



Received: 12-09-2025
Accepted: 22-10-2025

International Journal of Advanced Multidisciplinary Research and Studies

ISSN: 2583-049X

Hepatoprotective Effects of *Allium Cepa* (Onions) Juice Extract on Adriamycin - Induced Liver Damage in Rats

¹ Orji Joel Ekeokeh, ² Juliet Chimaobi Akpe, ³ Kelechi Immaculate Ezenagu, ⁴ Onyinye Elizabeth Nwachuya, ⁵ Abraham-Ajala Eunice, ⁶ Awe Boluwatife Samuel, ⁷ Ozoagu Chigozie Stephen, ⁸ Jonathan Joshua Danjuma, ⁹ Precious Chinonso Ugwuoke, ¹⁰ Ummusalma Abdulraheem, ¹¹ Nneji Chinasa Remigius, ¹² Ndukwe Chika Kalu, ¹³ Chigoziri Anthony Amugo, ¹⁴ Favour Uchechukwu Onwuasoanya, ¹⁵ Odaikaku Judith Nnaji, ¹⁶ Chiamaka Chrysolyte Orji, ¹⁷ Ariyo Yemisi Ayomiposi, ¹⁸ Onyia Chinasa Annastasia, ¹⁹ Obialor Oluchi peace, ²⁰ Ugwuanyi Amuche Louis, ²¹ Chidimma Sandra Ngwu, ²² Ahmed Yahia Mohamed Yahia, ²³ Nkechinyere Mercy Nwegbo

¹ Department of Medicine and Surgery, University of Nigeria, Enugu Campus, Enugu State, Nigeria
^{2, 4, 13, 14, 18, 20, 23} Department of Medical Laboratory Science, University of Nigeria, Enugu Campus, Enugu State, Nigeria
³ Department of Agricultural Economics and Extension, Nnamdi Azikiwe University, Awka, Anambra State, Nigeria
^{5, 12} Department of Medical Laboratory Science, Bowen University, Iwo, Osun State, Nigeria
⁶ F. Erismann Institute of Public Health, I.M Sechenov First Moscow State Medical University, Moscow, Russia
⁷ Department of Soil Science, Nnamdi Azikiwe University, Awka, Anambra State, Nigeria
⁸ Department of Medical Laboratory Science, Bayero University, Kano State, Nigeria
⁹ Department of Food Science and Technology, University: Federal Polytechnic Oko, Anambra State, Nigeria
¹⁰ Kuntau Science Academy, Kano State, Nigeria
¹¹ Department of Health Science, Imo State University, Imo State, Nigeria
¹⁵ Department of Biotechnology of the A.P. Nelyubin Institute of Pharmacy, First Moscow State Medical University Sechenov, Moscow, Russia
¹⁶ Department of Global Health and Infectious Disease Control Institute, Nasarawa State University, Nasarawa State, Nigeria
¹⁷ Department of Medical Laboratory Science, Achievers University Owo, Ondo-State, Nigeria
¹⁹ Department of Medical Laboratory Science, Abia State University, Uturu, Abia State, Nigeria
²¹ Department of Medical Laboratory Science Madonna University Elele Campus Rivers State Nigeria
²² Russian Language Learning Program for Students, The Agricultural University, Chiboxari, Russian Federation, Republic of Chuvashia

Corresponding Author: Orji Joel Ekeokeh

Abstract

Metabolic processes in the liver protect other organs and tissues in the body from harmful chemicals or toxins. Some byproducts of metabolic detoxification, if in excess, can cause damage to the liver and an example of such toxic chemical product is Adriamycin. Adriamycin (Doxorubicin) is one of the most effective chemotherapeutic agents against a variety of cancers, but its usefulness is seriously curtailed by the risk of developing heart failure. Doxorubicin is associated with adverse effects on organ like the liver. Acute liver injury is the loss of liver function that occurs rapidly in days or weeks usually in a person who has no pre-existing liver disease. Acute liver injury is less common than chronic liver injury. Onion is known for many medicinal properties. Onion contains antioxidants and anti-inflammatory compounds, decrease triglycerides and reduce cholesterol levels. The present study evaluated the protective effects of methanol extract of *Allium cepa* (MEAC) against Adriamycin-induced liver injury. Twenty-five (25) albino rats were randomly grouped into 5 groups A – E and received the following treatments daily for five days. Rats in group A (negative control) received intraperitoneal injection of Adriamycin (15mg/kg body weight) alone. Group B received Adriamycin (15mg/kg, i.p) and low dose of MEAC (200mg/kg, oral); group C received Adriamycin (15mg/kg, i.p) and high dose of MEAC (400mg/kg, oral);

group D received Adriamycin (15mg/kg, i.p) and vitamin C (200mg/kg, oral); Group E (Normal control) no treatment was administered to this group. Hepatotoxicity was assessed by measuring serum bilirubin, ALT, AST and ALP enzyme activities. Administration of the methanol extract of *Allium cepa* resulted in marked elevation of the biochemical parameters; ALT (29.45 ± 1.72 IU/L), AST (44.73 ± 3.41 IU/L), ALP (301.64 ± 25.37 IU/L), and total bilirubin (1.31 ± 0.25 mmol/L). Oral administration of high dose of MEAC showed significantly attenuated the serum concentration of ALT (22.47 ± 2.71 IU/L), AST (26.42 ± 2.09 IU/L), ALP (249.97 ± 26.01 IU/L) and bilirubin (0.99 ± 0.27 mmol/L), respectively. The phytochemical constituent in MEAC worked to maintain the structural integrity of the plasma membrane of the liver cells to protect it against breakage by the reactive metabolites formed from exposure to Adriamycin. Histopathological studies also supported the biochemical observations as liver sections of Adriamycin induced in the rats, deleterious alterations in the liver histoarchitecture. However, administration of high dose methanol extract of *Allium cepa* prevented liver damage induced by the Adriamycin. This study reveals that methanolic extract of *Allium cepa* (MEAC) at high doses, possesses strong antioxidant and liver protective ability.

Keywords: Allium Cepa, Adriamycin, Antioxidant, Hepatoprotective, Hepatotoxicity

Introduction

Liver is the largest visceral organ in the body, and it supports almost all other organs to a reasonable capacity. The liver performs numerous functions necessary for the body upkeep by maintaining the internal body milieu and also liver disease

conditions, performed by the liver macrophages [1]. Other functions of the liver includes: blood filtration, secretion of bile, protein synthesis (especially, vital proteins for blood clotting), lipolysis, storage of vitamins and minerals, enzyme production, generation of energy and detoxification of harmful chemicals or toxins. With respect to detoxification, the liver plays a pivotal role in the metabolism of drugs and other foreign substances that find their way into the body. This it does mainly through the cytochrome P450 group of isoenzymes; therefore the liver is mostly affected by toxic levels of drugs in the body which results in liver damage [2]. Inability of the liver to perform these functions, in liver disease conditions, can be investigated via series of tests generally known as the Liver Function Test (LFT).

Liver Function Tests help establish the health and function of the liver by estimating the levels of several biomedical markers. These biomarkers are: Alanine Transaminase (ALT), Aspartate aminotransferase (AST), Alkaline Phosphatase (ALP) and Bilirubin. Increased plasma level of any of these parameters is diagnostic of liver injury; for example, ALT and ALP both measure the enzymes released by the liver in response to damage or disease while ALP evaluates the bile duct system of the liver. Major symptoms of acute liver toxicity includes; weakness and fatigue, rapid onset of jaundice, abdominal pain, nausea and vomiting [3]. Although metabolic processes in the liver protects other organs and tissues in the body from harmful chemicals or toxins, however, the liver has been reported to be affected by several hepatotoxic drugs, one of which is doxorubicin, also known as adriamycin.

Adriamycin is a chemical substance with cycle-dependant and phase-specific mechanism of action consisting of the creation of irregular bonds between nucleobases of DNA and inhibition of key enzymes of DNA topoisomerase I and II as well as the formation of free radicals which damage DNA. Thanks to these highly selective properties, Adriamycin is widely used in oncology and hematology [4]. However, its anticancer mechanism of action is not fully understood. Certainly, inhibition of protein synthesis and DNA and RNA synthesis is responsible for most of the cytotoxic effects of Adriamycin.

One increasing limitation in the use of therapies involving Adriamycin is its hepatotoxicity, defined as the liver damage associated with dysfunction of this organ, caused by exposure to a drug or other non-infectious agent [5]. The liver plays an important role in numerous body functions – metabolism, detoxification, storing and elimination (neutralization of most drugs). The aetiology of the liver damage in Adriamycin-based chemotherapy is varied and has been linked to the adverse effects of cytostatic agents, viral infection, hepatitis sepsis, infiltration by tumour or a concomitant primary liver disease [6]. Other factors affecting the liver function include a reaction to antibiotics, analgesics, antiemetics, and other drugs, as well as concomitant diseases, immunosuppression, nutritional deficits or total parenteral nutrition [7]. The use of dietary extracts with antioxidant properties have been shown to reverse these effects (oxidative stress and others) caused by this toxic chemical. Many herbal plants play very important roles in the process of tissue repairs. Plant secondary compounds may exhibit more potent tissue repairs because they promote the repair mechanisms in a natural manner and are safer [8].

Allium cepa Linn is a member of the Liliaceae, which consists of over 250 genera and 3700 species. Because of their evergreen bulbs, tubers and rhizomes, these plants are able to survive under harsh conditions of wet or dry seasons. Different parts of the plant have been proven to possess antidiabetic, antioxidant, antihypertensive, antithrombotic, hypoglycemic and antihyperlipidemic effects [8]. The bulb contains Kampferol, β -sitosterol, ferulic acid, myricic acid, prostaglandins and several compounds of secondary metabolism [9]. Onion powder consumption has been reported to prevent non-alcoholic fatty liver disease (NAFLD) [10]. Furthermore, in traditional medicine in Eastern Nigeria, the bulb extract had reportedly been used to alleviate and improve side effects from liver problems.

Previous studies on the benefits and use of onion juice extracts in treatment of adriamycin-induced hepatotoxicity are very scarce, vague and unclear. This work is therefore required to provide a firm background on the use of onion juice extracts in treatment of adriamycin-induced liver damage or hepatotoxicity caused by any drug or chemical with similar mechanism of hepatotoxicity as Adriamycin. The aim of this study is to evaluate the hepatoprotective effects of *Allium cepa* (onions) juice extracts on adriamycin-induced liver damage in rats.

Materials and Methods

Plant Materials

The onion (*Allium cepa*) used for the study was obtained from Ogbete market, a local market in Enugu, Enugu state, Nigeria. The plant material was authenticated by a consultant taxonomist at the herbarium section of the Department of Plant Science and Biotechnology, University of Nigeria, Nsukka.

Chemicals Reagents and Drug

Adriamycin (Doxorubicin hydrochloride) from Calbiochem, UK; vitamin C (Alpha Pharmaceuticals, Enugu, Nigeria) to serve as an antioxidant. Reagent(s): AST, ALT, ALP and bilirubin laboratory kits (from Random laboratories Ltd, UK). Absolute methanol was of analytical grade and was purchased from Ogbete market.

Animals

Twenty-five (25) adult albino rats, weighing (100 - 160), were obtained from the animal house of the College of Veterinary Medicine, University of Nigeria. The animals were housed in metallic under standard conditions of temperature ($22 \pm 3^\circ\text{C}$) and a 12 h light, 12 h dark cycle they were adequately fed with chikun (Olam Animal feed mill ltd, Kaduna) The animals were kept under observation for about 14 days before the onset of the experiment for acclimatization. Experimental protocol and handling was according to Institutional guidelines describing the use of rats and in accordance with the American Physiological Society guiding principles for research involving animals and human beings [11].

Preparation of Plant Extracts

Fresh healthy 400g of onions (*A. cepa*) were washed, cut into small pieces and homogenized in a warring blender (Qasa blender, made in Nigeria). The resulting mixture was soaked in 2 L of 80% methanol. The mixture was allowed to stand for 24 h with intermittent shaking. Following filtration, the filtrate obtained was concentrated to dryness at

40°C using a rotary evaporator under reduced pressure. The dried Methanol extracts of *Allium cepa* (MEAC) were weighed and then stored in a refrigerator at 4°C.

Experimental Design

The twenty five (25) albino rats were grouped into grouped into (A-E) of five rats each and the received the following treatments which lasted for seven days:

Group A: (Negative Control): received intraperitoneal injection of Adriamycin (15 mg/kg body weight) daily for 5 days.

Group B: received Adriamycin (15 mg/kg, i.p) and low dose of MEAC (200mg/kg, oral) daily for 5 days.

Group C: received Adriamycin (15 mg/kg, i.p) and high dose of MEAC (400mg/kg, oral) daily for 5 days.

Group D: received Adriamycin (15 mg/kg, i.p) and vitamin C (200mg/kg, oral) daily for 5 days.

Group E: (Normal Control): No treatment was administered to this group.

Sacrificing of Animals and Sample Collection

Blood samples for the determination of serum analyses of bilirubin, AST, ALT, and ALP were taken by cardiac puncture of the left ventricle of the heart under chloroform anesthesia and the Liver was/were harvested for histopathological analyses.

Biochemical Analysis

The serum obtained was used for the analysis of bilirubin concentration (total and conjugated), and the liver enzyme markers (aspartate aminotransferase (AST, alanine aminotransferase (ALT), alkaline phosphatase (ALP) were analyzed using Rx Monza Analyzer and standard laboratory kit from Random laboratories Ltd.

Measurement of AST

AST was determined using colorimetric method as described by Reitman and Frankel [12].

Measurement of Bilirubin:

Bilirubin was determined using colorimetric method as described by Jendrassik and Groff [13].

Measurement of ALT:

ALT was determined using colorimetric method as described by Reitman and Frankel [12].

Measurement of ALP

ALP was determined using colorimetric method as described by Kind and King [13].

Histopathological analysis

The excised (Liver) was processed using the paraffin wax embedding technique, sectioned at 5 microns and stained using the Haematoxylin and Eosin [H and E] staining procedure [14]. (Baker *et al.*, 1998). The histological sections were examined using an Olympus TM light microscope.

Statistical analysis

Data analysis was done using GraphPad prism version 7.0 (GraphPad, San Diego, CA, USA). The results of the biochemical assays were reported as mean±SEM (standard error of mean). The level of significance was tested using

one way analysis of variance (ANOVA), followed by the Tukey post hoc analysis. Probability levels less than 0.05 ($p < 0.05$) was considered significant.

Results

Effects of treatments against adriamycin on body weight of Wister rats.

The effects of treatments against adriamycin (ADM) on body weight of Wister rats represented in fig. 4.1. It was observed that rats treated with only Adriamycin for 5 days had a significant weight loss; as the rats in the group loss appetite, were sluggish and responded slowly to stimulus. However, the reverse is seen in the normal control group rat, which had a significant weight gain over the 5 days period. Interestingly, the rats in the groups coadministered with adriamycin and low dose MEAC, High MEAC or vitamin C, separately, prevented weight loss in the rats.

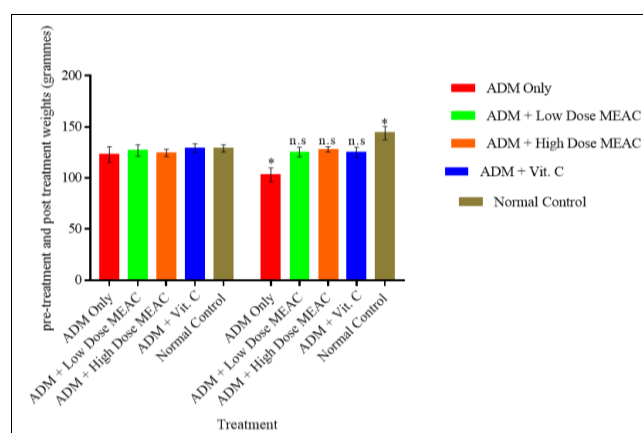


Fig 4.1: Effects of treatments against adriamycin on body weight of Wister rats

Histogram show the body weight of rats in the experimental groups. The preliminary data show intraperitoneal adriamycin for five days induced a significant weight loss when the post-weight is compared with pre-weight. However, oral administration of treatment with low dose MEAC, high dose MEAC or vitamin C separately ameliorated the abnormal weight loss when the post-weight is compared with pre-weight, separately for the individual treatment. The data are presented as mean ± SEM of body weight (gramme) for individual treatment. See Materials and Methods for experimental details. Statistical analyses were performed using ANOVA (* $p < 0.05$); n.s implies no significant difference ($p > 0.05$).

Biochemical Results

The functionality of the liver was established by estimating the serum level of the liver biochemical markers; Alanine transaminase (ALT), Aspartate transaminase (AST), Alkaline phosphatase (ALP) and Total bilirubin (TB) (Table 1). From the results, the treatment with vitamin C or high dose of MEAC showed significant antihepatotoxic effects ($P < 0.05$) in comparison with negative control (adriamycin only), while the low dose of MEAC offered a nonsignificant hepatoprotection against adriamycin. Furthermore, 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.

Table 1: Statistical comparison of Liver biochemical concentrations 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	1.31 ± 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 (Adriamycin only) is compared with all other groups.

Histopathological Results

The lobules of the liver of normal control rats (Group E) appeared structurally and functionally normal. The hepatocytes showed a well conserved morphology; 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 of low 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. Furthermore, photomicrograph of liver section from ADM + Vitamin C (group D), showed normal hepatocyte, there was mild periportal cellular infiltration and the sinusoids appeared dilated.

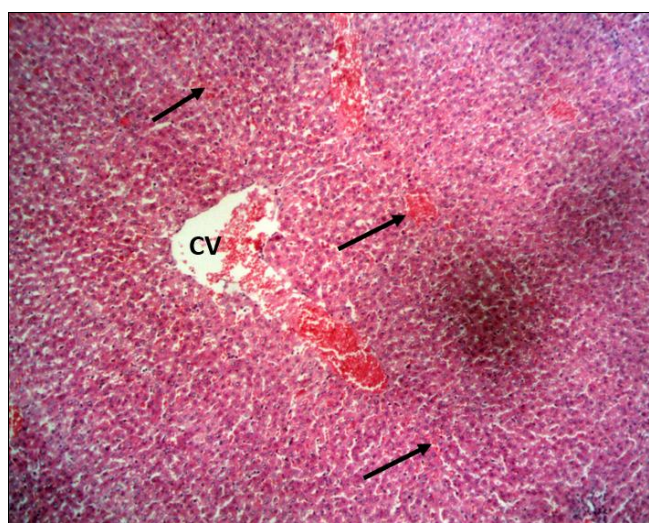


Fig 1: Photomicrograph of liver section from adriamycin treatment only (negative control group) (A). Features: The hepatocytes are normal; the central veins (CV) appear dilated and congested. There is also sinusoidal congestion (arrows). Stain: Haematoxylin and eosin. Magnification: X100.

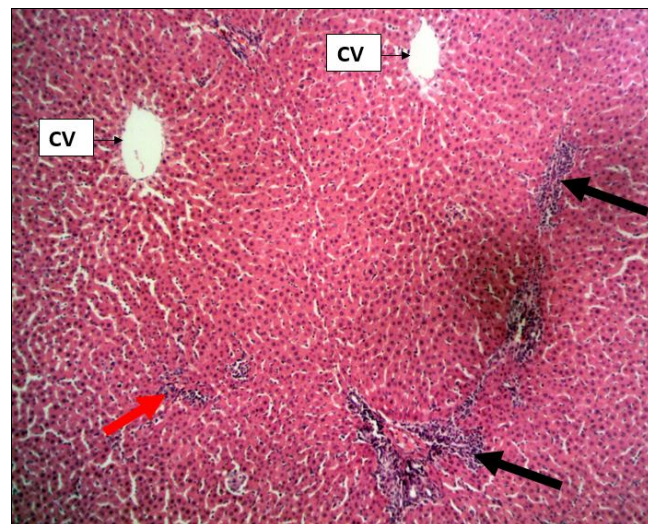


Fig 2: Photomicrograph of liver section from low dose MEAC treatment (group B). Features: There is mild periportal inflammation (black arrow) and areas of sinusoidal cellular infiltration (red arrow). Stain: Haematoxylin and eosin. Magnification: X100.

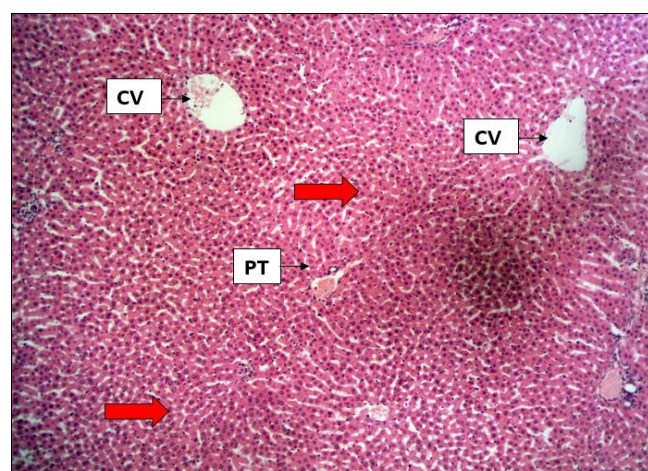


Fig 3: Photomicrograph of liver section from high dose MEAC treatment (group C). Features: Section has normal hepatocytes (arrow), central veins (CV) and congested portal venules in the portal triads (PT). Stain: Haematoxylin and eosin. Magnification: X100.

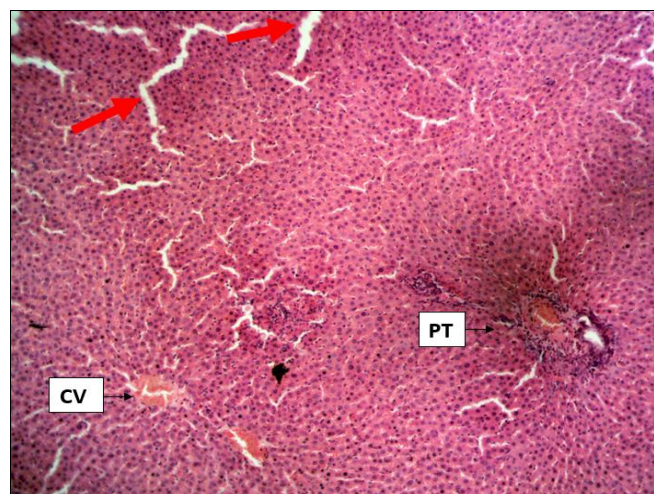


Fig 4: Photomicrograph of liver section from vitamin C treatment (group D). Features: Hepatocytes are normal, there is mild periportal cellular infiltration (PT) and the sinusoids appear dilated (arrows). Stain: Haematoxylin and eosin. Magnification: X100

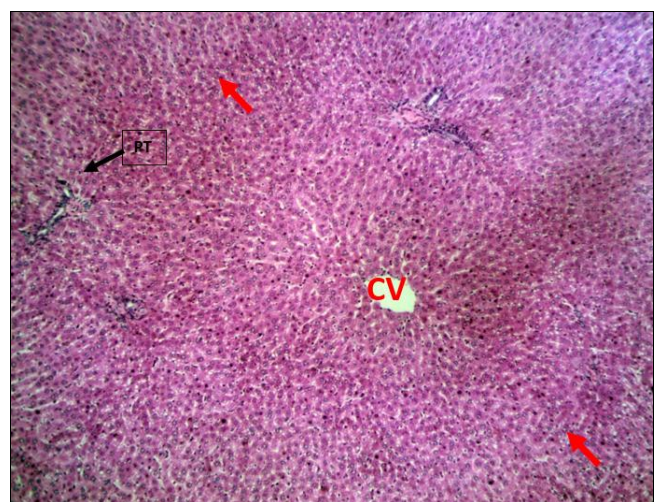


Fig 5: Photomicrograph of liver section from normal control rats (group E). Features: Liver section appears normal. Hepatocytes (red arrow); central vein (CV) and portal triad (PT) all appear normal. [Stain: H and E; $\times 100$]

Discussion

The liver is a large and vital organ whose main job, apart from filtering of blood from the digestive tract, is to also detoxify chemicals and drugs [16]. Doxorubicin is a very good example of such chemical being detoxified by the liver; as long term use is very harmful to the liver, causing hepatotoxicity, and other organs such as the kidney [17].

Doxorubicin is a widely used anticancer drug in the treatment of solid tumours and leukemia. However, valuable anticancer therapy with doxorubicin and other anthracyclines is severely limited by severe toxicities [18]. Doxorubicin toxicity is attributed to its prooxidant action. Free radical formation was suggested to be involved in Doxorubicin-induced hepatotoxicity [19]. Treatment with doxorubicin has been shown to cause hepatotoxicity in various animal species [20].

The mechanism of Adriamycin induced liver damage is that it involves the drug's ability to intercalate within DNA base pairs, causing breakage of DNA strands and inhibition of both DNA and RNA synthesis. Doxorubicin inhibits the enzyme topoisomerase II, causing DNA damage and induction of apoptosis. When combined with iron,

doxorubicin also causes free radical-mediated oxidative damage to DNA, further limiting the binding of doxorubicin with iron [21].

Nutrition and the liver interrelate in many ways and the possible effect of the slowing down liver damage has been considered. Several studies have emphasized on the importance of dietary composition in the treatment of liver injuries. The aim of this study was to investigate the hepatotoxicity protective effects of methanol extract of *Allium cepa* on Adriamycin induced liver damage in rats. The phytochemical components of the methanol extract of *Allium cepa* were analyzed and shown to include: Saponins, flavonoids, phenols, tannins, triterpenoids, steroids, cardiac glycosides, alkaloids, carbohydrates, proteins, oils and sugars. *Allium cepa*, a traditional medicinal plant, has been reported to possess antioxidant property and it is also widely used as food.

Medicinal plants are sources of important drugs for the treatment of diseases either alone or in combination with other plants [22]. Chemical compounds found in plants include alkaloids, glycosides, essential oils, saponins(+), tannins (+), steroids () resins, flavonoids (+), proteins, and others. These chemical compounds are potent bioactive compounds found in parts of medicinal plants which are useful for therapeutic purposes [23].

Phytochemicals are plant chemicals that have both harmful and therapeutic potentials. Studies have demonstrated that, in as much as plants produce these chemicals to protect themselves, they can also protect humans and other living things from diseases.

From biochemical results of this present study, negative control rats (Group A) that received Adriamycin alone when compared with the normal 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 confirmation of previous report of the hepatotoxicity of adriamycin [24]. 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 methanol extract of *Allium cepa* (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 agrees with the report of Baxla *et al.* [25], who reported the hepatoprotective effect of onion against lead-induced liver toxicity in Wistar rats [25]. This also agrees with previous report that onion has antioxidant and hepatoprotective properties [26]. The biochemical result is further backed by the histopathology by showing minor hepatocellular necrosis. The rats in group C administered with adriamycin and high dose methanol extract of *Allium cepa* prevented weight loss in rats. The functionality of the liver was established by estimating the serum level of the liver biochemical markers; Alanine transaminase (ALT), Aspartate transaminase (AST), Alkaline phosphatase (ALP) and total bilirubin (TB). From the results, the treatment with vitamin C or high dose of methanol extract of *Allium cepa* significant antihepatotoxic effects ($P < 0.05$) in comparison with the negative control (adriamycin only), while the low dose of methanol extract of *Allium cepa* offered a non-significant

hepatoprotective against adriamycin. Furthermore, 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 extracts. The hepatoprotective action of onion may be due to its antioxidant ability. The antioxidant action of onion may be attribute to the high contents of alkaloids (+) and flavonoids (+) as observed in the phytochemical result. The protective effects of flavonoids in biological system are ascribed to their ability to transfer free radical electrons, chelate metal catalyst, activate antioxidant enzyme, reduce alpha tocopherol radicals [27], and inhibit oxidases. Also, alkaloids have been known to possess antioxidant activity by their ability to quench superoxide anions and singlet oxygen [28]. In histological examination, as shown by the photomicrograph of all the groups it was observed that the lobules of the liver of normal control rats (Group E) appeared structurally and functionally normal. The hepatocytes showed a well conserved morphology; the central vein and portal traid 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 of low 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 methanol extract of *Allium cepa* (Group C), showed normal hepatocytes, normal veins and congested portal venule in the portal traid. Furthermore, photomicrograph of liver section from ADM + Vitamin C (Group D), showed normal hepatocyte; there was mild periportal cellular infiltration and sinusoids appeared dilated. The histological result is more effective to the liver damage caused by the Adriamycin induction than the low dose methanol extract of *Allium cepa*.

Conclusion

This study proves that the oral administration of adriamycin can cause severe liver damage; and however, *Allium cepa* extracts can protect the liver against Adriamycin induced liver damage.

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