Hepatoprotective and antioxidant activity of the methanolic extract of *Mucuna pruriens* Bak. roots

M Mujeeb^{1,*}, P Bagri¹, V Aeri¹ and SA Khan²

¹Department of Pharmacognosy and Phytochemistry, Faculty of Pharmacy, Jamia Hamdard, New Delhi-110062, India; ²Department of Pharmacy, Oman Medical College, Muscat, Sultanate of Oman

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SUMMARY

The methanolic extract of natural roots of *Mucuna pruriens* was screened for hepatoprotective and antioxidant effects in carbon tetrachloride (CCl₄) induced hepatotoxicity in Albino rats. The degree of protection was measured by estimating biochemical parameters like serum glutamate oxaloacetate transaminase, serum glutamate pyruvate transaminase, total protein and level of serum bilirubin (both total and direct). The extract also reduced CCl₄ induced lipid peroxidation both *in-vitro* and *in-vivo*. Hepatoprotective activity of methanolic extract at dose 300 mg/kg body weight, i.p., was comparable with standard drug Silymarin (25 mg/kg, i.p.). Furthermore, histopathological experiments were also carried out to support the study.

Key words: *Mucuna pruriens*; Hepatoprotective activity; Antioxidant activity; Carbon tetrachloride

INTRODUCTION

Mucuna pruriens Bak. (Family; Leguminosae) commonly known as Kiwanch in Hindi, is an annual twining herbaceous plant distributed throughout India in all plain districts to lower subtropical hills of Himalaya (Anonymous, 1995). The plant has been reported to contain 3,4 dihydroxy phenylalanine, alkaloids viz. nicotine, pruriene and prurienidine (Majumdar and Zalani, 1953; Niranjana and Katiyar, 1974), sterols, triterpenes and flavonoids (Aruna *et al.*, 1998).

In Ayurveda (An Indian Traditional system of medicine), the roots of *Mucuna pruriens* (MP) have been used in treatment of cholera, infertility,

snakebite and in worm infection (Anonymous, 1995). The other reported activities include Analgesic and Antipyretic (Jauk *et al.*, 1993), anticoagulant (Houghton and Shari., 1994), Antispasmodic (Dhar *et al.*, 1968) and Hypoglycaemic activity (Rathi, 2002). *In-vitro* lipid peroxidation and antimicrobial activity of MP seeds have also been reported (Yerra *et al.*, 2005).

Keeping in view the role of sterols, flavonoids and triterpenes in medicinal plants as hepatoprotective drugs (Handa and Kaur, 1996), it was thought worthwhile to conduct hepatoprotective studies on the roots of MP in scientific manner to validate its use in traditional system of medicine.

MATERIALS AND METHODS

Plant material

Roots of MP were procured from Baraut, U.P.,

*Correspondence: M Mujeeb, Department of Pharmacognosy and Phytochemistry, Faculty of Pharmacy, Jamia Hamdard, New Delhi-110062, India. E-mail: mujeeb_zaidi@yahoo.co.in, shahalamkhan@yahoo.com

India and authenticated by the taxonomist of Department of Botany, Faculty of Science, Hamdard University. The voucher specimen was deposited in the herbarium of university for future reference (JHFP-2075).

Preparation of extract

The air-dried and coarsely powdered roots of plant (3 kg) were soxhlet extracted with methanol for 72 h. The methanol extract was concentrated on a water bath and dried under reduced pressure to get a dark brown mass (200 g).

High performance thin layer chromatography (HPTLC) fingerprinting and preliminary phytochemical screening

Methanol extract of plant roots (1 g/ml) was applied (2 μ l) on silica gel G F₂₅₄ HPTLC plate in duplicate with bandwidth of 5 mm using Linomat V applying device. The chromatogram was developed in Twin trough chamber using solvent system Toluene: Ether (1:1) saturated with 10% acetic acid and scanned in scanner III at 366 nm wavelength using mercury lamp in fluorescence mode. On preliminary phytochemical screening of roots of MP, extract showed positive tests for glycosides, steroids, alkaloids and flavonoids.

Determination of phenolic compounds

Total phenols were estimated by Folin-Ciocalteu reagent according to the method of Gao *et al.* (2000). The total content was expressed as mg of Gallic acid equivalents/g extract.

Determination of total flavonoids

Total flavonoid content was estimated according to the Kosalec *et al.* (2004). The total content was expressed as mg of quercetin equivalents/g extract.

Animals

The hepatoprotective activity was carried out on Wistar Albino rats of either sex weighing (120 - 150 g), supplied by Central animal house facility of

Jamia Hamdard, New Delhi (Registration no. 173 / CPCSEA). They were maintained in a 12 h light/dark cycle at $25 \pm 2^\circ\text{C}$ and were allowed free access to standard pellet diet (Amrut Laboratory Rat Feed, Navamaharashtra, Pune, India) and water *ad libitum*. The study was approved by ethics committee CPCSEA and ethical norms were strictly followed during all experimental procedures.

Experimental protocol

Animals were divided into five groups; Group I (control), Group II (CCl₄ treated), Group III (CCl₄ + Silymarin treated), Group IV and V (CCl₄ + extract). Animals of group II, III, IV and V were administered 50% (v/v) CCl₄ in olive oil at a single dose of 2 ml/kg body weight per day for 4 days by S.C. route. Simultaneously but at different hours of the day, animals of group III, IV and V were fed with Silymarin suspension (25 mg/kg body weight, i.p.), methanol extract at a dose of 200 mg/kg and 300 mg/kg body weight, i.p. for 4 days respectively. Animals of group I was given distilled water in a volume of 10 - ml/kg-body weight.

Serum analysis and histopathological examination

On 5th day, after treatment period the animals of all groups were anaesthetized with ether and sacrificed. Blood was withdrawn from heart and serum was separated by centrifugation at 3,000 rpm at 30°C for 15 min and analysed for various biochemical parameters; Serum transaminases viz. Serum glutamate oxaloacetate transaminase (SGOT) (Frankel and Rietman, 1957), serum glutamate pyruvate transaminase (SGPT) (Frankel and Rietman, 1957), Total protein and Bilirubin (direct and total) (Evelyn and Malloy, 1937). The histopathological studies were also carried out by reported method (Luna, 2002). Liver was removed from sacrificed animal, sliced, and washed in normal saline. Liver pieces were processed in 10% formaldehyde solution. The pieces of liver were processed and embedded in paraffin wax. Sections made were 4-6 μ m in thickness. They

were stained with hematoxylin and eosin and lastly photographed.

***In-vitro* antioxidant activity by DPPH method**

The stable 1, 1-diphenyl-2-picryl hydrazyl radical (DPPH) (75 μ M) was used for determination of free radical-scavenging activity of the extract (Blois, 1958). 2.95 mg of DPPH was dissolved in 100 ml of methanol to give 75 μ M of DPPH solution.

The methanolic solution (0.5 ml) of plant extract in different concentration was mixed in a test tube with 2.5 ml of methanol containing 75 μ M DPPH, (stable free radical) which gave a distinctive absorbance at 517 nm. The reaction mixture was set-aside in the dark at room temperature for 90 min, and then absorbance was recorded at 517 nm. The corresponding blank readings were also taken and the remaining DPPH was calculated. The experiment was repeated for three times. Ascorbic acid and quercetin were used as standard controls.

***In-vivo* antioxidant activity**

The *in-vivo* antioxidant activity of methanolic extract was carried out in CCl₄ intoxicated rats. The liver sample collected were washed with chilled normal saline, weighed and 10% (w/v) liver homogenate were made in ice cold 0.15M KCl solution using motor driven Teflon pestle. The suspension was centrifuged at 2000 rpm at 4°C for 10 min and clear supernatant was used for the estimation of the following antioxidant markers.

Estimation of liver TBARS

The measurement of Thiobarbituric acid reactive substances (TBARS) was done as an index of lipid peroxidation and measured in terms of malondialdehyde (MDA) content following the method of Ohkawa *et al* (1979). The values of TBARS were presented as nmol MDA /mg protein.

Estimation of reduced glutathione (GSH)

Reduced Glutathione was estimated spectrophotometrically by using Ellman's reagent (5,5-dithiobis-2-

nitrobenzoic acid) (Ellman, 1959). Equal quantity of homogenate(W/V) and 10% trichloroacetic acid were mixed and centrifuged to separate the proteins. To 0.01 ml of this supernatant, 2 ml of phosphate buffer (pH 7.4), 0.5 ml of 5, 5-dithiobisnitro benzoic acid (DTNB) and 0.4 ml of distilled water was added. Absorbance was measured at 412 nm, and the values were expressed as μ mol/g of liver tissue.

Statistical analysis

Results of biochemical parameters are reported as mean $\pm$ S.E.M. Statistical significance was determined by one way analysis of variance (ANOVA) followed by Dunnet's 't' test (Woolson, 1987). *P* value < 0.05 was considered statistically significant.

RESULTS

HPTLC fingerprints of methanolic extract of MP roots showed presence of 18 spots, confirming the presence of different class of phytoconstituents as revealed by preliminary phytochemical screening. Total content of Phenolic and Flavonoid was also determined. The total phenolic and total flavonoid content was found to be 118 ± 8.8 mg (Gallic acid equivalent/g extract) and 52.81 ± 1.8 mg (Quercetin equivalent/g extract), respectively.

Administration of CCl₄ led to significant hepatocellular damage as evident from increase in serum activities of SGOT, SGPT and bilirubin concentration as compared to normal control group. Treatment of rats with methanolic extract of roots at a dose of 200 mg/kg and 300 mg/kg body weight, i.p. exhibited significant reduction (*P* < 0.05) in CCl₄ induced elevation of SGOT, SGPT, bilirubin and increased the level of TP (Total Protein) (Table 1). Treatment with Silymarin also reversed the hepatotoxicity significantly. However extract at a high dose (300 mg/kg body weight) produced better protection.

The IC₅₀ of the methanolic extract was found to be 64 μ g/ml. The value was found to be comparable to that of standard ascorbic acid and quercetin. In

Table 1. Effects of methanolic extract of *M. pruriens* roots on SGOT, SGPT, TP, Bilirubin in CCl₄ induced hepatotoxicity in rats

Group Treatment	SGOT (U/ml)	SGPT (U/ml)	Bilirubin (mg/dl)		TP (g/dl)
			Total	Direct	
I Control	46.23 ± 4.28	34 ± 2.38	0.68 ± 0.3	0.2 ± 0.05	7.42 ± 0.36
II CCl ₄ treated	268.39 ± 9.70	264 ± 11.01	3.36 ± 1.50	2.28 ± 0.19	2.16 ± 0.25
III CCl ₄ + Silymarin	53.06 ± 3.94*	42.4 ± 1.43*	1.03 ± 0.46*	0.37 ± 0.02*	6.82 ± 0.48*
IV CCl ₄ + extract (200 mg/Kg)	93.91 ± 4.25*	105.42 ± 2.23*	1.61 ± 0.72*	0.97 ± 0.11*	4.87 ± 0.36*
V CCl ₄ + extract (300 mg/Kg)	73.84 ± 2.54*	80.25 ± 2.79*	1.28 ± 0.57*	0.7 ± 0.13*	5.77 ± 0.35*

Values are mean ± S.E.M. (n = 5), **P* < 0.05 Vs CCl₄. one way analysis followed by Dunnet's *t* test.

Table 2. Effects of methanolic extract of *M. pruriens* roots on TBARS and GSH level in liver tissues of CCl₄ intoxicated rats

Treatment	TBARS (nmol MDA/mg protein)	GSH (mmol/g liver tissue)
Control	0.87 ± 0.05	65.45 ± 8.67
CCl ₄ treated	6.98 ± 0.53	15.17 ± 4.34
CCl ₄ + Silymarin	1.21 ± 0.07**	64.37 ± 7.88**
CCl ₄ + extract (200 mg/Kg)	4.33 ± 0.47*	50.88 ± 6.44*
CCl ₄ + extract (300 mg/Kg)	1.38 ± 0.22**	63.34 ± 8.36**

Values are mean ± S.E.M. (n = 5), **P* < 0.05, ** *P* < 0.001 Vs CCl₄. one way analysis followed by Dunnet's *t* test.

the present study, administration of methanolic extract at dose of 200 and 300 mg/kg body weight to the CCl₄ intoxicated rats caused a significant increase in the level of GSH and a significant decrease in the level of TBARS when compared to CCl₄ treated controlled group (Table 2). In liver tissues standard Silymarin at dose 25 mg/kg also showed significant antioxidant activity. The result of methanolic extract was comparable with that of standard drug Silymarin.

Fig. 1. Liver tissue of control rats showing normal histology.

Histopathological studies of liver sections in control animals showed normal hepatic cells with prominent nucleus and central vein (Fig. 1). In CCl₄ treated animals the sections showed hydropic changes in centrilobular hepatocytes with single cell necrosis, congestion of central vein and sinusoids were seen with acute and chronic inflammatory cells mainly in central zone (Fig. 2). Treatment of

Fig. 2. Liver tissue of CCl₄ treated animals showing hydropic changes and acute inflammatory cell infiltrate.

Fig. 3. Liver tissue of rats treated with CCl_4 and Silymarin showing almost normal histology.

Fig. 4. Liver tissue of rats treated with CCl_4 and *M. pruriens* (300 mg/kg) showing almost normal histology.

the animals with Silymarin and methanolic extract exhibited a significant recovery of hepatocytes in different sections of the liver (Figs. 3 and 4).

DISCUSSION

CCl_4 is one of the most commonly used hepatotoxin in the experimental study of liver diseases (Johnson and Kroening, 1998). The hepatotoxic effects of CCl_4 are largely due to its active metabolite, trichloro methyl radical (Srivastava *et al.*, 1990). These activated radicals bind covalently to the macromolecules and induce peroxidative degradation of membrane lipids of endoplasmic reticulum rich in polyunsaturated fatty acids. This leads to the

formation of lipid peroxides, which in turn give products like MDA that cause damage to the membrane. Lipid peroxidative degradation of biomembrane is one of the principle causes of CCl_4 toxicity, this is evidenced by the elevation of TBARS and decrease in the activity of free radicals scavenging enzyme (GSH). This is also evidenced by an elevation in the serum marker enzymes. The increase in the levels of serum bilirubin reflected the level of jaundice and increase of transaminases was the clear indication of cellular leakage and loss of functional integrity of cell membrane (Saraswat *et al.*, 1993). Methanolic extract has significantly reduced these liver enzyme levels and has increased the level of total protein in the serum in a dose dependent manner, which indicates hepatoprotection.

The DPPH test is very convenient method for screening small antioxidant molecule because the intensity of reaction can be analysed by simple spectrophotometric method (Soler-Rivas *et al.*, 2000). The DPPH radical is scavenged by antioxidant through the donation of their hydrogen to form the stable radical DPPH molecules. The antioxidant radicals formed are stabilized through the formation of non-radical product.

IC_{50} is considered to be a good measure of the antioxidant efficiency of pure compound and extract. The IC_{50} of the methanolic extract of MP roots (64 $\mu\text{g}/\text{ml}$) was found to be comparable to that of standard ascorbic acid and quercetin.

Lipid per-oxidation plays an important role in variety of disease like cancer, diabetes, aging, cardiovascular disorder, hepatitis, rheumatoid arthritis etc.

Lipid peroxidative degradation of biomembrane is one of the principle causes of CCl_4 toxicity. This is evidenced by the elevation of TBARS and decrease in the activity of free radicals scavenging enzymes like GSH in the CCl_4 intoxicated animals. Glutathione is a naturally occurring tripeptide, non-enzymatic biological antioxidant that is abundant in many living creature. It is widely known that a deficiency of GSH within living

organism can lead to tissue disorder and injury. Liver injury induced by consumption of alcohol and drugs, lung injury by smoking and muscle injury by intense physical activity (Kurata *et al.*, 1993), all are known to be correlated with decreased tissue level of GSH.

The increase in MDA level in liver induced by CCl₄ suggests enhanced lipid peroxidation leading to the tissue damage and failure of antioxidant defense mechanism to prevent formation of excessive free radicals. Treatment with methanolic extract (200 and 300 mg/kg) reduced the elevated level of TBARS (Thobarbituric acid reactive substances) in CCl₄ intoxicated liver tissues. It was also observed that GSH depletion due to the CCl₄ challenge was reversed by the test extract.

Histopathological studies revealed that CCl₄ caused steatosis and hydropic degeneration of the liver tissue. MP treatment exhibited protection, which confirmed the result of biochemical studies. Also all the effects of methanolic extract of MP were comparable with those of Silymarin, a proven hepatoprotective drug.

Preliminary phytochemical screening indicated the presence of phenolic and flavonoid glycosides. Free radical mediated process has been implicated in the pathogenesis of different diseases. Thus the antioxidant activity of the methanolic extract may be due to the presence of flavonoid and phenolic compounds and hepatoprotective activity is attributed due to its antioxidant property. Further isolation of active principles responsible for its hepatoprotective activity is currently under progress in our lab.

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