Trop J Nat Prod Res, October 2021; 5(10):1883-1888

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

Acute Effects of *Allium cepa* (onions) Methanol Extract on Adriamycin-induced Hepato-renal Damage in an Experimental Rat Model

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ARTICLE INFO

Article history:
Received 14September 2021
Revised 10October 2021
Accepted 21October 2021
Published online 02 November 2021

ABSTRACT

The liver's metabolic processes protect the body's other organs and tissues from toxic substances. Some metabolic detoxification byproducts, if present in excess, can harm the liver or kidneys. The effect of *Allium cepa* methanol extract (MEAC) on Adriamycin-induced acute liver and kidney damage was investigated in this study. A total of twenty-five albino rats were randomly assigned to one of five groups: For five days, Groups A-E were given the following treatments: Adriamycin (15 mg/kg) was administered intraperitoneally to Group A (negative control); Adriamycin (15 mg/kg) plus a low dose of MEAC (2000 mg/kg p.o.) were given to group B; Adriamycin (15 mg/kg) plus a high dose of MEAC (400 mg/kg p.o.) were given to group C; Adriamycin (15 mg/kg) plus vitamin C (200 mg/kg p.o.) were given to group D (positive control); and no treatment was given to group E. Standard biochemical procedures were used to determine hepatorenal toxicity by evaluating creatinine, urea, Na^+, K^+, bilirubin, ALT (alanine transaminase), AST (aspartate transaminase), and ALP (alkaline phosphatase) enzyme activity. The intraperitoneal administration of Adriamycin caused a marked elevation of the biochemical parameters; BUN (blood urea nitrogen), creatinine, K^+, serum bilirubin, ALT, AST and ALP enzyme activity, with reduced Na^+. Treatment with high dose of methanol extract of *Allium cepa* showed significantly attenuated hepatorenal dysfunction. Histopathological studies supported the biochemical observations as the liver and kidney sections of Adriamycin-induced rats showed significant recovery following administration of MEAC. The methanol extract of *Allium cepa* at high dose possesses strong hepatorenal protective ability.

Keywords: *Allium cepa*, Adriamycin, nephroprotection, hepatoprotection, nephrotoxicity, hepatotoxicity.

Introduction

Allium cepa, also known as onions, is a subspecies and primary member of the genus Allium. It has been used as a food and as a medicinal plant since ancient times.[1] Organic sulphur compounds are found in onions, which have an alliinase effect. It also has flavonoids like kaempferol and quercetin that are important as they possess anti-inflammatory, antiallergenic, vasodilatory, cardioprotective, anti-carcinogenic, antibacterial and antifungal properties.[2] Onions is traditionally used as a contraceptive, carminative, expectorant, emmenagogue, anthelminthic, tonic and aphrodisiac. It was also used in the treatment of bruises, cholera, colic, insect bites, bronchitis, tuberculosis, diabetes, dropsy, epileptic fits, catarrh, hysterical fits, fevers, hypertension, scurvy, pimples, jaundice and sores.[3]

With respect to detoxification, the liver and the kidneys are responsible for the metabolism and removal of drugs and other foreign substances that enter the body. The liver accomplishes this job primarily through the activity of the cytochrome P450 group of isoenzymes; thus, hazardous amounts of medicines in the body primarily affect the liver, resulting in liver damage.[4]The kidneys are not also spared from injuries due to chemical toxicity or their metabolic products.

Acute kidney injury (AKI) is a loss of renal function that occurs suddenly, and it can be detected within a few days due to damage in the kidney tissue. It can be brought on by a number of factors, including the ingestion of harmful substances, kidney ischemia (reduced blood flow to the kidney), or when inflammatory processes are induced and affect organs, causing electrolyte imbalance and waste product retention. A patient with AKI may produce small amount of urine (oliguric) or produce large amount of urine (non-oliguric).[5,6] Therefore urine output is among one of the criteria for defining Acute kidney injury.[7] The biochemical parameters used to assay this disease are serum electrolyte, creatinine, urea and uric acid.

Adriamycin (doxorubicin) is in the class of drugs called anthracycline and possesses strong anticancer activity.[8] Long term treatment with Adriamycin can result in toxicity or damage to vital organs. This toxicity is brought about by oxidative damage to cellular components.[9]Adriamycin is given intravenously or by intra-arterial drip infusion. When used to treat tumors, it can be given locally into the tumor site.[8] Adriamycin has been implicated to cause a shift between free oxygen radicals and antioxidant enzyme leading to tissue injury.[9]

Liver and kidney damages following toxic drugs-induced hepatoxicity and nephrotoxicity respectively have continued to pose a serious burden to patients and health care workers. The management and

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Citation: Uchendu IK, Ogbonna DK, Oguji EC, Omeh OJ, Ochi CJ, Udeh CA, Obigeorge CJ, Nnam CM, Omokungbe O, Ikekpeazu CA, Odeku AJ, Apara OP, Orisakwe OT. Acute Effects of *Allium cepa* (onions) Methanol Extract on Adriamycin-induced Hepato-renal Damage in an Experimental Rat Model.Trop J Nat Prod Res. 2021; 5(10):1883-1888. doi.org/10.26538/tjnpr/v5i10.29

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curative therapy of liver and kidney damage are massively expensive; so, there is need for cheaper treatment alternative using plant extracts. Despite the difficulties in detecting curative plant-based drugs, bioactive phytochemicals derived from plants will continue to be used in the search for additional novel treatments. In this work, biochemical indicators in relation to Adriamycin-induced hepatorenal damage were estimated.

Materials and Methods

Plant materials

The onion (*Allium cepa*) was purchased at Ogbete market, Enugu, Nigeria, in June 2020. The plant sample was recognized and authenticated by Ugwozor P.O. of Herbarium Section at Department of Botany and Plant Biotechnology, Faculty of Biological Sciences, University of Nigeria, Nsukka, Enugu. For future reference, a voucher specimen (UNH 455^{C}) was placed in the herbarium.

Chemical reagents and drug

Adriamycin (Doxorubicin hydrochloride) purchased from (Calbiochem, UK); vitamin C was purchased from Alpha Pharmaceuticals, Nigeria. Laboratory Reagent kits for the analyses of Na^{+}, K^{+}, Urea and Creatinine, ALT, AST,ALP and total bilirubin measurements were purchased from Random laboratories Ltd, UK. Absolute methanol was of analytical grade.

Preparation of vitamin C Solution

To make a stock concentration of 20 mg/mL, twenty 100 mg vitamin C tablets were ground to powder and dissolved in 100 mL distilled water (measured in a beaker).

Preparation of Adriamycin (doxorubicn) solution

To make a stock concentration of 1 mg/mL, 50 mg of Adriamycin was dissolved in distilled water and made up to 50 mL in a measuring cylinder.

Induction of hepatic and kidney injuries

Hepatorenal damage was induced in the animal by injecting Adriamycin solution (15 mg/kg) intraperitoneally every day for 5 days.

Conditioning of animals

From the animal house of the University of Nigeria's College of Veterinary Medicine, twenty-five (25) mature albino rats weighing 130±30 g were taken. The animals were kept in metal cages in the Department of Anatomy's animal house at regular temperature (22±3^{o}C) and a 12 hour light/12 hour dark cycle. They were given enough commercial rat pellets to eat (Chikun feed; Olam animal feed mill Ltd, Kaduna).Prior to the start of the experiment, the animals were kept under observation for 14 days, to acclimatize. The University of Nigeria Teaching Hospital's institution animal ethics committee authorized the experimental protocol (UNTH/CSA. 874/VOL. 21).

Extraction of Allium cepa

Fresh, healthy onions (*Allium cepa*) were washed, sliced into small pieces, and homogenized in a high-powered blender (Qasa blender made in Nigeria).The resultant mixture was then steeped in 2 liters of 80% methanol.The mixture was left to settle for 24 hours, with intermittent shaking. After filtration, the filtrate was concentrated to dryness at 40°C using a rotary evaporator operating at reduced pressure. The dried methanol extracts of *Allium cepa* (MEAC) were weighed,reconstituted in distilled water and stored at 4^{o}C in the refrigerator.

Animal treatment withthe extracts

A total of twenty-five (25) albino rats were randomly assigned to one of five (5) groups. The animals were weighed before and after the experiment. The gavage technique was used in the delivery of oral dosage to the laboratory rats.

Group A: (Negative Control): Received intraperitoneal injection of Adriamycin (15 mg/kgb.wt) daily for five days.
Group B: Received Adriamycin (15 mg/kg) plus MEAC (200 mg/kg, oral) daily for 5 days.
Group C: Received Adriamycin (15 mg/kg) plus MEAC (400 mg/kg, oral) daily for 5 days.
Group D: Received Adriamycin (15 mg/kg) plus vitamin C (200 mg/kg, oral) daily for 5 days.
Group E: No treatment was received by rats in this group.

Biochemical analysis

Under chloroform anesthesia, blood samples were drawn from the left ventricle of the heart for electrolyte, urea, creatinine, bilirubin, AST, ALT, and ALP determinations, and the kidneys and liver were excised for histological examinations. The following procedures were used to assess serum electrolyte, urea, and creatinine levels for renal function analyses:Serum urea level was estimatedaccording to the method described by Natelson *et al*.;[10] creatinine level determined colorimetrically using the Jaffe Reaction.[11]The serum ALT and AST activity were measured using the colorimetric method published by Reitman and Frankel for liver function testing.[12] Kind and King described a colorimetric method used for measuring ALP activity.[13]Total bilirubin was measured using the Colorimetric technique, as reported by Malloy and Evelyn.[14]

Histopathological analysis.

The kidneys and liver were removed and immersed in paraffin wax before being sectioned at 5 microns and stained with haematoxylin and eosin (H and E).[15]An Olympus TM light microscope was used to examine the histological sections.

Statistical analysis

GraphPad prism version 7.0 was used to examine the data. The biochemical assay findings were presented as mean±SEM (standard error of mean). An ANOVA was used to determine the degree of significance, followed by a Tukey post hoc analysis; the significance level was set at $p<0.05$.

Results and Discussion

Effects of treatments with Adriamycin on body weight of Wister rats.

The effect of treatments with Adriamycin (ADM) on body weight of Wistar rats is represented in Figure 1. It was observed that rats treated with only Adriamycin for 5 days had a significant weight loss; as the rats in the group lose appetite, were sluggish and responded slowly to stimulus. However, the reverse was seen in the normal control rat, which had a significant weight gain over the 5 days period. Interestingly, the rats in the groups co-administered with Adriamycin and low dose MEAC, High MEAC or vitamin C, separately, prevented weight loss in the rats

Rats in the experimental groups with different body weights are represented by a bar chart. When the pre-treatment weight is compared to the post-treatment weight, preliminary data reveal that intraperitoneal injection of Adriamycin for five days caused a considerable weight loss. However, cotreatment with oral administration of low dose MEAC, high dose MEAC or vitamin C separately ameliorated the abnormal weight loss when the pre-treatment weight is compared with post-treatment weight, separately for the individual treatment. For each treatment, the data is presented as a mean ± SEM of body weight (gram).

Biochemical results

The kidneys' functionality was determined by measuring the serum levels of biochemical indicators such asurea, creatinine, sodium (Na^{+}),potassium (K^{+}), and (Table 1). From the results, the treatment with vitamin C or high dose of MEAC showed significant anti-nephrototoxic effects ($P<0.05$) when compared with the negative control (Adriamycin only), while the low dose of MEAC offered a non-significant nephroprotection against Adriamycin. Furthermore, it was observed that the extract provided protection in a dose-dependent

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

manner, as high dose of extracts showed greater protection than low dose of the extract.

The liver's functioning was determined by measuring serum levels of the biochemical markers aspartate transminase (AST), alanine transminase (ALT), alkaline phosphatase (ALP), and total bilirubin (TB) (Table 2). From the results, the treatment with vitamin C or high dose of MEAC showed significant anti-hepatotoxic effects ($P< 0.05$) when compared with negative control (Adriamycin only), while the low dose of MEAC offered a non-significant hepatoprotection against Adriamycin. Again, it was observed that the extract provided protection in a dose-dependent manner, as high dose of extracts showed greater protection than low dose of the extract.

Histopathological results

Untreated control rats' kidney nephrons (Group E) appeared physically and functionally normal. The morphology of the nephrons was well preserved; the glomeruli and tubules appeared normal. In the kidney section of Adriamycin treatment only (negative control, group A), several Bowman's capsules were devoid of glomeruli while others were partly eroded. Moreover, in the kidney section of low dose-treated rats (group B) several Bowman's capsules were also devoid of glomeruli while some were constricted; the tubules appeared normal. While the rats administered with high dose of MEAC (group C), showed normal glomeruli and normal tubules. Furthermore, photomicrographs of ADM + Vitamin C (positive control group D) kidney sections revealed intact glomeruli and tubules. (Plate 1).

The lobules of the liver of untreated control rats (Group E) appeared structurally and functionally normal. The morphology of the hepatocytes was largely preserved; the central vein and portal triad all appeared normal. In the liver section of Adriamycin treatment only (negative control, group A), the hepatocytes appeared moderately normal; the central veins appeared dilated and congested. There was also sinusoidal congestion. In the liver section oflow dose-treated rats (group B), the hepatocytes appeared normal; there was mild periportal inflammation and areas of sinusoidal cellular infiltration. While the rats administered with high dose of MEAC (group C), showed normal hepatocytes, normal central veins and congested portal venules in the portal triads. A photomicrograph of a liver section from an ADM + Vitamin C (group D) rat revealed normal hepatocytes, there was mild periportal cellular infiltration and the sinusoids appeared dilated (Plate 2).

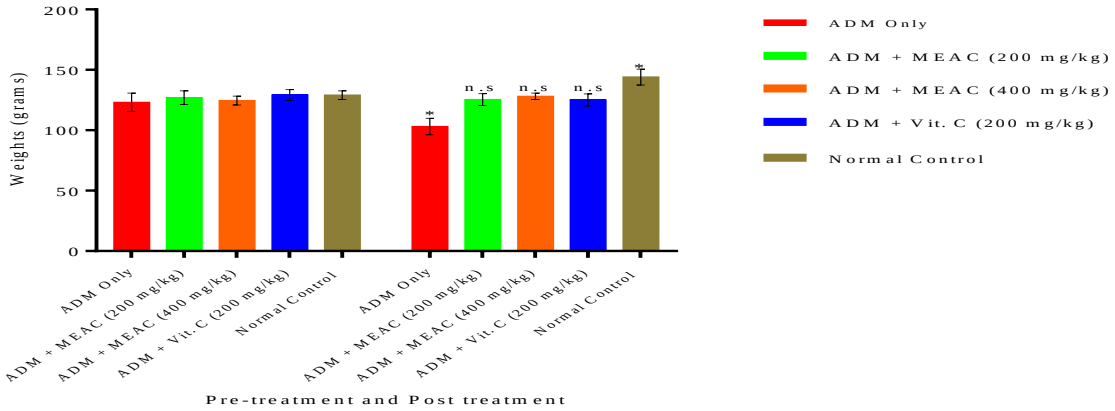

Figure 1: Effects of treatments with Adriamycin on body weight of Wister rats

A decrease in body weight of rat was recorded in the experimental groups. According to preliminary findings, intraperitonal injection of Adriamycin for 5 days induced a significant weight loss when the pre-treatment weight is compared with post-treatment weight. The weight loss could be due to the side effect of Adriamycin. The reduction in body weight agrees with the report by de Lima Junior[16], which stated that Adriamycin causes substantial weight loss and anorexia, as well as a disruption in systemic metabolism. However oral administration of treatment with low dose MEAC, high dose MEAC or vitamin C separately ameliorated the abnormal weight loss when pre-treatment weight is compared with post-treatment weight. Long term treatment with Adriamycin can result to toxicity or damage to vital organ such as kidney, liver and heart.[17-20] Adriamycin intoxication may have caused the significant loss of body weight, anorexia with disruption in system metabolism as observed in this study.

Adriamycin's toxicity is due to its prooxidant properties. It generates free radicals like superoxide and hydrogen peroxide, which are thought to be the cause of nephrotoxicity and hepatotoxicity. Furthermore, Adriamycin reduces the effectiveness of the cellular antioxidant defense system. Oxidative tissue damage is caused by an imbalance of reactive oxygen species between antioxidants. [21]

Kidney damage can be indicated consequently by high level of blood urea and creatinine. Damage to the kidney will make the kidney inept in excreting both urea and creatinine and cause their build up in the blood. When compared to the untreated control group, the biochemical data showed a significant rise in serum creatinine, BUN, and potassium, but a decrease in sodium in group A (Negative control) rats given Adriamycin alone. This indicates a decline in renal function, as indicated by lower creatinine and urea clearance.[22,23]

The harmful substance and a low dose of MEAC were administered to rats in group B, and the decreased metabolites and electrolytes showed a restorative effect, though not as drastic as in group C. When compared to group A rats, co-administration of Adriamycin and a high dose of MEAC resulted in a substantial reduction (p0.05) in blood urea nitrogen, creatinine, and potassium levels, indicating nephroprotection. The extract provided dose-dependent protection, with the high dose of MEAC providing stronger protection than the low dose, as demonstrated by improved creatinine and urea clearance levels.These findings could be due to the antioxidant and anti-inflammatory effects of phytoconstituents' mechanism of action. Phytochemicals are plant chemicals that have both harmful and therapeutic potentials. Plants produce these substances to protect themselves, but studies have shown that they can also prevent sickness in people and other living things.[24-26] According to Abduikadir *et al.*[27], the phytochemical examination of *Allium cepa* revealed the presence of flavonoids (+),tannins (+), alkaloids (+),and saponins (+), but no steroids. Flavonoids belong to a class of low molecular weight phenolic compounds.[28]They possess anti-oxidant activity via a number of processes which include; scavenging of reactive oxygen species, up to regulation of antioxidant defenses, and suppression of reactive oxygen species formation by inhibiting enzyme or chelating trace elements involved in generation of free radicals.[29]

From the biochemical results of this present study, negative control rats (Group A) that received Adriamycin alone when compared with the untreated control group showed significant increase in liver biochemical markers and serum total bilirubin and a marked decrease in body weight. The remarkable increase in the liver biomarker enzymes and bilirubin in Adriamycin administered rats is a

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

confirmation of previous report of the hepatoxicity of Adriamycin.[30]The histopathological result of the group A rats agrees with the biochemical results by showing marked hepatocellular necrosis when compared with the normal control.

The group that received Adriamycin and low dose MEAC (group B), showed a reduction in serum liver enzymes as well as total bilirubin levels and increase in body weight of the rats when compared to the Adriamycin alone-treated rats (group A). This is an indication of the hepatoprotective potential of onion against liver injury. This is consistent with the findings of Baxlaet al.[31], who found that onion has a hepatoprotective effect against lead-induced liver damage in Wister rats. This also agrees with previous report byHarmeet*et al*.,[32] that onion has antioxidant and hepatoprotective properties. The biochemical result is further backed by the histopathology by showing minor hepatocellular necrosis.

Table 1: Comparison of the concentration of biomarkers of kidney injury in different experimental animal groups

Groups	BUN (mg/dl)	Creatinine (mg/dl)	K^+ (mmol/l)	Na^+ (mmol/l)
A- ADM only	39.49 ± 3.74	1.51 ± 0.10	9.11 ± 0.03	133.16 ± 2.03
B- ADM +Low dose MEAC	26.21 ± 4.26	1.22 ± 0.24	8.69 ± 0.17	135.09 ± 0.19
C- ADM + High dose MEAC	23.07 ± 1.08*	1.01± 0.29*	6.13 ± 0.17*	142.78 ± 0.31*
D- ADM + Vitamin C	22.46 ± 3.72*	1.03± 0.17*	6.72 ± 0.21	137.09 ± 0.25
E- Normal Control	22.715 ± 1.50*	0.91 ± 0.26*	5.64 ± 0.10*	144.60 ± 1.62*

Values given as mean ± SEM. *$P < 0.05$ is significant when comparing (Adriamycin alone) to all other groups

Table 2: Comparison of the concentration of biomarkers of liver injury in different experimental animal groups

Groups	ALT (IU/L)	AST (IU/L)	ALP (IU/L)	TB (mg/dl)
A. ADM only	29.45 ± 1.72	44.73 ± 3.41	301.64± 25.37	.31 ± 0.25
B. ADM +Low dose MEAC	25.81 ± 2.65	32.8 ± 7.27	294.92 ± 25.95	1.19 ±0.34
C. ADM + High dose MEAC	22.47 ± 2.71*	26.42± 2.09*	249.97 ± 26.01*	0.99 ± 0.27*
D. ADM +Vitamin C	23.73 ± 1.18*	28.01 ± 2.64*	253.82 ± 32.46*	1.01±0.21*
E. Normal Control	22.42 ± 2.73*	24.65 ± 4.57*	230.34 ± 27.37*	0.96 ± 0.22*

Values given as mean ± SEM. **$P < 0.01$; *$P < 0.05$ is significant when comparing (Adriamycin alone) to all other groups

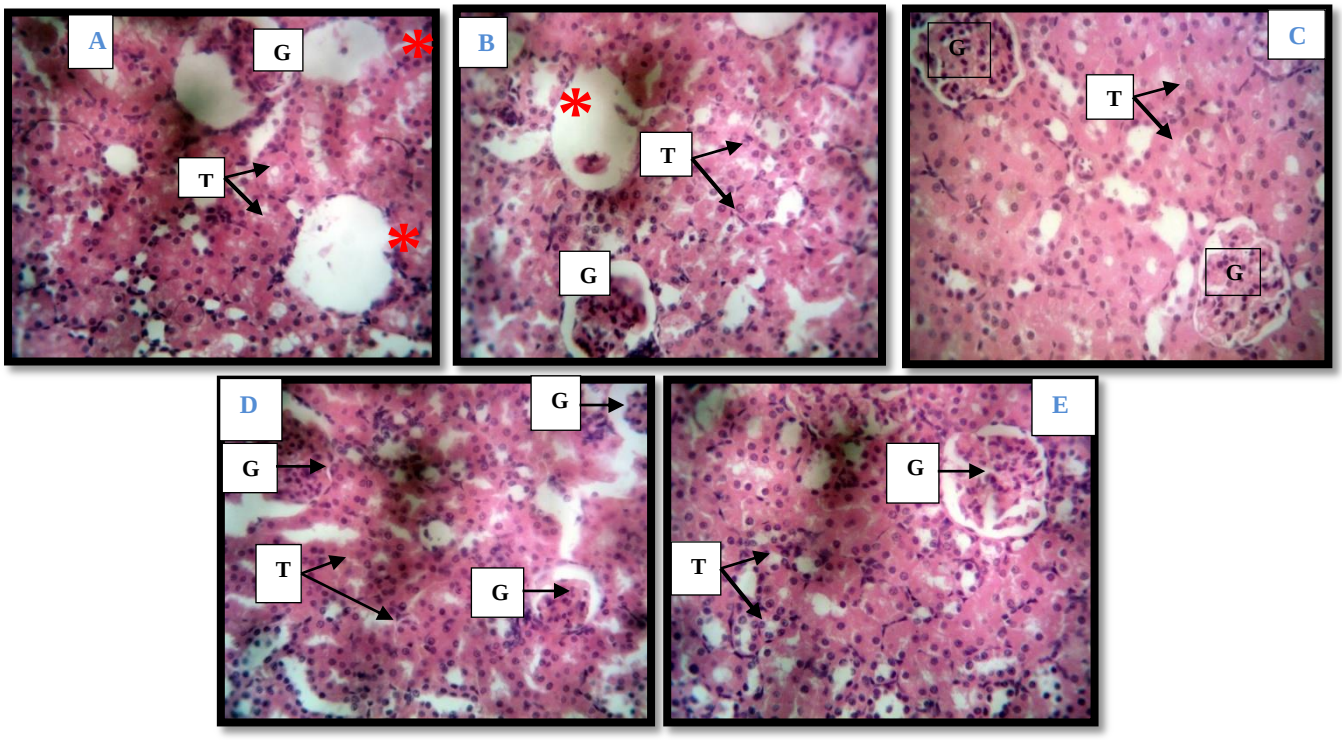

Plate 1: **(A)**Photomicrograph of kidney section from Adriamycin treatment only (negative control group). Features: Several Bowman's capsules are devoid of glomeruli (*) while others (G) are partly eroded; the tubules (T) appear normal. **(B)** Photomicrograph of kidney section from low dose MEAC treatment (group B). Features:Several Bowman's capsules are devoid of glomeruli (*) while some (G) are constricted; the tubules (T) appear normal.**(C)** Photomicrograph of kidney section from high dose MEAC treatment. Features: The glomeruli (G) and tubules (T) appear normal. **(D)** Photomicrograph of a vitamin C-treated kidney section. Features: The glomeruli (G) are slightly constricted while the tubules (T) are normal.**(E)** Photomicrograph of kidney section from normal control rats (group E). Features: The glomeruli (G) and tubules (T) appear normal. Stain: Haematoxylin and eosin. Magnification: X100.

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

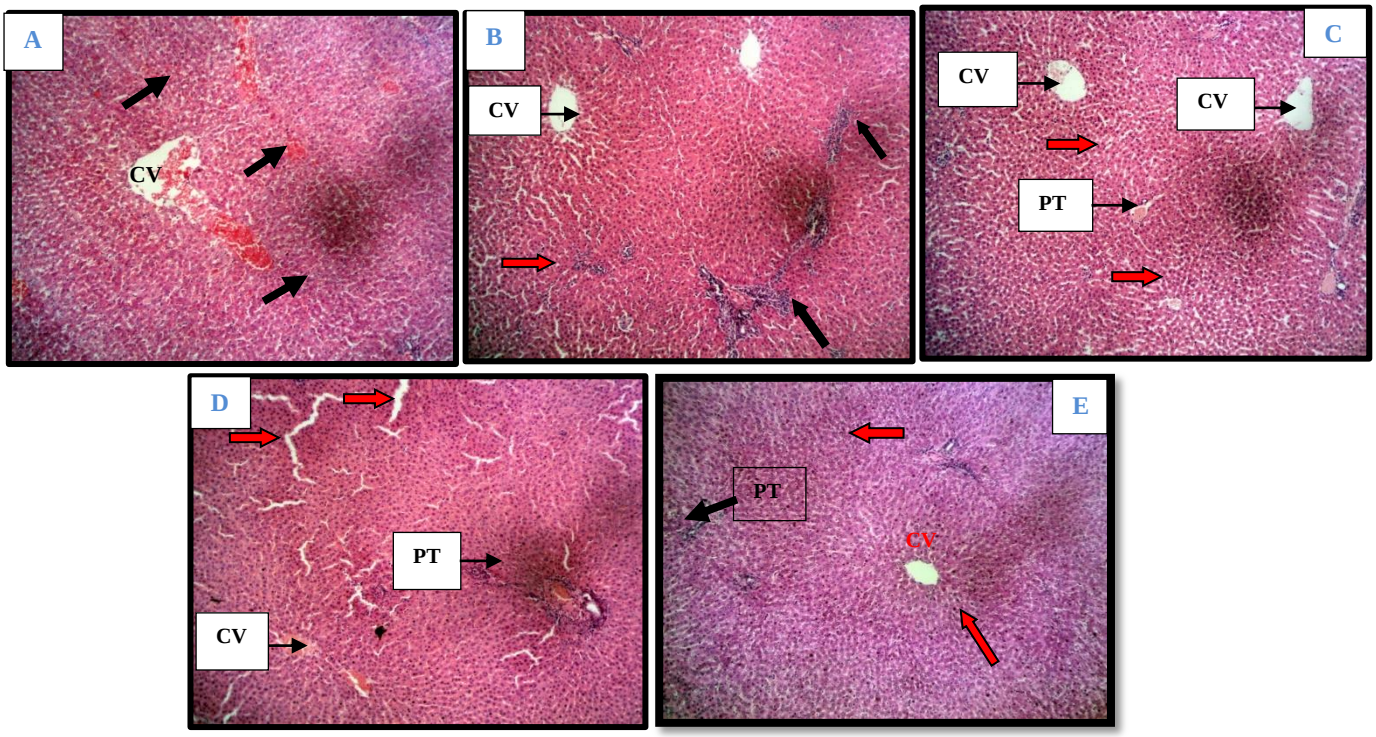

Plate 2: **(A)** Photomicrograph of liver section from Adriamycin treatment only (negative control group). Features: The hepatocytes are normal; the central veins (CV) appear dilated and congested. There is also sinusoidal congestion (arrows). **(B)** Photomicrograph of liver section from low dose MEAC treatment. Features: There is mild periportal inflammation (black arrow) and areas of sinusoidal cellular infiltration (red arrow). **(C)** Photomicrograph of liver section from high dose MEAC treatment. Features: Section has normal hepatocytes (arrow), central veins (CV) and congested portal venules in the portal triads (PT). **(D)** Photomicrograph of liver section from vitamin C treatment. Features: Hepatocytes are normal, there is mild periportal cellular infiltration (PT) and the sinusoids appear dilated (arrows). **(E)**Photomicrograph of liver section from normal control rats. Features: Liver section appears normal. Hepatocytes (red arrow); central vein (CV) and portal triad (PT) all appear normal. [Stain: H and E; ×100]

Conclusion

The present study shows that oral administration of methanol extract of *Allium cepa* could prevent the adverse effect caused by Adriamycin-induced acute hepatorenal toxicity. As a result, these findings suggest that *Allium cepa* extract may be beneficial to patients who may likely come down with acute kidney and/or liver injuries caused by toxic chemical agents like Adriamycin.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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