

Toxicological Assessments of *Piper nigrum* on Alloxan Induced Diabetic Rats

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Abstract: Black pepper, (*Piper nigrum*) is one of the plants used as spices in Africa and other parts of the world. The lethal toxicity and histology assessment of the organs of diabetic rats treated with different doses of *Piper nigrum* was analyzed. The acute toxicity (LD₅₀) test of the ethanol extract of *Piper nigrum* result showed that the leaves were not toxic up to 5000mg/kg body weight of the extract after 24 h of constant observation, indicating the safety of the leaves for human consumption. The histological sections of the liver and kidney of rats treated with the ethanol leaves extract of *Piper nigrum* showed no remarkable histological changes compared to group II (Diabetic untreated) which is an indication that *Piper nigrum* extract is safe and possess no threat to the organs of metabolism.

Key words: *Piper nigrum* • Toxicology • Lethal Dose • Acute Toxicity • Histology

INTRODUCTION

Diabetes mellitus (DM) is a chronic metabolic disorder characterized by high levels of glucose in the blood due to impaired secretion of insulin or insulin insensitivity [1]. Diabetes mellitus affects approximately 4% of the population worldwide and is expected to increase by 5.4% in 2025 [2]. Currently, the available therapy for diabetes includes insulin and various oral antidiabetic agents such as sulfonylureas, thiazolidinediones, α -glucosidase inhibitors etc. [3]. These drugs are used as monotherapy or in combination to achieve better glycaemic control. Each of the above oral antidiabetic agents are associated with a number of serious adverse effects [4], such as headache, loss of appetite, nausea, diarrhea, vomiting, hypoglycaemia, weight gain and heart failure [5]. Hence, the treatment of diabetes has shifted to the use of natural plant sources that has minimal side effects. Plants have played a major role in the introduction of new therapeutic agents and have received much attention as sources of biologically active substances including antioxidants, hypoglycaemic and hypolipidaemic agents [6].

Alloxan (2,4,5,6-pyrimidinetetrone) is an oxygenated pyrimidine derivative. It is present as alloxan hydrate in aqueous solution [7]. Alloxan is a toxic glucose

analogue, which selectively destroys insulin-producing cells in the pancreas (Beta cells) when administered to rodents and many other animal species [8]. This causes an insulin-dependent diabetes mellitus (Called "Alloxan Diabetes") in these animals, with characteristics similar to type I diabetes in humans. Alloxan is selectively toxic to insulin-producing pancreatic beta cells because it preferentially accumulates in beta cells through uptake via the GLUT-2 (Glucose transporter 2).

According to India Herbal Medicine (Ayurveda), *Piper nigrum* (Black pepper) possesses anti-tumorigenic, immuno-stimulatory, stomachic, carminative, anticholesterolaemic properties and again known for its strong phytochemical activities [9]. Piperine, a substance present in black pepper has been found to increase the absorption of selenium, B-complex vitamins, beta-carotene, curcumin as well as other nutrients from food. Piperine also inhibits pro-inflammatory cytokines that are produced by tumour cells. During that process, it interferes with the signaling mechanisms between cancer cells, thereby reducing tumor progression [10]. In respect to its numerous usage, the present study was undertaken to evaluate the toxicological effects of *Piper nigrum* ethanol leaves extract on alloxan induced diabetic rats.

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MATERIALS AND METHODS

Plant Material: The leaves of *Piper nigrum* were used for this study. The leaves were purchased from Ogige market in Nsukka and were identified by Mr. Alfred Ozioko of the Bioresources Development Centre and Conservation Programme (BDCP) Research Centre, Nsukka, Enugu State.

Extraction of Plant Materials: The leaves of *Piper nigrum* were air-dried at room temperature for four weeks after which it was grounded into fine powder. The powdered leaves (500g) were macerated in 1.5 L of absolute ethanol for 48 h. The solution was filtered with Whatman No.4 filter paper and the filtrate concentrated to a semi-solid residue in an oven at 60°C.

Experimental Animal: Thirty (30) adult albino rats were used for the study and eighteen (18) adult albino mice were used for the acute toxicity (LD₅₀) study. All the animals used were obtained from the Animal House of the Faculty of Biological Sciences, University of Nigeria Nsukka. The rats were fed with standard growers mash rat pellets (Grand Cereals Ltd, Enugu) and water. The animals were acclimatized for 7 days under standard environmental conditions, with a 12 hour light/dark cycle maintained on a regular feed (Top feed; grower mash) and water. The ethical procedures of the Department of Biochemistry for the care and use of laboratory animals approved the research.

Acute Toxicity Test of the Ethanol Extract of the *Piper nigrum* Leaves: The method of Lorke [11] was used for the acute toxicity test of the *Piper nigrum* leaves. Eighteen (18) adult albino mice were utilized in this study. The test was done in two stages. In stage one, the animals were grouped into three (3) groups of three rats each and were given 10,100 and 1000mg/kg body weight of the extract respectively orally. In the second stage, 1600, 2900 and 5000mg/kg body weight of the extract were administered to the animals which had been grouped as in stage one. The administration of the extract was done orally.

Experimental Induction of Diabetes: The baseline blood glucose levels were determined before the induction of diabetes. The rats were fasted overnight prior to injection of alloxan dissolved in iced cold normal saline at a dose of 150 mg/kg body weight and the route of administration was intraperitoneal. Blood samples were taken from the tail vein 72 h after the alloxan injection to measure the

blood glucose levels by ACCU-Check glucose meter. Animals with blood glucose levels (After fasting for 12 h) over 200 mg/dl were considered diabetic and used for the further study [12]. The treatment lasted for twenty one (21) days in which blood glucose levels of the animals were determined at the beginning and at the end of the study.

Experimental Design: Thirty (30) adult male Wistar albino rats weighing 125-220g were used for the study. They were acclimatized for fourteen (14) days with free access to feed and water. After acclimatization, they were evenly distributed into six (6) groups of five rats each. The baseline blood glucose levels were determined before the induction of diabetes. The rats were fasted overnight prior to injection of alloxan dissolved in iced cold normal saline at a dose of 150mg/kg body weight intraperitoneally. After 3 days, rats with blood glucose levels greater than 200mg/dl were considered diabetic and used for the investigation [13]. The treatment lasted for twenty one (21) days in which blood glucose levels and body weight of the rats were taken on day 0,7,14 and 21. The route of administration was via oral route with the aid of an oral intubation tube. The groups and doses administered are summarized below:

- Group I:** Control (Normal non-diabetic rats)
- Group II:** Positive control (Diabetic untreated rats)
- Group III:** Diabetic rats treated with 2.5mg/kg body weight of glibenclamide.
- Group IV:** Diabetic rats treated with 100mg/kg body weight of the ethanol extract
- Group V:** Diabetic rats treated with 200mg/kg body weight of the ethanol extract
- Group VI:** Diabetic rats treated with 300mg/kg body weight of the ethanol extract.

At the end of the experimental period the rats were starved for 12 h and then sacrificed under ether anaesthetized. Their organs (liver and kidney) was surgically removed and stored in 10% formalin for histological study. The method of Drury *et al.* [2] was followed for the histology examination of the various organs.

RESULTS

Acute Toxicity (LD₅₀) Test of Ethanol Extract of *Piper nigrum* Leaves: The acute toxicity test of the ethanol extract the *piper nigrum* leaves showed no death up to 5000mg/kg body weight.

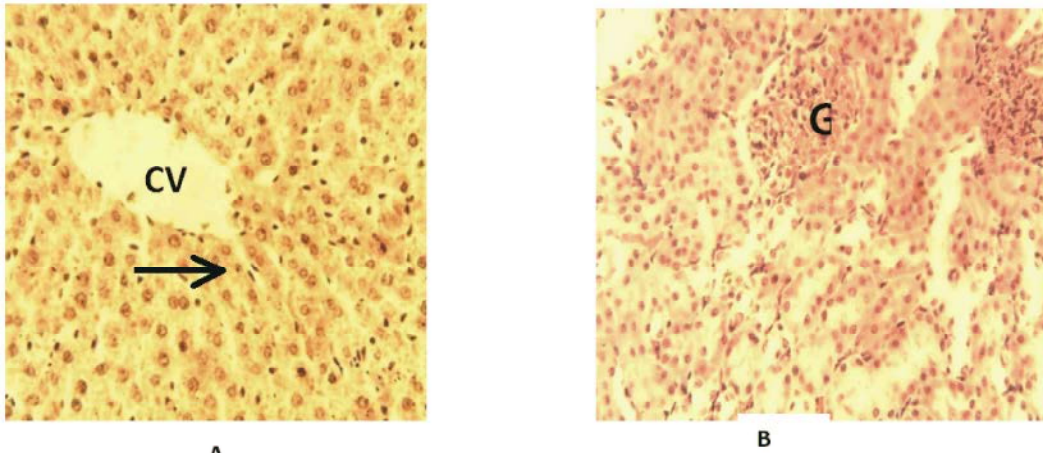


Plate 1: Photomicrograph of sections of organs from control rats showing, A- normal Liver with its central vein (CV) and Kupffer cells along the sinusoids (arrow), B- Kidney showing normal glomerulus (G) and renal tubules H&E $\times 400$

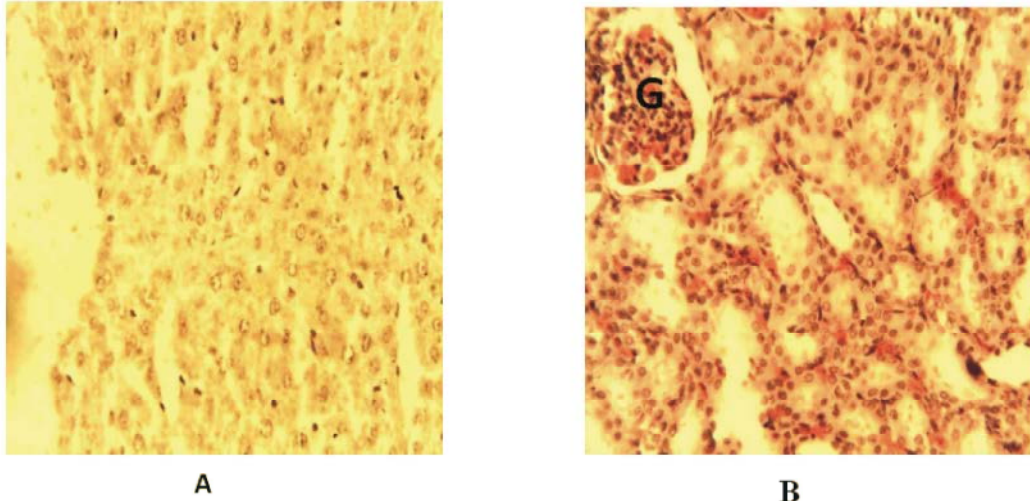


Plate 2: Histology sections of organs from untreated diabetic rats. A- liver with focal areas of hepatocyte degeneration, B- kidney having mild congestion of the glomerulus (G) H&E $\times 400$.

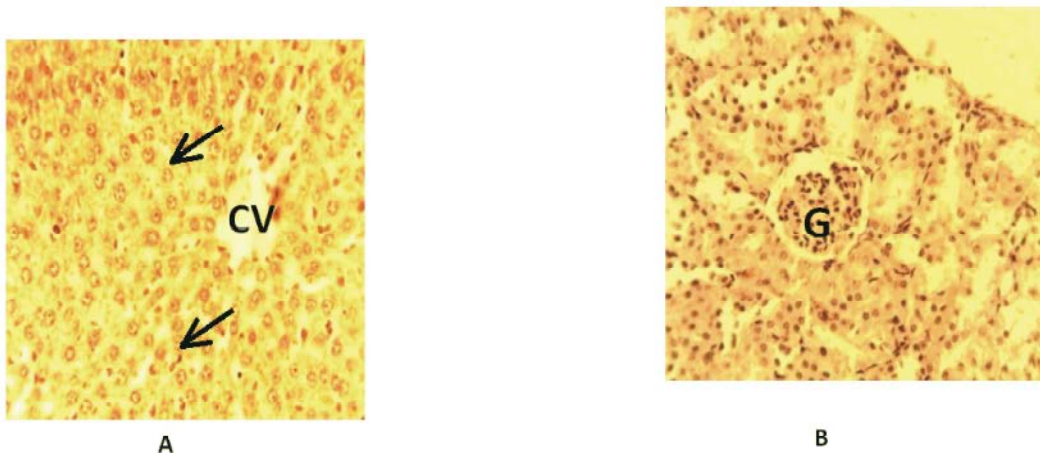


Plate 3: Photomicrograph sections of organs from rats treated with glibenclamide showing A- liver with the central vein (C) and B- the kidney with no observable histologic changes (see the glomerulus (G) and renal tubules). H&E $\times 400$

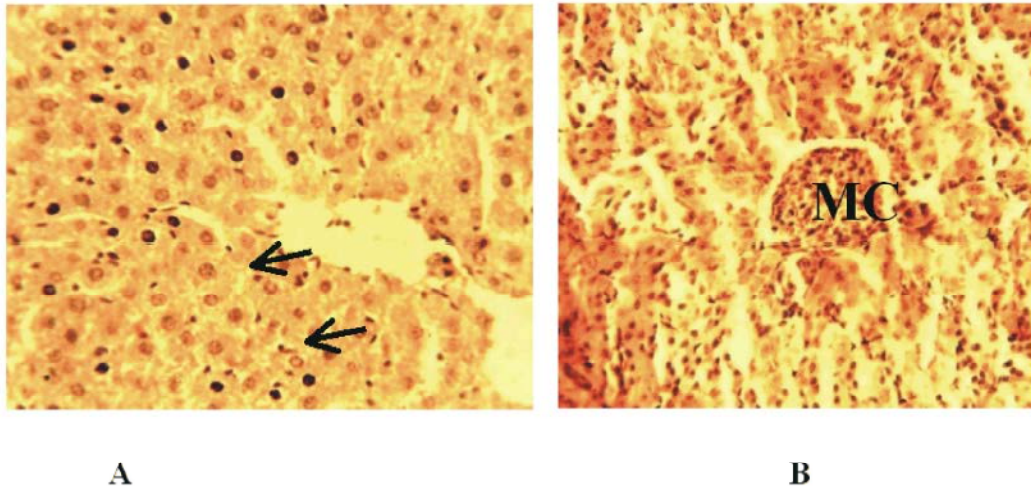


Plate 4: Histologic sections of organs from rats treated with 200mg/kg of leaves extract of *Piper nigrum* showing A- liver with partial restoration normal structure (arrow) and B- kidney with mild congestion (MC) of the glomerulus. H&E $\times 400$

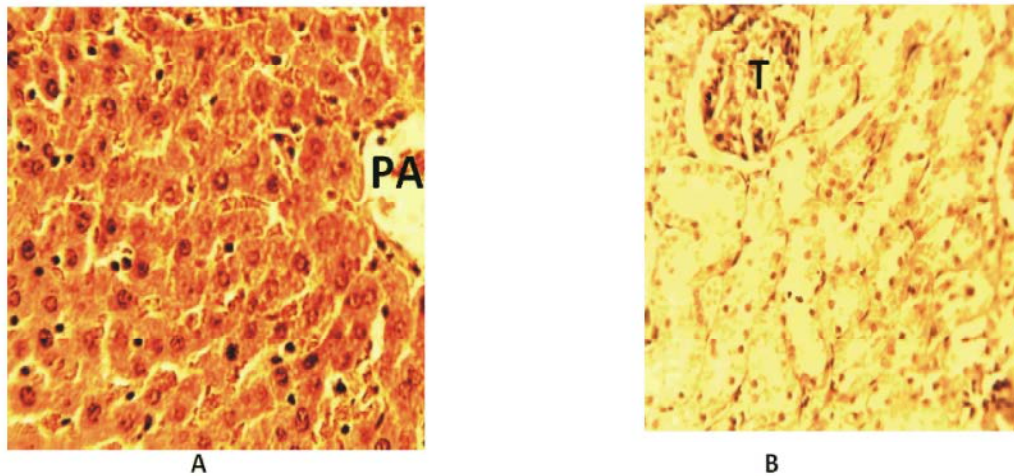


Plate 5: Photomicrograph of sections of organs from rats treated with 300mg/kg leaf extract of *Piper nigrum* showing; A- liver showing the portal area (PA) with no remarkable histologic change and B- kidney with normal tubules and glomerular tufts(T). H&E $\times 400$.

Table 1: Phase I and II of the acute toxicity (LD_{50}) test of *Piper nigrum* leaves.

	Dosage mg/kg	Mortality
Phase I		
Group 1	10	0/3
Group 2	100	0/3
Group 3	1000	0/3
Phase II		
Group 1	1600	0/3
Group 2	2900	0/3
Group 3	5000	0/3

DISCUSSION

The acute toxicity (LD_{50}) test of the ethanol extract showed that the plant was not toxic to the rats up to 5000mg/kg body weight of the extract, an indication that the leaves could be safe for human consumption and this report is in support of the report by Aqil *et al.* [1] and Szkudelski [14]. Taubes [15], also reported that *Piper nigrum* was devoid of toxicity up to 2000 mg/kg in experimental mice.

Microscopic examination of the liver and kidney sections of the control rat showed normal morphological structure of the central vein and kupffer cells respectively

(Plate 1). On the other hand, microscopic investigation of the liver and kidney section of diabetic untreated rat demonstrated various areas of hepatocyte degeneration of the liver and mild congestion of the glomerulus of the kidney (Plate 2). Investigation of the liver and kidney sections of diabetic treated with glibenclamide (Standard drug) revealed normal histological structure of the tissues as shown in Plate 3. Treatment with the leaves extract of *Piper nigrum* in the dose of 200mg/kg body weight showed partial restoration of normal histological structure of the liver and kidney with few disturbances in the liver and kidney cells arrangements (Plate 4). Treatment with the leaves extract of *Piper nigrum* in the dose of 300mg/kg body weight showed complete restoration of normal histological structure of the liver and kidney with no disturbance in the cell arrangements (Plate 5) when compared to group 2 (Diabetic untreated rat). The histology result indicated that *Piper nigrum* extract restored the damages caused by alloxan induction in experimental rats and posses no sign of toxicity to their organs. This finding complements established report by the Centre for Food Safety and Applied Nutrition, Food and Drug Administration as reported by Taubes [15] and Vijayakumar *et al.*[16].

CONCLUSION

This study indicates that *Piper nigrum* possess no threat to the organs of the experimental rats and its recommended safe for human consumption in the management and treatment of oxidative stressed diseases.

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