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Original Research Article

Consumption of Aqueous Leaf Extract of *Piper guineense* Alters Hematological and Biochemical Parameters in Wistar Rats

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Abstract

Much research has concentrated on the seed extracts of *Piper guineense* and not on the leaves which are widely consumed and hence this study. Fifteen male wistar rats weighing 150-250g were randomly divided into control, low dose (LD) and high dose (HD) groups of five rats each. LD and HD groups received 250mg/kg and 350mg/kg b.w. respectively of aqueous leaf extract of *Piper guineense* by gavaging. Duration of feeding was thirty days. The results showed significant increases ($p<0.001$) in serum levels of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) in both LD and HD groups compared with control. Serum total cholesterol (TC) was significantly ($p<0.001$) increased in both LD and HD groups compared with control. Serum triglyceride (TG) was significantly increased in HD group compared with control ($P < 0.001$) and LD ($P<0.001$) groups. High density lipoprotein (HDL-c) was significantly increased ($p<0.001$) in LD compared with control and decreased ($p<0.001$) in HD compared with control and LD groups. Low density lipoprotein (LDL-c) was significantly increased in LD ($p<0.001$) and HD ($p<0.01$) compared with control and decreased in HD ($p<0.001$) compared with LD. TG/HDL-c in the HD group was significantly ($p<0.001$) increased compared with control and LD groups. Aside RBC count and MCH which were significantly increased ($p<0.05$) and decreased ($p<0.05$) respectively in HD compared with LD group, all other haematological parameters (Hb, PCV, MCHC, MCV and WBC and platelet counts) did not differ significantly among the groups. In conclusion, consumption of aqueous leaf extract of *Piper guineense* causes dyslipidemia and elevation of liver enzymes but no significant effect on hematological indices.

Keywords: Haematological parameters, Lipid profile, Liver enzymes, *Piper guineense*, Wistar rat.

INTRODUCTION

Piper guineense is a widely consumed vegetable in the tropics especially in the south eastern part of Nigeria. This plant finds its usefulness in culinary, nutritional and medicinal purposes. However, most attention has focused on its fruit and seed extracts and hence the need to investigate the effects of its aqueous leaf extract. The west African species of *Piper guineense* (commonly called hot leaf) which is a climbing plant is commonly found in the tropics and subtropical regions. It is an important vegetable for food especially among the south easterners of Nigeria [1]. It is also used as food additive due to its spicy nature. The seeds and leaves of this plant also find their way in traditional herbal medicine for treatment of malaria [2], respiratory infections [3] and as an aphrodisic. The leaves are also used as a preparation for post-partum women to encourage uterine involution [4], have antifertility effects [5], antiparasitic and antifungal effects [2] as well as anticonvulsant effect [6]. The leaves extract has been shown to increase testicular weight [7] and elevates serum level of follicle stimulating hormone (FSH) [8].

Aqueous leaf extract of *Piper guineense* contains cardiac glycoside, alkaloids, sapponins, tannins, flavonoids, polyphenol [4], piperanine, dihydrowasanine [9]. From the foregoing, it can be understood that the leaf extract of Piper guineense has varied applications as a vegetable, additive and as a medicinal agent. Unfortunately, not much is documented on its hematological effects as well as its effect on serum liver enzymes and lipid profile hence this study.

MATERIALS AND METHODS

Experimental animals

Fifteen male Wistar rats weighing 150 to 250g were used for the experiment. They were housed at room temperature in metallic cages in the animal house

of the Department of Physiology, University of Calabar, Nigeria under a 12-hour light and 12-hour dark cycle. Ethical approval for the study was gotten from the Ethics Committee of the Faculty of Basic Medical Sciences, University of Calabar.

Preparation of aqueous leaf extract of *Piper guineense*

This was done as described by Dahiru *et al.*, [10]. Briefly, the leaves that were bought from a local market in Calabar, Nigeria, were washed and dried. They were grounded to fine powder, 300g of which was soaked in 2400ml of water for 24 hours. It was filtered with Whatman's filter paper and the filtrate oven-dried into a paste, 1g of which was dissolved in 10ml of water to form the stock from which the various doses were drawn.

Experimental design

The fifteen male Wistar rats were randomly grouped into three groups. Group 1 was a control group while groups 2 (LD) and 3 (HD) were test groups. LD and HD groups received low dose (250mg/kg) and high dose (350mg/kg) respectively of aqueous leaf extract of *Piper guineense* by gavaging. All rats had free access to rat feed and portable water daily for 30 days of the experiment.

Collection of blood samples

The rats were anesthetized using 3.5 % chloroform. Blood samples were collected via cardiac puncture using 5mL syringe attached to 21G needle. The collected blood samples were poured into pre labelled ethylenediaminetetracetate (EDTA) vials and plain capped sample bottles. The blood samples in the EDTA vials were used for measurement of haematological parameters while blood samples in the plain sample bottles were left for two hours to clot after which they were centrifuged at 10,000 rpm for 10 minutes and the serum collected for analysis of serum lipid profile and liver enzymes.

Determination of hematological parameters

Haematological parameters were measured using automated cell counter (Coulter Electronics, Luton, Bedfordshine, UK) having standard calibrations in line with the instructions of the manufacturer. Parameters measured were: red blood cell (RBC) count, haemoglobin (Hb) concentration, packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), white blood cell (WBC) count and platelet count.

Determination of serum AST and ALT

Serum AST and ALT were determined according to the method of Rietman and Frankel [11]. The absorbance was measured colorimetrically at a wave length of 520nm.

Evaluation of serum lipid profile

Total cholesterol was determined by enzymatic colorimetric method using Giesse Diagnostic kit and absorbance measured at 520nm wave length. Triglycerides concentration was accessed enzymatically using a series of coupled reactions in which triglycerides were hydrolysed to glycerol which is then oxidized. High density lipoprotein cholesterol was measured in a method where magnesium/dextran sulphate solution was first added to the specimen to form water soluble complexes with non-HDL-cholesterol fractions. Absorbance was measured at 520nm wave length, Low density and very low density lipoprotein cholesterol levels where computed.

Statistical Analysis

Data are expressed as mean±standard error of mean (SEM) and were analyzed by one way analysis of variance (ANOVA) followed with a post hoc test of least significant difference using statistical package for social science (SPSS). P-values of $p<0.05$ were considered statistically significant.

RESULTS

Comparison of hematological parameters in the different experimental groups

Red blood cell (RBC), hemoglobin (Hb) concentration and packed cell volume (PCV)

Table-1 shows red blood cell (RBC) count ($x10^6$ cell/µL), hemoglobin (Hb) concentration (g/dL) and packed cell volume (PCV) for control, LD and HD groups. RBC count was significantly ($p<0.05$) increased in HD group compared with LD group. RBC count in LD and HD groups was not significantly different from control. There was no significant difference in Hb concentration and PCV among the groups.

Table-1: Comparison of RBC count, Hb concentration and PCV in the different experimental groups

Groups	RBC ($x10^6$ cell/µL)	Hb (g/dL)	PCV (%)
Control	6.78±0.32	13.08±0.34	41.78±1.18
LD	$6.16±0.54^{ns}$	$12.34±0.99^{ns}$	$38.76±2.95^{ns}$
HD	$7.33±1.56^{ns, a}$	$13.72±0.34^{ns}$	$44.38±1.25^{ns}$

Values are expressed as mean±SEM, n=5
ns = not significant vs control
a = $p<0.05$ vs LD

Red blood cell absolute values (MCV, MCH and MCHC)

Table-2 shows red blood cell absolute values for control, LD and HD groups. MCV (fL) and MCHC (g/dL) were not significantly different among the groups. MCH in LD and HD groups was not significantly different compared with control. However, MCH (pg) was significantly ($p<0.05$) decreased in HD group compared with LD group.

Table-2: Comparison of RBC absolute values in the different experimental groups

Groups	MCV (fL)	MCH(pg)	MCHC (g/dL)
Control	61.82±1.46	19.36±0.42	31.30±0.25
LD	63.34±1.56^{ns}	20.12±0.25^{ns}	31.80±0.49^{ns}
HD	60.56±1.13^{ns}	18.64±0.36^{ns,a}	30.96±0.27^{ns}

Values are expressed as mean±SEM, n=5
ns = not significant vs control
a = p<0.05 vs LD

White blood cell (WBC) and platelet counts

Table-3 shows white blood cell (WBC) and platelet counts for control, LD and HD groups which were not significantly different among the groups.

Table-3: Comparison of WBC and platelet counts in the different experimental groups

Groups	WBC (x10^3 cell/µL)	Platelet (x10^3 cell/µL)
Control	7.60±1.39	836.80±145.43
LD	10.66±2.44	750.20±180.18
HD	7.58±1.2	846.20±66.05

Values are expressed as mean±SEM, n=5
ns = not significant vs control

Comparison of serum liver enzymes concentrations in the different experimental groups

Table-4 shows serum liver enzymes (AST and ALT) concentrations for control, LD and HD groups. Serum AST (mmol/L) and ALT (mmol/L) concentrations were significantly (p<0.001) increased in LD and HD groups compared with control. The concentrations of these enzymes were significantly (p<0.001) increased in HD group compared with LD group. Ratio of AST to ALT (AST/ALT) was significantly (p<0.001) decreased in LD and HD groups compared with control.

Table-4: Comparison of serum liver enzymes concentrations in the different experimental groups

Groups	AST(mmol/L)	ALT(mmol/L)	AST/ALT
Control	41.40±1.32	65.2±3.26	0.64±0.01
LD	64.8±3.91***	129.8±1.56***	0.50±0.02**
HD	81.21.60^{***,c}	164.4±2.51^{***,c}	0.49±0.00**

Values are expressed as mean±SEM, n=5
ns = not significant vs control
***p<0.001 vs control
c = p<0.001 vs LD

Comparison of serum lipid profile in the different experimental groups

Table-5 shows serum lipid profile for control, LD and HD groups. Serum TC concentration (mmol/L) was significantly increased (p<0.001) in LD and HD groups compared with control and decreased (p<0.001) in HD group compared with LD group. TG concentration (mmol/L) was significantly increased in HD group compared with control (p<0.001) and LD (p<0.001) groups. Serum HDL-c was significantly increased (p<0.001) in LD group and decreased (p<0.001) in HD group compared with control. Serum HDL-c was significantly (p<0.001) decreased in HD group compared with LD group. Serum LDL-c was significantly increased in LD (p<0.001) and HD (p<0.01) groups compared with control but significantly decreased (p<0.001) in HD group compared with LD group. Serum VLDL-c was significantly increased in HD group compared with control (p<0.001) and LD (p<0.001) groups. TC/HDL-c and TG/HDL-c were significantly increased (p<0.001) in HD group compared with control. TG/HDL-c was also significantly increased (p<0.001) in HD group compared with LD group. LDL-c/HDL-s was significantly increased in LD (p<0.01) and HD (p<0.001) groups compared with control.

Table-5: Comparison of serum lipid profile in the different experimental groups

Group	TC (mmol/L)	TG (mmol/L)	HDL-c (mmol/L)	LDL-c (mmol/L)	VLDL-c (mmol/L)	TC/HDL-c	TG/HDL-c	LDL-c/HDL-c
Control	1.02±0.05	0.26±0.02	0.49±0.03	0.41±0.05	0.12±0.01	2.08±0.11	0.53±0.14	0.85±0.12
LD	2.12±0.07***	0.36±0.04^{ns}	0.67±0.02***	1.35±0.05***,	0.17±0.01^{ns}	3.16±0.12^{ns}	0.54±0.11^{ns}	2.03±0.12*
HD	1.34±0.05^{***,c}	0.7±0.04^{***,c}	0.29±0.03^{***,c}	0.71±0.07^{*,c}	0.32±0.02^{***,c}	4.62±0.50***	2.41±0.40^{***,c}	2.57±0.39***

Values are expressed as mean±SEM, n=5
ns = not significant vs control
**p<0.01, ***p<0.001 vs control
c = p<0.001 vs LD

DISCUSSION

The effect of prolonged ingestion of aqueous leaf extract of *Piper guineense* on some hematological parameters, liver enzymes and lipid profile was investigated.

Aside RBC count and MCH which were significantly increased and decreased respectively in HD group compared with LD group, all other haematological parameters evaluated in this study did not demonstrate any significant differences among the groups (Table 1, 2 and 3). It can therefore be assumed that at the dosages and duration of treatment used in the present study, aqueous leaf extract of *Piper guineense* may not impair hematological functions.

Our results for serum liver enzymes strongly suggest that prolonged consumption of aqueous leaf extract of *Piper guineense* could have adverse effects on liver functions. The results demonstrated significant elevations in serum AST and ALT in the extract-treated groups in a dose related manner (Table-4). Both AST and ALT are sensitive indicators of hepatocellular injury though they lack specificity because they are present in other tissues like red blood cell, kidney, brain as well as skeletal and cardiac muscles in small amounts [12]. Nevertheless, Serum AST and ALT still remain the basic biochemical tests for assessing hepatocellular injury [13, 14]. Aspartate amino transferase is a cytosolic and mitochondrial enzyme [15] while alanine aminotransferase is a cytosolic hepatocyte enzyme. Hepatocellular injuries could arise from toxic or ischemic insults to the liver, acute hepatitis by hepatitis B and C viruses and metabolic insults to the liver. Because these enzymes are in the hepatocytes, damage to hepatocytes from these insults liberate the enzymes which "leak" into circulation [16]. They are normally higher in acute injuries. So the significant dose-dependent increase in serum AST and ALT in extract-fed rats could have resulted from hepatic injury since the liver is the major organ of origin of these enzymes [13]. The effect was worse in the HD group as serum AST and ALT concentrations were significantly ($p<0.001$) increased in this group compared with LD group. The ratio of AST to ALT was less than one (<1). This ratio is used clinically to differentiate alcoholic from other forms of liver toxicity [17, 18]. So our observed ratio of <1 suggests the possible effect of another toxic substance, the aqueous leaf extract of *Piper guineense*.

Evaluation of the lipid profile status is of utmost importance because dyslipidaemia is associated with arteriosclerosis and coronary heart diseases [19, 20]. Our results showed that consumption of this extract is associated with dyslipidaemia. Both doses of the extract caused a significant increase in serum TC compared with control but TC was lower in the HD group compared with the LD group (Table-5**)**. This may suggest the more serious effect of lower doses of this extract on cholesterol homeostasis. Though the significant increase in LDL-c appeared to have been countered by similar increase in HDL-c, the LDL-c/HDL-c ratio was significantly increased in the LD group compared with control suggesting that low doses of aqueous leaf extract of *Piper guineense* distort cholesterol metabolism which is implicated in cardiovascular diseases [20]. The increase in TC, TG, LDL-c, TG/HDL-c, TC/HDL-c and LDL-c/HDL-c as well as the observed decrease in HDL-c in the HD group compared with the control (Table-5) also suggest that at high doses, aqueous leaf extract of *Piper guineense* is associated with altered lipid metabolic state implicated in cardiovascular diseases [20, 21].

CONCLUSION

In conclusion, prolonged ingestion of aqueous leaf extract of *Piper guineense* has no untoward effect on blood indices but causes elevation of serum liver enzymes in a dose-related manner and dyslipidaemia at both low and high doses.

REFERENCES

1. Udoh, F. V., Akpan, J. O., & Ufaruna, N. (1996). Effect of Extract of Leaf and Seed of Piper guineense on Some Smooth Muscle Activity in Rat, Guinea-pig and Rabbit. *Phytotherapy Research*, *10*(7), 596-599.
2. Ekanem, A. P., & Obiekezie, A. I. (2000). Antiparasitic effects of leaf extract of Piper guineense (Husani) on the juveniles of Heterobranchus longifilis (Cuvier and Valencieness). *Afr. J. Fisher Aquacult*, *8*, 74.
3. Moundipa, F. P., Kamtchouing, P., Koueta, N., Tantchou, J., Foyang, N. P. R., & Mbiapo, F. T. (1999). Effects of aqueous extracts of Hibiscus macranthus and Basella alba in mature rat testis function. *Journal of ethnopharmacology*, *65*(2), 133-139.
4. Udoh, F. V. (1999). Uterine muscle reactivity to repeated administration and phytochemistry of the leaf and seed extracts of Piper guineense. *Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives*, *13*(1), 55-58.
5. Mbongue, F. G., Kamtchouing, P., Essame, O. J., Yewah, P. M., Dimo, T., & Lontsi, D. (2005). Effect of the aqueous extract of dry fruits of Piper guineense on the reproductive function of adult male rats. *Indian journal of pharmacology*, *37*(1), 30-32.
6. Abila, B., Richens, A., & Davies, J. A. (1993). Anticonvulsant effects of extracts of the West African black pepper, Piper guineense. *Journal of ethnopharmacology*, *39*(2), 113-117.
7. Okoye, C. N., Obasi, O. L., & Ezechukwu, O. (2016). Effects of methanolic leaf extract of Piper guineense on some reproductive parameters of male albino rats (Rattus norvegicus). *Animal Research International*, *13*(3), 2548-2553.
8. Agbai, E. O., Onyebuagu, P. C., Njoku, C. J., Ekezie, J., Eke, C. C., Nwanegwo, C. O., & Nwafor, A. C. (2017). Piper guineense leaf extract elevates serum follicle stimulating hormone level in the diestrus phase in non-pregnant female albino

Wistar rats. *Journal of Complementary and Alternative Medical Research*, *2*(4), 1-8.

9. Ekanem, A. P., Wang, M., Simon, J. E., Obiekezie, A. I., & Morah, F. (2004). In vivo and in vitro activities of the seed extract of Piper guineense Schum and Thonn against skin and gill monogenean parasites of goldfish (Carassius auratus auratus). *Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives*, *18*(10), 793-797.
10. Dahiru, D., Onubiyi, J. A., & Umaru, H. A. (2006). Phytochemical screening and antiulcerogenic effect of Moringa oleifera aqueous leaf extract. *African Journal of Traditional, Complementary and Alternative Medicines*, *3*(3), 70-75.
11. Reitman, S., & Frankel, S. (1957). A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. *American journal of clinical pathology*, *28*(1), 56-63.
12. Pratt, D. S., & Kaplan, M. M. (2000). Evaluation of abnormal liver-enzyme results in asymptomatic patients. *New England Journal of Medicine*, *342*(17), 1266-1271.
13. Giannini, E. G., Testa, R., & Savarino, V. (2005). Liver enzyme alteration: a guide for clinicians. *Cmaj*, *172*(3), 367-379.
14. Goorden, S. M., Buffart, T. E., Bakker, A., & Buijs, M. M. (2013). Liver disorders in adults: ALT and AST. *Nederlands tijdschrift voor geneeskunde*, *157*(43), A6443-A6443.
15. Johnston, D. E. (1999). Special considerations in interpreting liver function tests. *American family physician*, *59*, 2223-2232.
16. Haber, M. M., West, A. B., Haber, A. D., & Reuben, A. (1995). Relationship of Aminotransferases to Liver Histological Status in Chronic Hepatitis C 1. *American Journal of Gastroenterology*, *90*(8), 1250-1257.
17. Sorbi, D., Boynton, J., & Lindor, K. D. (1999). The ratio of aspartate aminotransferase to alanine aminotransferase: potential value in differentiating nonalcoholic steatohepatitis from alcoholic liver disease. *The American journal of gastroenterology*, *94*(4), 1018-1022.
18. Nyblom, H., Berggren, U., Balldin, J., & Olsson, R. (2004). High AST\ALT ratio may indicate advanced alcoholic liver disease rather than heavy drinking. *Alcohol and Alcoholism*, *39*(4), 336-339.
19. Luz, P. L. D., Favarato, D., Faria-Neto Junior, J. R., Lemos, P., & Chagas, A. C. P. (2008). High ratio of triglycerides to HDL-cholesterol predicts extensive coronary disease. *Clinics*, *63*(4), 427-432.
20. Ballantyne, C. M., & Hoogeveen, R. C. (2003). Role of lipid and lipoprotein profiles in risk assessment and therapy. *American heart journal*, *146*(2), 227-233.
21. Packard, C. J., & Shepherd, J. (1997). Lipoprotein heterogeneity and apolipoprotein B metabolism. *Arteriosclerosis, thrombosis, and vascular biology*, *17*(12), 3542-3556.