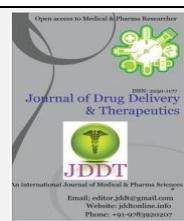


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Research Article

## Amelioration of Paracetamol-Induced Hepatotoxicity in Rat by the Administration of Chloroform extract of *Argemone mexicana*

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### ABSTRACT

**Aim:** The present study was undertaken to examine the effects of Chloroform extract of *Argemone mexicana* using the paracetamol-induced liver damage in rats as the animal model.

**Materials and methods:** Chloroform extract of *Argemone mexicana* (100 and 200 mg/kg body weight) was administered daily in experimental animals. The hepatoprotective efficacy of Chloroform extract of *Argemone mexicana* (100 and 200 mg/kg) was investigated against paracetamol-induced hepatotoxicity. The levels of serum glutamic oxaloacetic transaminase (SGOT), serum glutamic-pyruvic transaminase (SGPT), serum alkaline phosphatase (ALP), bilirubin and triglycerides were estimated. Moreover, chloroform extract of *Argemone mexicana* -aided antioxidant defense against hepatotoxic insult of paracetamol was measured by evaluating a number of anti-oxidative biomarkers including reduced malondialdehyde (MDA) in the serum.

**Results:** Oral administration of paracetamol (500 mg/kg b.wt.) resulted in a significant elevation of liver enzymes in serum such as SGOT, SGPT, ALP, bilirubin and triglyceride levels when compared with the results in the control group. As regards oxidative stress biomarkers, there were increased tissue levels of malondialdehyde in the group treated with paracetamol. All of these results were ameliorated by co-administration of chloroform extract of *Argemone mexicana*.

**Conclusions:** These results suggest that the protective role of Chloroform extract of *Argemone mexicana* in the prevention of PCM-induced hepatic toxicity in rats was associated with a decrease of oxidative stress in hepatic tissues.

**Keywords:** Antihepatotoxicity; Chloroform extract of *Argemone mexicana*; Paracetamol

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### INTRODUCTION

Liver is a vital organ that plays a role in controlling critical biochemical and physiological activities including homeostasis, growth, energy and nutrient supply, detoxification of drugs and other xenobiotics, and also combating infections<sup>1</sup>. Therefore, it is very susceptible to being damaged by hepatotoxic agents<sup>2</sup>.

Paracetamol (acetaminophen) is widely used as an antipyretic and analgesic, and it produces acute liver damage if administered in excess. Paracetamol is mainly metabolized in the liver to excretable glucuronide and sulphate conjugates. However, the hepatotoxicity of paracetamol has been attributed to the formation of toxic metabolites when part of it is activated by hepatic cytochrome P-450 to form the highly reactive metabolite N-acetyl-P-benzoquinone imine (NAPQI). NAPQI covalently

binds to cysteine groups on proteins to form 3-(cystein-S-yl) acetaminophen adducts. The glutathione protects hepatocytes by combining with the reactive metabolite of paracetamol, thus preventing covalent binding to liver proteins<sup>3</sup>.

Many newly developed drugs (e.g., rimonabant, propylthiouracil, or corticosteroids) have been used for treatment of liver diseases; however, these drugs possess harmful side effects such as insomnia, vomiting, constipation, and depression. For that reason, further research on plants and herbs that could potentially substitute the chemical-based drugs is very crucial as many medicinal plants have been found to possess hepatoprotective properties<sup>4</sup>.

Traditionally rhizome of *Nardostachys jatamansi* is used as antidiabetic activity, anti-cancer activity, anti-hiv activity, cns related activities, wound recovering action, anti

microbial activity, antioxidant activity, anti-inflammatory, analgesic, antipyretic activity, hepatoprotective activity, anti-fertility activity, antiallergic activity, nematicidal activity, allelopathic effect, antihelmintic activity, larvicidal activity, antifeedant action<sup>5</sup>. Therefore, the present study was aimed at determining the hepatoprotective activity of Chloroform extract of *Argemone mexicana* using the paracetamol-induced liver damage in rats as the animal model.

## MATERIALS AND METHODS

### Collection of plant materials

Selected whole plant materials of *Argemone mexicana* L. were collected and after the plant was collected they have been processed for cleaning in order to prevent the deterioration of phytochemicals present in plant.

### Preparation of Extract<sup>6-7</sup>

### Defatting of Plant Material

The shade dried Whole plant materials of *Argemone mexicana* L. were extraction with petroleum ether using maceration method. The extraction was continued till the defatting of the material had taken place.

### Extraction by maceration Method<sup>8</sup>

Whole plant material were extracted in four solvents of different polarity viz water, methanol, ethyl acetate and chloroform. Powdered plant material (100 gm) was extracted by maceration method. The resultant content was filtered with whatman filter paper no.1 and kept for evaporation of solvent to get the dry concentrated extract.

### Qualitative phytochemical analysis

Phytochemical examinations were carried out extracts as per the standard methods<sup>9-11</sup>.

### Animals

Wistar rats (150–200 g) were group housed (n= 6) under a standard 12 h light/dark cycle and controlled conditions of temperature and humidity (25±2 °C, 55–65%). Rats received standard rodent chow and water *ad libitum*. Rats were acclimatized to laboratory conditions for 7 days before carrying out the experiments. The study protocols were approved by the Institutional Animal Ethics Committee (IAEC).

### Drugs and Chemicals

Paracetamol and Silymarin (Sigma chemicals, USA) were used in present study. All other chemicals and other biochemical used in the experiments were of analytical grade from different firms.

### Experimental designs

#### Experimental design and treatment protocol

Rats were acclimated to animal laboratory conditions at 25°C, 55% humidity, and a 12 h:12 h light-dark cycle for seven days prior to testing. Water was supplied *ad libitum*, and the rats were fed a basal diet for the entirety of the study.

#### Paracetamol-induced hepatotoxicity<sup>12</sup>

**Group -I:** Normal control (0.5% CMC 1 ml/kg, p.o.)

**Group -II:** Rats were subcutaneously injected with Paracetamol (500 mg/kg b.wt.)

**Group -III:** Rats were subcutaneously injected with Paracetamol (500 mg/kg b.wt.) and silymarin 10 mg/kg.

**Group -IV:** Rats were subcutaneously injected with Paracetamol (500 mg/kg b.wt.) and Chloroform extract of *Argemone mexicana* 100mg/kg

**Group -V:** Rats were subcutaneously injected with Paracetamol (500 mg/kg b.wt.) and Chloroform extract of *Argemone mexicana* 200mg/kg

At the end of four weeks, food (but not water) was withheld from all animals for 12 h. All rats were sacrificed with isoflurane. Blood samples were collected in clean centrifuge tubes via cardiac puncture. The samples were centrifuged at 3000 rpm for 15 min to separate the serum. The serum was carefully removed and transferred into lavender test tubes and solidified at 20°C until utilization for biochemical experiments.

### Biochemical Evaluation in Serum

Blood samples were centrifuged for 10 min at 7000 rpm using micro-centrifuge to separate the serum. The levels of serum glutamic oxaloacetic transaminase (SGOT), serum glutamic-pyruvic transaminase (SGPT), serum alkaline phosphatase (SALP), bilirubin and triglycerides were estimated.

### Determination of antioxidant defense

The antioxidant activity of Chloroform extract of *Argemone mexicana* (100 and 200 mg/kg) was evaluated by investigating the levels of malondialdehyde (MDA) in the serum specimens of all experimental groups. The level of lipid peroxidation was determined by measuring the level of MDA using a previously established method<sup>13</sup>.

### Statistical analysis

The data were represented as mean ± SEM. Analysis of variance test was followed by individual comparison by Dunnett's test using Graph Pad Prism Software (Version 3.0) for the determination of the level of significance. The results were considered significant at P<0.05.

## RESULTS

### Phytochemical Screening

Preliminary phytochemical screening of *Argemone mexicana* all extracts revealed the presence of alkaloids, flavonoids, diterpenes, phenol and saponins among which flavones were the most prominent ones and the results are summarized in table 1. all extracts showed the presence of alkaloids, flavonoids, diterpenes, phenols and saponins.

### Acute toxicity

The extract was found to be safe in the dose used and no mortality up to a dose of 3000 mg/kg, b.w. for Chloroform extract was observed. Hence, 200 and 400 mg/kg b.w. p.o. were selected for the activity.

### Effect of Chloroform extract of *Argemone mexicana* (100 and 200 mg/kg) on paracetamol-induced hepatotoxicity and liver enzymes

The administration of PCM resulted in a marked increase in serum SGOT, SGPT, ALP, bilirubin and triglycerides. The protective actions of chloroform extract of *Argemone mexicana* at dose level of 100 and 200 mg/kg b.w on hepatotoxicity induced by PCM are summarized in Table 2. Pretreatment of the rats with chloroform extract of *Argemone mexicana* before PCM administration caused a

significant reduction in the values of SGOT, SGPT, ALP, bilirubin, and triglycerides.

**Effect of Chloroform extract of *Argemone mexicana* on MDA levels in the serum**

In Chloroform extract of *Argemone mexicana* 100 and 200 mg/kg/p.o. ( $1.55 \pm 0.40$ ;  $1.40 \pm 0.30$ ) treated group

malondialdehyde levels (MDA) level decreased significantly ( $p < 0.05$ ). In 10 mg/kg p.o. Silymarin ( $1.00 \pm 0.30$ ) treated group MDA level decreased significantly ( $p < 0.05$ ), respectively as compared with control group ( $2.65 \pm 0.08$ ), as shown in Table 3.

**Table 1: Result of Phytochemical screening of whole plant material of *Argemone mexicana* L**

S. No.	Constituents	Chloroform extract	Ethyl acetate extract	Methanol extract	Aqueous extract
1.	<b>Alkaloids</b> Hager's Test:	+ve	+ve	+ve	+ve
2.	<b>Glycosides</b> Legal's Test:	-ve	-ve	-ve	-ve
3.	<b>Flavonoids</b> Lead acetate Test:	+ve	+ve	+ve	+ve
4.	<b>Diterpenes</b> Copper acetate Test:	+ve	+ve	+ve	+ve
5.	<b>Phenol</b> Ferric Chloride Test:	+ve	+ve	+ve	+ve
6.	<b>Proteins</b> Xanthoproteic Test:	-ve	-ve	-ve	+ve
7.	<b>Carbohydrate</b> Fehling's Test:	+ve	-ve	+ve	-ve
8.	<b>Saponins</b> Froth Test:	+ve	+ve	+ve	+ve

**Table 2: Effect of Chloroform extract of *Argemone mexicana* on biochemical evaluation in serum in paracetamol induced hepatotoxicity in rats**

Group	Drug	Dose	SGPT (%)	SGOT (%)	Triglyceride (mg/dl)	ALP ( $\mu$ L)	Serum Bilirubin (g/dl)
I	Normal	0.5% CMC 1 ml/kg, p.o.	$90.0 \pm 9.74$	$90.0 \pm 9.74$	$70.00 \pm 8.00$	$90.0 \pm 10.00$	$90.0 \pm 6.50$
II	Paracetamol	500 mg/kg	$266.5 \pm 9.50$	$265.00 \pm 5.00$	$131.00 \pm 8.50$	$225.0 \pm 8.00$	$215.10 \pm 8.00$
III	Silymarin	10 mg/kg p.o.	$140.50 \pm 8.50^{***}$	$135.0 \pm 6.00^{***}$	$85.00 \pm 7.80^*$	$147.05 \pm 6.00^{**}$	$125.60 \pm 6.70^{**}$
IV	Chloroform extract of <i>Argemone mexicana</i>	100 mg/kg p.o.	$187.0 \pm 7.50^{**}$	$183.40 \pm 6.00^{**}$	$106.05 \pm 6.50^*$	$174.50 \pm 6.50^*$	$150.30 \pm 7.50^*$
V	Chloroform extract of <i>Argemone mexicana</i>	200 mg/kg p.o.	$171.0 \pm 6.50^{**}$	$168.0 \pm 5.50^{**}$	$98.00 \pm 7.00^*$	$163.50 \pm 6.50^*$	$143.50 \pm 6.50^*$

Values are expressed as the mean  $\pm$  SEM of six observations. \*\*\*  $P < 0.001$  vs. control treatment (One-way ANOVA followed by Dunnett's test)

**Table 3: Effect of Chloroform extract of *Argemone mexicana* on malondialdehyde levels (MDA) level in paracetamol induced hepatotoxicity in rats**

Group	Drug	Dose	MDA $\mu$ mol/L
I	Normal	0.5% CMC 1 ml/kg, p.o.	$0.65 \pm 0.07$
II	Paracetamol	500 mg/kg	$2.65 \pm 0.08$
III	Silymarin	10 mg/kg p.o.	$1.00 \pm 0.30^{**}$
IV	Chloroform extract of <i>Argemone mexicana</i>	100 mg/kg p.o.	$1.55 \pm 0.40^*$
V	Chloroform extract of <i>Argemone mexicana</i>	200 mg/kg p.o.	$1.40 \pm 0.30^*$

Values are expressed as mean  $\pm$  S.E.M. ( $n = 6$ ). Values are statistically significant at  $p < 0.05$  (One-way ANOVA followed by Dunnett's test).

## DISCUSSION

Paracetamol is a common antipyretic agent that is safe in therapeutic doses, but can produce fatal hepatic necrosis in man, rats and mice with toxic doses. PCM toxicity is due to the formation of toxic metabolites when a part of it is metabolized by cytochrome P-450. Introduction of cytochrome or depletion of hepatic glutathione is a prerequisite for PCM-induced hepatotoxicity. Due to liver damage, cellular leakage and loss of functional integrity results elevated serum enzymes levels<sup>14</sup>.

In the present study, PCM was caused significant elevation in the levels of SGOT, SGPT, SALP, bilirubin and triglycerides. Pretreatment with chloroform extract of *Argemone mexicana* was found to be significantly reversing PCM induced changes. Hence, a reduction in the levels of these enzymes demonstrates membrane stabilizing activity of the extract. Reduction in the levels of SGOT and SGPT towards the normal value is an indication of the regeneration process. The present data agreed with Nithianantham *et al.*<sup>15</sup> who found that paracetamol significantly increased SGPT, SGOT and bilirubin levels. Furthermore Abdel-Azeem *et al.*, said that acute paracetamol toxicity resulted in remarkable elevation in the activities of plasma SGPT, SGOT and ALP<sup>16</sup>.

Paracetamol administration in the last 5 progressive days induced oxidative stress in rat livers, as prove by a critical elevation in MDA generation. Lipid peroxidation influences the liver to more prominent degree causing the formation of high molecular mass protein aggregated with the membrane. Thus, the expansion in the level of MDA is a pointer of lipid peroxidation<sup>17</sup>. The reactive oxidative stress attacks polyunsaturated fatty acids and disturbs the cell membrane. It prompts oxidative lipid and form MDA, a product of lipid peroxidation. The Increase in production of liver MDA reported in our trials by paracetamol are coincided with previous study which reported that paracetamol increased extracellular MDA level<sup>18</sup>. The present results suggested that administration of chloroform extract of *Argemone mexicana* or silymarin prior and simultaneously with paracetamol resulted in critical reduction in activity of MDA compared with paracetamol group.

## CONCLUSION

Chloroform extract of *Argemone mexicana* has dose dependent hepatoprotective action upon PCM induced hepatotoxicity in rats, which may be attributed because of the presence of the phytoconstituents such as alkaloids, flavonoids, glycosides, etc., however, there is a need to identify the exact mechanism (s) and active phytoconstituent (s) involved in this effect for future studies.

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