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Pharma, Drug Discovery, Toxicology and Medicine

Highlights

- Diabetic Patients
- Toona Ciliata Leaves
- Anti-Ulcer Activity
- Activity of Rhus Coriaria

Discovering Thoughts, Inventing Future

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Anti-Ulcer Activity of Rhus Coriaria in Indomethacin and Water Immersion Restraint Induced Gastric Ulcer in Experimental Rats

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Abstract- The anti-ulcer activity of hydro alcoholic extract of Rhus coriaria Linn (HAERC) was investigated in Indomethacin and Water immersion-induced restraint gastric ulcer in Wistar rats. The assessment was carried out by using ulcer index, ulcer score and histopathological studies of the specimens. HAERC at doses of 145 and 248 mg/kg given orally produced significant inhibition of the gastric lesions induced by Indomethacin and Water Immersion restraint method and the results were comparable to the standard treatment regime. We observed that Rhus coriaria Linn extract exhibits significant anti ulcer activity and thus supports the Unani claims about the drug.

Keywords: rhus coriaria; indomethacin; water immersion induced ulcer model; ulcer index; post sumaq.

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Anti-Ulcer Activity of Rhus Coriaria in Indomethacin and Water Immersion Restraint Induced Gastric Ulcer in Experimental Rats

Haqeeq Ahmad [α], Abdul Wadud [σ], Nasreen Jahan [ρ] & Izharul Hasan [ω]

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I. Introduction

Peptic ulcer disease (PUD) is one of the most common gastro intestinal disorders, which causes a high rate of morbidity. An estimated 15,000 deaths occur each year because of PUD. The prevalence of duodenal ulcer is dominant in western population whereas gastric ulcer is more frequent in most Asian countries[1]. The lifetime prevalence of peptic ulcer disease is 5 to 10% with about equal prevalence in men and women. The incidence of ulcer increases with age because of excessive use of NSAIDs and the reduction in the tissue prostaglandins [2]. In India, PUD is common and the Indian pharmaceutical industries share 6.2 billion rupee and occupy 4.3% of the market share in consuming the antacids and antiulcer drugs [3]. Peptic ulcer which is usually an asymptomatic gastrointestinal disorder defined as a breach in the mucosa of the alimentary tract, which extends through the muscularis mucosa into the submucosa or deeper. Peptic ulcer disease commonly occurs when the linings of stomach or proximal duodenum are corroded by the acid-peptic juices which are secreted by the stomach cells [4,5]. Peptic ulcer is caused by *H. pylori* infection, long term and high doses of drugs such as non steroidal anti-inflammatory drugs (NSAIDs), diseases like Zollinger-Ellison syndrome, other factors such as smoking; emotional stress and excessive alcohol consumption also may contribute. In Unani Medicine, gastric ulcer is known as *Qarah-e-Medah*. Unani scholars mentioned its causes as, *Khilte Haad* (hot and irritant humour), Fuzlat (waste products), *intake* of hot and spicy foods, excessive intake of rotten food, alcohol and hard fibrous diet, desensitization of internal surface of stomach which causes excessive gastric secretions, chronic gastritis and indigestion, prolonged stress and strains and unabsorbed gastric secretions. The modern approach to control gastric ulceration is to inhibit gastric acid secretion, to promote gastro protection, to block apoptosis and to stimulate epithelial cell proliferation for effective healing [6]. Hence, Conventional medicine treats Peptic ulcer by proton pump inhibitors (PPI), H2-receptor antagonist, antacids and antibiotics for *H. pylori*. However, there are reports of adverse effects and relapse in the long run[7] that lead people to find the alternative medications. Furthermore, many of these drugs do not fulfill all the *beneficial necessities* [8]. The clinical evaluation of these drugs showed development of tolerance and incidence of relapse and side effects that make their efficacy debatable. This has been the rationale for the development of new, safe antiulcer drugs. Herbal drugs can provide lead for the development of such antiulcer drugs because these drugs are considered safer in view of their natural ingredients. In recent times, focus on plant research has increased all over the world and a large body of evidence has been collected to show immense potential of medicinal plants used in various traditional systems of medicine. More than 13,000 plants have been studied during the last few years [9].

Unani physicians in the treatment of gastritis, gastric ulcer and associated disorders due to its stomachic, astringent, desiccant, styptic, sedative and coolant activities [10] also use Post Sumaq (Fruit rind of Rhus coriaria Linn.) frequently. However, there is no scientific report regarding its efficacy in PUD. Therefore, the present study was carried out to examine the effect of Post Sumaq in gastric ulcer on animal model.

II. Material and Methods

Institutional Animal Ethics Committee (IAEC) of NIUM approved the present study. The test drug *Sumaq*

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(Rhus coriaria Linn) was procured from local market of Bangalore, and was identified by Dr. H.B. Singh, Chief Scientist and Head of National Institute of Science Communication and Information Resources (NISCAIR) New Delhi, vide Reference No. NISCAIR/RHMD 2030/38.

III. Preparation and Dose of the Test Drug

The fruits of test drug were dried in shade, the Post (rind) was peeled off, and its therapeutic dose (5gm) as in Unani medicine was used to calculate the dose for experiment [11]. Thus, dose was found to be 580 mg /kg. Since, the test drug was studied at two different doses; therefore a second dose was also calculated by the method of Miller and Tainter (1944) [12] and found to be 990 mg/kg. As the hydro alcoholic extract was used for the study, the dose of the extract was calculated with reference to the dose of crude drug after obtaining the 25% yield percentage of extract. The hydro alcoholic extract of the drug was used in the dose of 145mg/kg and 248mg/kg. Standard drug, Omeprazole (Manufactured in India by Dr Reddys Laboratories Ltd. Village Manuja Thana) was used in the dose of 20mg/kg.

IV. Animals

The study was carried out in healthy Wistar rats of either sex, weighing 150-250 gm. The animals were procured from Biogen Laboratory Animal Facility (Reg. No. 971/bc/06/CPCSEA), a registered breeder in Bangalore. They ware acclimatized to the laboratory condition for 7 days before the experimental studies. The rats were housed in polypropylene cages under controlled conditions of light (12/12) and temperature (23±20C) under strict hygienic conditions. The animals were given Standard food pellets (Hindustan Lever Ltd.) and tap water ad libitum.

V. Induction of Gastric Ulcer

This test was carried out by the method described by Vogel[13] with minor modification in the treatment schedule. The animals were divided into 8 groups of 6 animals each. The animals in group I were administered with distilled water throughout study and served as Plain control and after 36 hours they were sacrificed while the animals in group II (after 24 hours of fasting) were treated with Indomethacin 20 mg/kg, once daily, orally for 5 days and served as negative control. The animals in group III, IV and V were treated with standard drug Omeprazole and hydro alcoholic extract of test drug in the dose 20 mg/kg, 145mg/kg and 248 mg/kg, respectively and served as pre-treated standard, Pre-treated test group A and Pre-treated test group B, respectively. These treatments were continued for five days; however, on 6th day after 24 hours of fasting ulcer was induced by the administration of Indomethacin in the dose of 20 mg/kg, for the next five days. Food was withdrawn for two hours after Indomethacin administration. On 5th day after 12 hours of fasting, the animals were treated with the last dose of Indomethacin and after five hours of administration of Indomethacin, the animals including negative control were sacrificed. While in Post treated standard and test groups the animals were first kept on fasting for 24 hours and ulcer induced by the administration of Indomethacin in the dose 20 mg/kg, daily for 5 days, thereafter the animals were treated with standard and test drug for next 5 days in the same dose and same manner as described above. On 6th day after 12 hours of fasting, the animals were sacrificed.

The water immersion restraint induced gastric ulcer was done by the method of Hayaso and Takeuchi[14]. The animals in this model were also divided in to 8 groups of 6 animals each. The animals in Group I and II were treated with distilled water and were serve as Plain control and Negative control, respectively. While the animals in Group III, IV and V were treated with standard drug, and hydro alcoholic extract of the test drug in the dose of 20 mg/kg, 145mg/kg and 248mg/kg and served as Pre-treated standard, Pre-treated test group A and Pre-treated test group B, respectively. All the animals were treated in this way once daily for 5 days. They had free access to food and water during the treatment period. However, on 4th day they were kept on fasting for 12 hours with water ad libitum. On 5th day, 12 hour fasted rats were treated routinely and after one hour of treatment animals in Group I were sacrificed while in rest of the groups, ulcer was induced by water immersion restraint method. Then animals were sacrificed. The animals in Group VI, VII and VIII were also subjected to gastric ulceration in the same manner as mentioned above. After one hour of ulcer induction animals were treated with standard and test drug and served as *Post*-treated standard group (VI) and Post-treated test group A and group B (VII and VIII) , respectively. All the animals were treated in this manner orally, once daily for five days. On 5th day, 12 hours fasted animals were treated routinely and after one hour of treatment, they were sacrificed. In all the above methods, the animals were sacrificed under The opent one anesthesia (40 mg/kg, IP). Stomach was removed from the body and opened along with the greater curvature, washed with fresh water and spread on cardboard with the mucous surface upwards. The mucosal surface was examined for ulceration with the help of magnifying lens (10 fold magnification) and scored by the method of Brzozowski et al [15,21,22]

VI. Assessment of Extent of Ulceration

The parameters viz. Ulcer score, ulcer index and reduction percentage in ulcer were taken to assess the

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anti ulcerogenic effect. Histopathological studies were also carried out to determine the nature and amount of damage and the improvement after treatment.

VII. Statistical Analysis

The observations in various groups were expressed as Mean ± SEM. The ulcer score and index of various groups were compared with plain control group. The group comparison was analyzed by using ANOVA one way with Kruskall Wallis and Dunn's pair comparison test.

VIII. Results

Plain control (Group I), showed no pathological sign. In Group II (Negative control) where ulcer was induced by Indomethacin (20mg/kg) once daily for 5 days, the ulcer score was found to be 1.08±0.27. The ulcer scores in pre-treated standard and test groups where the animals were treated with Standard drug & test drug in low dose were found to be 1.16±0.30 &1.33±0.27 respectively when compared to negative control showed non- significant result. In pre-treated test Group B (Group V), the test drug was given orally in the dose of 248mg/kg ulcer score was found to be 0.66±0.27 with respect to negative control, showed non- significant decrease. In *Post* treated standard group (Group VI). Ulcer score was found to be 0.66±0.27 (insignificant) with respect to negative control. In Post treated test group A (VII) it was observed 1.08±0.27 (insignificant). In Post treated test group B (VIII) score was found to be 0.33±0.10 (insignificant). The ulcer index in Negative control Pre and Post treated standard, test group A and B were found to be 1.25, 1.63, 1.80, 0.44, 0.17, 1.67 and 0.22 respectively and percentage of ulcer reduction in Pre and *Post* treated standard, test group A and B were observed -7, -19, 39, 39, 0, and 47, respectively when calculated with Negative control. (Table no 1)

Table 1 : Effect of Hydro alcoholic extract of Post *Sumaq* on Indomethacin induced restraint gastric ulcer

Groups	Treatment	ADU(Mean± SEM)	%RU	Ulcer index	%Reduction
Group I Plain control	D W	0.08±0.08	17%	0.01	94
Group II Negative control	D W + IM 20mg/kg dissolved in CMC	1.08±0.27	100%	1.25	-----
Group III Pre-treated Stand	Omeprazole 20 mg/ kg + IM 20mg/kg Dissolved in CMC	1.16±0.30	100%	1.63	-7
Group IV Pre-treated test A	*Post Sumaq* 145mg/kg+ IM 20mg/kg dissolved in CMC	1.33±0.27	100%	1.80	-19
Group V Pre-treated test B	*Post Sumaq* 248mg/kg IM 20mg/kg dissolved in CMC	0.66±0.27	67%	0.44	39
Group VI *Post*-treated Stand	IM 20mg/kg. dissolved in CMC+ omeprazole 20 mg/kg	0.66±0.27	50%	0.17	39
GroupVII *Post*-treated test A	IM 20mg/kg. dissolved in CMC + *Sumaq*145 mg/kg	1.08±0.27	100%	1.67	0
Group VIII *Post*-treated B	IM 20mg/kg. dissolved in CMC + *Post Sumaq* 248 mg/kg	0.33±0.10	67%	0.22	47

(N=6 in each group. DW = Distilled water, IM = Indomethacin, CMC= Carboxy, methyl cellulose, %RU =Percentage of rats with ulceration, ADU = Average degree of ulceration)

Ulcer score in Negative control was found to be significantly increased ($p<0.01$) 1.16±0.21 when compared to plain control. The ulcer score in pre-treated standard, test group A and test group B, score was found to be 0.91±0.27, 0.66±0.16, and 0.83±0.21 respectively. No significant reduction was observed when compared to negative control. While in Post-treated Group VI, Group VII and Group VIII first ulcer was graded and ulcer score was found to be 0.41±0.08, 0.75±0.25 and 0.75±0.25 respectively, but no significant reduction was observed when compared to negative control. The ulcer index in Negative control, Pre and Post treated standard, test group A and B was observed 1.42, 0.76, 0.67, 0.83, 0.35, 0.76, and 0.83 respectively and percentage of ulcer reduction was found to be 45, 60, 50, 75, 55, and 55 respectively. (Table no 2).

Table 2 : Effects of hydro alcoholic extract of Post *Sumaq* on Water-immersion induced restraint gastric ulcer

Groups	Treatment	ADU(Mean± SEM)	%RU	Ulcer index	%Reduction
Group I Plain control	D.W	0.08±0.08	17%	0.01	93

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Group II Negative control	D.W+ Ulcer induction	1.16±0.21*	100%	1.42	---
Group III Pre-treated Stand	Omeprazole 20 mg/ kg + Ulcer Induction	0.91±0.27	83%	0.76	45
Group IV Pre-treated test A	*Post Sumaq* 145 mg/kg+ Ulcer induction	0.66±0.16	100%	0.67	60
Group V Pre-treated test B	*Post Sumaq* 248mg/kg + Ulcer induction	0.83±0.21	100%	0.83	50
Group VI *Post*-treated Stand	Ulcer Induction + Omeprazole 20 mg/kg	0.41±0.08	83%	0.35	75
GroupVII *Post*-treated test A	Ulcer induction + *Post Sumaq* 145 mg/kg	0.75±0.25	83%	0.76	55
Group VIII *Post*-treated B	Ulcer induction. + *Post Sumaq* 248 mg/kg	0.75±0.25	83%	0.83	55

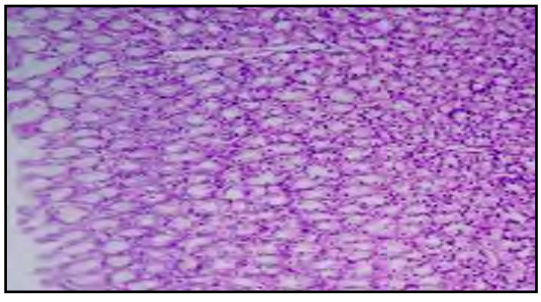
(N=6 in each group. Test used Kruskall Wallis test with Dunn, pair comparison test, N= 6 p<0.05 with respect to plain control, D.W= Distilled water)*

IX. Discussion

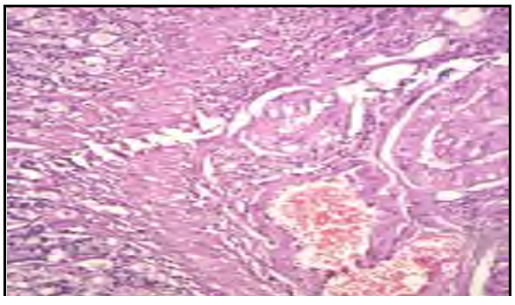
Gastric ulceration has long been viewed as the disease of stress, hence central nervous system may also play role in production of ulcer by causing hyperacidity. The techniques of restraint in albino rats provide a model for the study of stress induced gastric ulceration. Water immersion induced restraint gastric ulcer model is suitable to see anti stress effect of drugs. Therefore, the test drug was also evaluated by using this model. In Water Immersion Induced restraint gastric ulcer model the test drug was found both *precautionary and therapeutic in pre treated and Post treated test Groups at both dose level but the result was statistically non significant.* The histopathological findings are also in consonance. The findings indicate that the test drug does not possess anti anxiety properties and the same has not also been mentioned in Unani classics. However, it is clear from the result that the test drug has preventive & curative effect only at higher dose. Phytochemicals in *Rhus coraria* are ellagic acid, gallic acid isoquercitrin, myricitrin, myricetin, quercetin, quercitrin and tannic acid and flavinoids. Flavonoids protect the gastrointestinal mucosa from lesions produced by experimental ulcer models and different necrotic agents. Several mechanisms of action may be involved in this protective effect. Quercetin has an anti secretary mechanism of action. However, the most important mechanism of action responsible for the antiulcer activity of flavonoids is the antioxidant properties. Tannins are gastro protective which are present in the drug in sufficient amount[16,17].

As per the Unani theories it seems that the drug may have acted by temperament, as the *Mizaj* of the test drug is cold whereas that of diseases is hot[18,19,20]. But the anti ulcer mechanism cannot be just understood by *Mizaj* or Phytochemicals only as in case of herbal drugs only one or two or more Phytochemicals are not responsible for action. A number of chemicals and other interventions play the role in exerting actions and the ultimate effect is the cumulative effect. Further study is needed to establish the mechanism of anti ulcer effect of Post *Sumaq*.

Histopathological slides of different groups are shown below (Indomethacin ulcer model)



Group I : Normal mucosa inflammation and ulceration



Group II : Congestion, necrosis,

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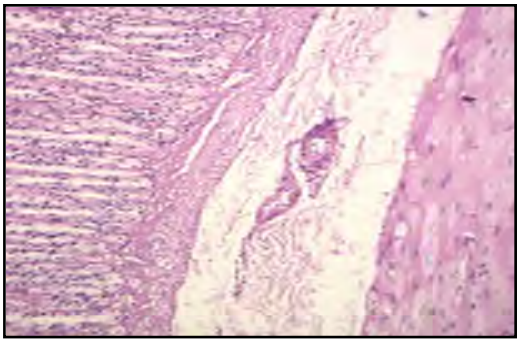
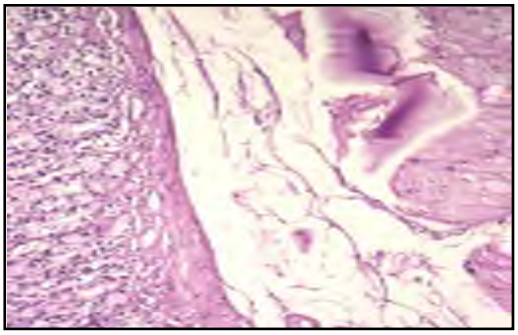
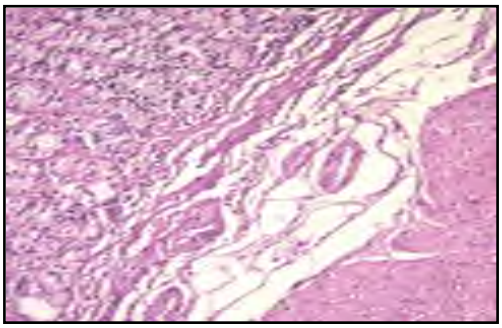


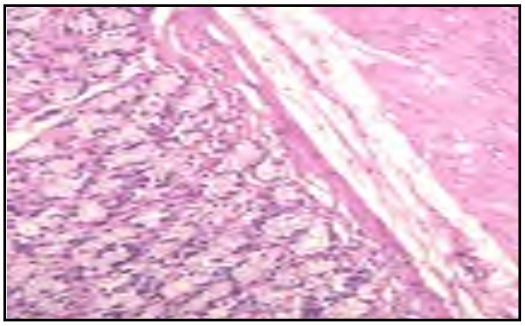
Group III : Oedema, necrosis, inflammation and ulceration



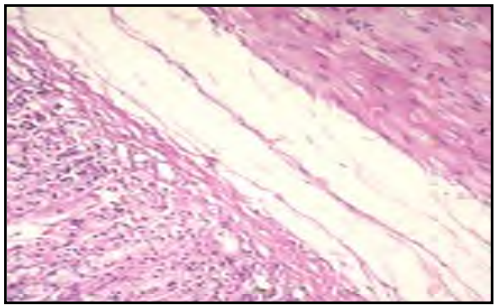
Group IV : Oedema, necrosis and ulceration



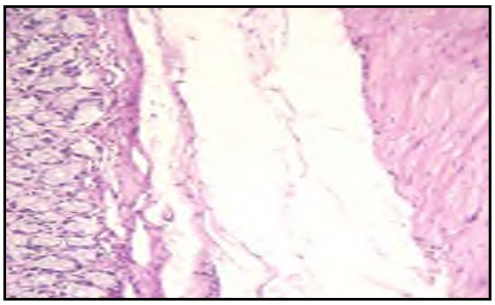
Group V : Inflammatory changes



Group VI : Inflammatory changes

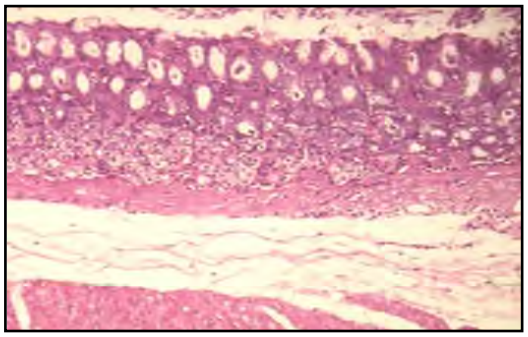


Group VII : Oedema, necrosis, inflammation and ulceration

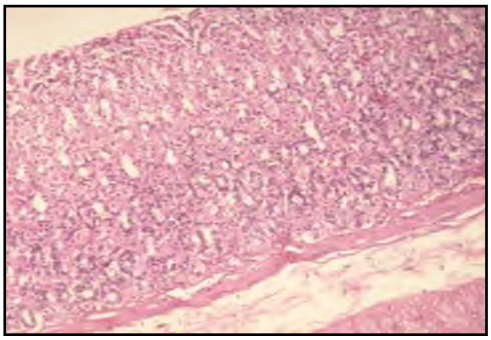


Group VIII : Inflammation and ulceration

Histopathological slides of different groups are shown below (Water immersion-induced restraint ulcer model)



Group I : Necrosis, inflammation and ulceration



Group II : Necrosis and inflammation

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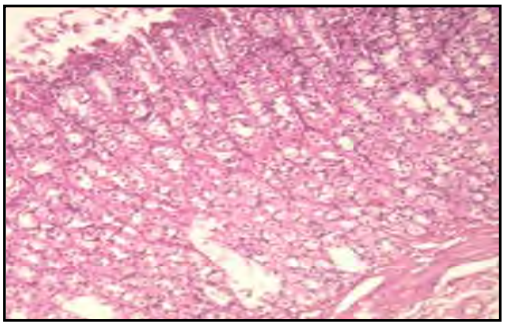
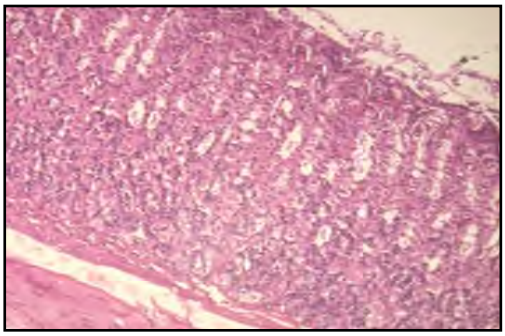
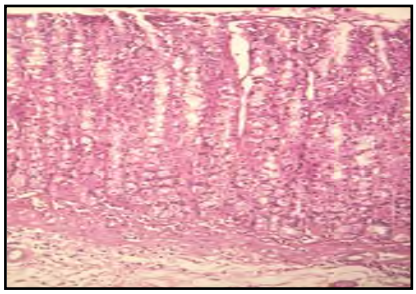


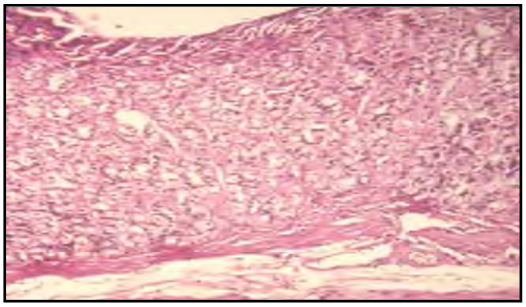
Group III : Necrosis and inflammation



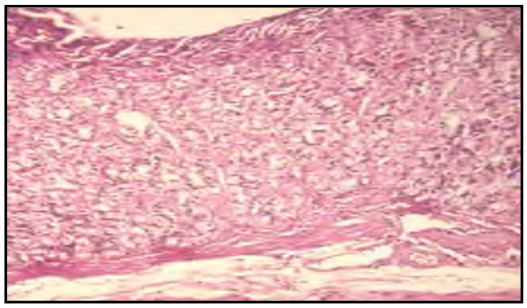
Group IV : Necrosis and inflammation



Group V : Necrosis and inflammation



Group VI : Inflammatory changes



Group VII : Inflammatory changes

X. Conclusion

Results of different experimental models revealed Post *Sumaq* to be a promising antiulcerogenic drug. The test drug possesses curative effect at higher dose against Indomethacin induced gastric ulcer. In Water Immersion-induced restraint gastric ulcer model the effect was less prominent therefore it can be concluded the test drug does not possess anti anxiety effect as this model produces ulcer due to stress. This is also evident from the literature that Post *Sumaq* is not used as an anti anxiety. The preventive effect of the test drug was more pronounced. This also validates the claim that herbal drugs are more preventive in nature. The drug is more effective at higher dose; therefore the dose of Post *Sumaq* should be revised after toxicity studies.

XI. Acknowledgement

The authors are highly thankful to the authorities of NIUM for providing necessary facilities to carry out this research work.

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Antidepressant and Anti-Inflammatory Activities of Cationic Amphiphilic Complexes of Sulphadiazine

By K.Hariprasath, I. Sudheer Babu, P.Venkatesh & U. Upendra Rao

Adarsa College of Pharmacy, India

Abstract- In our study we synthesized schiff' base of sulphadiazine on treating with aromatic aldehydes like para diethyl amino benzyldehyde and paradimethyl amino benzyldehyde. The synthesized schiff's bases were converted to its cationic amphiphilic bases by treating with methyl iodide. The cationic schiff bases were converted to metal complexes by treating with metals like copper chloride (CuCl2), zinc chloride (ZnCl2) and cadmium chloride (CdCl2). All the synthesized compounds were characterized by elemental analysis, IR and H1 NMR. Synthesized compounds were screened for anti-inflammatory and antidepressant activity. Copper metal complexes showed excellent anti-inflammatory activity and zinc metal complexes showed excellent anti-depressant activity.

Keywords: cationic amphiphilic bases, schiff' base, metal complexes, anti-inflammatory and antidepressant activity.

GJMR-B Classification : NLMC Code: QV 37.5

ANTIDEPRESSANTANDANTIINFLAMMATORYACTIVITIESOFCATIONICAMPHIPHILICCOMPLEXESOFSULPHADIAZINE

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Antidepressant and Anti-Inflammatory Activities of Cationic Amphiphilic Complexes of Sulphadiazine

K.Hariprasath [α], I. Sudheer Babu [σ], P.Venkatesh [ρ] & U. Upendra Rao [ω]

Abstract- In our study we synthesized schiff' base of sulphadiazine on treating with aromatic aldehydes like para diethyl amino benzyldehyde and paradimethyl amino benzyldehyde. The synthesized schiff's bases were converted to its cationic amphiphilic bases by treating with methyl iodide. The cationic schiff bases were converted to metal complexes by treating with metals like copper chloride (CuCl2), zinc chloride (ZnCl2) and cadmium chloride (CdCl2). All the synthesized compounds were characterized by elemental analysis, IR and H1 NMR. Synthesized compounds were screened for anti-inflammatory and antidepressant activity. Copper metal complexes showed excellent anti-inflammatory activity and zinc metal complexes showed excellent anti-depressant activity.

Keywords: cationic amphiphilic bases, schiff' base, metal complexes, anti-inflammatory and antidepressant activity.

I. Introduction

An amphiphilic substance exhibits a double affinity, which can be defined from the physico-chemical point of view as a polar-apolar duality. When a single surfactant molecule exhibit both anionic and cationic dissociations it is called amphoteric or zwitterionic. Cationic amphiphilic drugs (CADs) are widely used in chronic pharmacotherapies in spite of frequently observed side effects connected with lysosomal phospholipid (PL) storage. Cationic amphiphilic drugs (CADs) represent compounds of different therapeutic classes such as antidepressants, neuroleptics, and antiarrhythmics. In acidic cellular compartments these drugs become efficiently protonated and thus trapped in, e.g. lysosomes. As a result of pHdependent ion trapping, total lysosomal drug concentrations may exceed extracellular levels by orders of magnitude. Lysosomotropic drugs may inhibit lysosomal phospholipid (PL) metabolism leading to the formation of dense cytoplasmic granules, i.e. lysosomes filled with undegraded PLs. The formation of drug-PL complexes further enhances intracellular accumulation of drugs.

We all require iron, copper and zinc for normal brain function but metal metabolism becomes dysregulated in a variety of neurodegenerative diseases. Metals accumulate in Alzheimer's dementia and Parkinson's disease and are deficient in Menkes disease. Transition metals perform a wide range of biological functions in the brain. A common feature is their ability to exist in a variety of oxidation states and participate in redox reactions; thus copper, iron, and manganese are all catalytically active metals in a class of enzymes that sequester free radicals. It is useful to look at the common and varying functions of transition metals in the brain to better understand what mechanisms are disrupted in metal dyshomeostasis and how this may lead to cell death in diseases of the CNS (Tyszka, 2014).

Metal complexes are also known as coordination compounds, which include all metal compounds. Metal complex is a structure consisting of a central atom (or) ion (metal) bonded with anions (ligands). Compounds that contain a coordination complex are called coordination compounds. The bonding characteristics of complexes and alteration in size of the metal ion are related to thermodynamic aspects. The stability constants for the complexes formed from various metal ions and one ligand have a particular sequence (Banerjee, 2009, Shi, 2007).

The parent sulpha drugs Sulphadiazine is a well known antibacterial in olden days. But owing to their narrow spectrum of activity and side effects, now a days their usage is limited. In our research we improved the biological activity by different synthetic modifications, In the first step by converting the parent sulpha drug in the form of Schiff base, there by generating a lone pair of electrons in a sp2 hybridized orbital in the structure leading to the derivation of and different biological properties. In the second step of synthesis the Schiff bases were converted in to their cationic amphiphiles, which may alter their pharmacokinetic profile like distribution and binding parameters. This step helped us to improve the spectrum activity from narrow spectrum to broad spectrum activity, increasing the permeability of drug molecule in brain to cross blood-brain barrier, which improves the antidepressant property, and release of cationic lipids into the macrophage cytoplasm is a necessary step for anti-inflammatory activity. In the

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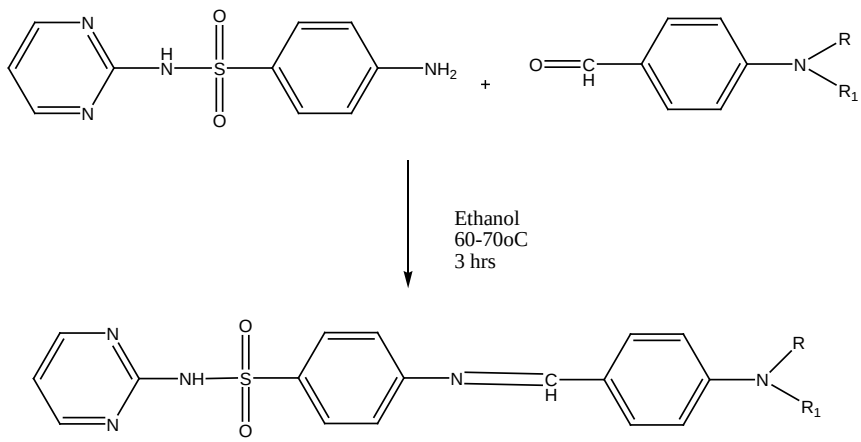
third step by deriving metal complexes of copper and zinc the biological properties like antidepressants and anti-inflammatory activities were strongly elucidated.

II. Materials and Methods

a) *Synthesis of Schiff's Base*

The Schiff's base has been synthesized by refluxing the reaction mixture of hot ethanolic solution (30 ml) of Sulphadiazine (0.01 mole) with hot ethanolic solution (30 ml) of different aromatic aldehyde (0.01 mole) for about 2-3 hours at 60-70^0 C (Fig-1). The mixture was allowed to stand over night. After that the colored solid product was filtered off, re-crystallized with ethanol and finally washed with petroleum ether. The final product was dried under reduced pressure over anhydrous calcium chloride (Panneerselvam, 2005).



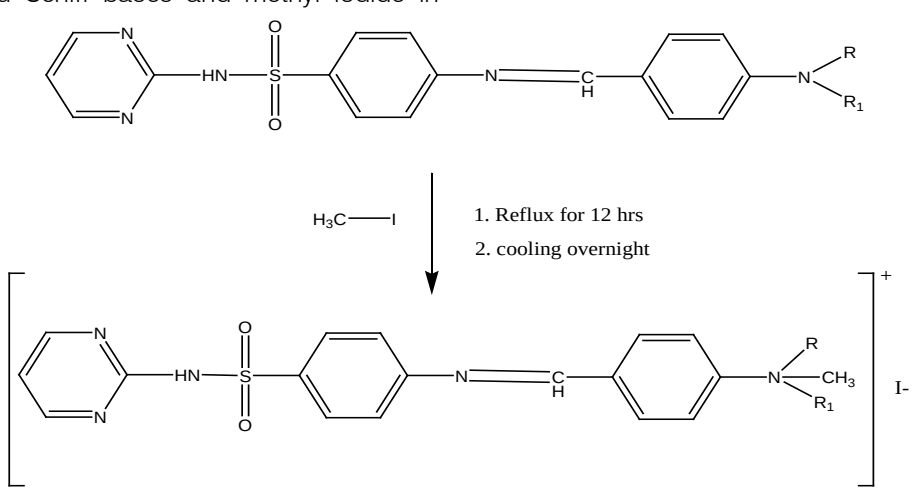
Compound A R-C_2H_5, R_1-C_2H_5

Compound B R-CH_3, R_1-CH_3

Figure 1: Scheme of synthesis of Sulphadiazine Schiff base

b) *Synthesis of cationic derivative of Schiff base*

Cationic derivatives of Schiff bases were obtained by direct reaction between equimolar amount of the synthesized Schiff bases and methyl iodide in 50ml ethanol (Fig-2). The reaction mixture was refluxed for 8 hours and left overnight. The precipitated products were filtered and recrystallized (Negm, 2010)



Compound A R-C_2H_5, R_1-C_2H_5

Compound B R-CH_3, R_1-CH_3

Figure 2 : Scheme of synthesis of cationic Schiff base

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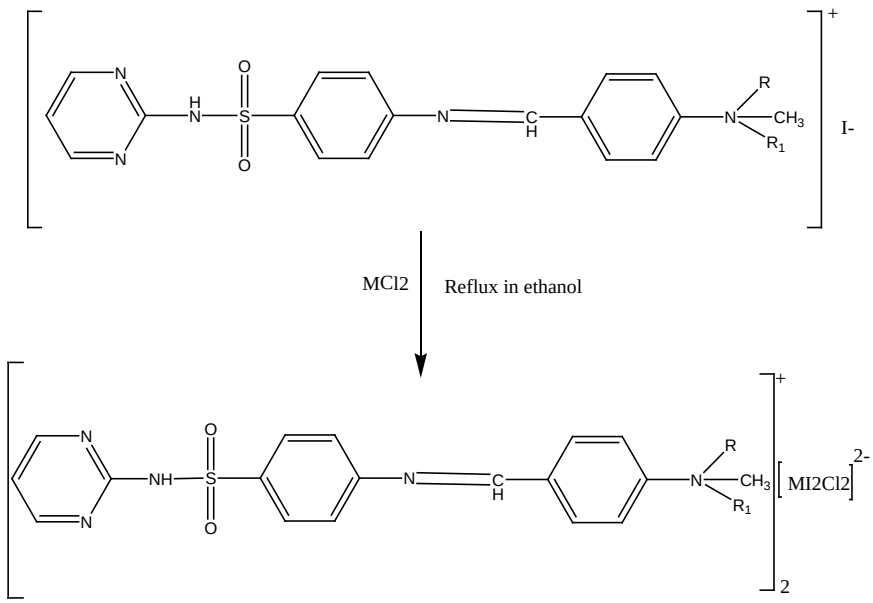
c) Synthesis of transition metal complexes.

Metal ion solutions of anhydrous $CuCl_2$, $ZnCl_2$ and $CdCl_2$ (0.0005 mol) in 50ml ethanol was added with synthesized cationic derivative of Schiff base separately and refluxed for 6 hours (Fig-3). The reaction mixture was left overnight to complete the precipitation of the products. The products were recrystallized with ethanol to obtain pure products (Ibotomba, 2012, Ajaykumar, 2009).



Compound A R-C_2H_5, R_1-C_2H_5

Compound B R-CH_3, R_1-CH_3

MCl_2- $CuCl_2$, $Zncl_2$ and $CdCl_2$

Figure 3 : Scheme of synthesis of cationic Schiff base

d) Determination of median lethal doses (LD50)

Animal's Swiss albino mice (20-25gm) and Male Sprague - Dawley rats (160-180) were maintained at standard diet and *ad libido.* The experiment protocol was approved from institutional ethical committee. The test compounds were dissolved in 3 % DMSO administered orally to different groups with increasing doses. Six animals were taken in each group. Mortality was determined after 24 hours of treatment. The dose, at which the 50 % mice survived, was considered as LD50 value of the compound (OECD, 2002).

e) Anti depressant activity

Male Sprague - Dawley rats weighing 160-180 grams were divided into eight groups of six animals each. The test groups received orally 20 mg/kg of each sample. The reference group received imipramine (5 mg/kg, p.o) while the control group received vehicle (1 % CMC). Naïve rats are individually forced to swim inside a vertical Plexiglas cylinder (height : 40 cm ; diameter : 18 cm ; containing 15 cm of water maintained at 25 oC). Floating behaviour during this 5 minutes period has been determined in different groups of rats. The percentage inhibition was calculated by the formula (Kulkarni, 2010).

$$\text{Percentage inhibition} = \frac{\text{Before treatment} - \text{After treatment}}{\text{Before treatment}} \times 100$$

f) Anti inflammatory activity

Swiss albino mice were divided into eight groups of six animals each. The test groups received orally 20 mg/kg of each sample. The reference group received diclofenac sodium (10 mg/kg, p.o) while the control group received vehicle (1 % CMC). All the animals should make a mark on both hind paws just beyond tibiofasial junction, so that every time the paw is dipped in mercury column up to fixed mark to ensure constant paw volume. After drug administration inject 0.1ml white egg portion to the plantar region of left paw of control as well as treated group. The right paw serve as reference non inflamed paw for comparison. The inflammation was quantitated in terms of ml i.e.

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replacement of water by edema using a Plethysmometer immediately before egg white injection and then 0, 1, 2 and 3 hours after egg white injection. The percent inhibition of edema as calculated for each group with respect to its vehicle treated control group. The anti-inflammatory activity was calculated by using the relation used by

% inhibition = (Vc-Vt/Vc) x 100

Whereas Vc was the average inflammation (hind paw edema) of the control group of mice at a given time, Vt was the average inflammation of the drug treated (i,e sample or reference diclofenac sodium) mice at the same time (Sathe, 2011).

g) Statistical analysis

The data were expressed as mean ±SEM. Statistical analysis was performed one-way ANOVA followed by Dunnett"s multiple comparison test using sigma stat software (version 2.0, Jandel Scientific Inc. USA

III. Results and Discussion

a) Characterization of synthesized compounds

A1- Copper metal complex of (E)- N-(4-(diethyl, methyl amino) benzylidene)-4-(pyrimidin -2-sulfonamidyl) benzenamine.

M.F: $C_{22}H_{26}Cl_2CuI_2N_5O_2S$. M. wt: 812.8. IR (KBr) cm-^{1}: NH bond stretching at 3400 cm-^{1}, C=N bond stretching at 1690 cm-^{1}, S=O stretching at 1140 cm-^{1}, C=C stretching at 1600 and 1475 cm-^{1}. H^1 NMR ($CDCl_3$) δ values: Multiplet at 7.44-8.38 for aromatic nucleus, singlet at 8.39 for N=CH peak, singlet at 4.0 for aromatic NH group, two triplet at 1.13 for two CH_3 groups in N-ethyl substitution, two quadret at 3.39 for two CH_2 group in N-ethyl substitution and singlet at 2.85 for N-methyl substitution. Elem Anal Calc: C, 32.51; H, 3.22; Cl, 8.72; Cu, 7.82; I, 31.2; N, 8.62; O, 3.94; S, 3.95. Elem Anal Found: C, 32.41; H, 3.32; Cl, 8.62; Cu, 7.92; I, 31.21; N, 8.60; O, 3.95; S, 3.96.

A2- Zinc metal complex of (E)- N-(4-(diethyl, methyl amino) benzylidene)-4-(pyrimidin -2-sulfonamidyl) benzenamine.

M.F: $C_{22}H_{26}Cl_2CuI_2N_5O_2SZn$. M. wt: 814.6. IR (KBr) cm-^{1}: NH bond stretching at 3400 cm-^{1}, C=N bond stretching at 1690 cm-^{1}, S=O stretching at 1140 cm-^{1}, C=C stretching at 1600 and 1475 cm-$^{1.}$ H^1 NMR (CDCl3) δ values: Multiplet at 7.44-8.38 for aromatic nucleus, singlet at 8.39 for N=CH peak, singlet at 4.0 for aromatic NH group, two triplet at 1.13 for two CH3 groups in N-ethyl substitution, two quadret at 3.39 for two CH2 group in N-ethyl substitution and singlet at 2.85 for N-methyl substitution. Elem Anal Calc: C, 32.44; H, 3.22; Cl, 8.70; I, 31.16; N, 8.60; O, 3.93; S, 3.94; Zn, 8.03. Elem Anal Found: C, 32.45; H, 3.23; Cl, 8.66; I, 31.14; N, 8.62; O, 3.83; S, 3.98; Zn, 8.13.

A3- Cadmium metal complex of (E)- N-(4-(diethyl, methyl amino) benzylidene)-4-(pyrimidin -2-sulfonamidyl) benzenamine.

M.F: $C_{22}H_{26}CdCl_2I_2N_5O_2S$. M.wt: 861.7. IR (KBr) cm-^{1}: NH bond stretching at 3400 cm-^{1}, C=N bond stretching at 1690 cm-^{1}, S=O stretching at 1140 cm-^{1}, C=C stretching at 1600 and 1475 cm-^{1}. H^1 NMR (CDCl3) δ values: Multiplet at 7.44-8.38 for aromatic nucleus, singlet at 8.39 for N=CH peak, singlet at 4.0 for aromatic NH group, two triplet at 1.13 for two CH_3 groups in N-ethyl substitution, two quadret at 3.39 for two CH_2 group in N-ethyl substitution and singlet at 2.85 for N-methyl substitution. Elem Anal Calc: C, 30.67; H, 3.04; Cd, 13.05; Cl, 8.23; I, 29.46; N, 8.13; O, 3.71; S, 3.72. Elem Anal Found: C, 30.77; H, 3.14; Cd, 13.00; Cl, 8.13; I, 29.36; N, 8.03; O, 3.78; S, 3.74.

B1- Copper metal complex of (E)- N-(4-(trimethyl amino) benzylidene)-4-(pyrimidin -2-sulfonamidyl) benzenamine

M.F: C20H22Cl2CuI2N5O2S. M.wt: 784.7. IR (KBr) cm-1: NH bond stretching at 3400 cm-1, C=N bond stretching at 1690 cm-1, S=O stretching at 1140 cm-1, C=C stretching at 1600 and 1475 cm-1. H1 NMR (CDCl3) δ values: Multiplet at 7.44-8.38 for aromatic nucleus, singlet at 8.39 for N=CH peak, singlet at 4.0 for aromatic NH group and three singlet at 2.85 for three N-methyl groups. Elem Anal Calc: C, 30.61; H, 2.83; Cl, 9.04; Cu, 8.10; I, 32.34; N, 8.92; O, 4.08; S, 4.09. Elem Anal Found: C, 30.59; H, 2.81; Cl, 9.14; Cu, 8.00; I, 32.14; N, 8.82; O, 4.18; S, 4.00.

B2- Zinc metal complex of (E)- N-(4-(trimethyl amino) benzylidene)-4-(pyrimidin -2-sulfonamidyl) benzenamine

M.F: $C_{20}H_{22}Cl_2I_2N_5O_2SZn$. M.wt: 786.6. IR (KBr) cm-^{1}: NH bond stretching at 3400 cm-^{1}, C=N bond stretching at 1690 cm-^{1}, S=O stretching at 1140 cm-^{1}, C=C stretching at 1600 and 1475 cm-^{1}. H^1 NMR (CDCl3) δ values: Multiplet at 7.44-8.38 for aromatic nucleus, singlet at 8.39 for N=CH peak, singlet at 4.0 for aromatic NH group and three singlet at 2.85 for three N-methyl groups. Elem Anal Calc: C, 30.54; H, 2.82; Cl, 9.01; I, 32.27; N, 8.90; O, 4.07; S, 4.08; Zn, 8.31. Elem Anal Found: C, 30.44; H, 2.92; Cl, 9.00; I, 32.26; N, 8.95; O, 4.17; S, 4.13; Zn, 8.21

B3- Cadmium metal complex of (E)- N-(4-(trimethyl amino) benzylidene)-4-(pyrimidin -2-sulfonamidyl) benzenamine.

M.F: $C_{20}H_{22}CdCl_2I_2N_5O_2S$. M.wt: 833.6. IR (KBr) cm-^{1}: NH bond stretching at 3400 cm-^{1}, C=N bond stretching at 1690 cm-^{1}, S=O stretching at 1140 cm-^{1}, C=C stretching at 1600 and 1475 cm-^{1}. H^1 NMR ($CDCl_3$) δ values: Multiplet at 7.44-8.38 for aromatic nucleus, singlet at 8.39 for N=CH peak, singlet at 4.0 for aromatic NH group and three singlet at 2.85 for three N-methyl groups. Elem Anal Calc: C, 28.82; H, 2.66; Cd, 13.48; Cl, 8.51; I, 30.45; N, 8.40; O, 3.84; S, 3.85. Elem Anal Found: C, 28.92; H, 2.56; Cd, 13.42; Cl, 8.57; I, 30.40; N, 8.45; O, 3.82; S, 3.87

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b) Anti inflammatory activity of synthesized compounds

Anti inflammatory activity was carried by paw oedema method using diclofenac sodium as standard. The results are given in the table-1. Currently used anti-inflammatory drugs are associated with some severe side effects. Therefore, the development of potent anti-inflammatory drugs with fewer side effects is necessary. A major factor limiting their use is gastrointestinal toxicity. In recent years, Schiff bases are widely used in formulating various types of drugs for their diversive biological activities. Metal complexes of Schiff bases have also been used as anti-inflammatory and antiarthritic agents. Anti- inflammatory of Zn (II) and Cu(II) complexes of indomethacin has been reported previously (Venugopala, 2003, Wei, 2006, Alam, 2012 & Sondhi, 2006), based on this we have evaluated anti-inflammatory activity of synthesized metal complexes. The down regulation of pro-inflammatory mediators through interaction of cationic lipids with the PKC pathway may explain this anti-inflammatory activity. Furthermore, since cationic lipids have intrinsic anti-inflammatory activity. Studies indicating that the release of cationic lipids into the macrophage cytoplasm is a necessary step for anti-inflammatory activity (Mario, 1997). Results revealed that the copper metal complexes (20mg/kg.b.wt) of Schiff bases of sulpha drugs A1, B1 showed excellent anti-inflammatory activity in carrageenan induced edema method by comparing with standard drug diclofenac sodium (10mg/kg.b.wt). It was thus confirmed that copper complexes, a unique class of potentially more therapeutically useful anti-inflammatory drugs. These results demonstrate that cationic lipids can be considered as novel anti-inflammatory agents.

Table1 : Results of *in vivo* anti inflammatory activity of metal complexes of schiff's base of Sulphadiazine

	Group	Dose	Paw volume (ml)				Difference Vc-Vt/Vc	Mean value	% anti-inflammatory activity
			0 hour	1 hour	2 hour	3 hour			
Group-I	Control	1% CMC	0.2	0.6	0.5	0.5	0.4	0.33	-
			0.2	0.6	0.6	0.7	0.4		
			0.2	0.5	0.6	0.6	0.3		
			0.2	0.5	0.6	0.7	0.3		
			0.2	0.5	0.5	0.8	0.3		
			0.2	0.5	0.7	0.8	0.3		
Group-II	Compound 1A1	(20mg/kg b.wt)	0.3	0.3	0.1	0.1	0	0.06	81***
			0.3	0.4	0.2	0.2	0.1		
			0.3	0.3	0.2	0.1	0		
			0.3	0.4	0.2	0.1	0.1		
			0.3	0.4	0.2	0.2	0.1		
			0.2	0.3	0.2	0.2	0.1		
Group-III	Compound 1A2	(20mg/kg b.wt)	0.3	0.5	0.4	0.3	0.2	0.20	39*
			0.3	0.6	0.4	0.3	0.3		
			0.3	0.5	0.4	0.3	0.2		
			0.2	0.4	0.2	0.2	0.2		
			0.3	0.4	0.3	0.2	0.1		
			0.3	0.5	0.4	0.4	0.2		
Group IV	Compound 1A3	(20mg/kg b.wt)	0.3	0.5	0.5	0.4	0.2	0.23	30*
			0.3	0.6	0.4	0.4	0.3		
			0.2	0.4	0.3	0.3	0.2		
			0.3	0.5	0.4	0.4	0.2		
			0.3	0.6	0.5	0.3	0.3		
			0.3	0.5	0.5	0.4	0.2		
Group V	Compound 1B1	(20mg/kg b.wt)	0.3	0.4	0.2	0.1	0.1	0.05	84***
			0.3	0.3	0.2	0.1	0		
			0.3	0.3	0.2	0.2	0		
			0.3	0.4	0.1	0.1	0.1		
			0.3	0.4	0.2	0.1	0.1		
			0.3	0.3	0.2	0.1	0		

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Group VI	Compound 1B2	(20mg/kg b.wt)	0.3	0.5	0.4	0.4	0.2	0.18	45**
			0.3	0.6	0.5	0.2	0.3		
			0.3	0.5	0.4	0.3	0.2		
			0.3	0.4	0.2	0.1	0.1		
			0.3	0.4	0.3	0.2	0.1		
			0.3	0.5	0.4	0.4	0.2		
Group-VII	Compound 1B3	(20mg/kg b.wt)	0.3	0.6	0.4	0.4	0.3	0.26	21*
			0.3	0.7	0.5	0.5	0.4		
			0.3	0.5	0.5	0.1	0.2		
			0.3	0.4	0.7	0.2	0.1		
			0.3	0.7	0.8	0.7	0.4		
			0.3	0.5	0.7	0.4	0.2		
Group VIII	Standard Diclofenac sodium	10mg/kg	0.2	0.2	0.2	0.1	0	0.05	84***
			0.2	0.3	0.1	0.1	0.1		
			0.2	0.3	0.2	0.1	0.1		
			0.3	0.4	0.1	0.2	0.1		
			0.2	0.3	0.1	0.1	0.1		
			0.2	0.3	0.1	0.16	0.1		

n = 6. Values are expressed as ± S.E.M.

***P< 0.001, **P< 0.01, *P< 0.05, ns P > 0.05 Vs Control (One way ANOVA followed by Dunnett's test).

c) Antidepressant activity of synthesized compounds

Anti depressant activity was evaluated by force swim test method, the animals which are immobile for less time considered as active. The results are given the table-2. Results revealed that the zinc metal complexes (20mg/k.b.wt) of Schiff bases of sulpha drug A2, B2 showed excellent anti-depressant activity in reducing the duration of depressed behavior in animal models by despair swim test when compared with the standard drug Imipramine (5mg/kg.b.wt).

Table 2 : Results of *in vivo* anti depressant activity of metal complexes of schiff's base of Sulphadiazine

S.No	Treatment	Immobile response in 5 minutes		Percentage response (%)
		Before treatment	After treatment	
1	Group-I Control (1% CMC)	3.20	3.66	-
		2.86	3.14	
		3.12	3.00	
		3.56	3.66	
		3.88	3.22	
		2.88	2.86	
2	Group-II Standard (Imipramine 5mg/kg.b.wt)	3.28	1.48	65***
		3.86	1.76	
		3.42	1.34	
		3.66	0.68	
		3.18	0.86	
		3.88	1.26	
3	Group-III A1 (20mg/kg.b.wt)	3.88	1.88	51**
		4.66	1.86	
		4.12	1.96	
		2.44	1.44	
		3.72	1.63	
		3.34	1.86	
4	Group-IV A2 (20mg/kg.b.wt)	4.20	1.66	75***
		3.86	0.88	
		4.12	1.22	
		3.44	0.86	
		4.66	0.66	
		2.88	0.46	
5	Group-V A3 (20mg/kg.b.wt)	3.88	1.88	37**
		2.88	1.42	
		3.56	2.88	
		4.66	3.68	

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		3.98	2.24	
		4.00	2.62	
6	Group-VI B1 (20mg/kg.b.wt)	4.20	2.61	34*
		2.86	2.02	
		3.72	1.68	
		3.48	2.82	
		3.88	2.86	
		4.88	2.92	
7	Group-VII B2 (20mg/kg.b.wt)	3.20	1.42	67***
		2.86	1.04	
		4.44	1.22	
		4.33	1.06	
		3.86	1.48	
		2.56	0.66	
8	Group-VIII B3 (20mg/kg.b.wt)	4.20	2.86	29*
		3.86	1.98	
		3.62	2.80	
		3.56	2.98	
		3.86	2.62	
		3.58	2.68	

n = 6. Values are expressed as ± S.E.M. ***P< 0.001, **P< 0.01, *P< 0.05, ns P > 0.05 Vs Control (One way ANOVA followed by Dunnett's test)

The most probable causes for depression are connected with the loss of homeostasis of the stress hormones, neurotransmitters, and disturbed trace elements levels. It has been reported that successful depression therapy can lead to zinc level normalization. It is reported that early life stress is a major risk factor for development of later depression due to affected neurogenesis in brain, especially in hippocampus. On the molecular level, these processes may be zinc-dependent via antioxidative activity changes and its influence on proper course of brain development process (Malgorzata, 2014, Chandramouli, 2012). There is an evidence for the role of mitochondrial dysfunction in the pathophysiology and treatment of neurodegenerative diseases, including mood disorders (Kato, 2000, Stork, 2005). Respiratory rate is a parameter characterizing functioning of the oxidative phosphorylation. The mitochondrial hypothesis states that impaired energy metabolism of brain cells is involved in the pathophysiology of antidepressants. The therapeutic or side effects of drugs administered in the treatment of depression may involve the targeted regulation of mitochondrial functions. In previous study it is reported that there is a direct mitochondrial targeting is involved in mechanisms of action of pharmacologically different antidepressants. Antidepressants are potent partial inhibitors of mitochondrial respiration (Jana, 2012).

IV. Conclusion

Cationic amphiphilic drugs (CADs) represent compounds of different therapeutic classes such as antidepressants, neuroleptics, and antiarrhythmics. In their neutral, lipophilic form cationic amphiphilic drug enter cells and their organelles. In acidic cellular compartments these drugs become efficiently protonated and thus trapped in, e.g. lysosomes. By increasing the permeability of drug molecule in brain to cross blood-brain barrier, which improves the antidepressant property and release of cationic lipids into the macrophage cytoplasm is a necessary step for anti-inflammatory activity.

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In Vivo Anti-Inflammatory and in Vitro Antioxidant Activities of Toona Ciliata Leaves Native to Bangladesh

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Abstract- The study was performed to assess the anti-inflammatory, antioxidant activities and identify the polyphenols of *Toona ciliata* grown in Bangladesh. Anti-inflammatory activity was examined by the methods of carrageenan and histamine-induced paw edema. At the dose of 400 mg/kg, effective anti-inflammatory activity ($P<0.01$) was observed in rats for both the test models of carrageenan and histamine-induced paw edema, compared to indomethacin. In ABTS scavenging assay, IC50 value was found significant (5.50µg/ml) compared to ascorbic acid (12.01µg/ml). The maximum absorbance of reducing power was obtained 0.4939 at 250µg/ml relative to ascorbic acid (1.1115µg/ml). Total antioxidant capacity, total phenolic and flavonoid content were found to be 357.1 mg/g ascorbic acid, 239.2 mg/g gallic acid, and 98.36 mg/g quercetin equivalent, respectively.

Keywords: toona ciliata, free radical scavenging, anti-inflammatory, hplc, epicatechin, p-coumeric acid, rutin hydrate.

GJMR-B Classification : NLMC Code: QV 766

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Hemayet Hossain [α], Proity Nayeeb Akbar [σ], Shaikh Emdadur Rahman [ρ], Tanzir Ahmed Khan [ω], M d. Mahfuzur Rahman [¥] & Ismet Ara Jahan [§]

Abstract- The study was performed to assess the anti-inflammatory, antioxidant activities and identify the polyphenols of *Toona ciliata* grown in Bangladesh. Anti-inflammatory activity was examined by the methods of carrageenan and histamine-induced paw edema. At the dose of 400 mg/kg, effective anti-inflammatory activity ($P<0.01$) was observed in rats for both the test models of carrageenan and histamine-induced paw edema, compared to indomethacin. In ABTS scavenging assay, IC50 value was found significant (5.50µg/ml) compared to ascorbic acid (12.01µg/ml). The maximum absorbance of reducing power was obtained 0.4939 at 250µg/ml relative to ascorbic acid (1.1115µg/ml). Total antioxidant capacity, total phenolic and flavonoid content were found to be 357.1 mg/g ascorbic acid, 239.2 mg/g gallic acid, and 98.36 mg/g quercetin equivalent, respectively. During HPLC analysis, catechin and ellagic acid were determined in considerable amounts (825.95 and 416.70 mg/100g extract, respectively). The findings suggest that *Toona ciliata* could be a potential source of natural antioxidant.

Keywords: toona ciliata, free radical scavenging, anti-inflammatory, hplc, epicatechin, p-coumeric acid, rutin hydrate.

I. Introduction

Toona ciliata *(T. ciliata)*, also commonly known as the red cedar, toon or toona, Burma cedar, Indian cedar or Indian mahogany, is a forest tree in the mahogany family (Meliaceae). It grows widely in the regions of southern Asia and Australia. (1, 2) These are usually large plants that grow up to a height of 25 to 35 m and the leaves are alternate and pinnetely veined with assymetrical base and an acute apex. [3]

Studies on the transverse section of the bark of *T. ciliata* revealed the presence of periderm, cortex, sclerides, mednllary rays and phloem fiber. [4] The barks were also found to contain tetranortriterpenoids, including toonacilin and the leaves hold a considerable amount of aromatic compounds like coumarin, glycoside, tannins, flavonoids, phenolic compounds, triterpenoids and steroids. [5] In addition, three new norlimonoids, two new tirucallane-type triterpenoids, and a new pimaradiene-type diterpenoid, along with two known limonoids and eight known tirucallane-type triterpenoids, were isolated from the leaves and twigs of *T. ciliata.* [5, 6]

The plant *T. ciliata* possess many important biological properties that account for it's traditional uses in medicinal treatments, construction purpose, dye preparation, etc. [7] The flowers are used to produce dye, which are worn around Asia as color silk. *T. ciliata* barks are useful in chronic dysentery, ulcer, leprosy, fever, headache, blood complaints, etc. [8]

The plant has been reported to exhibit significant antibacterial, antifungal, anticancer, anti-ulcer, anti-tumor, analgesic, anti-microbial, gastro protective and cytotoxic activity. [9, 10, 11] The ethanol leaf extract of *T. ciliata* was studied for its inhibitive effects on protein non-enzymatic glycation. [12]

The aim of the present work is to determine the anti-inflammatory, antioxidant activities and identify the bioactive polyphenolic compounds by HPLC in the ethanol extract of Toona ciliata leaves grown in Bangladesh.

II. Materials and Methods

a) Plant material

Fresh leaves were collected in May 2013 from Khulna, Bangladesh. Leaves of *T. ciliata* were washed, dried in the shade to minimize loss of volatile constituents and reduced to powder with a grinder.

b) Extraction

Collected fresh leaves were separated from undesirable materials and washed with water before letting it stand under the sun for a week. The dried leaves were coarsely powdered with the help of a grinder (Capacitor start motor, Wuhu motor factory, China). About 400g of the powered material was taken in a clean, flat-bottomed glass container and soaked in 1000 ml of ethanol. The container along with its contents

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was sealed and left to stand for a period of 7 days with continuous stirring by an orbital shaker. The mixture was first filtered in a clean cotton plug to remove any plant debris, and then through Whatman filter paper no. 1 (Bibby RE200, Sterilin Ltd., UK). The filtrate was concentrated by rotary vacuum evaporator (R-210, Buchi, Switzerland) and dried. The sample rendered 51g of greenish gummy concentrate (12.75%) and was designated as the crude ethanol extract.

c) Chemicals

Gallic acid (GA), (+)-catechin hydrate (CH), vanillic acid (VA), caffeic acid (CA), (-)-epicatechin (EC), p-coumaric acid (PCA), rutin hydrate (RH), ellagic acid (EA), quercetin (QU), ascorbic acid, ABTS, folin-ciocalteu's phenol reagent, carrageenan and histamine were purchased from Sigma–Aldrich (St. Louis, MO, USA). Acetonitrile (HPLC), methanol (HPLC), acetic acid (HPLC), ethanol, trichloroacetic acid (TCA), phosphate buffer (pH 6.6), potassium ferricyanide [K3Fe(CN)6], ferric chloride (FeCl3), sodium phosphate, EDTA, ammonium molybdate and sodium carbonate were of analytical grade and purchased from Merck (Darmstadt, Germany).

d) Test animals & drugs

For the screening of in vivo anti-inflammatory activity, male rats of Wister strain weighing 175-205 g were used. The animals were housed under standard laboratory conditions maintained at 25 ± 1°C and under 12/12 h light/dark cycle and feed with Balanced Trusty Chunts and water *ad libitum*. All experimental protocols were in compliance with Bangladesh Council of Scientific and Industrial Research (BCSIR) ethics committee on Research in animals as well as internationally accepted principles for laboratory animal use and care.

The standard drug, Indomethacin was used for this study and purchased from Square Pharmaceuticals Ltd, Bangladesh.

III. Anti-Inflammatory Activity Test

a) Carrageenan-induced oedema

The activity of *T. ciliata* ethanol leaf extract was evaluated using the carrageenan induced hind paw edema model. (13) The rats were divided into four groups (five rats per group). Group I (control) was given 1% tween 80 in normal saline (10 ml/kg), while Group II (positive control) received 10 mg/kg body wt. of indomethacin orally. Group III and IV were injected with 200 and 400 mg/kg body wt. of *T. ciliata* orally, respectively. Acute inflammation was induced in all the four groups by sub plantar injection of 0.1 ml of its suspension of carrageenan with 1% tween 80 in normal saline in the right paw of the rats, 1 h after the oral administration of the tested materials. The paw volume was measured with a micrometer screw gause at 1-hour interval after the administration of the drug and the extract. The percentage inhibition of inflammatory effect of the extract was calculated using the following expression:

Percentage inhibition of inflammation = [(Vc-Vt)/Vc] × 100, where Vc is the average degree of inflammation by the control group and Vt is the average degree of inflammation by the test group.

b) Histamine-induced oedema

The activity of the *T. ciliata* extract was evaluated with histamine-induced paw edema model. (14) The paw oedema was generated by injecting 0.1% histamine solution sub-plantarly into the left hind paw of each mice at a dose of 0.1 ml. Twenty rats were divided into four groups of five animals each. Group I (control) was supplied with 1% tween 80 in normal saline (10 ml/kg). Group II (positive control) received 10 mg/kg body wt. of indomethacin orally. Group III and IV were given 200 and 400 mg/kg body wt. of *T. ciliata* orally, respectively. Acute inflammation was induced in all the four groups by sub plantar injection of 0.1 ml of histamine with 1% tween 80 in normal saline in the right hind paw of the rats, 1 h after the oral administration of the tested materials. The paw volume was measured with a micrometer screw gause at 1, 2, 3 and 4 h after the administration of the drug and the extract. The percentage inhibition of inflammatory effect of the extract was calculated using the same formula as for calculating the carrageenan-induced paw oedema.

IV. Antioxidant Activity Test

a) ABTS radical scavenging activity test

The method of decolourisation of free radical $ABTS^+$ was performed according to Fan et al. with some modifications. (15) ABTS radical cation was prepared by mixing 7 mM ABTS solution with 2.45 mM potassium persulfate. The mixture was allowed to stand for 12-16 h at room temperature in the dark until reaching a stable oxidative state. The ABTS solution was diluted with ethanol to an absorbance of 0.70 ± 0.02 with pH 7.4 phosphate buffered saline (PBS) solution at 734 nm, before use. The reaction mixture was allowed to stand at room temperature for 6 min and the absorbance at 734 nm was immediately recorded. The ABTS scavenging activity was calculated as follows:

$$\text{ABTS scavenging effect} = I\ (\%) = (A_o - A_s / A_o) \times 100$$

Where, A_o = Absorbance of control and A_s = Absorbance of sample

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b) Reducing power assay

The reducing power of *T. ciliata* was studied using the method of Hemayet et al. and Dehpour et al. [16, 17] The extract at different concentrations was mixed with 1 ml ethanol, 2.5 ml phosphate buffer (0.2 M, pH 6.6), and 2.5 ml potassium ferricyanide [$K_3F_e(CN)_6$] (1%). The sample solutions were next incubated at 50°C for 20 min and a 10% solution of trichloroacetic acid (2.5 ml) was added to them. They were then centrifuged at 3000 rpm for 10 min. The top layer of the mixture (2.5 ml) was mixed with 2.5 ml distilled water and 0.5 ml of 0.1% $FeCl_3$. The absorbance was measured at 700 nm with a spectrophotometer. All determinations were carried out in triplicate.

c) Total antioxidant capacity

The total antioxidant capacity was measured by the method of Prieto et al. [18] The ethanol extract was prepared in its respective solvent and mixed with 1 ml of the reagent solution (0.6M H_2SO_4, 28 mM sodium phosphate, 4 mM ammonium molybdate mixture). The tubes were incubated for 90 min at 95°C. The mixture was cooled to room temperature and the absorbance was read at 695 nm against a blank sample. Ascorbic acid equivalents were calculated using the standard graph for ascorbic acid. The experiment was conducted in triplicates and values were expressed as equivalents of ascorbic acid in mg per gram of extract.

D. Total phenolic content

Total phenolic content of the extract was determined using the modified Folin-Ciocaltu method. [19, 20] After reacting 0.5 ml of extract (1 mg/ml), 5 ml Folin-Ciocaltu reagent (1:10 v/v distilled water) and 4 ml (75 g/l) of sodium carbonate, the sample solutions were mixed and left to stand at 40°C for the next 30 min for color development. The absorbance was read at 765 nm. The total phenolic content was calculated and expressed as mg of gallic acid equivalent per gram using the equation obtained from the standard gallic acid calibration curve, $y = 6.993x + 0.0379$, $R2 = 0.9995$.

d) E. Total flavonoid content

The total flavonoid content was determined by reactions of the aluminium chloride colorimetric method with some modifications. [21, 22] The absorbance of the reaction mixture was measured at 430 nm with a double beam Analykjena UV/Visible spectrophotometer (Model 205, Jena, Germany). Quercetin was used for calibration of a standard curve ($y = 6.2548x + 0.0925$; $R^2 = 0.998$) and the results were expressed as mg of quercetin equivalent per gram dry weight of sample.

V. HPLC Detection of Polyphenolics

Detection of selected polyphenolic compounds in the extract was carried out by HPLC as described by Ismet et al. [23] The analysis was performed on a Dionex UltiMate 3000 system equipped with quaternary rapid separation pump (LPG-3400RS) and photodiode array detector (DAD-3000RS). Separation was done using Acclaim® C_{18} (5μm) Dionex column (4.6 x 250 mm) at 30 °C with a flow rate of 1 ml/min and an injection volume of 20 μl. The mobile phase consisted of acetonitrile (solvent A), acetic acid solution pH 3.0 (solvent B), and methanol (solvent C) with the gradient elution program of 10%A/80%B/10%C (0-9 min), 20%A/60%B/20%C (10-19 min) and 100%A (20-30 min) with post run equilibration of the system with 5%A/95%B (5 min). The UV detector was set to 280 nm for 18.0 min, changed to 320 nm for 6 min, and finally to 380 nm and held for the rest of the analysis period while the diode array detector was set at an acquisition range from 200 nm to 700 nm. For the preparation of calibration curve, a standard stock solution was prepared in methanol containing gallic acid (GA), vanillic acid (VA), (+)-catechin (CH), (-)-epicatechin (EC), *p*-coumaric acid (PCA), rutin (R), ellagic acid (EA) (20 μg/ml each), caffeic acid (CA) (8 μg/ml) and quercetin (QU) (6 μg/ml). A solution of *T. ciliata* leaf extract was prepared in ethanol at 5 mg/ml. All solutions were filtered through 0.20 μm nylon syringe filter (Sartorius, Germany) and degassed in an ultrasonic water bath (Hwashin, Korea) for 15 min. Data acquisition, peak integration, and calibrations were performed with Dionex Chromeleon software (Version 6.80 RS 10).

VI. Statistical Aanalysis

Data were presented as mean ± Standard deviation (S.D). Statistical analysis for animal experiment was carried out using one-way ANOVA followed by Dunnet's multiple comparisons using SPSS Data Editor for Windows, Version 11.5.0 (SPSS Inc., U.S.A.). The results obtained were compared with the control group. P values < 0.05 were considered to be statistically significant.

VII. Results

a) Carrageenan-induced paw edema

The anti-inflammatory effect of the *T. ciliata* using carrageenan induced oedema test is expressed in Table 1. The paw edema was highly reduced by indomethacin ($p<0.05$; $p<0.01$) between the first and forth hour (50.48% to 64.46% inhibition). A maximum edema paw volume of 1.66 ± 0.08 mm was observed in the control group, four hours after the carrageenan injection. Rats which received 400 mg/kg body weight of the extract were observed to significantly decrease ($p<0.05$; $p<0.01$) the carrageenan-induced oedema paw volume between the 1 to 4 hour time interval, in comparison to that of the standard drug, indomethacin, at a dose of 10 mg/kg body weight. The highest reduction in the paw volume by the 400 mg/kg body weight of the extract at 4 h was 55.42%, while that by indomethacin was 64.46%, respectively.

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Table 1 : Effect of ethanol extract of *Toona ciliata* leaves and indomethacin on carrageenan-induced oedema paw volume in male wistar rats

Treatment Groups	Doses (mg/kg body weight)	Right hind paw volume (mm)			
		1 h	2 h	3 h	4 h
Control	2 ml/kg	1.03 ± 0.07	1.30 ± 0.09	1.50 ± 0.04	1.66 ± 0.08
Positive Control (Indomethacin)	10	0.51 ± 0.05* (50.48)	0.63 ± 0.06** (51.54)	0.70 ± 0.07* (53.33)	0.59 ± 0.03** (64.46)
Extract	200	0.95 ± 0.04* (7.77)	1.09 ± 0.05* (16.15)	1.16 ± 0.08** (22.67)	1.18 ± 0.09* (28.92)
Extract	400	0.59 ± 0.06** (42.72)	0.70 ± 0.03* (46.15)	0.76 ± 0.06** (49.33)	0.74 ± 0.04** (55.42)

Values in brackets denote percentage inhibition of the oedema paw volume.

Values are expressed as mean±SD; Values are calculated as compared to control using one way-ANOVA followed by Dunnet's Test; * indicates $P < 0.05$; ** indicates $P < 0.01$ vs. control; n = 5.

b) Histamine-induced paw edema

Table 2 gives information on the effect of *T. ciliata* extract on acute inflammation using histamine-induced paw edema test. A maximum edema paw volume of 1.59 ± 0.08 mm was observed in the control group at 4 h after histamine was injected. Rats that were pre-treated with 400 mg/kg body weight of the extract significantly compressed ($p<0.05$; $p<0.01$) the histamine-induced edema paw volume, in comparison to that by indomethacin. The percentage inhibition of the edema paw volume at 1, 2 and 3 h by the 400 mg/kg body weight of the extract was also found effective ($p<0.05$; $p<0.01$). The maximum reduction in the paw volume by the 400 mg/kg body weight of *T. ciliata* at 4 h was 56.60%, while that by the indomethacin declined to 65.41%, respectively.

Table 2 : Effect of ethanol extract of *Toona ciliata* leaves and indomethacin on histamine-induced oedema paw volume in male wistar rats

Treatment Groups	Doses (mg/kg body weight)	Right hind paw volume (mm)			
		1 h	2 h	3 h	4 h
Control	2 ml/kg	1.09 ± 0.06	1.29 ± 0.07	1.40 ± 0.05	1.59 ± 0.08
Positive Control (Indomethacin)	10	0.48 ± 0.08** (55.96)	0.56 ± 0.03* (56.59)	0.53 ± 0.08* (62.14)	0.55 ± 0.03** (65.41)
Extract	200	0.84 ± 0.05* (22.94)	0.87 ± 0.09* (32.56)	0.92 ± 0.07** (34.29)	0.99 ± 0.06* (37.73)
Extract	400	0.60 ± 0.04* (44.95)	0.67 ± 0.07** (48.06)	0.71 ± 0.05* (49.29)	0.69 ± 0.05** (56.60)

Values in brackets denote percentage inhibition of the oedema paw volume.

Values are expressed as mean±SD; Values are calculated as compared to control using one way-ANOVA followed by Dunnet's Test; * indicates $P < 0.05$; ** indicates $P < 0.01$ vs. control; n = 5.

c) ABTS radical scavenging activity

At minimum concentration (10 μg/ml), the highest activity obtained by the extract of *T. ciliata* was 98.22 ± 0.04 μg/ml (Table 3). The IC50 value of the extract was found to be 5.50 ± 0.16 μg/ml, which was similar to that of the ascorbic acid (12.01 ± 0.12 μg/ml).

Table 3 : ABTS radical scavenging activity of *T. ciliata* leaf extract with standard ascorbic acid

Concentration (μg/ml)	*T. ciliata* leaf extract	Ascorbic acid
10	98.22 ± 0.04	48.60 ± 0.17
20	98.60 ± 0.12	85.79 ± 0.25

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40	98.73 ± 0.08	99.19 ± 0.21
60	98.87 ± 0.41	99.25 ± 0.29
80	99.24 ± 0.10	99.53 ± 0.24
100	99.31 ± 0.13	95.58 ± 0.18
250	99.34 ± 0.11	99.85 ± 0.27
IC_{50}	5.50 ± 0.13	12.01 ± 0.12

The values are expressed as mean ± standard deviation (n=3).

d) Reducing power assay

The reducing power assay was determined based on the relative maximum absorbance of the extract of *T. ciliata* and was observed to increase with an increase in concentration (Table 4). At 250 µg/ml, the maximum absorbance for the ethanolic leaf extract of *T. ciliata* was found to be 0.4939 ± 0.029, while the standard ascorbic acid showed an absorbance of 1.1115 ± 0.009.

Table 4 : Reducing power assay of *T. ciliata* leaf extract with standard ascorbic acid

Concentration (µg/ml)	*T. ciliata* leaf extract	Ascorbic acid
10	0.0190 ± 0.028	0.3801 ± 0.012
20	0.0695 ± 0.071	0.4577 ± 0.017
40	0.0819 ± 0.017	0.5398 ± 0.023
60	0.1056 ± 0.041	0.6345 ± 0.037
80	0.1699 ± 0.062	0.7125 ± 0.013
100	0.1986 ± 0.041	0.7811 ± 0.029
250	0.4939 ± 0.029	1.1115 ± 0.009

The values are expressed as mean ± standard deviation (n=3).

e) Total antioxidant capacity

The ethanol extract of *T. ciliata* possessed a high total antioxidant capacity (Table 5). The total antioxidant capacity of the extract was obtained in significant quantity relative to the standard ascorbic acid per gram of extract (357.10 ± 2.02).

Table 5 : Total antioxidant capacity of ethanolic leaf extract of *T. ciliata*

Extract	Total antioxidant capacity
	mg of ascorbic acid equivalent (AAE) per g of dry extract
T. ciliata leaf extract	357.1 ± 2.02

The values are expressed as mean ± standard deviation (n=3).

f) Total phenolic content

Table 6 demonstrates the total phenolic content in the ethanol leaf extract of *T. ciliata.* High phenolic content was determined in the extract (239.2 ± 2.53 mg/g of gallic acid equivalent).

Table 6 : Total phenolic and flavonoid content of ethanolic leaf extract of *T. ciliata*

Extract	Total phenolic content	Total flavonoid content
	mg of gallic acid equivalent (GAE) per g of dry extract	mg of quercetin equivalent (QE) per g of dry extract
T. ciliata leaf extract	239.2 ± 2.53	98.36 ± 1.07

The values are expressed as mean ± standard deviation (n=3).

g) Total flavonoid content

Table 6 demonstrates the total flavonoid content in the leaf extract of T. ciliata. A considerably large amount of flavonoid was observed in the extract (98.36 ± 1.07 mg/g of quercetin).

h) HPLC assay of T. ciliata

The contents of the phenolic compounds in the leaf extract of *T. ciliata* were analyzed by RP-HPLC (Table 7). Based on the comparison of the retention times with those of the standard peaks, seven phenolic

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compounds: (+) catechin, vanillic acid, epicatechin, p-coumeric acid, rutin hydrate, ellagic acid and quercetin were identified, respectively (Figure 1). The most abundant phenolic compound obtained from the extract of *T. ciliata* was catechin (825.95 ± 5.39 mg/100 g dry extract) followed by ellagic acid (416.70 ± 3.58 mg/100 g dry extract). Next, there was epi-catechin, p-coumeric acid and rutin hydrate, which were also obtained in significant quantities, but in lower amounts than that of the first two (211.7 ± 2.36, 102.20 ± 1.87 and 77.57 ± 1.49 mg/100 g dry extract, respectively). Other polyphenolic compounds like vanillic acid and quercetin were also obtained in similar concentrations (34.05 ± 0.83 and 29.13 ± 0.65 mg/100 g dry extract).

Table 7 : Contents of polyphenolic compounds in the ethanolic leaf extract of *T. ciliata* (n=3)

Polyphenolic compound	Ethanol extract of *T. ciliata* leaf	
	Content (mg/100 g of dry extract)	% RSD
CH	825.95	5.39
VA	34.05	0.83
EC	211.7	2.36
PCA	102.2	1.87
RH	77.57	1.49
EA	416.7	3.58
QU	29.13	0.65

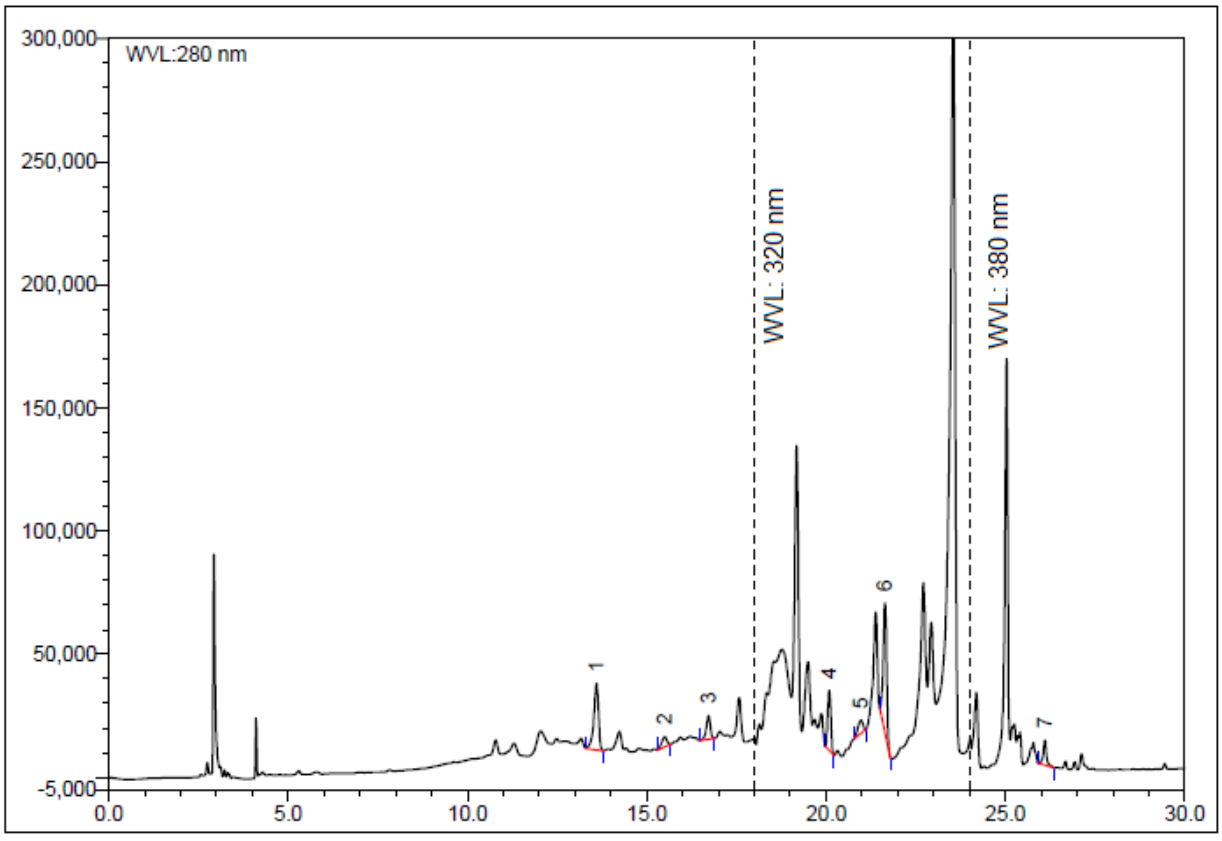

Figure 1 : HPLC chromatogram of ethanol leaf extract of *T. ciliata*. Peaks: 1, catechin hydrate; 2, vanillic acid; 3, epi-catechin; 4, *p*-coumeric acid; 5, rutin hydrate; 6, ellagic acid; 7, quercetin

VIII. Discussion

Carrageenan and histamine induced paw oedema were evaluated for their anti-inflammatory effect in *T. ciliata*. The carrageenan induced inflammatory response in rats is a biphasic response, which causes marked oedema formation that results from the rapid production of several inflammatory mediators such as histamine, serotonin, and bradykinins. The second step is the release of prostaglandins and nitric oxide with a peak at 3 h, which is produced by an inducible is of orm of cyclooxygenase (COX-2) and nitric oxide synthase

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(iNOS). [24] The present investigation was carried out in an attempt to reduce the oedomatogenic response in rats evoked by carrageenan. Results show that pre-treated oral administration of the extract was effective in the reduction of the response. Thus, a relationship can be inferred between the anti-inflammatory properties of the extract and the inhibition of intracellular signalling pathways in inflammatory mediators.

On injection, histamine acts as an inflammation mediator. [25] The liquid spreads out inside the body of the rat like a wheal and increases the permeability of the host capillary venules in the skin. Substances that inhibit the activity of histamine receptors shrink that particular area where the wheal was formed. This could be because the anti-inflammatory activity of the extract is supported by its anti-histamine activity. The antihistaminic effect of the extract increases with the concentration of the extract. The extract inhibits the formation and action of the inflammatory mediators, effectively suppressing the production of oedema by histamine. This study shows that *T. ciliata* has significant anti-oedematogenic effect ($P<0.01$) on paw oedema in rats induced by both carrageenan and histamine.

When subjected to reducing power assay, the extract causes the oxidation of ferricyanide complex to its ferrous form. This results in the extract to donate a hydrogen atom, which in turn helps to break the free radical chain and exert an antioxidant response. [26] The high phenolic content in the ethanol leaf extract of *T. ciliata* might be a reason for this reduction of Fe3+ to Fe2+, exhibiting stronger reducing power ability.

The total antioxidant capacity depends on the reduction of Mo (VI) to Mo (V) by the extract and the subsequent formation of green phosphate/Mo (V) complex at an acidic pH. [27])

Studies on the antioxidant activities of the leaf and flower extracts of *Toona ciliata* have previously been carried out using several different solvents i.e. petroleum ether, chloroform, ethyl acetate and methanol. Based on the results, all the extracts showed significant DPPH and ABTS radical scavenging activity in comparison with the standard, BHT (butylatedhydroxytoluene). The petroleum ether, chloroform, ethyl acetate and methanol extracts of *Toona ciliata* showed DPPH and ABTS significant activity with IC50 value of 150, 135.5, 105 and 92.5 µgml-1 for DPPH and 145, 120, 120.5 and 95 µgml-1 for ABTS scavenging activity, respectively, in comparison to standard BHT with an IC50 of 8 µgml-1 and 11.5 µgml-1, respectively. (12) Better results for the ABTS radical scavenging activity of *T. ciliata* ethanol extract relative to ascorbic acid were obtained from the present investigation (IC50 5.50 ± 0.130). Another study on the methanol and hexane fractions of *T. ciliata* leaves compared the plant's reducing power activity, which suggests a better activity for the methanol extract with a maximum relative absorbance of 0.440 ± 0.0001, which was very close to the absorbance of the standard drug, ascorbic acid (0.461 ± 0.0030). [28]

HPLC analysis of the ethanol leaf extract of *T. ciliata* was used to determine and quantify the phenolic compounds present in the extract. Several studies have shown that vanillic acid and quercetin possess antioxidant properties. In addition, catechin and ellagic acid compounds have been found to play a role in the anti-inflammatory activity [29, 30, 31] and rutin hydrate and quercetin are known to demonstrate good anti-inflammatory properties. [32, 33, 34] HPLC studies confirm the presence of relatively high concentration of these antioxidant chemicals in *T. ciliata*, which helps to explain the significant anti-inflammatory and antioxidant activity of this plant extract.

IX. Conclusion

The study demonstrates significant antioxidant and anti-inflammatory activity of the ethanol leaf extract of T. ciliata. Moreover phenolic compounds were detected with HPLC and a correlation can be suggested between the plant's antioxidant and anti-inflammatory properties and the high level of polyphenolic compounds present in its extract. Nevertheless, activity varies depending on several conditions including the plant type, its biome, growing conditions, etc. However, based on the results obtained, it can be asserted that the plant *T. ciliata,* grown in Bangladesh can be of great medicinal value in physiological processes and other cures, relieves and preventions.

X. Conflict of Interest

We declare that we have no conflict of interest.

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Glycemic Control and Self-Care Practice among Ambulatory Diabetic Patients in Ambo General Hospital, West Showa, Ethiopia

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Abstract- Background: The main goal in diabetes care is to improve the patient's quality of life, to maintain satisfactory metabolic control and to retain minimal complications caused by diabetes mellitus (DM). To accomplish these goals, self-care has a great role. However, most patients don't control their blood sugar label. The objective of this study was to investigate status of glycemic control and self-care practice among Ambulatory diabetes patients in Ambo General Hospital.

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Glycemic Control and Self-Care Practice among Ambulatory Diabetic Patients in Ambo General Hospital, West Showa, Ethiopia

Gobezie Temesgen Tegegne [α], Awoke Shiferaw [σ], Belayneh Kefale Gelaw [ρ], Amsalu Degu Defersha [ω], Minyahil Alebachew Woldu [¥] & Jimma Likisa Linjesa [§]

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Results: The study showed that 58.5% of the respondents had type 1 diabetes and the remaining 41.5% were type2 DM. Mean FBS of three consecutive month was183.28 mg/dL. Only23.4%, 34.2% and 28.8% of the respondents were able to control their Fasting Blood Sugar (FBS) to level below 126 mg/dL during their last first, second and third visit to the hospital. 20(18.01%) of the respondents do exercise daily and attend their follow up program as scheduled respectively.

Conclusion: The present study illustrates that the level of knowledge about diabetes and self care practices amongst diabetic patients was low. In addition, it showed that respondents' level of physical activity, their educational status and time of insulin injection was low. Type 1 DM is the most prevalent type identified during the study period. In general, self-care practice was inadequate, especially in terms of physical self-care activity and a deficit in terms of knowledge related to diabetes; this could be explained by factors such as limited education and low levels of economic status

Recommendation: Health care providers should educate and promote health to address the lack of information on a healthy diet, benefits of exercise and how exercise should be undertaken for ambulatory DM patients when they come for regular follow up regularly at the hospital. Primary care physician must interpret (preferably in patient's language), for each diabetic patient, the short and long-term benefits of adhering to diet and exercise recommendations, insulin storage site in the hospital as well as at home must be given special emphasis.

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Keywords: diabetes, self-care, fasting blood sugar, glycemic control, ambo general hospital.

I. Introduction

a) Background

Diabetes mellitus is not a single disease entity but rather a group of metabolic disorders sharing the common underlying feature of hyperglycemia. Hyperglycemia in diabetes results from defects in insulin secretion, insulin action, or, most commonly, both. The chronic hyperglycemia and attendant metabolic dysregulation of diabetes mellitus may be associated with secondary damage in multiple organ systems, especially the kidneys, eyes, nerves, and blood vessels. It also greatly increases the risk of developing coronary artery disease and cerebrovascular disease. In concert with great technologic advances, there have been pronounced changes in human behavior, with increasingly sedentary life styles and poor eating habits. This has contributed to the simultaneous escalation of diabetes and obesity worldwide, which some have termed the "diabesity" epidemic. (6)

Prevalence of both type 1 and type 2 DM is increasing worldwide, type 2 DM is rising much more rapidly, presumably because of increasing obesity, reduced activity levels as countries become more industrialized, and the aging of the population [1].

According to IDF diabetes Atlas, 5th edition 2012 report, currently, more than 80% of people with diabetes live in low and middle income countries. The African region is expected to experience the highest increase in coming years withestimated increase in prevalence rates of 98% for sub-Saharan Africa, and 94% for North Africa and the Middle East (10, 11, 12, 13).It also said regional prevalence of 3.8%. This would rise to 4.3% 2030.Based on the IDF Atlas 5th edition, 2012 report, number of cases of diabetes in Ethiopia to be estimated about 1.4 million in 2011 (7, 8).

The diagnosis involves evaluation of blood glucose levels which are normally maintained in a very narrow range; usually 70 to 120 mg/dl that is established by elevation of blood glucose by any one of the following three criteria (1, 3)

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A random blood glucose concentration of 200 mg/dL or higher, with classical signs and symptoms; A fasting glucose concentration of 126 mg/dL or higher on more than one occasion, or; An abnormal oral glucose tolerance test (OGTT), in which the glucose concentration is 200 mg/dL or higher 2 hours after a standard carbohydrate load (75 gm of glucose). (2)

b) Statement Of The Problem

DM is a life-long challenge that requires behavioral change and adequate self-care practices for better glycemic control. In the absence of appropriate self-care practice, the desired therapy targets are difficult, or even impossible, to achieve.

Thus, the aim of the present study was to assess self-care practices and glycemic control among diabetes patients in Ambo general hospital.

Non- communicable diseases including diabetes account for 60% of all deaths worldwide andmore than 80% of diabetes deaths occur in low- and middle-income countries. According to IDF Atlas 5th edition2012 report, Diabetes caused 4.6 million deaths in 2011 globally. World Health Organization projects that diabetesdeaths will double between 2005 and 2030. Statistics for medical complications from diabetes are also concerning. Proportions of patients with diabetic complications in sub Saharan region ranged from 7-63% for retinopathy, 27-66%for neuropathy and 10-83% for nephropathy. Diabetes is likely to increase the risk of several important infections inthe region, including tuberculosis, pneumonia and sepsis (14).Diabetes being a chronic illness requires continuous self-management practices bysufferers so that they can contribute meaningfully in the management of their lives. A situation where diabetes patients visit clinics regularly and their blood glucose levels still remain high despite the treatment they receive is problem that calls for attention. This is a very common observation in many diabetes patients. Severe complications, like gangrene that may lead to amputation and possible premature death, this might be because of lack of appropriate self-management practices (4).

Despite the benefits of engaging in a recommended self-management practice, research remains limited on determining recommended self-care practices level and its associated factors among diabetes patients. Researchers have suggested that self-care activities vary extensively according to the nature of the activity itself, with taking of medication often occurring as recommended and exercise frequently falling below recommended levels. For example, results from one study showed that 97% of respondents' with diabetes always or usually took their medication, whereas only 41% always or usually exercised, as cited by NancyE. Schoenberg (14). Furthermore, we currently lack an in-depth understanding of level and associated factors of type2 diabetes patients to ward diabetes self-care practices.

Although all forms of diabetes mellitus share hyperglycemia as a common feature, the underlying causes of hyperglycemia vary widely. The vast majority of cases of diabetes fall into one of two broad classes: (1)

Type1diabetes is characterized by an absolute deficiency of insulin secretion caused by pancreatic β-cell destruction, usually resulting from an autoimmune attack. It accounts for approximately 10% of all cases (5).

c) Significance Of The Study

The major problematic condition about diabetes self-care practices is that there is limited research findings on diabetic patients in our country, even there is no enough published material and little research is done on this areas. To address these deficits, this research explores for diabetes self-management practices. So the findings of this research can help diabetic patients to know how to control their sugar level and improve their self-care practice. Based on the findings of the research, patients of DM at Ambo hospital will be advised to strengthen those positive practices and will also be advised to practice appropriately by discouraging improper practices. The study can also serve as starting material for those who want to undertake further research on this area.

II. Objectives of The Study

a) General Objectives

- The aim of this study was to assess factors prevalence of glycemic control and self-management practices among ambulatory diabetic patients in Ambo general Hospital

b) Specific Objectives

- To determine prevalence of poor glycemic control.
- To assess dietary self-care practices among diabetic patients at Ambo General Hospital.
- To assess the physical activity and foot care practices among diabetic patients at Ambo
- General hospital.
- To assess blood glucose monitoring practice among the diabetic patients within three months.

III. Methods and Participants

a) Study area and period

This study was conducted in Ambo General Hospital, which is found in Ambo town, west showa, Ethiopia. Ambo has a total population of 260, 193 of whom 131, 922 are men. It is located in the west Showa zone of the Oromia region, 114 km West of Addis Ababa, the town has a latitude and longitude of 8.983O N 37.850O E and an elevation of 2101m. The town has an annual rain fall of 1012 mm with 18 CO average temperatures. The Hospital is found in 01 kebele of Ambo town near West Shewa zone prison House. The hospital give serves for about 10,000 People. The study was conducted from February 1/2014 to .May/2014

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b) Study design

Cross-sectional study was conducted from 01 February to May 30, 2014.

c) Source population

Source population includes all diabetes patients that attend their follow up schedule in Ambo general hospital.

d) Study population

Study population includes all diabetes patients that follow their diseases status in Ambo general Hospital for the last three months.

e) Sampling size Determination

All DM patients who came to Ambo general hospital for follow up during data collection period were considered to be included in the sample. Accordingly 111 patients fulfilled the inclusion criteria and were included in the study. Six of the patients that didn't fulfilled the inclusion criteria were excluded from the study.

f) Inclusion and exclusion criteria

i. *Inclusion criteria*

Patient that has been part of a follow-up program for at least three follow at Ambo General Hospital was included in the study.

ii. *Exclusion criteria*

Patients with mental health problems, hearing impairments or any other serious health problems and those patients who were unable to provide the appropriate information were excluded.

g) Study Variables

i. *Dependent variables*

Self-care practice among diabetic patients, glycemic control

ii. *Independent variables*

Socio demographic characteristics of study population

h) Data collection procedure and Instrument

Patients were interviewed using structured questionnaires and check lists was used to gain information from their card. The data collection was conducted by the joint collaboration of the investigator, nurses and health care professionals that are involved in delivery of care to the specified patients in the hospital by orienting them on how to collect the data. Questionnaires were prepared in English and translated into Amharic and Afan Oromo (local languages) and translated back into English to check its consistency. To identify the patterns of glycemic control, patients' charts was reviewed, retrospectively; the last three successive FBS or RBS results was recorded from the patient's card.

i) Data quality control

Pretest was done in 10 patients at Ambo Hospital to assure validity of the check list and questionnaire. Language experts who were qualified with second degree with linguistic and are Ambo University stuff members were used to translate the questionnaires from English to Amharic and Afan Oromo version. The questionnaires were revised for its completeness and consistency.

j) Data analysis

The data was cleaned, coded, entered and analyzed using SPSS version 20. Categorical variables were described by frequencies and percentages, and continuous variables were described by means and standard deviations. Figures and tables were used to summarize the results.

k) Ethical consideration

Formal letter was obtained from Research Ethics Committee of Ambo University and submitted to Ambo General Hospital, so the letter was given to the hospitals and they allowed us to do the research. Verbal consent was taken from the patient.

l) Operational definition

The level of glycemic control was indicated as 'adequate glycemic control' when FBS results were less than 126 mg/dL (7 mm/L) (i.e. an average of three visits), or when RBS results were less than 200/dL; 'inadequate glycemic control' takes place when a parameter is beyond the criteria of adequate glycemic.

i. *Knowledge*

Knowledge of patients' relating to diabetes and self-care practice was assessed by making use of 'yes/no' questions. A correct answer will be coded as '1' and an incorrect answer as '0'; the score is then computed. Respondents are labeled as having knowledge of diabetes and self-care practices if he or she scored ≥ the mean value, and having poor knowledge if he or she scored less than the mean.

ii. *Physical activity*

The levels of physical activity of the patients were classified into three levels based on their physical activities as light, moderate and heavy.

1. Light activity: Patients are in a sitting position most of the time, less than half of the time they are standing or walking, they seldom carry heavy things, and travel by car or motorbike.
2. Moderate activity: Patients are sitting, standing and walking about half of their time. They spend some time carrying heavy things and use public transport during non-leisure hours.
3. Heavy activity: Patients spend almost none of their time sitting and almost all of their time standing or walking, most of the time carrying heavy things, and they use public transport, cycle or walk everywhere.

Self-care

Self-care means looking after yourself in health way. This includes changes to your diet, different types

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of exercise or different types of medication you may need to make.

m) Limitations of the study

The findings from this situational analysis may not be generalized to the total population because of the lower sample size than the expected.

IV. Results

a) Socio-demographic characteristics of the participants

A total of 111diabetic patients were participated in the study giving a response rate of 100%. From total respondents 63 (56.75%) were male, regarding the age of participants, 33 (29.7%) of them were younger than 30 years of age and the remaining 78 respondents (70.27%) were above age of 30. Most of them completed grade 7-12 and 29 patients had monthly income of 500-800 birr.

Table 1 : socio-demographic characteristic of the patients in ambulatory diabetic patients

Characteristic type	Characteristic	Frequency (%)
Sex	Male	63(56.75)
	Female	48(43.25)
Age	1-29	33(29.7)
	30-44	31(27.9)
	45-64	37(33.3)
	Above 65	10(9)
Marital status	Single	42(37.8)
	Married	65(58.5)
	Widowed	4(3.3)
Religion	Orthodox	64(57.6)
	Muslim	8(7.2)
	Protestant	37(33.3)
	Others	2(1.8)
Ethnicity	Oromo	96(86.7)
	Amhara	14(12.6)
	Tigre	1(0.9)
Occupation	Farmer	38(34.2)
	Merchant	20(18)
	Civil servant	28(25.2)
	Others	25(22.5_)
Education level	Illiterate & non formal edu.	18(16.2)
	Grade 1-6	26(23.4)
	Grade 7-12	44(39.6)
	Above grade 12	23(20.7)
Income	<500	51(45.9)
	500-800	29(26.1)
	801-1500	16(14.4)
	>1500	15(13.5)

b) Clinical characteristics of the patients

Regarding the clinical characteristics of the patients, from the total of 111 patients 65 of them were diagnosed for type 1 DM (58.5%) and the remaining 46 of them (41.5%) were type 2 DM.70.27% say no family member with DM and the rest 29.73% say there is a family member with DM. Concerning the presence of other co morbidities 17. 11% have hypertension and 6.3% of the patients have CKD. When we look their respective drug use, 35 patients which contribute 31.53% use glabinclamide, 22 patients which contribute about19.81% use metformin, 6 patients which contribute 5.1% use both insulin & metformin, and 65 patients which contribute about 58.55% use insulin. Out of the 65 insulin users, 10 patients (15.38%) use refrigerator, 55(84.61%) use home prepared cool sites.

Of the patients who were using insulin, only 23 (20.7%) took meals 30 minutes after each insulin injection, and the remaining patients were used to eat before injection while 70.2% took meals after one hour of taking an injection. The majority of the respondents (104, 93.7%) follow their medication strictly to avoid

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raise in blood sugar level. From the total respondents, 96 of them (86.5%) attend their follow up program as per the schedule.10.8% patients have habit of alcohol or smoking and the remaining 99 respondents (89.2%) do not have any habit.

Means of communication was assessed to know how diabetic patients can obtain information or education from Medias and from newspapers as well as to know whether they have phone in case they face emergency conditions(hypoglycemia or hyperglycemia) to obtain health services. The results shows the majority of the patients have access to radio, TV and phone services and 91 respondents (82%) do not have the chance to get newspapers.

Table 2 : clinical characteristics of the patients

Variables	*Frequency (%)*
Type of DM	
Type1 DM	65(58.55)
Type 2 DM	46(41.44)
Time since DM d	
<2000	6(5.4)
2000-2002	33(29.7)]
2003-2006	72(64.89)
Hx of DM in the family	
Yes	33(29.73)
No	78(70.27)
Co morbidities	
CKD	7(6.3)
HTN	19(17.11)
CHF	1(0.9)
Stroke	0(0)
Others	13(11.71)
Pattern of drugs for DM	
Insulin	65(58.55)
Oral hypoglycemic agents	
Metformin	22(19.81)
Glabineclamide	35(31.53)
Insulin &metformin	6(5.4)
Source of information for DM	
Radio Yes	83(74.8)
No	28(25.2)
TV Yes	60(54)
No	51(46)
Phone Yes	58(52.2)
No	53(47.8)
Newspaper yes	20(18)
No	91(82)

c) Knowledge about diabetes

Participants were asked whether DM is a chronic disease or a curable disease and whether it is possible to control it by interventions, such as a healthy diet, exercise, and administering insulin and hypoglycemic drugs. Accordingly, 72 respondents (64.86%) responded that it is chronic disease 39 respondents (35.14%) said that DM is curable and 96respondents (86.48%) reported that it is possible to control diabetes. Furthermore, the majority (83.78%) of the respondents knows the sign and symptoms of DM and the remaining (16.22%) do not know the signs and symptoms of DM.

d) Self- care practice

i. *Dietary self -care practice*

Concerning to food items that they consume to control their sugar level, the majority of the respondents answered that injera (i.e. a stable food diet in Ethiopia made of Teff cereal), barely and kocho (i.e. a traditional staple food made of a false banana plant called enset or Ensete Scitamineae) have a low glycemic index and could be eaten freely by diabetic patients; Only 74 respondents (66.7%) stated that fibrous food (e.g.whole grain cereals) has a high glycemic index and similarly 29 respondents (26.1%) do not know the sign and symptoms of hypoglycemia.From those who know the sign and symptoms of hypoglycemia, 50% uses candy to control their sugar, 46% uses table sugar and the remaining uses soft drinks. 59.5% have regular time for meal whereas the remaining 41.5% do not have regular meal time. The majority of the respondents (103, 92.8%) eat three times per day.

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Table 3 : Type of food consumed by diabetic patients
Type of food they consume *Frequency (%)*

Type of food they consume	Frequency (%)
Injera (teff)	79(71.17)
Barely	73(65.76)
Kocho	13(11.71)
Others	17(15.31)
Food they take during hypoglycemia	
Table sugar	51(46)
Candy	55(49.54)
Soft drink	31(27.92)
Others	11(9.9)

ii. *Physical activity self- care practice*

The physical activity that the respondent performs was summarized in the table 4 below. Accordingly, 49.54%practice hard work such as farming, daily laborer, 21.62% perform aerobic exercise, and the rest 23.42%were involved other activities. Most of the respondents, 45.05% do physical exercise sometimes whereas 18.01% do exercise daily and the rest 36.94% never do physical exercise. With regard to practice of walking, 91 respondents (81.98%) do walk and the rest 20 respondents (18.02%) do not practice walking. 95.49 %) of patients has shoe wearing practice whereas the rest 5 do not. There is 110 (99.09 %) daily and 1(0.91%) twice a week foot washing practice among the respondents. Of the total respondents 99 (89.19%) have not sustained foot injury. Of those sustained foot injury 9get health care's and the rest 3get to traditional healers for support.

Table 4 : Physical activity self-care among DM patients of Ambo general Hospital, WestShoa, Ethiopia April –May 2004

Question	Response	Number of respondents (%)
1 What type of physical activity do you practice for your health	Hard work	55(49.54
	Aerobic exercise	24(21.62)
	Swimming	4(3.60)
	Driving bicycle	2(1.80)
	Others	26(23.42)
2 How often do you perform physical exercise	Daily	20(18.01)
	Sometimes	50(45.05)
	Never	41(36.94)
3 Practice of Walking on foot	Yes	91(81.98)
	No	20(18.02)
5 Do you wear shoe for your foot care	Yes	106(95.49)
	No	5(4.51)
6 How often do you wash your feet	Daily	110(99.09)
	Twice a week	1(0.91)
	Weekly	0(0.00)
	Do not wash	0(0.00)
7 Sustaining of foot injury	Yes	12(10.81)
	No	99(89.19)
8Actions taken in case of foot injury	Get health care	9(8.3)
	traditional healers	3(2.7)
	Heal spontaneously	0(0.00)

iii. *Self-Blood glucose monitoring, medication self-care, and Foot care practice*

There were 102 patients practicing blood glucose monitoring at home. 79.27% of the respondents do not forget to use their medication, whereas 98(88.28%) do not miss their medication intentionally. Most patients (106, 95.49%) do not interrupt their medication due to side effect or when feeling free of the disease but the rest 4.51 % (5 patients) interrupt their medication due to side effect or when feeling free of the disease. 99 of the respondents (89.19%) do not forget their medication while travelling but the rest 12(10.81%) do. With regard to the presence of health problem other than DM, 82 respondents (73.87%) say no and the rest 29 patients (26.13%) say yes. 78 patients(70.27%) say no family member with DM and the rest 33(29.73%) say there is a family member with DM.

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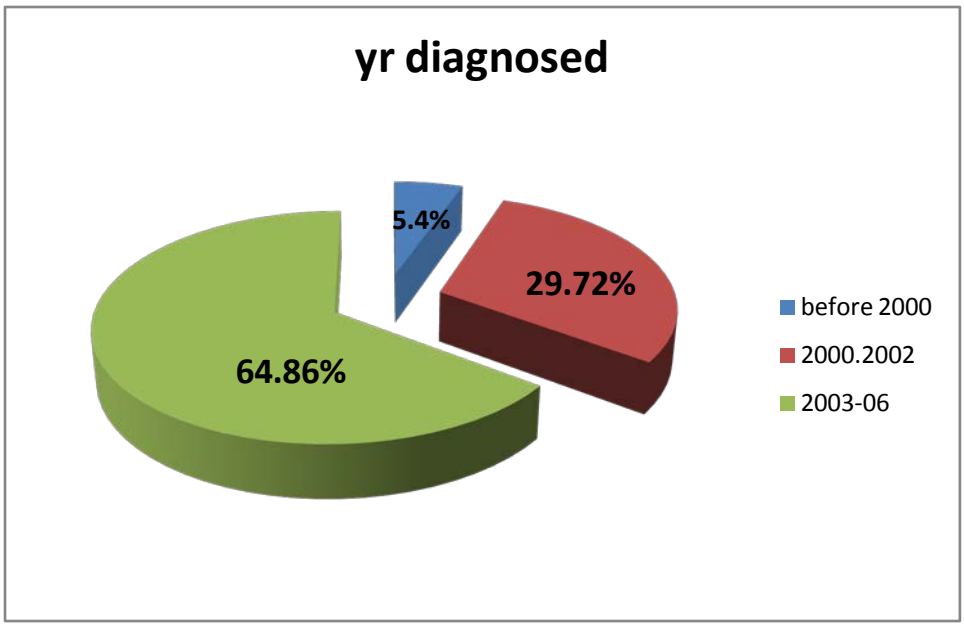
When we look their respective drug use, 35 patients which contribute 31.53% use glabinclamide, 22 patients which contribute about19.81% use metformin, 6 patients which contribute 5.1% use both insulin &metformin, and 63 patients which contribute about 56.76% use insulin. Out of the 69 insulin users, 10 patients (14.49%) use refrigerator, 53 patients (76.81%) use home prepared cool sites and 6 patients (8.70%) use other methods for storage of the drug at appropriate temperature.

Table 5 : Self- Blood glucose monitoring, medication self-care and Foot care practice among DM patients of Ambo general Hospital in West Shoa, Ethiopia April –May 2004

Question	Response	Number of respondents(%)
1-Blood glucose monitoring practice at home	Yes	9(8.11)
	No	102(91.89)
2-Forgetting medication use	Yes	23(20.73)
	No	88(79.27)
4- Medication missing for a reason other than forgetting	Yes	13(11.72)
	No	98(88.28)
6- Medication interruption due to side effect	Yes	5(4.51)
	No	106(95.49)
7- Forgetting medicine while traveling	Yes	12(10.81)
	No	99(89.19)
8-Interruption of medication when feeling free of the disease	Yes	5(4.51)
	No	106(95.49)

iv. *Glycemic control among respondents*

Check list was used to assess patients card to know their year of diagnosis, type of DM and their fasting blood sugar level for the last three months and the data obtained shows from the total of 111 patients 65 of them were diagnosed for type 1 DM (58.5%) and the remaining 46 of them (41.5%) were type 2 DM. Concerning the year of first diagnosis 72 of them (64.8%) were diagnosed after 2003, 33 of them (29.7%) were diagnosed from 2000-2002 and the remaining before 2000.



Figuer 1 : Year of first diagnosis of DM patients at Ambo General Hospital West Shoa, Ethiopia

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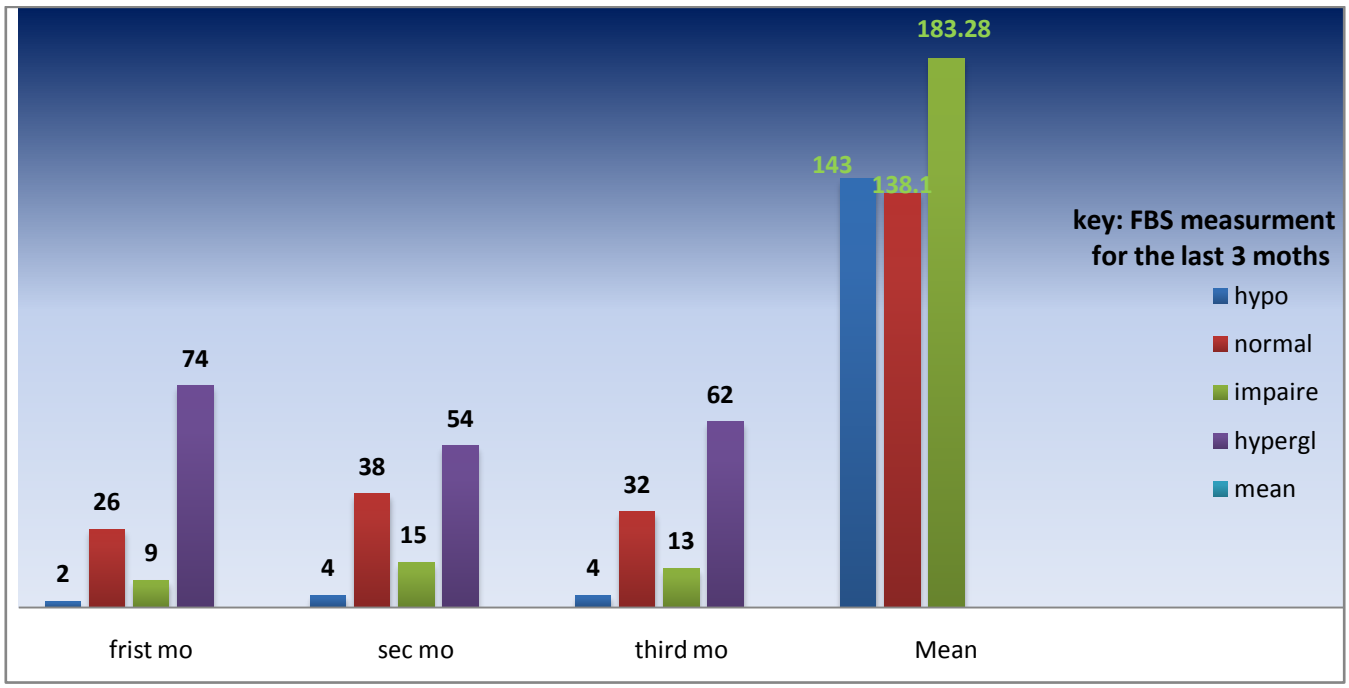
To know the sugar level of the patients each patient's card was revised to obtain their FBS level during their last three visits to Ambo Hospital and the result obtained was presented in the chart below

The chart below shows that the mean FBS of the respondents during the last visit to the hospital was 183.28. Out of the total 111 patients who visited Ambo Hospital during the third month 62 of them (55.8%) were tested to have FBS level of above 126, 13 (11.7%) have IFG level,32(28.8%) of them maintained their normal blood sugar level and 4 patients fall in hypoglycemia. During their second visit 38 patients (34.2%) maintained the FBS level within the normal range and 54 of them (48.6%) were in hyperglycemia. Similarly the FBS of the patients during their first visit shows 74 of them (66.65%) were tested to have FBS of above 126, 9 of them (8.1%) impaired sugar level,26 of them(23.4%) maintained their normal FBS.



Figuer 2 : FBS measurements for the last three months at Ambo general hospital West Shoa, Ethiopia April –May 2004

V. Discussion

The present study attempted to assess diabetes mellitus Patients' knowledge and self-care practices in terms of living with the disease. According to the findings of the study, most of the respondents know that DM is chronic disease. But 35.14% of the respondents think DM is curable and 16.22% of the respondents even do not know the sign and symptoms of DM. This is in agreement with the study conducted in Jima University [9] in which 34.21% of the patients responded DM is curable. The majority of the respondents consume teff, barely and kocho which they think have low glycemic index and could be eaten freely by diabetic patients. Diabetes self-management behaviors such as diet and exercise involve and depend on guidance from a health care provider, meal preparation in a family context and exercising with a partner [1].

The study also shows physical activity level of the patients were sub optimal and 41(36.42%) respondents never do any physical activity. This is in line with the study conducted at JimmaUniversity [8] in which most of the respondents has sub optimal physical exercise which could be because of having inadequate knowledge in terms of the benefits of regular physical exercise and a fear of hypoglycemia. Exercise has multiple positive benefits including cardiovascular risk reduction, reduced blood pressure, maintenance of muscle mass, reduction in body fat, and weight loss. For individuals with type 1 or type 2 DM, exercise is also useful for lowering plasma glucose (during and following exercise) and increasing insulin sensitivity [1]. In patients with diabetes, the ADA recommends 150 min/week (distributed over at least 3 days) of moderate aerobic physical activity. The exercise regimen should also include resistance training.

The study also showed that the majority of the respondents 95.49% wear shoe and 99.09% of the respondents wash their foot daily which is very important to decrease the complications associated with the disease. DM is the leading cause of non-traumatic lower extremity amputation. Patient education should emphasize careful selection of footwear, daily inspection of the feet to detect early signs of poor-fitting

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footwear or minor trauma, daily foot hygiene to keep the skin clean and moist, avoidance of self-treatment of foot abnormalities and high-risk behavior (e.g., walking barefoot), and prompt consultation with a health care provider if an abnormality arises (1).

Of the patients who were using insulin, only 23 (20.7%) took meals 30 minutes after each insulin injection, and the majority 70.2% of the patients were used to eat before injection. Regular insulin is given 30–45 min prior to a meal [1]. 6.3% of the respondents miss their medication and 13.5% do not attend their follow up as scheduled for them. More than half of the respondents 65(58.5%) were type 1 DM. Diabetes is managed via a regimen of control. Physicians advise adults living with type 2 diabetes to control blood sugar levels by controlling diet, maintaining regular exercise, and complying with medication. The extent to which individuals are able to adhere to such recommendations varies (5).

Regarding the FBS of the patients for the last three months the mean FBS of the last visit was found to be 183.28mg/dl which are far from the normal glucose homeostasis. Generally the results of their last three visit shows only, 32(28.8%) of them during the third month, 38 patients (34.2%) during the second month and 23.4% of them during the first visit maintained their sugar level within the normal range. This is consistent with other studies [10] who reported adequate glycemic control in 43.8% of type -2 diabetic patients. This indicates that most of the patients were not controlling their blood glucose level, despite most of them taking medication provided for them. Despite the increasing prevalence of diabetes, improved understanding of the disease, and a variety of new medications, glycemic control does not appear to be improving. Self-monitoring of blood glucose (SMBG) is one strategy for improving glycemic control; however, patient's adherence is suboptimal and a proper education and follow-up are crucial. SMBG should include post-prandial monitoring to identify glycemic excursions after meals, to indicate the need for lifestyle adjustments, and to provide patients' feedback on dietary choices (12).

Discussing the adequacy of glycemic control will be a handicap without mentioning glycosylated hemoglobin (HbAlc) determination; the ADA recommends that a patient should have glycosylated hemoglobin determination at least twice yearly. In addition, a study conducted in the United States of America (USA) showed that at least 77% of diabetic patients had at least one glycosylated hemoglobin determination in the two years preceding the study. However, none of the patients had glycosylated hemoglobin. Our findings, therefore, suggest that monitoring of glycemic control among DM patients at Ambo Hospital west Shoa Ethiopia may be less than optimal and this may be a probable contributory factor to late detection of patients at risk of complications and death from poorly controlled diabetes.

To know and follow self care practices anthropometric measurements are also important parameters for diabetic patents. But none of them have anthropometric measurement data.

Self-care activity in diabetic management includes medication self-care, dietary self-care, physical activity self-care and self-monitoring of blood glucose levels.

VI. Conclusion and Recommendation

a) *Conclusion*

In general, self-care practice was inadequate, especially in terms of physical self-care activity and a deficit in terms of knowledge related to diabetes. In some cases the patients do not attend their follow up strictly and sometimes miss taking their medications. Almost all the patients have good foot care hygiene. Regarding insulin injection time with respect to meal, majority of them take their medication after eating. The mean fasting blood sugar of the patients during the last visit was not controlled.

b) *Reccomandations*

The following recommendations are forwarded.

Health care providers: should educate and promote health to address the lack of information on a healthy diet, benefits of exercise and how exercise should be undertaken for ambulatory DM patients when they come for regular follow up regularly at the hospital

Primary care physician must interpret (preferably in patient's language), for each diabetic patient, the short and long-term benefits of adhering to diet and exercise recommendations, Insulin storage site in the hospital as well as at home must be given special emphasis.

Patients should learn to follow their medication strictly including the site of injection care for their diet with regular meal time and understand the benefit of physical exercise.

The hospital follow up schedule must not be longer than one month and patients must adapt to frequently visit the hospital for regular checkup. Patients can learn to control their blood sugar level at home by using glucometer.

Generally self -care practice including blood glucose monitoring is the back bone to control DM, hence further investigation by researchers is needed to strengthen diabetic self-care practice and promote health.

VII. List of Abbreviations

ADA = American Diabetic Association

ATP = Adenosine Triphosphate

DM = Diabetes Mellitus

DNA = DeoxyriboNucleic Acid

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FBS = Fasting Blood Sugar

HbA1c = Glycosylated Hemoglobin

OGTT = Oral Glucose Tolerance Test

RBS = Random Blood Sugar

IFG = Impaired fasting glucose

CKD = Chronic kidney disease

CHF = Congestive heart failure

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Conflict of interest : none declared

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Assessment of Knowledge, Attitude and Practices Regarding Life Style Modification among Type 2diabetic Mellitus Patients Attending Adama Hospital Medical College, Oromia Region, Ethiopia

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Abstract- Background: The person with diabetes mellitus has a chronic lifelong disease, the person must be knowledgeable to coordinate life style modification in to a daily routine of work to achieve and maintain normal physiological blood glucose level. The objective of this study was to determine the knowledge, attitude and practices of LSM management of type 2 diabetic patients in Adama Medical college Hospital.

Methodology: Across-sectional study was conducted to assess knowledge, attitude and practice of type 2 diabetic patients towards LSM management of DM in Adama Medical College Hospital. Data was cleaned and analysed by using SPSS version 16.0 and presented descriptively and analytically.

Keywords: type 2 diabetes mellitus, life style modification, knowledge, attitude, practice.

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Methodology: Across-sectional study was conducted to assess knowledge, attitude and practice of type 2 diabetic patients towards LSM management of DM in Adama Medical College Hospital. Data was cleaned and analysed by using SPSS version 16.0 and presented descriptively and analytically.

Result: Concerning knowledge of the patients towards LSM management of diabetic; majority of the patients were knowledgeable which accounts 90(77.59%) followed by 13(11.21%) patients fairly knowledgeable and the other 13(11.21%) patients were poorly knowledgeable. Regarding attitude of the patients 95(81.89%) patients had positive attitude and the other 21(18.11%) had fair attitude. In another way almost half of the patients 57(49.1%) had good practice. The other 39(33.62%) and 20(17.24%) have poor and average practice respectively.

Conclusion and Recommendation: The result of this study showed, majority of type2 DM patients had good knowledge, positive attitude and good practices towards LSM. The researcher recommend all stake holders (Ministry of health, Diabetic associations, Health institution, health professionals, caregivers and NGO) found around this area must cooperate to improve KAP of the patients towards LSM. Especial attention should be given to the practices of the patients and further research should also be done on this topic.

Keywords: type 2 diabetes mellitus, life style modification, knowledge, attitude, practice.

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I. Introduction

a) Background

Diabetes mellitus is a syndrome characterized by chronic hyperglycaemia, due to absolute or relative deficiency or diminished effectiveness of circulating insulin. It is the most common serious metabolic disease. Diabetes mellitus has been recognized as a clinical syndrome since ancient times and remains a crippling global health problem today [1]. Its clinical diagnosis indicated by presence of symptoms such as polyurea, polydipsia and unexplained weight loss; and confirmed when one of the following abnormal glucose measured: fasting plasma glucose (FPG) value of >= 7.0 mmol l-1 (126 mg dl-1), or the casual plasma glucose value >= 11.1 mmol l-1 (200 mg dl-1), or if the plasma glucose value 2 hours after a 75g oral load of glucose is>= 11.1 mmol l-1 (200 mg dl-1). However, in asymptomatic subjects, the test should be performed more than one occasion to confirm the diagnosis and treat the subject [2].

There are different categories of DM; but the two broad categories are type1 and type2.Both types of diabetes are preceded by a period of abnormal glucose classified as impaired fasting glucose (IFG) or impaired glucose tolerance (IGT). Type 1 DM is the result of complete or near-total insulin deficiency. Type 2 DM is a heterogeneous group of disorders characterized by variable degrees of insulin resistance, impaired insulin secretion, and increased glucose production [3].

The prevalence of type 2 diabetes is increasing in the world at high rate. For example according to the study of Diabetes Screening in Canada (DIASCAN) in the year 2000; the prevalence of Type 2 diabetes in individuals 40 years of age and older who see a general practitioner was 16.4% in Canada and nearly 20% in Québec. It was predicted that the number of Canadian diabetics will double within the next 15 years. The impact of diabetes on the health of populations and

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individuals afflicted with the disease is primarily related to late-stage complications. Diabetes is the primary cause of terminal kidney failure, blindness before the age of 65 and amputations [4].

The burden of diabetes is increasing in the worldwide including developing countries like Ethiopia. International diabetic federation association reported Ethiopia to be ranked 3rd in Africa with 1.4 million DM and prevalence of 3.32 by year 2012.The study done to determine prevalence of un diagnosized DM & its risk factor in institution of Bishoftu town, East Shoa at 95% confidence interval with (p=0.05 and margin of error 5%) in the year 2012/2013 reported the prevalence of undiagnosed DM to be 5% [5].

Ancient civilizations in Egypt, Greece, Rome and India recognized diabetes mellitus and recommended dietary modifications. During previous century's recommendation about dietary carbohydrates from diabetic individuals were based on theory rather than scientific facts. Prior to discovery of insulin, diabetes was treated with low carbohydrate, semi starvation diet. Even after insulin was discovered in 1921, most eastern diabetes specialists used low carbohydrates, high fat diets to treat diabetic individuals [6].

Today ;essential components of the treatment for diabetes include diabetes self management education, lifestyle interventions, and goal setting glucose management and pharmacologic management of hypertension and hyperlipidemia[7]. Life style modifications are considered the corner stone of management of diabetes mellitus and include the prescription of healthy diet, regular exercise, and avoidance of tobacco [8] .

Education regarding diabetes is very important to improve the life style of the patients which would be helpful in maintaining (controlling) blood glucose. Observation has been reported that improper guidance and communication can lead to poor compliance to both medication and life style [9].

Dietary recommendations for DM patients focus on the reduction of fat intake and increase of vegetable consumption with moderate calorie restriction and it should be individualized according to the patients' physical activity, co morbid condition and personal preferences. Da Qing study in China showed diet intervention alone associated with 31% reduction in the risk of developing type2 DM [10].

In the other way alcohol intake exacerbate neuropathy, dyslipidemia and obesity. Therefore it should be prohibited, if used it must be in moderation .Similarly smoking should be prohibited as it increases risk of complication [7].

Physical activity (exercise) also reduces the risk of developing DM type2 by 30-50%.Physical activity improves insulin sensitivity .eg It can reduces free fatty acid load to the liver; there by reduces hepatic insulin resistance .Moderate exercise as little up to 30min/day or 150min/week can show the differences [11].

b) Statement of the problem

Despite the availability of different treatment modalities, diabetes has remained a major cause of death. Now a day's 3.8 million deaths are attributable to the diabetes in each year worldwide. It is the 4th leading cause of global death. In 2005 there were 246 million people worldwide were affected with diabetes and are expected to affect 380 million by 2025, over a seven fold increase just over 20 years [12]. This indicates high burden of DM in today world and developing countries account for a substantially high proportion [13].

In Ethiopia; from hospital based studies; it was observed that the prevalence of diabetes admission had increased from 1.9% in 1970 to 9.5% in 1999 of all medical admissions [14]. World Health Organization (WHO) also estimated the number of diabetics in Ethiopia to be about 800,000 cases by the year 2000, and the number is expected to increase to 1.8 million by 2030 [15].

In another way, according to the 2011 report of the International Diabetes Federation (IDF), the number of adults living with diabetes in Ethiopia was 3.5%. A study done on urban Commercial Bank employees in Ethiopia showed a 6.5% prevalence of DM [16] which indicated the significance of lifestyle for DM aetiology and its burden to our country too.

Although, the diabetes is causing high wastage of life and resources the management is still low .Its management include pharmacologic and life style modification. However, LSM is ignored by many of the patients and care givers. For example Study done in Omani on type 2 DM patients in the year 2013 reported only less than 40% of the patients participate on regular exercise [17]. The other study done at USA in the year 2008 showed among 69 patients classified as elevated risk of diabetes only 17%,32% and 30% had received advice for weight loss ,exercise & diet modification [18].

Another similar cross sectional study done in four provinces of Kenya in the year 2010 found that; of the people participated in the study 75% had poor dietary practices, while 72%didn't participate in regular exercise & about 80% didn't monitor their body weight [19].

In another way, study done at Jimma University Specialised Hospital in the year 2011to assess quality of care given to diabetic patients showed that there was no attention given to diabetic education in Ethiopia. There were no diabetes nurse educators and diabetes dietician in the country and those who provide health services for diabetes had no special training for diabetes care [20].

In AMCH the diabetes patients appointed to follow up clinic according their disease status. Most patients come to the Hospital every month or every two month and refill their medication. They check up their

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blood glucose only when they come according to their follow up appointment and contact the physician for less than five minutes for refill. Most of the time they asked only for adherence to medication and do not checked awareness for life style intervention. In certain circumstances both health care giver and patients do not raise all about non pharmacologic management of diabetes including new diagnosed type2 DM patients. These observations raised the researcher' concern about the knowledge, attitude and practice of lifestyle modifications among diabetic patients at AMCH, which this study seeks to explore and document. Since there was no similar study in the area, this information gap about of LSM among AMCH type 2 DM patients could be answered by this study.

c) Significance of the study

It is obvious that there is no adequate information on knowledge, attitude and practice of non pharmacologic management of diabetes in our country; Ethiopia. Most of literatures are drafted from developed countries like USA, Russia and European countries.

Majority of the studies were done to evaluate the knowledge, attitudes and practices about diabetes mellitus among diabetics rather than on lifestyle modifications.

The result of this current study could be useful as a base line in implementing a community based awareness programme which will promote the importance of lifestyle modifications in the prevention and control of non-communicable diseases, particularly diabetes mellitus. Therefore it is helpful for all stake holders that involves in this areas i.e patients, caregivers, health care providers, health institution and policy makers (MOH, NGO, diabetic DM association)

The other benefit of this study is that the result of this study helps health care provider to give education and awareness on LSM management of DM. In addition DM patients' and family members realize the benefit of non pharmacologic management hand by hand with pharmacologic management for the success control of the disease and its complication. Thus the patients' care will improve and the patients' will be benefited.

Finally, Adama Medical College Hospital also uses this result for improving its services for DM patients and other chronic disease patients. It also helps to encourage good services of the clinics and modify the possible problems of between hospital mission and actual services given by diabetic follow up clinic .The hospital management can use this result to expand its services especially, this can help AMCH and diabetic association as feedback for the services they are giving for the patients

II. Objectives

a) General objective

- To assess knowledge, attitude and practice of Life Style Modification management of type 2 DM in Adama Medical College Hospital patients following diabetic clinic.

b) Specific objectives

To determine the knowledge, attitudes and practices of patients in relation to type 2 diabetes mellitus with reference to:

- Diet modification
- Importance of regular exercise
- To determine the demographic characteristics of type 2 diabetes mellitus patients attending diabetic clinic at Adama Hospital.

III. Method and Materials

a) Study area and period

The study was conducted in Adama Medical College Hospital (AMCH) located 99 km south east of Addis Ababa, Ethiopia, Oromiya region, East Shoa zone. It was established in 1946 by Italian Missionaries and named as HailemariamMammo memorial hospital little bit after establishment but its name was changed to Adama Referral Hospital in mean time and now it renamed AMC H by Oromiya regional state health bureau after it start to teach accelerated medicine, emergency surgery and anaesthesia nurses. The hospital gives services for about 5 million people east and southern parts of Oromia, Afar, Somali and Southern Nation Nationalities and People (SNNP).

Now the hospital has 465 different workers to give different services, of which 194 are administration workers. The other 271 workers are health professionals. There are specialist in different field(23),general practitioners(GP)36,Nurses(116),laboratory workers(20),x-ray(5), physiotherapy(2), sanitarians(2), Biomedical(1), Midwifery(16), Anaesthesia(9), Health officers(9),psychiatry nurses(3) and masters in different fields.

AMCH has different departments in it: Outpatient department (OPD) team case, internal medicine, dermatology, paediatrics, gynaecology/obstetric, surgery, dentistry, psychiatry, physiotherapy, ophthalmology, hospital pharmacy, anti retroviral therapy (ART) and tuberculoses (TB) Clinic. The study was conducted in OPD case team from April 1 to May 1.

The data from hospital management 2012/13 show the top 10 leading causes of outpatient visit were: Trauma, Pneumonia, Dyspepsia, Acute Upper Respiratory Tract Infection, Other or unspecified diseases of the eye and Adnexa, Urinary tract infection, Diarrhoea (non-bloody), Dental and gum diseases, Acute febrile Illness and Helmenthiasis. But the top 10 causes of admissions were: Other delivery , Trauma (Injury, Fracture etc), Pneumonia, AIDS and related diseases , Appendicitis ,Medical Abortion without complication (safe abortion), Other or unspecified obstetrics condition, Diarrhoea with dehydration, Diabetes mellitus and Severe acute malnutrition.DM

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ranked 19th from outpatient visit but 9th causes of hospital admission[24].

b) Study design

This study was a descriptive cross-sectional study

IV. Populations

a) Source population

All diabetic patients attending the diabetic clinic follow up were used as source population.

b) Study population

The study population consisted of all type 2 diabetes mellitus patients, aged 30 years and above, attending Adama Hospital for regular follow-ups from the 1st April 2014to the 1st May 2014. Age of 30 years was chosen as the cut-off age. One hundred sixteen (116) type 2 diabetes mellitus patients attended the diabetic clinic of AMCH during this period.

All diabetic patients who visited the follow up clinic within the limit of study period were included in the study.

c) Sample size and Sampling technique

The size of study population was limited by the number of diabetic patients visiting the clinic during the study. All diabetic patients those visited the hospital during study period were included depending on their consent.

d) Inclusion and Exclusion criteria

i. *Inclusion criteria:* Type 2 diabetes mellitus patients aged 30 and above attending the diabetic clinic at Adama Medical College Hospital for their regular follow up visits and had willing to participate in the study were included in the study.

ii. *Exclusion criteria:* Patients with type 1 diabetes mellitus, gestational diabetes, other specific types of diabetes mellitus and diabetes insipidus were excluded from the study. In addition, all type 2 diabetes mellitus patients with impaired memory or cognitive functions and those younger than 30 years were also excluded.

e) Variables

i. *Dependent variables:* knowledge, attitude and practice

ii. *Independent variables:* age, religion, Sex, educational status, marital status, ethnicity, monthly income, duration of disease.

V. Data Collection Process and Collection Technique

In this study a face-to-face interview using a structured questionnaire was carried out for data collection. The data was collected by the researcher and his assistant colleagues using a structured questionnaire. The researcher trained two qualified graduating students of clinical pharmacy, proficient in the local language (Amharic &Afan Oromo) as research assistants. They assisted him throughout the data collection processes through a face-to-face interview. During and after data collection principal investigator checked the consistence and completeness of the data.

a) Data Quality Assurance

In order to assure the quality of the data the following measures was under taken:

Data was collected by three of graduating class of clinical pharmacy students

- The data collectors were taken the training to check completeness of the data during the data collection and appropriate recording.
- The body mass index (BMI) of each participant was calculated by the researcher and his assistants using the formula BMI = Weight (kg)/Height (m2) after the weight and height were measured using a calibrated beam scale with height rod graduated in centimetres and participants were classified according to the WHO international classification of adult weight.
- The principal investigator supervised all field work, check for completeness and accuracy of data collection daily.

b) Data processing and analyzing

The collected data was coded and checked for completeness. Once data coded and checked for completeness, data processing was done by SPSS version 16.1 and, presentation of the data was done by using frequency distribution, percentage and rate.

c) Ethical consideration

Ethical clearance letter was written by Ambo University department of Pharmacy after approved the proposal of the study to request AMCH for the permission. AMCH management and research office approved the letter of study and requested different hospital departments and any help during the study. The purpose of the study was explained to the respondent and data was collected after ensuring their willingness to give their response. Confidentiality of participants maintained at all time .Participants were informed that the participation were voluntary.

d) Operational definitions

LSM- Non pharmacological management such as diet modification, and exercise design to treat problem of type 2 DM patients.

Knowledge- Understanding lifestyle modification in glycemic control and management of type 2 DM patients

Attitude- A patients' positive or negative feeling towards performing the defined behaviour i.e. (LSM)

Practice - Is a previous utilization of any of LSM

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VI. Results

a) Demographic Characteristics of Respondents

From total number of 116 type2 DM patients participated in the study 44(37.9%) male and 72(61.1%) were female. Regarding marital status of the patients; 16(13.8%) single, 80(69%) married, 10(8.6%) widow and 10(8.6%) patients were divorced. Concerning age of the clients 14(12.1%), and 52(44.83%) were in age group of 30-40, and >=61 respectively. The other 50 were in 1:1 ratio of 41-50 and 51-60 ages. Ethnicity of the patients participated in the study include Oromo (33.6%), Amhara (37.9%), Tigre (5.2%) and 27(23.3%) were other ethnic groups.

On the other hand 38(32.8%) patients do not read and write, and 24(20.7) patients read and write only. The other 28(24.1%), 23(19.8%), and 3 (2.6%) patients attended grade 1-8, grade9-12 and college respectively. When we consider employment status of the study participants; 27(23.8%) patients were unemployed (private employee) and other 11 (9.5%) were unable to do because of old age. Furthermore, 3(2.6%) were government employee, 17(14.7%) farmers, 34(29.3%) house wife and other 22(19%) had different jobs. In another way, about 41(35.3%) patients had monthly income less than 500 birr and 60(51.71%) patients had monthly income of 501-1500 birr. Only 14(12.1%) and 1(.9%) patients had monthly income of 1500-2500 and 2500-3000 birr per month respectively.

*Table1.*Distributions of socio demographic characteristics of diabetic patients at diabetic follow up clinic in AMCH in April, 2014

Socio demographic characteristics or variables		Number	Percent
Sex	Male	44	37.9
	Female	72	62.1
Age	30-40	14	12.1
	41-50	25	21.55
	51-60	25	21.55
	=>61	52	44.83
Marital status	Single	16	13.8
	Married	80	69
	Widow	10	8.6
	Divorced	10	8.6
Religion	Muslim	25	21.6
	Orthodox	65	56
	Protestant	18	15.5
	Other	8	6.9
Ethnicity	Oromo	39	33.6
	Amhara	44	37.9
	Tigre	6	5.2
	Other	27	23.3
Educational status	Not read write	38	32.8
	Read and write only	24	20.7
	Grade 1-8	28	24.1
	Grade 9-12	23	19.8
	College graduate	3	2.6
Employment status	Gov. Employee	3	2.1
	Un employed (Private employee	27	23.28
	Farmer	17	14.66
	House wife	34	29.31
	Not able to work	13	11.21
	Other	22	18.99
Monthly income in birr	<500 birr	41	35.3
	501-1500	60	51.7
	1501-2500	14	12.1
	2501-3000	1	0.9

b) Anthropometric Characteristics of Respondents

Among the study population only 5 (4.3%) patients were obese class I .The other 59(50.9%) patients and 52(44.8%) were in the range of normal weight and overweight respectively.

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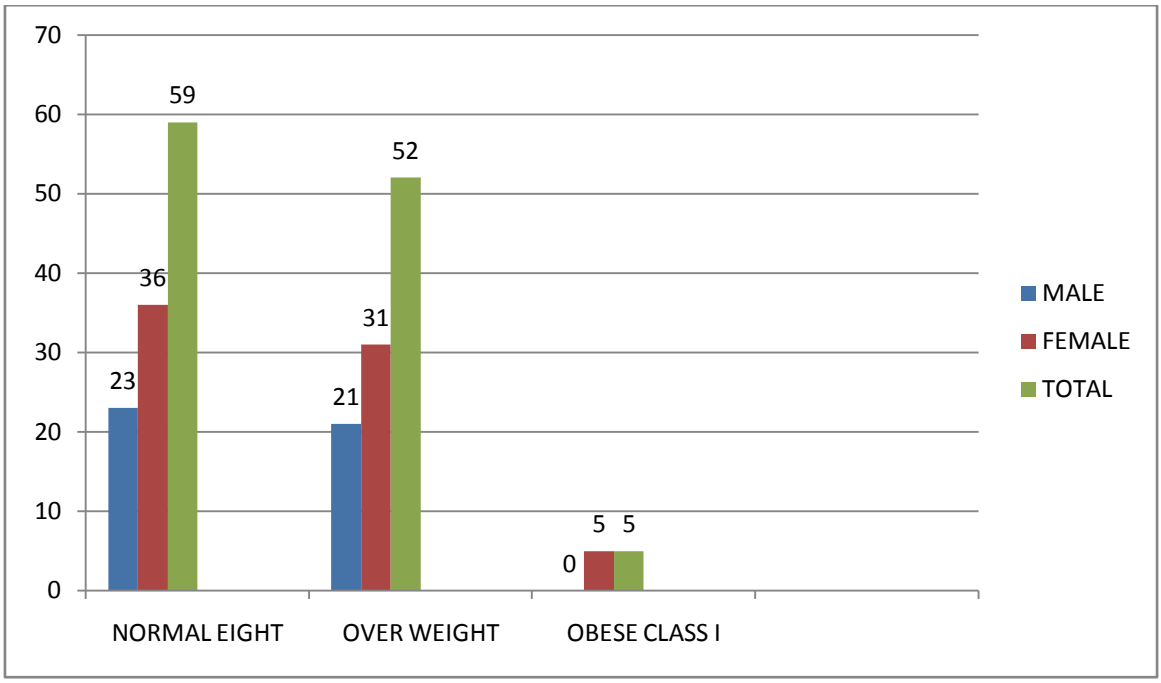

Figure1 : Distribution of type2 DM patients by their body weight in AMCH in April, 2014

c) Anthropometric Characteristics of Respondents

Regarding diabetic education such as attending group classes or having meeting with health professional half of the patients didn't get the opportunities while the other half got the chance one or more till now.

On the other hand, majority of the patients 59(50.9%) were 1-5 years with the disease followed by 6-10 years with the disease which were 42(36.2%).only one patient with disease for 25 years.

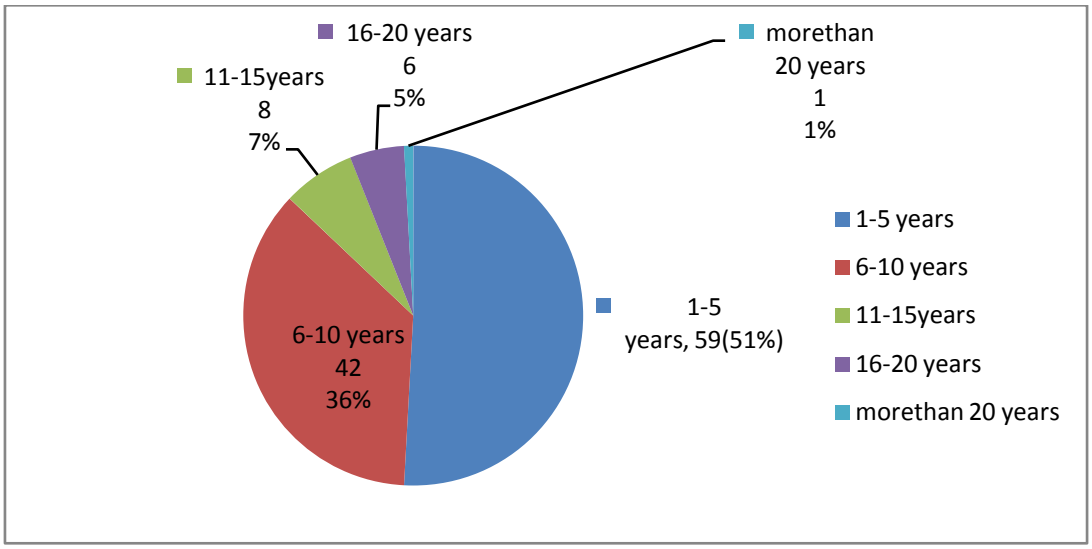

*Figure 2 .*Duration of disease of type 2 DM patients in AMCH in April, 2014.

d) Sources of life style modification

Majority of the patients 68(58.62%) following the diabetic clinic get the information from health personnel followed by those who get information from more than one source 25(21.55%).From the whole participants 12 patients reported that they have no information about LSM management of diabetes. See table 2 below

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Table 2 : Source of LSM Management of Type2 Dm Patients in AMCH in April,2014

Source of information	Frequency	Percent	Valid Percent
NO INFORMATION	12	10.3	10.3
HEALTH PERSONNEL	68	58.6	58.6
MEDIA	4	3.4	3.4
RELATIVE	2	1.7	1.7
FRIENDS	3	2.6	2.6
MORE THAN 1 OPTION	25	21.6	21.6
OTHER	2	1.7	1.7
Total	116	100.0	100.0

e) Types of treatment

When this study conducted; almost all patients were taking more than one types of treatment modalities. All patients attending the clinic were at least on one type of pharmacologic treatment and in parallel practice LSM partially or completely. Only 2 patients were taking one type of treatment modalities.

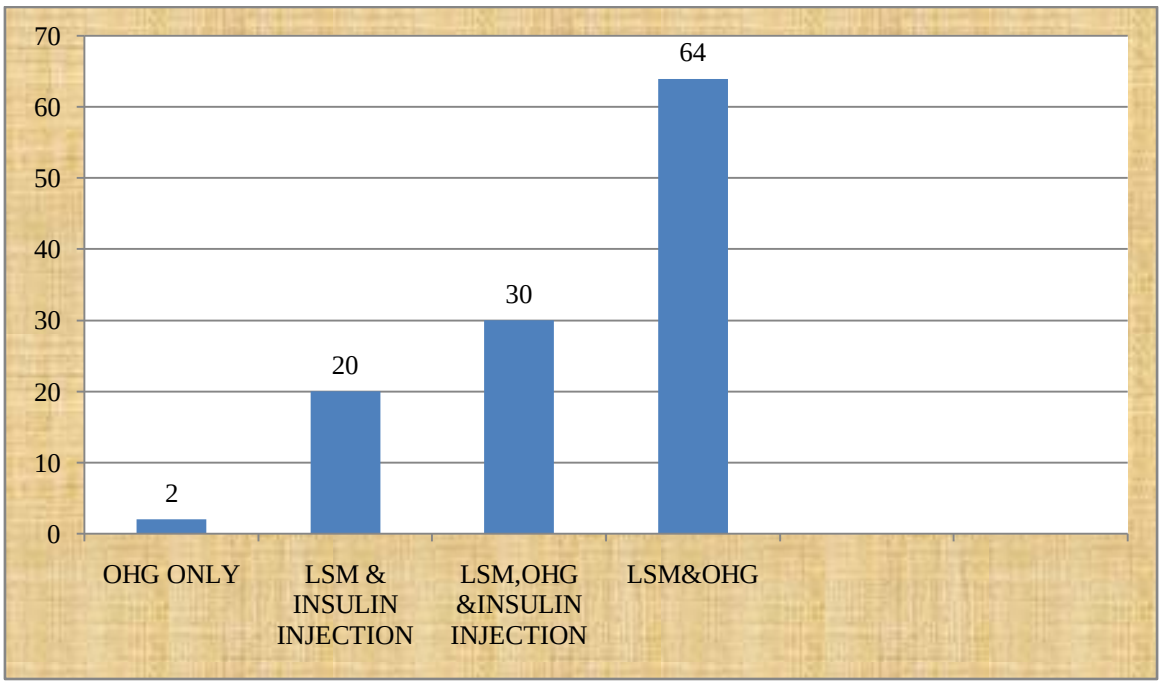

Figure 3 : Frequency distribution of type of treatment of diabetes patients in AMCH in April; 2014

f) Dietary habit

Regarding selected dietary practices of cigarette smoking, alcohol consumption, fruit and vegetable, sweet sugar, buttery and fatty meal; almost all patients do not smoke and consume alcohol always while 67(57.8%) patients never take sweaty food, 71 patients never take buttery and fatty meal, while only 3(2.6) patients never take fruit and vegetable because of lack of resource. About 54(46.6%) and 37(30.21%) patients take eggs usually and never respectively. Surprisingly only one patients use herbal medicine to control her diabetic symptom with her medication.

Table 3 : Selected Dietary Practice of Type2 Dm Patients at AMCH in April, 2014

Type of diet	Always	Some time	Occasionally	Never
Smoke	0	2	6	108
Alcohol	0	6	17	93
Chat	4	4	3	105
Fruit &vegetable	30	52	31	3
Sugar	2	13	34	67
Egg	0	27	54	35
Buttery and fatty meal	0	4	41	71

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g) Physical Activity

Regarding physical activity, 77(66.4%) patients exercise on regular programme or physical active and 39(33.6%) do not exercise on regular programme and were not physically active due to difference reasons. Of those who exercise regularly 19(16.4%) exercise less than 15 minute perday,21(18.1%) 15-30minute per day,14(12.1%) patients 30 minute per day ,11(9.5%) 30-45 minute per day ,2(1.7%) 46-1hour per day and only 8(6.9%) patients exercise for greater than 1 hour per day.

Table 4 : Frequency distribution of respondents with regard to physical activity

Variable	Number	Frequency (%)
Exercising regularly		
Yes	77	66.4
No	39	33.6
Total	116	100
If yes, how often		
Less than 15 min/day	21	18.1
Less than 30 min/day or less than 150 min/week	40	34.5
More than 30 min/day or more than 150 min/week	21	18.1

h) Knowledge Assessment of Respondents

Concerning knowledge of the patients towards LSM management of diabetic; majority of the patients were knowledgeable which accounts 90(77.59%). Of the left 26 patients, 13(11.21%) fairly knowledgeable and the other 13(11.21%) were poorly knowledgeable. Figure1 below summarize status of the knowledge of the patients.

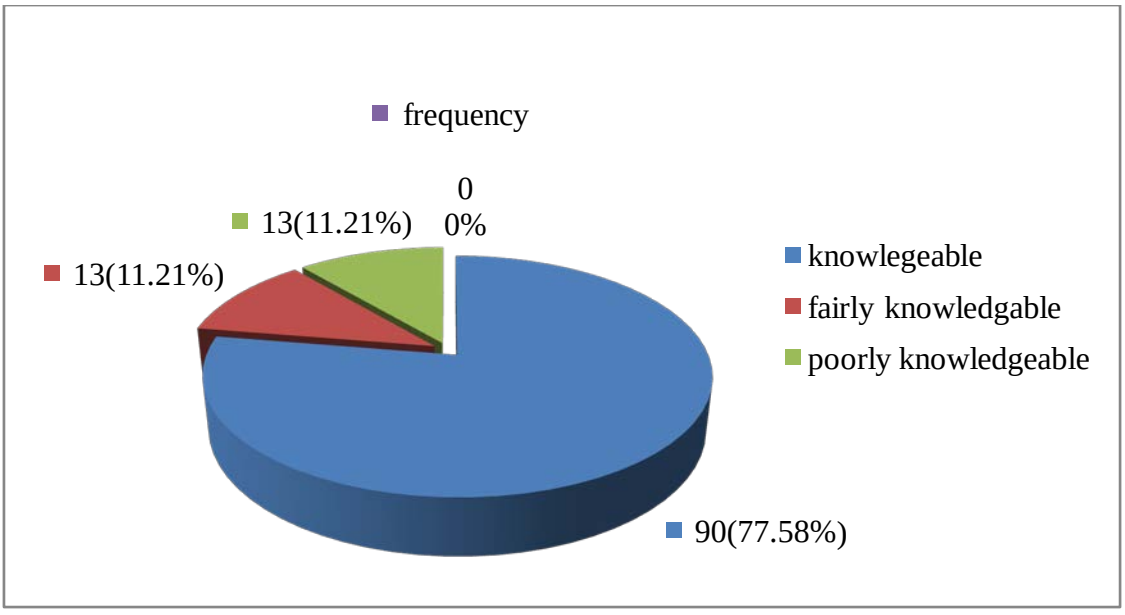

Figure 4 : Distribution of respondents according to their knowledge regarding the benefit of life style modification at diabetic follow up clinic AMCH April, 2014

i) Attitude Assessment of Respondents

Majority of the patients 95(81.89%) had positive (good) attitude toward s LSM management of diabetes. No patient had negative attitude to LSM but, 21(18.1%) patients had neutral attitude.

Table 5 : attitude status of the diabetic patients to ward LSM in AMCH April, 2014.

Attitude level	Number	Percent %
Positive	95	81.9
Neutral	21	18.1
Negative	0	0

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Regarding practice of LSM, almost half of the patients 57(49.1%) had good practice. The other 39(33.62%) and 20(17.24%) had low and average practice respectively. Table 3 below summarize the results.

Table 6 .Frequency distribution of diabetic patients by their practice of LSM management of diabetes AMCH April, 2014.

Level practice	Frequency	Percent
Good practice	57	49.13
Average practice	20	17.24
Low practice	39	33.62
Total	116	99.99

j) Correlation between Knowledge, Attitudes and Practices

Table 7 : Correlations between knowledge, attitude and practice level regarding lifestyle modifications

		knowledge of clients	level of attitude	level of practice
knowledge of clients	Pearson Correlation	1	.098	.184*
	Sig. (2-tailed)		.293	.048
	N	116	116	116
level of attitude	Pearson Correlation	.098	1	.517**
	Sig. (2-tailed)	.293		.000
	N	116	116	116
level of practice	Pearson Correlation	.184*	.517**	1
	Sig. (2-tailed)	.048	.000	
	N	116	116	116

*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).

Table7: shows the correlation of level of knowledge, attitude and practice towards LSM. It shows that there is positive Pearson correlation between level of knowledge and attitude(r=0.098, p=0.293), positive Pearson correlation of 0.184(p=0.048) between knowledge and practice, and positive Pearson correlation of 0.517(p=0.000) between level of attitude and practice level.

VII. Discussion

a) Demographic Characteristics of Respondents

There were more females (62.1%) than males (37.1%) participated in this study, a reflection of the gender ratio attendance of patients in the diabetic clinic at AHMC hospital. More recent reports from developing countries have found that DM and its risk factors are more common in women This finding is in keeping with the results from a study conducted in South Africa at Mamelodi Hospital in which 81.1 % were female and 18.9 % were male [25].

Majority of respondents in this study came from the age groups 41-50 years, 51-60 years and >61 years with 21.55%, 21.55% and 44.83% of respondents respectively, which add up to 87.93 % of respondents.. This is reflective of the fact that the ethnology of type 2 diabetes mellitus usually at old age [3, 17, and 25].

In this study respondents with no formal education consists (53.5%) and only (2.6%) respondents with higher education. This indicates that most respondents had little or no education. This result may be the direct consequence of scarcity of higher education system in Ethiopia in the past [26]. Additionally the results of this study reported less than half (45.7%) of the participants got diabetic education such as attending meeting with health professionals. This confirmed with the study conducted in 2011 at Jimma University Specialised Hospital which reported as there was no attention given to diabetic education in Ethiopia [20].

Majority of respondents in this study had income between 501 birr and 1500 birr (51.7%) followed by respondents in the less than 500 birr income (35.3%). This low income amongst majority of respondents could limit their accessibility and affordability of a well-balanced diet and healthy food and it was considered as the main factors (barrier) to their practice of life style modification especially diet modification.

This finding was in keeping with the results from a cross sectional study of Adherence to Diabetes Self-Management Practices among Type Ii Diabetic Patients in Ethiopia; in which majority of the study participants 139 (43%) had very low monthly income [27].

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Majority of respondents (50.9%) had normal weight, followed by 44.8% with overweight and only 4.3% had class I obesity. In the study class 2 obesity and class 3 (morbid obesity) were not found. This study had just demonstrated that lack of physical activities and poor dieting habit among respondents, seem to contribute to the development of type 2 DM rather than obesity. But about 45% of the patients in the study were overweight which increases the risk of obesity.

This finding is in contrast with many studies done on this area in which obesity was common in the representative sample of type 2 diabetes patients attending a diabetes clinic [28].

b) Knowledge Assessment of Respondents

In this study (77.59%) of respondent had adequate knowledge, (11.21%) of respondents had fair (average) knowledge and 11.21% of respondents had poor knowledge of the benefits of exercise, and healthy diet. Large numbers of the participants (68%) got information from health professionals may have contributed to this result. This relatively revealed similar result with study done in Nigeria at Kaduna in the year 2012 on 347 patients; 230 non diabetic and 117 diabetic patients' .The study recorded 56.4% score of knowledge for diabetic participants [23].

In contrast to this finding, IKOMBELE found in his study that no respondent had good knowledge and 92.6% of respondents had poor knowledge of the benefits of exercise, weight loss and healthy diet [25].

c) Attitude Assessment of Respondents

The majority of respondents (81.89%) had positive attitude towards lifestyle modifications, followed by 18.1% of respondents who had neutral attitude, and no respondents had a negative attitude. This revealed relatively similar results with study conducted on 100 patients attending diabetic clinic at kinikkashitan Seri Manjung which recorded 99% of patients answered greater than or equal to 50% of attitude question [29].
This finding is similar to those of studies done in South Africa at Mamelodi Hospital in which the majority of respondents (92.7% and 51.6% respectively) had positive attitude towards lifestyle modifications [25].

d) Practice Assessment of Respondents

The proportion of respondents with good practice (49.1%) and those with average practice (17.24%) and poor practice (33.62%).About one third, patients had poor practice of LSM and the result was not satisfactory as that of knowledge and attitude. That could be due to majority of the patients had limited resources and low income which limit their affordability for a well balanced dieting and necessary equipment to exercise. This result was similar with study conducted in Qatar and Omani which reported 49.5% of the respondents were not exercise regularly and 48% of the participants were not practices recommended diet [21] and less than 40% exercise regularly and only 56% of the patients were adhere to recommended diet respectively [19].

Regarding consumption of alcohol ,chat and smoking the results of this study is promising as 93.1%,90.51% and 80.2% patients never smoke, chew chat and take any type of alcohol respectively. This result is similar with study conducted in Western Nigeria in 2012 which reported all study patients neither consume alcohol nor smoke cigarette [22]. The other similar study conducted in Omani in the year 2013 also reported out of 106 study patients only 10.6% were smoker.

Additionally the result of this study showed as only one patient use herbal medicine. Surprisingly study in Omani also reported only 2 patient use herbal medicine regularly [17].

Correlation between Knowledge, Attitudes and Practices

There was a weak, non-significant positive correlation ($r = 0.098$, $p = 0.293$) between knowledge level and practice level of respondents. This means that being knowledgeable did not necessarily willingness to observe healthy lifestyle habits. In other way, there was a significant positive correlation ($r = 0.184$, $p = 0.048$) between the knowledge level and the practice level of respondents in this study. This means that the better respondents were knowledgeable, the better they were practice healthy lifestyle.

Finally, there was a very significant positive correlation ($r = 0.517$, $p = 0.000$) between attitude level and practice level. This means the better the patients had positive attitude toward LSM, the better they were practices healthy life style modification.

The result found in this study were opposite to the study conducted in South Africa at Mamelodi Hospital which reported significant positive correlation ($r = 0.171$, $p = 0.012$) between knowledge and attitude level, and weak positive non significant correlation ($r = 0.037$, $p = 0.587$) between attitude and practice level. In addition that study also reported weak positive non significant correlation($r = 0.102$, $p = 132$) between attitude and practice level [25].

VIII. Conclusion and Recommendation

a) Conclusion

The discussion on the findings of this study shows that the knowledge and attitude levels of lifestyle modifications among type 2 diabetes mellitus patients attending Adama Medical College Hospital were generally high. However practice of the patients regarding LSM still not sufficient as more than half of the patients had no good practices. The study also found out that there was significant positive correlation ($r = 0.0184$, $p = 0.048$) between knowledge and practice, very significant positive correlation ($r = 0.517$, $p = 0.000$) between attitude and practice and a weak non-significant correlation($r = 0.098$, $p = 0.293$) between knowledge and attitude.

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b) Recommendation

Lifestyle modification has important roles in prevention and management of chronic diseases like type 2 DM patients. But its prevalence is increasing worldwide at an alarming rate especially in developing countries due to different factors like sedentary life style (westernization), and deficits in the knowledge and practice of LSM.

Based on these facts and on our research findings, it was recommended that:

- ✓ Health education about life style modification (importance of exercise, physical exercise and weight loss) to the general society should be implemented by the responsible body.
- ✓ Medical nutrition intervention program should be implemented with a multidisciplinary team (Doctor, dietician, pharmacists…) and all stake holders (health institution, MOH, diabetic association other responsible nongovernmental organization) should work cooperatively on this issues
- ✓ Empower and train Adama hospital healthcare workers about this issue in order to promote behavioural change and adoption of healthy lifestyle practices by patients.
- ✓ Further research should be done on this area

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X. Acronyms and Abbreviations

AHMC = Adama Hospital Medical College

ART = Anti retroviral therapy

BMI = Body mass index

DM = diabetes mellitus

IDF = international diabetic federation

KAP = Knowledge, attitude and practice

LSM = Life style modification

OPD = outpatient department

OHG = Oral Hypoglycaemic

USA = United States of America

WHO = world health organization

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1. General,

2. Ethical Guidelines,

3. Submission of Manuscripts,

4. Manuscript's Category,

5. Structure and Format of Manuscript,

6. After Acceptance.

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- 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:

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Methods and Procedures	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
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References	Complete and correct format, well organized	Beside the point, Incomplete	Wrong format and structuring

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