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ASSESSING THE HEPATOPROTECTIVE POTENTIAL OF MUCUNA PRURIENS METHANOLIC EXTRACT IN MALE SPRAGUE DAWLEY RATS

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Abstract:

This study investigates the hepatoprotective and antioxidant effects of Mucuna pruriens methanolic extract (MPME) against paracetamol-induced liver toxicity in rats. Paracetamol (3 g/kg) was administered orally for 14 days to induce hepatotoxicity, followed by treatment with either Silymarin (100 mg/kg) or MPME at two doses (250 mg/kg and 500 mg/kg). Biochemical parameters including AST, ALT, ALP, bilirubin, LDH, and total protein were measured, along with antioxidant markers such as GSH, SOD, CAT, and MDA. Paracetamol significantly increased liver enzymes and MDA levels, while reducing antioxidant enzyme levels and total protein, indicating oxidative stress and liver damage. Co-administration of MPME significantly reversed these changes in a dose-dependent manner, with the 500 mg/kg dose showing effects comparable to Silymarin. The observed hepatoprotective activity is likely due to the presence of flavonoids, phenols, and other phytochemicals in the extract. These results suggest that MPME has strong potential as a natural hepatoprotective agent and support its traditional use in liver disorders.

Keywords: *hepatoprotective, antioxidant, MPME, Mucuna pruriens, AST, ALT, ALP*

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INTRODUCTION:

Medicinal plants play a key role in the human health care. About 80% of the world population rely on the use of traditional medicine which is predominantly based on plant materials [1]. The traditional medicine refers to a broad range of ancient natural health care practices including folk/tribal practices as well as Ayurveda, Siddha, Amchi and Unani. These medical practices originated from time immemorial and developed gradually, to a large extent, by relying or based on practical experiences without significant references to modern scientific principles.

These practices incorporated ancient beliefs and were passed on from one generation to another by oral tradition and/or guarded literature. Although herbal medicines are effective in the treatment of various ailments very often these drugs are unscientifically exploited and/or improperly used. Therefore, these plant drugs deserve detailed studies in the light of modern science.

It is estimated that about 7,500 plants are used in local health traditions in, mostly, rural and tribal villages of India. Out of these, the real medicinal value of over 4,000 plants is either little known or hitherto unknown to the mainstream population. The classical systems of medicine such as Ayurveda, Siddha, Amchi, Unani and Tibetan use about 1,200 plants [2]. A detailed investigation and documentation of plants used in local health traditions and pharmacological evaluation of these plants and their taxonomical relatives can lead to the development of invaluable plant drugs for many dreaded diseases. Random screening of plants has not proved economically effective [3].

The liver plays a central role in regulating various physiological processes, including metabolism, detoxification, and nutrient storage. Due to its key role in detoxifying exogenous substances, it is highly susceptible to damage from drugs, environmental toxins, alcohol, and infections. Liver diseases such as hepatitis, cirrhosis, and drug-induced liver injury are significant health concerns worldwide.

Hepatoprotective agents are substances that help prevent damage to the liver and promote the regeneration of hepatic tissue. Although synthetic drugs are available for liver disorders, they often come with adverse side effects. Therefore, there is growing interest in exploring natural products, especially medicinal plants, for their hepatoprotective potential.

Various plant extracts have demonstrated liver-protective effects due to their antioxidant, anti-inflammatory, and detoxifying properties. The present study aims to evaluate the hepatoprotective activity of a selected plant extract using experimental models of liver injury, with biochemical and histopathological assessments to support the findings.[4]

MATERIALS AND METHODS:

Collection and authentication of Plant material

The leaves of Mucuna Purine, was selected for investigation and were procured from the nearest area of our college. The plant material was taxonomically identified and authenticated by Dr. Madhava Chetty, Head of Department, Botany, Sri Venkateshwara academia, Tirupathi, Andhra Pradesh.

Preparation of plant extract

The fresh Leaf's was air dried in shade and extracted with water and alcohol in a ratio 50gm in 200ml of methanol 1:4 for Corchorus fascicularis extract, using a Soxhlet for 6-8 hrs at 55-600C. The supernatant was filtered through Whatman filter paper No.1 and concentrated under reduced pressure using vacuum at 44 ± 100C in a rotavapor The percentage of extract

yield was calculated by using the formula % of extract yield=(weight in gm of extract obtained)/(weight in gm of plant material taken)×100Qualitative phytochemical analysis

In order to detect the various constituents, present in the Corchorus Fascicular extracts, those were subjected to the tests[44, 45]. Phyto-chemical screening was performed for the detection of Alkaloids, Glycosides, Carbohydrates, Tannins, Resins, Flavanoids, Steroids, Proteins and Amino acids

Pharmacological Study

Animals

A total of 36 Wistar rats with an approximate age of 60 days were brought from our college On their arrival a sample of animals was chosen at random and weighed to ensure compliance with the age requested.. The animals were housed in metabolic cages (55 x 32.7 x 19 cm), with sawdust litter, in such a way that each cage contained a maximum of 5 animals of the same sex. All animals underwent a period of 7 days of observation and acclimatization between the date of arrival and the start of treatment. They were distributed among the experimental groups using a random distribution method. This procedure allows approximate equalization of initial bodyweights whilst allowing random allocation to experimental groups.

Acute Oral Toxicity Study[5]

Healthy Wistar rats (180-220 g) of both sex were used in acute toxicity studies as per OECD guidelines-425. The animals were fasted overnight and divided into 3 groups with 5 rat in each group. Extracts were administered at dose of 100, 500 and 2000 mg/kg, p.o. body weight. The mice were

observed continuously for behavioural and autonomic profiles for 2 hrs and for any signs of toxicity or mortality up to 48 hrs (OECD-425, 2001). The test substance was administered orally. The mice belonging to the control group were treated with the vehicle (distilled water) at the same administration volume as the rest of the treatment groups. The administration volume for oral administration was 10 ml/kg. The quantity of test substance administered to each animal was calculated from its body weight on the day of the treatment.

Experimental Design

Group 1: Receives (Distilled water) as control for 14 days.

Group 2: Receives a daily dose of Paracetamol (3g/ Kg of body weight, p.o) for 14 days (p.o)

Group 3: Receives a daily dose of Paracetamol (3g/ Kg of body weight) and after one hour a daily dosage of Standard Silymarin (100mg/kg) of body wieght for 14 days (p.o)

Group4: Receives a daily dose of Paracetamol (3g/ Kg of body weight) and one hour a daily dosage of MPME 250mg / Kg of body weight for 14 days (p.o)

Group 5: Receives a daily dose of Paracetamol (3g/ Kg of body weight) and one hour a daily dosage of MPME 500mg / Kg of body weight for 14 days (p.o)

Determination of Biochemical Parameters

Determination of aspartate aminotransferase (AST)/ SGOT

Aspartate aminotransferase, also known as Serum Glutamate Oxaloacetate Transaminase (SGOT)catalyses the transamination of L-aspartate and α keto glutarate to form oxaloacetate and L-glutamate. Oxaloacetate formed is coupled with 2,4-Dinitrophenyl hydrazine to form hydrazone, a brown coloured complex in alkaline medium which can be measured by colorimetry.

Procedure

Rietman and Frankle method was adopted for the estimation of SGOT[47]. The reaction systems used for this study included blank, standard, test (for each serum sample) and control (for each serum sample). 0.25 ml of buffered aspartate was added into all the test tubes. Then 0.05 ml of serum was added to the test group tubes and 0.05 ml of working pyruvate standard into the standard tubes. After proper mixing, all the tubes were kept for incubation at 37oC for 60 min, after which 0.25 ml each of 2,4-DNPH reagent was added into all the tubes. Then, 0.05 ml of distilled water and 0.05 ml of each serum sample was added to the blank and the serum control tubes respectively. The mixture was allowed to stand at room temperature for 20 min. After incubation, 2.5 ml of solution I was added to all test tubes. Mixed properly and optical density was measured in a spectrophotometer at 505 nm within 15 min.

The enzyme activity was calculated as:- AST (GOT) activity in IU/L) = [(Absorbance of test - Absorbance of control)/ (Absorbance of standard - Absorbance of blank)] x concentration of the standard.

Determination of alanine aminotransferase (ALT) or Serum Glutathione peroxidase (SGPT)

Procedure

Rietman and Frankle method (1957)[7] was adopted for the estimation of SGPT.

The reaction systems used for this study included blank, standard, test (for each serum sample) and control (for each serum sample). 0.25 ml of buffered alanine was added into all the test tubes. This was followed by the addition of 0.05 ml of serum into the test group tubes and 0.05 ml of working pyruvate standard into the standard tubes. After proper mixing, all the tubes were kept for incubation at 37oC for 60 minutes, after which 0.25 ml each of 2,4- DNPH reagent was added into all the tubes. Then, 0.05 ml of distilled water and 0.05 ml of each serum sample was added to the blank and the serum control tubes respectively. The mixture was allowed to stand at room temperature for 20 min. After incubation, 2.5 ml of solution I was added to all test tubes. Mixed properly and optical density was read against purified water in a spectrophotometer at 505 nm within 15 min.

The enzyme activity was calculated as:- ALT (GPT) activity in IU/L) = [(Absorbance of test - Absorbance of control)/ (Absorbance of standard - Absorbance of blank)] x concentration of the standard.

Determination of alkaline phosphatase (ALP)/serum alkaline phosphatase (SALP)

Procedure:

ALP was determined using the method of Kind and King[7].

The working solution was prepared by reconstituting one vial of buffered substrate with 2.2 ml of water. 0.5 ml of working buffered substrate and 1.5 ml of purified water was dispensed to blank, standard, control and test. Mixed well and incubated at 370C for 3 min. 0.05 ml each of serum and phenol standard were added to test and standard test tubes respectively. Mixed well and incubated for 15 min at 370C. Thereafter, 1 ml of chromogen reagent was added to all the test tubes. Then, added 0.05 ml of serum to control. Mixed well after addition of each reagent and the O.D of blank, standard, control and test were read against purified water at 510 nm. Serum alkaline phosphatase activity in KA units was calculated as follows

[(O.D. Test-O.D. Control) / (O.D. Standard- O.D. Blank)] x 10 Alkaline phosphate was expressed as U/l.

Alkaline phosphate was expressed as U/l.

Determination of bilirubin;

Procedure

Estimation of total bilirubin:

To 1.0 ml total bilirubin reagent, 0.02 ml of activator and 0.1 ml of serum were added, mixed well and incubated for exactly 5 minutes at room temperature. Sample blank was prepared by mixing 1.0 ml total bilirubin reagent with 0.1 ml of distilled water, mixed well and incubated for exactly 5 minutes at room temperature. The absorbance of each sample blank and test were measured at 532- 546 nm against distilled water blank. Total bilirubin and direct bilirubin level in serum was expressed as mg/dl.

The Bilirubin content was calculated using the following equation:

Total bilirubin (mg/dt) = Abs of the sample blank x 15.

Direct Bilirubin(mg/dt) = Abs of sample blank x 10.

Determination of Total proteins:

Procedure

Lowry method was adopted for the estimation of total protein[8].

To 0.1 ml of the liver homogenate, 0.9 ml of water, 4.5 ml of alkaline copper sulphate reagent were added and allowed to stand in the room temperature for 10 min. To this 0.5 ml of Folin's reagent was added. After 20 min, the blue colour developed was measured at 640 nm.

The level of protein present was expressed as mg/g tissue or mg/dl

Lactate Dehydrogenase Assay

The method described here is derived from the formulation recommended by the IFCC and was optimized for the performance and stability[9].

Determination of Antioxidant Enzymes and Lipid Peroxidation

Determination of superoxide dismutase:

Superoxide dismutase scavenges the superoxide radical (O● 2) and thus provides a first line defence against free radical damage. Superoxide dismutase is an endogenous enzymatic antioxidant which catalyses the dismutation of superoxide free radical. This method is based on the inhibition of the spontaneous oxidation of the adrenaline to adrenochrome by the enzyme superoxide dismutase. Superoxide anion (O2 -) interacts with peroxide to form hydroxyl radical (OH•) which causes damage in the absence of superoxide dismutase activity (R•)

Procedure

SOD was estimated as per the procedure described by Kakkaret al[10].

Liver homogenate (0.5 ml) was diluted with 0.5 ml of distilled water. To this, 0.25 ml ethanol and 0.15 ml of chloroform, all reagents chilled, were added. The mixture was shaken for 1 minute and centrifuged at 2000 rpm. The enzyme in the supernatant was determined. To 0.5 ml of the supernatant, 1.5 ml of buffer was added. The reaction was initiated by the addition of 0.4 ml epinephrine and change in optical density per minute was measured at 480 nm in a double beam UV-VIS spectrophotometer (UV 1700, Szhimadzhu)

SOD activity was expressed as U/mg.

Change in optical density per minute at 50% inhibition to adrenochrome transition by the enzyme is taken as one enzyme unit.

2. Determination of catalase:

In animals, catalase is present in all major body organs, especially being concentrated in liver and erythrocyte. During β-oxidation of fatty acids by flavoprotein dehydrogenase, hydrogen peroxide is generated, which is accepted upon by Catalase present in peroxisomes.

Catalase catalyses the rapid decomposition of hydrogen peroxide to water

Dichromate in acetic acid was converted to perchloric acid and then to chromic acetate when heated in presence of hydrogen peroxide. The chromic acetate thus produced is measured spectrophotometrically at 610 nm. The reaction is stopped at specific time interval by the addition of dichromate- acetic acid mixture and the remaining hydrogen peroxide is determined by measuring chromic acetate.

Procedure

The catalase activity was assayed by the method of Sinha (1972)[11]

Liver homogenate (0.1 ml) was taken, to which 1.0 ml of phosphate buffer and hydrogen peroxide were added. The reaction was arrested by the addition of 0.2 ml dichromate acetic acid reagent. Standard hydrogen peroxide in the range of 4 to 20 µl were taken and treated similarly. The tubes were heated in a boiling water bath for 10 min. The green color developed was read at 570 nm in a Double beam UVVIS spectrophotometer (UV 1700, Szhimadzhu). Catalase activity was expressed as U/mg.

Determination of glutathione peroxidase:

Glutathione was measured by its reaction with DTNB to give a compound that absorbs at 412 nm.

1.

Procedure

The glutathione peroxidase activity was measured according to the method of Rotrucket al., (1973)[11]

EDTA (0.2 ml each), sodium azide, reduced glutathione, H2O2; 0.4 ml of buffer and 0.1 ml of enzyme (liver homogenate) were mixed and incubated at 37°C for 10 min. The reaction was arrested by the addition of 0.5 ml of TCA and the tubes were centrifuged. To 0.5 ml of supernatant, 3.ml of sodium hydrogen phosphate and 1 ml of DTNB were added and the color developed was read

at 412 nm immediately in a Double beam UV-VIS spectrophotometer (UV 1700, Szhimadzhu.)
Glutathione peroxidase activity, in serum is expressed as µg/mg.

Determination of reduced glutathione:
Procedure
Reduced Glutathione was estimated by Ellman's procedure[12].
To 250 µL of tissue homogenate taken in 2 ml eppendroff tube, 1 mL of 5% TCA was added and the above solution was centrifuged at 3000 g for 10 min at room temperature. To 250 µL of the above supernatant, 1.5 ml of 0.2 M phosphate buffer was added and mixed well. 250 µL of 0.6 mM of Ellman's reagent (DTNB solution) was added to the above mixture and the absorbance was measured at 412 nm within 10 min. A standard graph was plotted using glutathione reduced solution (1 mg/ml) and GSH content present in the tissue homogenates was calculated by interpolation. Amount of glutathione expressed as µg/mg protein.

Determination of lipid peroxidation:
Procedure
Lipid peroxidation was estimated by the method of Okhawaet al., (1979)[13]
One ml of liver homogenate was mixed with 0.2 ml 4 % (w/v) sodium dodecyl sulfate, 1.5 ml 20% acetic acid in 0.27 M hydrochloric acid (pH 3.5) and 15 ml of 0.8% thiobarbituric acid (TBA, pH 7.4). The mixture was heated in a hot water bath at 85˚C for 1 h. The intensity of the pink colour developed was read against a reagent blank at 532 nm following centrifugation at 1200 g for 10 min. The concentration was expressed as n moles of MDA per mg of protein using 1,1,3,3,- tetra-ethoxy propane as the standard.

1) Collection of materials
Thin pieces of 3 to 5 mm, thickness were collected from tissues showing gross morbid changes along with normal tissue.

2) Fixation:
Kept the tissue in fixative for 24-48 hours at room temperature The fixation was useful in the following way

a. Serves to harden the tissues by coagulating the cell protein,
b. Prevents autolysis,
c. Preserves the structure of the tissue, and
d. Prevents shrinkage

Common Fixatives: 10% Formalin

3) Haematoxylin and eosin method of staining:
Deparaffinise the section by xylol 5 to 10 minutes and remove xylol by absolute alcohol. Then cleaned the section in tap water and stained with haematoxylin for 3-4 minutes and again cleaned under tap water. Allow the sections in tap water for few minutes and counter stained with 0.5% eosin until section appears light pink 15 to 30seconds), and then washed in tap water. Blotted and dehydrated in alcohol and cleared with xylol (15 to 30 seconds). Mounted on a Canada balsam or DPX Moutant and kept the slide dry and remove air bubbles.

STATISTICAL ANALYSIS
Statistical analysis was performed using GraphPad Prism version 7 for Windows (GraphPad Software, San Diego, CA, USA) and Microsoft Excel 2013. Raw data obtained from different wound models are expressed as mean ± SEM. Values less than 0.03 were considered to be statistically significant. The data were analyzed using GraphPad Prism version 7 for Windows and differences among groups were compared by one-way ANOVA followed by Dunnett's test.

5. RESULTS AND DISCUSSION:

Extractive Values and percentage yield of Crude Drugs

Sl. No.	Name of the drug	methanolic value (% W/W)	
		Theoretical	Obtained
1	Mucuna purine	>11	22.9±0.53

The methanolic extractive value indicated the presence of sugar, acids and inorganic compounds; the methanolic extractive value found to be 22.90±0.53. The alcohol soluble extractive values indicated the presence of polar constituents like phenols, alkaloids, steroids, glycosides, flavonoids. The alcohol soluble extractive value was found to be 14.86±0.12 which signify the nature of the phytoconstituents present in plant.

Table Percentage yield of Crude Drugs

Extract	Nature	Percentage of yield
MPME	Dark green	8.34

Preliminary qualitative phytochemical analysis of Mucuna purine

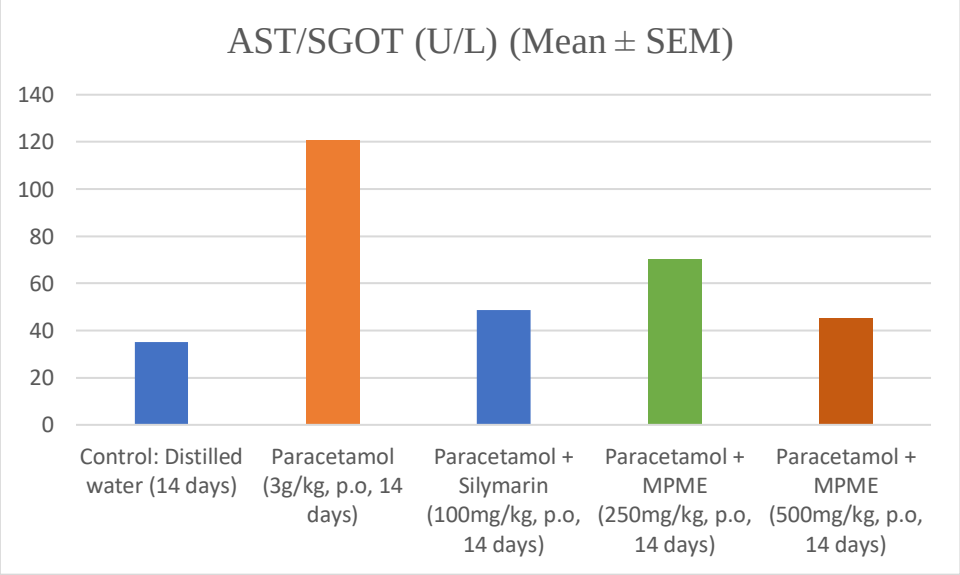
Sl. No.	Phytoconstituents	Test Result
1	Alkaloid	-ve
2	Glycosides	-ve
3	Carbohydrate	-ve
4	Protein	-ve
5	Amino acid	+ve
6	Steroids	-ve
7	Flavonoids	+ve
8	Terpenoids	+ve
9	Phenols	+ve
10	Saponins	-ve
11	Tannin	+ve

Determination of aspartate aminotransferase (AST)/ SGOT

Group	Treatment Description	AST/SGOT (U/L) (Mean ± SEM)
1	Control: Distilled water (14 days)	35.2 ± 2.1
2	Paracetamol (3g/kg, p.o, 14 days)	120.5 ± 5.8
3	Paracetamol + Silymarin (100mg/kg, p.o, 14 days)	48.7 ± 3.2
4	Paracetamol + MPME (250mg/kg, p.o, 14 days)	70.3 ± 4.0
5	Paracetamol + MPME (500mg/kg, p.o, 14 days)	45.1 ± 2.7

Group 2 (Paracetamol only) shows a significant increase in AST compared to the control group, indicating liver damage.
Groups 3 and 5 show reduced AST levels compared to Group 2, suggesting hepatoprotective effects of Silymarin and higher dose MPME.

Group 4 shows moderate reduction in AST levels.



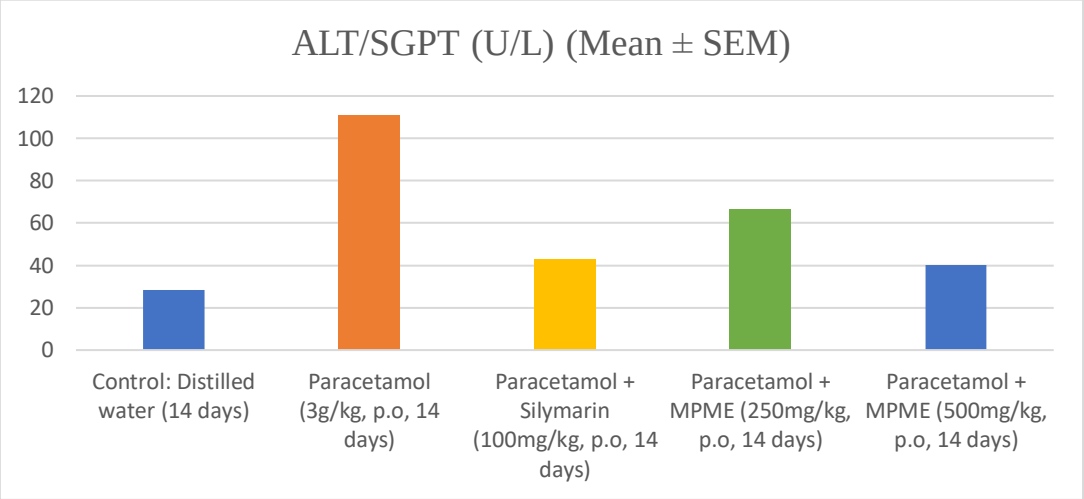
Determination of alanine aminotransferase (ALT) or Serum Glutathione peroxidase (SGPT)

Group	Treatment Description	ALT/SGPT (U/L) (Mean ± SEM)
1	Control: Distilled water (14 days)	28.4 ± 1.9
2	Paracetamol (3g/kg, p.o, 14 days)	110.7 ± 6.1
3	Paracetamol + Silymarin (100mg/kg, p.o, 14 days)	42.9 ± 2.8
4	Paracetamol + MPME (250mg/kg, p.o, 14 days)	66.5 ± 3.7
5	Paracetamol + MPME (500mg/kg, p.o, 14 days)	40.3 ± 2.3

Paracetamol causes a significant increase in ALT compared to control, indicating liver injury.

Both Silymarin and higher dose MPME (500mg/kg) show marked hepatoprotective effects by reducing ALT levels.

Lower dose MPME (250mg/kg) shows moderate reduction.



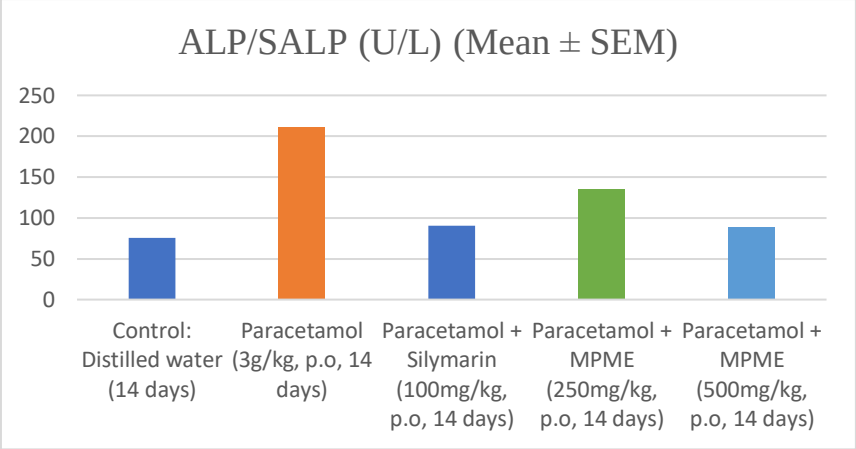
Determination of alkaline phosphatase (ALP)/serum alkaline phosphatase (SALP)

Group	Treatment Description	ALP/SALP (U/L) (Mean ± SEM)
1	Control: Distilled water (14 days)	75.4 ± 4.3
2	Paracetamol (3g/kg, p.o, 14 days)	210.8 ± 8.7
3	Paracetamol + Silymarin (100mg/kg, p.o, 14 days)	90.2 ± 5.1
4	Paracetamol + MPME (250mg/kg, p.o, 14 days)	135.6 ± 6.4
5	Paracetamol + MPME (500mg/kg, p.o, 14 days)	88.9 ± 4.7

Paracetamol significantly increases ALP levels, indicating liver dysfunction.

Silymarin and higher dose MPME (500 mg/kg) restore ALP closer to normal.

Lower dose MPME (250 mg/kg) shows partial protective effect.



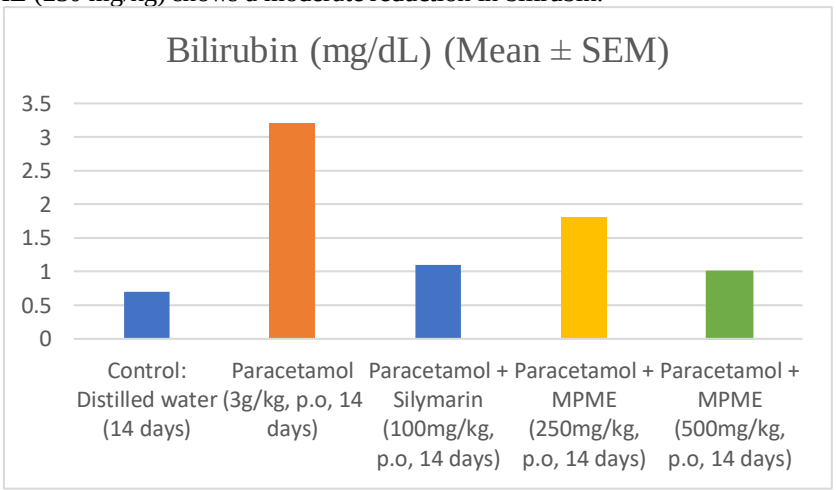
Determination of bilirubin

Group	Treatment Description	Bilirubin (mg/dL) (Mean ± SEM)
1	Control: Distilled water (14 days)	0.7 ± 0.05
2	Paracetamol (3g/kg, p.o, 14 days)	3.2 ± 0.18
3	Paracetamol + Silymarin (100mg/kg, p.o, 14 days)	1.1 ± 0.09
4	Paracetamol + MPME (250mg/kg, p.o, 14 days)	1.8 ± 0.12
5	Paracetamol + MPME (500mg/kg, p.o, 14 days)	1.0 ± 0.08

Paracetamol significantly increases bilirubin levels compared to control, indicating liver damage.

Treatment with Silymarin and higher dose MPME (500 mg/kg) significantly reduces bilirubin, suggesting hepatoprotective activity.

Lower dose MPME (250 mg/kg) shows a moderate reduction in bilirubin.



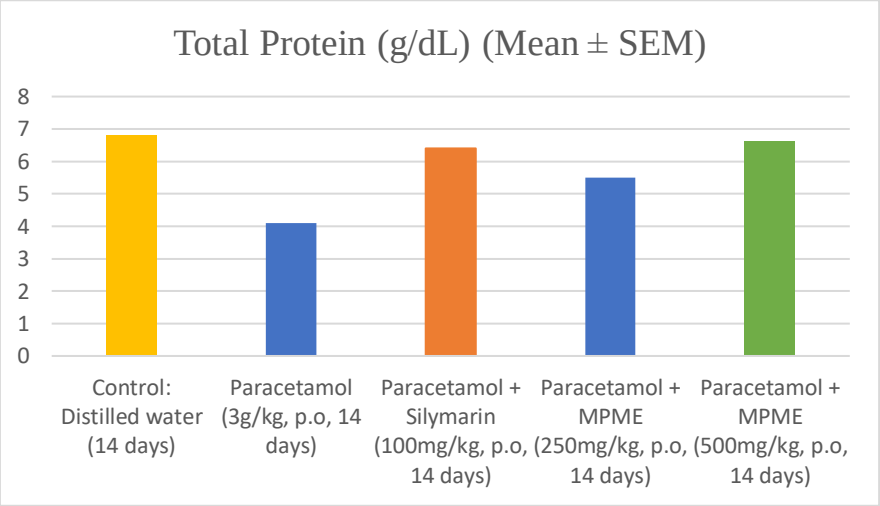
Determination of Total proteins

Group	Treatment Description	Total Protein (g/dL) (Mean ± SEM)
1	Control: Distilled water (14 days)	6.8 ± 0.15
2	Paracetamol (3g/kg, p.o, 14 days)	4.1 ± 0.20
3	Paracetamol + Silymarin (100mg/kg, p.o, 14 days)	6.4 ± 0.18
4	Paracetamol + MPME (250mg/kg, p.o, 14 days)	5.5 ± 0.16
5	Paracetamol + MPME (500mg/kg, p.o, 14 days)	6.6 ± 0.14

Paracetamol significantly reduces total protein, indicating impaired liver synthetic function.

Silymarin and MPME 500 mg/kg effectively restore total protein levels toward normal.

MPME 250 mg/kg shows a moderate protective effect.



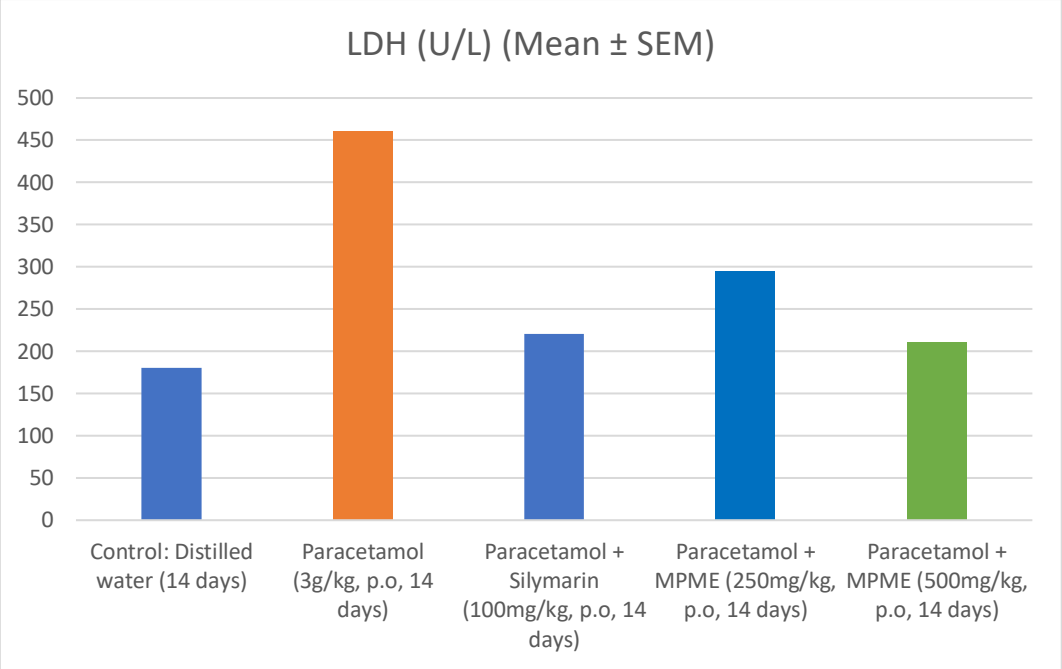
Lactate Dehydrogenase Assay

Group	Treatment Description	LDH (U/L) (Mean ± SEM)
1	Control: Distilled water (14 days)	180.6 ± 6.3
2	Paracetamol (3g/kg, p.o, 14 days)	460.2 ± 12.7
3	Paracetamol + Silymarin (100mg/kg, p.o, 14 days)	220.4 ± 7.8
4	Paracetamol + MPME (250mg/kg, p.o, 14 days)	295.1 ± 9.2
5	Paracetamol + MPME (500mg/kg, p.o, 14 days)	210.7 ± 6.9

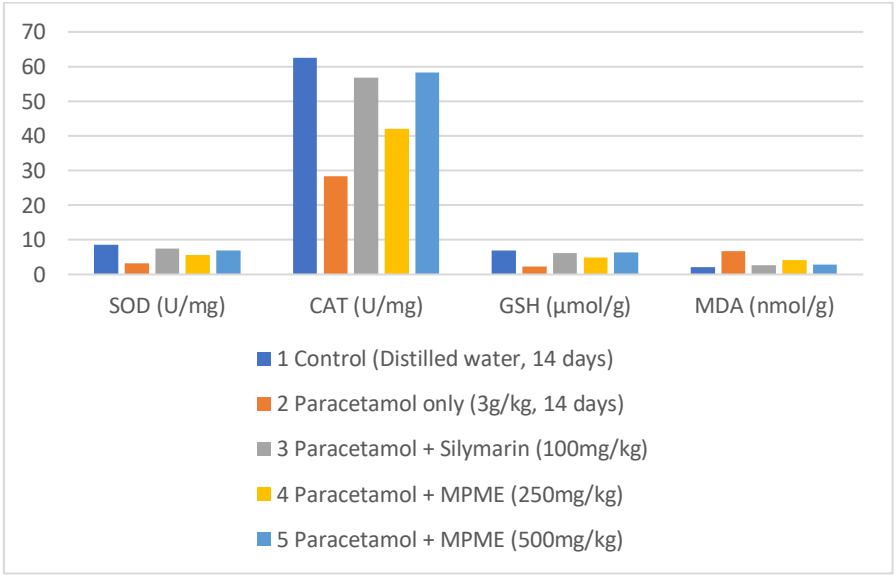
LDH levels are significantly elevated in the paracetamol-treated group, indicating cellular (especially hepatic) damage.

Silymarin and MPME 500 mg/kg groups show a strong reduction in LDH, suggesting protective effects.

MPME 250 mg/kg shows partial protection.



Group	Treatment Description	SOD (U/mg)	CAT (U/mg)	GSH (µmol/g)	MDA (nmol/g)
1	Control (Distilled water, 14 days)	8.6 ± 0.4	62.5 ± 2.1	6.9 ± 0.3	2.1 ± 0.1
2	Paracetamol only (3g/kg, 14 days)	3.2 ± 0.2	28.4 ± 1.8	2.3 ± 0.2	6.7 ± 0.3
3	Paracetamol + Silymarin (100mg/kg)	7.4 ± 0.3	56.8 ± 2.3	6.2 ± 0.3	2.6 ± 0.2
4	Paracetamol + MPME (250mg/kg)	5.6 ± 0.3	42.1 ± 2.0	4.8 ± 0.2	4.1 ± 0.2
5	Paracetamol + MPME (500mg/kg)	7.0 ± 0.4	58.2 ± 1.9	6.4 ± 0.3	2.8 ± 0.1



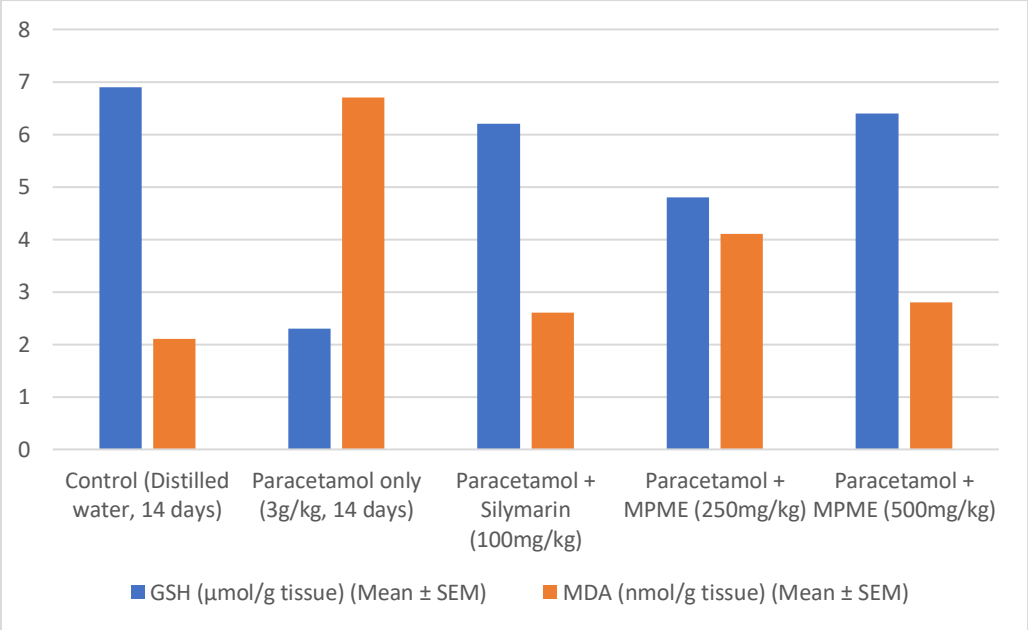
Determination of reduced glutathione

Determination of lipid peroxidation

Group	Treatment Description	GSH (µmol/g tissue) (Mean ± SEM)	MDA (nmol/g tissue) (Mean ± SEM)
1	Control (Distilled water, 14 days)	6.9 ± 0.3	2.1 ± 0.1
2	Paracetamol only (3g/kg, 14 days)	2.3 ± 0.2	6.7 ± 0.3
3	Paracetamol + Silymarin (100mg/kg)	6.2 ± 0.3	2.6 ± 0.2
4	Paracetamol + MPME (250mg/kg)	4.8 ± 0.2	4.1 ± 0.2
5	Paracetamol + MPME (500mg/kg)	6.4 ± 0.3	2.8 ± 0.1

GSH (Reduced Glutathione):
↓ Significantly decreased in Group 2 (Paracetamol), indicating oxidative damage.
↑ Restored in Groups 3 and 5, showing strong antioxidant protection from Silymarin and high-dose MPME.

MDA (Malondialdehyde):
↑ Significantly increased in Group 2, indicating lipid peroxidation.
↓ Reduced in Groups 3 and 5, suggesting inhibition of oxidative damage.



DISCUSSION

The present study was conducted to evaluate the hepatoprotective and antioxidant potential of Mucuna pruriens methanolic extract (MPME) against paracetamol-induced liver toxicity in experimental animals. Paracetamol is a widely used analgesic and antipyretic drug, but in high doses, it leads to acute liver damage primarily due to oxidative stress and depletion of hepatic glutathione reserves.

Biochemical Parameters

Administration of a high dose of paracetamol (3 g/kg) for 14 days resulted in a significant elevation in serum liver enzymes — AST (SGOT), ALT (SGPT), ALP (SALP), and bilirubin levels — in Group 2 animals compared to the control group. These elevated enzyme levels are indicative of hepatocellular membrane damage, allowing leakage of intracellular enzymes into the bloodstream. Additionally, total protein levels were markedly reduced, indicating impaired hepatic synthetic function.

Treatment with Silymarin (100 mg/kg) and MPME (250 and 500 mg/kg) showed a dose-dependent reversal of these biochemical changes. The higher dose of MPME (500 mg/kg) showed enzyme and bilirubin levels that were comparable to the

Silymarin-treated group, suggesting significant hepatoprotective potential.

Lactate Dehydrogenase (LDH)
LDH levels, a general marker of cellular damage, were significantly increased in the paracetamol-only group and normalized upon treatment with both Silymarin and MPME, further supporting the **cytoprotective effects** of the extract.

Oxidative Stress Markers
Paracetamol administration significantly altered antioxidant defense mechanisms. There was a marked reduction in GSH, SOD, and CAT levels along with a significant increase in MDA levels, indicating oxidative stress and lipid peroxidation. These findings align with the known mechanism of paracetamol toxicity involving reactive oxygen species (ROS) and glutathione depletion.

Co-treatment with MPME restored antioxidant enzyme levels and reduced lipid peroxidation in a dose-dependent manner. The 500 mg/kg MPME dose was particularly effective, showing results similar to the Silymarin group. This suggests that the hepatoprotective action of MPME is mediated, at least in part, through its antioxidant properties.

CONCLUSION:

The current study provides compelling evidence that Mucuna pruriens methanolic extract (MPME) possesses significant hepatoprotective and antioxidant properties, especially when administered against paracetamol-induced hepatotoxicity. Paracetamol, though widely used for its analgesic and antipyretic effects, is known to cause severe liver damage at high doses by generating reactive oxygen species (ROS) and depleting endogenous antioxidants such as glutathione. The liver injury was clearly observed in the form of elevated serum biomarkers such as AST, ALT, ALP, LDH, and bilirubin, alongside reduced total protein levels and marked oxidative stress, evidenced by increased malondialdehyde (MDA) levels and reduced activities of GSH, SOD, and catalase.

Treatment with MPME significantly reversed these toxic effects in a dose-dependent manner. The higher dose of MPME (500 mg/kg) was particularly effective, showing improvements nearly equivalent to the standard hepatoprotective agent, Silymarin. The extract restored liver enzyme levels to near-normal, improved antioxidant defense mechanisms, and reduced lipid peroxidation. These effects can be attributed to the rich presence of phytochemicals in MPME, especially flavonoids, phenols, terpenoids, and tannins, which are known for their free radical scavenging and hepatocellular protective activities.

This study not only supports the traditional use of Mucuna pruriens in liver disorders but also highlights its potential as a natural therapeutic agent against chemically induced hepatic injuries. Furthermore, the plant's safety profile, availability, and bioactive richness make it a promising candidate for further drug development.

However, while the preclinical data are promising, further studies are needed to:

- Isolate and characterize the active constituents of MPME.
- Determine the precise mechanisms of action at molecular and cellular levels.
- Conduct chronic toxicity and pharmacokinetic studies.
- Evaluate its efficacy through clinical trials in human subjects.

In conclusion, Mucuna pruriens methanolic extract offers strong potential as a hepatoprotective agent, capable of mitigating oxidative stress and liver damage caused by paracetamol toxicity. With further validation, it may serve as a cost-effective, plant-based alternative or complement to conventional liver-protective therapies.

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