

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

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ABSTRACT

Chronic alcohol administration is resulting the generation of reactive oxygen species, thereby leading to liver damage. There is a lack of reliable hepatoprotective drugs in modern medicine in the alcohol induced liver damage. Plant products play a vital role in the hepatoprotection by its antioxidants property. Natural products are the important source of remedies for the treatment of diseases including hepatic disorders. So the identification of a potential hepatotherapeutic agent for the protection of liver from various hepatotoxins will provide a useful way for the prevention of these liver related diseases. This study evaluates the hepatoprotective activity of *Mikania scandens (L.) willd.*(MS) in rats. Administration of alcohol at 40%v/v ethanol (2ml/100g body wt. p.o), for 21 days showed a significant elevated levels of aspartate aminotransferase (AST), alkaline phosphatase (ALP), alanine aminotransferase (ALT), total bilirubin (TB), triglycerides, cholesterol and lipid peroxidation(LPO). There was also a significant decreased levels of catalase, glutathione reductase and superoxide dismutase when compared to normal control rats. Pretreatment of rats with 500 mg/kg body weight of extract and fractions of *Mikania scandens (L.) willd.* or silymarin(100 mg/kg) was found to protect the rat from hepatotoxic action of ethanol as evidenced by significant reverse action when compared to group administered alcohol only. Histopathological studies show marked reduction in fatty degeneration and centrilobular necrosis in animals receiving *Mikania scandens (L.) willd.* along with ethanol as compared to the control group.

Keywords: Hepatotherapeutic, Ethanol, Fatty degeneration, *Mikania scandens (L.) Willd.*, Histopathological studies.

INTRODUCTION

Alcoholic liver disease is the hepatic manifestations of alcohol over consumption, including fatty liver, alcoholic hepatitis, and chronic hepatitis with hepatic fibrosis or cirrhosis¹. It is the major cause of liver disease in Western countries. About 12% of American adults had an alcohol dependence problem at some time in their life. Steatosis (fatty liver) will develop in any individual who consumes a large quantity of alcoholic beverages over a long period of time. Chronic alcohol consumption leads to various metabolic disorders including hepatic and extra hepatic diseases². Of all chronic heavy drinkers, only 15–20% develops hepatitis or cirrhosis.^{3,4}

Alcohol may be recognized as the second most widely used psychoactive substances in the world, after caffeine⁵. Near about 80–90% of alcohol is metabolized in the liver, where alcohol is oxidized to acetaldehyde⁶. The metabolic process is catalyzed by different enzymes like alcohol dehydrogenase(ADH), microsomal ethanol metabolizing system (MEOS) and acetaldehyde dehydrogenase (ALDH). Whereas the acetaldehyde is more toxic than alcohol, it is related with a larger number of the metabolic disorders in liver disease caused by alcohol⁷. Alcohol ingestions has been found to cause accumulation of reactive oxygen species which is the source of lipid peroxidation of cellular membranes and proteins as well as DNA oxidation, resulting in hepatocyte necrosis.^{8, 9} Scavenging of free radicals by antioxidants could reduce the necrosis process in the tissues.¹⁰ According to the various hypothesis that oxidative stress occurs only when the antioxidant capacity is insufficient. Many research models have focused on the alcohol-associated changes in the liver antioxidants. In spite of the advancement in allopathic system of medicine, the potent allopathic medicines are higher cost as well as associated with several adverse effects. Herbal drugs are known to play a vital role in the therapies of liver diseases. Now days the evaluation of hepatoprotective activity by antioxidant action is a burning focus in the herbal drugs research. The worldwide consumption of herbal remedies has been stimulated by several factors like those are free from adverse effects well as all herbal products are safe and pharmacologically active.¹¹ The liver has a great capacity for the metabolic homeostasis of the body including biotransformations, detoxification and excretion of many endogenous and exogenous compounds.^{12, 13} No scientific report is available relating the hepatoprotective potentials of *Mikania scandens*

(*L.*) Willd in alcohol induced liver damage. Therefore, the present work was planned to study the protective effect of *Mikania scandens (L.) Willd* extract in acute alcohol-induced hepatotoxicity 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 for 7 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. Then powdered materials were extracted with sufficient volume of ethyl alcohol to get the ethanolic extract. Then from the ethanolic extract different fractions like ethyl acetate, n-hexane and n-butanolic fractions were isolated. Then the solvent were removed under reduced pressure to get semisolid mass and dried in vacume dessicator.^{14,16}

Chemicals

Alcohol and silymarin were purchased from Titan Biotech Ltd, Kolkata. Aspartate aminotransferase (AST), alanine aminotransferase (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.^{17,18}

Experimental animals

Wistar albino rats, weighing about 180 – 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.

Treatment Protocol¹⁹⁻²³

In order to study the hepatotherapeutic effect of ethanolic extract and its fractions (ethyl acetate, n-hexane and n-butanolic) of *Mikania scandens* (L.) Willd.) in rat dose 500 mg/kg bw p.o were used respectively. 40% v/v ethanol (2ml/100g bw p.o.) was used as hepatotoxic chemical and Silymarin (100 mg/kg bw p.o) was used as a standard drug in this study. Rats were divided into eight groups as following protocol.

GROUP I: Normal control (n=6, the animals were given normal saline only for 21 days).

GROUP II: Hepatotoxic control (n=6, the animals were given alcohol for 21 Days).

GROUP III: Standard group (n=6, the animals were given alcohol +Silymarin for 21 days).

GROUP IV: Treatment group (n=6, the animals were given alcohol + MS extract for 21 days).

GROUP V: Treatment group (n=6, the animals were given alcohol + ethyl acetate fraction MS for 21 days).

GROUP VI: Treatment group (n=6, the animals were given alcohol + n-hexane fraction MS for 21 days).

GROUP VII: Standard group (n=6, the animals were given alcohol + n-butanolic fraction MS for 21 days).

GROUP VIII: Treatment group (n=6, the animals were given alcohol +Silymarin + MS extract for 21 days).

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

Biochemical Estimation

The levels of aspartate aminotransferase(AST), alanine aminotransferase(ALT), and alkaline phosphatase(ALP), Total Protein(TP), Total Billurubin(TB), cholesterol and triglycerides were estimated in the serum using standard kits from Span India Ltd, surat, India. The liver homogenate was centrifused by using high speed cooling centrifuse and supernatant was used for the assay of lipid peroxidation (LPO)²⁴, reduced glutathione (GSH)²⁵, super oxide dismutase (SOD)²⁶, and catalase(CAT)²⁷.

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 was stained with haemotoxylin for 5 min at room temperature. 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.^{28, 29}

Statistical Analysis

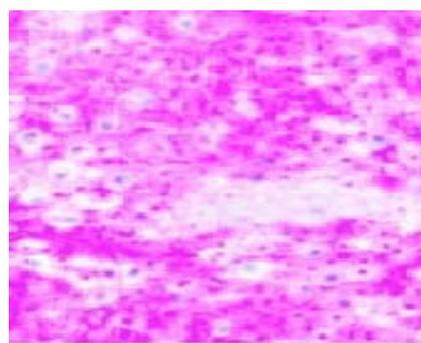
Data for hepatoprotective activity were expressed as Mean ± SEM from six rats in each group. Hepatoprotective activity were analyzed statistically using one way analysis of variance (ANOVA), followed by Tukey-Kramer Multiple Comparisons Test with the help of INTA soft ware. P value of < 0.05 was considered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

RESULTS

Exposure of rats to alcohol for 21 days showed significant (p<0.001) elevated levels serum biochemical parameters like AST, ALT, ALP, TB, Cholesterol and triglycerides. The protective effect of Ethanolic extract and its fractions of MS on serum-AST, ALT, ALP TB, Cholesterol and triglycerides in 40% v/v ethanol treated rats showed significant (p<0.001; p<0.05, respectively) decline as compared to 40% v/v ethanol treated groups. The degree of hepatoprotection by Ethanolic extract of *Mikania scandens* (L.) Willd. (500 mg/kg bw p.o.) was observed statistically near value with the standard drug (Table 1). The levels of total protein (TP) was significantly (p<0.05) decreased in hepatotoxic control rats. The Hepatoprotective effect of MS was observed in treatment groups. The levels of MDA in liver tissues of ethanol intoxicated rats were significantly (p<0.001) elevated when compared to the level of MDA in normal control animals. The administration of herbal drugs MS extract and its fractions at the therapeutic doses (500 mg/kg bw p.o.) showed maximum reduction in MDA level. Standard drug silymarin also maintained the similar result. In the GSH test, showed the much decreased level of glutathione in ethanol induced rats. Treatment with *Mikania scandens* (L.) Willd had showed significantly improved level. Similar activity also observed with the standard drug Silymarin. Rats treated with 40% v/v ethanol caused a significant (p<0.001) decline in the hepatic antioxidants such as SOD and CAT in comparison to normal control animals. Simultaneously, oral administration of MS extract and its fractions at the dose level 500mg/kg body weight/day showed significant (p<0.05; p<0.001) elevation in the activity of all antioxidant parameters like SOD and CAT near to normal value. In liver weight study, the liver weight of alcohol treated rats were highly increased. When treated with MS, weight of livers were significantly decreased (Table 3). In histological examinations, hepatocytes of the normal control group showed a normal cellular architecture of the liver. Whereas the liver section of rats treated with toxicant showing intense centrilobular necrosis and vacuolization. The liver sections of the rats treated with MS and silymarin along with ethanol toxicant showing a sign of protection as it was evident by the absence of necrosis and vacuoles.



Normal control (Group I)



Alcohol treated (Group II)

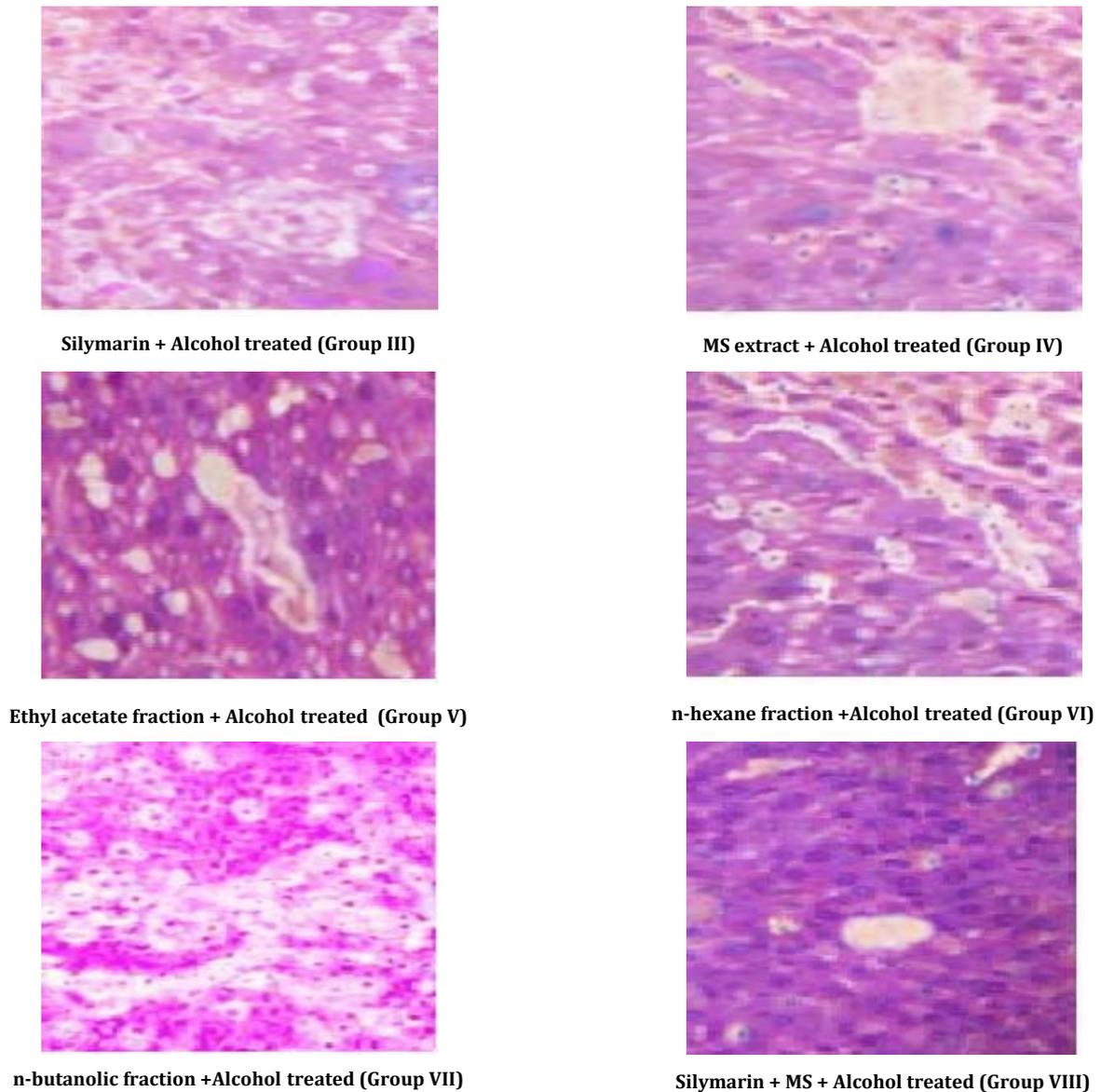


Fig. 1: Histopathological changes of liver for following administration of Alcohol, Silymarin, MS extract and its fractions in rats

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

Group	AST IU/L	ALT IU/L	ALP IU/L	TP gm/dl	TB mg/dl	Cholesterol mg/dl	Triglycerides mg/dl
Normal Control	27.41 ± 1.45	32.42 ± 0.72	46.63 ± 0.79	6.33 ± 0.69	0.35 ± 0.06	114.66 ± 0.68	130.33 ± 0.92
Alcohol	303.31 ± 1.03 ^{###}	287.39 ± 1.07 ^{###}	92.39 ± 0.86 ^{###}	3.44 ± 0.42 [#]	1.77 ± 0.02 ^{###}	187.51 ± 1.23 ^{###}	192.55 ± 1.54 ^{###}
Silymarin + Alcohol	45.38 ± 1.67 ^{***}	52.30 ± 0.52 ^{***}	50.65 ± 1.65 ^{***}	6.16 ± 1.29 [*]	0.39 ± 0.02 ^{***}	120.73 ± 0.63 ^{***}	136.53 ± 1.12 ^{***}
MS extract + Alcohol	48.27 ± 0.56 ^{***}	41.37 ± 0.40 ^{***}	48.70 ± 0.79 ^{***}	6.21 ± 0.72 [*]	0.36 ± 0.06 ^{***}	122.58 ± 2.00 ^{***}	135.42 ± 0.40 ^{***}
MS Ethyl acetate fraction + Alcohol	56.31 ± 1.75 ^{***}	66.35 ± 1.06 ^{***}	54.72 ± 0.62 ^{***}	5.24 ± 0.37 ^{ns}	0.60 ± 0.03 ^{***}	152.37 ± 0.42 ^{***}	149.71 ± 1.77 ^{***}
MS n-hexane fraction + Alcohol	86.23 ± 1.56 ^{***}	70.36 ± 0.88 ^{***}	58.69 ± 0.96 ^{***}	5.88 ± 0.69 ^{ns}	0.46 ± 0.01 ^{***}	150.56 ± 1.26 ^{***}	148.57 ± 0.67 ^{***}
MS n-butanolic fraction + Alcohol	293.29 ± 0.31 ^{ns}	260.44 ± 0.78 ^{ns}	79.73 ± 1.49 ^{***}	4.92 ± 0.75 ^{ns}	0.70 ± 0.02 ^{***}	186.48 ± 0.26 ^{ns}	165.48 ± 1.53 ^{***}
Silymarin + MS extract + Alcohol	44.43 ± 1.00 ^{***}	42.49 ± 1.19 ^{***}	55.67 ± 1.84 ^{***}	6.24 ± 0.52 [*]	0.37 ± 0.01 ^{***}	118.54 ± 1.60 ^{***}	135.36 ± 0.40 ^{***}

^{###}P<0.001 and [#]P<0.05 considered when Alcohol treated group compared to normal control group. ^{***}P<0.001, ^{*}P<0.05 are considered statistically significant and ^{ns}P>0.05 considered non significant when other groups are compared to Alcohol treated group.

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

Group	LPO nm of MDA/mg of protein	GSH µg/mg of protein	CAT U/mg of protein	SOD U/mg of protein
Normal Control	8.36 ± 0.69	12.66 ± 0.74	40.66 ± 1.89	33.60 ± 0.53
Alcohol	28.48 ± 1.12###	4.98 ± 0.63###	24.32 ± 1.12###	14.47 ± 1.10###
Silymarin + Alcohol	10.60 ± 0.96***	10.28 ± 0.76***	37.25 ± 1.08***	30.44 ± 1.09***
MS extract+ Alcohol	9.66 ± 0.45***	11.22 ± 1.13***	37.19 ± 1.09***	32.36 ± .82***
MS Ethyl acetate fraction + Alcohol	16.39 ± 0.58***	8.34 ± 0.77 ^{ns}	30.63 ± 0.96*	29.41 ± 0.77***
MS n-hexane fraction + Alcohol	18.39 ± 1.30***	8.30 ± 0.76 ^{ns}	28.64 ± 0.96 ^{ns}	29.39 ± 0.46***
MS n- butanolic fraction + Alcohol	22.64 ± 0.66***	5.80 ± 0.54 ^{ns}	24.28 ± 1.10 ^{ns}	27.45 ± 2.03***
Silymarin + MS extract + Alcohol	9.68 ± 0.45***	11.65 ± 0.77***	37.24 ± 1.40***	31.13 ± 0.76***

###P<0.001, considered when Alcohol treated group compared to normal control group. ***P<0.001, *P<0.05 are considered statistically significant and ^{ns}P>0.05 considered non significant when other groups are compared to Alcohol treated group.

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

Group	Liver weight (gm)
Normal Control	5.60 ± 0.24
Alcohol	7.86 ± 0.09###
Silymarin + Alcohol	5.95 ± 0.37***
MS extract+ Alcohol	5.98 ± 0.14***
MS Ethyl acetate fraction + Alcohol	6.31 ± 0.18***
MS n-hexane fraction + Alcohol	6.34 ± 0.14**
MS n-butanol fraction + Alcohol	7.31 ± 0.15 ^{ns}
Silymarin + MS extract + Alcohol	5.91 ± 0.37***

###P<0.001, considered when Alcohol treated group compared to normal control group. ***P<0.001, **P<0.01 considered statistically significant and ^{ns}P>0.05 considered non significant when other groups are compared to Alcohol treated group.

DISCUSSION

Herbal drugs are prescribed widely because of their effectiveness as well as fewer side effects and relatively low cost.^{30, 31} Hepatic cells appear to participate in a variety of enzymatic metabolic activities and also actively involved in alcohol induced marked liver damage.³² The therapeutic activity of a hepatoprotective drug to reduce the injurious effects due to hepatotoxins and to preserve the normal hepatic physiological mechanisms.³³ Alcohol treatment of rats is known to cause the translocation of fat from the peripheral adipose tissue to liver for accumulation. Formation of reactive oxygen species (ROS) oxidative stress and hepatic cell necrosis have been implicated to alcoholic liver disease. It has been reported that Kupffer cells are the major sources of ROS during chronic alcohol consumption, and these are activated for enhanced formation of pro-inflammatory factors.³⁴ The animals treated with alcohol (group 2) had a significant hepatic damage as indicated by the elevated (P<0.001) levels of serum enzyme markers like AST, ALT, ALP, TB, triglycerides and cholesterol (Table 1). The damage membrane releases the enzymes in to the circulation. ALT catalyses the conversion of alanine to pyruvate and glutamate, and is released in a same way. AST is more specific to the liver, and is a better parameter for detecting liver injury.³⁵ The rise in the ALT level is usually accompanied by an elevation in the levels of AST, which plays a role in the conversion of amino acids to keto acids. The present study reveals that the effect of pretreatment of ethanolic extract and fractions (ethyl acetate, n-hexane and n-butanol) of *Mikania scandens* (L.) Willd had been effective in offering protection which was comparable to Silymarin. These extracts and fractions were shown liver protective actions by lowering the levels of AST, ALT, ALP, TB, triglycerides and cholesterol (Table 1). The decrease in the levels of Total protein (TP) observed in the Alcohol treated rats suggested that the destruction in the number of hepatic cells which may result in decrease in hepatic capacity to synthesize protein. Treatment with MS marked elevated the total protein level which was compared with standard drug silymarin. The studies on lipid peroxidation, antioxidant enzymes like reduced glutathione, superoxide dismutase and catalase have been found to be of importance parameters in the assessment of liver damage.^{36, 37} Markedly elevated levels of MDA in liver intoxicated by alcohol 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. It was well accepted that a deficiency of GSH within living cells can lead to tissue injury. The liver injury produced by Ethanol (Alcohol) is known to be related with low tissue levels of GSH.³⁸ Decrease level of superoxide dismutase (SOD) is a 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.³⁹ In treatment of MS causes a significant increase in hepatic SOD and GSH activity. Catalase (CAT) is a haemoprotein and it protects cells from the accumulation of H₂O₂ by dismutating it to form H₂O and O₂ or by using it as an oxidant in which it works as a peroxidase.⁴⁰ In alcohol treated rats significantly (P<0.001) decreased levels of catalase but In the present study application of MS significantly increased the levels of CAT. In liver weight study it has been observed that alcohol treated rats gain more liver weight due to fatty cells deposition and inflations of the cells. When treated with MS and its fractions the liver weights were significantly lowered near to normal value. In histological study, hepatocytes of the normal control group was showing a normal histological architecture. Whereas the liver section of rats treated with ethanol was showing intense centrilobular necrosis, vacuolization fatty degeneration and inflammatory cells. The liver sections of the rats treated with MS and silymarin along with ethanol toxicant were showing a sign of protection.

CONCLUSION

In conclusion, Ethanolic extract and its fractions of *Mikania scandens* (L.) Willd. are effective against oxidative liver damage induced by alcohol administration. So this study will give the pharmacological support to use of folk medicine in the management of alcohol intoxicated hepatic damage.

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