





Original Article

## Effects of *Piper guineense* (African Black Pepper) Seeds on Lipid Profile, Renal Function Parameters, and Antioxidant Status of *Cavia porcellus*

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### ABSTRACT

**Background and objectives:** *Piper guineense* (African black pepper) is a widely consumed spice in some parts of Central and Western Africa, especially Nigeria, due to its nutritional and medicinal properties. This study investigated biochemical effects of extract of *P. guineense* seeds on lipid profile, renal function parameters, and antioxidant status of *Cavia porcellus*.

**Methods:** Forty guinea pigs (weighing 200–300g) were equally divided into a control group and three experimental groups. Animals in the control group received 1 mL of distilled water daily for 28 days, while animals in the experimental groups 1, 2, and 3 received 0.5 mL of distilled water along with 10, 20, and 20 mg/100 g body weight powdered seeds daily for 28 days.

**Results:** Phytochemical studies revealed the presence of flavonoids, alkaloids, tannins, saponins, and calcium. The median lethal oral dose of the aqueous extract of *P. guineense* seeds was greater than 5,000 mg/kg, indicating a high degree of safety. The extract at dose of 30 mg/100 g body weight had the most beneficial effects on lipid profile, renal function parameters, and antioxidant status of guinea pigs when compared with other groups.

**Conclusion:** Given the beneficial effects of *P. guineense* on lipid profile and renal function parameters, the plant can be incorporated in daily diets to ensure receiving adequate amount of essential vitamins, phytochemicals, and other nutrients present in the plant.

**Keywords:** [Piper](#), [Antioxidants](#), [Phytochemicals](#).

## INTRODUCTION

Nutraceutical is a food or part of a food that can modify and maintain normal physiological body functions in order to improve health and wellness of human beings (1). In recent years, food-derived substances have received a lot of attention due to their wide range of applications (2). Medicinal plants are the richest sources of drugs of traditional medicine, nutraceuticals, modern medicines, folk medicines, food supplements, pharmaceutical intermediates, and chemical entities for man-made drugs (3). Plant materials have the ability to synthesize a variety of chemical compounds with important biological functions (4). Bioactive constituents such as phenols, flavonoids, tannins, and alkaloids present in seeds, leaves, stems, and roots of plants have beneficial effects on the human body similar to chemical compounds present in conventional drugs (5). The kidneys are one of the most importantly examined organs because of their important role in filtration, metabolism, and excretion of compounds. The kidneys excrete a wide variety of metabolic waste products into the urine, regulate the body's acid-base balance and blood pressure, and finally influence the concentration of minerals, such as potassium, sodium, and calcium in the blood (6). Biochemical processes naturally lead to the formation of free radicals, which are controlled by the body under normal circumstances. However, if there is excessive free radical formation, cell or tissue damage can occur (7). To limit these harmful effects, an organism requires complex protection – the antioxidant system (8). An overview of epidemiological research suggests that individuals with the highest intake of natural antioxidants, whether through diet or supplements, tend to be less prone to diseases (9).

*Piper guineense*, commonly known as the West African black pepper, is a spicy plant from the family *Piperaceae* and the genus *Piper* (10). The plant is native to tropical regions of Central and Western Africa and is semi-cultivated in southern part of Nigeria. It has various culinary, nutritional, medicinal, cosmetic, and insecticidal applications (11). The plant is known as “Uziza” in Igbo and “Iyere” in Yoruba. Other common names are benin pepper, guinea pepper, and false cubeb (12). Given its anti-parasitic (13),

hematological (14), anti-tumor (15) and hepatoprotective properties (16), *P. guineense* can help to protect the body from development of diseases. The present study focused on biochemical effects of *P. guineense*, as a novel nutraceutical agent, on lipid profile, renal function parameters, and antioxidant status of guinea pigs.

## MATERIALS AND METHODS

Fresh mature seeds of *P. guineense* were obtained from New Benin market, Benin City (Nigeria) and authenticated in the Herbarium Center of University of Benin. The seeds were washed, sorted out, sundried, and finally milled into fine powder using a milling machine. Next, 60 g of the powdered seeds were separated into three different portions and mixed with the animal feeds.

Forty male guinea pigs (*Cavia porcellus*) were obtained from the animal house of the College of Medicine, Ambrose Alli University, Nigeria. The animals (weight: 200-300 g) were housed in metabolic cages under standard laboratory conditions, with access to feeds (pelletized growers mash) and water *ad libitum*. The experimental protocol was approved by the Ethics Committee of the University of Benin. The animals were equally divided into a control group and three experimental groups. The control group received 1 mL of distilled water daily for 28 days, while the experimental groups 1, 2, and 3 received 0.5 mL of distilled water along with 10, 20, and 30 mg/100g body weight/day powdered seeds for 28 days.

To obtain the *P. guineense* extract, 50 g of the powdered seeds were dissolved in 400 mL of distilled water and left to stand for 48 hours. Next, the mixture was filtered using a muslin bag and kept at room temperature. Presence of secondary metabolites in the aqueous seed extract was investigated using standard phytochemical screening procedures (7) and the Lorke's method (17) comprising two phases. In the first phase, nine animals were divided into three groups, each containing three guinea pigs. Groups 1, 2, and 3 received the aqueous extract of *P. guineense* seeds orally at 10, 100, and 1,000 mg/kg body weight, respectively. The animals were placed under observation for 24 hours to monitor their behavior as well as to confirm if mortality will occur. In the second phase, four animals were

distributed into a control group and three experimental groups, each containing one guinea pig. Groups 1, 2, and 3 received the aqueous extract of *P. guineense* seeds orally at 1,600, 2,900, and 5,000 mg/kg body weight, respectively. The lethal dose (LD<sub>50</sub>) of the extract was calculated using the formula  $LD_{50} = \frac{\sqrt{D_0 + D_{100}}}{2}$  where D<sub>0</sub> represents highest dose that gave no mortality and D<sub>100</sub> represents lowest dose that produced mortality. Blood samples were collected by cardiac puncture under mild ether or chloroform anesthesia. The samples were centrifuged at 3,500 rpm for 15 minutes to separate serum. High-density lipoprotein-cholesterol (HDL-C), triglyceride (TG), total cholesterol (TC), low-density lipoprotein-cholesterol (LDL-C), creatinine, urea, sodium (Na<sup>+</sup>), potassium (K<sup>+</sup>), bicarbonate (HCO<sub>3</sub><sup>-</sup>), superoxide dismutase (SOD), catalase (CAT), reduced glutathione (GSH), vitamin C, and vitamin E levels were measured.

Data were presented as mean ± standard error of the mean (SEM) and analyzed using one-way analysis of variance (ANOVA) with post-hoc Tukey HSD. Statistical analysis of data was carried out using the SPSS software (version 16) and at significance level of 0.05.

## RESULTS

Phytochemical screening of the aqueous extract of *P. guineense* seeds revealed the presence of flavonoids, alkaloids, tannins, saponins, and calcium at moderate concentrations, while carbohydrates, reducing sugars, and glycosides were present at low concentrations.

In the control group, serum levels of TC, TG, and LDL-C increased significantly, while HDL-C decreased significantly ( $p < 0.05$ ) when compared with other groups. Serum levels of TC, TG, and LDL-C decreased significantly, and HDL-C increased significantly in group 3 ( $p < 0.05$ ) compared with groups 1 and 2 ([Table 1](#)).

Table 1- Lipid profile of animals in the control and experimental groups

Groups	Lipid profile			
	Total Cholesterol (mmol/L)	Triglyceride (mmol/L)	HDL-C (mmol/L)	LDL-C (mmol/L)
Control	122.23 ± 1.12 <sup>a</sup>	100.56 ± 1.98 <sup>a</sup>	18.23 ± 2.98 <sup>a</sup>	135.42 ± 4.21 <sup>a</sup>
Group 1 (10 mg/100 g bwt)	101.66 ± 2.00 <sup>b</sup>	97.00 ± 4.64 <sup>b</sup>	22.00 ± 0.02 <sup>b</sup>	111.26 ± 2.00 <sup>b</sup>
Group 2 (20 mg/100 g bwt)	97.44 ± 6.22 <sup>c</sup>	88.00 ± 2.00 <sup>c</sup>	29.12 ± 1.30 <sup>c</sup>	90.99 ± 7.22 <sup>c</sup>
Group 3 (30 mg/100 g bwt)	90.32 ± 1.00 <sup>d</sup>	75.91 ± 1.72 <sup>d</sup>	35.11 ± 3.00 <sup>d</sup>	79.00 ± 2.11 <sup>d</sup>

Values are presented as mean ± SEM of 10 animals in each group. <sup>a</sup> significant difference with groups 1,2, and 3; <sup>b</sup> significant difference with the control group; <sup>c,d</sup> significant difference with group 1.

[Table 2](#) depicts the effects of *P. guineense* seeds on the renal function parameters of guinea pigs. Mean urea and creatinine levels increased significantly in the control group when compared with other groups. Mean urea

and creatinine levels decreased significantly in group 3 compared with groups 1 and 2. In addition, mean levels of Na<sup>+</sup>, K<sup>+</sup>, and HCO<sub>3</sub><sup>-</sup> decreased significantly in the control group compared with groups 1, 2, and 3, respectively.

Table 2- Effects of *P. guineense* seeds on renal function parameters of animals in the control and experimental groups

Groups	Renal function parameters				
	Urea (mg/dL)	Creatinine (mg/dL)	Na <sup>+</sup> (mmol/L)	K <sup>+</sup> (mmol/L)	HCO <sub>3</sub> <sup>-</sup> (mmol/L)
Control	52.11 ± 0.01 <sup>a</sup>	2.30 ± 0.55 <sup>a</sup>	100.88 ± 2.00 <sup>a</sup>	6.6 ± 1.86 <sup>a</sup>	18 ± 1.88 <sup>a</sup>
Group 1 (10 mg/100 g bwt)	40.77 ± 2.00 <sup>b</sup>	1.00 ± 2.33 <sup>b</sup>	95.88 ± 2.33 <sup>b</sup>	6.0 ± 2.54 <sup>a,b</sup>	16 ± 2.00 <sup>b</sup>
Group 2 (20 mg/100 g bwt)	33.21 ± 2.11 <sup>c</sup>	1.00 ± 1.99 <sup>b,c</sup>	80.60 ± 0.19 <sup>c</sup>	5.1 ± 0.03 <sup>c</sup>	13 ± 2.39 <sup>c</sup>
Group 3 (30 mg/100 g bwt)	33.00 ± 1.99 <sup>c,d</sup>	0.89 ± 0.21 <sup>d</sup>	69.20 ± 3.91 <sup>d</sup>	4.8 ± 3.00 <sup>d</sup>	11 ± 1.09 <sup>d</sup>

Values are presented as mean ± SEM of 10 animals in each group. <sup>a</sup> significant difference with groups 1,2, and 3; <sup>b</sup> significant difference from group 1; <sup>c</sup> significant difference from group 2; <sup>d</sup> significant difference with groups 1, 2, and control.

[Table 3](#) illustrates the effects of *P. guineense* seeds on the enzymatic and non-enzymatic antioxidant status of guinea pigs. Mean levels of SOD, GSH, vitamin C, and vitamin E

decreased significantly in the control group compared with other groups. Mean level of catalase increased significantly in the control group compared with other groups.

**Table 3- The effects of *P. guineense* seeds on the enzymatic and non-enzymatic antioxidant status of animals in the control and experimental groups**

Groups	Superoxide dismutases (U/mL)	Catalase (U/mL)	Glutathione (mg/L)	Vitamin C (μmol/L)	Vitamin E (μmol/L)
Control	2.93 ± 1.11 <sup>a</sup>	15.00 ± 2.93 <sup>a</sup>	21.88 ± 3.00 <sup>a</sup>	0.28 ± 1.77 <sup>a</sup>	0.19 ± 0.42 <sup>a</sup>
Group 1 (10 mg/100 g bwt)	5.40 ± 4.77 <sup>b</sup>	12.33 ± 0.90 <sup>b</sup>	24.47 ± 0.44 <sup>b</sup>	1.63 ± 0.21 <sup>b</sup>	0.44 ± 3.30 <sup>a,b</sup>
Group 2 (20 mg/100 g bwt)	6.29 ± 0.00 <sup>c</sup>	12.32 ± 3.99 <sup>b,c</sup>	29.10 ± 1.21 <sup>c</sup>	1.90 ± 0.39 <sup>b,c</sup>	0.82 ± 2.87 <sup>a,c</sup>
Group 3 (30 mg/100 g bwt)	6.00 ± 2.00 <sup>c,d</sup>	9.01 ± 3.11 <sup>d</sup>	32.60 ± 0.30 <sup>d</sup>	2.21 ± 0.11 <sup>d</sup>	1.00 ± 0.00 <sup>d</sup>

Values are presented as mean ± SEM of 10 animals in each group. <sup>b</sup> significant difference with the control group; <sup>c</sup> significant difference with groups 1 and 2.

## DISCUSSION

The present study examined the biochemical effects of *P. guineense* seeds aqueous extract as a nutraceutical agent on the lipid profile, renal function parameters and antioxidant status of guinea pigs. The phytochemical investigation showed the presence of flavonoids, calcium, tannins, alkaloids, and saponins at moderate concentrations, while carbohydrate, reducing sugars, and glycosides were present at low concentrations. Similarly, Okwu also reported the presence of these bioactive constituents in the leaves of *P. guineense* (18). This shows that both the leaves and seed extracts of *P. guineense* contain phytochemical compounds with medicinal properties and are a rich source of nutrients and dietary fibers that could protect against diseases (19). The toxicity of plant-derived substances for safety of usage has received a lot of attention. The present study showed that *P. guineense* at all tested doses had no significant toxic effects as no mortality was recorded in both phases after 24 hours. The median LD<sub>50</sub> (oral) of the aqueous extract of *P. guineense* seeds was greater than 5,000 mg/kg, which confirms its safety. In line with this finding, Oguwike et al. reported that the aqueous extract of piper seeds was safe for use in albino mice over 30 days (20).

Serum levels of TC, TG, and LDL-C increased significantly, while HDL-C decreased significantly compared with other groups. Serum levels of TC, TG, and LDL-C decreased significantly, and HDL-C increased

significantly in group 3 compared with groups 1 and 2. These results suggest the extract of *P. guineense* seeds may have lipid-lowering properties due to the presence of flavonoids. This finding is consistent with findings of Nwachi and Igbinobaro (21).

A renal function test identifies the presence of renal disease, monitors the kidneys' response to treatment, and determines disease progression (22). In this study, mean urea and creatinine levels increased significantly in the control group compared with other groups. Mean urea and creatinine levels decreased significantly in group 3 compared with groups 1 and 2. In addition, mean levels of Na<sup>+</sup>, K<sup>+</sup>, and HCO<sub>3</sub><sup>-</sup> decreased significantly in the control group compared with groups 1, 2, and 3, respectively. These findings confirm the beneficial effects of extract of *P. guineense* seeds on renal function. Okoye and Igwilo also reported significant changes in renal function parameters after administration of *P. guineense* leaves extract to Wistar albino rat (23). Organisms have evolved sophisticated mechanisms of antioxidant defense for protection against free radicals-induced damages (24-26). There are a number of enzymatic and non-enzymatic antioxidants that can help to protect against oxidative damage (27). In our study, mean levels of SOD, GSH, vitamin C, and vitamin E decreased significantly in the control group compared with other groups. Mean level of catalase increased significantly in the control group

compared with other groups. These results indicate that the extract of *P. guineense* seeds have free radical scavenging properties, which stabilizes the plasma membrane of hepatocytes. Moreover, the bioactive components (flavonoids, phenols,  $\alpha$ -tocopherols, etc.) of the *P. guineense* extract possess free radical scavenging properties that help prevent oxidative damage, cell injury, and cell death. In a previous study, the extract of *P. guineense* leaves exhibited free radical scavenging properties.

## CONCLUSION

The results indicated that the aqueous extract of *P. guineense* seeds is an important source of dietary nutrients and diverse phytochemicals with therapeutic potentials. Therefore, it is recommended to further investigate the application of this plant and its components for therapeutic purposes.

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### Ethics approvals and consent to participate

The experimental protocol was approved by the Ethics Committee of the University of Benin, Benin City, Nigeria.

## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding publication of this article.

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