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Pharma, Drug Discovery, Toxicology & Medicine

Highlights

- New Antineoplastic Therapies
- Evaluation of the Quality Indicators
- Utilisation of Products Labelled
- Gastroprotective Effect of Tadalafil

Discovering Thoughts, Inventing Future

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Evaluation of the Quality Indicators of the Drug "Bralekord" Solution for Infusions

By Azamat Ibragimovich Abdunazarov & Azizakhon Dilshodovna Tashpulatova

Tashkent Pharmaceutical Institute

Abstract- The results of standardization and quality control of the drug "Bralecord" solution for infusions are given, according to such quality indicators as: description, authenticity, transparency, color, pH, mechanical inclusions, impurities, osmolality.

Keywords: quality indicators, standardization, quality control, injection solution, pharmacopoeia, normative document.

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Evaluation of the Quality Indicators of the Drug "Bralekord" Solution for Infusions

Azamat Ibragimovich Abdunazarov [α] & Azizakhon Dilshodovna Tashpulatova [σ]

Abstract- **The results of standardization and quality control of the drug "Bralecord" solution for infusions are given, according to such quality indicators as: description, authenticity, transparency, color, pH, mechanical inclusions, impurities, osmolality.**

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I. Introduction

Processes of globalization of the pharmaceutical market; a high level of competition, as a result of which there is a shift in competitiveness factors from the level of the product to the level of the organization as a whole; an increase in the number of interactions in the process of drug circulation; social responsibility of business and its customer orientation; the characteristics of pharmaceutical products, as well as the requirements of regulatory legal acts, are the main reasons for the development and implementation of quality assurance systems or, at a higher level of development, quality management in the implementation of pharmaceutical activities.

An essential condition for the functioning of the sphere of drug circulation, as well as one of the main mechanisms for ensuring the required level of quality and safety of pharmaceutical products and services in the interests of the consumer, is the standardization procedure [1-2].

Standardization is the activity of establishing rules and characteristics for the purpose of their voluntary reuse, aimed at achieving orderliness in the areas of production and circulation of products and increasing competitiveness.

To date, one of the pressing issues of healthcare in the Republic of Uzbekistan is the provision of vital drugs that meet the high requirements of modern medicine. The quality of infusion solutions must meet the stringent requirements of modern standards. Only in the conditions of pharmaceutical production, it is possible to eliminate the influence of the human factor as much as possible and introduce multi-stage quality control. The control of the main indicators of the quality of the finished product and the parameters of the technological process plays an important role in obtaining drugs of guaranteed quality. An increased risk in the parenteral route of administration of large volume solutions (100 ml or more) or infusion solutions causes high requirements for their quality [3].

Quality standards for medicines should ensure the development of a high quality, effective and safe medicine, and should be revised in a timely manner, taking into account new achievements in medical, pharmaceutical and other sciences and the requirements of leading foreign pharmacopoeias.

An objective assessment of the quality of medicines depends not only on the merit of the methods, but also on the fact that their use in different laboratories allows obtaining identical results. This is ensured by the standardization of quality assessment methods, the preparation of solutions, reagents and indicators, the standardization of instruments, etc. The standard as ND establishes a set of norms or requirements for the object of standardization [4].

Bralecord is a combined drug containing in its composition: sodium citicoline, L-arginine hydrochloride, levocarnitine, used as a nootropic and metabolic agent.

Purpose of the study. The purpose of these studies is to develop methods for assessing the quality and establishing indicators of the quality of the combined drug "Bralecord" solution for infusion.

II. Experimental Part

a) Materials and research methods

As objects of study, 5 series of pilot samples of the drug "Bralecord" solution for infusions were used. During the study, solvents, reagents and consumables from MERCK (Germany) were used. The following auxiliary equipment was also used in the tests: magnetic stirrers, BP-310S electronic analytical balance from Sartorius (Germany), HS 32 AC sterilizer with automation, Seven Easy pH meters from Mettler Toledo (Switzerland) and 766 Calimatic Knick (Germany), Julabo water thermostat (Germany), Osmomat 010 type osmometer, Gonotek (Germany), PAMAS SVSS liquid particle counters (Germany).

b) Results and its discussion

The evaluation of the quality indicators of the study drug was carried out in accordance with the modern requirements of the national and foreign pharmacopoeias [5-7], as well as in accordance with the general technical regulation on the safety of medicines [8] in terms of such indicators as:

Author: Tashkent Pharmaceutical Institute. e-mail: author.uzb@mail.ru

description, authenticity, transparency, color, pH, mechanical inclusions, impurities, osmolality, sterility, bacterial endotoxins, abnormal toxicity, quantitation.

The initial stage of work is organoleptic control, which is a mandatory type of control and consists in checking the medicinal product in terms of appearance, smell, mixing uniformity, and the absence of mechanical impurities in liquid dosage forms.

c) Description

The drug should be a clear, colorless or slightly yellowish solution (visual method). All studied five series of the drug meet the requirements of the pharmacopoeia.

d) Authenticity

Identification was carried out by a chemical method using qualitative reactions to the main components of the drug: sodium citicoline, arginine, levocarnitine and chlorides.

Sodium citicoline was determined by the spectrophotometric method at an absorption maximum at 280 nm. 1.0 ml of the drug was placed in a volumetric flask with a capacity of 100 ml, the volume of the solution was brought to the mark with a 0.01 mol/l solution of hydrochloric acid and mixed. 10 ml of the resulting solution was placed in a volumetric flask with a capacity of 50 ml, the volume of the solution was brought to the mark with a 0.01 mol/l solution of hydrochloric acid and stirred (test solution).

The optical density of the resulting solution was measured on a spectrophotometer at the absorption maximum at a wavelength of 280 nm in a cuvette with a layer thickness of 10 mm, using a 0.01 mol/l hydrochloric acid solution as a reference solution. In parallel, the optical density of the RSO solution of sodium citicoline was measured at the same wavelength.

Arginine. 15 ml of water was added to 5 ml of the preparation. To 2 ml of the resulting solution was acidified 1 ml of α-naphthol solution and 2 ml of a mixture of equal volumes of 3% sodium hypochlorite solution and water; a red color is formed.

Levocarnitine. 2 ml of the drug was transferred into a test tube, 5 ml of 1 mol/l hydrochloric acid solution and a few drops of ammonium rheinecate solution were added, and a red-violet precipitate formed.

Preparation of ammonium rheinecate solution: about 500 mg of ammonium rheinecate was mixed with 20 ml of water, shaken periodically for 1 hour and filtered. The solution is used within 2 days.

Chlorides. 2.0 ml of the drug was acidified with dilute nitric acid, 0.4 ml of a silver nitrate solution was added, and a white curdled precipitate (chlorides) was formed upon standing.

All studied five batches of the drug confirmed the presence of citicoline sodium, arginine, levocarnitine and chlorides in the solution of the study drug.

Transparency. The solution of the drug should be transparent compared to water for injection.

Chromaticity was determined in accordance with the European or British Pharmacopoeia. It was found that the color of the preparation should not be more intense than the reference solution Y7.

The pH was set potentiometrically. It was found that the pH of the drug solution should be in the range from 5.0 to 7.5.

All studied five batches of the drug met the requirements of pharmacopoeias in terms of mandatory indicators: transparency, color, pH.

Foreign impurities

Citicoline sodium. High performance liquid chromatography method.

Chromatographic system and determination conditions

Instrument liquid chromatograph "ShimadzuLC-6A"

Steel column, size 30 cm x 4.0 mm

Octadecylsilane filler chemically bonded to porous silica gel or ceramic microparticles 10 μm in diameter (LI, USP)

Flow rate 2 ml/min

Detection 275 nm

Injection volume 20 μl

A column filled with octadecylsilane silica gel and phosphate buffer solution was used (equal volumes of 0.1 mol/l potassium dihydrogen phosphate and tetrabutylammonium phosphate solution were mixed, the pH value of 0.01 mol/l tetrabutylammonium hydroxide was adjusted to 4.5 with phosphoric acid), methanol (95 : 5) as mobile phase.

1 ml of the drug was carefully transferred into a 10 ml volumetric flask and diluted with water to the mark (test solution).

Dissolve the required amount of citicoline sodium RSO and levocarnitine RSO in water to obtain a solution of 1.045 mg/ml sodium citicoline and 2.0 mg/ml levocarnitine (control solution).

Transfer 1.25 ml of the control solution to a 100 ml volumetric flask. Dilute to the mark with water and mix well (system suitability solution).

Introduced 20 μl of reference solution 1 into the column. The dilutions were adjusted so that the main maximum in the chromatogram was 20-25% of the full scale of the chart. Add 20 μl of each test solution, system suitability solution, and control solution separately to the column. Chromatography was continued at 2.5 times the retention time of the citicoline peak.

Calculations:

The content of an individual non-identifying or identifying impurity, in percent:

$$X = \frac{AT \times 100\%}{As}$$

where: AT = Peak area of an individual impurity in the test solution

As = Sum of all peak areas in control solution

At the same time, it was established that the individual impurity should be no more than 0.5%, and the total impurity - no more than 2.0%. All five batches of the study drug met the established norm.

Substances detected by ninhydrin. The determinations are carried out by thin layer chromatography.

System suitability solution: 0.4mg/ml each of arginine hydrochloride and L-lysine hydrochloride with water

Sample solution: 10 mg/ml RSO arginine hydrochloride with water

Standard solution: dilute 1 ml of sample solution with water to 100 ml. Dilute 5 ml of the resulting solution with water to 10 ml (0.05 mg/ml)

Note: The concentration of the solution is approximately 0.5% of this sample solution.

Test solution: 100 µl of the drug is added to 320 µl of water (10 mg/ml)

Chromatography System Mode: TLC

Adsorbent: 0.25 mm layer of chromatographic silica gel mixture

Applied sample volume: 5 µl

Mobile solvent system: isopropyl alcohol and ammonium hydroxide (70:30)

Nebulizer: 2 mg/ml ninhydrin in butyl alcohol and 2N acetic acid (95:5)

Drying: at a temperature from 100 °C to 105 °C until complete removal of ammonia. The plate is sprayed with a solution of 2 mg/ml ninhydrin in a mixture of butyl alcohol and 2 N acetic acid (95:5) and heated at 100°C to 105°C for 15 minutes. Examine the plate under daylight. The system suitability chromatogram shows two completely separated spots.

Norm: any spot, except for the main one, should not be more intense than the main spot on the chromatogram of the standard solution (0.5%). Individual impurities: no more than 0.5%, total impurities: no more than 2.0%.

The results of the analysis are considered reliable if two clearly separated spots appear on the chromatogram of the system suitability solution.

All studied five series of the drug corresponded to the established norm.

Levocarnitine. High performance liquid chromatography method.

Reagents: 2M sodium hydroxide solution. Solution A: 6.81 g of KH_2PO_4 are dissolved in 800 ml of water, 2M sodium hydroxide solution is added to obtain a pH of 4.7, the volume of the solution is adjusted to the mark with water and mixed; Acetonitrile.

Chromatography conditions. Column: Aminopropyl methylsilangel (USP L8), 250x4.6mm, 5µm; Column temperature: 30^0C+1^0C; UV detector: 205nm. Mobile phase: A mixture of 35 volumes of solution A and 65 volumes of acetonitrile; flow rate: 1.0ml/min; injection volume: 25µl.

Standard solutions and test solution are introduced into the chromatograph and chromatograms are recorded within 20 minutes. The sensitivity of the system is determined by the height of the main peak on the chromatogram of the standard solution (c), which is at least 20% of the full scale of the recording device. The test is considered invalid if the resolution between the peaks of levocarnitine and levocarnitine impurity A in the chromatogram of the standard solution (c) is 0.9. On the chromatogram of the test solution: the area of any peak of levocarnitine impurity A is not more than the main peak of the chromatogram of the standard solution (c) (1%); the area of any peak, except for the main peak and the peak of levocarnitine impurity A, does not exceed the area of the main peak in the chromatogram of the standard solution a (0.2%); the sum of the areas of all peaks, except for the main peak and the peak of impurity A, does not exceed 2.5 times the area of the main peak in the chromatogram of standard solution a (0.5%).

Preparation of standard solution "a". 100 mg of the drug solution is transferred into a 100 ml volumetric flask, the volume is adjusted to the mark with solution A. 1 ml of the resulting solution is diluted to 10 ml with the same solvent (0.2%).

Preparation of standard solution "c". 20.0 mg of the working standard sample of levocarnitine impurity A is dissolved in water, the solution is adjusted with water to 100 ml. 2.5 ml of the resulting solution is diluted to 10 ml with solution A (1.0%).

Preparation of standard solution "c". 10 mg of the working standard sample of levocarnitine impurity A is dissolved in water and diluted with water to 25 ml and 2.5 ml of the resulting solution is diluted to 10 ml with solution A.

Preparation of standard solution "d". Dissolve 100 mg of the working standard of levocarnitine in 10 ml of standard solution C.

Preparation of the test solution. 2.5 ml of the preparation solution is transferred into a volumetric flask with a capacity of 100 ml and the volume is brought to the mark with solution A.

Norm: impurities A should be no more than 1.0%; other impurities - no more than 0.2%; the sum of impurities, except for impurity A - no more than 0.5%.

According to the results of the studies, all five series of the drug under study corresponded to the established norm.

Osmolality. The determination of the osmolality of the solution is carried out by the cryoscopic method using a Beckmann thermometer. The determination of the freezing point is carried out on the installation shown in Figure 1.

The main part of the setup is a test tube with a side branch. Its upper opening is tightly closed with a cork through which the Beckmann thermometer and a wire stirrer pass, one end of which is bent in the form of a ring freely covering the lower part of the thermometer. This test tube is inserted into a wider test tube, which acts as an air jacket that prevents the liquid from cooling too quickly. The assembled apparatus is placed in a Bunsen beaker, which is filled with a cooling mixture before the experiment.

The role of the cooling mixture is performed by ice chips, to which crystalline sodium chloride is added to reduce the temperature. A stirrer is used to stir the cooling mixture. The temperature of the cooling mixture should be (4-5)°C below the freezing point of the test liquid.

The zero point of the instrument is set to water for injection. The instrument is calibrated using standard sodium chloride solutions. The determination is carried out three times and the average value is taken.

As needed, prepare standard solutions based on the data in Table 1.

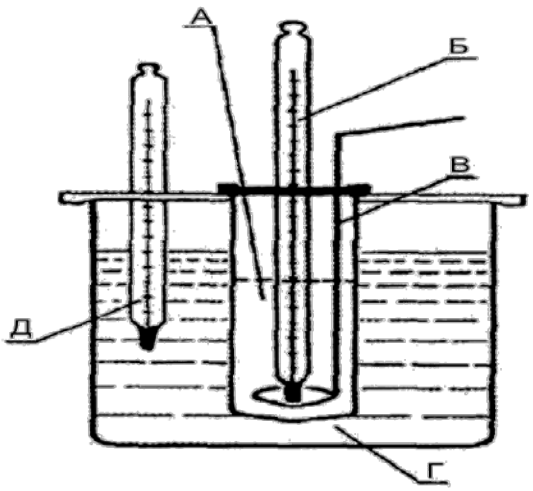
To determine the freezing point of a solvent or a test solution, 28-30 g of liquid is placed into an inner small test tube through a side hole, a stirrer and a Beckmann thermometer are also placed here. This tube is placed through an air jacket into the cooled mixture and the test liquid is evenly mixed by raising and lowering the stirrer. When determining the freezing point of a liquid, the mercury column in the thermometer begins to fall as the liquid cools. Usually, before freezing, the liquid is supercooled, and the temperature of the liquid drops below the freezing point. As soon as the crystallization process begins, the temperature of the solution rises beyond the freezing point.

Table 1: Data for osmometer instrument

Mass of sodium chloride, in grams per 1 kg of water	Actual osmolarity (mosm/kg)	Theoretical osmolality (ideal)	Molar osmotic coefficient (mosm/kg)	Freezing temperature drop ΔTzam.
3,087	100	105,67	0,9463	0,186
6,260	200	214,20	0,9337	0,372
9,463	300	323,83	0,9264	0,558
12,684	400	437,07	0,9215	0,744
15,916	500	544,66	0,9180	0,930
19,147	600	655,24	0,9157	1,116
22,380	700	765,86	0,9140	1,302

The increase in temperature occurs due to the release of open heat of solidification.

After that, the test tube is removed from the liquid, the crystals are melted by heating the test tube with the hand, and the determination is repeated again. The experiment is carried out three times. The discrepancy between the definitions should be no more than 0.01°C. In case of hypothermia, it is necessary to add a solvent crystal to the liquid. The accuracy of determination with this method is +5%.



The device of the Beckman device: A - a vessel for the test solution; B - Beckman thermometer; B - stirrer; G - container with coolant; D - thermometer for measuring the temperature of the cooling mixture.

Before each measurement, the tube with the lateral process is rinsed with the solution intended for the study, and the measurement is also made with the test samples.

The instrument is ready to measure the test product if the value obtained for the calibration solutions is within two values of the calibration scale. To reduce the error and check the reproducibility, it is recommended to repeat measurements with several samples from the same sample, averaging the results. The measurement error should not exceed ±2%.

The calculation of osmolality is carried out according to the following formula:

$$S_{osm}, \text{ mOsmol/kg (mOsmol / l)} = \frac{(T2\text{-}T1) \times 1000}{1.858},$$

where: 1.858 is the molar cryoscopic constant of water, corresponding to the decrease in freezing point that occurs as a result of dissolution

1 mole of a substance in 1 kg of water;
1000 - Conversion factor osm/kg to mosm/kg;
T_2 - freezing point of pure solvent (0C);
T_1 is the freezing point of the solution (0C).

Based on the measurements carried out, it was found that the osmolality should be in the range from 410 mOsmol/kg to 590 mOsmol/kg. All studied five series of the drug corresponded to the established norm.

Mechanical inclusions: Mechanical inclusions in dosage forms for parenteral use are extraneous mobile insoluble particles, with the exception of gas bubbles, accidentally present in drug solutions. The test for the presence of visible and invisible particulate matter is intended for visual evaluation of liquid parenteral dosage forms, including infusion solutions, and is a mandatory pharmacopoeial quality indicator.

Based on the foregoing, as a result of the research, visible and invisible mechanical particles were determined. To detect visible particles in accordance with the SP RUz., Eur.F., 2.9.20, OFS 42 Uz-0006-3341-2018, during visual inspection, the test preparation should not contain visible particles (visible particles should be absent).

To detect invisible particles in accordance with the SP RUz., Eur.F., 2.9.19, OFS 42 Uz-0005-3340-2018, invisible particles per 50 ml and 100 ml bottle for particles ≥ 25 microns should be no more than 600 pieces, for particles≥10 microns should be no more than 6000 pieces (Ev.F., 2.9.19, OFS 42 Uz-0005-3340-2018).

For 200 ml vial:

Particle size	Number of particles in 1 ml
10 micronsormore	Not more than 25 h/ml
25 micronsormore	Not more than 3 h/ml

All studied five series of the drug corresponded to the established norm in terms of "mechanical inclusions".

The results of studies on the quality indicators of the drug "Bralecord" solution for infusion are presented in table 2.

Table 2: Research results on some quality indicators drug "Bralecord" solution for infusion

The name of indicators	Norms	Series 1	Series 2	Series 3	Series 4	Series 5
Description	Clear, colorless or slightly yellowish solution.	Corresponds	corresponds	corresponds	corresponds	corresponds
Authenticity	SF-metry, qualitative reactions	confirmed	confirmed	confirmed	confirmed	confirmed
Transparency	The drug must be transparent	transparent	transparent	transparent	transparent	transparent
Chroma	The color of the preparation should not be more intense than the reference solution Y_7	no more intense than reference solution Y_7	no more intense than reference solution Y_7	no more intense than reference solution Y_7	no more intense than reference solution Y_7	no more intense than reference solution Y_7
pH	5,0-7,5	6,05	6,10	6,08	6,12	6,02

Foreign matter:						
Citicoline sodium:	no more than 0,5%	no more than 0,5%	no more than 0,5%	no more than 0,5%	no more than 0,5%	no more than 0,5%
Individual impurity total impurity	no more than 2,0%	no more than 2,0%	no more than 2,0%	no more than 2,0%	no more than 2,0%	no more than 2,0%
Levocarnitine admixture A: others: the sum of impurities, except for impurity A:	no more than 1,0% no more than 0,2% no more than0,5%	no more than 1,0% no more than 0,2% no more than0,5%	no more than 1,0% no more than 0,2% no more than0,5%	no more than 1,0% no more than 0,2% no more than0,5%	no more than 1,0% no more than 0,2% no more than0,5%	no more than 1,0% no more than 0,2% no more than0,5%
Substancesdetectedbyninhydrin	Any spot, except for the main one, should not be more intense than the spot on the chromatogram of the standard solution (0.5%)	no more than0,5%	no more than0,5%	no more than0,5%	no more than0,5%	no more than0,5%
Osmolality	410-590 mosmol/l	475mosmol/l	478 mosmol/l	476 mosmol/l	477 mosmol/l	480 mosmol/l
Mechanicalinclusions (visible)	Visible particles must be absent	missing	missing	missing	missing	missing
Mechanicalinclusions (invisible)	One vial for particles ≥ 25 microns should contain no more than 600 pieces; Particles ≥ 10 microns should be no more than 6000 pieces	corresponds	corresponds	corresponds	corresponds	corresponds

III. Conclusion

On the basis of the conducted experimental studies, the main indicators of the quality of the drug "Bralecord" solution for infusions were established in terms of: description, authenticity, transparency, color, pH, impurities, osmolality, mechanical inclusions. The limits of their normalization in accordance with the pharmacopoeias have been established. Approved quality indicators are included in the regulatory documentation for the study drug.

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From the Histological Model to the Mutational Model: The Study of Heavy Metals and Other Substances in New Antineoplastic Therapies

By Pasquale Ruffolo, Osvaldo Acquaviva, Pierpaolo Capece, Ferdinando Mazzei, Bruno Ruffolo, Manuela Panunzio, Alessandra Paraggio & Andrea Ruffolo

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Keywords: tumour disease, therapeutic response, complications, genomic modification, genomic and genetic tests, trace elements, immune response, nutritional supplementation, heavy metals, dioxins, hyperglycaemia, and hyperinsulinemia.

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From the Histological Model to the Mutational Model: The Study of Heavy Metals and Other Substances in New Antineoplastic Therapies

Pasquale Ruffolo [α], Osvaldo Acquaviva [σ], Pierpaolo Capece [ρ], Ferdinando Mazzei [ω], Bruno Ruffolo [¥], Manuela Panunzio [§], Alessandra Paraggio [χ] & Andrea Ruffolo [ν]

Abstract- The study of genetic mutations in tumours changes the therapeutic approach; in fact, we move into a different advanced and strategic phase by entering personalized precision medicine and shifting the focus from the tissue study of the tumour to the modification and proliferation of neoplastic cells. The therapeutic programs, especially in oncology, are changing. We are in a phase of transition from the histological model to the model of genetic mutation (mutation). When a person's immune defences do not respond to a disease it is because the body cannot produce or activate specific lymphocytes and antibodies against that disease. This causes of a lack of therapeutic response or recurrence of the disease. A fundamental role is played by genomic tests, which are carried out directly on the neoplastic tissue and other modifies tissues, and the dosage of toxic substances (heavy metals, dioxins, furans, PCBs, etc.) analysed 'primarily' in the tumour and subsequently in the various biological matrices (tissues, modified, blood, urine, hair, nails, breast milk, saliva, etc.) as well as genetic tests. A significant role is a search for toxic substances and therapeutic integration of trace elements beneficial for cellular metabolism, especially for defence mechanisms, absent or deficient in these patients, such as Copper, Selenium, Zinc, Cobalt, Iron, and Manganese to improve or modify a lack of therapeutic response. Another parameter to consider is hyperglycaemia in diabetic subjects, which according to various scientific research, is considered a personal risk factor not so much in the formation phase of the neoplastic disease, but in the healing response phase.

Keywords: tumour disease, therapeutic response, complications, genomic modification, genomic and genetic tests, trace elements, immune response, nutritional supplementation, heavy metals, dioxins, hyperglycaemia, and hyperinsulinemia.

Author α: Scientific Manager C.M.O.-Napoli-Italia, President of ISDE-Pompei-Italia.
Author σ: Pharmacologist expert in nutraceuticals-Salerno-Italia.
Author ρ: Chemist, director of the study of chemical and environmental analysis.
Author ω: Department otolaryngology resident, University of Siena-Italia.
Author ¥: Doctor of medicine Center C.M.O.
Author §: Psychologist expert in psychology of environmental damage.
Author χ: Prevention Technician in the environmental and in the workplace.
Author ν: Labor and Health Lawyer.
e-mail: nino.ruffolo100@gmail.com

Introduction

Environmental pollution is today one of the major global problems with significant repercussions on human health. Despite this, little has been done to highlight the possible correlations between the different forms of pollution and the many related diseases, especially neoplastic ones. Unfortunately, little attention is paid to the importance of having a healthy, complete, and balanced diet to prevent neoplastic and chronic-degenerative diseases and to support the various therapeutic approaches. In this context, Nutrigenomics plays a fundamental role, which studies how nutrients can induce a modification or suppress genetic expressions, consequently acting on the individual phenotype. Nutrition (www.cestraecologia.it), with the possible introduction of toxic substances and a few substances useful for our body's defence mechanisms, such as vitamin D and vitamin C as well as Zinc and Copper, etc., as well as the environment in which we live, both due to soil, water and atmospheric pollution and the presence of electromagnetic fields and industrial sites (Sima Study; Rapporti ISTISAN 10/22, Ambiente salute, www.iss.it), can affect our DNA. Among the toxic substances with which we come into contact, both for environmental pollution and food, we have taken into consideration some dioxins because they are linked to immune defence deficits since they cause a reduced production of B and T lymphocytes as well as the PAHs which especially damage the lungs and nervous system.

We must start from the assumption that the genome is not a rigid structure, but interacts dynamically and evolutionarily with the environment, so that pollution, global warming, and climate change will bring about phenotypic changes as an expression of the new genetic, with loss of biodiversity, but also of fertility. The many energy sources, now used for decades, further contribute to polluting our environment by acting accordingly on the genetic structure. A world that evolves with little respect for the environment will always make recent changes to our DNA which unfortunately can hardly be co-managed or controlled by the many increasingly current therapeutic approaches, denying us the right to health if there is no due respect for the

environment, we live in. In this article, our main objective is to analyse the presence in cancer patients, not only of nutritional deficiencies, but also of food toxicity that causes the accumulation of toxic substances that act by modifying, reducing, or blocking the immune response. For this reason, we must start from the analysis of the genetic mutations of tumours, changing the therapeutic approach; in fact, we move from the histological study of the tumour to a different advanced and strategic phase, entering a personalized precision medicine, shifting the focus to genetic modifications and neoplastic cell proliferation. The therapeutic programs, especially in oncology, are changing, as we are in a phase of transition from the histological model to the model of genetic mutation, called mutational model. To this new diagnostic model, according to our studies, it is essential to add the in-depth analysis of certain substances, such as heavy metals, dioxins, PCBs, etc., in the tumour and in the biological matrices and which can reduce or modify the therapeutic response. When a person's immune defences do not respond to a specific disease, it is because the body cannot produce or activate specific lymphocytes and antibodies against that disease. This causes the lack of therapeutic response or recurrence of the disease. A fundamental role is played by genomic tests, which are carried out directly on the neoplastic tissue, and modified tissues, but also the dosage of toxic substances (heavy metals, dioxins, furans, PCBs, etc.) analysed "firstly" in the tumour and subsequently in the various biological matrices (tumoral tissues, blood, urine, hair, nails, mother's milk, saliva, etc... skin appendages), as well as specific genetic tests. A key role is also the therapeutic integration in case of the absence of trace elements useful for cellular metabolism, especially for the defence mechanisms, such as Zinc, Copper, Selenium, Magnesium, Cobalt, Iron and Manganese and essential vitamins to improve or modify lack of therapeutic response.

The lack of trace elements could be seen as a possible "predisposition" to lack of response to immunological therapies and, therefore, to a greater risk of aggravation of the disease due to lack of therapeutic response. It is reported in the literature that the lack of Selenium, Zinc, Copper, Magnesium, Manganese, Iron, and Cobalt can be correlated to states of immunodeficiency.

In fact, in several studies, it appears that an absent or reduced therapeutic response of the cancer patient has been related to the lack of activation of specific genes associated with the immune defences, which are not activated. Their non-activation could be referred as low concentrations (or values), especially of Copper and Zinc, but also of Selenium, Cobalt, Manganese, and Iron. For example, Zinc participates, through the Zinc Finger Protein, in the DNA repair processes, and therefore, in the recovery of the immune defences.

Even the altered dosage of these trace elements in different organs and systems finds some correspondences in the possible organ-specific complications, giving us the possibility of predicting in which patients a rapid and aggressive evolution of the disease could arise with an individual-specificity and organ-specificity, but also in which patients a severe or pauci-symptomatic form.

It is important to clarify that the dosage of heavy metals in the tumour and in the various biological matrices does not and cannot be a methodology for preventing neoplastic disease, but a helpful method for improving the therapeutic response of the several therapeutic approaches.

Another parameter to consider is hyperglycaemia in diabetic subjects which, according to various scientific research, is considered a personal risk factor not so much in the formation phase of the neoplastic disease, but in the healing response phase. Recent studies have shown that hyperglycaemia is the cause not only of a delayed and reduced immune response but also of an increase in co-morbidity and mortality. (Updates of the FNOMCeO).

Hyperglycaemia, accompanied by insulin resistance, and consequent hyperinsulinemia, push towards rapid cell proliferation, while the high number of sugars and lipids in the blood act as metabolic fuel for the spread of the tumour. The high insulin-glucose ratio in people with diabetes causes the loss of control of the DNA regulatory genes in some cells, starting a transformation mechanism, as occurs in cancers of the gastrointestinal tract. This brings to a high proliferation, migration, and infiltration of tumour cells that make the disease particularly aggressive.

Following our mutational therapeutic approach, it should be emphasized that even the deficiencies of some substances in diabetic subjects can compromise the extent of the response and modify it. This is the case of Zinc deficiency which could damage the immune response due to reduced production of Zinc Finger Protein.

According to our conclusions, therefore, in the diabetic oncological patient treated with immunotherapy, the therapeutic program must be adjusted with the dosage, both in the tumour and in the various biological matrices, of Zinc and with careful control of the glucose/insulin ratio to avoid a reduced or no response therapeutic.

Once again, environmental pollution has proven to be a severe danger to human health, since many neoplastic diseases have shown a greater incidence, diffusion, aggressiveness, and mortality in the most polluted areas.

This research, still in an early stage, requires considerable resources and more patients, and is meant

to be a further invitation to start a multi-center clinical-scientific program for a broader study of heavy metals, dioxins, PAHs, polychlorinated biphenyls in cancer patients, being treated with immunotherapy, but also for many patients who do not respond to the several standard therapeutic programs. In fact, this program allows to study in these patients, in addition to the genetic mutations, also the presence or deficiency of some substances. Then we pass from the histological model to the model of genetic mutation (mutation) both in the cancer and in the biological matrices, and we can positively affect the therapeutic approach even in cancer patients who do not respond to immunotherapy or who have had a recurrence of the disease after immunotherapy, so as to implement precision and personalized therapeutic programs.

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- *Pasquale Ruffolo,* C.M.O. scientific director, former full professor at the National Cancer Institute - Naples, President of ISDE Pompeii.
- *Osvaldo Acquaviva,* pharmacologist expert in nutraceuticals.
- *Pierpaolo Capece,* chemist, director of the study of chemical and environmental analysis.
- *Bruno Ruffolo,* Doctor of Medicine Center.
- *Manuela Panunzio,* psychologist, expert in psychology of environmental damage.
- *Alessandra Paraggio,* prevention technician in the environment and in the workplace.
- *Andrea Ruffolo,* health lawyer.

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Qualitative Studies on the Factors Influencing the Utilisation of Products Labelled "Food for Special Medicinal Use" (FSMP)

By Chereches Marius Calin, Finta Hajnal, Popa Cristian Olimpiu, Stefanescu Daniela & Muntean Daniela Lucia

Tashkent Pharmaceutical Institute

Abstract- This study aimed to investigate Romanian physicians' awareness, recommendation practices, and opinions regarding using Foods for Special Medical Purposes (FSMPs) products. A total of ten physicians were interviewed using a structured questionnaire, and their responses were analysed using thematic content analysis. The study found that physicians were aware of FSMPs and recommended them to their patients based on nutritional deficits, weight loss, or deglutition impairments. In addition, disease stage, treatment scheme, taste, affordability, and availability were identified as factors influencing the recommendation and use of FSMPs. While physicians generally did not consult clinical trials, clinical experience was deemed essential for recommending FSMPs to patients. Patients' feedback regarding the usage and sourcing of FSMPs was generally positive, with some expressing concerns about the availability of different flavours and the costs of purchasing the products.

Keywords: food for special medical purposes, FSMP, qualitative research, nutritional needs for oncology patients.

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Abstract- This study aimed to investigate Romanian physicians' awareness, recommendation practices, and opinions regarding using Foods for Special Medical Purposes (FSMPs) products. A total of ten physicians were interviewed using a structured questionnaire, and their responses were analysed using thematic content analysis. The study found that physicians were aware of FSMPs and recommended them to their patients based on nutritional deficits, weight loss, or deglutition impairments. In addition, disease stage, treatment scheme, taste, affordability, and availability were identified as factors influencing the recommendation and use of FSMPs. While physicians generally did not consult clinical trials, clinical experience was deemed essential for recommending FSMPs to patients. Patients' feedback regarding the usage and sourcing of FSMPs was generally positive, with some expressing concerns about the availability of different flavours and the costs of purchasing the products. The study concludes that physicians play a vital role in recommending FSMPs to patients and ensuring they have the necessary nutritional support during treatment. However, more patient education materials and collaboration with nutritionists may be required to improve patient outcomes and reduce patient financial burden.

Keywords: food for special medical purposes, FSMP, qualitative research, nutritional needs for oncology patients.

I. Introduction

Food for special medical purposes (FSMPs) are foods designed for people with particular medical disorders and nutritional needs. FSMPs address the dietary requirements of individuals who cannot eat a typical diet because of ailments like malabsorption, metabolic abnormalities, and food allergies. They are often only available with a prescription and are meant to be used under medical supervision.

FSMPs may be used as the sole source of nutrition or as a supplement to a regular diet, depending on the individual's medical needs. These products can come in different forms, such as powders, liquids, or semi-solids and can be used in various settings, including hospitals, long-term care facilities, and home settings.

Inborn errors of metabolism, severe food allergies, and gastrointestinal illnesses like Crohn's disease or ulcerative colitis are a few examples of medical conditions that might call for the usage of FSMPs.

To ensure their effectiveness and safety, FSMPs are governed by regulations and are required to adhere to specific standards. The FSMP industry is regulated by various national and international bodies, such as the European Food Safety Authority (EFSA) and the U.S. Food and Drug Administration (FDA), which set standards for the safety, quality, and efficacy of FSMP products [8, 16, 28, 31].

FSMPs are defined according to Article 2(2)(g) of Regulation 609/2013[10]as follows:

"*food specially processed or formulated and intended for the dietary management of patients, including infants, to be used under medical supervision; it is intended for the exclusive or partial feeding of patients with a limited, impaired or disturbed capacity to take, digest, absorb, metabolise or excrete ordinary food or certain nutrients contained therein, or metabolites, or with other medically-determined nutrient requirements, whose dietary management cannot be achieved by modification of the normal diet alone*".

Particularly in Romania, Regulation 128/2018 updated the Regulation 609/2013 mentioned above (named within most of the documents as "FSG Regulation"), and the food notification procedure for special medical purposes (FSMP) was set by Order of the Ministry of Health nr.820/2019[19]. Furthermore, specific standards which apply to FSMP are also outlined within Codex Alimentarius by World Health Organisation [8, 31].

The Commission wanted to clarify the distinctions between FSMP and food supplement products and other ideas relevant to the legislative framework that applies to this category of products through the classification of FSMP, which was subject to specific notice [8].

Author α σ ¥: Faculty of Pharmacy, "George Emil Palade" University of Medicine, Pharmacy, Science and Technology of Târgu Mureș, 540142 Târgu Mureș, Romania. e-mail: mariuscalinchereches@mac.com
Author ρ: Faculty of European Studies, Babeș-Bolyai University, 400090 Cluj-Napoca, Romania.
Author ω: Faculty of Economics and Law, "George Emil Palade" University of Medicine, Pharmacy, Science and Technology of Târgu Mureș, 540142 Târgu Mureș, Romania.

FSMPs are divided into three groups [8]: (a) nutritionally complete foods with a standard nutrient formulation, (b) nutritionally complete foods with a nutrient-adapted formulation unique to a disease, disorder, or medical condition, and (c) nutritionally incomplete foods with either a standard formulation or a nutrient-adapted formulation unique to a disease, disorder, or medical condition. The first two categories can serve as a patient's only source of nutrition, whereas the third can only increase intake to supplement other sources of nutrients. The FSG Regulation and Codex Alimentarius both clearly define the composition of products, which are defined as FSMP that must contain specific nutrients from the List (vitamins, minerals, amino acids, carnitine-taurine, nucleotides, and coline-inositol) or meet specific requirements (meet food standards, provide scientific support regarding the contribution of ingredients to satisfying nutritional needs and to comply with conditions related to pesticides) [10, 16, 31].

Some writers believe that FSMPs intended for oncology patients may not be adequate for some critical elements such as Zn, Cu, Se, Fe, or Mn, despite the regulatory standards regarding the composition of FSMP implying that the formulations are based on commonly accepted scientific facts [11]. In addition, similar studies indicated sample determination for Se, Zn, K, Mg, and vitamin C differed from (E.U.) 2016/128 requirements for enteral tube feed formulae, raising questions about the bioavailability of synthetic vitamins and minerals in comparison to those from natural sources [23].

Products classified as FSMP should be usable by humans and effectively supply nutritional support to the people they are designed for. Other authors put up a different classification that is more workable and acceptable. This classification explains some product categories that fall under the FSMP range and is being developed by an interdisciplinary group of German industry [10, 29].

All regulatory bodies stress the significance of accurately labelling FSMP products, and there are now clear guidelines and explanations. The labelling should provide information regarding energy value, the content of proteins, carbohydrates and fat (per 100 g or ml), the content of vitamins and minerals (per 100 g or ml), osmolarity and/or acid-base balance and the origin and nature of proteins (animal or plants). The manufacturers should disclose the nature of the product, its purpose, and specific usage instructions in sufficient detail. The material shouldn't mislead people or include any characteristics or qualities that imply these items may cure or treat certain illnesses. Additionally, references to usage guidelines should be made, such as use only under medical supervision, information about how to store or retain a container after it has been opened, not suited for parenteral use, etc. It is important to note that while general public advertising should be outlawed, providing educational materials and appropriate resources to healthcare professionals is paramount [8, 9, 31].

Stomatitis and oral mucositis are common in oncology patients receiving chemotherapy or radiation therapy for various types of cancer. These conditions significantly negatively impact the duration, complications, and cost of the entire treatment and the toll they take on the patients' and families' quality of life. Several papers and studies support the value of nutrition and its impact on lowering patient stress and oncology treatment expenses.

Medical costs for patients receiving radiotherapy and developing mucositis or pharyngitis during the treatment are much greater than for those without such conditions, according to a study on patients with head and neck cancer (HNC) and non-small cell lung cancer ($39,313 vs $20,798) [21]. Another study on HNC patients receiving radiotherapy found that 91 percent of patients experienced oral mucositis, and 66 percent of those cases were severe (>Grade 3). Depending on the severity, oral mucositis was associated with increased expenses of $1700–$6000[6]. In addition, the side effects of cancer treatment, such as nausea, vomiting, oral mucositis, and exhaustion, create numerous logistical difficulties and significantly strain the patient and the healthcare system, especially when cancer is treated as a chronic condition [3]. According to a different study, oral or/and G.I. mucositis occurred in 51% of chemotherapy-treated cancer patients with solid tumours or lymphoma. If a patient has oral mucositis or both G.I. and oral mucositis, the projected cost of chemotherapy each cycle is 1.6 times higher and 2.3 times higher, respectively. For patients receiving a hematopoietic cell transplant, an additional point in the peak mucositis score resulted in an additional $25,000 in hospital expenses [17]. In treating several cancers, surgery, chemotherapy (including immunotherapy or target therapy), and radiotherapy may be employed, and the expenditures involved with all these efforts are substantial [13].

Early nutrition support and prevention of oral mucositis or other radiochemotherapy-related side effects for cancer patients determine benefits on all levels (patient, physician, and cost management) by reducing weight loss, fewer radio/chemotherapy breaks, fewer delays in completing radio/chemo cycles, fewer unplanned hospitalisations or shorter lengths of stay in hospitals, and ultimately a higher rate of treatment completion [7, 17, 22, 24].

National healthcare systems reimburse FSMP products in several countries. The E.U. allows for regional or provincial variations in granting FSMP reimbursement. France, Germany, Italy, Spain, and the U.K. are some nations that reimburse FSMPs. Also,

France and Brazil are the countries that have a formal HTA process for medical nutrition [25]. However, the FSMP type and content may affect the reimbursement criteria and procedure. It's critical to remember that depending on the country and the patient's medical condition, different restrictions may apply for FSMP product reimbursement ([2, 25, 27]. Chinese researchers and authorities place a high value on FSMP and HTA evaluation for products under this category. Several papers have been written about creating rules, clinical trial prerequisites, and HTA evaluation standards [15, 20, 26].

We examined numerous sources to estimate the size of the worldwide FSMP market, and the results are consistent. The market is estimated to be worth $11.2 billion in 2019 or $13.48 billion in 2021, depending on the source, and it might reach $19.67 billion in 2028 or $19.41 billion in 2030. For the timeframes mentioned above, a CAGR of 5.6 percent to 6.5 percent is anticipated for the growth rate. The key drivers of the growth mentioned by the sourced consulted are the increased awareness of the benefits of this category of products, the increased prevalence of chronic diseases, the increased demand of older adults, the proliferation of new private label manufacturers and the expansion of new distribution channels. More than 65 percent of the market share in the worldwide FSMP market is held by the top 10 companies. Nestle, Danone (Nutricia), Abbott, Bayer, Mead-Johnson, Ajinomoto, Fresenius-Kabi, Lenus Pharma, GFI-Gruppo Farmaimpresa, Galen Limited, BOSSD, and Leskon & EnterNutr are among the companies mentioned. In addition, the 2020 acquisition by Danone of the Murray Goulburn Dairy company in China has indicated a market concentration [5, 18, 30].

An important issue is related to the information regarding the FSMP category. The regulation stipulates that using FSMP needs medical supervision, which is part of this category's definition. Healthcare professionals give medical supervision, and they can help patients with FSMPs. By healthcare professionals (HCPs), the regulatory body means "persons having qualifications in medicine, nutrition, pharmacy or for other healthcare professionals responsible for maternal care and childcare" according to FSG Regulation, art 9.Without the limits typically imposed on communication to the general public, it is crucial that HCPs may receive complete information regarding the product composition, the clinical justification and supporting data, the appropriate usage, preparation, and the intended target group [2, 10]. However, the legal responsibilities of pharmacies and pharmacists for dispensing FSMPs must be investigated within the current legal framework for the entire pharmacy practice, given that the majority of products in this category are obtained from pharmacies, and there are no formal prescription requirements capable of tracing the recommendation process as a whole [1].

Public outreach efforts should adhere to the aforementioned regulations. Therefore, they are limited to labelling, educational content, and possibly using digital techniques like SEO or SEM. Nevertheless, the digitalisation of healthcare systems is a fact and will significantly impact all future issues. One possible connection between healthcare digitalisation and FSMPs is that digital tools can help monitor, assess, and manage food safety hazards and offer consumers and health professionals information and transparency. Another possible connection is that digital technologies can assist in customising FSMPs based on an individual's needs, preferences, and circumstances [12].

II. Material and Methods

The research technique examined was in-depth interviews with medical professionals with specialities in the sectors that suggest patients with demands for such products - cancer, ENT, radiation, or surgery - to evaluate the factors influencing the recommendation and use of FSMPs. The in-depth interview method allows the researcher to approach the subject with common questions. It enables us to decide which issues, during a quantitative research phase, the participants should be questioned about. Using this strategy, we can get a wealth of descriptive information on people's attitudes, behaviours, and perceptions [14].

The following benefits of in-depth interview methods [13] are beneficial for this study:

- Since respondents are dispersed, bringing them all together for a focus group study would be difficult and expensive.
- While a respondent has approximately 10 minutes in a focus group setting, and the other participants may influence his perspective, in an in-depth interview, only 30 to 45 minutes are set aside for the conversation with one respondent.
- The debate may disclose previously assumed habits since the participants fully engage with the topic and tell the whole tale.
- Since using FSMPs requires specialised expertise, the study process necessitates thorough explanations of some subjects.
- Later on, the research agenda incorporates respondents' viewpoints.

The operators were given instructions on conducting themselves before and during the interview. The logic of the research was emphasised, as well as simple instructions such as the need for a preparatory discussion before the interview, how to conduct the interview itself and to follow the guide as well as how notes should be made and finally, the extraction of answers and the final document for each subject.

The interview's outline contained several subjects for conversation with the issues, including:

- Is the subject recommending any FSMPs? Do they know the category of FSMP? How did they become aware of this category?
- Which factors are influencing the subject' recommendation? Clinical proof, the stage of the disease, correlation with the underlying treatment, relationships with other specialities?
- Which particular names or brands are popular or used? What features should the items that the subject recommends or uses have?
- Does the subject know what laws apply to FSMPs? Did the topic have any thoughts on how to make clarity more precise?
- Price: are FSMPs expensive or affordable? Who covers the cost of the products?
- Which are the suppliers and the channels to procure FSMPs products?
- Patients' feedback about the use of FSMPs
- Subject' feedback, in their medical capacity
- Few data about the subject of the interview to analyse the answers, such as speciality, type of medical unit in which they work, city and age, experience or associations with academic work.

The operators were equipped additionally with the following:

- Script of introductory talk with the subject about the research, a short description of the research aims and a little information about FSMPs.
- A list of the most popular brands/products available to help the subjects put the questions in context.

Three operators conducted in-depth interviews, and answers from 10 interviews were collected. The interviews were done from October 2022 to January 2023.

We expected doctors to evaluate several considerations before recommending Food for Special Medical Purposes (FSMPs), including:

- The patient's medical condition: FSMPs are designed for those with particular medical conditions with unique dietary requirements. Doctors will assess the patient's condition to decide whether an FSMP is necessary.
- Nutrient requirements: FSMPs are created to satisfy the patient's unique nutrient requirements. Doctors will consider the patient's nutritional demands and determine whether an FSMP can fulfil those needs.
- Digestive capabilities: FSMPs are designed for those whose medical circumstances prevent them from following a typical diet. Doctors will assess the patient's digestibility and decide whether an FSMP is necessary.
- Allergies or intolerances: Patients with severe food allergies or intolerances may take FSMPs. If an FSMP is necessary, doctors will assess the patient's allergies or food intolerances.
- Medical history: The doctor's recommendation of an FSMP may also consider the patient's medical background. For instance, to guarantee enough nutrition, a patient with a history of malabsorption may need an FSMP.
- Accessibility: Doctors will also consider the patient's ability to access the FSMP. This encompasses elements like price, accessibility, and usability.

III. Results and Discussions

Ten in-depth interviews were conducted to gather information, and the distribution of specialities and places of residency is shown in the accompanying table:

Table I: Demographics for the respondents

Residence		Specialities	
Brasov	2	ENT's	1
Cluj Napoca	2	Oncology	4
Constanța	1	Pneumology	1
București	1	Radiotherapy	3
Oradea	1	Surgeron	1
Sibiu	1		
Târgu Mures	2		

Key findings were grouped following this article's interview guidelines and narrative logic.

a) Awareness about the FSMPs category of products

All of the subjects interviewed were aware of FSMPs products.

When asked where they first learned about FSMPs, most respondents (7/10) reported they got their information from the producers' medical personnel, and only one said they found out on their own through an internet search. Two respondents stated that they knew these items from their training period for their internship or residency more than ten years prior. The other doctors - their colleagues, who learned from medical reps, were an additional source of information. The statement made by one respondent that information about FSMPs is provided at the medical committee's weekly meetings, where medical representatives attend and present various goods, was also noteworthy. Other respondents addressed the distribution of samples to

patients. The Fresibin, Nutridrink, and Nutricia lines were cited in response to brands.

When we inquired about FSMP information sources, the responses were consistent. They all listed the same kind of resources: medical representatives, inserts and pamphlets ordered by the manufacturers, documents found online through searches, and various manufacturing company presentations at medical congresses and events.

b) How are these products recommended?

The conditions surrounding the recommendation of FSMPs were crucial to our study. Respondents mentioned patients with specific illnesses (pancreatic cancer, gastric cancer, oesophagal cancer, bronchopulmonary cancer, and any cancer of ENT kind) are candidates of choice to benefit from the FSMPs use. Due to the prolonged length of combo treatment, nutritional support with FSMPs is especially necessary for cancer of the ENT type (chemotherapy and radiotherapy).According to one respondent's experience, patients who utilised FSMPs during their treatment likely improved the treatment and had greater success. Several responders mentioned nutritional deficits, weight loss, or deglutition impairments (dysphagia or xerostomia). According to that respondent, one responder further links the usage of FSMPs with the clinic's nutritionist, who suggests such products be used. All respondents mentioned they recommended FSMPs products to their patients. Patients were encouraged to use FSMPs after being discharged from the hospital for those who require such indication.

c) Which factors influence the recommendation?

Usually, the doctor makes the recommendation, and the availability of products in the pharmacies in the neighbourhood of the hospital or patient's residence is considered.

Another respondent was more specific, saying that in ENT cancers, the standard is to recommend FSMPs starting with 2nd week of treatment, which takes six weeks and the patient is monitored every week concerning weight and alimentation.

In addition, it was reported that cancer patients and those with Chronic obstructive pulmonary disease (COPD) had difficulty with deglutition or nutrition and appeared cachectic. Therefore, these products are also advised for their use.

Most respondents mentioned that patients with cancers in ENT, oesophagus, bronchopulmonary or patients with widespread metastasis and palliative needs require FSMPs to keep their nutritional status, weight and general condition satisfactory.

d) Are clinical trials available and influence the recommendations?

Most respondents mentioned they did not consult clinical trials, some justifying due to lack of time. However, some respondents emphasise that clinical experience is essential for recommending FSMP products to patients. At the same time, one respondent considers that there is no significant difference between various brands within this category of products. It is worth mentioning that one respondent considers that clinical trials are available and results are good ones and recommend using such products in line with the stage of the disease. However, the same doctor recommends keeping the patient without medicinal products and with ordinary alimentation as long as possible.

e) Does the disease stage influence the choice of the FSMPs?

Respondents generally indicated weight loss and body mass index (BMI) as principal triggers for recommending FSMP to patients under treatment. The primary need is to have the patient well balanced from the nutritional point of view to support chemo or radiotherapy treatment for the whole duration. FSMP products are helping patients to achieve this objective. The appearance of problems with deglutition or food absorption also triggered the recommendation of using these products, as well as certain medical conditions (such as gastrectomy, oesophagectomy, acute pancreatitis or complete dysphagia for solid food).

A subject stressed that, when making a suggestion, the affordability must be addressed alongside medical reasons.

f) Is the recommendation correlated with the treatment scheme?

FSMPs are recommended for cancers like colon cancer or pancreatic cancer with the condition that makes ordinary alimentation challenging to achieve, according to one respondent or the recommendation being linked with BMI or weight loss.

Another respondent considers that the administration has nothing to do with the treatment scheme, but with the comfort or the patient's needs, so the FSMPs might be recommendable to those in need.

The linkage with the treatment is that doctors must ensure that the patient can follow the treatment scheme. Particularly in radiotherapy, postponing or delaying treatment is not an option. Thus FSMPs products may help the patient to cope with the side effects of chemo or radiotherapy and enable him to keep up with and complete the whole treatment scheme successfully.

g) Which products or brands are used or recommended?

The respondents spontaneously mentioned four brands, as per the following table:

Table II: FSMP brands mentioned by respondents (count of mentions)

Brand	Mentions, out of total
MediDrink	1/10
Nutridrink	3/10
Fresubin	9/10
Nutricia	1/10

In addition to brand names, several respondents cited other requirements for items to be utilised, including hypercaloric or normocaloric content, high protein content, and various tastes and flavours.

h) What features and characteristics of products make them useful?

The taste and flavour are the most significant and commonly cited characteristics. According to the feedback received by the physicians in our panel, some patients claimed that certain products were overly sweet or had a high level of acidity. In contrast, others disapproved of the products that tasted like bananas. Conversely, patients more readily accepted FSMPs when they tasted strawberries and chocolate.

One respondent gave a more detailed description, emphasising the demand for lactose-free, gluten-free products, high in vitamins, minerals, and proteins and hypercaloric.

i) Is the pertinent legal framework known? Are there any necessary changes that need to be made?

With one exception, our respondents were unaware if there is in place specific legislation governing FSMPs and how this product category differs from food supplements. One participant had in-depth knowledge of E.U. regulations, informational requirements, informational requirements for the public and specialists, classification, quality standards, labelling, indication, and precautions. No change to the current legislation was mentioned as necessary, identified or proposed by participants in this research.

j) Price and affordability

Participants paid careful attention to the concerns regarding the pricing and accessibility of FSMPs, which were identified as an important topic affecting the use and availability of FSMPs for in-need patients.

Three respondents indicated their awareness of prices; one accurately identified the average bottle price as 15 RON, but the other two knew a price range of 30–50 RON. One even brought up the substantial distinction between Nutridrink, an example of a more reasonably priced product with 30 RON/bottle, and Fresubin, an example of an expensive product with 50 RON/bottle. According to respondents' calculations, a patient would require products costing between 30 and 50 RON for every bottle (4-5,000 RON or 800- 1,000 Euro), which is out of reach for many patients, given the overall cost of cancer therapy.

We gathered some pricing for products in this category from online pharmacies or wholesalers and their spot prices to explain the mathematics of the price problem. The following table displays the findings.

Table III: Price information about brands mentioned by respondents and sources of information

Product	List price per pack	Source of information	bottles per pack	price per bottle	price per 7 weeks treatment (2 bottles/day)
Fresubin Protein Energy, 4x200ml	62.88 RON	https://alimentespeciale.ro/fresubin-protein-energy-drink-fructe-tropicale-x-200ml-fresenuis-kabi/103593.htm	4	15.72 RON	1,540.53 RON
Bautura cu aroma de vanilie Fresubin 2kcal, 4x200ml - hypercaloric	80.02 RON	https://alimentespeciale.ro/fresubin-2kcal-drink-x-200ml-vanilie-fresenius-kabi/100992.htm	4	20.01 RON	1,960.49 RON
Fresubin Pro Drink Alune de padure 4x200ml-hypercaloric, hyper-proteic	120.33 RON	https://alimentespeciale.ro/fresubin-pro-drink-alune-de-padure-4x200ml-fresenius-kabi/161630.htm	4	30.08 RON	2,948.09 RON

Fresubin, 4x200 ml, Fresenius Kabi Germania	57.50 RON	https://comenzi.farmaciatei.ro/vitamine-si-suplimente/digestie/nutritie-speciala/bautura-energizanta-cu-proteine-aroma-de-vanilie-fresubin-4x200-ml-fresenius-kabi-germania-p323667	4	14.38 RON	1,408.75 RON
MediDrink plus vanilie x200ml	17.00 RON	https://www.farmaciilenapofarm.ro/nutritie-speciala/medidrink-plus-vanilie-x200ml-39202.html	1	17.00 RON	1,666.00 RON
Nutridrink banane x 200ml Nutricia	14.62 RON	https://alimentespeciale.ro/nutridrink-banane-x-200ml-nutricia/103021.htm	1	14.62 RON	1,432.76 RON

Sources for prices have been accessed on March 2, 2023

Using the price data from the above sources, the cost for a patient using two bottles daily for seven weeks would range from 1,433 to 2,948 RON (i.e. 290-590 Euro). Furthermore, the National Institute of Statistics, cited by Statista, said that the average salary in Romania was 3,416 RON in 2021, which is necessary to make the previous information pertinent.

The responses from the other respondents were less detailed. At the same time, 1/3 of them said that these products are generally accessible and affordable, and the other third claimed that they are far too expensive for patients to afford to be used as they should be.

k) Who is paying for FSMPs products?

In Romania, FSMPs are not reimbursed; consequently, the patient, their family, or the hospital covers the expense of these items during hospitalisation. At the same time, most treatments are also administered in an outpatient setting, and most respondents to our survey said that the patient is responsible for purchasing these goods. There were two mentions of the hospital purchasing such products for hospitalised patients, out of the general budget of the healthcare unit, or it received donations. Respondents also emphasised that for some patients, the cost of purchasing the required quantity of FSMP's products may exceed their financial means.

l) How do patients source FSMP products?

As far as our respondents learned from personal experience, buying these products from pharmacies, online pharmacies, or online shops doesn't present any significant problems. However, some stressed the necessity of placing a preorder to guarantee that the required versions will be available. One participant spoke about successfully obtaining Medidrink from the online pharmacy Spring Farma and buying FSMP products online rather than from traditional pharmacies or the neighbour country, ungary, which may be more affordable in some cases. Pharmacy, online shopping, and charity donations were the supply methods highlighted. One participant brought up a hospital-organised tender for the purchase of Fresubin.

Additionally, we examined the market data from the research firm Cegedim [4], which compiles data from hospitals and retail pharmacies in Romania, as shown in the following table.

Table IV: Sales (units and value) for FSMP on pharmacy channel in Romania for the year 2022 (source: Cegedim)

MANUFACTURER	PRODUCT NAME	Sales 2022 (units)	Value 2022 (PPP, RON)
FRESENIUS-KABI		**106,178**	**4,807,822**
	FRESUBIN	92,786	4,032,172
	DIBEN	4,692	203,146
	SUPPORTAN	4,220	279,809
	SURVIMED	2,722	165,364
	PROVIDEXTRA DRINK	663	44,565
	CALSHAKE	502	19,499
	FREBINI	453	18,469
	KABI GLUTAMINE	140	44,798
NESTLE		**21,532**	**790,416**
	OPTIFIBRE	20,971	749,973
	NUTREN OPTIMUM	420	20,571
	MODULEN	141	19,872
NUTRICIA		**2,408**	**25,558**
	NUTRIDRINK	2,408	25,558

For 2022, we identified reports of relatively low sales and only a few brands. Although this is the only source we could find, it is improbable that only 106,178 units were sold through retail pharmacies in Romania. We can only speculate that online pharmacies are not counted and that most online shops did not report sales to Cegedim, thus underestimating the totals. Only Fresubin indicates large sales among the important manufacturers, who are present on the market with more brands than respondents to our research reported (over 90 percent of the total).

m) What feedback did the responders receive from their patients?

We can group the feedback on two main aspects – how patients use the products and how they can source them.

Patients are generally happy with the products. However, patients are concerned about the availability of different flavours to suit specific needs in terms of usage, especially in the case of some patients whose tastes are altered during chemotherapy or radiotherapy. In addition, it is important to note that certain patients have expressed concerns about items being either too sweet or acidic in some circumstances.

Regarding sourcing, some patients expressed the necessity to place an order in advance, ask family members for information, or look up sources online. However, the biggest problem mentioned is that most patients need help to secure the funding necessary to buy the supplements during their therapy, especially in cases of a 6-7 weeks treatment scheme associated with the condition requiring FSMPs aid.

n) The opinion of the physicians regarding FSMPs

The most important objective for the participants in this research concerning the use of FSMP products is to help patients to go through the course of treatment in an acceptable physical shape, and nutritional status is paramount for such an aim. For example, one participant in the study reported that FSMP users were less likely to have stomatitis or mucositis. In addition, most survey respondents said that patients were better equipped nutritionally to withstand the effects of chemotherapy and/or radiotherapy over their whole course.

Since some patients cannot drink coffee during therapy, suggestions such as adding a coffee-like flavour were made. Since the financial burden currently exists for cancer patients, the added expense of FSMP products causes enormous obstacles for patients. Hence respondents to our poll emphasised the necessity for a method to pay these costs externally through reimbursement or a similar mechanism.

Doctors emphasise that these expenses (i.e. for FSMP) are less than what would be needed for chemotherapy or radiation therapy. Since this keeps the patients well enough to complete the course successfully, the treatments are unquestionably worthwhile.

o) Extra details provided by respondents

We asked respondents about any specific subjects they would like to bring up after the interview, and the following we believe merits discussion:

- Using FSMP would lower the cost of patient care while being necessary for finishing the treatment.
- It might be beneficial to commission more patient education materials on FSMP.
- The help and collaboration of a nutritionist are critical since they free up the doctor from this responsibility and assist patients at risk.
- In some extreme cases (i.e. N.G. tube feeding), using FSMPs would lower the burden on the budget necessary for treatment and the patient's comfort.

IV. Conclusions

The study's findings provide insight into Romanian physicians' awareness, recommendation practices, and opinions regarding the use of FSMPs. Physicians recommend FSMPs to patients based on specific medical conditions, treatment schemes, taste preferences, and affordability. Disease stage and treatment scheme were identified as factors that influence the recommendation and use of FSMPs. Clinical experience was deemed essential for recommending FSMPs to patients, while patients' feedback regarding the usage and sourcing of FSMPs was generally positive. The study concludes that physicians play a crucial role in ensuring that patients have the necessary nutritional support during treatment. However, more patient education materials and collaboration with nutritionists may be required to improve patient outcomes and reduce patient financial burden. Overall, the study highlights the importance of FSMPs in patient care and the need for further research and collaboration between healthcare professionals to optimise the use of these products.

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Gastroprotective Effect of Tadalafil on Wistar Rat of Stress-Induced Gastric Ulcer

By Doris N. Ajibo, Udeme O. Georgewill & Owunari A. Georgewill
University of Port Harcourt

Abstract- This study was carried out to investigate the gastro protective property of tadalafil in stress – induced gastric ulcer. The wistar rats of male sex (wt =180 – 252 g) were divided into 6 groups (n= 5) and pretreated with the drugs for two weeks prior to gastric ulcer induction. Food was withdrawn 24 h and water 2 h before the commencement of the experiment. Group 1 received control (distilled water); Group 2 and 3 received Tadalafil (50 and 100mg/kg); Group 4 and 5 received tadalafil (50 and 100mg/kg) + Omeprazole 1.75mg/kg) and Group 6 received positive control/ standard Omeprazole (30mg/kg). The ulceration was induced with0.5 ml of 95% ethanol and 0.25 g/kg reserpine respectively on end the 14 days pretreatment course, 1h after the last dose. The ulceration was induced by immersion and mobilization at the end of the 14 days pretreatment course, 1h after the last dose of drugs was given. All drugs were administered through the aid of orogastric cannula.

Keywords: gastric ulcer, tadalafil, ulcer scores, histopathology.

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Strictly as per the compliance and regulations of:

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Abstract- This study was carried out to investigate the gastro protective property of tadalafil in stress – induced gastric ulcer. The wistar rats of male sex (wt =180 – 252 g) were divided into 6 groups (n= 5) and pretreated with the drugs for two weeks prior to gastric ulcer induction. Food was withdrawn 24 h and water 2 h before the commencement of the experiment. Group 1 received control (distilled water); Group 2 and 3 received Tadalafil (50 and 100mg/kg); Group 4 and 5 received tadalafil (50 and 100mg/kg) + Omeprazole 1.75mg/kg) and Group 6 received positive control/ standard Omeprazole (30mg/kg). The ulceration was induced with0.5 ml of 95% ethanol and 0.25 g/kg reserpine respectively on end the 14 days pretreatment course, 1h after the last dose. The ulceration was induced by immersion and mobilization at the end of the 14 days pretreatment course, 1h after the last dose of drugs was given. All drugs were administered through the aid of orogastric cannula. 6 h later, the animals were sacrificed by cervical dislocation, dissected and the stomachs were isolated and carefully opened along the greater curvature. Mucous secretion was evaluated. Gastric tissues were obtained and fixed with 10% formaldehyde in saline. Macroscopic examination was carried out with a hand lens and scored for the presence of lesions. The stomach were removed and opened along the greater curvature. The tissues were fixed with 10% formaldehyde in saline. . The Ulcer score, Ulcer index, and Preventive ratio of drugs were calculated. From the study, tadalafil 50mg/kg and 100mg/kg reduced significantly ($P < 0.05$) the level of ulcer index with elevation in percentage protective ratio. The histopathology revealed normal stomach tissue wall and absence of distortion with tadalafil 50mg/kg and 100mg/kg and their various combinations with standard, omeprazole.

Keywords: gastric ulcer, tadalafil, ulcer scores, histopathology.

I. Introduction

Drug repositioning is a system whereby drugs already in use is redirected and channeled for another therapeutic use. It is the system of redirecting the use of drugs already in existence for another clinical indication. Drug repurposing involves the utilization of a drug for a totally different therapeutic application. It is also known as drug rechanneling, re-profiling, or re-routing (Emdormi et al., 2020). Drug repurposing is necessitated by the fact that traditional drug discovery and development process has become quite expensive taking an average of US$1.8billion and a long duration of time with an average of (13-15) years. The long development process, high cost, drug resistance, toxicity and a very low success rate have revealed the unavoidable need for drug repurposing of old conventional drugs for a new therapeutic application (Wuerth et al., 2016).

Gastric mucosa is frequently opened to so many substances ranging from food, vital nutrients and many other deleterious agents. These substances given via the oral route can lead to destruction of gastric-mucosal integrity. Some of these substances have deleterious impact on the gastric mucosa which is the cause of some Gastric ulcers and acute mucosal damage (Chavez- Pina et al., 2010). Such substances could be ethanol/alcohol, Nicotine, ingestion of non-steroidal anti-inflammatory drugs (NSAIDs- Ibuprofen, Indomethacin), pepsin, smoking etc. The gastric membrane protective system that maintains and upholds its integrity include: epithelial cells secreting mucus, endogenous prostaglandin (Takeuchi, 2014), bicarbonates, normal gastric blood flow (Zhu and Kaunitz, 2008). Gastric lesions are the detectable effects of these aggressive factors which are linked to cellular influx, release of free radicals such as reactive oxygen species, cytokines and growth factors.

Tadalafil is PDE5 inhibitors just like sildenafil and vardenafil. It hampers with cGMP breakdown by the PDE5 (Phosphodiesterase enzyme 5), thereby leading to the buildup of cGMP which invariably bring about the dilation of smooth muscle of the blood vessels. Elevation of cGMP level enhances PDE5 actions (Cruz-Burgos et al., 2021). cGMP builds up to excite its metabolism; however, the pharmacological PDE5 inhibition hinders this negative feed-back procedure. Tadalafil is particular for PDE5 and in a lesser percentage inhibits PDE6 (Ahmed, 2019), which functions for visual transduction in the retina. In Ajiboye and Oluwole, (2012), they proposed that Tadalafil significantly reduced indomethacin-linked gastric ulcer compare to control at high doses. They recorded significant variations in (area, depth & width) of the ulcer when Tadalafil (10 mg kg -1 BW) group were compared to the control. This study seeks to find out the effect of tadalafil on stress-induced gastric ulcer by immersion on ulcer scores, ulcer index, lipid profile, hematology

Corresponding Author α: Department of Experimental Pharmacology and Toxicology, Faculty of Pharmaceutical Sciences, University of Port Harcourt, Rivers State. e-mail: doris.ajibo@uniport.edu.ng
Author σ ρ: Department of Pharmacology and Therapeutics, Faculty of Basic Clinical Sciences, University of Port Harcourt, Rivers State.

parameters, biochemical parameters and stomach tissue wall (looking at the photomicrograph).

II. Methods

a) Effect of Tadalafil on Water Immersion-Stress Model of Gastric Ulceration in Rats

This procedure was carried out according to Senay and Levine (1967) with modifications. Wistar rats of male sex (weighing 180-220g) were divided into 5 groups (n=6). Food was removed 24 hrs and water 2hrs prior the experiment.

The wistar rats were pretreated with distilled water, Omeprazole 1.75mg/kg, tadalafil 50mg/kg, tadalafil 100mg/kg, tadalafil 50mg/kg/Omeprazole 1.75mg/kg and tadalafil 100mg/kg and Omeprazole 1.75m/kg daily for two weeks prior to gastric ulcer induction of stress with cold water bath immersion method.

They were vertically immersed individually in a compartment of cage water tank containing water and the temperature of the tank sustained between 15 – 20 °C using ice pack to generate stress ulceration.

Group 1 received distilled water, no drugs

Group 2 received Omeprazole 1.75mg/kg;

Group 3 and 4 received Tadalafil 50 & 100mg/kg respectively;

Groups 5 and 6 received Tadalafil 50 & 100mg/kg/ Omeprazole 1.75mg/kg

The ulceration was induced by immersion and mobilization at the end of the 14 days pretreatment course, 1h after the last dose.

All drugs were administered through the aid of orogastric cannula.

6h later, the animals were sacrificed by cervical dislocation. The stomach were removed and opened along the greater curvature. The tissues were fixed with 10% formaldehyde in saline. Macroscopic examination was carried out with a hand lens and scored for the presence of lesions. The Ulcer score (Lau and Ogbe, 1981), Ulcer index, and Preventive ratio of drugs were evaluated.

Macroscopic examination was carried out with a hand less and scored for the presence of lesions Calculation of Ulcer Index

This was carried out as described by (Martin-aragon et al., 1994). It is shown below:

M XN 100

Where: M = Mean number of ulcers per rat in the group N= Percentage of rats with ulcer in the group.

b) Determination of adherent gastric mucus content

The extraction of mucus was done by the method described by Ettarh and Okwari, (1999). The rats were fasted overnight and after administration of anesthesia, their abdomens were opened and the stomachs cut and washed in saline and opened along the greater curvature and slightly stretched and supported with dissecting pins on a corkboard. The accumulated mucus was removed using a blunt scalpel into a pre-weighed beaker holding 4ml of water (M). The final weight of the beaker plus the mucus (N) minus M gives the weight of the mucus (Z) for each animal in all the groups, i.e. (N –M = Z g).

c) Determination of Antioxidant activity

Sample preparations

Prior to homogenization, the stomach tissues were washed with distilled water to reduce the effect of hemoglobin with free radicals and to get rid of blood attached to the mucous membrane. The tissues were sliced into piece and homogenized using Teflon homogenizer (Polytron, Heidolph RZR 1 Germany) with the right buffer and centrifuged at 18,000rpm for 15 minutes at 4◦C. The supernatant was collected in a beaker for further analysis.

d) Determination of superoxide dismutase (SOD)

This procedure was carried out according to Sun et al. (1988). The activity of the enzyme was investigated by estimating its ability to suppress the photochemical reduction of nitro-blue tetrazolium (NBT).

In a dark chamber, I mL of the reactant (50mM phosphate buffer, 100nM EDTA and 13mM 1-methionine, pH 7.8) was blended with 30 µ l of 2 µm riboflavin. The resulting solution in a tube was exposed to fluorescent light bulbs (15W) for 15 minutes and completely read using spectrophotometer at 560nM.

Note: In this assay, the photochemical reduction of riboflavin produces O^{2-} which breakdown the NBT to yield formazan salt that absorbs at a wavelength of 560nM.

e) Determination of membrane lipid per oxidation, MDA

Gastric content of lipid peroxidation was carried out following the Ohkawa et al. (1979). Determination of the rate of lipoperoxidation in the gastric mucus membrane was evaluated by assaying of malondialdehyde (MDA) using the thiobarbituric acid reactive substances (TBARS) test. Supernatant obtained after homogenization was mixed with 400 µl of 0.6% thiobarbituric acid, and incubated at 95 -100◦C for I h and absorbance read at 532 nM. Using 1, 1,3,3-tetramethoxypropane, a standard curve was obtained. Consequently, the result was expressed as nmol of MDA/mg protein (Bradford, 1976).

f) Determination of Alanine aminotransferase (ALT)

ALT was assayed using (Reitman & Frankel, 1957).

Principle

a-oxoglutarate + L-Alanine L- Glutamate + Pyruvate Alanine amino transferase was determined by regulating the concentration of pyruvate hydrazone developed with 2, 4- dinitrophenylhyd-

razine. Reagents (R) R1: Buffer (phosphate buffer 100mmol/L, pH 7.4, L- alanine 200mmol/L, a-oxoglutarate 10mmol/L) R2: 2, 4-dinitrophenylhydrazine 2.0mmol/L

Procedure

Two test tubes which contained blank and samples were adequately labeled.

R1 (at 0. 5ml) was introduced into the both test tubes containing both the blank and samples. Distilled water of (0.1ml) was also introduced to the sample alone. The two test tubes were kept warm for (30 min) at (37°C) after which (0.5ml) of R2 was added to each of the tubes, blend thoroughly and left to settle for (20 min) at 25°C.

At this point, (5ml) of Sodium hydroxide was introduced to both tubes, mixed carefully and the sample read was determined against the blank after (5 min) at (546nm).

g) Determination of Aspartate Amino Transferase (AST)

The AST concentration assessment was carried out using Reitman and Frankel (1957) method. Principle AST was assayed by regulating the concentration of oxaloacetate hydrazone created with 2, 4-dinitrophenylhydrazine. α-oxoglutarate + L-aspartate L-glutamate + oxaloacetate Reagent (R) R1: Buffer (phosphate buffer 100mmol/L, pH 7.4, L-aspartate 100mmol/L and a-oxoglutarate 100mmol/L) R2 : 2,4-dinitrophenylhydrazine (2mmol/L)

Procedure

Two test- tubes, the blank and samples were adequately labeled. R1(0.5ml) was introduced into tubes containing the blank and samples.

Distilled water (0.1ml) was also introduced to the sample alone. The (2) test- tubes were warmed for (0.5hr) at (37°C). Then R2 (0.5ml) was put in both tubes containing blank and samples, mixed appropriately, permitted to stay for (20 min) at (25°C). At this point, Sodium hydroxide (5ml) was introduced to the two test-tubes containing the blank and samples. The tubes were properly mixed and the sample read/determined against the blank after (5 min) at 546nm.

h) Determination of Alkaline Phosphate (ALP)

ALP concentration examination was executed by the aid of Randox kit following Deutsche Gesellschaft fur Klinische Chemmie (GSCC) i.e. German Association of Clinical Chemistry recommendation.

Principle

P-nitrophenylphosphate is being hydrolyzed by ALP to yield phosphate and P-nitrophenol P-nitrophenylphosphate + H20 phosphate + P-nitrophenol

Reagent 1a: Buffer (Diethanolamine buffer 1mol/pH9.8, MgCl2 0.5mmol/L) Reagent 1b: Substrate (p-nitrophenylphosphate 10mmol/L)

Procedure

0.05 ml of sample and 3.00ml reagent 1a (Diethanolamine buffer 1mol/pH9.8, MgCl2 0.5mmol/L) and reagent 1b (p-nitrophenylphosphate 10mmol/L) were pipette into a cuvette. Absorbance of the mixture was read at time (0, 1, 2, & 3 at 405nm).

i) Determination of Urea (Jung et al., 1975)

The reagents for urea assay were arranged following Jung et al., (1975) directives. Jung working reagents consist of:

Working reagents: (100 mg/L) o-phthal-aldehyde, (215 mg/L) N-(1-naphthyl) ethylenediamine, (2.5 mol/ L) sulfuric acid, (2.5 g/L) boric-acid, and (0.03%) Brij-35. Modified reagents: These include; (100 mg/L) o-phthal-aldehyde, (513 mg/L) primaquine bis-phosphate, (2.5 mol/ L) sulfuric-acid, (2.5 g/L) boric-acid, and (0.03% Brij-35). Standard: Double-distilled water and (5.00 mg/dL) urea

Procedure

Water (50 μL), standard of (50 μL and 5.00 mg/dL), and (50 μL) of each sample was moved into distinct well of a clear flat-bottom 96-well plate. 200 μL of newly arranged working reagent was included and combined by shaking the plate. At room temperature, the reaction was incubated. Measurement of optical densities was done at 430nm and 505nm on the plate reader for determination utilizing the modified reagent and the standard Jung reagent inclusively.

j) Determination of serum creatinine concentration (Roscoe, 1953)

Reagent 1 (R1) working solution: Sodium hydroxide: 0.20 mol/l

Reagent 2 (R2) working solution: Picric acid Standard solution of creatinine Procedure

Preparation of alkaline creatinine picrate was done by adding 2ml of 0.75N NaOH and 2ml of saturated picric acid to 6ml of a Standard solution consisting of 0.25-1.0mg of creatinine per 100mls. Linear color response (orange) was seen on a logarithmic scale. The absorbance was read at 500nm against a blank prepared with distilled water (in place of standard solution), NaOH and Picric acid. For the various blood samples, precipitation of the serum was done by adding 2 volumes of a serum dilution, one part of sodium tungstate 5% and one part of either 0.33N H2SO4. The fundus section of the treated animals' stomach was homogenized (5%) in ice cold 0.9% saline. The mitochondrial fraction was collected via centrifugation and utilized for the Analysis of the enzymatic Antioxidants such (SOD & CAT). The protein was analyzed in mucosal homogenate to reveal the activities of SOD and CAT per milligram (mg) of protein.

k) Hematology Assay

Blood specimens were acquired from the treated reserpine-induced, ethanol-induced and stress-induced gastric ulcers rats via cardiac puncture after sacrificing the animal using anti-coagulant EDTA bottle. Hematological parameters and indices were determined from unclotted blood samples using standard protocols (Jain, 1986). Hematological analysis was performed for parameters such as; WBC, RBC, Platelet count, Hematocrits.

l) Measurement of serum lipid profile

The serum lipid profile of total cholesterol (Roeschlau et al., 1974), serum triglyceride (Buccolo and David, 1973) high density lipoprotein (Burstein et al., 1970) were measured while very low density lipoprotein (VLDL) was calculated as triglyceride/5 and low density lipoprotein (LDL) was calculated using the equation: LDL = total cholesterol – (HDL + VLDL).

m) Histopathological Analysis

The stomach tissues obtained from the animals was subjected to histopathological examination using the method of Drury (1983).

n) Fixation and washing

To preserve the tissues, formalin (10%) was utilized. A minute portion of the tissues (1- 2cm in diameter) were sliced using a razor blade that is sharp. Small pieces of tissues that were kept in the 10% formalin and the container mixed quietly to ensure that the reagent penetrated all the tissues and also to avoid them gumming to the surfaces. At 25°C they were incubated and allow to be properly fixed. Subsequently they were washed with running water for 24 hours to wash off to much of the fixatives.

o) Dehydration

It was ensured the tissues were without H2O before embedding them in paraffin. Tissues were submerged in automatic tissue processor consisting of 12 jars in other to attain the dehydration. 70, 90 and 95% absolute alcohol was introduced in the first three containers respectively. The essence of this is to get rid of the water content in the tissues. Fresh absolute alcohol was reintroduced to ascertain complete water elimination. Similar procedure was done in the other jars of the automatic tissue processor.

p) Clearing

At his point, Xylene solutions were utilized in the clearing of the tissue sections. This procedure was indicated in the other jars of the automatic tissue processor. Xylene solution was preferred because it is miscible with both alcohol and paraffin before penetration occurs. The essence of carrying out clearing was to get rid of opacity from dehydrated tissues. The tissue stayed in the solution for 15 minutes before it was removed.

q) Infiltration with paraffin

The tissues were infiltrated with paraffin wax for 50-52°C. They were moved to a bath with molten paraffin. They were incubated for 30-60 minutes in the first bath and thereafter, transferred to a fresh dish containing paraffin in fourth jars containing automatic tissue processor for the same duration of time.

r) Embedding (Blocking) with Paraffin

The tissues were completely soaked with paraffin and the paraffin allowed to solidify in and out of the tissues.

s) Paraffin Sectioning

The soaked sections of the tissues were sliced into squares and fixed in the microtome knives for partitioning and thereafter passed through the water bath.

t) Mounting

Thin layer of the albumen fixative was prepared on a clean glass slides. The slides were used to obtain the required section from the other partitions in the water. The partitions on the glass slides were moisturized before staining was carried out.

u) Staining with Haematoxylin

Series of Jars containing alcohols of reducing strength and different staining solutions were brought and the slides passed through each of them. Microscopic Observation of Slide Slides were made ready and viewed under the photomicroscope one after the other at 400 magnification power of the microscope. Each of the slides was photographed. Various rats were obtained after carrying out Ethanol-induced and indomethacin-induced gastric acid ulcer models. They were dissected through the large curvature. The injuries were located, sliced and fixed in ALFAC solution for (24 hr) at 4 °C. Processing of the samples was carried out through embedding in paraplast. They were cut into 7u thick sections of which they were stained with periodic acid- schiff (PAS), (Vacca, 1985) and hematoxylin-eosin (Behmer et al., 1976).

Analysis of the sample was done with a Leica microscope in conjunction with Leica Qwin software (Leica England).

v) Methods of Data Collection

At the end of each experiment both the test animals and the controls were sacrificed under chloroform anesthesia. One milliliter (1ml) of blood was collected from each rat via cardiac puncture. Blood samples were obtained in anticoagulant bottle (EDTA) bottle for lipid, biochemical and hematological assessments. The stomach were carefully harvested and fixed with formalin for histo-pathological assessment.

w) Statistical Analysis

The data obtained from this research were reported as mean ± SEM. Statistical significance were also computed by the aids of One-Way Variance of Analysis followed by Turkey's Honesty Significant Difference (HSD) test at the level of significance ($p < 0.05$).

x) Ethical Approval

This research was approved by the UPH Research Ethical Committee. The set guidelines and regulations pertaining to experimental animal management were accurately followed.

III. Results

a) Calculation of Ulcer score, Ulcer Index and Percentage Preventive Ratio

Ulcer scored for the presence of gastric lesion following rating scale of Lau and ogbe (1981) as follows: 0.0 = usual color of, 1.5 = hemorrhagic lines, 2.0 = ulcers having (>3 but =5mm2) area, 3.0= ulcers > 5mm2,

Ulcer index was calculated using severity scores and average number of ulcers per animal. Severity was scored as: 0 - Normal stomach, 0.5 - Red coloration, 1 - Spot ulcers, 1.5 - Hemorrhagic streaks, 2 - Ulcer > 3 mm but ‹ 5 mm, 3 - Ulcers > 5 mm

Ulcer index (UI) = UN + US + UP × 10,

Where UI = ulcer index, UN = average number of ulcers per animal, US = average of severity score, UP = percentage of animals with ulcer.

$$\text{Percentage protective ratio} = 100 - \frac{[\text{UI pretreated}]\ \text{X}\ 100}{[\text{UI control}]}$$

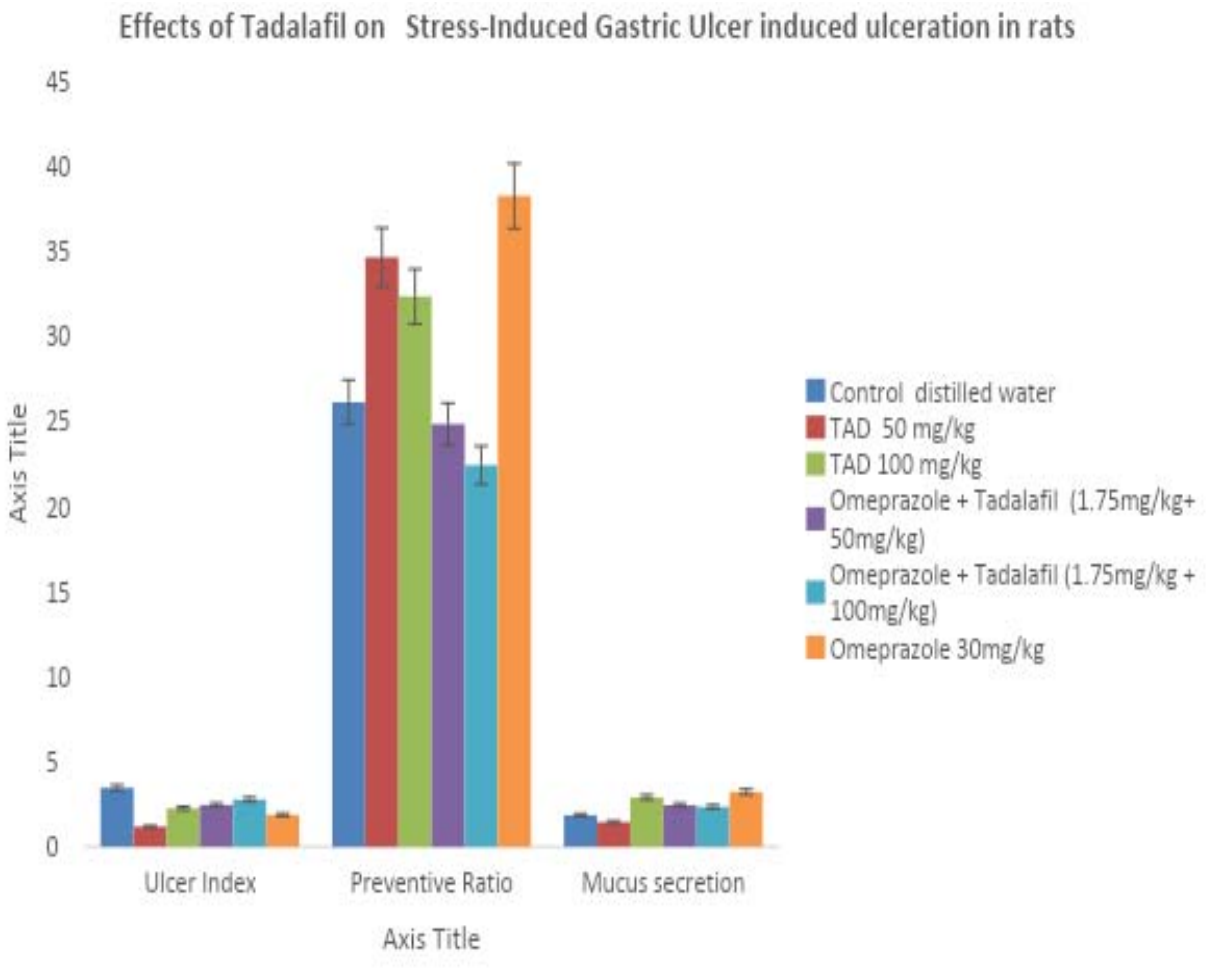
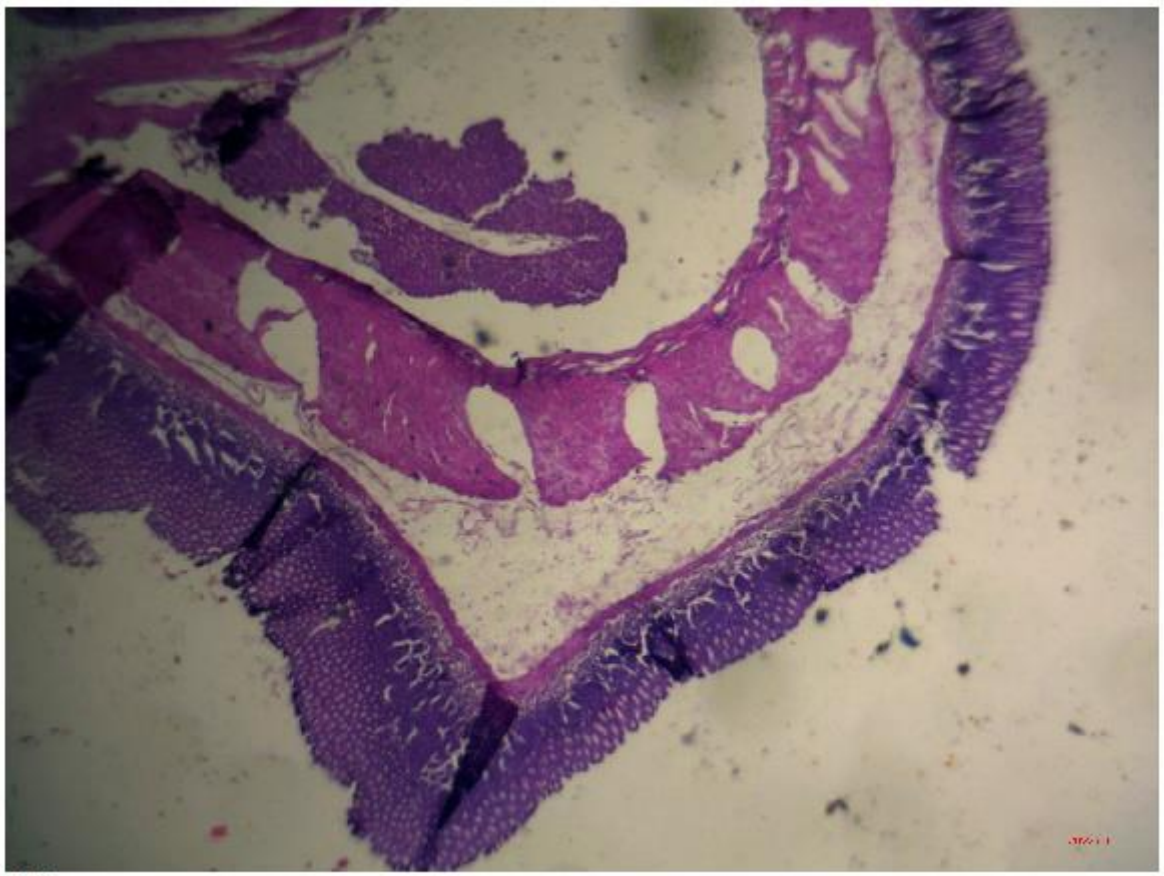


Fig. 4.1: Bar chart representing ulcer index, preventive ratio and mucous secretion in stress- induced model. In this model of gastric ulceration, tadalafil 50mg/kg and tadalafil 100mg/kg + omeprazole 1.75mg/kg revealed a significant reduction in ulcer index. The percentage protective ratio is 26.10, 34.60, 24.80, 22.40 and 38.20 respectively. The mucous secretion is significantly

elevated ($p<0.05$) with tadalafil 100mg/kg and omeprazole 1.75mg/kg + tadalafil 100mg/kg as compared to standard.

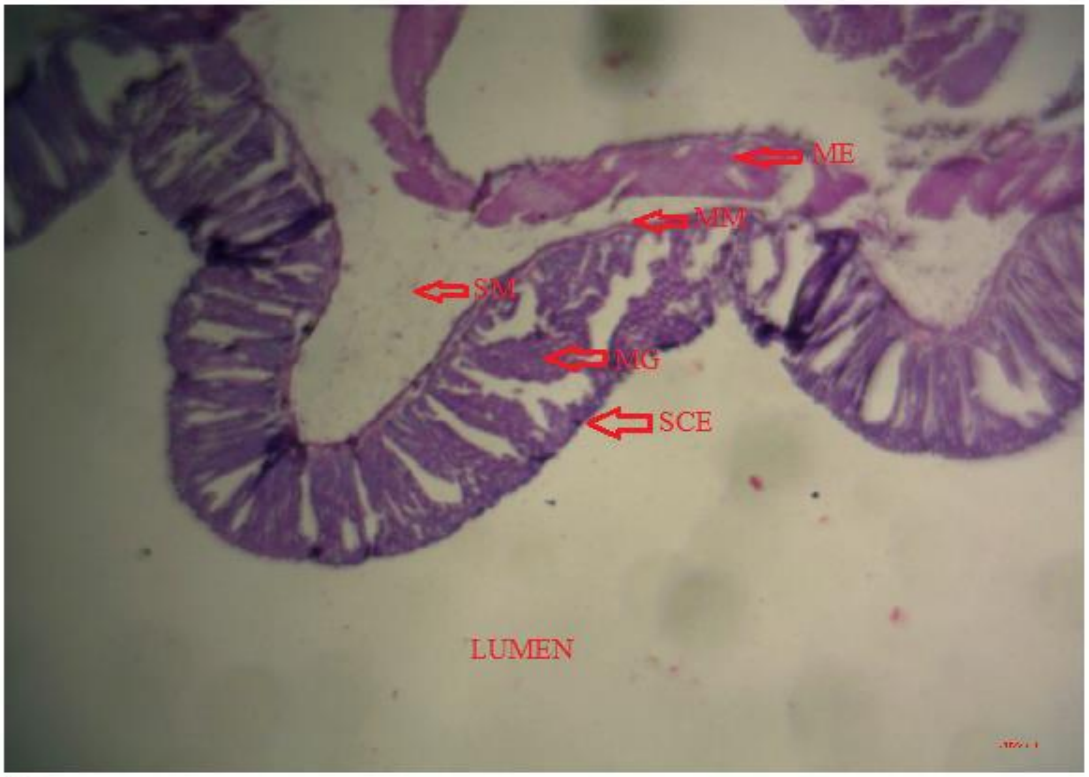
b) Investigation of effect of tadalafil on stress- induced ulcer

This investigation was executed on six groups of adult male wistar rats of five rats in each group. The wistar rats were pretreated with distilled water, Omeprazole 1.75mg/kg, tadalafil 50mg/kg, tadalafil 100mg/kg, tad 50mg/kg/Omeprazole 1.75mg/kg and tad 100mg/kg and Omeprazole 1.75m/kg daily for two weeks prior to gastric ulcer induction with stress. The group pretreated with distilled water functioned as the negative control whereas the group pretreated with only Omeprazole served as a positive control. The wistar rats were sacrificed under diethyl ether anesthesia 4 hrs after ulcer generation. The gastric tissues obtained from each of the rats were correctly prepared for histological examination via a microscope. The representative photomicrographs achieved from the study are presented below, showing the negative control and revealed some v-shaped histological abrasions, ulcer, indicated with arrows. Also, a figure obtained from the positive control (Omeprazole pretreated group) has normal histological. The subsequent two plates; figures 4.17 are the representative photomicrographs gotten from the wistar rats exposed to tadalafil 50mg/kg for 14 days. The two plates also depicted some distortions. More so, figures 4.18 which represent the tadalafil 100mg/kg pretreated group retained the appearance of a histologically normal gastric tissue.



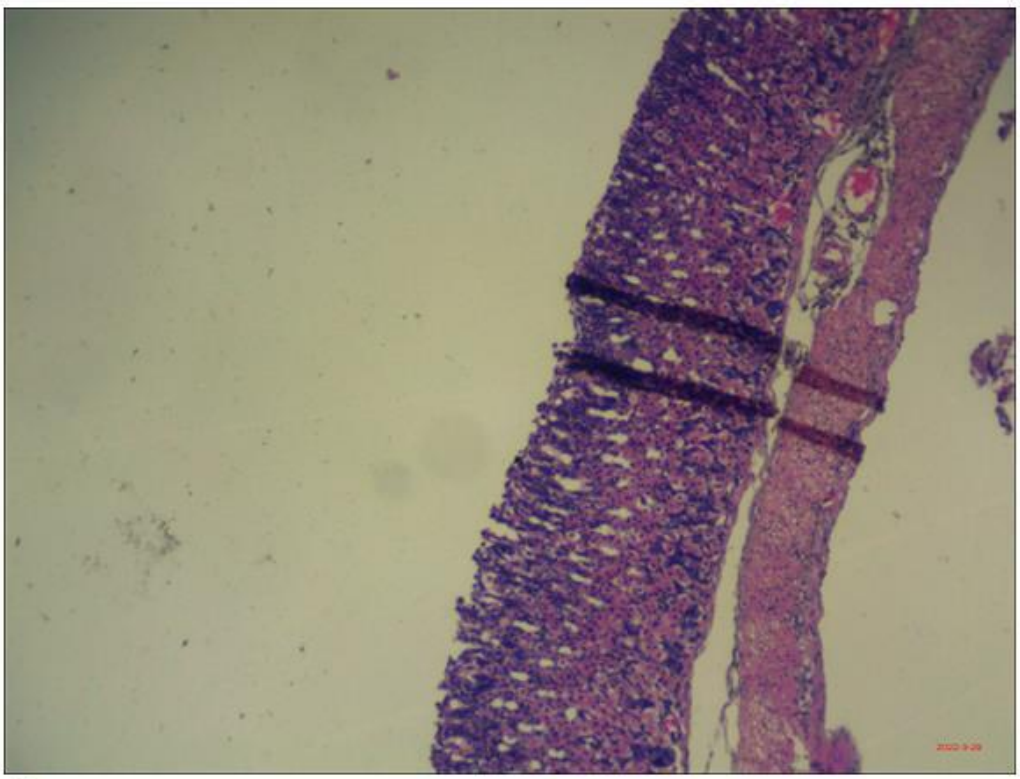
15

Photomicrograph of rat's stomach tissue showing the effect of stress (water immersion) on the stomach tissue through water immersion. The photomicrographs obtained from this group retained the features of histological distorted stomach tissue which include; mucosa lined with intact Simple columnar epithelia (SCE) containing epithelial gland (EG), muscularis mucosa (MM), muscularis externa (ME) and blood vessels (BV). There is no disruption or distortion to the Simple columnar epithelium (SCE) with neither edema nor leucocytes infiltration of the submucosal later (H&E stain, 20X magnification)



24

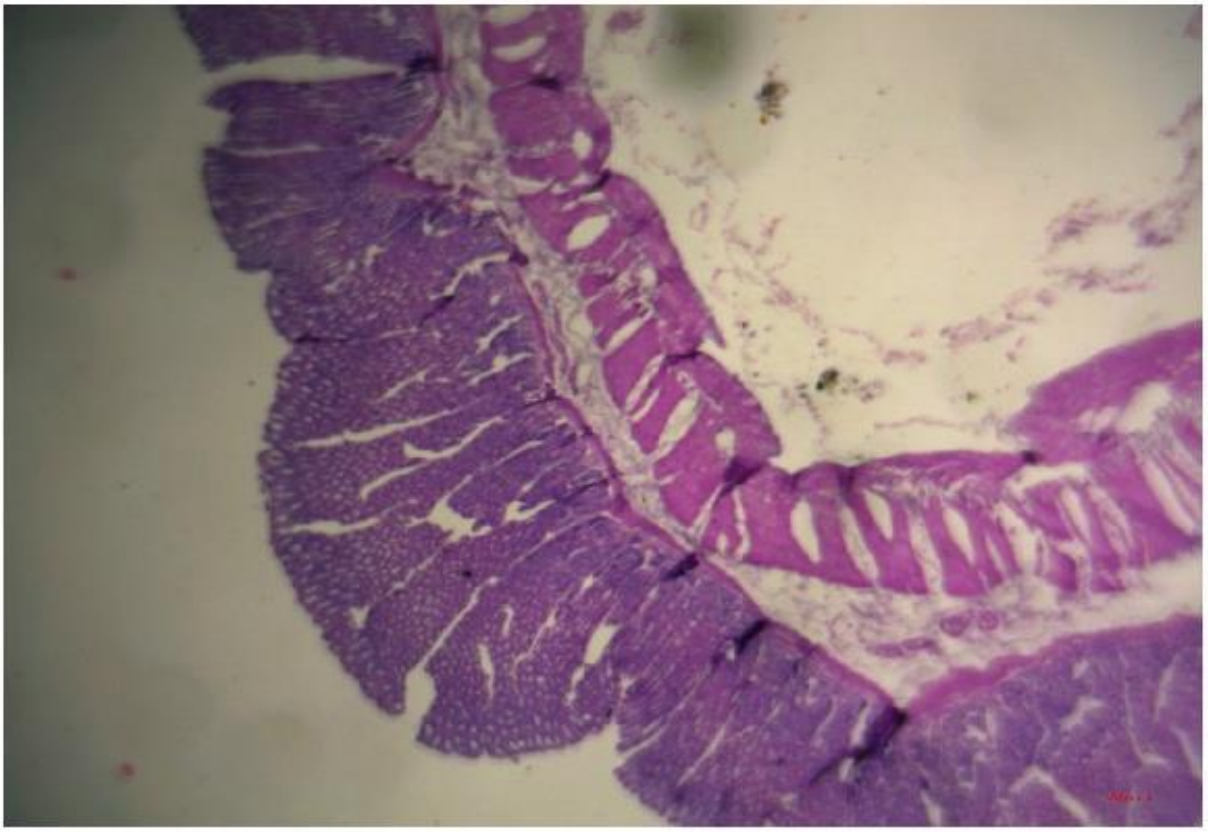
Photomicrograph of rat's stomach tissue showing the effect of omeprazole 1.75mg/kg on stress – induced ulcer. The photomicrographs obtained from this group has normal stomach lining which include; mucosa lined with intact Simple columnar epithelia (SCE) containing epithelial gland (EG), muscularis mucosa (MM), muscularis externa (ME) and blood vessels (BV). There is no disruption or distortion to the Simple columnar epithelium (SCE) with neither edema nor leucocytes infiltration of the submucosal later (H&E stain, 20X magnification).



3

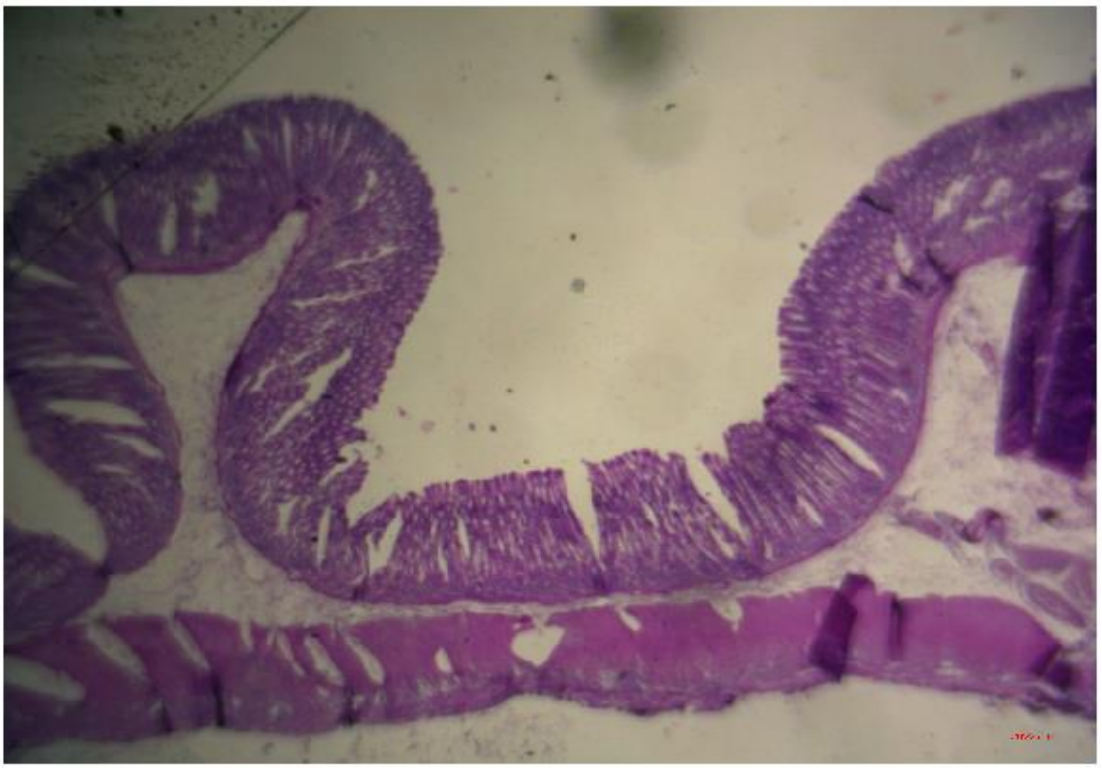
Photomicrograph of rat's stomach tissue showing the effect of omeprazole 1.75mg/kg on stress – induced ulcer. The photomicrographs obtained from this group has normal stomach lining which include; mucosa lined with intact Simple columnar epithelia (SCE) containing epithelial gland (EG), muscularis mucosa (MM), muscularis externa (ME) and blood vessels (BV).

There is no disruption or distortion to the Simple columnar epithelium (SCE) with neither edema nor leucocytes infiltration of the submucosal later (H&E stain, 20X magnification)



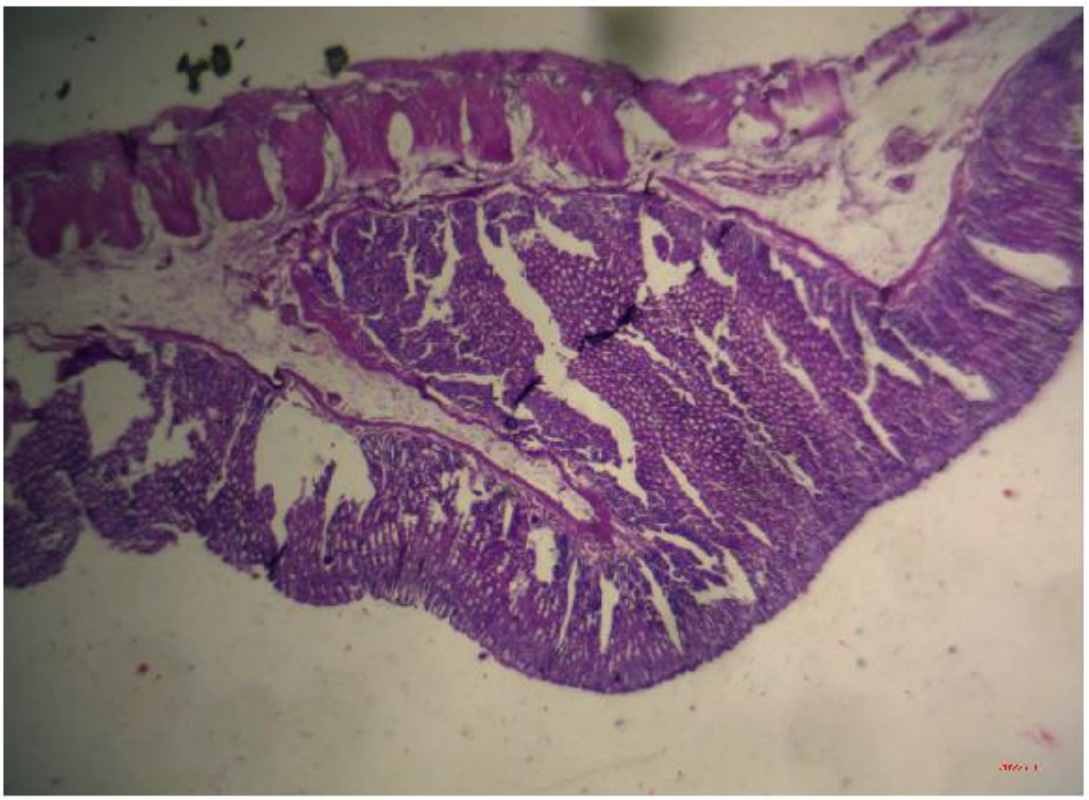
5

Photomicrograph of rat's stomach tissue showing the effect of tadalafil 50mg/kg on stress – induced ulcer. The photomicrographs obtained from this group has normal stomach lining which include; mucosa lined with intact Simple columnar epithelia (SCE) containing epithelial gland (EG), muscularis mucosa (MM), muscularis externa (ME) and blood vessels (BV). There is no disruption or distortion to the Simple columnar epithelium (SCE) with neither edema nor leucocytes infiltration of the submucosal later (H&E stain, 20X magnification)



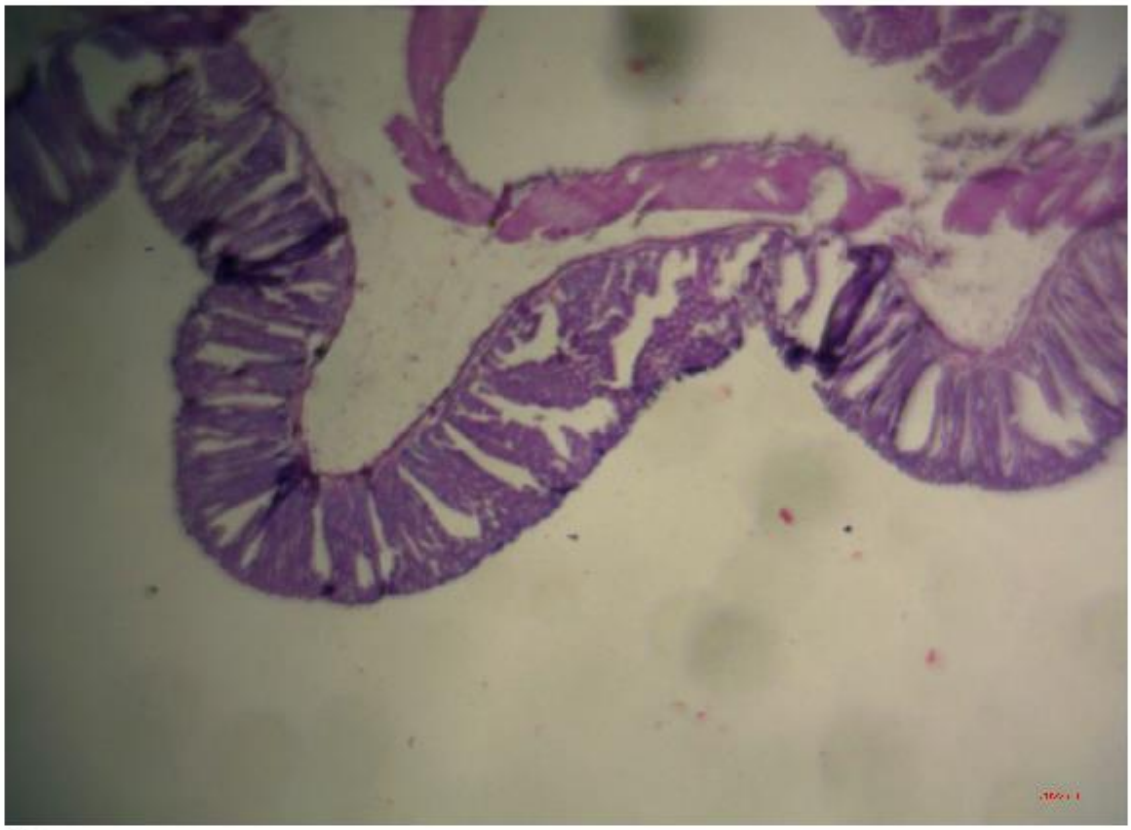
11

Photomicrograph of rat's stomach tissue showing the effect of tadalafil 100mg/kg on stress – induced ulcer. The photomicrographs obtained from this group has normal stomach lining which include; mucosa lined with intact Simple columnar epithelia (SCE) containing epithelial gland (EG), muscularis mucosa (MM), muscularis externa (ME) and blood vessels (BV). There is no disruption or distortion to the Simple columnar epithelium (SCE) with neither edema nor leucocytes infiltration of the submucosal later (H&E stain, 20X magnification)



12

Photomicrograph of rat's stomach tissue showing the effect of tadalafil 50mg/kg / Omeprazole on Stress –induced ulcer. The photomicrographs obtained from this group has normal stomach lining which include; mucosa lined with intact Simple columnar epithelia (SCE) containing epithelial gland (EG), muscularis mucosa (MM), muscularis externa (ME) and blood vessels (BV). There is no disruption or distortion to the Simple columnar epithelium (SCE) with neither edema nor leucocytes infiltration of the submucosal later (H&E stain, 20X magnification)

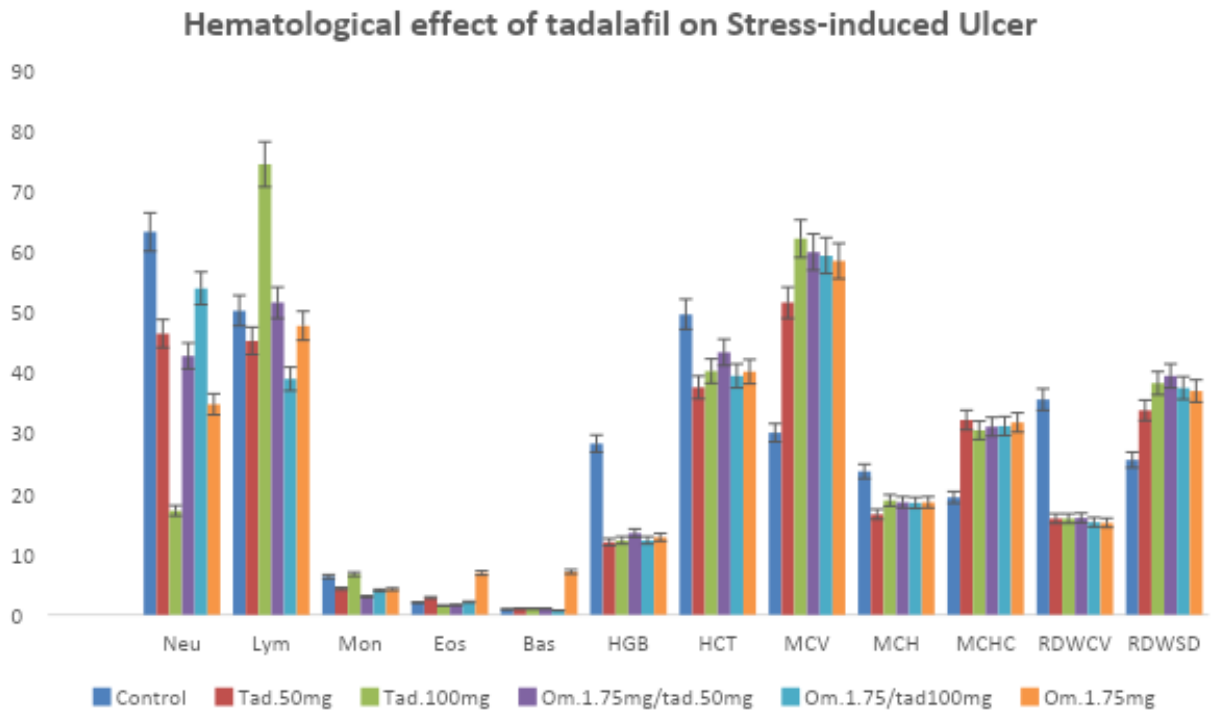


24

Photomicrograph of rat's stomach tissue showing the effect of tadalafil 100mg/kg and omeprazole on Stress –induced ulcer. The photomicrographs obtained from this group has normal stomach lining which include; mucosa lined with intact Simple columnar epithelia (SCE) containing epithelial gland (EG), muscularis mucosa (MM), muscularis externa (ME) and blood vessels (BV). There is no disruption or distortion to the Simple columnar epithelium (SCE) with neither edema nor leucocytes infiltration of the submucosal later (H&E stain, 20X magnification)

c) *Assessment of hematological effect of tadalafil in stress- induced ulcer*

This study was performed on six groups of adult male wistar rats of 5 rats each. The Wistar rats were pretreated with distilled water, Omeprazole 1.75mg/kg, tadalafil 50mg/kg, tadalafil 100mg/kg, tad 50mg/kg/Omeprazole 1.75mg/kg and tad 100mg/kg and Omeprazole 1.75m/kg daily for two weeks prior to gastric ulcer induction with stress. The group pretreated with distilled water functioned as the negative control whereas the group pretreated with only Omeprazole served as a positive control. The wistar rats were sacrificed under chloroform anesthesia 4 hrs after ulcer generation. Blood samples obtained from the wistar rats were used to assess the hematological effect of tadalafil on the animals. The result obtained from this investigation is presented on table below



The ANOVA result revealed a significant alteration in the white blood cells counts with ($P = 0.003 < 0.005$). The Means comparison of hematological parameters gathered from different groups of the wistar rats equally demonstrated a significant change ($P = 0.001 < 0.005$). From the analysis, ethanol produced the following effect on the hematology profile of the rats: The neutrophils, and lymphocytes has an elevated mean score values of the treated groups as compared to the control as compared to the control while eosinophil, monocytes and basophils which are component of WBC, has reduced mean score value compared to the standard. The hemoglobin level is low when compared to the control whereas the hematocrits /red cell component of the blood was highly elevated in comparison to the control.

d) Investigation of biochemical effect of tadalafil in stress induced ulcer

This investigation was executed on six groups of adult male wistar rats of 5 rats each. The Wistar rats were pretreated with distilled water, Omeprazole 1.75mg/kg, tadalafil 50mg/kg, tadalafil 100mg/kg, tad 50mg/kg/Omeprazole 1.75mg/kg and tad 100mg/kg and Omeprazole 1.75m/kg daily for two weeks prior to gastric ulcer induction with stress. The group pretreated with distilled water functioned as the negative control whereas the group pretreated with only Omeprazole served as a positive control. The wistar rats were sacrificed under chloroform anesthesia 4 hrs after ulcer generation. Blood samples obtained from the wistar rats were used to assess the biochemical effect of tadalafil on the animals. The result obtained from this investigation is shown on below 4.45.

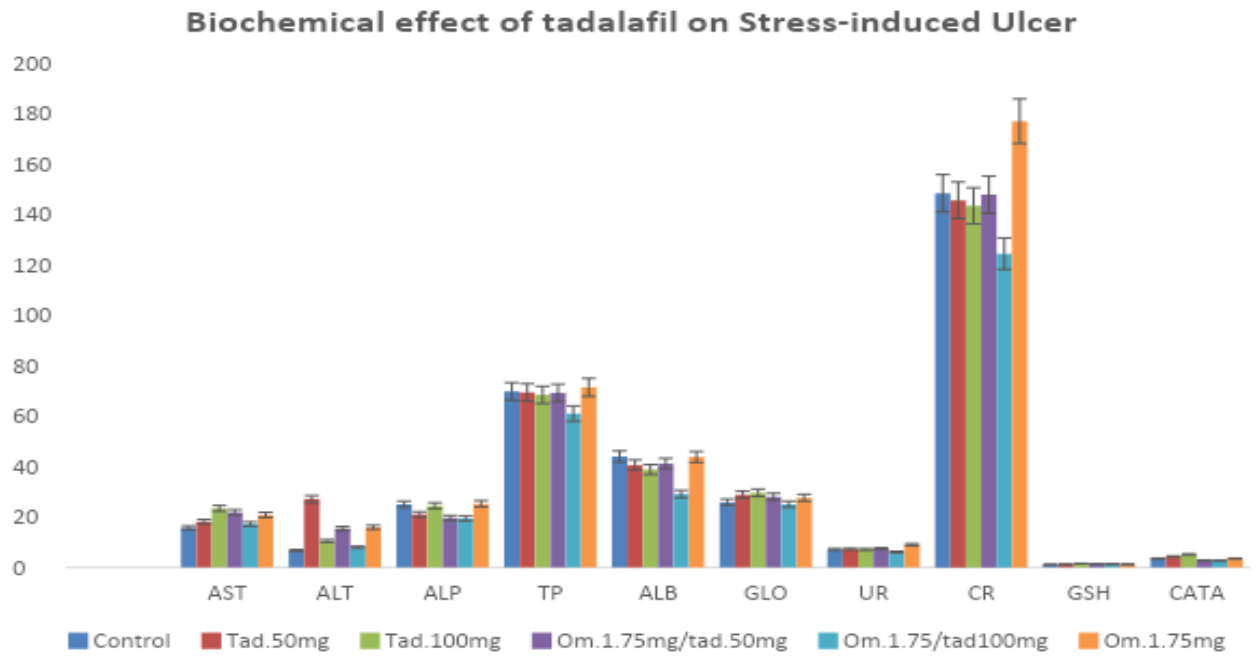


Fig. 4.45: The ANOVA result showed a significant change in the means of ALT, GLO, UR and CR with P= 0.001, 0.001, 0.001, 0.001and 0.001 < 0.05 respectively compared to the negative control. From the analysis, ethanol produced the following effect on biochemical parameters.

Aspartate transferase, AST: From the result chart, tadalafil 100mg/kg, omeprazole 1.75mg/kg+tadalafil 50mg/kg and omeprazole alone yielded a higher mean score value when compared to control. Alanine transaminase, ALT: The result demonstrated that tadalafil 50mg/kg produced a higher mean score value than other groups, followed by omeprazole 1.75mg/kg +tadalafil 50mg/kg and omeprazole alone. Alanine phosphatase, ALP: Here, tadalafil 100mg/kg and omeprazole standard drugs produced a lesser mean score value compared to control. Total protein, TP: From the bar chart, there was an elevated mean score value from all the groups with the standard drugs omeprazole yielding higher value. Albumin, ALB: The result obtained from the various group from the analysis induced an increase in the albumin mean score value with the standard drugs omeprazole showing a higher level of effect. Globulin, GLO: The result revealed that the various groups treated possess equipotent or similar mean score value which is comparable to control. Urea, UR: There was a reduced mean score value of urea level obtained by the various groups as compared to standard. Creatinine, CR: From the bar chart, there was an elevated mean score value from all the groups treated as compared to the standard with the highest peak. Glutathione (GSH) and Catalase (CAT): There was a reduced mean score value of glutathione and catalase level obtained by the various groups studied.

e) *Examination of effect of tadalafil on lipid profile in stress induced ulcer*

This investigation was performed on six groups of adult male wistar rats of 5 rats each. The wistar rats were pretreated with distilled water, Omeprazole 1.75mg/kg, tadalafil 50mg/kg, tadalafil 100mg/kg, tad 50mg/kg/Omeprazole 1.75mg/kg and tad 100mg/kg and Omeprazole 1.75m/kg daily for two weeks prior to gastric ulcer generation with ethanol. The group placed on only distilled water served as the negative control while the group pretreated with only omeprazole played the role of a positive control. The wistar rats were sacrificed under chloroform anesthesia 4 hours after ulcer induction. Blood samples obtained from members of various treatment groups of wistar rats were used to examine the biochemical effect of tadalafil on the rats.

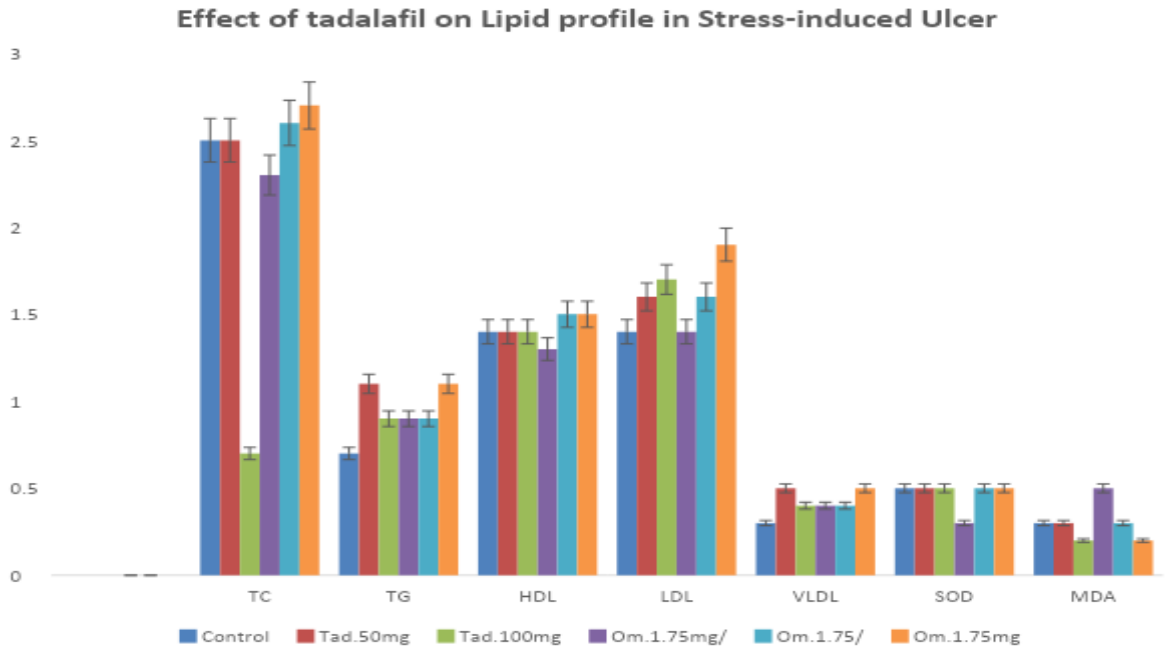


Fig. 4.48: The ANOVA result revealed a significant alterations in the means TG, LDL, VLDL and MDA, with P-value = 0.001, 0.001, 0.001, 0.001, < 0.05 correspondingly compared to the negative control. From the result obtained, the TC, TG, HDL, LDL and VLDL produced an extremely elevated mean score value with all the treated groups (NOTE: TG, total glyceride, TC, total cholesterol, HDL, high density lipoprotein, LDL, low density lipoprotein, VLDL, very low density lipoprotein, VLDL,)

IV. Discussion

Prior to generation of ulcers with stress model in the different treated groups of wistar rats, the animals were pretreated with distilled water, Omeprazole 1.75mg/kg, tadalafil 50mg/kg, tadalafil 100mg/kg, tad 50mg/kg/Omeprazole 1.75mg/kg and tad 100mg/kg and Omeprazole 1.75m/kg daily for 14 days.

The mechanism behind stress gastric ulcer model involves the release of histamine and elevation of gastric acid secretion and output with diminished mucus production and poor gastric blood flow (Dejban et al., 2020). This model is known to reduce the amount and content of mucus secretion and production through the diminished synthesis of its component, mucin. This makes this model important in investigating mucosal and cyto-protective properties (Kuna et al., 2019). The stress equally activates gastrointestinal motility with elevated vagal activity.

The reduction in ulcer index, elevation of preventive protective ratio and reduced mucus secretion seen above, revealed that tadalafil have the potential to counteract the activities of stress triggered on the mucosal wall by protecting the gastric mucosa layer and reduce gastric acid secretion.

Meanwhile, tadalafil acts by increasing the blood flow to gastrointestinal tissues following increased cGMP levels. As a phosphodiesterase inhibitor, tadalafil enhances the endogenous synthesis of NO (Ahmed, 2019) and this will invariably produce anti-inflammatory effects by enhancing cGMP production as shown in figure above. Tadalafil bring about production of more NO. NO is widely known as a vasodilator via its capacity to enhance blood flow in GIT tissues thereby reducing tissue breakdown.

The photomicrographs of the negative control (stress with water immersion) revealed a v-shaped histological abrasions representing ulcers. This is a confirmation of the ulcerogenic activity of stress (Chuang et al., 2021). Also, plates obtained from the positive control (Omeprazole pretreated group) equally retained normal histological features. Photomicrographs pretreated with tadalafil 50mg/kg and 100mg/kg showed no histological disruption. The gastric mucosa integrity was maintained. This suggests that tadalafil exhibits cyto-protective effect especially at high dose (100mg/kg) (Abd Al Haleem et al., 2021).

Investigation of impact of tadalafil and omeprazole combination regimen on stress induced ulcer revealed photomicrographs from wistar rat gastric tissue pretreated with a combined regimen of tadalafil 50mg/kg and Omeprazole 1.75mg/kg. The photomicrographs revealed the characteristics of a histologically normal wistar rat gastric tissue. Interestingly, Omeprazole/tadalafil 50mg/kg combination regimen completely protect the wistar rats against stress induced gastric ulceration.

It therefore suggests that even tadalafil at 50mg/kg has some intrinsic cyto-protecting activity which may not be observable in mono-therapy (Abd Al

Haleem et al., 2021). However, tadalafil 50mg/kg synergizes with Omeprazole to produce a greater or a detectable gastro-protective effect. Also, photomicrographs gotten from the stomach tissue of wistar rats pretreated with tadalafil 100mg/kg/ Omeprazole 1.75mg/kg combination treatment are equally without histological abrasions. This proposes that although tadalafil exhibits its observable cyto-protective effect at high dose (100mg/kg) when used a monotherapy, in a combination regimen with Omeprazole satisfactory gastro-protective effect can be produced even with tad 50mg/kg instead of tad 100mg/kg.

Assessment of hematological effect of tadalafil in stress induced ulcer exposed the result obtained from this investigation. The result revealed a significant alteration in the white blood cells counts for the group pretreated with only distilled water (negative control). The Means comparison of hematological parameters gathered from different treatment groups of the wistar rats equally demonstrated a significant change in the white blood cell count and red blood cell count. The significant elevation in the white blood cell count of the negative control appears to be a validation that stress really is an ulcerogenic substance; the increase in white blood cell count is a normal system reaction to injury or inflammation. The effect on red blood cell was insignificant, implying that each or both of the drugs (tadalafil and Omeprazole have no direct impact on erythrocyte (Wang et al., 2017).

Investigation of biochemical effect of tadalafil in stress induced ulcer exposed the significant changes in the means of AST, ALT, GLO, UR and CR respectively compared to the negative control. The statistics points to hepatotoxic and nephrotoxic effects of the test substances (tadalafil and Omeprazole).

However, examination of effect of tadalafil on lipid profile in stress induced ulcer was carried out .The result obtained from this investigation revealed significant alterations in the means TG, LDL, VLDL and MDA, correspondingly compared to the negative control. The significant elevation in the total protein may be an indication of inflammatory state following the ulcer. But a significant increase in the plasma level of LDL caused by the tadalafil appears to command some apprehensions.

Investigation on the effect of tadalafil both single administration 50mg/kg and 100mg/kg and the various combination with standard drug omeprazole on the photomicrographs, revealed that the various combinations has protective effects on the gastric mucosa wall by producing normal stomach lining which include; mucosa lined with intact Simple columnar epithelia (SCE) containing epithelial gland (EG), muscularis mucosa (MM), muscularis externa (ME) and blood vessels (BV). There is no disruption or distortion to the Simple columnar epithelium (SCE) with neither edema nor leucocytes infiltration of the sub-mucosal later. This effect is produced by the ethanol model, reserpine model and stress-induced model. Tadalafil is a phoshodiesterase V inhibitor that act by increasing blood flow to tissues in response to increased cGMP levels. They are also Nitric Oxide (NO) donors. NO is a potent vasodilator which increases blood flow in tissues where present thus preventing tissue damage (Ajiboye and Oluwole, 2012).

V. Summary of Findings

The study shows that Omeprazole pretreatment can offer full gastro-protection against stress linked ulcer. More so, tadalafil mono-therapy and tadalafil/Omeprazole combination pre-therapy can also provide a satisfactory gastro-protection against stress-related gastric ulcer.

More so, tadalafil100mg/kg and both tad 50mg/k and tad 100mg/kg /Omeprazole combined treatment regimens all showed full gastro-protective effects against stress-linked gastric ulcer.

The result obtained from the effect of tadalafil on hematological parameters on ethanol induced ulcer investigation documented a significant increase in the white blood cell counts.

The result obtained from the investigation of the impact of tadalafil on the hematological parameters of stress induced wistar rats model of ulcer recorded a significant elevation in the white blood cells counts.

Furthermore, the result gathered from the study for determination of activity of tadalafil and tadalafil Omeprazole combined treatment also revealed a significant alteration in the white blood cells counts. Again, the result also recorded significant different in AST, ALT, GLO, UR and CR respectively compared to the negative control in stress induced wistar rats gastric ulcer model.

VI. Limitations

This study has triggered the urge for realization of the time/effect relationship of tadalafil only and tadalafil/Omeprazole combination pretreatment on different gastric ulcer models. It would have been interesting to know if tadalafil/Omeprazole combination pretreatment can provide the required gastro protective effect within a shorter course than either of the agents used alone. Nevertheless, this study failed to consider this in its design.

VII. Conclusion

Tadalafil at the doses of 50 and 100mg/kg used alone or in combination with Omeprazole 1.75mg/kg possess satisfactory gastro-protective impact against stress-induced gastric ulcers. Nevertheless, these pre-treatment regimens (tadalafil and Omeprazole appear to be nephrotoxic and hepatotoxic as evidenced by their

impacts on the liver enzymes and other biochemical parameters.

VIII. Recommendations

Tadalafil at the doses of 50 and 100mg/kg may be used alone or in combination with Omeprazole to safeguard people who are at risk against gastric ulcer.There is an important demand for agents that can protect the liver and kidney from the deleterious effect of tadalafil and Omeprazole. A further study shall reveal the time/effect relationship of tad/omeprazole combined therapy compared to either drug used alone.

Contributions to Knowledge

Tadalafil at the doses of 50 and 100mg/kg pretreatment can guard against stress induced ulcer in wistar rats.The alterations in leucocytes count following tadalafil and Omeprazole treatment may not be a direct impact of the test drugs but part of the normal gastrointestinal tract protective mechanisms against some deleterious activities of the drug at other regions. Tadalafil alone or in combination with Omeprazole seem to be nephrotoxic and hepatotoxic. Tadalafil alone or in combination with Omeprazole appear to have insignificant effect on lipid profile in wistar rats.

Conflict of Interest

There was no conflict of interest among the authors while carrying out this research.

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Tables: Tables should be cautiously designed, uncrowned, and include only essential data. Each must have an Arabic number, e.g., Table 4, a self-explanatory caption, and be on a separate sheet. Authors must submit tables in an editable format and not as images. References to these tables (if any) must be mentioned accurately.

Figures

Figures are supposed to be submitted as separate files. Always include a citation in the text for each figure using Arabic numbers, e.g., Fig. 4. Artwork must be submitted online in vector electronic form or by emailing it.

Preparation of Eletronic Figures for Publication

Although low-quality images are sufficient for review purposes, print publication requires high-quality images to prevent the final product being blurred or fuzzy. Submit (possibly by e-mail) EPS (line art) or TIFF (halftone/ photographs) files only. MS PowerPoint and Word Graphics are unsuitable for printed pictures. Avoid using pixel-oriented software. Scans (TIFF only) should have a resolution of at least 350 dpi (halftone) or 700 to 1100 dpi (line drawings). Please give the data for figures in black and white or submit a Color Work Agreement form. EPS files must be saved with fonts embedded (and with a TIFF preview, if possible).

For scanned images, the scanning resolution at final image size ought to be as follows to ensure good reproduction: line art: >650 dpi; halftones (including gel photographs): >350 dpi; figures containing both halftone and line images: >650 dpi.

Color charges: Authors are advised to pay the full cost for the reproduction of their color artwork. Hence, please note that if there is color artwork in your manuscript when it is accepted for publication, we would require you to complete and return a Color Work Agreement form before your paper can be published. Also, you can email your editor to remove the color fee after acceptance of the paper.

Tips for writing a good quality Medical Research Paper

1. Choosing the topic: In most cases, the topic is selected by the interests of the author, but it can also be suggested by the guides. You can have several topics, and then judge which you are most comfortable with. This may be done by asking several questions of yourself, like "Will I be able to carry out a search in this area? Will I find all necessary resources to accomplish the search? Will I be able to find all information in this field area?" If the answer to this type of question is "yes," then you ought to choose that topic. In most cases, you may have to conduct surveys and visit several places. Also, you might have to do a lot of work to find all the rises and falls of the various data on that subject. Sometimes, detailed information plays a vital role, instead of short information. Evaluators are human: The first thing to remember is that evaluators are also human beings. They are not only meant for rejecting a paper. They are here to evaluate your paper. So present your best aspect.

2. Think like evaluators: If you are in confusion or getting demotivated because your paper may not be accepted by the evaluators, then think, and try to evaluate your paper like an evaluator. Try to understand what an evaluator wants in your research paper, and you will automatically have your answer. Make blueprints of paper: The outline is the plan or framework that will help you to arrange your thoughts. It will make your paper logical. But remember that all points of your outline must be related to the topic you have chosen.

3. Ask your guides: If you are having any difficulty with your research, then do not hesitate to share your difficulty with your guide (if you have one). They will surely help you out and resolve your doubts. If you can't clarify what exactly you require for your work, then ask your supervisor to help you with an alternative. He or she might also provide you with a list of essential readings.

4. Use of computer is recommended: As you are doing research in the field of medical research then this point is quite obvious. Use right software: Always use good quality software packages. If you are not capable of judging good software, then you can lose the quality of your paper unknowingly. There are various programs available to help you which you can get through the internet.

5. Use the internet for help: An excellent start for your paper is using Google. It is a wondrous search engine, where you can have your doubts resolved. You may also read some answers for the frequent question of how to write your research paper or find a model research paper. You can download books from the internet. If you have all the required books, place importance on reading, selecting, and analyzing the specified information. Then sketch out your research paper. Use big pictures: You may use encyclopedias like Wikipedia to get pictures with the best resolution. At Global Journals, you should strictly follow here.

6. Bookmarks are useful: When you read any book or magazine, you generally use bookmarks, right? It is a good habit which helps to not lose your continuity. You should always use bookmarks while searching on the internet also, which will make your search easier.

7. Revise what you wrote: When you write anything, always read it, summarize it, and then finalize it.

8. Make every effort: Make every effort to mention what you are going to write in your paper. That means always have a good start. Try to mention everything in the introduction—what is the need for a particular research paper. Polish your work with good writing skills and always give an evaluator what he wants. Make backups: When you are going to do any important thing like making a research paper, you should always have backup copies of it either on your computer or on paper. This protects you from losing any portion of your important data.

9. Produce good diagrams of your own: Always try to include good charts or diagrams in your paper to improve quality. Using several unnecessary diagrams will degrade the quality of your paper by creating a hodgepodge. So always try to include diagrams which were made by you to improve the readability of your paper. Use of direct quotes: When you do research relevant to literature, history, or current affairs, then use of quotes becomes essential, but if the study is relevant to science, use of quotes is not preferable.

10. Use proper verb tense: Use proper verb tenses in your paper. Use past tense to present those events that have happened. Use present tense to indicate events that are going on. Use future tense to indicate events that will happen in the future. Use of wrong tenses will confuse the evaluator. Avoid sentences that are incomplete.

11. Pick a good study spot: Always try to pick a spot for your research which is quiet. Not every spot is good for studying.

12. Know what you know: Always try to know what you know by making objectives, otherwise you will be confused and unable to achieve your target.

13. Use good grammar: Always use good grammar and words that will have a positive impact on the evaluator; use of good vocabulary does not mean using tough words which the evaluator has to find in a dictionary. Do not fragment sentences. Eliminate one-word sentences. Do not ever use a big word when a smaller one would suffice.

Verbs have to be in agreement with their subjects. In a research paper, do not start sentences with conjunctions or finish them with prepositions. When writing formally, it is advisable to never split an infinitive because someone will (wrongly) complain. Avoid clichés like a disease. Always shun irritating alliteration. Use language which is simple and straightforward. Put together a neat summary.

14. Arrangement of information: Each section of the main body should start with an opening sentence, and there should be a changeover at the end of the section. Give only valid and powerful arguments for your topic. You may also maintain your arguments with records.

15. Never start at the last minute: Always allow enough time for research work. Leaving everything to the last minute will degrade your paper and spoil your work.

16. Multitasking in research is not good: Doing several things at the same time is a bad habit in the case of research activity. Research is an area where everything has a particular time slot. Divide your research work into parts, and do a particular part in a particular time slot.

17. Never copy others' work: Never copy others' work and give it your name because if the evaluator has seen it anywhere, you will be in trouble. Take proper rest and food: No matter how many hours you spend on your research activity, if you are not taking care of your health, then all your efforts will have been in vain. For quality research, take proper rest and food.

18. Go to seminars: Attend seminars if the topic is relevant to your research area. Utilize all your resources.

19. Refresh your mind after intervals: Try to give your mind a rest by listening to soft music or sleeping in intervals. This will also improve your memory. Acquire colleagues: Always try to acquire colleagues. No matter how sharp you are, if you acquire colleagues, they can give you ideas which will be helpful to your research.

20. Think technically: Always think technically. If anything happens, search for its reasons, benefits, and demerits. Think and then print: When you go to print your paper, check that tables are not split, headings are not detached from their descriptions, and page sequence is maintained.

21. Adding unnecessary information: Do not add unnecessary information like "I have used MS Excel to draw graphs." Irrelevant and inappropriate material is superfluous. Foreign terminology and phrases are not apropos. One should never take a broad view. Analogy is like feathers on a snake. Use words properly, regardless of how others use them. Remove quotations. Puns are for kids, not grunt readers. Never oversimplify: When adding material to your research paper, never go for oversimplification; this will definitely irritate the evaluator. Be specific. Never use rhythmic redundancies. Contractions shouldn't be used in a research paper. Comparisons are as terrible as clichés. Give up ampersands, abbreviations, and so on. Remove commas that are not necessary. Parenthetical words should be between brackets or commas. Understatement is always the best way to put forward earth-shaking thoughts. Give a detailed literary review.

22. Report concluded results: Use concluded results. From raw data, filter the results, and then conclude your studies based on measurements and observations taken. An appropriate number of decimal places should be used. Parenthetical remarks are prohibited here. Proofread carefully at the final stage. At the end, give an outline to your arguments. Spot perspectives of further study of the subject. Justify your conclusion at the bottom sufficiently, which will probably include examples.

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

Informal Guidelines of Research Paper Writing

Key points to remember:

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

Final points:

One purpose of organizing a research paper is to let people interpret your efforts selectively. The journal requires the following sections, submitted in the order listed, with each section starting on a new page:

The introduction: This will be compiled from reference matter and reflect the design processes or outline of basis that directed you to make a study. As you carry out the process of study, the method and process section will be constructed like that. The results segment will show related statistics in nearly sequential order and direct reviewers to similar intellectual paths throughout the data that you gathered to carry out your study.

The discussion section:

This will provide understanding of the data and projections as to the implications of the results. The use of good quality references throughout the paper will give the effort trustworthiness by representing an alertness to prior workings.

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

General style:

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

To make a paper clear: Adhere to recommended page limits.

Mistakes to avoid:

- Insertion of a title at the foot of a page with subsequent text on the next page.
- Separating a table, chart, or figure—confine each to a single page.
- Submitting a manuscript with pages out of sequence.
- In every section of your document, use standard writing style, including articles ("a" and "the").
- Keep paying attention to the topic of the paper.
- Use paragraphs to split each significant point (excluding the abstract).
- Align the primary line of each section.
- Present your points in sound order.
- Use present tense to report well-accepted matters.
- Use past tense to describe specific results.
- Do not use familiar wording; don't address the reviewer directly. Don't use slang or superlatives.
- Avoid use of extra pictures—include only those figures essential to presenting results.

Title page:

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

Abstract: This summary should be two hundred words or less. It should clearly and briefly explain the key findings reported in the manuscript and must have precise statistics. It should not have acronyms or abbreviations. It should be logical in itself. Do not cite references at this point.

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

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

Reason for writing the article—theory, overall issue, purpose.

- Fundamental goal.
- To-the-point depiction of the research.
- Consequences, including definite statistics—if the consequences are quantitative in nature, account for this; results of any numerical analysis should be reported. Significant conclusions or questions that emerge from the research.

Approach:

- Single section and succinct.
- An outline of the job done is always written in past tense.
- Concentrate on shortening results—limit background information to a verdict or two.
- Exact spelling, clarity of sentences and phrases, and appropriate reporting of quantities (proper units, important statistics) are just as significant in an abstract as they are anywhere else.

Introduction:

The introduction should "introduce" the manuscript. The reviewer should be presented with sufficient background information to be capable of comprehending and calculating the purpose of your study without having to refer to other works. The basis for the study should be offered. Give the most important references, but avoid making a comprehensive appraisal of the topic. Describe the problem visibly. If the problem is not acknowledged in a logical, reasonable way, the reviewer will give no attention to your results. Speak in common terms about techniques used to explain the problem, if needed, but do not present any particulars about the protocols here.

The following approach can create a valuable beginning:

- Explain the value (significance) of the study.
- Defend the model—why did you employ this particular system or method? What is its compensation? Remark upon its appropriateness from an abstract point of view as well as pointing out sensible reasons for using it.
- Present a justification. State your particular theory(-ies) or aim(s), and describe the logic that led you to choose them.
- Briefly explain the study's tentative purpose and how it meets the declared objectives.

Approach:

Use past tense except for when referring to recognized facts. After all, the manuscript will be submitted after the entire job is done. Sort out your thoughts; manufacture one key point for every section. If you make the four points listed above, you will need at least four paragraphs. Present surrounding information only when it is necessary to support a situation. The reviewer does not desire to read everything you know about a topic. Shape the theory specifically—do not take a broad view.

As always, give awareness to spelling, simplicity, and correctness of sentences and phrases.

Procedures (methods and materials):

This part is supposed to be the easiest to carve if you have good skills. A soundly written procedures segment allows a capable scientist to replicate 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 to give the least amount of information that would permit another capable scientist to replicate 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 section, mention the specific item describing the way, but draw the basic principle while stating the situation. The purpose is to show 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:

Materials may be reported in part of a section or else they may be recognized along with your measures.

Methods:

- Report the method and not the 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—detail how procedures were completed, not how they were performed on a particular day.
- If well-known procedures were used, account for the procedure by name, possibly with a reference, and that's all.

Approach:

It is embarrassing to use vigorous voice when documenting methods without using first person, which would focus the reviewer's interest on the researcher rather than the job. As a result, when writing up the methods, most authors use third person passive voice.

Use standard style in this and 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 as 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. Use statistics and tables, if suitable, to present consequences most efficiently.

You must clearly differentiate material which would usually be incorporated in a study editorial from any unprocessed data or additional appendix matter that would not be available. In fact, such matters should not be submitted at all except if requested by the instructor.

Content:

- Sum up your conclusions in text and demonstrate them, if suitable, with figures and tables.
- In the manuscript, explain each of your consequences, and point the reader to remarks that are most appropriate.
- Present a background, such as by describing the question that was addressed by creation of an exacting study.
- Explain results of control experiments and give 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 manuscript.

What to stay away from:

- Do not discuss or infer your outcome, report surrounding information, or try to explain anything.
- Do not include raw data or intermediate calculations in a research manuscript.
- Do not present similar data more than once.
- A manuscript should complement any figures or tables, not duplicate information.
- Never confuse figures with tables—there is a difference.

Approach:

As always, use past tense when you submit 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 section.

Figures and tables:

If you put figures and tables at the end of some details, make certain that they are visibly distinguished from any attached appendix materials, such as raw facts. Whatever the position, each table must be titled, numbered one after the other, and include a heading. All figures and tables must be divided from the text.

Discussion:

The discussion is expected to be the trickiest segment to write. A lot of papers submitted to the journal are discarded based on problems with the discussion. There is no rule for how long an 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 implications of the study. The purpose here is to offer an understanding of your results and support all of your conclusions, using facts from your research and generally accepted information, if suitable. The implication of results should be fully 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 the prospect, and let it drop at that. Make a decision as to whether each premise is supported or 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 an experiment might be personalized to accomplish a new idea.
- Give details of all of your remarks as much as possible, focusing on mechanisms.
- Make a decision as to whether the tentative design sufficiently addressed the theory and whether or not it was correctly restricted. Try to present substitute explanations if they are sensible alternatives.
- One piece of research will not counter an overall question, so maintain the large picture in mind. Where do you go next? The best studies unlock new avenues of study. What questions remain?
- Recommendations for detailed papers will offer supplementary suggestions.

Approach:

When you refer to information, differentiate data generated by your own studies from other available information. Present work done by specific persons (including you) in past tense.

Describe generally acknowledged facts and main beliefs in present tense.

The Administration Rules

Administration Rules to Be Strictly Followed before Submitting Your Research Paper to Global Journals Inc.

Please read the following rules and regulations carefully before submitting your research paper to Global Journals Inc. to avoid rejection.

Segment draft and final research paper: You have to strictly follow the template of a research paper, failing which your paper may get rejected. You are expected to write each part of the paper wholly on your own. The peer reviewers need to identify your own perspective of the concepts in your own terms. Please do not extract straight from any other source, and do not rephrase someone else's analysis. Do not allow anyone else to proofread your manuscript.

Written material: You may discuss this with your guides and key sources. Do not copy anyone else's paper, even if this is only imitation, otherwise it will be rejected on the grounds of plagiarism, which is illegal. Various methods to avoid plagiarism are strictly applied by us to every paper, and, if found guilty, you may be blacklisted, which could affect your career adversely. To guard yourself and others from possible illegal use, please do not permit anyone to use or even read your paper and file.

Criterion for Grading a Research Paper (Compilation) by Global Journals

Please note that following table is only a Grading of "Paper Compilation" and not on "Performed/Stated Research" whose grading solely depends on Individual Assigned Peer Reviewer and Editorial Board Member. These can be available only on request and after decision of Paper. This report will be the property of Global Journals.

Topics	Grades		
	A-B	**C-D**	**E-F**
Abstract	Clear and concise with appropriate content, Correct format. 200 words or below	Unclear summary and no specific data, Incorrect form Above 200 words	No specific data with ambiguous information Above 250 words
Introduction	Containing all background details with clear goal and appropriate details, flow specification, no grammar and spelling mistake, well organized sentence and paragraph, reference cited	Unclear and confusing data, appropriate format, grammar and spelling errors with unorganized matter	Out of place depth and content, hazy format
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
Result	Well organized, Clear and specific, Correct units with precision, correct data, well structuring of paragraph, no grammar and spelling mistake	Complete and embarrassed text, difficult to comprehend	Irregular format with wrong facts and figures
Discussion	Well organized, meaningful specification, sound conclusion, logical and concise explanation, highly structured paragraph reference cited	Wordy, unclear conclusion, spurious	Conclusion is not cited, unorganized, difficult to comprehend
References	Complete and correct format, well organized	Beside the point, Incomplete	Wrong format and structuring

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