Original Article

Hepatoprotective Activity of Aqueous Extract of *Portulaca oleracea* (Nunia sag) on Carbon Tetrachloride Induced Hepatotoxicity in Rats.

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ABSTRACT:

Introduction: *Aqueous extract of entire plant of Portulaca oleracea has hepatoprotective activity in Long-Evans rats by inducing hepatic injury with carbon tetrachloride (CCL_4). CCL_4 induced hepatic damage was manifested by a significant increase in the activities of marker enzymes. Biochemical data exhibited significant hepatoprotective activity of aqueous extract of Portulaca oleracea at oral dose of 500 mg/kg against CCL_4 induced hepatic injuty. Silymarin was used as reference standard also exhibited significant hepatoprotective activity against CCL_4. The biochemical observations were supplemented with histopathological examination of rat liver sections.* ***Results:*** *Treatment with aqueous extract of Portulaca oleracea in both CCl_4 pretreated and CCl_4 co-administered group resulted in significant decrease serum bilirubin, ALT, AST, Alk.P* ***Conclusion:*** *Extract of Portulaca oleracea has a good hepatoprotective effect on CCL_4 induced hepatotoxicity in experimental rats. However further studies should be carried out to determine the active principles responsible for the hepatoprotective effects and its cellular mechanism of action. Toxicological studies of Portulaca oleracea in animals should also be carried out before any clinical trial for suitability of using in human.*

Keywords: *Portulaca oleracea; Silymarin; aqueous extract; Carbon tetrachloride; hepatoprotective activity.*

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INTRODUCTION:

Liver is the key organ regulating homeostasis in body. Because of its unique metabolism and relationship to the gastrointestinal tract, the liver is an important organ of target for toxicity produced by the drugs, xenobiotics and oxidative stress.[1]

The most common liver diseases are various types of acute hepatitis, chronic hepatitis, fatty changes, cirrhosis and malignancy. Severe acute liver disease is encountered in clinical practice leading to fulminant or acute hepatic failure. Most common cause of fulminant hepatic failure include drugs or toxins induced hepatic injury or viral hepatitis.[2]

Viral hepatitis has become a menace to public health in Asia and Africa, making development of inexpensive control measures urgent.[3] Apart from viral hepatitis and other causes are drugs, and there are more than 900 drugs that can cause liver damage. Drugs account for 20-40% of all instances of fulminant liver failure.[1] Drug induced injury to the liver can mimic any form of acute or chronic liver diseases. Acetaminophen, a most commonly used analgesic agent, may cause hepatic injury when

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used in overdose or in therapeutic dose chronically.[4] Ingestion of large amount of alcohol is also a cause of hepatitis with focal necrosis of liver cells.[5]

Many drugs and toxins can be detoxified by conjugation with glutathione. When the levels of these drugs or toxins, however, exceed the liver concentration of reduced glutathione, such components become acutely hepatotoxic e.g. acetaminophen intoxication and in alcoholics with liver failure.[5]

In developed countries like United states, approximately 2000 cases of acute liver failure occur annually of which 50% are acetaminophen induced.[6] In an underdeveloped country like Bangladesh about 35 million people are suffering from liver disease.[7] Out of the total death from liver disease, 80% is due to fulminant hepatic failure.[8] Statistics for drug related hepatic injury is not available in our country but the rate seems to be certainly alarming due to indiscriminate and inappropriate use of drugs particularly, commonly used analgesics.

Despite tremendous development in the field of medical science, liver diseases are still the threatening problems to our health sector. In absence of reliable liver protection drugs in modern medicine, a large number of medicinal preparations are recommended for the treatment of liver disorders and quite often claimed to offer significant relief.[1]

Different herbal plants have hepatoprotective effect such as *Silybum marianum*,[9] *Andrographis paniculata*,[10] *Nigella sativa*,[11] *Carica papaya*,[12] *Curcuma longa*,[13] *Moringa oleifera*,[14] *Tamarindus indica,*[15] *Plantago major* L,[16] *Eugenia jambolana*.[17]

Attempts are being made globally to get scientific evidence for these traditionally reported herbal drugs. This scenario provides a severe necessity to carry out research in the area of hepatotoxicity. So it is necessary to find out a suitable herbal drug which is easily available, least toxic, and less expensive. Study suggest that *Portulaca oleracea* has hepatoprotective effect.[1]

METHODS AND MATERIAL:

This experimental study on rats was done in the Department of Pharmacology, in collaboration with department of Pathology, Dhaka Medical College and Centre for Advanced Research in Science (CARS) of University of Dhaka; from January 2012 to December 2012.

Plant material: The whole plant of *P. oleracea* was collected from local market (Dhaka). The plant was identified and authentified by Plant Taxonomy unit of Bangladesh National Herbarium, Mirpur, Dhaka, with voucher specimen No. 37908. Extract was prepared in Centre for Advanced Research in Science (CARS) of University of Dhaka. The plants were cleaned, dried in shadow. Powdered by mechanical grinder.The powder were then decocted in purified boiling water in the ratio of 1:9 for 30 min. This decoction was kept for an overnight and filtered. The filtrate was concentrated and evaporated to dryness in vaco at 40℃, using rotary evaporator**.** The yield was calculated and the dry extract was stored in a refrigerator at 2-8^{0}C. During the experiment the extract was dissolved in distilled water and administered to the animals at 500 mg/kg body weight (bw). **Animals:** Long-Evans rats of both sexes, weighing between 180 to 200 grams were used for the study. The animals were obtained from animal house of BCSIR, Dhaka. The rats were well accommodated in metallic cages (6 rats in each cage), at room temperature and well ventilated room, in animal house of Dhaka Medical College. Saw dust was used as bedding and changed every alternate day and proper cleaning measures were taken regularly. Rats were fed with standard pellet diet (10 to15 gm/rat/day) (ICDDRB, Dhaka, Bangladesh) and allowed drinking water ad libitum. **Drugs and chemicals: Silbin (Silymarin)-** Used as the standard drug and provided by Square pharmaceuticals. **Carbon tetrachloride:** Purchased from local market of scientific goods. **Olive oil:** Purchased from local market. **Kits:** Global's bilirubin kit (UK)**,** Global's SGPT (ALT) kit (UK)**,** Global's SGOT (AST) kit (UK)**,** Global's Alk.P kit (UK) were used for biochemical examination**.** Acute oral toxicity study was performed. The *Portulaca oleracea* (PO) extract was administered

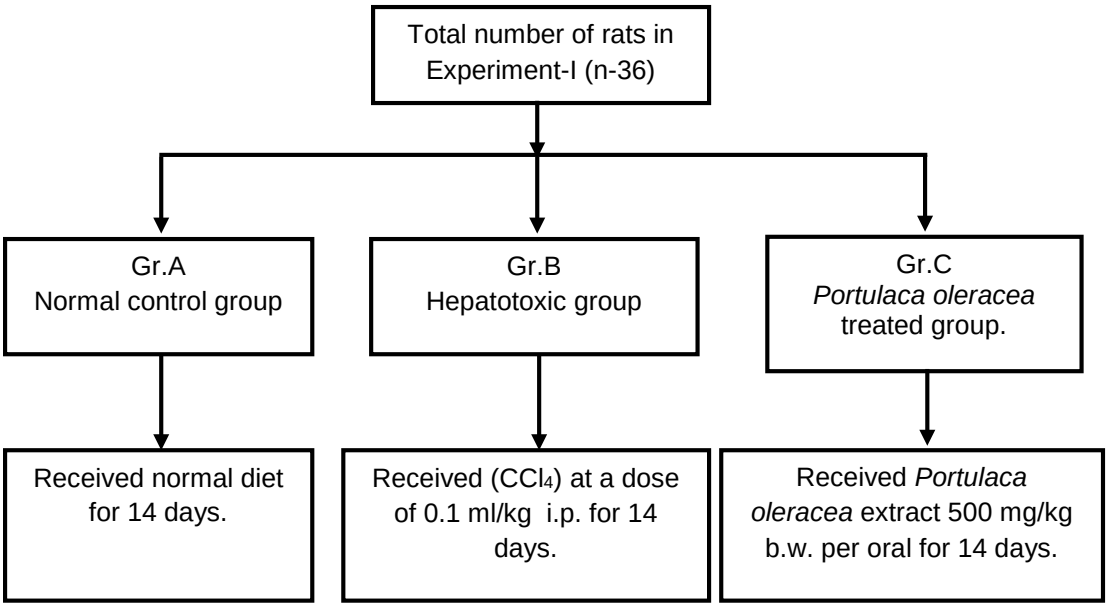
orally in doses of 5, 50, 300, 2000 mg/kg body weight to groups of rats (n=3) and the percentage mortality was recorded over a period of 24 h. During the first 1 h of drug administration, rats were observed for gross behavioral changes.[1] The extract was devoid of any toxicity in rats when given in dose up to 2000 mg/kg by oral route. Hence, for further studies 500 mg/kg doses of extract were used.

Study design or experimental design: This study included 108 Long-Evans rats divided into 9 subgroups which were included within 3 major experimental groups:

1. Experiment-I
2. Experiment-II
3. Experiment-III

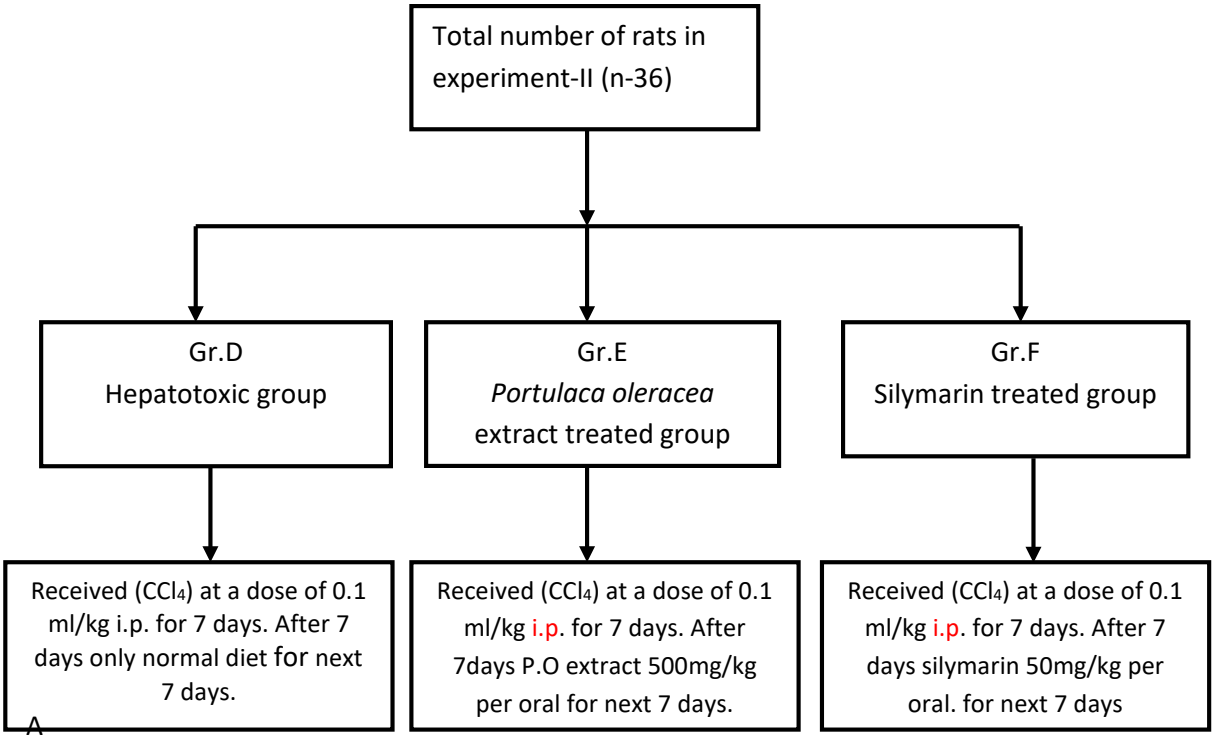
Each subgroup consisted of 12 rats.

EXPERIMENT-I



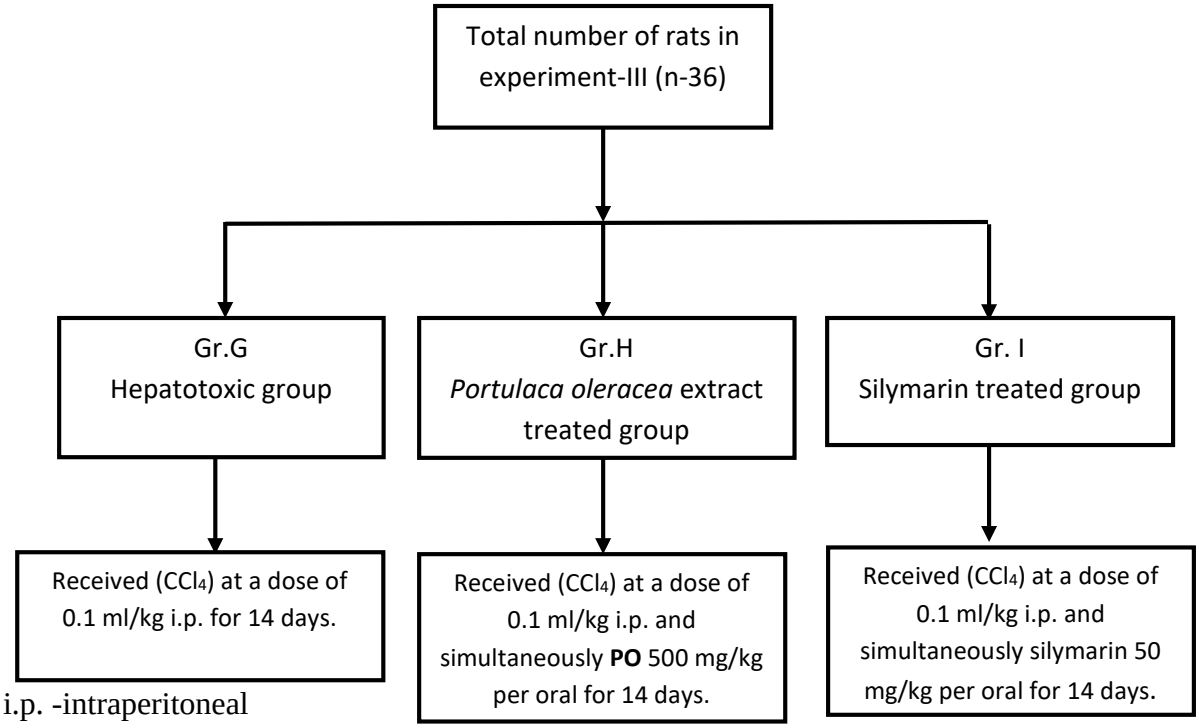
i.p.-intraperitoneal,
PO- *Portulaca oleracea* extract

EXPERIMENT-II



A

EXPERIMENT-III



i.p. -intraperitoneal

PO - *Portulaca oleracea* extract

On the 15th day rats were anaesthetized with light chloroform and on average 3cc blood was collected from each animal by cardiac puncture for serum analysis of Total bilirubin **(TB),** Aspartate transaminase **(AST),** Alanine transaminase **(ALT),** Alkaline phosphatase **(Alk.P).** The rats were then sacrificed and their livers were removed. One lobe was then fixed in 10% formalin for histopathological studies. One lobe of liver was fixed in 10% formalin and then processed by automated tissue processor. Liver tissues was embedded, sectioned by a microtome and was stained with Haematoxyline and Eosin stain. Each section was examined by light microscope with magnification of 10x, 40x and gross histopathological changes was noted**.**

Statistical Analysis: Collected data were tabulated and expressed as mean ± SD. Statistical analysis was done by Student's unpaired *t*- test. After appropriate statistical analysis P<0.05 was taken as level of significance.

RESULTS:

Table-1: Effect of carbon tetrachloride and *Portulaca oleracea* extract on serum biochemical parameters in experimental rats.

Groups	**TB (mg /dl)**	**ALT (U/ml)**	**AST (U/ml)**	**Alk.P** (K.A.U)
Group A (Normal control)	0.55 ± 0.044	33.27± 2.23	43.28 ± 2.9	11.77 ± 0.72
GroupB (Hepatotoxic)	0.90 ± 0.03***	136.22±4.02***	147.28 ±4.41***	44.88±1.12***
Group C (PO extract)	0.53 ± 0.043	32.38±2.35	41.76± 2.93	11.28±0.69

All values are shown as mean± SEM and *n*= 12 rats, *** indicate P<0.001 when compared between Gr.A vs Gr.B and P>0.05 when compared between Gr.A vs Gr.C.

Table-1 shows the increase in mean serum bilirubin, ALP, AST and Alk.P level in the carbon tetrachloride treated group B was highly significant (P< 0.001) in comparison with the normal control group A. The mean values of serum bilirubin, ALT, AST, Alk.P were not significant in Gr.A (Normal Control) vs Gr.C (PO extract treated group).

Table-2: Effects of *Portulaca oleracea* and silymarin on serum biochemical parameters in CCl_4 pretreated rats.

Groups	TB (mg/ dl)	ALT(U/ ml)	AST(U/ ml)	Alk.P (K.A.unit)
Group-D (Hepatotoxic)	0.85 ± 0.043	121.70 ± 3.06	122.33 ± 4.13	38.58 ± 1.36
Group–E (PO extract)	0.72 ± 0.038*	69.9 ± 3.02***	90.1 ± 3.17***	19.6 ± 0.93***
Group–F (Silymarin)	0.68 ± 0.029**	65.45± 3.51***	85.55 ± 2.63***	18.88 ± 0.53***

All values are shown as mean± SEM and *n*=12. * indicate P<0.05, ** indicate P<0.01, *** indicate P <0.001 when compared between Gr.D (Hepatotoxic) vs Gr.E (PO extract treated group); Gr.D vs Gr.F (Silymarin treated).

Table-2 shows the decrease in mean serum bilirubin level in group E and F was significant (P<0.05), (P<0.01) respectively in comparison with group D. At the same way the decrease in mean serum ALT, AST, Alk.P level in group E and F were significant (P<0.001) in comparison with group D.

Table-3: Effects of *Portulaca oleracea* and silymarin on serum biochemical parameters in CCl_4 co-treated rats.

Groups	TB (mg/dl)	ALT(U/ml)	AST(U/ml)	Alk.P(K.A.unit)
Group-G (Hepatotoxic)	0.85 ± 0.043	136.22 ± 1.16	147.28 ± 4.41	44.88 ± 1.12
Group – H (PO extract)	0.68 ± 0.038 **	66.38 ± 3.25 ***	87.1 ± 2.59 ***	19.20 ± 0.58***
Group – I (Silymarin)	0.67 ± 0.028 ***	64.27 ± 3.58 ***	85.48 ± 2.63 ***	18.87 ± 0.56***

All values are shown as mean± SEM and *n*=12. * indicate P<0.05, ** indicate P<0.01, *** indicate P <0.001 when compared between Gr.G (Hepatotoxic) vs Gr.H (PO extract treated); Gr.G vs Gr.I. (Silymarin treated).

Table-3 shows the decrease in mean serum bilirubin level in group H and I was significant (P<0.01), (P<0.001) respectively in comparison with group G. At the same way the decrease in mean serum ALT, AST, Alk.P level in group H and I were significant (P<0.001) in comparison with group
G.

Table-4: Effects of *Portulaca oleracea* and silymarin on serum biochemical parameters in CCl_4 treated rats.

Groups	TB (mg/dl)	ALT (U/ml)	AST (U/ml)	Alk.P (K.A.unit)
Group – E (PO extract)	0.72 ± 0.038	69.9 ± 3.02	90.1 ± 3.17	19.6 ± 0.93
Group – F (Silymarin)	0.68 ± 0.029	65.45 ± 3.51	85.55 ± 2.63	18.88 ± 0.53
Group – H (PO extract)	0.68 ± 0.038	66.88 ± 3.25	87.10 ± 2.59	19.20± 0.58
Group – I (Silymarin)	0.67 ± 0.028	64.27 ± 3.58	85.48 ± 2.63	18.87 ± 0.56

All values are shown as mean± SEM and *n*=12. P>0.05, when compared between Gr.E vs Gr.F; Gr.H vs Gr.I.

Table-4 shows the mean values of serum bilibubin, ALT, AST, Alk.P were not significant (P>0.05) in between the (Gr.E vs Gr.F; Gr.H vs Gr.I). So it seems that both the drugs are equally effective in CCl_4 induced hepatic injury.

Histological observations:

The following gross histological changes were observed:

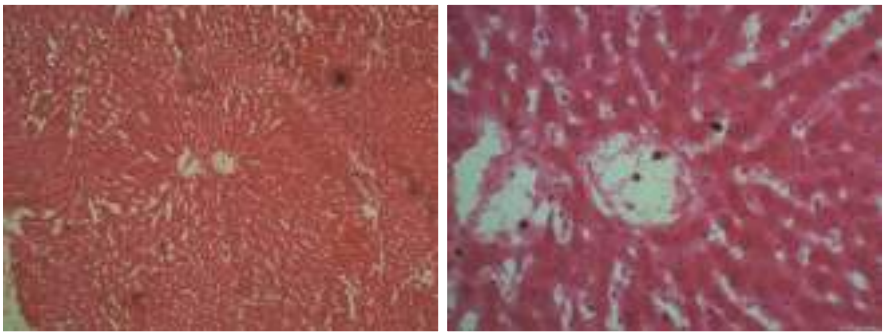
Histological slides of the normal control rat liver showed normal hepatic architecture consisting of central vein in the center and portal tracts at the periphery of the central vein. Plates of liver cell radiating from the central vein to the portal tract. The nucleus and cytoplasm of the liver cells were essentially normal in appearance (slide-1).

Histological slides of the CCl_4 treated rat's liver showed distortion of the hepatic architecture. The hepatocytes in the hepatic lobule showed hydropic changes, centrilobular necrosis, steatosis and diffuse hepatocyte necrosis. Infiltrations of small

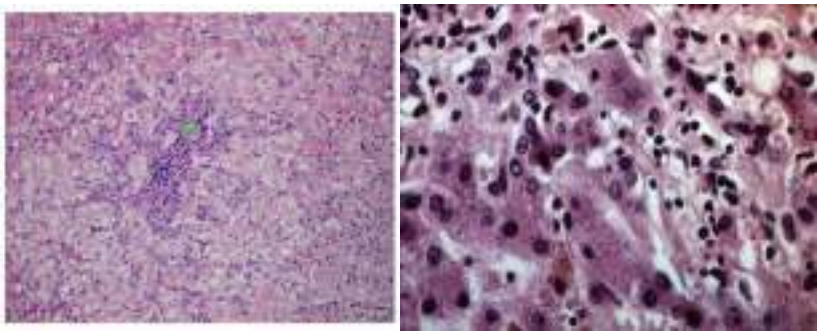
number of inflammatory cells were also found (slide-2).

Histological slides of the *Portulaca oleracea* extract treated rat's liver showed normal hepatic architecture (slide-3).

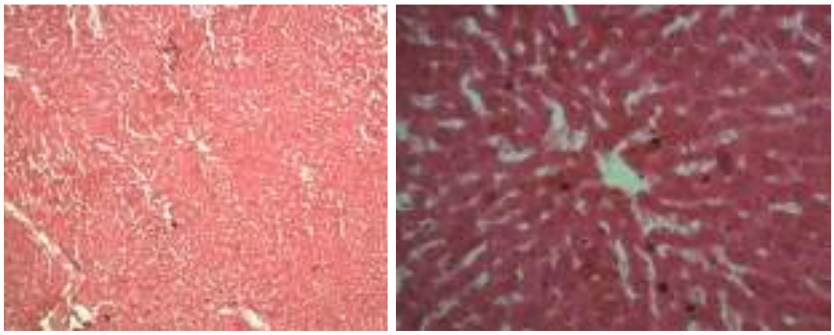
Histological slides of the *Portulaca oleracea* extract treated and silymarin treated rat's liver in CCl_4 pretreated and CCl_4 co-treated groups showed a protective effect by decreasing the extent of centrilobular necrosis and steatosis when compared to CCl_4 group (slide-4); (slide-5); (slide-6); (slide-7) respectively.



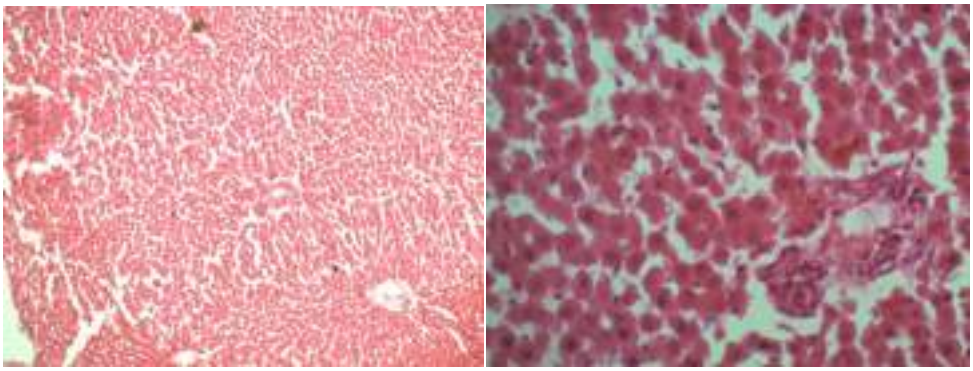
Slide-I: Photomicrographs (Magnification at 10x and 40x objectives) showing the normal hepatic architecture.



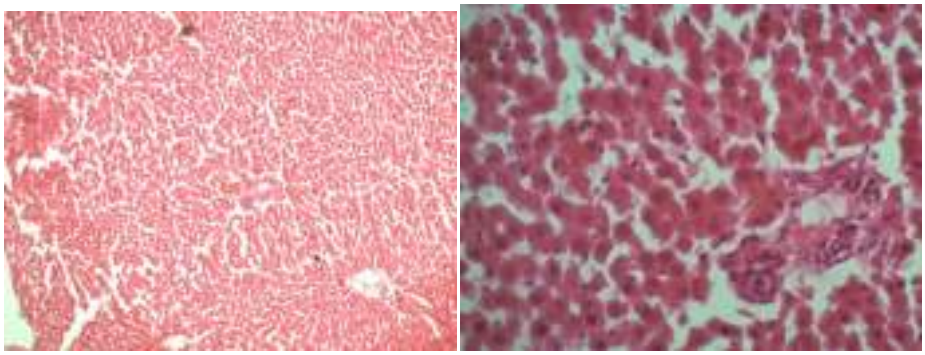
Slide-2: Photomicrographs (Magnification at 10x and 40x objectives) of carbon tetrachloride treated group. Hepatocytes showing hydropic changes, centrilobular necrosis, steatosis and diffuse hepatocyte necrosis.



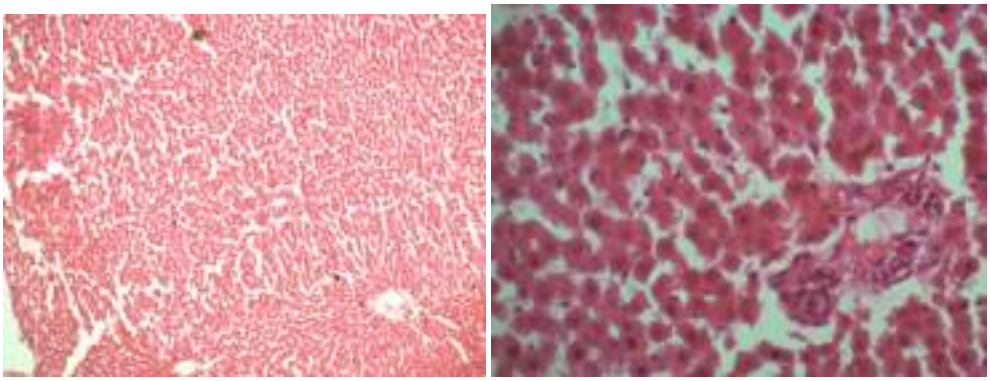
Silde-3: Photomicrographs (Magnification at 10x and 40x objectives) showing the normal hepatic architecture (*Portulaca oleracea* treated group).



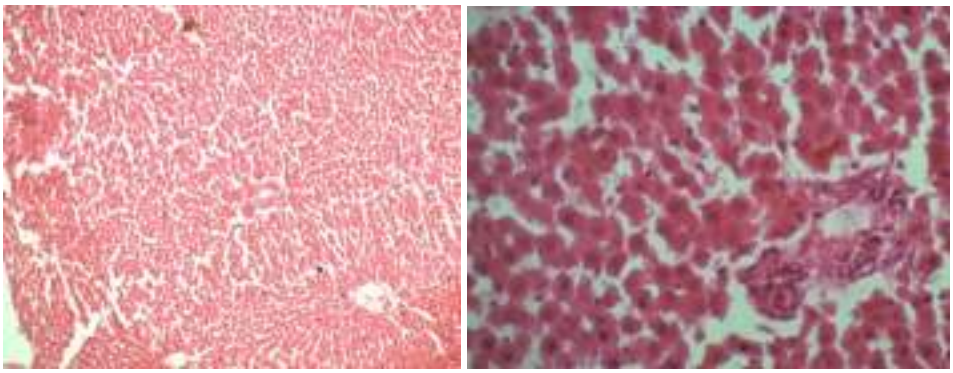
Slide-4: Photomicrographs (Magnification at 10x and 40x objectives) showing decreasing the extent of centrilobular necrosis and steatosis (*Portulaca oleracea* treated group in CCl_4 pretreated group) when compared to CCl_4 group.



Slide-5: Photomicrographs (Magnification at 10x and 40x objectives) showing decreasing the extent of centrilobular necrosis and steatosis (silymarin treated group in CCl_4 pretreated group) when compared to CCl_4 group.



Slide-6: Photomicrographs (Magnification at 10x and 40x objectives) showing decreasing the extent of centrilobular necrosis and steatosis (*Portulaca oleracea* treated group in CCl_4 co-treated group) when compared to CCl_4 group.



Slide-7: Photomicrographs (Magnification at 10x and 40x objectives) showing decreasing the extent of centrilobular necrosis and steatosis (silymarin treated group in CCl_4 cotreated group) when compared to CCl_4 group.

DISCUSSION:

In the present study hepatotoxicity was induced by administration of carbon tetrachloride (0.1ml/kg b.w.). The hepatotoxicity was evidenced by a significant increase ($P<0.001$) in mean serum ALT, AST and Alk.P levels. Increase in liver enzyme levels is an indicator of hepatotoxicity and amount of liver damage can be assessed by determining serum ALT, AST. Serum ALP and total bilirubin levels are related to function of hepatocytes. Increase in serum ALP is due to increased synthesis, in presence of increasing biliary pressure.[1] Histopathological examination of liver tissue also revealed gross cellular damage. Similar results were also found in rats by Mehmet et al., Vaghela et al., Idris et al., Sisodia et al. [11,12,16, 17]

Carbon tetrachloride is a routinely used hepatotoxin for experimental study of liver diseases. Administration of CCl_4 causes acute liver damage that mimics natural causes. It mediates changes in liver function that ultimately leads to destruction of hepatocellular membrane. Cytochrome P-450 activates CCl_4 to form various free radicals (trichloromethyl, hexachloroethane, phosgene etc.) which are involved in the pathogenesis of liver damage in chain reactions resulting in peroxidation of lipids, covalent binding of macromolecules, disruption of metabolic mechanisms in mitochondria, decreasing levels of phospholipids, increasing triglyceride levels, inhibition of calcium pumps of microsomes thus leading to liver necrosis.[1]

In the present study, treatment with aqueous extract of *Portulaca oleracea* in both CCl_4 pretreated and CCl_4 co-administered group resulted in significant decrease serum bilirubin, ALT, AST, Alk.P and histopathological study of liver tissue showed a protective effect by decreasing the extent of centrilobular necrosis and steatosis when compared to CCl_4 group. Anusha et al. in their study showed a significant reduction in serum ALT, AST, Alk.P, total bilirubin levels in the groups treated with aqueous extract of *Portulaca oleracea.* The enzyme levels were almost restored to normal.[1] The findings of their study is in agreement with the present study. Prabhakaran et al. in their study showed that the methanol and petroleum ether extracts of entire plant of *Portulaca oleracea* in carboxy methyl cellulose (CMC) had hepatoprotective activity in Wister albino rats by inducing hepatic injury with D-galactosamine.[18] D-galactosamine induced hepatic damage was manifested by a significant increase in the activities of marker enzymes. The hepatoprotective activity was observed by the biochemical observations, were supplemented with histological examination of rat liver section.[18] These findings correlate with the present study. Kulkarni et al. showed the protective effect of hydro-alcoholic extract of *Portulaca oleracea* L. leaf has hepatoprotective activity against ethanol induced hepatotoxicity in rats. Ethanol feeding resulted in

liver injury as indicated by the serum activities of marker enzymes such as AST, ALT, Alk.P, and TB. Post treatment of *Portulaca oleracea* L. extract reversed these alterations to near normal.[19] In another study Kulkarni et al. showed the hydro-alcoholic extract of *Portulaca oleracea* exhibited significant hepatoprotective activity (P<0.01) close to silymarin. The rats intoxicated with rifampicin, elevated significantly (P<0.01) the serum biochemical parameters. The AST, ALT, ALP level increased by rifampicin treatment was significantly (P<0.01) reduced by hydro-alcoholic extract of *Portulaca oleracea* (140 and 280 mg/ kg) after 48 hr. Total bilirubin and total protein level was also restored to the normal level. Histopathology of the liver gives the evidence for the protection imparted by the hydro-alcoholic extract. The hydro-alcoholic extract has a definite hepatoprotective and regenerative activity.[20] Though the study designs and or extract used in the mentioned above studies were different from the present one, similar results clearly indicate hepatoprotective effect of *Portulaca oleracea*.

The reversal of increased serum enzymes in CCl_4 induced liver damage by the extract may be due to the prevention of the leakage of intracellular enzymes by its membrane stabilizing activity.[1] Amino transferases contribute a group of enzymes that catalyze the interconversion of amino acids and α-keto acids by the transfer of amino groups. These are liver specific enzymes and are considered to be very sensitive and reliable indices for necessary hepatotoxic as well as hepatoprotective or curative effect of various compounds. Both AST and ALT levels increase due to toxic compounds affecting the integrity of the liver cells.[1] Decreased levels of transaminases indicate stabilization of plasma membrane and protection of hepatocytes against damage caused by hepatotoxin. This is in agreement with the commonly accepted view that serum levels of transaminases return to normal with the healing of hepatic parenchyma and the regeneration of hepatocytes.[20]

Alkaline phosphatase is a membrane bound glycoprotein enzyme with a high concentration in sinusoids and endothelium. This enzyme reaches the liver mainly from the bone. It is excreted into the bile; therefore, its elevation in serum occurs in hepatobiliary diseases.[1] Serum alkaline phosphatase is related to the functioning of hepatocytes and increase in its activity is due to the increased synthesis in presence of biliary pressure Table-1. The results of the present study indicate that the test groups probably stabilize the hepatic plasma membrane from CCl_4 induced damage which is evident fromTable-2, Table-3.

The efficacy of any hepatoprotective drug is dependent on its capacity of either reducing the harmful effect or restoring the normal hepatic physiology that has been disturbed by a hepatotoxin.[1] Phytoconstituents like the flavonoids, triterpenoids, saponins, and alkaloids[21] are known to possess hepatoprotective activity. The presence of flavonoids in our extract may be responsible for its antioxidant and thus hepatoprotective activity.

The potential usefulness of the extract in clinical conditions associated with liver damage is still to be demonstrated. Investigations are needed for the isolation of the active principle responsible for hepatoprotective activity and research works are needed to be carried out with regard to intoxication with other models such as iron, alcohol etc to prove its efficacy.

CONCLUSION:

Extract of *Portulaca oleracea* has a good hepatoprotective effect on carbon tetrachloride induced hepatotoxicity in experimental rats. However further studies should be carried out to determine the active principles responsible for the hepatoprotective effects and its cellular mechanism of action. Toxicological studies of *Portulaca oleracea* in animals should also be carried out before any clinical trial for suitability of using in human.

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