Journal of Medical Sciences

ISSN 1682-4474

science alert

ANSInet
an open access publisher
http://ansinet.com

Research Paper

JMS (ISSN 1682-4474) is an International, peer-reviewed scientific journal that publishes original article in experimental & clinical medicine and related disciplines such as molecular biology, biochemistry, genetics, biophysics, bio-and medical technology. JMS is issued eight times per year on paper and in electronic format.

For further information about this article or if you need reprints, please contact:

Salim S. Al-Rejaie
Department of Pharmacology and Toxicology, College of Pharmacy, P.O. Box 2457, King Saud University, Riyadh 11451, Saudi Arabia

J. Med. Sci., 12 (1): 1-9
1st January, 2012
DOI: 10.3923/jms.2012.1.9

Effect of Oleo-gum-resin on Ethanol-induced Hepatotoxicity in Rats

Salim S. Al-Rejaie

Numerous plant resin content essential oils with terpenes and sesquiterpenes have shown hepatoprotective activity. The aim of the present study was to investigate the hepatoprotective activity of Oleo-gum-resin (OGR) in rats. The hepatoprotective activity of OGR was evaluated in rats by assessing the ethanol-induced oxidative stress estimating alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphates (ALP), acid phosphatase (ACP), triglycerides (TG) and Total Cholesterol (TC) in plasma. In liver, glutathione (GSH), malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT), nucleic acids (DNA and RNA), total protein, TG and TC levels were estimated. Enzymatic activities were increased in plasma after ethanol administration and that was dose dependently decreased in OGR treated alcoholic animals. Similar effect was seen in lipid and MDA levels. Nucleic acids, GSH levels, SOD and CAT activities decreased by alcohol and protected with OGR treatments in dose dependent manner. The observed attenuation to ethanol's effect may be related to the biochemical changes induced, possibly, under the influence of different constituents (cinnamaldehyde, eugenol and terpenoids) of the OGR. However, OGR can contribute to alleviate the adverse effect of ethanol ingestion by enhancing the lipid metabolism and the hepatic antioxidant defense system.

Key words: *Commiphora molmol*, oleo-gum-resin, ethanol, hepatic injury

ANSInet
Asian Network for Scientific Information

Department of Pharmacology and Toxicology, College of Pharmacy, P.O. Box 2457, King Saud University, Riyadh 11451, Saudi Arabia

INTRODUCTION

The OGR of *Commiphora molmol* (*C. molmol*) Engler (Burseraceae) known as 'mur' or 'myrrh' and used traditionally as a perfume and for the treatment of different diseases. Many reports have expressed the medicinal importance of the OGR as anti-inflammatory, anti-spasmodic, anti-rheumatics and anti-dormice (Evans, 1989). Experimentally, it has been proven as an anti-gastric ulcer (Al-Harbi *et al*., 1997) and has anti-oxidative, cytotoxic and non-mutagenic properties (Qureshi *et al*., 1993; Al-Harbi *et al*., 1994). Abdel-Aziz *et al*. (2006) reported that, OGR can improve the cellular immunity of schistosomiasis-induced infections in mice. Chemical analysis of OGR showed that it contains volatile oils (up to 17%), resins (up to 40%) and gum (up to 60%) (Brieskorn and Noble, 1982; Tripathi *et al*., 1984; Evans, 1989). Fractionation of the volatile oil revealed the presence of different terpenes, sesquiterpenes, esters, cinnamaldehyde, cuminaldehyde, cumic alcohol, eugenol, heerabolene, limonene, dipentene, pinene, m-cresol and cadinene. The resins contain α-, β-and γ-commiphoric acids, commiphorinic acid, α-and β-herrabomyrrhols, heeraboresene, commiferin, ketosteroids, campesterol, β-sitosterol, cholesterol, α-amyrone and 3-epi-α-amyrin. On hydrolysis, the gum yielded arabinose, galactose, xylose and 4-0-methylglucuronic acid (Brieskorn and Noble, 1982; Tripathi *et al*., 1984; Evans, 1989).

Significant antioxidant property was observed by several bioactive triterpenes, such as ursolic acid and oleanolic acid (Ramachandran *et al*., 2008; Rios *et al*., 2000). They were effective in protecting against chemically induced liver injury in laboratory animals and processed an anti-inflammatory and anti-hyperlipidemic property. These bioactive substances were proposed for skin cancer prevention since they were used in cosmetic products for many years and found to have less toxic potentials (Ramachandran *et al*., 2008; Rios *et al*., 2000). Indeed, acute toxicity test for OGR exhibited no visible signs of toxicity and no mortality was observed up to 3 g kg^{-1} (Shah *et al*., 1989). Eugenol, another OGR constituent, has been found to be cytotoxic in isolated rat hepatocytes (Thompson *et al*., 1991).

Alcohol abuse and alcoholism are serious current health and socioeconomic problems throughout world (Bunout, 1999; Singh *et al*., 2007). However, the development of suitable treatment of alcoholism remains a challenging aim for ethanol research (Bunout, 1999; McDonough, 2003; Singh *et al*., 2007). Ethanol is a fat-soluble non-electrolyte, which is readily absorbed from the skin and gastrointestinal tract, diffuses rapidly into circulation and distributed uniformly throughout the body (McDonough, 2003). Administration of ethanol chronically results in hepatic lipids accumulation as well as lipid peroxide which can also cause autooxidation of hepatic cells either by acting as a pro-oxidant or by reducing the antioxidant levels leading to a significant hepatotoxicity (Crawford and Blankenhorn, 1991). Hepatic oxidative stress of the lipid peroxidation by ethanol has been identified to play a pathogenic role in Alcoholic Liver Disease (ALD) (Bunout, 1999). In alcohol abusers, thiol groups are decreases and pro-oxidative enzymes increases (Ebuehi and Asonye, 2007; Prakash *et al*., 2008). It was reported that herbal supplementation are one of the alternatives to minimize diseases induced by alcoholism (Singh *et al*., 2007; Saka *et al*., 2011; Ogunlade *et al*., 2012; Baranisrinivasan *et al*., 2009). Thus, the present study aimed to investigate the effect of OGR on liver damage induced by ethanol and the possible mechanisms of OGR hepatoprotection using male Wistar albino rats as an animal model.

MATERIALS AND METHODS

Animals: Forty-eight male Wistar albino rats, home bred, roughly same age (8-weeks) and weighing 180-200 g were used in the present study. All animals were maintained under controlled conditions of temperature (22±1°C), humidity (50-55%) and light (12 h dark and 12 h light). Animals were provided free access to Purina rat chow (Manufactured by Grain Silos and Flour Mills Organization, Riyadh, Saudi Arabia) and water. All experimental procedures including euthanasia were conducted in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals, Institute for Laboratory Animal Research (NIH Publications No. 80-23; 1996) as well as the Ethical Guidelines of the Experimental Animal Care Center, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia.

Plant material: The authenticated OGR of *C. molmol* was purchased from the local market in Riyadh, Saudi Arabia. For the purpose of recoding, the voucher specimen of the OGR was kept in the herbarium, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia.

Phytochemical screening: Chemical tests were carried out on the aqueous extract using standard procedure to identify the constituents (Sofowora, 1982; AOAC, 1990). Essential oil obtained by steam distillation (23.17%) from that volatile oil was yield 9.72%. The presence of terpenes, sesquiterpenes, easter, cuminic, aldehyde and eugenol was identified.

Experimental procedure: The animals were randomly divided into eight groups by taking six rats in each group: (1) Control (vehicle), (2) OGR (125 mg kg^{-1}), (3) OGR (250 mg kg^{-1}), (4) OGR (500 mg kg^{-1}), (5) Ethanol (5 g kg^{-1}), (6) OGR (125 mg kg^{-1})+Ethanol (5 g kg^{-1}), (7) OGR (250 mg kg^{-1})+Ethanol (5 g kg^{-1}) and (8) OGR (500 mg kg^{-1})+Ethanol (5 g kg^{-1}). The selection of the higher dose (500 mg kg^{-1}) was based from other publication (Al-Harbi *et al*., 1994; Al-Harbi *et al*., 1997; Rao *et al*., 2001).

Every day morning, required doses of OGR were prepared by suspending crushed powder in distilled water and administered orally (gavage) in between 9 to 10 a.m. to the rats for five consecutive weeks. Ethanol dose (25% v/v; 5 g kg^{-1} body weight) was calculated according the body weight and administered (gavage) daily one hour after the each drug treatment (Lee, 2004; Alsaif, 2007). All animals were kept with free access to rat pellet and drinking water during the entire experimental period. Body weight of each animal was recorded every week on Saturday before treatments start. At the end of the treatment period, animals were slightly anaesthetized by using ether, blood samples were collected through cardiac puncture in heparin containing tubes. The blood samples were centrifuged at 3000 rpm for 10 min and the suppurated plasma samples were labeled and kept in freezer at -20°C till analysis. Immediately, whole liver was dissected, weighed, dipped in liquid nitrogen for a minute and then kept in ultra-deep freezer at -70°C till analysis.

Enzymatic assays: In plasma, AST, ALT, ACP, ALP, were estimated in plasma by using commercially available diagnostic kits (Randox diagnostic reagents, Randox Laboratories, USA).

Plasma and hepatic lipid analyses: The plasma total cholesterol and triglycerides levels were determined by using commercially available enzymatic kits (Randox diagnostic reagents, Randox Laboratories, USA). The hepatic lipids were extracted using the procedure of Folch *et al.* (1957). The dried lipid residues were dissolved in 1 mL ethanol. Triton X-100 and sodium cholate solutions (in distilled water) were added to 200 μL of the dissolved lipid solution to produce a final concentration of 5 g L^{-1} and 5 mmol L^{-1}, respectively. The hepatic cholesterol and triglycerides were analyzed with the same enzymatic kit (Randox diagnostic reagents, Randox Laboratories, USA).

Estimation of malondialdehyde (MDA) in liver: The method described by Ohkawa *et al.* (1979) was used for MDA analysis. Briefly, liver tissues (200 mg) were homogenized in aqueous 0.15 M KCl solution and 1 mL of homogenate was mixed with 1 mL of 10% TCA and centrifuged at 3,000 rpm for 15 min. One milliliter supernatant was mixed with 1 mL of 0.67% 2-thiobarbituric acid then placed the tubes at boiling water bath for 15 min. Optical density of the clear pink supernatants was measured at 532 nm.

Estimation of glutathione (GSH) in liver: The concentration of GSH was measured using the method described by Sedlak and Lindsay (1968). Liver tissues (around 200 mg) were homogenized in ice-cold 0.02 M ethylenediaminetetraacetic acid (EDTA), 0.5 mL homogenate was mixed with 0.2 M tris buffer, pH 8.2 and 0.1 mL of 0.01 M Ellman's reagent, [5,5'-dithiobis-(2-nitro-benzoic acid)] (DTNB). Each sample tube was centrifuged at 3000 g at room temperature for 15 min. The absorbance of the clear supernatants was measured using spectrophotometer at 412 nm in one centimeter quarts cells.

Estimation of superoxide dismutase (SOD) activity in liver: SOD activity in liver tissues was assayed using the method described by Kakkar *et al.* (1984) with the aid of nitroblue tetrazolium as the indicator. Liver tissues (200 mg) were homogenized with 10 times (w/v) 0.1 sodium phosphate buffer (pH 7.4). The reagents: sodium pyrophosphate buffer 1.2 mL (0.052 M) pH 8.3, 0.1 mL phenazine methosulphate (186 μM), 0.3 mL nitro blue tetrazolium (300 μM) and 0.2 mL NADH (780 μM) were added to 0.1 mL of processed tissue sample. The sample mixture was incubated for 90 min at 30°C. Four mL of n-butanol and one mL of acetic acid were then added to each sample and the mixture was shaken vigorously. Samples were centrifuged at 4000 rpm for 10 min and the organic layer was withdrawn and absorbance was measured at 560 nm using a spectrophotometer (LKB-Pharmacia, Mark II, Ireland).

Estimation of catalase (CAT) activity in live: The CAT activity was measured by the method of Aebi (1983) using hydrogen peroxide as substrate. The disappearance of H_2O_2 was measured at 240 nm. The activity was expressed as μmole min^{-1} mg^{-1} protein using the extension coefficient of 0.0436 mM mg^{-1}. To elaborate, CAT exerts a dual-function decomposition of H_2O_2 to give H_2O and O_2 and oxidation of H donors. In the ultraviolet range, H_2O_2 shows a continual increase in absorption with decreasing wavelength. The decomposition of H_2O_2 can be followed directly by the decrease in absorbance at 240 nm. The difference in absorbance per unit time is a

measure of CAT activity. Liver tissues (200 mg) were homogenized in 8 mL of 0.05 M phosphate buffer at pH 7.0. The tissue homogenates were centrifuged at 4°C for 15 min at 1500 g. The supernatants were removed into separate test tubes and kept on ice until the enzyme assay. Sample was measured against a blank containing 2.8 mL (1:500 v/v) phosphate buffer instead of H_2O_2 (30 mM hydrogen peroxide) and 0.2 mL enzyme solution. The reaction was started by addition of H_2O_2. The initial absorbance should be A = 0.500 followed by the decrease in absorbance for about 30 sec.

Estimation of total proteins and nucleic acids in liver: Total proteins were estimated by the modified Lowry method of Schacterle and Pollack (1973). Bovine plasma albumin was used as standard. The method described by Bregman (1983) was used to determine the levels of nucleic acids (DNA and RNA). Liver tissues (1 g) were homogenized in ice-cold distilled water and the homogenates were suspended in 10% ice-cold trichloroacetic acid (TCA). Pellets were extracted with 95% ethanol twice. DNA levels were determined by treating the nucleic acid extract with diphenylamine reagent and measuring the intensity of blue color at 600 nm. For quantification of RNA, the nucleic acid extract was treated with orcinol reagent and the green color was recorded at 660 nm on spectrophotometer (LKB-Pharmacia, Mark II, Ireland).

Statistical analysis: All data were presented as the Mean±Standard Error of the Mean (SEM). The data were evaluated by a one-way ANOVA using SPSS program and the differences between the means were assessed using Student Newman-Keuls. The differences were considered statistically significant at $p<0.05$.

RESULTS

Mean body and liver weights were not significantly altered in groups treated with different doses (125, 250 and 500 mg kg^{-1} day^{-1}) of OGR as compared to controls. Ethanol treatment for 5 consecutive weeks significantly ($p<0.01$) decreased the body weights and increased ($p<0.001$) the liver weight compared to control group. OGR treatment at high dose (500 mg kg^{-1} day^{-1}) showed significant ($p<0.05$) increase in body weights in animals that received ethanol in compared to animals received ethanol alone. Ethanol increased liver weights were significantly decreased by OGR treatments in a relative dose-dependent manner (Table 1).

The enzymatic activities of ALT, AST, ALP and ACP in control and treated with different doses of OGR alone groups found similar. All the above enzymes were significantly ($p<0.001$) increased by ethanol treatment as compared to controls. The increase in AST activity by ethanol was significantly and dose-dependently inhibited by OGR. The ethanol-induced increase in ALT, ALP and ACP was significantly reduced by high doses (250 and 500 mg kg^{-1} day^{-1}) of OGR treatments, respectively (Table 2).

Table 1: Effect of OGR (125, 250 and 500 mg kg^{-1} day^{-1}) on body and liver weights in ethanol (5 g kg^{-1} day^{-1}) administered rats

Oral dose of OGR (gavage; mg kg^{-1} day^{-1})	Body weight (g)	Liver weight g/100 g b.wt.
Control (Vehicle)	163.3±5.2	4.0±0.1
OGR (125)	157.4±7.2	3.8±0.2
OGR (250)	174.6±6.4	3.5±0.3
OGR (500)	166.4±7.1	3.7±0.3
Ethanol (5 g kg^{-1} day^{-1})	123.8±8.7**a	5.1±0.2***a
OGR (125)+Ethanol	127.4±5.7	4.5±0.1*b
OGR (250)+Ethanol	142.7±7.9	3.5±0.4**b
OGR (500)+Ethanol	154.2±5.6*b	3.6±0.2**b

a: Ethanol group was statistically compared to control groups. b: OGR treated groups were statistically compared to ethanol treated group. *$p<0.05$, **$p<0.01$ and ***$p<0.001$. Values were expressed as Mean±SEM and analyzed using one-way ANOVA followed by Student Newman-Keuls method as post hoc test. Six rats were used in each group

The lipids levels in plasma and hepatic cells were not significantly affected by the different doses of OGR compared to the vehicle group. The total cholesterol and triglycerides concentrations in plasma were significantly ($p<0.001$) increased in ethanol treated animals compared to control rats. Similar increase was found in hepatic lipids of rats supplemented with ethanol. The high doses (250 and 500 mg kg^{-1}) of OGR suppressed the increase in plasma cholesterol and triglycerides ($p<0.01$ and $p<0.001$, respectively) induced by ethanol. Higher doses (250 and 500 mg kg^{-1} day^{-1}) of OGR treatments to ethanol treated rats also significantly attenuated ethanol effect on hepatic cholesterol ($p<0.05$ and $p<0.001$) and on triglycerides ($p<0.05$ and $p<0.01$), respectively (Table 3).

The concentrations of hepatic GSH and MDA in OGR (125, 250, 500 mg kg^{-1} day^{-1}) treated rats showed no significant alterations as compared to control group. In liver, the GSH levels found decrease while MDA levels increase significantly ($p<0.001$) in ethanol administered rats. OGR treatment with the doses of 125, 250 and 500 mg kg^{-1} day^{-1} significantly inhibited these changes induced by ethanol in a dose dependent manner ($p<0.05$, $p<0.01$ and $p<0.001$), respectively (Table 4).

OGR treatments with different doses to normal rats for 5 weeks did not cause any significant changes in liver SOD and CAT activities while compared to control group of rats. However, SOD and CAT activities in hepatic cells of the ethanol-treated animals were significantly ($p<0.01$ and $p<0.001$, respectively) lowered compared to control animals (Table 5). Higher doses (250 and

Table 2: Effect of OGR (125, 250 and 500 mg kg^{-1} day^{-1}) on serum AST, ALT, ALP and ACP in ethanol (5 g kg^{-1} day^{-1}) administered rats

Oral dose of OGR (gavage; mg kg^{-1} day^{-1})	AST (U L^{-1})	ALT (U L^{-1})	ALP (U L^{-1})	ACP (U L^{-1})
Control (Vehicle)	60.2±5.9	24.4±2.2	328.1±18.1	95.4±5.4
OGR (125)	72.5±7.2	23.5±2.6	341.3±17.6	102.3±6.9
OGR(250)	68.4±6.4	21.8±3.1	336±14.7	89.6±7.5
OGR (500)	59.1±5.7	24.2±3.4	354±17.5	92.7±6.7
Ethanol (5 g kg^{-1} day^{-1})	124.5±7.9*** a	65.4±6.7***a	487.9±23.1***a	141.3±6.1***a
OGR (125)+Ethanol	95.9±6.8*b	56.4±9.3	423.7±29.5	128.1±3.4
OGR (250)+Ethanol	87.8±6.5**b	39.2±2.9*b	371.5±23.7**b	117.2±4.9*b
OGR (500)+Ethanol	72.5±4.7***b	32.1±2.7***b	341.3±17.7***b	102.4±3.9***b

a: Ethanol group was statistically compared to control groups. b: OGR treated groups were statistically compared to ethanol treated group. *p<0.05, **p<0.01 and ***p<0.001. Values were expressed as Mean±SEM and analyzed using one-way ANOVA followed by Student Newman-Keuls method as post hoc test. Six rats were used in each group

Table 3: Effect of OGR (125, 250 and 500 mg kg^{-1} day^{-1}) on serum and hepatic lipids (total cholesterol and triglycerides) levels in ethanol administered rats

Oral dose of OGR (gavage; mg kg^{-1} day^{-1})	Serum lipids		Hepatic lipids	
	Total cholesterol (mmol L^{-1})	Triglycerides (mmol L^{-1})	Total cholesterol (mmol g^{-1})	Triglycerides (mmol g^{-1})
Control(Vehicle)	3.0±0.1	1.8±0.1	0.2±0.1	0.4±0.1
OGR (125)	2.9±0.2	1.7±0.2	0.25±0.1	0.4±0.1
OGR (250)	3.1±0.2	1.6±0.2	0.2±0.1	0.4±0.1
OGR (500)	2.8±0.4	1.7±0.2	0.2±0.2	0.4±0.1
Ethanol (5 g kg^{-1} day^{-1})	4.2±0.1*** a	3.1±0.2*** a	0.4±0.1*** a	0.6±0.1** a
OGR (125)+Ethanol	3.9±0.1	2.4±0.3* b	0.2±0.1	0.6±0.1
OGR (250)+Ethanol	3.7±0.1**b	2.1±0.2**b	0.3±0.1*b	0.43±0.1*b
OGR (500)+Ethanol	3.2±0.1*** b	1.6±0.2*** b	0.2±0.1*** b	0.4 ± 0.1**b

a: Ethanol group was statistically compared to control groups. b: OGR treated groups were statistically compared to ethanol treated group. *p<0.05, **p<0.01 and ***p<0.001. Values were expressed as Mean ±SEM and analyzed using one-way ANOVA followed by Student Newman-Keuls method as post hoc test. Six rats were used in each group

Table 4: Effect of OGR (125, 250 and 500 mg kg^{-1} day^{-1}) on hepatic GSH and MDA concentrations of normal and ethanol (5 g kg^{-1} day^{-1}) administered rats

Oral dose of OGR (gavage; mg kg^{-1} day^{-1})	GSH (nmol/100 mg wet tissue)	MDA (nmol g^{-1} wet tissue)
Control(vehicle)	193.9±6.3	256.4±11.9
OGR (125)	188.4±7.24	267.3±12.5
OGR (250)	201.5±10.2	270.4±9.8
OGR (500)	198.7±9.8	264.2±8.7
Ethanol (5 g kg^{-1} day^{-1})	133.1±5.6*** a	384.6±14.9*** a
OGR (125) + Ethanol	153.3±5.6* b	324.8±17.3* b
OGR (250) + Ethanol	177.2±6.5** b	310.5±13.9** b
OGR (500) + Ethanol	189.2±5.8*** b	278.6±14.3*** b

a: Ethanol group was statistically compared to control groups. b: OGR treated groups were statistically compared to ethanol treated group. *p<0.05, **p<0.01 and ***p<0.001. Values were expressed as Mean±SEM and analyzed using one-way ANOVA followed Student Newman-Keuls method as post hoc test. Six rats were used in each group

Table 5: Effect of OGR (125, 250 and 500 mg kg^{-1} day^{-1}) on hepatic SOD and CAT activity of normal and ethanol (5 g kg^{-1} day^{-1}) administered rats

Oral dose of OGR (gavage; mg kg^{-1} day^{-1})	Liver SOD (U mg^{-1} of proteins)	Liver CAT (µmole/min/mg of proteins)
Control(Vehicle)	12.9±0.7	519.3±13.3
OGR (125)	13.4±1.0	546.4±18.7
OGR (250)	12.6±0.7	537.6±19.2
OGR (500)	12.7±0.6	528.1±15.4
Ethanol (5 g kg^{-1} day^{-1})	9.5±0.6** a	413.2±17.2*** a
OGR (125)+Ethanol	10.9±0.8	453.9±12.3
OGR (250)+Ethanol	11.9±0.7** b	487.4±11.8** b
OGR (500)+Ethanol	13.0±0.1*** b	507.2±14.7*** b

a: Ethanol group was statistically compared to control groups. b: OGR treated groups were statistically compared to ethanol treated group. *p<0.05, **p<0.01 and ***p<0.001. Values were expressed as Mean±SEM and analyzed using one-way ANOVA followed by Student Newman-Keuls method as post hoc test. Six rats were used in each group

500 mg kg^{-1} day^{-1}) treatments of OGR to ethanol-treated rat bring back the SOD and CAT activities to normal respectively (Table 5).

Nucleic acids (DNA and RNA) and total protein in liver of rats treated with different doses of OGR suspension remained same as controls. Ethanol treatment for five consecutive weeks induced significant (p<0.001) reduction in hepatic DNA and RNA concentration as compared to the animals in control group. This decrease was significantly protected by the higher doses (250 and 500 mg kg^{-1}) of OGR (p<0.01 and p<0.001), respectively. Total hepatic protein levels was also significantly (p<0.01) decreased by ethanol treatment. OGR administration at doses 250 and 500 mg kg^{-1} to ethanol-treated animals showed significant (p<0.05) increase in total protein

Table 6: Effect of OGR (125, 250 and 500 mg kg^{-1} day^{-1}) on hepatic nucleic acids (DNA and RNA) and total protein levels of normal and ethanol (5 g kg^{-1} day^{-1}) administered rats

Oral dose of OGR (gavage; mg kg^{-1} day^{-1})	DNA (μg/100 mg)	RNA (μg/100 mg)	Total protein (mg/100 mg)
Control (Vehicle)	210.4±8.6	643.2±15.2	16.6±0.6
OGR (125)	217.1±7.2	651.3±21.4	15.9±0.5
OGR (250)	198.2±8.1	621.4±23.5	16.2±0.7
OGR (500)	224.5±9.4	633.3±24.5	16.0±0.6
Ethanol (5 g kg^{-1} day^{-1})	148.2±4.9***a	502.89±19.8***a	13.1±0.8**a
OGR (125)+Ethanol	164.6±8.4	546.4±39.2	14.9±0.7
OGR (250)+Ethanol	181.1±6.7**b	589.7±15.5**b	15.6±0.6*b
OGR (500)+Ethanol	204.6±8.4***b	616.4±39.2***b	15.9±0.7*b

a: Ethanol group was statistically compared to control groups. b: OGR treated groups were statistically compared to ethanol treated group. *$p<0.05$, **$p<0.01$ and ***$p<0.001$. Values were expressed as Mean±SEM and analyzed using one-way ANOVA followed by Student Newman-Keuls method as post hoc test. Six rats were used in each group

concentrations as compared to the animals administered with ethanol alone (Table 6).

DISCUSSION

The present study was designed to investigate the possible preventive effect of OGR on oxidative hepatic damage following ethanol exposure using male Wistar albino rats as an animal model. Most of the alcohol related diseases including cancer, hepatotoxicity, myopathy and cerebellar atrophy induces oxidative stress (Ishii *et al*., 1997). These findings are justified several epidemiological evidences that ethanol has linked as a risk factor for Alcoholic Liver Disease (ALD), which may cause fatty liver, hepatitis, fibrosis, cirrhosis and hepatocellular carcinoma (Corrao *et al*., 2004; Russo *et al*., 2004). Several reports have been conformed that the critical role of oxidative stress to induced pathological changes for developing ALD (Bailey and Cunningham, 2002; Corrao *et al*., 2004).

In present study, ethanol ingestion (5 g kg^{-1} day^{-1}), decreased the body weights and increased the liver weights, similar results were reported in earlier studies. (Tadic *et al*., 2002). Higher dose of OGR (500 mg kg^{-1} day^{-1}) significantly increased the body weight while liver weights were protected in dose dependent manner. In present study, ethanol-induced changes on oxidative biomarkers are similar as described before (Crawford and Blankenhorn, 1991; Bunout, 1999; Albano, 2006). Herbal supplementations are one of the alternatives to minimize alcohol-induced diseases (Singh *et al*., 2007). Indeed, OGR in the current study dose-dependently attenuated ethanol-induced increase in the activity of intra-cellular enzymes (AST and ALT). Levels for plasma ALT are frequently used as a laboratory index for hepatotoxicity (Amacher, 1998; Sheweita *et al*., 2001). AST levels, on the other hand, are considered a less specific biomarker for liver function (Sheweita *et al*., 2001; Zeng *et al*., 2008). Ethanol-induced oxidative stress may have damaged hepatocellular biomembrane and the subcellular liver organelles including mitochondria, which possibly led to the elevation of ALT and AST (Amacher, 1998; Sheweita *et al*., 2001; Zeng *et al*., 2008). Our results have also shown the significant increase in the activities of both ACP and ALP. This may be due to some alteration in lysosomal enzyme activities (Handa and Sharma, 1990). Furthermore, ethanol-induced increase in the activity of plasma ACP and ALP were inhibited by OGR in the current study. ACP and ALP are distributed in the lysosomal fraction of cells and considered an indicator for toxicity in metabolic organs (Handa and Sharma, 1990). Overall, OGR may have normalized the activity of ALT, AST, ACP and ALP in the ethanol treated groups and thus protected the hepatic tissues from ethanol-induced injury.

Chronic consumption of ethanol causes the accumulation of lipids in the liver and lipid peroxide in other tissues (Bunout, 1999). In the present study, the plasma lipids (total cholesterol and triglycerides) were significantly increased by ethanol supplementation in accordance with other reports (Lee, 2004). However, the plasma total cholesterol levels were lowered by the OGR supplement compared to the ethanol alone treated rats. Indeed, the regulating property as a hypolipidemic of *Commiphora mukul* (Guggul), a close relative to OGR, was discovered by earlier reports (Malhotra *et al*., 1977; Nityanand *et al*., 1989).

In this study, alcohol treatment had reduced levels of liver GSH and reduced the activity of SOD and CAT as compared to the experimental control, which corresponds to the results reported by Lee (2004). However, OGR dramatically restores the levels of GSH, SOD and CAT, which were inhibited by ethanol. The liver tissue harbours a host of antioxidant defense mechanisms to prevent free radical-induced cellular damage (Zeng *et al*., 2008). These defense system include the non-enzymatic (most abundantly GSH) and enzymatic antioxidant defenses (SOD and CAT) (Wu *et al*., 2004; Zeng *et al*., 2008). In addition, GSH plays an important role in the antioxidant defense system, metabolism of nutrient and regulation of cellular events (Wu *et al*., 2004). Through non-enzymatic and enzymatic process, GSH scavenges free radicals and other oxygen species (e.g., hydroxyl radical, lipid peroxyl radical, peroxynitrite and H_2O_2) (Fang *et al*., 2002). Ethanol (4 g kg^{-1} body weight) was found to decrease the hepatic GSH content five hours after 35% ethanol administration (Speisky *et al*., 1985). Recently, Vogt and Richie (2007)

have showed that ethanol (5 mg kg^{-1} b.wt.) significantly decreased GSH two and 6 h after exposure, which was recovered to the normal level after 24 h.

In ethanol supplemented rats, SOD and CAT activities were significantly lowered in present study and that is in agreement with earlier reports (Jurczuk *et al*., 2004; Nencini *et al*., 2010). It is known that the SOD and CAT help to scavenge superoxide ions and hydroxyl ions, respectively (Antonenkov and Panchenko, 1988; Reddy and Lokesh, 1992; Sheela and Augusti, 1992). Lowered activities of SOD and CAT would result in the accumulation of highly reactive free radicals, such as superoxide (O_2^-) and hydroxyl radical, leading to deleterious effects such as loss of cell membrane integrity and membrane function (Reddy and Lokesh, 1992). Indeed, Antonenkov and Panchenko (1988) also reported that hepatic SOD and CAT activity were evidently decreased in ethanol-fed rats. Such decrease in SOD activity may be linked with the elevation of the intracellular concentration of H_2O_2 as a result of CAT inactivation (Antonenkov and Panchenko, 1988). It has been reported that CAT may to be responsible for the detoxification of H_2O_2, which is an effective inhibitor of SOD (De Duve and Baudhuin, 1996). Our results showed that administration of OGR to ethanol treated animals significantly elevated SOD and CAT activities as compared with those in ethanol treatment alone. Thus, it is possible that these antioxidant enzymes as well as the above mentioned GSH work synergistically following OGR administration to cope with the oxidative stress induced by ethanol.

In the present study, ethanol treatment significantly increased the MDA levels and reduced protein and nucleic acid concentrations in hepatic tissues. Ethanol is metabolized in the body to produce free radicals (Halliwell, 1991). OGR pretreatment provided a relatively dose-dependent protection against the action of ethanol on nucleic acids and protein contents and significantly and dose-dependently reduced MDA concentrations. This may indicate the presence of some phytoconstituents with antioxidative nature in it. MDA has long been used as a particular biomarker of oxidative stress (Mates *et al*., 1999; Lykkesfeldt, 2007) and any increase of MDA may reflect the enhancement of the chain reactions for Lipid Peroxidation (LPO). LPO is initiated following Reactive Oxygen Species (ROS) attack on the poly-unsaturated fatty acid in the biomembrane, which ultimately leads to dysfunction of biomembrane and damage to membrane enzymes (Zeng *et al*., 2008). Antioxidants are well known to play a major role in the protection of cellular damage by scavenging free radical formation (Albano, 2006). Therefore, the cytoprotective and antioxidative effect of OGR in the present study was possibly attributed to the presence of cinnamaldehyde, eugenol and some terpenoids (Pettit, 1978; Akira *et al*., 1986; Shah *et al*., 1989). These reported antioxidative constituents may, at least in part, be responsible for the observed protection by pretreatment with OGR.

In conclusion, ethanol-induced oxidative stress led to liver dysfunction, which was dramatically attenuated by OGR pretreatment. OGR restored the hepatic activates of ALT, AST, ALP, ACP, SOD and CAT as well as the levels of lipids, MDA, DNA, RNA and protein possibly leading to its preventive activity. However, although detoxification action of OGR was clearly beneficial for ethanol-treated rats, the detoxification mechanism at the pharmacological and biochemical level still needs to be elucidated; therefore, further studies to identify the effective components of the this resin appear be warranted.

ACKNOWLEDGMENTS

This study was funded by the Deanship of Scientific Research at King Saud University through the research group project No. (RGP-VPP-142). This work was partially supported by Global Research Network for Medicinal Plants (GRNMP) and King Saud University.

REFERENCES

AOAC, 1990. Official Methods of Analysis of the Association of Official Analytical Chemists. Association of Official Analytical Chemists, Washington, USA.

Abdel-Aziz, M.M., A.T. Abbas, K.A. Elbakry, E.A. Toson and M. El-Sherbiny, 2006. Immune response on mice infected with *Schistosoma mansoni* and treated with myrrh. J. Medical Sci., 6: 858-861.

Aebi, H., 1983. Catalase. In: Methods of Enzymatic Analysis, Bergmeyer, H. (Ed.). Verlag Chemie, Weinheim, Gemany, pp: 273-277.

Akira, T., S. Tanaka and M. Tabata, 1986. Pharmacological studies on the antiulcerogenic activity of Chinese cinnamon. Planta Med., 52: 440-443.

Al-Harbi, M.M., S. Qureshi, M.M. Ahmed, S. Rafatullah and A.H. Shah, 1994. Effect of *Commiphora molmol* (Oleo-gum-resin) on the cytological and biochemical changes induced by cyclophosphamide in mice. Am. J. Chin. Med., 32: 77-82.

Al-Harbi, M.M., S. Qureshi, M. Raza, M.M. Ahmed, M. Afzal and A.M. Shah, 1997. Gastric antiulcer and cytoprotective effect of *Commiphora molmol* in rats. J. Ethanolpharmacol., 55: 141-150.

Albano, E., 2006. Alcohol, oxidative stress and free radical damage. Proc. Nutr. Soc., 65: 278-290.

Alsaif, M.A., 2007. Effect of thymoquinone on ethanol-induced hepatotoxicity in Wistar rats. J. Med. Sci., 7: 1164-1170.

Amacher, D.E., 1998. Serum transaminase elevations as indicators of hepatic injury following the administration of drugs. Regul. Toxicol. Pharmacol., 27: 119-130.

Antonenkov, V.D. and L.F. Panchenko, 1988. Effect of chronic ethanol treatment under partial catalase inhibition on the activity of enzymes related to peroxide metabolism in rat liver and heart. Int. J. Biochem., 20: 823-828.

Bailey, S.M. and C.C. Cunningham, 2002. Contribution of mitochondria to oxidative stress associated with alcoholic liver disease. Free Radical Biol. Med., 32: 11-16.

Baranisrinivasan, P., E.K. Elumalai, C. Sivakumar, S. Viviyan Therasa and E. David, 2009. Hepatoprotective effect of *Enicostemma littorale* Blume and *Eclipta alba* during ethanol induced oxidative stress in albino rats. Int. J. Pharmacol., 5: 268-272.

Bregman, A.A., 1983. Laboratory Investigation and Cell Biology. John Wiley and Sons, New York, USA., ISBN-10: 047186241X, pp: 51-60.

Brieskorn, C.H. and P. Noble, 1982. Constituents of the essential oil of myrrh. Planta Med., 44: 87-90.

Bunout, D., 1999. Nutritional and metabolic effects of alcoholism: Their relationship with alcoholic liver disease. Nutrition, 15: 583-589.

Corrao, G., V. Bagnardi, A. Zambon and C. La Vecchia, 2004. A meta-analysis of alcohol consumption and the risk of 15 diseases. Prev. Med., 38: 613-619.

Crawford, D.W. and D.H. Blankenhorn, 1991. Arterial wall oxygenation, oxyradicals and atherosclerosis. Atherosclerosis, 89: 97-108.

De Duve, C. and P. Baudhuin, 1996. Peroxisomes (microbodies and related particles). Physiol. Rev., 46: 323-357.

Ebuehi, O.A.T. and C.L. Asonye, 2007. Gender and alcohol consumption affect human serum enzymes, protein and bilirubin. Asian J. Biochem., 2: 330-336.

Evans, W.C., 1989. Trease and Evans Pharmacognosy. 13th Edn., Balliere Tindall, London, ISBN: 0702013617, pp: 378-689.

Fang, Y.Z., S. Yang and G. Wu, 2002. Free radicals, antioxidants and nutrition. Nutrition, 18: 872-879.

Folch, J., M. Lees and G.H.S. Stanley, 1957. A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem., 226: 497-509.

Halliwell, B., 1991. Introduction to free radicals in human disease. Saudi Med. J., 12: 13-19.

Handa, S.S. and A. Sharma, 1990. Hepatoprotective activity of andrographolide from *Andrographis paniculata* against carbon tetrachloride. Indian J. Med. Res., 92: 276-283.

Ishii, H., J. Kurose and S. Kato, 1997. Pathogenesis of alcoholic liver disease with particular emphasis on oxidative stress. J. Gastroenterol. Hepatol., 12: S272-S282.

Jurczuk, M., M.M. Brzoska, J. Moniuszko-Jakoniuk, M. Galazyn-Sidorszuk and E. Eulikowska-Karpinska, 2004. Antioxidant enzymes activity and lipid peroxidation in liver and kidney of rats exposed to cadmium and ethanol. Food Chem. Toxicol., 42: 429-438.

Kakkar, P., B. Das and P.N. Viswanathan, 1984. A modified spectrophotometric assay of superoxide dismutase. Indian J. Biochem. Biophys., 21: 130-132.

Lee, J.S., 2004. Supplementation of *Pueraria radix* water extract on changes of antioxidant enzymes and lipid profile in ethanol-treated rats. Clin. Chim. Acta, 347: 121-128.

Lykkesfeldt, J., 2007. Malondialdehyde as biomarker of oxidative damage to lipids caused by smoking. Clin. Chim. Acta, 380: 50-58.

Malhotra, S.C., M.M. Ahuja and K.R. Sundaram, 1977. Long term clinical studies on the hypolipidemic effect of *Commiphora mukul* guggulu and clofibrate. Indian J. Med. Res., 65: 390-395.

Mates, J.M., C. Perez-Gomez and I.N. De Castro, 1999. Antioxidant enzymes and human diseases. Clin. Biochem., 32: 595-603.

McDonough, K.H., 2003. Antioxidant nutrients and alcohol. Toxicology, 189: 89-97.

Nencini, C., G.G. Franchi, F. Cavallo and L. Micheli, 2010. Protective effect of *Allium neapolitanum* Cyr. versus *Allium sativum* L. on acute ethanol-induced oxidative stress in rat liver. J. Med. Food, 13: 329-335.

Nityanand, S., J.S. Srivastava and O.P. Asthana, 1989. Clinical trials with gugulipid. A new hypolipidaemic agent. J. Assoc. Physicians India, 37: 323-328.

Ogunlade, B., L.C. Saalu, O.S. Ogunmodede, G.G. Akunna, O.A. Adeeyo and G.O. Ajayi, 2012. The salutary role of *Allium cepa* extract on the liver histology, liver oxidative status and liver marker enzymes of rabbits submitted to alcohol-induced toxicity. Am. J. Biochem. Mol. Biol., 2: 67-81.

Ohkawa, H., N. Ohishi and K. Yagi, 1979. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal. Biochem., 95: 351-358.

Pettit, G.R., 1978. Biosynthetie Produetsjbr Cancer Chenlotherapy. Plenum Press, New York, USA.

Prakash, M., J.K. Shetty, S. Tripathy, M. Verma, S. Vasudev and P.V. Bhandary, 2008. Serum total Thiol status in alcohol abusers. Asian J. Biochem., 3: 48-51.

Qureshi, S., M.M. Al-Harbi, M.M. Ahmed, M. Raza, A.B. Giangreco and A.H. Shah, 1993. Evaluation of genotoxic, cytotoxic and antitumor properties of *Commiphora molmol* using normal and Ehrlich ascites carcinoma cell-bearing Swiss albino mice. Cancer Chemother. Pharmacol., 33: 130-138.

Ramachandran, S., N. Rajendra Prasad, K.V. Pugalendi and V.P. Menon, 2008. Modulation of UVB-induced oxidative stress by ursolic acid in human blood lymphocytes. Asian J. Biochem., 3: 11-18.

Rao, R.M., Z.A. Khan and A.H. Shah, 2001. Toxicity studies in mice of *Commiphora molmol* oleo-gum resin. J. Ethnopharmacol., 76: 151-154.

Reddy, A.C.P. and B.R. Lokesh, 1992. Studies on spice principles as antioxidants in the inhibition of lipid peroxidation of rat liver microsomes. Mol. Cell. Biochem., 111: 117-124.

Rios, J.L., M.C. Recio, S. Manez and R.M. Giner, 2000. Natural Triterpenoids as Antiinflammatory Agents. In: Studies in Natural Products Chemistry: Bioactive Natural Products, Rahman, A. (Ed.). Elsevier, Amsterdam, The Netherlands, pp: 93-143.

Russo, D., V. Purohit, L. Foudin and M. Salin, 2004. Workshop on alcohol use and health disparities 2002: A call to arms. Alcohol, 32: 37-43.

Saka, W.A., R.E. Akhigbe, O.S. Ishola, E.A. Ashamu, O.T. Olayemi and G.E. Adeleke, 2011. Hepatotherapeutic effect of aloe vera in alcohol-induced hepatic damage. Pak. J. Biol. Sci., 14: 742-746.

Schacterle, G.R. and R.L. Pollack, 1973. A simplified method for the quantitative assay of small amount of protein in biological material. Anal. Biochem., 51: 654-655.

Sedlak, J. and R.H. Lindsay, 1968. Estimation of total, protein-bound and nonprotein sulfhydryl groups in tissue with Ellman's reagent. Anal. Biochem., 25: 192-205.

Shah, A.H., S. Qureshi, M. Tariq and A.M. Ageel, 1989. Toxicity studies on six plants used in the traditional Arab.. system of medicine. Phytother. Res., 3: 25-29.

Sheela, C.G. and K.T. Augusti, 1992. Antidiabetic effects of S-allyl cysteine sulphoxide isolated from garlic *Allium sativum* Linn. Indian J. Exp. Biol., 30: 523-526.

Sheweita, S.A., M. Abd El-Gabar and M. Bastawy, 2001. Carbon tetrachloride-induced changes in the activity of phase II drug-metabolizing enzyme in the liver of male rats: Role of antioxidants. Toxicology, 165: 217-224.

Singh, P.K., S. Roy and S. Dey, 2007. Protective activity of andrographolide and arabinogalactan proteins from *Andrographis paniculata* Nees. against ethanol-induced toxicity in mice. J. Ethnopharmacol., 111: 13-21.

Sofowora, A., 1982. Medicinal Plants and Traditional Medicine in Africa. Spectrum Books Limited, Ibadan, Nigria.

Speisky, H., A. MacDonald, G. Giles, H. Orrego and Y. Israel, 1985. Increased loss and decreased synthesis of hepatic glutathione after acute ethanol administration. Biochem. J., 225: 565-572.

Tadic, S., M.S. Elm, H.S. Li, G.J. Van Londen, V.M. Subbotin, D.C. Whitcomb and P.K. Eagon, 2002. Sex differences in hepatic gene expression in a rat model of ethanol-induced liver injury. J. Applied Physiol., 93: 1057-1068.

Thompson, D.C., D. Constantin-Teodosiu and P. Moldeus, 1991. Metabolism and cytotoxicity of eugenol in isolated rat hepatocytes. Chem. Biol. Interact., 77: 137-147.

Tripathi, Y.B., O.P. Malhotra and S.N. Tripathi, 1984. Thyroid stimulating action of z-guggulsterone obtained from commiphora mukul. Planta Med., 50: 78-80.

Vogt, B.L. and J.P. Richie Jr., 2007. Glutathione depletion and recovery after acute ethanol administration in the aging mouse. Biochem. Pharmacol., 73: 1613-1621.

Wu, G., Y.Z. Fang, S. Yang, J.R. Lupton and N.D. Turner, 2004. Glutathione metabolism and its implications for health. J. Nutr., 134: 489-492.

Zeng, T., F.F. Guo, C.L. Zhang, S. Zhao, D.D. Dou, X.C. Gao and K.Q. Xie, 2008. The anti-fatty liver effects of garlic oil on acute ethanol-exposed mice. Chem. Biol. Interact., 176: 234-242.