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Hepatoprotective activity of different extracts of *Pterospermum acerifolium* against paracetamol induced hepatotoxicity in albino rats

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Abstract
The petroleum ether and hydro alcoholic extracts of *Pterospermum acerifolium* belonging to the Sterculiaceae family were studied for hepatoprotective activity against albino rats with liver damage induced by paracetamol. The petroleum ether extract at 50 mg/kg was having best activity as it decreased the mean level of bilirubin from 2.3645 ± 0.07 to 0.2975 ± 0.13. Petroleum ether extract was found to highly protective at both the dose of 25 mg/kg and 50 mg/kg for ALP, SGOT, and SGPT. For LPO level, SOD and Catalase level it was observed that both extract were having significantly protection at 25 mg/kg and 50 mg/kg dose.

Keywords: *Pterospermum acerifolium*, Herbal drugs, Hepatoprotective activity, paracetamol

Introduction
It is not unreasonable to believe that the plant kingdom should yield safe and effective drugs for most of the human ailments. Though India has a rich tradition in the use of medicinal plants, the effort to develop drugs from plants has had limited success. Hence, plant have returned to the fore front in drug development as medicines, as source of active molecules and as lead to the discovery of new drugs. The use of medicinal plants to treat various diseases in India dates back to the times of Rig-Veda (3500-1800BC).Later, the monumental Ayurveda works like charak samhita and Sushruta samhita followed by other Ayurveda drugs entering in to several medicinal preparation were in the management of health care. In fact these systems have been in practice even in remote areas of our country for centuries. World Health Organization (WHO) estimates that 80% of the people living in developing countries almost exclusively using traditional medicine and about 119 secondary metabolites of plants are used globally as drugs [1].

Herbal drugs constitute only those traditional medicines, which primarily use medicinal plants preparation for therapy. The earliest recorded evidence of their use in in Indian, Chinese, Egyptian, Greek, Roman and Syrian text dates back to about 5000 years. The classical Indian texts include Rigveda, Atharvaveda, Charak Samhita and Sushruta Samhita. The herbal medicines have traditions of ancient civilization and scientific heritage [2]

Liver has the great capacity to detoxicate toxic substance and synthesize useful chemical principal. Therefore, damage to the liver infected by hepatotoxic agent is of great concern. Liver diseases are mainly caused by toxic chemicals, excess consumption of alcohol, infection and autoimmune disorders. Most of the hepatotoxic chemicals damage liver cells prim artily by producing reactive species which form covalent bond with the lipids of the tissue. However, inbuilt protective mechanism combat the hazardous reaction associated with the free radicals. Due to excessive exposure to hazardous chemicals, sometimes the free radical generated are so high that they overpower the natural defense system leading to hepatic damage and cause jaundice, cirrhosis, and fatty liver. Production of the reactive species manifesting tissue thiol depletion, lipid peroxidation, plasma membrane damage etc., culminating into severe hepatic injury). Certain medicinal agent when taken in overdoses and sometimes even when introduced within therapeutic ranges may injure organ. Liver is the most important organ in maintaining the homeostasis of the body. It is involved with almost all the biochemical pathways related to growth, fight against disease, nutrient supply, energy provision and reproduction. It plays a pivotal role in regulating metabolism of endogenous as well as xenobiotic substances, secretion and storage [3].

Pterospermum acerifolium (Sterculiaceae family) is well distributed in India, particularly in sub-Himalayan tract and outer Himalayan valleys.

Common name is kanakchampa or muchukunda. Externally the leaves are variable in shape and size, 25-35 cm (l), 15-30 cm (w), orbicular or oblong, entire or various lobed, cordate, glabrous above and clothed beneath with whitish floccose tomentum (Kirtikar K.R, 1996). Flavonoids in the leaves include luteolin-7-O-β-D glucuronide, luteolin-7-O-β-D glucoside, kaempferol-3-O- β-D galactoside, friedeline, barurenol [4]). Traditionally it is used for ear pain, Small pox, leucorrhoea, inflammation, ulcers, leprosy, blood disorders, tumours, diabetics [5].

However, no work has been reported on the hepatoprotective properties of *Pterospermum acerifolium*. So the present study has been undertaken to investigate hepatoprotective activity of the petroleum ether and hydro alcoholic extract of *Pterospermum acerifolium* leaves on paracetamol induced liver damage in rats.

Materials and Methods

Collection of plant material

Fresh leaves of *Pterospermum acerifolium* (Sterculiaceae family) collected in the month of August 2010 from Bhopal, dried under shade and powdered in a mixer grinder. The powdered leaves were packed in air bags and stored in air tight containers until use.

Authentication

The botanical identity was confirmed by department of Botany, Safia College of Science, Bhopal (M.P) where voucher specimen no (148/BOT/SAFIA/2010) has been deposited for further references.

Drugs and chemicals

Polyethylene glycol-400, sodium dodecyl sulphate, Sodium dihydrogen phosphate dehydrate, Disodium hydrogen phosphate anhydrous, ethylene diamine tetra chloric acid (EDTA), trichloroacetic acid, Tris base (merck), Formaldehyde (LR 37-41%), Acetic acid, Sodium pyrophosphate, Phenazine methosulphate, Thiobarbituric acid, (Sunchem AR), Ellman's reagent (Sigma-Aldrich), Sodium ctrate, Nitro blue tetrazolium (NBT), Nicotinamide Adenine dinucleotide(sd fine –chem limited), 30% Hydrogen peroxide (Rankem LR), Silymarin and Paracetamol (Alfa Remedies, Ambala). All other solvents/reagents were of analytical grade from Merk-India.

Extraction

120g dried powdered drug was taken and extracted with petroleum ether using hot continuous extraction method at 40-50 ^{0}C. After extraction the extract was dried in oven at 50 ^{0}C. The percentage yield of petroleum ether extract was 4.12% which was refrigerated at 18^{o}C. 100g of the dried powder marc was extracted with methanol: water (7:3) by soxhlation method at 10-20oC. The percentage yield of hydro alcoholic extract was 13.62% which was refrigerated at 18^{o}C.

Phytochemical screening

The preliminary phytochemical screening was done by following standard qualitative chemical tests for phytoconstituents and the hydroalcoholic leaf extract of on preliminary phytochemical screening showed the presence of tannins, phenolics and flavonoids.

Experimental Animals

Albino rats (Sprague Dawley strain) of either sex, 3-4 months old and weight around 100-150gm. The animals were housed in an animal room with alternating with light- dark cycle of 12 hreach. The animals were acclimatized for at least 7 days to the laboratory condition before conducting experiments in PBRI having institutional animal ethical committee approval number 1283/C/O9/CPCSEA and PBRI /IAEC/2003/PN33.

Acute Toxicity Studies

20 Animals were divided into 5 groups of 4 each & were administrated with aliquot doses of extract orally (100, 150, 200, 250, &300mg/kg) Mortality was not noticed in the dose of 300 mg/kg. The LD50 of extract was found to be 250 (mg/kg) body weight. One tenth of this dose was selected as the therapeutic dose for the evaluation.

Paracetamol-induced hepatotoxicity in rats

Paracetamol (acetaminophen), a widely used analgesic-antipyretic drug on accidental over dosage (which may occur in alcoholics and elderly), produces acute hepatic damage. The covalent binding of an oxidation product of paracetamol i.e., N-acetyl-p-benzoquinoneimine and sulphydryl groups of protein results in cell necrosis and lipid peroxidation which causes hepatotoxicity leading to increased levels of serum marker enzymes like SGOT, SGPT, ALP and total bilirubin

Experimental method

Animals were divided into 7 groups and each group were comprised of 4 rats.

Group 1 served as Normal control which was treated with vehicle only. Group 2 served as paracetamol Control and the animal received paracetamol (500mg/kg) orally once daily for 7 days and then treated with vehicle only. Group 3 received paracetamol (500mg/kg) orally once daily and treated with petroleum ether extract (25mg/kg) conjointly for 7 days. Group 4 received paracetamol (500mg/kg) orally once daily and treated with petroleum ether extract (50mg/kg) conjointly for 7 days. Group 5 received paracetamol (500mg/kg) orally once daily and treated with hydroalcoholic extract (25mg/kg) conjointly for 7 days. Group 6 received paracetamol (500mg/kg) orally once daily and treated with hydroalcoholic extract (50mg/kg) conjointly for 7 days. Group 7received paracetamol (500mg/kg) orally once daily and treated with Silymarin (25mg/kg) conjointly for 7 days [3].

The biochemical parameters were estimated after an 18h fast following the last dose.

Biochemical studies

The blood was obtained from all animals by puncturing retro-orbital plexus. The blood samples were allowed to clot for 45 min at room temperature. Serum was separated by centrifugation at 2500rpm at 30 ^{0}C for 15 min and utilized for the estimation of various biochemical parameters namely SGOT, SGPT, SALP, and serum bilirubin. After collection of blood samples the rats in different groups were sacrificed and their livers were excised immediately and washed in ice cold normal saline, followed by 0.15 M Tris-Hcl (pH 7.4) blotted dry and weighed. A 10%w/v of homogenate was prepared in 0.15 M Tris-Hcl buffer and processed for the estimation of lipid peroxidation. A part of homogenate after precipitating proteins with Trichloroacetic acid (TCA) was used for estimation of glutathione. The rest of the homogenate was centrifuged at 1500 rpm for 15 min at 4 ^{0}C. The supernatant thus obtained was used for estimation of SOD and CATALASE activities [6].

Serum hepatospecific markers

Activities of serum glutamate oxaloacetate transaminase (SGOT) and serum glutamate pyruvate transaminase (SGPT) were estimated by the method of Reitman and Frankel. 0.05 ml of serum with 0.25 ml of substrate (aspartate and α-ketoglutarate for SGOT; alanine and α -ketoglutarate for SGPT, in phosphate buffer pH 7.4) was incubated for an hour in case of SGOT and 30 min. for SGPT. 0.25 ml of DNPH solution was added to arrest the reaction and kept for 20 min in room temperature. After incubation 1 ml of 0.4N NaOH was added and absorbance was read at 505 nm in uv-vis spectrophotometer. Activities were expressed as IU/L.

Alkaline phosphatase activity was assayed using sodium phenyl phosphate as substrate. The colour developed was read at 510 nm in uv-vis spectrophotometer after 10 min. Activities of ALP was expressed as IU/L. Serum total bilirubin level was estimated based on the method of Malloy and Evelyn. D Diazobenzenesulfonic acid (0.25 ml) reacts with bilirubin in diluted serum (0.1 ml serum + 0.9 ml distilled water) and forms purple colored azobilirubin, which was measured at 540 nm in uv-vis spectrophotometer. Activities of total bilirubin were expressed as mg/dl [7].

Determination of Thiobarbituric Acid Reactive Substances (TBARS)

Lipid peroxidation in liver tissues were estimated colorimetrically by measuring thiobarbituric acid reactive substances (TBARS) [8]. To 0.2ml of sample, 0.2ml of 8.1% Sodium dodecyl sulfate, 1.5 ml of 20% acetic acid and 1.5 ml of 0.8% TBA were added. The volume of the mixture was made up to 4ml with distilled water and then heated at 95 ^{0}C in a water bath for 60 min. After incubation the tubes were cooled to room temperature and the final volume was made upto 5 ml in each tube. Then 5 ml of n-butanol: Pyridine mixture was added and the contents were vortexed thoroughly for 2 min. After centrifugation at 3000 rpm for 10 min the upper organic layer was taken and its OD was read at 532 nm against an appropriate blank without the sample. The levels of lipid peroxides were expressed as milimoles of thiobarbituric acid reactive substances (TBARS)/100gram of liver tissue using an extinction co-efficient of 1.56x10 ^{5}M^{-1}cm^{-1}

Determination of reduced glutathione (GSH)

Reduced glutathione (GSH) was determined [9]. To 0.1 ml of different tissue homogenate 2.4 ml of 0.02M EDTA solution was added and kept on ice bath for 10 min. Then 2 ml of distilled water and 0.5 ml of 50 % TCA were added. This mixture was kept on ice for 10-15 min and then centrifuged at 3000 rpm for 15 min. 1 ml of supernatant was taken and 2ml of Tris-Hcl buffer was added. Then 0.05ml of DTNB solution (Ellman's reagent) was added and vortexed thoroughly. OD was read within 2-3min after the addition of DTNB) at 412 nm against a reagent blank. Absorbance values were compared with a standard curve generated from known GSH.

Assay of super oxide dismutase (SOD)

Superoxide dismutase (SOD) activity was determined [10]. The assay mixture contained 0.1ml of sample, 1.2ml of sodium pyrophosphate buffer (pH 8.3, 0.052 M), 0.1 ml of phenazine methosulphate (186µm), 0.3 ml of nitro blue tetrazolium (300µm), 0.2 ml of NADH (750µm). Reaction was started by addition of NADH. After incubation at 30^0 C for 90 sec, the reaction was stopped by the addition of 0.1 ml of glacial acetic acid. The reaction mixture was stirred vigorously with 4.0 ml of n-butanol. The mixture was allowed to stand for 10 min, centrifuged and butanol layer was separated. The colour intensity of the chromogen in butanol layer was measured at 560 nm against n-butanol and concentration of SOD was expressed as units/g of liver tissue. Absorbance values were compared with a standard curve generated from known SOD.

Assay of Catalase (CAT)

Catalase estimation was done spectrophotometrically following the decrease in absorbance at 230 nm. The liver tissue was homogenized in M/150 phosphate buffer (pH 7.0) at 1-4 ^{0}C and centrifuged at 5000 rpm. The reaction mixture contained 0.01 M phosphate buffer (pH 7.0), 2 mM H2O2 and the enzyme extract. The specific activity of catalase was expressed in terms of units/gram of liver tissue. Absorbance values were compared with a standard curve generated from known CAT [11].

Statistical analysis

The experimental results were expressed as the Mean ± SEM for six animals in each group. The biochemical parameters were analysed statistically using one-way analysis of variance ANOVA, followed by Bonferroni t-test. P value of < 0.05 was considered as statistically significant.

Results and Discussion

Different extracts of *Pterospermum acerifolium* was investigated for its protective potential against paracetamol induced toxicity on various parameters like total bilirubin, SGOT, SGPT, ALP, SOD, LPO, GSH and catalase level (Table no. 1).

Effect of petroleum ether and hydroalcoholic extract of *Pterospermum acerifolium* was studied on total bilirubin and both of the extracts were having significant protection against increased bilirubin at both the dose. The petroleum ether extract at 50 mg/kg was having best activity as it decreased the mean level of bilirubin from 2.3645 ± 0.07 to 0.2975 ± 0.13 on the other hand hydroalcoholic extract at 25 mg/kg dose was least effective. In both cases silymarin decreased the total bilirubin significantly.

Effect of different extracts of *Pterospermum acerifolium* was studied on hepatic parameters like ALP, SGOT, SGPT in paracetamol intoxicated rats. Paracetamol increased ALP, SGOT and SGPT significantly. Petroleum ether extract was found to highly protective at both the dose of 25 mg/kg and 50 mg/kg for ALP, SGOT, and SGPT. The level of all three parameters decreased significantly as compared to that of induction due to paracetamol intoxication. The hydroalcoholic extract was having no significant protection at 25 mg/kg for ALP, SGOT, and SGPT leve. Although the extract was having significant protection at 50 mg/kg for ALP and SGOT level but the extract was having no significant protection for SGPT level at 50 mg/kg dose.

Table 1: Effect of different extracts of *Pterospermum acerifolium* on hepatic parameters like total bilirubin, SGOT, SGPT, ALP

Groups	Total bilirubin (mg/100ml)	ALP(IU/L)	SGOT(IU/L)	SGPT(IU/L)
1.Vehicle50%PEG	0.1115 ± 0.082359	20.35 ± 4.720261	39.6575 ± 7.403357	19.9075 ± 1.992843
2. Paracetamol + Vehicle 50%PEG	2.3645 ± 0.078139**	37.725 ± 1.499744**	70.8475 ± 6.399179**	24.385 ± 3.869716**
3. Paracetamol +Pt.Ether extract 25mg/kg	0.63525 ± 0.150659*	20.84 ± 4.60936*	53.3225 ± 7.916019*	13.37 ± 3.663814*
4. Paracetamol +Pt.ether extract50mg/kg	0.2975 ± 0.135136*	16.0775 ± 4.150754*	39.785 ± 3.259719*	12.995 ± 4.670607*
5. Paracetamol + Hydroalcoholic extract 25mg/kg	1.7175 ± 0.43739*	34.0325 ± 11.22993***	62.2425 ± 7.778249***	19.86 ± 4.433982***
6. paracetamol +hydroalcoholic extract 50mg/kg	0.55075 ± 0.272204*	12.685 ± 4.698294*	32.9475 ± 5.091276###	18.955 ± 2.677742***
7. Paracetamol + Silymarin 25mg/kg	0.33975 ± 0.131378*	4.97 ± 1.404825###	40.0875 ± 2.542405###	21.4475± 5.14864

Effect on LPO and GSH level of different extracts of *Pterospermum acerifolium w*as studied and it was observed that the Paracetamol treatment significantly increased the level of LPO and significantly decreased the GSH level. For LPO level it was observed that both extract were having significantly protection at 25 mg/kg and 50 mg/kg dose. The Hydro alcoholic extract at 50 mg/kg was having best activity as compared to other dose and pet ether. The pet ether extract was having non-significant effect at both doses for GSH level. The hydroalcoholic extract was also not having any significant effect at 25 mg /kg dose although the extract was having significant protection at 50 mg/kg dose.

The effect of petroleum ether extract and hydro alcoholic extract on the SOD and Catalase level was observed for protection against paracetamol induced damage. It was observed that both of the extract were having significant effect on the SOD and Catalase level as compared to that of the paracetamol treated groups at 25 mg/kg dose.

Table 2: Effect of different extracts of *Pterospermum acerifolium* on hepatic parameters like SOD, LPO, GSH and catalase level

Groups	LPO Nm MDA/mg protein	GSH µg/mg protein	SOD Unit/mg liver protein	CATALASE Units/mg Liver protein
1.Vehicle50%PEG	1.25 ± 0.05	6.04745 ± 0.909192		0.5145 ± 0.281484
2. Paracetamol + Vehicle 50%PEG	7.32 ± 0.28**	2.911175 ± 0.477035****	0.074275 ± 0.070832**	0.23475 ± 0.15195**
3. Paracetamol +Pt.Ether extract 25mg/kg	6.68 ± 0.95*	3.7654 ± 0.663493***	1.958975 ± 0.164216*	1.2789 ± 0.41125*
4. Paracetamol +Pt.ether extract50mg/kg	4.25 ± 0.69*	3.668375 ± 1.844724***	0.71235 ± 0.258093*	1.176 ± 0.759105*
5. Paracetamol + Hydroalcoholic extract 25mg/kg	3.125 ± 0.07*	3.513225 ± 0.250375***	0.327225 ± 0.242532*	2.1315 ± 0.773207*
6. paracetamol +hydroalcoholic extract 50mg/kg	3.38 ± 0.21*	2.006425 ± 0.167773*	2.674575 ± 0.488408*	1.818 ± 0.795786*
7. Paracetamol + Silymarin 25mg/kg	3.43 ± 0.66*	4.209975 ± 0.699386*	0.818825 ± 0.251701*	0.882 ± 0.831558*

Conclusion

The results indicate that both petroleum ether extract and hydro alcoholic extract of *Pterospermum acerifolium* have significant hepato protective activity. This may be probably due to the flavonoids as the flavonoids possess antioxidant properties and found to be useful in the treatment of liver damage.

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