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Hepatoprotective potential of dichloromethan extract of *Cassia alata* L. (Caesalpiniaceae) leaves from Burkina Faso against carbon tetrachloride-induced hepatic damage in rats

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Abstract

Cassia alata L. is an herbal plant that has been used as hepatoprotective and anti-inflammatory agent in Burkina Faso medicine. The purpose of this study is to finalize a traditionally improved drug (MTA) that will serve in the treatment of the hepatic disorders. Rats were orally fed with CF-AECal (50 and 100 mg/kg bw) along with administration of CCl_4 (20% CCl_4/ olive oil; 0.5 ml/kg bw) for 6 weeks.The protection level of hepatic cells by CF-AECal is evaluated by blood rate of SGPT, SGOT, APL and TB and also tissues rate of MDA, GSH, SOD and CAT. This protection rate is observed on the tissues through histological sections.The results showed that the levels of SGPT (12.64 ± 0.25), SGOT (13.13 ± 0.88), ALP (20.39 ± 0.44), TB (12.21 ± 0.30), were significantly increased in CCl_4 treated rats when compared with the normal group, but CF-AECal (50 and 100 mg/kg) treated rats showed maximum reduction of SGPT (6.71 ± 0.58 and 6.42 ± 0.49), SGOT (11.25 ± 0.45 and 10.79 ± 0.51), ALP (18.50 ± 0.76 and 16.08 ± 1.49), total bilirubin (10.95 ± 0.62 and 10.36 ± 0.13). Also CF-AECal significantly reduced liver MDA, GSH, SOD and CAT content when compared with control. Histopathological examination of liver sections confirmed that, treatment with CF-AECal decreased the degree of hepatic damage induced by CCl_4. Conclusion: these results suggest that the extract of *C. alata* leaves at various doses protects the rats from CCl_4 effects due to its polyphenol and particularly its flavonoids.

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Introduction

The liver is the organ in the body weighing 1200-1500g. It is a key organ in regulating homeostasis within the body. It regulates several important functions including protein synthesis, storage and metabolism of fats and carbohydrates, detoxification of drugs and other toxins, metabolism of hormones and excretion of bilirubin. Liver diseases are associated with distortion of these metabolic functions (Wolf *et al.*, 1999; Ward *et al.*, 2005). Liver diseases such as jaundice, cirrhosis and fatty liver diseases are very common in large public health problem in the world (Guillouzo *et al.*, 1989). Jaundice and hepatitis are two major hepatic disorders that account for a high death rate (Pang *et al.*, 1992; Ross *et al.*, 1996). Hepatotoxicity is defined as an injury to the liver that is associated with impaired liver function caused by exposure to a drug or another non-infectious agent (Navarro and Senior, 2006). Carbon tetrachloride (CCl_4) is one of the most commonly used hepatotoxins in the experimental study of liver diseases. The hepatotoxic effect of CCl_4 is largely due to its active metabolite, trichloromethyl radical. The administration of CCl_4 in rats enhances hepatic protein oxidation and leads to an accumulation of CCl_4 oxidized proteins in the liver (Premitha *et al.*, 1999). The resulting hepatic injury was characterized by leakage of cellular enzymes into the blood stream and by centrilobular necrosis (Recknagel *et al.*, 1989; Muriel *et al.*, 2001). Many of the modern drugs mainly based on synthetic chemical compounds however have been found to have harmful side effects on human system. This has triggered off extensive research and development in the field of herbal medicine. In fact there is a growing demand for herbal medicine in most of the developed and developing countries of the world nowadays (Handa *et al.*, 1986). Extracts from the leaves of *Cassia alata* have shown several pharmacological properties such as antimicrobial and antifungal activities (Somchit *et al.*, 2003; Crockett *et al.*, 1992), antiseptic (Esimone *et al.*, 2008), anti-inflammatory and analgesic (Palanichamy *et al.*, 1990) and anti-hyperglycemic (Palanichamy *et al.*, 1988) properties. In Burkina Faso, some ethnobotanical studies carried out by Nacoulma/Ouedraogo (1996) on " medicinal plants and traditional medicine practices in Burkina Faso: cases of the Plateau Central Region" showed that the leaves of *Cassia alata* contain an anti-inflammatory and hepatoprotective effect. In order to consolidate the findings of this research, we have then tested these effects on animal models. The study of hepatoprotective effects of *Cassia alata* on wistar rats is a first experiment in Burkina Faso. The fact that this test on wistar rats is the first test in Burkina Faso makes this study original. *Cassia alata* is an exotic plant that has a great geographical distribution; however it undergoes the effects of abiotic factors which characterize its habitat. In our study, we had recommended the count back method. Thus, we split our extract with solvents of increasing polarity including hexane and dichloromethane. This makes it possible to obtain extracts which show a variable chemical composition, since these solvents do not dissolve the same secondary metabolites from plants. Our previous studies enabled us to know that the fractioning with dichloromethane presented the best activity. Since the purpose of these pharmacological tests is to work out a traditionally improved drug (MTA); we have chosen the most active extract (Extract from *Cassia alata* with dichloromethane).

Materials and methods

Preparation of the plant extract

The leaves of *Cassia alata* have been harvested in Ouagadougou in september 2013. The leaves were dried at room temperature and crushed into powder. It was identified by Dr Ouedraogo of University Ouaga1 Pr Joseph KI-ZERBO where a voucher specimen n°15965 was deposited. 1kg of the powder of *Cassia alata* was macerated and soaked at room temperature in alchol 80% for 48 hours. Preparation was filtered using a whatman paper, and concentrated in a rotary evaporator under reduced pressure and then lyophilized. The return was 13.75 %. This extract (10g) was dissolved in distilled water (75 ml). The aqueous extract obtained was introduced into a separating funnel. At this volume

the hexane is added (75 ml). The mixture is shaken vigorously. The agitation is marked by periods of lower pressure in the bulb. After decantation time (30 min), hexane fraction which was distinguished clearly from aqueous fraction, was collected. The same procedure was repeated three times with the aqueous fraction that remained. All fractions in the hexane were concentrated in the rotary evaporator to obtain the final fraction in the hexane (HF-AECal). Dichloromethane (75 ml) was added to the aqueous fraction. The same procedure described above was followed to obtain the final fraction of dichloromethane (CF-AECal).

Animals

Wistar rats weighing 180-250g were housed in conventional cages with free access to tap water and food *ad libitum*. These animals were submitted at 22 ± 2° C with a 12 hours light-dark cycle. All procedures involving laboratory animal use were in accordance with the guidelines of the Faculty of Science at the University of Yaoundé I.

Treatment of the animals

Thirty rats weighing between 180 and 230 g had been shared in five groups of six. They were treated, by oral route, with carbon tetrachloride (CCl_4 20%) at the dose 0.5 ml/kg bw. The rats were treated two days per week (monday and thursday) during six weeks. Among the groups of addicted animals, one only didn't receive a treatment (negative control). The groups of addicted animals remaining received four days of treatments per week (tuesday, wednesday, friday and saturday) of the CF-AECal 50 mg/kg and 100 mg/kg (test groups) then to the silymarin 200 mg/kg (positive groups). To these groups of animals was added another none addicted that received 1 ml of olive oil only (normal group). The treatment lasted six weeks (Hernandez-Munoz *et al.*, 2001). At the end of the experimentation, the animals were anesthetized, sacrificed by cervical decapitation and blood was taken for proteins and biochemical analyses (SGPT, SGOT, TB, ALP). By laparotomy, the liver was collected to assess the rate of the superoxyde dismutase (SOD), of the reduced glutathione (GSH), of malondialdehyde (MDA), and the catalase activity (CAT).

Assessment of liver function

After six weeks of drug treatment, the animals were dissected under ether anesthesia. Blood from each rat was taken from carotid artery at the neck and collected in previously labeled centrifuging tubes and allowed to clot for 30 min at room temperature. Serum was separated by centrifugation at 3000 rpm for 15 min. The separated serum were used for the estimation of some biochemical parameters such as SGPT (Cypress diagnostics kits code HBE07), SGOT (Cypress diagnostics kits code HBE06), Bilirubin (total and direct) (Fortress diagnostics kits code BXC0193) and ALP (Fortress diagnostics kits code BXC0184). Concentration of the biochemical constituents was calculated according to the manufacture instruction. Immediately after sacrifice, the liver was excised from each experimental mouse and washed in ice-cold saline solution (0.9% NaCl). A 10% w/v of the liver homogenate was prepared in Tris-HCl buffer (0.1M, pH.7.4). The homogenate was centrifuged at 3000 rpm x 25 min at 4°C. Supernatant was collected into sterilized tubes and stored at -20°C until analysis of reduced glutathione (Ellman, 1959), catalase (Sinha, 1972), superoxide dismutase (Misra et Fridovish, 1972) activity and lipid peroxidation (MDA) (Wilbur *etal.*, 1949).

Histopathological studies

Autopsy samples were taken from liver of sacrificed rats in both control and experimental groups and fixed in 10% formalin saline solution. Serial alcohol (50-100%), were used for dehydration of the tissue samples. Tissue specimens were cleared in xylene and embedded in paraffin. The paraffin blocks were sectioned at 5 µm thickness by sledge microtome Reichert-jung 2030. The obtained tissue sections were collected on the glass slides and stained by hematoxylin and eosin. These tissues are finally observed to the photo-microscopic Olympus CH-2 and are shot while adapting one camera Olympus 101 on the microscope. So will be appreciated the degree of necrosis, fatty changes, ballooning degeneration

and lymphocyte infiltration of the liver (Banchroft *etal*., 1996; Adewusi *et al*., 2010).

Statistical analysis

The experimental results were expressed as the mean ± SEM for six animals in each group. The biochemical parameters were analyzed statistically using one-way ANOVA followed by Newman-keuls post hoc test with the help of Graph Pad Prism 5.03 software. P value of < 0.05 was considered as statistically significant.

Results

Relative weights of the organs

The results of relative liver and kidney weight in CCl_4 induced hepatotoxicity are given in Table 1.

Table 1. Effect of CF-AECal on CCl_4 induced hepatotoxicity in rat organs.

Purifiers organs		Treatments (mg/kg bw)				
		Standard	Control	CF-AECal 50	CF-AECal 100	Silymarin 200
Weight (mg)	Liver	2.74 ± 0.11	3.38 ± 0.13 *	3.03 ± 0.07	2.98 ± 0.20	2.96 ± 0.06
	Kidney	0.51 ± 0.01	0.6 ± 0.02 *	0.56 ± 0.01	0.57 ± 0.01	0.56 ± 0.01
	Spleen	0.29 ± 0.02	0.39 ± 0.02	0.38 ± 0.03	0.37 ± 0.04	0.33 ± 0.01

The values are expressed as mean ± SEM, (n = 5). * P<0.05, ** p< 0.01, ***p<0.001.

The relative liver weight in control group increased (P < 0.05) as compared to standardgroup.

Assessment of liver function

CCl_4 (20%) caused a significant increase of serum marker enzyme in particular the transaminase (SGOT and SGPT) and alkaline phosphatase (ALP). Similarly, a significant increase is observed of the total serum bilirubin (TB) and proteins. This phenomenon is especially observed with rats of the control group (CCl_4 0.5 mg/kg bw) in comparison with standard group rats as shown in Fig. 1. CF-AECal 100 mg/kg resulted in a significant decline in the rate of serum enzymes (SGOT, SGPT) and protein compared with CCl_4 groups. CF-AECal 50 mg/kg induced a significant decrease of SGOT, SGPT, ALP, TB and proteins compared with CCl_4 group rats (Fig. 1).

Scorers of the anti-oxidant enzymes of the liver

CF-AECal 50 and 100 mg/kg had significantly inhibited the increase of SOD (p< 0.001) due to the CCl_4 (20%). CF-AECal 100 mg/kg had significantly increased glutathione level in the liver compared to the CCl_4 groups. All doses of CF-AECal caused a significant restoration of the catalase by report to the CCl_4 groups. The increase in the concentration of MDA and proteins in CCl_4 (20%) has been inhibited by CF-AECal 50 and 100 mg/kg in the liver (p< 0.001). Silymarin group nearly normalized the levels of the anti-oxidant enzymes (Fig. 2).

Histopathological studies of the liver

The Fig. 3A shows normal histological structure of hepatic lobule which consists of central vein surrounded by normal hepatocytes. Livers of CCl_4-intoxicated show extensive area of necrosis, severe fatty degeneration of hepatocytes and infiltration of leucocytes in hepatic sinusoid and congestion (Fig. 3B). Liver section pretreated with silymarin 200 mg/kg nearly normalized histology of the hepatic lobule (Fig. 3C). Livers pretreated with CF-AECal 50mg/kg show a congestion and little vacuolar degeneration of hepatocytes (Fig. 3D). Livers pretreated with CF-AECal 100 mg/kg show reduced inflammation and degenerative changes (Fig. 3E).

Discussion

Phytochemical studies revealed the presence of flavonoids, tannins, phlobatannins, alkaloids, anthraquinones, cardiac glycosides and saponins in the leaves of *Cassia alata* (Kingsley *et al*., 2011; Baravalia *et al*., 2010).

Relative weights of the organs

The liver weight significantly increased among the rats exposed to the CCl_4.

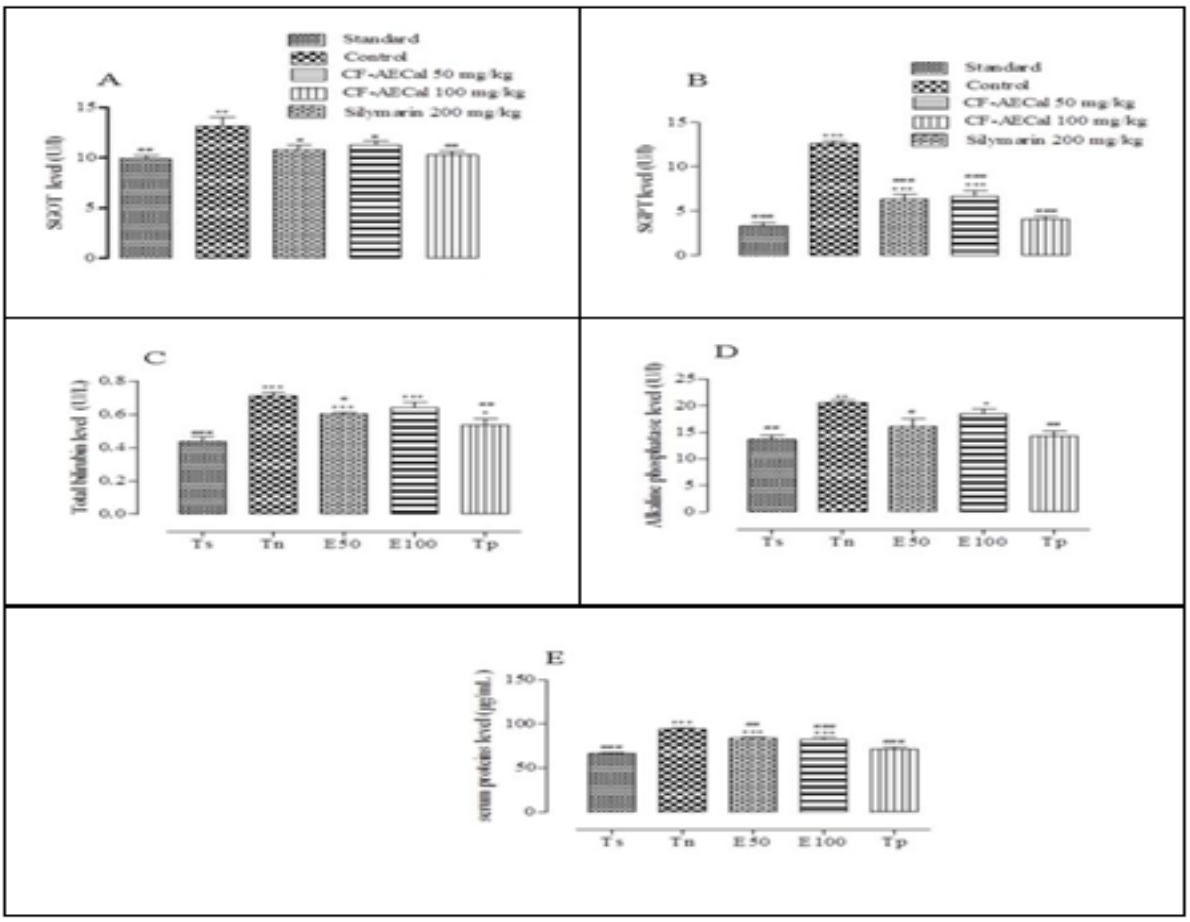

Fig. 1. Effect of CF-AECal on the hepatic scorers among the rats intoxicated in the CCl_4 20% A: serum aspartate aminotransferse level (U/I), B scrum alanine aminotransferase level (U/I), C: total bilirubin level (U/I), D: alkaline phosphatase activity (U/I), E: scrum protein level (μg/ml). The values are expressed on average ± SEM, n=5 *p<0.05, **p<0.01,***p<0.001.

Treatment of CF-AECal decreased the liver weight significantly indicating the recovery of liver tissue from damage. The key role of the glutathione in the defense of the body against the free radicals is known (Boyd *et al.*, 1981). Excessive peroxidation causes increased glutathione consumption. Glutathione plays a major protective role as a cleaner of free radicals that combine with non-protein thiols to abolish free radical toxicity (Vane *et al.*, 1994; Swierkosz *et al.*, 1995). Anti-oxidation by glutathione protects the body from many diseases due to H_2O_2, ethanol and numerous other toxins (Choi *et al.*, 2009).

Scorers of the anti-oxidant enzymes of the liver

Oral administration of dichloromethane extracts of *Cassia alata* leaves (CF-AECal) significantly brought back the lipid peroxidation, antioxidant enzymes such as SOD and CAT values to near normal level. The potential activity exhibited by the CF-AECal action against CCl_4 toxicity make them potential agents to treat liver diseases and oxidative stress. These results were similar to the protective effect of the aqueous extract of *Cassia fistula* in albino wistar rats (Parthasarathy *et al.*, 2009), and the evaluation of *in vivo* antioxidant and hepatoprotective activity of *cassia occidentalis*against paracetamol-induced liver toxicity in rats (Sheeba *et al.*, 2010). The hepatoprotective activity of *C. alata* would be to prevent the reduction of the rate of the GSH and this grace to the presence of the flavonoids in its leaves (Scevola *et al.*, 1984; Wegener *et al.*, 1999). Flavonoids, alkaloids, saponins, glycosides are known for their hepatoprotective effect (Manjunatha *et al.*, 2006; Tran *et al.*, 2001; Vijayan *et al.*, 2003).

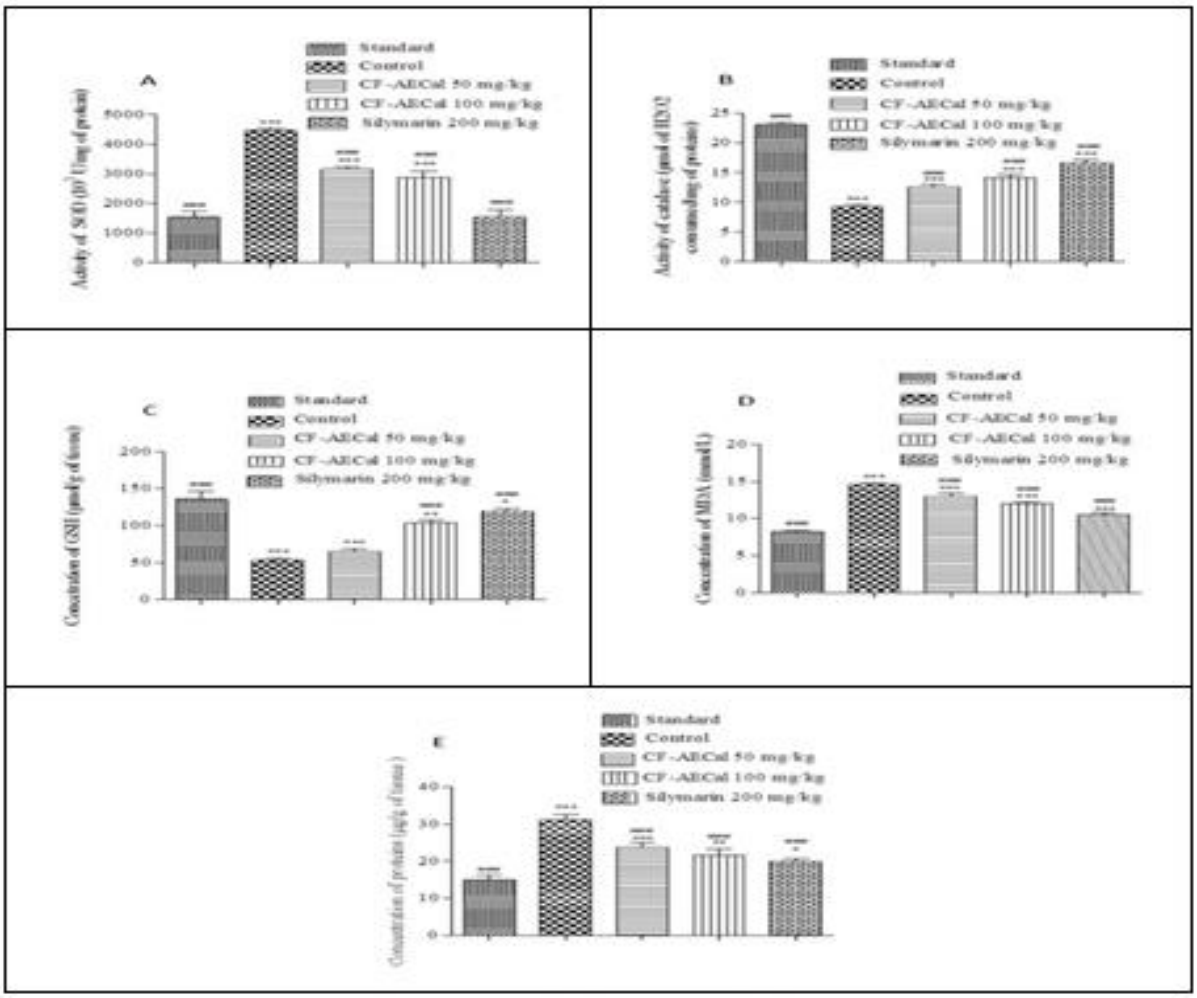

Fig. 2. Effect of CF-AECal on CCl_4 induced oxidative enzymes in rats, A: superoxyde dismutase activity (U/mg of protein).B: activity of catalase (μmol of H_2O_2 Consumed/mg of tissue), C: concentration of glutathione(μmol/g of tissue), D: concentration of (malondialdehyde (mmol/L), E: concentration of proteins (μg/g of tissue). The values are expressed on average, SEM, n= 5, *p<0.05, **p<0.01, ***p<0.001.

Assessment of liver function

The increased level of SGOT, SGPT, ALP and TB is conventional indicator of the liver injury. In the present study, it is observed that administration of CCl_4 elevates the levels of these blood enzymes. Levels of total proteins are increased. Dichloromethane extracts of *Cassia alata* leaves and reference drug silymarin-treated groups exhibited lower levels of SGOT, SGPT, ALP, and bilirubin as compared to CCl_4 treated groups. The treatment with CF-AECal also significantly lowered total protein levels. The stabilization of serum SGOT, SGPT, ALP and bilirubin by CF-AECal is clear indication of the improvement of the functional status of the liver cells. The characteristic feature of experimental hepatic damage observed is significant decrease in protein level. The rats which received CF-AECal showed restoration of protein levels. These results are in agreement with those of Arijit *et al.*(2012) who valued the hepatoprotective effect of leaves of *Cassia sophera*. The increase of the transaminases can be due to the passage of these enzymes in blood following the change of the cellular membranes. CF-AECal would initiate the processes of regeneration and hepatic damage repair led by CCl_4 (Suresh Kuma *et al.*, 2008; Moselhy *et al.*, 2009), sense of the reduction of its enzymes. Thabrew *et al.* (1987) retained that the reduction in the blood of the transaminases is bound by the hepatic parenchyma recovery and the regeneration of hepatocytes. Our findings corroborate the work of Kingsley *et al.*(2011) on the hepatoprotective effect of crude methanolic

extract and fractions of Ring worm plant *Senna alata* leaves from Nigeria against carbon tetrachloride – induced hepatic damage in rats. The mechanism of action may be the activation of the constitutive androstane receptor (CAR), a key regulator in bilirubin clearance in the liver (Moore *et al.*, 2004). The restoration of the concentration in protein can be due to the increase of the synthesis of the proteins. Indeed, the stimulation of synthesis of proteins accelerates the mechanism of regeneration of the hepatic cells (Awang *et al.*, 1993).

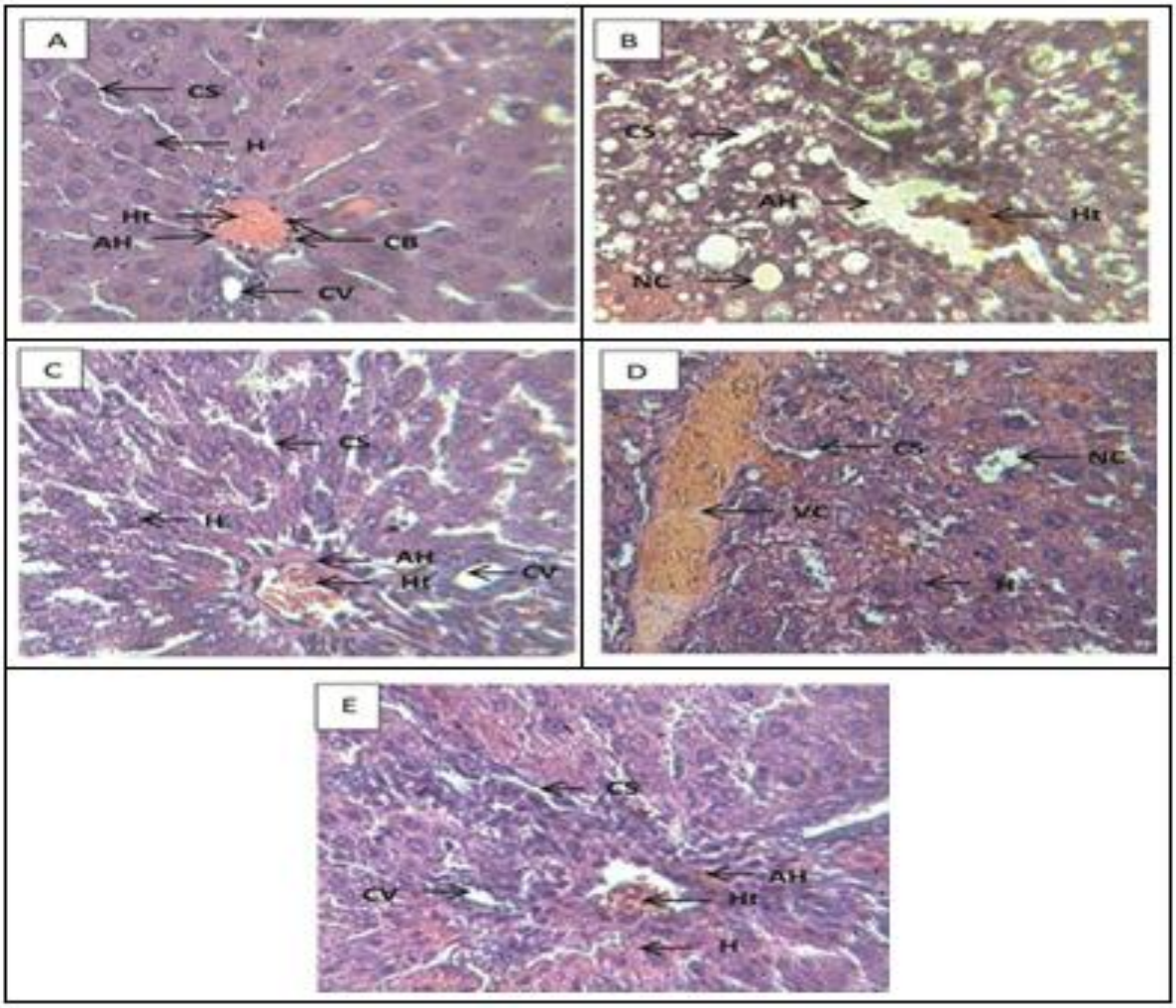

Fig. 3. Histopathology of liver sections of animals from different groups (H & E x 400). A: liver of the rat treated to olive oil, B: liver of the rat intoxicated with CCl_4 20%, C: liver of the rat treated with silymarin 200 mg/kg, D: liver of the rat treated with CF-AECal 50 mg/kg, E: liver of the rat treated with CF-AECal 100 mg/kg. *AH*: hepatic artery. *CV*: central vein, *H*: hepatocytes hacmatite, *NC*: Cellular necrosis, *VC*: vascular congestion.

Histopathological studies of the liver

The histopathological hepatic lesions induced by administration of CCl_4 was remarkably improved by the treatment with dichloromethane extract of *Cassia alata*, and showed protective effects by decreasing hepatocellular degeneration and necrosis. The protective effects were also observed in silymarin treated animals. This was in conformity with the results of serum aminotransferase activity and hepatic oxidative stress level. This result is comparable to the results of Mohammed *et al.*(2013) that demonstrated that the ethanolic extracts of *Melia azedarachCatharanthus Rosea* and *Brassica oleracea .var.capitata* (300 mg/kg and 500mg/kg, *p.o*) significantly warned the damages of the liver due to the Simvastatin. The recovery towards

normalization of serum enzymes and liver histological architecture caused by dichloromethane extract of *C. alata* was almost similar to that caused by silymarin, in the present study. Similar results have been reported (Morazzoni *et al.*, 1995; Pradeep Kumar Samal, 2013). Silymarin is a known hepatoprotective compound. It is reported to have a protective effect on the plasma membrane of hepatocytes (Ramellini *et al.*, 1976).

Conclusion

In conclusion, the results of present study demonstrate that *Cassia alata* leaves(50 mg/kg and 100mg/kg,) has potent hepatoprotective activity, against carbon chloride induced liver damage. Our study also implies that the hepatoprotective effects of Cassia alata may be due to its antioxidant property of the phenolic compounds. Further investigation is in progress to determine the exact constituents responsible for hepatoprotective effect.

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