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EFFECT OF METHANOL MORINGA OLEIFERA LEAVES EXTRACT ON HEMATOLOGICAL PARAMETERS OF ALLOXAN INDUCED DIABETIC WISTAR RATS

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Abstract: **This study was designed to investigate the effects of *Moringa oleifera* leaves in the alloxan treated diabetic rats. The rats with body weights (120-200g) of either set were used for the study. Alloxan was given by single intraperitoneal injection (150mg/kg) and was administered orally, once a day for 21 days. The blood glucose, body weight and hematological parameters was evaluated in alloxan induced diabetic rats after 21 days daily treatment with *Moringa oleifera* leaves extract. The result of the study showed decrease in blood glucose level at the end of 21days, *Moringa oleifera* leaves also improved the body weight of alloxan treated rats. The result of the study proved the hypoglycemic effects of *Moringa oleifera* leaves in allotan treated diabetic rats. It may be due to the presence of flavoniods, which helps to speed up the natural healing process and may improve the glycemic control in diabetic rats.**

Keywords: **Alloxan, Hematological, Diabetic and Methanol.**

1. INTRODUCTION

Many oral synthetic anti-diabetic agents have developed. Hyperglycemia can be handled, initially with oral agents and insulin therapy, which sometimes required achieving targeted glycemia levels. However, these synthetic agents produce some serious side effects and relatively expensive for developing countries. *Moringa Oleifera* has been employed in many industrial processes. The seed oil which is known as ben seed oil for lubrication of machines and in the manufacture, of perfume and hair care products. Other uses of *Moringa* are; feed for farm animals, it promote better milk production. The seed is used in water purification and plant growth stimulator. Its extract have been extensively used in the manufacturing of creams, ointments oils and moisturizers, *Moringa* seed cake remaining after the extraction of the oil is commonly used as a fertilizer. (Fuglie, 2000) The leaves have been used in the production of domestic cleaning agent. (Fuglie, 2000). (Foidl et al 2001). Diabetes is disease in which blood glucose (sugar) levels rises higher than normal. Without treatment, diabetes is a progressive disease that gradually “wear out” critical body functions, including nerves, vision, muscles and vital body organs, such as the liver and pancreas. Untreated diabetes can lead to limb amputation, blindness, fatty liver disease, kidney disease and a variety of cardiovascular disease as well as premature, preventable death. Almost 95% of diagnosed diabetes among adults is type 2 diabetes if you have type 2 diabetes, your body does not use insulin properly. Blood is one of the

sensitive target for toxic compounds and an important index of physiological and pathological state in man and lower animals (Edoga *et al*, 2013). Haemoglobin gives red blood cells their colour. Each red cells contain approximately 640 million haemoglobin molecules (Otitoju *et al*, 2014). Below 4500 is low WBC and this can be caused by fewer, bone marrow deficiency or failure high WBC may be due to anemia leukemia (Fahmy T. Ali, Nahla S. Hassan and Rehab R. Abdrabou, (2015). The aim is to evaluate the effect of methanol moringa oleifera leaves extracts on hematological parameters of alloxan induced wister rats. The use of *Moringa oleifera* leaf extract in the treatment of virtually all ailments calls for further research to support the claim for its ability to boost blood (Otitoju *et al*, 2014). Medicinal plants constitute an important source of potential therapeutic agents for diabetes (Edoga *et al*, 2013). *Moringa oleifera* has been regarded as a food substance since ancient times and has also been used for treatment of many diseases such as diabetes, hyperlipidemia and cardiovascular disease. The aim of this study is to evaluate the antidiabetic activity of *Moringa oleifera* leaf extract and three of its active ingredients (moringinine, quercetin and chlorogenic acid) Fahmy T. Ali, Nahla S. Hassan and Rehab R. Abdrabou, (2015).

2. MATERIALS AND METHODS

Sample Collection

Moringaoleifera leaves were collected from southern part of Nigeria at Abuja campus around botanical garden of University of Port Harcourt, Rivers State, Nigeria and *Moringa oleifera* leaves were authetificated by Dr Chimezie with herbarium number UPH/P/105 of the University Department of Plants and Science Biotechnology, Faculty of Science, University of Port-Harcourt.

Preparation of extract

The leaves of *Moringa oleifera* were separated from the stem for complete shade drying. The leaves were shade dried for 21 days. The dried leaves were grinded into powdered form using pulverizer. The grinded leaves were then taken to the Malaria Research Laboratory of the University of Port-Harcourt for extraction using the Rotary Vane Extractor.

Experimental Animals

Thirty two (32) Wistar rats weighing between 140-180g were obtained from the animal house of the pharmacology department of the University of Port-Harcourt. The rats where divided into 5 groups of 8 rats each according to their weight and sex.

Experimental Design

Thirty two Wistar rats where used for the study. The thirty two Wistar rats where acclimatized for a period of 3 weeks (21 days) with the Ugo Basile non-invasive blood recorder machine, their weight where checked using an analogue weighing scale and where maintained with standard rat feed and water. At the end of the acclimatization period there was an increase in the weight of the rats ranging from 160 – 200g and their blood pressure ranging from 100mmHg systolic and 77mmHg diastolic to 130mmHg systolic and 80 diastolic.

Induction of Diabetics

Thirty two (32) animals were fasted overnight (12 hours) and their glucose levels were checked with Accu-check machine (glocometer) before induction of diabetes. blood sample was collected from the tail vain of the animal to check the glucose level, Diabetes was induced by slow intraperitioneal injection of alloxan solution at a dose of (150mg/kg body weight) from literature review, 1g of alloxan was dissolved in 10ml normal saline and administered within few minutes of its preparation, and 0.2ml/kg of the solution were given to the rats to cause diabetes. Diabetes were confirmed on the second day using glucose determination device.

Group 1: This group were not induce with alloxan but had access to feed and water for 21-days.

Group 2: This group were induce with alloxan but was not treated with any drug or extract, but were given free access to feed and water only for 21- days.

Group 3: This group were induced with alloxan and treated with glucophage drug for 21 days

Group 4: This group were induced with alloxan and treated with methanol extract of *Moringa* for 21 days.

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Methods of collection of blood sample

All the experimental animals were sacrificed after 21 days of treatment using chloroform fume as anaesthesia. Blood samples were collected through cardiac puncture in heparinised anticoagulant bottles. These were taken to a research laboratory for analysis.

Determination of haematological parameters

The procedure for the haematology test was carried out using the Auto Haematology Analyzer.

Procedure

1. Sample (blood) was collected and placed in an EDTA bottle and inverted to mix properly.
2. The Haematology machine was switched on and allowed to complete the on process displaying the parameters on the screen and with the commencement of a prob (tube).
3. The sample was introduced into the tube (prob) by placing the EDTA bottle containing the blood under the prob to make sure it touches the blood and the aspirator was pressed.
4. The machine dispensed the sample into the various counting chamber compartments and each of the chamber aspirates the respecting three solution (E-Z cleanser, cell lyse and diluents).
5. Each of the reagent mixes with the aspirated sample at the counting chamber for proper dilution of the aspirated blood samples.
6. The counting was done by the machine automatically within it seconds and the machine ends counting process and display the result value.
7. The value was printed by pressing the printing button and the printing rollers rolled out the result accordingly.
8. The result was compared with the normal international value inbuilt in the machine.

3. RESULTS AND DISCUSSION

Table 1: Effects of methanol *Moringa oleifera* leaves extracts on leukocytes parameters in alloxan induced diabetic wister rats.

GROUPS	WBC	Neu%	Lym%	Mon%	Eos%	Bas%
Negative Control	4.55±1.16	29.17±2.51	53±6.13	16.2±10.93	3.65±0.85	0.05±0.05
Normal Control	5.08±1.19	31.5±3.09	56.5±2.83	7.88±1.92	3.9±0.9	0 ± 0
Glucophage	5.64±2.12	31.6±2.54	56±2.81	5.08±2.15	5.86±1.89	0.2±0.2
Methanol Extract	5.1±03	5±06	7±04	1±02	±00	±0

Table 2: Effects of methanol *Moringa oleifera* leaves extracts on erythrocytes parameters on the study groups

GROUPS	RBC	HGB	HCT	MCV	MCH	MCHC
Negative Control	3.73±0.79	10.75±1.70	32.55±4.91	74.42±7.12	39.63±12.61	33.4±0.14
Normal Control	4.11±0.38	13.13±0.85	39.38±2.54	99.45±7.93	33.14±2.65	33.3±0.03
Glucophage	4.24±0.28	12.08±0.76	36.2±2.27	85.72±2.82	28.6±0.96	33.34±0.04
Methanol Extract	4.7±0	14.7±0	44±0	93.6±0	31.3±0	33.4±0

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Table 3: Effects of methanol *Moringa oleifera* leaves extracts on thrombocytes parameters in alloxan induced diabetic wister rats.

GROUPS	RDW-CV	RDW-SD	PLT	MPV	PDW	PCT
Negative Control	17.05±3.43	37.75±15.95	236.7±98.46	62.32±53.94	8.66±1.86	2.80±2.64
Normal control	12.91±0.43	29.1±4.24	154.8±21	8.29±0.53	14.76±0.62	2.23±2.00
Glucophage	12.18±0.21	21.86±2.12	205.8±27.66	7.36±0.15	10.1±0.94**	0.2±0.04
Methanol extract	12.5±0	25.3±0	680±0*	6.7±0	12.3±0	0.23±0

Table 4: Effects of *Moringa Oleifera* Leaves Extract on Body Weight in Alloxan Induced Diabetic Rats

Groups	Dayo	2 Days After Induction			One Week Of Treatment			Two Weeks Of Treatment			Three Weeks Of Treatment		
Noramal C.	160	1	6	0	1	8	0	1	9	0	2	0	0
Diabetic C.	160	1	4	0	1	4	0	1	6	0	1	8	0
Glucophage	200	1	6	0	1	8	0	2	0	0	2	2	0
Methanol	200	1	6	0	1	6	0	1	8	0	2	0	0

Moringa has become popular as a leaf powder supplement, it is used as a traditional remedy for many ailments, and *Moringa* helps to reduce some diabetes symptoms. *Moringa* leaf powder has been effective at reducing lipid and glucose levels and regulating oxidative stress in diabetic patients which means it lowers blood sugar and cholesterol and improves protection against cell damage. (Brenda Godinez, 2014). Diabetes mellitus is increasing in the developing world, with an increase in the number of diabetes patients in younger age groups; the therapeutic management of diabetes without any side effects remains a challenge. In response, there is a growing interest in evaluating herbal remedies, which are seen to be less toxic and to have negligible side effects. Therefore herbal and natural products with anti-diabetic activity and fewer side effects are strongly needed. Acute toxicity study revealed the non-toxic nature of the extract (mol) at maximum dose of 5000mg/kg indicating the dosage selected 100mg/kg is safe. Blood glucose was measured in different groups over the six-week period. At week 0, 2 days after induction of diabetes all animals in the untreated diabetic, glucophage treated and *Moringa* treated diabetic groups remained hyperglycemic (FBG>250mg/dl). After one week of the treatment 30.8± 11.8 and 20.2± 5.0. the animals on glucophage and *Moringa* treated diabetic groups, respectively had a reduction in their blood glucose and was close to the control group ($p>0.05$). The blood glucose level progressively normalized as the administration continued to the six week. Weekly changes in body weight of animals from various groups showed that at week 0, the initial body weight of various groups were normal (160-200g) but after the animals made diabetic at week 2, three was decrease in the body weight of the animals those of (160-140, 200-160 and 200-180) both untreated diabetic, *Moringa* treated group and glucophage treated group, but as the treatment continued to 3 and 4th week there was an increase in their weights again when compared with their weight at week 0. The ability of *Moringa oleifera* leaf to protect body weight loss seems to be a result of its ability to reduce hyperglycemia. The aim of this study was to investigate the effect of *Moringa* on blood parameters as well as changes in the animal's body weights, at the end of the study, it was found that mean values of all the parameters were within normal range (Brenda Godinez, 2014). The body weights of the rats increased significantly, this could support earlier reports that *M. oleifera* is of a high nutritional values (Ram, 1994, Anwar et al, 2007). The increase in body weights of the rats might be due to the fact that *M. oleifera* is rich in amino acids, vitamins and minerals, particularly iron (Anwar *et al*, 2007). Findings from this study showed that administration of *Moringa oleifera* leaves extract has no significant effects on RBC, HGB, HCT, MCV, MCH, MCHC. WBC, NEU, LYM, MON, EOS, BAS. RDW-CV, RDW-SD, PLT, MPV, PDW and PCT. Comparing the results in table 4.1, between the leukocytes parameters, the groups negative control, normal control, glocophage, and methanol extract, showed no significant effects, there was a decrease in the negative control, after the animals were made diabetic, but after treatment both the *Moringa* extract group and the glucophage group were having the same valued with the normal control group. In table 2, the erythrocyte parameters, in the groups here, the blood parameters reduced in the negative groups, but after treatment the levels became almost normal compared with the normal group. But table 3, the thrombocytes parameters, after the animals were made diabetic, most of the parameters in the negative control group increased, but later became normalized after treatment with methanol *Moringa*

extract and glucophage drug as well when compared with the normal control group. This corresponds with the report of Adeeyo et al, (2013), where *Moringa oleifera* leaves extract was used at doses 100, 200 and 300mg/kg. And *Moringa* treated hyperglycemic groups normalized to near normal, showing that *Moringa* possess hypoglycemic properties that can be very useful in the management of diabetes. There was no significant effect on the leukocytes parameters. Also in table 2 there was no significant effect on the erythrocytes parameters compared with all the groups. And in table 4.3 showed no significant effect on the thrombocytes parameters compared with the groups, except on PDW ($p<0.01$). Reduction in the glucose levels from high to normal in the *Moringa* and glucophage treated groups, showed that *Moringa oleifera* leaves is almost as effective as the standard drug (glucophage). The absence of significant changes on the levels of blood indices suggests that the extract is safe with no deleterious effect on the hematological function. This is in agreement with the report of Anwar *et al*, (2007) that *Moringa oleifera* maintain the levels of blood glucose in diabetic patients. Also Adeeyo *et al*, (2013) reported that dietary components of *M. Oleifera* have a measurable effect on blood constituents and mean values of each parameter were within the normal range. It is also in agreement with the findings of Adeeyo et al, (2013). Where *Moringa oleifera* leaves extract was used at doses 100, 200, and 300mg/kg. And *Moringa* treated hyperglycemic groups normalized to near normal, showing that moringa possess hypoglycemic properties that can be very useful in the management of diabetic hyperglycemia. Edoga *et al*, (2013). Also reported that aqueous extract of the leaf of *Moringa oleifera* (100, 200, and 300mg/kg) exhibited 23.14, 27.05 and 33.18% reduction of the blood glucose levels in rats.

4. CONCLUSION

In conclusion, the result of this study supports the report about *M. oleifera* in having medicinal effect in curing some health problems associated with nutritional status and this was indicated in this study by its positive effects on most blood parameters, glucose levels and body weights of the experimental animals.

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DISCLOSURE OF CONFLICT OF INTEREST

No conflict of interest exist between the authors.

STATEMENT OF ETHICAL APPROVAL

Ethical approval was received from the Departmental Head and her Committee

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