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THE HEPATO-PROTECTIVE EFFECT OF CASSIA FISTULA (AMALTAS) LEAVES IN ISONIAZID AND RIFAMPICIN INDUCED HEPATOTOXICITY IN RODENTS

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Certain medicinal plants have been reported to have their effect on various experimentally induced diseases. Drug induced hepatitis (DIH) is one of them. The purpose of this study was to assess the effect of ethanolic extract of Cassia fistula leaves in experimentally induced drug hepatitis (DIH) in rodents. The rats were divided into four groups, i.e. a control group (A), antituberculous (ATT) group (B), and the remaining two groups (C and D) served as experimental therapy groups. They received Cassia fistula extract as hepatoprotective agent. Rats having normal liver functions were included in this study. Group C experimental rats received (INH/RIF) (50 mg/kg) each and ethanolic extract of Cassia fistula at 400 mg/kg of body weight. On the other hand group D experimental rats received (INH/RIF) (50 mg/kg) each and ethanolic extract of Cassia fistula at 500 mg/kg of body weight. Blood samples were taken at 30th day and liver in each was taken out for microscopical examination on day 30th. The (ATT) group rats showed variable increase in serum ALT, AST, ALP and total bilirubin levels. Group C treated with 400 mg/kg of body weight Cassia fistula treatment decreased the level of these parameters in rats. On the other hand group D rats treated with 500 mg/kg body weight of Cassia fistula dose significantly decreased levels of these biochemical parameters. The morphological examination of experimental group C rats showed slight recovery whereas the rats in experimental group D showed a significant recovery. Cassia fistula constituents, especially flavonoids and anthraquinones have strong anti-oxidant activity which provides hepato-protection against drug-induced hepatitis (DIH). In ***conclusion,*** *high dose of Cassia fistula ethanolic leaves extract (500 mg/kg) body weight showed hepato-protection against INH/RIF induced hepatitis in rats.*

Key Words: *Hepato-protection, isoniazid (INH), rifampicin (RIF), Cassia fistula, drug-induced hepatitis (DIH), oxidative stress.*

INTRODUCTION

Mycobacterium tuberculosis (MT) causes infection allover the world and results in more deaths than any other microbial agent. Approximately one third of World's population is infected with *M.tuberculosis*. It is estimated that each year three million people die of tuberculosis and eight million new cases occur.[1] It has been estimated that tuberculosis ranks seventh among illnesses as a cause of disability adjusted life years (DALYs) lost. In the estimate of disease morbidity, it is projected that ranking is unlikely to change through early part of the 21st century.[2]

Isoniazid, and Rifampicin (INH and RIF), being the first line drugs used as antituberculous chemotherapy, are known to be associated with hepatotoxicity.[3] The rate of hepatic damage has been reported to be much higher in developing countries (8-30%). Oxidative stress produced by INH and RIF causes hepatic injury.[4] The frequency of hepatotoxicity is increased when these drugs are used in combination. Majority of normally formed free radicals are removed by the action of reduced glutathione. This causes the initiation of lipid peroxidation (LPO) resulting in tissue injury.5

Various experimental animals are used to investigate hepatitis; however INH and RIF induced hepatitis rat models have been used in model of drug induced hepatitis.[23]

Cassia fistula belonging to the family Leguminosae Casesalpinaceae is commonly called as Amaltas an Indian Labernum and is native to India, the Amazon, Sri Lanka and is extensively diffused in various countries.[6] Its main property being that of a mild laxative suitable for children and pregnant women. It is also a purgative due to the wax aloin[7] and has been used to treat many intestinal disorders like ulcers.[8,9] The plant has a high therapeutic value and it exerts antipyretic and analgesic effects.[10] Cassia fistula Linn is used as an anti-periodic agent

in the treatment of rheumatism[8,9] and the leaf extract is also used for its anti-tussive and wound healing properties.[11,12] It has been concluded that plant parts could be used as a therapeutic agent in the treatment of hypercholesterolaemia partially due to their fibre and mucilage content.[13] It has been reported to possess antitumor,[14] hepatopro-tective,[15] antifertility,[16] antioxidant properties.[17-19] In addition its action on the central nervous[20] and in-hibitory effect on leukotriene biosynthesis has also been suggested.[21] *Cassia fistula* plant organs are known to be an important source of secondary metabolites, notably phenolic compounds.

The purpose of the present study was to see the effect of *Cassia fistula* on INH and RIF induced hepatitis in wistar rats.

MATERIALS AND METHODS

Animals

Forty adult male wistar rats weighing 200-250g were procured for this study. They were kept in the experimental research laboratory of University of Health Sciences, Lahore. Rats were divided into 4 groups, each having 10 animals. Before the commencement of the experiment, all animals were kept for one week under the same laboratory conditions, at a temperature of 22 ± 2°C, relative humidity of 70 ± 4% and 12 hour light / dark cycle. They received nutritionally standard diet and tap water. The care and handling of rats were in accordance with the internationally accepted standard guidelines for use of animals.

Plant materials and preparation of the extract

Leaves of *Cassia fistula* were collected from Gulshan-e-Iqbal Park, Lahore and authenticated from a botanist. Ethanolic leaf extract (ELE) was prepared from *Cassia fistula*. Freshly collected leaves were washed in tap water and then distilled water and were shade dried for about 1 week. The dried leaves were crushed into a coarse powder. One hundred gram of the powder was soaked in 1 liter of ethanol for 30 days with occasional shaking. After 30 days this ethanolic leaf extract (ELE) was filtered and evaporated to dryness over a water bath at 60°C. The yield of ethanolic leaf extract was 19-20%.[22] This (ELE) of *Cassia fistula* was got standardized from Applied Chemistry Research Centre (ACRC) at PCSIR laboratories, Lahore, Pakistan.

Experimental Procedure

After acclimatization, 10 rats were labeled as control. All other rats were given INH and RIF (50 mg/kg) each orally to produce hepatotoxicity.[23] The pH of RIF solution was adjusted 30 3.0 with 0.1 mol/l.[24] The rats Group A (control A) were fed on standard diet with tap water and received no drug. Group B (INH and RIF) rats received 50 mg/kg of INH and RIF orally once daily for 30 days and were fed on standard diet and tap water. Group C (experimental group 1) rats received 50 mg/kg of INH and RIF each orally through insulin syringe once daily and ethanolic extract of *Cassia fistula* 400 mg/kg orally once daily for 30 days.[15]

Group D (experimental group 2) rats received 50 mg/kg of INH and RIF each orally through insulin syringe once daily and ethanolic extract of *Cassia fistula* 500 mg / kg orally once daily for 30 days.[22]

Blood Sample collection

A day after administration of the last dose of extract, the animals were anaesthetized under ether vapours and their blood samples were collected by performing cardiac puncture in sterile vacuotainer with gel. Serum samples were separated from the clot after centrifugation at 3000 rev/min for 10 min, using bench top centrifuge. Serum samples were stored in eppendorf tubes and at -20°C until used for biochemical estimation.[25]

Biochemical Analysis

ALT, AST, ALP and total bilirubin levels were estimated by commercially available kits (Randox of UK). Serum ALT, AST, ALP was estimated by IFCC method.[26-30] Total bilirubin was estimated according to Calorimetric method.[31]

Liver tissue for morphology

When anaesthetized, the livers of all animal were exposed and a wedge was removed after their gross examinations and they were preserved.

Statistical Analysis

The data was entered and analysed using SPSS 17.0 (Statistical Package for Social Sciences). All data are shown as mean ± S.E.M. One way ANOVA was applied to observe group mean differences. Post Hoc Tukey test was applied to observe mean differences among the groups. A p-value of <0.05 was considered as statistically significant.

RESULTS

Effect of Cassia fistula on the serum ALT levels:
We found a significantly increased (P<0.05) level of serum ALT in rats of group B as compared to compared to group A (control due to INH and RIF, but these levels were significantly reduced (P<0.01) in rats of group C and D treated with *Cassia fistula*.

Effect of Cassia fistula on the serum AST levels:
Results showed a significantly increased (P<0.05) level of serum AST in rats of group B as compared to compared to group A (control) due to INH and RIF, but these levels were significantly reduced (P<

0.01) in rats of group C and D treated with *Cassia fistula.*

Table 1: *Mean ± SEM values of different ALT, AST, ALP, total serum bilirubin in all groups (n = 10).*

	Group A	Group B	Group C	Group D
Serum ALT (u/l)	41.61 ± 671	79.03 ± 1.67*	46.22 ± 814**	38.29 ± 571**
Serum AST (u/l)	111.46 ± 2	293.22 ± 5.27*	135.06 ± 1.68**	109.22 ± 1.09**
Serum ALP (u/l)	108.44 ± 1.58	403.84 ± 3.15*	174.51 ± 1.61**	99.45 ± 2.18**
Serum total bilirubin (mg/dl)	0.373 ± 0033	0.607 ± 0052*	0.480 ± 0042**	0.479 ± 0029**

* $p<0.05$
** $p<0.01$

Effect of Cassia fistula on the serum ALP levels: We observed that INH and RIF caused significantly increased ($P<0.05$) level of serum ALP in rats of group B as compared to compared to group A (control) due to INH and RIF, but these levels were significantly reduced ($P<0.01$) in rats of group C and D treated with *Cassia fistula.*

Effect of Cassia fistula on the serum total bilirubin levels: Our results showed that INH and RIF caused significantly increased ($P<0.05$) level of serum total bilirubin in rats of group B as compared to compared to group A (control) due to INH and RIF, but these levels were significantly reduced ($P<0.01$) in rats of group C and D treated with *Cassia fistula.*

Histopathological examination

In histopathological studies of liver, the control group A showed normal gross appearance i.e. dark maroon color of liver having smooth surfaces, microscopically normal lobular appearance having normal central vein, radiating cords of heptocytes, normal portal tract in most of them (Fig?). Group B rats who were given INH and RIF, showed moderate to severe liver damage characterized by clear cytoplasm, vascular congestion, fatty changes, apoptosis and focal areas of necrosis and vacuolation of cytoplasm as a feature of ballooning degeneration (fig?). Group C (Experimental 1) rats were given INH and RIF and low dose of *Cassia fistula* extract (400 mg/kg) showed slight recovery and evidence of regeneration in some hepatocytes (Fig?). Group D (Experimental 2) rats were given INH and RIF and high dose of *Cassia fistula* extract (500 mg/kg) showed vascular congestion and evidence of regeneration with few apoptotic bodies (Fig?).

DISCUSSION

Isoniazid (INH) and rifampicin (RIF) are the most important first line drugs, used for the treatment of tuberculosis. Isoniazid (INH) can cause hepatotoxicity in 20% of patients and is usually associated with an inflammatory response.[32] INH and RIF are reported to induce hepatotoxicity judged by elevated serum ALT, AST, ALP and total bilirubin levels, presence of focal hepatocytic necrosis and portal triaditis.[33]

Plant-derived antioxidants such as vitamin E, vitamin C, polyphenol including phenolic acids, phenolic diterpenes, flavonoids, catechins, procyanidins, and anthocyanins are being increasingly suggested as important dietary factors. Supplementation with berry juice,[34] flavones from skullcap, catechins from green tea, anthocyanins from chokeberry, and condensed tannins from faba beans[35] are indeed protective of oxidative stress indices in rats.[19]

The protective action of antioxidants is usually due to the inhibition of free radical chain reaction and the resultant prevention of peroxidative deterioration of structural lipids in membranous organelles. Circulating antioxidants mainly vitamin C and vitamin E and tissue enzymatic and non-enzymatic such as superoxide dismutase (SOD), catalase (CAT) and reduced glutathione (GSH) play important role in alleviating tissue damage due to the formation of free radicals.[36]

In this study, we observed the hepatoprotective effect of *Cassia fistula* in INH and RIF induced hepatotoxicity in rats. A significant elevation was observed in the levels of serum ALT, AST, ALP and total bilirubin in group B which received INH and RIF as compared to group A rats who received no medicines. Elevated levels of these parameters in serum are presumptive markers of hepatotoxic lesions in the liver. Co-administration of high dose (50 mg/kg) *Cassia fistula* ethanolic extract with INH and RIF in group D, maintained the levels of ALT, AST, ALP, serum total bilirubin towards normalcy as compared to group B rats. This was most likely due to the anti oxidant effect of *Cassia fistula* constituents. In group C rats, who received a low dose of 400 mg/kg of *Cassia fistula* along with INH and RIF, biochemical levels of enzymes and bilirubin

decreased significantly but did not reach the normal. Our results are in accordance with some previous studies.[22,37]

On morphological examination in group C (Experimental 1), low dose *Cassia fistula* showed partial recovery in some liver slides. While in group D (Experimental 2), high dose *Cassia fistula* showed a significant recovery towards normal, this result shows hepatoprotection after a high dose *Cassia fistula* extract in experimentally drug induced hepatitis (DIH) in rats. Our results are in accordance with some previous studies.[23,37]

The main constituents in *Cassia fistula* are potent phenolic antioxidants such as anthraquinones, flavonoids and flavan-3-ol derivatives.[6] Therefore, in our study flavonoids in *Cassia fistula* might have a role in the recovery in INH and RIF induced hepatotoxicity in rats.

In **conclusion,** the results of the present study indicate that the co-treatment of *Cassia fistula* leaf extract prevents INH and RIF induced hepatotoxicity in rats. The high dose *Cassia fistula* leaf extract, showed better results as compared to low dose, both biochemically and morphologically. The overall hepatoprotective effect of *Cassia fistula* is probably due to a counteraction of free radicals by its antioxidants i.e. flavonoids.

Further studies are needed to see if a higher dose and different routes of administration of *Cassia fistula* have a hepatoprotective effect.

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