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Research Article

# HEPATOTHERAPEUTIC EFFECTS OF *MIKANIA SCANDENS* (L.) WILLD. ON PARACETAMOL INDUCED HEPATOTOXICITY IN RATS

**TARASANKAR MAITY[a]*, AYAZ AHMAD[b], NILANJAN PAHARI[a]**

[a] Department of Pharmacy, Calcutta Institute of Pharmaceutical Technology and AHS. Uluberia, Howrah-711316, West Bengal, [b] Department of Pharmaceutical science, NIMS University, Shobha Nagar, Delhi Highway, Jaipur 303121, Rajasthan, India.
Email: ts_maity@indiatimes.com

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**ABSTRACT**

The present study is aimed to evaluating the hepatoprotective effect of *Mikania scandens* (L.) Willd.(MS) by paracetamol(PCM) induced liver damage in rats. Hepatoprotective action of MS extract and its fractions were evaluated using animal model of hepatotoxicity induced by Paracetamol. Liver marker enzymes were assayed in serum and antioxidant status was assessed in liver tissue. Hepatotoxicity in rats was achieved by intraperitoneal dose of 2 gm/kg of paracetamol for 14 days. The extract and the fractions of MS (500mg/kg) were administered orally to the animals with hepatotoxicity induced by paracetamol (2gm/kg). Silymarin (100mg/kg) was given as reference standard and studied by comparing parameters such as aspartate aminotrasferase(AST), alanine aminotrasferase(ALT), alkaline phosphatase (ALP),total bilirubin (TB), total protein (TP), triglycerides, cholesterol and the hepatic oxidative stress parameters like as levels of, malondialdehyde(MDA), reduced glutathione (GSH), catalase(CAT),super oxide dismutase (SOD) in rats. The results showed that plant extract and its fractions were effective in protecting the liver against the injury induced by paracetamol in rats. Histopathological studies also confirmed the hepatoprotective activity of these extracts when compared with Paracetamol treated groups. It can be concluded from the result that the plant *Mikania scandens* (L.)Willd possesses hepatoprotective activity against paracetamol induced hepatotoxicity in rats.

**Keywords**: Hepatotoxicity, Hepatoprotective, *Mikania scandens* (L.) Willd, Histopathological study, Paracetamol.

## INTRODUCTION

Liver is the key organ, which plays a vital role in regulating various physiological processes in the body. It is involved in various vital functions, such as metabolism, secretion, storage supply of nutrients and energy. It has great capacity to detoxication and deposition of endogeneous substances.[1,2] The liver is expected not only to perform physiological functions but also to protect against the hazards associated with harmful drugs and chemicals[3-5]. It is widely exposed to xenobiotics, hepatotoxins, and chemotherapeutic agents that lead to impairment of its functions. Most common causes of liver diseases are viral infections, [6,7] drugs,[8,9] toxic chemicals, excess consumption of alcohol and autoimmune disorders. Most of the hepatotoxic chemicals damage liver cells due to lipid peroxidation as well as other oxidativedamages.[10,11] Liver diseases are regarded as one of the serious health disorders. Steroids, vaccines and antiviral drugs that are recommended as therapy for liver diseases. Those therapies have many adverse effects especially when administered for long periods of time. There is a worldwide trend for use of traditional system herbal medicines for the treatment of several liver diseases. Natural remedies from plant sources have been found as potential hepatoprotective agents with diverse chemical structures. *Mikania scandens* (L.) Willd. is an apical twiner that climbs dextrally (from left to right). Dextral climbing is unusual in this genus. Leaves simple, acuminate, coarsely dentate or shallowly, unevenly lobed (sinuate), 1.5-8 cm wide and 2.5-14 cm long with a deeply cordate base. Leaves bear 3-7 strong basal veins, giving them a palmate venation and are arranged oppositely at swollen nodes along the slightly four-angled, glabrous stem. Petioles are slender and shorter than the leaf blades, 1 to 10 cm long. The plant *Mikania scandens* (L.) Willd. has been reported Analgesic and Antioxidant activity. This plant contains various chemical constituents like alkaloids, flavonoids, saponins, proteins, tannins, carbohydrates etc[12]. A review of literatures has no information on the hepatoprotective effects of this plant against paracetamol induced hepatotoxicity in rats. So the present study is therefore, assess the potency of this important herb for its hepatoprotective nature against paracetamol induced toxicity model in rats.

## MATERIALS AND METHODS

### Plant Material

The whole plant with leaves, stems and roots were collected from rural areas of East Medinipur, West Bengal. The plants were thoroughly washed with water; roots and stems were discarded and the leaves were dried in hot air woven at 35°C for7 days. The authentication of the plant was done by Central National Herbarium, Botanical Garden, Howrah, Voucher no. CNH/124/2011/Tech.II/614.

### Extraction of the leaves of *Mikania scandens* (L.) Willd

The leaves of *Mikania scandens* (L.) Willd. were dried and powdered. The coarse powdered materials were defatted with petroleum ether (60-80$^0$C). Then powdered materials were extracted with sufficient volume of ethyl alcohol (95%) to get the ethanolic extract. Then from the thanolic extract different fractions like ethyl acetate and n-hexane fractions were isolated. Then the solvent were removed under reduced pressure to get semisolid mass and dried in vacuum dessicator.[13-15]

### Chemicals

Paracetamol and silymarin were supplied from Institute. Aspartate aminotrasferase(AST), alanine aminotrasferase(ALT), alkaline phosphatase(ALP), Total Protein, Total Billurubin,Total cholesterol and triglycerides etc kits were obtained from Span Diagnostic Lab, India. Other chemicals used in this experiment were also of analytical grade.

### Phytochemical screening

Specific methods were used for preliminary phyto chemical screening of those extracts. It was found that extracts contains alkaloids, flavonoides, glycosides, steroid, tannins etc. Following tests are performed. Carbohydrates with Benedict's test, Proteins with Biuret test, Alkaloids with Dragendorff's test, tannins with ferric chloride and potassium dichromate solutions test, saponins with foam test,steroids with Libermann- Burchard test and flavonoids with the use of Mg and HCl.[16,17]

### Experimental animals

Wistar albino rats, weighing about 150 – 200g were obtained from institute animal center and used in the experiments. The protocol was approved by the Institute's Animal Ethical Committee. Animals were kept in animal house at an ambient temperature of 25°C and 45 – 55% relative humidity, with 12 h each of dark and light cycles. Animals were fed pellet diet and water *ad-libitum*. All the

experiment procedures were performed according to the purpose of control and supervision of experiments on animal (CPCSEA), ministry of social justice and empowerment Government of India.

**Assessment of hepatoprotective activity**[18- 24]

The rats were divided into the seven groups each containing 6 rats. The weight range of the animals was equally distributed in all groups. They were acclimatized to housing conditions for at least one week prior to use.

**Group-I:** control rats,which fed normal diet and water.

**Group-II:** rats treated with Paracetamol (2gm/kg, i.p.) for 14 days.

**Group-III:** rats treated with Paracetamol (2gm /kg, i.p.) + Silymarin (100 mg/kg) orally once daily for 14 days.

**Group-IV:** rats treated with Paracetamol (2gm / kg, i.p.) + crude extract of Mikania scandens (500mg/kg) once daily for 14 days.

**Group-V:** rats treated with Paracetamol (2gm /kg, i.p.) + Ethyl acetate fraction MS (500mg/kg) orally once daily for 14 days.

**Group-VI:** rats treated with Paracetamol (2gm / kg, i.p.) + n-hexane fraction of MS (500mg/kg) orally once daily for 14 days.

**Group-VII:** rats treated with Paracetamol (2gm/ kg, i.p.)+ MS(500mg/kg)+ Silymarin(100 mg/kg)

At the end of the treatment, rats were sacrificed by cervical dislocation, blood samples were collected by direct cardiac puncture. The serums were used for the evaluation of marker enzymes. Livers were dissected out and washed with ice-cold saline and homogenates were prepared in 0.1N Tris HCL buffer (pH 7.4). The homogenates were used for the assay of antioxidant marker enzymes.

**Biochemical Estimation**

The levels of aspartate aminotrasferase(AST), alanine aminotrasferase(ALT), and alkaline phosphatase(ALP), Total Protein(TP), Total Billurubin(TB), cholesterol,and triglycerides were estimated in the serum using standardl kits from Span India ltd, surat,India. The liver homogenate was centrifused for 25 minutes using high speed centrifuse and supernatant was used for the assay of lipid peroxidation (LPO)[25], reduced glutathione (GSH)[26], super oxide dismutase(SOD) [27], and catalase(CAT)[28].

**Histopathological studies**

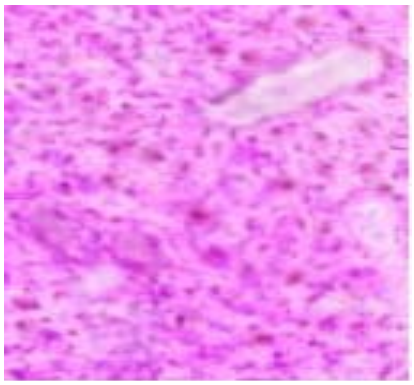
The livers were excised quickly and fixed in 10% formalin and paraffin embedded. Sections of about 4- 6 µm were stained with haemotoxylin and eosin(H&E) for Histological evaluation. In brief 4-6 µm thick sections of paraffin embedded rat liver were dewaxed with distilled water for 2min. Then the section were stained with haemotoxylin for 5 min at room tempature. After 15 min,the section were counterstained with eosin for 2min, dehydrated with alcohol, washed with xylene and blocked by eosin. Hemotoxylin and eosin stained studies were observed under microscope.

**Statistical Analysis**

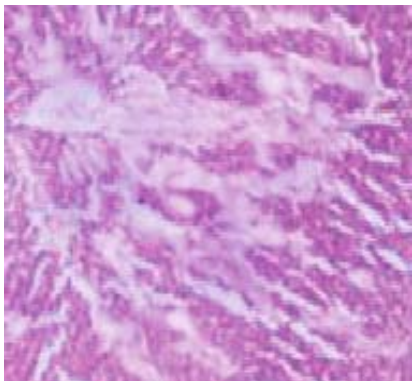
Data for hepatoprotective activity were expressed as Mean ± SEM from six rats in each group. Hepatoprotective activity were analysed statistically using one way analysis of variance (ANOVA), followed by Tukey-Kramer Multiple Comparisons Test with the help of INTA soft ware. P value of < 0.05 was considered as statistically significant.

**RESULTS**

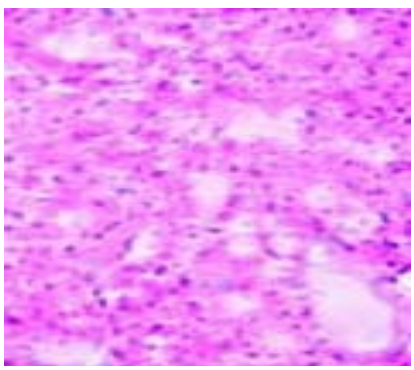
The activities of serum hepatic marker enzymes namely aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), total billirubin(TB), Cholesterol and triglycerides showed a significant (P<0.001) increase in Paracetamol treated rats as compared to normal control group (Table 1). The extract and fractions of MS treatments significantly(P<0.001) reversed the levels of AST, ALT, ALP, TB, triglycerides, cholesterol as compared to Paracetamol treated rats. The total protein concentration was significantly (p<0.001) lowered in Paracetamol treated group as compared to normal control group (Table1).



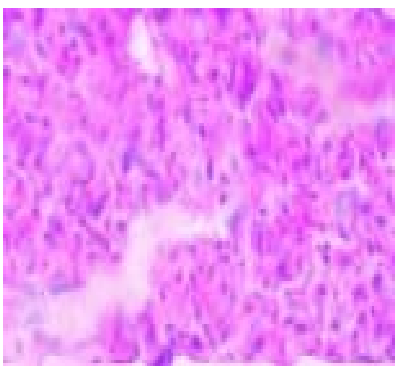
**Normal control (Group I)**



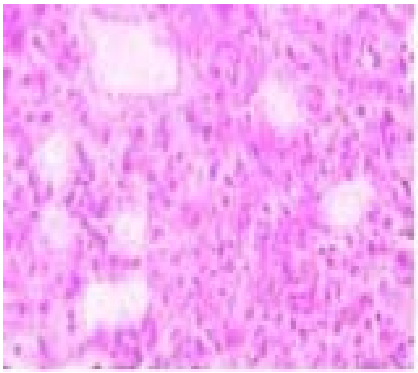
**PCM treated (Group II)**



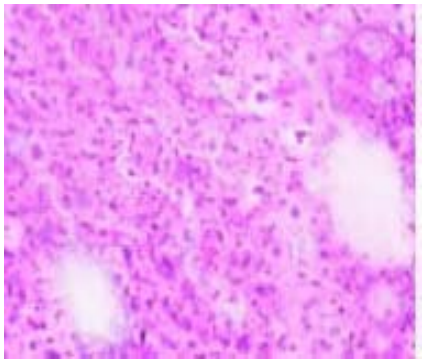
**Silymarin + PCM treated (Group III)**



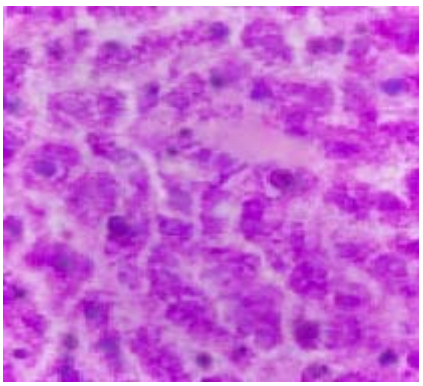
**MS extract + PCM treated(Group IV)**



**Ethyl acetate fraction + PCM treated (Group V)**



**N-hexane fraction +PCM treated (Group VI)**



**Silymarin + MS + PCM treated (Group VII)**

**Fig. 1: Histopathological changes in liver for following administration of Paracetamol, Silymarin, MS extract and its fractions in rats**

**Table 1: Effect of Paracetamol (PCM), silymarin, *Mikania scandens (L.) Willd* (MS) and its fractions on serum biochemical parameters**

| Group | AST U/L | ALT U/L | ALP U/L | TP gm/dl | TB mg/dl | Cholesterol mg/dl | Triglycerides mg/dl |
|---|---|---|---|---|---|---|---|
| Normal Control | 37.53 ± 0.56 | 30.71 ± 0.34 | 59.26 ± 0.54 | 8.23 ± 0.55 | 0.83 ± 0.01 | 126.56 ± 1.07 | 131.53 ± 0.36 |
| PCM | 135.55 ± 0.25### | 169.32 ± 0.55### | 172.47 ± 0.38### | 5.66 ± 0.26### | 1.66 ± 0.08### | 191.32 ± 0.84### | 182.50 ± 0.30### |
| Silymarin +PCM | 91.61± 0.57*** | 55.42 ± 0.30*** | 89.53 ± 0.34*** | 7.45 ± 0.54* | 0.92 ± 0.02*** | 155.60 ± 0.57* | 146.44 ± 0.29*** |
| MS extract + PCM | 70.73 ± 0.75*** | 66.49± 0.63*** | 98.34 ± 0.49*** | 7.5 ± 0.15* | 0.85 ± 0.02*** | 142.48 ± 0.23** | 155.52 ± 0.81*** |
| MS Ethyl acetate fraction + PCM | 101± 0.62*** | 65.26 ± 0.50*** | 115.45 ± 0.57*** | 6.44 ± 0.25ns | 0.99 ± 0.01*** | 150.42 ± 1.33* | 159.35 ± 0.72*** |
| MS n-hexane fraction + PCM | 80.50 ± 1.03*** | 78.24 ± 0.37*** | 105.46 ± 0.81*** | 6.95 ± 0.52ns | 1.02 ± 0.02*** | 170.52 ± 0.85ns | 160.42 ± 1.31*** |
| Silymarin + MS extract + PCM | 68.40 ± 1.06*** | 67.43 ± 0.36*** | 71.39 ± 1.28*** | 7.52 ± 0.29* | 0.84 ± 0.01*** | 151.44 ± 0.63* | 139.35 ± 0.32*** |

###P<0.001, considered when PCM treated group compared to normal control group. ***P<0.001, **P<0.01, *P<0.05 considered statistically significant when other groups are compared to PCM treated group

**Table 2: Effect of Paracetamol (PCM), silymarin, *Mikania scandens (L.) Willd* (MS) and its fractions on hepatic oxidative stress parameters**

| Group | LPO ηm of MDA/mg of protein | GSH µg/mg of protein | CAT U/mg of protein | SOD U/mg of protein |
|---|---|---|---|---|
| Normal Control | 6.89 ± 0.27 | 16.88 ±0.57 | 37.08 ± 0.33 | 24.39 ± 1.07 |
| PCM | 16.56 ± 0.38### | 6.03 ± 0.04### | 18.50 ± 0.60### | 16.47 ± 0.54### |
| Silymarin + PCM | 11.56 ± 0.38*** | 12.64 ± 0.29*** | 31.46 ± 0.78*** | 20.52 ± 0.29*** |
| MS extract+ PCM | 7.56 ± 0.27*** | 11.37 ± 0.30*** | 32.42 ± 0.15*** | 21.90 ± 0.39*** |
| MS Ethyl acetate fraction + PCM | 8.58 ± 0.26*** | 7.5 ± 0.29* | 25.42 ± 0.79*** | 19.95 ± 0.26*** |
| MS n-hexane fraction + PCM | 8.37 ±0.61*** | 8.50± 0.15*** | 23.41±1.05*** | 19.50 ± 0.13** |
| Silymarin + MS extract + PCM | 6.93 ± 0.35*** | 13.39 ± 0.13*** | 34.39 ±0.83*** | 22.27 ± 0.28*** |

###P<0.001, considered when PCM treated group compared to normal control group. ***P<0.001, **P<0.01, *P<0.05 considered statistically significant when other groups are compared to PCM treated group

**Table 3: Effect of paracetamol, silymarin, *Mikania scandens (L.) Willd* (MS) and its fractions on rat's liver weight study**

| Group | Liver weight (gm) |
|---|---|
| Normal Control | 4.45 ± 0.23 |
| PCM | 6.04 ± 0.08### |
| Silymarin + PCM | 5.25 ± 0.22* |
| MS extract+ PCM | 5.03 ± 0.03*** |
| MS Ethyl acetate fraction + PCM | 5.22 ± 0.07** |
| MS n-hexane fraction + PCM | 5.15 ±0.03** |
| Silymarin + MS extract + PCM | 5.11 ± 0.12** |

###P<0.001,considered when PCM treated group compared to normal control group. ***P<0.001, **P<0.01, *P<0.05 considered statistically significant when other groups are compared to PCM treated group

Administration of MS markedly elevated the concentration of TP levels. Malondialdehyde (MDA) level was significantly (p<0.001) increased in Paracetamol treated rats when compared to normal control group. Administration of MS remarkably lowered the level of MDA. The levels of GSH, SOD and CAT were significantly (p<0.001) decreased in Paracetamol treated rats when compared to normal control group. Whereas application of MS treatments significantly ( P< 0.001) reversed the levels of GSH, SOD and CAT(Table 2). In liver weight study, the liver weight of paracetamol treated rats was highly increased. When treated with MS, weight of livers were significantly decreased (Table 3). The above all results are compared with the Silymarin + paracetamol treated group (Table 1,2 & 3). Histopathological studies of liver tissue of the normal animal showed normal hepatocytes with central vein, cytoplasm, and nucleus. Damage of parenchymal cells,hemorrhagic necrosis of hepatocytes, and necrosis seen around central vein were observed in paracetamol treated rats. The liver sections of the rats treated with both silymarin as well as MS followed by Paracetamol intoxication showed a sign of protection.

## DISCUSSION

Now days, many studies have been developed with traditional medicines, in an attempt to invent new drugs for hepatitis.[29] The present study investigated the hepatoprotective effect of *Mikania scandens* (L.) Willd on experimental liver injury using PCM induced hepatotoxicity models in rat. Silymarin is used as a standard drug in various experimental and clinical studies due to its hepatoprotective effects. PCM results hepatotoxicity in men as well as in experimental animals. So the PCM -induced hepatotoxicity was selected as experimental models of liver injury in present study. PCM is metabolized to a toxic reactive metabolite N-acetyl-p-bezoquinone imine (NAPQI) by cytochrome P-450 which is further reported to cause massive oxidative stress and finally liver cell death.[30-32]The elevated levels of serum enzymes are indication of cellular leakages and loss of functional capacity of cell membrane in liver. It has been established that serum biochemical parameters such as AST, ALT, ALP etc levels were elevated in paracetamol-induced hepatotoxicity.[33] During the assessment of liver damage by paracetamol the determination of enzyme levels such as AST, ALT is widely used. AST found in mitochondria of hepatocytes. Necrosis of liver cells release the enzyme into circulation and it can be measured in the serum. High concentrations of AST show liver damage. ALT is more specific to the liver, and it is a better parameter for finding hepatic injury. High levels of AST indicate the cellular leakage as well as loss of functional ability of cell membrane in liver[34]. Serum ALP and bilirubin is also related with liver cell damage. High concentrations of ALP and TB were shown serious hepatic damage in Paracetamol treated rats. The decrease in the levels of Total protein (TP) observed in the Paracetamol treated rats suggested that the decrease in the number of hepatocytes which may result in decrease in hepatic capacity to synthesize protein. Cholesterol and triglycerides levels also increased in the paracetamol treated rats. In the present study, Treatment with both silymarin and MS (500mg/kg, p.o,) reduced the serum levels of AST, ALT, ALP, TB, triglycerides, cholesterol and elevated the level of TP, near normal value. This experimental results indicate that the plant extract and its fractions have stabilizes the plasma membrane as well as helped in healing of the hepatic tissue damage. Lipid peroxidation has been indicated to be the destructive process in liver injury due to Paracetamol administration. The increase in MDA level in liver induced by paracetamol suggests that enhanced lipid peroxidation leading to tissue damage. This phenomenon postulates failure of antioxidant defense mechanism to prevent formation of excessive free radicals. Treatment with MS and its fractions significantly reduced the levels of MDA. GSH removes free radical species such as hydrogen peroxide, superoxide radicals and maintains membrane protein thiols. The lowering GSH level in hepatic mitochondria is considered the most important mechanism in the paracetamol induced hepatotoxicity. Reduced GSH level was lowered in paracetamol treated group may be due to conjugation of GSH with NAPQI to form mercapturic acid[35]. Treatment with MS markedly increased the level of glutathione in a dose dependent manner. Decreased level of superoxide dismutase (SOD) is an important hepatic oxidative stress parameter during the assessment of liver damage. It scavenges the superoxide anion to form hydrogen peroxide and thus preventing the toxic effect caused by this radical[36]. In application of MS causes a significant increase in hepatic SOD activity.So this plant extracts and its fractions preventing reactive free radical induced oxidative damage to liver. Catalase (CAT) is the most sensitive enzymatic parameter in liver injury caused by oxidative stress. Catalase(CAT) is a haemoprotein and it protects cells from the accumulation of $H_2O_2$ by dismutating it to form $H_2O$ and $O_2$[37]. So reduction in the activities of these enzymes may indicate the toxic effects produced by toxicants. In the present study administration of MS significantly increased the levels of CAT. Histopathological changes observed in the liver of PCM treated rats having reduced oxygen perfusion. The development of liver disease may progress as a function of the rate of cell death. Treatment of rats with 500 mg/kg ethanolic extract and its fractions of *Mikania scandens* (L.) Willd. were more effective in protecting livers against necrosis.

## CONCLUSION

Bassed on the results, it can be concluded that decrease levels of TP, SOD, GSH, catalase activity, and increased serum marker enzymes and lipid peroxidation level in paracetamol treated rats was due to hepatocellular damage. The ethnolic extract and its ethyl acetate and n-hexane fraction afforded protection from such paracetamol induced liver damage, whereas the ethnolic extract of *Mikania scandens* (L.) Willd has shown the most pronounced hepatoprotective effect. So the plant *Mikania scandens* (L.) Willd possesses a competent hepatoprotective activity against Paracetamol intoxication in rats. Further detailed investigations may confirm the utility profile of this drug.

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