HEPATOPROTECTIVE ACTIVITY OF *ARGEMONE MEXICANA* LINN AGAINST TOXIC EFFECTS OF CARBON TETRACHLORIDE IN RATS

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ABSTRACT

The aim of this study was to evaluate the hepatoprotective effects of ethyl acetate extract of *Argemone mexicana* Linn in carbon tetrachloride (CCl₄)-treated rats. Animals were pretreated with the ethyl acetate extract (200 & 400 mg/kg body weight) for one week and then challenged with CCl₄ (1ml/kg bw) in olive oil (1:1, v/v) on 7th day. Serum marker enzymes (ALP, AST, ALT and Total Bilirubin) were estimated in all the study groups. Alteration in the levels of biochemical markers of hepatic damage like AST, ALT, ALP and Total Bilirubin were tested in both CCl₄ treated and extract treated groups. CCl₄ has significantly (P<0.001) enhanced the AST, ALT and ALP levels in liver. Treatment of ethyl acetate extract of *Argemone mexicana* Linn (200 & 400 mg/kg) produced significant (P<0.001) hepatoprotective effects as evidenced by decreased serum enzyme activities, AST, ALT, ALP and serum Bilirubin and an almost normal histological architecture of the liver, in treated groups, compared to the controls. The experimental results indicate that, ethyl acetate extract has excellent hepatoprotective effect. A similar experimental result was also observed by histological parameters.

Key words: Hepatoprotective, Carbon tetrachloride, Histology, Biochemical markers.

INTRODUCTION

The liver is a vital organ of paramount importance involved in the maintenance of metabolic functions and detoxification from the exogenous and endogenous challenges, like
xenobiotics, drugs, viral infections and chronic alcoholism. If during all such exposures to the above mentioned challenges the natural protective mechanisms of the liver are overpowered, the result is hepatic injury. Liver damage is always associated with cellular necrosis, increase in tissue lipid peroxidation and depletion in the tissue GSH levels. In addition serum levels of many biochemical markers like SGOT, SGPT, triglycerides, cholesterol, bilirubin, alkaline phosphates, are elevated (Setty et al., 2007). However, inbuilt antioxidant systems like super oxide dismutase SOD, tissue glutathione GSH etc. protect the tissue from free radical attack. Excessive release of reactive oxygen species power over this system resulting in organic damage (Kumar et al., 2008).

The plant *Argemone mexicana* belongs to family Papaveraceae, commonly known as prickly poppy or maxican pop, is an indigenous herb. It is traditionally used as analgesic antispasmodic, antitussive, demulcent, emetic, expectorant, hallucinogenic, purgative, sedative, skin, warts.

The present pharmacological investigation focuses on evaluation of the efficacy of ethyl acetate extract of *Argemone mexicana* Linn for their protection against CCl₄- induced hepatotoxicity.

**MATERIALS AND METHODS**

**Plant material**

The plant of *Argemone mexicana* Linn was collected by uprooting the whole plant from the local surroundings at Bhopal city of M.P, during the month of September to October 2011. The plant was acknowledged by Dr. Zia-Ul-Hassan Head of the PG Department of Botany, Safia Science College Bhopal MP. The voucher specimens (herbarium; V. No. 05EA, 25/2010) are kept in the herbarium of Bhoj Mahavidyalaya Bhopal (M.P) future reference.

**Preparation of extract**

The live plants collected were washed thoroughly under running tap water and then were rinsed in distilled water; they were allowed to dry for some time. Then these plants were shade dried without any contamination for about 3 to 4 weeks. The plant powder is extracted according Harbone (Harborn and Baxter., 1995). The dried powdered of *Argemone mexicana* Linn (2kg) was successively Soxhlet extracted using Petroleum Ether, ethyl acetate extract for 72 hours. The extracts were dried under reduced pressure using rotator evaporator to get the crude and were stored below 4°C until further used. When needed, the extract was
suspended/dissolved in desired solvent and used.

**Experimental animals**

A total number of 30 albino Wistar rats were obtained from the animal house of Pinnacle Biomedical Research institute PBRI (regd no. 1283/c/09/ (PCSEA). Male Wistar rats weighing between 150 – 220 gm were used for this study. The animals were placed at random and allocated to treatment groups in polypropylene cages with paddy husk as bedding. Animals were housed at a temperature of 24±2°C and relative humidity of 30 – 70%. A 12:12 light: day cycle was followed. All animals were allowed to free access to water and fed with standard commercial pelleted rat chaw. All the experimental procedures and protocols used in this study were reviewed by the Institutional Animal Ethics Committee (IAEC) and were in accordance with the guidelines of the IAEC. All animals were subjected to tests for toxicity and hepatoprotective study.

**Acute oral toxicity study**

Acute oral toxicity refers to those adverse effects occurring following oral administration of a single dose of a substance, or multiple doses given within 24 hours. The acute oral toxicity study was carried out according OECD (Organization for Economic Co-operation and Development) 423 guideline which is based on a stepwise procedure with the use of a minimum number of animals per step. Healthy, young, adult albino Wister rats (180-200 g) were used for this study and kept in their cages for at least 5 days prior to dosing to allow for acclimatization to the laboratory conditions. Animals were fasted prior to dosing (only water was withheld over night). On next day, the fasted body weight of each animal was determined and the dose was calculated according to the body weight.

24 animals were divided into four groups for giving dose 5, 50, 300 and 2000 mg/kg. The test substance was administered in a single dose by gavage using a stomach tube or a suitable intubation cannula. First a dose of 5 mg/kg was administrated to the animals and all animals survived (means dose was tolerated) so subsequent doses were increased. Maximum dose is 2000 mg/kg. Subsequently doses of 50, 300, 2000 mg/kg were administrated and no lethality was observed. Animals were observed individually at least once during the first 30 minutes after dosing, periodically during the first 24 hours (with special attention given during the first 4 hours), and daily thereafter, for a total of 14 days.

**Dose selections**
On the basis of acute toxic study, two test doses were selected for further animal study. As there were no lethality observed up to 2000mg/kg in the animals. Thus 2000 mg/kg was considered as NOAEL (Not Observed Adverse Effect limit). Hence 1/10th and 1/5th of NOAEL (i.e. 2000 mg/kg p.o. body weight) were calculated as 200 mg/kg and 400 mg/kg p.o. body weight and these were selected as doses for in vivo hepatoprotective activity of ethyl acetate extract of *Argemone mexicana* Linn.

**Preparation of plant extract, Hepatotoxic & standard drug**

**Preparation of extract solution**

The ethyl acetate extract of plant *Argemone mexicana* Linn was dissolved in DMSO for oral administration. The solution of ethyl acetate extract was prepared at a dose of 200 and 400 mg/kg body weight.

**Hepatotoxin**

CCl₄ was suspended in Olive oil (CCl₄: Olive oil 1:1.1ml/kg p.o) body weight orally.

**Standard Drug**

Silymarine at a dose of 100 mg/kg bodyweight administered by oral route. It is a polyherbal formulation used as a standard drug (*Debasish et al., 2001*).

**Experimental design for evaluation of Hepatoprotective activity**

The animals were divided into five groups consisting of six animals in each. The animals were then subjected to either one of the following treatments for 7 days.

**Group I:** Vehicle (10 ml/kg, p.o.)

**Group II:** Distilled water for 7 days + CCl₄ (1 ml/kg, p.o.) on seventh day

**Group III:** Silymarin (100 mg/kg/day, p.o.) for 7 days + CCl₄ (1ml/kg, p.o.) on seventh day

**Group IV:** Ethyl acetate extract (200 mg/kg/day, p.o.) for 7 days + CCl₄ (1 ml/kg, p.o.) on seventh day

**Group V:** Ethyl acetate extract (400mg/kg/day, p.o.) for 7 days + CCl₄ (1 ml/kg p.o.) on seventh day

The CCl₄ was administered after dilution with olive oil in the ratio of 1:1. Food was withdrawn 12 h before carbon tetrachloride administration to enhance liver damage in animals of groups 2, 3, 4 and 5. At the end of the drug treatment period, all the animals were anaesthetized by application of Diethyl ether 24 h after the administration of CCl₄. Blood
samples were collected from a group of animals from dorsal aorta by heparinized syringe in vacationer tubes. Plasma was separated from the collected blood by centrifugation at 3000g for 15 minutes. After the collection of blood the animals were sacrificed and the blood samples were collected and serum was used for assay of marker enzymes such as aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and serum bilirubin according to the reported methods (Reitman and Frankel., 1957, Copeland., 1985, Thomas.,1998, Jendrassik and Gruff ., 1938).

**Histopathological Examinations**

A portion of liver tissue from each group was carefully dissected out, washed with 0.9% normal saline solution and preserved in a 10% Formalin solution for fixation. 50 micro sections were cut using Microtome (Scientech) and stained with hematoxylin and Eosin staining. Sections (4-5-mm thick) were observed under microscope (Olympus ‘CH20I’ Trinocular) at 40X and 100X. Photographs of slides were taken using Sony digital camera attached to microscope. The liver was then subjected to histopathological examination.

**Statistical analyses**

The statistical significance was assessed using one way analysis of variance (ANOVA) followed by Bonferroni’s multiple comparison test. The values are expressed as mean ± SEM and p<0.001 was considered significant.

**RESULTS AND DISCUSSION**

**Acute toxicity**

The purpose of this study is to allow selection of the appropriate starting dose for the main study. Acute oral toxicity study of was performed to determine dosage safety of the of the ethyl acetate extract of *Argemone mexicana* L. During the first 4 hr. after the drug administration, animals were continuously observed for gross behavioral changes & then observation is continued for 24 hr & 72 hr in regular intervals for 14 days. The parameter such as hyperactivity, grooming, convulsions, sedation, hypothermia, fur colour, mortality and death were observed. The animal did not show any signs of toxicity and behavioral changes after 24 hrs and 72 hrs and no animals were found to have been dead in studies (Table 1)
Table No.1: Assessment of Acute toxicity studies according to OECD 423 guide lines

<table>
<thead>
<tr>
<th>S.NO</th>
<th>Dose (Methanolic extract)</th>
<th>Lethality</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>24hours</td>
</tr>
<tr>
<td>1.</td>
<td>5 mg/kg</td>
<td>0/3</td>
</tr>
<tr>
<td>2.</td>
<td>5 mg/kg</td>
<td>0/3</td>
</tr>
<tr>
<td>3.</td>
<td>50 mg/kg</td>
<td>0/3</td>
</tr>
<tr>
<td>4.</td>
<td>50 mg/kg</td>
<td>0/3</td>
</tr>
<tr>
<td>5.</td>
<td>300 mg/kg</td>
<td>0/3</td>
</tr>
<tr>
<td>6.</td>
<td>300 mg/kg</td>
<td>0/3</td>
</tr>
<tr>
<td>7.</td>
<td>2000 mg/kg</td>
<td>0/3</td>
</tr>
<tr>
<td>8.</td>
<td>2000 mg/kg</td>
<td>0/3</td>
</tr>
</tbody>
</table>

Liver Function test

The present study had been attempted to investigate the role of hepatoprotective activity of crude ethyl acetate extract of *Argemone mexicana* Linn against CCl₄ induced rats. The results indicate that after CCl₄ administration there was significant increase in serum marker enzymes like alanine aminotransferase (ALT), aspartate aminotransferase (AST) alkaline phosphatase (ALP) and Bilirubin of rats is shown in Group II (Table II). The table shows that in normal control rats, the serum levels of ALT, AST, ALP and Bilirubin was only 32.1±4.8451 IU/L, 28.8±3.746 IU/L, 105.4±8.0160 IU/L and 0.76±0.048 mg/dl respectively. Administration of CCl₄ (1 ml/kg, p.o.) induced a significant (P < 0.001) marked increase in the serum hepatic enzyme levels, SGOT (136.6±5.680 IU/L), SGPT (32.6±2.581 IU/L), ALP (302.3±9.872 IU/L) and Total bilirubin (1.81±0.111mg/dl) as compared to normal controls indicating liver damage (Table II). The table also showed the comparison of effects among the untreated (normal control) and carbon tetrachloride treated (induction control or standard) group with the drug treated group of rats. The results were represented as Mean± Standard Error of Mean (M±SEM). The statistical significance was computed using Bonferroni’s multiple comparison tests. The value of obtained for exceeded the limit for significance. That is in all cases ‘p’ was less than 0.001.
Table II: Effect of ethyl acetate extract of *Argemone mexicana* on biochemical parameters of liver in rats after CCl₄ administration

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Treatment</th>
<th>Dose</th>
<th>SGOT (IU/L)</th>
<th>SGPT (IU/L)</th>
<th>ALP (IU/L)</th>
<th>BILIRUBIN (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Vehicle saline</td>
<td>10 ml/kg</td>
<td>32.1±4.845</td>
<td>28.8±3.746</td>
<td>105.4±8.016</td>
<td>0.76±0.048</td>
</tr>
<tr>
<td>2</td>
<td>Control (CCl₄)</td>
<td>-</td>
<td>136.6±5.680</td>
<td>32.6±2.581</td>
<td>302.3±9.872</td>
<td>1.81±0.111</td>
</tr>
<tr>
<td>3</td>
<td>Silymarine</td>
<td>100 mg/kg</td>
<td>44.1±3.920</td>
<td>38.5±8.336</td>
<td>125.1±7.833</td>
<td>0.80±0.054</td>
</tr>
<tr>
<td>4</td>
<td>Extract</td>
<td>200 mg/kg</td>
<td>84.6±4.226</td>
<td>78.6±6.377</td>
<td>223.5±9.027</td>
<td>1.08±0.057</td>
</tr>
<tr>
<td>5</td>
<td>Extract</td>
<td>400 mg/kg</td>
<td>69.1±4.792</td>
<td>63.1±6.177</td>
<td>161.8±5.344</td>
<td>0.93±0.031</td>
</tr>
</tbody>
</table>

Each group consist of six animals (N=6)

**P<0.001 as compared to CCl₄ treated group**

Treatment of ethyl acetate extract at the dose level of 200mg/kg significantly (P < 0.001) reversed the levels of SGOT, SGPT, ALP and Total bilirubin by 84.6±4.226, 78.6±6.377, 223.5±9.027 IU/L and 1.08±0.057mg/dl respectively, while at higher doses of 400mg/kg bw, causing a reduction of 69.1±4.792 IU/L, 63.1±6.177 IU/L, 161.8±5.344 IU/L and 0.93±0.031 mg/dl respectively when compared to CCl₄ treated rats. Silymarine (100 mg/kg) treated animals also showed significant decrease (P < 0.001) in SGOT, SGPT, ALP and bilirubin levels when compared to CCl₄ alone treated rats. Thus the results showed that the ethyl acetate extract at different concentrations (200 and 400mg/kg bw) reduced the serum marker levels comparable to the standard drug silymarin. A significant rise in cytoplasmic transaminases (SGOT and SGPT), alkaline phosphatases (ALP) in circulation was a clear indication of cellular leakage, loss of functional integrity of the cell membrane and necrosis in the liver (*He and Aoyama., 2003*) and the rise in the level of serum total bilirubin (TB) is most sensitive tool that reflects the severity of jaundice (*Sturgill and Lambert., 1997*).

**DISCUSSION**

In Indian system of medicine certain herbs are claimed to provide relief against liver disorders. The claimed therapeutic reputation has to be verified in the scientific manure. In the present study one such drug *Argemone mexicana* was taken for the study.

CCl₄-induced hepatic injuries are commonly used animal models for the screening of hepatoprotective plant extracts and the magnitude of hepatic damage is assessed by measuring the level of released cytosolic transaminases including SGOT, SGPT and ALP in
circulation along with damaged liver architecture (Agarwa et al., 2006). The CCl₄ is biotransformed by the cytochrome P450 system (CYP₂E₁) in the endoplasmic reticulum to produce trichloromethyl free radical (*CCl₃) trichloromethyl free radical when combined with cellular lipids and proteins in the presence of oxygen form trichloromethyl peroxide radical (*OOCCl₃) which may attacks lipids on the membrane of endoplasmic reticulum faster than trichloromethyl free radical. Thus, trichloromethyl peroxyl free radical leads to cell death (Recknagel et al., 1989). These processes are known as lipid peroxidation, leading to functional and structural disruption of hepatocytes (Hogade et al., 2010). Assessment of liver damage can be made by estimating the activities of serum ALT, AST, ALP, TB, which are originally present in higher concentration in cytoplasm. When there is hepatopathy, these enzymes leak into the blood stream in conformity with the extent of liver damage. The elevated levels of these entire marker enzymes observed in the CCl₄ treated group II rats in this present study corresponded to the extensive liver damage induced by toxin. The tendency of these marker enzymes to return towards a near-normalcy in Group IV and V (Argemone mexicana extract 200 and 400 mg/kg bw) treated rats was a clear manifestation of hepatoprotective effect of Argemone mexicana.

The activity levels of AST, ALT and ALP levels in serum are the markers to indicate the structural integrity of the liver tissue. The increased levels of these enzymes in CCl₄ treated animals might be due to the leakage of enzymes into the serum. The significant decrement in the levels of the ALT, AST and ALP in plant extract fed animals might be due to decreased leakage from the liver cells. This suggests that plant extract was able to repair the probable hepatic injury and/or restore the cellular permeability; thus reducing the toxic effect of CCl₄ in the liver tissue. The results are in agreement with the commonly accepted view that serum level of transaminase returns to normal with healing of hepatic parenchyma and the regeneration of hepatocytes (Thabrew et al., 1987).

The bar diagrams representation for the enzyme levels of SGOT, SGPT, ALP and bilirubin (Figure 1-2). It is concluded that ethyl acetate extract have almost equivalent, properly reduced the elevated level biochemical markers when compared with Silymarine.
Figure 1: Effect of ethyl acetate extract of *Argemone mexicana* on SGOT, SGPT, ALP and billirubin of CCl₄ induced hepatotoxicity in rats

Figure 2: Effect of ethyl acetate extract of *Argemone mexicana* on bilirubin of CCl₄ induced hepatotoxicity in rats

**Histopathology**

Histological studies also confirmed the hepatoprotective effect of the ethyl acetate extract of *E. alba*. Histopathological liver sections of Vehicle group showed normal cellular architecture with distinct hepatic cells, sinusoidal spaces, and central vein. (Fig A) The histopathological examination showed typical disarrangement of normal hepatic cells with necrosis, cloudy swelling, fatty degeneration of hepatocytes and vacuolization are observed in Carbon tetrachloride intoxicated liver (Fig B). The liver sections of the rat treated with 200 and 400 mg/kg bodyweight p.o of ethyl acetate extract *Argemone mexicana* followed by carbon tetrachloride intoxication showed less vacuole formation and absence of necrosis and
overall less visible changes observed (Fig D and E) and these changes were comparable with standard Silymarine (Fig C), supplementing the protective effect of the ethyl acetate extract and the standard hepatoprotective drug. Although at lower dose 200mg/kg bw signs of inflammation were present a little bit, but at 400 mg/kg protection was more prominent Thus, histological examination clearly demonstrated the protection of liver by acetate extract *Argemone mexicana* against Carbon tetrachloride cytotoxicity.

![Fig. A: Vehicle Group](image)

![Fig. B: CCl₄ induced group](image)

![Fig. C: Silymarin treated group](image)

![Fig. D: Extract -200mg/kg treated group](image)

![Fig. E: Extract-400 mg/kg treated group](image)
CONCLUSION
In the present study, it was proved that the ethyl acetate extract of *A mexicana* was found to be non toxic to rats up to the dose of 2000 mg/kg.p.o The above study revealed that CCl₄ induced hepatotoxicity in male albino rats, was significantly reduced after being supplemented with the ethyl acetate extract of the folklore medicinal plant *A mexicana*, which revealed its restorative effect. However, to know the exact mechanism of action of *A mexicana*, further studies with purified fractions/bioactive compounds warranted.

REFERENCES