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Effects of Ethanol Leaf Extract of *Piptadeniastrum africanum* on Biochemical Parameters of Diethylnitrosamine-Induced Liver Cancer Mice

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Abstract

Cancer is one of the leading causes of death in the world. *Piptadeniastrum africanum* is famous in traditional medicine with a wide range of clinical treatments. The objective of this study was to evaluate the effects of ethanol extract of the leaves of *Piptadeniastrum africanum* on the biochemical parameters of diethylnitrosamine-induced (DEN) liver cancer. Ethanol extract was prepared from *Piptadeniastrum africanum* leaves using cold maceration with 70% ethanol solution. Phytochemical analysis identified the main bioactive components. The study involved administering different doses of the extract (200 mg/kg, 400 mg/kg, 600 mg/kg) to mice that had been exposed to diethylnitrosamine. Repeated doses of diethylnitrosamine, DEN (200 mg/kg for six weeks) were used to induce liver cancer. The control group consisted of normal controls (normal saline 0.1 ml/kg) untreated group and the doxorubicin (50 mg/kg) group. There was statistically significant decrease ($p \leq 0.05$) in liver parameters including ALT, AST, ALP, TP and ALB in the group receiving ethanol extract of the leaves *Piptadeniastrum africanum* compared to the negative control. In the same way, levels of urea, BUN, and creatinine in the group receiving ethanol leaf extract of *Piptadeniastrum africanum* decreased ($p \leq 0.05$) compared to the negative group. The extract also reduced the levels of oxidative stress parameters ($p \leq 0.05$) compared to the negative control. Similarly, the extract restored kidney and liver tissue to a relatively normal architecture when compared to the negative control group. In conclusion, ethanol leaf extract of *Piptadeniastrum africanum* significantly regulates liver, kidney, and oxidative stress parameters of diethylnitrosamine-induced (DEN) liver cancer mice.

Keywords: *Cancer, Traditional medicine, Ethanol extract, Diethylnitrosamine (DEN), Liver cancer, Biochemical parameters.*

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1. Introduction

Human anatomy is characterized by an important functional factor: the liver, which is responsible for many functions (metabolism, detoxification, protein and bile production gluteal synthesis) is important for the general sustainability of life (Ahmed *et al*., 2023; Sharma *et al*., 2022). Liver also has the ability to restore damaged areas which are occurred due to injuries induced by overexposure to harmful substances such as pollution, infection, extreme conditions etc. (Huang *et al*., 2023; Wang *et al*., 2022). Liver injury can be acute liver injury and chronic depending on the exposure time. It is often associated with other medical conditions, such as chemical toxicity in the liver, liver fibrosis, liver cirrhosis, or hepatocellular carcinoma (Kim *et al*., 2023; Jones and Smith, 2023). Liver injury is often the result of oxidative stress as there is an increase in reactive oxygen species and a decrease in the level of protective factors (Singh *et al*., 2023; Zhou *et al*., 2023).

Piptadeniastrum africanum, a tree native to Africa, is considered to have great medicinal importance by traditional African health providers (Adetutu *et al*., 2023; Ekanem *et al*., 2022). Tree parts such as bark and leaves are widely used for their medicinal properties (Okafor *et al*., 2022; Ajayi *et al*., 2023). It is said to contain a variety of active components, such as flavonoids, tannins, steroids, and glycosides, which are expected to explain properties such as anti-helminth activity, anti-inflammatory and antibacterial (Yawanawa *et al*., 2016; IJAAR, 2022).

For example, methanolic extracts of *P. africanum* show effective enzyme inhibitory properties which is relevant in treating diseases such as diabetes and neurodegenerative diseases due to the effect of the extract on enzymes α-glucosidase and acetylcholinesterase (MDPI, 2022; Kwaku *et al*. 2023; Folashed *et al*. 2022). Moreover, the plant has the ability to alleviate oxidative stress (Chidiebere *et al*., 2023). This makes it an option for treating many medical conditions, hence highlighting its increasing pharmacological (Ekanem *et al*., 2022; Ajayi *et al*., 2023; Okafor *et al*., 2022). These among other medicinal values of *P. africanum* justify the further exploration of its pharmaceutical applications (Oboh *et al*., 2022; Folashade *et al*., 2022).

Hepatocellular carcinoma (HCC) is a main cause of cancer-related mortality globally, accounting for good sized morbidity due to its aggressive development and late diagnosis (Wang *et al*., 2023; Liu *et al*., 2023). The number one risk elements for HCC include continual liver diseases along with hepatitis B and C, alcoholic liver disease, and non-alcoholic steatohepatitis, which predispose to cirrhosis and malignant transformation (Kim *et al*., 2023; Sharma *et al*., 2022). Among experimental models, diethyl nitrosamine (DEN)-triggered liver cancer is broadly used to study hepatic carcinogenesis because of its capacity to generate oxidative pressure, DNA harm, and continual infection, main to tumor improvement (Zhou *et al*., 2023; Patel *et al*., 2023). DEN initiates carcinogenesis by means of forming DNA adducts, impairing genome balance, and triggering molecular pathways that sell tumorigenesis (Ahmed *et al*., 2023; Huang *et al*., 2023).

Herbal compounds with antioxidant and anti-inflammatory houses have proven promise in mitigating DEN-prompted hepatotoxicity, paving the way for revolutionary therapeutic techniques (Singh *et al*., 2023; Dube *et al*., 2022). Continued studies into DEN-brought about liver most cancers give important insights into the mechanisms of hepatocarcinogenesis and the improvement of powerful remedies.

2. Materials and Methods

Chemicals/reagents

All the chemicals used were of analytical grade. Diethyl nitrosamine (Sigma Chemical Co, St Louis, Mo, USA). Doxorubicin and normal saline (Nacl 0.9% w/v), Dragendorff's reagent (alkaloids), frothing test reagents (saponins), ferric chloride (tannins), aluminum chloride (flavonoids) and Salkowski reagent (steroids) were used.

The leaves of *Piptadeniastrum africanum* Brennan were collected in March from Bauchi, Bauchi State and identified at the Herbarium of the Department of Biological Sciences, Sa'adu Zungur University, Bauchi. The collected leaves were washed with distilled water. It was dried indoors at room temperature for six days (144 hours) and ground into a coarse powder using a laboratory mill. The powder was cold extracted using a mixture of 70% ethanol and 30% distilled water as a solution. Phytochemical analysis was performed according to established methods (Sofowara, 1993; Trease & Evans, 1989; Harborne, 1973). Acute toxicity of the oral ethanol blue extract was assessed by a dose-limiting test as described in OECD guideline 425.

Thirty male and female rats weighing between 19 and 30 g were obtained from the Ministry of Medicine and Pharmacy, ABU Zaria. Animals were housed in well-ventilated cages. They had access to food and water with adjusted growth media for 10 days before starting the experiment. Ethical clearance certificate with Ref. No. BASUG/FBMS/REC/ VOL. 4/0049 number was obtained from the Bauchi State University. Mice were exposed to repeated doses of diethylnitrosamine (DEN) (200 mg/kg) for six weeks to induce liver cancer. Enzyme linked immunosorbent assay using the UBI MAGIWELL (USA) enzyme immunoassay kit was used to quantify the cancer marker alpha fetoprotein (AFP) antigen (CEA) (Sell *et al*., 1983). The rats were then divided into six groups (n = 4 per group), including a control group (negative, positive [doxorubicin, 50 mg/kg], and normal control group) and an experimental group. Treated with 1 mg, 200 mg/kg, 400 mg/kg, and 600 mg/kg leaf extract for an additional 6 weeks. At the end of the study Mice were weighed and humanely euthanized with ketamine, and collect blood and tissue samples for biochemical and histological analysis. Serum concentration of aspartate Amino-transferase (AST) and alanine amino-transferase (ALT) were determined according to Reitman and Frankel (1957). Alkaline phosphatase (ALP) activity was determined by the method of King and Armstrong (1980), while plasma bilirubin concentration was determined by the method of Jendrassik and Grof (1938). Serum albumin was determined by the Bromocresol Green (BCG) method (Doumas *et al*., 1997) and total protein was determined by the Biuret method (Lowry *et al*., 1951). Serum creatinine Catalase activity was determined according to the method of Bartels *et al*. (1972) and urea concentration was determined using a modified Berthelot reaction (Faweett & Scout, 1960). Catalase activity was quantified using the method described by Aebi *et al*. (1984), while superoxide dismutase (SOD) activity was determined according to Martin *et al*. (1987) in the field. Lipid peroxidation was determined by determination of malondialdehyde concentration. (MDA) using the method of Fraga *et al*. (1988) in the field.

Data are presented as mean ± standard deviation (SEM), and statistical comparisons between groups were performed using one-way analysis of variance (ANOVA) with Bonferroni's post hoc test (SPSS 27.0 for Windows).

3. Results

The results in this section (Tables 1-6, Fig. 1 and Plates 1-2) detail the phytochemical composition, toxicity evaluation, and therapeutic effects of ethanol leaf extract of *P. africanum* and its fractions. The data include analyses of biochemical, liver, kidney, oxidative stress, and histological parameters in DEN-induced cancer models. Significant effects are noted across various treatment groups, as detailed below.

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Table 1: Phytochemical composition of ethanol leaves extract of *P. africanum*

Phytochemicals	**Results**
Alkaloids	-
Tannins	+
Saponins	+
Flavonoids	+
Steroids	+

Table 2: Oral Median Lethal Dose (LD_{50}) of Alkaloid and Flavonoid-Rich Fractions of *Detarium microcarpum* Stem Bark in Wistar Rats

Treatment Groups (mg/kg)	**Toxicity sign t/n**	**Mortality d/a**	**Gross pathology l/nl**
10ml/kg N/Saline	0/5	0/5	0/5
EEPA 5000	0/5	0/5	0/5

Table 3: Effect of ethanol leaves extract of *P. africanum* on liver parameters of the DEN-induced liver cancer mice

Groups(n=7)	AST(IU/L)	ALT(IU/L)	ALP(IU/L)
NC 1mg/ml	6.20 ± 0.01	5.20±0.01	18.41±0.01
DEN 20mg/ml	18.01± 0.04	43.28±0.01	13.33±0.01
L1 200mg/kg	10.50± 0.01	34.80± 0.01	11.00±0.01 *
L2 4000mg/kg	7.80± 0.01	31.20. ± 0.01	11.80±0.01*
L3 600mg/kg	7.40±0.01	32.80± 0.15	9.80± 0.15
DOX 50 mg/kg	7.30±0.01	12.80±0.15	7.21±0.01

Results presented SEM, * = P ≤ 0.05, L1-L3(Leaves extract), DEN (Diethyl nitrosamine), AST Aspartate aminotransferase), ALT (Alanine aminotransferase, ALP (Alkaline phosphate), One-way ANOVA, Bonferroni *Post hoc* test

Table 4: Effect of ethanol leaves Extract of *P. africanum* on TB and ALB of the DEN- Induced liver cancer mice

Groups(n=7)	TP(g/dl)	ALB(g/dl)
NC 1mg/ml	2.40 ± 0.01	2.42 ± 0.01
DEN 20mg/ml	5.25 ± 0.01	4.35 ± 0.01
L1 200mgkg	3.80 ± 0.01*	0.89 ± 0.03*
L2 400mg/kg	3.34 ± 0.12*	0.91 ± 0.05*
L3 600mg/kg	3.60 ± 0.12*	0.95 ± 0.01 *
DOX 50 mg/kg	4.35 ± 0.01	0.8 ± 0.01

Results presented SEM, * = P ≤ 0.05, L1-L3(Leaves extract), DEN (Diethyl nitrosamine), TP (Total protein), ALB (Albumin), One-way ANOVA, Bonferroni *Post hoc* test

Table 5: Effect of ethanol leaves extract of *P. africanum* on kidney parameter on DEN-induced kidney cancer mice

Groups(n=7)	Urea (mg/dl)	Creatinine (mg/dl)	BUN (mg/dl)
NC 1mg/ml	27.03 ± 0.01	0.95 ± 0.01	34.56 ± 0.01
DEN 20mg/ml	54.81 ± 0.01	4.54 ± 0.01	45.36 ± 0.01
L1 200mg/kg	39.76 ± 0.67*	1.15 ± 0.05*	41.80 ± 0,69*
L2 4000mg/kg	36.52 ± 0.24*	1.50 ± 0.10*	39.50 ± 0.23 *
L3 600mg/kg	33.40 ± 0.59*	0.99 ± 0.02*	38.758 ± 0.31*
DOX 50 mg/kg	30.21±0.74	0.880±0.01	19.44±0.01

Results presented SEM, * = P ≤ 0.05, L1-L3 (Leaves extract), DEN (Diethyl nitrosamine), Urea, Creatinine, and Blood Urea Nitrogen (BUN) One-way ANOVA, Bonferroni *Post hoc* test

Table 6: Effect of ethanol leaves extract of *P. africanum* on oxidative stress parameter on DEN-induced mice

Groups(n=7)	MDA (u/l)	**SOD** (u/l)	CAT(u/l)	LDH(u/l)
NC 1mg/ml	3.55 ± 0.01	4.97 ± 0.01	47.58 ± 0.01	319.05 ± 0.01
DEN 20mg/ml	18.19 ± 0.01	143.75 ± 0.01	135.14 ± 0.01	30.13 ± 0.01
L1 200mg/kg	7.73 ± 0,02*	98.55 ± 0.24*	107.02 ± 0.46*	459.04 ±0.41*
L2 4000mg/kg	7.74 ± 0,02*	85.08 ± 0.33*	96.97 ± 0.20*	424.80 ±0.62*
L3 600mg/kg	8.37 ± 0.01*	74.88 ± 0.21*	77.48 ± 0.20*	361.31 ±0.63*
DOX 50 mg/kg	4.97±0.01	63.95±0.01	51.75±3.11	329.05±0.63

Results presented SEM, * = P ≤ 0.05, L1-L3 (Leaves extract), DEN (Diethyl nitrosamine), malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT) and lactate dehydrogenase (LDH), One-way ANOVA, Bonferroni *Post hoc* test

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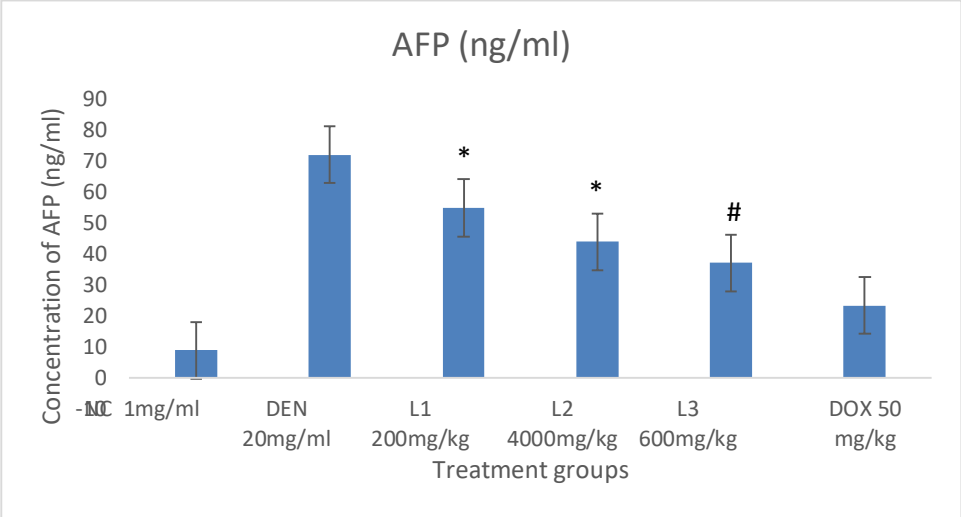

Figure 1: Effect of ethanol leaves extract of *P. africanum* on Alpha Fetoprotein on DEN-induced mice
Results presented SEM, $^{\#}$ = P ≤ 0.01, L1-L3 * = P ≤ 0.05, L1-L3 (Leaves extract), DEN (Diethyl nitrosamine), AFP (Alpha Fetoprotein), One-way ANOVA, Bonferroni *Post hoc* test

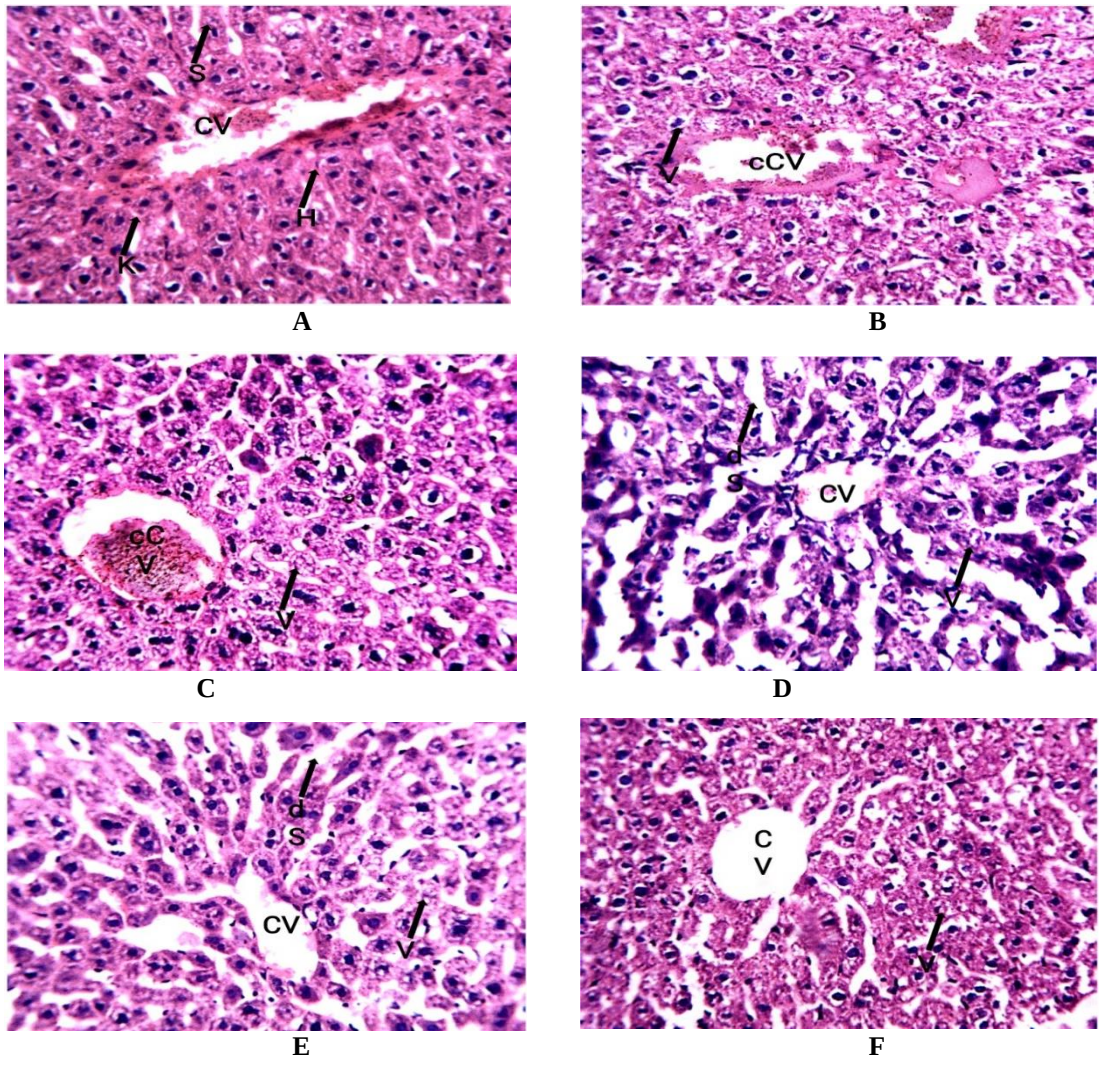

Plate I: Microphotograh of liver tissue showing the effect of ethanol leaves extract of *P. africanum* in the DEN-induced liver cancer mice.

A: normal control, B: diethyl nirosamine, C, D, and E are the leaves extract of *P. africanum* at the doses of 200, 400 and 600 mg/kg respectively, F: Doxorubincin Sinusoid (S), Central vein (CV), Hepatocyte (H), Kupffer cell (K) (H&E x250)

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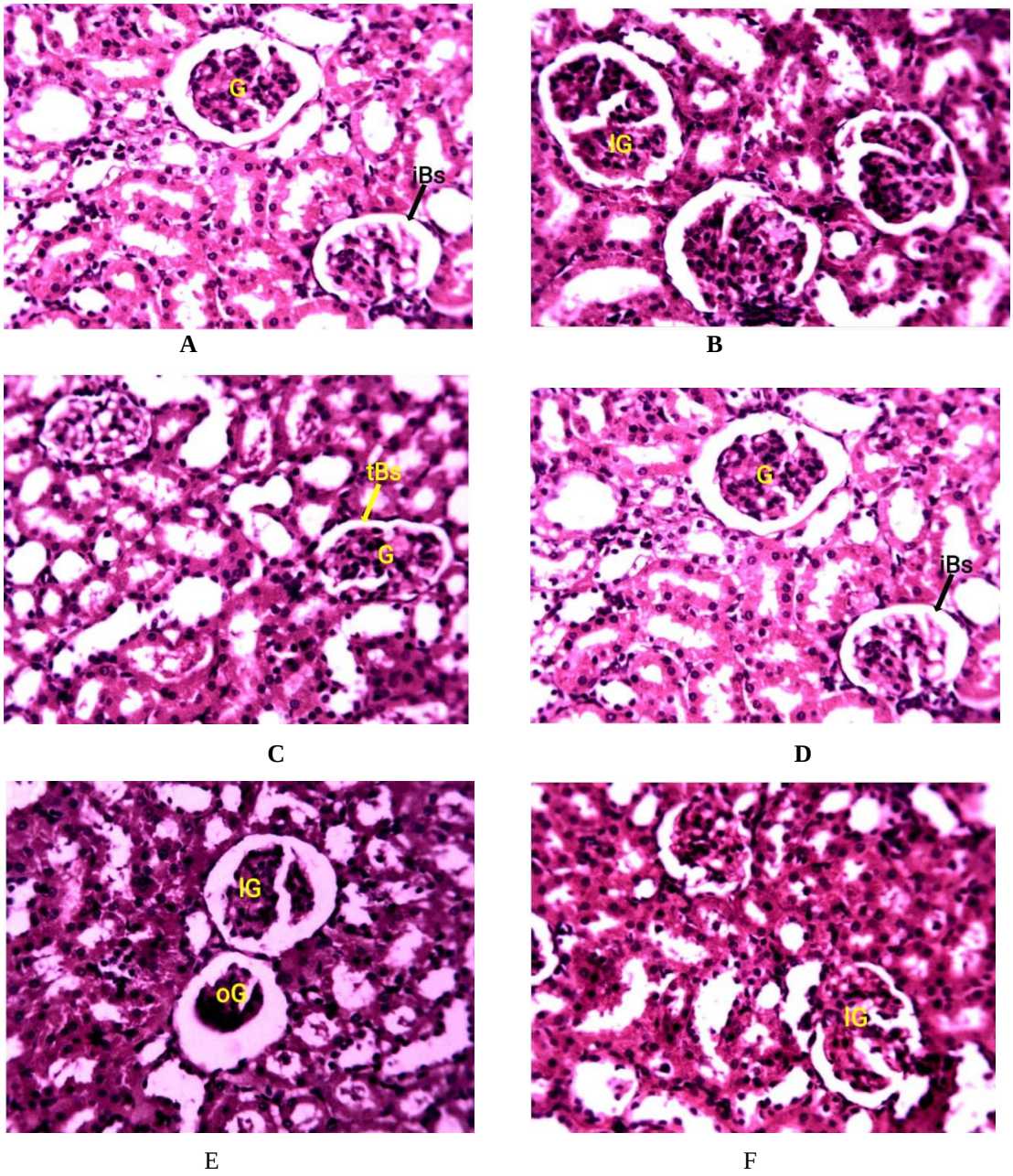

Plate 2: Microphotograh of kidney tissue showing the effect of ethanol leaves extract of *P. africanum* in the DEN-induced liver cancer mice.

A: normal control, B: diethyl nirosamine, C, D, and E are the leaves extract of *P. africanum* at the doses of 200, 400 and 600 mg/kg respectively, F: Doxorubincin, Glomerulus (G), Lobulated Glomerulus (lG), Obliterated Glomerulus (oG) (H&E x250)

4. Discussion

The presence of flavonoids and saponins in *Piptadeniastrum africanum* is associated with a well-known therapeutic role. Especially in its antioxidant and anti-inflammatory activities. Flavonoids, which are prominent in plants such as *Moringa oleifera*, have been well documented to scavenge free radicals and alleviate oxidative stress-related diseases such as liver and kidney injury (Singh *et al*., 2020; Anwar *et al*., 2007). Similarly, saponins, which are known for their cell membrane stabilization and immune modulating properties, demonstrated hepatoprotective effects in liver damage models (Akinmoladun *et al*., 2021; Nwankwo *et al*., 2023).

The absence of alkaloids in *P. africanum* increases its suitability as a treatment to reduce the risk of side effects such as neurotoxicity. This is a concern seen in alkaloid-rich plants such as *Datura stramonium* (Ogundipe *et al*., 2016; Bamidele *et al*., 2020). This is in contrast to plants that balance biological activity with toxicity risk (Nkukwana *et al*., 2021).

According to OECD guidelines, ethanol extracts of *P. africanum* leaves have a high LD50 value, with no observed mortality at doses of 5000 mg/kg. This wide safety margin provides a solid basis for use, therapeutically (OECD, 2008; Mohammed *et al*., 2020). Elevated levels of aspartate

aminotransferase (AST) and alanine aminotransferase (ALT), which indicates liver cell membrane damage and enzyme deficiency together with decreased alkaline phosphatase (ALP) levels are consistent with a DEN-induced hepatotoxicity model (Ahmed *et al*., 2021; Ugbaja *et al*., 2023). Administration of ethanol extract of *P. africanum* significantly reduced AST, ALT, and ALP levels. This indicates the stabilizing and anti-inflammatory ability of liver cell membranes. This reflects the discovery of other phytochemical-rich plants, such as *Curcuma longa* and *Vernonia amygdalina*, which can similarly reduce liver biomarkers through antioxidant and anti-inflammatory mechanisms (Egbung *et al*., 2023; Akinmoladun *et al*., 2021). Biomarkers of impaired kidney function, such as urea, creatinine, and blood urea nitrogen (BUN), reflect taxa's reduction in glomerular filtration and dysfunction of normal tubular function caused by oxidative stress and inflammation. DEN-induced nephrotoxicity, which is associated with excess reactive oxygen species (ROS), reflects these patterns (Ahmed *et al*., 2021; Zhou *et al*., 2019). Treatment with *P. africanum* extracts significantly reduces these biomarkers which indicates an improved kidney. This effect is due to the antioxidant and anti-inflammatory properties of two phytochemicals, including flavonoids and tannins (Bamidele *et al*., 2020; Mohammed *et al*., 2022).

Malondialdeide (MDA), a marker of lipid oxidation, together with superoxide dismutase (SOD) and catalase (CAT), reflect the level of oxidative stress. Sustained elevation of these enzymes in the DEN-treated group indicates disruption of redox homeostasis and widespread organ damage (Ugbaja *et al*., 2023; Singh *et al*., 2020). Significant decreases in MDA, SOD, CAT, and lactate dehydrogenase (LDH) levels were seen in the *P. africanum*-treated group, indicating its ability to restore redox balance and reduce Oxidative damage (Egbung *et al*., 2023; Zhou *et al*., 2019).

DEN, a well-known liver toxin and carcinogen induces liver damage through oxidative stress, inflammation, and DNA alkylation (Ahmed *et al*., 2021). The hepatocyte damage and disruption of liver tissue architecture observed in this study support previous findings (Ahmed *et al*., 2021; Mohammed *et al*., 2020). Treatment with *P. africanum* extract resulted in a marked histological improvement and to some extend restored normal liver structure. Similar effects were found in Phyllanthus niruri, where flavonoid-rich extracts improved liver cell regeneration (Zhou *et al*., 2019; Singh *et al*., 2020). The excellent efficacy of the ethanolic leaf extract of *P. africanum* is due to its phytochemical composition, include saponins, flavonoids, steroids and tannins, which have been shown to work together. For example, flavonoids from Camellia sinensis (green tea) regulate antioxidant enzymes in Similarly, positive oxidants such as SOD and CAT reduce oxidative stress (Akinmoladun *et al*., 2021; Nkukwana *et al*., 2021). It has been shown that tannins in Terminalia chebula contain pro-inflammatory cytokines such as TNF-α and IL-6, which reduce inflammation in kidney tissue (Egbung *et al*., 2023; Ogundipe *et al*., 2016).

5. Conclusion

The ethanol extract of the leaves *Piptadeniastrum africanum* shows significant liver-protective and kidney-protective properties. This is mainly due to the rich phytochemical components. The ability of the extract to restore biochemical, oxidative, and histopathological parameters highlights its potential to address specific organ damage caused by toxins such as DEN. This makes the plant a promising candidate for medicinal applications. Further studies focused on molecular mechanisms and clinical trials will be necessary to develop integration with evidence-based medicine.

Authors Contribution

Study concept and design: Ahmad M.M; Laboratory experiments: Ahmad, M.M; Analysis and interpretation of data: Abba M. U.; Drafting the manuscript: Ahmad, M.M; Reviewing the manuscript: Kura, A.U.; All authors approved the final version of the manuscript.

Conflict of Interests

The authors declare no conflict of interest.

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