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ANTI-HEPATOTOXIC AND RENAL PROTECTION ACTIVITIES OF METHANOL ROOT EXTRACT OF *Combretum dolichopetalum*

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ABSTRACT

High incidence of drug-induced liver injury has necessitated the search for potent and cost-effective herbal management models. Hence, this study was aimed at evaluating the hepatoprotective effect of methanol root extract of *Combretum dolichopetalum (MECD)* against paracetamol (Acetaminophen)-induced hepatotoxicity in male albino Wistar rats. The liver function markers: alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and kidney function markers: Creatinine (Cr) and Urea (Ur) were determined to evaluate the hepatoprotective and renal protective effects of *MECD*. The result showed that ALT activity was significantly (*P<0.05*) elevated compared to the normal (81.00±1.53 IU/L) and positive (75.67±2.60 IU/L) controls. The AST activity at 500 mg/kg (137.33±1.20 IU/L) was significantly (*P<0.05*) higher compared to all the control groups. The Cr and Ur concentrations of the negative control group were significantly (*P<0.05*) elevated compared to all the other groups. However, administration of 200 – 500 mg/kg of MECD caused a significant (*P<0.05*) reduction in the creatinine and urea concentrations. There was no significant (*P>0.05*) difference in the urea concentration of the extract-treated groups compared to the normal and positive controls, whereas the reductions of Cr concentration were dose-dependent. Liver sections of the animals treated with 200 mg/kg and 300 mg/kg of the root extract showed mild centrilobular hepatocellular degeneration while those treated with 500 mg/kg of the extract showed the normal histo-architecture of the laboratory rodents. The results of this study suggest that the methanol root extract of *Combretum dolichopetalum* protects the liver against liver toxicity induced by acetaminophen in a dose-dependent manner.

KEYWORDS: **Hepatoprotection; *Combretum dolichopetalum*; Acetaminophen-induced hepatotoxicity; Renal function indices; Liver enzymes; Paracetamol.**

INTRODUCTION

The problems in predicting and preventing drug-related idiosyncratic liver damage have been addressed extensively in recent review articles [1-3]. Drug-induced liver injury is a significant clinical problem worldwide [4]. Most drug-induced liver injury and acute liver failure occur due to either accidental or intentional overdose of acetaminophen (N-acetyl-p-aminophenol, paracetamol, APAP) [5,6]. When used at therapeutic doses, APAP is metabolized by glucuronidation or sulfation by the cytochrome p450 system into the reactive metabolite N-acetyl-p-benzoquinone imine (NAPQI). Under normal circumstances, NAPQI is rapidly converted to nontoxic metabolites by glutathione (GSH). However, at large doses of APAP, NAPQI levels increase and may react with hepatic proteins,

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resulting in liver injury [7,8]. Because of its dose-dependent toxicity, APAP-induced hepatic damage can be studied in animal models and most mechanisms are translatable to humans [7]. Despite the great scientific achievements in the field of hepatology, management of liver diseases is still a challenge to modern medicine. Natural products are known to be used as alternative remedies in the treatment of liver toxicity. Silymarin (SLM) is a standard drug used almost in liver protection all over the world.

Substantial progress has been made in understanding the interaction between herbal drugs and the liver. However, our knowledge of the potentials and risks of botanical drugs is still limited and efforts to elucidate them should be intensified. In Nigeria, the aqueous extract of *Combretum dolichopetalum* is used in folklore medicine for the relief or cure of stomachache, blood in stool, diarrhoea and related gastrointestinal disorders. The herbalists and native doctors use aqueous extracts of various parts of the plant for the treatment of gastrointestinal and liver diseases. In "Ogwu" a town in Imo State Nigeria; the roots popularly known as "*Oroboli*" are boiled together with yam tubers and the aqueous extracts is usually taken by patients with stomachache. In Oba Idemili North local government of Anambra state, it is known as "*mmanwuefi*" and the aqueous extract of the leaves is used in the treatment of diarrhoea. The extract, which contained tannins, alkaloids, glycosides, flavonoids and saponins, protected rats from gastric and duodenal ulcer [9]. Following the various claims of the therapeutic potentials of *Combretum dolichopetalum,* coupled with the dearth of information on protective effects of its methanol extract, this study was designed to evaluate the anti-hepatotoxic and renal protection activity of the methanolic root extract of *Combretum dolichopetalum* on APAP-induced liver damage.

MATERIALS AND METHODS

Plant collection and identification

Fresh roots of *Combretum dolichopetalum* were collected between January and February, 2019 from Orba, Udenu Local Government Area of Enugu State. The Botanical Identification was confirmed by a Taxonomist at Bioresources Development and Conservation Programme (BDCP) Centre and deposited in the Herbarium with voucher specimen number BDCP 0094.

Extraction of the Plant Material

The roots of *Combretum dolichopetalum* were air-dried for 7 days. Thereafter, the dried roots were ground to coarse powder which was extracted by macerating in methanol. The filtrate was dried *in vacuo* using a rotary evaporator to obtain the methanol extract of *Combretum dolichopetalum* (MECD). The filtrate was concentrated in a water bath at 40 °C. The methanolic root extract was further air-dried for about 48 hours and after that, it was stored in a refrigerator until it was ready for use.

Chemicals

Paracetamol powder (Acetaminophen) and Silymarin were purchased from a local pharmacy in Umuahia, Abia State. All other chemicals and other reagents used in the experiment were of analytical grade.

Phytochemical analysis

The total methanolic extract of *C. dolichopetalum* root was assessed for the presence of phytoconstituents according to the standard procedures described by Harbone [10].

Experimental animals

Thirty (30) male albino Wistar rats with average weight of 55 g were sourced from the Laboratory Animal Unit of the Department of Veterinary Physiology, Pharmacology and Biochemistry University of Nigeria Nsukka Enugu, Enugu State. Prior to the experiments, the animals were fasted overnight. Animals were housed in aluminum cages at room temperature and under natural light/dark cycles. The rats were supplied with clean drinking water and fed *ad libitum* with standard commercial pellet Grower's feed. The rats were acclimatized for two weeks prior to study.

Experimental design

The animals were divided into six (6) groups of five (5) rats each as shown below. Toxicity was induced by oral administration of 400 mg/kg body weight of paracetamol (PCM) followed by treatment with methanol extract of *C. dolichopetalum* roots (MECD) for 14 days.

Group 1: Normal control – received distilled water, feed and normal saline.

Group 2: Positive control – received 400 mg/kg PCM and 200 mg/kg Silymarin (SLM)

Group 3: Negative control – received 400 mg/kg PCM and left untreated

Group 4: received 400 mg/kg PCM and 200 mg/kg MECD

Group 5: received 400 mg/kg PCM and 300 mg/kg MECD

Group 5: received 400 mg/kg PCM and 500 mg/kg MECD

Sample collection

After fourteen days of administration, animals in all groups were starved overnight and sacrificed by cardiac puncture on the fifteenth day. Blood sample and liver tissues of each group were collected separately into sterilized plain bottles. Blood samples were collected and centrifuged at 3000 ×g for 15 minutes at 4 ^{0}C to obtain the serum. The liver tissues were preserved in formalin prior to use.

Determination of hepatic and renal function indices

Using Randox test kits, activities of serum alanine transaminase (ALT) and aspartate transaminase (AST) activities were analyzed by means of the methods of Reitman and Frankel [11], while the serum alkaline phosphatase (ALP) activity was determined as per the colourimetric method designated by Englehardt [12]. Likewise, the urea concentration was assayed using urease Berthelot as described by Fawcett and Scott [13], while the creatinine concentration was assayed via direct endpoint according to the method of Henry [14].

Histopathological examination

Tissue preparation

Gross lesions were recorded as observed during the post mortem examination. Sections of the liver were collected for histopathological examination. The collected samples were fixed in 10% phosphate buffered formalin for 48 hours. The tissues were subsequently trimmed, dehydrated in 4 grades of alcohol (70, 80, 90 and 100 %), cleared in 3 grades of xylene and embedded in molten wax. On solidifying, the blocks were sectioned, 5µm thick with a rotary microtome, floated in water bath and incubated at 60 ˚C for 30 minutes. The 5 µm thick sectioned tissues were subsequently cleared in 3 grades of xylene and rehydrated in 3 grades of alcohol (90, 80 and 70 %). The sections were then stained with Hematoxylin for 15 minutes. Blueing was done with ammonium chloride. Differentiation was done with 1% acid alcohol before counterstaining with Eosin. Permanent mounts were made on degreased glass slides using a mountant – DPX.

Statistical analysis

Data were analysed using analysis of variance (ANOVA) to test for significant differences between means at $P<0.05$. The statistical significance of difference of the means was evaluated by Duncan's Post Hoc test.

RESULTS

Phytochemical constituents

The root of *Combretum dolichopetalum* contains moderate levels of alkaloids, flavonoids, tannins, sterols and saponins (Table 1).

Effects of MECD on hepatic indices of paracetamol-intoxicated rats

There was a significant ($P<0.05$) elevation in the ALT activity of rats in Groups 3 (Figure 1), which was induced with toxicity and left untreated, when compared to the rest groups. Treatment with the standard drug, 200, 300 and 500 mg/kg b.w of MECD caused a significant ($P<0.05$) reduction in the ALT activity in relation to the negative control. There was no significant ($P>0.05$) difference between the ALT activity of Group 6 (treated with 500 mg/kg of MECD) when compared with the positive control, while it was significantly ($P<0.05$) lower when compared to Groups 4 and 5 (treated with 200 and 300 mg/kg MECD respectively). There was a dose-dependent reductive effect of the MECD on ALT activity. There was a significant ($P<0.05$) elevation in the ALT activity of rats in Groups 3, which was induced with hepatotoxicity and left untreated, when compared with the control and treatment groups. The AST activity of Group 6 (treated with 500 mg/kg MECD) was significantly ($P<0.05$) reduced when compared to the control and treatment groups (Figure 2). There was no significant ($P>0.05$) difference in AST activity of Groups 4 and 5 when compared to the normal and positive control groups. There was a significant ($P<0.05$) increase in ALP activity in Group 3 (untreated) when compared to the treatment groups (Groups 4-6). However, treatment with different doses of MECD caused a significant ($P<0.05$) dose-dependent reduction in ALP activity (Figure 3). The ALP activity (Figure 3) of the negative control was markedly ($P<0.05$) elevated when compared with the control and treatment groups.

Effects of MECD on the renal function indices of paracetamol-intoxicated rats

There was no significant ($P>0.05$) difference in the urea concentration of the treatment groups when compared to the normal and positive control groups (Figure 4). As for creatinine concentration (Figure 5), there was a significant ($P<0.05$) upsurge in the creatinine concentration in Group 2 (Negative control) when compared to the other groups. This was followed by a significant ($P<0.05$) dose-dependent reduction in the concentration of creatinine by MECD. There was non-significant ($P>0.05$) difference in creatinine concentration between the normal and positive control groups, and between Groups 5 and 6 (treated with 300 and 500 mg/kg MECD respectively). The creatinine

concentration in Group 4 (treated with 200 mg/kg MECD) was significantly ($p < 0.05$) greater than that of normal and positive control groups.

Histopathological examination results

Figures 6–11 show the effects of the methanol extract of *C. dolichopetalum* (MECD) on liver histomorphology of paracetamol-induced albino rats. Sections of the liver in Group 1 (Figures 6a & b) showed normal hepatic histo-architecture of the Wistar rat. It showed hepatic lobules composed of normal hepatocytes arranged in radiating cords around the central veins (CV). The hepatic cords are separated by the hepatic sinusoids as the hepatic cords radiate towards the portal areas which consist of normal hepatic artery (A), hepatic vein (V) and bile duct (B). Sections of the liver collected from Group 2 (Figures 7a & b) showed the normal hepatic histo-architecture of the laboratory rodent with few fatty globules. It showed that hepatic lobules composed of normal hepatocytes arranged in radiating cords around the central veins (CV). The hepatic cords are separated by the hepatic sinusoids as the hepatic cords radiate towards the portal areas (P) which consist of normal hepatic artery, hepatic vein and bile duct. Sections of the liver collected from the negative control (untreated) group showed a widespread, random, individual necrosis of the hepatocytes and random multifocal aggregates of inflammatory cells (Figures 8a & b).

Liver sections collected from Group 4 showed a mild centrilobular hepatocellular degeneration and random widespread individual necrosis of the hepatocyte (Figures 9a &b). The degenerate hepatocytes appear slightly swollen and contain numerous minute clear vacuoles in their cytoplasm. Sections of the liver from Group 5 showed a mild vacuolar hepatocellular degeneration of the hepatocytes in the centrilobular and mid-zonal areas. The hepatocytes around the portal areas (periportal hepatocytes) appeared apparently normal (Figures 10a & b). Notice that the cells around the central vein are paler than those around the portal area. Liver sections of Group 6 (Figures 11a & b) showed the normal hepatic histo-architecture of the laboratory rodent just as observed in the normal control group. It showed hepatic lobules composed of normal hepatocytes arranged in radiating cords around the central veins (CV). The hepatic cords are separated by the hepatic sinusoids as the hepatic cords radiate towards the portal areas (P) which consist of normal hepatic artery, hepatic vein and bile duct.

DISCUSSION

Although paracetamol is remarkably safe in therapeutic dose, excessive amount causes hepatic necrosis leading to acute liver failure. The hepatotoxicity of paracetamol has been attributed to the formation of highly reactive electrophile, *N*-acetyl-*p*-benzoquinoneimine (NAPQI) by cytochrome P-450 [15]. Phytochemical studies demonstrated that the root extract of the *Combretum dolichopetalum* are rich in flavonoids and alkaloids. Flavonoids are a large class of phenolic compounds and constitute one of the largest groups of secondary metabolites in plants. Moreover, these compounds significantly protect cells against the damaging effects of reactive oxygen species (ROS). In general, the hepatoprotective activity of plants can be considered as an expression of the functional improvement of hepatocytes that results from accelerated cellular regeneration [16-18]. Therefore, silymarin that has been employed as a protective treatment of liver disease by its antioxidant properties deriving from the phenolic nature of flavonolignans [16,19,20].

The root decoction of *Combretum dolichopetalum* is widely used in Nigeria and other countries around the world in traditional medicine to treat stomachache, diarrhea, gastric ulcer, cough and liver disorders. The phytonutrients inherent in the root of *C. dolichopetalum* such as tannins, saponins, alkaloids, flavonoids and phenols are known for their numerous therapeutic activities including hepatoprotective effect [21].

ALT and AST are used to assess liver function; ALT is highly precise in monitoring hepatocellular status, and AST is a sensitive indicator of mitochondrial problems, particularly in centrilobular areas of the liver. Increases in the levels of cytoplasmic enzymes such as AST, ALT, and ALP are considered indicators of hepatic dysfunction and damage [22]. The leakage of cytosolic contents into the systemic circulation alters liver function. In our study, the activities of AST, ALT, and ALP in serum increased after paracetamol was introduced. These increases can be attributed to hepatic damage resulting in an increased rate of synthesis or release of functional enzymes from bio membranes [23]. However, these increases were significantly ameliorated by treatment with 200 mg/kg and 300 mg/kg of the methanol extract of the root of *Combretum dolichopetalum,* which is suggestive of its hepatoprotective effect.

Urea is major nitrogenous end-product of protein and amino acid catabolism, produced by liver and distributed throughout intracellular and extracellular fluid. In kidneys, urea is filtered out of blood by

Table 1: Quantitative phytochemical composition of *C. dolichopetalum* (bush willow) roots

Phytochemical	Concentration
Flavonoids	2.87±0.50
Alkaloids	4.17± 0.01
Sterols	1.24 ± 0.01
Tannins	1.69 ± 0.01
Glycosides	4.06 ± 0.00
Phenols	0.78 ± 0.00
Anthraquinones	0.59 ± 0.01

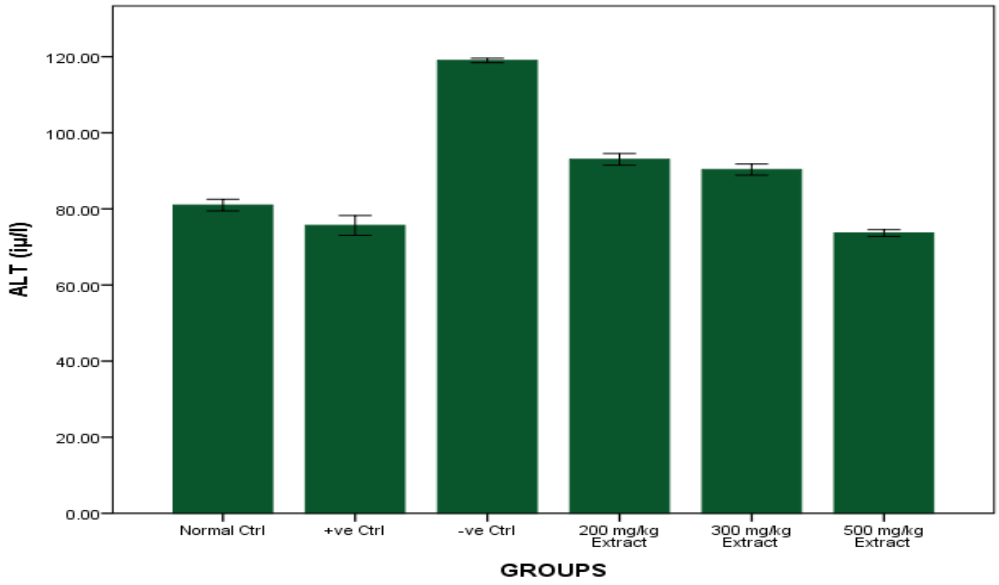

Figure 1: Effect of the extract (MECD) on ALT activities of paracetamol-intoxicated albino rats. PCM = Paracetamol; SLM = Silymarin.

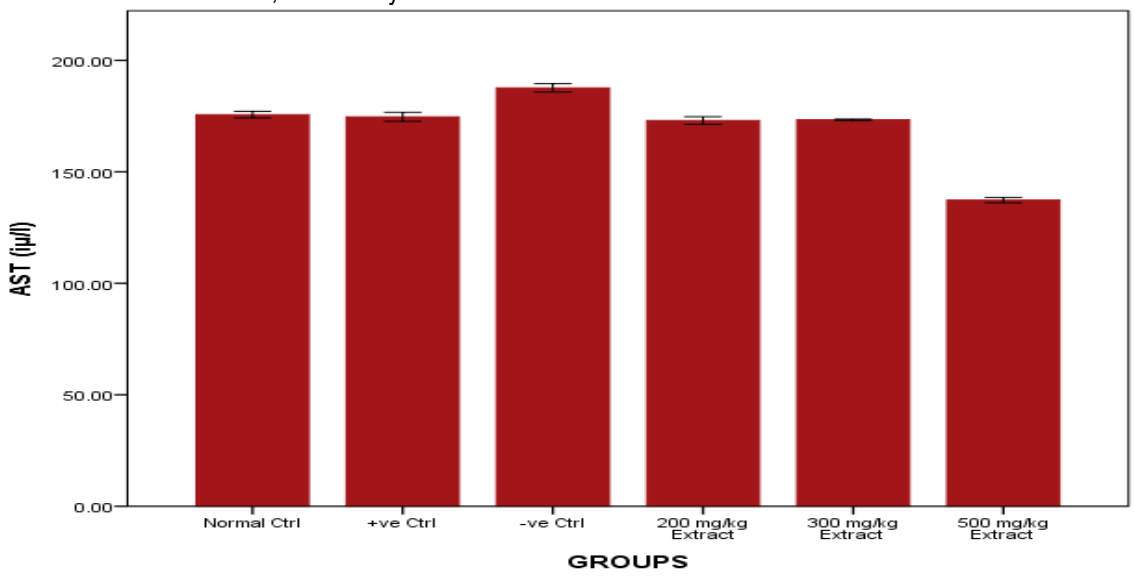

Figure 2: Effect of the extract (MECD) on AST activities. PCM = Paracetamol; SLM = Silymarin.

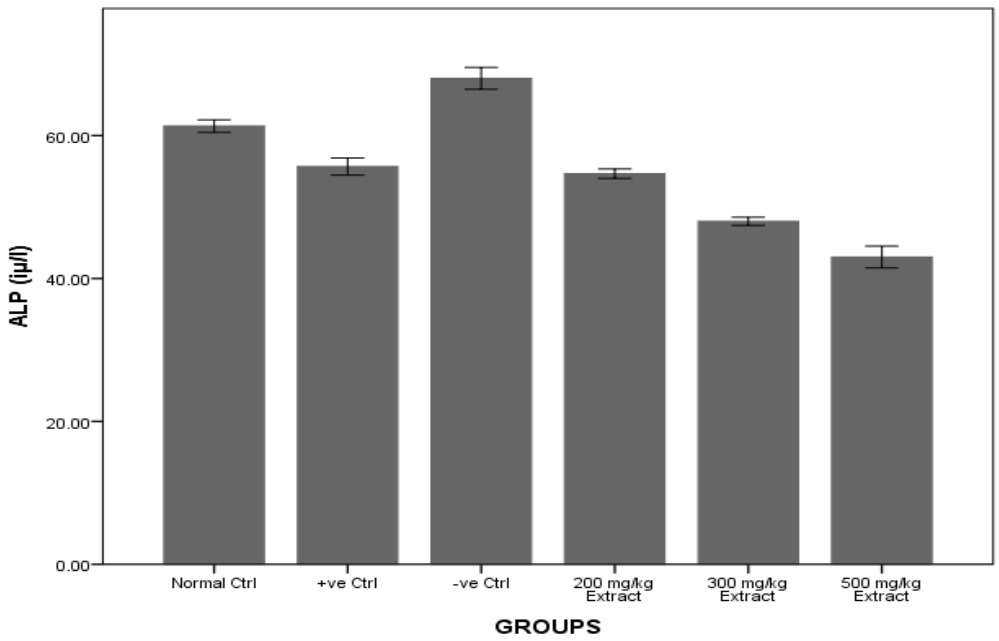

Figure 3: Effect of the extract (MECD) on ALP activities. PCM = Paracetamol; SLM = Silymarin.

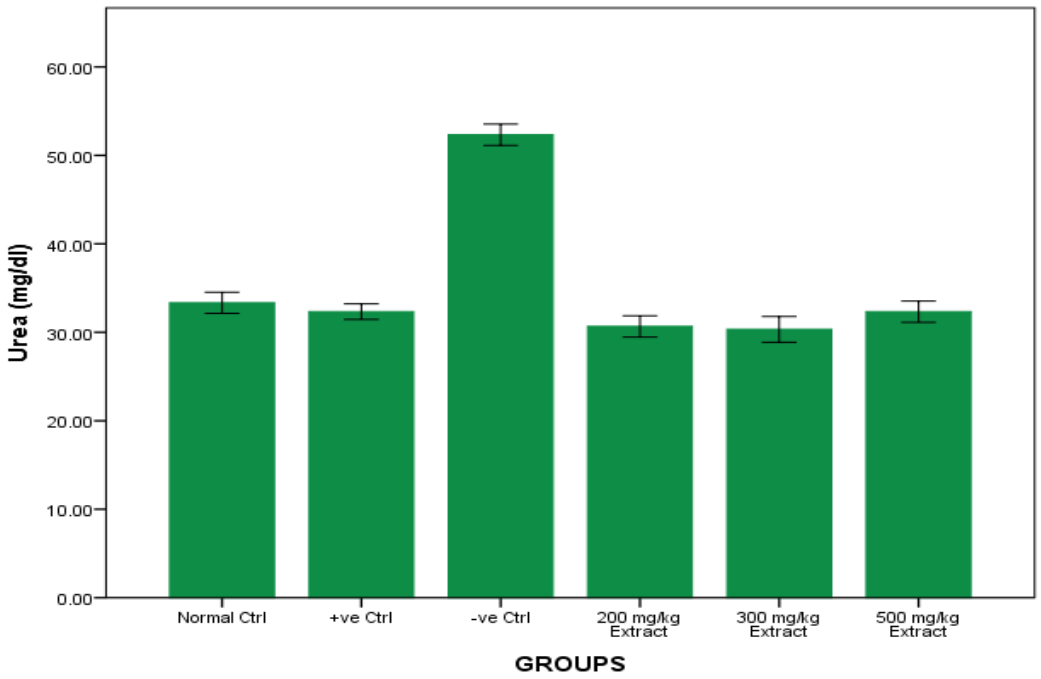

Figure 4: Effect of the extract (MECD) on urea concentrations. PCM = Paracetamol, SLM = Silymarin.

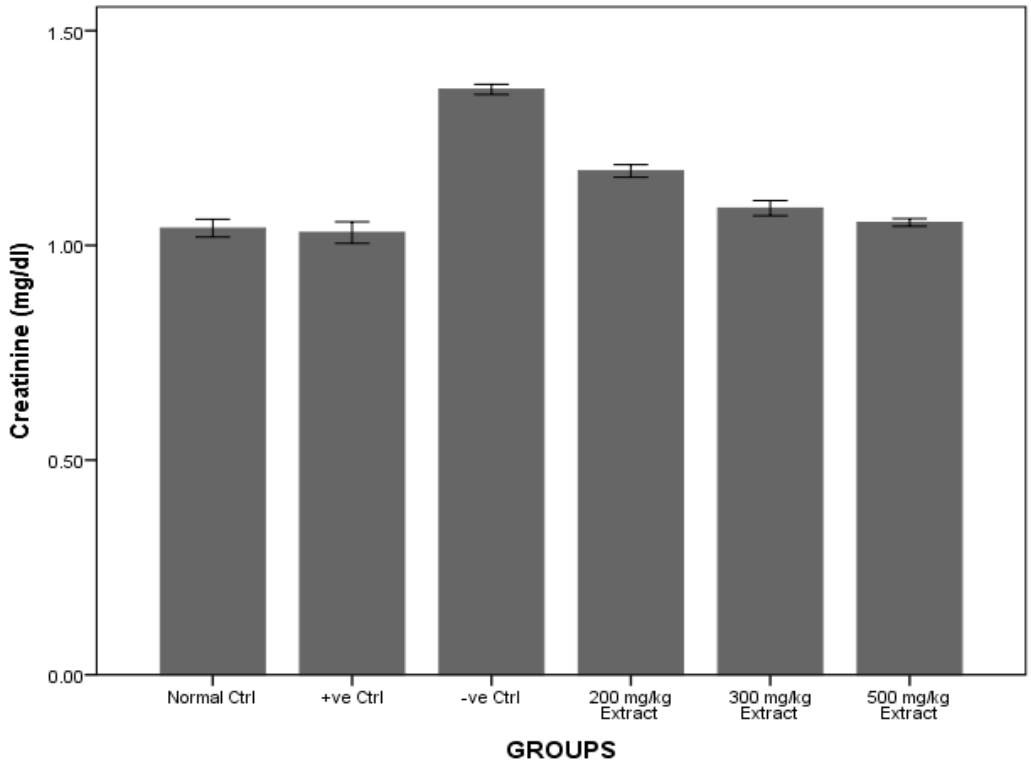

Figure 5: Effect of the extract (MECD) on Creatinine concentrations. PCM = Paracetamol; SLM = Silymarin.

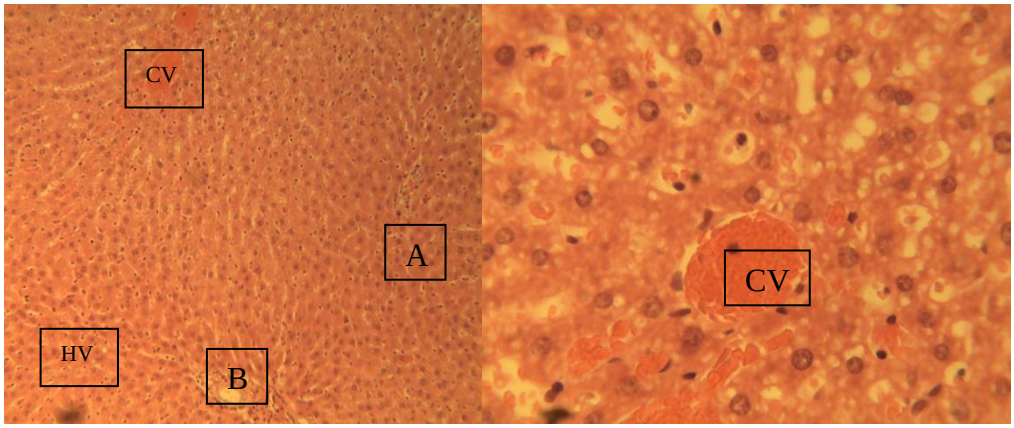

Figure 6: Histomorphology of liver section from normal control rats.

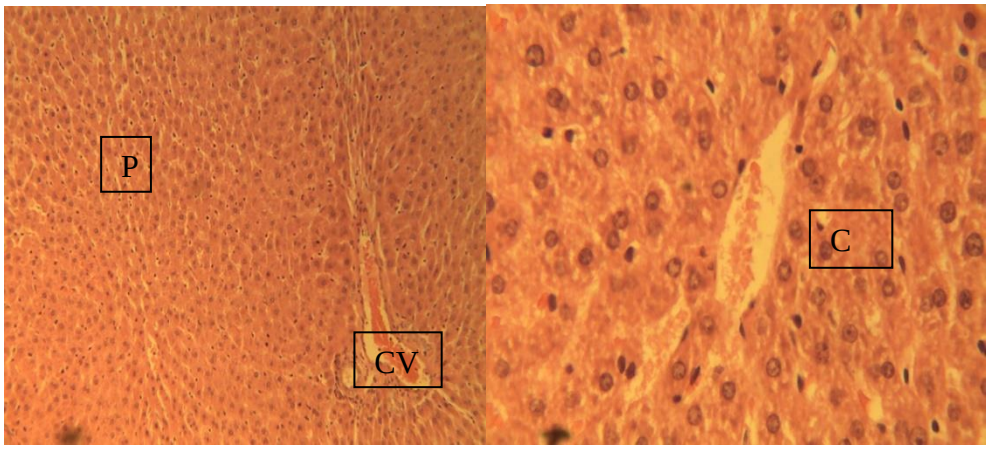

Figure 7: Histomorphology of liver section of paracetamol-intoxicated rats treated with Silymarin (Group 2).

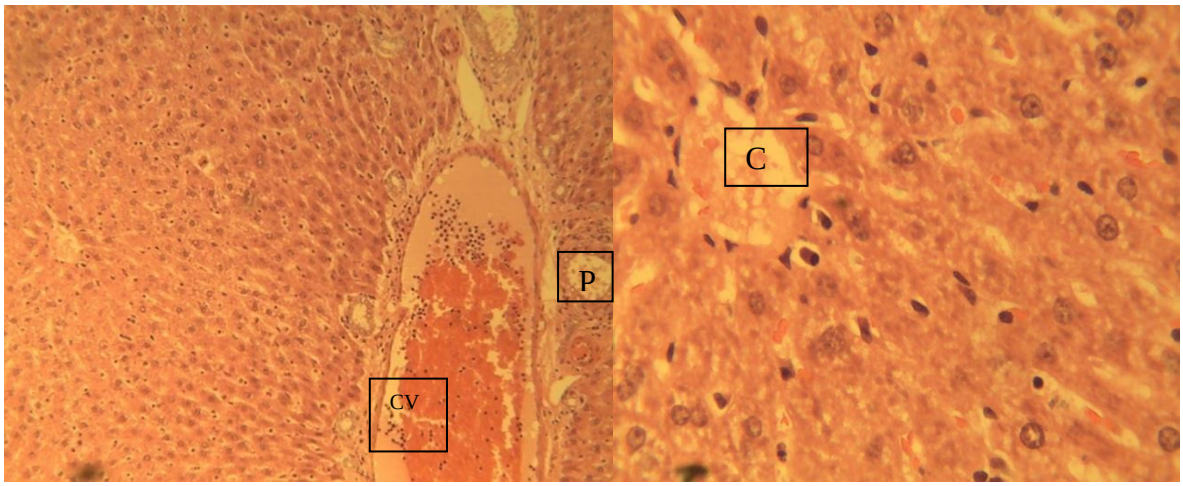

Figure 8: Histomorphology of liver section of paracetamol-intoxicated rats (untreated).

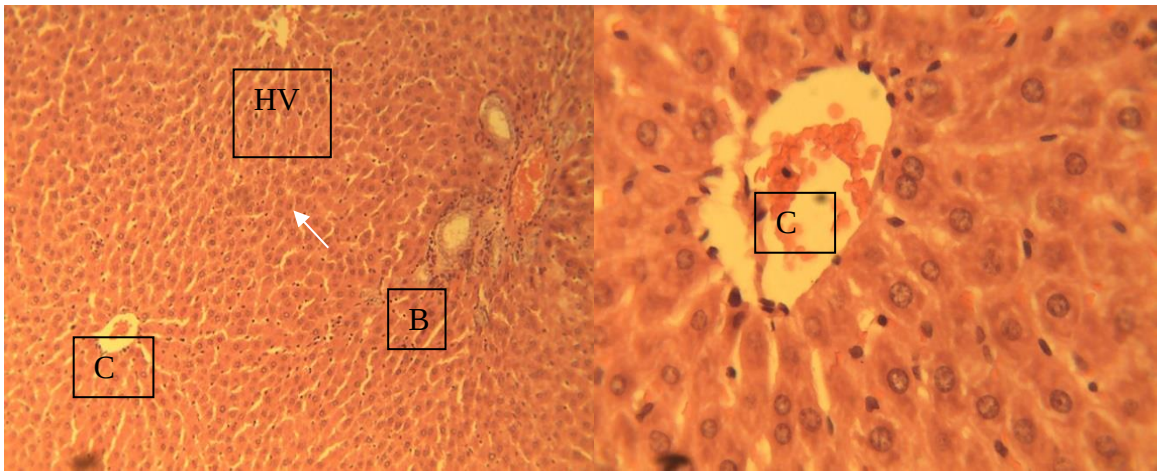

Figure 9: Histomorphology of liver section of paracetamol-intoxicated rats treated with 200 mg/kg MECD.

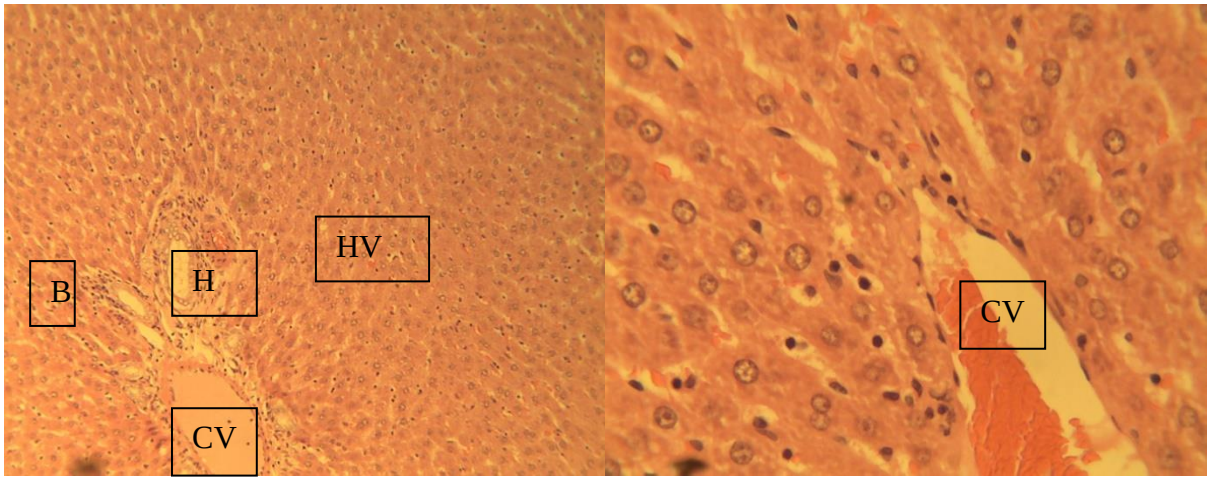

Figure 10: Histomorphology of liver section of paracetamol-intoxicated rats treated with 300 mg/kg MECD.

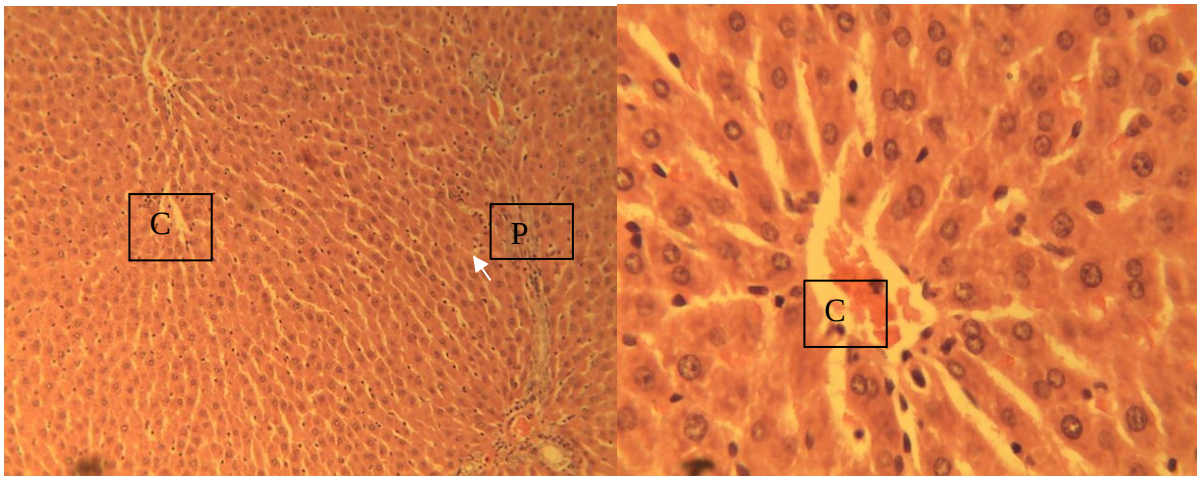

Figure 11: Histomorphology of liver section of paracetamol-intoxicated rats treated with 500 mg/kg MECD.

glomeruli and is partially being reabsorbed with water [24]. Creatinine is a breakdown product of creatine phosphate in muscle and is usually produced at a constant rate by the body depending on muscle mass [25]. The rationale for the use of creatinine and/or urea measurement to assess renal function is that plasma/serum levels of both reflect glomerular filtration rate (GFR), the parameter that defines kidney function. Irrespective of its cause, kidney disease is associated with decrease in GFR, and the severity of kidney disease correlates closely but inversely with GFR. However, in this study, concentrations of creatinine and Urea in serum increased after paracetamol was introduced in the negative control. The treatment with various doses of the MECD extracts reduced the Creatinine and urea concentrations in the serum – indicating a stabilization of the kidney function by the MECD root extract.

The degenerations including random and individual necrosis of the hepatocytes, and random multifocal aggregates of inflammatory cells after paracetamol intoxication were seen to be dose-dependently ameliorated by the MECD root extract. The 500 mg/kg of MECD root extract restored the normal histological architecture of the hepatocytes as seen in the normal control group.

Based on the biochemical and histopathological findings, the current study demonstrated the potential hepatoprotective properties of MECD against paracetamol-induced hepatotoxicity in Wistar rats, which is also consistent with its folkloric use in the treatment of liver disorders and ailments. In that context, the reduction of paracetamol-elevated ALT and AST levels caused by MECD root extract suggests stabilization of plasma membranes as well as alleviation or repair of hepatocellular damage, whereas the concomitant diminishing of the raised ALP levels indicates the improvement of biliary dysfunction, which is consistent with previous report [26].

CONCLUSION

The hepatoprotective screening of the methanolic root extract of *C. dolichopetalum* revealed that it possesses an ameliorative potential against drug-induced toxicity in the liver. Considering the positive effect of the methanolic root extract of *C. dolichopetalum* on the liver by decreasing the activities of the liver function enzymes which implies a healthy and functional liver; increased consumption of the plant is therefore encouraged but in a moderate dose as a high dose of it could be harmful to the liver.

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