HEPATOPROTECTIVE EFFECTS OF KYLLINGA BREVFOLIA ON CARBON TETRACHLORIDE INDUCED LIVER DAMAGE IN RATS

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ABSTRACT

To investigate the hepatoprotective activity and acute oral toxicity of methanolic extract of whole plant of Kyllinga brevifolia (MEKB) in male Wistar albino rats by using CCl₄ induced hepatotoxicity. The MEKB at doses of 200 and 400mg/kg, p.o and the standard drug Silymarin (100mg/kg, p.o) were administered three times at 12h intervals and then CCl₄ (1ml/kg) was administered to all the groups except normal control for 2 days. The hepatoprotective activity was assessed by using various biochemical parameters like SGOT, SGPT, ALP, γ-GT, TP and total bilirubin along with histopathological studies were observed after 36h of CCl₄ treatment. The MEKB at the doses of 200 and 400mg/kg inhibited CCl₄ induced liver toxicity in Wistar albino rats as assessed by the biochemical changes and histopathological studies. The methanolic extract of whole plant of Kyllinga brevifolia afforded significant protection against CCl₄ induced hepatocellular injury.

Key words: Kyllinga brevifolia, Hepatoprotective, CCl₄, Silymarin, Hepatotoxicity.

INTRODUCTION

The liver is the largest organ in the body weighing 1200-1500g. It is a key organ in regulating homeostasis within the body. It regulates several important functions including protein synthesis, storage and metabolism of fats and carbohydrates, detoxification of drugs and other toxins, metabolism of hormones and excretion of bilirubin. Liver diseases are associated with distortion of these metabolic functions [1,2]. Although viruses are the main cause of liver diseases, the liver lesions arising from xenobiotics, excessive drug therapy, environmental pollution and alcoholic intoxication are not uncommon [3]. Every year about 20,000 deaths are found due to liver disorders [4]. Thus to maintain a healthy liver is a crucial factor for overall health and well being [5]. Thus, liver diseases remain one of the serious health problems and its disorders are numerous with no effective remedies [6-8]. There is no rational therapy available for treating liver disorders and management of liver diseases is still a challenge to the modern medicine [9,10]. In the absence of reliable liver protective drugs in allopathic medical practices, herbs play a role in the management of various liver disorders [6]. The use of natural remedies for the treatment of various hepatic diseases has a long history and medicinal plants and their derivatives are still used all over the world [4].

Kyllinga brevifolia Rottb. (Family - Cyperaceae) is perennial sedge arising from a thin creeping rhizome bearing three angled stems up to 30 cm high. Leaves are basal, in three ranks, long-linear (grass-like), about the same length as stems. Flowers minute, borne in a terminal white globose head (occasionally two smaller lateral heads may also be present) up to 8 mm in diameter, subtended by three spreading leaf-like bracts up to 15 cm long. Fruit a minute achene up to 1.5 mm long. Flowers and fruits are usually...
available throughout the year. It is common in damp, disturbed places such as pastures, cane fields, stream sides, etc. from near sea -level up to over 1000 m elevation. It is distributed widely distributed throughout the tropics and common throughout in the South Pacific. Traditional Used for treat liver disease [11,12].

MATERIALS AND METHODS

Plant collection

The whole plant of Kyllinga brevifolia was collected from Tirupati, Andhra Pradesh, in the month of August 2010. The plant was authenticated by Prof. P. Jayaraman, Director of National Institute of Herbal Science, W.Tambaram, Chennai. The voucher specimen of the plant was deposited at the college, for further reference.

Preparation of plant extract

The whole plant of Kyllinga brevifolia were dried in shade and pulverized in grinder-mixer to obtain a coarse powder. It was then passed through the 40 mesh sieve. A weighed quantity (210gm) of the powder was subjected to continuous hot extraction with methanol in Soxhlet apparatus for 48h. The extraction was evaporated under reduced pressure using rotary evaporator until all the solvent has been removed to give an extract sample. The percentage of yield of methanolic extract of Kyllinga brevifolia was found to be 12.5%w/w.

Animals used

The animals were maintained in a well-ventilated room with 12:12 hour light/dark cycle in polypropylene cages. The animals were fed with standard pellet feed (Hindustan Lever Limited., Bangalore) and water was given ad libitum. Ethical committee clearance was obtained from IAEC (Institutional Animal Ethics Committee) of CPCSEA.

Acute Toxicity Study

The acute toxicity methanolic extracts of Kyllinga brevifolia was determined as per the OECD guideline no. 423 (Acute Toxic Class Method). It was observed that the test extract was not mortal even at 2000mg/kg dose. Hence, 1/10th (200mg/kg) and 1/5th (400mg/kg) of this dose were selected for further study [13].

Carbon tetrachloride induced hepatotoxicity in rats

The liver protective effect was evaluated using the carbon tetrachloride (CCl₄) model described by Rao and Mishra [14]. Wistar albino rats (150-200g) were divided into five groups and were subjected to the following treatments; group-I served as normal control; received vehicle only. Group-II served as untreated group; received only CCl₄, to assist assessing the severity of toxicity produced by carbon tetrachloride administration. Groups III-V served as treated groups; received MEKB at the dose of 200 and 400mg/kg, p.o. and standard drug Silymarin at a dose of 100mg/kg, p.o. were administered orally to rats of the respective groups three times at 12h intervals. Carbon tetrachloride diluted with liquid paraffin (1:1) was administered in dose of 1ml/kg, p.o. for 2 days to all animal groups except for normal control. After 36h of carbon tetrachloride treatment, blood was collected from all groups of rats by puncturing the retro-orbital sinus. Serum was separated by centrifugation at 2500rpm at 37°C for 15min and analyzed for various biochemical parameters.

Biochemical estimation

The separated serum was subjected to estimate SGOT and SGPT by Reitman and Frankel method [15], alkaline phosphatase (ALP) by Kind and King method [16], and bilirubin by Malloy and Evelyn method [17].

Statistical analysis

The data were expressed as mean ± standard error mean (S.E.M). The Significance of differences among the group was assessed using one way and multiple way analysis of variance (ANOVA). The test followed by Tukey-Kramer multiple comparison tests, the p values less than 0.05 were considered as significance.

RESULTS

Acute toxicity study

In the acute toxicity study, the animals treated with the MEKB at a higher dose of 2000 mg/kg did not manifest any significant abnormal signs, behavioral changes, body weight changes, or macroscopic findings at any time of observation. There was no mortality in the above-mentioned dose at the end of the 14 days of observation.

Effect of MEKB on CCl₄ – induced hepatotoxicity

The results of MEKB on Carbon tetrachloride-induced hepatotoxicity were represented in Table 1. The animals treated only with CCl₄ exhibited a significant increase (P<0.001) the levels of SGOT, SGPT, ALP, γ-GT and total bilirubin as well as decrease in the levels of TP when compared to the normal control group after 36h of CCl₄ treatment, indicating hepatocellular damage. The MEKB at tested doses (group-III & IV) produced a significant reduction (P<0.001) in the CCl₄ induced elevated levels of SGOT, SGPT, ALP, γ-GT and total bilirubin as well as increases the TP when compared to the animals treated only with CCl₄ (group-II) after 36h of CCl₄ treatment. Overall, MEKB at tested doses significantly reduced the levels of hepatic enzymes and total bilirubin.
Table-1: Effects of MEKB on alternation of hepatic enzyme and serum bilirubin in rat after 36h. of CCl₄ treatment

<table>
<thead>
<tr>
<th>Groups (n=6)</th>
<th>Biochemical Parameters</th>
<th>Total Bilirubin (mg/dl)</th>
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<tbody>
<tr>
<td></td>
<td>SGOT (U/L)</td>
<td>SGPT (U/L)</td>
</tr>
<tr>
<td>Group-I (Normal Control)</td>
<td>28.27 ± 0.12**</td>
<td>17.24 ± 0.02**</td>
</tr>
<tr>
<td>Group-II (CCl₄: 1ml/kg)</td>
<td>60.45 ± 0.14</td>
<td>35.15 ± 0.12</td>
</tr>
<tr>
<td>Group-III (MEKB 200mg/kg)</td>
<td>42.19 ± 0.14**</td>
<td>30.46 ± 0.04**</td>
</tr>
<tr>
<td>Group-IV (MEKB 400mg/kg)</td>
<td>37.28 ±0.05**</td>
<td>23.54 ± 0.14**</td>
</tr>
<tr>
<td>Group-V (Silymarin 100mg/kg)</td>
<td>31.47 ±0.04**</td>
<td>21.33 ± 0.42**</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM of 6 rats in each group. ** p<0.001, as compared to CCl₄-treated group. SGOT = Serum glutamate oxaloacetate transaminase, SGPT = Serum glutamate pyruvate transaminase, ALP = Alkaline phosphatase, γ-GT = Gamma glutamyl transpeptidase, TP = Total proteins.

DISCUSSION AND CONCLUSION

Liver is the vital organ of metabolism and excretion. It produces and secretes bile; it also produces fibrinogen, prothrombin, heparin and sulfuric acid ester. The liver converts sugar into glycogen [18]. Any changes in anatomy or functions of liver are characterized by cirrhosis, jaundice, tumors, liver cell necrosis and hepatitis, metabolic and degenerative lesion etc. The management of hepatic diseases is still a challenge to the modern medicines [10,19]. Herbal medicines play a major role in the treatment of liver disorders. A number of medicinal plants and their formulations are widely used for the treatment of these disorders [20,21]. However, there were not enough scientific investigations on the hepatoprotective activities conferred to these plants. One of the plants from Indian flora is Kyllinga brevifolia. The present studies were performed to investigate the hepatoprotective activity of methanolic extract of whole plant Kyllinga brevifolia in rats against carbon tetrachloride as hepatotoxin to prove its claims in folklore practice against liver diseases.

Carbon tetrachloride (CCl₄) is one of the most commonly used hepatotoxins in the experimental study of liver diseases [22]. CCl₄ is potent hepatotoxin producing centrilobular hepatic necrosis. It is accumulated in hepatic parenchyma cells and metabolized to trichloromethyl free radicals (CCl₃.) by liver cytochrome P-450 dependent monooxygenases. This CCl₃ free radical combined with cellular lipids and proteins in the presence of oxygen to produce lipid peroxides [23]. Thus, antioxidant or free radical generation inhibition is important in protection against CCl₄ induced liver lesion [24]. The flavonoids constituents possess free radical scavenging properties [25].

In general, the extent of liver damage is assessed by histopathological evaluation and levels of hepatic enzymes such as ALP, SGOT, SGPT and also Bilirubin release in circulation [26,27]. The estimation of gamma glutamyl transpeptidase (γ-GT) is an important screening test with a high negative predictive value for hepatic disease [28].

Administration of hepatotoxins CCl₄ elevated the serum levels of SGOT, SGPT, ALP, γ-GT and bilirubin as well as decreases total serum proteins (TP) significantly [29,30]. The rise in serum enzymes level and bilirubin has been attributed to the damaged structural integrity of the liver, because they are cytoplasmic in location and released into circulation after cellular damages [31].
In our investigation, the biochemical changes were observed after 36h. of CCl₄ treatment. Thereby, it was found that the animal groups which are pretreated with MEKB at the dose of 200 and 400mg/kg (groups-III and IV) as well as silymarin at the dose of 100mg/kg (group-V) for three times at 12h. intervals, resulted in significantly decreases the hepatic enzymes such as SGOT, SGPT, ALP and γ-GT and also total bilirubin; as well as increases the total serum proteins (TP) as compared to animals treated only with CCl₄ (group-II). These results give us the suggestion that, the animals which are pretreated with MEKB as well as silymarin, showed a protection against the injurious effects of CCl₄ that may results from the interference with cytochrome P-450. These biochemical restoration may be due to the inhibitory effects on cytochrome P-450 or/and promotion of its glucuronidation [32,33]. Silymarin is a known hepatoprotective drug. It is reported to have a protective effect on the plasma membrane of hepatocytes [34].

It is well documented that the phytoconstituents comes under the category of flavonoids, alkaloids, glycosides, carotenoids, phenols, coumarins, lignans, essential oil, lipids, monoterpenes, xanthenes and organic acids are reported to have hepatoprotective activity [35].

Literature review revealed that various chemical investigations were carried out with this plant. William Carey Mamidipalli et al., have been reported the preliminary phytochemical screening of the methanolic extract of Antigonon leptopus revealed that presence of steroids, flavonoids, tannins, alkaloids and glycosides [36]. Mulabagal vanisree et al., have been reported that the purification of the methanolic extract yielded n-hentriacontane, ferulic acid, 4-hydroxycinnamic acid, quercetin-3-rhamnoside and kaempferol-3-glucoside; along with beta-sitosterol, beta-sitosterol-glucoside and d-mannitol [37]. The hepatoprotective activity of Kyllinga brevifolia may be attributed due to presence of these constituents. This study supports the traditional claims and the MEKB could be added in traditional preparations for the various liver diseases.

It is concluded from the data, that the methanolic extract of whole plant of Kyllinga brevifolia possesses significant hepatoprotective activity and may prove to be effective for the treatment of liver disorders. However, longer duration studies on chronic models are necessary to elucidate the exact mechanism of action so as to develop it as a potent hepatoprotective drug.

REFERENCES