

Hepatoprotective Potential and Histological Studies of Effects of *Celosia Argentea* L. on Paracetamol-Induced Liver Damage

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Abstract: *Celosia argentea* L. is a common vegetable known to possess anti-oxidative and other therapeutic properties. This study evaluates the hepatoprotective activities and histological effects of aqueous extract of *Celosia argentea* L. on acetaminophen-induced liver damage in rats, compared to the effects of a standard drug –silymarin. Twenty-five male rats were used in this study. These were divided into five groups of five animals each. Animals in group 1 were given 1ml/kg body weight (b.w) distilled water (control [C]), group 2 were given 100mg/kg b.w silymarin for 4 days plus acetaminophen for 3 days [SL], groups 3 and 4 were given 250 and 500mg/kg b.w aqueous extract of *C. argentea* for 4 days plus acetaminophen for 3 days (CA1 and CA2, respectively) and group 5 were given 1 ml/kg b.w. distilled water for 4 days and 1g/kg b.w acetaminophen (PCM) for 3 days. Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and total bilirubin activities were assessed on day 8, values of mean and standard error were compared at significance level of $p < 0.05$. Overall, mean ALT, AST and ALP levels in CA2 (21.8 ± 1.4 , 84.2 ± 8.2 and 175.9 ± 36.9 U/L, respectively) was lower than PCM group and similar to SL group (37.6 ± 3.9 , 97.2 ± 5.2 and 151.1 ± 21.91 , respectively, $p > 0.05$). Mean values in control group were similar to CA2 but significantly lower than PCM and CA1. Total bilirubin was higher but not significantly different compared to C group, suggesting a lack of effect on total bilirubin. *C. argentea*

ameliorates and protects against acetaminophen-induced liver damage in rats, with a comparable effect with silymarin at a dose of 500mg/kg b.w. A regular consumption of the vegetable can play a role in sustaining health and can be used in place of long term therapy in individuals with compromised liver or actively exposed to chemotherapeutic drugs with adverse effects on liver.

Keywords: *Celosia argentea*, liver damage, silymarin, hepatoprotective

Introduction

Liver diseases which include liver cirrhosis, fibrosis, liver cancer, hepatitis etc. are common causes of death worldwide [1] and have been linked to a number of factors which include excess alcohol intake, metabolic syndromes, hepatitis B and C infection, free radicals, overdose of non-steroidal anti-inflammatory drugs, chemicals such as carbon tetrachloride (CCl₄), halothane etc. Liver injury caused by chemicals and drugs is a major toxicological issue due to limited therapeutics for this condition without side effects. Medicinal plants have been acknowledged as a rich source of bioactives for prevention and treatment of ailments [2]. They serve as alternative for medicines with little side effects and are good sources for development of new drugs for safe treatment of diseases.

Treatment of diseases with high doses of some drugs e.g. antibiotics, acetaminophen, methotrexate, is often associated with toxicity causing hepatic damage [3]. As a result of this shortcoming, research is directed towards discovery and use of active chemicals from medicinal plants which are commonly consumed foods that produce protective and therapeutic effects, demonstrating comparable outcomes with standard drugs and showing minimal side effects [4]. Studies are required to screen commonly consumed indigenous

vegetables for their antioxidative and protective properties to serve as natural remedies for many ailing individuals. Medicinal plants have made significant contributions to the prevention and treatment of hepatotoxicity [5] one of which is *Celosia argentea*.

Celosia argentea L. is a vigorous, broad leaf, edible annual vegetable belonging to the family Amaranthaceae. It is popularly called silver cock's comb in English, *shoko* in Yoruba, Lagos spinach in Lagos, Nigeria. It is grown as edible vegetable and also for medicinal uses in Africa, Southeast Asia and other regions of the world [6]. Many bioactive compounds such as flavonoids, carotenoids, polyphenols and vitamins have been identified in *C. argentea*, which confer many biological properties due to their free radical scavenging activities [7]. Hepatoprotective properties have been reported from other regions using ethanolic extracts on CCl₄ or paracetamol-induced liver damage [7-9]. Root, stem and leaves of *C. argentea* have also been reportedly used for rapid healing of wounds, immune-stimulating, curing of kidney stones, antipyretic, antioxidant, anticancer, diuretic and antibacterial and anti-hepatotoxic effects, [10].

The aim of this study is to investigate the hepatoprotective effects of *C. argentea* L. in its edible form (aqueous extract) on paracetamol-induced liver damage in rats and compare these

effects with a standard drug – silymarin.

Materials and Methods

Plant materials: *Celosia argentea* fresh leaves were acquired from the local market at Ota in Ogun State. Plant identification was carried out by a botanist in the Department of Biological Sciences, Covenant University. The fresh leaves were washed to remove dust particles and air dried for three weeks after which they were blended into fine powder using a blender. Forty grams of the powdered leaves was put in a 500ml conical flask and 320ml of distilled water was added, and the mixture was left for 24hours and was filtered using a whatman No. 1 filter paper to obtain the filtrate. The mash obtained was again reconstituted in 320ml of distilled water and the maceration was repeated. The combined filtrate was then evaporated under reduced pressure at 80°C in a rotary evaporator and the crude extract was obtained with a yield of 5g.

Experimental animals: Male albino rats with a mean weight of 140g were obtained from a commercial animal house and kept in clean cages and placed in a well- ventilated room at the animal house of the Department of Biological Sciences, Covenant University, Ogun State, Nigeria at optimum temperature and relative humidity. They were acclimatized to the laboratory condition for one week and were given food and water *ad libitum*. All animals were treated in accordance with the recommendations of National Institute of Health (NIH) guidelines for the care and use of laboratory animals [11].

Study groups: The rats (n = 25) were divided into 5 groups of five animals

in each group. The animals in group 1(C) served as normal control group and were given only vehicle (distilled water, 1ml/kg b.w.) for 7 days, animals in group 2(SL) served as positive control and were given Silymarin (100mg/kg b.w.) for 4 days and PCM for 3 days, group 3(CA1) and 4(CA2) received 250 and 500mg/kg b.w aqueous extract of *C. argentea* respectively for 4 days plus paracetamol on days 5 – 7. Group 5 (PCM) served as the negative control and were administered with only vehicle (1ml/kg b.w.) for 4 days plus paracetamol (1g/kg b.w.) on days 5 – 7.

Blood collection and preparation of tissue sample:

Animals were anaesthetized 24hours after the last treatment on the 7th day with diethyl ether prior to dissection and blood samples were collected through cardiac puncture into lithium heparinized bottles for aspartate aminotransferase (AST), alkaline phosphatase (ALP), alanine aminotransferase (ALT) and total bilirubin (TB) assays. Serum was obtained by centrifuging the blood at 4000 rpm for 15 minutes into 2ml tubes and stored at -20°C until required for the assays. The liver was excised and washed in normal saline (0.9% NaCl) and a portion fixed in 10% formaldehyde for histopathological examination.

Analysis of biochemical parameters:

Commercial test kits for AST, ALT, ALP and total bilirubin (Randox® Laboratories, United Kingdom) were purchased and used for liver function tests following the protocol from the supplier's specifications from the standard kits.

Histological analysis: A section of the liver was fixed in 10 % formalin immediately after sacrifice. The fixed liver sections were embedded in paraffin, 5-6µm thick liver section was stained in hematoxylin-eosin; this was examined under compound microscope for determination of histopathological changes

Statistical analysis

Data is presented as mean \pm standard error of mean (SEM) for continuous data and was compared using one way ANOVA followed by Least square difference (LSD) post hoc test, all test of significance was taken at $p < 0.05$.

Results

Twenty five healthy animals with mean weight of 140g on day 0 were used for the experiment. There was no significant difference in the weight of the animals after treatment on day 7 ($p > 0.05$). There was also no change in the physical appearance of the animals.

Effects of treatment on liver function tests

The mean values of 2 doses of *Celosia argentea* on serum alkaline phosphatase, aspartate aminotransferase, alanine aminotransferase and total bilirubin in the experimental animals are shown in figures 1 - 4. There was a significantly higher serum AST level in group CA1 105.18 ± 7.26 U/L, this was similar to group PCM with mean value of 113.11 ± 6.20 compared to control group with a mean value of 84.58 ± 11.22 ($p < 0.05$). The corresponding mean and SEM values in group CA2 (84.21 ± 8.25) was similar to SL (97.24 ± 5.23) and control. Figure 1 shows the serum AST levels in all groups after treatment.

There was also a significantly higher serum ALP level in group PCM with a

mean value of 385.02 ± 38.62 compared to the control group with a mean value of 128.34 ± 20.7 U/L, indicating liver damage. Animals in group SL, CA1 and CA2 had significantly lower mean values of 151.11 ± 29.92 , 226.32 ± 33.67 and 175.95 ± 36.94 U/L respectively. Mean values of groups SL and CA2 were similar ($p > 0.05$). Figure 2 shows the ALP levels in all groups after treatment.

Similarly, there was a significantly higher serum ALT level in group PCM with a mean value of 55.40 ± 3.48 U/L. The corresponding values in Groups SL, CA1 and CA2 are 37.60 ± 3.93 , 30.00 ± 5.69 and 21.80 ± 1.49 U/L respectively. The result is shown in figure 3. There was no significant change in the total bilirubin of the animals in groups CA1 and CA2 suggesting that the plant has no significant effect on total bilirubin (figure 4).

Histological analysis

The liver of control rats (figure 5) showed hepatocytes arranged in plates, a fibrocollagenous connective tissue stroma without necrosis or any area of infiltration by lymphocytes. This is in contrast with the features observed in the animals in group PCM (figure 6) that shows infiltration of the portal tracts and interface by lymphocytes (Portal & Interface Hepatitis), presence of necrosis within which are cellular debris, extensive bile regurgitation i.e. cholestasis and bile ductular proliferation. However, the animals in groups CA1 (figure 7) showed infiltration of portal tracts with small areas of necrosis while animals in groups CA2 (figure 8) and SL (figure 9) showed similar features of infiltration of portal tracts without any areas of necrosis.

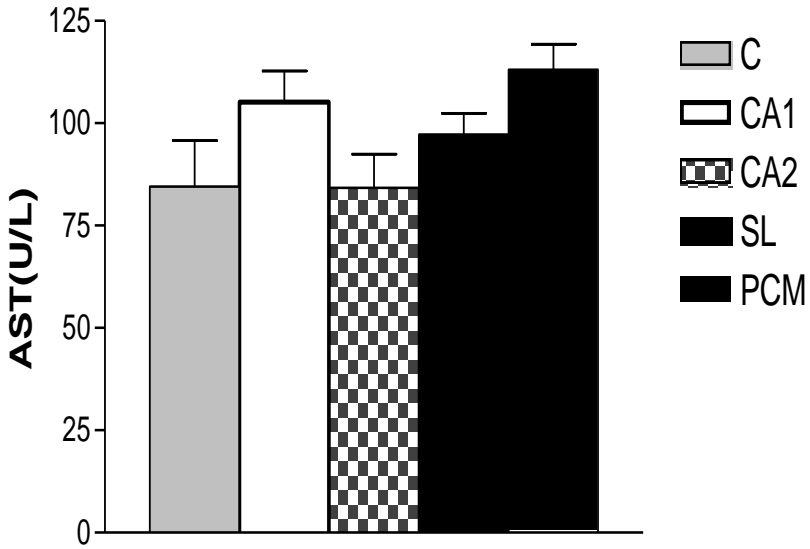


Figure 1: Histogram showing changes in AST mean values (with SEM) in the experimental animals after treatment

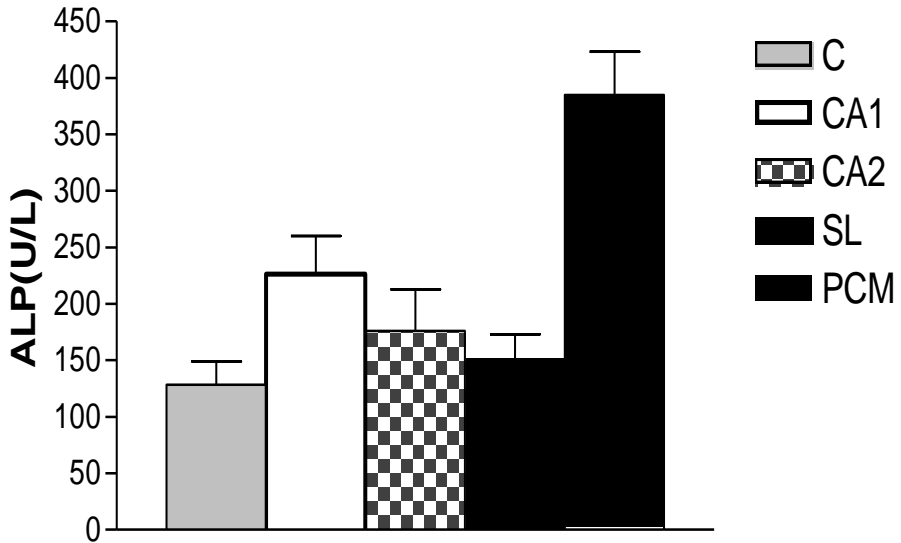


Figure 2: Histogram showing changes in ALP mean values (with SEM) in the experimental animals after treatment

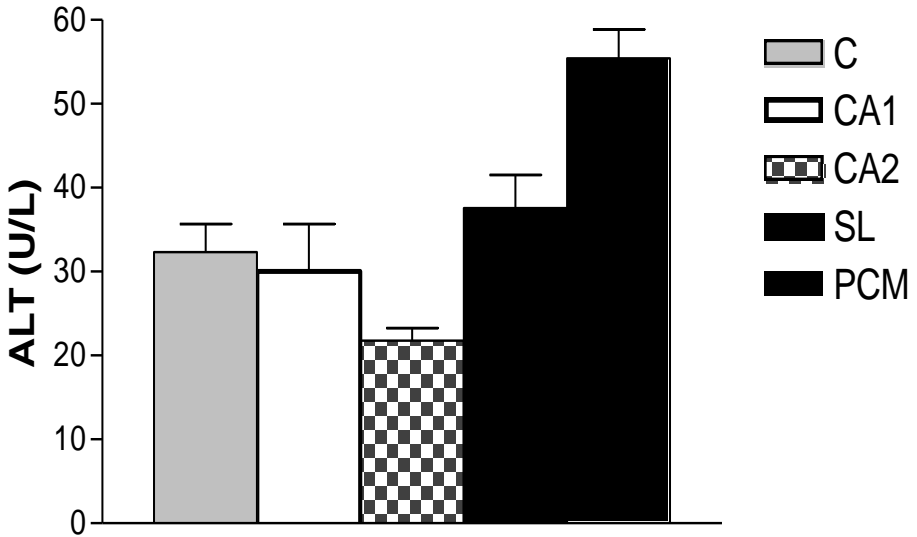


Figure 3: Histogram showing changes in ALT mean values (with SEM) in the experimental animals after treatment

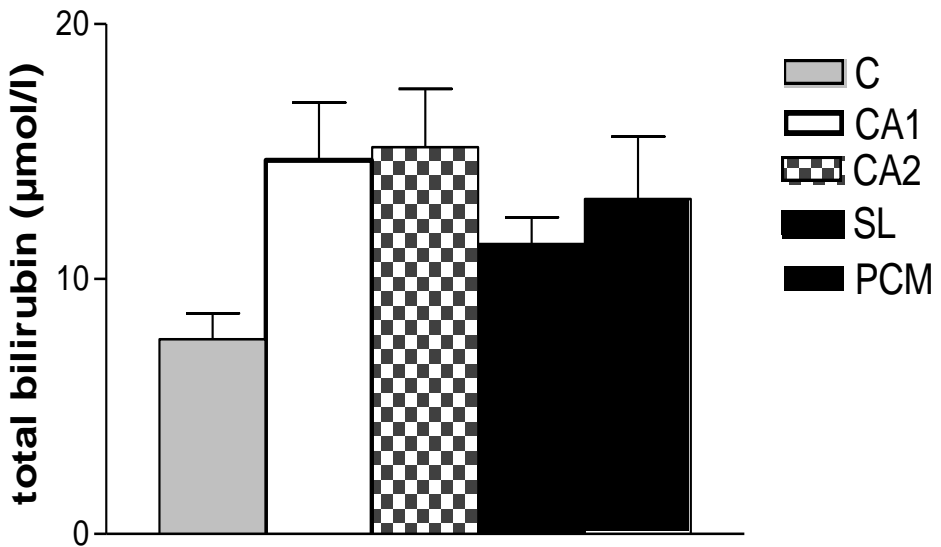


Figure 4: Histogram showing changes in total bilirubin mean values (with SEM) in the experimental animals after treatment.

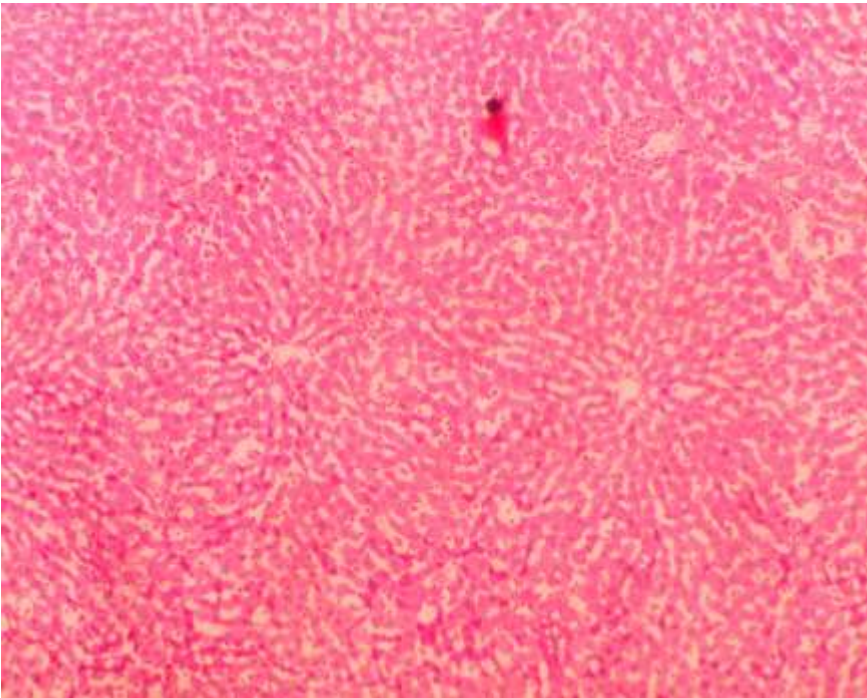


Figure 5: Control group showing hepatocytes (light arrow) arranged in plates within a fibrocollagenous connective tissue stroma (Haematoxylin & Eosin stain, x 40 magnification).

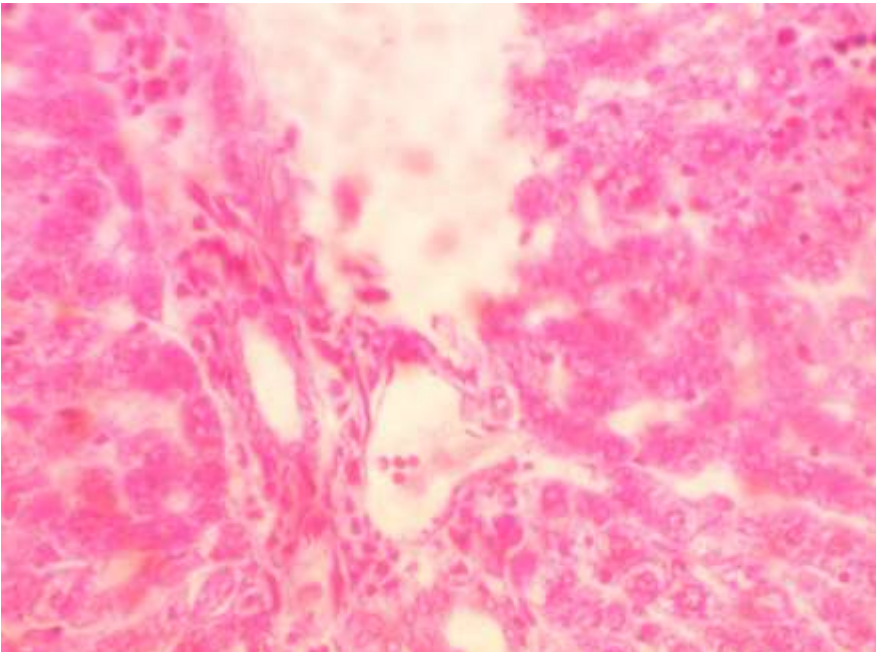


Figure 6: PCM group showing bile ductular proliferation (light arrows) within the portal tract (Haematoxylin & Eosin stain, x 100 magnification)

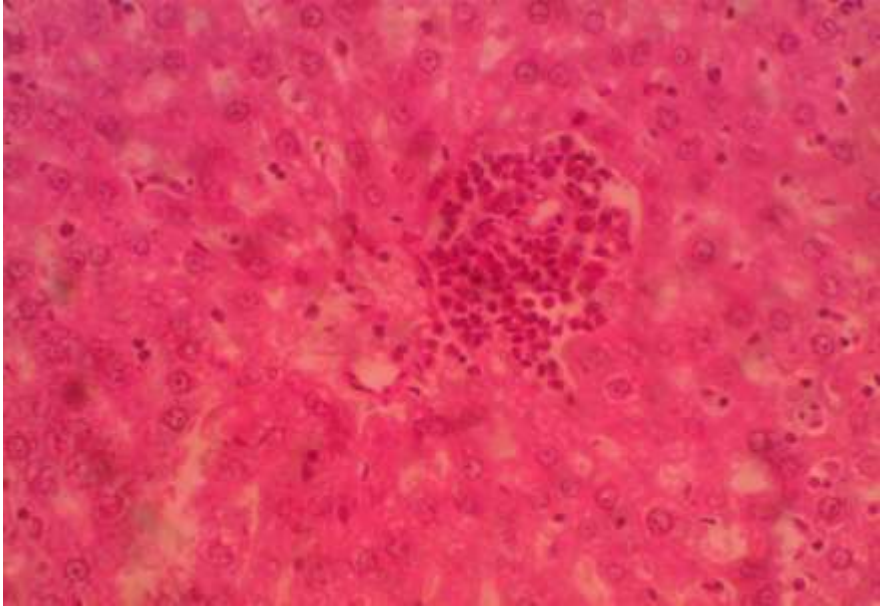


Figure 7: CA1 showing lymphocyte aggregation (light arrow) in the interface (Haematoxylin & Eosin stain, x 100 magnification).

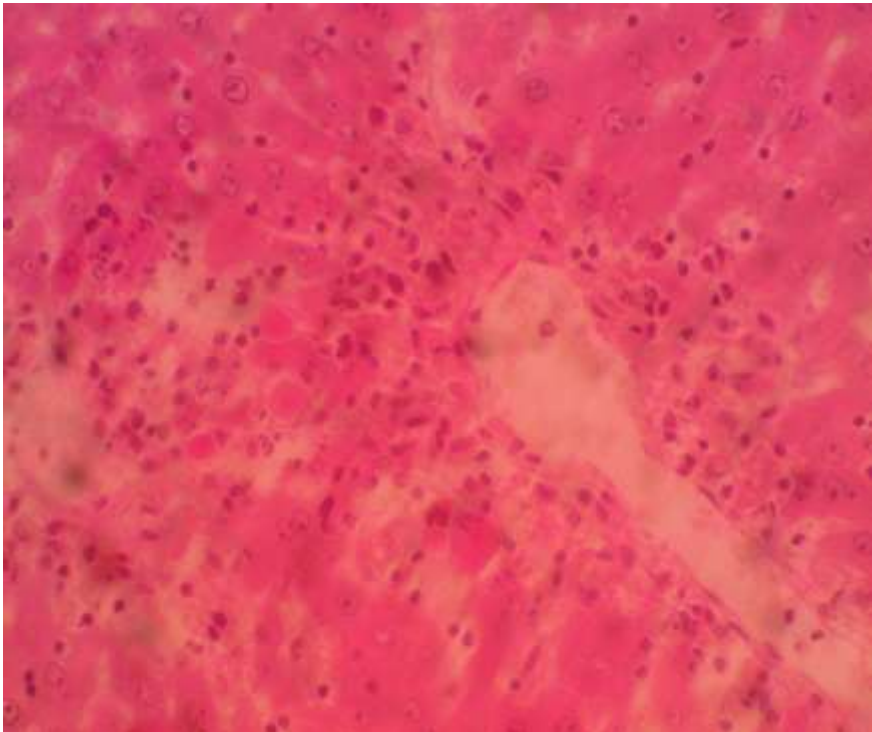


Figure 8: CA2 showing florid lymphocyte infiltration (light arrow) within the interface {interface hepatitis} (Haematoxylin & Eosin stain, x 100 magnification).

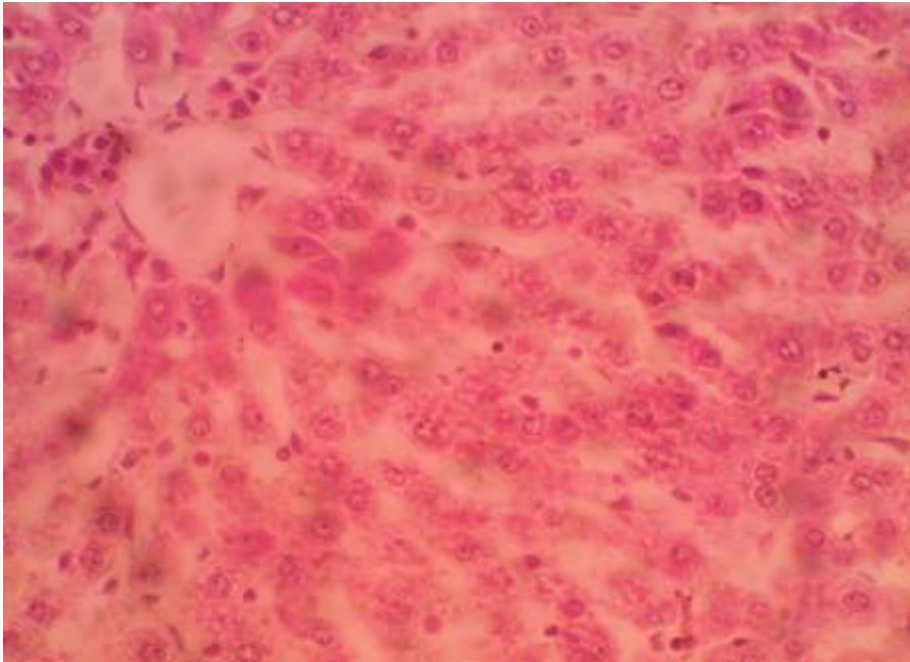


Figure 9: Silymarin group showing infiltration of lymphocytes (light arrow) within the interface {interface hepatitis} (Haematoxylin & Eosin stain, x 100 magnification).

Discussion

Medicinal plants have been acknowledged as a rich source of bioactives for prevention and treatment of ailments [2]. The ability of any medicinal plant to ameliorate harmful effects or restore physiologic state of hepatocytes after exposure to a hepatotoxic agent makes it a hepatoprotective plant. The results obtained from this study is in conformity with induced liver damage previous literatures with overdose of acetaminophen [12-13] as evidenced by the increased activities of serum ALT, ALP, AST and total bilirubin compared to animals that were not treated with the toxic doses of the drug. Acetaminophen-induced liver damage is marked by hepatocellular necrosis and leads to an increase in the

activities of serum ALT, AST, ALP and total bilirubin [14]. The increased level of serum ASP and ALT indicates loss of functional integrity of liver cell membrane and cell leakage and ALP is related to functioning of the hepatocytes and its increased level in the serum indicates obstructive jaundice and intra-hepatic cholestasis [15].

Liver is a vital organ of the body that plays important roles in metabolism of xenobiotics, endogenous compounds, and is involved in many biochemical processes thus receives the most toxicological assault resulting from oxidative stress. Necrosis of the liver cells is one of the most common effects of paracetamol toxicity [16]. The pathogenesis of liver damaged has been shown to be due to highly

reactive metabolite, N-acetyl-p-benzoquinone imine (NAPQ1) formed in excess as well as other oxidants e.g. nitric oxide which covalently binds to cysteine residues of proteins to form adducts, in hepatic centrilobular cells that develop into necrosis [17]. This occurs in the presence of oxidative stress.

Oxidative stress, a condition in which there is an imbalance between the concentrations of reactive oxygen species (ROS) and physiological antioxidants, resulting in oxidative damage to many biomolecules within the cell. It is well established as a major risk factor for the development of several diseases including atherosclerosis, liver diseases, cardiovascular disease, cancer, etc. The histology results of the liver in acetaminophen treated animals showed necrosis, infiltration of portal tracts by inflammatory cells, cholestasis. However animals pre-treated with 500mg/kg aqueous extract of *C. argentea* were protected against necrosis by acetaminophen. The biochemical assays also corroborate this finding showing reduced serum ALT, AST and ALP activities in CA2 group. The results are in agreement with previous studies which reported that ethanolic extracts of *Celosia argentea* seeds or plant exhibited similar hepatoprotective activity against carbon tetrachloride-induced and paracetamol-induced hepatotoxicity in rats [8-9].

References

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Silymarin, a flavonoid containing drug is known to protect against liver diseases [13, 18] at a dose range of 25 to 200mg/kg b.w. The hepatoprotective effect of *C. argentea* was demonstrable at a dose of 500mg/kg b.w and this was similar to effects observed in silymarin group, however there was very limited effect when administered at 250mg/kg b.w. Leaf extract of *C. argentea* contains phytochemicals such as alkaloids, saponins, flavonoids and tanins [9, 19]. These hepatoprotective potentials are indeed due to abundant phytochemicals in the plant but studies are needed to determine the actual or combination of phytochemicals responsible for the hepatoprotective activity observed.

This in turn can be increased in newly developed drugs to enhance their activities.

It can be concluded from our studies that *Celosia argentea* has hepatoprotective property in a dose-dependent manner and with comparable effect with silymarin, a standard drug for treating liver diseases. It is highly recommended that *Celosia argentea* should be included regularly in diets for prevention of liver diseases and toxicity of the liver because of the proven health benefits. It is also readily available as an indigenous vegetable that is relatively cheap and shows no toxic effects compared to treatment with standard drugs.

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