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EFFECTS OF DICHLOROMETHANE FRACTION OF ETHANOL EXTRACT OF *PHYLLANTHUS AMARUS* LEAF ON 1, 2-DIMETHYLHYDRAZINE INDUCED HEPATO-RENAL DYSFUNCTION IN SWISS ALBINO MICE

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ABSTRACT

The objective of this study was to assess the impact of the Dichloromethane (DCM) fraction of the ethanol extract from *Phyllanthus amarus* leaves on hepato-renal toxicity induced by 1,2-dimethylhydrazine (DMH) in Swiss albino mice. A total of twenty-five male Swiss albino mice, each weighing between 21-25g, were used in a 90-day (12-week) experiment. The mice were divided into five groups: Group 1 (Control), Group 2 (25 mg/kg DMH + 250 mg/kg DCM fraction of *P. amarus*), Group 3 (25 mg/kg DMH + 350 mg/kg DCM fraction of *P. amarus*), Group 4 (25 mg/kg DMH + 450 mg/kg DCM fraction of *P. amarus*), and Group 5 (25 mg/kg DMH). Each cage, made of mental steel, housed five animals. The mice had free access to food and clean water and were kept under a 12-hour light/dark cycle. DMH was administered orally at a dose of 25 mg/kg body weight using a dolphin gavage, three times a week for a duration of eight weeks. Following the end of DMH treatment, the DCM fraction of the ethanol extract of *P. amarus* leaves was administered orally for an additional four weeks. The animals were fasted overnight and were sacrificed after being anesthetized using urethane. Blood was collected into plain and EDTA containers for biochemical investigation. Liver and kidneys were excised and homogenized for the assay of some antioxidant biomarkers. Liver function indices were assayed as well as kidney function biomarkers. Portions of the liver and kidney were used for histological analysis Results obtained showed that DCM fraction of crude ethanol extract of *P. amarus* has healing effect against 1,2- dimethylhydrazine induced hepato-renal toxicity.

Keywords: *Phyllanthus amarus*, 1, 2 –dimethylhydrazine (DMH), Dichloromethane (DCM), Antioxidants, Liver, Kidney function biomarkers

INTRODUCTION

Over the years, plant materials as sources of medicinal compounds have continued to play a dominant role in the maintenance of human health. The term medicinal plant includes a variety of plants used in herbal therapy owing to their medicinal properties. Medicinal plants are considered to be a rich source of phytochemicals and bioactive components which can be used in drug development and synthesis; hence they are recommended for their therapeutic value (Rasool, 2012). Over 50% of all modern chemical drugs are of natural plant product origin, and is essential in drug development programs of the pharmaceutical industry (Burton *et al*., 2008). The presence of various life sustaining constituents in plants made scientists to investigate these plants for their uses in treating certain infectious diseases and management of chronic wounds, an impressive number of modern drugs have been isolated from natural sources. However, since a single plant contains widely diverse phytochemicals, the effects of using a whole plant as medicine are uncertain (Yaya *et al*., 2012). Approximately 80% of the world total population depends exclusively on plants for their health and healing. Recently there has been an upsurge of interest in the therapeutic potential of medicinal plants as antioxidants in reducing such free radical-induced tissue injury (Pourmorad *et al*., 2006).

The Genus Phyllanthus (Family: Phyllantheae) consists of approximately 1000 species, spread over the American, African, Australian and Asian Continents (Tasheen and Mishra, 2013). Phyllanthus amarus is one of the most pharmacologically important species of the Phyllanthus family. It is commonly found in forest areas, arid land, savannah areas, leached and exhausted soil in many countries including China, India, Nigeria, Cuba and Philippines amongst others (Bharatiya, 1992; Burkill, 1994). Due to its impressive preclinical therapeutic properties, extracts of species of the Phyllanthus have been evaluated to treat hypertension, jaundice, and diabetes. Other studies revealed preclinical pharmacological activity and therapeutic potential of phytochemicals isolated from P amarus (Araujo *et al*., 2012). The powdered leaves of P. amarus were used in clinical studies evaluating its usefulness in patients suffering from chronic damage to the liver due to protracted hepatitis B virus infection. Based on its very useful medicinal properties, *P. amarus* is very frequently utilized in traditional medicine (Joseph and Raj, 2011).

The Phyllanthus species known locally as "stone breaker" (believed to break up kidney stones), belong to Euphorbiaceae, one of the largest and commonest plant families in the world (326 genera, 7750 species). These plants are used traditionally to treat urolithiasis (eliminating the renal calculi), inflammatory bowel disease, diabetes and hepatitis B. One of the species of "stone breaker" (quebrapedra), Phyllanthus amarus is a small herb, usually under 30 cm tall, with numerous small oblong-elliptic or squarish leaves, glabrous, about 6-12 mm long; flowers very small, in cymules hidden under the leaves; cymules bisexual, of 1 male and 1 female flower; calyx-lobes 5, acute; pedicels 2 mm long; capsule small, depressed-globose; seeds 5-7-ribbed" (Isah and Ibrahim, 2014). This plant has a long history in medical herbalism system to treat kidney and urinary bladder disturbances. Thus, substantial studies on Phyllanthus regarding their chemistry, pharmacological activity and clinical effectiveness have been carried out. The extracts of these plants have been reported to have pharmacological

effects such as antiviral activity against hepatitis B and related hepatitis viruses, anti-bacterial activity, anti-hepatotoxic or liver-protecting activity, and hypoglycaemia properties (Etta, 2008; Mazumder *et al*., 2006; Kloucek *et al*., 2005).

Dimethylhydrazine

(DMH) occurs as 1, 1-dimethylhydrazine and 1, 2-dimethylhydrazine isomers. They are acutely toxic and also it causes cancer in animals after cutaneous or oral exposure, both are colorless, clear, liquids, they are a component of rocket fuel.(Al Hamdany *et al*, 2022). Hydrazine was used recently in the preparation of agricultural chemicals, corrosion inhibitor and in heat systems as oxygen scavenger, about 45,000 metric tons were annually synthesized as hydrazine, 1,1-dimethylhydrazine and monomethyl hydrazine . The mechanism underlying DMH-induced colon carcinogenesis involves a series of metabolic reactions, including methylation reactions. DMH, together with its metabolite azoxymethane (AOM), begins as procarcinogens, necessitating metabolic activation to create DNA-reactive substances. Both DMH and AOM, as alkylating agents, exert their mutagenic effects by methylating guanine in DNA at the N-7 position. This alkylation leads to the replacement of thymidine (paired with the alkylated guanine) with cytosine, facilitated by proton donation, ultimately modifying the DNA bases. During subsequent replication, mispairing emerges—guanine pairs with thymine, and cytosine pairs with adenine—resulting in DNA mutations. The metabolism of these procarcinogenic compounds involves various enzymes, including xenobiotic-metabolizing enzymes, and encompasses several stages of N oxidation hydroxylation, culminating in the formation of the ultimate carcinogen methylazoxymethanol (MAM).Regarding the administration of DMH, the primary route involves subcutaneous injection (Aranganthan and Nalini, 2013). Intraperitoneal injections have also successfully induced colon tumors (Ghadi *et al*., 2013). Furthermore, even single injections directly into the rectum of germ-free mice have led to colon tumors. Additionally, oral administration via gavage methods has been shown to generate colon tumors in mice. DMH administration results in 100% epithelial dysplasia and the development of precancerous lesions. Notably, DMH is responsible for a broad spectrum of tumors within the gastrointestinal tract of Swiss Albino mice. The aim of this study was to investigate the healing effects of DCM fraction of crude ethanol extract of *P.amarus* leaf in hepato-renal dysfunction induced by DMH in Swiss albino mice.

MATERIALS AND METHODS

Plant preparation

Collection and Identification of Phyllanthus Amarus Plant

The leaves of *P. amarus* were collected from the botanical garden of University of Calabar, Nigeria and was identified by an expert in the Department of Botany, University of Calabar, Nigeria and a voucher number (288BCM3424) assigned.

Preparation Procedures for Plant Extraction

The plant was kept exposed at room temperature to be air-dried at the Biochemistry Advanced lab for a period of 7 days. The dry leaves were further pulverized using an electric blender till a fine powder was obtained, which was stored in an air-tight container.

Extraction from Plant Leaves

The pulverized *Phyllanthus amarus* plant was weighed (1650g) and was placed in a jar, 7.5L of Ethanol was further added to the jar. The plant was macerated for 72 hours, at intervals of 4 hours with a glass stirrer. The mixture was separated with a fine cheese-cloth to get the extract required to carry out the experiment and the residue was eliminated. The extract gotten was then concentrated in a rotary evaporator to get a paste, which was place in a desiccator for further dryness. Percentage yield was calculated.

% Yield = (Mass of crude extract of plant) / (Mass of dry weight of plant) x 100

Fractionation of Crude Extract

1000g of the crude extract was placed in a separating funnel and 2.5L of DCM was added to the crude extract mixed in a little water, this mixture was mixed by inversion of the separating funnel with the cork and tap tightly closed. The mixture in the separating funnel was de-gassed by releasing the cork, and allowed to settle after which two distinct layers were formed (an aqueous layer at the bottom and a pure layer of DCM on the top). The DCM fraction of *P. amarus* was obtained by gentling running off the aqueous mixture from the separating funnel, while the DCM fraction was decanted into a round bottom flask. This process was repeated until a clear solution of DCM was obtained.

Animals Study

Twenty-five male Swiss albino mice of weight 21-25g were purchased from Kene-God Venture, at the department of animals and environmental biology, University of Benin, Benin City, Edo State. They were acclimatized to diet and environment for 1 week after arrival. They were housed in a density of 5 animals per rack mounted plastic with detachable steel aerated covered cages and were given clean drinking water ad libitum. The temperature (20-22°C) and lighting (12-hour light/dark cycle) were constantly controlled.

Chemical

DMH was dissolved in distilled water and was administered (25 mg/kg body) orally. The volumes of gavage were from 0.21-0.25 ml. Oral administration of DMH lasted for 8 weeks (3 times a week).

Administration of DMH

25mg/kg body weight of DMH was administered orally with the aid of a dolphin gavage 3 times weekly for 8 weeks (24 doses).

Experimental design

The animals were randomly divided into 5(five) groups: Group 1(Control), Group2 (25mg/kg DMH +250mg/kg DCM fraction of *P.amarus*), Group 3 (25mg/kg DMH + 350mg/kg DCM fraction of *P.amarus*), Group 4(25mg/kg DMH+450mg/kg DCM fraction of *P.amarus*) and group 5 (25mg/kg DMH).

Animal Sacrifice/Sample Collection

The animals were handled according to the guidelines for the treatment of laboratory animals. They were sacrificed at the end of the 28-day administration period of *P.amarus* fractions (on the 29th day) after an overnight fast. Each mouse was anaesthetized using urethane (1200mg/kg body weight) intraperitoneally and its blood sample collected via a heart puncture. The blood samples were collected in labeled Lithium Heparin and Ethylene Diamine Tetra-Acetic Acid, (EDTA) tubes. The kidney and liver were collected in an organ bag with formalin for histopathology. Section of the kidney and liver was placed in an organ bag containing 0.9%

NaCl in distilled water; this was later homogenized for antioxidant assay.

Biochemical Analysis

Liver function test

Aspartate and Alanine amino transferases activities were estimated by the method of Reitman and Frankel, (1957).

Kidney function test

Urea and creatinine concentrations were estimated by the method described in their kits (Fortrex, U.S.A.)

Electrolytes (Sodium, Potassium and Bicarbonate ions concentration was assayed using their respective kits (Randox, U.K.)

Antioxidant biomarkers

Superoxide dismutase (SOD) activity was assayed by the method of Misra and Fridovich, (1972). Catalase (CAT) activity was assayed by the method of Cohen *et al*, (1970). Glutathione peroxidase (GPx) activity was estimated by the method of Rotruck *et al*, (1973). Reduced Glutathione (GSH) concentration was estimated by the mothed of Ellman, (1959) and Malondialdehyde (MDA) level was estimated by the method of Buege and Aust, (1978).

Histological Examination of the tissues

Portion of the liver and kidney were sectioned and fixed in 10% formalin for 48hrs. The specimen was then dehydrated through a graded series of alcohol and cleared in three changes of xylene before being embedded in paraffin. Serial sections, each of 4 µm thickness, were made and stained with hematoxylin and eosin according to standard method. Histological assessment was performed under light microscopy. In every H and E section, a minimum of 25 circular tubule were measured in two axes drawn perpendicular to each other using an image analyzer (Image Proplus 3.0)

RESULTS AND DISCUSSION

The results are presented as mean ± SEM. They were analyzed statistically via one-way Analysis of Variance (ANOVA) using Graph Pad Prism Package, version 10.3.0 and the significance of differences was determined using the Turkey-Kramer Multiple Comparisons Test as the post test for determination of significant difference between means. They were considered significant at $p<0.05$.

Table 1: Antioxidant biomarkers in the liver of mice exposed to DMH treated with DCM fraction of crude ethanol extract of *P.amarus*

	Antioxidant Biomarkers				
Groups	**SOD (Units/mg protein)**	**CAT (Units/mg protein)**	**GSH (µM/mg wet tissue)**	**GPx (Units/mg protein)**	**MDA (mol/mg wet tissue)**
Group 1 (Control)	4.157 ± 0.84	13.66 ± 0.93	3.230 ± 0.08	2.037 ± 0.01	2.209 ± 0.01
Group 2 (25 mg/kgDMH + 250 mg/kg DCM)	2.273 ± 0.33	5.353 ± 0.75	2.713 ± 0.19	2.099 ± 0.04	2.481 ± 0.09
Group 3 (25 mg/kg DMH + 350 mg/kg DCM)	3.071 ± 0.04	5.017 ± 1.08	3.130 ± 0.10	1.490 ± 0.07	1.859 ± 0.02
Group 4 (25 mg/kg DMH + 450 mg/kg DCM)	3.614 ± 0.35	6.020 ± 0.97	3.920 ± 0.29	1.964 ± 0.05	2.769 ± 0.16
Group 5 (25 mg/kg DMH)	0.499 ± 0.09*	2.187 ± 0.46*	4.413 ± 0.20	1.046 ± 0.03*	5.201 ± 0.14*

Data are expressed as mean±SEM. n=5

* Significant difference at P<0.05

Table 2: Antioxidant biomarkers in the kidney of mice exposed to DMH treated with DCM fraction of crude ethanol extract of *P.amarus*

	Antioxidant Biomarkers				
Groups	**SOD (Units/mg protein)**	**CAT (Units/mg protein)**	**GSH (µM/mg wet tissue)**	**GPx (Units/mg protein)**	**MDA (mol/mg wet tissue)**
Group 1(Control)	2.061 ± 0.02	7.290 ± 1.02	1.530 ± 0.04	0.991 ± 0.04	3.797 ± 0.04
Group 2 (25 mg/kg DMH + 250 mg/kg DCM)	0.911 ± 0.10	3.291 ± 0.99	1.022 ± 0.01	0.410 ± 0.01	1.964 ± 0.02
Group 3 (25 mg/kg DMH + 350 mg/kg DCM)	1.445 ± 0.30	3.710 ± 0.50	0.720 ± 0.08	0.730 ± 0.08	3.118 ± 0.02
Group 4 (25 mg/kg DMH + 450 mg/kg DCM)	2.020 ± 0.01	3.301 ± 0.02	1.303 ± 0.03	0.971 ± 0.09	3.006 ± 0.09
Group 5 (25 mg/kg DMH)	1.207 ± 0.12	0.995 ± 0.02*	0.941 ± 0.08	0.397 ± 0.07	7.910 ± 0.09*

Data are expressed as mean±SEM. n=5

* Significant different at *P<0.05*

Table 3: Liver function test for mice exposed to DMH treated with DCM fraction of crude extract of *P. amarus*

Groups	**Liver Biomarkers**	
	ALT (U/L)	**AST (U/L)**
Group 1 (Control)	168.20 ± 24.57	250.61 ± 0.53
Group 2 (25 mg/kg DMH + 250 mg/kg DCM)	175.18 ± 01.54	290.10 ± 2.33
Group 3 (25 mg/kg DMH + 350 mg/kg DCM)	137.40 ± 03.03	320.59 ± 2.29
Group 4 (25 mg/kg DMH + 450 mg/kg DCM)	245.00 ± 58.02	300.85 ± 0.58
Group 5 (25 mg/kg DMH)	156.60 ± 11.68	180.96 ± 0.66*

Data are expressed as mean±SEM, n=5

* Significant different at *P<0.05*.

Table 4: Kidney function test for mice exposed to DMH treated with DCM fraction of crude extract of *P. amarus*

Groups	Kidney Biomarkers	
	Urea (mmol/L)	Creatinine (mg/dL)
Group 1 (Control)	0.72 ± 0.03	2.13 ± 0.36
Group 2 (25 mg/kg DMH + 250 mg/kg DCM)	1.22 ± 0.18	1.45 ± 0.13
Group 3 (25 mg/kg DMH + 350 mg/kg DCM)	1.25 ± 0.25	1.60 ± 0.19
Group 4 (25 mg/kg DMH + 450 mg/kg DCM)	0.58 ± 0.18	1.34 ± 0.13
Group 5 (25 mg/kg DMH)	1.73 ± 0.09	1.80 ± 0.20

Data are expressed as mean±SEM. n=5

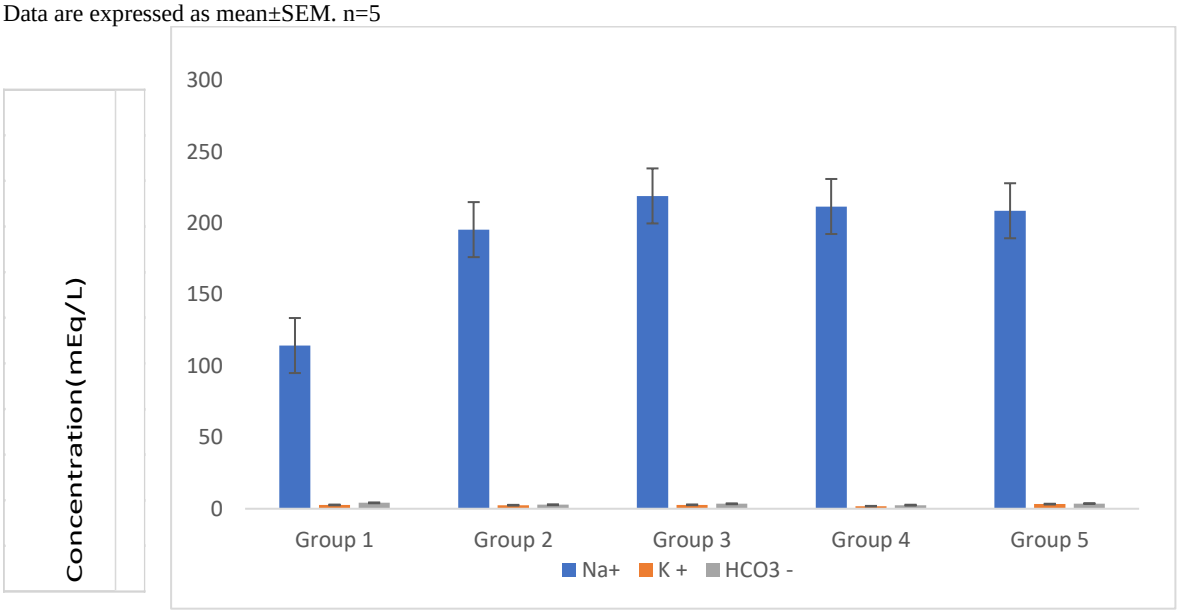
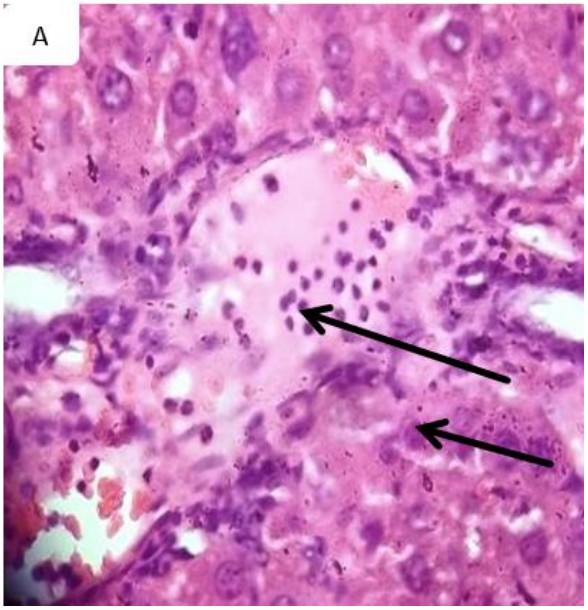


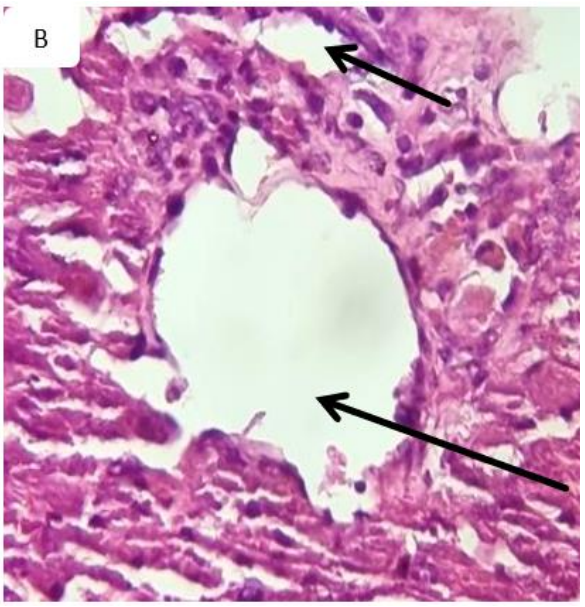
Figure 1: Showing results for electrolytes in mice exposed to DMH treated with DCM fractions of crude ethanol extract of *P.amarus*

Photomicrographs of the liver and kidney exposed to DMH treated with DCM fraction of crude ethanol extract of *P.amarus*



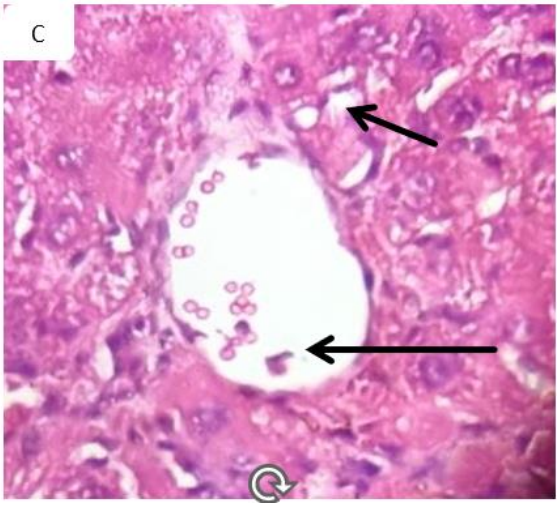
250mg/kgbwt DCM Fraction. X400 Magnification

Plate 1: Liver histology reveals congested central vein (long arrow) filled with mononuclear cells and surrounded by inflammatory and mononuclear exudates. The hepatocytes reveals enlarged vacuolated nucleus (short arrow).



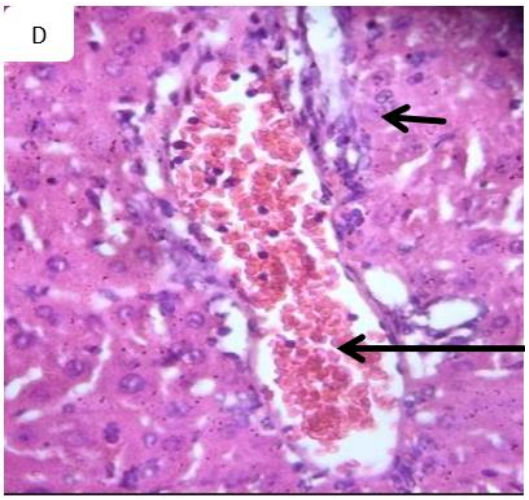
350mg/kgbwt DCM Fraction. X400 Magnification

Plate 2: Liver histology reveals visible central vein (long arrow) and well fenestrated sinusoids and hepatocytes with vacuolated nucleus (short arrow)



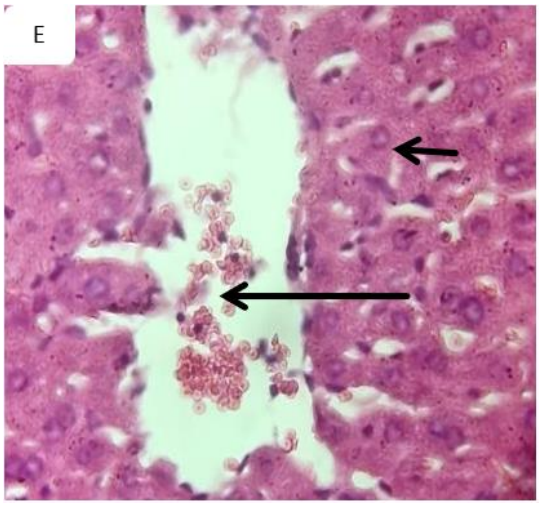
450mg/kgbwt DCM Fraction. X400 Magnification

Plate 3: Liver histology reveals visible central vein (long arrow) and well fenestrated sinusoids and hepatocytes with mild steatosis (short arrow)



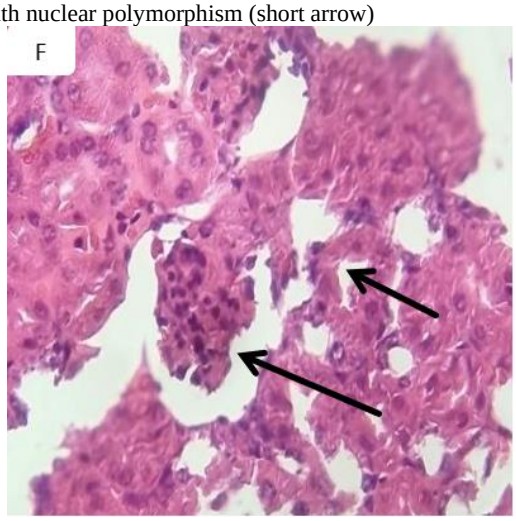
DMH Only. X400 Magnification

Plate 4: Liver histology reveals congested dilated central vein (long arrow) surrounded by pools of mononuclear exudates and well fenestrated sinusoids and hepatocytes with nuclear polymorphism (short arrow)



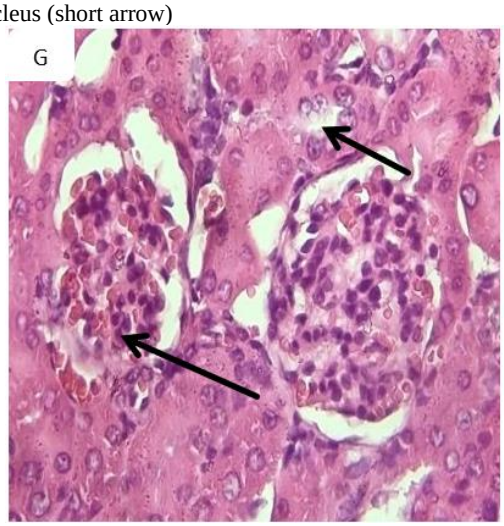
Control. X400 Magnification

Plate 5: Liver histology reveals central vein (long arrow) and well fenestrated sinusoids and hepatocytes with pyknotic nucleus (short arrow)



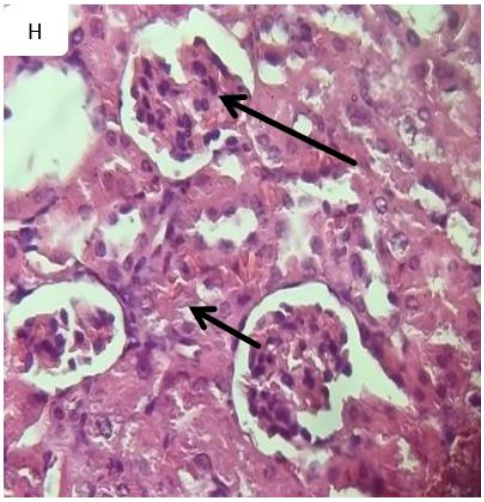
250mg/kgbwt DCM Fraction. X400 Magnification

Plate 6: Kidney reveals unremarkable renal corpuscle with atrophied glomerulus (long arrow) with not so prominent tubules dilated (short arrow) and interstitial.



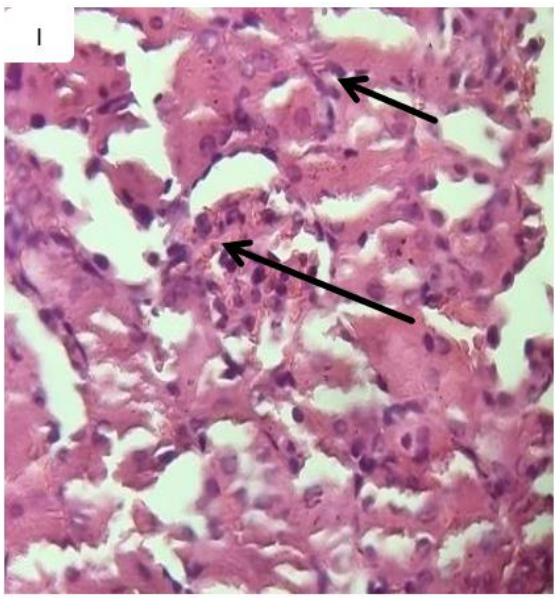
350mg/kgbwt DCM Fraction. X400 Magnification

Plate 7: Kidney reveals unremarkable renal corpuscle with enlarged glomerulus (long arrow) with not so prominent tubules (short arrow) and interstitial.



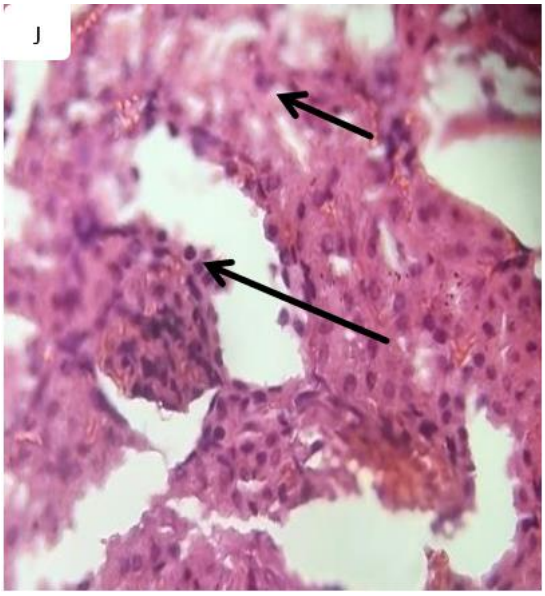
450mg/kgbwt DCM Fraction. X400 Magnification

Plate 8: Kidney reveals unremarkable renal corpuscle with glomerulus (long arrow) with prominent tubules (short arrow) and interstitial.



DMH Only. X400 Magnification

Plate 9: Kidney reveals unremarkable renal corpuscle with distorted glomerulus (long arrow) with distorted tubules (short arrow) and interstitial.



DMH Only. X400 Magnification

Plate 10: Kidney reveals unremarkable renal corpuscle with distorted glomerulus (long arrow) with distorted tubules (short arrow) and interstitial.

Discussion

Medicinal plants are considered a rich source of ingredients which can be used in drug development and synthesis hence they are recommended for their therapeutic value (Rasool, 2012). The bioactive compounds found in plants such as alkaloids, flavonoids, lignin, phenols, tannins and terpenes have the potential to control and prevent human diseases. Many of the groundbreaking drugs developed in the last five decades, which have transformed modern medicine, have their origins in plants. These chemical compounds exhibit therapeutic properties similar to both plant and animal-based medicines (Refaz *et al*., 2017). An example of such species which have wide range of use in various treatment of disease is *Phyllanthus amarus*. This plant has a long history of use in traditional medicine dating back over 3000 years and has been the subject of numerous scientific investigations that explored its phytochemical constituents and pharmacological uses (Sonia *et al*., 2014). Evidences from research have proven that these plant substances may be helpful, playing essential roles in the prevention and management of cellular anomalies. The genus *Phyllanthus* of the Phyllantaceae family was described for the first time by Linnaeus in 1737, and is of substantial medicinal significance (Aparupa *et al*., 2022). In the current study, the activities of SOD and CAT in the liver homogenate were significantly different when control was compared with positive control group (DMH Only) at P< 0.05 but there was no significant difference when compared to plant treated groups. There was significant decrease in the activity of SOD and CAT in diseased group (DMH Only) when compared to plant treated groups. For GSH and GPx, there was no significant difference in the concentration and activity across all the experimented groups. Invariably, the level of MDA concentration in liver homogenate of disease group was markedly elevated when compared to, control group and plant treated groups, indicating lipid peroxidation. The degree of elevation was significant at P<0.05. This report is in synergy with findings of Frank *et al*, (2017), which reported decrease in the activity of liver SOD and CAT, increase in the concentration of MDA in disease condition. Also, in a study by Karuna *et al* (2011), the antioxidant activity of aqueous extract of whole plant of *P. amarus* at the dose of 200mg/kg body weight/day was evaluated in streptozotocin (STZ)-induced diabetic male Wistar albino rats; the activities of antioxidant enzymes; glutathione reductase, glutathione peroxidase and glutathione-s-transferase, catalase and superoxide dismutase showed a significant decrease in renal lipid peroxidation, protein oxidation and a significant increase in glutathione content and glutathione reductase, glutathione peroxidase and glutathione-s-transferase activities when compared with STZ-induced diabetic rats. The activities of catalase and superoxide dismutase were normalized in the diabetic treated group but lowered in the diabetic group. Raphael *et al.*, in 2002, also established that the plant could inhibit lipid peroxidation, and scavenge hydroxyl and superoxide radicals' *in-vitro*. The healing effect of *P. amarus* DCM fraction can be attributed to the presence of antioxidant biomarkers such as Flavonoids and phenols. Results of antioxidants in the kidney was also evaluated in the current study. SOD activity in control and plant treated groups were not significantly different from group administered DMH only, but the activity of SOD was downregulated in DMH treated group. For CAT activity, there was significant difference at P< 0.05 when control and plant treated groups were compared to DMH treated group. Like the liver, the kidney homogenate showed no significant difference in the concentration and activity of GSH and GPx across all the groups. ALT (Alanine amino transferase) activity was not significantly altered by the effect of DHM across all groups. In the case of AST (Aspartate amino transferase), the activity was significantly different when control was compared to DMH treated, whereas it was not significantly different from plant treated. This potentiates the hepato protective potential of DCM fraction of crude extract of *P.amarus* leaf. Research has it that *P.amarus* aqueous leaf extract protects the liver from CCl_4 induced hepatotoxicity (Krithika and Verma, 2009). The activity of ALT and AST was high in the control and plant treated group. This is due to the nature of the sample used (Liver and kidney homogenate), indicating that both enzymes are liver based. Reverse will be the case when blood plasma is used. During liver damage, liver enzymes leak into

the blood thereby causing offshoot in its concentration in the blood. Unlike the liver, kidney biomarkers (Urea and Creatinine) concentrations were not significantly altered across all the experimented groups, but there was slight alteration in DMH treated group. This research also is in concordance with report of Adeneye and Adokiye, (2008). They revealed that "single oral dose (100–400 mg/kg/day) of the leaves and seed aqueous extracts of *P. amarus* protected against the effects of acetaminophen and gentamicin-induced nephrotoxicity in Wistar rats for 14 days". The extracts significantly attenuated elevations in the serum creatinine and blood urea nitrogen levels in a dose-related fashion. Similar trend was observed in electrolyte (K^+ and $HCO_3^{2})^-$ concentrations. The concentration of Na^+ in groups 2 (250mg/kgbwt DCM fraction) was not significantly different from control (group 1). Also, photomicrographs of the liver and kidney explained the protective potentials of DCM fraction of the crude ethanol extract of *P.amarus* leaf when compared with control and DMH Treated groups.

CONCLUSION

The preliminary study was to evaluate the hepato-renal protective potentials in DCM fraction of crude ethanol extract (CEE) of *P.amarus* leaf on 1, 2-dimethylhdrazine induced hepato-renal dysfunction in Swiss albino mice. The overall results suggested that the plant fraction has hepato–renal protective potentials due to its ability to regulate in-vivo antioxidant biomarkers, AST activity in the liver, electrolytes concentration in the kidney. The plant healing potentials can be attributed to the presence of bioactive molecules. Histological examination also revealed the ameliorative potentials of DCM fraction of CEE of *P.amarus* leaf. Notably, DCM fraction (350mg/kgbwt and 450mg/kgbwt) had a better protective potential.

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REFERENCES

Aparupa, B. M., Anindita B., and Sharmila, C., (2022). An insight into the potent medicinal plant Phyllanthus amarus. *The Nucleus*. 65, 437-472. https://doi.org/10.1007/s13237-022-00409-z

Araujo, R.E., Luiz, A.S., Cinthia, R.C., Ranniere, G.F., Hugo, C.G., Pedro, R.P. (2012). Growth Inhibitory effects of Phyllanthus niruri extracts in combination with cisplatin on cancer cell lines. *World J of Gastro*. 18: 4162-6168. https://doi.org/10.3748/wjg.v18.i31.4162 .

Aigner, B.A., Darsow, U., Grosber, M., Ring, J., Plötz, S.G. (2010) multiple basal cell carcinomas after long-term exposure to hydrazine: Case report and review of the literature. *Dermatol*. ; 221:300-302. https://doi.org/10.1159/000321338 . Epub 2010 Nov 22.

Bharatiya, V.B. (1992). Selected medicinal plants of India Bombany. *Tafa press*. 2: 235-237.

Binyamin, Y., Frenkel, A., Brotfain, E., Koyfman, L, Shliom, O, Klein, M. (2018).Elevated CPK levels afterhydrazine inhalation exposure in an F16 aircraft technician. *Toxicol*.5:927-928. https://doi.org/10.1016/j.toxrep.2018.08.023 .

Burkill, H.M. (1994). The useful plant of West Tropical Africa. *Royal bot.* 2: 234-237.

Banerjee, A., & Chattopadhyay, S. (2022). An insight into the potent medicinal plant Phyllanthus amarus Schum. and Thonn. The Nucleus: an International Journal of Cytology and Allied Topics, 1-36.Buege, J.A. and Aust, S.D. (1978).Microsomal lipid peroxidation. *Methods Enzymol*.52: 302-310. https://doi.org/10.1007/s13237-022-00409-z .

Cohen, G., Dembiec, D. and Marcus, J. (1970).Measurement of catalase activity in tissue extract. *Ann.Biochem*.34:30-38. https://doi.org/10.1016/0003-2697(70)90083-7 .

Etta, H. (2008). Effects of *Phyllanthus amarus* on litter traits in albino rats. *Scientific Research and Essay.* 3(8):370–372.

Isah, M. B. and Ibrahim, M. A. (2014). The role of antioxidants treatment on the pathogenesis of malarial infections: a review. *Parasitology Research*. 113: 801–805. https://doi.org/10.1007/s00436-014-3804-1 .

Joseph, B. and Raj, S. J. (2011). An Overview: Pharmacognostic Property of *Phyllanthus amrus li nn. Int Jour of Pharm.* 1: 41–45.

Karuna, R., Bharathi, V. G., Reddy, S. S., Ramesh, B. and Saralakumari, D. (2011). Protective effects of *Phyllanthus amarus* aqueous extract against renal oxidative stress in Streptozotocin-induced diabetic rats. *Ind Jour of Pharm.* 43:414–418. https://doi.org/10.4103/0253-7613.83112 .

Keshavarz, M.H., Ramadan, A., Mousaviazar, A., Zali, A., Shokrollahi, A. (2011)..Reducing dangerous effects of unsymmetrical dimethyl hydrazine as a liquid propellant by addition of hydroxyl-ethylhydrazine, part II,performance with several oxidizers. *J Energ Mater*. 29(3):228-240.

Kloucek, P., Polesny, Z., Svobodova, B., Vlkova, E. and Kokoska, L. (2005). Antibacterial screening of some Peruvian medicinal plants used in Calleria District. *Jour of Ethnopharma.* 99 (2): 309-312. https://doi.org/10.1016/j.jep.2005.01.062

Kolumbaeva, S.Z., Shalakhmetova, T.M, Begimbetova, D.A., Bersimbaev, R.I., Kalimagambetov, A.M. (2007).Mutagenic effect of the rocket fuel component unsymmetrical dimethylhydrazine on rats of different age. *Russ J Genet*. 43(6):608-12

Krithika, A. R. and Verma, R. R. J. (2009b). Mitigation of carbon tetrachloride induced damage by *Phyllanthus amarus* in liver of mice. *Acta Poloniae Pharma – Drug Res.* ***66****: 439–445.*

Lia, W., Zhoua, M., Xua ,N., Hua, Y., Wanga Ch, Lib, D., Liuc, L., Lia, D.(2016).Comparative analysis of protective effects of curcumin, curcumin-b-cyclodextrin nanoparticle and nanoliposomal curcumin on unsymmetrical dimethyl hydrazine poisoning in mice. *Bioengineered*.7 (5):334-341. https://doi.org/10.1080/21655979.2016.1197029

Makarovsky, I., Markel, G., Dushnitsky, T., Eisenkraft, A. (2008). Hydrazine: The space era agent. *Isr Med Assoc* J. 2008; 10 (4):302-6

Mazumder, A., Mahato, A. and Mazumder, R. (2006). Antimicrobial potentially of Phyllanthus amarus against drug resistant pathogens. Natl Prod Res. **20** (4): 323-326.Mena, S., Ortega, A. and Estrela J.M. (2009). Oxidative stress in environmental- induced carcinogenesis. *Mutation Research*. **674** (1-2): 36–44.

Misra, H.P. and Fridovich, I. (1972). The role of superoxide anion in the autoxidation of epinephrine and single assay for superoxide dismutase. *Journal of Biological Chemistry*.247:3170-3175. PMID: **4623845**

Omoregie,F.O., Okolie, N.P. and Abu O.D.(2017). Effects of aqueous extract of Annona muricata leaves on cyanide-induced toxicity in New Zealand Rabbits. *NISEB Journal*.16 (4):142-147. http://www.nisebjournal.org

Pourmorad, F., Hosseinimehr, S. J. and Shahabimajd, N. (2006). Antioxidant activity, phenols,flavanoid contents of selected Iranian medicinal plants. *S Afri J of Biotech*. **5**:1142-1145.

Raphael, K.R., Ajith, T.A., Joseph, S. and Kuttan, R. (2002). Anti-mutagenic activity of Phyllanthus amarus Schum & Thonn in-vitro as well as in vivo. *Teratog. Carcinog. Mutagen.* 22: 285-291. https://doi.org/10.1002/tcm.10021 .

Rasool, H. (2012). Medicinal Plant (Importance and uses). *Pharma Anal Acta*. **3**: 2153-2435.

Refaz, A. D., Mohd, S., and Parvaiz, H.Q. (2017). General overview of medicinal plants. *Journal of Phytopharmacology*. 6(6):349-351. www.phytopharmajournal.com

Reitman, S. and Frankel, S. (1957). A colorimetric method for the determination of SGOT and SGPT. *Am. J. Clin.Path.*28:56-58. https://doi.org/10.1093/ajcp/28.1.56

Ritz, B., Zhao, Y., Krishnadasan, A., Kennedy, N., Morgenstern, H. (2006).Estimated effects of hydrazine exposure on cancer incidence and mortality in aerospace workers. *Epidemiol*. 17(2):154-161. https://doi.org/10.1097/01.ede.0000199323.55534 .

Sadik, N.A.(2012). Chemopreventive efficacy of green tea drinking against 1,2-dimethyl hydrazine-induced rat colon carcinogenesis. *Cell Biochem Funct*. 31(3):196-207. https://doi.org/10.1002/cbf.2873 .

Shebbo, S., El Joumaa, M., Kawach, R., Borjac, J. (2020). Metrics Hepatoprotective effect of Matricaria chamomilla aqueous extract against 1,2-Dimethylhydrazine-induced carcinogenic hepatic damage in mice. *Heliyon*. 6(6):1-3. https://doi.org/10.1016/j.heliyon.2020.e04082 .

Sonia, V., Hitender, S., Munish, G. (2014). Phyllanthus Amarus: A Review. *Journal of Pharmacognosy and Phytochemistry* 3 (2):18-22. https://www.phytojournal.com/archives/2014.v3.i2.348/phyllanthus-amarus-a-review

Tasheen, M. and Mishra, G. (2013). Ethnobotany and Diuretic Activity of some Selected Indian Medicinal plants. *The Pharma Innov*. **2**: 112. www.thepharmajournal.com

Umeaku, U., Ofusori, D.A., Umeaku, O.P., Edward, T.A. (2020). The effect of aqueous extract of Ocimum gratissium (Linn) on 1, 2 - dimethylhydrazine induced colon cancer in male Wistar rats. *IJHA*.2 (1):22-35. https://doi.org/10.14302/issn.2577-2279.ijha-20-3180

Walia, S., Kamal, R., Dhawan, D.K., Kanwar, S.S. (2018). Chemoprevention by probiotics during 1,2-dimethylhydrazineinduced colon carcinogenesis in rats. *Dig Dis Sci*. 63:900-909. https://doi.org/10.1007/s10620-018-4949-z

Yaya, G., Benjamin, K. A. B., Fanté, B., Sorho, S., Amadou, T. S. and Jean-Marie, C. (2012). *Med Bact RASĀYAN J of Chem*. **5**(3): 332 – 340.