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Effect of *Carduus marrianus* and *Chelidonium majus* in hepatic damage

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Abstract
The present study was undertaken to investigate hepatoprotective activity of *Carduus marrianus* and *Chelidonium majus* against paracetamol intoxicated rats and supplemented by histopathological studies of liver tissues. The Homoeopathic Potencies of *C. marianus* and *C. majus* have significantly altered the Paracetamol induced liver damages. Hepatic collagen content as evident from decreased (p<0.05) hydroxyproline levels and hepatic mast cell infiltration were significantly decreased in pre-treated animals. In addition, *C. marianus* and *C. majus* potencies significantly (p<0.05) reduced hepatic lipid peroxidation as evident in the liver tissues. The findings of study suggested that potencies of *C. marianus* and *C. Majus* heal the Paracetamol induced Hepatic tissue damages. Hence it is have more potential effect in hepatoprotective activity.

Keywords: *Carduus marrianus, Chelidonium majus,* hepatoprotective, Silimarin

Introduction

Carduus marianus

The part used in medicine is the fruit known as *Fructus silybi*, or *Semen carduimariae*. Homoeopathic mother tincture is prepared by covering the whole ripe seeds (1 part by weight) with diluted alcohol (2 parts), and letting stand in a dark, cool place in a well-stoppered bottle for 8 days, shaking twice a day. It is then decanted, strained, and filtered. Congestion of the liver, spleen and kidneys is relieved by its use [1]. Bilious states, with stitches in the side and pain in the abdomen, hard and tender right hypochondrium, gall stones, jaundice, hepatic pain and swelling, vomiting of pregnancy, and leucocythemia, are conditions in which it is reported useful.

Chelidonium majus

This (Papaveraceae) is a plant of great interest for its use in various diseases in European countries and in Chinese herbal medicines. Crude extracts of various parts such as the root, shoot and leaves have been reported to have several isoquinoline alkaloids, such as, sanguinarine, chelidonine, chelerythrine, berberine and coptisisine. Both crude extracts of *C. majus* and purified compounds derived from it have been reported to exhibit interesting anti-viral, anti-inflammatory, anti-tumor and anti-microbial properties both *in vitro* and *in vivo* [2-4].

In the homeopathic mode of treatment, various micro doses (potencies) of Chelidonium herb extract are routinely used against several forms of liver disorders, But, to our knowledge, whether ultra-low doses of *C. majus*, namely, Chelidonium-30 (Ch-30) and Chelidonium-200 (Ch-200), & Chelidonium 1M could also have similar activities had not been experimentally tested so far in mice *in vivo*. The recent investigation was therefore undertaken primarily to examine if Ch-30 and Ch-200, Chelidonium prepared as per homeopathic procedure ameliorating effects in the activities of some marker enzymes like acid and alkaline phosphatases, and peroxidase in various tissues during Paracetamol induced hepatic damage in Wister albino rats [5].

Materials and methods

Test substance

a) Paracetamol, b) *Carduus marianus,* and *Chelidonium majus,* c) Distilled water, d) Drachm bottle

Test system and management

Species : Albino rats
Sex : Both
Numbers : 120
Body weight range : 150 to 180gms
Identification : By cage card and corresponding picric acid colour.
Number of animals : 06 each group
Acclimation : One month in experimental room.
Selection of animals : After acclimatization the animals will be subjected to a gross observation, to ensure that the selected animals are in good state of health. Animals will be then randomly selected for final allotment to the study.

Environmental Conditions: Air conditioned room with optimal air changes per Hour, relative humidity, temperature and illumination cycle set to 12 h light and 12 h dark.

Accommodation: Groups housed in polypropylene cages with stainless steel grill top, facilities for food and water bottle and bedding of clean paddy husk.

Diet: Brand feed pellet was provided *ad libitum*

Water: U.V.Purified and filtered water was provided *ad libitum* in polypropylene bottles with stainless Steel sipper tubes.

Principle of the Experiments

In Homoeopathic system and traditional system of medicine, there are a number of plants which are used in the treatment of Liver diseases and its complication [6]. *C. marrianus* and *C. majus* exhibited marked action on liver, which could be related to their protective and curative activity against liver related complication.

Study design

Albino Rats weighing about 150gms are marked. Animals are kept to overnight fasting. Divide the animals into ten groups and 6 animals in each group.

Group's treatment

This experimental study was conducted in hygienic conditions with normal food (made up of powdered wheat, gram and milk) without any other animal/plant protein supplement and water *ad libitum*, were divided into various sets of 06 rats in each group.

Group I: Normal control for estimate normal enzymatic level and normal hepatic texture

Group II: Negative control (Paracetamol 750mgs/kg was given orally once and kept on placebo)

Group III: Paracetamol 750mgs/kg was given orally once, after 36 hours *C. marianus*12X (aqueous preparation) potency 0.5ml was given orally once a day for 10days.

Group IV: Paracetamol 750mgs/kg was given orally once, after 36 hours *C. marianus* 30 potency 0.5ml was orally given once a day for 10days.

Group V: Paracetamol 750mgs/kg was given orally once, after 36 hours *C. marianus* 200 potency 0.5ml was given orally once a day for 10days.

Group VI: Paracetamol 750mgs/kg was given orally once, after 36 hours *C. marianus* 1M potency 0.5ml was given orally once a day for 10days.

Group VII: Paracetamol 750mgs/kg was given orally once, after 36 hours *C. majus*12x potency 0.5ml was given orally once a day for 10days.

Group VIII Paracetamol 750mgs/kg was given orally once; after36 hour's *C. Majus* 30 potency 0.5ml was given orally once a day for 10days.

Group IX: Paracetamol 750mgs/kg was given orally once, after 36 hours *C. Majus* 200 potency 0.5ml was given orally once a day for 10days.

Group X: Paracetamol 750mgs/kg was given orally once, after 36 hours *C. Majus* 1M potency 0.5ml was given orally once a day for 10days

After 10 days all the rats were sacrificed under light ether anesthesia. Blood samples were collected in sterile centrifuge tube and allowed to clot. Serum was separated by centrifuging at 2,500 rpm for 15minutes and used to estimate the serum oxylopyride transaminase, Serum Glutamo oxylo Transaminase, serum alkaline phosphate, Total protein and Total Bilirubin.

After the animals were sacrificed, the abdomen of each is cut opened and the liver is removed. The ratio of wet liver weight per 100g of animal is calculated. The livers were preserved in neutral buffered formalin and were processed for paraffin embedding, following the standard micro-technique. Five-micron section of liver, stained with alum hematoxylin and eosin were observed under microscope for histo-pathological changes.

After evaluating enzymatic changes in paracetamol induced hepatic damage all the groups were analyzed by appropriate statistical method.

Procedure

1. Animals were divided into 10 groups each group of 06 animals
2. For comparison,
   I. was designated as normal control group,
   II. was designated as disease induced non-treated group,
   III. To X was designated as treated groups.
1. The hepatic damage was by inducing paracetamol 750 mg/kg body weight.
2. The animals were maintained for 36 hours for the development of hepatic damage and its complications.
3. Treatment was started from the end of 36 hours and continued till the end of the 10th day. The animals of group III to VI were treated with *C. marianus* 12x, 30, 200 & 1m respectively and the animals of group VII to X were treated by *C. majus* 12x, 30, 200 & 1m respectively.
4. At the end of the 10th day all the rats were sacrificed under light ether anesthesia. Blood samples were collected in sterile centrifuge tube and allowed to clot. Serum was separated by centrifuging at 2,500 rpm for 15minutes and used to estimate the serum oxylopyride Transaminase, serum Glutamooxylo Transaminase, serum alkaline phosphate, Total protein and Total Bilirubin.
5. After the experiment animals were sacrificed and liver was removed for histo- pathological studies.

Dose formulation

C. marianus 12x, 30, 200 & 1m were treated respectively to the animal groups, III to VI. Group VII to X were treated

with *Chelidonium majus* 12x, 30, 200 & 1m respectively

Administration of test substance

The test substance was administered by oral gavage to each animal as a single dose, using an incubation needle fitted on to a syringe of appropriate size. The dose administered to individual animal will be calculated according to its body weight recorded from the day of administration of test substance.

Statistical analysis

Results of biochemical estimations were expressed as means of six animals in each group. The statistical analysis was carried out using one way ANOVA. The difference in values at $P \leq 0.05$ was considered as statistically significant.

Histopathological studies

The liver samples were excised from the animals of each group after draining the blood and washed with normal saline. Initially the materials were fixed in 10% buffered neutral formalin for 48 hrs. They were processed for paraffin embedding. The sections were taken at 5 µm thickness, processed in alcohol-xylene series and were stained with alum- haematoxylin and eosin [7]. The sections were examined microscopically for the evaluation of histo-pathological changes.

Table 1: Enzyme level of control, toxic control and standard

Groups	Biochemical parameters					
	SGPT (IU/L)	SGOT (IU/L)	SALP (IU/L)	Bilirubin Direct (mg/dL)	Bilirubin Indirect (mg/dL)	Triglycerides (mg/dL)
Control	65.05±0.74	108.16±0.06	74.71±1.06	0.16±0.004	0.58±0.01	132.40±1.28
Toxic Control	124.34±1.90*	242.66±0.34*	188.60±2.04*	0.96±0.02*	1.88±0.12*	194.20±1.88*
Standard	72.62±0.90***	120.12±12***	78.66±1.20***	0.20±0.02***	0.66±0.04***	146.80±0.8***

*P<0.05 denote value significant from control,
*P<0.05, **P<0.01, ***P<0.001 denote value significantly different from toxic control

Table 2: Level of liver enzymes treated with *Carduus marianus* 12X, 30X, 200X & 1M

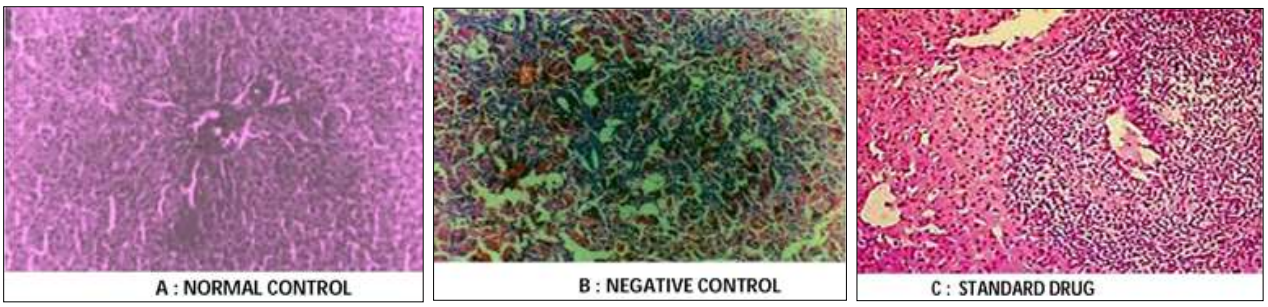
Groups	Biochemical parameters					
	SGPT (IU/L)	SGOT (IU/L)	SALP (IU/L)	Bilirubin Direct (mg/dL)	Bilirubin Indirect (mg/dL)	Triglycerides (mg/dL)
Paracetamol 750mgs/kg + cardusmarianus12X potency 0.5ml	106.54±0.68**	156.60±0.84**	96.26±1.26**	0.38±0.004**	0.78±0.02**	160.6±0.36**
Paracetamol 750mgs/kg + cardusmarianus30X potency 0.5ml	98.08±0.28*	145.82±0.12*	92.06±1.08*	0.36±0.04*	0.74±0.02*	158.06±2.28*
Paracetamol 750mgs/kg + cardusmarianus200X potency 0.5ml	78.06±0.36*	128.62±0.22*	80.04±1.06*	0.24±0.06*	0.68±0.03*	150.02±2.26*
Paracetamol 750mgs/kg +cardusmarianus1M potency 0.5ml	90.06±0.36*	135.92±0.12*	84.04±1.06*	0.28±0.04*	0.70±0.03*	156.02±2.26*

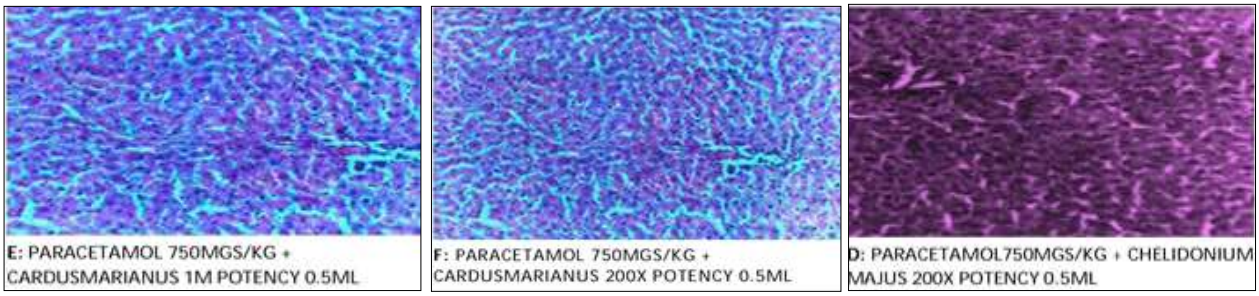
*P<0.05 denote value significant from control,
*P<0.05, **P<0.01, ***P<0.001 denote value significantly different from toxic control

Table 3: Level of liver enzymes treated with *Chelidonium majus 12 X, 30X, 200X & 1M*

Groups	Biochemical parameters					
	SGPT (IU/L)	SGOT (IU/L)	SALP (IU/L)	Bilirubin Direct (mg/dL)	Bilirubin Indirect (mg/dL)	Triglycerides (mg/dL)
Paracetamol 750mgs/kg + *Chelidonium majus*12X potency 0.5ml	110.24±0.42**	168.20±0.82**	108.28±1.28**	0.42±0.008**	0.82±0.06**	176.8±0.38**
Paracetamol 750mgs/kg + *Chelidonium majus*30X potency 0.5ml	102.06±0.28*	150.82±0.12*	98.06±1.06*	0.40±0.08*	0.80±0.02*	160.06±2.22*
Paracetamol 750mgs/kg + *Chelidonium majus*200x potency 0.5ml	82.08±0.38*	132.60±0.26*	88.02±1.08*	0.30±0.08*	0.76±0.06*	154.06±2.28*
Paracetamol 750mgs/kg + *Chelidonium majus*1M potency 0.5ml	116.28±0.40**	170.22±0.86**	112.22±1.26**	0.46±0.006**	0.92±0.08**	180.8±0.36**

*P<0.05 denote value significant from control,
*P<0.05, **P<0.01, ***P<0.001 denote value significantly different from toxic control





A: Histopathology of normal liver of control animals showed normal hepatic lobules.B: Histopathology of Paracetamol (750mg/kg bw) liver showing hepatocyte degeneration, obstruction of hepatic sinusoids with pervasive of hepatocellular necrosis C: Histopathology of Standard drug showed mild hepatocellular necrosis. Both D and E: Histopathology of paracetomol (750mg/kg bw), *Chelidonium majus* 200x patency 0.5 ml and *Cardus marianus* 200x showing reduce damage of hepatic sinusoids and mild regeneration of hepatocellular necrosis. F: Histopathology of paracetamol (750mg/kg bw) and *Cardus marianus* 1M potency 0.5 ml showing moderately reduced damaged hepatic lobules, regeneration of hepatocellular necrosis.

Fig 1: Effect of *Carduus marianus* and *Chelidonium majus* in hepatic damage

Results

The results of the acute toxicity study indicated that there was no toxicity observed. In addition, the total protein level, and urea level were decreased reflecting the liver injury due to the toxic effect of Paracetamol [8, 9, 10]. The blood samples of the animals treated drug showed significant reduction in the levels of liver function serum markers and bilirubin and increased the total protein, and urea level to restore the normal condition. The effect was more pronounced in the animals treated with our drug more effective than the effect of the standard drug silymarin. The histological profile of control animal showed normal hepatocytes (Fig. 1a). The section of liver of the animals treated with parcetamol exhibited intense centrilobular necrosis, vacuolization and macro vesicular fatty changes. The liver sections of silymarin treated animals showed normal hepatic architecture (Fig. 1c). Significant accumulation of fatty lobules was observed in the liver sections (Fig. 2d) of S5 treated animals. The liver sections of the animals treated with Livome exhibited significant liver protection against CCl4 induced liver damage as evident by the presence of normal hepatic cords, absence of necrosis and fatty infiltration (Fig. 2e).

Discussion

Homeopathy medicines are gaining popularity in developing countries because of its natural and harmless effect. This increase in popularity and the scarcity of scientific studies on their safety and efficacy have raised concerns regarding toxicity and adverse effects of these remedies [14]. These medicines in its original state contain measurable bioactive principles with the potential to cause adverse effects [15]. In India there are large numbers of electro homoeopathic practitioners exist and millions of publics are receiving benefits from the electro homoeopathic mode of treatment for acute and chronic diseases [16].

In the present investigation, the development of toxin-induced necrotic liver injury was noticed in rats by the administration of the toxin paracetamol. The results of biochemical parameters revealed the elevation of enzyme level in paracetamol-treated group, indicating that paracetamol induced damage to the liver. A significant reduction was observed in SGPT, SGOT, ALP, total bilirubin and increase in protein level in the groups treated with standard drug silymarin and the groups treated with *C. marrianus* 12X,30,200,1M and *C. majus*, 12X,30,200,1M.

Conclusion

The results of our investigation revealed that the homoeopathic preparations *C. marrianus*12X, 30, 200, 1M and *C. majus* 12X, 30, 200, 1M do not exhibit any toxicity and were more effective hepatoprotective at par with the standard drug as manifested by restoration of (Paracetamol induced liver toxicity). More scientific research is required in understanding basic principles, phyto-constituents of active principle, and mechanism of action of homoeopathic medicines by the application of current advanced techniques and tools for the benefit of future generations.

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