



IJPPR

INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals

ISSN 2349-7203




Human Journals

Research Article

August 2021 Vol.:22, Issue:1


© All rights are reserved by P.Amudha et al.

Evaluation of Hepatoprotective and Antioxidant Activity Using Hydroalcoholic Leaf Extract of *Mukia maderaspatana* (Linn.) M. Roem Against Paracetamol Induced Liver Damage in Wistar Albino Rats



IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals

ISSN 2349-7203



P.Amudha^{1*}, P.Subathiradevi², S.S Pragathishri³

¹ Professor, Department of Pharmacology, C.L.Baid Metha College of Pharmacy, Rajiv Gandhi Salai, Old Mahabalipuram Road, Jyothinagar, Thorappaikam, Chennai -600 097, Tamil Nadu, India.

² Department of Pharmacology, C.L.Baid Metha College of Pharmacy, Rajiv Gandhi Salai, Old Mahabalipuram Road, Jyothinagar, Thorappaikam, Chennai -600 097, Tamil Nadu, India.

³ Department of Pharmacology, C.L.Baid Metha College of Pharmacy, Rajiv Gandhi Salai, Old Mahabalipuram Road, Jyothinagar, Thorappaikam, Chennai -600 097, Tamil Nadu, India.

Submitted: 23 July 2021
Accepted: 29 July 2021
Published: 30 August 2021

Keywords: Hepatoprotective, antioxidant, *Mukai maderaspatana* (L), *Mukai Roeam*, Paracetamol induced liver damage, Biochemical parameters

ABSTRACT

Hepatoprotection or antihepatotoxicity is the ability of a chemical substance to prevent damage to the liver. Antioxidants are substances that can prevent or slow damage to cells caused by free radicals, unstable molecules that the body produces as a reaction to environmental and other pressure. The purpose of the present study is to investigate the phytoconstituents and hepatoprotective and antioxidant activities of the Hydroalcoholic Extract of *Mukia maderaspatana* (Linn.) M. Roem (HEMM) leaves on Wistar albino rats. Preliminary phytochemical analysis of HEMM showed the presence or absence of Alkaloids, Carbohydrates, Flavonoids, Saponins, Tannins, Proteins, Steroids, Phenols, Gums and mucilage, Glycosides, Terpenes, and sterols. HEMM at the doses of 200mg/kg body weight and 400mg/kg body weight orally was administered to evaluate hepatoprotective and antioxidant activity against paracetamol-induced liver damage for 21 days in Wistar albino rats. Treatment with HEMM leaves showed hepatoprotective activity by significantly decreasing SGOT, SGPT, ACP, TB and showed an increase in TP. HEMM leaves showed antioxidant activity by significantly decreasing SOD, CAT and showed an increase in LPO when compared to silymarin.



HUMAN JOURNALS

www.ijppr.humanjournals.com

INTRODUCTION:

All living being including man has been troubling by many ailments from past so many centuries and most often nature has provided the cure. Plants, one of the important members of nature has played a great role for years in combating the disease of the human race ^[1]. Throughout the ages, humans have relied on nature for their basic needs, for the production of food, shelter, clothing, transportation, fertilizers, flavors and fragrances, and medicines ^[2]. Natural products originated from plants, animals, metals, and minerals serving as the basis for the treatment of human disease. Medicinal plants based on the traditional system of medicine have been playing an incredible role in providing diagnosis and treatment of human beings, especially in developing countries. The utilization of herbal drugs has also increased in developed countries ^[3]. The Greek word for liver is hepar, so medicinal terms related to the liver often start with 'hepato' or 'hepatic'. The liver plays a pivotal role in metabolism, secretion, and storage and is sometimes referred to as the "Great chemical factory" of the body, because the body depends on the liver to regulate, synthesize, store and secrete many important proteins, nutrients, chemicals and to purify and clear toxins or unnecessary substances from the body ^[4]. The liver alters exogenous and endogenous chemicals (e.g. drugs), foreign molecules, and hormones to make them less toxic or less biologically active. This process, called metabolic detoxification, diminishes intestinal or renal tubular reabsorption of potentially toxic substances and facilitates their intestinal and renal excretion ^[5]. Hepatotoxin is a toxic chemical substance that damages the liver. The hepatoprotective effect was studied against chemicals and drugs that induced hepatotoxicity in rats like alcohol, carbon tetrachloride, galactosamine, paracetamol, isoniazid, and rifampicin, antibiotics, peroxidized oil, aflatoxin, etc. The severity of hepatotoxicity is greatly increased if the drug is continued after symptoms develop. Arsenic, phosphorus, copper, and iron are among the various inorganic compounds producing hepatotoxicity. The organic agents include certain naturally occurring plant toxins such as pyrrolizidine alkaloids, mycotoxins, and bacterial toxins ^[6]. The antioxidant is any substance that delays prevents or removes oxidative damage to a target molecule ^[7]. A scabrous scandent or prostrate much-branched climbing annual with very hispid angular stem and simple tendrils; leaves simple, alternate ovate or sub deltoid, entire or 3-5 lobed, minutely denticulate, very scabrid above, cordate at the base with a wide sinus; flowers small, yellow, male in small fascicles on very short peduncles, females almost sessile solitary or sub fasciculate; fruits globose, brownish yellow, finally turning red, seeds ovoid-oblong, compressed ^[8]. The leaves of *Mukia maderaspatana*

contain several phytochemicals, including spinasterol, 22, 23-dihydrospinasterol, β -sitosterol, decosaenoic acid, triterpenes, phenolic compounds, and multiple glycosides (22, 23-dihydrospinasterol-3-O- β -d glucoside) [9].

MATERIALS AND METHODS

Plant materials:

Freshly collected leaf materials of *Mukia maderaspatana* (Linn.) *M. Roam* was thoroughly washed under running water to remove adherent impurities. The leaves were dried under shade drying at room temperature. The shade dried leaves were subjected to pulverization to get a coarse powder. Fines were collected by sieving. 160gms of the shade dry coarsely powdered leaves was placed in the soxhlet apparatus using 500ml of 1:1 ratio of water and ethanol. The obtained extract was concentrated under reduced pressure to obtain a green color semi-solid mass. The obtained extracts were weighed and stored at low temperatures (4 to 8°C) for future analysis. The percentage yield was calculated by using the following formula.

$$\text{Percentage yield (\%w/w)} = \frac{\text{Weight of extract obtained (g)}}{\text{Weight of plant material used}} \times 100$$

Preliminary Phytochemical Analysis:

HEMM was subjected to preliminary phytochemical screening for the presence or absence of Phytoconstituents like Alkaloids, Carbohydrates, Flavonoids, Saponins, Tannins, Proteins, Steroids, Phenols, Gums and mucilage, Glycosides, Terpenes, and Sterols [10].

Experimental animal studies:

Wistar albino rats of either sex weighing 150-200g were used for this study. The animals are divided into five groups. They were housed six per cage under standard laboratory conditions at a room temperature at $12 \pm 2^\circ\text{C}$ with 12hr light/dark cycle. The animals were provided with standard pellet chow ad. libitum. Animals acclimatized to laboratory conditions one week before the initiation of experiments. Ethical committee clearance was obtained from IAEC (Institutional Animal Ethics Committee) of CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals).

IAEC Reference no:

Reg No: 12/321/PORe/S/01 CPCSEA Date: 12.10.2018.

Acute toxicity study:

OECD guidelines 423 were followed to carry out acute toxicity study at the dose level of 2000mg/kg body weight, by oral route in three rats. Since most of the crude extracts possess an LD50 value of more than 2000mg/kg, p.o. So starting dose 2000mg/kg, p.o. was used. Dose-volume administered was 1ml/100gm bodyweight to each rat which were fasted overnight with water and ad. libitum. Food was withheld for a further 24 hours after administration of the drug and observed for the signs of toxicity. No toxicity or death was observed in the experimental rats when they were subjected to toxicity study [11].

Experimental Design:

Paracetamol Induced Liver Damage:

Group I: Control animal treated with distilled water orally.

Group II: Treated with Paracetamol (2g/kg, p.o) on 21st day

Group III: HEMM leaves (200mg/kg, p.o) + Paracetamol (2g/kg, p.o) on 21st day

Group IV: HEMM leaves (400mg/kg, p.o) + Paracetamol (2g/kg, p.o) on 21st day

Group V: Standard silymarin (50mg/kg, p.o) + Paracetamol (2g/kg, p.o) on 21st day

Procedure:

The animals were randomly divided into 5 groups of 6 animals in each and treated orally as below for 21 days. The first group of six animals was treated with distilled water and considered as the control for 21 days. The second group of six animals was treated with Paracetamol 2g/kg body weight p.o. suspended in 1% CMC only on the 21st day. The third group of six animals was treated with HEMM leaves 200mg/kg body weight p.o. suspended in 1% CMC for 21 days and treated with Paracetamol 2g/kg body weight p.o. suspended in 1% CMC only on the 21st day. The fourth group of six animals was treated with HEMM leaves 400 mg/kg body weight p.o. suspended in 1% CMC for 21 days and treated with Paracetamol 2g/kg body weight p.o. suspended in 1% CMC only on the 21st day. The fifth group of six animals was treated with standard Silymarin 50mg/kg body weight p.o. suspended in 1% CMC for 21 days and treated with Paracetamol 2g/kg body weight p.o. suspended in 1% CMC only on the 21st day. 24 hrs after the intoxication of paracetamol, all the animals were sacrificed, and samples were collected for various biochemical analyses [12].

Biochemical parameters:

All the 5 groups of animals were sacrificed on the 22nd day under light ether anesthesia. The blood samples were collected separately by cardiac puncture into sterilized dry centrifuge tubes and allowed to coagulate for 30mins at 37°C. The clear serum was separated at 3000rpm. For 10mins and biochemical investigations were carried out to assess liver function viz., SGPT [13], SGOT [13], ALP [14], Total bilirubin [15], Total protein [16] and antioxidant enzyme function viz., SOD [17], CAT [18], LPO [19].

RESULTS

Phytochemical investigations:

Hydroalcoholic leaf extract of *Mukia maderaspatana* (Linn.) M. Roem showed the presence of alkaloids, carbohydrates, flavonoids, saponins, tannins, proteins, steroids and showed the absence of phenols, gums, and mucilage, glycosides, terpenes, and sterols.

Acute oral toxicity study:

There was no considerable change in body weight before and after treatment and no sign of toxicity was observed. (Table 1)

Paracetamol Induced Liver Damage:

There was a significant increase in SGOT in groups II, III, IV, V ($P < 0.001$) when compared to group I. There was a significant decrease in SGOT in groups III, IV, V ($P < 0.001$) when compared to group II. There was a significant increase in SGOT in group III ($P < 0.001$), IV ($P < 0.05$) when compared to group V. There was a significant increase in SGPT in groups II, III, IV, V ($P < 0.001$) when compared to group I. There was a significant decrease in SGPT in group III, IV, V ($P < 0.001$) when compared to group II. There was a significant increase in SGPT in group III, IV ($P < 0.001$) when compared to group V. Results are shown in Table 2 and Figure 1.

There was a significant increase in ALP in groups II, III, IV, V ($P < 0.001$) when compared to group I. There was a significant decrease in ALP in groups III, IV, V ($P < 0.001$) when compared to group II. There was a significant increase in ALP in group III ($P < 0.001$), IV ($P < 0.05$) when compared to group V. There was a significant decrease in Total protein in groups II, III, IV, V ($P < 0.001$) when compared to group I. There was a significant increase in

Total protein in group III ($P<0.01$), IV, V ($P<0.001$) when compared to group II. There was a significant decrease in Total protein in group III ($P<0.001$), IV ($P<0.01$) when compared to group V. Results are shown in Table 3 and Figure 1. There was a significant increase in Total bilirubin in groups II, III ($P<0.001$), IV ($P<0.01$), V ($P<0.05$) when compared to group I. There was a significant decrease in Total bilirubin in groups III, IV, V ($P<0.001$) when compared to group II. There was a significant increase in Total bilirubin in groups III, IV (ns) when compared to group V. Results are shown in Table 3 and Figure 2.

There was a significant decrease in SOD in groups II, III, IV ($P<0.001$), V (ns) when compared to group I. There was a significant increase in SOD in groups III, IV, V ($P<0.001$) when compared to group II. There was a significant decrease in SOD in Group III, IV ($P<0.001$) when compared to group V. There was a significant decrease in CAT in groups II, III, IV, V ($P<0.001$) when compared to group I. There was a significant increase in CAT in group III, IV, V ($P<0.001$) when compared to group II. There was a significant decrease in CAT in group III ($P<0.001$), IV ($P<0.01$) when compared to group V. There was a significant increase in LPO in groups II, III, IV, V ($P<0.001$) when compared to group I. There was a significant decrease in LPO in group III, IV, V ($P<0.001$) when compared to group II. There was a significant increase in LPO in groups III, IV ($P<0.001$) when compared to group V. Results are shown in Table 4 and Figure 2.

Table 1: Acute oral toxicity studies of HEMM (OECD 423 guidelines)

Sl. No	Treatment Group	Dose	Weight of Animal in 'g' Before the test	Weight of Animal in 'g' After the test	Signs of toxicity	Onset of toxicity	Duration
1	HEMM	2g/kg	198	200	No	Nil	14 days
2	HEMM	2g/kg	200	200	No	Nil	14 days
3	HEMM	2g/kg	200	200	No	Nil	14 days

Table 2: Effect of HEMM leaves on SGOT and SGPT in Paracetamol Induced Liver Damage

Group	SGOT (IU/L)	SGPT (IU/L)
I	33.98± 0.887	28.6±0.8
II	121.04±1.400a***	121.12±1.453a***
III	80.72±3.049a***b***c***	71.54±2.454a***b***c***
IV	63.7±1.664a***b***c*	59.54±1.436a***b***c***
V	55.56±1.904a***b***	49.54±1.436a***b***

Table 3: Effect of HEMM leaves on ALP, Total protein and Total bilirubin in Paracetamol Induced Liver Damage

Group	ALP (IU/L)	TOTAL PROTEIN (IU/L)	TOTAL BILIRUBIN (mg/dl)
I	33.16±1.011	6.248±0.074	0.532±0.016
II	122±1.917a***	2.524±0.175a***	4.1±0.331a***
III	68.56±2.338a***b***c***	3.248±0.074a***b***c***	1.574±0.017a***b***c ^{ns}
IV	59.16±2.653a***b***c*	4.524±0.175a***b***c**	1.378±0.027a***b***c ^{ns}
V	49.16±2.653a***b***	5.248±0.074a***b***	1.232±0.016a*b***

Table 4: Effect of HEMM leaves on SOD, CAT, and LPO in Paracetamol Induced Liver Damage

Group	SOD (U/mg protein)	CAT (Moles Of H ₂ O ₂ Consumed/min)	LPO (nmol MDA/mg protein)
I	10.12±0.185	1.03±0.007	20.71±0.154
II	6.2±0.114a***	0.444±0.026a***b***	99.26±0.212a***b***
III	7.68±0.185a***b***c***	0.728±0.008a***b***c***	57.05±0.558a***b***c***
IV	8.38±0.128a*** b***c***	0.816±0.009a***b***c**	54.29±0.545a***b***c***
V	10.02±0.106a ^{ns} b***	0.912±0.019a***b***	39.02±0.185a***b***

The values are expressed as mean ± SEM of 6 animals;

Comparisons were made between:

- a. Group I vs Group II, III, IV, V is considered as “a”.
- b. Group II vs Group III, IV, V is considered as “b”.
- c. Group V vs Group III, IV is considered as “C”.

Statistical significance test for comparison was done by One way ANOVA followed by Dunnett’s test.

Symbols represent statistical significance $p^* < 0.05$, $p^{**} < 0.01$, $p^{***} < 0.001$.

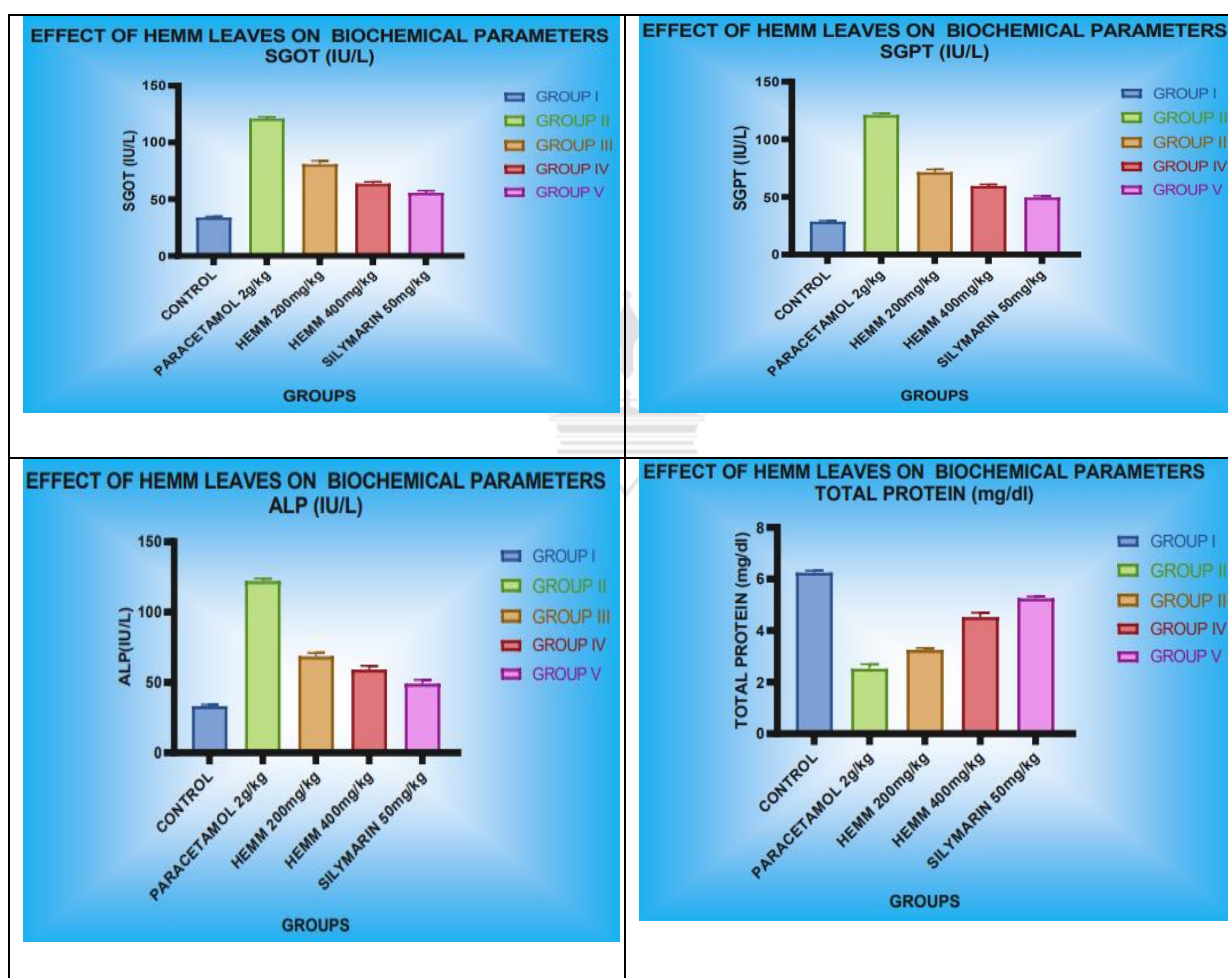


Figure 1: Effect of HEMM leaves on SGOT, SGPT, ALP, and Total protein in Paracetamol Induced Liver Damage

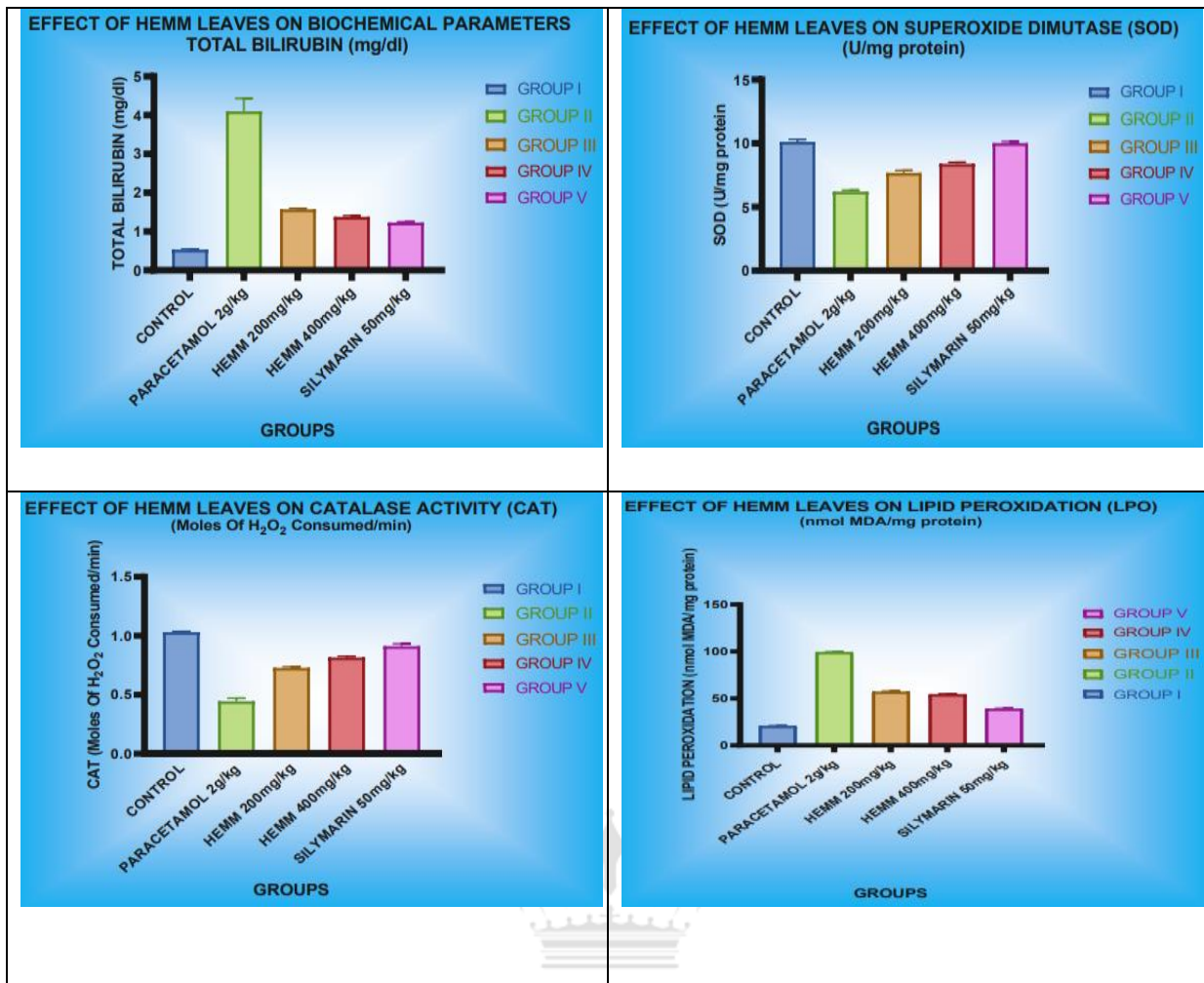


Figure 2: Effect of HEMM leaves on Total bilirubin, SOD, CAT, and LPO in Paracetamol Induced Liver Damage

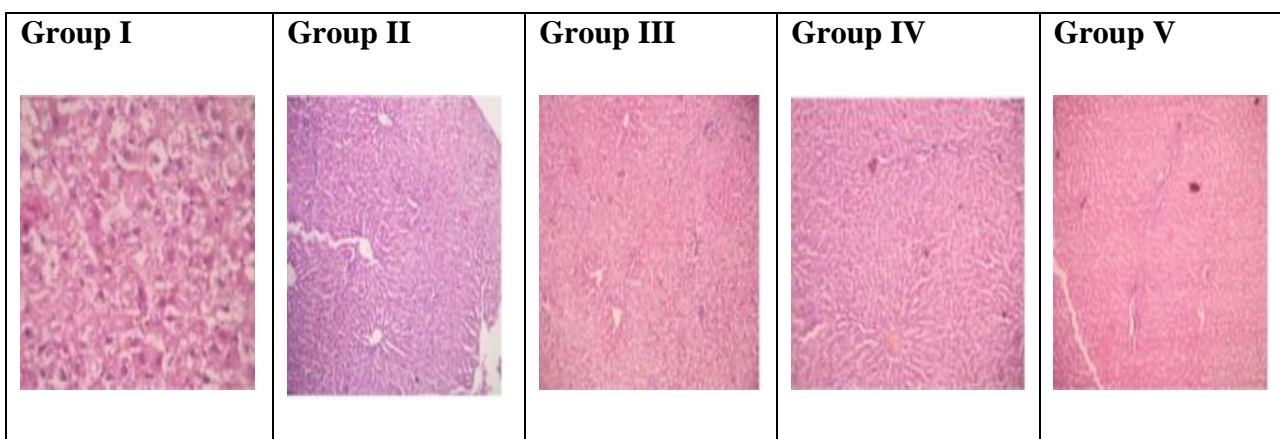


Figure 3: Histopathological slides of different groups are shown below (Paracetamol Induced Liver Damage)

DISCUSSION AND CONCLUSION

Liver disorders are the ninth biggest killer in India and rank 27th in the list of countries affected by various liver disorders. More than 900 drugs, toxins, and herbs have been reported to cause liver injury. Approximately 75% of the idiosyncratic drug reactions result in liver transplantation or death. In the United States, approximately 2000 cases of acute liver failure occur annually and drugs account for over 50% of them (37% are due to acetaminophen, 13% are idiosyncratic reactions due to other medications). Drug-induced hepatotoxicity is a major cause of liver damage. Rifampicin, Isoniazid, Pyrazinamide, Acetaminophen, Nimesulide, Diclofenac, Ibuprofen, Sulinac, Ritonavir, Indinavir, Atorvastatin, Phenytoin, etc; are the common drugs that cause hepatotoxicity [20].

Mukia maderaspatana (L.) M.Roem. of the family, Cucurbitaceae is an important traditional medicinal plant generally practiced in western districts of Tamil Nadu. The plant is bitter, sweet, refrigerant, carminative, vulnerable, expectorant, and tonic and it is useful in vitiated conditions of pitta, burning sensation, dyspepsia, flatulence, colic, constipation, ulcers, cough, asthma, and vertigo. Further, it is reported to have the properties viz., Anti-rheumatic, Anti-flatulent, Anti-inflammatory, Anticancer, Anti-diabetic, Diuretic, and Stomachic also. The squeezed plant is applied to treat scabies of animals [21].

Phytochemical screening of HEMM leaves showed the presence of Flavonoids as one of the constituents in this study. Several scientific studies indicated that flavonoids have a protective effect on the liver. Flavonoids are the lead for our present hepatoprotective studies. Acute oral toxicity study of HEMM leaves did not exhibit mortality or any profound toxic reactions at a dose of 2000mg/kg p.o.

Acetaminophen is a well-known antipyretic and analgesic drug, which is safe in therapeutic doses but can produce fatal hepatic necrosis in man, rats, and mice with toxic doses. It is employed as an experimental hepatotoxic agent. Acetaminophen is metabolized in the liver via three pathways. Acetaminophen is converted to a toxic metabolite. The toxicity occurs because of its reactive metabolite, N-acetyl-P-benzoquinone imine (NAPQI). NAPQI exerts its toxicity primarily via its oxidative effect on cellular proteins.

Sulfhydryl compounds are among the most important endogenous antioxidants. Glutathione (GSH) is the main intracellular nonprotein sulfhydryl and it plays an important role in the maintenance of cellular proteins and lipids in their functional states. NAPQI binds to GSH,

forming a conjugate which results in the conversion of GSH to an oxidized form of glutathione. When GSH is lowered, the toxic effects of oxidative insult are exacerbated, resulting in increased membrane and cell damage.

When liver cell plasma is damaged, a variety of enzymes located normally in the cytosol is released into the blood, thereby causing increased SGOT, SGPT, ALP, TB enzyme levels in the serum whereas TP level in the serum was markedly decreased.

Acetaminophen-induced liver damage was inhibited significantly by which confirms the protective action of the HEMM leaves against experimentally induced liver damage in rats. SGOT, SGPT, ALP, TP, and TB were the most sensitive tests employed in the diagnosis of hepatic disease. The elevated levels of SGOT, SGPT, ALP, TB parameters were significantly reduced by the treatment of HEMM leaves.

SGOT, SGPT, ALP, TP, and TB are the biomarkers of hepatoprotective activity. After the treatment of paracetamol, there was an increase in SGOT, SGPT, ALP, and TB levels and a decrease in TP level. After the treatment of HEMM leaves there was a decrease in SGOT, SGPT, ALP, and TB level and an increase in TP level indicates that the HEMM leaves have Hepatoprotective activity.

The body has an effective defense mechanism to prevent and neutralize the free radical-induced damage. This is proficient by a set of endogenous antioxidant enzymes such as SOD, and catalase. The significantly reduced activities of SOD and CAT pointed out the hepatic damage in the rats administered with paracetamol drugs, but on treatment with HEMM leaves groups showed a significant increase in the level of these enzymes due to the ability of the administered compounds to scavenge reactive oxygen species.

The significantly increased activities of LPO pointed out the hepatic damage in the rats administered with paracetamol drugs, but on treatment with HEMM leaves groups showed a significant decrease in the level of these enzymes due to the ability of the administered compounds to scavenge reactive oxygen species.

The hepatoprotective effect of HEMM leaves was further confirmed by histopathological examination of the liver. The liver sections of the rats treated with HEMM leaves and intoxicated with acetaminophen (Group-4) and rats treated with Silymarin and intoxicated with acetaminophen (Group-5) showed less vacuole formation, reduced sinusoidal dilation,

and less disarrangement and degeneration of hepatocytes, indicating marked regenerative activity. The intensity of centrilobular necrosis was less.

It is suggested that HEMM leaves act by its stabilizing effect on the plasma membrane as was reported in the case of Silymarin.

CONCLUSION

In conclusion, the present study provided preliminary data for the first time that the leaf of *Mukia maderaspatana* (L.) M. Roem. possesses significant hepatoprotective activity by decreasing SGOT, SGPT, ALP, and TB level and increasing TP level and it also has antioxidant activity by decreasing SOD and CAT level and by increasing LPO level against Paracetamol induced liver damage in rats, which is comparable with that of Silymarin. Thus, it is proved that *Mukia maderaspatana* (Linn.) M. Roem has potent hepatoprotective activity and antioxidant activity. Further work is necessary to elucidate the mechanism of action of *Mukia maderaspatana* (Linn.) M. Roem.

ACKNOWLEDGEMENT

We are very thankful to the Department of Pharmacology, Principal, and Management of C.L. Baid Metha College of Pharmacy for providing the facilities to conduct the research.

REFERENCES

1. Balunas MJ, Kinghorn AD. Drug discovery from medicinal plants. Life sciences. 2005;78(5):431-41.
2. Cragg GM, Newman DJ. Plants as a source of anti-cancer agents. Journal of ethnopharmacology. 2005;100(1-2):72-9.
3. Balunas MJ, Kinghorn AD. Drug discovery from medicinal plants. Life sciences. 2005;78(5):431-41.
4. Yunusa U, Lawal UB, Idris A, Garba SN. Occupational health hazards among commercial motorcyclists in Ahmadu Bello University, Zaria. International Organization of Scientific Research Journal of Nursing and Health Science. 2014;3(17):4652.
5. Al-Howiriny T. Evaluation of hepatoprotective activity of broccoli _Brassica oleracea in Rats. Hungarian Medical Journal. 2008; 1;2(1):145-56.
6. Singh R, Kumar S, Rana AC, Sharma N. Different model of hepatotoxicity and related liver diseases: a review. Int Res JPharm. 2012;3:86-94.
7. Halliwell B, Gutteridge JM. The definition and measurement of antioxidants in biological systems. Free radical biology & medicine. 1995;18(1):125-6.
8. Singh MP, Panda H. Medicinal herbs with their formulations. Daya Books; 2005;45- 65.
9. Petrus AJ. *Mukia maderaspatana* (L.) M. Roemer-A Review of Its Global Distribution, Phytochemical Profile, and Antioxidant Capacity. Asian Journal of Chemistry. 2011;24(6).
10. Chand S, Manokaran S, Saravanan S. Anxiolytic Activity of Hydro-Alcoholic Extract of *Mukia Maderaspatana* Linn: Leaves on Experimental Animals. Jordan Journal of Pharmaceutical Sciences. 2012;108(405):1-6.
11. OECD Guidelines for the testing of chemicals. Test no.423:A.

12. Gomathy G, Venkatesan D, Palani S. Gastroprotective potentials of the ethanolic extract of *Mukiamaderaspatana* against indomethacin-induced gastric ulcer in rats. *Natural product research*. 2015;29(22):2107-11.
13. Reitman S, Frankel S. Determination of serum glutamate oxaloacetate and glutamic pyruvic acid transaminases. *American Journal of Clinical Pathology*. 1957; 28, 56–66.
14. Marsh WH, Fingerhut B, Kirsch E. Adaptation of an alkaline phosphatase method for automatic colorimetric analysis. *Clinical chemistry*. 1959;5(2):119-26.
15. Perry B, Doumas BT, Buffone G, Glick M, Ou CN, Ryder K. Measurement of total bilirubin by use of bilirubin oxidase. *Clinical chemistry*. 1986;32(2):329-32.
16. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. *Journal of biological chemistry* 1951;193:265-75
17. Kakkar P, Das B, Viswanathan PN. A modified spectrophotometric assay of superoxide dismutase. 1984;76-86.
18. Sinha P, Zoltners AA. The multiple-choice knapsack problem. *Operations Research*. 1979;27(3):503-15.
19. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by the thiobarbituric acid reaction. *Analytical biochemistry*. 1979;95(2):351-8.
20. Yunusa U, Lawal UB, Idris A, Garba SN. Occupational health hazards among commercial motorcyclists in Ahmadu Bello University, Zaria. *International Organization of Scientific Research Journal of Nursing and Health Science*. 2014;3(17):4652.
21. Singh MP, Panda H. *Medicinal herbs with their formulations*. Daya Books; 2005;45- 65.

