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RESEARCH ARTICLE

ASSESSMENT OF PHYTOCHEMICAL COMPOSITION AND EFFECTS OF AQUEOUS EXTRACT OF PIPER UMBELLATUM LEAVES ON BLOOD BIOMARKERS AND LIVER AND KIDNEY HISTOLOGY IN WISTAR RATS

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Abstract

Medicinal plants are used in traditional and alternative medicine for the management and prevention of various diseases. Piper umbellatum is used in Côte d'Ivoire to treat numerous kidney ailments and arterial hypertension. The aim of this study is to evaluate these effects on biomarkers and the histology of the kidney and liver. In this experimental study, 20 male and 20 female rats were divided randomly into 4 groups by gender (n=5) as control and three experimental groups receiving different doses of EAPu (400, 800 and 1000 mg/kg). At the end of the treatment, blood samples were taken from caudal vein, and serum urea, creatinine, ASAT and ALAT and blood count were analyzed and compared in control. All the animal's kidneys and liver were exposed after dissection, and tissue sections were prepared for histopathological evaluation. Phytochemical analysis identified the presence of saponins, flavonoids, sterols, triterpenoids, coumarins, tannins, and phenolic compounds, whereas alkaloids were not detected. A significant decrease in urea and creatinine levels was observed only in males, compared to the control group, at the dose of 1000 mg/kg BW ($p < 0.05$). No evidence of damage to renal and liver tissue was observed in this study. In conclusion, the aqueous extract reduced serum urea and creatinine levels, likely due to the identified phytochemicals of therapeutic interest, without inducing nephrotoxicity.

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Introduction:-

Piper umbellatum (Linn) (Piperaceae) is a tropical dicotyledonous herbaceous species widely distributed across Africa, including Côte d'Ivoire, as well as in Central and South America. The plant is extensively recognized for its broad application in traditional medicine, where it is used to manage a variety of conditions, particularly

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inflammatory, infectious, and renal disorders (Roersch, 2010). Its therapeutic potential is largely attributed to the presence of diverse bioactive compounds, including flavonoids, terpenoids, alkaloids, sterols, tannins, and saponins (Kouadio, 2023). These phytochemicals are known to exert multiple biological activities that underpin the medicinal relevance of the species. Collectively, these compounds are associated with antioxidant and anti-inflammatory activities and may contribute to the plant's potential nephroprotective effects, thereby supporting its widespread use in traditional healthcare systems. Several studies have highlighted the diverse pharmacological properties of *Piper umbellatum*, including its antimicrobial and antiparasitic properties, alongside potential diuretic and nephroprotective effects consistent with its traditional application in the treatment of urinary and renal conditions (Roersch, 2010).

In Côte d'Ivoire, aqueous preparations of *Piper umbellatum* are routinely consumed as traditional remedies. However, their use remains essentially empirical, with no standardized dosing regimen and limited consideration of inter-individual physiological variability. (Kouadio, 2023). While accumulating scientific evidence supports the pharmacological potential of *P. umbellatum*, particularly its antioxidant and anti-inflammatory properties, its safety profile remains inadequately characterized. Indeed, studies on medicinal plants indicate that, despite their perceived safety, improper or prolonged use may lead to adverse effects on vital organs, including the liver and kidneys (Bwanbale, 2026). This highlights the critical need for rigorous toxicological assessments and well-designed clinical studies to ensure the safe and effective use of *P. umbellatum* in both traditional and modern medical contexts. The present study aimed to evaluate the effects of acute administration of an aqueous extract of *P. umbellatum* on renal histological architecture and key biochemical indices of kidney function.

Materials and Methods:-

Plant collection and extract preparation:-

Piper umbellatum was gathered in Yakassé-Mé, Ivory Coast. The botanical sample was taxonomically verified on July 10, 1980, by Professor Ake-Assi Laurent, a plant scientist at the Centre National de Floristique (UFR Biosciences, Félix Houphouët-Boigny University, Abidjan, Côte d'Ivoire). Recent leaves of *Piper umbellatum* were dried in the air at ambient temperature in a shaded area to avoid photodegradation. The leaves were subsequently crushed into a fine powder utilising a laboratory micro-mill (IKA Labortechnik, Type A10). One hundred grams of the powdered substance were extracted using one litre of distilled water by boiling for 15 minutes. The obtained mixture was filtered repeatedly using hydrophilic cotton and Whatman filter paper to yield a clear filtrate. The filtrate was then evaporated under reduced pressure at 60°C with a Büchi rotary evaporator, followed by drying in an oven at 50 ± 5°C. The resulting product was a fine, water-soluble powder known as the aqueous extract of *Piper umbellatum* (EAPu).

Phytochemical screening:-

Freshly prepared extracts of *Piper umbellatum* and its constituents were subjected to standard phytochemical analysis methods (Harborne, 1998 ; Zague, 2009 ; Kokate, 2000) to detect the presence of flavonoids, alkaloids, xanthoproteins, carboxylic acid, carbohydrates, glycosides, saponins, tannins, proteins, etc..

Animals used in the study:-

Rattus norvegicus strain females (nulliparous and non-pregnant) and males, healthy young adults aged 8–12 weeks, and albino rats weighing 200-250 g were used. They were obtained from the Vivarium (animal house), Ecole Nationale Supérieure (ENS), and Abidjan, Côte d'Ivoire. Animals were housed in metabolic cages and maintained under standard laboratory conditions ($T^{\circ} = 25 \pm 2^{\circ}\text{C}$) with a dark and light cycle (12/12 hours). They were allowed free access to a standard dry pellet diet and water ad libitum.

Experimental Design:-

In this study, twenty-four male and female Wistar rats were employed. They were randomly assigned to five groups, each of which had six rats—three females and three males—in various cages:

Group I: Control;

Group II : EAPu 400 mg/kg of BW;

Group III: EAPu 800 mg/kg of BW;

Group IV: EAPu 1000 mg/kg of BW.

Each group of rats received the same dose repeatedly throughout a 14-day period. Group I rats were given distilled water (vehicle) until the end of the trial. All experimental protocols were conducted in accordance with the protocols for the protection of experimental animals of the European Council on Legislation 2012/707.

Analytical procedures:-

At the end of the treatments (15th days), the animals were sacrificed, blood samples were taken, centrifuged, and the serum was extracted for urea and creatinine assays. The colorimetric and kinetic technique allows for the measurement of these parameters. Blood's Electrolytes analysis of Na⁺, K⁺ and Cl⁻ concentrations are measured using flame photometer (Janssens, 2015). Blood samples collected in EDTA tubes were used for the determination of red blood cell (RBC) count, total and differential white blood cell (WBC) counts, and platelet count using standard laboratory procedure (Sanderson, 1981). The blood count (hemogram) is performed using a Mindray BC-30 automatic analyzer (China).

Histopathological examination:-

The kidneys and liver specimens from each rat were removed and immediately stored in 10%v/v formalin in normal saline after gross histological examination. They were dehydrated using increasing concentrations of isopropyl alcohol (80–100%). Paraffin sections at 5 µm thickness were made from the paraffin-embedded organs using a Leica rotary microtome (Leica® RM2125 RTS, Germany). This was followed by routine staining with hematoxylin and eosin which involved the process of deparaffinization, hydration, staining, rinsing and clearing in xylene. Slides were viewed under light microscope (Olympus® CK41SF, Philippines).

Statistical Analysis:-

The data and graphical representations were statistically analysed using GraphPad Prism 8.4.3 software (La Jolla, CA, USA). Analysis of variance (ANOVA) and the Tukey-Kramer test were used to determine the significance of variations found between concentrations. The findings and data are presented as mean ± SEM (standard error of mean). Inter group comparison were carried out and a P value of < 0.05 was considered statistically significant.

Results:-**Extract yield and phytochemicals:-**

The extract derived from 100 grams of dried Piper umbellatum leaves yielded 18.78 grams, which is a 18.78 % yield. According to the analyses related to the phytochemical composition, extracts of Piper umbellatum leaves contain saponins, flavonoids, Sterols and Triterpenoids, coumarins, tannins and phenolic compounds without alkaloids (Table 1).

Table 1 : Phytochemical screening of the aqueous extract of Piper umbellatum

Secondary metabolites	Reactant/Tests	Results
Tanins and Phenolic compounds	FeCl ₃	+
Flavonoids	Réactif de Neu	+
Alkaloids	Dragendorff; Bouchardat	-
Saponosids	Physical	+
Coumarins	KOH 5%	+
Sterols and Triterpenoids	Vanillin-sulfuric acid	+

(+) : presence of Secondary metabolites;

(-) : absence of Secondary metabolites.

Effect of the extract on body weight evolution of rats:-

Administration of the extract to the male and female rats caused no-dose dependent, and no significant reduction in the percentage weight gain only on female compared to the control (Fig. 1)

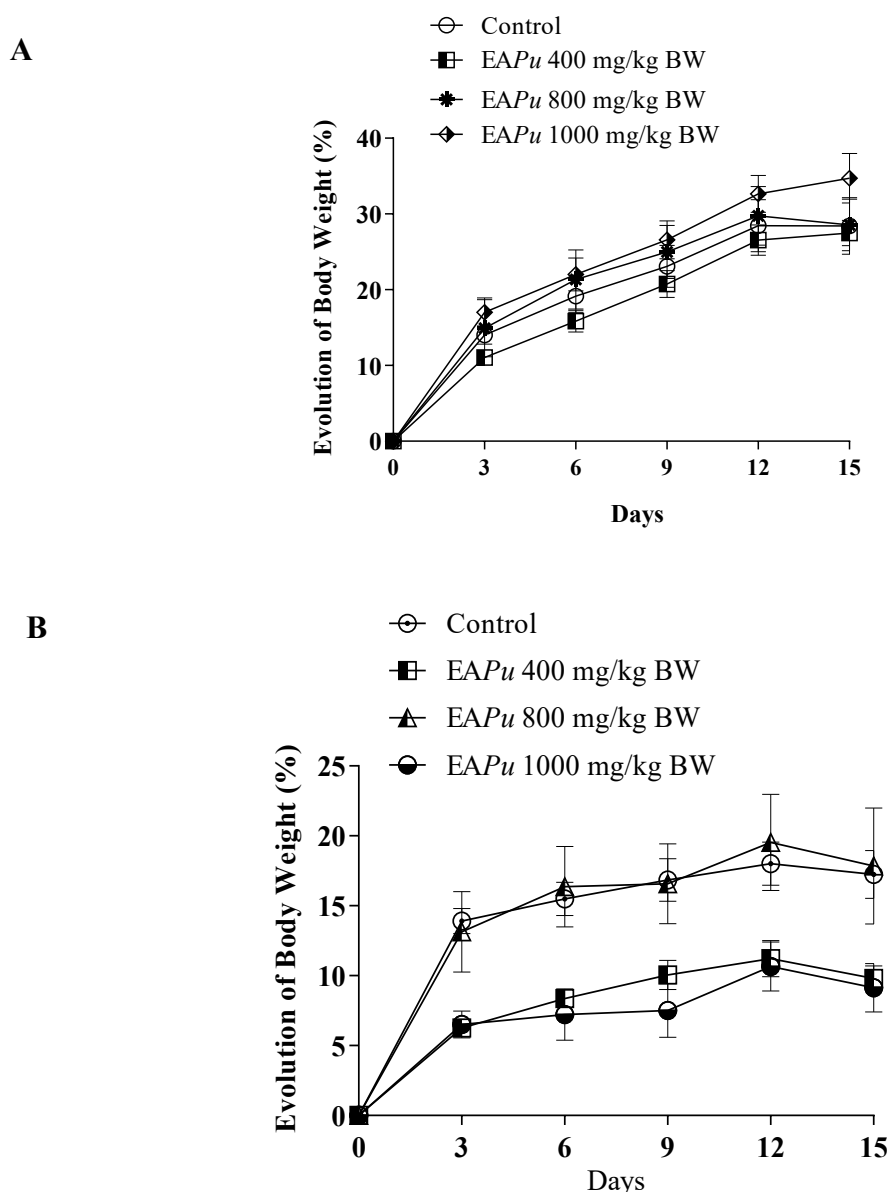


Figure 1: Body weight evolution of male (A) and female (B) rats during EAPu administration.

n = 5 in each case, All values are presented as mean + Standard Error of Mean (ESM). No significant difference compare to control (distilled water) $p > 0,05$; DW = Distilled water (10 mL/kg); EAPu = Piper umbellatum aqueous extract (400, 800 and 1000 mg/kg).

Effect of Piper umbellatum on the liver and kidney biomarkers on wistar rat:-

The effect of EAPu extract, administered at doses of 400, 800, and 1000 mg/kg BW, on creatinine and urea levels is shown in Table 2. No significant difference was observed in urea levels in male and female rats at doses of 400 and 800 mg/kg BW compared to the control group ($p > 0.05$). Administration of the extract had no effect on the concentrations of these parameters. In contrast, a significant decrease in urea (0.51 ± 0.006 vs 0.40 ± 0.009) and creatinine (13.88 ± 0.30 vs 12.28 ± 0.48) levels was observed only in males, compared to the control group, at the dose of 1000 mg/kg BW ($p < 0.05$). Furthermore, no significant reduction in serum AST and ALT levels was observed in the groups of male and female rats treated with the EAPu extract at doses of 400, 800, and 1000 mg/kg, respectively, compared to the control groups ($p > 0,05$).

Table 2. Effect of acute oral administration of Piper umbellatum aqueous extract on liver and kidney biomarkers on wistar rat

Parameters/ Groups	Gender	Control	EAPu 400 mg/kg B.W	EAPu 800 mg/kg B.W	EAPu 1000 mg/kg B.W
	Male	151.9 ± 9.16	172.8 ± 6.96	184.9 ± 19.25	175.8 ± 18.78
ASAT (UI/L)	Female	159.7 ± 10.28	153.6 ± 18.48	149.1 ± 2.83	160.2 ± 6.37
	Male	57.01 ± 0.80	90.12 ± 8.22	78.3 ± 6.78	90.73 ± 19.47
ALAT (UI/L)	Female	62.53 ± 3.61	58.07 ± 7.40	62.92 ± 6.6	43.47 ± 1.11
Urea (g/l)	Male	0.51 ± 0.006	0.56 ± 0.04	0.44 ± 0.01	0.40 ± 0.009*
	Female	0.40 ± 0.03	0.39 ± 0.02	0.38 ± 0.01	0.37 ± 0.02
Creatinine (mg/l)	Male	13.88 ± 0.30	13.39 ± 0.61	12.46 ± 0.04	12.28 ± 0.48*
	Female	12.85 ± 0.41	12.64 ± 0.19	11.05 ± 0.30	10.55 ± 0.13

n = 5 in each case, All values are presented as mean + Standard Error of Mean (ESM). Values with superscripts * means significant difference at P<0.05 when compared to control. DW = Distilled water (10 mL/kg); EAPu = Piper umbellatum aqueous extract (400, 800 and 1000 mg/kg).

Effect of Piper umbellatum on the blood electrolytes levels:-

Table 3 shown the results obtained in rats orally administered with aqueous extract of EAPu (400, 800 and 1000 mg/kg of BW) for up to two weeks. No significant effects in serum sodium, potassium and chloride levels when compared with the control (p > 0,05).

Table 3. Effect of oral administration of Piper umbellatum aqueous extract on serum concentration of Na⁺, K⁺ and Cl⁻ ions in wistar rats

Parameters/ Groups	Gender	Control	EAPu 400 mg/kg B.W	EAPu 800 mg/kg B.W	EAPu 1000 mg/kg B.W
Na ⁺ (mEq/L)	Male	141 ± 0.98	138.3 ± 2.89	141 ± 2.56	137.7 ± 0.13
	Female	140.7 ± 1.30	140 ± 1.12	138 ± 1.50	139 ± 0.95
K ⁺ (mEq/L)	Male	7.06 ± 0.42	8.23 ± 0.35	5.56 ± 0.68	5.73 ± 0.80
	Female	6.73 ± 0.49	5.4 ± 0.62	5 ± 0.70	7.2 ± 0.22
Cl ⁻ (mEq/L)	Male	112 ± 3.02	118 ± 1.29	117 ± 1.34	116.3 ± 1.60
	Female	118.3 ± 2.49	119.7 ± 2.14	114.3 ± 2.48	122 ± 2.03

n = 5 in each case. All values are presented as mean + Standard Error of Mean (ESM). No significant difference compare to control (distilled water) $p > 0.05$; DW = Distilled water (10 mL/kg); EAPu = Piper umbellatum aqueous extract (400, 800 and 1000 mg/kg).

Effect of Piper umbellatum aqueous extract on haematological parameters:-

As shown in Table 4, oral administration of the aqueous EAPu extract to rats at doses of 400, 800, and 1000 mg/kg body weight over a two-week period did not induce any statistically significant effect on not significantly alter the red blood cell count (RBC), haemoglobin (Hb) and white blood cell count (WBC) levels when compared with the control group ($p > 0.05$).

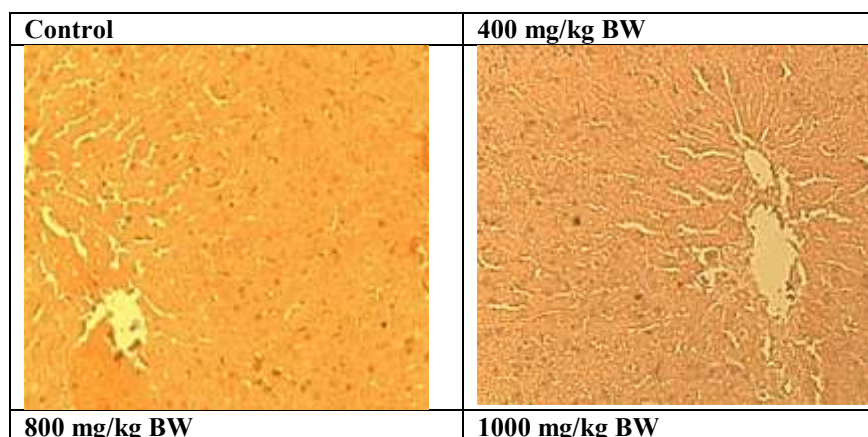
Table 4– Effect of Piper umbellatum aqueous extract on haematological parameters of male and female rat

		Distilled Water		EAPu mg/kg of BW		
Haematological parameters	Gender	Control		400	800	1000
RBC (x10) ⁶ /mm ³	Male	7.29 ± 0.19	6.96 ± 0.20	5.90 ± 1.17	7.53 ± 0.09	
	Female	6.73 ± 0.42	5.44 ± 0.02	6.87 ± 0.27	6.34 ± 0.29	
Hb (g/dl)	Male	13.87 ± 0.55	13.20 ± 0.69	11.07 ± 0.50	14.13 ± 0.87	
	Female	12.67 ± 0.15	11.47 ± 0.06	13 ± 0.17	12.67 ± 0.25	
WBC (x10) ³ /mm ³	Male	12.2 ± 1.97	14.03 ± 0.90	10.60 ± 0.96	12.43 ± 1.21	
	Female	9.10 ± 0.10	11.57 ± 0.15	10.77 ± 0.06	12.23 ± 0.15	
Platelet (10) ³ /mm ³	Male	580.33 ± 0.58	634 ± 3.61	513.67 ± 1	639.67 ± 2.52	
	Female	510 ± 1	553 ± 1	674 ± 4.36	552.33 ± 4.93	

n = 5 in each case. All values are presented as mean + Standard Error of Mean (ESM). No significant difference compare to control (distilled water) $p > 0.05$; DW = Distilled water (10 mL/kg); EAPu = Piper umbellatum aqueous extract (400, 800 and 1000 mg/kg).

Effect of oral administration of aqueous extract of Piper umbellatum on the histology of the liver and kidney of rats:-

In the histopathological study, microscopic examination of the liver and the kidney revealed showed no sign of abnormality in the tissue architecture (Fig. 2 & 3).



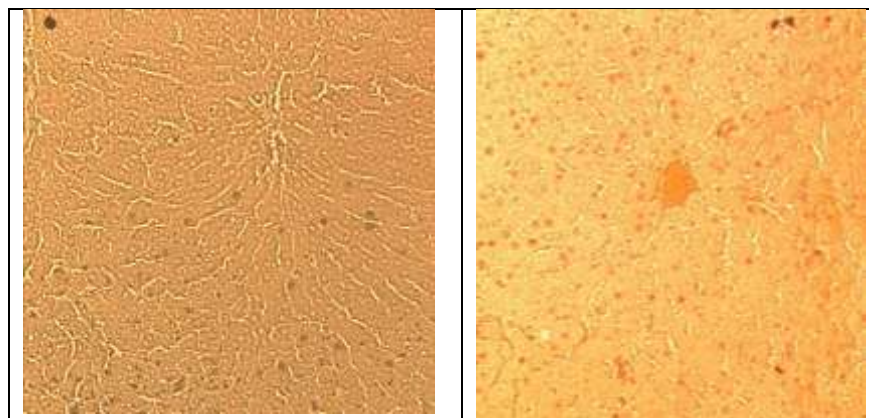


Figure 2: Histology of liver sections showing the effect of aqueous extract of Piper umbellatum after a 14-day administration in rats

Coloring : eosin hepathoylin ; x 100

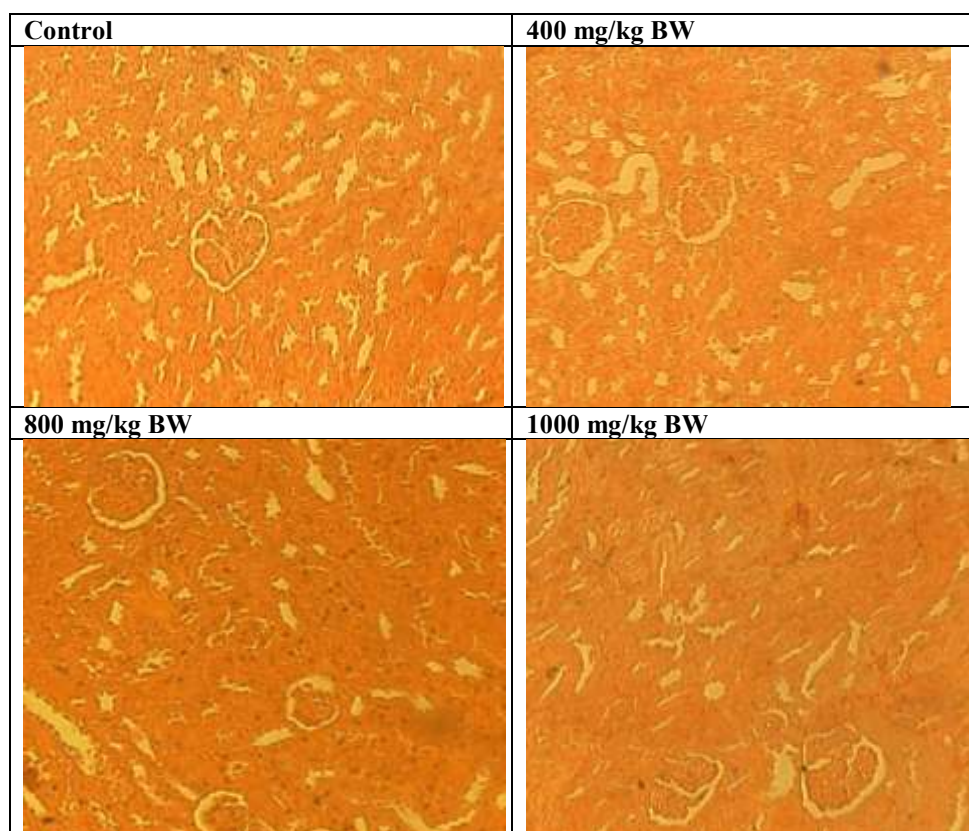


Figure 3: Histology of kidney sections showing the effect of aqueous extract of Piper umbellatum after a 14-day administration in rats

Coloring : eosin hepathoylin ; x 100

Discussion:-

The aim of this study was to evaluate the phytochemical composition and the effects of acute administration of an aqueous extract of Piper umbellatum leaves on biomarkers of hepatic and renal function, as well as on the histological architecture of the liver and kidney, in Wistar rats. Phytochemical screening revealed the presence of saponins, flavonoids, sterols, triterpenoids, coumarins, tannins, and phenolic compounds, while alkaloids were absent. This composition aligns with studies on Piper umbellatum leaves, which demonstrated a notable abundance of phenolic compounds, flavonoids, and tannins (Peters et al., 2022). It is also consistent with phytochemical reviews of Piper species, which highlight the prevalence of polyphenols, terpenes, and other biologically active

secondary metabolites (Salehi et al., 2019; Carsono et al., 2022). Flavonoids and phenolic compounds are widely recognized for their antioxidant properties, primarily stemming from their ability to scavenge free radicals and mitigate cellular oxidative damage (Alsawaf et al., 2022; Carsono et al., 2022). Saponins, triterpenoids and coumarins also contribute to the anti-inflammatory and cytoprotective effects observed in many tropical medicinal plants (Salehi et al., 2019).

Thus, the observed chemical composition could form the pharmacological basis for the traditional uses attributed to *Piper umbellatum*. The absence of significant variation in weight gain among rats treated with the aqueous extract of *Piper umbellatum* indicates that the extract did not alter the animals general physiological state. In the evaluation of natural substances with therapeutic potential, changes in body weight serve as a sensitive indicator for detecting systemic toxicity, reduced food intake, or treatment-induced metabolic disturbances (OECD, 2001). The results obtained are consistent with available pharmacological data on *Piper umbellatum*, which generally characterize the plant as having a favorable toxicological profile and multiple biological activities, with no evident signs of acute toxicity at standard experimental doses (Iwamoto et al., 2015; Cota & Romualdo, 2026). The absence of weight loss therefore suggests that the aqueous extract is well tolerated by the animals.

Gowda et al. (2010) demonstrated that urea and creatinine are key biochemical markers of renal functional integrity. An increase in their serum concentrations generally reflects a decrease in the glomerular filtration rate or damage to renal structures. In the present study, no significant increase in these parameters was observed in the treated animals, indicating that the aqueous extract of *Piper umbellatum* did not exert any detectable nephrotoxic effect. Conversely, a significant reduction in urea and creatinine levels was observed in male animals receiving the 1000 mg/kg dose.

This decrease may reflect a potential nephroprotective effect of the extract. Such a hypothesis is biologically plausible given the presence of flavonoids and phenolic compounds in *Piper umbellatum* leaves. Indeed, flavonoids are capable of reducing renal oxidative stress, modulating inflammatory responses, and preserving the function of tubular and glomerular cells (Alsawaf et al., 2022; Cao et al., 2022). Several studies have also shown that these compounds can act via the Nrf2/Keap1 pathway, a key molecular mechanism in protecting renal tissue against oxidative damage (Alsawaf et al., 2022; Cao et al., 2022). The effect observed solely in males could be attributed to sex-related differences in the metabolism of bioactive compounds or in the physiological regulation of renal function. However, this hypothesis requires further investigation. ALT and AST enzymes serve as standard markers for assessing hepatocellular integrity. Elevated serum activity levels are generally associated with increased hepatocyte membrane permeability or hepatic cytolysis (Hall et al., 2021). In this study, no significant differences in ALT and AST activities were observed between the treated and control groups. This enzymatic stability suggests that the aqueous extract of *Piper umbellatum* does not induce any measurable liver damage.

These results are consistent with the anti-inflammatory and antioxidant properties previously reported for *Piper umbellatum* (Iwamoto et al., 2015). They also align with existing data on *Piper* species, many of which exhibit protective effects against oxidative stress-induced cellular damage (Salehi et al., 2019; Carsono et al., 2022). Microscopic examination of the liver and kidneys revealed no structural abnormalities following the administration of the aqueous *Piper umbellatum* extract at doses of 400, 800 and 1000 mg/kg of body weight. Glomerular and tubular architecture remained intact in treated animals, while the liver maintained normal histological organization. The preservation of both renal and hepatic structures suggests that the compounds present in the extract do not induce major inflammation, cellular degeneration, or tissue necrosis under the conditions of this study. Future research will aim to evaluate the nephroprotective properties of the aqueous *P. umbellatum* extract using a pathological model.

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