Research Paper

Antihepatotoxic effect of grape seed oil in rat

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ABSTRACT

Objectives: To study the effect of oral administration of grape seed oil (GSO) against carbontetrachloride (CCl_4)-induced hepatotoxicity in rats.

Methods: Liver damage was induced in male Wistar rats (150–250 g) by administering CCl_4 (0.5 ml/kg, i.p.) once per day for 7 days and the extent of damage was studied by assessing biochemical parameters such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) in serum and concentrations of malondialdehyde (MDA), hydroperoxides, glutathione (GSH), catalase (CAT), superoxide dismutase (SOD), and total protein (TP) in liver. The effect of co-administration of GSO (3.7 g/kg, orally) on the above parameters was further investigated and compared with a vitamin E (100 mg/kg, orally) treated group. Histopatholgical studies of the experimental animals were also done.

Results: Oral administration of GSO (3.7 g/kg, body weight orally) for 7 days resulted in a significant reduction in serum AST, ALT, and ALP levels and liver MDA and hydroperoxides and significant improvement in glutathione, SOD, CAT, and TP, when compared with CCl_4 damaged rats. The antioxidant effect of GSO at 3.7 g/kg for 7 days was found to be comparable with vitamin E (100 mg/kg, orally) in CCl_4-treated rats. Profound fatty degeneration, fibrosis, and necrosis observed in the hepatic architecture of CCl_4-treated rats were found to acquire near – normalcy in drug co-administered rats.

Conclusion: The GSO has protected the liver from CCl_4 damage. Probable mechanism of action may be due to the protection against oxidative damage produced by CCl_4.

KEY WORDS: Antihepatotoxic effect; glutathione; grape seed oil.

Grape seed oil (GSO) is obtained from grape seeds after the wine pressing in Italy and France. The GSO contains 75% linoleic acid, 15% oleic acid, 6% palmitic acid, 3% stearic acid, and 1% linolenic acid.[1] Studies revealed the beneficial HDL effect of GSO and research shows that subjects were instructed to use up to 45 ml of GSO in their daily diet as a substitute for their usual oil and within 2 weeks there was 13–14% increase in HDL level.[2] The GSO has a very high level of antioxidant vitamin E (60–120 mg/100 g), which makes the oil very stable. The antioxidant property is claimed to be the mechanism of hepatoprotective activity.[3] The GSO exhibits a variety of interesting properties such as reducing platelet aggregation, prevents hypertension caused by sodium excess, normalizes lesions occurring from obesity and diabetes.[4]

Among the various mechanisms involved in the hepatotoxic effect of carbontetrachloride (CCl_4), one is oxidative damage through free-radical generation[5] and antioxidant property is claimed to be one of the mechanisms of hepatoprotective effect of indigenous drugs.[6] The GSO has antioxidant properties.[2],[3] Hence, the objective of the study was to evaluate the effect of GSO on CCl_4-induced hepatotoxicity.

Materials and methods

Drugs and chemicals

The GSO is a kind gift from LoDuca Bros Inc., Milwaukee, USA. Carbontetrachloride (CCl_4) was obtained from E. Merck (India) Ltd., Mumbai. Thiobarbituric acid (TBA), 5,51-dithiobis-2-nitrobenzoic acid (DTNB), and glutathione (GSH) were obtained from Sigma, USA. Vitamin E was obtained from Hi Media Pvt., Ltd., Mumbai. All chemicals used in the study were of analytical grade.

Experimental animals

Male Wistar albino rats (150–250 g) were used. The animals were acclimatized to laboratory conditions for 5 days prior to the experiments and had access to food and water *ad libitum*. Before commencing the work, permission from Institutional Animal Ethics Committee was obtained.

Selection of dose of GSO

The human dose of GSO was converted in to the animal dose using the standard dose-converting table.[7] Further, the dose for the hepatoprotective studies was adjusted based on the observation during the toxicity studies. The GSO at a dose of 3.7 g/kg (4 ml/kg) was administered orally to study the hepatoprotective activity. An emulsion of GSO was prepared using 2% gum acacia by wet gum method.

Experimental design

Acute toxicity studies

Wistar Albino rats (150–250 g) maintained under standard laboratory conditions were used. A total of five animals

were used which received a single-oral dose (2000 mg/kg, body weight) of GSO. Animals were kept overnight fasting prior to drug administration. After the administration of GSO, food was with held for further 3–4 h. Animals were observed individually at least once during the first 30 min after dosing, periodically during the first 24 h (with special attention during the first 4 h) and daily thereafter for a period of 14 days. Once daily cage side observations included changes in skin and fur, eyes and mucous membrane (nasal), and also respiratory rate, circulatory (heart rate and blood pressure), autonomic (salivation, lacrimation, perspiration, piloerection urinary incontinence, and defecation), and central nervous system (ptosis, drowsiness, gait, tremors and convulsion)[8] changes.

Hepatoprotective studies

Animals were divided into five groups, consisting of six animals each. Group I served as control, which received 2% gum acacia orally for 7 days. Group II received GSO (3.7 g/kg, orally) for 7 days. Group III received CCl_4 0.5 ml/kg, i.p. for 7 days.[9]–[11] Group IV received CCl_4 0.5 ml/kg, i.p. and GSO (3.7 g/kg, orally) simultaneously for 7 days. Group V received $CC1_4$ 0.5 ml/kg, i.p. and vitamin E (100 mg/kg, orally)[12] simultaneously for 7 days. After 7 days of treatment, the rats were kept overnight fasting and killed by cervical dislocation. At the end of the treatment, blood samples were collected by direct cardiac puncture under ether anaesthesia and the serum was used for the assay of marker enzymes viz., aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP).[13],[14] The enzyme levels were assayed using the standard kits from Lupin laboratories. The results were expressed as units/liter (U/l). Liver samples were dissected out and washed immediately with ice-cold saline to remove as much blood as possible. Liver homogenates (5% w/v) were prepared in cold 50 mM potassium phosphate buffer (pH 7.4) using a Remi homogenizer. The unbroken cells and cell debris were removed by centrifugation at 1000 rpm for 10 min using a Remi C-24 refrigerated centrifuge. The supernatant was used for the estimation of GSH,[15] malondialdehyde (MDA),[16] hydroperoxides,[17] superoxide dismutase (SOD),[18] catalase (CAT)[19], and total protein (TP)[20] levels.

Histopathological studies

A portion of liver tissue in each group was preserved in 10% formaldehyde solution for histopathological studies. Haematoxylin and eosin were used for staining and later the microscopic slides of the liver cells were photographed.[21],[22]

Statistical analysis

Values were represented as mean±SD. Data were analyzed using one-way analysis of variance (ANOVA) and group means were compared using Duncan's multiple range test. P values <0.05 were considered significant.[23]

Results

In acute toxicity study, no signs, and symptoms of toxicity and mortality were observed. There was a significant ($P<0.05$) increase in the serum hepatic enzyme levels after CCl_4 treatment, which was prevented with GSO. The GSO when administered alone did not alter the enzyme levels when compared to the control values. The MDA and hydroperoxide levels were found to be elevated after the administration of CCl_4, which was significantly ($P<0.05$) prevented by GSO. There was a significant ($P<0.05$) rise in GSH, SOD and CAT contents of liver after treatment with GSO. There was a significant decrease in TP level after CCl_4 treatment, which was prevented with GSO (Table 1 and 2).

Table 1

Effect of GSO on serum ALT, AST, and ALP in rats after 7 days treatment

Groups	*ALT (U/l)*	*AST (U/l)*	*ALP (U/l)*
Control (2% gum acacia)	71.3±4.3*	286.3±1.7*	1101.3±6.5*
GSO (3.7 g/kg)	79.9±5.7*	295.6±3.0*	1195.0±4.5*
CCl_4 (0.5 ml/kg, i.p.)	259.6±2.4**	775.3±3.6**	1569.4±3.6**
CCl_4 + GSO (3.7 g/kg)	91.3±1.3***	312.8±3.0***	1224.3±2.6***
CCl_4 + VE (100 mg/kg)	84.3±7.3*,***	301.3±3.4*,***	1206.8±4.0*,***
F	58.3	117.9	245.9
d.f.	4, 25	4, 25	4, 25
P	<0.05	<0.05	<0.05

Values are mean±SD; n=6 in each group. Values with different superscripts (*. ** and ***) differ significantly from each other at $P<0.05$ (Duncan's multiple range test).

Histopathological examination

Histoathological examination of CCl_4-treated rat liver revealed fatty degeneration, necrosis, and fibrosis (Fig. 1). Concurrent administration of GSO preserved the histological structure of liver though there was mild congestion and regeneration of liver tissue (Fig. 2).

Discussion

The CCl_4 is one of the most commonly used hepatotoxins in the experimental study of liver diseases.[24] The lipid peroxidative degradation of biomembranes is one of the principal causes of hepatotoxicity of CCl_4.[25] This is evidenced by an elevation in the serum maker enzymes, namely AST, ALT, and ALP. The GSO has significantly reduced these liver enzyme levels. Further, GSO has increased the level of TPs, which indicates hepatoprotective activity. Stimulation of protein synthesis accelerates the regeneration process and the production of liver cells.

In our study, elevation in the levels of end products of lipid peroxidation in CCl_4-treated animals was observed. The increase in MDA and hydroperoxide levels in liver suggests enhanced lipid peroxidation leading to tissue damage and failure of antioxidant defence mechanisms. Treatment with GSO significantly prevented these changes. Hence, the mechanism of hepatoprotection of GSO may be due to its antioxidant effect. Since GSO has significantly increased the glutathione, SOD and CAT contents of the liver, it may also be useful in hepatotoxicity induced by other agents. The antioxidant enzyme levels of the CCl_4-treated group were decreased whereas that of GSO-treated group is almost similar to that of the control and vitamin-E-treated groups.

Histopathological studies showed that CCl_4 caused fatty degeneration and necrosis of the liver tissue. Pretreatment with GSO exhibited protection, which confirmed the results of

Figure 1. Liver tissue of CCl_4-treated animals showing fatty degeneration and necrosis (haematoxylin and eosin x 100)

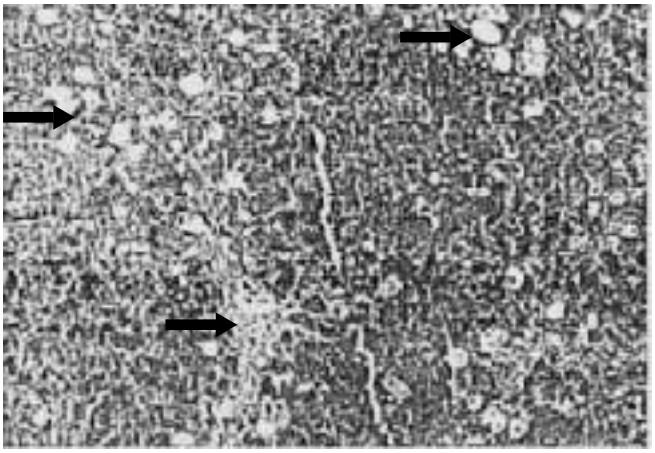

Figure 2. Liver tissue of rats treated with CCl_4 and GSO showing almost normal histology and mild congestion (haematoxylin and eosin x 100)

Table 2

Effect of GSO on liver MDA, hydroperoxides, GSH, SOD, CAT, and TP in rats after 7 days treatment

Groups	*MDA nmol/mg protein*	*Hydroperoxides mmol/100 g tissue*	*GSH mg/100 g tissue*	*SOD units/mg protein*	*CAT units/mg protein*	*Total mg/ml protein*
Control (2% gum acacia)	198.7 ± 2.2*	72.3 ± 3.1*	46.8 ± 1.8*	6.80 ± 0.25*	75.2 ± 1.46*	20.35 ± 1.34*
GSO (3.7 g/kg)	220.5 ± 5.7*	70.6 ± 6.6*	44.0 ± 2.2*	6.91 ± 0.22*	76.7 ± 1.13*	19.42 ± 3.55*
CCl_4 (0.5 ml/kg, i.p.)	461.1 ± 4.0**	97.6 ± 5.3**	20.8 ± 5.1**	3.41 ± 0.09**	44.9 ± 1.30**	9.10 ± 0.70**
CCl_4 + GSO (3.7 g/kg)	293.3 ± 2.9***	79.2 ± 2.8***	35.6 ± 4.5***	5.01 ± 0.34***	58.7 ± 2.94***	17.0 ± 0.16***
CCl_4 + VE (100 mg/kg)	236.3 ± 1.4*	75.4 ± 3.5*	41.3 ± 4.1*	6.48 ± 0.24*	69.9 ± 3.10*	19.36 ± 5.60*
F	81.9	12.4	22.4	4.67	11.12	3.47
d.f.	4, 25	4, 25	4, 25	4, 25	4, 25	4, 25
P	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05

Values are mean±SD; n=6 in each group. Values with different superscripts (*, ** and ***) differ significantly from each other at P<0.05 (Duncan's multiple range test).

biochemical studies. These results of our study indicate that simultaneous treatment with GSO protects the liver against CCl_4-induced hepatotoxicity.

The GSO offers vast possibilities in the treatment of various liver disorders. This may be due to the high level of antioxidant vitamin E, which was claimed to be the mechanism of hepatoprotection. Further studies on any other models and extensive clinical trials are needed to confirm these results.

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