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Some Biochemical Changes in Serum of Female Albino Rats Administered Aqueous Extract of *Piper guineense* Schumach Seeds

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Authors' contributions

This work was carried out in collaboration of all authors. Author FOU conceived and designed the study. Authors FOU and CI wrote the protocol and first draft of the manuscript. Author AEU collected processed and prepared the plant seeds extract and also collected the albino rats. Authors FOU and CI managed the literature search, animal experiments and administration of plant extract and collection of biochemical samples from the sacrificed animals. Authors CI and AEU performed the statistical analysis, proofread and formatted the final manuscript. All authors read, corrected and approved the final manuscript.

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ABSTRACT

This study examined the biochemical changes in serum of female albino rats administered aqueous extract of *Piper guineense* Schumach seeds extract. The test animals (groups 2, 3 and 4) received 25 mg/kg, 50 mg/kg and 75 mg/kg of the extract respectively for 21 days, while group 1 served as control and received a placebo of 0.9% physiological saline which is the vehicle for administration. On the 22nd day the test animals which have been fasted overnight were sacrificed with chloroform anesthesia. Blood was collected through cardiac puncture. Results show that serum protein, albumin and packed cell volume concentration significantly ($p \leq 0.05$) increased. Creatinine and urea levels decreased ($p \leq 0.05$) significantly, 95% confidence level, while cholesterol profile decreased

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(p≤0.05) significantly, except HDL. Superoxide dismutase, glutathione-s-transferase, increased (p≤0.05) significantly, while lipid peroxidation decreased. This study has shown that nursing mothers in South East of Nigeria who use the *Piper guineense* Schumach seeds may derive immense benefit from its effect on the biochemical parameters assayed in this study.

Keywords: Spices; lipid; antioxidants; protein; creatinine; urea; Piper guineense.

1. INTRODUCTION

Spices are aromatic vegetable substances that are used in small quantities for seasoning of foods and also as preservatives or flavoring [1]. Spices enhance the taste of food, beverage and drug [2]. Spices contain phytochemicals which give them their characteristic odor, flavor and other properties [3].

Piper guineense (Schumach.et Thonn / Piperaceae) (Uziza or black pepper) is a spice, a West African tropical plant that is widely consumed and is considered medicinal by some people [4]. The aroma, flavor and preservative properties of *Piper guineense* Schumach are probably due to the nutritional and non-nutritional factors of the plant. It is rich in protein, fat, fiber and carbohydrate. The vitamin content is also high, especially vitamin C, which is an antioxidant. Peroxide value and free fatty acid level are generally low. The essential oils content is fairly high and range from 0.1 to 5.2%. The fruits of *Piper guineense* Schumach (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 [5]. Juliani et al. [6] reported that *Piper guineense* Schumach is an important plant used in traditional medicine and as a spice. As a medicinal plant, *Piper guineense* Schumach seeds are used to relief discomfort in the stomach caused by excess gas, while the leaves are used to treat respiratory infections, rheumatism and syphilis [7]. The leaves and seeds of the plant are used to flavor soup, stew and food generally [8]. The presence of an alkaloid – piperine has also been reported. The piperine contains strong anticonvulsant called anti- epileparine [N- 3-4- methylene dioxycinamyl piperidine] which has been synthesized for the treatment of epilepsy (Abila et al. [9], Oleifa et al. [10], Amosan et al. [11]. Udoh et al. [12] and Echo et al. [5] have also reported that the plant has therapeutic effect for the management of convulsion, leprosy, inflammation and rheumatoid pains. Bacteriostatic and bactericidal properties of *Piper guineense* Schumach has also been reported by Zollo et al. [13], Okigbo and Igwe [14], while Echo et al. [5] reported the molluscidal properties of the methanolic seed extract of the plant, and suggested it could be used for the treatment of Bilharzia. Mbongwe et al. [15] reported that the leaves of *Piper guineense* Schumach seeds are used for treating female infertility, while the seeds are used as an aphrodisiac. Hence the Igbos of the South Eastern part of Nigeria use the seeds to prepare soups for nursing mothers from the first day of delivery to prevent post partum contractions and to aid the fast return of the uterine muscles of the uterus to their original shape. It is also believed that it aids the increase in breast milk production and flow.

The present study is therefore aimed at investigating the biochemical changes that may occur in female albino rats due the administration of aqueous extract of *Piper guineense* Schumach seeds.

2. MATERIALS AND METHODS

2.1 *Piper guineense* Schumach Seeds

The *Piper guineense* Schumach seeds were purchased from the Eke Okigwe market 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.

2.2 Preparation of Extract

The *Piper guineense* Schumach 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. 40 g of the powder was dissolved in 300 ml of distilled water and left to stand for 48 hrs with occasional shaking. The mixture was then filtered using a muslin bag. 263 ml of the filtrate was recovered. The concentration of the extract was determined to be 10.15 mg/ml.

2.3 Experimental Animals and Design

Forty female albino rats, 7 weeks old and weighing between 128 g-142 g were used for the study. The animals were randomly placed into four groups of 10 animals each. Group 1 served as control, Group 2, 3 and 4 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.

2.4 Treatment of Animals

The animals were starved overnight prior to the commencement of the administration of the *Piper guineense* Schumach seeds extract**.** The administration of the *Piper guineense* Schumach seeds extract was by oral intubation once daily at 25 mg/kg body weight, 50 mg/kg body weight and 75 mg/kg body weight for group 2, 3 and 4 animals respectively for 21 days.

2.5 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 10 mL 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 (serum) was harvested by simple aspiration with Pasteur pipette and stored in clean tubes at -4°C until analysis.

2.6 Estimation of Parameters

2.6.1 Determination of total cholesterol

Total cholesterol was determined by the enzymatic colorimetric cho-PAP method, High density lipoprotein (HDL) Very low density lipoprotein (VLDL), and Low density lipoprotein (LDL) were determined as described by Burstein et al. [16]. Triacylglycerol and cholesterol were assayed as described by Fletcher [17], Richmond [18] and Barlett [19], using Biosystem kits. The measurements were performed according to the manufacturers' instruction.

2.6.2 Determination of total protein

Total protein test kits produced by Randox laboratories were used. This test involves formation of colored complex between cupric ions in alkaline medium with peptide bonds. The intensity of the color formed is proportional to the concentration of the protein.

2.6.3 Determination of albumin

Albumin test kit produced by Biosystem kits were used to estimate albumin. The principle of this test is based on albumin quantitatively binding to bromocresol green (BCG). The complex of albumin-BCG so formed absorbs maximally at 578 nm, and the absorbance is directly proportional to the concentration of albumin in the sample.

2.6.4 Determination of lipid peroxidation

Thiobarbituric acid reactive substances (TBARS) method as described by Buege and Aust [20] was used to determine lipid peroxidation,

2.6.5 Determination of antioxidant enzymes

Glutathione-s- transferase (GST) activity was determined according to the method described by Habig et al. [21], while Superoxide Dismutase was measured as described by Kakkar et al. [22].

2.6.6 Hematological parameters

Commercial kits (Biosystem kits) were used to estimate packed cell volume, creatinine and urea. Packed cell volume and creatinine were assayed as described by Dacie and Lewis [23].

2.7 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 ($p<0.05$).

3. RESULTS

The results of the analysis is presented in Tables 1-4 below.

4. DISCUSSION

Body weight of experimental animal increased ($p \leq 0.05$) significantly ($p \leq 0.05$) compared to control (Table 1). Animals treated with 75 mg/kg body weight of extract showed significant increase in body weight. The effect of the extract on body weight seems to be dose dependent, as shown on Table 1. The increase in weight may be due to the high content of protein in the extract and the phytochemicals which could elicit increase in protein synthesis.

Triacylglycerol, total cholesterol, Low density and very low density cholesterol decreased ($p \leq 0.05$) non-significantly compared to control; while high density cholesterol increased ($p \leq 0.05$) significantly (Table 2). Cholesterol is a precursor of other steroids such as sex hormones. It is also an important constituent of cell membranes and bile acids [24,25]. Low density and high density cholesterol are involved in the transport of cholesterol to and from the liver. The reduction of these fatty acids may show that the *Piper guineense* seed extract may have hypocholesterolmic effect. The increase in High density cholesterol could be of immense benefit to the animals, since HDL is also known as the 'good' cholesterol and is involved in transport of other lipids to the liver for disposal in bile [26].

Lipid peroxidation decreased ($p \leq 0.05$) significantly (Table 3) compared to control. The significant decrease in lipid peroxidation values in the experimental animals may be as a result of the extract aiding in the reduction of lipid oxidation since the *Piper guineense* seeds are rich in vitamin C which is an antioxidant. Lipid peroxidation is due to the oxidation of lipids especially polyunsaturated lipids to generate peroxides and aldehydes which have been associated with the development of diseases [27,28]. The aldehydes and peroxides are long-lived and can easily spread to distant sites through blood circulation to cause further peroxidation of cells, hence they have been reported to be more dangerous than reactive oxygen species (ROS) [27]. Lipid peroxidation causes cytotoxicity and injury that leads to cell damage due to its ability to form malondialdehyde (MDA) [29].

The antioxidant enzymes superoxide dismutase (SOD) and glutathione s-transferase (GST) increased ($p \leq 0.05$) significantly (Table 3) compared to control. The increase in SOD and GST could be attributed to the presence of phytochemicals in the *Piper guineense* seeds extract especially flavonoids. The seeds of *Piper guineense* are rich in flavonoids, which may have induced detoxification enzymes through up-regulation of their genes by interacting with antioxidant response elements [30-32]. Prestera et al. [33] reported that substances like flavonoids that posses electrophilic centers are able to react with sulfhydryl groups through oxido-reduction or alkylation. Pantuck et al. [34] also reported that an increase in GST activity translates to increased capacity to conjugate and excrete toxic intermediates that can cause diseases such as diabetes. The significant increase in antioxidant enzymes further confirms the high antioxidant capacities of the seeds of *Piper guineense.*

In humans the damaging effects of oxidants and lipid peroxidation can be checked by an organized antioxidant defense mechanism. Several enzymatic and non-enzymatic processes are involved that protect cells against oxidative damage. Farombi et al. [35] reported that the major enzymatic anti-oxidant systems include superoxide dismutase (SOD), glutathione peroxidase (GSH), glutathione – s- transferase (GST) and catalase, while the non – enzymatic antioxidants include glutathione and dietary constituents such as vitamins, flavonoids and carotenoids. The antioxidants protect cells from the damaging effects of free radicals and lipid peroxidation. Opara et al. [36] recorded significant ($p \leq 0.05$) increase in total protein, albumin and globulin, while cholesterol and glucose levels decreased significantly ($p \leq 0.05$) in biochemical responses of adult rabbits to aqueous extract of *Ocimum gratissium* leaves. Aziza et al. [37] reported a decrease in triglycerides, total cholesterol and malondialdehyde level, while GSH and HDL-cholesterol levels increased in rats administered *Gymnema sylvestre* R.Br. leaves extract.

Serum protein concentration increased ($p \leq 0.05$) significantly, while albumin increased non-significantly (Table 4). This could be attributed to the high protein content of the *Piper guineense* seeds [5]. However, the non-significant increase in albumin content could infer a possible preservation of the stability of the liver. Packed cell volume increased ($p \leq 0.05$) significantly, while creatinine and urea decreased ($p \leq 0.05$) significantly (Table 4). The increase in PCV may be contributed by the rich phytochemical content of *Piper guineense* seeds which increased the rate of hemoglobin synthesis in the experimental animals, while increasing the excretion of creatinine and urea [38].

Table 1. Effect of *Piper guineense* seed extract on animal weight (g)

	Group 1 (Control)	Group 2	Group 3	Group 4
Body weight	92.56±0.65*	98.74±0.35	101.46±0.86*	102.15±0.63*

**Values are mean±SD of triplicate determinations (n=10)*
*Values with * are statistically significant (p<0.05) compared with the control*

Table 2. Effect of *Piper guineense* seed extract on fatty acid levels (mg/dL)

Parameter	Group 1 (Control)	Group 2	Group 3	Group 4
Triacylglycerol	73.46±3.28*	72.95±4.72	70.54±2.35*	68.42±3.82*
Total cholesterol	1.43 ±0.18	1.37±0.09	1.26±0.10	1.20±0.12*
HDL	35.56±0.86*	36.43±0.16*	37.76±0.24*	38.57±0.11*
LDL	32.68±2.14	31.82±2.06	30.32±2.21*	28.54±2.16
VLDL	11.56±0.85*	11.14±0.14*	10.76±0.56*	10.25±0.43*

**Values are mean±SD of triplicate determinations (n=10)*
*Values with * are statistically significant (p<0.05) compared with the control*

Table 3. Effect of *Piper guineense* seed extract on lipid peroxidation levels and antioxidant enzymes

Parameter	Group 1 (Control)	Group 2	Group 3	Group 4
Lipid peroxidation (Mol/L)	4.69±0.65*	3.28±0.16	3.11±0.72	3.01±0.52*
SOD (IU/L)	68.76±6.74	104.57±5.64*	108.26±5.96*	112.39±6.86*
GST (IU/L)	6.86±1.64	7.56±1.22	8.73±1.45*	9.28±1.32*

**Values are mean±SD of triplicate determinations (n=10)*
*Values with * are statistically significant (p<0.05) compared with the control*

Table 4. Effect of *Piper guineense* seed extract on some macromolecules

Parameter	Group 1 (Control)	Group 2	Group 3	Group 4
Serum protein(mg/dl)	246.45±12.25	252.25±10.24	265.53±9.65*	278.48±9.82*
Albumin (mg/dl)	2.16±0.13*	2.37±0.17	2.52±0.15*	2.58±0.22*
PCV (L/L)%	30.25±0.56*	33.14±1.05	34.68±1.27*	34.94±1.21*
Creatinine (mg/dl)	0.65±0.03	0.52±0.02	0.46±0.06	0.41±0.04
Urea (mg/dl)	38.74±1.43*	38.14±1.69	36.45±1.36	36.16±1.76*

**Values are mean±SD of triplicate determinations (n=10)*
*Values with * are statistically significant (p<0.05) compared with the control*

The kidney is a major organ for excretion and its stability is estimated by the creatinine clearance, which is a function of the glomerular filtration rate.

5. CONCLUSION

This study has been able to show that the *Piper guineense* seeds increased the body weight of the experimental animals, hence may elicit and increase protein synthesis. Total cholesterol and triacylglycerol were significantly reduced, hence the seeds may also have the ability to control cholesterol levels. Lipid peroxidation levels were reduced while antioxidant enzymes increased; albumin non-significantly increased, pack cell volume significantly increased, while creatinine and urea were reduced. The reduction in urea, creatinine and cholesterol levels as observed in this study, shows that *Piper guineense* seeds can be used to improve kidney function and for the management of hypercholesterolemia. These observations have lend credence to the claims of alternative medicine practitioners that nursing mothers who use these spices may benefit from its effect on some biochemical indices as reported in this study.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTEREST

Authors have declared that no competing interest exists.

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