Statistical analysis: The results were expressed as Mean \pm SD. Student's t-test was used to examine the correlation of oxidative stress parameters (MDA, PC and GSH). Significant variation was considered when P-value was less than 0.05. Linear regression analysis was used to explore the correlation between the values of the oxidative stress parameters and serum iron in miscarriage women.

III. RESULTS

In table 1 serum iron concentration and ferritin level both were raise in PIS group women if compared to the control group (PINS group) with p value < 0.005. The level of malondialdehyde MDA and the serum protein carbonyl PC were significantly enhanced $p < 0.005$ in pregnant women that taken iron supplements (PIS GROUP) in comparison to the age matched control group that didn't take any iron supplementation during pregnancy. The reduced glutathione GSH show a significant decrement $P < 0.005$ in pregnant women that taken iron regularly unlike those didn't take iron supplementation during pregnancy.

It is obvious from table 2 that serum iron concentration and ferritin level both were raise in MISG women if compared to the PIS GROUP women with p value < 0.025. The level of malondialdehyde MDA and the serum protein carbonyl PC were significantly enhanced $p < 0.005$ in miscarriage women that taken iron supplements (MISG) in comparison to the age matched pregnant women that taken a regular oral doses of iron supplementation during pregnancy (PIS GROUP). The reduced glutathione GSH show a significant decrement $P < 0.025$ in miscarriage women that taken iron regularly unlike the high level of GSH in pregnant group (PIS GROUP).

Table (1) : Blood hemoglobin conc., serum iron, ferritin, malondialdehyde, protein carbonyl groups and reduced glutathione in iron supplemented and non supplemented pregnant women.

Parameters	PINS GROUP NO = 31 (Control group)		PIS GROUP NO = 35		P <
	Mean \pm SD	Range	Mean \pm SD	Range	
Hb (g/dl)	11.50 \pm 2.40	7.1-13.5	11.2 \pm 1.70	7.3 -13.1	N.S
S.iron (μ M)	16.50 \pm 3.58	10.40- 24.02	21.03 \pm 3.72	11.17-26.4	0.005
S.ferritin (ng /ml)	35.53 \pm 15.25	14.75-65.99	54.11 \pm 17.18	28.67-79.5	0.005
MDA (μ M)	9.50 \pm 2.43	5.16-13.96	13.43 \pm 3.22	7.51-18.30	0.005
P.C nmol / mg . protein	2. 62 \pm 0.72	4.41 - 1.60	3.41 \pm 0.87	1.31-5.10	0.005
GSH (μ M)	173.6 \pm 20.34	135 - 225	154.4 \pm 13.35	124-187	0.005

Table (2) : Blood hemoglobin conc., serum iron, ferritin, malondialdehyde, protein carbonyl group and reduced glutathione in pregnant iron supplemented and miscarriage iron supplemented women.

Parameters	PIS GROUP NO = 35		MIS GROUP NO = 30		P <
	Mean \pm SD	Range	Mean \pm SD	Range	
Hb (g/dl)	11.60 \pm 1.70	7.30 -13.60	10.40 \pm 1.76	7.60 -13.71	0.025
S.iron (μ M)	21.03 \pm 3.72	11.17-26.40	23.24 \pm 3.41	14.17-28.30	0.025
S.ferritin (ng /ml)	54.11 \pm 17.18	28.67-79.5	57.78 \pm 14.57	30.57-82.20	0.025
MDA (μ M)	13.43 \pm 3.22	7.51-18.30	16.59 \pm 3.28	8.10 -20.73	0.005
P.C nmol / mg prot	3.41 \pm 0.87	1.31-5.10	4.96 \pm 0.98	1.65 - 6.25	0.005
GSH (μ M)	154.4 \pm 13.35	124-187	149.3 \pm 11.62	125.3-160.7	0.025

In figure 1 below the regression analysis was used to show the linear relationship between serum iron concentration as a dependent variable and the MDA as the independent variable, however $r = 0.63$ and $r^2 =$

0.80. The correlation was positive and significant as shown from the plotted regression line and its equation.

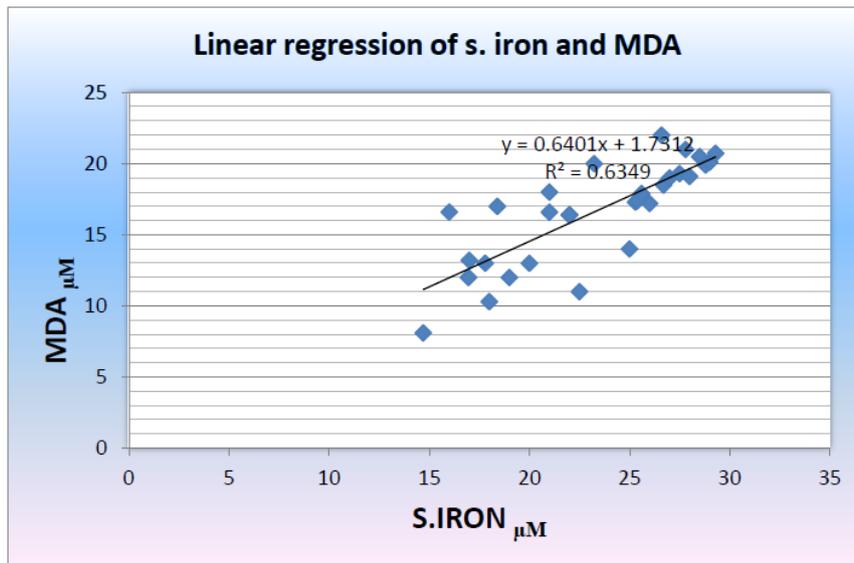


Figure 1 : Regression line of serum iron and malondialdehyde in miscarriage women.

In figure 2 the regression analysis was used to show the linear relationship between serum iron concentration as a dependent variable and protein carbonyl groups as the independent variable, however

$r = 0.74$ and $r^2 = 0.55$. The correlation was positive and progressive as shown in fig.2 from the regression line and its equation.

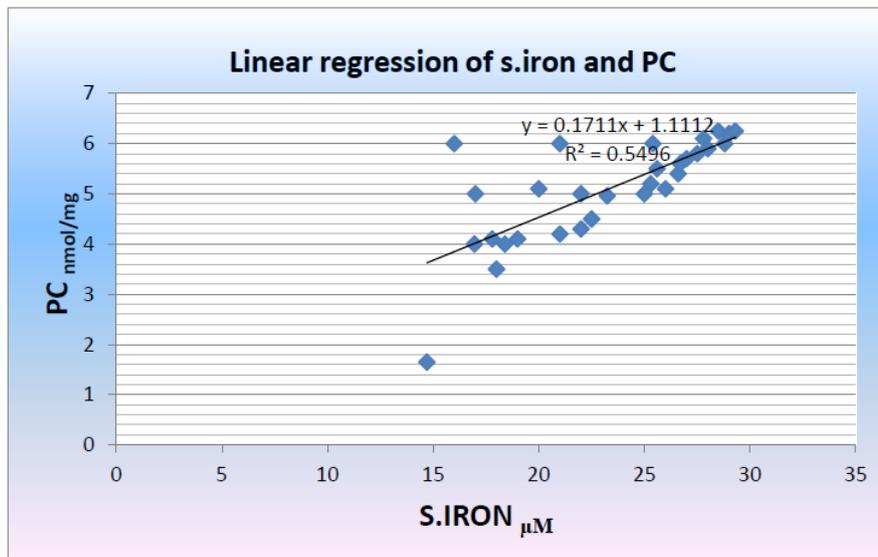


Figure 2 : Regression line of serum iron and protein carbonyl groups in miscarriage women.

In figure 3 the regression analysis was used to show the linear relationship between serum iron concentration as a dependent variable and serum reduced glutathione as the independent variable, however $r = -0.84$ and $r^2 = 0.70$. The correlation was negative and descendent as shown below in the regression line and its equation.

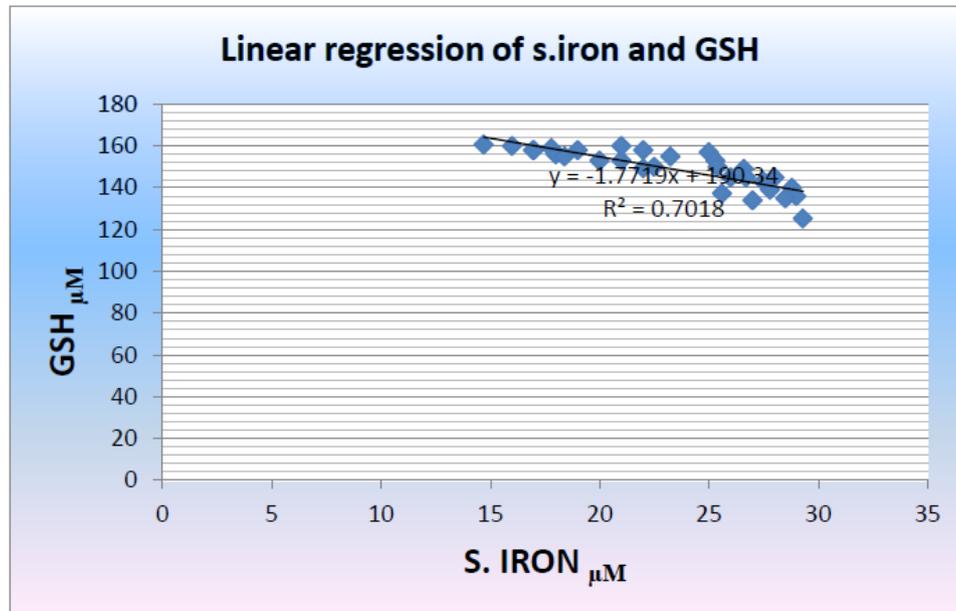


Figure 3 : Regression line of serum iron and reduced glutathione in miscarriage women.

IV. DISCUSSION

In the current study the oxidative stress parameters were considerably employed to explore the role of iron supplementation in pregnant and miscarriage women and to realize the consequences of oxidative stress load complications in those individuals. Pregnancy is a physiological state accompanied by higher energy demand and an increase in oxygen requirement, with various compensate enzyme adaptation (43). The changes occur with advancing pregnancy to met the increase demands for proper body function of mother to fulfill the requirement of fetus (44,45). The increase in oxygen demand is met by increase ventilation. Such conditions are responsible for raised oxidative stress in normal pregnancy (46).

The results in table 1 revel that there is a general decrement in oxidative stress in the pregnant women that not taken any supplementation of iron when compared with those supplemented with iron tablets and this fact may be related to the physiological changes that associated to the process of gestation itself. However Adiga U. *et al* in 2009 reported that the Pregnancy is a state, which is more prone for oxidative stress. Various studies reported that there is a development of a strong defense mechanisms against free radical damage, as the pregnancy progresses (47).

The enhancement of serum iron and ferritin levels in PIS group in table 1 is attributed to the supplements of iron tablets that taken orally. Hillman and Henderson in 1969 reported that with oral iron supplementation, patients were able to achieve serum iron values between 12.5 μM and 27.0 μM, and red blood cell production was able to increase to four or five times normal (48).

In table 1 the levels of MDA and PC in PIS group were significantly ($p < 0.005$) higher than that of PINS group this result were in consistent with the outcomes of many studies for example Minic oka in 2005(49), noticed that during the periods of intravenous iron therapy the amount of iron promotes iron – mediated formation of free radical species which could result in lipid peroxidation which give raise to MDA and oxidation of plasma proteins. He concluded an elevation in serum P.C groups after intravenous iron supplementation, through a study on evaluation of oxidative stress after intravenous iron supplementation.

The same upturn was noticed in table 2, the protein carbonyl groups (PC) in sera of miscarriage women (MIS group) and serum MDA both were significantly ($p < 0.005$) higher than that of pregnant women in (PIS group) The attribution involve the catalyzing role of iron as in Fenton reaction. Fenton reaction lead to generation of highly reactive hydroxyl radicals which in turn would attack preferentially the proteins as a target molecules. This reaction will result in the enhancement of protein carbonyl content (50,51). Iron serves as a catalyst for protein oxidation and the formation of reactive oxygen species that in turn cause side chain modification of many amino acid and ultimately to carbonyl groups formation (52). The higher serum iron concentration were in coordinate with higher oxidative stress and raise in serum MDA concentration. This can be further demonstrated by using the linear regression analysis as in figure 1. The linear regression analysis indicated a significant ($r = 0.63$) positive and progressive correlation between serum iron and MDA levels in MIS group similar finding of such positive correlation has been shown by Sarah et al in 2008 who

concluded that there is a positive correlation between MDA level and serum iron status in young women (53).

Figure 2 shows a significant correlation between serum iron and PC groups in the circulation of the miscarriage women where $r = 0.74$ and $r^2 = 0.55$. This finding was also supported by M.J.Davies *et al* who mentioned that the protein carbonyls are formed early during oxidative stress conditions and are not a result of one specific oxidant, thus they can be called a marker of overall protein oxidation (54).

In table 1 GSH decreased in PIS group when compared to PINS group and this may be related to the differences in serum iron concentrations in the two groups. In the same trend the results in table 2 reveal that the serum GSH in miscarriage group was lower than that of pregnant women by $\approx 3.4\%$ and the correlation between serum iron and serum GSH was shown in figure 3 which display a significant negative regression linearity where $r = -0.84$ and $r^2 = 0.70$. This decrement may be in coincidence with the speculations of Rajdl D. *et al* who documented that the lower GSH levels may be due to the increased turnover of GSH for preventing oxidative damage in iron supplemented group (55). The generation of free radicals is dependent on the presence of various transition metal ions and the most important transition metal in vivo is believed to be the iron (56). Paik *et al* in 1999, demonstrated that the higher level of ferritin have lower levels of GSH (negative correlation), and this conclusion was obtained through a study on enhanced oxidative stress in hemodialysis patient receiving intravenous iron therapy (57). Mustafa *et al* in 2010 detected markedly higher levels of MDA and significantly lower GSH levels in the maternal blood of pregnant women (58).

Finally the oxidative stress that induced by iron supplementation in miscarriage as shown in table 2 is often referred to by many other investigators. Many scientists indicated that Oxidative stress has also been implicated in early miscarriage. Jauniaux *et al* (59) suggested that early pregnancy loss may result from premature oxygenation of the early embryonic environment. By using an O_2 probe in women before first-trimester termination, they observed a steep rise in placental pO_2 between 8 and 12 wk of gestation.

Thus, it is well established that maternal metabolic disorders such as diabetes, which are associated with an increased generation of oxygen free radicals, are known to be associated with a higher incidence of miscarriages and fetal structural defects (60), indicating that the mammalian conceptus can be irreversibly damaged by oxidative stress (61).

Ruder EH in 2008 reported that in vitro fertilization is also affected by excessive ROS in embryo culture media, and the routine practice of incubating embryos at low oxygen tension can prevent embryo arrest and enhance the chances of successful fertilization (62).

V. CONCLUSION

Miscarriage in many pregnant women especially in the early first weeks of gestation affected significantly by the iron that frequently and routinely prescribed to these women in Iraq by most physicians in the form of different types of tablets and formulas. The oxidative stress that induced by the uptake of this transition metal (Fe) in the circulation may be the real causative factor in the spontaneous abortion and many other pregnancy complications. The iron supplements should be given to those who actually suffering from iron deficiency after accurate diagnosis by the suitable biochemical tools. In addition to that the administration of excess iron should be followed by supplying the necessary amount of antioxidant to neutralize the probable oxidation potential of iron during pregnancy.

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Kinetic Spectrophotometric Procedure for Investigation of Furosemide Drug in Pharmaceuticals Preparations

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Abstract- A simple, rapid, sensitive, inexpensive, and easy to perform kinetic Spectrophotometric procedure for the investigation of trace quantities of the drug of Furosemide (FRO) as bulk and in the Pharmaceuticals Preparations has been improved. The method was depended on the fashioning of the Schiff's base by the reaction of aldehyde group present in the 5-sulfo salicylaldehyde reagent and the primary amino group present in Furosemide, which acts as a ligand for the formation of intense colored complex with Co(II) in the acidic medium with maximum absorption at 608nm. the Kinetic Spectrophotometric Method was by using the Fixed Time method for investigation of drug was studied. the law of Beer is applied on the range of concentration between (5 – 100) ppm, the molar absorptivity and the sensitivity of Sandell were $3.9295 \times 10^4 \text{ l.mol}^{-1}.\text{cm}^{-1}$, $0.008 \mu\text{g.cm}^{-2}$ subsequently. the detection limit (LOD) was $2.133 \mu\text{g/ml}^{-1}$. LOQ was $1.105 \mu\text{g/ml}^{-1}$. The perfect circumstances for all colour improvement are portrayed and the suggested procedure has been effectively connected for the investigation of amounts of Furosemide (FRO) in bulk form and in pharmaceutical preparations (tablets, injection sample) The additives and general excipients materials did not affect in the studied method.

Keywords: *kinetic; spectrophotometric; method; determination; furosemide drug; pharmaceuticals preparations.*

GJMR-B Classification : *NLMC Code: QV 55*



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Abstract- A simple, rapid, sensitive, inexpensive, and easy to perform kinetic Spectrophotometric procedure for the investigation of trace quantities of the drug of Furosemide (FRO) as bulk and in the Pharmaceuticals Preparations has been improved. The method was depended on the fashioning of the Schiff's base by the reaction of aldehyde group present in the 5-sulfo salicylaldehyde reagent and the primary amino group present in Furosemide, which acts as a ligand for the formation of intense colored complex with Co(II) in the acidic medium with maximum absorption at 608nm. the Kinetic Spectrophotometric Method was by using the Fixed Time method for investigation of drug was studied. the law of Beer is applied on the range of concentration between (5 – 100) ppm, the molar absorptivity and the sensitivity of Sandell were $3.9295 \times 10^4 \text{ l.mol}^{-1}.\text{cm}^{-1}$, $0.008 \mu\text{g}.\text{cm}^{-2}$ subsequently. the detection limit (LOD) was $2.133 \mu\text{g/ml}^{-1}$. LOQ was $1.105 \mu\text{g/ml}^{-1}$. The perfect circumstances for all colour improvement are portrayed and the suggested procedure has been effectively connected for the investigation of amounts of Furosemide (FRO) in bulk form and in pharmaceutical preparations (tablets, injection sample) The additives and general excipients materials did not affect in the studied method. The statistical comparison between the results that obtained from the reference method gave good agreement.

Keywords: kinetic; spectrophotometric; method; determination; furosemide drug; pharmaceuticals preparations.

I. INTRODUCTION

Furosemide drug is widely used in medicine because of it was giving as a drug diuretic given to many patients[1] furosemide chemical name is 5-(aminosulfonyl)-4-chloro-2-[(2-furanyl methyl)amino] benzoic acid] figure(1) [2]. It has the following generic names: Frusemide, Fursemide, Aisemide, Beronald, Desdimin, Lasilix and others. The empirical formula is $\text{C}_{12}\text{H}_{11}\text{ClN}_2\text{O}_5\text{S}$ according to the molecular weight of 330.77. Furosemide is a white to slightly yellow, odorless, almost tasteless crystalline powder, soluble slightly in water, chloroform and ether [3]. soluble in acetone, methanol, dimethyl formamide [2] and in solutions of alkali hydroxides [3]. Its melting point is about 210 C° with decomposition. and crumble when exposed to air or light [4]. The pH of the aqueous solution is in the range 8.9 to 9.3. The UV spectrum of furosemide (0.01 mg/ml) in (0.1N) (NaOH) was scanned

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from 190 to 400 nm using DMS solvent spectrophotometer it exhibited two maxima absorption at 226 and 272 nm[2].

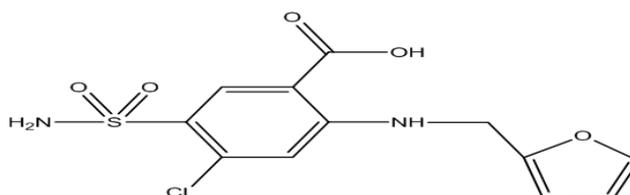


Figure (1) : The chemical structure of Furosemide Drug (FRO)

Furosemide is a sulphonamide derivative [1]. (FRO) is using as a strongly diuretic potent associated to the thiazides but it have strong effective than thiazides [5]. The Preparations for (FRO) were injection and tablets samples[6]. Methods that used to investigation (FRO) are as most depended on HPLC[7-12]. However a number of spectrophotometric, they have been mentioned for furosemide too[13-16], and related drugs [17,18]. A modern procedure for the spectrophotometric investigation of (FRO) in there pharmaceuticals. by this article given a newly, rapid, sensitive and simple kinetic Spectrophotometric procedure for the investigated of trace quantities of (FRO) drug as bulk and in the Pharmaceuticals Preparations depending on the Schiff's base formation by the primary amino group found in Furosemide and aldehyde group found in the 5-sulfo salicylaldehyde reagent which acts as a ligand for the formation of intense colored complex with Co(II) in the acidic medium to form an intense colored product and the perfect circumstances were studied for this reaction. the method was applied on some different kinds of pharmaceutical Preparations with high accuracy and precision by using different doses and forms.

II. MATERIALS AND METHODS

a) Apparatus

- Every absorbance and spectral estimations were performed on double - beam applied UV-Visible 160 digital recording spectrometer (japan).
- Heating-cooling water bath (Haake, Fe3).
- Analytical balance (Sartorius BL 210S).
- pH meter, Jenway 3020.

b) Material and reagents

The Chemicals which were used in the procedure with highly degree of purity and did not need to purification, their solutions were prepared by the following :-

- 1- *Furosemide (FRO) (500 ppm)*:- Pure substance was processed from the (SDI) (the company state for drug industries and medical appliances) Samara – Iraq.The The (500) ppm standard concentration solution of(FRO) was given by dissolution(0.05 gm) of a bluk substance in (100 mL) methanol as solvent in the volumetric flask . It was transferred to a dark flask and it is stable for at least one week the solution was stayed stable for more of one month The stock solution was taken Almost to perpare working concentrations.
- 2- *Ethanolic sulphuric acid solution (2%)*:- It was prepared by adding(2ml) form concentrated sulphuric acid from (GCC)at percentge (%98) inl (100ml) volumetric flask, mixed well and completed the volume by Abselute ethanol from(BDH Chemicals Ltd, %99.9) .
- 3- *5-sulfo salicylaldehyde (5SSA)(0.01) M*:- The reagent was given from (BDH) Chemicals Ltd, reagent Laboratory) company by dissolution (0.202) gm of pure material in (100 ml) methanol as solvent by (BDH Chemicals Ltd,%99.9).

4- *Cobalt chloride $CoCl_2 \cdot 6H_2O$ (0.003) M*:- it was supplied from (BDH) Chemicals Ltd, reagent Laboratory) company given by dissolution 0.071 g in 100 mL of deionzed water.

c) Suggested Procedure [19]

In a progression of flasks of (25ml), equal volumes of the solutions standard with concentrations of (10 – 100) ppm, individually in conclusive volume were included independently, taken after with the addition of (5ml) from the Reagent (5SSA) (0.01M), and complete the volumes to (15ml) by Ethanolic sulphuric acid solution and ,now heating and mixing well in boiling water bath at room temperature for (50) min, for the formation the Schiff base then we leave the solutions for ten minutes for cooling, the absorbance was obtained at (360nm), individually against ethanol solvent as blank, (1.5) mL of Co(II)solution was added and complete the volumes by using ethanol. The absorbance of the complex green colour was obtained at(608) nm against blank reagent, After(10) min, The quantity of (FRO) found in the samples was examined from the calibration curve.

d) Assay Procedure for Furosemide (FRO) in Pharmaceutical Preparations.

A number of preparations giving the Furosemide (FRO) as ingredient active were gotten and it incorporated the next table:-

Table (1) : Pharmaceutical preparation studied

Pharmaceutical preparation	Declared composition	Company
Furosemide Tablets(5)	Per tablet 40 mg furosemide	Actavis, Branstaple company (U.K)
Lasix, furosemide tablets(5)	Per tablet: 40 mg furosemide	Sanofi-Aventis Deutschland, Sanofi Winthrop Industrie company (France)
Furosemide Tablets BP (5)	Per tablet 40 mg furosemide	Bristol Laboratories, Berkhamsted, Herts company (U.K)
Furosemide bosi injection	Per 2 mL: ampoule 20 mg furosemide	Sanofi-aventis Guildford, Surrey, company (U.K)

1- Tablets Procedure [4]

Five tablets were taken and weighing, soft powdered from every kind of tablets independently, precisely measured the powder segment for identical to (0.05)gm of (FRO) that relies on upon the kind of tablets which be utilized, dissolvable after that sifting to particular the non-disintegrated segments. At that point moved into a (100ml) flask calibrated and weakened to the last volume by methanol solvent. Taken after take the reasonable measure of every record arrangement and treated in the same conditions that were utilized as a part of the based procedure for making was to discover a concentration relying on a calibration curve.

e) Injection Procedure [1]

(5ml) ampoule which have (0.05 gm) of (FRO) was moved into 100 ml flasks volumetric and weakened up to the imprint with methanol solvent. At that point we

computed the concentration by using the calibration curve.

III. RESULTS AND DISCUSSION

a) The perfect circumstances were studies for reaction

Various circumstances were contemplated that are influencing on the absorbance for the result compound that lead to increase it.

b) Effect of (5SSA) Reagent Concentration[20]

To study the reagent effect of (5SSA) concentration on the absorbance. It was making by utilizing (2) ml of (500) ppm Furosemide (FRO) drug were transferred into a sequence of 25 mL volumetric flasks, varying volumes for (5SSA)(0.01) M from (0.5 - 8)ml was taking and complete the volumes to (15ml) by Ethanolic sulphuric acid solution and. heating and mixing well in boiling water bath temperature for (50) min, for the formation the Schiff

base, (1.5) mL of Co(II)solution was added and complete the volumes by utilizing ethanol, the perfect volume was (5 mL) for the reagent, that produces the

largely absorption, that was utilized in the next experiments. and appearing in figure(2)



Figure (2) : The volume Effect of (5SSA) (0.01) M

c) Acid Effect [19]

The results were appear that the nearness of acid was making expanding in the highly absorbance for the result product, in this manner a few acids, for example HCl, CH₃COOH, H₂SO₄ and HNO₃ are inspected at (2%) as concentration they were gave that every one of the studied acids obtained the absorbance of colour product, sulphuric acid was the best acid that obtains the largely absorption which chose in the accompanying experiments. which was used as Ethanolic sulphuric acid solution, that used for dilution all solutions for drug.

d) Effect of Cobalt chloride concentration [21]

The effect of different volumes (0.1- 3 mL) of Cobalt chloride (0.003M) on the formation of the complex was also studied (Figure (3)). It was observed an expansion in the absorbance of complex formed up to 1.5 mL it was remaining constant in bigger volumes. Therefore, 1.5 mL of 0.003M Co(II)solution was used for the determination of drug, since it gives high sensitivity and minimum reagent blank.

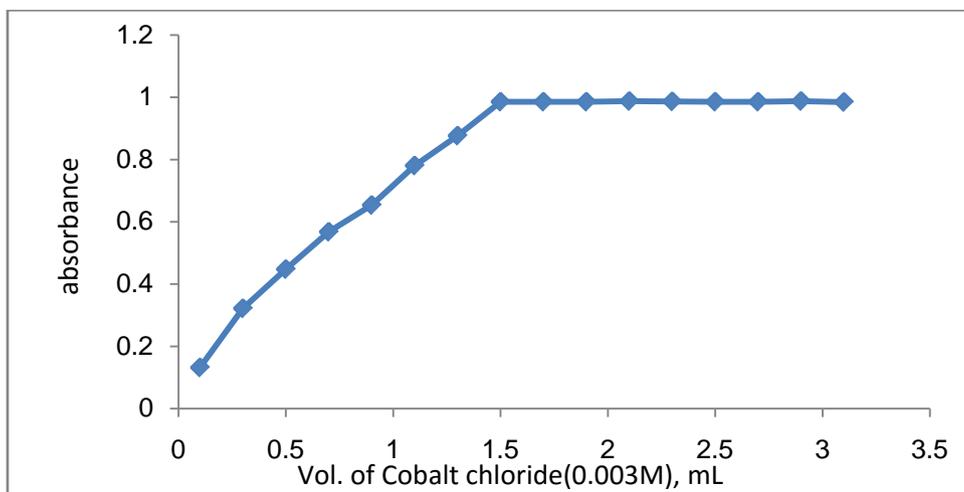


Figure (3) : The volume Effect of cobalt chloride (0.003M)

e) Effect of Temperature[19]

The temperature effect was examined for the range of (20- 120) °C It was found that the color intensity expanded with temperature increasing up to 25 °C, and it was slightly increased at higher temperatures.

The highest absorbance was at 100 °C, the next experiments were performed by boiling water bath at the temperature of room that need to Schiff base formation to simplify the analytical procedure.

f) Effect of order addition [20]

Different orders of addition of the reagents had been done. The results table (3) indicate that the following order (No.1): (FRO) drug, (5SSA) reagent, Ethanolic sulphuric acid and Co(II)solution gave the highest intensity and was selected in the subsequent experiments. (where D =Drug, R=Reagent, M=Co(II) A=Acid).

Table (2) : Effect of addition order

No.	Order of addition	Abs.
1	D+R+A+M	0.788
2	R+M+D+A	0.566
3	M+D+A+R	0.493
4	A+R+M+D	0.202

g) Effect of the time for completing the Schiff base reaction [22]

After heating and mixing well in boiling water bath at room temperature for drug and reagent It was found (50) minutes that the best time for completing the Schiff base reaction which selected in subsequent experiments.

h) Reaction Time Effect [23]

The intensity colour for the product was shown on the maximum after the drug (FRO) had been

responded with the Schiff base, and Co(II)solution and got stabilized after 10 minutes. In this manner ten minutes advancement time was chosen as ideal in the suggested procedure. The resulted colour was stabilized at over than 24 hour.

i) Absorption Spectra

The spectral scan was directed to get the more absorption wavelength of coming about the Schiff base compound subsequent to introducing the perfect circumstances for this reaction. a gainst blank solution (ethanol solvent), after that, The spectral scan was obtained the greater wavelength absorption for the colour complex which was resulting from the reaction between the Schiff base and Co(II)solution.

Figure (4) shows the spectra of colour complex formed by the reaction between the Schiff base and Co(II)solution .against blank (Schiff base solution), the absorption was maximum at 608 nm where (A) spectrum gives the colour for complex resulting by the reaction, (B) is obtaining the spectrum of blank (Schiff base solution). (C) spectrum represents the spectrum for pure drug (FRO).

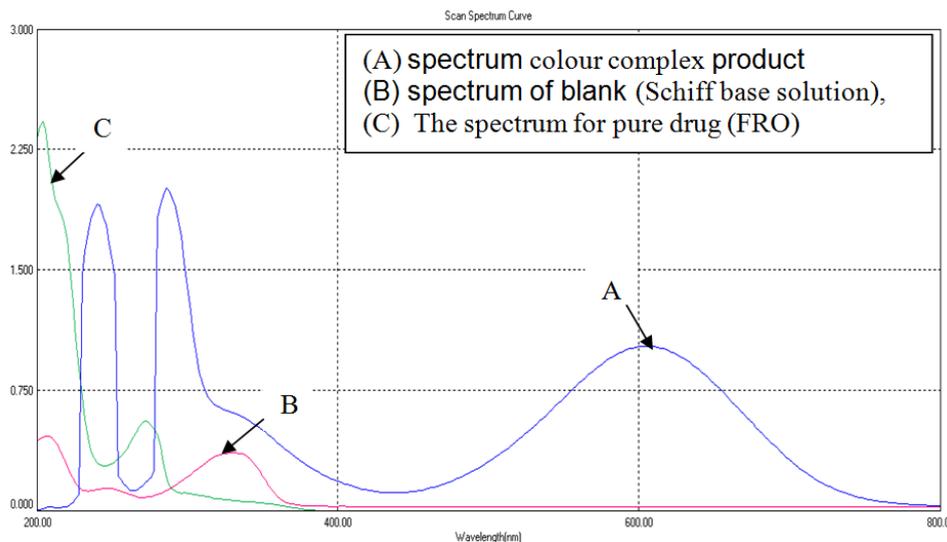


Figure (4) : Appears the spectra of green product at(40 ppm) of (FRO) (A) , the blank(B) (Schiff base solution) (C) the spectrum for pure drug (FRO)

j) The Kinetic study and Calibration curve [24-27]

The Fixed Time Kinetic Spectrophotometric procedure for investigation of drug was studied In this procedure, the absorbance for the reaction solutions which have different quantities of (FRO) drug were calculated at a pre-chose fixed time at intervals of 2 min. The absorbance between the times t_1 (2 min) and t_2 (4, 6, 8, 10, 12, 14, 16 and 18 min) was recorded and plotted against the concentration of drug The corresponding linear regression equations with values of r^2 are

summarized in Table (4). Plainly t the slope expands by the time, the most satisfactory values of r^2 and the intercept were gotten for an altered time of(10) min for the (FRO) which was therefore selected as the more accepted time interval for the estimation and demonstrated wider concentration ranges for quantification. for this reason, on the foundation of expanding concentration range and the analysis less time.

Table (3) : The equations Regression for the the considered drugs of various concentrations at various time intervals using fixed time procedure

Reaction time (min)	Linear range(ppm)	Regression equation	r ²
2	12 - 50	y= 0.0076 x + 0.321	0.9912
4	10 - 50	y= 0.0105 x + 0.211	0.9933
6	10 - 60	y= 0.1154 x + 0.123	0.9943
8	5 - 80	y= 0.1179 x + 0.093	0.9986
10	5 - 100	y= 0.1188 x + 0.071	0.9994
12	5 - 100	y= 0.1191x + 0.128	0.9989
14	5 - 90	y= 0.1173x + 0.099	0.9980
16	5 - 70	y= 0.1145 x + 0.137	0.9977
18	5 - 60	y= 0.1112 x + 0.154	0.9969

Utilizing the circumstances depicted in the strategy, a calibration curve was linear for Furosemide (FRO) is acquired (Figure 3), that appears that law of Beer is obeyed into the range of concentration

between (5 – 100) ppm, the other Spectral and Statistical information for the investigation of (FRO) by the purposed procedure were summarized at Table (5).

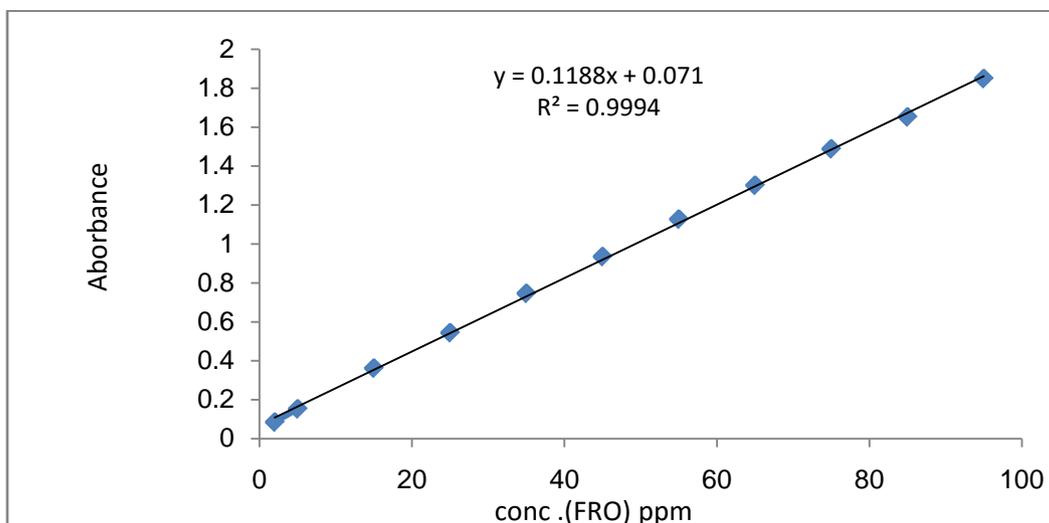


Figure (5) : Shows the Calibration curve of (FRO)

Table (4) : Analytical Data for the time fixed procedure of the kinetic spectrophotometric procedures for investigation of furosemide(FRO)drug

Parameter	Value
λ _{max} , nm	608
Correlation coefficient ,r ²	0.9994
Slope(b)	0.0188
The Molar absorptivity (L .mol ⁻¹ .cm ⁻¹)	3.9295 x10 ⁴
The law of Beer limits (μg/ml)	(5 – 100)
the sensitivity of Sandell (μg.cm ⁻²)	0.008
Intercept (a)	0.071
The detection Limit (LOD) (μg/ml ⁻¹)	1.105
The quantification Limit (LOQ) (μg/ml ⁻¹)	2.336

k) Precision and Accuracy

The precision and accuracy for the studied kinetic Spectrophotometric procedure were investigated at three various concentrations from furosemide (FRO) drug breaking down five repeat tests of every concentration by the proposed method. percentage relative error (E%) as accuracy and Percentage relative standard deviation (RSD%) as precision for the proposed procedure were computed. The results in Table (6) depict good accuracy and precision as illustrated by the low values of RSD%. and low values of E%, proving the repeatability and reproducibility of theproposed method. (All the results were calculated for five determinations).

Table (5) : Precision and Accuracy of the studied method

Conc. Of (FRO) ppm present	Conc. Of (FRO) ppm Found	% Error	% Recovery	% R.S.D
10.00	9.85	-1.50	98.50	0.89
40.00	39.88	- 0.30	99.70	0.56
80.00	80.15	0.18	100.18	0.21

l) Stoichiometry of reaction[28-31]

The Schiff base formation was apparent from the literatures, that a mole ratio between (FRO) drug and (5SSA) was (1:1) that leading to form a new ligand having low absorbance. The absorbance sensitivity has been increased by its reaction with Co(II) to give an intense colored complexes. The stoichiometry of the reaction of (FRO) drug with (5SSA) and Co(II) was performed by mole ratio method and Job's method. The methods applied by using (1×10^{-3})M solution of

each of the drug, the reagent and Co(II) salt to determine the stoichiometry of complex product. The absorbance of the solutions were measured at maximum wave length (608 nm) colour product. from these methods (figure 4) the results are showing that 2:1 new ligand (Drug with (5SSA): Co(II) complex was formed at 608nm. The product formed was ethanol soluble, A reaction mechanism based on the above reaction is shown in Scheme fig(5) [24-26].

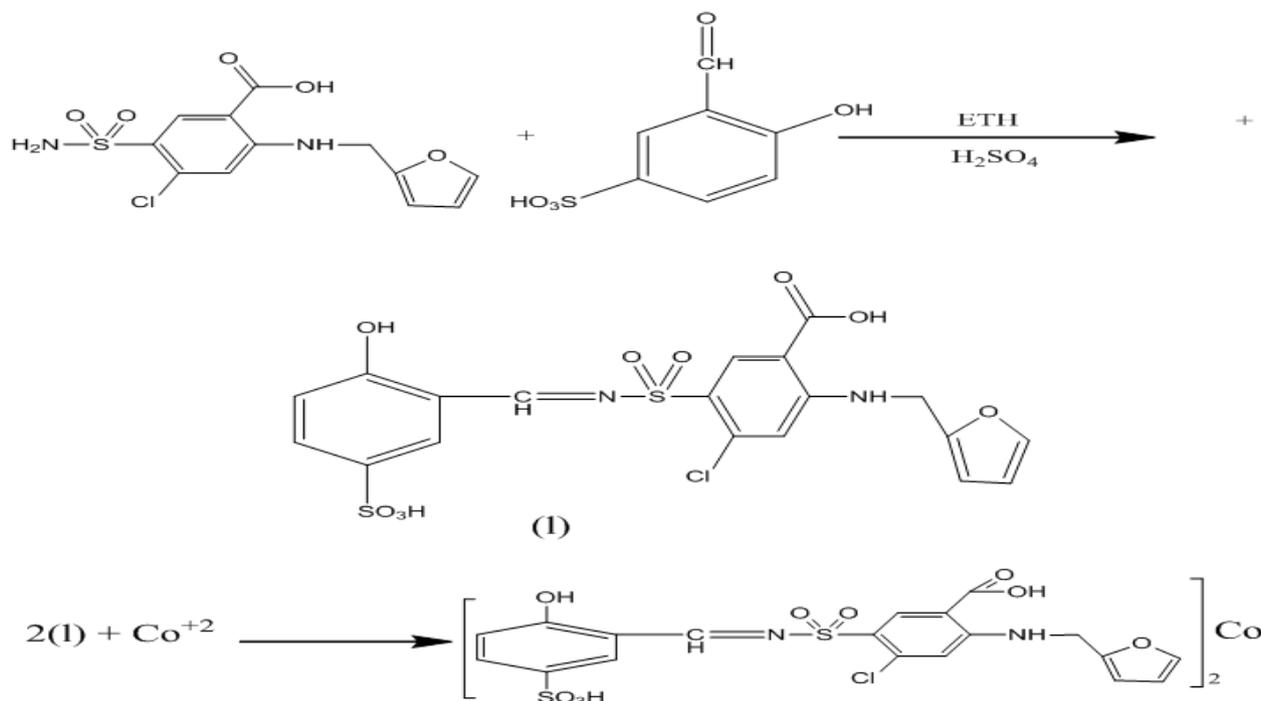


Figure (6) : Scheme of probable reaction pathway for the formation of complexes of (FRO) drug with (5SSA) and Co(II)

The stability constant for the product complex was figured by looking and measuring the absorbance of a solution which including stoichiometric measure of new ligand (Drug with (5SSA) and Co(II) with that of solution which was having the perfect quantity (1ml of 2×10^{-3} M). and utilizing the solution of Co(II) ion at five times concentration from the initial concentration. The average for calculated stability constant for the colour result in ethanol under the characterized experimental circumstances was $2.38 \times 10^6 \text{ l}^2 \cdot \text{mol}^{-2}$

Effect of solvent [32]:- The type of solvent that was dissolving the drug substance employed affects both the wavelength and intensity of maximum absorption. Table (2) shows the effect solvent, methanol was the best solvent, which giving very high intensity of maximum absorption which was in the case of using (5SSA) as the aromatic aldehyde. methanol is appeared to be a decent solvent from the point perspective of economy and sensitivity. (Solvent utilized for the dilution to the mark of (25ml) conical flask).

Table (6) : Spectrophotometric attributes of the colored product in different organic solvents

Solvent	λ_{max} , nm	ϵ , $\text{L} \cdot \text{mol}^{-1} \cdot \text{cm}^{-1}$
Acetone	580	3.176×10^3
chloroform	530	1.3368×10^3
2- propanol	436	8.4271×10^2
Acetic acid	345	1.8710×10^2
Dimethyl sulphoxide	570	2.9010×10^2
CCl_4	510	Two layers
Dioxine	480	Two layers
Dimethyl formamide	510	1.0320×10^3
Ethanol	560	6.1010×10^3
Benzene	530	2.3210×10^2
Methanol	608	3.9295×10^4
Teri butyl alcohol	555	Two layers
Formic acid	430	2.8733×10^2
Pyridine	360	Turbid
Di ethyl ethe	420	1.8761×10^2

m) Interferences

The Specificity of the suggested kinetic spectrophotometric procedure (fixed time procedure) for investigation of the (FRO) drugs within the sight of Often experienced excipients like, talc, lactose, Acacia, starch, Glucose Sucrose, polyvinylpyrrolidone (PVP), magnesium stearate, and aspartate. by the presented study, solution of (FRO) was framed and everyone from the studied excipients was given independently by ten-times concentrations more than of (FRO) were prepared by the self procedure in the Calibration curve. (2 ml) of (100)ppm (FRO) and (2ml) of every excipients was connected for the study of interferences after that dilution to the sign of volumetric flask (25ml). grade of impedance was believed to make the procedure more satisfactory if the mistake was not more than $\pm 2\%$ in respect to the normal No obstructions were seen on the investigation of (FRO) within a sight of the studied excipients (Three determinations Average) Table(7)

Table (7) : Excipients Effect at (400) ppm on the recovery of (FRO) drug at (40)ppm for fixed time method

Interference	% Error	% Recovery
Talc	- 2.150	97.850
lactose	+ 2.140	102.140
Acacia	+ 1.150	101.150
starch	- 2.660	97.340
Glucose	- 4.100	95.900
Sucrose	+ .3.150	103.150
magnesium stearate	- 2.200	97.800
Aspartate	+ 1.110	101.110
PVP	- 3.450	96.550

n) Application of the procedure

The procedure was applied for the assay of pharmaceutical preparations of the drug, was studied. The consequence of examine for accessible formulations of Furosemide (FRO) drugs are appeared by following Table (8).

Table (8) : Furosemide (FRO) drug in as pure substance and forms of dosage

Pharmaceutical preparations including (FRO)	Average recovery %	
	Proposed procedure	Standard procedure ⁽⁴⁾
Pure (FRO)	98.410	98.230
Furosemide tablets (40) mg (FRO)	100.360	99.660
Lasix , furosemide tablets (40)mg (FRO)	100.420	100.170
Furosemide tablets BP (40) mg (FRO)	101.220	99.450
Furosemide bosi injection (20) mg (FRO)	98.860	98.340

Wherever the three normal determinations. also, the standard strategy were gotten from (2009)British Pharmacopeia. The outcomes were Replicable and the determination procedure of formulations was through examined by the Standard procedure

IV. CONCLUSION

The present spectrophotometric procedure was A rapid, easily, sensitive and precise procedure has been created for the investigation of trace quantities of Furosemide (FRO) and relevant for the investigation of (FRO) in tablets and injection, The proposed procedures are free from basic exploratory conditions and muddled systems, for example, extraction step. The reagents utilized as a part of the procedures are shabby, promptly accessible and the methods don't include any dreary sample preparation. These points of interest empower the utilization of the presented techniques in quality routine control investigation of Furosemide (FRO) in pharmaceutical preparations.

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Abstract- Aims/hypothesis: The aqueous extracts of purple sweet potatoes contained highly anthocyanin and has a hypoglycemic effect and prevent oxidative stress. The pharmacological mechanisms of this anthocyanin is not clear. The antioxidant effect of anthocyanin is proposed could protect the oxidative stress of pancreatic β -cell and recovery their function.

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Results: The injection of streptozotocin afforded a damaging of pancreatic β -cell of rats from 50 to 14.8 cells / 5 field views and introduced hyperglycemic rats. The blood glucose levels of this diabetic rat were ranged between (200 - 600) mg/dL in average 422 mg/dL. The aqueous the purple sweet potato extract prevented this pancreatic β -cell damaging and decreased the blood glucose level into the normal level.

Keywords: *purple sweet potato, pancreatic β -cell, diabetic rat.*

GJMR-B Classification : *NLMC Code: QV 37*



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The Pharmacological Mechanisms of Anthocyanin in Aqueous Extract of Purple Sweet Potato as Antihyperglycemic Herbal Remedy

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Abstract- Aims/hypothesis: The aqueous extracts of purple sweet potatoes contained highly anthocyanin and has a hypoglycemic effect and prevent oxidative stress. The pharmacological mechanisms of this anthocyanin is not clear. The antioxidant effect of anthocyanin is proposed could protect the oxidative stress of pancreatic β -cell and recovery their function.

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Conclusions/interpretation: The acetylated anthocyanin on purple sweet potato tuber could protected the damaging of pancreatic β -cells by induced streptozotocin, reduced the blood glucose into normal level.

Keywords: purple sweet potato, pancreatic β -cell, diabetic rat.

I. INTRODUCTION

The diabetes mellitus (DM) is a chronic disease, which need lifelong therapy. Herbal remedies are most popular choice for this condition. Purple sweet potatoes (*Ipomoea batata* L) tuber is an interlude snacks for Balinese people. The aqueous extracts of purple sweet potatoes, which are ingested into rats, introduced a hypoglycemic effect and prevent oxidative stress [1]. This extract could reduce a blood glucose level on rats, which were administrated high dose

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glucose [2]. The antioxidant agents of this potatoes increased antioxidant enzymes, which is superoxide dismutase (SOD) [3]. The pharmacological mechanism of this hypoglycemic effect is still studied. One of the proposed mechanisms is a protection of pancreatic β -cells by the antioxidant agents of this sweet potato.

The consequence of hyperglycemia on DM patients is a vulnerable pancreatic β -cells to the reactive oxidative stress (ROS). In this condition, the β -cells are not able to compensate the insulin resistance. The hyperglycemia can be a trigger for the free radical production [4]. This state can also lead to glycation reactions, which could be increase, the β -cell apoptosis [5], and also lower levels of antioxidant enzymes in pancreatic tissue such as: catalase, SOD and glutathione peroxidase [6]. This condition introduces the β -cell dysfunction.

On in vitro and in vivo studies were reported that the acetylated anthocyanins in purple sweet potato tubers are the potential antioxidant agent to overcome ROS reaction (7- 10). The aqueous extract of purple sweet potatoes, which were harvested in Bali, contained relatively high anthocyanin [11] and evinced a as prevent oxidative stress and anti-hyperglycemic effect [1,10]. The aim of this study was to describe the mechanism of the acetylated anthocyanins in purple sweet potato tubers to prevent the ROS on pancreatic β -cells of hyperglycemia rats.

II. MATERIALS AND METHODS

Materials: The male Wistar rats obtained from animal house facility of Pharmacology Laboratory, Medicine Faculty, Udayana University, Denpasar-Bali-Indonesia, streptozotocin (Sigma, St. Louis, Mo, USA), animal laboratory, citrate buffer (pH 4.5), sweet potato tubers from Balinese farmers, 5% glucose solution, formalin, ethanol, xylene, aldehyde, phosphate buffered (the chemicals were analysis grade from Merck, Germany), distilled water, commercial pellet diet.

Instruments: Histoplast (Thermo Scientific, UK), microtome Leica 820 (Germany), Gomori's Aldehyde Fuschine stain and counterstaining with Nuclear Fast Red. Briefly (Sigma-Aldrich, USA), blood glucose level control (Roche, Germany), Olympus CX41 Microscope and Optilab camera (Optilab, Indonesia) for five

Langerhans Island each slide at 400X-600X magnification. Quantification was done by ImageRaster software (Optilab, Indonesia).

Aqueous Extract of Purple Sweet potato: The washed and peeled purple sweet potato tubers from Balinese farmers were trimmed into cubical a form 2.0-2.5 cm3. One kg of these cubical forms was mixed with 1 liter water and then was blended. This doughy was filtered through three layers of gauze. The filtered aqueous extract was boiled up for 30 minutes. The extract was keep sterile till the administration.

The male Wistar rats: The study was proved and allowed by the ethic commission of Medicine Faculty - Udayana University on number:792/UN.14.2/Litbang 2012. The 15 male Wistar rats were 3-4 months, 175-225 g weigh. They lived under standard laboratory conditions at $25 \pm 2^{\circ}\text{C}$, relative humidity ($50 \pm 15\%$) and normal photoperiod (12-hour light-dark cycle). Commercial pellet diet and water were provided ad libitum. The rats were divided into 3 groups and each consisted of 5 rats. Group 1 was a diabetic group, which's induced by intraperitoneal injection single dose of 40 mg/kg streptozotocine in freshly dissolved of citrate buffer (pH 4.5). After injection, the rats had free access on food and water and were administrated 5% glucose solution to drink overnight to counter hypoglycaemic shock. Group 2 was the treated group. The rats on this group were administrated orally aqueous extract of purple sweet potato tuber at the dose of 4 ml/day for 2 weeks along, before intraperitoneal injection single dose of streptozotocin. The administration of aqueous extract of purple sweet potato tuber was continued till 60 days after diabetic induced. Group 3 was a control group, which were administrated a placebo (citrate buffer injection) and normal diet. This study was conducted on randomized control group post-test design.

Blood glucose levels: The blood samples were collected from the retro-orbitalis sinus and the blood glucose level was determinated by orthotoluidine method. The blood glucose levels were controlled in every week of treatment.

Pancreatic β -cell Examination: This test was done at histology labor of medicine faculty of Udayana University, Denpasar, Bali, Indonesia. After the treatment all rats were sacrificed and the pancreas was immediately removed for the examination of β -cell structure. The removed pancreas were immersed in phosphate buffered-formalin 10% for 24 hours, after that dehydrated using serial ethanol 50%, 70%, 80%, 95%, 100% for 2 hours each phase respectively. The Clearing was done using xylene two times for 1 hour respectively. Embedding was done using Histoplast (Thermo Scientific, UK) at 60°C two times for 30 minutes respectively and finally blocking was done. The specimen was trimmed using rotary microtome Leica

820 for 5 um sections and mount on glass microscope slide. Staining for β -cells was done by Gomori's Aldehyde Fuschine stain and counterstaining with Nuclear Fast Red. Briefly, the paraffin section were rehydrated by using xylene two times for 5 minutes each phase, 100% ethanol one times for 2 minutes, 95% ethanol two times for 2 minutes each phase, 70% ethanol for 2 minutes and distilled water for 2 minutes. Immerse slide in filtered aldehyde fuschin stain for 10 minutes and wash in 95% ethanol two times for 30 seconds - 1 minute each phase. Wash slide again briefly in 70% ethanol and distilled water. Immerse slide in Nuclear Fast Red for 5 minutes then washed with distilled water. Dehydrated the slide by immersing in 70% ethanol 20 seconds, 95% ethanol two times each phase 20 seconds, 100% ethanol 20 seconds, xylene two times each phase 2 minutes. Coverslips were mounted on sections using xylene-based mounting medium (DPX). Cells with pink nucleus and purple granules in their cytoplasm are pancreatic β -cells. Photomicrograph was done using Olympus CX41 Microscope and Optilab camera (Optilab, Indonesia) for five Langerhans Island each slide at 400X-600X magnification. Quantification was done by ImageRaster software (Optilab, Indonesia).

Statistical analysis: Statistical analysis was carried out using SPSS for Window 15.0. All data were expressed as mean \pm SD. Groups of data were compared with one way ANOVA. Values were considered statistically significant, when $p < 0.05$.

III. RESULTS

The body weight of rats before treatment was statistically similar ($p > 0.05$). The body weight outgrowth of rats along observation is presented on Fig. 1. The diabetic group rats were losing their body weight significantly compared to other groups ($p < 0.05$).

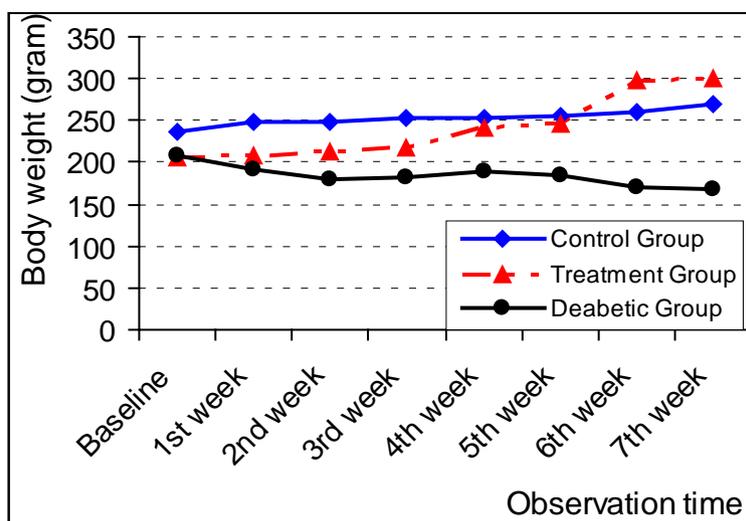


Figure 1 : The outgrowth of rat-body weight during observation

The blood glucose levels of rats before treatment were ranged 115-117 mg/dl. These outgrowth levels are presented on Fig. 2. The blood glucose levels of diabetic group were ranged between (200 - 600) mg/dl with average 422 mg/dl, between (102 - 361) mg/dl with average 152 mg / dl for administrated aqueous purple sweet potato extract group, and

between (113 - 118) mg/dl with average 116 mg/dl for control group, respectively. The blood glucose levels of diabetic group were significantly higher then other groups. The treatment group was presented a significantly lower blood glucose levels then diabetic group ($p < 0.5$).

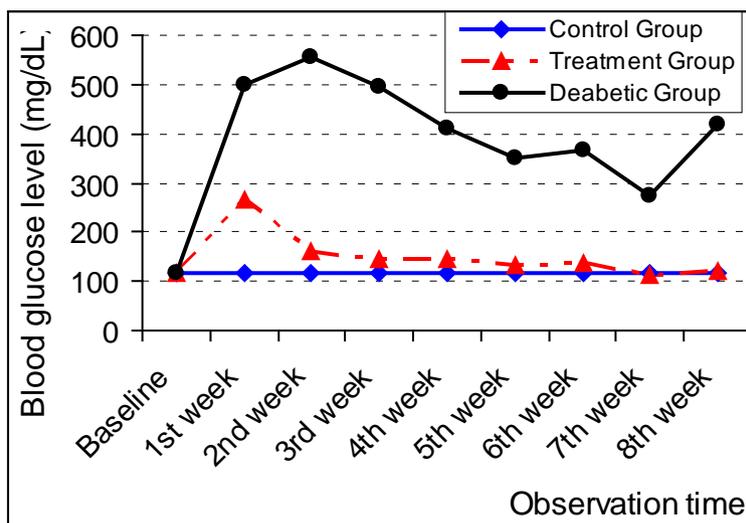


Figure 2 : The outgrowth of blood glucose level during observation

The figure 3 shows the number of pancreatic β -cells of rats after 60 days blood glucose level observation time. The pancreatic histological pictures of all rat-groups are presented in figure 4. The average of β -cell was 50 ± 2.5 cells/5 fields-views for control group, 40 ± 2 cells/5 fields-views for treatment group, and 14.8 ± 0.8 cells/5 fields-views for diabetic group, respectively. The amount of pancreatic β -cells of rats in diabetic group was lowest then other groups and significantly difference ($p < 0.5$).



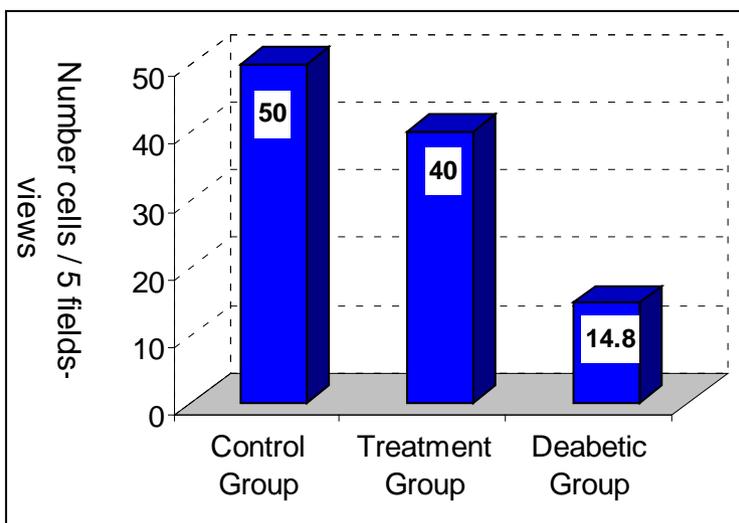


Figure 3 : Comparison of the number of pancreatic β -cells of rats

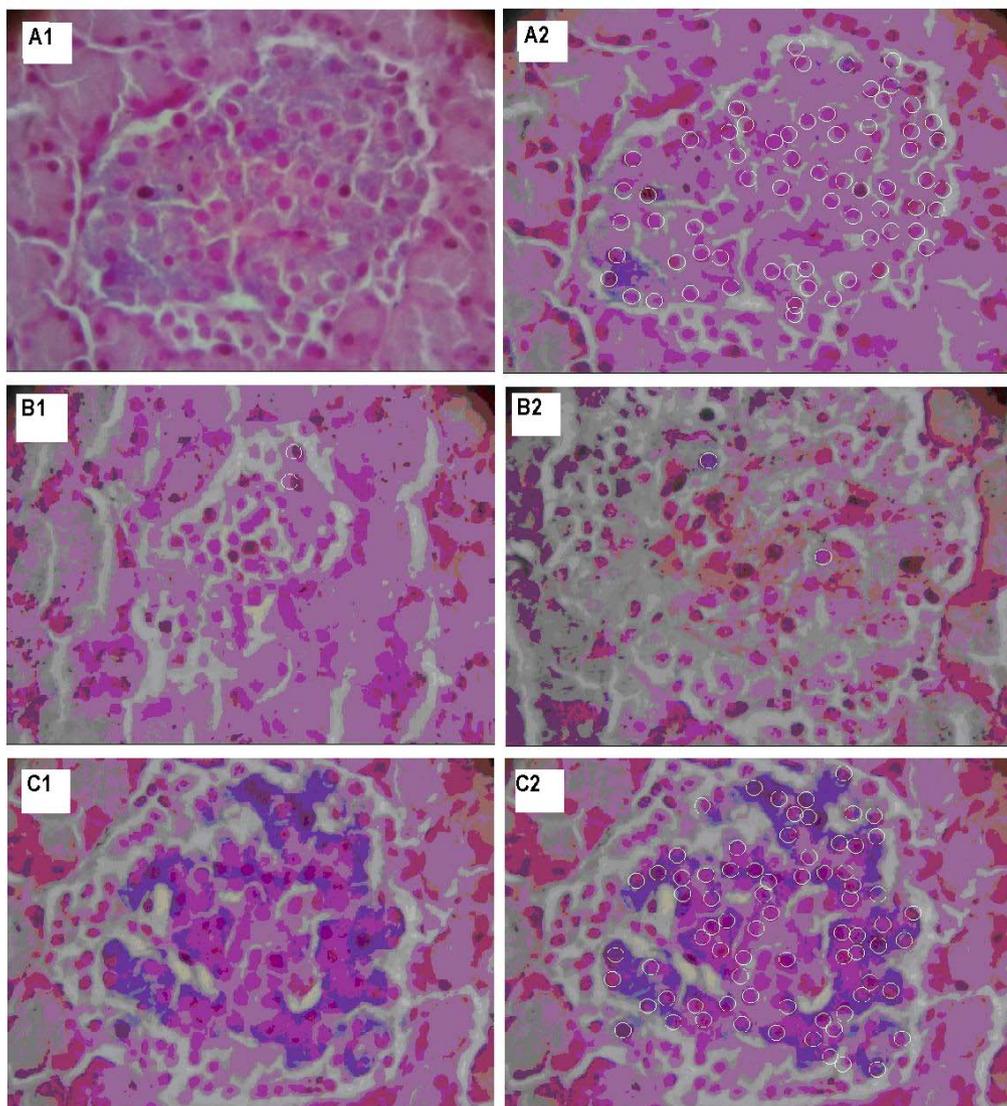


Figure 3 : The pancreatic β -cells of rats in three experimental groups, A: control group, B: diabetic group, C: treatment group, 1: unsigned pancreatic β -cells, 2: the signed pancreatic β -cells,with circle.

IV. DISCUSSION

The induction of intraperitoneal streptozotocin could decrease the amount of pancreatic β -cells and increase the blood glucose level of rats significantly ($p < 0.5$), which are compared to the control group. Administration of streptozotocin induced an oxidative stress in the pancreatic tissue, which due to increased formation of free radical [12]. The streptozotocin was reported has a diabetogenic effect in rats by cytotoxic action on the pancreatic β -cells and increasing free radical generation in pancreatic tissue [13].

The administration of aqueous extract of purple sweet potato tuber before and after induced streptozotocin presented the protection of ROS in pancreatic tissue of rats. The purple sweet potato tuber from Balinese farmers was reported containing a high anthocyanins [11] and has well anti oxidant activity [3]. The protective of ROS on rats-treatment group could due to the contained anthocyanins in aqueous extract of purple sweet potato tuber. The anthocyanin has reported, has ability to prevent apoptosis induced by streptozotocin on pancreatic cells through regulation of caspase-3, Bax, and Bcl-2 proteins [14]. Based on this study, we concluded that the contained anthocyanins on purple sweet potato from Balinese farmers could be covered for ROS after induced streptozotocin in pancreatic β -cells in rats.

The anthocyanin could regulate the blood glucose level by inhibit the alpha-glucosidase [15] and could also increase the phosphorylation of insulin receptor [14]. These effects could prevent the increasing of blood glucose after meal and increase the concerting the blood glucose into glycogen. The administration of the ethanol extract of purple sweet potato tuber from Balinese farmers could maintain the blood glucose level of mice after administrated a high doses glucose load [2]. The high blood glucose level increased intracellular peroxide levels in the pancreatic β -cell, this also will be introduced the intensive ROS in the pancreatic tissue [16]. The study presented, that the blood glucose level of treatment rats group increased to maximum at the first week after induced streptozotocin then decreased along observation time to normal level after the continuing administration of the aqueous extract of purple sweet potato. The study showed that the administration of aqueous extract of purple sweet potato on this study could reduce the blood glucose level into a normal level. The effects of anthocyanin in this potato on damaging protection of pancreatic β -cells, inhibiting the alpha-glucosidase and induction of converting blood glucose into glycogen were the proposed pharmacological mechanisms to the observed outgrowth blood glucose level in the treatment rats group.

The anthocyanin is an important part of the human diet, which can be highly found in purple sweet

potato. This potato can be used as herbal medicine for against ROS-induced degenerative diseases.

V. CONCLUSION

The acetylated anthocyanin on purple sweet potato tuber could protect the damaging the pancreatic β -cells by induced streptozotocin and reduced the blood glucose into the normal level.

Abbreviations

DM	diabetes mellitus
SOD	superoxide dismutase
ROS	reactive oxidative stress
DPX	xylene-based mounting medium
SPSS	statistical package for the social sciences

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Training Needs Assessment and Professional Development of Pharmacists in Dawacom Pharmaceutical Chain, Jordan

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Abstract- This thesis explores the soft skills that a pharmacist needs in order to perform effectively. In order to be a good professional, a pharmacist should possess "a combination of comprehensive therapeutic knowledge, experience, problem-solving skills, and judgment" (Burke, et al., 2008). Throughout the research we expect to identify skills such as: teamwork skills, communication skills, patient oriented attitude, risk management skills, empathy, patience, reliability and professionalism, patient relation management, analytical skills, decision making among the employees of Dawacom.

Knowing that managerial skills include technical skills, human skills and conceptual skills, it is hypnotized that, although pharmacists possess strong technical skills, they register a deficit in the human and conceptual skills areas. The research will show that if the managerial skills of the Dawacom's employees are correctly identified, the management team/HR department will be able to focus the trainings towards those areas where the employees have difficulties, thus helping the pharmacists perform efficiently.

GJMR-B Classification : NLMC Code: QV 21.5



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Training Needs Assessment and Professional Development of Pharmacists in Dawacom Pharmaceutical Chain, Jordan

Aref Ibrahim Alabed ^α & Prof. Dr. Salameh Malik ^σ

Abstract- This thesis explores the soft skills that a pharmacist needs in order to perform effectively. In order to be a good professional, a pharmacist should possess "a combination of comprehensive therapeutic knowledge, experience, problem-solving skills, and judgment" (Burke, et al., 2008). Throughout the research we expect to identify skills such as: teamwork skills, communication skills, patient oriented attitude, risk management skills, empathy, patience, reliability and professionalism, patient relation management, analytical skills, decision making among the employees of Dawacom.

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I. INTRODUCTION

Recent researches have brought to scholar's attention the soft skills for a better performance of employees. As a consequence, there is a continuous need for research in effective soft skill training. The term "soft skills" has received a great attention among practitioners while the importance of soft skills in management grew in recent researches. Throughout this paper we will refer to attitudes and behaviours displayed in work relations as "soft skills". Often human resources professionals have turned their attention to appropriate training programs to improve soft skills of their employees with the purpose of enhancing their performances.

If we talk about a better understanding and mastering of soft skills by using training we should have a closer look at professions that require deep soft skills, such as pharmacists. Workplace training on soft skills such as communication, stress management, conflict management, leadership, team work, etc.

The profession of pharmacist has a special importance in society; the pharmacist is the one who, along with other health professionals, offers products and pharmaceutical services, following its use as

judiciously in order to obtain maximum therapeutic effect.

The pharmaceutical activity can take place in units of the drug industry and consists in:

- Preparing dosage forms of drugs, manufacturing and drug control, storage, preservation and distribution of medicines,
- Drug control activities and preparation, and
- Providing information and advice on drugs.

The pharmacist must be informed of all developments in the science and legislation in force, to maintain a level of professional competence appropriate to the performance of professional duties and to comply with the Code of Ethics developed by the College of Pharmacists. The pharmacist is responsible, according to academic training, to exercise other professional activities such as:

- Working with your doctor to establish and follow the patient's therapy,
- Marketing and pharmaceutical management and health education activities or administration.

In pharmacy a great importance is given to informing the patient as inaccurate information or a misunderstanding of some aspects may lead to treatment failure and, consequently, to an increase in health costs. Information is formulated and presented according to the understanding of each patient, being informed about the benefits and risks of each drug administration are mandatory. The pharmacist must call on all the information they hold to ensure the safe, proper and effective use of a medicine. The pharmacist has moral and professional obligation to ensure that services provided to each patient are of adequate quality and that has an important role with other health professionals to optimize drug treatment outcomes.

Improving the performance of the human resources is a goal of any company. The performance of an organization is settled according to the course in which human, material, informational and financial resources are used in order to achieve the set objectives, at the desired level. An important part of the performance is the quality of the employees. The professional competences and knowledge in the field of pharmacy are decisive in regard to obtaining the

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efficiency of the administrative system through resource management, employee motivation, forecasting the future evolutions and control of the ongoing processes. The organizations which understand and are aware of the human behaviour and the importance of knowledge and skills implement policies to enhance the skills of their employees. According to (Ernst, et al., 2012) a number of articles regarding the training and competencies of pharmacists have been published recently. Unfortunately these documents do not clearly recommend training requirements for pharmacists.

The purpose of this paper is to assess the training needs of the pharmacists working within Dawacom Pharmaceutical Chain and to provide recommendations for future training of pharmacists in order to enhance their competencies. These recommendations have been developed based on a thorough assessment of the training needs of the employees of Dawacom.

We hope that these recommendations will be used by other companies in the future when they seek to define the qualifications of their pharmacists with a focus on the soft skills. The ability to assess the soft skills is the key to improving them. A training needs assessment is used to determine whether training is the right solution to a workplace problem (Cekada, 2010). The assessment is an ongoing process of gathering data to determine what training needs exist in order to develop training that can help the organization meet its objectives (Brown, 2002).

In order to be a good professional, a pharmacist should possess "a combination of comprehensive therapeutic knowledge, experience, problem-solving skills, and judgment" (Burke, et al., 2008). Throughout the research we expected to identify skills such as: teamwork skills, communication skills, patient oriented attitude, risk management skills, empathy, patience, reliability and professionalism, patient relation management, analytical skills, decision making among the employees of Dawacom; and we intended to answer the following questions:

1. What are the current common skills of the pharmacists at Dawacom?
2. How can the current common skills of the pharmacists at Dawacom be assessed?
3. What critical skills (other than technical skills) need the pharmacists in order to be able to perform efficiently?
4. What is the link between the critical skills and pharmacists' performance?
5. Towards what skills should the training efforts be channeled?

Knowing that managerial skills include technical skills, human skills and conceptual skills, it is hypnotized that, although pharmacists possess strong technical skills, they register a deficit in the human and

conceptual skills areas. The research will show that if the managerial skills of the Dawacom's employees are correctly identified, the management team/HR department will be able to focus the training towards those areas where the employees have difficulties, thus helping the pharmacists perform efficiently. There will be identified a need for improving the communications skills of pharmacist and challenges to effective communication.

Since human capital is a mix of knowledge, skills and abilities, that help the employees, in this case the pharmacists, enhance performance and productivity we aimed to identify the degree in which it contributes to the competitive advantage of a company. There are with respect to this issue, but our purpose was to assess the need for soft skills of pharmacists.

II. LITERATURE REVIEW

Due to the continuous economic changes in the pharmaceuticals field of activity appeared a new set of challenges:

- Reduced growth rate in global mature markets such as the USA and Western Europe. Only "pharmerging" markets such as China, India, Russia, Turkey, Brazil, Korea and Mexico registered a significant increase.
- Due to the fact that at global level the awareness of maintaining health increased, generic products rapidly gaining ground. These lead to lower profits of the players in the market who see the protection of brand products threatened by a much shorter life cycle.
- There is a growing need to demonstrate the economic viability of products and solutions - with a cost-benefit analysis being able to consider all aspects of the therapeutic process.
- New health policies such as e-prescribing and strict forms are introduced to more advanced markets. Treatment protocols specify which drugs are safe, effective and cost-effective for different patients.
- New stakeholders influence purchasing decisions, including payers, patients, governments, distribution channels, pressure groups and the power of doctors is decreasing.
- Increased responsibility - all stakeholders must be prepared to justify decisions and be accountable for the results.
- The Internet gives patients more access to health information through Web sites, blogs, and forums. On this basis, patients exert an increasing influence on treatment decisions of physicians.

Exploring the performances of pharmacists of Dawacom has the purpose of understanding the transfer of soft skills through training and it represents the central subject of this thesis. The aim is not only to contribute from a scientific point of view, but also to

enhance the professional practice especially in the area of soft skills.

Understanding the concept of "soft skills" together with identifying the most influential authors and models is an important first step prior to identifying them in a company and training setting, more specifically in a training setting relying on performing the profession of pharmacists. The notion of soft skills often tends not to be defined by what it means, but rather by what it does not mean: soft skills are positioned as the opposite of hard skills, which are defined as the technical ability and the factual knowledge needed to do a job (Klaus, 2008). Other authors avoided giving a clear definition of "soft skills" by providing examples (Balcar, 2014), (Rajabi, et al., 2013). Muir makes an actual transition to defining the term actively by stating that soft skills are "attitudes and behaviours displayed in interactions among individuals that affect outcomes of such encounters" quoted in (Myers & Larson, 2005).

(Schulz, 2008) offers a list of examples of soft skills:

- Communication skills
- Critical and structured thinking
- Problem solving skills
- Creativity
- Teamwork capability
- Negotiating skills
- Self-management
- Time management
- Conflict management
- Cultural awareness
- Common knowledge
- Responsibility
- Etiquette and good manners
- Courtesy
- Self-esteem
- Sociability
- Integrity / Honesty
- Empathy
- Work ethic
- Project management
- Business management

To be successful in this tough environment, candidates for jobs have to bring along a "competitive edge" that distinguishes them from other candidates with similar qualifications and comparable evaluation results (Schulz, 2008).

Changes in the healthcare system affect all aspects including pharmacy services, pharmacists' role, and expectations and patients' behaviour (Volmer, et al., 2009). In a recent survey soft skills were rated as more important than the hard skills (Aasheim & Williams, 2009). Soft skills have become a crucial and increasingly sort after quality for careers in corporate world, irrespective of the sector (John, 2009). Soft skills are essentially people's skills or personality specific skills

(John, 2009). Hewitt Sean (2008) and Tobin (2006), quoted in (John, 2009), state that soft skills are non-technical, intangible, personality specific skills which determines an individual's strength as a leader, listener and negotiator, or as a conflict mediator. Soft skills are the traits and abilities of attitude and behaviour rather than of knowledge or technical aptitude.

Community pharmacists today are involved in a wide variety of professional activities which may be considered as either product or patient-oriented (Al-Arifi, 2012). Through this role, the pharmacist gained a direct interaction with the patient. This experience generates opinions and views for both the patients and pharmacists (Al-Arifi, 2012). Several studies have investigated patients' satisfaction and attitudes to community pharmacy services (Al Hajji, et al., 2011), (Catic, et al., 2013), (Wirth, et al., 2011).

Superior soft skills play an important role in this commercial era, and the pharmacy field makes no exception. Employees with superior soft skills represent a competitive advantage for any organization. Pharmacy profession has undergone a significant paradigm shift with movement away from a traditional distributive role toward a clinical and patient oriented practice, which has intensified the focus on teamwork and the importance of inter professional relationships (Salman, et al., 2013) quoting (Kritikos et al. 2003). Due to the public's growing awareness of the complexities of the healthcare delivery system, rapid developments in technology, constantly increasing health related issues, and cost of the therapy changed the roles and functions of pharmacists (Salman, et al., 2013).

According to (Simpson, 2006) the term "soft skill" originated from employers identifying the need for non-traditional skills which relate to the individual and how they interact with others (Simpson, 2006). The purpose of soft skill training is to make it more accessible to those who require it and to promote adaptability and employability, at the same time maintaining employee retention (Redha Al Abduwani, 2012).

In establishing effective relationships with patients, the pharmacists' responsibility to achieve desired outcomes must be kept in mind (Gupta, 2011). Communication competence is one of the generic competencies health care professionals are expected to have (Kubota, et al., 2011). Because communication competence can be improved through training, programs to develop better communication skills in pharmacist should be developed. According to (Brown, 2002) there are four main reasons why training needs assessment is necessary before the actual training:

- To identify specific problem areas in the organization
- To obtain management support
- To develop data for evaluation

- To determine the costs and benefits of training

The needs assessment can also be used to diagnose the learning and performance needs of individuals, teams, functional units, and whole organizations (Gupta, 2011).

Communication is important for pharmacists because:

- It establishes an ongoing relation between the pharmacist and the patients
- It provides the exchange information necessary to assess the patients' health conditions, reach decisions on treatment plans, implement the plans, and evaluate the effects of the treatment on the patients' quality of life (Gupta, 2011).

Traditionally, pharmacists are expected to have technical skills related to medicine needed for fulfilling their duties towards patients. However, with changing patient needs, the role of pharmacists has evolved from a purely technical one, to include managerial one as well. Developments in the discipline of pharmacy over the last decade in the developed nations have led to emergence of the term "pharmacy business management" (Davies, et al., 2013). Thus, business management can be termed an essential component of pharmacy training with changing pharmaceutical industry. (Frenk, et al., 2011) found out that competencies development and skill development is necessary for personnel in medical profession including pharmacy, nursing, dentistry, and psychology, to cope with the challenges of this century. Since pharmacists deal with patients on a regular basis, it is essential that they acquire people management skills as well.

Research in UK indicates that with changing business model of pharmacy businesses, educational institutes have realized the need to equip pharmacy students with the managerial skills to run pharmacies (Davies, et al., 2013).

A study performed by (Paraidathathu, et al., 2011) identified a set of seven soft skills necessary to pharmacists in order to perform effectively:

- Ability to communicate clearly, sensitively and effectively with patients (including counselling and providing information on medications)
- Ability analyse and make decision to solve problems involving ethical issues
- Ability to recognise and respect the attitudes, actions and belief of others (patients)
- Ability assume responsibility
- Ability to receive constructive criticism in a positive manner
- Ability to maintain a proper attitude to work
- Ability effectively in a team and can collaborate well with other healthcare professionals.

When dealing with soft skills one may be tempted to come to the conclusion that soft skills are a phenomenon of the practical management area and the

academic world considers this notion a competence. This happens, on one hand, due to the popularity of soft skills among practitioners and on the other due to the fact that the academics use this concept in a conservative manner.

(Maize, et al., 2010) stated, in a work published in a review of remediation programs in pharmacy and other health professions programs, that data in the pharmacy literature are scarce. They also mentioned that if a set of preventive measures are taken the need for remediation will be minimized.

Success in pharmacy professional programs and in professional practice requires not only advanced scientific knowledge and technical skill but also a number of abilities (communication skill, empathy, professionalism, etc.) that may not be easily characterized by cognitive measures, i.e. "soft" skills and experiences that may become more evident the pharmacists' performance (Clavier, 2013). The ultimate goal of the pharmacy profession is to render pharmaceutical care in providing medication services to patients (American Association of Colleges of Pharmacy, 2011).

We must admit that there is great controversy regarding the acquisition of soft skills in general and throughout this paper we will try to show to what degree the soft skills can be enhanced in order to achieve performances. We chose the pharmacy area because little evidence regarding the soft skills was produced both in academia and private sector. If we are to understand the findings of our study first we must understand the importance of soft skills and how they develop. The literature gives us evidence regarding the importance of soft-skills development and what causes the acquisition of such skills, but we will try to focus more on the pharmacy field of activity.

We consider that the soft skills training should be continuous and in order for these to enhance they need to be practiced as often as possible.

Throughout the paper we analysed the following soft skills (Tietze, 2011) among the pharmacist of Dawacom:

- Communication skills
- People skills
- Leadership skills
- Customer skills
- Analytical skills
- Courage and integrity

a) *Communication skills*

Communication skills and our behaviour in society are quite important, and sometimes they can play a very important role in the life and / or our career, especially if we choose a profession where we deal with people, as is the case of the pharmacists.

If pharmacists manage to communicate effectively, they seem more organized; more informed

and will increase their chances of getting where they want in terms of career path. People who can effectively communicate easily explain useful and interesting ideas and are able to accept answers to others - whatever they may be.

Communication, if effective, was considered in some studies as one of the most important managerial skills (Rajabi, et al., 2013). Various competency models include issues related to communication. Many academic models related to communication make reference to: expression in the voice and face and emotional intelligence (Goleman, 2006). We can consider the communication one of the most conceptualised term in the academic world and it denotes a process where a sender transmits messages over a channel to a receiver whereas the message can be distorted by some noise (Robbins & Coulter, 2002).

The ability to communicate is a characteristic to every human being and is necessary to any profession. Because they deal with different categories of people the pharmacists have to be able to adapt their communication style to any patient they come across (Majzub, et al., 2010). They have to be able to overcome all barriers that may appear while interacting with patients and colleagues, they must be able to not to make negative assumptions regarding a patient or a doctor who prescribed a treatment, they must not be subjective in relation to patients and peers, and must not draw quick and undocumented conclusions. While communicating orally the pharmacists must meet all the characteristics of a good communicator:

- Be a good listener
- Transmit a clear message
- Use a language common to the interlocutor
- Provide an appropriate feed-back
- The non-verbal language has to be coordinated with the verbal one
- Ask the right questions (Beardsley & Kimberlin, 2012).

Communication skills are a fundamental component of this approach to care. A substantial amount of evidence demonstrates that patient-centered communication has a positive impact on important outcomes, including patient satisfaction, adherence to recommended treatment, and self-management of chronic disease (Levinson, et al., 2010).

b) People skills

In this category we included emotional intelligence (Goleman, 2006), generally defined as the overlap between emotion and intelligence, or more simply, the intelligent use of emotions (Galal, et al., 2012), teamwork and receiving criticism. According to (Westli, et al., 2010) teamwork has the following dimensions: coordination, information exchange, use of authority and assertiveness, assessing capabilities and, supporting behaviour.

The emotional intelligence includes a set of competences:

- *Emotional Awareness:* Recognizing their emotions and their effects. Those who hold this competency: know exactly what emotions feel and why, understand the links between their feelings and what they think, do and say, understand how feelings affect their performance, acknowledge and are guided according to their core values and goals.
- *Corect Self-Evaluation:* Knowing the personal limits and strengths. Those who hold this competency: are aware of their own strengths and weaknesses, learn by experience, praise the received feed-back, learn continuously and are interested in the personal development.
- *Self Esteem:* Those who poses this competency are self-confident, fight for what is right even if they risk to be criticised, are able to make decisions despite uncertainty.
- *Self Control:* Controlling the emotions and impulses. Those who possess this competence are able to successfully control their impulsive feelings and negative emotions, are optimistic and firm even during critical times, think clearly during stressful times.
- *Correctness:* Keeping the honesty and integrity standards. Those who possess this competence: act in an ethic manner, build relationships based on trust, are aware of their own mistakes and signal others' unethical behaviour.
- *Conscientiousness:* Taking responsibility for personal performances. Those who possess this competence: live by their commitments and keep their word, take responsibilities for meeting their goals, are organised and pay attention while working.
- *Adaptability:* Flexibility to change. Those who possess this competence: are multi-tasking, have a good reaction to change, are adaptable to any circumstance.
- *Innovativeness:* Are open and feel comfortable when they deal with new ideas and situations. Those who possess this situation: look for new opportunities in wide range of sources, find original solutions to their problems, and generate new ideas.
- *The Desire To Achieve:* The continuous effort to improve oneself and to reach a certain excellence standard. Those who possess this competence: are results oriented, set competitive goals, search for information in order to minimize the uncertainty, look for ways to improve their performances.
- *Loyalty:* Alignment to the company's goals and values. Those who possess this competence: are

able to make sacrifices for the good of the company, find a purpose in the company's mission.

- *Initiative*: Availability to opportunities. Those who possess this competence: are ready to take advantage of opportunities, cross the line when necessary.
- *Optimism*: Perseverance in meeting the objectives despite the obstacles and roadblocks. Those who possess this competence act due to hope and not to fear of failure [adapted from: (Goleman, et al., 2013), (Urch Druskat, et al., 2013), (Ryback, 2012), (Zeidner, 2009), (O'Boyle., et al., 2011), (Ciarrochi & Mayer, 2012)].

c) *Leadership skills*

The divers set of challenges and opportunities facing the pharmaceuticals sector leads to the conclusion that new sales models, approaches and practices are needed. Reality is not the same everywhere in the world, but the increasing complexity of markets requires new skills and strategies to benefit from the new opportunities. Renewed marketing strategies must consider carefully each stakeholder group and prepare effective action. Pharmacists must truly understand that stakeholder concerns are to influence, develop new offers for sale approaches and find ways to reach them.

Leadership competencies in community service, in our case pharmacy, include not just knowledge and attitudes but also a broad set of skills such as coalition building, policy advocacy, fundraising, program planning, motivation, and facilitation—skills not taught in the medical curriculum (Goldstein, et al., 2009). In a study performed in 2009 Goldstein et al. identified the following leadership frameworks:

- Understanding the importance of developing a transforming vision to guide leadership goals;
- Understanding the value of different leadership styles;
- Using self-reflection to identify personal strengths, weaknesses, values, and ethics;
- Identifying and connecting with appropriate role models and mentors and practicing networking skills;
- Focusing on team-building skills (i.e., facilitation, giving and receiving constructive feedback, conflict resolution);
- Learning and applying media and policy advocacy skills necessary for structural change (i.e., preparing and giving presentations, organizing community activism, using the media);
- Demonstrating collaboration and fundraising skills needed for successful leadership; and
- Demonstrating commitments to community-based leadership by applying learned skill sets to service projects rooted in the community (Goldstein, et al., 2009).

The leadership skills of pharmacists include: conducting regular meetings with direct reports to discuss performance and achievement of objectives, giving others direct, constructive feedback which can be used to achieve goals, leading people and allocating tasks to achieve goals, empowering others to achieve results and holds them accountable for actions, motivating others in order to reach organizational goals, influencing others in a way that results in acceptance, agreement, or behaviour change, customer skills (Johansen & Johansen, 2012), (Lindegaard, 2010).

The customer skills are reflected in the customer satisfaction, which has become one of the key issues for companies in their efforts to improve quality in the competitive marketplace (Kärnä, 2004) and the pharmacy industry makes no exception. The pillars of the customer skills analysed in this paper are: understanding who the department's customer is and communicates that priority consistently, becoming more effective in satisfying customer needs, pursuing the best customer-focused responses that add value to the business.

d) *Analytical skills*

For this section we considered the followings dimensions: selecting the appropriate techniques for analysis, interpreting financial data, reports, balance sheets, and cash flow analysis and generating alternative solutions to problems and challenges. By default, due to their profession the pharmacists have analytical skills. These are characterised by their orientation towards prevention and anticipation.

The analytical skills of pharmacists can be developed by tasks involving action. Analytical skills help the practitioner identify the problems, plan interventions and conduct evaluations (Hardina, 2002). According the literature the analytical skills of pharmacists are developed during college years and have been identified as an essential outcome of pharmacy education (Cisneros, 2009), this would mean if there are pharmacists who don't possess such a set of skills than, with appropriate training they could acquire analytical skills.

The ability to think, including logical thinking, analytical thinking, problem solving and decision making, was identified as an important outcome for pharmacy education (Oderda, et al., 2010). The use of critical thinking, identified by the commission as a competency of the thinking ability outcome, is assumed to result in pharmacy practitioners that are better able to solve problems and think as experts (Oderda, et al., 2010).

e) *Business skills*

For this section we considered the following dimensions: aligning resources to meet the business needs of Dawacom, understanding the costs, profits, markets, and added value of the department and how

those contribute to the success of Dawacom, looking at the "big picture" of the company's goals, rather than individual department's needs and anticipating marketplace opportunities and supports speed to market.

The role of pharmacists has expanded from that of compounder and dispenser of medicines to encompass clinical elements around safe and effective medicines use (Jacobs, et al., 2011). The organisation of community pharmacies varies between countries; however, they commonly offer a range of healthcare and non-healthcare products alongside dispensing medicines, and operate in a business environment whilst regulated by professional and governmental policies and statute (Jacobs, et al., 2011).

The business skills for pharmacists refer to the ability of a pharmacist to put into practice his/her ideas. These competences depend on the creativity, innovation, taking risks, the ability to plan and manage projects in order to meet the goals. These also include motivation and determination to meet the objectives. Business skills are also useful to avoid errors in pharmacies. A strong set of business skills leads to good performance on the job.

f) *Courage and Integrity*

While the ancient Greeks described courage as a desired response to physical danger, contemporary scholars more frequently address courage as the commitment to stand up for/act upon one's ethical beliefs - an essential virtue for all healthcare professionals today (Murray, 2010).

Before identifying problems, a pharmacist must have a thorough knowledge of the values and ethics associated to their practice (Hardina, 2002). We consider the concept of "integrity" in the pharmaceuticals area to refer to being honest and not to use false information, to keep the confidentiality of data both when dealing work related issues both at work and in personal life. The ethical values and principles create a framework for an honest behaviour. Throughout the paper we will consider the following pillars supporting the integrity: moral values (e.g. honesty), motivation (e.g. desires, interests and ideals), commitments (e.g. both spoken and to one's self), qualities (e.g. perseverance), firm position (even in front of oppositions), obeying the law, open-minded and always ready to analyse new information.

From all the above we can conclude that the activity of a pharmacist must not be compromised by personal or third parties' interests, or by financial reasons. Also a pharmacist should never find himself/herself in association with reports, communications or other information when these are considered to contain: false or wrong declarations, information delivered in an incautious manner, etc.

An important characteristic of the integrity is accountability and considering the public good (in this

case of the patient), and in order for the pharmacist to gain the trust of the patients the integrity must be in the centre of the professional life due to the need of aligning the performances to the needs of the society. The integrity refers not only to the professional life, but to the professional life, too, as sometime the behaviour in the personal life if scandalous can damage the professional activity.

The culture of an organization has impact on the manner in which the employees behave, as integrity breeches generate unethical practices and have a devastating effect on the activity of a pharmacy and on its relation with the patients. The integrity is not only a personal value it also is an organizational value. An upright pharmacist will not act only according to his/her personal values. An upright person is aware and takes into account the needs of the organization during the decision making process.

An extension of integrity is the courage, which we will refer to as doing what needs to be done regardless of the consequences.

g) *Training and development*

In most organization the training takes place on the job because this is easier to implement them and the costs are lower (the managers have to assign a person to train the juniors which is cheaper than paying a trainer to perform the training). Training on the jobs has disadvantages, too, such as: disrupting the workplace, errors that take place while learning. There also are skills that cannot be acquired through on the job training and they have to take place in organized setting, outside the work site. (Robbins & Coulter, 2002) proposed a list of training examples, both on-the-job and outside-the-job:



Table 1 : Employee training methods (Robbins & Coulter, 2002)

Examples of On-the-Job Training Methods	Job rotation	Lateral transfers allowing employees to work at different jobs. Provides good exposure to a variety of tasks
	Understudy assignments	Working with a seasoned veteran, coach, or mentor. Provides support and encouragement from an experienced worker. In the trades industry, this may also be an apprenticeship
Examples of Off-the-Job Training Methods	Classroom lectures	Lectures designed to convey specific technical, interpersonal, or problem-solving skills
	Films and videos	Using media to explicitly demonstrate technical skills that are not easily presented by other training methods
	Simulation exercises	Learning a job by actually performing the work (or its simulation). May include case analyses, experiential exercises, role playing, and group interaction
	Vestibule training	Learning tasks on the same equipment that one actually will use on the job but in a simulated work environment.

Often training is directed towards enhancing the technical skills and job-specific competencies of the employees ignoring the soft skills or giving them a secondary importance.

The literature and academic researches bring evidence that if the training programs are comprehensive the employees have better performances on the job. One such study was performed in by Jukova and Constantine (1998) quoted in (Björkman, 2000).

The field of human resources development is a complex one and includes: informal education and formal education. The human resources development could be generally defined as a set of practices within an organization which have the purpose of enhancing the contribution of the human resource to the success of the organization. The human resources development became a priority for any organization. The human resources development is based on a variety of human resources processes such as: assessing the performances, attracting the resources, identifying the individuals who have potential, etc. The beneficiaries of the human resources programs are: the employees, who grow on the career path, promote within the enterprise, receive raises, acquire valuable knowledge, and the organization who directly gains better human capital. The human resources development involves: employee training and development, career management and organizational development. The human resource is vital for the organization because in this competitive environment, where the organizations have to face different challenges, the human capital became a valuable asset in the continuous struggle to obtain a certain competitive advantage. The human

resources development is important for the organization because the innovative ideas, the quality and the continuous improvement, as well as other elements, necessary to compete in the modern business environment, come from human beings and not from machines. We consider two important factors that make it difficult to attract new skills in the company and using them:

- The human resources professionals have to foresee and to rise to the expectations of the stakeholders (employees, employers, unions, manager, etc.)
- The human resources professionals face difficulties in imposing themselves as decision makers in the development of the organization, as the vast majority of the organizations outsource the human resources services.

Competency in pharmacy is the ability of the pharmacist to make deliberate choices from a repertoire of behaviors for handling situations and tasks in the specific context of professional pharmacy practice by using and integrating knowledge and personal values in accordance with professional role and responsibilities (Meštrović, et al., 2012). By assessing the differences between the current and desired levels of pharmacist competencies, it is possible to develop an individual education plan aimed at achieving the desired level of competency (Meštrović, et al., 2012). Knowledge, skills, attitudes, and personal pharmacist values thereby receive real significance through experience in practice. Educational programs therefore should focus on integrating knowledge and practice (Meštrović et al., 2012).

Perhaps ironically, concerns about the interface between pharmacy practice and business were not an especially significant issue during much of the twentieth century (Tootelian, et al., 2012). The "drugstore" was a business. It was engaged in many activities in addition to dispensing prescriptions. The pharmacist and his or her employees did everything from dispensing prescriptions to selling household wares to making ice-cream sodas (Tootelian, et al., 2012). To be sure, there were some who considered all non-clinical activities to be unprofessional (Tootelian, et al., 2012). Yet, while clinical functions have always been an integral part of the business, most recognized the fact that the drugstore owner-pharmacist served in many varied roles (Tootelian, et al., 2012).

(Tootelian, et al., 2012) stated that usually within communities pharmacists are considered one of the most respected professionals for four reasons:

- First, they are more accessible than other health professional (e.g. they do not need appointments)
- Second, they have experience in consulting with patients regarding major issues in the medication administration (e.g. side effects, dosage)
- Third, they do not charge for their services
- Forth, they are less intimidating than doctors and don't have a history a baring bad news
- Finally, they are closer to common people, as they are perceived both as heath care professionals and business people.

Being closer to the community makes it imperative for the pharmacists to possess a strong set of soft skills and if they don't have it, they need training to enhance these skills. Often pharmacists are associated to self-medication and due to this fact pharmacists need to be able to recognize the symptoms, choose an appropriate product and explain the direction for use to the patients and advise them on monitoring the desired effects and side effects (Wilbur, et al., 2010). A study performed by (Wilbur, et al., 2010) showed that eighty-percent of patients consider that pharmacists give reliable advice about drugs as compared to other health-care professionals (e.g. nurses), seventy-six percent would be comfortable receiving medication directly from a pharmacist. Pharmacists are a key component of health care systems and they need to work with other health care professionals to develop the best plan for the patient (AbuRuz, et al., 2012) and in order to perform well on the job they need to communicate very well with the patients, peers and other care givers, they need to possess strong analytical skills in order to identify problems, they need strong people skill in order to link with the patients and peers, they need to be honest about their work, and leadership and business skills if they are in charge with a pharmacy. When they do not have these skills measures to acquire them are to be

taken. As showed in a study performed by (Hertig, et al., 2011) practitioners prefer clinical functions with greater direct contact with the patient and are willing to accept change and, therefore, will be integral to developing and implementing practice models, which will make it easier for Dawacom to train its employees in order to acquire more soft skills and perform better on the job.

h) Knowledge management and the learning organization

The "learning organization" is perhaps best known challenge of postmodern approaches within organizations. Peter Senge consider by the vast majority of scholars to be the father of this concept stated that the values of the learning organization and the competitive advantage derive from the continuous learning, both individual and collective. He recommends the leaders to challenge the individuals to continuously consider the needs of the organization. The purpose is to induce in them a powerful intrinsic motivation. By applying these ideas referring to the performance of the individuals and groups, the leaders can create incentives through which the employees' affection towards the organization is increased. The learning organization is that organization where people continuously develop their capacity of obtaining the results they want, where new models are encouraged and where the employees learn together how to learn (Cors, 2003).

Such an organization should have the following characteristics:

- To offer continuously new opportunities to learn to its employees
- To use the training programs to meet its goals
- To find the link between individual and organizational performance
- To encourage the dialogue and to create an environment that encourages the employees present their opinions and take risks
- To use creative tensions as a source of energy and re-engineering
- To always be aware of the environment where it has its activity.

The learning organization involves new managerial directions in terms of: long and short term planning, team work, cooperation, diversity, power of attorney, focus on employees and leadership. The organizations which don't create an organizational culture based on learning, will not adapt quick enough and will not face the challenges from their environment. While fro many organizations the concept is clear and put into practice, for others it creates confusion. The learning organization is a philosophy, not a program. The learning organization is continuously evolving. One cannot say: I'm here! I've created such an organization!

Inside an organization, Knowledge Management is the management of organizational knowledge in order to create value in the business and generate benefits in a competitive environment that is in continuous change. Knowledge Management refers essentially to obtain knowledge by the right person at the right time. It also means the ability of an organization to learn and to use the knowledge when needed. This may not sound so complex, but requires a strong link with the corporate strategy and understanding of forms in which the information exists. In the organizations where we find it, knowledge management involves creating organizational processes and functions accepted and supported by all members. Knowledge Management may include the creation of new knowledge, and can focus solely on sharing knowledge, sharing and refining them. So, the organizational processes involved in the knowledge management include the ability to process the data and information with the help of technology and the employees' ability to innovate and be creative. The purpose of knowledge management is to use the existing and available knowledge within an organization in the most efficient manner in order to create new useful knowledge.

The knowledge management helps the organization:

- To improve and focus its development efforts according to its needs
- To learn from its past mistakes and success
- To better explore its knowledge and to use them in the area where the organization needs them
- To promote a long term approach regarding the skills development and eliminating the old knowledge
- To innovate
- To protect its knowledge and key competences.

III. METHODOLOGY

The present research can be defined at a primary level approach as a practical study designed to diagnose a fact. The central objective of this paper is to investigate the effects of soft skills upon the performance of the employees at Dawacom. Qualitative research involves the collection, analysis, and interpretation of data that are not easily reduced to numbers. These data relate to the social world and the concepts and behaviors of people within it (Anderson, 2010). Health care involves complex human interactions and these demand complex understanding and qualitative research can be extended by the use of qualitative methods (Anderson, 2010). The final aim of administering this survey to the employees of the Dawacom was to be able to propose the right training that could help the employees enhance their performances. Empirical evidence is similarly important as it indicates whether the training and education approach works in practice, which is the ultimate goal of

training. A training program that does not work in practice is of limited value (Puhakainen & Siponen, 2010). In order to obtain accurate data we used both open questions and multiple choice questions. Most of the multiple choice answers were on the Likert scale (Boone & Boone, 2012), (Chomeya, 2010), (Chimi & Russell, 2009,). The Likert scale is the most known and used ordinal scale in studies. It is widely used in research studies and is sometimes referred to as rating scale although it is not the same thing. The main feature of an ordinal scale is that it allows measuring the difference but not the specific difference between the measured quantities. Ordinal scale does not allow arithmetic operations on categories. We aren't able to specify what is the size difference between the two categories because, we cannot quantify the clear difference between "satisfied" and "very satisfied" in the mind of the subjects.

We used dichotomist questions (e.g. with only two answers to choose from) and multi dichotomist questions (three or more answers to choose from). In the last category are also included the Likert scale questions mentioned above. We included questions to identify facts, knowledge, opinions and also questions to check the accuracy of the answers. The questions are intended to generate both one variable and more variable in order to offer us a wider range to interpret and analyze the data. We decided to alternate the questions in order to prevent the subject from abandoning before completing all the questions, especially because there are a great number of questions.

The questionnaire was composed of four parts, from A to D. In section A by using 30 questions we intended to identify the general perception of the subjects regarding the training they attended at Dawacom. The opinions of the employees matter that is why we used the first section to find out the level of training needs in the organization. We tried to measure key dimensions of employee opinions, attitude, engagement, satisfaction, loyalty, training needs, and organizational effectiveness. In section B the purpose of the four questions was to find out whether the employees at Dawacom considered the training to have helped them improve their performances. The purpose of section C was to find out what is the subjects' point of view regarding the soft skills of pharmacists: their needs and their self- assessment; for this section we used 47 questions. In this section of the questionnaire the employees were asked to rate the importance of different work-related skills. After indicating the importance of each skill, they were asked to select which skill they would like to receive training on. They are also asked to provide suggestions on how this skill can be improved. The purpose of these questions was to identify what the employees need and if they can be included in those of the company, also we tried to

involve the employees in the assessment process by requesting suggestions. The last section, i.e. section D, is complementary to the previous section by its purpose, that offering the subjects another opportunity to assess themselves in terms of frequency and effectiveness of soft skills, i.e. how often they observe the use of certain skills and how useful they were to their activity at Dawacom. This section includes 15 questions. Continuing from the previous section, in the last one we tried to investigate the needs of the human resources within Dawacom, and explore what can be done to provide them with the resources and materials they need in order to do their job the most effective way possible.

In elaborating the questionnaire we used in our research we followed the indications of (Dörnyei & Taguchi, 2010) and (Ebrahim & Bowling, 2005). The technique used was by sending the questionnaire online. We chose this technique because: of the low costs, eliminating the operator's possible influences, the correct registration of the answers, keeping the subjects anonymous, giving the subjects enough time to think and ensuring a greater number of employees who filled in the survey.

The general purpose of the survey used in our research was to assess the training needs of the employees at Dawacom. In general the assessment process is used to diagnose the need of training that is to take place in an organization. Our survey gathers data that helped us determine what kind of training is needed in order to help the employees and the organization meet their goals and objectives. We tried to identify any gaps that there are between what soft skills an employee at Dawacom has and what kind of soft skills the company needs in order to be competitive. In developing a research project it is also required a reflective approach coming from the researcher's part. She/he will have to identify him/her personal interests in relation to the studied subject, resources available, and skills with which he/she can create research instruments. We will conclude by saying that the creation of a research design supports a personalized approach that leaves room for new approaches, and this is also the case of the research on Dawacom.

We must mention here that this piece of academic research is principally focused on the Pharmacy sector only due to the limited word count and time constraints; but we intend to expand it in the future to include "benchmarking against training excellence in other industries".

IV. FINDINGS

Initially we questions a sample of 65 employees of Dawacom's employees, but out of these only 50 answers were validated; the 15 answers invalidated were either incomplete or belonged to other categories

of employees than the pharmacists, which are our target group. The data was gathered online, after having talked to the subjects in advance.

Below are the centralized results:

- Demographic results:
 - 26% of the subjects had leadership responsibilities
 - Most of the employees have less than 5 years' experience in the field
 - Almost half of the employees underwent training within the company
- Statistics regarding the employees who underwent training:
 - Only 10% employees underwent training on soft skills; out of whom only 7% were confident about the necessity of the training
 - 8% employees underwent external training while the rest of them took part in in-house training
 - 5% of the employees are familiar with the procedures that the company uses to identify the training needs
 - All the employees consider the training to be necessary for them improve their performance
 - The employees took part in both on the job (50%) and off the job training (50%)
 - On the job training involved job rotation
 - Off the job training involved lectures
 - The majority of training sessions were 1 day long
 - Only 20% of the employees stated that their manager help them in selecting the training they need
 - None of the employees was totally satisfied with the training sessions and most of the consider that the duration of the training is not sufficient
 - 90% consider that the training techniques are not effective
 - 90% are neutral towards introducing training that include programs for developing interpersonal skills and human values
 - 30% of the employees stated that the training helped them change the department, 50% stated that the training helped them face the challenges, and 20% stated that the training helped them rectify day to day errors
 - 80% of the employees consider that the training should take every month
 - All of the employees said that the basis of the training was to appraise performance
 - Only 20% of the employees stated that they were totally aware of the objectives of the training
 - All of the employees stated that the training did not meet their expectations and considered that

- the trainer had little knowledge about the objective of the training
- o All of them considered that the training had little relevance to their situation, 90% considered that the training did not remain relevant in time, 100% considered that the training was not important to their development
- o 100% considered that their improvement was partially noticed by the colleagues and managers
- o 70% rated the training as being of a fair quality
- o 90% of the subjects said that they feel more competent professionally after the training and that the training program enhanced self-confidence to take challenges; their status was also improved in the company
- o Most of the employees enjoyed the role plays
- o All the employees would recommend the training they attended to their peers
- o Time management and communication skills are the skills considered to be the most beneficial to the pharmacists' activity but the employees failed to explain why they consider these skills important
- o Most of the employees considered that the pharmacists, in order to perform effectively, need pharmacy skills and patient skills
- o 30% of the subjects considered that self-confidence is the skills they already have, 20% said pharmacy knowledge and the rest of them stated skills related to human interaction
- o When asked what other skills they need, the subjects stated that they need the following skills:
 - Stress management
 - Patience
 - Listening skills
 - Better reaction to customers' needs
- o When asked about the logistical requirements, the subjects gave answers out of the topic (e.g. patience, smart, etc.)
- o 80% of the subjects consider that it is important to establish and monitor goals
- o 70% consider that it is important to encourage collaboration among fellow employees and departments to achieve results, to seek and use opportunities for continuous learning and self-development, to understand and achieve company's mission and values, to respond to the company's changes, take appropriate and timely action to overcome unexpected hurdles or obstacles to a plan or project, express loyalty and dedication to the company in interactions with others, to set up and monitor time frames
- o 70% of the employee who took part in our study considered encouraging collaboration among fellow employees and departments to achieve results to be the most important skills, 10% seeking and utilizing opportunities for continuous learning and self-development, 10% Responding to a changing organization, and 10% understanding and seeking to achieve the company's mission and values
- o In order to improve these skills the subjects made the following suggestions: choose good leaders facilitate the communication between the managing department and other departments opportunities are coming once so getting benefit from them is an important training to take team work is an important way to achieve goals coordinate with team and divide the tasks among all employees will help in getting results coordinate with team and divide the tasks among all employees will help in getting results hiding all problems that annoy customers rereading the plan every day help in accomplish goals wrote in it encouraging employees will make them give their better for the company rewards always make employees increase their faith to the company cooperation between employees and good team working will give excellent results
- o 70% of the subjects consider listening to be very important in their line of activity and suggested the following measures: how to have an interest to help people and offering all what they need is a valuable training listening to customers will encourage them to come fluently to the pharmacy when you give attention to patients, they feel that you care about them when you give attention to patients, they feel that you care about them listening and give attention to customer will make them feel of care giving attention to customers and show them that you are care will make them come again and again
- o 70% of the subjects considered that recognizing and rewarding people for doing their best is important
- o constructively receiving criticism and suggestions from others is important for 70% of the subjects, who also think that recognizing and rewarding people for doing their best is a skill that they need to improve
- o giving feedback is important for only 50% of the subjects
- o Leading people and allocating tasks to achieve goals is important for 80% of the subjects
- o Empowering others to achieve results and holds them accountable for actions is important for 70% of the subjects

- Motivating others in order to reach organizational goals is important for 60% of the subjects
- Influencing others in a way that results in acceptance, agreement, or behavior change is important for 80%
- 40% feel that empowering others to achieve results and holds them accountable for actions is the leader skill most important to receive training on
- 90% of the pharmacists are aware understanding who the department's customer is and communicates that priority consistently is important
- 70% consider that becoming more effective in satisfying customer needs is important and they need to improve this customer skill
- 90% consider that pursuing the best customer-focused responses that add value to the business is important
- 70% are aware of the importance of understanding the costs, profits, markets, and added value of the department and how those contribute to the success of the company and prefer looking at the bigger picture than the individual goals
- Interpreting financial data, reports, balance sheets, and cash flow analysis is considered the most important analytical skills by 70% of the subjects

Below we present the results in terms of frequency of skills usage:

- Required technical knowledge - most occasions (70%)
- Understating of client environment - most occasions (80%)
- Communicating the importance of knowledge of roles and responsibilities and standards to team members - many occasions (40%), most occasions (40%)
- Demonstrating the importance of quality by reviewing work products of others and making necessary modifications - most occasions (70%)
- Demonstrating critical thinking skills to understand business issues relevant to client and project - 70% most or moderate occasions
- Presenting information and data candidly, accurately and completely 60% most or moderate occasions
- Recognizing and taking action when issues require additional consultation and/or escalation - 70% most or moderate occasions
- Willingness to take a position that challenges the prevailing opinion - 70% most or moderate occasions

- Understanding professional standards and the importance of regulatory responsibilities - 80% most or moderate occasions
- Setting a positive example in timely adherence to: internal and external compliance responsibilities - 80% most or many occasions
- Preparing concise, well written documents using appropriate business and technical language - 90% most or many occasions
- Understanding and conveying the importance of communications - 80% most or many occasions
- Providing clear and concise instructions to others - 70% most occasions
- Sharing insights to enhance process and results - 10% all occasions, 60% most occasions
- Playing an active role in discussions and meetings, providing substantive input delivered at the appropriate time - 80% most and many occasions

Below are the results in terms of effectiveness:

- Required technical knowledge - moderate effectiveness (70%)
- Understating of client environment - moderate effectiveness (60%)
- Communicating the importance of knowledge of roles and responsibilities and standards to team members - moderate effectiveness (70%)
- Demonstrating the importance of quality by reviewing work products of others and making necessary modifications - moderate effectiveness (70%)
- Demonstrating critical thinking skills to understand business issues relevant to client and project - moderate effectiveness (60%)
- Presenting information and data candidly, accurately and completely - moderate effectiveness (70%)
- Recognizing and taking action when issues require additional consultation and/or escalation - moderate effectiveness (60%)
- Willingness to take a position that challenges the prevailing opinion - moderate effectiveness (60%)
- Understanding professional standards and the importance of regulatory responsibilities - moderate effectiveness (60%)
- Setting a positive example in timely adherence to: internal and external compliance responsibilities - moderate effectiveness (60%)
- Preparing concise, well written documents using appropriate business and technical language - moderate effectiveness (80%)
- Understanding and conveying the importance of communications moderate effectiveness (70%)
- Providing clear and concise instructions to others - moderate effectiveness (70%)



- Sharing insights to enhance process and results - effectiveness (70%)
- Playing an active role in discussions and meetings, providing substantive input delivered at the appropriate time - moderate effectiveness (80%)

In order to make sure that the maximum value is extracted from the results presented above we adopted a validator approach to our primary research. This approach helped us identify a number of contradictions (as mentioned above); these contradictions create opportunities for business improvement and organizational development such as: addressing the customer's "latent needs" or organizational "hidden norms".

V. DISCUSSIONS

a) Analysis of internal and external environment of Dawacom

From the management point of view the assessment of the needs at organizational level is the solution to harmonize the needs and ambitions of the employees with those of the organization. The assessment can be seen as a tool to support the decision taken process in order to allocate the resources. We must take into consideration the fact the resource allocation is a central part of the organizational planning. The purpose of the needs assessment is to:

- Provide the necessary information to develop and improve a training program
- Reengineer the organization in order to improve the performance function of the identified objectives
- Establish the criteria to contract the training services / human resources development services
- Identify the solutions to some complex problems.

The SWOT analysis is one of the methods most often used to analyze and assess the training needs of an organization. The name of this analysis model means: strengths, weaknesses, opportunities and threats. The SWOT analysis was successfully implemented as methods of organizational assessment. The strengths and weaknesses make reference to the internal environment of the organization; these represent intrinsic characteristics of the organization's internal state of affairs. The opportunities and the threats make reference to the external environment, i.e. what happens outside the organization and influences its activity. The context may either favor or block the activity of the organization.

Analyzing the results of our research we identified the following strengths, weaknesses, opportunities and threats regarding the training and human resources development within Dawacom:

- Strengths
 - Training are both on the job and off the job

- The employees enjoy parts of the training, e.g. role plays
- 90% of the employees feel confident regarding their professional development as a result of training
- Weaknesses:
 - Few employees are familiar with the company's procedures of identifying the training needs
 - The training is not very varied
 - The training techniques are not effective
 - Employees are not aware of the objective of the training
 - Employees don't consider the training relevant to their role in the company on the long run
 - Training is considered to be of very low quality
- Opportunities:
 - Training helps the employees face challenges and promote
 - The employees are open to attending training
 - The employees are open to express their need by offering suggestions on how to improve certain sets of skills
- Threats:
 - Few employees understand the necessity of training on soft skills (7%)
 - Employees are neutral to introducing training meant to enhance soft skills
 - Employees consider self-confidence to be a skill

Usually the training needs assessment in an organization has as main objective solving certain problems. The above analysis offers information which can be used to make decisions and valid recommendations regarding the training. The SWOT analysis of the training needs helped us assess the degree of satisfaction of the employees within Dawacom with respect to their skills enhancement. This analysis also helped us assess the quality of previous training suggesting areas of improvement. A logical utility of such an analysis is also to make sure that the direct costs related to the training are justified.

b) Meaning of the results

Due to the percentage of subjects we consider the results to be representative for Dawacom. As we can see in Figure 1 half of the employees have been working at Dawacom for approximately 3 years which can suggest either a high fluctuation of employees or that the company grew year by year and the growth is reflected in the increase of the number of employees. Due to the limited access to the company's files and limited time to undergo a more thorough research we could not state for sure what is the case of Dawacom when it comes to its employees' seniority in the company.

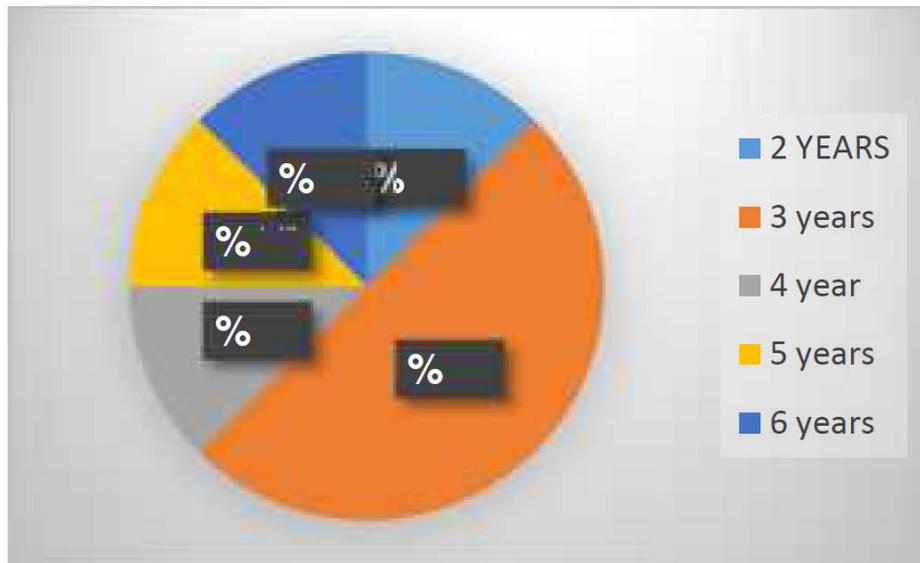


Figure 1 : The experience in years of Dawacom's employees

When analyzing the number of the employees who underwent training within the company we observed that only 10% of them took part in training on soft skills. As we stated in the literature review chapter the soft skills (irrespective of their kind) are very important for the pharmacists or any other kind of professionals. The fact that such a small number of employees within Dawacom attended training on soft skills suggests that the management team is not very aware of the importance of enhancing the soft skills of its employees; and having only 7% of the employees who consider that the training were necessary suggests that the training did not meet their objectives. An explanation regarding this issues might be that often the training is in-house training and the trainers might not be professionals.

The 5% of the employees who are familiar with the company's procedures of identifying the training needs, could mean that the company is not open towards the employees with respect to this issue. The communication from the management team to the employees is filtered and important information is not passed to the latter group.

We also identified a paradox: on one hand the employees stated that they are not able to identify the necessity of the training they attended and on the other hand they also stated that the training are necessary for them to improve their performance. This suggests that either the employees were not honest when they answered the questions and gave the answers they were supposed to give or they simply were not satisfied by the training they attended. The latter possibility is supported by the answers of a set of questions: on one hand the employees stated that they were not satisfied with the quality of the training they attended and on the other hand they feel that the length was not enough. We

this that the employees gave this answers for two reasons:

- Due to the short length of the training they did not gather enough knowledge
- The quality of the training was not a satisfactory one

The answers regarding the type of training they attended suggest that the subjects were not very familiar with a wide variety of training categories. When we analyzed their open answers we observed that the answers were similar stating that they attended job training or lectures; and most of these being one day long would explain why they employees feel that they don't find the training useful: the training might just scratched the surface regarding the enhancement of the employees' skills.

The fact that only 20% of the employees were helped by their managers in selecting the training they attended makes us wonder what is the procedure of selecting and attending a training within Dawacom.

For this situation we identified two possible explanations:

- There is not a clear procedure regarding the training within Dawacom
- The employees don't want to give credit to their managers regarding their professional development.

The questionable quality of the training was also supported by the fact that 90% of the subjects considered that the training techniques were not effective in their situation, 90% don't consider it to be useful to introduce other training sessions, not even for developing interpersonal skills and human values.

We also identified a set of answers that contradict the opinion regarding the poor quality of the training that the subjects have regarding the training

they attended at Dawacom: the employees stated that the training helped them change the department, face the challenges and rectify day to day errors. When someone states that he/she did not find a training useful and latter states exactly the opposite it rises a pertinent question: did the subjects gave accurate answers?

The fact that the training length is short is also supported by the answers of 80% of the subjects who consider that the training, in order to be effective, should be organized every month, Unfortunately the subjects did not understand the main core of the training; they consider that the training are to appraise performance and only 20% of them are aware of the objectives of the training they attended. What is even more serious is that the trainers made the impression that they are not aware of the objectives of their own training.

We also observed a pattern regarding the training organized by Dawacom for its employees, i.e. the employees consider the training to be irrelevant for their activity, 90% of the subjects stated that the training didn't prove to be relevant in time and all of them stated that the training did not help them in their professional development. Although the literature brings arguments to the importance of training, at Dawacom the management seems not to be aware of this fact. We could say that if a training is not important for a certain job, then soon that job won't be important anymore, is the company ready to give up pharmacists?

The questioned employees don't feel confident about their improvement as a result of the training they attended; this can be explained in two possible ways:

- They did not used the new skills at all
- The training did not help them improve themselves from a professional point of view.

We also tried to identify what the employees feel about time management and communication skills. According to the results of our investigation it seems that although they heard about the two concepts, the subjects don't clearly understand their meaning. A testimony to this affirmation is that they could not explain why they need time management and communication skills and why are these skills important for a pharmacist. Another issue that all the subjects agreed on is the category the skills they need. When asked what other skills they need in order to perform effectively they gave examples of soft skills.

Unfortunately the subjects do not distinguish between character traits and skills, 30% of them consider self-confidence a skill. According to Jeffries (2005) cited by (Ma, 2013) "self-confidence is conceptually defined as trusting the soundness of one's own judgment and performance", whilst a skill is a learned ability. Another area of confusion for the subject was the related to the logistical requirements; they consider patience and intelligence a logistic requirement, which is not the case since according to

(Merriam-Webster, 2004) the logistics requirement means: the procurement, maintenance, distribution, and replacement of personnel and materiel. The purpose of such questions was to identify what other needs the subjects had. Their answers suggest that they did not pay the necessary attention to the questions and since they knew that the main focus was on the training within Dawacom they imagined that we were expecting a training related answer.

If we are to create the profile of the employee at Dawacom based on the date we obtained he/she should look like this: very good pharmacy skills, good soft skills, patient, team-player and able to manage time effectively. Unfortunately at this point the questioned pharmacists don't meet all the criteria especially due to the lack of soft skills.

A more complete view on the skills of the pharmacists of Dawacom results from the analysis of the frequency of using some soft skills and the effectiveness of this usage. We quantified these in Figure 2 and Figure 3. By analyzing the above mentioned figures we conclude the following: although the profession of pharmacist requires a solid set of pharmacy knowledge, the subjects accept the prevalence of soft skills in their work. We consider that a training that would connect the pharmacy skills with the soft skills would be highly appreciated. The data analysis also shows a certain consistency of the employees' options for a variety of soft skills, most of them similar.

Most of the questioned subjects stated that the skills that they most frequently use are preparing concise, well written documents using appropriate business and technical language, understanding the client's needs, understanding professional standards and the importance of regulatory responsibilities, setting a positive example in timely adherence to: internal and external compliance responsibilities, understanding and conveying the importance of communications and playing an active role in discussions and meetings, providing substantive input delivered at the appropriate time are the skills that they use most frequently during their work. As we can see these skills are mainly related to the client - pharmacist interaction, and the involvement of the company in this relation by providing training that could enhance these skills could increase the company's competitiveness. Our analysis does not reveal the significant differences between the skills and a classification of their importance in the activity of a pharmacist.

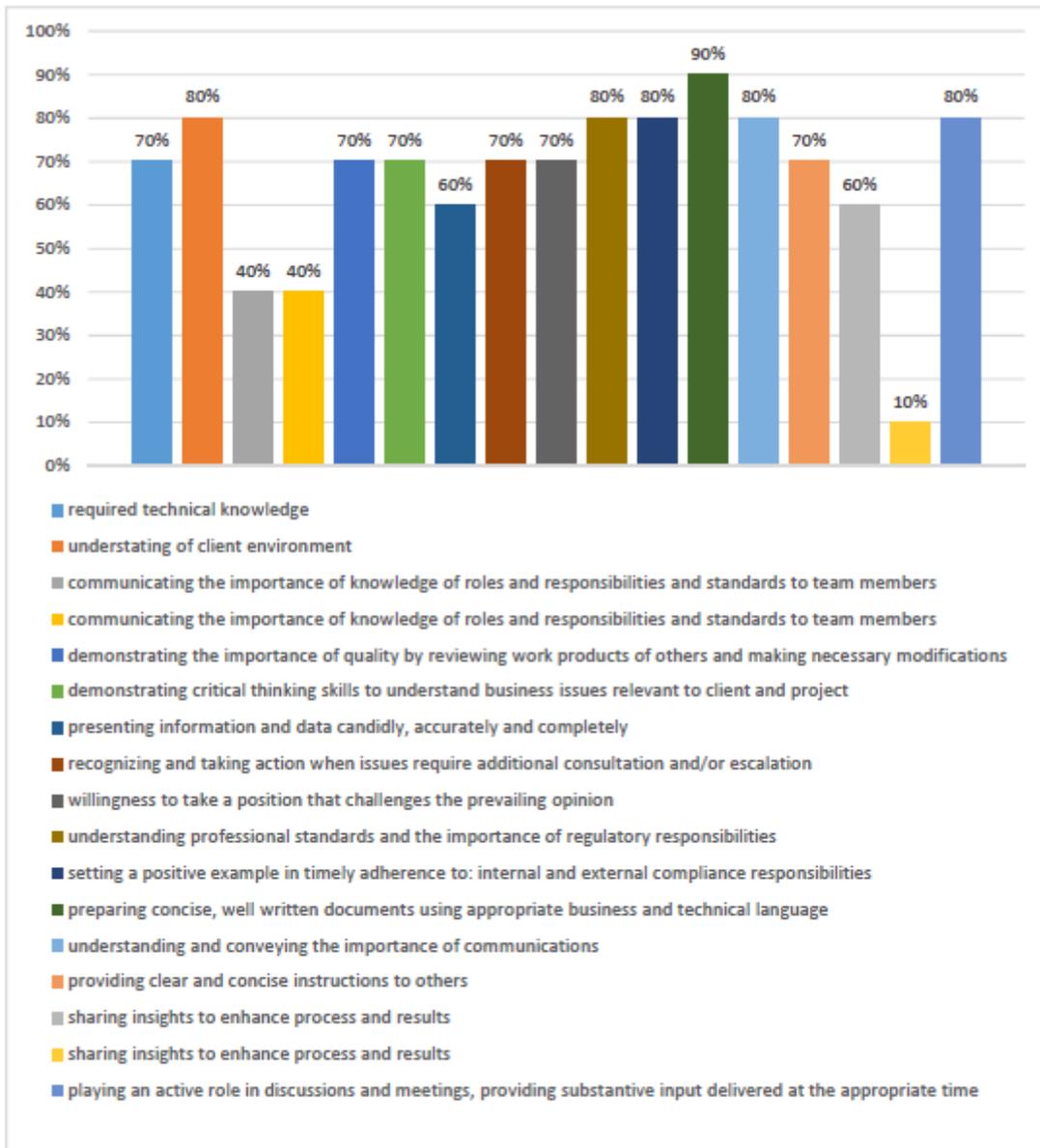


Figure 2 : Frequency of skills use for Dawacom's employees



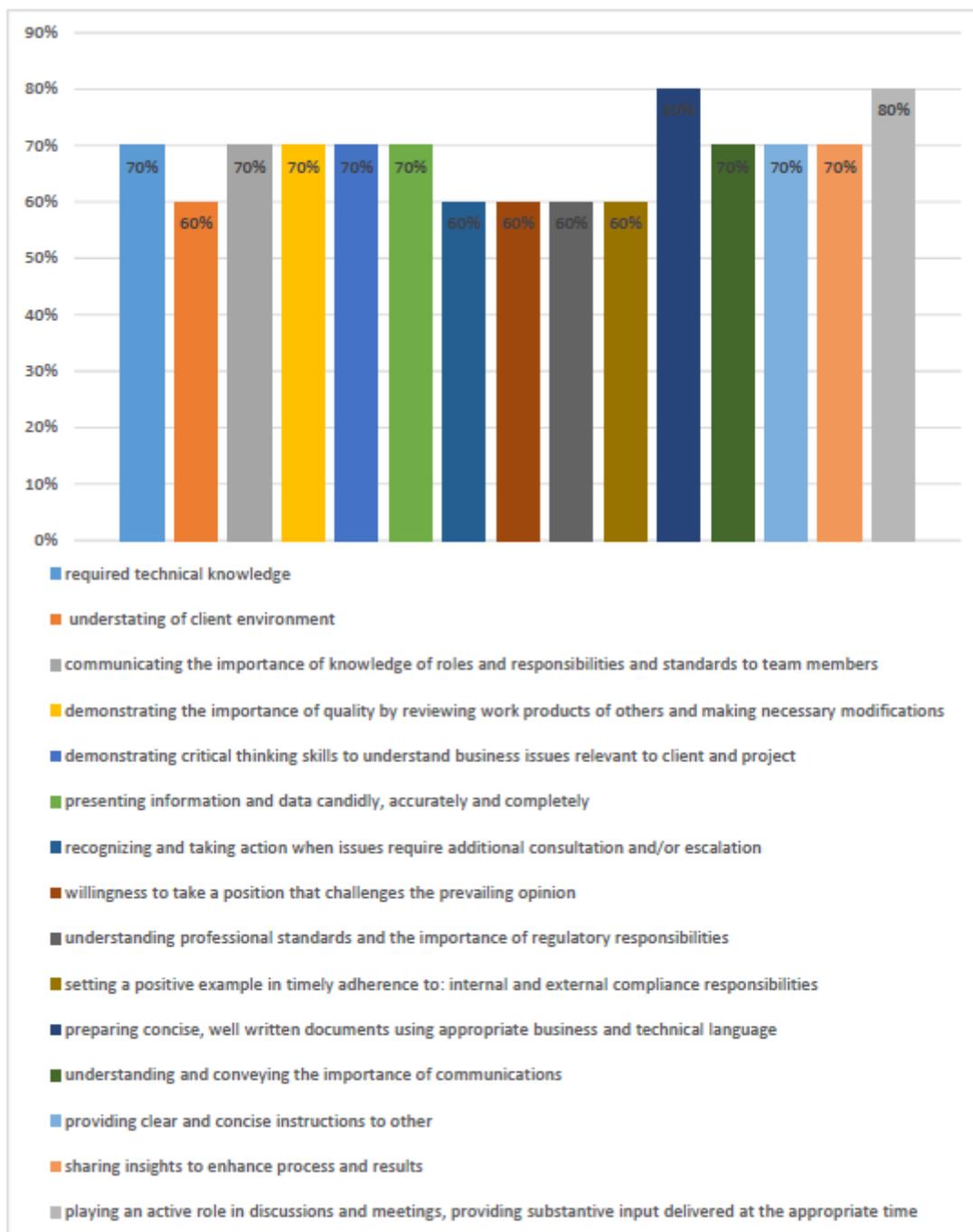


Figure 3 : Effectiveness of skill use for Dawacom's employees

c) *Suggestions for Dawacom*

We consider the training to be important for two reasons:

- The company is under pressure to provide high quality services and value, which needs new skills and these skills are built up through training
- Experience on the job is not enough anymore. Due to the increased competition in this continuously changing market, people can't rely only on time and experience to build the needed skills. Experience is outdated while knowledge is the trend.

Based on the results of our research we propose the following actions for Dawacom in terms of training:

- Correlate the training with the company's strategy - this would help the management team reduce the costs generated by bad and ineffective training and it can also help in reducing recruitment costs and attracting the right talent, whilst reducing the risk of staff leaving with those all- important skills which then are lost
- Connect the responsibilities of the pharmacists with the necessary skills in order to meet the company's

goals - this would help the company reduce the risk of having another ineffective training and also reduce the risks of work related accidents

- Analyze each training organized by the company and identify the parts that were not satisfactory and why
- Identify the learning style of each employee in order to group the employees according to their learning style and create the training according to this style in order to maximize the effects of the training programs
- Also, the company needs to consider that older employees learn differently from the younger ones. Perhaps the younger employees are more familiar with mobile-device-based training, while older employees may react better to an in-person training.
- By using different approaches, the company could develop training programs that resonate with all the employees, thus avoiding the lack of satisfaction among them. Besides various approaches will not only introduce skills and processes, but also create desired behaviors and results, and encourage an attitude favorable continuous learning
- In order to avoid the situation to become worst within the company, the management team should start working on a professional development for the employees.
- In order to make sure that the training is relevant to the company needs and those of the employees, the management team should consider the following:
 - Link the training to the strategy of the company
 - Link the expected results of the training to the job responsibilities, the overall strategic goals of the company and the desired professional development of the employees and involve the employees in the training programs. If the company makes sure that its employees understand their role in the training program, they will support it
 - Make sure that the content of the training is relevant and delivered through the right methods and techniques. When developing the training program the company should do it with the end user's needs in minds. The company should select a trainer able to create a content easy to digest by the employees and relevant to their job responsibilities.
 - The delivery method is also very important. The trainer has to make sure that the training will "reach" its target user whether he/she chooses an online tool, smartphone app, workshop or snail mail
 - As our research revealed, the speed of a training is very important. The quicker the trainees apply what they learned in real life

situation, the quicker the organization will reach its goals

- Training must also be sustained. What we mean by this is that if the skills that the employees are supposed to apply are challenging or are very different from what they were previously doing, it is very important to have a check list to see how well the learning is working and strengthen the areas that might have not been immediately applied
- Understand what can be measured and what cannot. There is the possibility not to be able to quantify the effectiveness of training. In such cases the behavior of the employee needs to be observed over time and identify the effects of the training.

The collected answers represent either the expectations of the sample employees or certainties resulted from their experience as attendees of the training organized by the company. By studying these answers we understand that the training need to change the focus from information to formation (professional development), by applying modern training methods and approaching the trainees differently, having as purpose the development and improvement of the soft skills of all the pharmacist in the company. The manner in which the results are distributed to most of the questions allows us to observe the frequency of a set of personal traits that can be seen in most of the respondent employees, traits specific to creative people: curiosity - described by the desire to learn and grow professionally, ingenuity - describe by the way some of the respondent flourished the answers, passion - observed in the passion they have for their work and self-confidence.

It is impossible to identify all the challenges that the development programs at Dawacom had to face over time. This "territory" is misleading and it makes a difficult road to cross for those who want to cross it. Training should not be treated as an act of faith, but should be supported by a positive philosophy and realistic about how it will contribute to the success of the organization. Not all training is necessary and, inevitably, a good thing. Today, when resources are limited and must be used with care and trainers are asked to justify their position and realize the activities they undertake. Misguided and inappropriate training does not serve anyone: neither the trainer nor the employee and the organization. The organization may also decide that the most advantageous approach, at some point, is to hire people with experience, not to invest in training. But any decision is taken considering the strategy.

The contemporary literature provides us with information on the human resources development with the involvement of the organization and on the skills and



competences of the employees. After carefully analyzing the available literature we came to the conclusion that our research is unique in the pharmaceutical area. As it can be seen in the literature review chapter, the scientific world provides us information on soft skills for different fields of activity (c2d website, 2009), (Kaipa, et al., 2010), (Heckman & Kautz, 2012), even in the academia with focus on students (Hendarman & Tjakraatmadja, 2012), (Zhang, 2012) and medical field with focus on doctors and nurses, but not on pharmacists.

Even though the soft skills are the same for any activity, the needed set of skills depends from one job to another, function of the responsibilities and work environment. Because the profession of pharmacists was neglected by the scholar in terms of soft skills, we decided to pay attention to it. We used the questionnaire to have a general inside view over the skills of the pharmacists, with focus on the soft skills that they need in order to perform effectively. We tried to touch various areas regarding the soft skills of pharmacists: feedback on training attended, relation performance - feedback, needed soft skills to have an easier job.

VI. CONCLUSIONS AND RECOMMENDATION

Throughout the paper we intended to analyze what skills are and what skills the pharmacists need in order to be preformat, and what are the dimensions specific to this field of activity. Starting from the concepts of skills and professional development of the human resources within a company, as presented in the available literature we could observe that the existing data is not enough to offer a clear and holistic view on the skills needed by pharmacists. Starting from the existing research we performed our own research whose purpose was to fill in some missing parts.

In this thesis we studied in depth the skills needed by pharmacists at Dawacom in order to perform effectively in their line of duty. The research present in this thesis was oriented towards identifying the opinions of Dawacom's employees with respect to the training they attended and were organized by the company they work for. The needs identified through the critical investigation we performed and the rigorous examination of the training programs of the company and the professional development of the employees, if taken into consideration by the management team of the company could impact in a positive manner the future strategies.

A skills represents the harmonious combination and use of knowledge, habits and attitudes in order to obtain the expected results at work. Being a skilled person means to apply specialized knowledge, use specific behavior, analyze and take decisions, being creative, and working with the team members, communicating effectively, adapting to a specific environment, coping with challenges.

The research revealed the following:

- In order to perform effectively pharmacists need pharmacy skills and patient skills (soft skills)
- The subjects were not very reliable; it was very difficult to gather their answers and code the data due to their chaotic answers
- The company should set goals and monitor them
- The employees treasure the team work and are aware about its importance
- The employees don't identify themselves with the mission and values of Dawacom

Our study reveals the fact the individuals who work or are interested in their education, can grow professionally, can help the company they work for to obtain human capital that can be converted to economic capital and thus increase the competitiveness of the company on the market. Also, we consider that positive measures to persuade the pharmacist to be involved in the development of the company are important; the present paper being one of these possible measures. Being able to distinguish between an effective and useful training and one at the opposite pole is very important for a company, both for the morale of the employees and for the efficient use of its budget.

We could observe during the research that the subjects were reluctant to provide accurate data or even to fill in the questionnaire. Due to the fact that we divided the questions into sections and from time to time we repeated some questions under a different form we were able to identify the contradictory nature of the Dawacom's employees. On one hand they said that they promoted or improved their skills due to training and on the other hand they stated that the training they attended was of poor quality.

A future research could follow the positive vs. negative dialectics and identify the themes considered to be irrelevant by the employees. Thus we would be able to understand the ability of the employees to make critical evaluations in terms of the utility value oriented towards the own professional development.

With respect to the limits and recommendations for possible future research, first of all we distinguish a lack of representativeness of the studied sample, the purpose of our research being mainly to identify the existence of a set of skills useful in the line of work of the pharmacists, the selection we performed was not a very rigorous one. Due to this reason, we were cautious in generalizing the conclusion, the results and the interpretation of the obtained data. Though, this shortcoming was compensated by the statistical significance of most of the obtained data, besides, we considered only those data that had a significant statistical importance at Dawacom's level, following that in further research to achieve a more rigorous selection involving other professionals such as a sociologist and / or a statistician. Also, an important issue regarding our

paper is the presentation and the analysis of the data obtained through different sets of questions whose purpose was to eliminate those answers that are not reliable. A major contribution of our scientific paper is the applicative research. As it can be observed in the analysis and interpretation of the obtained results, it completes the theoretical discourse, by embodying the image of the soft skills possessed by the employees, on one hand, and needed, on the other hand.

Based on the obtained results we recommend Dawacom to:

- Develop a "core training" for pharmacist.
- Design competency assessment tools for development and training purposes
- Provide training and development for managers, especially those who will need to adapt to new roles as coaches, facilitators, and resources to teams.

By "core training" we mean that the company should set a training whose purpose is to develop the soft skills identified through our research as being the most important for the activity of a pharmacist. We consider that a strong core training would enhance the soft skills of pharmacists and would help the company focus its training resource in the right direction. In our opinion a core training has the following stages:

- Identify the strengths of each employee
- Provide a training to enhance the existing skills
- Identify the missing skills
- Provide training to develop missing skills.

In order for a training to be effective first of all the company should identify the competencies of its employees and to support this approach we recommend that the assessment should use at least two assessment tools: competency-based interviews (these are effective for targeting specific competencies), competency assessment tests (they can be used for certifications, performance management, and for the validation of knowledge and skills).

Manager should have people skills. Our research revealed that the manager at Dawacom are not involved in the development of their subordinates which led to a sense that the managers are not very competent. Managers need to be able to interrelate with the employees "to feel the pulse" of their teams. Most employees promoted to managers due to their knowledge and technical skills, but once they hold this position their performances will be measured according to the strength of their soft skills.

The benefits of the training can be measured by:

- Costs savings vs. costs generated by training
- Efficiency
- Performance to schedule
- Income received
- The extent to which trainees mix
- Changes in the behavior and performance

- Labor savings
- Productivity increases
- Payback period

A conclusion that we can draw, after going through this research is that we cannot neglect the employee's feelings toward the company they work for; the myth according to which we need to only focus on the results and the employee is just a pawn to be run, is no longer topical! If you want results, you have to focus on developing the employees' skills, but also on creating an organizational environment that fosters both performance and employee happiness.

Another conclusion that we can draw from our research is that our project should run for a longer period than originally expected and will have to call for actions of the most diverse and attractive, so that the chances of success to be higher. In addition, it is clear that the company need to work on the relation it has with its employees, it has to encourage honesty and explain its values to all of them and make sure that they accept them and act accordingly. Also the company need to work on disseminating the information and changing the attitudes and behaviors among the employees.

Therefore if a company wants performance, effectiveness and increased productivity, it has to take into consideration the skills its employees possess and to create an environment that encourages skills enhancement.

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Amer: "You will be amazing with the Mba"

Asma'a: "No body knows your limit, you are over energetic"

Mohammad: "You did not surprize me, because you are always have a plan "B" and ambitious"

Orwa: "Very smart aref if you make it, but think twice you are our dentist"

Sukainah: "My full support to you my twin"

Hamzah: "No one can predict what is your next step Aref, I am Jealous that you have this spirit"

Moath: "I will follow you after you finish, be ready brother"

Anas: You will be a super Dr. Aref when you finish from this great university"

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VII. APPENDIX

Personal Data

Name

Department

Designation

Experience

Thank you for taking your time to fill in this survey. We are examining our human resources needs and exploring what can be done in order to provide our employees with knowledge and infrastructure to help them perform effectively.

Please carefully read each question and choose your answer from the dropdown list (where appropriate) or type your answer as detailed as possible.

Section A

Nr.	Question	Answer	√	
1	Have you undergone any training program?	Yes		
		No		
		If yes specify?	Technical skills	
			Soft skills	
		Process training		
		All		
2	Do you think training is necessary?	Required		
		Somewhat required		
		Must		
		Not at all required		
3	What kind of training is given in the organization?	In-house training		
		External training		
		Both		
4	Does your organization have a set procedure for identifying training needs?	Yes		
		No		
		Not aware		
5	Is training necessary for improving your performance?	Yes		
		No		
		If no specify why		



6	What are the training programs that you have attended?		On the Job	
			Off the job	
7	What is the average duration of the training sessions?		1 day only	
			1 week	
			2-5 days	
			More than a week	
8	Does your manager help you in selecting the training program you require?		Yes	
			No	
9	Are you satisfied with the way the training session was organized?		Satisfied	
			Not Satisfied	
			Average	
10	Is the duration of the training program sufficient?		Yes	
			No	
11	Do you think the teaching methodology adopted is effective?		Yes	
			No	
12	Do you think training should include programs for developing interpersonal skills & Human Values?		Relevant	
			Irrelevant	
			Neutral	
13	How has training helped you?		Rectifying day-to-day errors	
			Changing Departments(projects)	
			Face new Challenges	
14	How frequently do you think training programs should be conducted?		Monthly	
			Quarterly	

			Annually	
15	What is the basis on which the training process is initiated?		Performance appraisal	
			When project is going on	
16	The extent to which you were aware of the objectives of the program at the time of your nomination.		Very little	
			Little	
			Fully	
			Not aware	
17	The extent to which the program was in line with your expectation		Very little	
			Little	
			Fully	
			Not aware	
18	The extent to which the trainer had knowledge of the objective of the training program.		Very little	
			Little	
			Fully	
			Not aware	
19	The extent to which program was relevant to your work situation.		Very little	
			Little	
			Fully	
			Not aware	
20	The extent to which the program remained relevant over a period of time		Very little	
			Little	
			Fully	
			Not aware	
21	Has your improvement in the training program been noticed by your colleagues and manager?		Yes	
			No	
			Partially	



22	What is your overall rating of the training program?		Outstanding	
			Excellent	
			Very good	
			Fair	
23	I feel more confident & better equipped to act as a leader & handle conflicting situations.		Strongly Agree	
			Disagree	
			Undecided	
			Agree	
			Strongly Agree	
24	After attending the program I have become more competent professionally		Strongly Agree	
			Disagree	
			Undecided	
			Agree	
			Strongly Agree.	
25	The program has infused in me, a lot of self-confidence to handle new challenges.		Strongly Agree	
			Disagree	
			Undecided	
			Agree	
			Strongly Agree.	
26	Has the Managers/team lead's supervisions & instructions reduced after the training?		Strongly Agree	
			Disagree	
			Undecided	
			Agree	
			Strongly Agree.	
27	Attending the program has improved my status in the organization.		Strongly Agree	
			Disagree	
			Undecided	
			Agree	
			Strongly Agree.	

27	Attending the program has improved my status in the organization.		Strongly Agree	
			Disagree	
			Undecided	
			Agree	
			Strongly Agree.	
28	In your opinion, how could we improve the training program organized by Dawacom?			
29	Any Suggestion? Please Specify.			
30	What aspect of training program did you enjoy the most?			

Section B

Nr.	Question	Answer	√
1	Thinking about the technical area of environmental health where you currently work. Please list any training you believe would be beneficial to you and is not, to the best of your knowledge, currently being planned? By technical we mean any subjects or topics specific to EH core function work: e.g. food control; health and safety; environmental protection; consumer protection; housing; pest control; dog control, licensing; water quality. Or any other technical/specialist area: e.g. nutrition, diet, community development, etc.		
2	Thinking about crosscutting topics / issues within the context of future training. Please choose	Time management	
		Project management	
		Managing organizational change	
	the 3 areas from the list below that you feel would be most beneficial to your wider professional development (i.e. apart from any specific technical topics listed in the preceding question).	Improving service quality	
		Leadership	
		Managing budgets	
		Effective EH business planning	
		Developing effective policy	
		Community/stakeholder engagement	

			Effective partnership working	
			How to advocate effectively	
			Team building	
			Motivation	
			Dealing with stress	
			Communication skills	
			Community Planning	
			The use and relevance of social marketing in behavioral change	
			Emergency planning	
			Planning effective interventions - evidence and evaluation	
			Cultural diversity in the work place	
			Dealing with the media	
			Future career development opportunities	
			Getting your ideal job	
			Creating the best impression	
3	From the 3 areas/topics you identified in question 15, please further expand on these answers	1		
		2		
		-		
4	Thinking back over the last 12 – 18 months, have you attended any unique or distinctive training courses that you feel were of value and that you would recommend to others? Please state the name of the course/s, training provider/s and give a brief description of the course/s			

Section C

Nr.	Question	Answer	√
1	What skills do you think that a pharmacist needs to have in order to perform effectively?		
2	What skills do you already have?		

3	What skills do you still have to acquire?		
4	What logistical requirements do you have in order to perform effectively?		
	How important are the following skills to your job?		
5	Establishing and monitoring goals and objectives	Not Applicable	
		Not Very Important	
		Somewhat Important	
		Important	
		Very Important	
6	Encouraging collaboration among fellow employees and departments to achieve results	Not Applicable	
		Not Very Important	
		Somewhat Important	
		Important	
		Very Important	
7	Seeking and utilizing opportunities for continuous learning and self-development	Not Applicable	
		Not Very Important	
		Somewhat Important	
		Important	
		Very Important	
8	Understanding and seeking to achieve [Dawacom]'s mission and values	Not Applicable	
		Not Very Important	
		Somewhat Important	
		Important	
		Very Important	
9	Responding to a changing organization	Not Applicable	
		Not Very Important	
		Somewhat Important	
		Important	
		Very Important	
10	Taking appropriate and timely action to overcome unexpected hurdles or obstacles to a plan or project	Not Applicable	

Work skills



		Somewhat Important	
		Important	
		Very Important	
18	Which communication skill do you feel is most important to receive training (select one)		
19	Suggestions on how to improve these skills		
	How important are the following skills to your job?		
20	Encouraging teamwork and collaboration as a method to accomplish tasks and achieve objectives	Not Applicable	
	People skills	Not Very Important	
		Somewhat Important	
		Important	
		Very Important	
21	Recognizing and rewarding people for doing their best	Not Applicable	
		Not Very Important	
		Somewhat Important	
		Important	
		Very Important	
22	Constructively receiving criticism and suggestions from others	Not Applicable	
		Not Very Important	
		Somewhat Important	
		Important	
		Very Important	
23	If you needed training to improve your people skills, which one would you want training (select one)		
	How important are the following skills to your job?		



24	Leader Skills	Conducting regular meetings with direct reports to discuss performance and achievement of objectives	Not Applicable		
			Not Very Important		
			Somewhat Important		
			Important		
			Very Important		
25			Giving others direct, constructive feedback which can be used to achieve goals	Not Applicable	
				Not Very Important	
				Somewhat Important	
				Important	
				Very Important	
26			Leading people and allocating tasks to achieve goals	Not Applicable	
				Not Very Important	
				Somewhat Important	
				Important	
				Very Important	
27			Empowering others to achieve results and holds them accountable for actions	Not Applicable	
				Not Very Important	
			Somewhat Important		
			Important		
			Very Important		
28		Motivating others in order to reach organizational goals	Not Applicable		
			Not Very Important		
			Somewhat Important		
			Important		
			Very Important		
			Not Applicable		
29		Influencing others in a way that results in acceptance, agreement, or behavior change	Not Applicable		
			Not Very Important		
			Somewhat Important		
			Important		
			Very Important		

30		Which leader skill do you feel is most important to receive training (select one)			
31		Suggestions for how you would improve leader skills:			
How important are the following skills to your job?					
32	Customer Skills	Understanding who the department's customer is and communicates that priority consistently	Not Applicable		
			Not Very Important		
			Somewhat Important		
			Important		
			Very Important		
33			Becoming more effective in satisfying customer needs	Not Applicable	
				Not Very Important	
				Somewhat Important	
				Important	
				Very Important	
34		Pursuing the best customer-focused responses that add value to the business	Not Applicable		
			Not Very Important		
			Somewhat Important		
			Important		
			Very Important		
35		Which customer skill do you feel is most important to receive training (select one)			
36		Suggestions for how you would improve customer skills:	Type your answer here		
How important are the following skills to your job?					

37	Business Skills	Aligning resources to meet the business needs of [Dawacom]	Not Applicable		
			Not Very Important		
			Somewhat Important		
			Important		
			Very Important		
38			Understanding the costs, profits, markets, and added value of the department and how those contribute to the success of [Dawacom]	Not Applicable	
				Not Very Important	
				Somewhat Important	
				Important	
				Very Important	
39			Looking at the "big picture" of [Dawacom]'s goals, rather than individual department's needs	Not Applicable	
				Not Very Important	
				Somewhat Important	
				Important	
			Very Important		
40		Anticipating marketplace opportunities and supports speed to market.	Not Applicable		
			Not Very Important		
			Somewhat Important		
			Important		
			Very Important		
41		Which business skill do you feel is most important to receive training (select one)			
42		Suggestions for how you would improve business skills:			
		How important are the following skills to your job?			
43		Selecting the appropriate techniques for analysis	Not Applicable		
			Not Very Important		
			Somewhat Important		
			Important		
			Very Important		

How important are the following skills to your job?			
43		Selecting the appropriate techniques for analysis	Not Applicable
			Not Very Important
			Somewhat Important
			Important
			Very Important
44	Analytical Skills	Interpreting financial data, reports, balance sheets, and cash flow analysis	Not Applicable
			Not Very Important
			Somewhat Important
			Important
			Very Important
45		Generating alternative solutions to problems and challenges.	Not Applicable
			Not Very Important
			Somewhat Important
			Important
			Very Important
46		Which analytical skill do you feel is most important to receive training (select one)	
47		Suggestions for how you would improve analytical skills:	

Section D

Nr	Focus	Question	Frequency	√	Effectiveness of use	√
1		I demonstrate required technical knowledge in my work	No opportunity to observe		Cannot Say	
			Never even though situations needed it		Poor effectiveness: The behavior when used was never effective.	





	Focusing on the Client		Few occasions even though situations needed it	Marginal Effectiveness: Behavior when used was not usually effective.	
			Many occasions when needed	Moderate Effectiveness: Behavior when used was usually effective.	
			Most occasions when it was needed	Very Effective: Behavior was always effective when used.	
			All occasions when needed		
2	I demonstrate understanding of client's organization and business/regulatory environment.	No opportunity to observe		Cannot Say	
			Never even though situations needed it	Poor effectiveness: The behavior when used was never effective.	
			Few occasions even though situations needed it	Marginal Effectiveness: Behavior when used was not usually effective.	
			Many occasions when needed	Moderate Effectiveness: Behavior when used was usually effective.	
			Most occasions when it was needed	Very Effective: Behavior was always effective when used.	
			All occasions when needed		

3		I communicate the importance of knowledge of roles and responsibilities and standards to team members.	No opportunity to observe		Cannot Say
			Never even though situations needed it		Poor effectiveness: The behavior when used was never effective.
			Few occasions even though situations needed it		Marginal Effectiveness: Behavior when used was not usually effective.
			Many occasions when needed		Moderate Effectiveness: Behavior when used was usually effective.
			Most occasions when it was needed		Very Effective: Behavior was always effective when used.
			All occasions when needed		
4		I demonstrate importance of quality by reviewing work products of others and making necessary modifications.	No opportunity to observe		Cannot Say
			Never even though situations needed it		Poor effectiveness: The behavior when used was never effective.
			Few occasions even though situations needed it		Marginal Effectiveness: Behavior when used was not usually effective.

			Many occasions when needed	Moderate Effectiveness: Behavior when used was usually effective.	
			Most occasions when it was needed	Very Effective: Behavior was always effective when used.	
			All occasions when needed		
5		I demonstrate critical thinking skills to understand business issues relevant to client and project.	No opportunity to observe	Cannot Say	
			Never even though situations needed it	Poor effectiveness: The behavior when used was never effective.	
			Few occasions even though situations needed it	Marginal Effectiveness: Behavior when used was not usually effective.	
			Many occasions when needed	Moderate Effectiveness: Behavior when used was usually effective.	
			Most occasions when it was needed	Very Effective: Behavior was always effective when used.	
			All occasions when needed	Cannot Say	
			No opportunity to observe		

6	Demonstrating Courage and Integrity	I represent information and data candidly, accurately and completely.	No opportunity to observe	Cannot Say	
			Never even though situations needed it	Poor effectiveness: The behavior when used was never effective.	
			Few occasions even though situations needed it	Marginal Effectiveness: Behavior when used was not usually effective.	
			Many occasions when needed	Moderate Effectiveness: Behavior when used was usually effective.	
			Most occasions when it was needed	Very Effective: Behavior was always effective when used.	
			All occasions when needed		
7	Demonstrating Courage and Integrity	I recognize and take action when issues require additional consultation and/or escalation.	No opportunity to observe	Cannot Say	
			Never even though situations needed it	Poor effectiveness: The behavior when used was never effective.	
6	Demonstrating Courage and Integrity	I represent information and data candidly, accurately and completely.	No opportunity to observe	Cannot Say	
			Never even though situations needed it	Poor effectiveness: The behavior when used was never effective.	



	Demonstrating Courage and Integrity		Few occasions even though situations needed it	Marginal Effectiveness: Behavior when used was not usually effective.	
			Many occasions when needed	Moderate Effectiveness: Behavior when used was usually effective.	
			Most occasions when it was needed	Very Effective: Behavior was always effective when used.	
			All occasions when needed		
7		I recognize and take action when issues require additional consultation and/or escalation.	No opportunity to observe	Cannot Say	
			Never even though situations needed it	Poor effectiveness: The behavior when used was never effective.	
			Few occasions even though situations needed it	Marginal Effectiveness: Behavior when used was not usually effective.	
			Many occasions when needed	Moderate Effectiveness: Behavior when used was usually effective.	
			Most occasions when it was needed	Very Effective: Behavior was always effective when used.	
8			I am willing to take a position that challenges the prevailing opinion.	No opportunity to observe	Cannot Say

			Never even though situations needed it	Poor effectiveness: The behavior when used was never effective.	
			Few occasions even though situations needed it	Marginal Effectiveness: Behavior when used was not usually effective.	
			Many occasions when needed	Moderate Effectiveness: Behavior when used was usually effective.	
			Most occasions when it was needed	Very Effective: Behavior was always effective when used.	
			All occasions when needed		
9	I understand professional standards and the importance of regulatory responsibilities.	No opportunity to observe		Cannot Say	
		Never even though situations needed it		Poor effectiveness: The behavior when used was never effective.	
		Few occasions even though situations needed it		Marginal Effectiveness: Behavior when used was not usually effective.	
		Many occasions when needed		Moderate Effectiveness: Behavior when used was usually effective.	

			Most occasions when it was needed	Very Effective: Behavior was always effective when used.	
			All occasions when needed		
10		I set a positive example in timely adherence to: internal and external compliance responsibilities	No opportunity to observe	Cannot Say	
			Never even though situations needed it	Poor effectiveness: The behavior when used was never effective.	
			Few occasions even though situations needed it	Marginal Effectiveness: Behavior when used was not usually effective.	
			Many occasions when needed	Moderate Effectiveness: Behavior when used was usually effective.	
			Most occasions when it was needed	Very Effective: Behavior was always effective when used.	
			All occasions when needed		
11		I prepare concise, well written documents using appropriate business and technical language.	No opportunity to observe	Cannot Say	

	Communicating with Impact		Never even though situations needed it		Poor effectiveness: The behavior when used was never effective.		
			Few occasions even though situations needed it		Marginal Effectiveness: Behavior when used was not usually effective.		
	12		Many occasions when needed		Moderate Effectiveness: Behavior when used was usually effective.		
			Most occasions when it was needed		Very Effective: Behavior was always effective when used.		
			All occasions when needed				
			I understand and convey the importance of communications.	No opportunity to observe		Cannot Say	
				Never even though situations needed it		Poor effectiveness: The behavior when used was never effective.	
				Few occasions even though situations needed it		Marginal Effectiveness: Behavior when used was not usually effective.	
				Many occasions when needed		Moderate Effectiveness: Behavior when used was usually effective.	

			Many occasions when needed	Moderate Effectiveness: Behavior when used was usually effective.
			Most occasions when it was needed	Very Effective: Behavior was always effective when used.
			All occasions when needed	
13	I provide clear and concise instructions to others;	No opportunity to observe		Cannot Say
		Never even though situations needed it		Poor effectiveness: The behavior when used was never effective.
		Few occasions even though situations needed it		Marginal Effectiveness: Behavior when used was not usually effective.
		Many occasions when needed		Moderate Effectiveness: Behavior when used was usually effective.
		Most occasions when it was needed		Very Effective: Behavior was always effective when used.
		All occasions when needed		
14	I share insights to enhance process and results.	No opportunity to observe		Cannot Say
		Never even though situations needed it		Poor effectiveness: The behavior when used was never effective.

			Few occasions even though situations needed it		Marginal Effectiveness: Behavior when used was not usually effective.	
			Many occasions when needed		Moderate Effectiveness: Behavior when used was usually effective.	
			Most occasions when it was needed		Very Effective: Behavior was always effective when used.	
			All occasions when needed			
15		I play active role in discussions and meetings, providing substantive input delivered at the appropriate time.	No opportunity to observe		Cannot Say	
			Never even though situations needed it		Poor effectiveness: The behavior when used was never effective.	
			Few occasions even though situations needed it		Marginal Effectiveness: Behavior when used was not usually effective.	
			Many occasions when needed		Moderate Effectiveness: Behavior when used was usually effective.	
			Most occasions when it was needed		Very Effective: Behavior was always effective when used.	
			All occasions when needed			

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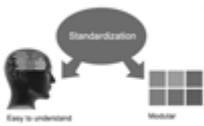
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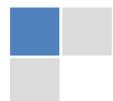
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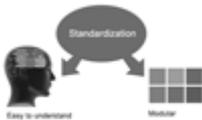


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Note :

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- In case, the chairperson needs to be replaced then consent of 2/3rd board members are required and they are also required to jointly pass the resolution copy of which should be sent to us. In such case, it will be compulsory to obtain our approval before replacement.
- In case of “Difference of Opinion [if any]” among the Board members, our decision will be final and binding to everyone.

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3. Submission of Manuscripts,
4. Manuscript's Category,
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32. Never oversimplify everything: To add material in your research paper, never go for oversimplification. This will definitely irritate the evaluator. Be more or less specific. Also too, by no means, ever use rhythmic redundancies. Contractions aren't essential and shouldn't be there used. Comparisons are as terrible as clichés. Give up ampersands and abbreviations, and so on. Remove commas, that are, not necessary. Parenthetical words however should be together with this in commas. Understatement is all the time the complete best way to put onward earth-shaking thoughts. Give a detailed literary review.

33. Report concluded results: Use concluded results. From raw data, filter the results and then conclude your studies based on measurements and observations taken. Significant figures and appropriate number of decimal places should be used. Parenthetical remarks are prohibitive. Proofread carefully at final stage. In the end give outline to your arguments. Spot out perspectives of further study of this subject. Justify your conclusion by at the bottom of them with sufficient justifications and examples.

34. After conclusion: Once you have concluded your research, the next most important step is to present your findings. Presentation is extremely important as it is the definite medium through which your research is going to be in print to the rest of the crowd. Care should be taken to categorize your thoughts well and present them in a logical and neat manner. A good quality research paper format is essential because it serves to highlight your research paper and bring to light all necessary aspects in your research.

INFORMAL GUIDELINES OF RESEARCH PAPER WRITING

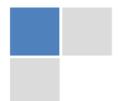
Key points to remember:

- Submit all work in its final form.
- Write your paper in the form, which is presented in the guidelines using the template.
- Please note the criterion for grading the final paper by peer-reviewers.

Final Points:

A purpose of organizing a research paper is to let people to interpret your effort selectively. The journal requires the following sections, submitted in the order listed, each section to start on a new page.

The introduction will be compiled from reference matter and will reflect the design processes or outline of basis that direct you to make study. As you will carry out the process of study, the method and process section will be constructed as like that. The result segment will show related statistics in nearly sequential order and will direct the reviewers next to the similar intellectual paths throughout the data that you took to carry out your study. The discussion section will provide understanding of the data and projections as to the implication of the results. The use of good quality references all through the paper will give the effort trustworthiness by representing an alertness of prior workings.



Writing a research paper is not an easy job no matter how trouble-free the actual research or concept. Practice, excellent preparation, and controlled record keeping are the only means to make straightforward the progression.

General style:

Specific editorial column necessities for compliance of a manuscript will always take over from directions in these general guidelines.

To make a paper clear

- Adhere to recommended page limits

Mistakes to evade

- Insertion a title at the foot of a page with the subsequent text on the next page
- Separating a table/chart or figure - impound each figure/table to a single page
- Submitting a manuscript with pages out of sequence

In every sections of your document

- Use standard writing style including articles ("a", "the," etc.)
- Keep on paying attention on the research topic of the paper
- Use paragraphs to split each significant point (excluding for the abstract)
- Align the primary line of each section
- Present your points in sound order
- Use present tense to report well accepted
- Use past tense to describe specific results
- Shun familiar wording, don't address the reviewer directly, and don't use slang, slang language, or superlatives
- Shun use of extra pictures - include only those figures essential to presenting results

Title Page:

Choose a revealing title. It should be short. It should not have non-standard acronyms or abbreviations. It should not exceed two printed lines. It should include the name(s) and address (es) of all authors.



Abstract:

The summary should be two hundred words or less. It should briefly and clearly explain the key findings reported in the manuscript-- must have precise statistics. It should not have abnormal acronyms or abbreviations. It should be logical in itself. Shun citing references at this point.

An abstract is a brief distinct paragraph summary of finished work or work in development. In a minute or less a reviewer can be taught the foundation behind the study, common approach to the problem, relevant results, and significant conclusions or new questions.

Write your summary when your paper is completed because how can you write the summary of anything which is not yet written? Wealth of terminology is very essential in abstract. Yet, use comprehensive sentences and do not let go readability for brevity. You can maintain it succinct by phrasing sentences so that they provide more than lone rationale. The author can at this moment go straight to shortening the outcome. Sum up the study, with the subsequent elements in any summary. Try to maintain the initial two items to no more than one ruling each.

- Reason of the study - theory, overall issue, purpose
- Fundamental goal
- To the point depiction of the research
- Consequences, including definite statistics - if the consequences are quantitative in nature, account quantitative data; results of any numerical analysis should be reported
- Significant conclusions or questions that track from the research(es)

Approach:

- Single section, and succinct
- As an outline of job done, it is always written in past tense
- A conceptual should situate on its own, and not submit to any other part of the paper such as a form or table
- Center on shortening results - bound background information to a verdict or two, if completely necessary
- What you account in an abstract must be regular with what you reported in the manuscript
- Exact spelling, clearness of sentences and phrases, and appropriate reporting of quantities (proper units, important statistics) are just as significant in an abstract as they are anywhere else

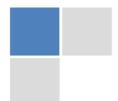
Introduction:

The **Introduction** should "introduce" the manuscript. The reviewer should be presented with sufficient background information to be capable to comprehend and calculate the purpose of your study without having to submit to other works. The basis for the study should be offered. Give most important references but shun difficult to make a comprehensive appraisal of the topic. In the introduction, describe the problem visibly. If the problem is not acknowledged in a logical, reasonable way, the reviewer will have no attention in your result. Speak in common terms about techniques used to explain the problem, if needed, but do not present any particulars about the protocols here. Following approach can create a valuable beginning:

- Explain the value (significance) of the study
- Shield the model - why did you employ this particular system or method? What is its compensation? You strength remark on its appropriateness from a abstract point of vision as well as point out sensible reasons for using it.
- Present a justification. Status your particular theory (es) or aim(s), and describe the logic that led you to choose them.
- Very for a short time explain the tentative propose and how it skilled the declared objectives.

Approach:

- Use past tense except for when referring to recognized facts. After all, the manuscript will be submitted after the entire job is done.
- Sort out your thoughts; manufacture one key point with every section. If you make the four points listed above, you will need a least of four paragraphs.



- Present surroundings information only as desirable in order hold up a situation. The reviewer does not desire to read the whole thing you know about a topic.
- Shape the theory/purpose specifically - do not take a broad view.
- As always, give awareness to spelling, simplicity and correctness of sentences and phrases.

Procedures (Methods and Materials):

This part is supposed to be the easiest to carve if you have good skills. A sound written Procedures segment allows a capable scientist to replacement your results. Present precise information about your supplies. The suppliers and clarity of reagents can be helpful bits of information. Present methods in sequential order but linked methodologies can be grouped as a segment. Be concise when relating the protocols. Attempt for the least amount of information that would permit another capable scientist to spare your outcome but be cautious that vital information is integrated. The use of subheadings is suggested and ought to be synchronized with the results section. When a technique is used that has been well described in another object, mention the specific item describing a way but draw the basic principle while stating the situation. The purpose is to text all particular resources and broad procedures, so that another person may use some or all of the methods in one more study or referee the scientific value of your work. It is not to be a step by step report of the whole thing you did, nor is a methods section a set of orders.

Materials:

- Explain materials individually only if the study is so complex that it saves liberty this way.
- Embrace particular materials, and any tools or provisions that are not frequently found in laboratories.
- Do not take in frequently found.
- If use of a definite type of tools.
- Materials may be reported in a part section or else they may be recognized along with your measures.

Methods:

- Report the method (not particulars of each process that engaged the same methodology)
- Describe the method entirely
- To be succinct, present methods under headings dedicated to specific dealings or groups of measures
- Simplify - details how procedures were completed not how they were exclusively performed on a particular day.
- If well known procedures were used, account the procedure by name, possibly with reference, and that's all.

Approach:

- It is embarrassed or not possible to use vigorous voice when documenting methods with no using first person, which would focus the reviewer's interest on the researcher rather than the job. As a result when script up the methods most authors use third person passive voice.
- Use standard style in this and in every other part of the paper - avoid familiar lists, and use full sentences.

What to keep away from

- Resources and methods are not a set of information.
- Skip all descriptive information and surroundings - save it for the argument.
- Leave out information that is immaterial to a third party.

Results:

The principle of a results segment is to present and demonstrate your conclusion. Create this part a entirely objective details of the outcome, and save all understanding for the discussion.

The page length of this segment is set by the sum and types of data to be reported. Carry on to be to the point, by means of statistics and tables, if suitable, to present consequences most efficiently. You must obviously differentiate material that would usually be incorporated in a study editorial from any unprocessed data or additional appendix matter that would not be available. In fact, such matter should not be submitted at all except requested by the instructor.



Content

- Sum up your conclusion in text and demonstrate them, if suitable, with figures and tables.
- In manuscript, explain each of your consequences, point the reader to remarks that are most appropriate.
- Present a background, such as by describing the question that was addressed by creation an exacting study.
- Explain results of control experiments and comprise remarks that are not accessible in a prescribed figure or table, if appropriate.
- Examine your data, then prepare the analyzed (transformed) data in the form of a figure (graph), table, or in manuscript form.

What to stay away from

- Do not discuss or infer your outcome, report surroundings information, or try to explain anything.
- Not at all, take in raw data or intermediate calculations in a research manuscript.
- Do not present the similar data more than once.
- Manuscript should complement any figures or tables, not duplicate the identical information.
- Never confuse figures with tables - there is a difference.

Approach

- As forever, use past tense when you submit to your results, and put the whole thing in a reasonable order.
- Put figures and tables, appropriately numbered, in order at the end of the report
- If you desire, you may place your figures and tables properly within the text of your results part.

Figures and tables

- If you put figures and tables at the end of the details, make certain that they are visibly distinguished from any attach appendix materials, such as raw facts
- Despite of position, each figure must be numbered one after the other and complete with subtitle
- In spite of position, each table must be titled, numbered one after the other and complete with heading
- All figure and table must be adequately complete that it could situate on its own, divide from text

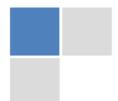
Discussion:

The Discussion is expected the trickiest segment to write and describe. A lot of papers submitted for journal are discarded based on problems with the Discussion. There is no head of state for how long a argument should be. Position your understanding of the outcome visibly to lead the reviewer through your conclusions, and then finish the paper with a summing up of the implication of the study. The purpose here is to offer an understanding of your results and hold up for all of your conclusions, using facts from your research and generally accepted information, if suitable. The implication of result should be visibly described. Infer your data in the conversation in suitable depth. This means that when you clarify an observable fact you must explain mechanisms that may account for the observation. If your results vary from your prospect, make clear why that may have happened. If your results agree, then explain the theory that the proof supported. It is never suitable to just state that the data approved with prospect, and let it drop at that.

- Make a decision if each premise is supported, discarded, or if you cannot make a conclusion with assurance. Do not just dismiss a study or part of a study as "uncertain."
- Research papers are not acknowledged if the work is imperfect. Draw what conclusions you can based upon the results that you have, and take care of the study as a finished work
- You may propose future guidelines, such as how the experiment might be personalized to accomplish a new idea.
- Give details all of your remarks as much as possible, focus on mechanisms.
- Make a decision if the tentative design sufficiently addressed the theory, and whether or not it was correctly restricted.
- Try to present substitute explanations if sensible alternatives be present.
- One research will not counter an overall question, so maintain the large picture in mind, where do you go next? The best studies unlock new avenues of study. What questions remain?
- Recommendations for detailed papers will offer supplementary suggestions.

Approach:

- When you refer to information, differentiate data generated by your own studies from available information
- Submit to work done by specific persons (including you) in past tense.
- Submit to generally acknowledged facts and main beliefs in present tense.



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<i>Introduction</i>	Containing all background details with clear goal and appropriate details, flow specification, no grammar and spelling mistake, well organized sentence and paragraph, reference cited	Unclear and confusing data, appropriate format, grammar and spelling errors with unorganized matter	Out of place depth and content, hazy format
<i>Methods and Procedures</i>	Clear and to the point with well arranged paragraph, precision and accuracy of facts and figures, well organized subheads	Difficult to comprehend with embarrassed text, too much explanation but completed	Incorrect and unorganized structure with hazy meaning
<i>Result</i>	Well organized, Clear and specific, Correct units with precision, correct data, well structuring of paragraph, no grammar and spelling mistake	Complete and embarrassed text, difficult to comprehend	Irregular format with wrong facts and figures
<i>Discussion</i>	Well organized, meaningful specification, sound conclusion, logical and concise explanation, highly structured paragraph reference cited	Wordy, unclear conclusion, spurious	Conclusion is not cited, unorganized, difficult to comprehend
<i>References</i>	Complete and correct format, well organized	Beside the point, Incomplete	Wrong format and structuring



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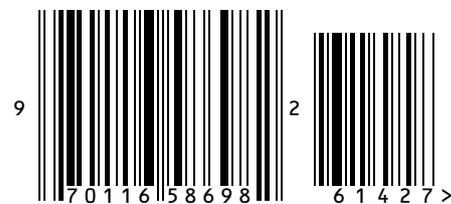
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