Hepatoprotective activity of *Indigofera aspalathoides* extract against CCl4-induced liver damage

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SUMMARY

The plant *Indigofera aspalathoides* are used by a large number of tribes in India for the treatment of various hepatic disorder. The methanol extract of *Indigofera aspalathoides* (MEIA) was evaluated for its effect on carbon tetrachloride (CCl4) induced liver damage. Biochemical parameters such as serum glutamine oxaloacetate trasaminase (SGOT), serum glutamine pyruvate transaminase (SGPT), serum alkaline phosphatase (ALP), total serum protein (TP), thiobarbituric acid reactive substances (TBRS) and glutathione content of the liver were estimated to assess liver function and metabolism. Biochemical observations suggest that methanol extract of *Indigofera aspalathoides* (MEIA) significantly restored the liver function and metabolism towards normal condition in CCl4-induced hepatic damage.

Key words: *Indigofera aspalathoides*; Hepatoprotective; Carbon tetrachloride; Silymarin

INTRODUCTION

In the traditional medicinal system, the leaves, flowers and shoot *Indigofera aspalathoides* (Leguminosae) are used cooling and demulcent. They are used in the form of decoction for leprosy and cancerous affections. The leaves are also applied to abscesses. The root is chewed in toothache and aphthae. The whole is used in edematous tumors and the ashes are used in the preparations for dandruffs (Kirtika and Basu, 2001). In the present study effect of methanol extract of *Indigofera aspalathoides* (MEIA) was evaluated for its effect on CCl4 induced liver damage. Biochemical parameters such as serum glutamine oxaloacetate trasaminase (SGOT), serum glutamine pyruvate transaminase (SGPT), serum alkaline phosphatase (ALP), total serum protein, thiobarbituric acid reactive substances, bilirubin and glutathione content of the liver were estimated to assess liver function and metabolism.

Preparation of plant extract

The plant of *Indigofera aspalathoides* was collected in the month of March from Tamilnadu, identified by Botanical Survey of India, Shibpur, Howrah. After shade dried it is powdered in mechanical grinder. The powdered material was then extracted with petroleum ether and methanol in a Soxhlet extraction apparatus. The solvent was completely removed under reduced pressure and stored in a vacuum desiccator. The yield of pet ether extract is 7.5% and methanol extract is 4.5%.

Reagents for estimation of biochemical markers were obtained from Sigma Chemical Co., St. Louis Solvents (AR grade) were obtained from E-Merck, Mumbai.

Treatment protocol

Male Swiss Albino mice weighing (22-26 g) were maintained in identical laboratory conditions and given standard food pellets (Hindustan Lever, Mumbai) and water *ad libitum*. The mice were divided into six groups (n=10). Group I received only normal saline, intraperitoneally (i.p) (Ashok et
al., 2001) and served as normal control. Group II received CCl₄ 0.5 ml/kg, i.p. once daily for seven days (Rao et al., 1993). Group III, IV and V were received CCl₄ 0.5 ml/kg, i.p. and 100, 200 and 400 mg/kg body weight of methanol extract of Indigofera aspalathoides (MEIA) simultaneously for 7 days (Tosaki et al., 1994). Group VI received standard drug silymarin 25 mg/kg body weight p.o and 0.5 ml/kg of CCl₄ i.p. for 7 days (Morazzoni and Bombardelli, 1995). Twenty-four hours after the administration of CCl₄ the mice of each group were anaesthetized in ether chamber to obtain liver and blood was collected by cardiac puncture. The blood samples were allowed to clot for 45 min at room temperature. Serum was separated by centrifugation at 2500 rpm for 15 min and analyzed for various biochemical parameters.

Biochemical estimations
Serum glutamine oxaloacetate transaminase (SGOT) (Bergmeyer and Brent, 1974), serum glutamine pyruvate transaminase (SGPT) (Bergmeyer and Brent, 1974), serum alkaline phosphatase (ALP) (King, 1965), bilirubin (Sanyal) and total protein (Lowry et al., 1951) were estimated in the serum of the mice. Glutathione (GSH) was estimated by 5,5-dithio bis-2-nitro benzoic acid by the method of Elman et al. (Ellman, 1959) and expressed as mmol/g of liver. Thiobarbituric acid reactive substances (TBARS) were measured in the liver by the method of Okhawa et al (Ohakawa et al., 1979).

Statistical analysis
Results shown are expressed as ± SEM and the test of significance of the results was evaluated by Student’s t test.

RESULTS AND DISCUSSION
Results are summarized in Table 1 and 2. CCl₄ is fungicide that was recognized as potent hepatotoxic and carcinogens in rats. It was found that chronic administration of CCl₄ produced cirrhosis in rats. CCl₄ toxicity is mainly due to the formation of CCl₅⁺ which is responsible for the change in cell permeability. Increase in intracellular concentration of calcium causes increase the nucleus and causes enlargement of the size of the nuclei. It also inhibits mitochondrial activity, which ultimately lead to the cell death. It has been well established that elevated levels of SGOT and SGPT are indicative of cellular leakage and loss of functional integrity of the cell membrane in liver. The methanol extract of Indigofera aspalathoides decrease the elevate serum

| Table 1. Effect of methanol extract of Indigofera aspalathoides on CCl₄ induce liver damage in mice |
|-----------------------------------------------|-------------------------------|-------------------------------|-----------------|-----------------|
| Treatment                        | SGOT (IU/L) | SGPT (IU/L) | ALP (IU/L) | Total Protein | Bilirubin |
| Control (5 ml/kg) | 78.07 ± 0.11 | 63.39 ± 0.10 | 32.72 ± 0.80 | 7.32 ± 0.13 | 1.05 ± 0.17 |
| CCl₄ (0.5 ml/kg) | 189.32 ± 0.59 | 142.37 ± 0.43 | 72.84 ± 0.72 | 3.19 ± 0.45 | 2.59 ± 0.69 |
| CCl₄ + MEIA (100 mg/kg) | 175.72 ± 0.95 | 131.42 ± 0.98 | 64.32 ± 1.52 | 4.22 ± 0.59 | 2.12 ± 0.36 |
| CCl₄ + MEIA (200 mg/kg) | 141.12 ± 0.92 | 108.42 ± 0.77 | 49.13 ± 0.65 | 4.91 ± 0.33 | 1.69 ± 0.91 |
| CCl₄ + MEIA (400 mg/kg) | 103.32 ± 0.27 | 82.33 ± 0.17 | 41.12 ± 0.33 | 5.22 ± 0.13 | 1.54 ± 0.35 |
| CCl₄ + Silymarin (25 mg/kg, i.p.) | 84.67 ± 0.23 | 72.32 ± 0.16 | 54.42 ± 0.32 | 6.32 ± 0.89 | 1.2 ± 0.29 |

IU/L. International Units per Litre.
All p<0.05 denotes high significance of the experimental values when compared to CCl₄ treated group.

| Table 2. Effect of Indigofera aspalathoides on TBARS and glutathione levels in CCl₄ treated mice |
|-----------------------------------------------|-------------------------------|-------------------------------|
| Treatment                        | TBARS (mole/g of wet tissue) | Glutathione (U/mg of protein) |
| Control (5 ml/kg) | 0.75 ± 0.05 | 13.75 ± 0.51 |
| CCl₄ (0.5 ml/kg) | 1.57 ± 0.19 | 10.05 ± 0.32 |
| CCl₄ + MEIA (100 mg/kg) | 1.29 ± 0.11 | 11.12 ± 0.41 |
| CCl₄ + MEIA (200 mg/kg) | 1.05 ± 0.08 | 13.72 ± 0.76 |
| CCl₄ + MEIA (400 mg/kg) | 0.87 ± 0.06 | 16.45 ± 0.46 |
| CCl₄ + Silymarin (25 mg/kg, i.p.) | 0.71 ± 0.19 | 18.89 ± 0.89 |

TBARS—Thiobarbituric acid reactive substances
All p<0.05 denotes high significance of the experimental values when compared to CCl₄ treated group.

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enzyme level, in CCl₄ fed rats when compared to the untreated animals. It seems that the extract preserve the structural integrity of the hepatocellular membrane which is evident form the significant reduction in CCl₄ induced rise in serum enzymes in rats. The reversal of increased serum enzymes may be due to the prevention of leakage of the intracellular enzymes by its membrane stabilizing activity.

Lipid peroxidation is a natural phenomenon involved in peroxidative loss at unsaturated lipids thus bringing about lipid degradation and membrane disordering (Lappei, 1973). Peroxidised lipid has been considered to play a significant role in the pathogenesis of several diseases and may be taken as the molecular mechanism of cell injury under pathological conditions (Kuaiimoto et al., 1981).

Oxidative stress is one of the mechanism involved in acute hepatotoxicity by CCl₄. This hepatotoxin is rapidly transformed to free radical trichloromethyl (CCl₃⁺) by cytochrome p 450-2e1 (CYP2e1) in the microsomal compartment of liver CCl₃ (Recknagel and Ghosal, 1966) and its highly reactive derivative the trichloromethyl propoxy radical (Cl₃COO⁻) may interact with membrane lipids leading to their peroxidation, which was resolved in malondialdehyde (MDA) among other metabolites.

Free radicals initiate and promote the propagation of lipid peroxidation that is usually measured through its catabolite MDA. MEIA showed ability to prevent CCl₄ induced increment of MDA levels it suggesting that MEIA inhibit lipid peroxidation and its propagation in the liver.

The free radicals in the multisteps process of carcinogenesis is well established (Fisher et al., 1998) antioxidants are reported to offer a protective effect against cancer and hepatitis (Uddin and Ahmed, 1995; Schwartz et al., 1994).

Glutathione is the natural antioxidant in our body system and it is protective chemically induced hepatic damage and oxidative stress by antioxidant mechanism (Videla and Valenzuela, 1982). GSH level decreased with increased level of lipid peroxidation in CCl₄ treated rats indicates that CCl₄ is the highly hepatotoxic. MEIA significantly increased the level of GSH, which means the extract itself having antioxidant property and it prevent the CCl₄ and other free radicals.

There has been well established that elevated levels of SGOT, SGPT are indicative of cellular leakage and loss of CCl₄ induces fatty liver and cell necrosis. These parameters play a significant role in inducing triacylglycerol accumulation, depletion of GSH, increased lipid peroxidation, membrane damage, depression of protein synthesis and loss of enzyme activity.

It has been shown that protective agents exert their action against CCl₄ induced liver injury by impairment of CCl₄ mediated lipid peroxidation either through decreased production of free radical derivatives or due to the antioxidant activity of the protective agent itself.

Our present study suggested that MEIA restore the altered biochemical parameters and it was found to be a potent hepatoprotective agent.

REFERENCES


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