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. Group 1 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 intraperitonially 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≤ 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≤ 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.
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 a 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 dioxycuraminyl piperidine] and has been synthesized for the treatment of epilepsy [14]. Echo et al.; [6] reported that methanolic extracts of the plant have molluscicidal 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 eupetic. 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, venereal 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°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. Hematological 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≤0.05).

3. Results

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

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Group 1 (Control)</th>
<th>Group 2</th>
<th>Group 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALT</td>
<td>45.25 ± 0.31*</td>
<td>40.67 ± 0.67*</td>
<td>36.69 ± 0.33*</td>
</tr>
<tr>
<td>AST</td>
<td>33.10 ± 0.43</td>
<td>31.56 ± 0.08</td>
<td>27.48 ± 0.27</td>
</tr>
<tr>
<td>ALP</td>
<td>105.10 ± 0.24*</td>
<td>96.86 ± 0.54*</td>
<td>89.19 ± 0.14*</td>
</tr>
</tbody>
</table>

*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≤0.05) significantly compared to control, while AST decreased (p≤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≤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.

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≤ 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<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 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.

Acknowledgement

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References


