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HAEMATOLOGICAL PARAMETERS OF ALLOXAN-INDUCED DIABETIC RATS TREATED WITH LEAF ESSENTIAL OIL OF *HOSLUNDIA OPPOSITA* (VAHL)

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ABSTRACT

The effect of leaf essential oil of *Hoslundia opposita* (Vahl) on the haematological parameters of alloxan-induced diabetic rats was investigated. Forty-eight albino rats (*Rattus norvegicus*), of average weight 132.5 g, were randomly selected into normal and diabetic groups, each with four sub-groups. The rats were treated with 110 and 220 mg/kg body weight (b. wt.) of the essential oil. 14.2 mg/kg body weight of metformin (Glucophage) was used as a reference drug. All treatments were administered, intraperitoneally, once a day for four days. Haematological parameters like haemoglobin (HGB), red blood cell (RBC) count, white blood cell (WBC) count, percentage lymphocytes (LYM) and neutrophils (NEU) were analysed. There were no significant differences ($p > 0.05$) in the erythrocyte indices of all the normal (non-diabetic) rats, both treated and untreated. However, there was a significant increase ($p < 0.05$) in the WBC count and a significant reduction ($p < 0.05$) in the lymphocyte (LYM) percentages of the normal (non-diabetic) rats administered with higher dose of the essential oil. The results also revealed a significant reduction ($p < 0.05$) and a significant increase ($p < 0.05$) in the RBC counts of untreated diabetic rats and diabetic rats administered 110 mg/kg b. wt. of the oil respectively. A significant increase ($p < 0.05$) in the LYM of diabetic untreated rats was also observed, while administration of metformin and 110 mg/kg b. wt. *Hoslundia opposita* leaf essential oil (HOLEO) to diabetic rats significantly ($p < 0.05$) reduced the LYM percentages to values within range of the normal control animals. Overall, administration of the oil has significant ameliorative effect on alloxan-induced anaemia in diabetic state and this may be of immense benefits in the management of type 2 diabetes and its associated haematological complications.

Keywords: *Hoslundia opposita,* leaf, essential oil, diabetic rats, haematology

INTRODUCTION

Diabetes mellitus describes a metabolic disorder of multiple aetiology, which is characterized by chronic hyperglycemia (WHO, 1999). Recurrent or persistent hyperglycemia during diabetes causes glycation of body proteins which in turn leads to secondary complications affecting eyes, kidneys, nerves and arteries (Sharma, 1993). In humans, glycosylation of tissue has been described not only for haemoglobin but also in red blood cell membranes, serum albumin, serum globulins and other plasma proteins, as well as collagen and elastic tissues. Glycosylation and stiffening of red blood cells, may be responsible for, or associated with, large vessel disease in diabetes (Guthrie and Guthrie, 2002).

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In diabetes, reduced haemoglobin has been reported (Mansi, 2006). Reduction in haemoglobin may be accompanied by a fall in the red blood cell count and packed cell volume (Moss, 1999; Muhammad and Oloyede, 2009). Very low readings of RBC, haemoglobin and hematocrit could indicate anaemia (Muhammad and Oloyede, 2009).

Conventionally, type 2 diabetes and its complications are treated with synthetic oral hypoglycemic agents like sulphonylureas, thiazolinedione and biguanides (Rosac, 2002). However, not all synthetic drugs can serve as curative agents for complications of diabetes and most do produce adverse health effects (Cheng and Fantus, 2005). A main side effect of thiazolinedione is anaemia (Cheng and Fantus, 2005). Thus, coupled with the high cost of management and the adverse side effects associated with various available clinical antihyperglycemic agents, there is need to explore alternatives offered by traditional phytotherapies. Common advantages of herbal medicines are effectiveness, safety, affordability and acceptability (Momin, 1987).

Hoslundia opposita Vahl. (Lamiaceae) is an herbaceous perennial shrub native to Africa and wildly grown in Nigeria, where it is commonly known as "Oke ota" by the Igbos and "Efirin odan" by the Yorubas (Iwu, 1993). Infusions of its leaf are widely used in African traditional medicine for treating various ailments including diabetes (Abbiw et al., 2002). Usman et al. (2010) reported 1,8-cineole (Eucalyptol) as the main constituents of North-Central Nigerian grown leaf essential oil of this plant. Previous studies in our laboratory had established the antimicrobial (Saliu et al., 2011) and glucose lowering effects of the essential oil extracted from the leaves of *H. opposita* (Muhammad et al., 2011). However, there is paucity of information on its effect on diabetes-induced anaemia or its blood relating functions in non-pathological state. Thus, the present study was carried out to evaluate the effects of leaf essential oil of *H. opposita* on the haematological parameters in normal (non-diabetic) and alloxan-induced diabetic rats.

MATERIALS AND METHODS

Sources of materials

Alloxan monohydrate and dimethylsulfoxide (Sigma Chemical Company, St. Louis, Mo, USA), Accu-chek active glucometer and strips (Roche Diagnostic, Mannheim, Germany) and OHAUS analytical balance (Ohaus Corporation, NJ, USA), were used.

Fresh leaves of *Hoslundia opposita* were obtained from the Parks and Gardens Unit of the University of Ilorin, Nigeria. Identification of the leaf was carried out at the herbarium of the Forestry Research Institute of Nigeria (FRIN), Ibadan, Oyo State, where a voucher specimen was deposited (FH10086637-0).

Albino rats (*Rattus norvegicus*) were obtained from the Animal House of the Department of Biochemistry, University of Ilorin, Nigeria.

Oil isolation and standardization

Pulverished leaves of *Hoslundia opposita* (500 g) were hydrodistilled for 3 h in a Clevenger- type apparatus, according to the British Pharmacopeia specification (1980). Five (5) percent and ten percent (5 and 10 %) v/v of the resulting oil were prepared, using saline solution of dimethylsulphoxide (DMSO) (Lahlou, 2004).

Animal grouping and management

Forty-eight (48) albino rats (*Rattus norvegicus*) with an average weight of 132.5 g were maintained under standard laboratory conditions (12-h light/dark cycle, 25±2 °C). Prior to experimentation, the rats were acclimatized to laboratory conditions for one week. They were then randomly selected into two large groups (non-diabetic and diabetic), each with four sub-groups.

Induction of experimental diabetes

After fasting for 18 h, animals in the diabetic group were subjected to a single intraperitoneal injection of alloxan monohy-

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drate, 160 mg/kg body weight, freshly dissolved in sterile distilled water. 48 h after alloxan injection, fasting blood glucose (FBG) was determined using a glucose oxidase-based commercial glucometer (Accu-chek active, Roche Diagnostic). Rats showing FBG above 250 mg/dl were considered diabetic (Aruna et al., 1999).

Administration of oil

All treatments were intraperitoneally (IP) administered to rats once a day as shown below:

Non-diabetic rats

- Non-diabetic and untreated control rats (NC)
- Non-diabetic rats treated with 200 mg/kg body weight (b. wt.) of DMSO (NDMSO)
- Non-diabetic rats treated with 110 mg/kg b. wt. of essential oil (NT1)
- Non-diabetic rats treated with 220 mg/kg b. wt. of essential oil (NT2)

Diabetic rats

- Diabetic control and untreated control rats (DC)
- Diabetic rats treated with 14.2 mg/kg b. wt. of metformin (DM)
- Diabetic rats treated with 110 mg/kg b. wt. of essential oil (DT1)
- Diabetic rats treated with 22 mg/kg b. wt. of essential oil (DT2)

Determination of haematological parameters

The Automated Haematologic Analyzer (Sysmex KX – 21) was used to analyze the haematological parameters such as RBC, PCV, HGB, WBC, NEU and LYM. The analyses were carried out based on standard methods (Dacie and Lewis, 1991).

Statistical analysis

All data are expressed as the mean of six replicates $\pm$ standard error of mean (S.E.M). Statistical evaluation of data was performed by Graph pad prism version 5.02 using one way analysis of variance (ANOVA), followed by Dunett's posthoc test for multiple comparism. Values were considered statistically significant at $p < 0.05$ (confidence level = 95 %).

RESULTS

The effects of intraperitoneal (IP) administration of *Hoslundia opposita* leaf essential oil (HOLEO) (110 and 220 mg/kg b. wt.), metformin (14.2 mg/kg b.wt.) and vehicle (200 mg/kg b. wt.) on haematological parameters in non-diabetic and alloxan-induced diabetic rats are shown in Tables 1 and 2.

In normal, non-diabetic rats treated with HOLEO, considerable increases in RBC and PCV and reduction in HGB were observed. However, the alterations were not significantly different ($p < 0.05$) when compared with the normal untreated rat. The red blood indices in diabetic control were significantly lower than normal (non-diabetic) control. The red blood cell count (RBC) of diabetic untreated rat was reduced significantly ($p < 0.05$), while there were significant ($p < 0.05$) increases in RBC count of diabetic rats treated with the reference drug (metformin) and 110 mg/kg b.wt. of HOLEO. Appreciable elevations that were not significantly ($p > 0.05$) different were also observed in the PCV and HGB levels in HOLEO and metformin treated rats.

The WBC and NEU of the normal (non-diabetic) rats treated with HOLEO significantly increased ($p < 0.05$) while LYM was significantly reduced ($p < 0.05$). In contrast, LYM percentage significantly ($p < 0.05$) increase in diabetic untreated rats, however, treatment with metformin and 110mg/kg b. wt. significantly reduced ($p < 0.05$) the LYM percentages to values within the range of the normal (non-diabetic) control.

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Table 1: Effect of intraperitoneal administration of leaf essential oil of *Hoslundia opposita* on some erythrocyte indices in non-diabetic and alloxan-induced diabetic rats

Parameters	RBC (x10^{12} µ/L)	PCV %	Hb (g/dL)
Non-diabetic			
NC	3.57±0.14^{bcd}	33.83±1.17^{ab}	6.35±0.28^{ab}
NDMSO	3.53±0.27^{bcd}	33.50±1.40^{ab}	5.87±0.21^{ab}
NT1	3.98±0.06^{cd}	31.33±1.05^{ab}	5.71±0.15^{a}
NT2	4.06±0.23^{d}	34.67±2.29^{b}	5.78±0.18^{ab}
Diabetic			
DC	2.83±0.11^{a}	29.33±0.95^{a}	5.96±0.32^{ab}
DM	3.38±0.12^{bc}	33.67±1.30^{ab}	6.82±0.36^{b}
DT1	3.37±0.13^{bc}	33.00±1.23^{ab}	6.62±0.36^{ab}
DT2	3.17±0.16^{ab}	30.83±1.17^{ab}	6.18±0.33^{ab}

Values are expressed as mean of six replicates ± S.E.M.
Values with different superscripts along a column are statistically different (P<0.05).
NC = normal control; NDMSO = normal+200 mg/kg b.wt. DMSO; NT1 = normal+110 mg/kg b.wt. HOLEO; NT2 = normal+220 mg/kg b.wt. HOLEO; DC = diabetic control; DM = diabetic+14.2 mg/kg metformin; DT1= diabetic+110 mg/kg b.wt. HOLEO; diabetic+110 mg/kg b.wt. HOLEO

Table 2: Effect of intraperitoneal administration of leaf essential oil of *Hoslundia opposita* on some leukocyte indices in non-diabetic and alloxan-induced diabetic rats

Parameters	WBC (x10^{12}µ/L)	Lymphocytes %	Neutrophils %
Non-diabetic			
NC	7.03±0.41^{ab}	63.50±1.52^{b}	38.50±1.62^{ab}
NDMSO	6.80±0.13^{a}	56.00±1.77^{a}	43.00±2.00^{b}
NT1	7.67±0.13^{abc}	57.83±1.72^{ab}	39.50±2.13^{ab}
NT2	8.80±0.42^{c}	56.17±1.25^{a}	43.33±0.99^{b}
Diabetic			
DC	8.37±0.49^{bc}	70.67±1.67^{c}	35.33±1.50^{a}
DM	8.17±0.47^{abc}	58.33±1.38^{ab}	39.50±1.63^{ab}
DT1	7.68±0.44^{abc}	60.33±1.38^{ab}	43.50±1.86^{b}
DT2	7.23±0.41^{ab}	71.67±1.67^{c}	40.00±1.83^{ab}

Values are expressed as mean of six replicates ± S.E.M.
Values with different superscripts along a column are statistically different (P<0.05).
NC = normal control; NDMSO = normal+200 mg/kg b.wt. DMSO; NT1 = normal+110 mg/kg b.wt. HOLEO; NT2 = normal+220 mg/kg b.wt. HOLEO; DC = diabetic control; DM = diabetic+14.2 mg/kg metformin; DT1 = diabetic+110 mg/kg b.wt. HOLEO; diabetic+110 mg/kg b.wt. HOLEO

DISCUSSION

Reports have shown that administration of medicinal compounds or drugs can alter the normal range of haematological parameters (Ajagbonna et al., 1999). These alterations could either be positive or negative (Adeneye, 2008). Assessment of haematological parameters can be used to determine the extent of deleterious effect on blood constituents of an animal (Muhammad et al., 2004; Ashafa et al., 2009). It can also be used to explain blood relating functions of chemical compounds/plant extract (Yakubu et al., 2007). This is because it plays a role in physiological, nutritional and pathological state of an organism (Muhammad et al., 2000).

In non-diabetic (normal) rats, administration of HOLEO did not elicit any changes in the haematological parameters, however, the treatment, especially at higher dosage (220 mg/kg b. wt.), significantly

increase WBC count and LYM index. This might be due to the fact that there was no destruction of matured red blood cells by the extracts. The extract, therefore at the dosages administered, have no deleterious effect on oxygen-carrying capacity of the blood. This is because HGB, a major constituent of erythrocytes, which functions in oxygen transport and is used as an index to evaluate physical condition of an animal (Suchantabud et al., 2008), was not altered. Although, selective immune modulatory effect and localized toxicity could occur as recorded in lymphocyte index of the HOLEO treated non diabetic rats (Yakubu et al., 2007).

The appreciable decrease in PCV and HGB and the significant reduction in RBC count observed in the untreated alloxan induced diabetic rats correlates with the findings of Mansi (2006). Reactive oxygen species have also been implicated in the mechanism of red cells damage (Rao et al., 2003). The cytotoxic action of diabetogenic agent such as alloxan is mediated by reactive oxygen species (Szkudelski, 2001). Hyperglycemia results in glycosylated haemoglobin, thus total haemoglobin level is decreased in alloxan induced diabetic rats (Sheela and Augusti, 1992). Reduction in haemoglobin may be accompanied by a fall in the red blood cell count and packed cell volume (Moss, 1999; Muhammad and Oloyede, 2009), thus correlating with decreased level of red blood indices observed in the diabetic untreated rats in this study (Mansi, 2006; Mohammed et al., 2009). Very low readings of RBC, haemoglobin and hematocrit can indicate anaemia (Muhammad and Oloyede, 2009). It was suggested that patients with diabetes mellitus often have autonomic dysfunction (Lishner et al., 1987).

However, treatment of diabetic rats with HOLEO led to increase in RBC indicating ameliorative effect of the extract on alloxan induced anaemia. Also, HOLEO extracts effected increase in neutrophil percentages in diabetic treated rats and this may indicate an anti-infective effect of the extract (Mohammed et al., 2009). It may however, not be able to act as a general boost to the immune system since it exerted a reduction in other leukocyte parameters in rats as well.

CONCLUSION

Overall, data from this study revealed that intraperitoneal administration of the leaf essential oil of *Hoslundia opposita* has significant ameliorative effect on alloxan-induced anaemia. This may be of immense benefits in the management of type 2 diabetes and its associated haematological complications, although, administration of higher dosage (220 mg/kg body weight) of the oil might have deleterious effect in non-pathological condition.

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