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STUDY THE BIOCHEMICAL PARAMETERS AND HISTOPATHOLOGICAL CHANGES IN LIVER OF ALBINO RATS TO FIND OUT THE EFFECT OF METHANOLIC EXTRACT OF *MIMOSA PUDICA* LEAVES AGAINST PARACETAMOL INDUCED HEPATIC DAMAGES

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ABSTRACT:

The present study was conducted to evaluate the hepatoprotective activity of Methanolic extract of *Mimosa pudica* Linn.against paracetamol induced liver damage in rats. The Methanolic extract of *Mimosa pudica* (300mg/kg & 600mg/kg) wasadministered orally to the animals with hepatotoxicity induced by single dose of paracetamol (2gm/kg). Silymarin (25mg/kg) was givenas reference standard. All the test drugs were administered orally by suspending in 0.5% Carboxy methyl cellulose (CMC)solution. The methanolic extract of leaves of plant *Mimosa pudica* in the doses of 300mg/kg, 600mg/kgbody wt, reduced the levels of serum SGPT, SGOT, ALP and Total bilirubin and increased the Total protein level significantly. In the histopathological studies of the liver section of rats showed the significant recovery with the Methanolic extract of *Mimosa pudica* when compared with Control group and Paracetamol treated group.

KEY WORDS: *Mimosa pudica*, Paracetamol, Hepatoprotetive and Hepatotoxicity.

INTRODUCTION:

The liver plays a central role in transforming and clearing chemicals and is susceptible to the toxicity from these agents. Hepatotoxicity implies chemical-driven liver damage. Certain medicinal agents when taken in overdoses and sometimes even when introduced within therapeutic ranges may injure the organ. Other chemical agents such as those used in laboratories and industries, natural chemicals (e.g. microcystins) and herbal remedies can also induce hepatotoxicity. Chemicals that cause liver injury are called hepatotoxins. More than 900 drugs have been implicated in causing liver injury and it is the most common reason for a drug to be withdrawn from the market. Chemicals often cause subclinical injury to liver which manifests only as abnormal liver enzyme tests. Drug induced liver injury is responsible for 5% of all hospital admissions and 50% of all acute liver failures.[1]

Liver disease is still a worldwide health problem. Unfortunately, conventional or synthetic drugs used in the treatment of liver diseases are inadequate and

sometimes can have serious side effects[2]. In the absence of a reliable liver protective drug in modern medicine there are a number of medicinal preparations in Ayurveda recommended for the treatment of liver disorders. In view of severe undesirable side effects of synthetic agents, there is growing focus to follow systematic research methodology and to evaluate scientific basis for the traditional herbal medicines that are claimed to possess hepatoprotective activity.[3] Traditionally it is reported that the plant *Mimosa pudica* Linn. from Mimosaceae family used for the treatment of dysentery, various vaginal problems and inflammations, burning sensation, fatigue, various liver disorder, Jaundice etc.[4] So in the present study of the plant belongs to Mimosaceae family has been investigated for hepatoprotective activity against Paracetamol induced hepatotoxicity in rats.

MATERIALS AND METHODS:

Plant Materials:

The entire plant Mimosa pudica Linn. was collected from Jalpaiguri district of West Bengal during the month of June-July. They were thoroughly washed in running water, segregated from the grass and other extraneous material and the field data of the plant like its height, flower color and soil condition were noted in the note book. The authentication was carried out by the help of **Botanical Survey of India (BSI)**, Sikkim Himalayan Regional Centre, Gangtok, Sikkim- 737103. Then the leaves were taken and dried in shade for 30 days. The shade-dried leaves were powdered about 1kg and were extracted with methanol in a Soxhlet extractor for 72 hours. Extract was evaporated and taken for the pharmacological studies.[5, 6]

Drugs and Chemicals:

Silymarin was purchased from Micro labs, Tamilnadu. India SGOT, SGPT, ALP, Bilirubin and Total Protein kits were procured from Span Diagnostics, Surat, India & Transasia Bio-Medicals Ltd. Paracetamol(Pure) was purchased from AGAPE pharmaceuticals, Sikkim. All other chemicals were obtained from local sources and were of analytical grade.

Animals:

Albino rats (Wistar strain) weighing 100-120g of either sex were used for the study. The animals was procured and housed in the animal house of Himalayan Pharmacy Institute, Sikkim at least 2 weeks prior to the study, So that animal could adapt to the new environment. Animal house was well maintained under the standard hygienic conditions, at a temperature (22 ±20c), room humidity (60 ± 10%) with 12 hrs day and night cycle, with food and water. Total 30 Rats were housed in 5 groups of 6 per cage. Cleaning and sanitation was done on alternate days. Paddy husk was provided as bedding material which was cleaned every day. The cages were maintained clean. All the pharmacological work was carried out after obtaining the approval from the Institutional Animal Ethical Committee of Himalayan Pharmacy Institute, Sikkim.

Working Protocol

Group I: Control group (Vehicle)

Group II: Normal saline (5ml/kg) for 6 days + single dose of Paracetamol (2gm/kg) orally on day 7th.

Group III: Standard group treated with Silymain 25 mg/kg orally for 6 days + single dose of Paracetamol (2gm/kg) orally on day 7th.

Group IV: Test group treated with alcoholic extract (300 mg/kg) + single dose of Paracetamol (2gm/kg) orally on day 7th.

Group V: Test groups treated with alcoholic extract (600 mg/kg body weight) orally for 6 days + single dose of Paracetamol (2gm/kg) orally on day 7th.

Experimental Details

Animals were randomized and divided into five

groups (I-V) of six animals in each group. **I** served as untreated control and fed orally with 0.5% CMC of 5ml/kg body weight daily for seven days. **Group II** rats were treated orally with the standard drug Silymain (25 mg/kg) orally for 6 days and single dose of Paracetamol (2gm/kg) orally on 7th day. **Group III** rats were treated with Normal saline (5ml/kg) orally for 6 days and single dose of Paracetamol (2gm/kg) orally on day 7th. **Group IV (**Test group) rats were treated with alcoholic extract (300 mg/kg) for 6 days and single dose of Paracetamol (2gm/kg) orally on day 7th. Similarly**, Group V**(test group) rats were treated with alcoholic extract (600 mg/kg body weight) orally for 6 days and single dose of Paracetamol (2gm/kg) orally on day 7th .For the proper dissolving of Silymarin, Paracetamol and Methanolic extracts with purified water respectively the 0.5% CMC (Carboxy methyl cellulose) were used. Hepatotoxicity was induced by oral administration of a single dose of paracetamol (2 gm/kg body weight). After 48 hours of treatment, blood was collected by intracardiac puncturing and was allowed to coagulate at room temperature for 30 minutes. Serum was separated by centrifugation at 3000 rpm for about 5 minutes. The clear straw colored serum was the collected and stored at 2-8ºC for the measurement of marker enzymes levels to assess the liver functions.[7,8,9]

Assessment of biochemical parameters

Estimation of **SGOT, SGPT and ALP** were done with the reagents supplied in the kits (Span Diagnostic Ltd.) which were reconstituted, mixed with serum as directed. The SGOT and SGPT were measured at 340nm and expressed as IU/L. The serum alkaline phosphatase (ALP) was estimated by mixing with the reagent (*p*-nitro phenyl phosphate, magnesium, buffers and stabilizers) with serum, estimated at 405 nm and expressed as IU/L. **Total Protein** concentration was measured by using standard method of Lowery. The total protein was estimated at 555nm and expressed as mg/dl. **Total Bilirubin** was estimated by Diazo method of Pearlman and Lee with the help of reagents in the kits (Transasia Bio-Medicals Ltd.) measured at 546 nm and expressed as mg/dl.[10,11]

AST was estimated by Modified UV (IFCC), Kinetic assay method[11, 12]

Tab 1. Procedure

Pipette into tube marked	Test
Serum/Plasma	100µL
Working AST regent	1000µL

1. Mix well and aspirate immediately for measurement.
2. Programme the analyser as per assay parameters.
3. Blank the analyzer with purified water.
4. Read absorbance after 60 seconds. Repeat reading after every 30 seconds i. e. upto 120 seconds at 340 nm wavelength.
5. Determine the mean absorbance change per minute.

ALT was estimated by Modified UV (IFCC), Kinetic assay method[12, 13]

Tab 2. Procedure

Pipette into tube marked	Test
Serum/Plasma	100µL
Working ALT regent	1000µL

1. Mix well and aspirate immediately for measurement.
2. Programmed the analyzer as per assay parameters.
3. Blank the analyzer with purified water.
4. Read absorbance after 60 seconds. Repeat reading after every 30 seconds i. e. upto 120 seconds at 340 nm wavelength.

5. Determine the mean absorbance change per minute.

ALP was estimated by *p*NPP-AMP (IFCC), Kinetic assay method[12, 13]

Tab 3. Procedure

Pipette into tube marked	Test
Serum/Plasma	20µL
Working ALP regent	1000µL

1. Mix well and aspirate immediately for measurement.
2. Programmed the analyzer as per assay parameters.
3. Blank the analyzer with purified water.
4. Read absorbance after 30 seconds. Repeat reading after every 30 seconds i. e. upto 120 seconds at 405 nm wavelength.
5. Determine the mean absorbance change per minute.

Total protein estimation done by standard method of Lowery[14]

Protein concentration was measured by using standard method of Lowery.

Preparation of Lowry solution

It was prepared by mixing the following solutions i.e. Solution A, Solution B and Solution C in a ratio of 100: 1: 1 respectively .

Solution A: 2.8598g NaOH was dissolved in 200 ml distilled water then in it 14.3084g Na2CO3 was added, the volume was made up to 500 ml with distilled water.

Solution B: 1.4232g of CuSO45H2O was dissolved in 80 ml distilled water and the volume was made up to 100 ml with distilled water.

Solution C: Dissolve 2.85299g Na2 –tartarate 2H2O was dissolved in 80 ml distilled water and the volume was made up to 100 ml with distilled water.

Preparation of Folin reagent

5 ml of 2N Folin Ciocalteu's Phenol reagent was mixed with 6 ml of distilled water. This solution is light sensitive so it was prepared at the last 5 minutes of the first sample incubation and was kept in an amber colour container.

Procedure

The sample was taken out from freezer to thaw. The samples were vortexes well and 0.5 ml was transferred in 10 ml glass tube. Then in it 0.7 ml Lowry solution was added and caped well. The tubes were vortex briefly for mixing. It was then incubated for 20 min at room temperature in dark. After 20 min of incubation the samples were taken out and 0.1 ml Folin reagent were added to each tube. The tubes were caped and vortex for mixing. The tubes were incubated once more for 30 min. After 30 min the samples were vortex again. The samples were transferred in a cuvette and measure the absorbance against blank. From the standard curve of BSA (Bovine serum albumin), the protein concentration were calculated.

Histopathological examination[15, 16]

The blood was collected by intracardia puncture and the liver was removed, sliced and washed in saline. Liver pieces were preserved in 10% formasal (10% formaldehyde diluted using normal saline) for histopathological study. The pieces of liver were processed and embedded in paraffin wax. Sections were made about 4-6 ?m in thickness, stained with haematoxylin and eosin. They were mounted and observed under light microscope for histological changes.

Statistical analysis

The data are expressed as Mean ± SEM. Where, N=6(rats). Statistical analysis was made by one way ANOVA followed by Dunnett multiple comparison test; p values <0.01 are considered as significant when compared with Control group **(Group I)** and p values <0.01 are also considered as significant when compared with Paracetamol treated group **(Group II).** Highest significant difference test has been performed with Graph pad instat software.

RESULTS:

Tab 4. Biochemical Parameters of Liver

Treatment mg/kg,p.o	SGPT / ALT (IU/L)	SGOT / AST (IU/L)	ALP (IU/L)	Total Bilirubin (mg/dl)	Total Protein (mg/dl)
Vehicle Control	41.55±0.30	69.50±0.80	52.13±0.41	0.806±0.003	7.660±0.447
Paracetamol (2gm/kg)	222.65±0.86**a**	273.13±1.2**a**	232.71±0.87**a**	2.688±0.170**a**	3.852±0.092**a**
Silymarin 25mg/kg + Paracetamol	62.15±0.50**a,b**	83.26±0.62**a,b**	70.94±0.29**a,b**	0.988±0.005**a,b**	6.750±0.057**a,b**
MtOH ext 300mg/kg + Paracetamol	148.59±0.61 **a, b**	182.71±0.75 **a, b**	170.21±0.49 **a, b**	1.898±0.027**a,b**	4.503±0.140**a,b**
MtOH ext 600mg/kg + Paracetamol	75.35±0.34**a,b**	97.08±0.40**a,b**	87.52±0.73**a,b**	1.460±0.141**a,b**	5.733±0.097**a,b**

Where, MtOH = Methanolic Extract of *Mimosa Pudica*

All Values are expressed as Mean ± SEM. n = 6 rats in each group. a, P<0.01 compared to control group; b , P<0.01 compared to Paracetamol-treated group.

Graphical representation of Biochemical Parameters

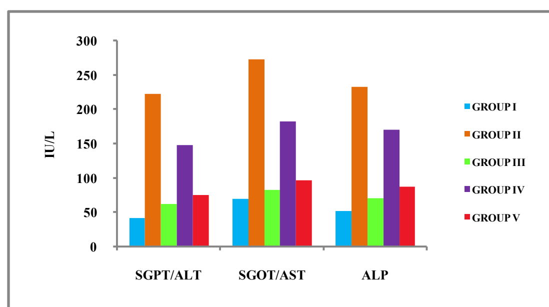


Fig 1. Graphical representation of **SGPT, SGOT, ALP** level of all groups (**Group I**=Control (Vehicle**)** group, **Group II**=Paracetamol-induced group, **Group III**= Silymarin treated group, **Group IV**= Methanolic extract, 300mg/kg, **Group V**= Methanolic extract, 600mg/kg) in Paracetamol induced Hepatotoxicy

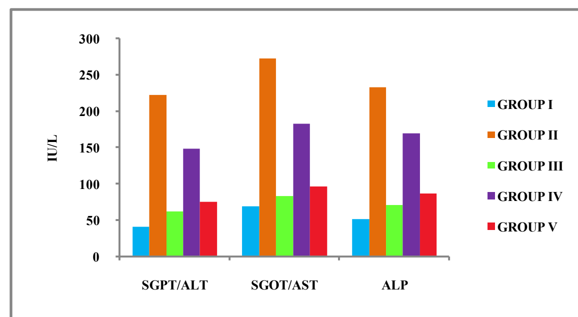
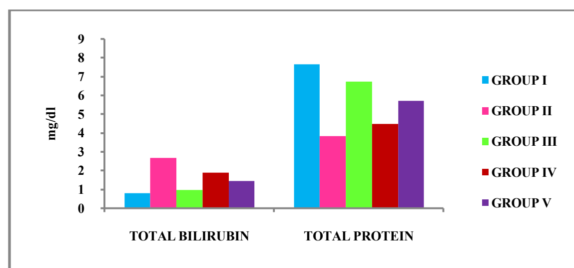


Fig 2. Graphical representation of **Total Bilirubin and Total Protein** level of all groups (**Group I**=Control (Vehicle) group, **Group II**=Paracetamol-induced group, **Group III**= Silymarin treated group, **Group IV**= Methanolic extract, 300mg/kg, **Group V**= Methanolic extract, 600mg/kg) in Paracetamol induced Hepatotoxicy



Histopathological changes in Liver

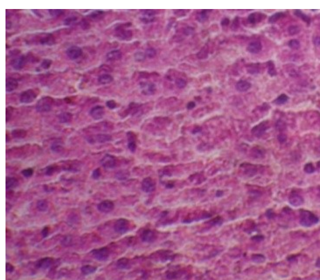


Fig 3. Treated with CMC (Control group)

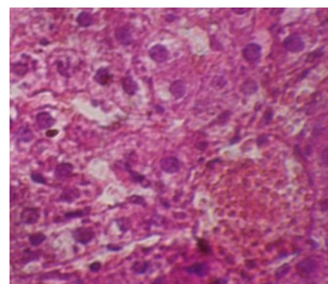


Fig 4. Treated with Paracetamol

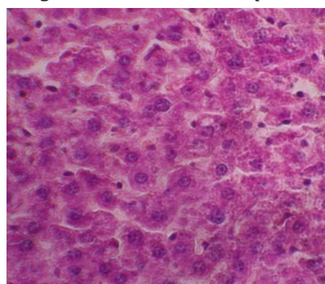


Fig 5. Treated with Silymarin

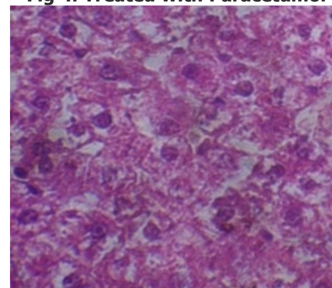


Fig 6. Treated with MtOH ext.(300mg/kg)

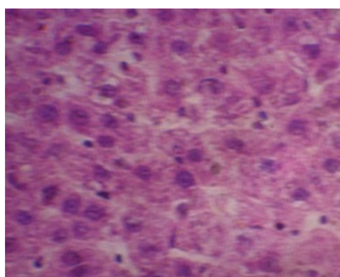


Fig 7. Treated with MtOH ext. (600mg/kg)

DISCUSSION

Treatment with paracetamol by administering 2g per kg of body weight at a single dose in albino rats caused considerable increase of **SGOT** (also known as aspartate transaminase, AST), SGPT (also known alanine transaminase, ALT), ALP(Alkaline phosphatase), Total bilirunin and and decrease of Total protein. Estimation of the serum level revealed that paracetamol induced a remarkable increase of SGPT, SGOT, ALP, Total bilirubin and decrease the Total protein level by comparison to the control animals. Methanolic extract at dose 300mg/kg body weight reduced these paracetamol induced level of **SGOT, SGPT, ALP & Total bilirubin** by 33.26%,

33.11%, 26.86% and 29.72% respectively and increase the Total protein by 12.82% where as the dose at 600mg/kg body weight reduced these paracetamol induced level of SGPT, SGOT, ALP & Total Bilirubin by 66.16%, 64.46%, 32.39% and 48.11% respectively and increase the Total protein by 21.54%. The results were found to be statistically significant and the '*p*' values were less than 0.01%. These observations inferred Alcoholic extract (methanol) might contain hepatoprotective principles and the higher dose (600mg/kg body weight) of the extract shows more significant result than the dose 300mg/kg body weight. Paracetamol at higher doses causes hepatic necrosis due to increased formation of reactive intermediate such as *N*-acetyl-*p*-benzo-quinone imine (NAPQI) by oxidation through the cytochrome P-450 mixed function oxidase system and NAPQI is then irreversibly conjugated with the SH groups of glutathione. Attenuation of the necrotic effect of paracetamol by the extract might be due the reversible inhibition of the oxidative enzymes. Histo-pathological study of liver sections of control group showed normal cellular architecture with distinct hepatic cells, sinusoidal spaces and central vein **(Fig 3.)**. In the liver sections of paracetamol intoxicated rats **(Fig 4.)**, there was disarrangement and degeneration of normal hepatic cells with intense centrilobular necrosis, sinusoidal hemorrhages, fatty changes, cytoplasomic vacuolization and inflammatory changes. Treatment with Standard drug silymarin showed the prominent protection of liver **(Fig 5.)**.Treatment with the methanolic extract (300mg/kg) showed less amount protection**(Fig 6.)** ,but the methanolic extract(600mg/kg) exhibited prominent protection against paracetamol intoxication, which was evidenced by less centrilobular necrosis, less vacuole formation, reduced sinusoidal dilation, and less disarrangement and degeneration of hepatocytes **(Fig 7.)**. These studies suggest that the methanolic extract of *M. pudica* protected the hepatic necrosis by inhibiting enzymatic oxidation.

CONCLUSION:

The Methanolic extract of leaves of plant *Mimosa pudica* found to have significant hepatoprotectiveactivity against Paracetamol induced hepatotoxicity in Wistar rats. The effect is almost comparable to silymarin orslightly less. Methanolic extract of leaves of plant *Mimosa pudica* in the doses of 300mg/kg and 600mg/kgbody wt, reduced the levels of serum SGPT, SGOT, ALP and Total bilirubin and increased the Total protein level. These observations inferred that Methanolic extract at higher dose (600mg/kg body wt) shows more significant result than the dose 300mg/kg body wt. In the histopathological studies of the liver section of rats showed the significant recovery with the leaves of Methanolic extracts of *Mimosa Pudica* against Paracetamol induced hepatotoxicity. Though the Methanolic extract of*Mimosa pudica* showed significant hepatoprotective activity against Paracetamol induced hepatotoxicity, it isalso needed further research to isolate the compound and exact mechanism responsible forhepatoprotective activity of the plant to rationalize its use as a drug to give more emphasis on this plant for the development of medicinal value.

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