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 hepatoprotective agent for the protection of liver from various hepatotoxins will provide a useful way for the prevention of these liver related diseases. This study evaluated the hepatoprotective activity of Mikania scandens (L) Willd.(MS) in rats. Administration of alcohol at 40%v/v ethanol (2ml/100g body wt. po), 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 showed 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: Hepatoprotective, 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 cirrhosis. 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 diseases. Of all chronic heavy drinkers, only 15–20% develops hepatitis or cirrhosis.

Alcohol may be recognized as the second most widely used psychoactive substances in the world, after caffeine. Near about 80–90% of alcohol is metabolized in the liver, where alcohol is oxidized to acetaldehyde. The metabolic process is catalyzed by different enzymes like alcohol dehydrogenase (ADH), microsomal ethanol metabolizing system (MMEs) 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 alcohol. 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. Scavenging of free radicals by antioxidants could reduce the necrosis process in the tissues. 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 allopatic system of medicine, the potent allopatic 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. The liver has a great capacity for the metabolic homeostasis of the body including biotransformations, detoxification and excretion of many endogenous and exogenous compounds. 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 oven 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.I/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-butanol fractions were isolated. Then the solvent were removed under reduced pressure to get semisolid mass and dried in vacuum desiccator.

Chemicals

Alcohol and silymarin were purchased from Titan Biotech Ltd. Kolkata. Aspartate aminotrasferase (AST), alanine aminotrasferase (ALT), alkaline phosphatase (ALP), Total Protein, Total Bilirubin, 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, flavonoids, 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 Lberman- Burchard test and flavonoids with the use of Mg and HCl.
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 Protocol 19-23

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 Bilirubin (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 dismutate (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 haematoxylin 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 haematoxylin 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. Hematoxylin 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 software. 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 Ethanol 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.) Wild. (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.) Wild 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.
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Silymarin + Alcohol treated (Group III)

MS extract + Alcohol treated (Group IV)

Ethyl acetate fraction + Alcohol treated (Group V)

n-hexane fraction + Alcohol treated (Group VI)

n-butanol fraction + Alcohol treated (Group VII)

Silymarin + MS + Alcohol treated (Group VIII)

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

<table>
<thead>
<tr>
<th>Group</th>
<th>AST IU/L</th>
<th>ALT IU/L</th>
<th>ALP IU/L</th>
<th>TP gm/dl</th>
<th>TB mg/dl</th>
<th>Cholesterol mg/dl</th>
<th>Triglycerides mg/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Control</td>
<td>27.41 ± 1.45</td>
<td>32.42 ± 0.72</td>
<td>46.63 ± 0.79</td>
<td>6.33 ± 0.69</td>
<td>0.35 ± 0.06</td>
<td>114.66 ± 0.68</td>
<td>130.33 ± 0.92</td>
</tr>
<tr>
<td>Alcohol</td>
<td>303.31 ± 1.03</td>
<td>287.39 ± 1.07</td>
<td>92.39 ± 0.86</td>
<td>3.44 ± 0.42</td>
<td>1.77 ± 0.04</td>
<td>187.51 ± 1.23</td>
<td>192.55 ± 1.54</td>
</tr>
<tr>
<td>Silymarin + Alcohol</td>
<td>45.38 ± 1.07</td>
<td>52.30 ± 1.07</td>
<td>50.65 ± 1.07</td>
<td>6.16 ± 0.04</td>
<td>0.39 ± 0.01</td>
<td>120.73 ± 1.07</td>
<td>136.53 ± 1.37</td>
</tr>
<tr>
<td>MS extract + Alcohol</td>
<td>48.27 ± 1.07</td>
<td>52.30 ± 1.07</td>
<td>50.65 ± 1.07</td>
<td>6.16 ± 0.04</td>
<td>0.39 ± 0.01</td>
<td>120.73 ± 1.07</td>
<td>136.53 ± 1.37</td>
</tr>
<tr>
<td>MS Ethyl acetate fraction + Alcohol</td>
<td>56.31 ± 1.07</td>
<td>66.35 ± 1.07</td>
<td>54.72 ± 1.07</td>
<td>6.24 ± 0.04</td>
<td>0.39 ± 0.01</td>
<td>120.73 ± 1.07</td>
<td>136.53 ± 1.37</td>
</tr>
<tr>
<td>MS n-hexane fraction + Alcohol</td>
<td>86.23 ± 1.07</td>
<td>70.36 ± 1.07</td>
<td>58.69 ± 1.07</td>
<td>5.88 ± 0.04</td>
<td>0.46 ± 0.01</td>
<td>150.56 ± 1.07</td>
<td>149.71 ± 1.37</td>
</tr>
<tr>
<td>MS n-butanol fraction + Alcohol</td>
<td>1.56 ± 1.07</td>
<td>0.88 ± 1.07</td>
<td>0.96 ± 1.07</td>
<td>0.69 ± 0.04</td>
<td>0.01 ± 0.01</td>
<td>1.26 ± 1.07</td>
<td>0.67 ± 1.37</td>
</tr>
<tr>
<td>Silymarin + MS extract + Alcohol</td>
<td>44.43 ± 1.07</td>
<td>42.49 ± 1.07</td>
<td>55.67 ± 1.07</td>
<td>6.24 ± 0.04</td>
<td>0.37 ± 0.01</td>
<td>118.54 ± 1.07</td>
<td>135.36 ± 1.37</td>
</tr>
<tr>
<td>Alcohol</td>
<td>1.00 ± 1.07</td>
<td>1.19 ± 1.07</td>
<td>1.84 ± 1.07</td>
<td>0.52 ± 0.04</td>
<td>0.01 ± 0.01</td>
<td>1.60 ± 1.07</td>
<td>0.40 ± 1.37</td>
</tr>
</tbody>
</table>

###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.
4.9
2
54x189
ALP, TB, triglycerides and cholesterol (Table 1). The decrease in the 
was comparable to Silymarin. These extracts and fractions were 
releases the enzymes in to the circulation. ALT catalyses the 
triglycerides and cholesterol (Table 1). The damage membrane
37Markedly elevated levels of MDA in liver intoxicated by alcohol 
was compared with standard drug silymarin. The studies on lipid 
may result in decrease in hepatic capacity to synthesize protein.

Silymarin + MS extract + Alcohol 
MS n
MS Ethyl acetate fraction + Alcohol 
MS n-hexane fraction + Alcohol 
MS n-butanol fraction + Alcohol 
Silymarin + MS extract + Alcohol

###P<0.001, considered when Alcohol treated group compared to normal control group. ***P<0.001, *P<0.05 are considered statistically significant and +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

<table>
<thead>
<tr>
<th>Group</th>
<th>LPO nm of MDA/mg of protein</th>
<th>GSH μg/mg of protein</th>
<th>CAT U/mg of protein</th>
<th>SOD U/mg of protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Control</td>
<td>8.36 ± 0.69</td>
<td>12.66 ± 0.74</td>
<td>40.66 ± 1.89</td>
<td>33.60 ± 0.53</td>
</tr>
<tr>
<td>Alcohol</td>
<td>28.48 ± 1.12***</td>
<td>4.98 ± 0.63***</td>
<td>24.32 ± 1.12***</td>
<td>14.47 ± 1.0***</td>
</tr>
<tr>
<td>Silymarin + Alcohol</td>
<td>10.60 ± 0.96***</td>
<td>10.28 ± 0.76***</td>
<td>37.25 ± 1.08***</td>
<td>30.44 ± 1.09***</td>
</tr>
<tr>
<td>MS extract+ Alcohol</td>
<td>9.66 ± 0.45***</td>
<td>11.22 ± 1.13***</td>
<td>37.19 ± 1.09***</td>
<td>32.36 ± 2.82***</td>
</tr>
<tr>
<td>MS Ethyl acetate fraction + Alcohol</td>
<td>16.39 ± 0.58***</td>
<td>8.34 ± 0.77***</td>
<td>30.63 ± 0.96*</td>
<td>29.41 ± 0.77***</td>
</tr>
<tr>
<td>MS n-hexane fraction + Alcohol</td>
<td>18.39 ± 0.130***</td>
<td>8.30 ± 0.76***</td>
<td>28.64 ± 0.96*</td>
<td>29.39 ± 0.46***</td>
</tr>
<tr>
<td>MS n-butanol fraction + Alcohol</td>
<td>22.64 ± 0.66***</td>
<td>5.80 ± 0.54***</td>
<td>24.26 ± 1.10**</td>
<td>27.45±2.09***</td>
</tr>
<tr>
<td>Silymarin + MS extract + Alcohol</td>
<td>9.62 ± 0.45***</td>
<td>11.65 ± 0.77***</td>
<td>37.24 ± 1.40***</td>
<td>31.13 ± 0.76***</td>
</tr>
</tbody>
</table>

Discussion

Herbal drugs are prescribed widely because of their effectiveness as well as fewer side effects and relatively low cost.10, 11 Hepatic cells appear to participate in a variety of enzymatic metabolic activities and also actively involved in alcohol induced marked liver damage.12 The therapeutic activity of a hepatoprotective drug to reduce the injurious effects due to hepatotoxins and to preserve the normal hepatic physiological mechanisms.32 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 alcholic 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 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-butanolic) 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 damage membrane releases the enzymes in to the circulation. ALT catalyses the conversion of alanine to pyruvate and glutamate, and is released in a similar 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 consumption, and these are activated for enhanced formation of pro-inflammatory factors. The animals treated with MS and its fractions the liver weights were significantly lower than 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 cirrhotic nodules, 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, Ethanol 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.

References


