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Extraction and hepatoprotective activity of *Pterospermum acerifolium* on antitubercular drug induce toxicity in Swiss albino mice

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ABSTRACT

Present study deals with the extraction and hepatoprotective activity of *Pterospermum acerifolium* on antitubercular drug (Isoniazid and Rifampicin) induce toxicity in swiss albino mice. The leaves of *Pterospermum acerifolium* were subjected to maceration using hydro alcohol as solvent in the ratio 3:7 for 7 days. Toxicity study was performed on swiss albino mice at the different dose 1/20th, 1/10th and 1/5th of 100, 200 and 400 mg/kg body weight. The effect of extract on biochemical parameters SGOT, SGPT, ALP and Bilirubin against isoniazid and rifampicin (INH+RIF) induced hepatotoxicity in mice was studied using distilled water as control and silymarin as standard. The biochemical studies were done span diagnostic kits. In animals treated with INH+RIF the level of SGOT, SGPT, ALP and Bilirubin was found to significantly high ($P<0.05$) as compared to vehicle treated group. In extract treated group level of SGOT, SGPT, ALP and Bilirubin was found to be significantly less ($P<0.05$) as compared to INH+RIF treated group. It can be concluded that hydroalcoholic extract of leaves of *Pterospermum acerifolium* possess significant heptoprotective potential against INH + RIF induced hepatotoxicity. The extract of leaves of *Pterospermum acerifolium* can be used in future in combination with antitubercular drug to prevent the acute hepatic damage produced on accidental over dosage.

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INTRODUCTION

Herbals are the main source of the treatment used from ancient time. The medicinal properties in plants are mainly due to the presence of secondary metabolites. *Pterospermum acerifolium* belonging to the family Sterculiaceae is commonly found in subtropical countries which have been traditionally used for various disease and disorders. As per reported literature the extract of *Pterospermum acerifolium* contains flavanoids, polyphenol, tannins and lipoic acid which are having good antioxidant property. The antitubercular synthetic drugs have been observed to have toxicity for long term and acute overdose use hence in the present study an attempt was made for assessing the hepatoprotective potential of *Pterospermum acerifolium* against antitubercular drug induced hepatotoxicity.[1-6]

MATERIAL AND METHODS

Extraction

Pterospermum acerifolium leaves were collected authenticated and pulverized into moderately coarse powder and were macerated using hydro alcohol as solvent in the ratio 3:7. for 7 days. The filtrates were dried and subjected to phytochemical evaluation.

Toxicity study[7]

The test guideline 423 was followed for oral toxicity studies. Five swiss albino mice 200g ± 20 gm weight was treated with oral drug administration with fasting for 3-4 hours (Pre-experimentation) and 1-2 hours (Post-experimentation). The Hydroalcoholic extract of *Pterospermum acerifolium* at a dose of 2000 mg/kg body weight did not produce any mortality and other toxic effects during entire duration of study and all animals survived till 14 days of observation period. Hence 1/20th, 1/10th and 1/5th of this dose i.e. 100, 200 and 400 mg/kg body weight were used for further studies. Swiss albino mice were grouped in four, each group containing of 5 animals. The first group was considered as control group receiving only distilled water. Food was withdrawn for 16 hrs before Isoniazide & Rifampicin administration to group 2, 3 and 4 to enhance the acute liver toxicity. The dose of Isoniazide (75mg/kg, orally) & Rifampicin (150mg/kg, orally) was administered till seven day. On 7th day group 2 receive Isoniazide & Rifampicin only while group 3 and group 4 after Isoniazide & Rifampicin treated receive silymarine (standard) and extracts of *Pterospermum acerifolium* respectively. After 6 hrs of treatment all the animal were anesthetized using anaesthetic ether and blood sample were collected by retro-orbital puncture method and

serum was used for estimation of AST, ALT, ALP and bilirubin.[7]

Biochemical Estimation [8-10]

Assay of Aspartate aminotransferase (AST) or (SGOT) Alanine aminotransferase (ALT) or (SGPT) and alkaline phosphatase (ALP)

Aspartate aminotransferase, Alanine and alkaline phosphate in serum were assayed using Span Diagnostic Kit. [58] The serum and working reagent (enzymes) were mixed in 1:10 (AST & ALT) & 1:50 (ALP) ratio in different well plates and immediately absorbance was taken at 405 nm (AST & ALP) & 340 nm (ALT) wavelength followed by repeat reading after every 30 seconds upto 120 sec. The mean absorbance change per min (ΔA /minute) was determined and used for calculations (Table 1)

Total and Direct Bilirubin

Equal volumes of caffeine sodium benzoate and purified water were mixed and used as working reagent. 100 µl of Sulphanilic acid, 50 µl of serum sample and 1 ml of working reagent were mixed and labelled as Sample blank (AB_1). In another test tube, mix 50 ul of Sodium nitrite, 50 ul sulphanilic acids, 50 ul of Serum sample, 1 ml of working reagent were mixed and labelled the test tube as Test. Both the test tubes were incubated at room temperature for 5 minutes and the absorbance was measured at 546 nm. Absorbance of Sample blank (AB_1) was subtracted from absorbance of test (AT_1) (table 1). The differences were then multiplied by the conversion factor 26.312 to obtain concentration of total bilirubin in mg/dL.

RESULTS AND DISCUSSION

The effect of extract on biochemical parameters against Isoniazide & Rifampicin (INH+RIF) induced hepatotoxicity in mice was studied. The level of biochemical parameters such as SGOT, SGPT, ALP and bilirubin in the INH+RIF treated group were increased as compared to the normal group. After given treatment with extract at doses of 400 mg/kg and Silymarin (standard) at 100 mg/kg had significantly restored the level of SGOT, SGPT, ALP and bilirubin as compared to INH+RIF treated groups and vehicle treated group. The use of antitubercular drug produces acute hepatic damage on accidental over dosage. In Isoniazide & Rifampicin treated group SGOT, SGPT, ALP and Bilirubin level was significantly high ($P<0.05$) as compared to only vehicle treated group. When animals were administered extract at the dose of 400 mg/kg it was observed that level of all marker enzymes was significantly less ($P<0.05$) as compared to that of control treated group. The result has clearly highlighted the hepato-protective potential of the extract against Isoniazide & Rifampicin induced toxicity.

Table 1: Effect of the Extract on biochemical parameters (Isoniazide+ Rifampicin)

Treatment Group	Bioassay parameters			
	SGOT	SGPT	ALP	BILIRUBIN
Vehicle	45.49±1.396	52.52±1.712	18.47±1.245	1.914±0.1802
INH+RIF	136.66±5.68[a]	132.66±2.58[a]	302.33±9.87[a]	1.81±0.111[a]
Silymarin	47.16±3.76 [ab]	38.5±8.36[ab]	182.16±11.7[ab]	0.808±0.056[ab]
S(400mg/kg)+INH+RIF	79.833±6.67[ab]	64.5±5.08[ab]	214.16±11.9[ab]	0.893±0.048[ab]

All data are presented in mean ± SD (n =6)

a- $P<0.05$ as compared to vehicle treated group, b- $P<0.05$ as compared to Vehicle + INH+RIF group

CONCLUSION

The metabolism of human beings is influence by the function of liver. It can be concluded with the biochemical results that the extracts of *Pterospermum acerifolium* has hepatoprotective action. The production and elimination of the toxic metabolites depends on the activities of several enzymes, such as N-acetyl transferase 2 (NAT2), cytochrome P450 oxidase (CYP2E1) and glutathione S-transferase (GSTM1). Hence the role of extract on the enzyme involve in production and elimination of the toxic metabolites can be studied and the extracts can be used in future in combination with antitubercular drug to prevent the acute hepatic damage produced on accidental over dosage

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