Research Article

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Hepatoprotective Activity of *Cassia fistula* Seeds against Paracetamol-Induced Hepatic Injury in rats.

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Methanolic extract of *Cassia fistula* seeds was prepared and tested for its hepatoprotective effect against paracetamol induced hepatitis in rats. Alteration in the level of biochemical markers of hepatic damage like SGOT, SGPT, ALP and Billirubin were tested in both treated and untreated groups. Paracetamol (2g/kg) has enhanced the SGPT, SGOT, ALP and billirubin level reduced. Treatment with Methanolic extract of *Cassia fistula* seeds (200mg/kg and 400mg/kg) has brought back the altered level of biochemical markers to the near normal levels in the dose dependant manner.

Keywords:- *Cassia fistula*, Paracetamol, Hepatoprotective.

INTRODUCTION

The liver is the vital organ of paramount importance involved in the maintenance of metabolic function and detoxification from the exogenous and endogenous challenges, like xenobiotic, drugs, viral infection 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 liquid peroxidation and depletion in the tissue GSH levels. In addition serum levels of many biochemical markers like SGOT, SGPT, ALP and billirubin are elevated [1, 2].

In spite of phenomenal growth of modern medicine, there are no synthetic drugs available for hepatic disorder. However there are several herbs/herbal formulation claimed have possess beneficial activity in treating hepatic disorder. In one of our field survey we found that a widely grown plant *Cassia fistula* which has claimed to possess hepatoprotective property, it was found that this plant contains flavonoids, alkaloids, cardiac glycosides, tannins [1]. There are reports showed that seeds possess antiinflammatory, antipyretic, analgesic, antimicrobial properties and larvicidal activity [2,3]. The flower of the plant was reported to possess wound healing activity [4,5]. The roots are reported to have anti-fertility and anti-ulcer activities [6,7]. However there are no scientific bases or reports in the modern literature regarding its usefulness as hepatoprotective agent. Thus the present study was conducted to evaluate

The hepatoprotective activity of the methanolic extract of the cassia fistula seeds by using paracetamol-induced hepatic injury in rats.

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MATERIALS AND METHODS

1. Plant

The seeds were collected in September from the local habital faizpur, Authenticated and a Herbarium specimen was deposited in the Botanical survey of India, Pune with No. BSI/WC/Tech/2007/733/NBC-1

2. Preparation of extract [8, 9]

The shade-dried powder of seeds extracted in a Soxhlet extractor by exhaustive extraction using methanol as solvent and it gives 17 % yield of extract.

3. Animals

Wistar rats (125–175 g) of either sex breed in the animal house of TVES College of Pharmacy, Faizpur (**652/02/a/CPCSEA**), were used for this study were kept in standard environmental conditions, fed with standard rodent diet and with water ad libitum. Approval from the institutional animal ethical committee for the usage of animals in the experiments was obtained.

4. Hepatoprotective activity [10]

The method according to Bhakta T. *et.al.* has been used in this study[4, 8]. Both extracts were administered to group of 6 female albino Wistar rats, weighing about 180-200g, enzymatic levels and histopathology were recorded during the evaluation. Paracetamol (2 ml/kg) i.p. used to induce hepatotoxicity. Marked increased in the

serum levels like SGOT, SGPT, SALP and Serum Bilirubin were taken as indication of hepatotoxicity. The procedure consists of, Group A – Served as Control and received single daily dose of 1 ml/kg i.p. of sucrose solution,Group B – Also received single daily dose of 1 ml/kg i.p.

Table No 1 : Effect of *Cassia fistula* leaf extract and liver tonic on serum biochemical parameters during Paracetamol -induced acute liver damage in rats (n = 10).

Sr. No	SGOT	SGPT	ALP	BELLIRUBIN
Control (Normal)	44.500 ± 1.517	36.833 ± 2.639	25.483 ± 1.167	2.173 ± 0.1178
Paracetamol Treated	145.50 ± 10.015	128.00 ± 11.100	89.817 ± 7.360	12.517 ± 1.681
Standard (Liv-52)	60.000 ± 5.099	43.500 ± 5.992	43.433 ± 2.198	5.252 ± 1.336
Methanolic Extract	62.667 ± 6.593	47.667 ± 4.633	43.817 ± 2.041	5.517 ± 1.413
Aqueous Extract	70.333±5.465	56.667±2.944	57.150±9.779	5.983±1.408

sucrose solution for 7 days and on 7th day received 2 ml/kg of Paracetamol by i.p. route, Group C– Received standard drug Liv-52 as a single daily dose of 5 ml/kg of oral route for 7 days and on 7th day 2 ml/kg of Paracetamol by i.p. route, Group D, E received single daily dose of 400 mg/kg of extracts by oral route for 7 days respectively and on 7th day 2 ml/kg of paracetamol by i.p. route, All the rats in above groups were sacrificed on 8th day under light anaesthetic ether. Blood from each rat was collected through cardiac puncture under ether anaesthesia for biochemical investigation i.e. SGOT, SGPT, SALP and serum Bilirubin estimation. Blood was allowed to coagulate at 37^{0}C for 30 min and the serum was separated by centrifugation at 2500 rpm for 10 minutes. The liver of all the experimental animals were removed and processed immediately for histological investigation.

5. Biochemical analysis

Assay of serum GOT and GPT activities [11, 12]

All rats were killed under light ether anaesthesia after 36 h of CCl_4 administration and blood withdrawn from the carotid artery was centrifuged at 300 rpm for 10 min (Lin and Lin, 1995) to separate the serum. Serum transaminase activity was measured according to the method of Reitman and Frankel(1957).

Assay of serum bilirubin and serum alkaline phosphatase [13, 14]

Serum bilirubin concentration was estimated following the method of Malloy and Evelyn (1937). Serum alkaline phosphatase was estimated following the method of Kind and King`s method (1976).

6. Histopathological Study

Histopathological examination of hepatocytes [15]

Each rat was laprotomized to obtain the liver immediately after collecting blood under ether anaesthesia. Small fragments of the rat liver were fixed in 10% formalin solution, dehydrated with ethanol solution from 50% to 100%, embedded in paraffin and cut into 5 µm thick sections which were stained using haemotoxylineosin dye for photomicroscopic observation including necrosis, steatosis and fatty change of hepatic cells.

Statistical analysis

The data are expressed as mean + SEM and the statistical significance were evaluated using the student's t-test. [16]

RESULTS AND DISCUSSION

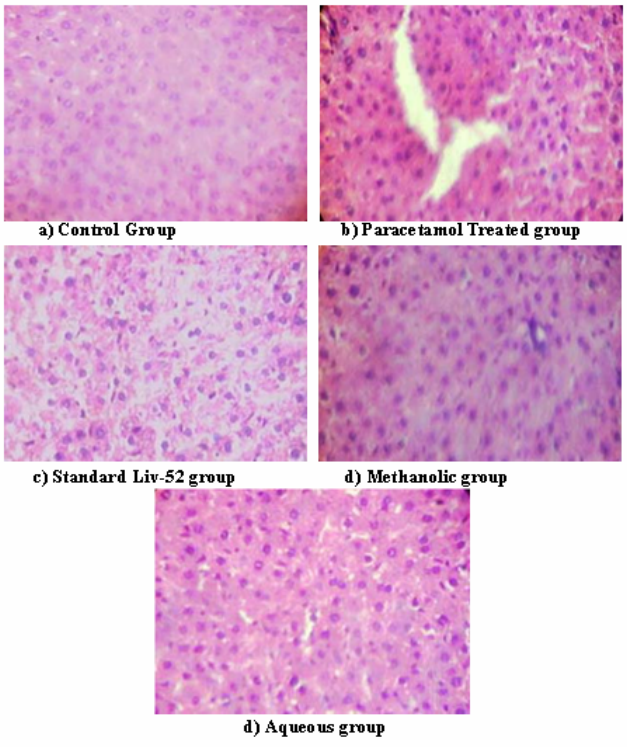
Hepatotoxicity is clear that when paracetamol was used to induce liver toxicity there is a substantial increase in enzyme activity of SGOT, SGPT, SALP and Serum Bilirubin. Any decrease in the

activity of above enzymes would indicate reversed of induced liver toxicity.

Table 2 : Histopathological Study of liver of albino rats.

Control (A)	Observed under 100 x H.E of magnification, showed liver tissue with typical lobular arrangement. Individual lobules consist of hepatocytes arranged as cords radiating around centrally placed terminal hepatic veins. Hepatocytes seen are uniform in size, polyherbal in shape, with centrally located large nuclei. The cytoplasm is strongly eosinophilic with a fine basophilic granularity. Portal tracts containing terminal branches of the hepatic portal vein and hepatic artery at the periphery in fibrous stroma are also seen. Impression : Normal liver tissue
Paracetamol Treated (B)	Observed under 100 x H.E of magnification showed liver tissue with disturbances in the lobular arrangement. Degenerative and early necrotic changes extending across lobules. Hepatocytes show ballooning degeneration and steatotic changes. Some amount of fibrosis seen in portal tracts. Impression: Liver with cytotoxic injury showing mild necrosis and fibrotic changes.
Standard Liv-52 (C)	Observed under 400 x H.E of magnification, showed liver tissue with typical lobular arrangement. Hepatocytes show variable size. There is a mild increase in fibrous connective tissues. Impression: Liver with mild sign of hepatotoxicity.
Methanolic Extract (D)	Observed under 100 x H.E of magnification, showed liver tissue with typical lobular arrangement. Few hepatocytes shows steatotic accumulation. Impression: Liver with minimal sign of hepatotoxicity.
Aqueous Extract (E)	Observed under 400 x H.E of magnification, showed liver tissue with typical lobular arrangement. Hepatocyte shows variable size. Ballooning changes, steatotic accumulation. There is a mild increase in fibrous connective tissues. inflammatory cells are seen within the parenchyma. Impression: Liver with sign of hepatotoxicity.

Fig 1: Histopathological Microphotograph of Rat liver tissue



a) Control Group b) Paracetamol Treated group

c) Standard Liv-52 group d) Methanolic group

d) Aqueous group

The results indicated that the methanolic extract showed significantly reduced the elevated levels of SGOT, SGPT, SALP and Serum Bilirubin when compared with aqueous test extracts. Aqueous extract has reduced the elevated levels of SGOT, SGPT, SALP and Serum Bilirubin to lesser extent compared with methanolic extract. The methanolic extract has reduced the increased SGOT levels from 145.50 IU/L to 62.667 IU/L, SGPT levels from 128.00 IU/L to 47.67 IU/L, SALP levels from 89.82 K.A. units to 43.82 K.A. units and serum bilirubin levels from 12.52 mg/dl to 5.517 mg/dl. Aqueous extract has reduced the increased SGOT levels from 145.5 IU/L to 70.33 IU/L, SGPT levels from 128.0 IU/L to 56.67 IU/L, SALP levels from 89.82 K.A. units to 57.15 K.A. units and Serum bilirubin levels from 12.52 mg/dl to 5.983 mg/dl. While the standard drug Liv-52 has reduced increased SGOT levels from 145.50 IU/L to 60.33 IU/L, SGPT levels from 128.00 IU/L to 43.5 IU/L, SALP levels from 89.82 K.A. units to 43.43 K.A. units and Serum bilirubin levels from 12.52 mg/dl to 5.252 mg/dl. The enzymatic levels of SGOT, SGPT, SALP and serum billirubin are indicated in the Table no.1.

CONCLUSION

In the present study, seeds of *Cassia fistula* Linn. Were evaluated for its pharmacognostical, phytochemical and pharmacological aspects. Powder of seeds of *Cassia fistula* Linn gives loss on drying (Not more than 6.3%) and ash value (Not more than 4.2%), which showed that the crude drug used are of good quality.

Pharmacological Screening

Acute toxicity testing of both extracts were determined as per OECD guidelines and its ambenment time to time. LD_{50} of both test extracts were found to be 2000 mg/kg. The degree of hepatotoxicity development can be known by elevated levels of SGOT, SGPT, SALP and Serum Bilirubin enzymes which is attributed due to its covalent bonding N-acetyl-p-benzoquinoneimine. Oxidation product of paracetamol to sulphydryl group of protein resulting in cell necrosis and lipid peroxidation induced by decrease in glutathione in the liver, causes hepatotoxicity. Both extracts were evaluated for hepatoprotective activity in albino rats (wistar strains). Methanolic extract showed significantly reduced the elevated levels of SGOT, SGPT, SALP and Serum Bilirubin

when compared with Liv-52 used as standard drug. Aqueous extract has reduced the elevated levels of SGOT, SGPT, SALP and Serum Bilirubin to lesser extent compared with Liv-52 used as standard drug.

With aid of enzyme levels and histopathological studies of rat liver we can concluded that methanolic extract have shows better hepatoprotective activity as compared with standard. (Liv-52) While Aqueous extract has exhibited moderate hepatoprotective activity.

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