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Effect of methanol *Moringa oleifera* leaves extract on the hematological parameters of cadmium chloride induced hypertensive Wistar rats

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Abstract

This study was carried out to evaluate the effect of methanol *Moringa oleifera* leaves extract on the hematological parameters of cadmium chloride induced hypertensive wistar rats. 32 wistar rats weighing between 160-200kg where used and grouped into four groups of 8 rats each. All groups except group one where induced with both cadmium chloride to cause hypertension according to their body weight group 1 which served as normal control took only water and feed for 21 days. Group 2 which served as hypertensive control took only water and feed for 21 days. Group 3 took 0.26ml/kg body weight of a standard drug (Nifedipine) for 21 days and group 4 which served as test group took 0.16ml/kg body weight of *Moringa oleifera* leaves extract for 21 days. At the end of which the animals where sacrifice 3ml of blood samples were collected through cardiac purcture and were taken to laboratory for analysis. The hematology analysis was done using an Auto Haematology Analyzer. The parameters measured include the leucocyte parameters, erythrocyte parameters, thrombocyte parameters and the hematological indices. The data analysis was determined by one-way Analysis of Variance (ANOVA) followed by Duncan post-hoc test and P value<0.05 was considered significant. The effect of methanol *Moringa oleifera* leaves extract shows no significant difference on leucocyte parameters at 7.27±2.39 (WBC), for Erythrocyte parameters it showed a significant difference of 46.50±1.67 (HTC) and for hematological indices and thrombocyte parameters, it shows a significant difference of 66.25±24.30 (RDW-SD). The result reveals that the extract has an effect on hematological parameters that lead to hypotension when compared to both normal and hypertensive control.

Keywords: Hematological; Methanol; Hypertensive; Cadmium Chloride

1. Introduction

The use of medicinal plant in most developing nations is a development that has attracted more concerns among health workers and researchers. The practice of traditional medicine is as old as the origin of man (Doughari *et al*., 2009). The use of traditional medicine in the treatment and management of diseases in the Africa continent cannot fade away and this could be attributed to the socio-cultural, socio-economic, lack of basic health care and qualified personnel (Fasuyi, 2006). Vegetables vary considerably in their nutrient contents and are good source of vitamins, essential amino acids, protein as well as mineral and antioxidants (Fasuyi, 2006). These are included in meals mainly for their nutritional value and some are best reserved due to their medicinal properties for the sick (Doughari *et al*., 2009). *Moringa oleifera* leaves used as vegetables in various countries of the world have been shown to have positive effect on some haematological parameters (Adedapo *et al*., 2009). *Moringa* preparations (e.g extractions, decoctions, poultices, creams, emollients, salves, powders, porridges) have been use for the treatment and prevention of disease or infection either through dietary or topical administration (Palada, 1996). It has been shown that *Moringa* increases glucose tolerance in diabetic patients (Palada, 1996). The root, seed, bark, fruit, leaves, flower and immature pods act as cardiac and circulatory drugs and possess antitumour activity (Palada, 1996). *Moringa* leaves contain flavonoids such as quercetin and kaempferol

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which have been identified as the most potent antioxidant in the leaves (Siddhuraju and Becker, 2003). Some of the compounds that have been isolated from *Moringa* preparations which are reported to have hypotensive, anticancer and antibacterial activities include 4-(4-0-acetyl-a-l-rhamnopyranosyloxy) benzyl isothiocyanate, 4-(α-L-rhamnopyranosyloxy) benzyl isothiocyanate, niazimicin, pterygospermin, benzyl isothiocyanate, and 4-(α- L-rhamnopyranosyloxy) benzyl glucosinolate (Fahey *et al*., 2001). The aim is to determine the possible effect of methanol *Moringa oleifera* leave extract on the haematological parameters of cadmium chloride induced hypertensive wistar rats.

The main cause of hypertension is the increased level of sodium in the blood which results in abnormal systolic and diastolic pressure. Left untreated, high blood pressure hastens the deterioration of the circulatory system and can cause serious illness or death.

High blood pressure is classified as either primary hypertension or secondary hypertension. Haematological parameters (blood parameters) are the major indices of physiological, pathological, and nutritional status of an organism and changes in the constituents of blood when compared to normal values could be used to interpret the metabolic state of an animal (Fahey *et al*., 2001). (Fahey *et al*., 2001) investigated the renal protective effect of nifedipine (2-nitrophenyl derivative BAY a 1040) in streptozotocin (STZ)-induced spontaneously hypertensive rats (SHRs, 8 weeks of age). Author suggested that nifedipine inhibits the development of albuminuria and glomerular enlargement in STZ-induced diabetic SHRs.

2. Materials and methods

2.1. Sample Collection

Moringa oleifera leaves were collected from southern part of Nigeria at Omuoko Aluu in Ikwere Local Government Area of Rivers State, Nigeria and *Moringa oleifera* leaves were authetificated by Dr Chimezie with herbarium number UPH/P/105 of the University of Port Harcourt, Department of Plants and Biotechnology, Faculty of Science, University of Port-Harcourt, Nigeria.

2.2. 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.

2.3. Experimental Animals

Thirty two (32) wistar rats weighing between 100-140g 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.

2.4. Experimental Design

Thirty two wistar rats where used for the study. The thirty two wistar rats where acclimatized for a period of 1 week (7 days) with the Ugo Basile non-invasive blood recorder device, 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 100 – 200g and their blood pressure ranging from 100mmHg systolic and 77mmHg diastolic to 130mmHg systolic and 80 diastolic.

2.5. Induction of hypertension

0.8g of cadmium chloride (CdCl2) was dissolved in 100ml of distilled water to prepare a stock solution of 0.8mg/ml. A single administration of 3 doses of the cadmium chloride was given to each three groups (group 2- group 4) intraperitoneal according to their body weight which were 3 dose of 0.2ml for 100g body weight, 0.23ml for 185g body weight and 0.25ml for 200g body weight to cause hypertension in the wistar rats after a fasting period of 24 hours. Hypertension was confirmed 48 hours after inducement and the animals were treated daily as follows;

- **Group 1**: Were given tap water and feed as normal control for 21 days.
- **Group 2**: Hypertensive rats were given tap water and feed as hypertensive control for 21 days.
- **Group 3**: Hypertensive rats treated with 0.26ml/kg of Nifedipine (standard drugs) for 21 days.
- **Group 4**: Hypertensive rats treated with 0.16ml/kg of *Moringa oleifera* leaves methanol extract for 21 days.

2.6. 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.

2.7. Determination of haematological parameters

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

2.7.1. Procedure

- Sample (blood) was collected and placed in an EDTA bottle and inverted to mix properly.
- 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).
- 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.
- 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).
- Each of the reagent mixes with the aspirated sample at the counting chamber for proper dilution of the aspirated blood samples.
- The counting was done by the machine automatically within it seconds and the machine ends counting process and display the result value.
- The value was printed by pressing the printing button and the printing rollers rolled out the result accordingly.
- The result was compared with the normal international value inbuilt in the machine.

3. Results and discussion

Table 1 Effect of *Moringa oleifera* leaves extract on Leucocyte Parameters of the study groups.

Parameters	**Groups**			
	Normal Control	**Hypertensive Control**	**Known drug (Nifedipine)**	***Moringa* leaves methanol extract**
WBC ($10^{\wedge}9$/Ls)	5.25±1.18	3.50±0.50	4.64±0.90	7.27±2.39
Neutrophil ($10^{\wedge}9$/L)	2.14±0.29	1.51±0.50	1.48±0.36	2.67±0.46
Lymphocyte ($10^{\wedge}9$/L)	1.38±0.56	1.33±0.99	0.86±0.46	1.00±0.52
Monocyte ($10^{\wedge}9$/L)	0.13±0.13	0.33±0.21	0.14±0.21	0.02±0.027
Eosinophil ($10^{\wedge}9$/L)	0.04±0.04	0.00±0.00	0.01±0.01	0.00±0.00
Basophil ($10^{\wedge}9$/L)	0.00±0.00	0.00±0.00	0.00±0,00	0.00±0.00
Neutrophil (%)	31.5±3.09	28.33±4.42	24.86±4.01	27.50±2.09
Lymphocyte (%)	56.50±2.83	58.50±5.29	65.00±3.77	63.33±2.06

Medicinal plant are of great importance to the health of individuals and communities and their medicinal values lie in some chemical substances that provides definite physiological actions on the human body (Edeoga et al.,2005). *Moringa oleifera* plant is very important for its medicinal value. Some compounds that have been isolated from Moringa preparations are reported to have hypertensive activities (Fahey, 2005). Various part of the plants such as the leaves act as cardiac and circulatory stimulant and possesses anti-hypertensive activities (Anwar et al., 2007).

Blood provides the basic connections between the various organs and cells of the body and to maintain a constant cellular environment by circulating through every tissue delivering nutrient to them and removing waste product (Ganong, 2003). The blood cells (Erythrocytes or Red Blood Cells, Leucocytes or white Blood Cells, and Thrombocytes or platelets) are regulated so that excessive variation in the quality and quantity is prevented. This regulation is via some specialized feedback mechanism for the cells (Guyton and Hall, 2004).

Table 2 Effect of *Moringa oleifera* leaves Methanol extract on Erythrocyte Parameters of the study groups**.**

PARAMP PARAMETERS	GROUPS			
	Normal control	**Hypertensive control**	**Known drug (Nifedipine)**	***Moringa* leaves methanol extract**
Monocyte (%)	7.88±1.92	9.17±2.24	5.43±1.85	5.50±1.38
Eosinophil (%)	3.88±0.92	4.00±0.73	3.43±1.04	3.50±0.89
Basophil (%)	0.00±0.00	0.00±0.00	0.14±0.14	0.17±0.17
RBC (10^9/L)	4.13±0.48	3.83±0.83	5.29±0.42	4.83±0.17
HGB (g/dL)	13.13±0.90	10.72±2.33	13.53±1.69	15.45±0.54 b
HCT	39.38±2.54	33.83±7.36	47.43±2.36 b	46.50±1.67 b
MCV (fL)	99.50±7.91 b	68.68±14.34	91.53±4.03	98.65±5.17 b
MCH (pg)	33.00±2.65 b	22.78±4.77	30.34±1.32	32.85±1.70 b

Table 3 Effect of *Moringa oleifera* leaves Methanol extract on Haematological Indices and Thrombocyte parameters of study groups.

PARAMETERS	GROUPS			
	Normal control	**Hypertensive control**	**Known drug (Nifedipine)**	***Moringa* leaves Methanol extract**
MCHC(g/dL)	33.00±0.00	27.50±5.50	33.00±0.00	29.37±3.67
RDW-CV (%)	12.88±0.44	12.83±0.40	13.00±0.31	13.82±0.82
RDW-SD (Fl)	29.13±4.25	26.50±2.88	29.14±3.10	66.25±24.30 a, b
PLT (10^9/l)	154.75±21.00	254.50±48.86	157.71±36.69	142.00±53.83
MPV(FI)	8.25±0.56	7.33±0.21	7.53±0.48	10.28±1.61 b
PDW	14.50±0.65	10.83±1.11	13.76±0.90	12.88±2.61
PCT (%)	2.00±2.00	0.17±0.17	0.02±0.02	0.06±0.06

This research work was design to investigate the effect of *Moringa oleifera* leaves extract on the haematological parameters of cadmium chloride induced hypertensive wistar rats.

The white blood cell indices are lymphocyte, neutrophils, monocytes, eosinophils and basophils which makes up for the toatal white blood cell count. From the result in the table 1, the total white blood cell count which include neutrophil, lymphocyte, monocyte, eosinophil, basophil and their percentage respectively of the group treated with *Moringa* leaves extract shows no significant difference ($p>0.05$) when compared to normal control and hypertensive control respectively statistically. There is also no significant difference on the group treated with a known drug (Nifedipine) when compared with to both normal control and hypertensive control respectively. This findings is in agreement with the study done by Anwar et al., (2007) which suggest that a raised WBC count may indicate increased catecholamine level or enhanced sympathetic nervous system activity thus causing an increase in blood pressure and eventually resulting in sustained hypertension. But visually there is a slight increase in the percentage of both neutrophil, monocyte and eosinophil of the *Moringa oleiera* group compared to the normal control, hypertensive control and the known drug group which means that the extract is capable of increasing the immune system of the body. This is in agreement with Anwar et al., (2007) that extract from *Moringa oleifera* leaves have been shown to modulate humoral and cellular immunity in rats and mice.

From the result in table 2, RBC of *Moringa oleifera* leaves extract statistically shows no significant difference ($p>0.05$) when compared with both normal control and hypertensive control. Recently, it has been shown that RBC measured by

nuclear magnetic resonance is significantly decreased in hypertensive and that treatment of hypertension partially restores RBC level (Al-Muhana *et al*., 2006). This observation confirms the treatment of the group treated with *Moringa oleifera* leaves extract with the statistical value of 4.84±0.17 compared to the normal control (4.13±0.48) and the hypertensive control (3.83±0.83).

According to the findings by Giacomo et al., (1986), Massimo et al., (1992) and Al-Muhana et al., (2006). They stated that two ionic systems are involved in cell volume regulation, namely, a loop diuretic-sensitive Na+ -k+ symport and intra-cellular calcium, are reported to be altered in hypertension. The result of HGB as shown in table 2 shows that there is a significant difference of the *Moringa oleifera* group compared to the normal control and hypertensive control group. This observation is in agreement with earlier works reported by Adedapo et al., (2009) who reported a slight increase in Hb count among rats given 400mg/kg body weight of *M. oleifera* leaves extract.

Haematocrit (HTC) is a determinant of whole blood viscosity. Viscosity affect peripheral resistance to blood flow, and peripheral resistance affect blood pressure (Rampling MW 1999). From the result in table 2, there is a significant difference in HCT of the *Moringa oleifera* group compared to the normal control and hypertensive control but there is no significant difference when compared to the known drug group. This is in contrast with Adedapo et al., (2009) that most hypertensive patients exhibit increased blood viscosity compared with healthy ones.

Comparing the Mean Corpuscular Volume (MCV) of *Moringa oleifera* leaves extract group with that of normal control group, there is no significant difference but when compared with the hypertensive control and known drug there is a significant difference. The significant difference seen in *Moringa oleifera* group when compared with the hypertensive control is in constituent with the studies reported by Massimo et al., (1992) and Al-Muhana et al (2006) that if MCV is decreased in hypertensive group, then the hypothesis can be made to show how the increased blood pressure leads to decrease MCV. Comparing Mean Corpuscular Haemoglobin (MCH) of *Moringa oleifera* leaves extract group (32.85±1.70) with that of normal control (33.00±2.65) there is no significant difference (p>0-05). Also comparing the normal control with the hypertensive control and known drug there is a significant difference. The result for the Mean Corpuscular Haemoglobin concentration (MCHC) and RDW-CV shows no significant difference in all the groups while RDW-SD of the *Moringa oleifera* group shows a significant difference when compared to both the normal control and hypertensive control respectively. RDW has been recently determined to be associated with hypertension and it was indicated of poor prognosis in heart failure and acute myocardial infarction (Al-Muhana et al 2006). Although the mechanism of the relationship between hypertension RDW was nt clearly understood, increase inflammation was the most reasonable theory (Al-Muhana *et al.,* 2006).

Platelet indices comprise Platelet Distribution Width (PDW), Mean Platelet Volume (MPV), Plateletcrit (PCT), and Blood Platelet Count (PLT). The result on MPV in *Moringa* leaves extract group (10.28±1.61) shows a significant difference ($p<0.05$) when compared with normal control (8.25±0.56) and the hypertensive control (7.33±0.21).

Statistically, PLT, PDW and PCT show no significant difference in the *Moringa oleifera* group compared to the normal control and hypertensive control. But visually there is a decrease in their values when compared to the normal control and hypertensive control. According to Al-Muhana *et al*., (2006)), there are two possible mechanisms of that may contribute to increase platelet parameter levels during high blood pressure such as firstly; pulmonary vascular endothelial dysfunction was linked with the path mechanism of hypertension, which might lead to platelet activation and thrombosis. And secondly, systemic inflammation and immune dysfunction in patients with high blood pressure might cause platelet activation (Massimo *et al*., (1992)). This statement shows that there are no such in the group treated with *Moringa* extract.

4. Conclusion

In conclusion, the result indicates that *Moringa oleifera* leaves extract has a positive effect on the haematological parameters of hypertensive Wistar rats. This suggests that *Moringa oleifera* leaves extract has hypertensive or anti-hypertensive properties and can be used for the treatment of hypertension.

Compliance with ethical standards

Disclosure of conflict of interest

There was no conflict of interest to be disclosed.

Statement of ethical approval

Ethical approval was sought from Animal Ethics Committee and Departmental Head and was received.

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