JOURNAL OF SCIENCE AND TECHNOLOGY
ISSN: 2229-8460 e-ISSN: 2600-7924

JST

Vol. 16 No. 1 (2024) 84-94
https://publisher.uthm.edu.my/ojs/index.php/jst

Protective Effects of Aqueous Extract of *Piper nigrum* Whole Fruits Against Tramadol-induced Hepato-and Nephrotoxicity in Wistar Rats

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DOI: https://doi.org/10.30880/jst.2024.16.01.009

Article Info

Received: 28 December 2023
Accepted: 2 June 2024
Available online: 23 June 2024

Keywords

Black pepper, biochemical parameters, piperaceae, toxicity, tramadol

Abstract

Tramadol, a widely used analgesic, is becoming more abused in most countries. Hence, this study aims to investigate the protective effects of aqueous extract of *Piper nigrum* whole fruits on tramadol-induced toxicity in male Wistar rats. Six groups (A-F) of male Wistar rats were exposed to a 14-day oral treatment. Specifically, the control group, induced group (tramadol at 60 mg/kg), reference drug group (Vitamin C at 250 mg/kg) and the extract groups (250, 500, and 1000 mg/kg). The levels of liver cellular markers ALT, AST and ALP as well as the kidney function parameters (urea, creatinine and uric acid) showed a significant (p<0.05) increase following the administration of 60 mg/kg of tramadol and a significant reversal was observed following the administration of extract in a dose dependent manner. However, K^+ and HCO_3^- showed a significant (p< 0.05) decrease and were also reversed following the administration of extract in a dose dependent manner. Tramadol also showed a significant (p<0.05) increase in the concentration of WBC and LYM, and a significant (p< 0.05) decrease in the levels of RBC, HGB, HCT, RDW-SD, RDW-CV, and MPV. The reference drug and the extract showed significant (p< 0.05) reversal in the levels of these biochemical parameters. Histological examination revealed normal liver and kidney architecture in all groups, indicating no signs of inflammation or damage. These findings revealed that the aqueous extracts from whole fruits of *P. nigrum* at concentrations of 250 and 500 and 1000 mg/kg may potentially offer protection and general improvement in the overall health status and function of the hematopoietic system.

1. Introduction

Across the world, people employ a variety of traditional medical systems, such as herbal remedies, acupuncture, and indigenous traditional medicine, to treat medical conditions involving medicinal plants [1]. Traditional medicine has been using medicinal plants for thousands of years to treat illnesses. Modern science has recognized the active qualities of medicinal plants and has incorporated a variety of plant-based medications into contemporary pharmacotherapy [2]. These drugs have been utilized for millennia by ancient civilizations [3]. Despite the fact that medicinal plants have helped advance contemporary medicine, it is crucial to ensure their safe and efficient usage. In order to inform and maximize medicinal plant usage, this necessitates the proper administration, identification, and preparation of the plants in addition to the collection of up-to-date toxicological or safety information. The safety of medicinal plants is an important element to consider, given that their toxicity might vary due to variations in their chemical makeup and other reasons. The notion that they are intrinsically safe, basically due to their long history of usage is no longer valid considering the wealth of evidence demonstrating the negative consequences of herbal treatments [4,5].

Tramadol, a prescribed analgesic, has been linked to a variety of consequences, extending beyond its primary analgesic function [6]. It is a cyclohexanol derivative having monoaminergic properties, used in treating pain issues that are both acute and chronic. It reduces serotonin and noradrenaline reuptake by acting on opioid receptors [7]. Numerous studies have linked long-term tramadol exposure to the production of oxidative damage, inflammation, and apoptosis. In a study by Mohamed and Mahmoud [8], the effects of long-term tramadol treatment on rats' cerebrum were examined and the results showed that the drug caused oxidative damage by increasing cerebral lipid peroxidation, nitric oxide, and reduced glutathione (GSH) concentration. Additionally, the study found that rats given tramadol had higher levels of pro-inflammatory cytokines in their serum and that the rats' brains expressed inflammatory markers, indicating an inflammatory response. Furthermore, Long-term tramadol usage has also been linked to a significant risk of liver and renal damage [7,8].

Piper nigrum, commonly known as black pepper in English, while locally known as *Iyere* in Yoruba, *Uziza* in Igbo and *Barkono baki* in Hausa, is a popular spice worldwide [9]. Black pepper has been historically one of the most important spices and herbal medicines, with various potential health benefits. Numerous pharmacological characteristics of *P. nigrum* have been discovered, which may add to its possible health advantages. Black pepper contains many significant components, for instance, essential oils and piperine. These compounds have been linked to several biological advantages, such as antibacterial activity, antioxidant and anti-inflammatory properties, and more. [9]. Apart from these characteristics, *P. nigrum* could also be able to control or lessen oxidative stress and inflammation. Hence, this study aims to examine the effects of aqueous extracts of *Piper nigrum* whole fruits on liver and kidney damage following repeated use of tramadol using wistar rats as experimental models. Specifically, the Liver Cellular Markers, Kidney Function Parameters, Hematological Parameters will be assayed for; Histopathological examination will also be carried out

2. Materials and Methods

2.1 Materials

2.1.1 Collection and Authentication of Plant Material

Fresh *P. nigrum* whole fruits were bought from an herb seller at a market (*Oja Ipata*) in Ilorin West local government, Kwara State, Nigeria. The leaves were identified and authenticated by a botanist at the University of Ilorin Herbarium, Ilorin, Nigeria. A voucher sample was deposited under "UILH/001/1575/2023".

2.1.2 Experimental Animals

A total of 48 male Wistar rats (*Rattus norvegicus*) that weighed 139.20 ± 3.25 g were provided by Markeen Global Ventures at Ilorin, Nigeria. Clean aluminum cages with enough ventilation (temperature: 25℃ – 27℃; photoperiod: 12 hours' light and dark cycle; relative humidity: 45% – 50%) were utilized to house and care for the animals. Rat pellets (by the Nigerian Company, Top Feeds Nigeria Limited) and distilled water were freely available to the animals except where otherwise stated. The study was carried out in accordance with the National Institutes of Health's standards (NIH Publication No. 80-23) for the care and use of laboratory animals, with approval from the ethical review committee of Al-Hikmah University (HUI/UERC/2023/015). The handling and care of the animals was provided by strictly adhering to the institution's policy on animal usage and care.

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2.1.3 Drugs, Assay Kits and Chemicals Used

Tramadol was a product of Peace Standard Pharmaceutical Ind. Ltd, Ilorin, Kwara State, while Vitamin C was obtained from Unicure Pharmaceutical Ltd, Ijebu-ode, Ogun State. Assay kits for serum electrolyte, creatinine, urea, total protein, alkaline phosphatase, alanine aminotransferase and aspartate aminotransferase are products of Sigma-Aldrich Inc., located at St. Louis, Missouri, USA. The remaining chemicals utilized in this experiment were of analytical grade and were made using calibrated measuring flasks and distilled water in compliance with the guidelines.

2.2 Methods

2.2.1 Preparation of Extract

The whole pepper fruits were rinsed under running tap water and air-dried at room temperature for 96 hours. Prior to extraction, the dried fruits were pulverized using an electric blender (Master Chef Blender, MC-BL 1980, Guangdong, China), and kept in an airtight container. 1000 milliliters of distilled water were used to extract one known gram (100 g) of the powdered sample (1/10 w/v) at 25^{o}C and was continuously shaken for 48 hours, and then filtered using Whatman No. 1 filter paper. A rotary evaporator (Model RE 52A Zhengzhou, Henan, China) was used to concentrate the filtrate that was produced, yielding 16 g. To provide the necessary dosages of 250, 500, and 1000 mg/kg body weight, the yield was reconstituted in distilled water (estimated based on data from the ethnobotanical survey). The percentage (%) yield was calculated mathematically as;

% Yield = (Weight of the Crude Extract (g)/Weight of Dried Powdered Sample (g) ×100

2.2.2 Animal Grouping and Administration of Extract

A total of 48 male rats were divided into six groups (A to F) in a complete randomized design, with 8 animals per group as follows:

- Group A: Rats received 0.5ml of distilled water
- Group B: Rats treated with tramadol and administered 0.5 mL of distilled water
- Group C: Rats treated with tramadol and administered 0.5 mL of 250 mg/kg of Vitamin C
- Groups D: Rats treated with tramadol and administered 0.5 mL of 250 mg/kg of the extract
- Groups E: Rats treated with tramadol and administered 0.5 mL of 500 mg/kg of the extract
- Groups F: Rats treated with tramadol and administered 0.5 mL of 1000 mg/kg of the extract

Using a plastic oropharyngeal cannula, the different animal groups were treated as described above once daily (08:00–08:45 h) for 14 days.

2.2.3 Preparation of Serum and Tissue Supernatants

Yakubu et al. [10] instructions for preparing the serum were followed. On the 15th day, rats were briefly anaesthetised with diethyl ether fumes. When they become unconscious, the jugular veins were cut and 5 mL of the blood was collected into sterile, dry centrifuge tubes. For fifteen minutes, the samples were stored at room temperature to allow the blood to coagulate. Using a Uniscope Laboratory Centrifuge (Model SM800B, Surgifriend Medicals, Essex, UK) centrifuged at 503 × g for 10 minutes, clear serum was collected and stored at -20°C. Furthermore, the tissue supernatants were prepared as described by Yakubu *et al*. [10]. The liver and kidney were homogenized in an ice-cold solution of 0.25 M sucrose (1:5 w/v) after blotting with blotting paper, and thinly sliced. The homogenates were centrifuged at 894 *× g* for 15 min and the supernatant was frozen at -20°C before biochemical assays were carried out within 24 h of preparation.

2.2.4 Determination of Biochemical Parameters

The concentrations of aspartate aminotransferase activity (AST) and alanine aminotransferase (ALT) was determined according to the method given by Reitman and Frankel [11], alkaline phosphatase (ALP) was carried by adopting Wright *et al.* [12] method. Serum urea was determined as described by Langenfeld *et al.* [13], serum creatinine by Bartels *et al*. [14], and serum uric acid was done following the method of Tietz [15]. Serum potassium ion and sodium ion were assayed with method of Rastegar [16], while serum bicarbonate ion was determined as described by Libório *et al.* [17]. The method described by Dacie and Lewis [18] was utilized to evaluate the hematological parameters including hemoglobin (HGB), packed cell volume (PCV), white blood cells

(WBC), red blood cells (RBC), the mean corpuscular hemoglobin (MCH), the mean corpuscular hemoglobin concentration (MCHC), the mean corpuscular volume (MCV), platelets, neutrophils, and lymphocytes (LYM) using the Sysmex KX 21 N hematology analyzer.

2.2.5 Histopathological Examination

The method described by Drury and Wallington [19] was employed. The organs of interest (liver and kidney) tissues were fixed in 10% (v/v) formalin, dehydrated through different grades of ethanol, and xylene embedded in paraffin. The tissues were then sectioned (5 µm thick) and stained with Hematoxylin and Eosin stain (H&E). The histology slide was examined using an acuscope® (China) microscope withTSView® Software (China) to observe the pathological changes. Using the Canon Image Folio software program, cross sections of 12 liver and kidney were taken at an enlargement of 400 pixels (Model: Powershot A2500, Japan).

2.2.6 Statistical Analysis

The data generated from the study were presented as the mean ± standard error of the mean of eight replicates and a one-way analysis of variance (ANOVA) was performed. The data were considered statistically different at (p< 0.05) using GraphPad Prism version 6.01 (GraphPad Software, Inc., San Diego, California, United States).

3. Results

3.1 Effects of Aqueous Extract of P. nigrum Whole Fruits on Liver Cellular Markers in Male Rats' Serum

The Liver cellular markers' activity, ALT, AST and ALP showed significant (p < 0.05) increase when administered tramadol. Administration of the reference drug (Vitamin C), 250, 500 and 1000 mg/kg of the extract shows a significant (p < 0.05) reversal in the levels of the liver cellular markers (Table 1).

3.2 Effects of Oral Administration of Aqueous Extract of P. nigrum Whole Fruits on Kidney Function Parameters of Rats

When treated with tramadol, urea levels of, creatinine and uric acid showed significant (p < 0.05) increase. In contrast, K^+ and HCO_3^- levels showed significant (p < 0.05) decrease, whereas Na^+ shows no significant difference (p> 0.05). However, with the extract administration at 250, 500 and 1000 mg/kg, the levels of creatinine, uric acid, K^+ and HCO_3^- shows a significant (p < 0.05) reversal in a dose dependent manner that is favorably compares to the group administered with the reference drug (Vitamin C) (Table 2).

3.3 Effects of Oral Administration of Aqueous Extract of Piper Nigrum Whole Fruits on Hematological Parameters of Male Wistar Rats

WBC and LYM level showed significant (p < 0.05) increase when administered tramadol while RBC, HGB, HCT, RDW-SD, RDW-CV and MPV showed significant (p < 0.05) decrease. However, MCV, PDW and P-LCR showed no significant (p> 0.05) difference. When administered the reference drug, and the extract, the concentrations of the hematological indices showed a significant (p < 0.05) reversal except LYM (Table 3).

Table 1 *Liver cellular markers in male rats' serum administered P. nigrum whole fruits aqueous extract*

Group/ Parameters (U/L/mg protein)	A (Control)	B (60 mg/kg Tramadol)	C (Tramadol + 250 mg/kg Vitamin C)	D (Tramadol + 250 mg/kg *P.nigrum*)	E (Tramadol + 500 mg/kg *P.nigrum*)	F (Tramadol + 1000 mg/kg *P.nigrum*)
ALT	118.23± 3.99^a	135.55± 2.28^b	117.51± 7.85^c	130.25± 4.00^b	116.76± 9.56^a	111.28± 11.16^a
AST	293.48± 7.72^a	331.78± 8.05^b	304.47± 15.23^a	326.35± 22.31^c	315.66± 23.37^c	291.79± 11.59^a
ALP	1.89± 0.04^a	2.79 ± 0.04^b	1.85± 0.09^a	2.74 ± 0.09^b	2.05± 0.10^c	1.96± 0.18^a

Values are means of eight replicates ± SEM; Values with different superscripts across the row shows a significant (p < 0.05) difference

3.4 Histopathological Examination of the Liver and Kidney

Histological examination of the liver and kidney across the groups were presented with preserved architecture composed of cords of normal hepatocytes, normal portal tracts, and central vein. There is no significant inflammation or features of acute or chronic damage (Fig. 1A - 1F). Similarly, the medullary and cortical

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architecture in the kidney of all the treated animals were preserved with no evidence of deformity on the proximal as well as the distal convoluted tubules [Fig. 2A-2F].

Table 2 *Kidney function parameters in male rats' serum administered P. nigrum whole fruits aqueous extract*

Group/ Parameters (mg/dl)	A (Control)	B (60 mg/kg Tramadol)	C (Tramadol + 250 mg/kg Vitamin C)	D (Tramadol + 250 mg/kg *P. nigrum*)	E (Tramadol + 500 mg/kg *P. nigrum*)	F (Tramadol + 1000 mg/kg *P. nigrum*)
Urea	155.05± 7.07^{a}	267.17± 3.94^{b}	171.71± 6.2^{c}	200.57± 4.40^{d}	198.02± 6.14^{d}	174.24± 2.31^{c}
Creatinine	0.47± 0.09^{a}	0.94± 0.52^{b}	0.46± 10.08^{a}	0.47± 0.09^{a}	0.59± 0.03^{a}	0 .47± 0.54^{a}
Uric Acid	2.81± 0.06^{a}	4.84± 0.04^{b}	2.74± 0.08^{a}	2.81± 0.08^{a}	2.87± 0.27^{a}	2.96± 0.49^{a}
K^+	0.69± 0.06^{a}	0.23± 0.21^{b}	0.66± 0.29^{a}	0.22± 0.18^{b}	0.32± 0.07^{c}	0.63± 0.46^{a}
Na^+	71.43± 1.62^{a}	73.42± 1.60^{a}	73.97± 1.04^{a}	72.80± 1.55^{a}	73.70± 0.50^{a}	74.11± 0.79^{a}
HCO_3^-	81.95± 3.74^{a}	71.31± 3.97^{b}	79.87± 2.20^{a}	74.91± 6.32^{a}	74.69± 5.67^{a}	79.92± 3.17^{a}

Values are means of eight replicates ± SEM; Values with different superscripts across the row shows a significant ($p < 0.05$) difference

Table 3 *Hematological parameters in male rats' plasma administered P. nigrum whole fruits aqueous extract*

Group/ Parameters	A (Control)	B (60 mg/kg Tramadol)	C (Tramadol + 250 mg/kg Vitamin C)	D (Tramadol + 250 mg/kg *P. nigrum*)	E (Tramadol + 500 mg/kg *P. nigrum*)	F (Tramadol + 1000 mg/kg *P. nigrum*)
RBC ($\times 10^6$)	5.46 ± 0.80^{a}	3.93± 0.86^{b}	6.26± 0.63^{a}	5.275± 0.05^{a}	5.29± 0.91^{a}	5.06± 0.10 ^{a}
HGB (g/dl)	8.25± 1.85 ^{a}	5.50 ± 1.60^{b}	7.75± 1.15^{a}	7.90 ± 0.30^{a}	8.25± 0.65^{a}	8.05± 0.05^{a}
WBC (10^3/µL)	11.95± 0.95^{a}	15.70 ± 3.40^{b}	11.25± 4.55^{a}	11.10 ± 0.70^{a}	11.45± 0.75^{a}	10.65± 0.85^{a}
HCT (%)	32.40 ± 6.20 ^{a}	20.75± 5.95^{b}	30.45± 3.25^{a}	30.00 ± 1.30^{a}	36.55± 6.35^{a}	28.15± 0.15^{a}
MCV (fL)	57.70 ± 1.50^{a}	58.60± 1.30^{a}	46.65± 12.45^{a}	56.85± 1.95^{a}	57.80 ± 1.80^{a}	56.1± 20.4^{a}
MCH (pg)	14.60 ± 0.90 ^{a}	10.10 ± 0.40 ^{b}	15.55± 0.25 ^{a}	14.95± 0.45^{a}	14.90 ± 1.10 ^{a}	15.9± 0.20 ^{a}
MCHC (g/dL)	25.30 ± 0.90^{a}	20.75± 0.15^{b}	25.95± 0.85^{a}	26.35± 0.15^{a}	25.80 ± 2.7 ^{a}	26.6± 0.04^{a}
PLT (10^3/µL)	650.50 ±50^{a}	456.00 ± 10.00^{b}	673.00 ±52.00^{a}	666.50 ± 28.50^{a}	696.50 ± 92.5^{a}	630.5± 29.5^{a}
RDW-SD (fL)	50.35± 9.95 ^{a}	43.50 ± 4.50^{b}	45.55± 0.85^{b}	47.05± 4.65^{a}	48.15± 1.05^{a}	49.55± 3.45^{a}
RDW-CV (%)	25.75± 5.75^{a}	21.50 ± 2.30^{b}	25.50 ± 0.10^{a}	24.25± 1.85^{a}	25.00 ± 0.60^{a}	24.15± 1.45^{a}
PDW (fL)	5.15± 5.13^{a}	6.12 ± 3.01^{a}	5.40 ± 5.40^{a}	5.12 ± 2.19^{a}	5.19± 5.13^{a}	5.26 ± 4.73^{a}
MPV (fL)	3.65± 3.65 ^{a}	3.20 ± 2.05^{b}	3.80 ± 3.80^{a}	3.50 ± 2.10^{a}	3.50 ± 3.21^{a}	3.59 ± 2.29^{a}
P-LCR (%)	13.28 ± 5.70 ^{a}	14.50 ± 3.12^{a}	15.25± 7.25^{a}	16.80 ± 3.91^{a}	11.20 ± 0.40^{a}	10.07 ± 3.20^{a}
LYM (%)	75.40± 6.10^{a}	86.50 ± 0.90^{b}	84.80 ± 2.80^{b}	85.5± 15.10^{b}	88.75± 15.05^{b}	85.6± 3.50^{b}

Values are means of eight replicates ± SEM; Values with different superscripts across the row shows a significant ($p < 0.05$) difference

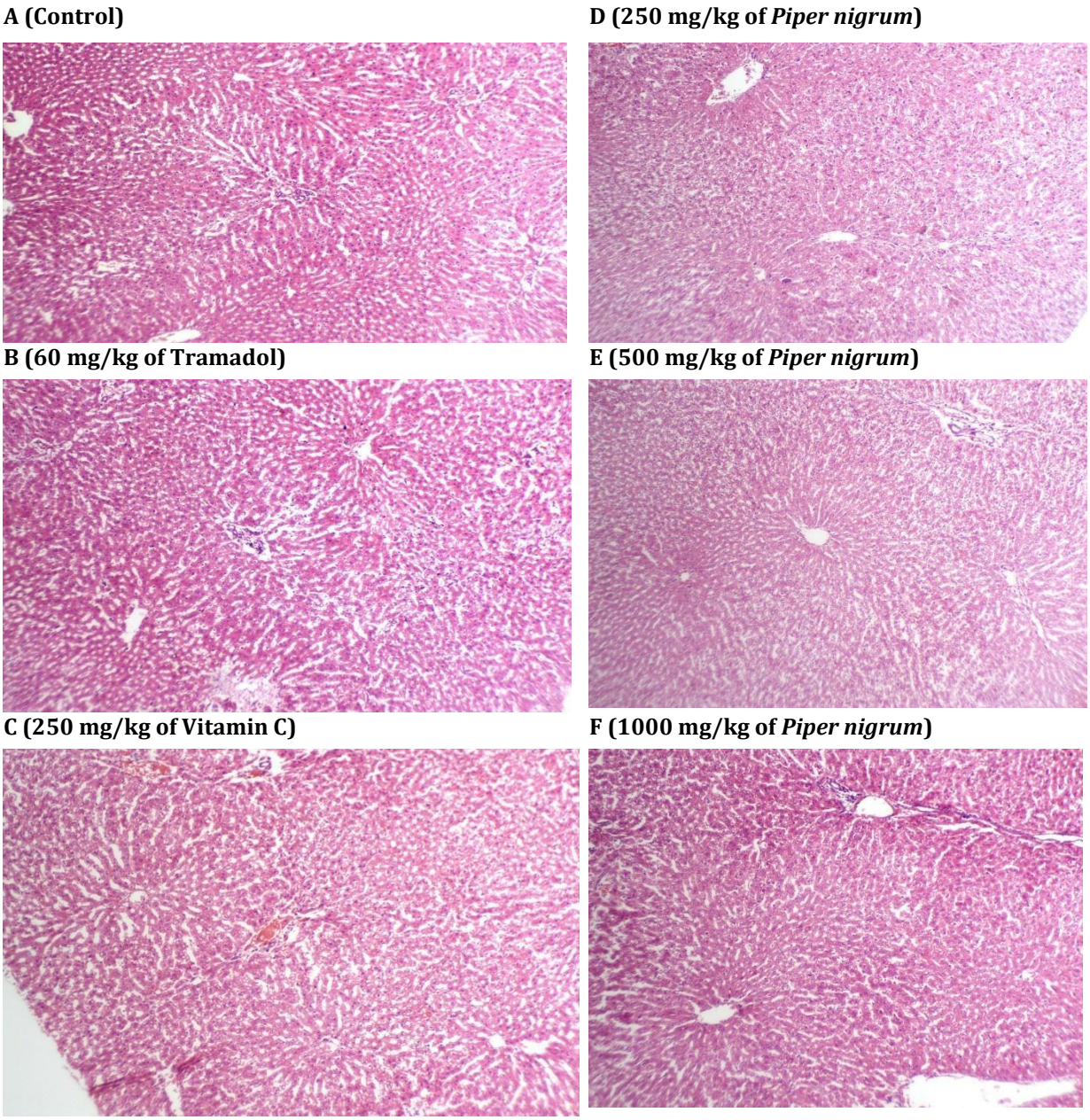

Fig. 1: *Histopathological examination of the liver by hematoxylin-eosin staining reveals normal cytostructure (magnification ×400)*

4. Discussion

The use of herbal remedies is growing in popularity, yet there are concerns about their effective dosage and safety. Herbal products are not inherently safe because of active ingredients that may alter drug metabolism and interact with the body's physiology [20,21]. It is therefore critical to assess the safety as well as the efficacy of herbal products before employing them therapeutically. Taking this action ensures that the benefits of utilizing herbal products outweigh any potential risks and ensures their safety and effectiveness. One of the primary concerns with using herbal products is how they affect the liver and kidney, two main organs involved in detoxification and biotransformation [21]. Examining the activity of enzymes in tissue and plasma is essential to identify potential tissue damage [22].

4.1 Effects of Aqueous Extract of *P. Nigrum* Whole Fruits on Liver Cellular Markers

Alanine aminotransferase (ALT) is an enzyme that catalyzes the reductive transfer of an amino group from alanine to α-ketoglutarate to yield pyruvate and glutamate [23]. ALT is primarily localised in the liver and plays a crucial part in the metabolism of amino acids. AST, an enzyme found primarily in the liver, catalyzes the

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reductive transfer of amino groups from aspartate to α-ketoglutarate to yield oxaloacetate and glutamate [23]. ALP hydrolyzes monophosphates at alkaline pH and is a membrane integrity marker [24].

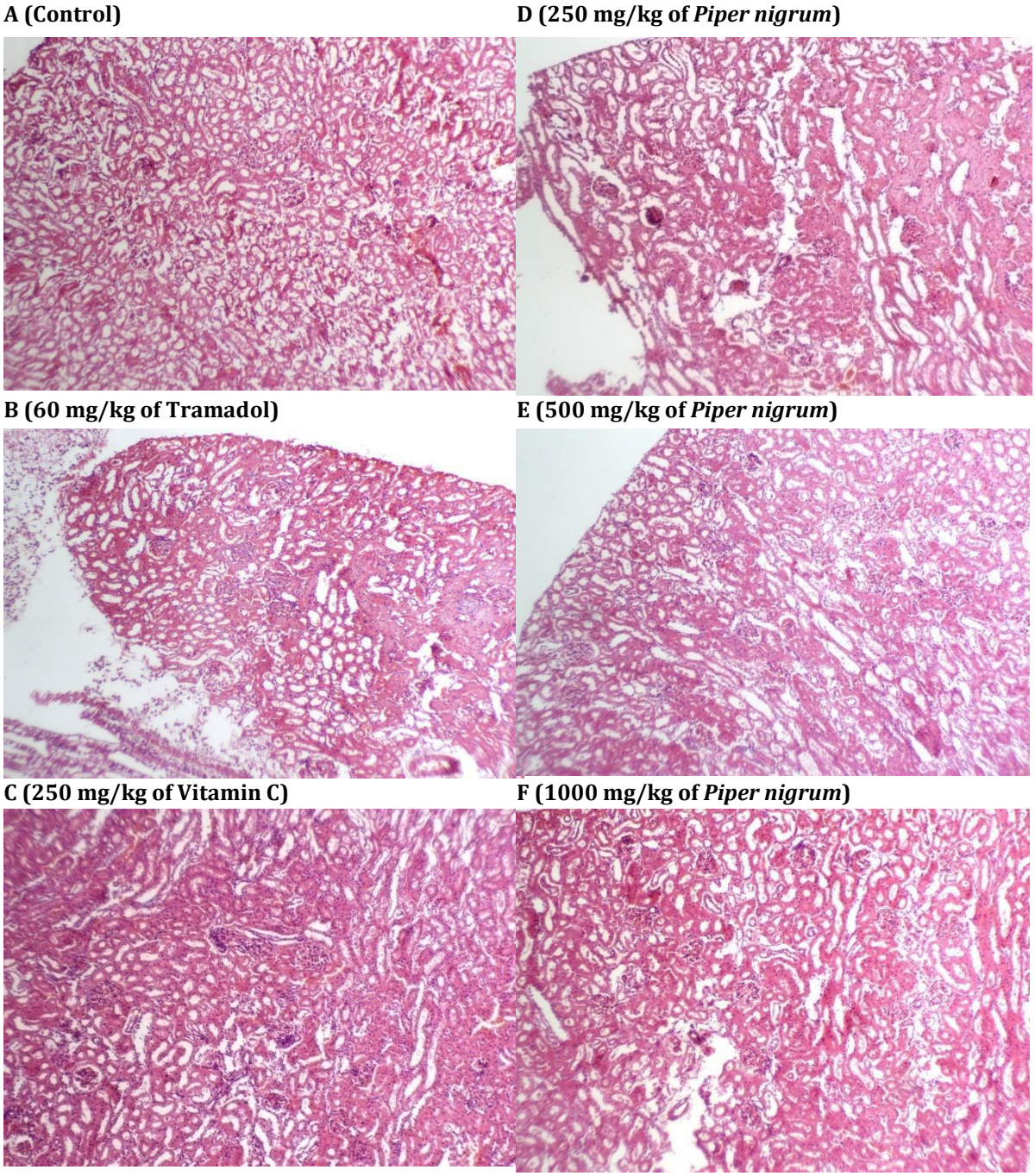

Fig. 2 *Histopathological examination of the kidney by hematoxylin-eosin staining reveals normal cytostructure (magnification ×400)*

ALT, AST and ALP activity increase show that the administration of tramadol may have hepatotoxic effects or indicate potential liver and heart damage [22]. Increased ALT, AST and ALP activity in the bloodstream are often associated with liver damage or stress as ALT is released to the bloodstream as liver cells get injured. ALP activity in the blood can also indicate bone issues, among other conditions. Administration of extracts resulted in ALT, AST and ALP levels which compares favorably to the control group suggesting that the extract of *P. nigrum* is capable of reversing tramadol induced toxicity, hence, its liver-protective properties [24].

4.2 Effects of Aqueous Extract of *P. Nigrum* Whole Fruits on Kidney Function Parameters

Serum urea, creatinine, uric acid and electrolytes are indicators of renal excretory function useful in diagnosing impaired renal function. These tests provide valuable data on the kidneys' ability to filter and excrete waste products, regulate fluid and electrolyte balance and maintain overall renal function [25]. Urea is a waste product produced when the liver breaks down proteins. It is normally present in small amounts in the blood, but the levels can increase in injured liver or when there's excessive protein breakdown. As a byproduct of muscular action, creatine and phosphocreatine combine to become creatinine [26]. It is normally present in the blood at constant levels, unless there's a problem with kidney function. Purines, which are present in a variety of foods, break down to create uric acid as a waste product. It is the primary byproduct of protein metabolism, generated by the liver and eliminated by the kidney tubules; decreased excretion is a sign of renal illness [27]. High levels of uric acid in the blood can lead to conditions such as gout or kidney stones. Increase creatinine urea plus the uric acid levels after administering tramadol indicates that the drug has affected kidney function, muscle activity and protein metabolism and purine metabolism. The significant decrease in urea, creatinine and the uric acid levels at all dosages of the extract may suggest that the extract may have helped reduce the amount of nitrogenous waste products and excessive protein breakdown in the body, lessening tramadol's negative effects. The plant could also be beneficial for individuals with conditions like gout, where elevated uric acid levels are a concern.

Inorganic electrolytes are normally present in significant quantities in both extracellular and intracellular fluids. They play a crucial role in maintaining the balance of fluids and ions within the body, ensuring the proper functioning of cells and tissues [28]. Because of their propensity to quickly split into their component ions or radicals, they comprise the single most important factor in the transfer and movement of water and electrolytes between three divisions of the extracellular and intracellular compartment [28]. Potassium ions have a significant impact on how nerve impulses are propagated along the nerve cells and transmitted to receptor cells [29]. Treatment with tramadol resulted in a significant decrease in serum potassium concentration, suggesting that the drug either increases potassium excretion or decreases potassium reabsorption resulting in hypokalemia. A condition known as hypokalemia is characterized by low blood potassium levels [30]. Numerous physiological consequences, such as heart arrhythmias and muscular weakness, can result from hypokalemia. In addition, bicarbonate is vital for the body's acid-base equilibrium and for preserving the appropriate blood pH. Bicarbonate (HCO^{3-}) decrease causes metabolic acidosis, typically accompanied by a similar drop in carbon dioxide partial pressure (PCO2). HCO^{3-} levels were significantly reduced after adminitering tramadol, indicating that this dosage may cause respiratory acidosis and hypercapnia, which may then indirectly impact the body's acid-base balance and cause metabolic acidosis. When the body either creates too much acid or is unable to adequately eliminate it, metabolic acidosis results. The changes in the kidney function indicators were significantly mitigated by the extract, which may suggest the extract ability to restore nephron's capacity to function normally by ensuring that nitrogenous waste products are being excreted and also maintains an equilibrium in osmotic pressure [31,32].

4.3 Effects of Aqueous Extract of *P. nigrum* Whole Fruits on Hematological Parameters

The hematological results provide insights into the hematological profile of tramadol, reference drug and *P. nigrum* aqueous extract on various blood parameters. Numerous hematological characteristics were investigated in the study, such as the counts of white blood cells (WBC), red blood cells (RBC), platelet (PLT) parameters, hemoglobin (HGB), hematocrit (HCT), and lymphocyte (LYM) levels, among others. The findings reveal a complex interrelationship between these substances and blood parameters, shedding light on potential implications for clinical practice. The study showed that administering tramadol led to a significant rise in WBC and LYM levels. The activation of defense mechanisms (immune system) in rats treated with tramadol was shown by this significant increase in white blood cell count. Because of the rats' cell-mediated immune response, this activation of white blood cells is a beneficial reaction for survival. Increase in white blood cells will respond lead to the production of more monocytes, granulocytes, and lymphocytes [33]. Tramadol's ability to boost immunological response and increase natural killer cell activity might potentially be the cause. On the other hand, metrics measuring red cell distribution width (RDW-SD and RDW-CV) and mean platelet volume (MPV) have significantly decreased, as well as RBC, HGB, HCT, MCH, MCHC, PLT, and RDW after tramadol treatment. The possible explanation for this might be that tramadol's inhibition of the morphological differentiation in red blood cells led to the decrease in these blood components, which in turn affects other blood components including hemoglobin concentration and platelet count. As a result, the body's metabolic processes will create less energy overall. The negative consequences of the body's blood clotting mechanism may possibly be the cause [33]. Tramadol may influence the sympathetic nerve's decreased activity or function during splenic blood circulation. It might therefore change HCT's volume and explain the volume decrease. This will result in a drop in hemoglobin concentration and obstruction of bone marrow iron production, which will then have an impact on MCH and MCHC. Previous studies have demonstrated that when an animal is given an injection of tramadol, bodily fluids move from areas outside the blood vessels to the inside of the blood vessels. This causes a decrease in HCT [7,8]. Alternatively, increased aldosterone and antidiuretic hormone release may originate from it, which

leads to salt and water retention and extracellular fluid expansion, hemodilution, and a drop in HCT. Red blood cell (RBC) counts were also generally lower in patients using tramadol. The inhibitory effect of Tramadol on erythropoiesis may account for this outcome. Low energy production resulted from hemoglobin (HGB) reduction in and red blood cell count, which decreased the oxygen delivery to various organs.

4.4 Histopathological Examination of the Liver and Kidney

Additional support for the biochemical assessment might come from histological alterations in tissue sections [34]. The rats' kidney and liver retained their structural integrity after taking the dosages of the extract of *Piper nigrum* whole fruits. The results of the histological analysis show that the kidney and liver tissues in Groups A–F have normal histology and comparable characteristics, suggesting that the tissues don't appear to have any significant anomalies or pathological changes. Furthermore, there are no obvious signs of injury or inflammation, and the cellular structure, arrangement, and organization of the liver and kidney tissues are within the anticipated range. This implies that the tissues have maintained their physiological integrity.

5. Conclusion

This study underscores the preventive benefits of *P. nigrum* aqueous extract against the liver and kidney damage that may arise due to excessive use of tramadol. The significant decrease in urea, creatinine and uric acid levels following the administration of the extract showed the capability of the extract to reduce the amount of nitrogenous waste products and excessive protein breakdown, thereby reducing tramadol negative effects. The plant could also be beneficial for individuals with conditions like gout, where elevated uric acid levels are a major concern. Furthermore, this study reported that all the treated animals' kidneys retained their medullary and cortical architecture, with no indication of deformation on the proximal and distal convoluted tubules. Additionally, the lobular architecture, hepatocytes, central vein, and portal tracts of all experimental animals were within normal physiology, with no evidence of adhesion, inflammation, degenerative changes, or necrosis, thereby showing a convincing evidence of extract protective effects in shielding the kidney and liver by attenuating the alterations in their functioning indices arising from the abuse of tramadol.

Acknowledgement

The authors appreciate the contribution of Mr. Dele Aiyepeku of the Department of Biochemistry, University of Ilorin, Ilorin, Nigeria, for the technical assistance provided in the course of this study.

Conflict of Interest

Authors declare that there is no conflict of interests regarding the publication of the paper.

Author Contribution

The authors confirm contribution to the paper as follows: ***study conception and design:*** *Author QON, Author SA;* ***data collection:*** *Author SA, Author MBF, Author MRA, Author MAD, Author OTO;* ***analysis and interpretation of results:*** *Author QON, Author MBF, Author MAD, Author OTO;* ***draft manuscript preparation:*** *Author SA, Author MBF, Author MRA. All authors reviewed the results and approved the final version of the manuscript.*

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