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HEPATOPROTECTIVE ACTIVITY OF HERBAL FORMULATION AGAINST PARACETAMOL-INDUCED HEPATOTOXICITY IN RATS

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ABSTRACT

The present research work is directed towards the study of hepatoprotective activities of herbal formulation against paracetamol-induced hepatotoxicity in rats. The study was designed to evaluate the hepatoprotective activity of herbal formulation in acute experimental liver injury induced by paracetamol (750mg/kg) in Wistar albino rats. The effect of formulation was also studied on serum glutamate oxalate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), serum alkaline phosphatase (SALP), total bilirubin, and total protein. The histological study exhibited reduced signs of paracetamol-induced hepatotoxicity. The hepatoprotective activity of formulation (100mg/kg and 200mg/kg) was also substantiated by significant ($p<0.05$) decrease in levels of SGOT, SGPT, SALP, and Total bilirubin. The effects of formulation were comparable to that of standard drug, Silymarin. From the study, it can be concluded that the herbal formulation has hepatoprotective activity.

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INTRODUCTION

Liver is the most important organ in the body and plays a pivotal role in regulating various physiological processes. It is also involved in several vital functions, such as metabolism, storage and secretion. It has great capacity to detoxicate toxic substances and synthesize useful principles. It is involved with almost all the biochemical pathways to growth, fight against disease, nutrient supply, energy provision, reproduction. [1] Liver diseases are the most serious ailments and are mainly caused by toxic chemicals (excess consumption of alcohol, high doses of paracetamol, anti-tubercular drugs, chemotherapeutic agents, etc) [2,3] Paracetamol hepatotoxicity is caused by the reaction metabolite N-acetyl-p-benzoquinoneimine (NAPQI), which caused oxidative stress and glutathione depletion [4]. In the absence of reliable liver protective drugs in allopathic medical practices, herbs play a role in the management of various liver disorders [5,6,7]. Numerous medicinal plants and their formulations are used for liver disorders in ethno medicine practice as well as traditional system of medicine in India.

Silymarin has been used for 20 years in clinical practice for the treatment of toxic liver diseases [8]. Silymarin is a flavonolignan and extracted from the seed and fruit of the plant *Silybum marianum*, also called "milk thistle". Silymarin consist of four flavonolignan isomers namely-silybin, isosilybin, silychristin and silydianin. Silymarin is orally absorbed and is excreted mainly through bile as sulphates and conjugates [9,10]. In this study, Silymarin was used as a positive control against PCM-induced acute hepatic damage in rats. In the present study, polyherbal formulation developed by Dr. Mahesh Kshirsagar of Shrirampur, Dist. Ahmednagar. Maharashtra State, an Ayurvedic Physician, containing *Curcuma longa, Embelica officinalis, Termenalia chebula, Terminalia belirica, Myrica negi* and bees wax was used against paracetamol-induced hepatotoxicity in rats.

MATERIALS AND METHODS

Formulation contents

The polyherbal formulation contained equal parts of rhizomes of *Curcuma longa*, fruits of *Embelica officinalis, Termenalia chebula, Terminalia belirica*, and *Myrica negi* and bees wax.

Preliminary phytochemical investigation

The preliminary phytochemical screening of formulation was carried out for qualitative identification of phytoconstituents as described by [11,12]

Experimental Animals:

Studies were carried out using Wistar albino rats (200-250g) obtained from the animal house, National Toxicity Pune (NTC), Pune, India. Rats were grouped and housed in polyacrylic cages (38 x 23 x 10 cm) with not more than six animals per cage and maintained under standard laboratory conditions (temperature 25 ± 2ºC) with dark and light cycle (12/12h). The animals were fed with the standard pellet diet supplied by M/s Prashant Enterprises, Pune, India and fresh water *ad libitum*. All animals were acclimatized to laboratory conditions for a week before commencement of experiment. All procedures described were reviewed and approved by the University Animals Ethical Committee.

Drugs and Chemicals

Silymarin was purchased from Loba Chemie, Mumbai-400 002, India. SGOT, SGPT, SALP, Total protein and Total bilirubin kits were purchased from Pathozyme Diagnostics, Kolhapur, India. Ketamine was purchased from Sigma Aldrich, Mumbai, India. Paracetamol, Na-CMC and other chemical utilized were of analytical grade and were obtained from Loba Chemie, Mumbai, India.

Paracetamol-induced hepatotoxicity in rats (Acute model)

Animals were randomized and divided into six groups (Six animals in each group). The animals were then subjected to the following treatments for 7 days.

Group I: 0.5% CMC (1 ml/kg, po) for 7 days.
Group II: 0.5% CMC (1 ml/kg, po) for 7 days + Paracetamol (750 mg/kg, po) on 7th day.
Group III: Formulation (100 mg/kg, po) for 7 days.
Group IV: Formulation (100 mg/kg, po) for 7 days + Paracetamol (750mg/kg, po) on 7th day.
Group V: Formulation (200 mg/kg, po) for 7 days + Paracetamol (750mg/kg, po) on 7th day.
Group IV: Silymarin (25 mg/kg, po) for seven days + Paracetamol (750mg/kg, po) on 7th day.

Biochemical Studies:

The animals were anesthetized using ketamine (100 mg/kg, ip) and blood samples were collected by cardiac puncturing in to sterilized dry centrifuge tubes and allowed to coagulate for 45 min at room temperature. Serum was separated by centrifugation at 3000 rev/min at room temperature for 15 min and utilized for the estimation of various biochemical parameters namely SGOT, SGPT, SALP, Total protein, and Total bilirubin.

Histopathology study:

The animals were sacrificed by decapitation method and the abdomen was cut open to remove the liver. The liver sample was washed with normal saline. Initially the materials were fixed in 10% buffered neutral formalin and then with Boucin's solution (mixture of 75 ml of saturated picric acid, 25 ml of 40% formaldehyde and 5 ml of glacial acetic acid) for 12 hr, then embeded in

paraffin and cut into 5 µm thick section and stained using hematoxylin-eosin dye and finally mounted in di-phenyl-xylene. The sections were then observed under microscope for histopathological changes in liver architecture and their photomicrographs were taken for the evaluation of histopathological changes.

STATISTICAL ANALYSIS

The experimental results were expressed as the Mean ± SEM for animals in each group. The biochemical parameters were analyzed statistically using one-way analysis of variance ANOVA, followed by Dunnett's multiple comparison test. P value of < 0.05 was considered as statistically significant.

RESULTS

Preliminary Phytochemical Screening

The preliminary phytochemical screening of formulation indicated presence of carbohydrates, flavonoids, protein, tannin, alkaloids, saponins, steroids, and volatile oil.

Biochemical Study

The effect of formulation on amount of serum transaminase, alkaline phosphatase, bilirubin and total protein in paracetamol induced hepatotoxicity in rats is given in Table-1. Administration of paracetamol (750mg/kg, p. o.) after 18 hr of intoxication resulted a significant ($p<0.05$) elevation of hepatospecific serum markers SGOT, SGPT, SALP, Total bilirubin and Total protein as compared to the vehicle control group. On administration of formulation at the dose level of 100 and 200 mg/kg and Silymarin (25mg/kg), the level of these enzymes was found reverting towards normal. Group receiving only formulation at the dose level of 100 mg/kg did not changed the level of enzyme significantly. The effect of the formulation in both the doses was comparable to that of standard reference drug Silymarin.

Table-1 Effect of formulation at the dose level of 100mg/kg and 200mg/kg and Silymarin on Serum enzymes (SGOT, SGPT and SALP), Total protein and Total bilirubin on Paracetamol induced liver on damage on rats.

Groups		Dose (mg/kg)	SGOT (IU/L)	SGPT (IU/L)	SALP (IU/L)	Total Protein	Total Bilirubin
Vehicle (0.5% CMC)		1ml/kg	87.84 ± 2.52	41.01 ± 2.56	22.28 ± 1.90	8.67 ± 0.42	1.17 ± 0.17
Paracetamol		750mg/kg	155.7 ± 3.81#	81.26 ± 2.29#	45.73 ± 4.2#	4.33 ± 0.21#	5.5 ± 0.22#
Formulation		100mg/kg	81.34 ±2.40	36.49 ± 1.36	17.19 ± 1.47	9.33 ± 0.21	0.83 ± 0.17
Formulation	+	100mg/kg	111.54 ±	60.49	31.4	7.33	2.5
Paracetamol		750mg/kg	2.14*	± 2.97*	± 2.56*	± 0.21*	± 0.22*
Formulation	+	200mg/kg	105.93	53.34	29.1	7.83	2.17
Paracetamol		750mg/kg	± 3.10*	± 3.56*	± 1.12*	± 0.31*	± 0.17*
Silymarin	+	25mg/kg	96.13	46.19	26.62	8	1.83
Paracetamol		750mg/kg	± 3.39*	± 1.59*	± 1.20*	± 0.26*	± 0.17*

Values are expressed as mean ± SEM, n = 6 rats. Data was analyzed by one way ANOVA followed by Dunnets test. *$p<0.001$ when compared with paracetamol treated group. # $p < 0.001$, compared with vehicle treated group using students 't' test.

Histopathological Study

The hepatoprotective effect of formulation was confirmed by histopathological examination of the liver tissue of control and the treated groups. The histological architecture of control group (Figure-1) showed normal cellular architecture with distinct hepatic cells and sinusoidal space. The liver section of rats intoxicated with paracetamol (Figure-2) showed disarrangement and degeneration of hepatic cells with severe necrosis and disappearance of nuclei. The animals treated with formulation and intoxicated with paracetamol showed no visible changes confirming the safety of the formulation and moderate hepatoprotective activity at the dose level of 100mg/kg (Figure-4) and 200mg/kg (Figure-5). Rats treated with Silymarin and intoxicated with paracetamol showed less disarrangement and degeneration of hepatocytes, indicating marked regeneration activity (Figure - 6).

Histopathology of Rats liver in Vehicle and Treated groups.

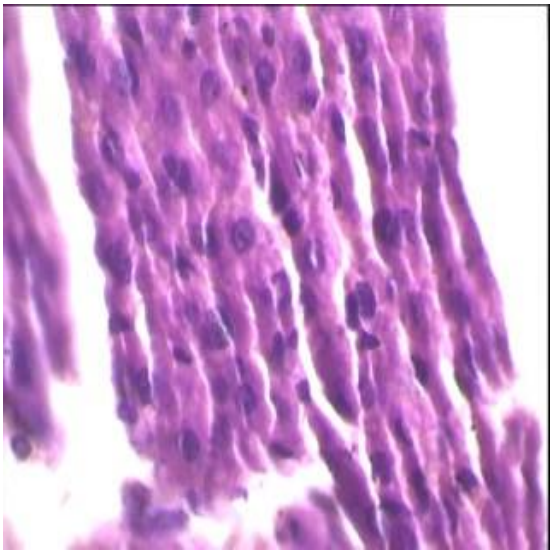

Fig 1 Vehicle, 0.5% CMC (1ml/kg)

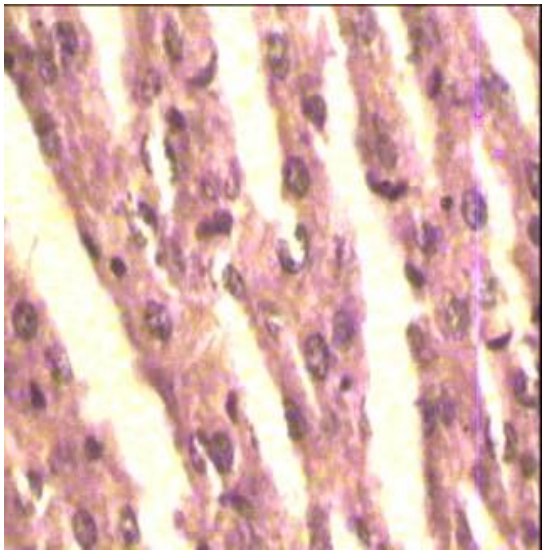

Fig 2 Paracetamol (750mg/kg)

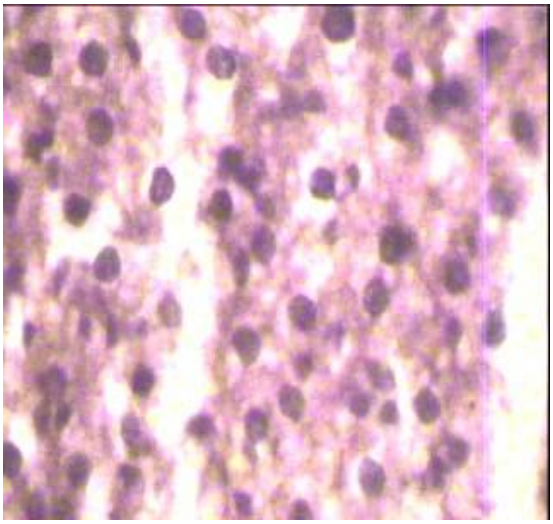

Fig 3 Formulation (100mg/kg)

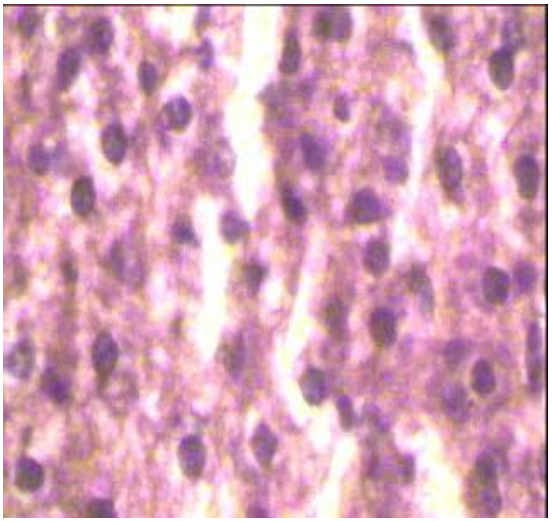

Fig 4 Form (100mg/g) + para (750mg/kg)

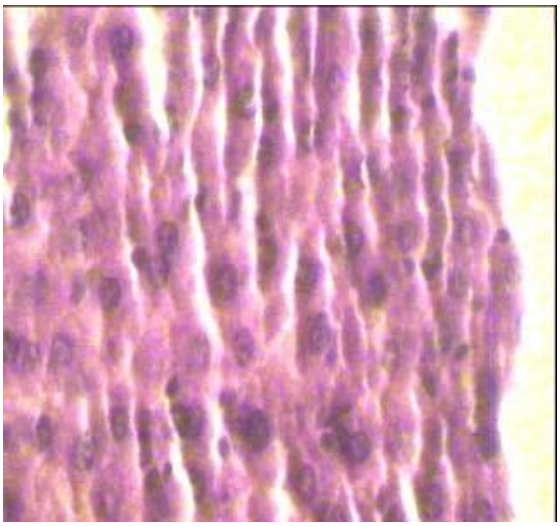

Fig 5 Form (200mg/g) + para (750mg/kg)

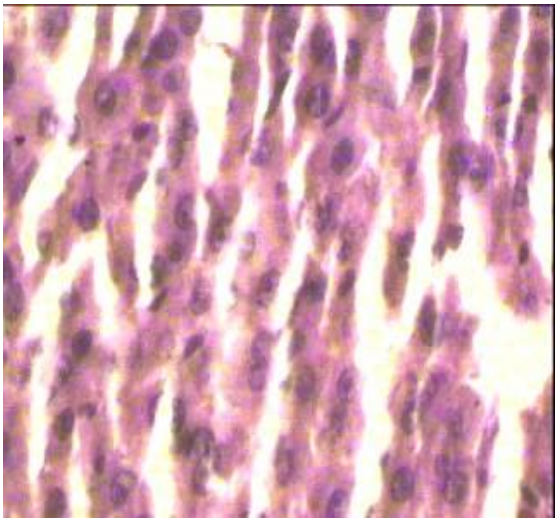

Fig 6 Silymarin (25mg/g) + para (750mg/kg)

DISCUSSION

Paracetamol is widely used as analgesic and antipyretic drug in clinical practice. At low dose, about 80% of ingested paracetamol is eliminated mainly as sulfate and glucoronoide conjugate before oxidation and 5% is oxidized by hepatic cytochrome P_{450} to a highly reactive and toxic eletrophile, i.e. N-acetyl-p-benzoquineimine (NAPQI). Administration of toxic dose of paracetamol causes saturation of sulfation and glucuronidation and so the higher percentage of paracetamol molecule is oxidized to a highly reactive species known as N-acety-p-benzoquineimine (NAPQI) by cytochrome P_{450}**.** Semiquinone radicals, obtained by the reduction of one electron from N-acety-p-benzoquineimine, can covalently bind to macromolecules of cellular membrane and increase lipid peroxidation resulting in tissue damage. Higher dose of paracetamol and NAPQI can oxidize and alkylate intracellular glutathione (GSH), which results in reduction of liver GSH pool and subsequently lead to increased in lipid peroxidation and liver damage.[13] Girish et al., (2009) have reviewed plants reported to possess hepatoprotective activity. The plants used in this formulation have been reported to possess hepatoprotective activity.[14] Jose & Kuttan (2000) reported hepatoprotective activity of *E. officinalis* and showed that the extract normalized the changes induced by paracetamol.[15] In the present study, it was observed that the animals treated with the paracetamol resulted in significant hepatic damage as shown by the elevated levels of serum markers. The changes in the level of these markers reflect on the hepatic structure integrity. The rise in the SGOT is usually accompanied by an elevation in the levels of SGPT, which plays important role in the conversion of amino acids to keto acids [16].

When the hepatic cell membrane is damaged, the enzymes SGOT, SGPT and SALP which are normally located in the cytosol, leak into circulation from hepatocytes leading to increased serum level of SGOT, SGPT and SALP. Paracetamol induced liver injury results in decreased serum total protein level and an elevated the level of SGOT, SGPT, SALP and Total bilirubin. The pretreatment with formulation at both the dose levels significantly attenuated the elevated level of the serum markers. The normalization of serum markers by formulation suggests that the formulation protect the membrane integrity against paracetamol induced leakage of marker enzymes in to the circulation. The above changes can be considered as an expression of the functional improvement of the hepatocytes, which may be caused by the accelerated regeneration of parenchyma cells.

CONCLUSION

In present studies the formulation produced significant hepatoprotective activity by preventing the elevation of serum biochemical parameters like SGOT, SGPT, SALP and Total bilirubin in paracetamol induced hepatotoxicity in rats. From the histopathological studies it is confirmed that the formulation is hepatoprotective.

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