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Research Article

Investigation of Histopathological Effect of *Piper nigrum* Consumption on Selected Organ of Male Wistar Rats

Nosa Terry Omorodion[1]* and Wilson Ogbonna Ajanwachuku[2]

[1]Department of Medical Laboratory Sciences, Faculty of Health Science and Technology, University of Nigeria, Enugu campus, Enugu, Nigeria

[2]Health Services Department, Ugbowo, University of Benin, Benin city, Edo State, Nigeria

***Corresponding Author:** Nosa Terry Omorodion, Department of Medical Laboratory Sciences, Faculty of Health Science and Technology, University of Nigeria, Enugu campus, Enugu, Nigeria.

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Abstract

Piper nigrum (Black pepper) plant is a flowering woody perennial climbing vine that belongs to Piperaceae family. Pepper plants easily grow in the shade on supporting trees, trellises or poles up to a maximum height of 13 feet or 4 meters and roots may come out from leaf nodes if vine touches the ground. The plants have heart shape alternate leaves with typically large size of 5 - 10 cm in length and 3 - 6 cm across, with 5 to 7 prominent palmate veins. The flowers are small, monoecious with separate male and female flowers but may be polygamous which contain both male and female flowers. Piper species significantly inhibited the atherogenic diet induced increased lipid profile and alteration in antioxidant enzymes activities. The micrograph of the kidney sections in control group I showed normal histological cytoarchitecture with well-defined glomeruli. However, at 4 weeks of black pepper administration, cellular infiltration and severe lobulated and distorted glomeruli were observed in rat fed 0.62 ml, also at the 6th weeks of black pepper glomeruli shrinkage was seen. Conclusively, it could be inferred from the findings that black pepper administration can cause architectural distortions as evident in the kidneys, liver and testes sections above.

Keywords: *Piper nigrum*; Histopathological; Liver; Testes

Introduction

Piper nigrum (Black pepper) plant is a flowering woody perennial climbing vine that belongs to Piperaceae family. Pepper plants easily grow in the shade on supporting trees, trellises or poles up to a maximum height of 13 feet or 4 meters and roots may come out from leaf nodes if the vine touches the ground [1]. The plants have heart shape alternate leaves with typically large size of 5 - 10 cm in length and 3 - 6 cm across, with 5 to 7 prominent palmate veins. The flowers are small, monoecious with separate male and female flowers but may be polygamous which contain both male and female flowers [2]. The small flowers are borne on pendulous spikes at the leaf nodes that are nearly as long as the leaves. The length of spikes goes up to 7 - 15 cm. The black pepper's fruits are small (3 to 4 mm in diameter) called a drupe and the dried unripe fruits of *Piper nigrum* are known as a peppercorn [4]. The fully mature fruits are dark red in color and approximately 5 mm in diameter. Many investigators isolated different types of compounds viz Phenolics, flavonoids, alkaloids, amides and steroids, lignans, neolignans, terpenes, chalcones etc. and many other compounds [1]. Some of the compounds are Brachyamide B, Dihydro-pipericide, (2E,4E)-N-Eicosadienoyl-piperidine, N-trans-Feruloyl Tyramine, N-Formyl piperidine, Guineensine, pentadienoyl as piperidine, (2E,4E)- Nisobuty-ldecadienamid, isobutyl-eicosadienamide, Tricholein, Trichostachine, isobutyl-eicosatrienamide, Isobutyl-octadienamide, Piperamide, Piperamine, Piperettine, Pipericide, Piperine, Piperolein B, Sarmentine, Sarmentosine, Retrofractamide. Khan and Siddiqui [3] evaluated the antibacterial potential of aqueous decoction of *Piper nigrum* L. (black pepper), *Laurus nobilis* L. (bay leaf), *Pimpinella anisum* L. (aniseed), and *Coriandum sativum* L. (coriander) against different bacterial isolates from oral cavity of two hundred individual volunteers. Plants are important source of antioxidants [5]. The memory enhancing and antioxidant proprieties of the methanolic extract of *Piper nigrum* L. fruits at a doses of 50 and 100 mg/kg, orally, for 21 days in amyloid beta (1-42) were investigated in Alzheimer's disease model in rats [5]. Piper species significantly inhibited the atherogenic diet induced increased lipid profile and alteration in antioxidant enzymes activities. This study showed an antioxidant protective role of the extracts of Piper species against atherogenic diet induced oxidative stress in renal, cardiac and hepatic tissues [6].

Aim of the Study

The aim of this study was to investigate the histopathological effect *Piper nigrum* consumption as aphrodisiacs on selected organ of male wistar rats.

Materials and Method

Substance Preparation

Black pepper

This was extracted according to the method described by Ebeye., *et al* [7].

Acute toxicity (LD50) test of black pepper (Piper nigrum) and ginger (Zingiber officinale)

The method of Locke [8] was used for this work.

	Dosage (mg/kg)	Observation	Remark	Mortality
Phase I				
Group 1	10	Weakness	Negative	0/4
Group 2	100	Fur/skin color change	Negative	0/4
Group 3	1000	diarrhoea	Negative	0/4
Phase II				
Group 1	1,600	Weakness	Negative	0/4
Group 2	2,900	Fur/skin color change	Negative	0/4
Group 3	5000	diarrhoea	Negative	0/4

Table 1: Physical observation of experimental rats used for acute toxicity test of *Piper nigrum*.

Observations	Control Group A	Test groups		
		B (Pn)	C (Zo)	D (Pn/Zo)
Fur colour	-	+	+	+
Behavioural Changes	-	-	-	-
Skin surfaces	-	-	-	-
Diarrhoea	-	-	-	-
Death	-	-	-	-

Table 2: Notable physical observations of rats administered Piper nigrum.

Key: +: Present; -: Negative; Pn: Piper nigrum; Zo: Zingiber officinale.

Research Design

Thirty adult Wistar rats aged 2 - 3 months were used for the purpose of this study. They were procured from the animal house of University of Benin, Faculty Agriculture, University of Benin, Benin city, Edo state and after acclimatization were divided into three groups of ten rats each. Group I served as the control and the rats were given distilled water. Group II were fed with *Piper nigrum* aqueous extract, Group III were fed with adequate amount of fresh fruits of *Piper nigrum* in Edo State and authenticated by a botanist in the Department of Botany, University of Benin, Edo State.

The substance administration was given daily for 42 days (6 weeks) and the weights of both the test animal and control monitored every 2 week starting from. After the administration, the rats were sacrificed through cranial dislocation and the kidney, liver and testis harvested. They were observed macroscopically and processed for light microscopy. Analysis of Variance (ANOVA) was used to analyze the results of the weight and differences were considered significant at $P < 0.05$ level of confidence. All data were expressed as Mean ± Standard error of mean (SEM). The results were presented in tables and comparisons made statistically.

Histological Processing

The tissues were processed using automatic tissue processor according to the processing schedule used in University of Benin Teaching Hospital (UBTH), Edo State, Nigeria. The fixed plastic cassette tissues in 10% formalin were automatically processed by passing them through different grades of alcohol as follows.

After the last timing, the tissues were removed from their plastic cassettes and placed at the centre of the metallic tissue mould and then filled with molten paraffin wax. They were also left to solidify after which they were now placed in the refrigerator at 5°C for 15 minutes. After the blocks were cooled in the refrigerator for the time stated above (15 minutes), the blocks were then removed from the metallic case using a knife and after which the paraffin wax at the side of the blocks were removed [9].

The blocks were then trimmed and cut serially at 5µ using a rotary microtome. The sections were floated in water bath at 55oC and picked up by the use of a clean frosted end slides. The frosted end slides were now placed on the hot plate for 40 minutes for adequate attachment of the sections on the slides after which the sections were de-waxed, hydrated, air dried and stored in a slide box ready for staining process.

Staining Procedure

Sections for general tissue structure were stained by Haematoxylin and Eosin technique [10].

The slides were examined under a light microscope and photomicrographs were taken.

Result

Result of acute toxicity test of black pepper (*Piper nigrum*)

Physical observation of experimental rats used for acute toxicity test of *Piper nigrum*

Physical observation of the experimental rats used for the LD_{50} showed that there was no weakness, no diarrhea, no changes in skin surfaces, no changes in fur colour and no death in the test animals administered with single doses of aqueous extract of *Piper nigrum* in phase I and II. This suggests that there was no apparent sign of toxicity in the test animal at a single dose of up to 5000 mg/kg.

Results of notable physical observations of experimental rats

The test groups presented change in fur colour compared to the control and that before the study. On the other hand, there were no comparable changes in behavioral pattern, skin surfaces on the feet, hand, tail, mouth, ears and eyes. Similarly, fecal nature (output, texture and quantity) were not different in the entire groups.

Histological observations

The histological observations indicated that black pepper administration for a duration of two (2) weeks resulted in mild vacuolar congestion and glomerular degeneration and as seen in the kidney cytoarchitecture in the treated group fed with 0.62 ml (which added up to 25 mg/100g) of black pepper. These changes are at variance with that of the control group A. The micrograph of the kidney sections in control group l showed normal histological cytoarchitecture with well-defined glomeruli. However, at 4 weeks of black pepper administration, cellular infiltration and severe lobulated and distorted glomeruli were observed in rat fed 0.62 ml. At 6 weeks of black pepper administration, glomeruli shrinkage was seen. The histological findings in the testis tissue sections showed that the control testis present normal cytoarchitecture with intact seminiferous tubules and well defined interstitial spaces. In group

2 fed with black pepper, sperm cell degeneration was observed after 2 weeks of administration. Haemorrhagic congestion and sperm cell degeneration was observed in testis of rats administered with black pepper after 4 weeks. At the end of six weeks, haemorrhagic congestion and seminiferous tubule shrinkage was observed in the testis.

Plate 1: Photomicrograph of control kidney tissue section (H and E: X200) showing normal cytoarchitectural features with intact glomeruli.

Plate 2: Photomicrograph of control liver tissue section (H and E: X400) showing normal cytoarchitectural features with intact central vein.

Plate 3: Photomicrograph of control testis tissue section (H and E: X100) showing normal cytoarchitectural features with well-defined seminiferous tubules.

Plate 4: Photomicrograph of kidney tissue section (H and E: X100) of rats fed with black pepper for 2 weeks showing mild glomerular shrinkage (black arrow) with vascular congestion.

Plate 5: Photomicrograph of kidney tissue section (H and E: X100) of rats fed with black pepper for 2 weeks showing mild glomerular shrinkage (black arrow) with vascular congestion.

Plate 6: Photomicrograph of kidney tissue section (H and E: X100) of rats fed with black pepper for 4 weeks showing severe glomerular damage (black arrow) with cellular infiltration (circle).

Plate 7: Photomicrograph of kidney tissue section (H and E: X100) of rats fed with black pepper for 6 weeks showing severe glomerular shrinkage (arrow).

Plate 8: Photomicrograph of liver tissue section (H and E: X100) of rats fed with black pepper for 2 weeks showing vascular congestion.

Plate 9: Photomicrograph of liver tissue section (H and E: X100) of rats fed with black pepper for 4 weeks showing vacuolation (black arrow) and vascular congestion (blue arrow).

Plate 10: Photomicrograph of liver tissue section (H and E: X100) of rats fed with black pepper for 6 weeks showing severe vascular congestion (blue arrow).

Plate 11: Photomicrograph of liver tissue section (H and E: X400) of rats fed with black pepper for 6 weeks showing severe vascular congestion (black arrow), nuclear vacuolation (blue arrow) and basophilic cells (circle).

Plate 12: Photomicrograph of testis tissue section (H and E: X100) of rats fed with black pepper for 4 weeks showing haemorrhagic congestion (black arrow).

Plate 13: Photomicrograph of testis tissue section (H and E: X400) of rats fed with black pepper for 6 weeks showing haemorrhagic congestion (black arrow) and seminiferous tubule shrinkage (blue arrow).

Discussion

The pathologic changes observed in this study may be attributed to the chemicals contained in the study substances. In fact, it has been documented that black pepper (*Piper nigrum*) contain several amine alkaloids, sterols and lignans. Lignans are phenolic compounds found mainly in plants and are believed to protect humans from tumors and viruses. They however also produce side effect such as irritation, scarring and tissue necrosis thus may be responsible for the observed lobulated glomeruli, vascular congestion, cellular infiltration, glomerular shrinkages, nuclear vacuolation, tissue vacuolation, sperm cell degeneration, seminiferous tubule degeneration and interstitial haemorrhage as seen in sections through the kidney, liver and testis. Although the emphasis of this study was not on the effect on weight but on the histopathological changes weight variations were monitored considering its significance and thus, reemphasizes the opinion that increased concentration of active compounds in plants extracts are not always beneficial and can even promote adverse biological effects [11].

Conclusion

Conclusively, it could be inferred from the findings that black pepper administration can cause architectural distortions as evident in the kidneys, liver and testes sections above.

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