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HEPATOPROTECTIVE ACTIVITY OF ETHYL ACETATE OF *MIMOSA PUDICA* LEAVES AGAINST PARACETAMOL INDUCED IN ALBINO WISTER RATS

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Abstract

Bilirubin is generally thought to exist in three major forms in serum as unconjugated bilirubin, as the monoglucuronide, or as the diglucuronide. This exclusivity and dearth of any noticeable adverse efficacy propose the opportunity of using *Mimosa pudica* L as an efficient adjuvant in the treatment of liver damage. The objective of this study is to find out qualitatively and quantitatively phytochemical constituents and antioxidant activities, in the leaves of *Mimosa pudica* extracts. The extracts from, ethanol were investigated for the presence of alkaloid, glycoside, tannin, flavonoid, terpenoids. Particular reagents were used to screen phytochemicals in the samples and their presence was indicated by the changes of color, precipitation. Histopathological examination of the liver tissues of control and treated groups also confirmed the hepatoprotective activity. The present study aimed to explore the hepatoprotective effects of ethanolic extract of *Mimosa pudica L* against paracetamol-induced liver injury in experimental rats. Liver injury induced by a single dose of paracetamol (100 mg/kg b.w.) in male Wistar rats. *Mimosa pudica* administration to paracetamol treated Wistar rats significantly reduced the levels of Histopathological examination to near normal range. The present study thus explores the modulating effect of *Mimosa pudica* in paracetamol induced hepatoprotective Activity.

***Keywords*:** Hepatotoxicity, paracetamol, *Mimosa pudica* and phytochemical

Introduction

Bilirubin is generally thought to exist in three major forms in serum as unconjugated bilirubin, as the monoglucuronide, or as the diglucuronide. Hepatocellular jaundice - a type of jaundice that occurs as a result of liver disease or injury. Hemolytic jaundice -a type of jaundice that occurs as a result of hemolysis (an accelerated breakdown of erythrocytes- red blood cells) leading to an increase in production of bilirubin. The common causes of jaundice include viral infections of the liver (hepatitis A, hepatitis B, hepatitis C, hepatitis D, and hepatitis E), parasitic infections of the liver, gallstones, pancreatic cancer, metabolic disorders present from birth that makes it makes it hard for the body to breakdown bilirubin. (*Porchezhian*, and Ansari, 2005) Jaundice is characterized by the yellow color of the skin, mucous membranes, or eyes. As red cells are broken in the body to be replaced by new ones, the liver removes the old blood cells, forming bilirubin in the process. Bilirubin is next to broken down in the liver and removed from the body via the feces (Rothermundt, 2011)

Drug-induced liver injury is responsible for 5% of all hospital admissions and 50% of all acute liver failures (Friedman *et al*., 2003). The past 30 years have witnessed major progress in the knowledge and management of liver disease yet approximately 29 million persons in the European Union still suffer from a chronic liver condition. Difficulties in accessing data from individual countries hinder the comprehensive evolution of the burden of liver disease in Europe and comparison with other diseases (Rahmatullah, 2010). Paracetamol (acetaminophen) is a pain reliever and a fever reducer. The extract mechanism of action is not known. Paracetamol is used to treat many conditions such as headache, muscle aches, arthritis, backache, toothache, colds, and fevers. It relieves pain in mild arthritis but does not affect the underlying inflammation and swelling of the joint (Vasudevan *et al*., 2000).

Indian Traditional Medicine like Ayurveda, Sidha, and Unani are predominantly based on the use of plant materials. The association of medical plants with other plants in their habitat also influences their medicinal values in some cases. One of the important and well-documented uses of plant products is their use as hepatoprotective agents. Despite tremendous strides in modern medicine, there are hardly any drugs that stimulate liver function, offer protection to the liver from damage, or help regeneration of hepatic cells (Kuruvilla, 2010). Plants have been a rich source of medicines because they produce a host of bioactive molecules, most of which probably evolved as chemical defenses against predation or infection (Sharma Upendrakumar, 2009).

The *Mimosa pudica* invites the attention of the researchers worldwide for its pharmacological activities such as antidiabetic, antitoxin, wound healing activities, and antihepatotoxic. The plant is characterized by the presence of an array of phytochemicals including alkaloids, glycosides, flavonoids, and carbohydrates. The medicinal plant has and dislocation, isolated from it has demonstrated significant effects in animal studies. The review summarizes pharmacognostic and pharmacological investigations carried out on the plant (Baby Joseph *et al*., 2013). The present study has evaluated the modulating effect of *Mimosa pudica* on tissues in paracetamol induced hepatocellular Activity.

Materials and Methods

a) Collection of plant material: The fresh healthy leaves *Mimosa pudica* were collected from Puliyancheri village, Krishnagiri district, Krishnagiri taluk, Tamilnadu, India.

b) Preparation of extract: The leaves of *Mimosa pudica* was collected and washed thoroughly in distilled water. The leaves were dried at room temperature. Dried leaves pieces were uniformly using a mechanical grinder to make a fine powder. 25gm powder was macerated in 250ml ethyl acetate

for 3 days at room temperature with occasional stirring. After 3 days, ethyl acetate extract was filtered Whatman No.1 filter paper. The concentrated extract was collected in a Petri dish and allowed to air dry for complete evaporation of ethyl acetate.

c) Experimental Animals: All the experiments were done with male Albino Wister rat weighing 140-150g obtained from the Venkateshwara enterprises, Bangalore were used in this study. The healthy male Swiss rats were collected from Bangalore, Karnataka, India.

d) Experimental Design: Paracetamol tablet was then made to powder with the help of mortar and pestle. The paracetamol powder was administrating at concentrations of 100mg/kg was dissolved in distilled water given to rats through oral incubations for a period of 7 days. In the experiment a total of 20 rats were used in the study.

The rats were divided into following four groups of five rats each.

Group-1: Normal control rats

Group-2: Paracetamol control rats

Group-3 : *Mimosa pudica* + Paracetamol

Group-4: Normal rats treated with *Mimosa pudica*

e) Collection of blood: After the last treatment, all the rats were sacrificed by cervical deception. Blood was collected for the estimation of blood glucose. Serum and plasma were separated from blood after centrifugation. The liver tissue was excised immediately from the rats, washed off the blood with ice-cold physiological saline. A known weight of the liver tissue was homogenized in an appropriate buffer solution. The homogenate was centrifuged and the supernatant was used for the estimation of various biochemical parameters.

Phytochemical Analysis

Qualitative analysis phytochemical analysis activity: To identify the Phytochemical in plant extracts tests were carried out. The stock concentration of plant extracts 10mg/ml was used.

Test for Phytosterols

A small quantity of the extract was suspended in 5ml chloroform separately. The above-obtained chloroform solution was subjected to the following test.

- **Liebermann Bur chard's test:** The 1ml of the prepared chloroform solution was treated with few drops of concentrated sulfuric acid and observed for the bluish-green color.
- **Salkowski test:** To 1ml of the prepared chloroform solution was treated with few drops of concentrated sulfuric acid and observed for the color.

Test for Phenol

- **Ferric chloride test:** Five mg of extract was taken and 0.5 ml of 5% ferric chloride was added and observed for dark bluish-black color.

Test for Glycosides

Extracts were hydrolyzed with dilute HCL and then subjected to the test for glycosides.

- **Modified Borntrager's test:** To extracts were treated with ferric chloride solution and immersed in boiling water for above 5 minutes. The mixture was cooled and extracted with equal volume benzene. The benzene layer was separated and treated with ammonia solution and observed for rose-pink color in the ammonia layer.
- **Bromine water test:** To test solution was dissolved in bromine water and observed for the formation of the yellow precipitate.

Test for flavonoides

- **Alkaline reagent test:** To extracts were treated with few drops of sodium hydroxide solution and observed of intense yellow color.
- **Lead acetate:** To extracts were treated with a few drops of lead acetate solution and observed the yellow color precipitate.
- **Shinoda test:** To 2-3 ml of extract, few fragments of magnesium metal were added in a test tube followed by drops wise addition of concentrate HCL and observed for magenta color.

Test for Alkaloids

To a small quantity of the extract of the treated with few drops of dilute hydrochloric acid and filtered.

- **Mayer's test:** To filter was treated with Mayer's reagent (potassium mercury chloride) was observed a yellow colored precipitate.
- **Wager's test:** To filter was treated with wager's reagent (Iodine in Potassium iodide) was observed of brown, reddish precipitated.
- **Dragendroff's test:** To filter was treated with Dragendroff's reagent (solution of potassium bismuth iodide) was observed of the red precipitate.
- **Hager's test:** To filter was treated with Hager's reagent (saturated picric acid solution) presence of alkaloids confirmed by the formation of a yellow-colored precipitate.

Test for Tannins

- **Lead acetate test:** To extract was taken and 0.5ml of 1%lead acetate solution was added and the observed for the precipitate.

Test for Saponin

- **Froth test:** To extract was diluted with distilled water to 20ml and this was shaken in a graduated cylinder for 15 minutes and observed for the 1 cm layer of foam.
- **Foam test:** To 0.5gm extract was shaken with 2ml of water and observed for the produced persists for 10 minutes presences.

Test for Carbohydrate

- **Benedict's test:** 1ml of test solution equal volume of Benedict's reagent and test solution in the test tube were observed green-yellow precipitate.
- **Fehling's test:** 1ml of Fehling's A and Fehling's B solutions were mixed and boiled for a 1minute boiling water bath for 5-10 minutes and tubes were observed for a yellow, then brick-red precipitate.

Test for Terpenoid

- **Salkowski test:** To 5ml of the extract was mixed with 2ml of chloroform and 3ml of concentrated sulfuric acid was carefully added to form a layer. A reddish-brown coloration of the interface was formed to show positive results.

Test for Protein

- **Biuret test:** To test solution few drops 4% NaOH and $CuSO_4$ solution were added and observed for the violet or pink color.
- **Millon's test:** To 3ml test solution 5ml of Millon's reagent was added and observed for white precipitate.
- **Xantho protein test:** To 3ml of test solution 1ml of concentrated H_2SO_4 was added and observed for white precipitate.

Test for Amino Acid

- **Ninhydrin test:** To 3ml test solution and 3 drops 5% Ninhydrin solution was heated in a water bath for 10 minutes, observed for purple or bluish color.

Estimation ff Glucose

To estimate the amount of blood glucose present in the given serum sample by the Orthotoludine method. Sasaki & Matsui, 1972). Various concentrations of working standard solution (0.2 to 1.0 ml) were taken in a series of test tubes labeled as S1 to S5 and made up to 1 ml with distilled water. For blank, 1ml of distilled water was taken. 0.4 ml of unknown solution and 1 ml of supernatant was taken in a test tube marked as T1 and T2 and made up to 1 ml with distilled water. Then added 4 ml of orthotoludine reagent to all the test tubes and kept in boiling water bath for 10 minutes and then cooled. The greenish-blue color developed was read calorimetrically at 620 nm.

Estimation of Blood Urea

To estimate the amount of urea present in the given serum sample by Kitamura 1959 method. Pipette out 0.5-2.5 ml of working standard in different solutions with the concentration range of 10-50 µg respectively 0.2 ml of serum sample was taken in duplicate in the tubes marked as T1 and T2. The volume of all the tubes was made up to 3ml with distilled water and 3ml of distilled water serves as blank. Then added 3ml of DAM reagent, followed by 3ml of the acid mixture. Tubes were mixed well and heat vigorously in a boiling water bath for 20 minutes. Then the tubes were cooled and read measured calorimetrically at 540nm. A standard calibration graph was drawn by plotting concentration of urea on 'x' and optical density on 'y' axis from the standard calibration curve the amount of urea present in the serum sample was calculated

Estimation of Serum Creatinine

To estimate the amount of certain in the given serum sample. Pipette out 3ml of the diluted serum into graduated centrifuge tubes and added 1.0ml of 0.04N picric acid noted the level of the liquid placed in a beaker off briskly boiling water both for 1 hour. The mouths of the tubes were converted with marbles to minimize evaporation at the same time placed in a blank tube containing 3ml of water and 1ml of picric acid in the boiling water at the end of 1 hour cooled at room temperature and made up to the original level with the water. 1ml of 0.74N sodium hydroxide and completed the estimation as for certain the reading on the + colorimeter now including the certain present in the difference between this above gives the certain formed from certain. The production of mahogany red color with an alkaline picric solution. The intensity of color developed is read in the colorimeter against a reagent blank at 540nm. A standard graph was drawn plotting colorimeter reading on the 'y' axis and concentration of certain on the 'x' axis.

Estimation of Total Protein

To determine the A/G ratio of the given serum sample by the Burette method. Pipette out 6.0ml of the sulfate-sulfite solution into a 90% 15mm centrifuge tube and on to it layer 0.4ml of serum. Invert to mix then remove at once 2ml of the mixture and into 5ml of the burnet mixture shake the mixture tubes well place the tubes in a water bath 370c for 10 mines allow to cool for absorption measure at 555 million microns (540-560). The amount of globulin can be calculated from this A/G ratio is measured.

Determination of Serum Bilirubin

To determine the amount of bilirubin present in the given serum sample by Malloy and Evelyn, (1937). Weigh 10mg into a 100ml volumetric flask. Working away from bright light, dissolve the bilirubin in a minimum (about 5ml) of 0.1N sodium carbonate solution as quickly as possible, since it is unstable in alkaline solution. Made the volume with methanol or with human citrated plasma obtained from blood bank from out-dated bottles. It must not be employed or lipemic. It should be stored at 40c and not frozen. Plasma is left in sunlight for some hours before use to destroy bilirubin present. This is to be kept frozen in small aliquots. The underlying principle is the conversion of bilirubin to the purple-colored bilirubin when coupled with diazotized sulphanilic acid. A standard graph was drawn by plotting bilirubin concentration on X-axis and optical density on the Y-axis and the amount of bilirubin present in the sample was calculated

Estimation of Uric Acid

To estimate the amount of uric acid present in the given serum sample by Caraway method 1955. Into different tubes pipette out 0.5 to 2.5ml of the working standard corresponding to mg values 3, 6, 9, 12, 15 respectively and made up to 3.0ml and with water, in the same way, 3.0ml of the diluted serum and 3ml of water as blank were taken in separate tubes. To all the tubes added 1.0ml of the serum reagent followed by 1.0ml of 14% sodium carbonate solution and let N stand for 15 minutes for the color to develop. The color was read in a colorimeter measured at 540nm against reagent blank.

Histopathology observation of liver and tissues

Liver tissue in form animals of each group were preserved in 10% formalin, embedded in paraffin. Sections of 5-6 mm were routinely stained with hematoxylin and (H & E) and examined under a light microscope. Any alterations compared to the normal structure were recorded.

Results and Discussion

The present study is on hepatoprotective activity of ethyl acetate extract of *Mimosa pudica* leaves against

paracetamol-induced in albino rats shade dried and powder leaf sample was successively extracted with ethyl acetate the extracts were filtrated and concentrated using vacuum distillation. The different solvent extracts were subjected to quantitative tests for the identification of various Phytochemical constituents per the standard procedure.

The result preliminarily qualitative Phytochemical analysis on leaves of ethyl acetate solvent extract of *Mimosa pudica* was showed in (Table 1) the result showed the presence of compounds Alkaloids, Flavanoids, Glycosides, and Carbohydrates. The absence of compounds Steroids, Tannins, Amino acid, Phenols, Phytosterols and Proteins.

Table 1 : Phytochemical analysis of crude leaf extracts of *Mimosa pudica*

Components	Different types of solvent extract
	Ethyl Acetate
Alkaloids	+
Flavanoids	+
Steroids	_
Tannins	_
Saponins	_
Glycoside	+
Carbohydrates	+
Amino acid	_
Phenols	_
Phytosterols	_
Proteins	_

(+) presence solvent (-) absence solvent

Table 2: Represent the effect of biochemical parameters on ethyl acetate extract of *Mimosa pudica.*

Bio Chemical Parameters	GROUP-I	GROUP-II	GROUP-III	GROUP-IV
Urea mg/dl	15.58±4.71	38.50±5.17	17.83±4.47	15.58±8
Uric acid mg /dl	5.27±1.77	17.47±1.86	7.21±0.78	4.99±1.66
Blood glucose mg/dl	99.99±27.1	183.33±16.67	144.44±15.71	83.33±16.56
Total protein mg/dl	1.94±0.64	5.48±0.93	2.82±0.52	1.6±0.48
Creatinine mg/dl	2.26±0.61	5.95±0.57	3.03±0.36	2.35±0.25
Total bilirubin mg/dl	2.75±0.93	7.75±0.70	3.91±0.93	2.12±0.37
Direct bilirubin mg/dl	2.5±0.61	5.93±0.62	3.25±0.61	2.62±0.37

Value is expressed as the mean ± SD for six animals in each group. Comparisons are made between group I with group II(A), group I with group IV(B) and group II with group-III(C)

The same result for the preliminary screening of the leaf extract of *Mimosa pudica*. Presence of suggests the ability of this plant to play a major role as anti-diarrhea and antihaemorrhagic agents. Further the extract showed the absence of saponins, tannins, and of fixed oil and fats.

The result preliminarily biochemical analysis on an extract of *Mimosa pudica* leaves was shown in (Table 2) the result showed the presence of urea, uric acid, blood glucose, Total protein, Creatinine, total bilirubin, and direct bilirubin.

Table 2 Presents the changes in biochemical activities: urea and in the serum of *Mimosa pudica* leaf extract treated and experimental rats. In Group II [Paracetomal induced] rats urea activities were significantly ($p<0.05$) increased when compared to that of control (Group I) rats. On administration of Ethyl acetate extract activity has decreased enzyme activities that were reversed significantly ($p<0.05$) to near normal in treated (Group IV) animals. No significant changes were observed in the Ethyl acetate extract administered control (Group III) animals when compared to control rats.

The presents the changes in biochemical activities: glucose and uric acid in the serum of *Mimosa pudica* leaf extract treated and experimental rats. In Group II [Paracetomal induced] rats glucose and uric acid activities were significantly ($p<0.05$) increased when compared to that of control (Group I) rats. On administration of Ethyl acetate extract activity has decreased biochemical activities that were reversed significantly ($p<0.05$) to near normal in treated (Group IV) animals. No significant changes were observed in the Ethyl acetate extract administered control (Group III) animals when compared to control rats.

The changes in biochemical activities: protein and certain in the serum of *Mimosa pudica* leaf extract treated and experimental rats. In Group II [Paracetomal induced] rats activities were significantly ($p<0.05$) increased when compared to that of control (Group I) rats. On administration of Ethyl acetate extract activity has decreased biochemical activities that were reversed significantly ($p<0.05$) to near normal in treated (Group IV) animals. No significant changes were observed in the Ethyl acetate extract administered control (Group III) animals when compared to control rats.

The drug-induced hepatic jaundice is often associated with marked elevation in blood urea nitrogen, cause acute tubular hepatic. These biochemical parameters have been used to investigate drug-induced hepatic jaundice in animals the blood urea level registered a significant increase in the monosodium treated rats. The values reverse to near normal values in the extract, serum levels of urea were used as indicators of renal function. Elevated blood urea is known to be correlated with increased protein catabolism in mammals and the conversion of ammonia to urea as a result of increased synthesis of the arginase enzyme involved in urea production. Urea is the main end product of protein catabolism. Renal diseases that diminish the glomerular filtration lead to urea retention and decrease in urea are seen in severe seen liver disease with the destruction of cells leading to impairment of the urea cycle.

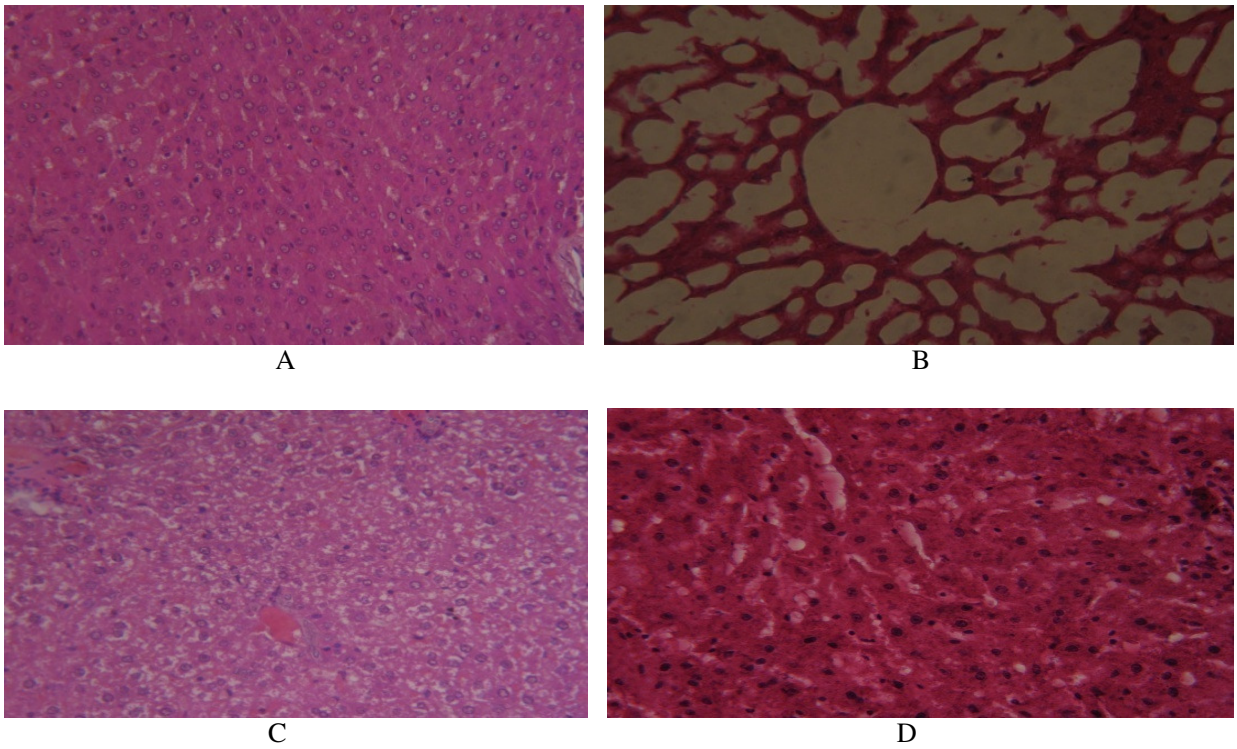
Uric acid is the major product of purine nucleotides, adenosine, and guanosine. Uric acid, one of the major endogenous water-soluble antioxidants of the body, has been thought to be a metabolically inert end product of purine metabolism. Elevated leaves of uric acid are due to either an increase in uric acid production or a decrease in its excretion.

Certain is a byproduct of the breakdown of certain and phosphor certain which is considered as an energy storage compound in muscle. The serum certain concentration is based on several factors including diet composition, muscle mass, and gender. Serum certain value also depends on the

ability of the kidney to excrete certain. An elevation in certain usually occurs simultaneously with an increase in blood urea nitrogen. Certain concentration is often used as a variable not only to asses impairment of kidney function but also as a clinical endpoint to detect the treatment-related toxic effect of compounds on the kidney in experimental animals.

Histopathological studies in liver

Histopathological studies control rats (Group I) H and E section show structure of the liver, 1/2 liver showing normal hepatocytes with uniform sinusoidal space (Fig. A). The paracetamol-induced rats (Group II) H and E section show structure of the liver, nearby 1/2 Liver showing severe disruption of hepatic cords, massive necrosis of hepatocytes, and wide distension of sinusoidal space around the central vein (Fig B). The paracetamol + *Mimosa pudica* Extract sample induced rats (Group III) H and E section shows the structure of the liver, 1/2 Liver showing moderate vacuolar degeneration and mild congestion of central vein suggesting the moderate efficacy of the extract (fig C). The *Mimosa pudica* extract sample induced rats (Group IV) H and E sections show the structure of liver, ½ liver showing the normal pattern of hepatic cords with mild vacuolar degenerative changes (Fig D).



A. Control rats: Liver showing normal hepatocytes with uniform sinusoidal
B. Paracetamol Induced rats: The liver showing severe disruption of hepatic cords, massive necrosis of hepatocytes, and wide distension of sinusoidal space around the central vein.
C. Paracetamol+ *Mimosa pudica* leaves extract induced rats: Liver showing moderate vacuolar degeneration and mild congestion of central vein suggesting the moderate efficacy of the extract.
D. *Mimosa pudica* leaves extract induced rats: Liver showing a normal pattern of hepatic cords with mild vacuolar degenerative changes.

Conclusion

The extract prepared from the *Mimosa pudica* leaves sample was subjected primarily to Phytochemical screening used to a different reagent to identify the Alkaloids, Flavanoids, carbohydrates, and steroids. Paracetamol (acetaminophen), a widely used antipyretic and analgesic drug produces acute liver damage in accidental overdoses are consumed. The covalent bind ring of the n-acetyl-p-benzoquinone imines, an oxidation product of paracetamol to sulphydryl groups of protein resulting jaundice and hepatotoxicity induced by a decrease in the liver as the cause of hepatotoxicity in jaundice. Lipid peroxidation has been postulated as being the destructive process in liver injury due to paracetamol administration. Elevation in the levels of superoxide radical in hepatic jaundice of treated with paracetamol was observed. The increased superoxide radical levels in the liver suggest enhanced lipid peroxidation leading to tissue damage and failure of antioxidant defense mechanisms to prevent the formation of excessive free radicals. Treatment with *Mimosa pudica* significantly reversed these changes. So result in the leaves extract of *Mimosa pudica* to protective activity of hepatocellular and damage the reduced side effect of the plant was found.

In the present study, oral administration of ethyl acetate extracted of *Mimosa pudica* leaves significantly prevented the bilirubin in paracetamol painted albino wister rats, which indicates its potent hepatocellular damage in experimental hepatoprotective activity *Mimosa pudica* leaves not only prevented the hepatoprotective formation but also inhibited the abnormal synthesis of hepatocellular damage as evidenced by decreased levels of tissue damage in paracetamol + *Mimosa pudica* treated rates. The modulating effect of *Mimosa pudica* on cellular damage in hepatitis could probably be due to its inhibitory role in hepatocellular synthesis or on the activity of the hepatocellular. The present study thus demonstrates the protective efficacy of *Mimosa pudica* leaves on abnormal hepatocellular tissue expression during paracetamol induced oral hepatoprotective activity.

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