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Hepatoprotective Activity of Ethanolic Extract of *Mukia maderaspatana Linn. M. Roem* Against Paracetamol Induced Hepatotoxicity in Albino Rats

Thiruppathi M*, Natarajan P, Abdul Rahman Basith M, Achara K, Arunkumar L and Abinesh S

Department of Pharmacology, Sankaralingam Bhuvaneswari College of Pharmacy, Anaikuttam, Sivakasi – 626130, Tamilnadu, India.

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***Corresponding Author Email:** sbcpthiruppathi@gmail.com

Abstract

Introduction: *Mukia maderaspatana* (Family: Cucurbitaceae) is used to treat malarial fever and liver disorders. This study aims to investigate possible hepatoprotective activities of ethanolic extract of *Mukia maderaspatana* against paracetamol-induced hepatotoxicity. **Methods:** Hepatotoxicity was induced in Wistar albino rats by oral administration, 2 g/kg body weight on 7th day after the administration of ethanolic extract of *Mukia maderaspatana* and silymarin (100 mg/kg). Ethanolic extract of *Mukia maderaspatana* was administered orally at doses of 200 mg/kg and 400 mg/kg body weight daily for 7 days. Several serum enzyme markers, aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), bilirubin and total protein was measured to assess the effect of the extract on paracetamol (acetaminophen)-induced hepatic damage. The study included histopathological examination of liver sections. **Results:** Blood samples from rats treated with ethanolic extract of *Mukia maderaspatana* (200 mg/kg body weight and 400 mg/kg body weight) had significant reductions in serum markers in paracetamol administered rats, signifying the effect of the plant extract in restoring the normal functional ability of hepatocytes. Silymarin (100 mg/kg, p.o.) was used as a standard drug. **Conclusion:** The ethanolic extract of *Mukia maderaspatana* exhibits protective effects against paracetamol-induced hepatotoxicity.

Keywords

Mukia maderaspatana, Paracetamol, Silymarin, Hepatoprotection, Histopathology.

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INTRODUCTION

Nowadays Herbal plants have been used throughout the world for the treatment of various diseases. Medicinal plants derived drugs are known to play a vital role in the management of liver diseases. In India, about 40 polyherbal formulations having hepatoprotective action are being used. It has been reported that 160 phytochemicals from 101 plants have hepatoprotective activity[1]. Hepatotoxicity is the most serious condition and is mainly caused by toxic agents like excess consumption of alcohol, high doses of paracetamol, carbon tetrachloride, antitubercular drugs, anesthetic agents, and some chemotherapeutic agents etc. *Mukia maderaspatana* (Family: Cucurbitaceae) contains numerous phytoconstituents such as Flavonoids, Alkaloids, Carbohydrates, Proteins, Tannins, Coumarines, Polyphenols, vitamin C, E and carotenoids. Mostly Flavonoids reveal antihypertensive, vasodilatory, antihyperglycemic,

antihyperlipidemic, hepatoprotective, immunomodulatory, anti-inflammatory, antirheumatic, antiulcer, anxiolytic, antimicrobial, and antiplatelet aggregation properties[2]. Acetaminophen, a widely used analgesic and antipyretic drug commonly used for pain and fever relief. It is commonly considered a "safe drug" when taking within the suggested therapeutic dose. But in higher doses, it causes hepatotoxicity in humans and experimental models[3]. Herbal formulations (Liv 52, Livergen, Livokin, Octogen, and Silymarin) and have been effectively produced pharmacological, biochemical and histopathological parameters against hepatotoxicity in a rat model induced by paracetamol (PCM). Although of tremendous progress in modern medicine, there are no effective drugs available that offer protection to the liver from damage[4].Our literature review exposed that no attempt has been made to this date to study the hepatoprotective activity of *Mukia maderaspatana* leaves. Thus, we take this opportunity to study the hepatoprotective activity of ethanol extract of *Mukia maderaspatana* leaves using the paracetamol- (PCM-) induced hepatotoxicity in rats as the animal model.

MATERIALS AND METHODS

PLANT COLLECTION AND AUTHENTICATION

The leaves of the plant *Mukia maderaspatna* were collected from the local area at Sivakasi, Virudhunagar during December 2019. The species for the proposed study was positively identified and authenticated by Dr N.Senthilkumar, Associate Professor and Head, Dept. of Botany, Ayyanadar Janakiammal College of Arts and Science, Sivakasi, Virudhunagar Dist.

DRUGS AND CHEMICALS

Paracetamol was procured from XYKAA 650, TROKIAA PHARMACEUTICALS LTD., AHEMADABAD. The standard drug Silymarin (SILYBON 140MG TAB) was procured from the local medical shop. All reagents procured were analytical grade.

PREPARATION OF ETHANOLIC EXTRACT

About 400gm of *Mukia maderaspatna* Linn. air-dried powdered material was taken in 1000ml Soxhlet apparatus and extracted with 95% ethanol for 72hrs. At the end of 3rd day, the powder was taken out and it was dried. After drying it was again packed and extracted by using ethanol as solvent, till the colour disappeared. The temperature was maintained at 55ºC-65ºC. After that, the plant extract was concentrated by distillation process and solvent was recovered. The extract was stored in a desiccator. A reddish-brown residue was obtained. The percentage yield was calculated[5].

PRELIMINARY PHYTOCHEMICAL ANALYSIS

The ethanolic extracts of *Mukia maderaspatana* obtained were subjected to qualitative analysis to test the presence of various phytochemical constituents like alkaloids, carbohydrates, glycosides, flavonoids, steroids, aminoacid, phenols, proteins, tannins etc [6,7,8].

SELECTION OF ANIMALS

Healthy Albino Wister strain rats of 150-200gm of both sexes were used for this study. The rats were maintained in a controlled room temperature (25± 2°c) on 12 hr light-dark cycle (lights on 7.00 am) with free access to sterile food and water *ad libitum*.[37] The rats were placed in polypropylene cages with three animals per cage and were allowed to acclimatize one week before treatments. The study was approved by the Institutional Animal Ethical Committee (IAEC), which follows the guidelines of Committee for the Purpose of Control and Supervision of Experimental Animals (CPCSEA). The studies were conducted by the ethical committee Ref No: SBCP/2020-21/CPCSEA/IAEC/I(1)/F16/159.

ACUTE TOXICITY STUDIES [9, 10]

An acute oral toxicity test was carried out as per the Organization for Economic Co-operation and Development (OECD-423) guidelines for testing of chemicals. Three randomly selected male rats were used for the acute toxicity study of extract. For a sighting study, a single rat was fasted overnight and administered a starting dose of 2000 mg/kg body weight/p.o. Then, the rats also fasted for 4 h with no access to food after extract administration. Immediately after dosing, the animals were observed continuously for the first 4h with 30 min intervals and until 24 h for any behavioral changes and signs of toxicity. Since no death was observed within 24 h, additional two animals were added for the extract and administered the same dose 2000mg/kg body weight/po. The animals were observed continuously for 4 h with 30 min intervals and then for 14 consecutive days with an interval of 24 hrs.

HEPATOPROTECTIVE ACTIVITY

The rats were divided into five groups of four animals each. Group I received normal saline (5 mL/kg body weight) as a control, daily for 7 days. Group II received the hepatotoxic group and were treated paracetamol 2g/kg. Group IV and Group V received ethanolic extract of *Mukia maderaspatana* (200 and 400 mg/kg body weight per day, respectively) for 7

days. Group III received silymarin, standard drug (100 mg/kg body weight daily) for 7 days. On the 7th day, paracetamol suspension (2 g/kg body weight) was given orally to all the rats except Group I.[11] At the end of the experimental study, Blood samples were collected by retro-orbital plexus route. Serum was separated by centrifuging at 2500 rpm for 15 min and used for the analysis of various biochemical parameters, including AST, alanine aminotransferase (ALT), Alanine phosphate (ALP)[12] & total bilirubin[13]. All rats were sacrificed by using ketamine injection. Liver samples were collected for histopathological studies.

HISTOPATHOLOGY STUDY OF THE LIVER [14]

A portion of liver tissue in each group was fixed in 10% formalin and proceeded for histopathological studies for evaluation of hepatocytes cells, sinusoidal congestion, and mononuclear inflammatory cells of different groups.

STATISTICAL ANALYSIS:

Results were expressed as Mean ± SEM. The total variation was analyzed by one-way analysis of variance (ANOVA). Differences among mean values were analyzed by Dunnet's test with a P value < 0.05 significant.

RESULTS

PHYTOCHEMICAL SCREENING

The Percentage yield of ethanolic extract of *Mukia maderaspatna* Linn was found to be 8.53% and Ethanolic extract shows Flavonoids, Alkaloids, Carbohydrates, Proteins, Tannins, Coumarines, Polyphenols, vitamin C, E and carotenoids. The results were shown in table.1

Table 1: Preliminary phytochemical screening of ethanolic extract of *Mukia maderaspatana*

Plant Constituents	Ethanolic extract of *Mukia maderaspatana*
Steroids	**-**
Flavonoids	**+**
Alkaloids	**+**
Carbohydrate	**+**
Protein	**+**
Tannins	**+**
Glycosides	**-**
Saponins	**-**
Triterpenes	**-**
Fixed oil	**-**
Coumarines	**+**

ACUTE TOXICITY TEST

Table 2 reports the ethanolic extract of *Mukia maderaspatana* did not result in any mortality of rat up to the dose of 2000 mg/kg body weight.

Table 2: Acute toxicity studies in rat treated with *Mukia maderaspatana*

Observation	Effect					
Gross behavior activity	**1hr**	**4 hrs**	**24 hrs**	**48 hrs**	**7 days**	**14days**
Sense of touch and sound	N	N	N	N	N	N
Writhing	-	-	-	-	-	-
Tremor	-	-	-	-	-	-
Convulsions	-	-	-	-	-	-
Hind limb paralysis	-	-	-	-	-	-
Respiration	N	N	N	N	N	N
Salivation	N	N	N	N	N	N
Urination	N	N	N	N	N	N
Diarrhoea	-	-	-	-	-	-
Mortality	-	-	-	-	-	-

(-) No Effect (N) Normal effect

HEPATOPROTECTIVE ACTIVITY

Table 3: Serum biochemical parameters of *Mukia maderspatana* on paracetamol induced hepatotoxicity in rats

S.No	Group	SGPT (units/ml)	SGOT (Units/ml)	Alkaline Phosphates (U/L)	Bilirubin (mg/dl)
1	Control	65.21±1.41	71.05±1.32	15.41±0.14	1.21±0.27
2	Standard sillymarin (100mg/kg p.o.)	70.42±1.47*	79.11±2.02*	16.87±0.11*	1.29±0.19*
3	Paracetamol 2g/kg p.o.	128.21±2.37*	136.12±2.43*	56.21±0.20*	2.57±0.11*
4	Ethanolic extract 200mg/kg p.o.	98.12±0.78*	92.21±0.31*	41.26±0.31*	1.68±0.17*
5	Ethanolic extract 400mg/kg p.o.	74.21±0.12*	84.10±0.01*	21.03±0.03*	1.32±0.11*

Values are expressed as mean ± Standard Error Mean. **Data differed significantly at p<0.005 when compared with the normal control group in the relevant column. *Data differed significantly at p<0.005 when compared with the Paracetamol with the relevant (negative control) group in the relevant column.

HISTOPATHOLOGICAL STUDIES

Histopathological studies of rat liver tissue portion from the control group (Group I) showed normal hepatic cells with central vein and sinusoidal dilation (Figure 1).

In the paracetamol group (Group II), severe hepatotoxicity was observed in the form of severe necrosis and disappearance of nuclei (Figure 2).

Liver tissue from paracetamol + silymarin group (Group III) had normal hepatic cells with portal vein and portal artery (Figure 3).

Normal hepatocytes with regenerating hepatocytes and mild inflammation in the portal area were observed in groups IV and V, treated with ethanolic extract of *Mukia maderaspatana*, 200 and 400 mg/kg body weight, respectively (Figures 4 and 5).

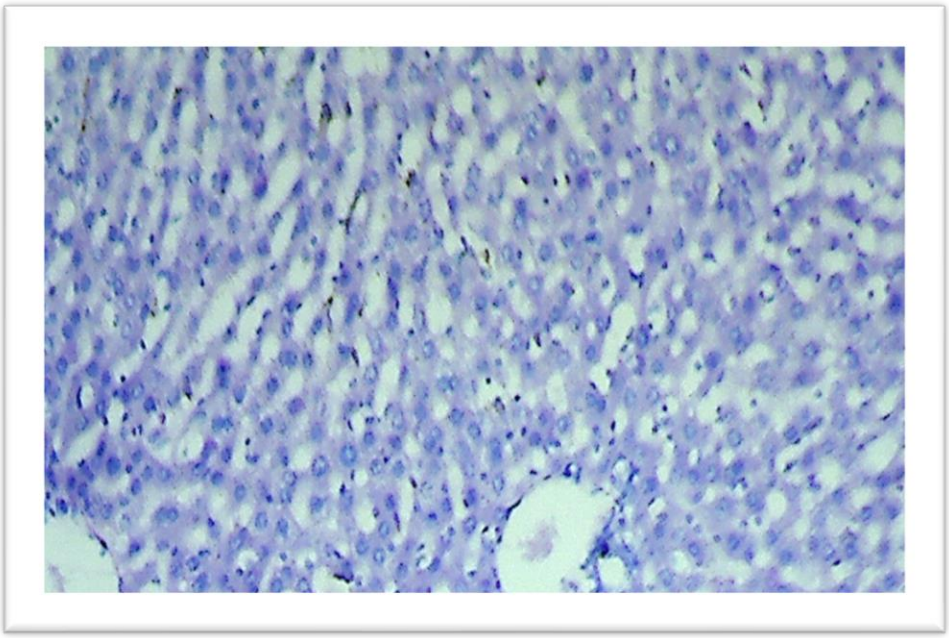

Figure 1: Control group showing moderate sinusoidal and central vein dilatation and congestion with prominent nucleus.

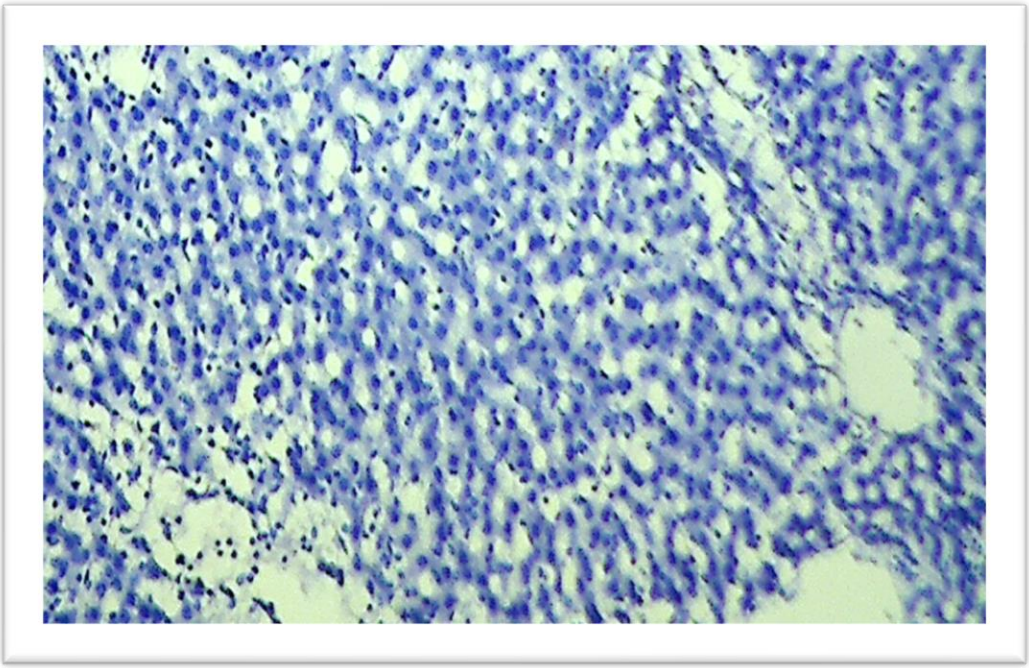

Figure 2: Liver section of paracetamol (2 g/kg) treated rats showing hydropic degeneration and focal necrosis.

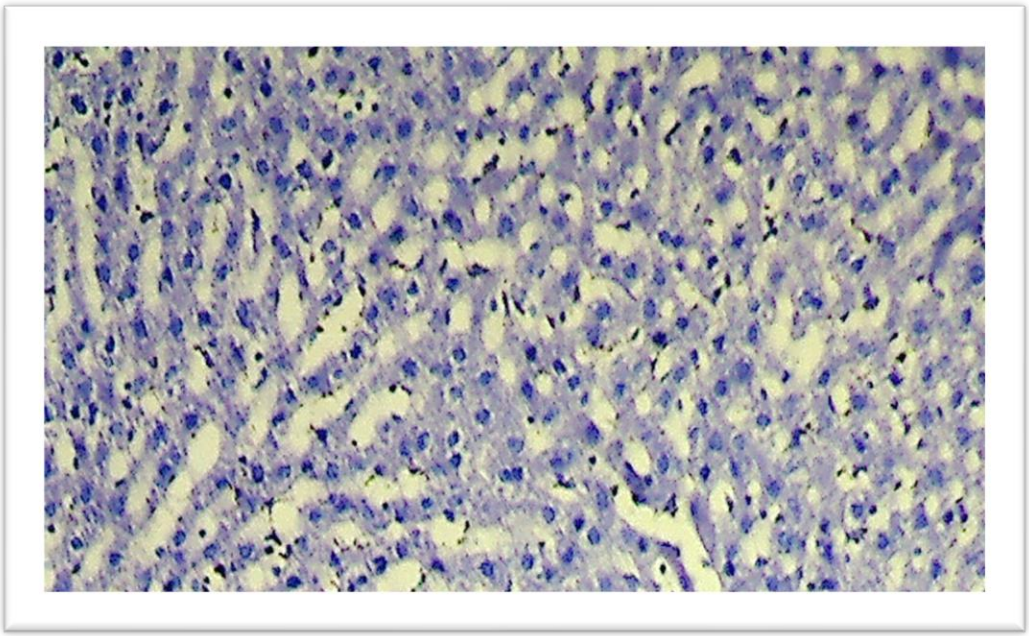

Figure 3: Liver section of rats treated with paracetamol (2 g/kg, p.o) + silymarin (100 mg/kg, p.o) × 14 days (Group III) showing normal histological appearance IV) showing with no signs of necrosis.

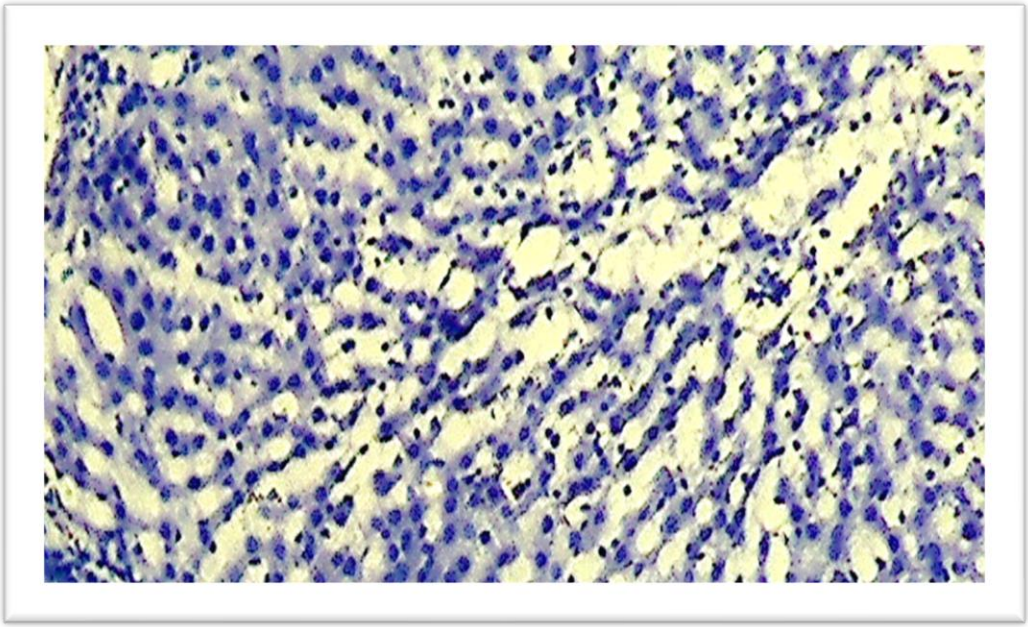

Figure 4: Liver section of rats treated with paracetamol (2 g/kg, p.o) + ethanolic extract (200 mg/kg, p.o) × 14 days (Group IV) hydrophobic lesions with congestion and mild signs of necrosis.

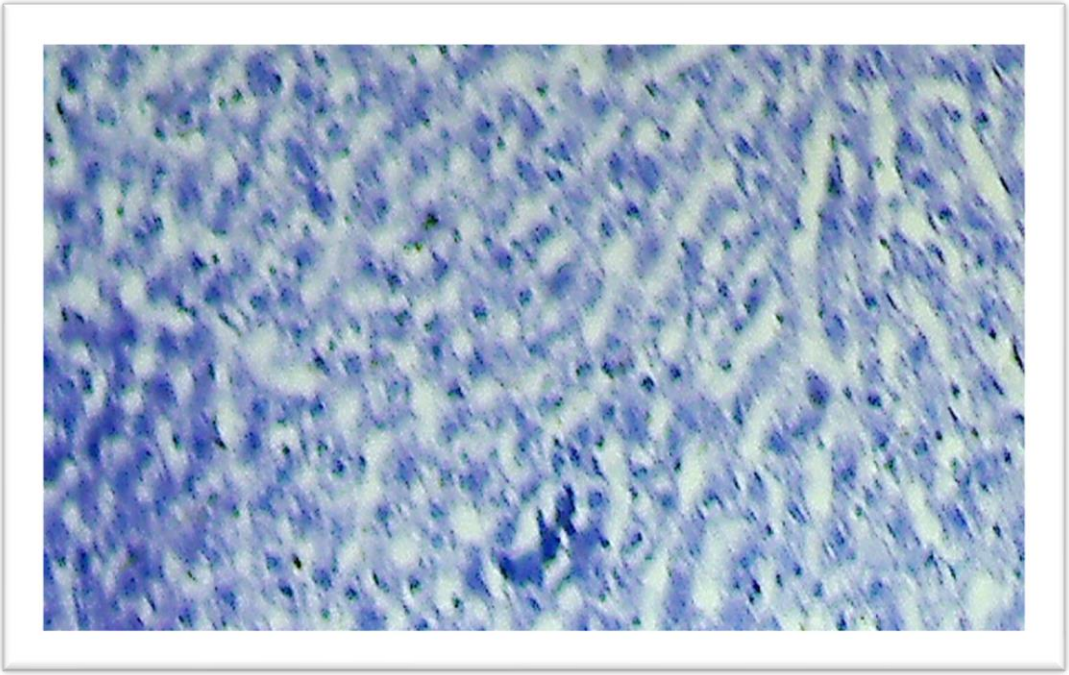

Figure 5: Liver section of paracetamol (2 g/kg, p.o) + ethanolic extract (400 mg/kg, p.o) × 14 days (Group V) showing mild congestion with no signs of necrosis.

DISCUSSION

The present study explored the ability of ethanolic extracts of *Mukia maderaspatana* to offer protection against hepatotoxicity induced by paracetamol as an over the counter drug. Liver is a main metabolic organ affected by several chemicals, enzymes, toxins and liver injuries induced by various toxins have been recognized as a major toxicological problem for years[15]. In the absence of dependable liver protective drugs in modern medical sciences, herbal plants play an important role in the controlling of various liver disorders. A various plants show hepatoprotective activity[16]. *Mukia maderaspatana* is used extensively in the Indian traditional system of

medicine as a hepatoprotective and hepatostimulative agent. The ethanolic extract of *Mukia maderaspatana* inhibited paracetamol induced liver toxicity in Wister albino rats [17].

Earlier research showed that *Mukia maderaspatana* at a dose 2000 mg/kg is safe for animals[18]. Evaluation of Histopathological studies of rats administered by paracetamol induced showed severe necrosis and disappearance of nuclei. This can be due to the formation of reactive metabolites (e.g. NAPQI), because of high dose administration of paracetamol. All these histopathological changes were suggestively reduced in rat liver treated with ethanolic extract of *Mukia maderaspatana*. The study of biochemical parameters such as SGOT, SGPT, ALP, and bilirubin, and total protein has been found to be of great value of evaluate to clinical and experimental liver damage [19]. In the present investigation, the rats suffered substantial hepatic injury from treatment with paracetamol, as indicated by high levels of serum markers. An increase in SGOT is usually accompanied by an increase in ALT, which plays a important role in the conversion of amino acids to ketoacids[20]. Prior treatment with ethanolic extract of *Mukia maderaspatana* at 200 mg/kg body weight and 400 mg/kg body weight, significantly decreased elevated levels of serum markers. This proves that ethanolic extract of *Mukia maderaspatana* so as to protect the integrity of the hepatocytes membrane from paracetamol-induced serum enzyme markers into blood circulation. These changes can be considered a efficient improvement of hepatocytes and may be caused by augmented restoration of parenchyma cells. Serum ALP and bilirubin are associated to hepatic cell damage [21]. Elevation in serum ALP is due to increased synthesis in the presence of increasing biliary pressure [22]. The decrease in the levels of both ALP and bilirubin could be due to the presence of flavonoids and its have antioxidant effects which may protect the hepatotoxicity induced by paracetamol.

CONCLUSION

The present study analyzed the hepatoprotective potential of ethanolic extracts of *Mukia maderaspatana*. The results indicated the potential of these plant extracts to offer protection against the acute hepatotoxicity induced by paracetamol. In support of this study, histopathological results also show significant activity of the plant extract. Liver section of plant *Mukia maderaspatana* of ethanolic extract 400mg/kg p.o. treated groups clearly showed normal hepatic cells and central vein when compared with 200mg/kg p.o. thereby conforming the hepatoprotective effect. But in the ethanolic extract of *Mukia maderaspatana* shows regeneration of hepatocytes was observed, which confirms the hepatoprotective activity and supports the traditional application of the same under light of modern science.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

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