IOSR Journal Of Pharmacy And Biological Sciences (IOSR-JPBS)
e-ISSN:2278-3008, p-ISSN:2319-7676. Volume 17, Issue 2 Ser. III (Mar. –Apr. 2022), PP 18-22
www.Iosrjournals.Org

Pharmacological, Haemopoeitic and Biochemical Effect of *Moringa Oleifera* on Male Diabetic Albino Wistar Rats.

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Abstract

The pharmacological, haemopoietic and biochemical effect of aqueous extract of Moringa oleifera on male albino wistar rats were investigated. A total of thirty (30) male wistar rats were used. They were grouped into 3 groups namely; the control group A (n=10) and Test groups B (n=10) and B2 (n=10). After inducing diabetes and anaemia in the test animals, they were fed with the Moringa extract for 28 days.

Blood samples and urine samples collected from them were analyzed. Results showed that the extract of M. oleifera can influence insulin positively and thus reduce the blood sugar (FBS) level of test rats (Group B1) from 205 ±0.8mg/dl to 75±1.4mg/dl compared with their corresponding control.

The extract of M. oleifera also demonstrated positive haemopoietic effect in the body of the test rats by elevating the haemoglobin concentration and Packed cell volume of the test animals from 6.5±0.2mg/dl and 18.6±0.4% to 14.5±0.2 and 43.5±0.5% respectively. The volume of urine sample produced by the diabetic rats were also reduced at the end of the extract feed compared to the test rats on Dianil solutions.

It could be deduced that extract of M. oleifera could possess some elements that can affect sugar and blood levels in rats.

Key Words: *Haemopoiesis, diabetes, M. oleifera, biochemical extract.*

Date of Submission: 29-03-2022 Date of Acceptance: 10-04-2022

I. Introduction

In most developing countries especially the Sub-saharan part of Africa, people mostly depend on herbal medical cure. Some plants have been discovered to have medicinal value (Olaleye et al, 2000). These plants have been used as a source of various drugs where man can get a relief from almost all ailments. One of such plants is*M. oleifera*, This have been made easy by the use of recently developed photochemical and phytopharmacological techniques. Substances called active principles have been implicated by extraction from these natural herbs. These active principle are mostly phytochemicals like alkaloids. The ability of this plants to cure different ailments have been traced to these essential/active principles (Ukairo et al, 2001).

M. oleiferaLam is a tree that grows widely in many tropical and subtropical countries. It is grown commercially in India, Africa, South and Central America, Mexico, Hawaii and throughout Asia and South East Asia (Stobs et al 2015). It is known as drumstick tree based on the appearance of its immature seed pods.Seeds, leaves, oil, sap, bark, roots and flowers are widely used in traditional medicine.

Chemical contents of *M. oleifera*:

Leaves of *M. oleifera* have been characterized to contain a desirable nutritional balance containing Vitamins, minerals, amino acids and fatty acids (Mayo et al, 2011). Additionally, the leaves are reported to contain various types of antioxidant compounds such as ascorbic acid, flavennoids, phenolics and carotenoids (Alhakmani et al, 2013).

According to several commentaries, (Anwar et al, 2007, Mbikay 2012), Various preparations of *M. oleifera* are used for their anti-inflammatory, antihypertensive, diuretic, antimicrobial, anti-oxidant, antidiabetic, antihyperlipidaemic, antineoplastic, antipyretic, antiulcer, cardioprotectant and hepatoprotectant activities. The therapeutic potential of *M. oleifera* leaves in treating hyperglycaemia and dyslipidemia was reviewed by Mbikay (2012).

Diabetes mellitus (DM) is a metabolic disease that involves problem with the hormone called Insulin. It is characterized by hyperglycemia, glycosuria, increased protein breakdown, polyuria, weight loss, ketosis, acidosis and coma. The pathological condition can lead to a life threatening rise in the blood levels of the Ketonic bodies (Berg et al, 2002). Treatment of patient is based on the axis of control or management than total cure. Some herbs have been screened to show that they have some hypoglycaemic effects in both normal and diabetic animals.

To alleviate the suffering of diabetic subjects with anaemic symptoms using simple plants around our reach is what prompted our curiosity to embark on this study using animal model.

II. Materials And Methods

ANIMALS:

Thirty (30) healthy male albino wistar rats weighing 180-200g were selected for the study. They were housed in wire mesh cages under standard conditions (temperature 22-25^{o}c, 12 hour light and 12 hour darkness cycle). They were allowed free access to water and food (produced by Pfizer Nigeria Limited Benin; Edo state) throughout the period of experiments. However the study was conducted in accordance with the recommendation from the declaration of Helsinki on guiding principles in care and use of animals.

EXPERIMENTAL DESIGNS:

The animals were randomly distributed into three (3) groups of 10 rats per cage. Group A (10 rats) served as control group (Extract free), Group B (20 rats) served as test rats which was later shared into two groups B1 (10 induced anaemic and diabetic rats on dianil solution),and B2(10 induced diabetic rats on Moringa extract). The test animals(B1 andB2) were placed on acute feeding on the extract for 28 days (One month). All the rats received rat feed and water ad libitum.

PREPARATION OF EXTRACT OF MORINGA OLEIFERA:

Fresh leaves of *M. oleifera* were plucked from the botanical garden of pharmacognosy dept, Faculty of Pharmaceutical Science Chukwuemeka Odumegwu Ojukwu Unversity Anambra State Nigeria and the species was identified by a taxonomist. The leaves were washed, weighed and spread in the sun for two weeks to dry. They were further oven dried and later pulverized to fine powder. Later, 2.0gram of the extract was dissolved in 100ml of distilled water to give 20mg. the solution was allowed to stand for hours after which it was filtered through cheese cloth. The filterate was further filtered with Whatman's No. 1 filter paper and stored under 4^{o}c to minimize the activity of microorganism. 1.0ml was administered daily for each of the test rats in group B1 and B2 using blunt syringe while water was given to control rats instead of the extracts.

PHYTOCHEMICAL ANALYSIS OF LEAVES:

The procedure for examination of the elements present in*M. oleifera* is as described by Harbourne (1973), Trease and Evans (1996). The extract was tested for alkaloids, flavonoids, reducing sugars, saponins, glycosides, resins, calcium, carbohydrates, steroids, fats and oil, acidic compounds.

TOXICITY STUDY:

The lethal dose (LD_{50}) of the extract in albino mice was determined using Lorke's method (1983). The procedure of determining the lethal dose is by increasing the concentration of the extract given to the mice (per body weight) in each group consisting of eight (8) mice per group for five (5) days. The concentration used are 400mg/kg, 500mg/kg, 1000mg/kg, 1500mg/kg, 2000mg/kg, 3000mg/kg, 4000 mg/kg and 5000mg/kg. The mortality rate was determined and a graph plotted to determine the lethal dose (LD_{50}).

INDUCTION OF ANAEMIA AND DIABETES (HYPERGLYCEMIA) IN RATS:

Hyperglycemia and anaemia were caused to appear in male albino wister rats by starving the rats and giving them intraperitoneal administration of alloxan monohydrate (150mg/kg body weight) dissolved in normal saline given at the rate of 0.4ml at 48 hours intervals. The rats were fasted for 18 hours before the first injection. Since alloxan is capable of producing fatal hypoglycaemia as a result of massive pancreatic insulin release, the rats were treated with 30 percent glucose solution orally at different time intervals after six hours of alloxan induction and 5% glucose solution was kept in bottles in their cages for the next 24 hrs to prevent hypoglycaemia. After 7 days, the rats with diabetes mellitus showing glycosuria (indicated by urinalysis and

FBS using BG meter) and hyperglycemia with glucose range of 250mg to 350mg/dl and haemoglobin concentration of 6mg/dl (42%) were used for the test. Induction of diabetes was accomplished by three doses of alloxan injection that was administered at 3rd injection the rats were fasted again and the blood glucose levels were recorded.

ORAL FEEDING OF THE *MAGNIFERA OLEIFERA* EXTRACTS:

Water extract of *M. oleifera* was given to the test rats using blunt 1.0ml syringe. The control rats received clean water ad libitum. The group 3 rats (B2) on diabetic solution were administered 1.0ml of the diabetic tablet dissolved in 5ml water for injection. All the rats were administered the solutions with blunt needle and syringe. Extract feed lasted for 28days.

TESTS:

DETERMINATEION OF HAEMOPOEITIC AND BIOCHEMICAL PROFILES IN ALBINO RATS:

2.0ml of blood samples were collected from all the animals in the 3 groups by cardiac puncture into anticoagulant bottles (EDTA sequestrene) and fluoride bottles. They were used within hours of sample collection urine samples were collected from the rats by spreading clean white waterproof on the table where the rats were kept during the urine output analysis and Pasteur pipettes were used to pick the samples when they are dropped by the animals into graduated test tubes. Blood samples and urine samples were collected before the extract feed and 28 days after the extract feed.

HAEMOPOIETIC TESTS:

- Haemoglobin concentration and Packed cell volume were estimated according to the method described by Alexander and Griffit (1993).
- The white blood cell count and Platelet counts were estimated using the visual method of Dacie and Lewis (1991).
- Biochemical Analysis: The fasting blood and Random blood sugar test were carried out using B.G meter. The Fasting blood sugar test was carried out with the test animals in groups B1 & B2 after 12hrs overnight fast. Samples were collected from them before giving them their feed and after eating around 30 minutes interval, the random blood sample was collected from the test rats for blood sugar test using BG meter.
- The cholesterol estimation was carried out as described by Baker (1998).

STATISTICAL ANALYSIS:

The data obtained from the research were expressed as Mean and Standard deviation (Mean ± S.D) while students' T- test was used to compare the result of the control and the test. A P-value of less than or equal to ($P<0.05$ or $P=0.05$) was considered statistically significant.

III. Results:

Table 1: Phytochemical analysis of *Moringa oleifera.*

Constituents of *Moringa oleifera*.					
	Calcium, Phosphorus	Tannins, Saponins, Carbohydrate.	Alkaloids	Flavenoids, Terpenes, Glycosides.	Resins, Steroids, Fats & Oil, Anthraquinones.
Degree of concentration	++	+	+++	++	-

Key: - (Negative) + (Present in small quantity) ++ (Present in moderate quantity, +++ (Present in large quantity).

Table 2: Indicates the haemopoietic profile of induced male albino rats feed on aqueous extract of *Moringa oleifera* and their corresponding control.

Groups	**Hbg/dl ± S.D**	**PCV% ± S.D**	**WBC/mm^3 ± S.D**	**Platelets * 10^9/L ± S.D**
Group A (control) n=10, Extract free	13.2± 0.3	39 ±1.0	5,360 ±42	160 ± 15
Test rats Group B, n-20 Day1 before induction of diabetes.	13.4 ± 0.7	40.2 ± 2.0	5,200 ± 40	159 ± 17
Group B2 during Anemia	6.5 ± 0.2	18.6 ± 0.4	2,600 ± 50	110 ± 15
Group B1, n=10. Induced diabetic rats 28days after extract feed.				

	14.5 ± 0.2	43.5 ± 0.5	5,800 ± 84	169 ± 28
Group B2 n=10. Induced diabetic and anaemic rat on Dianil tablets for 5days. No Extract	8.7 ± 0.4	25.1 ± 0.6	3380 ± 13	141 ± 29
P. Values	P<0.05	P<0.05	P<0.05	P<0.05

Table 3: Biochemical profile and urine output of induced anaemic and diabetic male albino wistar rats on the extract feed of *Moringa oleifera* and their corresponding controls.

Groups	**FBS mg/dl ± S.D**	**RBS mg/dl ± S.D**	**Cholesterol mg/dl ± S.D**	**Urine output ml ± S.D**
Group A (control) n=10, extract free	68 ± 2.4	76 ± 3.4	264 ± 22	4.0 ± 0.5
Test Rats Group B, n=20 Day 1 before diabetes and anaemia induction	70 ± 1.5	82 ± 1.7	278 ± 10	3.5 ± 1.4
Group B1, n=10 during diabetes	205 ± 0.8	290 ± 2.8	280 ± 15	18 ± 0.4
Group B1, n=10 Diabetic and anaemic rats 28 days after Moringa extract.	75 ± 1.4	82 ± 0.6	190 ± 3.5	5.0 ± 0.2
Group B2, n=10 Diabetic and anaemic rats 5 days after Dianil tabs	62 ± 1.4	65 ± 2.1	210 ± 12	7.0 ± 3.0
P. Value	P<0.05	P<0.05	P<0.05	P<0.05

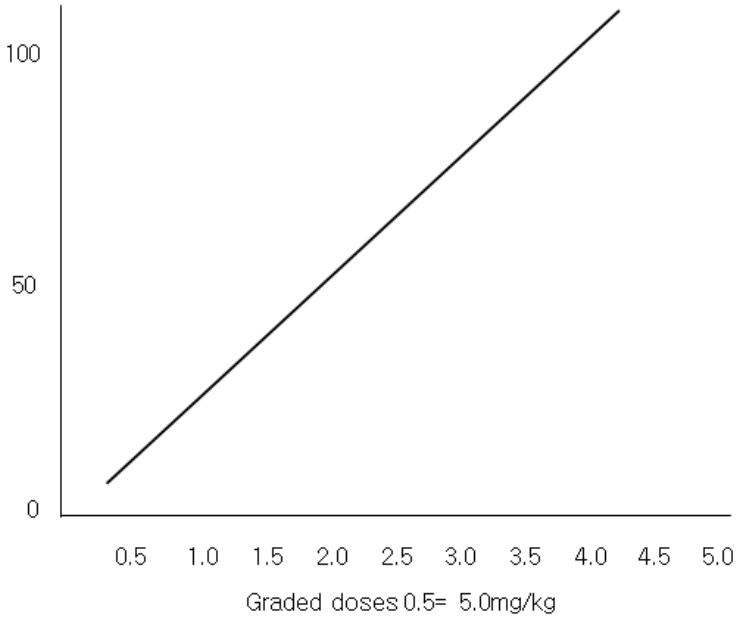

Figure 1: Lethality study (LD_{50}) of the effects of administering graded doses of (500-500mg per kg 1 P rat) *Moringa oleifera* against the percentage mortality.

IV. Discussion

The pharmacological, haemopoietic and biochemical effect of aqueos extract of *M. oleifera* (Drum stick tree) on male diabetic albino wistar rats has been studied. The phytochemical studies indicates that *M. oleifera* extract contains real plant elements. Substances found in the phytochemical studies include calcium, phosphorous, tannins, saponins, carbohydrate, alkaloids, flavennoids, terpenes, glycosides but substances such as resins, steroids, fats and oil and anthraquinones were absent. The extract of *M. oleifera* contains some principle elements that could act as antihyperglycemic agent that influenced the sugar level and brought the fasting blood sugar level from 205±0.8mg/dl to 75±1.4mg/dl and also reduced random blood sugar level 290±2.8mg/dl to 82±0.6mg/dl (Tables 1 and 2). This is almost in the same range in the group B2 rats given dianil tabs. Several reports exist concerning the anti-hyperglycaemic effects of*M. oleifera* leaf products in rats.

Ndong et al (2007) administered 2g of a leaf powder per kilogram to rats and demonstrated that the leaf powder decreased blood glucose levels by 23% relative to controls. Jasw et al, (2009) demonstrated that an aqueous extract of *M. oleifera* leaves decreased blood glucose levels in a dose dependent manner when using doses of 100300mg/kg. The anti-hyperglycaemic effects of an aqueous leaf extract may be due in part to presence of an intestinal sucrose inhibitor (Adisakwattata and Chanathong, 2011).

The result of the lethality study (LD_{50}) shows that LD_{50} in rats using *M. oleifera* is 3500mg/kg (Fig 1) hence the volume (1ml) containing 5mg/kg concentration of the extract was safe throughout the period of study. An antioxidant found in *M. oleifera* called chlorogenic acid is known to stabilize blood sugar levels. It may help process sugar better and affect insulin.

The research studies also indicates that the aqueous extract of *M. oleifera* had a positive effect on the haemopoietic system (Table 2). The result showed that anaemic and diabetic rats with haemoglobin conc of 6.5g/dl and packed cell volume of 18.6±0.4% were increased to 14.5±0.2g/dl and 43.5±0.5% due to the extract feed for 28 days.

Anemia, a reduction in normal haemoglobin concentration is one of the causes of still birth, preterm and low birth weight babies causing cognitive disabilities during the later years can be corrected by the use of *M. oleifera* leaves for its rich supply of Iron and ascorbic acid.

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PROF. F.N OGUWIKE, et. al. "Pharmacological, Haemopoeitic and Biochemical Effect of Moringa Oleifera on Male Diabetic Albino Wistar Rats.." *IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS)*, 17(2), (2022): pp. 18-22.