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Effect of Aqueous Extract of Piper Guineense Seeds on Some Liver Enzymes, Antioxidant Enzymes and Some Hematological Parameters in Albino Rats

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Abstract

The effect of aqueous extract of *Piper guineense* seeds on some liver enzymes, antioxidant enzymes and some hematological parameters in albino rats was investigated. Thirty female albino rats, aged 7 weeks and weighing between 128g to 142g were randomly placed into three groups of 10 animals each. Group1 served as control and received no extract except 5ml of physiological saline as a placebo, while group 2 and 3 received the aqueous seed extract intraperitonally with 5ml of physiological saline as the vehicle at 10mg/kg body weight and 20mg/kg body weight respectively. Results show that the liver enzymes: alanine transaminase (ALT), aspartate transaminase (AST) and alkaline phosphatase (ALP) decreased ($p \leq 0.05$) significantly. Catalase (CAT) and glutathione peroxidase also decreased significantly. ALT, AST and ALP decreased from 45.25 ± 0.31 to 36.69 ± 0.33 (IU/L); 33.10 ± 0.43 to 27.48 ± 0.27(IU/L) and 105.10 ± 0.24 to 89.19 ± 0.41 (IU/L) respectively. CAT and GSH decreased from 0.33 ± 0.11 to 0.17 ± 0.12 (IU/L) and 38.08 ± 0.31 to 33.07 ± 0.27 (IU/L) respectively. Red blood cell count, white blood cell count and hemoglobin concentration increased ($p \leq 0.05$) significantly from 241.33 ± 2.93 x 10^6 to 298.10 ± 4.28 x 10^6 µ/l; 129.67 ± 2.10 x 10^3 to 194.67 ± 4.82 x 10^3 µ/l and 10.33 ± 0.20 to 12.04 ± 0.22 g/dl respectively. The effect of the extract on these parameters is dose dependent. These findings are of clinical importance. Nursing mothers in the South East of Nigeria are usually placed on diets prepared with this spice. It is believed this spice aid in the fast return of the uterine muscles to the original shape and effect increase in the flow of the nursing mothers' breast milk.

Keywords

Medicinal Plant, Spice, Hepatoprotective, Antioxidant, Seeds, Piper Guineense

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1. Introduction

Piper guineense (Uziza or black pepper) is a West African tropical plant, widely consumed as spice and is considered medicinal by some people [1; 2]. Spices are aromatic vegetable substances that are used in small quantities for seasoning of foods and also used as preservatives or flavouring and thus enhancing the taste of food, beverage and drugs [3]. Spices contain essential oils which are complex mixtures of organic compounds called phytochemicals. These compounds give spices their characteristic odors, flavors and other properties [4; 5]

Piper guineense have nutritional and non nutritional factors which are responsible for its aroma, flavor and preservative properties. It is rich in protein, fat, fiber and carbohydrate. Proximate analysis results showed that on dry weight basis *Piper guineense* contains crude protein from 4.6 to 22.1%, fat (ether extract) ranged from 7.5 to 36.0%, while total carbohydrate content ranged from 34.6 to 71.9%. The vitamin content is also high especially vitamin C. Peroxide

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value and free fatty acid levels are generally low. The essential oils content are fairly high and range from 0.1 to 5.2% [6]. Juliani *et al*.; [7] reported that *Piper guineense* is an important plant used in traditional medicine and as spice. The fruits of *Piper guineense* (which is the part of the plant that is traditionally used) are rich in a wide range of natural products including volatile oils, lignans, amides, alkaloids, flavonoids and polyphenols.

Bep, [8] reported that the seeds of *Piper guineense* is used to relieve discomfort in the stomach caused by excess gas. The leaves of the plant are used to treat respiratory infections, rheumatism and syphilis. Okigbo and Igwe [9]; Kim-Kabari *et al*.; [10] reported that *Piper guineense* is used as preservative, while Agoha, [11] reported that the leaves and seeds of the plant is used to flavour soup, stew and food generally. Abila *et al*.; [12]; Oleifa *et al*.; [13] also reported the presence of an important alkaloid - pipereine. The pipereine contains strong anticonvulsant called anti-epileparine [N-3-4- methylene dioxycinamyl piperidine] and has been synthesized for the treatment of epilepsy [14]. Echo *et al*.; [6] reported that methanolic extracts of the plant have molluscidal properties and may be useful in the treatment of bilharzia. The plant is claimed to be therapeutically useful in the management of convulsion, leprosy, inflammation and/or rheumatoid pains [15; 6]. Amvam-Zollo *et al*.; [16] and Okigbo and Igwe [9] also reported the bacteriostatic and bacteriocidal effects of *Piper guineese* on some bacteria and the leaves are considered aperitive, carminative and eupeptic. The leaves are also used for female infertility while the fruits are used as an aphrodisiac [17].

Generally, *Piper guineense* seeds are used in the preparation of drugs for treatment of cough, intestinal diseases, veneral diseases, cold and rheumatism. It is also used for preparation of non-toxic insecticides and perfumes [18; 19].

In the South Eastern parts of Nigeria, the fruits are used to prepare soups for mothers from the first day of delivery to prevent post partum contraction and to aid in the fast return of the uterine muscles to the original shape, and increase the flow of the nursing mothers' breast milk.

The present study is therefore aimed at investigating the effect of aqueous extract of *Piper guineense* seeds on some liver enzymes, antioxidant enzymes and some hematological parameters in female Albino rats. The results will help reveal the likely clinical effects of the spice on these parameters on consumers.

2. Materials and Methods

Piper guineense seeds

The Piper guineense seeds were bought from the Eke Okigwe market, Okigwe, Imo State as sold. The seeds were identified and authenticated at the Department of Plant Science and Biotechnology, Abia State University, Uturu Nigeria and voucher specimens deposited at the departmental herbarium.

2.1. Preparation of Extract

The *Piper guineense* seeds were cleaned and sorted for healthy seeds. The seeds were sun dried for five days, and milled into fine powder using a milling machine. 40g of the powder was dissolved in 300ml of distilled water and left to stand for 48hrs with occasional shaking. The mixture was then filtered using a muslin bag. 263ml of the filtrate was recovered. The concentration of the extract was determined to be 10.15mg/ml.

2.2. Experimental Animals and Design

Thirty female albino rats, 7 weeks old and weighing between 128g-142g were use for the study. The animals were randomly placed into three groups of 10 animals each. Group 1 served as control, Group 2, and 3 served as test animals. The animals were allowed feed and water *ad libitum*. Standard laboratory protocols for animal studies were maintained. Approval for animal studies was obtained from the Animal Ethics Committee of the College of Medicine and Health Sciences, Abia State University, Uturu, Nigeria.

2.3. Treatment of Animals

The animals were starved overnight prior to the commencement of the administration of the *Piper guineense* extract**.** The administration of the *Piper guineense* seeds extract was by intraperitonal injection at 10mg/kg body weight and 20mg/kg body weight for group 2 and 3 animals respectively for 21 days.

2.4. Collection of Blood Samples and Serum Preparation

At the 22nd day the animals were sacrificed after overnight starving. Incisions were made into their thoracic cavity. Blood samples were collected by heart aorta puncture using a 10mL hypodermic syringe and allowed to clot in sample vials. The samples were centrifuged at 3000 rpm for 5 min. using the Bran Scientific and Instrument Company England centrifuge. The supernatant was harvested by simple aspiration with Pasteur pipette and stored in clean tubes at

- 4^{o}C until analysis.

Chemicals:

All chemicals used were of analytical grade.

2.5. Analysis

Assay for Antioxidant enzymes activity of test animals

The antioxidant enzymes: Catalase and Glutathione peroxidase were assayed as described by Weydert and Cullen [20] using Biosystem kits.

2.5.1. Assay for Liver Enzymes

Alanine aminotransferase (ALT), Aspartate transferase (AST) and Alkaline phosphatase (ALP) tests were carried out using ultra violet (UV) kinetic test kits produced by Cypress diagnostics**.** The test is based on photometric determination of rate of nicotinamide adenine dinucleotide (NADH) consumption by pyruvate and oxaloacetate which is directly related to ALT, AST and ALP activities, respectively. The test is based on the procedure of Reithman and Frankel [21].

2.5.2. Hemalotological Parameters

Hemoglobin was determined according to the method of Alexander and Griffths [22].

White blood cell and red blood cell count was determined as described by Dacie and Lewis [23] using Biosystem kits.

2.5.3. Statistical Analysis

Values were represented as Mean ± SD. Data obtained were subjected to one way Analysis of Variance (ANOVA) and group means were compared using Duncan's new multiple range tests. Differences were considered to be significant at ($p \leq 0.05$).

3. Results

Table 1. Liver enzymes activity (IU/L) of test animals.

Enzyme	Group 1 (Control)	Group 2	Group 3
ALT	45.25 ± 0.31*	40.67 ± 0.67*	36.69 ± 0.33*
AST	33.10 ± 0.43	31.56 ± 0.08	27.48 ± 0.27
ALP	105.10 ± 0.24*	96.86 ± 0.54*	89.19 ± 0.14*

*Values are mean ±SD of group determinations (n=10)
Values with * are statistically significant ($p<0.05$)

Table 2. Antioxidant Enzyme activities (IU/L) of test animals.

Enzyme	Group I (Control)	Group 2	Group 3
Catalase	0.33 ± 0.11*	0.28 ± 0.67	0.17 ±0.12*
Glutathione peroxidase	38.08 ± 0.31*	36.32 ± 0.84	33.07 ± 0.27*

*Values are mean ±SD of group determinations (n=10)
Values with * are statistically significant ($p<0.05$)

4. Discussion

Liver enzymes ALT and ALP decreased ($p \leq 0.05$) significantly compared to control, while AST decreased ($p \leq 0.05$) non-significantly as shown in table 1. The decrease in liver enzyme concentration in the serum of the test rats may be an indication that there may not be liver damage [24]. This result shows the extract may not have any apparent toxic effect on the liver of the experimental animals and could be a hepatoprotective agent. This result also agrees with the report of Nwozo *et al.;* [25] who reported that *Piper guineense* seed extract showed hepatoprotective effect on ethanol induced toxicity in male rats. Echo *et al*.; [6] also reported that the seeds of *Piper guineense* contain flavonoids, hence the seeds can offer some levels of hepatoprotection possibly due to flavonoids which exert a membrane-stabilizing action that protects the liver cells from injury [26].

The antioxidant status of the animals was evaluated by measuring the concentration of catalase and glutathione peroxidase enzymes. Catalase and glutathione peroxidase decreased ($p \leq 0.05$) significantly compared to control (Table 2). The significant reduction in catalase concentration is indicative that catalase is probably being used [27]. SOD converts the harmful superoxide radical to hydrogen peroxide, which catalase converts to water and oxygen which is not harmful. This reaction most likely is taking place in the experimental animals, hence the reduction in catalase level.

Table 3. Hb, WBC and RBC Count of test animals.

	Group 1 (Control)	Group 2	Group 3
RBC (µ/l)	241.33 ± 2.93 x 10^6 *	265.46 ± 3.45 x 10^6	298.10 ± 4.28 x 10^{6*}
WBC (µ/l)	129.67 ± 2.10 x 10^3 *	164.47 ± 4.64 x10^3*	194.67 ± 4.82 x 10^3*
Hb (g/dl)	10.33 ± 0.20 *	10.97 ± 0.46	12.04 ± 0.22 *

*Values are mean ±SD of group determinations (n=10)
Values with * are statistically significant ($p<0.05$)

The significant reduction of glutathione peroxidase in the test animals is suggestive that the *Piper guineense* seed extract may offer protection against lipid peroxidation. Peroxidase is involved in the reduction of lipid and hydrogen peroxide to eliminate oxidative stress. Chae *et al*.; [28] reported that induction of peroxidase provides an important chemopreventive strategy against carcinogens, as well as diseases caused by oxidative stress. Oxidative stress is caused by imbalance in the oxidant versus the anti-oxidant levels and is implicated in the development of diseases such as cancer, diabetes, ageing etc [29].

Hemoglobin (Hb), White blood cell and Red blood cell counts increased ($p \leq 0.05$) significantly compared to control (Table 3). Increase in hemoglobin concentration may be as a

direct consequence of increased consumption of plants with high content of minerals and vitamins that may stimulate synthesis of hemoglobin [30]. The *Piper guineense* seeds are rich in minerals and vitamins as reported by [6; 7].

White blood cell and red blood cell count increased ($p \leq 0.05$) significantly compared to control (Table 3). The increase in WBC and RBC count may have been caused by the nature and quantity of the protein content of the *Piper guineense* seeds extract. In this study, the experimental rats could be considered to be adequately hemoglobinized as shown by the significant increase in hemoglobin concentration of the test animals, hence the significant increase in WBC and RBC count. The level of WBC is used as an index of immune function and there was significant increase. The plant seeds are rich in phytochemicals, vitamins and minerals; hence these could influence the synthesis of white blood cells and red blood cells in the experimental animals [7].

5. Conclusion

This study has been able to show that the *Piper guineense* seeds have no toxic effects on the liver of the test animals. The seeds have antioxidant activity. Hemoglobin content is improved which suggests that blood cells are also not destroyed. It is therefore possible that nursing mothers who use this spices may benefit from its effect on post partum contraction as claimed by alternative medicine practitioners and may also benefit from its effect in increasing hemoglobin, white blood cell, red blood cell count and antioxidant enzymes levels.

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References

[1] Iwu M, (1986). Empirical investigation of dietary plants used in Igbo ethnomedicine. In: Plants in indigenous medicine and diet. Nina Etkined Redgroove Publishing Company, New York pp131-150

[2] Iwu M., (1993). Hand Book of African medicinal plants.CRC Press, Boca Raton, Flip, pp: 183-184.

[3] Marcel KK, Akhanovna MJ, Yves-Alain B, Gabin DB, Jérôme ZB (2010). *In vitro* antioxidant activities of total flavonoids extracts from leaves and stems of *Adenia lobata* (Jacq.) Engl. (Passifloraceae). *Journal Pharmacog Phytother.* 3 (1): 8-12.

[4] Olusegun Ekundayo, Intolaakso Rita Marie, Babajide Oguntimein and Abayomi Sofowora (2000) Essential oil constituents of Ashanti pepper (*Piper guineense*) fruits. *J. Agric. Food Chem*. 26(5): 880-882

[5] Hafner UC, John DA and Mickey CB (2003). Nutrition for Developing countries.*Journal of Nutrition.* 6: 18-25

[6] Echo IA, Osuagwu AN, Agbor RB, Okpako EC, Ekanem, B.E. (2012) Phytochemical Composition of *Aframomum melegueta* and *Piper guineense* Seeds *World Journal of Applied Environmental Chemistry*. 2 (1): 17-21

[7] Juliani Rodolfo H, Koroch AR, Giordano L, Amekuse L, Koffa SJ, Asante-Dartey SE ,Simon JE (2013). *Piper guineense* (Piperaceae): Chemistry, Traditional Uses and Functional Properties of West African Black Pepper. African Natural Plant Products. Volume II: Discoveries and Challenges in Chemistry, Health and Nutrition. *ACS Symposium series,* Vol.1127 pp 33-48

[8] Bep EO (1999). Medicinal Plants in Nigeria. *J. of Agric and Food Sci.* 7: 218-225

[9] Okigbo RN and Igwe DI (2007). Antimicrobial effects of *Piper guineense* 'Uziza', and *Phyllantus amarus* 'Ebe-Benizo' on *Candida albicans* and *Streptococcus faecalis*.*Acta Microbiologica et Immunologica Hungarica*. 54(4): 353-366

[10] Kim-kabari DB, Barimala IS, Achinewhu SC and Adeniji TA (2011). Effect of extracts from three indigenous spices on the chemical stability of smoked-dried catfish during storage. *African J. of Food, Agriculture, Nutrition and Development.* 11(6):174-181

[11] [Agoha AE (2002). Uses of *Piper guineense* fruits. *Journal of Food Sci*. 10 (3): 231-238

[12] Abila B, Richens A, Davies JA (1993) Anticonvulsant effects of extracts of the West African black pepper, Piper guineense. Journal of Ethnopharmacology 39 (2): 113-117

[13] Olaifa JI, Eehum WO, Akingbohungbe EA (2002). Insecticidal activity of some Nigerian Plants *Insect Science Application* 8: 221-224

[14] Amosan CA, Okerie TG and Mike BC. (2002). The use of *Piper guineense* fruit oil as a protectant of dried fish against dermatis and maculates. *Global J. Pure Sci*. 8 (2): 197-201

[15] Udo FVI, Lot TY, Braide VB (1999) Effects of extracts of seed and leaf of Piper guineense on skeletal muscle activity in rat and frog. *Phytother Res*. 13 (2): 106-10.

[16] Amvam Zollo, P. H.; Biyiti, L.; Tchoumbougnang, F.; Menut, C., Lamaty, G.; and Bouche, PH. (1998) Aromatic plants of Tropical Central Africa. Part XXXII. Chemical composition and antifungal activity of thirteen essential oils from aromatic plants of Cameroon. *Flavour and Fragrance Journal*. 13 (2): 107 - 114

[17] 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 Pharmacol*. 37:30-32

[18] Irvine GH (1965) Medical Laboratory Manual for Tropical countries. 2nd Edn. Buffer Wort and Company USA. 50: 529-531.

[19] Purseglove JN, Brown EG and robin RJ (1996) Spices. Longmans Scientific and Technical Publishers Vol. 2: 1-6

[20] Weydert CJ and Cullen JJ (2010). Measurement of superoxide dismutase, catalase and glutathione peroxidase in cultured cell and tissues. *Nature Protocols* 5: 51-60

[21] Reithman S and Frankel S (1957). A colorimetric method for the determination of serum oxaloacetic and glutamic pyruvic transaminases. *Am. J. Clin. Pathol.* 28(1): 56-63

[22] Alexander RR and Griffths JM (1993). Hemoglobin determination by the cyanomethaemoglobin method. In Basic Biomedical methods. 2nd Edn. John Wiley and Sons Inc. New York. Pp 188-189.

[23] Dacie JV and Lewis SM (1990). Practical Hematology. Churchill Livingstone.New York

[24] Chanchal K. Roy and Das, Amit Kumar (2010). Comparative evaluation of different extracts of leaves of *Psidium guajava* linn. for hepatoprotective activity.*Pakistan Journal of Pharmaceutical Sciences.* 23 (1): 15.

[25] Nwozo Sarah O, Ajagbe Adeyinka A, and Oyinloye Babatunji E. (2012) Hepatoprotective effect of *Piper guineense* aqueous extract against ethanol-induced toxicity in male rats. *Journal of Exp. Integr Med* 2 (2): 71-76

[26] Schnell, Zoanne Burgess; Van Leeuwen, Anne M.; Kranpitz, Todd R. (2003). In: Davis's Comprehensive Handbook of Laboratory & Diagnostic Tests. 22.

[27] Del RD, Stewart AJ and. Pellegrini N (2005). A review of recent studies on malondialdehyde as toxic molecule and biological marker of oxidative stress. *Nutr. Metab Cardiovasc. Dis*.15 (4): 316-328

[28] Chae YH, Rosa J, Williams LK, Desai D, Amin SG, Fiala E and El-Bayoumy K (1998). Induction of oxidative DNA damage by 7, 12-dimethylaminobenzo [a] anthracene and 2-amino-1-methyl-6-phenylimidazo[4, 5, 6] pyridine in rats. *Proc. Am. Assoc. Cancer Res.* 39: 490

[29] Nordmann R (1993). Free radicals, oxidative stress and antioxidant vitamins. *C R Seances Soc. Biol. Fil*. 187: 277-285

[30] Alada ARA (2000). The hematological effect of *Telfaria occidentalis* diet preparation. *Afr. J. Biomed. Res*., 3: 185-186