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Research Article

The hematological action of ethanol extracts of *Gongronema latifolium* and *Ocimum gratissimum* in Alloxan induced Diabetic rats

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ABSTRACT

Hematological indices after treatment with low dose concentration of individual extract of *gongronema latifolium* (200 mg/kg), *ocimum gratissimum* (200 mg/kg) and combined extracts of *gongronema latifolium* and *ocimum gratissimum* (400 mg/kg) in alloxan induced diabetes mellitus was studied in rats for thirty days. The PCV, Hb concentration, RBC count, MCV, MCH and MCHC were evaluated. The results showed a significant increase ($P < 0.05$) in PCV, Hb concentration and RBC count in groups administered with individual extracts and combined extracts without statistically significant difference ($P > 0.05$) in MCV, MCH and MCHC. It is concluded that low dose concentration of ethanol extracts *gongronema latifolium*, *ocimum gratissimum* and/or combined extracts increased PCV, Hb concentration and RBC count without change in MCV, MCH and MCHC levels.

Keywords: *Gongronema latifolium, Ocimum gratissimum*, Blood, Alloxan.

INTRODUCTION

Gongronema latifolium (Asclepiadaceae) is a climbing perennial plant.[1] The plant is harvested from forest in Southeastern States of Nigeria[2] and is commonly locally called "Utazi" and "Arokeke" in the Southeastern and Southwestern States in Nigeria.[3] It is an edible rainforest plant native to the South Eastern part of Nigeria, has been widely used in folk medicine as a spice and vegetable[4]. Several studies have reported that aqueous and ethanol extract of the plant exhibited hypolipidemic[5], anti-lipid peroxidative[6], hypoglycemic[5,6], antidiabetic[7], hepatoprotective[8-11].

Ocimum gratissimum (lamiaceae) is believed to originate from Asia and Africa[12] is a perennial plant that is woody at the base with an average height of 1-3 m high. In Nigeria, this plant is called "effinrin-nia" by the Yoruba's, "Nchumou" in Igbo and "diadoya" in Hausa[13]. It is used extensively used throughout West Africa as a febrifuge, antimalarial and anti-convulsant[14], treatment of respiratory tract infection[15] and diarrhea[16] and possess antioxidant activity[17-19].Administration of aqueous leaf extract caused a statistically significant reduction in plasma glucose[20-22]. One of the most potent methods to induce

experimental diabetes mellitus is chemical induction by alloxan[23]. It is a well known diabetogenic agent that is used to induce type-I diabetes in experimental animals[24]. Although diabetic condition produces alteration in hematological indices, there has been conflicting reports on the effects of *gongronema latifolium* and *ocimum gratissimum* on hematological indices in experimental rats[25, 26]. Thus we evaluated firstly the effect of these extracts on PCV, Hb concentration, RBC count, MCV, MCH and MCHC.

MATERIALS AND METHODS

Animal models

Twenty-five male albino wistar rats weighing (160-250 g) were used in the study. The animals were obtained from the Animal House of Department of Pharmacology, College of Medicine and Health Sciences, University of Port Harcourt, Nigeria. They were kept under standard laboratory condition and fed with commercial Growers mesh (Top Feeds Ltd.) and water *ad libitum*. The animals were kept in plastic cages and allowed to acclimatize for 2 weeks. The rats were divided into five groups namely groups I, II, III, IV and V. Twenty overnight fasted rats from groups II, III, IV and V rats were made diabetic using single intraperitoneal injection (i.p.) of freshly prepared solution of alloxan monohydrate (100 mg/kg body weight) dissolved in physiological solution. The alloxanized rats were kept for two days with free access to food and water. The rats were fasted on the 3rd day for 12 hours and their blood glucose levels were determined using Finetest glucometer and its corresponding strips. The rats that exhibited glucose level above 200 mg/dl were used for the study.

Extraction of plant material

Gongronema latifolium and *ocimum gratissimum* were purchased from the local market in Elele, Rivers State. The fresh leaves were washed ad sundried for 7 days. The dried leaves were grounded into fine powder and packed separately. About 200 g of the fine powder of the two leaves each were extracted with 1000 ml of ethanol by cold maceration for 48 hours and filtered. The preparation was filtered using Whatman No. 1 filter paper and the filtrate was dried in a hot air oven to obtain the ethanol extract (100 g). This method was used in the extract of the two plants respectively. From the stock solution appropriate volumes were taken.

Study protocol

The extracts of *ocimum gratissimum* and *gongronema latifolium* was administered twice daily by gavage. Group I (5 rats) were used as controls. Group II (5 rats) received food and water only. Group III (5 rats) received 200 mg/kg of *ocimum gratissimum* extract. Group IV (5 rats) received 200 mg/kg of *gongronema latifolium* extract. Group V (5 rats) received 100 mg/kg of *ocimum gratissimum* and *gongronema latifolium* respectively.

Blood sample collection and analysis

Blood glucose level was monitored simultaneously as the administration of extract progressed throughout the duration of the experiment. At the end of the experiment, the rats were anaesthetized under chloroform and sacrificed. 5 ml of blood was collected via cardiac puncture from each rat and put into EDTA container. From the blood samples collected, red blood cell (RBC) count was done using the methods by Dacie and Lewis[27] and Antai et al[28]. Blood was diluted to 1:200 with Hayem's fluid which preserved the corpuscles and then counted with Neubauer counting chamber under a light microscope. Sahli's hemoglobinometer was employed for estimation of hemoglobin (Hb) content of the blood while the packed cell volume (PCV) was done using the microhaematocrit method[27].

Calculation of mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC).

The different absolute values: mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were calculated from values of RBC, PCV and Hb as follows:

MCV (μm) = PCV (%) x 10/RBC count (x 10^6/mm^3)

MCH (pg) = Hb (g/dl) x 10/RBC count (x 10^6/mm^3)

MCHC (pg) = Hb (g/dl) x 100/PCV (%)

Statistical analysis

The data obtained was analyzed using the Statistical Package for Social Sciences (SPSS version 16.0 for windows). Analysis of variance (ANOVA) was used to compare means, and values were considered significant at $P < 0.05$.

RESULTS

Table 1 shows the blood glucose level before and after administration of alloxan induced diabetes mellitus. The experimental groups II, III, IV and V exhibited blood glucose levels ($P < 0.05$) above 200 mg/dl respectively. Table 2 shows that blood glucose level of groups III, IV and V was significantly reduced ($P < 0.05$) at the 3rd week of experiment compared to group II. There was significant difference ($P < 0.05$) in the blood glucose at the 3rd week between group I compared to group III, IV and V respectively.

Effect of with *ocimum gratissimum* (O. G) and *gongronema latifolium* (G. L) on hematological parameters

Table 3 shows the effect of *ocimum gratissimum* and *gongronema latifolium* extract on PCV, Hb, RBC, MCV, MCH and MCHC. The table shows that treatment of III, IV and V respectively caused a significant increase ($P < 0.05$) in PCV, Hb, RBC, MCV, MCH and MCHC levels compared to group II. There was no significant difference in PCV, Hb, RBC, MCV, MCH and MCHC levels ($P > 0.05$) between group I compared with group III, IV and V respectively.

DISCUSSION

The significant reduction in the blood glucose level after treatment with *ocimum gratissimum* and *gongronema latifolium* observed in this study agrees with previous reports in rats[5, 6, 20-22, 29-31]. This significant reduction in blood glucose may be due to the antidiabetic properties of individual extracts. Several studies have showed that *ocimum gratissimum* reduced blood glucose level in diabetic induced rats[20-22] as well as *gongronema latifolium* extract[5, 6,29,30]. The reduction in the blood glucose of group IV and V, although not statistically significant could explain the antidiabetic potency of *gongronema latifolium* extract. Udoh et al.[31] have reported insulin-like activity of *gongronema latifolium*. The phytochemical, polyphenol in *gongronema latifolium* has being reported to possess antidiabetic activity[32]. Therefore, it is credible to suggest that this antidiabetic activity (table 2) may be solely dependent on the activity of *gongronema latifolium* extract and to a lesser degree on the activity of *ocimum gratissimum*.

Result (table 3) revealed that 200 mg/kg of *ocimum gratissimum*, 200 mg/kg of *gongronema latifolium* and 400 mg/kg of both extracts significantly increased PCV, Hb concentration and RBC count. Conversely, several studies have shown that *ocimum gratissimum* extract significantly reduced hematological parameters[33] and at high dose concentration[34]. As observed in the present study, the increase in these hematological parameters may be dose-related since at low dose (200mg/kg of *ocimum gratissimum*) these hematological parameters significantly increased. Studies have shown that low dose concentration of *ocimum gratissimum* extract, increased RBC count in dose and duration-related[35], increased PCV level, an effect dependent on sex[36] and increased PCV level, Hb concentration and RBC count[37]. These reports are in corroboration with our result (table 3) suggesting that *ocimum gratissimum* extract increased PCV level, Hb concentration and RBC count at low dose concentration. *Gongronema latifolium* extract (200mg/kg) significantly increased the levels on PCV, Hb and RBC count (table 3). These results obtained in the present study agree with early studies[38], which reported increase in RBC count, PCV and Hb levels following administration of *gongronema latifolium* extract. This increase in these hematological parameters may be dose-related because data have showed that PCV and Hb levels were significantly increased at low dose concentration with little or no effect on the RBC count[25], whereas increased dose concentration of extract decreased RBC count, Hb and PCV levels[38-39]. Insulin-induced hypoglycemia increased venous hematocrit and decreased plasma volume[40]. Reports have also suggested a direct action of insulin on erythroid progenitors, indicating that insulin stimulated the formation of colony forming unit-erythroid and burst forming unit-erythroid[41], and directly stimulated the proliferation of the late stage of primitive erythroid progenitor cells and mature erythroid progenitor cells through the sharing of receptors[42]. Thus, it is credible to suggest that low dose concentration of *gongronema latifolium* extract increased RBC count, PCV and Hb levels which could be insulin-dependent. Thus it is not surprising in the present study that combined extract of *ocimum latifolium* and *gongronema latifolium* extracts increased the hematological parameters so much than individual extracts although not statistically significant. The levels of MCV, MCH and MCHC were unaltered at low dose concentration of *ocimum gratissimum* and *gongronema latifolium* extracts corroborating with earlier studies by Antai et al.[25] who reported that *gongronema* latifolium extract did not alter MCV, MCH and MCHC. Although studies[38] have showed that *gongronema latifolium* extract reduced MCH and MCHC in high dose groups. Therefore, it can be concluded that at low dose concentration, individual extract of *ocimum gratissimum* or/and *gongronema latifolium* extracts increased hematological parameters in alloxan induced diabetic rats.

Table 1
Blood glucose level before and after administration of diabetes mellitus

Groups	Blood glucose before induction of diabetes (mg/dl)	Blood glucose after induction of diabetes (mg/dl)
I Control	77.25 ± 8.70	77.25 ± 8.70
II Diabetic Control	63.50 ± 4.09	245.50 ± 20.99
III 200mg/kg O.G.	78.75 ± 6.66	308.75 ± 53.07
IV 200mg/kg G.L.	86.25 ± 2.56	290.75 ± 43.67
V 400mg/kg of O.G + G. L	78.25 ± 3.61	382.00 ± 27.36

Table 2
Blood glucose level at different weeks and at the end of experiment after treatment with *ocimum gratissimum* (O. G) and *gongronema latifolium* (G. L)

Treatment groups	Blood glucose level after first week of treatment (mg/dl)	Blood glucose level after second week of treatment (mg/dl)	Blood glucose level at the third week of experiment (mg/dl)
I Control	69.50 ± 4.21	79.25 ± 2.29	72.50 ± 2.02
II Diabetic control	262.25 ± 20.30[a]	266.75 ± 20.37[a]	284.00 ± 32.88[a]
III 200mg/kg O.G.	237.50 ± 34.96[a]	192.75 ± 54.29[ab]	128.75 ± 28.81[ab]
IV 200mg/kg G.L.	250.25 ± 41.96[a]	142.75 ± 23.48[ab]	96.75 ± 7.44[ab]
V 400mg/kg of O.G+ G.L	283.50 ± 40.47[a]	141.88 ± 45.23[ab]	107.25 ± 4.96[ab]

Data represented as mean + SEM; ([a]) P < 0.05 significant difference between control ([b]) P < 0.05 significant difference between diabetic control

Table 3
Some hematological parameters after treatment with *ocimum gratissimum* (O. G.) and *gongronema latifolium* (G. L.) at the end of the experiment

Parameters	I Control	II Diabetic control	III 200mg/kg of O.G	IV 200mg/kg of G.L	V 400mg/kg of O.G + G.L
PCV (%)	34.25 ± 0.85	24.25 ± 2.53[a]	38.00 ± 0.41[ab]	38.50 ± 0.65[ab]	39.50 ± 0.65[ab]
Hb (g/dl)	11.38 ± 0.19	5.93 ± 0.70[a]	12.30 ± 0.17[ab]	12.70 ± 0.13[ab]	13.23 ± 0.13[ab]
RBC (x 10^6/cubic mm)	5.56 ± 0.03	2.08 ± 0.61[a]	6.30 ± 0.04[ab]	6.65 ± 0.11[ab]	6.82 ± 0.06[ab]
MCV (pg/cell)	61.43 ± 1.38	43.04 ± 2.24[a]	60.35 ± 0.48[b]	57.98 ± 1.85[b]	58.78 ± 0.11[b]
MCH (pg/cell)	20.30 ± 0.24	17.50 ± 1.04[a]	19.53 ± 0.18[b]	19.13 ± 0.49[b]	18.98 ± 0.19[b]
MCHC (mg/cell)	32.85 ± 0.40	18.50 ± 2.95[a]	32.45 ± 0.54[b]	33.18 ± 0.12[b]	32.25 ± 0.10[b]

Data represented as mean + SEM; ([a]) P < 0.05 significant difference between normal control ([b]) P < 0.05 significant difference between diabetic control,

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