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Evaluation of Hepatoprotective effect of roots of Paeonia officinalis Linn. in Paracetamol induced Hepatotoxicity

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Abstract: The hepatoprotective effect of methanolic extract of root of *Paeonia officinalis* was evaluated against Paracetamol induced hepatic damage in rats. Paracetamol (2 g/kg) increases the serum levels of SGOT, SGPT, Bilirubin (total and direct), ALP and decrease in serum levels of GSH. The dose of 400mg/kg/day of *Paeonia officinalis* was found to be much more hepatoprotective than 200mg/kg/day dose as evidenced by biochemical parameters and marked regenerative activity observed in rat that received 400mg/kg/day dose. However, Silymarin was found to be more effective as a hepatoprotective than the aqueous extracts of the roots of *Paeonia officinalis*.

Keywords: *Paeonia officinalis*; Hepatotoxicity; Liver; Root.

Introduction: The liver is a vital organ of paramount importance involved in the maintenance of metabolic function and detoxification from the exogenous and endogenous challenges, like xenobiotics, drugs, viral infection and chronic alcoholism. If during all such exposures to these challenges, the natural protective mechanisms of the liver are overpowered, the result is a hepatic injury. Damage to liver is always associated with cellular necrosis and increase in serum levels of many biochemical markers like SGOT, SGPT, ALP and bilirubin.[1]

Medicinal plants are gaining importance in the fields of research. Medicinal plants originate from almost every part of the globe. Such plants serve the primary healthcare needs of up to 80 % of people in developing countries where there is increasing awareness of and demand for medicinal plants for healthcare and dietary supplements that often help to save lives.[2]

Unavailability of rational therapy in modern medicine and no or very less positive influence of synthetic drugs in liver damage have urged researchers in this field to look for herbal drugs with better hepatoprotective action. Numerous medicinal plants and their formulations are used for liver disorders in ethno medical practices and in traditional system of medicine in India.[3] About 160 phytoconstituents from 101 plants have been reported to possess hepatoprotective activity.[4] In India, about 40 polyherbal commercial formulations are available and prescribed by physicians to treat hepatic disorders.[5]

The root of *Paeonia officinalis*, (Ood Saleeb) has been used in Unani, Ayurvedic and Homoepathic systems of medicine for years.[6, 7] However, no phytochemical investigation, toxicity study or anti-hepatoprotective study has been carried out on this plant.[7]

So the present study was undertaken to study the antihepatotoxic profile of the roots of *Paeonia officinalis* against Paracetamol induced hepatotoxicity in albino rats.

Paracetamol (PCM) is a widely used industrial chemical and a potent hepatotoxin. Ingestion of high doses leads to acute liver failure accompanied by centri-lobular degeneration and necrosis in the liver of both man and experimental animals. Toxicity of paracetamol is thought to be produce by N-acetyl-p-benzoquinineimine, a reactive electrophilic metabolite of a cytochrome P-450 mediated reaction.[8]

2. Materials and Methods

Plant material

Dried roots of *Paeonia Officinalis* were obtained from a local area of Orissa. A sample of the plant material was deposited in the herbarium of the Department of Pharmaceutical Sciences for future reference.

Preparation of extract

Methanolic extract of root of *Paeonia officinalis* (APO) was prepared by the method given by Alkofahi.[9] Dried Paeonia officinalis roots were pulverized the powdered material (1kg) was macerated in distilled water for 24 hours with occasional shaking and then it was allowed to stand for 18 hours. The contents were kept for elution and then filtered. The extract was evaporated to dryness under reduced pressure and controlled temperature (40- 50 ºC) (yield 10%, w/w).

Animals

Albino rats of Wistar strain, both sexes, weighing 125-150gm, were procured from the animal house of Indian Institute of Integrative Medicine (IIIM) canal road, Jammu. The animals were kept in polypropylene cages (6 in each cage) under standard laboratory conditions (12 hour light and 12 hour dark: day and night cycle) and had a free access to commercial pelleted diet (Ashirwad Industries) and tap water ad libitum. All studies were performed in accordance with the guide for the care and use of laboratory animals, as adopted and promulgated by the Institutional Animal Care Committee, CPCSEA, India (Reg. No. IAEC/PHARM.S/CL/KU/2012). All the chemicals used were of the analytical grade from standard companies and the water used was always the double distilled water.

PCM induced hepatotoxicity

Hepatotoxicity was induced by orally administered paracetamol (2g/kg). The animals were divided into five groups of 6 animals each.

Group I- Served as control group received vehicle (1% CMC).

Group II- Served as toxic control received 1% CMC (1ml/kg) for one week and administration of Paracetamol (2g/kg orally) on 5th day.

Group III- Served as standard received Silymarin at a dose of 100 mg/kg., once a day for seven days and administration of Paracetamol (2g/kg orally) on 5th day.

Group IV- Served as test-1 received leaf and bark methanolic extract at a dose of 200 mg/kg., once a day for seven days and administration of paracetamol (2g/kg orally) on 5th day.

Group V- Served as test-2 received leaf and bark methanolic extract at a dose of 400 mg/kg., once a day for seven days and

administration of paracetamol (2g/kg orally) on 5th day.

On the seventh day after 2 hour of respective treatments all rats were sacrificed by cervical dislocation after collecting the blood from retro-orbital plexus using fine glass capillary and collected in plain sterile microcentrifuge tubes under ether anesthesia for biochemical estimations. The blood serum was separated by centrifugation at 7000 rpm for15 min. The liver from all the animals were collected, washed, weighed and used for the histopathological study.

Assessment of Hepatoprotective activity

The following biochemical investigations were carried out in serum: Serum alanine transaminase (ALT)[10],Serum aspartate transaminase (AST)[11], Serum alkaline phosphatase (ALP)[12], Total bilirubin[13], and Total serum protein[14].

After collecting the blood samples, the animals from all groups were sacrificed by cervical dislocation. The abdomen of the animals was cut open to remove the liver which was washed with normal saline and then fixed in 10% neutral formalin solution to be processed separately for histological observation.

Histopathological studies

Thin sections (5 µM) were cut and stained with routine hematoxylin and eosin stain for photo microscopic assessment. The initial examination was qualitative, with the purpose of determining histopathological lesions in liver tissue.[15]

Statistical Analysis

All the results were expressed as mean ± SEM. One-way Analysis of Variance (ANOVA) was used for the statistical analysis of data. Student's't' test was used for determining the significance. A probability value of $p<0.05$ was considered as significant and $p<0.01$ was considered highly significant.

3. Results

PCM treated rats showed significant increase in the serum levels of SGOT, SGPT, Bilirubin (total and direct), ALP and decrease in serum levels of GSH (Table number 1).

Table number: 1

Effect of the methanolic extract of *Paeonia officinalis* on different biological parameters.

Group	Dose	SGOT (IU/L)	SGPT (IU/L)	ALP (IU/L)	GSH (IU/L)	Direct bilirubin mg/dl	Total bilirubin mg/dl
Normal Control	-	36.10 ±1.34	46.19 ± 0.63	50.10 ± 1.44	7.92 ± 0.55	1.16 ± 0.05	1.42 ± 0.02
PCM Only	2 g/kg	105.4 ± 2.85	120.7 ± 1.02	81.13 ± 1.51	4.90 ± 0.36	3.12 ± 0.10	6.29 ± 0.09
Silymarin + PCM	100 mg/kg	48.28 ± 1.19**	54.00 ± 1.09*	51.44 ± 1.23**	7.23 ± 0.12**	1.46 ± 0.03**	1.79 ± 0.02**
Extract + PCM	200 mg/kg	68.81 ± 1.78*	81.52 ± 0.61**	76.63 ± 1.42**	6.54 ± 0.22**ns**	2.32 ± 0.09*	2.41 ± 0.15**
Extract + PCM	400 mg/kg	65.31 ± 3.09**	64.94 ± 1.00*	65.04 ± 1.13**	7.05 ± 0.31**	2.17 ± 0.06**	2.21 ± 0.08**

Results expressed as mean ± SEM and **P<0.001 as compared to toxic control, *P<0.01 compared to toxic control & ns (non-significant) as compared to toxic control.

Figure number 1: Graph showing variation in different parameter levels of SGOT, SGPT and ALP against Paracetamol (PCM) Toxic group.

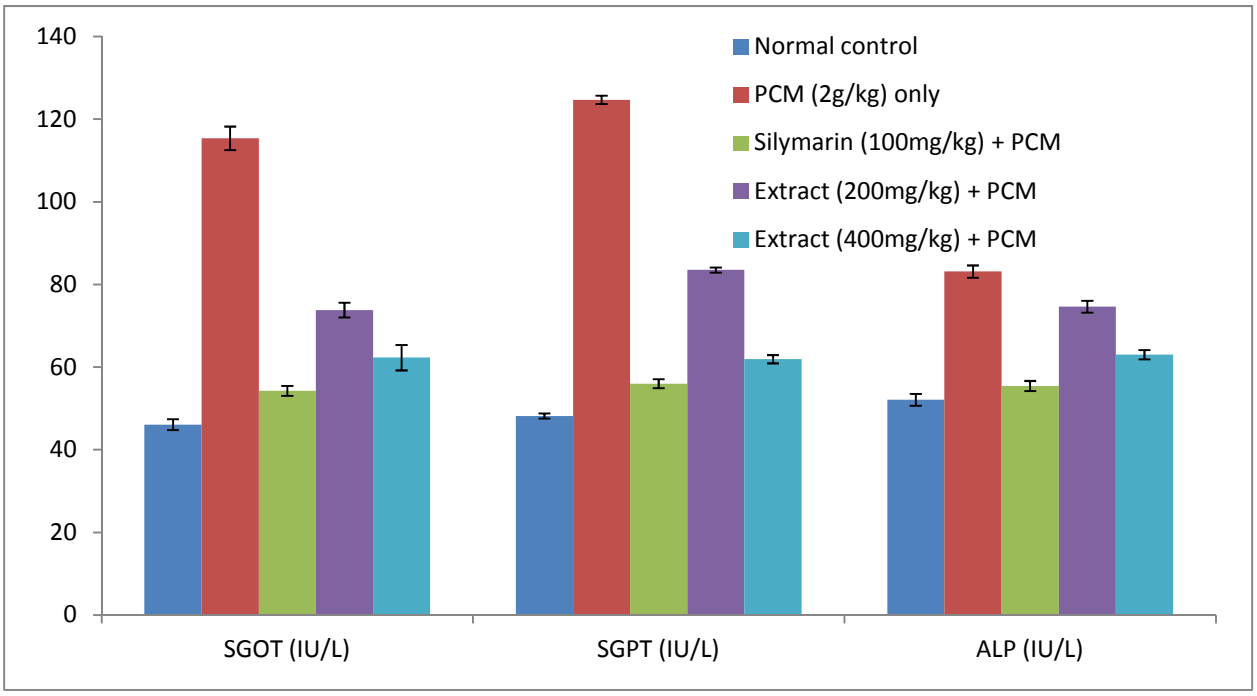
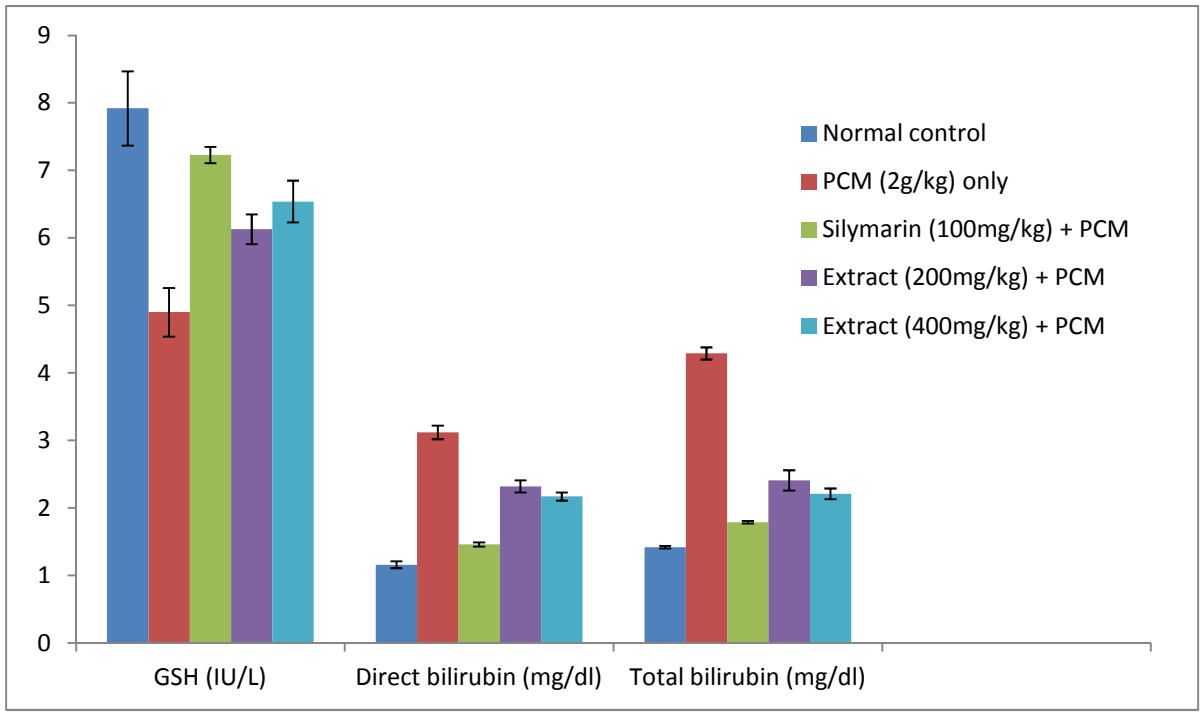


Figure number 2: Graph showing variation in different parameter levels of GSH, Direct bilirubin and Total bilirubin against Paracetamol (PCM) Toxic group.



The treatment with Silymarin significantly reduced the SGOT, SGPT, Bilirubin (total and direct), ALP and increased the levels of GSH while extract of root of *Paeonia officinalis* plant also showed significant hepatoprotective activity. The 400 mg/kg of plant extract was more effective than 200 mg/kg. PCM (2 g/kg) led to extensive necrosis in hepatic tissues. Low dose of *Paeonia officinalis* produced partial recovery and with higher dose, there was near complete recovery. The mean weight of the liver was decrease in PCM treated group significantly. While the doses of plant extract increased mean weight.

4. Discussion

Paracetamol is a known antipyretic and an analgesic, which produces hepatic necrosis in high doses and after that undergoing bio-

activation to a toxic electrophile, N-acetyl-p-benzoquinone- imine (NAPQI) by cytochrome P450 monooxygenase[16]. NAPQI binds to macromolecules and cellular proteins, and also oxidizes lipids and alters homeostasis of calcium after depletion of glutathione. Pretreatment with methanolic *Paeonia officinalis* extract restored the depleted GSH concentration near normalcy and brought down the elevated levels of SGOT, SGPT, ALKP and Bilirubin. These biochemical restorations may be due to the inhibitory effects on cytochrome P450 or/and promotion of its glucuronidation.[17, 18]

This is evident from the fact that there is elevation in the levels of various biochemical markers of hepatic damage like SGPT, SGOT, bilirubin, and ALP. Treatment with Silymarin and *Paeonia officinalis* has increased tissue GSH level and the elevated levels of above mentioned biochemical markers to the near healthy levels. The treatment has also demonstrated the reduced hepatic damage.

Histopathological studies also confirm the hepatoprotective role of the methanolic extract of the roots of *Paeonia officinalis* in antagonizing the deleterious effect of PCM on the histology of liver. While PCM treated rats showed extensive histological changes, the animals treated with PCM and the extract of *Paeonia officinalis* concurrently at the doses of 200 and 400mg/kg/day showed only moderate to mild changes. The dose of 400mg/kg/day of the methanolic extract was found to be more effective than the dose of 200mg/kg/day in protecting the liver against the hepatocellular injury caused by PCM.

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