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Full Length Research Paper

Hepatoprotective potentials of *Butea monosperma* stem bark extract against carbon tetrachloride induced hepatotoxicity in albino rats

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Carbon tetrachloride (CCl_4) pharmacological tool was used to produce liver damage in rats. Silymarin (100 mg/kg) and extract of *Butea monosperma* (shown to be hepatoprotective substances) prevented the CCl_4 induced toxicity. Hydroalcholic extract of the stem bark of *B. monosperma* was evaluated for its hepatoprotective. This *in vitro* efficacy was reinforced by a significant dose dependent hepatoprotection (at 100 and 200 mg/kg dose) by decreasing the activity of serum enzymes, bilirubin, and lipid peroxidation while it significantly increased the reduced Glutathione levels of tissue in a dose dependant manner. The hepatoprotective activities of the extract are being comparable to standards Silymarin. The results obtained in the present study indicate that stem bark extract of *B. monosperma* is a potential source of natural hepatoprotective. The hepatoprotective property may be attributed to the antioxidant potential and the phytochemical constituents of the plant. The present study justifies the claim of the native practitioner that the decoction of the plant is useful in treating jaundice and find out the clinical efficacy of the *B. monosperma.*

Key words: Carbon tetrachloride, *Butea monosperma*, silymarin, alanine amino transferase, glutathione, serum glutamate oxaloacetate transaminase, serum glutamate pyruvate transaminase, hepatoprotection.

INTRODUCTION

Hepatic system is a very vital organ system involved in the body's metabolic activities. As a result the chemical reactions in the liver may generate several reactive species like free radicals. These reactive species form covalent bond with the lipids of the tissue. However inbuilt protective mechanisms combat the hazardous reactions associated with the free radicals. Due to excessive exposure to hazardous chemicals, the free radicals generated will be so high such that they overpower the natural defensive system leading to hepatic damage and cause jaundice, cirrhosis and fatty liver, which remain one of the serious health problems. Carbon tetrachloride (CCl_4) is one of such hazardous chemicals which induces hepatopathy through membrane lipid peroxidation by its free radical derivatives, trichloromethyl radical (CCl_3•) and trichloromethylperoxy radical (CCl_3O_2•). Excessive production of the reactive species manifests in tissuethiol depletion, lipid peroxidation, plasma membrane damage etc., culminating into severe hepatic injury (Chrungoo et

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Abbreviations: **CCl_4**, Carbon tetrachloride; **CCl_3**, trichloromethyl radical; **CCl_3O_2,** trichloromethylperoxy radical; **SGOT,** serum gluataic oxaloacetate transaminase; **SGPT,** serum glutamic pyruvic tranaminase; **MDA,** malonaldehyde; **ALP,** alkaline phosphate; **GR,** glutathione reductase; **TCA,** tissue glutathione; **DTNB,** 5, 5 Dithio-bis 2- nitrobenzoic acid; **ANOVA,** analysis of variance; **TCA,** trichloroacetate; **TBA**, tribromoanisole; **HCl,** hydrochloric acid; **GPx,** glutathione peroxidase; **SOD,** superoxide dismutase.

al., 1997). The traditional systems of medicine together with homoeopathy and folklore medicine continue to play a significant role largely in the health care system of the population. *Butea monosperma* (Lam.) Taub (Palas) belonging to the family leguminoceae grown wildly in many parts of India. The plant is regularly used by the rural and tribal people in curing various disorders (Jhade et al., 2009). The bark of the plant is an appetiser, lessens inflammation, dysmenorrhoea used in liver disorders, fractures, and gonorrhoea, topically in piles and hydrocele purifies the blood. Leaf is appetiser, very astringent, carminative, anthelmintic, aphrodisiac, tonic; lessen inflammation and lumbago, cures boils and piles. Gum is acrid, astringent, aphrodisiac, tonic to the liver, used in the diseases of the chest and lungs useful in syphilis. The flower is bitter, aphrodisiac, expectorant, and tonic, emmenagogue, diuretic, astringent, and good in inflammation, burning urine and gonorrhoea. The fruit and seeds are bitter and oily, anthelmintic, useful in piles, eye diseases and inflammation. The lye is useful in enlargement of spleen (Kirtikar and Basu, 1991). *B. monosperma* (Lam.) is commonly known as flame of forest, belongs to the family Fabaceae (Patil et al., 2006). It is locally called palas, palash, mutthuga, bijasneha, dhak, khakara, chichra, bastard teak, Bengal Kino, Nourouc and it is common throughout India, Burma and Ceylon except in very acrid parts. Generally it grows gregariously on open grasslands and scattered in mixed forest. Plantations can be raised both on irrigated and dry lands. The pods should be collected and sown before the commencement of rains, root suckers are freely produced and help in vegetative propagation. In India, palas ranks next to kusum (*schleichera trijuga*) as a host tree for lac insect (Kirtikar and Basu, 1935; Kapoor, 2005). Almost all the parts of the plant are being used since decades in medicine and for other purposes. These days' herbal medicines are more popular than modern medicine because of their effectiveness, easy availability, low cost and for being comparatively devoid of side effects. Nature always stands a golden mark to exemplify the outstanding phenomenon of symbiosis and it has provided the storehouse of remedies to cure all ailments of mankind, only the thing is that there is a need to evaluate them scientifically. Stem bark powder is used to stupefy fishes. Young roots are used for making ropes (7). Green leaves are good fodder for domestic animals. Leaves are used for making platters, cups, bowls and beedi wrappers (7, Ambasta, 1994). Leaves are also used for making Ghongda to protect from rains and are eaten by buffaloes and elephants. Similarly there were claims from a local native practitioner that the decoction of the test plant is highly useful in treating jaundice. Since the pharmacological profile of the plant is not completely established. Therefore this plant is taken for the present study. With this scientific information, the present study was designed with an aim to assess the hepatoprotective activity of the stem bark extract of *B. monosperma*, against CCl_4 induced liver damage.

MATERIALS AND METHODS

Plant source

Healthy disease free, mature fresh plant root sample were collected locally from Bilaspur, Chhattisgarh, India. Fresh plants were washed thoroughly 2-3 times with running tap water and once with sterile water, shade-dried without any contamination. The dried stem barks were then powdered using a grinder.

Preparation of extracts

The *B. monosperma* collected locally from Bilaspur, Chhattisgarh, India. The fresh plants were detached from the stems and dried at room temperature (27°C) for a week. They were then weighed several times until the weight was constant. The dried stem bark then ground into a fine powder with the help of grinder and the powder kept in an airtight amber container, for extraction procedure. The dried powder (25 g) stem bark of *B. monosperma* was extracted with a mixture of water and methanol in the ratio of 50:50, respectively. Extraction was continued at the temperature of 27°C till clear solvent was observed in siphon tube. Extract was concentrated in water bath at 40°C. Concentrated extract was dried at 40°C in hot air oven. Dried extract was packed in an air tight container.

Preliminary phytochemical investigation

All the extracts were subjected to preliminary phytochemical tests (Kokate, 1985). All the tests reveal that the plant possesses steroids, glycosides, triterpenoids, tannins and flavonoids. Since hydroalcholic extract has shown the better results for the presence of polyphenolic compounds and triterpenoids, this extract was selected for further study.

Animals

Male swiss albino rats (Animal house of Pinnacle Biomedical Research Institute, Bhopal, India.) weighing between 120-150 g were used. They were housed in polypropylene cages under standard conditions (23 ± 2 .C, humidity 60–70%, 12 h light/dark cycles). They were given standard pellet diet (Lipton India Ltd. pellets) and tap water ad libitum. The experiments were performed during day (08:00-16:00 h). The institutional animal ethical committee approved to the study protocol.

Carbon tetrachloride induced toxicity

The method of Ko et al. (1993) was used for screening the hepatoprotectivity of the test extract. The animals were randomly assigned into 5 groups of 6 animals. Groups 1 and 2 served as normal and intoxicated control and received only the vehicle (normal saline). Group 3 served as standard, was treated with Silymarin (100 mg / kg / day BW for 3 days) The animals of Groups 4 and 5 received stem bark extract of *B. monosperma* (100 mg/kg BW and 200 mg / kg BW, respectively) for 3 days. 24 h after the last dosing, animals (except Group1) were treated orally with CCl_4 (11% *v/v* in olive oil) at a dose of 1 ml / kg BW. Animals were sacrificed 24 h, after CCl_4 treatment, hepatic tissue and heparinized blood sample were taken and assessed for serum enzyme and Glutathione estimation. Serum enzymes, which were assessed, include Serum gluataic oxaloacetate transaminase (SGOT) and Serum glutamic pyruvic tranaminase (SGPT) (Retiman and Frankel, 1957), total bilirubin and direct bilirubin (Malloy and Evelyn, 1937), and

Table 1. Effect of stem bark extract of *B. Monosperma* and CCl_4-induced hepatoxicity (n=6).

Group	SGOT Level (U/L) Mean ±SE	SGPT (U/L) Mean ±SE	ALP (mg/dl) Mean ±SE	Total bilirubin (mg/dl) Mean ±SE	Direct bilirubin (mg/dl) Mean ±SE
Group 1	112.9±0.71	49.5±0.05	217.6±0.12	0.890±0.001	0.179±0.001
Group 2	281.9±0.61	171.2±0.60	890.8±0.57	8.47±0.061	3.91±0.006
Group 3	179.1±0.46	80.1±0.55	398.1±1.07	3.53±0.115	0.185±0.002
Group 4	227.2±1.18	154.2±0.63	520±0.64	5.86±0.577	0.532±0.002
Group 5	199.2±0.51	114.6±1.80	669.7±0.55	4.52±0.105	0.439±0.001

Values are the Mean ± SEM of six rats/ treatment. Group 1, Normal animals (untreated); Group 2, CCl_4 (1ml/kg) treated animals; Group 3, CCl_4 + Silymarin (100mg/kg BW) treated animals; Group 4, CCl_4 + *B. monosperma* (100mg/kg BW) treated animals; Group 5, CCl_4 + *B. monosperma* (200mg/kg BW) treated animals.

Alkaline phosphate (ALP) content. Tissue glutathione measurements were performed using a modification of the Ellman procedure (GeorgeEllman, 1959; Aykae, 1985). Tissue samples were homogenized in ice cold trichloroacetate (TCA) (l gm tissue plus 10 ml 10% TCA) in a homogeniser. Briefly, after centrifugation at 3000 rpm for 10 min, 0.5 ml supernatant was added to 2 ml of 0.3 M disodium hydrogen phosphate solution. A 0.2 ml solution of 5, 5 Dithio-bis 2- nitrobenzoic acid (DTNB), (0.4 mg in 1 ml of 1% Sodium nitrate) was added and the absorbance at 412 nm was measured immediately after mixing. Extent of lipid peroxidation was done by combining 1.0 ml of biological sample (0.1 – 2.0 mg of membrane protein or 0.1–0.2 µmol of lipid phosphate) with 2.0 ml of TCA- tribromoanisole (TBA)-hydrochloric acid (HCl) and thoroughly mixed. The solution was heated for 15 min in a boiling water bath. After cooling, the flocculent precipitate was removed by centrifugation at 1000 rpm for 10 min. The absorbance of the sample is determined at 535 nm against blank that contains all the reagents without the lipid (John and Steven, 1978).

Statistical analysis

Data were analyzed by analysis of variance (ANOVA) followed by Bonferroni's multiple variance test. Results with $P<0.05$ were considered statistically significant.

RESULTS AND DISCUSSION

The estimated values of serum GOT, GPT, ALP, Total bilirubin and direct bilirubin values in control (saline + vehicle) group of rats were found to be 112.9±0.71, 49.5±0.05, 217.6±0.12, 0.890±0.001, 0.179±0.001, respectively (Table I). A remarkable elevation was observed in serum GOT, GPT, ALP, total bilirubin and direct bilirubin values in CCl_4 intoxicated rats (Toxic control group). In the groups treated with 100 mg/kg and 200 mg/kg of the stem bark of *B. monosperma* extract, the above biochemical markers of hepatotoxicity were found to be decreased when compared to CCl_4 treated control group. Evidently, the hepatoprotective effects of higher dose of *B. monosperma* (200 mg/kg) were near to that of standard i.e. Silymarin (100 mg/kg). Both the doses of stem bark extract of *B. monosperma* used in the study showed significant protective property than control. However the test extract was found to be less potent than that of standard drug. The tissue glutathione was found to be depleted upon CCl_4 intoxication, indicate that the tissue damage is due to over powering the inbuilt free radical scavenger mechanisms. This tissue GSH depletion was inhibited by the pretreatment with test extract in a dose dependant manner. Similarly lipid peroxidation induced by CCl_4 treatment was reversed by test extract in a dose dependant manner. The results are compiled in Table 2.

CCl_4 is a pharmacological tool used to produce liver damage in animal models; its hepatotoxic action begins with changes in endoplasmic reticulum which results in loss of metabolic enzymes located in the intracellular structure (Wagner and Wolff, 1977; Recnagel, 1983). The stem bark extract of *B. monosperma* was taken for assessing the *in vivo* hepatoprotective properties. Pretreatment with the test extract has reduced the elevated levels of biochemical markers of hepatoxicity. Further it was also observed that the tissue GSH depletion due to CCl_4 challenge was reversed by the test extract and also reduced the extent of lipid peroxidation. Most of the mammals have an effective mechanism to prevent and neutralize the free radical induced damage, which is accomplished by a set of endogeneous substances such as superoxide dismutase (SOD) catalase, glutathione peroxidase(GPx/) and glutathione reductase (GR). CCl_4 undergo hepatic metabolism to give rise to trichloro methyl radicals, which upon reacting with reactive oxygen species yields CCl_3O_2, which forms covalent bond with membrane lipids and destroy the membrane integrity. The observation of increased malonaldehyde (MDA) formation in hepatic cells after CCl_4 challenge is in accordance with the earlier report, which suggests involvement of CCl_3 and CCl_3O_2 in the propagation of peroxidation process (Indu and Aruna, 1993). The pretreatment with extract has prevented oxygen free radicals and thereby prevented the formation of peroxy radicals. This aspect of test extract also contributes to the hepatoprotectivity. Thus, from the results of the present investigation it may be concluded that the stem bark extract of *B. monosperma* possess significant hepatoprotective activity. It appears that hepatoprotective activity of the test extract is due to their phytochemical constituent which contains kino-tannic acid, gallic acid, pyrocatechin. The plant also contains palasitrin, and major glycosides as

Table 2. Effect of stem bark extract of *B. monosperma* on hepatic GSH status in rats (n=6) and carbon tetrachloride-induced peroxidation in rats (n=6).

Treatment	Dose (mg/Kg BW)	GSH (Abs. 412 nm)	Lipid peroxidation (Abs. 535 nm)
Normal Saline	-	0.122±0.0001	0.090±0.002
Normal Saline-CCl_4	-	0.576±0.002	0.305±0.001
Sylamarin- CCl_4	100	0.791±0.003	0.072±0.004
B. monosperma -CCl_4	100	0.429±0.002	0.214±0.003
B. monosperma -CCl_4	200	0.659±0.001	0.147±0.001

Abs: Absorbance

butrin, alanind, allophanic acid, butolic acid, cyanidin, histidine, lupenone, lupeol, (-) - medicarpin, miroestrol, palasimide and shellolic acid (Sharma and Deshwal, 2011) and antioxidant potential. The antioxidant potential may be attributed to the presence of polyphenolic compounds. Further studies like isolation and characterization of the active principal(s) responsible for such activity are needed to confirm. However the present study justifies the claim of the native practitioner that the plant is used as a hepatoprotective.

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