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Evaluation of Hepatoprotective Effect of Hydroalcoholic Extract of Momordica Charantia Leaves in Carbon Tetrachloride-Induced Liver Toxicity in Wistar Rats

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Liver carries out a variety of physiological functions and protects against damaging drugs and chemicals. Herbs have been shown to play a major role in the management of various liver disorders. Due to the lack of effective liver protective medication in modern medicine, several herbal options for the treatment of liver diseases in Ayurveda are suggested. In this current study, we evaluated the hepatoprotective action of *Momordica charantia*leaf extract in comparison to Liv-52, a standard hepatoprotective drug.In Wistar rats, hepatotoxicity was induced by administering carbon tetrachloride (CCl_4) 1ml / kg body weight subcutaneously on alternate days for a week in a suspension of liquid paraffin.Rats were grouped into 5 groups with group I as control, group II - CCl_4 treatment only, group III receiving a mixture of Liv-52 orally (5 ml / kg) and CCl_4, and group IV and group V receiving Momordica charantia leaf extract administered orally to rats at doses of 100 and 200 mg / kg respectively, together with CCl_4 for 1 week.. Indices of liver functions (lipid profile) were evaluated in the serum of the rats. Animals were sacrificed after the study period and liver tissue was isolated for histopathological changes. The mean results for groupsI to Vfor SGOT levels in IU/L were:53.57±1.19, 167.72±5.57, 54.72 ±0.83, 69.41±2.35 and 60.72 ±1.5 respectively; for SGPT in IU/L were 37.00±1.77, 118.16±2.91, 61.41±1.25, 47.92±1.71and58.59±1.81 respectively; for ALPin IU/L were165.44± 4.84, 281.33±7.11, 206±6.95, 190.62±5.47and188.86±2.5 respectively and for totalbilirubinlevels in mg/dl were 0.71±0.66, 1.57± 0.1, 0.80 ±0.20.88±0.02&0.77±0.03respectively. The findings from this study showed a decrease in the liver enzymes and therefore suggests protective activityof Momordica *charantia* leaf extract against CCl_4 induced hepatic toxicity.

Keywords: CCl_4; Hepatotoxicity; Momordica charantia; Lipid profile.

The liver is the essential organ and has many critical functions, such as metabolism, excretion, detoxification and haematological functions (Fetal life).[1] Hepatic injury is linked with these functions being impaired. The liver is also an essential organ that regulates the homeostasis of the body.[2] It is involved in nearly all biochemical development processes, illness control, nutrient supply, power supply and reproduction.[3] Liver not only performs the above-mentioned physiological

functions but also protects against harmful drugs and chemicals. Thus, because of its strategic positioning in the body, the liver is continuously and variedly exposed to toxins.[4] Toxins from the intestinal tract first go to the liver resulting in a variety of liver ailments. Thus, liver ailment remains one of the most serious health problems. Despite tremendous scientific advancements in the field of hepatology in recent years, liver problems are on the rise. Jaundice and Hepatitis are the two major hepatic disorders which account for high death rates.[5]

Western medicine has little to give for hepatic disease therapy, and it is primarily plant-based preparation that is used to treat hepatic disorders.[6,7] Therefore, many folk remedies of plant origin are being evaluated for their possible hepatoprotective effects.

In ethnomedical practice, several medicinal plants and their preparations are used for hepatic diseases as well as the traditional system of medicine in India.[8] Herbs play a crucial role in managing different liver diseases. A variety of herbal preparations for hepatic disease treatment is suggested in Ayurveda due to the absence of secure and efficient hepatic protective medication in modern medicine.[9]

Momordica charantia (bitter melon) belongs to the family of Cucurbitaceae, which is a food as well as medicine. The plant possesses antimicrobial activity[10] and contains antioxidant compounds such as vitamin C, calcium, magnesium, sulphur and other trace elements.11 Although Momordica charantia has been commonly used as folk medicine and health food, no scientific research has been reported on its in-vivo antioxidant efficacy to hepatic damage caused by carbon tetrachloride.

Different chemical-induced hepatotoxicity models were commonly used to investigate drug and plant extract hepatoprotective effects. In this study, we assessed Momordica charantia's hepatoprotective effect against carbon tetrachloride-induced liver damage in Wistar rats.

MATERIALS AND METHODS

Experimental Animals

For evaluation of the hepatoprotective activity of Momordica charantia; Thirty (30) Wistar rats of both sexes weighing between 150-200 gm body weight were used. The animals were obtained from the central animal house of SVS Medical college. Rats were kept in polypropylene cages provided with paddy husk bedding. Free access to food (standard pellets) and water ad libitum was provided to them. Under these conditions, the animals were acclimatized for a week with 12/12 hours of light and dark cycle.

Materials

Carbon tetrachloride (CCI4) a hepatotoxic drug was obtained from Accord lab's Hyderabad. Liquid paraffin used as a vehicle for this study was obtained from Prem lab's Hyderabad, India.

Liv-52 Liver tonic was used as a standard control and was manufactured by Himalaya Drugs Company, Bangalore, India. The test drug, Momordica charantia leaf hydroalcoholic extract (MCLHE), was obtained from Chemiloids Company, Vijayawada, India.

CCl4 induced hepatotoxicity8

Liver toxicity was induced in rats by administering CCI4 subcutaneously in a suspension of liquid paraffin (LP: CCL4=1:2 v/v) at a dose of 1 ml per kg body weight on alternate days for 7 days.3,12

Experimental Design

Thirty (30) Wistar rats of either sex were divided into five (5) experimental groups (n=6 per group). Separate cages were allotted for each group and the cages were marked with the group number (I-V). The groups were as follows:

Group I: Rats were served as a control and got 1 ml/kg fluid paraffin (LP).

Group II: Ras received CCL4 (1:2 v/v).

Group III: Rats received Liv-52 orally (5 ml/kg) daily and CCL4.

Group IV and V: Extract of Momordica charantia (MCHLE) 100 and 200 mg/day respectively by oral route and CCL4.13,14

CCl4 administered in a suspension of liquid paraffin (1:2 v/v) at a dose of 1 ml per kg body weight. All the drugs administered via subcutaneous route for a week on alternative days.

Sample Collections

Blood samples from the retro-orbital plexus were collected on the eighth day using heparinised capillary tubes. After the study period, animals were sacrificed by cervical dislocation through an incision made on the jugular vein and the liver tissues were collected and preserved in

10% formalin for histopathological study.

Blood samples were centrifuged, serum was collected and used for the estimation of biochemical parameters such as glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT) by MOD IFCC method,15 serum alkaline phosphatase (ALP) by PNPP kinetic method and Total Bilirubin was estimated by Mod. Jendrassic and Groff's method.16 All the kits were manufactured by crest Biosystems Goa – 02, India.

RESULTS

In the present study, hydroalcoholic extract of Momordica charantia leaves was assessed for its hepatoprotective activity in carbon tetrachloride-induced hepatotoxicity in rats.

The serum glutamate oxaloacetate transaminase (SGOT) in control animals (Group I) varied from 50.11 – 58.11 with a mean of 53.57 ±1.19 IU/L. This increased to 167.72±5.57 IU/L after administration of CCL4 in Group II. In Group III, which received Liv-52 along with CCL4, the serum SGOT was reduced to 54.72 ±0.83 IU/L. Whereas, with groups IV and V receiving Momordica charantia in doses of 100 and 200 mg/kg respectively along with CCL4, serum SGOT decreased to 69.41 ± 2.35 and 60.72 ± 1.5 IU/L respectively. The effect of Momordica charantia in reducing the elevated levels SGOT as a result of CCL4 appeared to be slightly less than that of standard drug Liv-52.

The serum glutamate pyruvate transaminase (SGPT) in control animals varied from 31.01 – 41.30 IU/L with a mean value of 37.00 ± 1.77 IU/L. After administration of CCL4 to animals in group II, the SGPT raised to 118.16± 2.91 IU/L. In group III, the Liv-52 given to animals along with CCL4 reduced the SGPT levels to 61.41 ± 1.25 IU/L. The animals in groups IV and V receiving Momordica charantia in doses of 100

Table 1. Effect of Hydroalcoholic Extract of Momordica charantia leaves on serum enzymes and Total Bilirubin

GROUP (n=6)	SGOT (IU/L)	SGPT (IU/L)	ALP (IU/L)	Total bilirubin (IU/L)
Group I	53.57 ± 1.19	37.00 ± 1.77	165.44 ±4.84	0.71±4.84
Group II	167.72 ± 5.57	118.16 ± 2.91	281.33 ± 7.11	1.57 ±0.1
Group III	54.72±0.83	61.41 ± 1.25	206.00 ± 6.95	0.80 ± 0.2
Group IV	69.41 ± 2.35	47.92 ± 1.71	190.62 ± 5.47	0.88 ± 0.02
Group V	60.72 ± 1.5	58.59 ± 1.81	188.86 ± 2.53	0.77 ± 0.03

Data were presented as Mean ± SEM

Table 2. Post ANOVA least significant difference chart

Variables	D.F	t – value	Std. Error Difference	p-value
SGOT of Group III Vs II	10	20.46	5.63	0.001
SGPT of Group III Vs II	10	17.873	3.17	0.001
ALP of Group III Vs II	10	7.57	9.945	0.001
Total Bilirubin of Group III Vs II	10	0.804	0.112	0.001
SGOT of Group IV Vs II	10	16.245	6.051	0.001
SGPT of Group IV Vs II	10	20.75	3.383	0.001
ALP of Group IV Vs II	10	9.479	8.977	0.001
Total Bilirubin of Group IV Vs II	10	6.204	0.1118	0.01
SGOT of Group V Vs II	10	18.53	5.774	0.001
SGPT of Group V Vs II	10	17.33	3.435	0.001
ALP of Group V Vs II	10	12.245	7.551	0.001
Total Bilirubin of Group V Vs II	10	6.078	0.114	0.01

and 200 mg respectively, displayed reduced levels of SGPT to 47.92 ± 1.71 IU/L and 58.59 ± 1.81 IU/L respectively. When compared with Liv-52, Momordica charantia in both the test doses were found to be more effective in protecting the liver from CCL4 induced damage. Momordica charantia in a dose of 100 mg/kg was found to be more effective than 200 mg in reducing SGPT levels.

In the animals in Group I, the serum alkaline phosphatase (ALP) level was 165.44± 4.84 IU/L with a variation of 150.33 – 178.00 IU/L. CCL4 increased ALP levels to 281.33 ± 7.11 IU/L in animals in Group II. The level of ALP was reduced to 206.00± 6.95 IU/L in a Group III animals receiving Liv-52. The ALP levels were lowered to 190.62±5.47 and 188.86±2.5 IU/L in animals belonging to a Groups IV (100 mg/ kg Momordica charantia) and Group V (200 mg/ kg Momordica charantia). This suggests that the Momordica charantia's effect on ALP levels is slightly better than the effect of Liv-52.

The total bilirubin levels were elevated to 1.57 ± 0.1 mg/dl after CCL4 administration in Group II when compared to the normal value of 0.71+ 0.66 mg/dl in Group I animals. The Liv-52 and Momordica charantia (100 mg/kg and 200 mg /kg) groups reversed the elevated levels of total bilirubin to very close to normal values i.e. 0.80±0.2, 0.88 ± 0.02 & 0.77 ± 0.03 mg/dl respectively with p-values found to be significant, i.e. $p < 0.001$.

DISCUSSION

Carbon tetrachloride is an experimental hepatotoxicant that is commonly used. Carbon tetrachloride's mechanism of action is complicated, multifactorial and not fully understood. Carbon tetrachloride, which is metabolized to free radical CCL3, accumulates in hepatic parenchymal cells. CCl4 is biotransformed into the trichloromethyl-free radicle (CCL3) that triggers lipid peroxidation by CYP2E1, CYP2 and possible CYP3A mechanism. These free radicals respond with molecular oxygen to generate peroxy radicals (H2O2, O2 and OH- owing to the incomplete decrease of molecular oxygen), which cause oxidative destruction of endoplasmic reticulum membrane lipids that are rich in polyunsaturated fatty acids and therefore damage the liver.[17–19] Carbon tetrachloride generates dose-dependent hepatotoxicity, causing lipid peroxidation directly in the liver.[18,20]

One of the main causes of hepatoxicity is lipid peroxidant degradation of biomembranes.[21] An increase in the serum concentrations of glutamate pyruvate transaminase (GPT) in chronic hepatic necrosis is accompanied by a rise in the rate of glutamate dehydrogenase (GDH), which is indicative of mitochondrial liver injury.[22] This is demonstrated by an elevation of the serum marker enzymes SGOT, SGPT, ALP in CCL4 treated rats. When liver cell plasma membrane is damaged, a range of enzymes in the cytosol will release into the blood; their evaluation is useful quantitative markers of the extent and type of hepatic cell damage.

In the present study, treatment with different doses of Momordica charantia extract (100 mg, 200 mg/kg orally) significantly reversed all such elevated marker enzymes such as SGOT, SGPT ALP, and total bilirubin, and the results obtained were comparable to those of the Liv-52 group treated.

The decrease in total bilirubin after one week of Momordica charantia administration suggests that it enhances bilirubin clearance. The quicker regression of raised transaminase values due to the treatment of Momordica charantia (100 mg, 200 mg) also suggests its hepatoprotective activity.

Liv-52's protective effects have been shown earlier in reducing lipid peroxidation in hepatotoxic conditions and are attributed to the action of Liv-52 in reducing tocopherol levels.[23] Although there is insufficient information to determine the mechanism of action of Liv-52 protection, this may be due to its antiperoxidant activity, which is either dependent on reduced production of radical CCL4 derivatives or its antioxidant action.

Several scientific reports indicated certain flavonoids, ascorbic acid, phenols, beta carotene, caffiec acid, catechin, and gallic acid, have a protective effect on the liver due to their antioxidant properties.[24,25] Presence of those compounds in Momordica charantia may be responsible for the protective effects on CCL4 induced liver damage.

The outcome of our research indicates that Momordica charantia's hepatoprotective activity

may be similar to Liv-52 (l.o.c cit) owing to the reduced formation of CCL4 radical derivatives.

Momordica charantia is found to be hepatoprotective because it avoids lipid peroxidation or potentially irreversible binding of CCL4 to vital cellular proteins for its metabolism. In the present study, Momordica charantia leaf hydroalcoholic extract showed protection against CCL4 toxicity, as there is a significant reduction in all biochemical parameters. MCLHE 200mg / kg showed stronger hepatoprotective activity on CCL4-induced liver damage in rats than 100mg / kg. The plant extract's observed protective effects against carbon tetrachloride can be attributed to flavonoids, ascorbic acid, etc.

CONCLUSION

Carbon tetrachloride (CCL4) induces hepatic damage by causing lipid peroxidation due to its metabolite free radical CCL3. The Momordica charantia extract 200 mg/kg showed hepatoprotective activity in rats similar to Liv-52, probably because it contains flavonoids and other phytochemical agents. Further studies are needed to elicit the active component responsible for its hepatoprotective effect.

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