Protoplasma (2013) 250:483–493
DOI 10.1007/s00709-012-0431-5

ORIGINAL ARTICLE

Hepatoprotective effect of *Origanum vulgare* in Wistar rats against carbon tetrachloride-induced hepatotoxicity

Mohammad Sikander · Shabnam Malik · Kehkashan Parveen · Maqsood Ahmad · Deepak Yadav · Zubair Bin Hafeez · Manish Bansal

Received: 4 May 2012 / Accepted: 26 June 2012 / Published online: 7 July 2012

Abstract The effect of an aqueous extract of *Origanum vulgare* (OV) leaves extract on CCl_4-induced hepatotoxicity was investigated in normal and hepatotoxic rats. To evaluate the hepatoprotective activity of OV, rats were divided into six groups: control group, *O. vulgare* group, carbon tetrachloride (CCl_4; 2 ml/kg body weight) group, and three treatment groups that received CCl_4 and OV at doses of 50, 100, 150 mg/kg body weight orally for 15 days. Alanine amino transferase (ALT), alkaline phosphatase (ALP), and aspartate amino transferase (AST) in serum, lipid peroxide (LPO), GST, CAT, SOD, GPx, GR, and GSH in liver tissue were estimated to assess liver function. CCl_4 administration led to pathological and biochemical evidence of liver injury as compared to controls. OV administration led to significant protection against CCl_4-induced hepatotoxicity in dose-dependent manner, maximum activity was found in CCl_4+OV3 (150 mg/kg body weight) groups and changes in the hepatocytes were confirmed through histopathological analysis of liver tissues. It was also associated with significantly lower serum ALT, ALP, and AST levels, higher GST, CAT, SOD, GPx, GR, and GSH level in liver tissue. The level of LPO also decreases significantly after the administration of OV leaves extract. The biochemical observations were supplemented with histopathological examination of rat liver sections. Thus, the study suggests *O. vulgare* showed protective activity against CCl_4-induced hepatotoxicity in Wistar rats and might be beneficial for the liver toxicity.

Keywords Hepato toxicity · Carbon tetrachloride · *Origanum vulgare* · Oxidative stress · Lipid peroxidation

Handling Editor: Peter Nick

M. Sikander (✉) · S. Malik · Z. B. Hafeez
Department of Biotechnology, Hamdard University,
Hamdard Nagar,
New Delhi 110062, India
e-mail: sikanderbiochem@gmail.com

K. Parveen
Department of Biochemistry, Jamia Hamdard,
New Delhi 110062, India

M. Ahmad
Department of Pharmacognosy & Phytochemistry,
Faculty of Pharmacy, Hamdard University,
New Delhi 110062, India

D. Yadav
Department of Ilmul Adivia Faculty of Medicine,
Hamdard University,
New Delhi 110062, India

M. Bansal
National Research Center On Equines,
Sirsa Road,
Hisar 125001, India

Introduction

Liver regulates many important metabolic functions. Hepatic injury is associated with distortion of these metabolic functions (Wolf 1999). In absence of a reliable liver protective drug in the modern system of medicine, a number of medicinal preparations in Ayurveda, the Indian system of medicine, are recommended for the treatment of liver disorders (Chatterjee 2000). Natural remedies from medicinal plants are considered to be effective and safe treatments for hepatotoxicity. Liver ailments are mainly caused by toxic chemicals (Eesha et al. 2011). The free radical reactions responsible for the pathogenesis of liver injury have been investigated in a few defined experimental systems using carbon tetrachloride, excess iron, or ethanol as pro-oxidant agents. The damaged and injured hepatocytes have been investigated to be the sequential progression of activated oxygen species, covalent binding, and lipid peroxidation (Geesin et al. 1990). *Origanum vulgare* Linn., family

Lamiaceae, posseses a remarkable medicinal potential to combat the many physiological disorders. The genus *Origanum* is represented by over 44 species, 6 subspecies, 3 botanical varieties, and 18 naturally occurring hybrids. Only the species, *O. vulgare*, found in India (Rao et al. 2011). The essential oil along with other secondary metabolites approve its wide application for antihyperglycaemic (Lemhadri et al. 2004), anti-inflammatory (Kelm et al. 2000), cytotoxic (Sivropoulou et al. 1996), antioxidant (Sahin et al. 2004; Milos et al. 2000), antifungal (Ertas et al. 2005), antibacterial (Bayder et al. 2004; Avadhani et al. 1999; Viuda-martos et al. 2008), antithrombin (Goun et al. 2002), antimutagenic and anticarcinogenic effects (Lozano et al. 2004). Some reports deal with the presence of three DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavengers-4′-*O*-β-D-glucopyranosyl-3′, 4′-dihydroxybenzyl protocatechuate, 4′-*O*-β-D-glucopyranosyl-3′,4′-didehydroxybenzyl, 4′-dihydroxybenzyl 4-*O*-methylprotocatechhuate, 4′-*O*-β-D-glucopyranosyl-4′hydroxybenzyl protocatechuate (Matsura et al. 2003), and five antioxidant phenolic compounds (Kikuzaki and Nakatani 1989). The phytochemical studies confirm the presence of large number of polar constituents which support antioxidant potential (Koukoulitsa et al. 2006). *O. vulgare* (OV) essential oil is reported to have the major compounds as carvacrol and thymol (Tian and Lai 2006). Nowadays, approximately 80 % of the world's population prefers the traditional, plant-based medicines for their primary health care (Cosge et al. 2009).

The literature survey revealed the insufficient validated scientific reports on hepatoprotection, hence the aim of the present study was to evaluate the hepatoprotective effect of aqueous extract of leaves of *O. vulgare*. In view of this, the present study was undertaken to investigate the hepatoprotective activity of *O. vulgare* leaves extract against CCl4-induced hepatotoxicity in Wistar rats.

Materials and method

Collection and authentication of plant

Kilmora herbs were collected at authenticated by Kumaun Grameen Udyog, PO Kasiyalekh, Nanital District, Uttarakhand, India.

Preparation of the aqueous extract

One-gram oregano leaves were minced in water, boiled in distilled water until half of volume, and then filtered to obtain the aqueous extract. The extract is concentrated under vacuum until dry and dissolved in water to the final desired concentration.

Experimental animals

Male Wistar rats (130±10 g), 4–6-week-old, were obtained from Central Animal House of Hamdard University, New Delhi. They were housed in polypropylene cages in groups of eight rats per cage and kept in a room maintained at 25±2 °C with a 12-h light/dark cycle. They were allowed to acclimatize for 1 week before the experiments and were given free access to standard laboratory feed (Amrut Laboratory, rat and mice feed, Navmaharashtra Chakan Oil Mills Ltd, Pune, India) and water ad libitum. Approval to do animal experimentation was obtained from Institutional Animal Ethics Committee registered under the Committee for the Purpose of Control and Supervision of Experimental Animals (173/CPCSEA).

Chemicals and reagents

TBA (thiobarbituric acid) and TCA (trichloroacetic acid) were purchased from Merck (Germany). Carbon tetrachloride (CCl_4) and all other chemicals and reagents were of the analytical grade, supplied by S. Merck (India). 1-chloro-2,4-dinitrobenzene (CDNB) was purchased from Himedia (India) and bovine serum albumin was purchased from Sigma Chemical Company USA.

Experimental protocols

Rats were randomly divided into six groups with eight animals in each group. The experimental design and treatment protocol were as follows:

Control (C): Normal control, animals were orally administered saline only.
C+OV: Only OV was given orally (150 mg/kg body weight) for 15 days.
CCl_4: Single dose of CCl_4 was given intraperitoneally (2 ml/kg body weight).
CCl_4+OV1: CCl_4+50 mg/kg body weight OV was given orally for 15 days.
CCl_4+OV2: CCl_4+100 mg/kg body weight OV orally was given orally for 15 days.
CCl_4+OV3: CCl_4+150 mg/kg body weight OV orally was given orally for 15 days.

Hepatotoxicity was induced in CCl_4, CCl_4+OV1, CCl_4+OV2 and CCl_4+OV3 groups by an injection of CCl_4 (2 ml/kg body weight, 1:1 with Olive oil i.p.). The aqueous extract of OV was administered orally for 15 days through gavage.

Tissue preparation

At the end of experimental periods, the rats were anesthetized by ether inhalation and blood was collected from the

dorsal aorta. Serum was separated by centrifugation at 4,000 rpm for 10 min and stored at −80 °C before analysis. Rats were then sacrificed, and their livers were excised

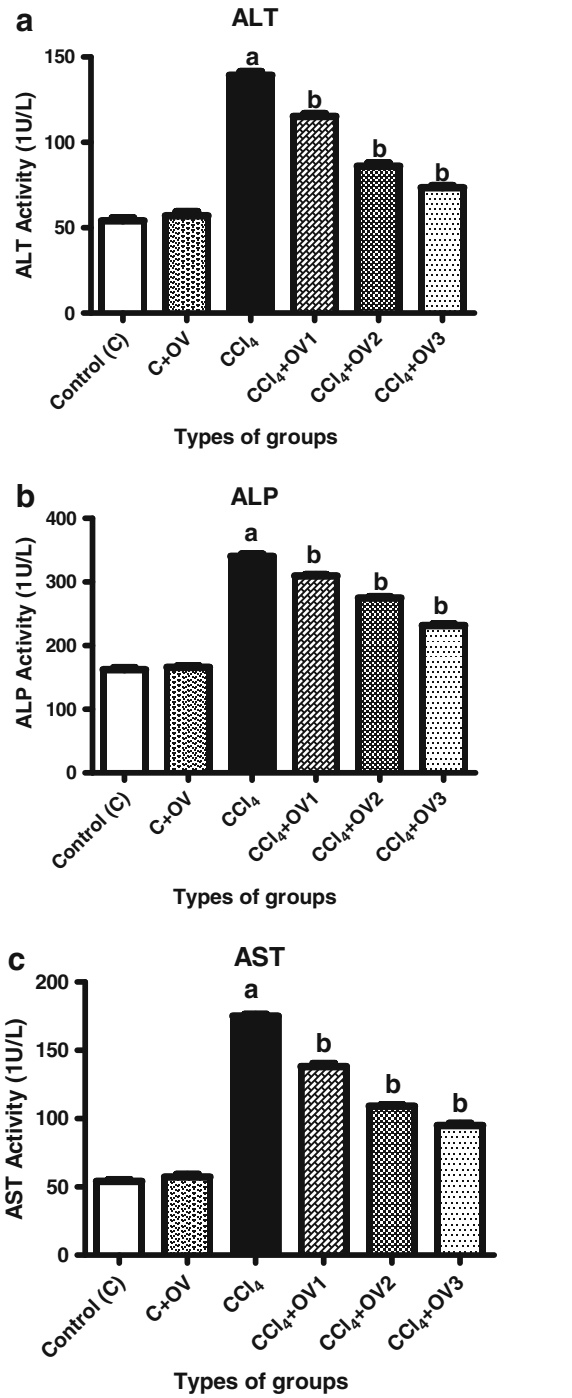


Fig. 1 **a**, **b**, **c** Effect of OV supplementation on ALT, ALP, and AST levels. Values are expressed as mean±S.E.M. (n=8). CCl_4 group showed significant increase in ALT, ALP, and AST levels compared to the control group (a, P<0.05 CCl_4 vs. control group). *O. vulgare* supplementation significantly decreased ALT, ALP, and AST levels in the CCl_4+OV*3* (150 mg/kg) group compared to the CCl_4-induced hepatotoxic group (b, P<0.05 CCl_4+ OV*3* vs. CCl_4 group)

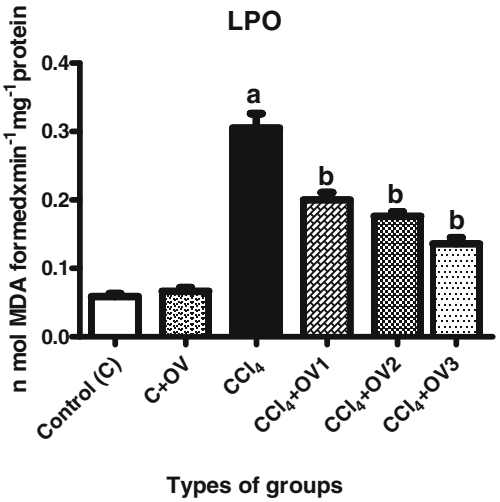


Fig. 2 Effect of OV supplementation on MDA levels. Values are expressed as mean±S.E.M. (n=8). CCl_4 group showed significant increase in MDA levels compared to the control group (a, P<0.05 CCl_4 vs. control group). *O. vulgare* supplementation significantly decreased MDA levels dose dependently in the CCl_4+OV*3* (150 mg/kg) groups compared to the CCl_4-induced hepatotoxic group (b, P< 0.05 CCl_4+ OV*3* vs. CCl_4 group)

immediately and perfused with ice-cold saline. To prevent auto-oxidation or ex vivo oxidation of the tissue, homogenization was carried out at 4 °C in 0.1 M phosphate-buffer (pH 7.4) containing protease inhibitors: 5 mM leupeptin, 1.5 mM aprotinin, 2 mM phenylethylsulfonylfluoride (PMSF), 3 mM pepstatin A, and 10 mM EDTA, 0.1 mM EGTA, 1 mM benzamidine, and 0.04 % butylated hydroxytoluene. The homogenate was centrifuged at 800×g for 5 min at 4 °C to separate the nuclear debris and supernatant was used for estimation of thiobarbituric-reactive substances (TBARS). The supernatant was further centrifuged at 10,000×g for 20 min at 4 °C to get the post-mitochondrial supernatant (PMS), which was used for various biochemical assays.

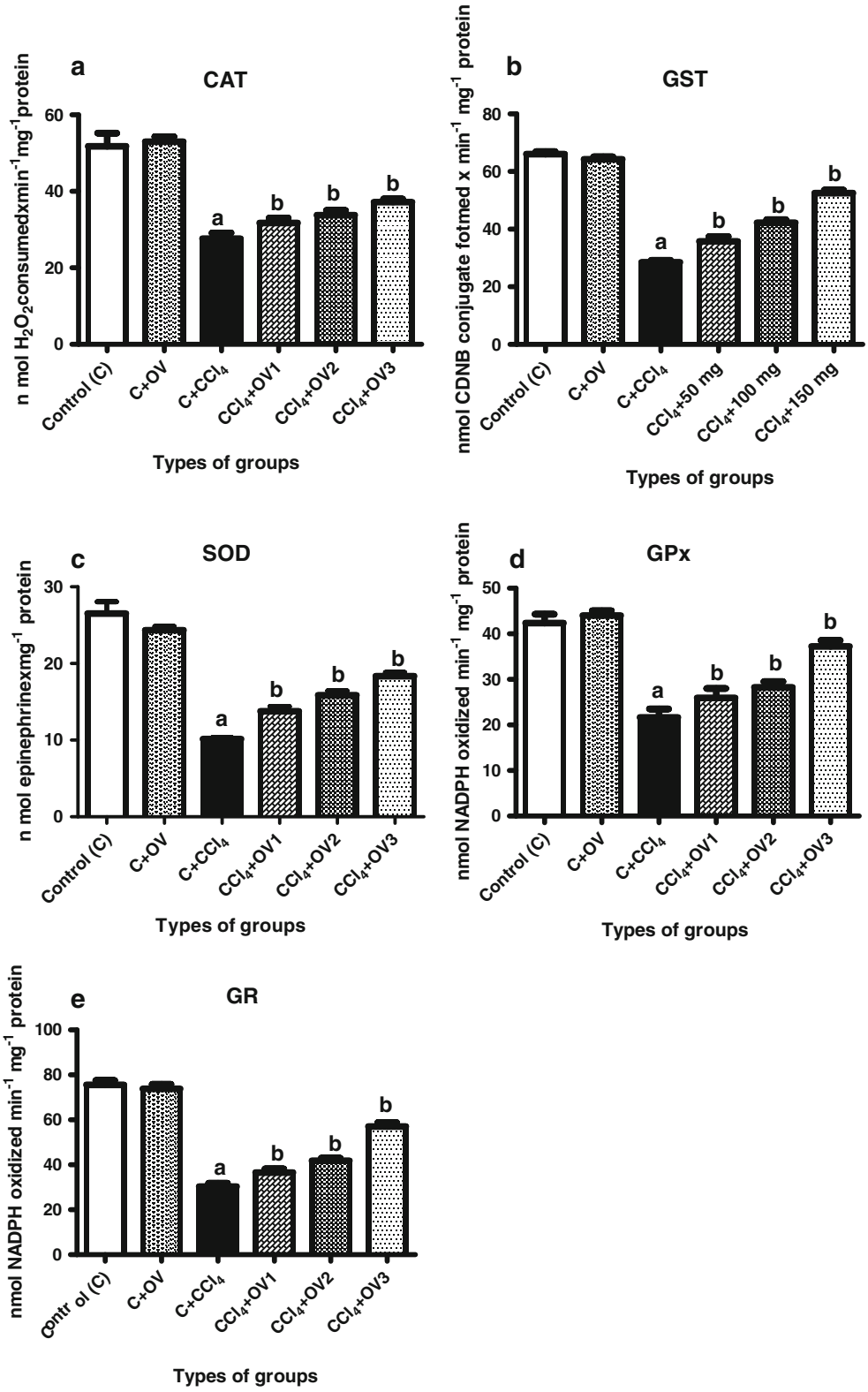
Biochemical estimations

Assay for serum ALT and AST activity

The serum enzymes were assayed using diagnostic kits provided by Span, and the procedure was followed as described by Reitman and Frankel (1957).

Assay for alkaline phosphatase activity

Alkaline phosphatase (ALP) activity was determined by the Span diagnostic kit, and procedure was followed as described by Kind and King (1954).



Assay for malonaldehyde

Malonaldehyde (MDA) is a measure of the end product of lipid peroxidation. It was measured as described by Ohkawa et al. (1979). Briefly, the reagents: 1.5 ml acetic acid (20 %) pH 3.5, 1.5 ml thiobarbituric acid (0.8 %), and 0.2 ml sodium dodecyl sulfate (8.1 %) were added to 0.1 ml of PMS sample. The mixture was then heated at 100 °C for 1 h. The mixture

◀ **Fig. 3** **a** Effect of OV supplementation on CAT activity. Values are expressed as mean±S.E.M. (n=8). CCl_4 hepatotoxic group showed significant decrease in CAT activity compared to the control group (a, P<0.05 CCl_4 vs. control group). *O. vulgare* supplementation significantly increased CAT activity in the CCl_4+ OV*3* (150 mg/kg) group as compared to the CCl_4-induced hepatotoxic group (b, P<0.05 CCl_4+ OV*3* vs. CCl_4 group). **b** Effect of OV supplementation on GST activity. Values are expressed as mean±S.E.M. (n=8). CCl_4 group showed significant decrease in GST activity compared to the control group (a, P<0.05 CCl_4 vs. control group). *O. vulgare* supplementation significantly increased GST activity in the CCl_4+OV*3* (150 mg/kg) group compared to the CCl_4-induced hepatotoxicity (b, P<0.05 CCl_4+ OV*3* vs. CCl_4 group). **c** Effect of *O. vulgare* supplementation on SOD activity. Values are expressed as mean±S.E.M. (n=8). CCl_4 hepatotoxic group showed significant decrease in SOD activity compared to the control group (a, P<0.05 CCl_4 vs. control group). OV supplementation significantly increased SOD activity in the CCl_4+ OV*3* (150 mg/kg) group compared to the CCl_4-induced hepatotoxic group (b, P<0.05 CCl_4+OV*3* vs. CCl_4 group). **d** Effect of *O. vulgare* treatment on GPx activity. Values are expressed as mean±S.E.M. (n=8). CCl_4 hepatotoxic group showed significant decrease in GPx activity as compared to the control group (a, P<0.05 CCl_4 vs. control group). OV treatment significantly increased GPx activity in the CCl_4+ OV*3* (150 mg/kg) group compared to the CCl_4-induced hepatotoxic group (b, P<0.05 CCl_4+OV*3* vs. CCl_4 group), **e** Effect of *O. vulgare* treatment on GR activity. Values are expressed as mean±S.E.M. (n=8). CCl_4 hepatotoxic group showed significant decrease in GR activity as compared to the control group (a, P<0.05 CCl_4 vs. control group). OV treatment significantly increased GR activity in the CCl_4+ OV*3* (150 mg/kg) group compared to the CCl_4-induced hepatotoxic group (b, P<0.05 CCl_4+OV*3* vs. CCl_4 group)

was then cooled, 5 ml of *n*-butanol/pyridine [15:1 %, *v*/*v*] and 1 ml of distilled water was added and shaken vigorously. After centrifugation at 4,000 rpm for 10 min, the organic layer was separated, and the absorbance was measured at 532 nm using a spectrophotometer (Shimadzu-1601, Japan). The amounts of MDA formed in each of the samples were expressed as the nmol MDA formed/min/mg protein by using a molar extinction coefficient of 1.56×10^5 $M^{-1}cm^{-1}$.

Assay for catalase

CAT activity was assayed by the method of Claiborne (1985). Briefly, the assay mixture consisted of 0.05 M phosphate-buffer (pH 7.0), 0.019 M hydrogen peroxide (H_2O_2), and 0.05 ml PMS in a total volume of 3.0 ml. Changes in absorbance were recorded at 240 nm. CAT activity was expressed as nanomoles of H_2O_2 consumed per minute per milligrams of protein.

Assay for GST

The activity of GST was measured by the method of Habig et al. (1974). The reaction mixture consisted of 1.0 mM GSH, 1.0mM CDNB, 0.1 M phosphate buffer (pH 7.4), and 0.1 ml of PMS in a total volume of 3.0 ml. The change in absorbance was recorded at 340 nm and enzyme activity was calculated as nanomoles of CDNB conjugate formed per minute per milligram of protein using molar extinction coefficient of 9.6×103 M^{-1} cm^{-1}.

Assay for superoxide dismutase

SOD activity was measured with some modifications (Stevens et al. 2000). The reaction mixture contained 0.8 ml of 50 mmol/l glycine buffer (pH 10.4), and 0.2 ml PMS. The reaction was initiated by the addition of 0.02 ml of a 20 mg/ml solution of (−)epinephrine. Absorbance was recorded at 480 nm in a spectrophotometer. SOD activity was expressed as nanomoles of (−)epinephrine protected from oxidation by the sample compared with the corresponding readings in the blank cuvette. The molar extinction coefficient of 4.02×10^3 $M^{-1}cm^{-1}$ was used for calculations.

Assay for GPx

GPx (EC 1.11.1.9) activity was measured by the coupled assay method of Wheeler et al. (1990) in which oxidation of GSH was coupled to NADPH oxidation, catalyzed by GR. The reaction mixture consisted of 0.2 mM H2O2, 1 mM GSH, 1.4 unit of GR, 1.43 mM NADPH, 1 mM sodium azide, PMS (0.1 ml), and PB (0.1 M) in total volume of 2.0 ml. GPx activity was defined as nanomoles NADPH oxidized per minute per milligram of protein, using a molar extinction coefficient of 6.22×103 M^{-1} cm^{-1}.

Assay for GR

GR (EC 1.6.4.2) activity was measured by the method of Carlberg and Mannerviek (1975). The assay system consisted of 0.1 MPB (pH 7.6), 0.5 mM EDTA, 1 mM GSSH, 0.1 mM NADPH, and PMS (0.1 ml) in a total volume of 2.0 ml. The enzyme activity was quantitated at 25 °C by measuring the disappearance of NADPH at 340 nm, and calculated as nmol NADPH oxidized min^{-1} mg^{-1} protein, using a molar extinction coefficient of 6.22×103 M^{-1} cm^{-1}.

Assay for GSH

Reduced GSH content was determined by the method of Jollow et al. (1974), with slight modification. PMS was mixed with 4.0 % sulfosalicylic acid (*w*/*v*) in a 1:1 ratio (*v*/*v*). The samples were incubated at 4 °C for 1 h, and later centrifuged at 1,200×*g* for 15 min at 4 °C. The assay mixture contained 0.1 ml of supernatant, 1.0 Mm DTNB, and 0.1 MPB (pH 7.4) in a total volume of 1.0 ml. The yellow color that developed was read immediately at 412 nm in a spectrophotometer (Shimadzu-1601, Japan). The GSH content was calculated as millimolrs of GSH per milligram of protein, using a molar extinction coefficient of 13.6×103 M^{-1} cm^{-1}.

Estimation of proteins

The protein concentration in all samples was determined by the Lowry method (Lowry et al. 1951), using bovine serum albumin as standard.

Histological examinations

For histological examinations, liver sections from different groups were stained with hematoxylin and eosin (H and E). Briefly, at end of experiment, the rats were anesthetized with ether and perfused transcardially with saline. Livers were removed quickly and postfixed in buffered formalin (10 %) for 24 h. After fixation was completed, slices (3–4 mm) of these tissues were dehydrated and embedded in paraffin. At least four cross-sections were taken from each tissue in 5-μm thickness and stained with H and E. Following two washings with xylene (2 min each), tissue sections were mounted with DPX mountant. The slides were observed for histopathological changes and microphotographs were taken using an Olympus BX50 microscope system (Olympus, Japan).

Statistical analysis

All the result are expressed as mean±SEM (n=8). Statistical analysis of the data was obtained via analysis of variance, followed by Tukey's test. P<0.05 was considered statistically significant.

Results

The hepatoprotective effects were revealed after experimental protocol of 15 days. Animals were treated with carbon tetrachloride and showed a significant hepato-necrosis and oxidative stress. When the stress and damage to hepatocytes for this group were compared to the normal group biochemically, the increased levels of serum alanine amino transferase (ALT), aspartate amino transferase (AST), ALP, and lipid peroxide (LPO) levels were found (P<0.05); whereas the levels of CAT, GST, SOD, GPx, GR, and GSH were found to be decreased (P<0.05).

Effect of OV leaves extarct on serum ALT, ALP, and AST activity

The effects of OV leaves extract on ALT, ALP, and AST levels were measured to demonstrate the activities of these enzymes in liver of CCl_4-induced hepatotoxic groups. There were no significant changes in ALT, ALP, and AST levels in the control+OV-treated group as compared to control group. These parameters were significantly (P<0.05) increased in the CCl_4 group compared to the control group. Levels of ALT, ALP, and AST in the CCl_4 group decreased significantly (P<0.05) with OV supplementation in the CCl4+ OV3 group (Fig. 1a, b, and c).

OV leaves extract supplementation decreased MDA levels in the CCl_4-induced hepatotoxicity

The effects of OV extract on MDA levels were measured to demonstrate the rate of LPO in liver of CCl_4-induced hepatotoxic group. There were no significant changes in MDA levels in the control+OV-treated group compared to control group. These parameters were significantly (P<0.05) increased in the CCl_4 group as compared to the control group. Levels of MDA in the CCl_4 group decreased significantly (P<0.05) with OV supplementation in the CCl_4+OV3 group (Fig. 2).

Effect of OV leaves treatment on the activity of antioxidant enzymes in the liver of control and experimental groups

Effects of OV leaves extract on the activity of CAT, GST, SOD GPx, and GR in the CCl_4-induced hepatotoxic group and control groups (Fig. 3a–e). The activity of GST, CAT, SOD GPx, and GR in control+OV group did not change significantly as compared to the control group. On the other hand, the activities of these enzymes were depleted significantly (P<0.05) in the CCl_4-induced hepatotoxic group as compared to the control group. The OV significantly (P<

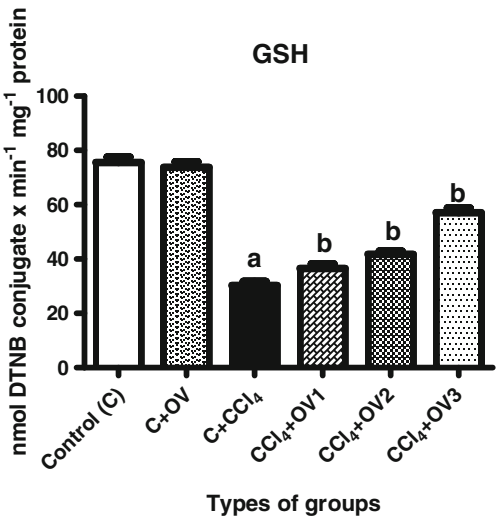


Fig. 4 Effect of OV supplementation on GSH content in the liver. Values are expressed as mean±S.E.M. (n=8). The CCl_4 group showed significant decrease in GSH content compared to the control group (*a*, P<0.05 CCl_4 vs. control group). *O. vulgare* supplementation significantly increased GSH content in the CCl_4+OV*3* (150 mg/kg) group compared to the CCl_4-induced hepatotoxicity (*b*, P<0.05 CCl_4+ OV*3* vs. CCl_4 group)

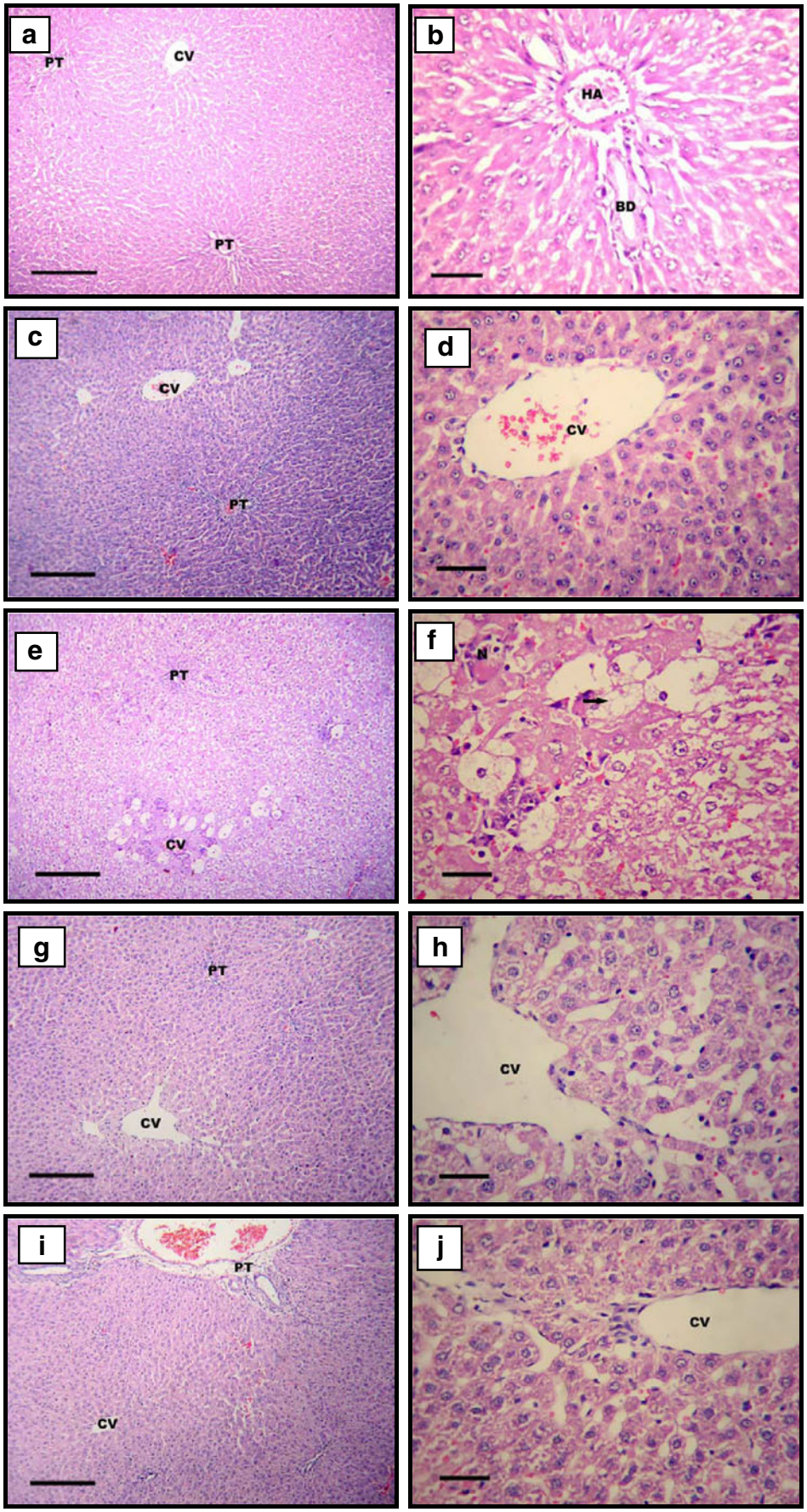
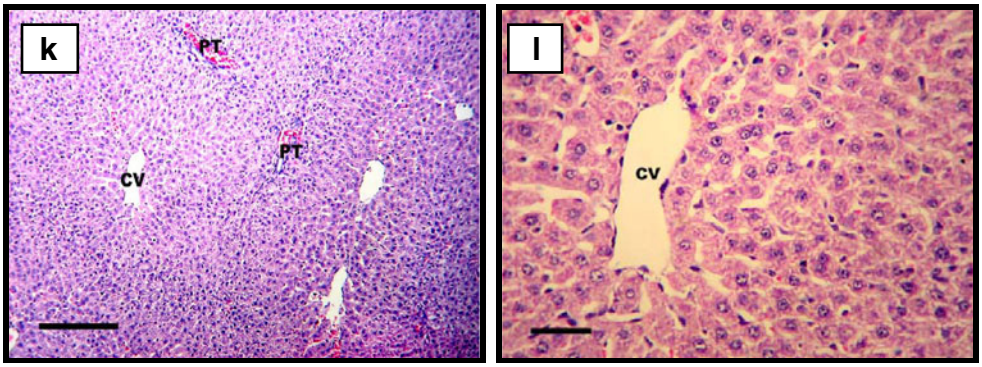


Fig. 5 **a**, **b** Photomicrographs showing histopathological changes in liver tissue. Control group (×100) showing normal liver architecture. *PT* portal triad, *CV* central vein. Same section at ×400 showing details of a normal PT, *PV* portal vein, *BD* bile duct, *HA* hepatic artery. **c**, **d** Liver group (C+OV), at low power (×100) showing normal arrangement of cells in the liver lobule. *PT* portal triad and *CV* central vein. Same section at high power (×400) showing centrizonal area. **e**, **f** Liver Group (CCl_4), at low power (×100) showing vacuolation of hepatocytes and focal necrosis in the centrizonal area. *PT* portal triad and CV central vein. Same section at high power (×400) showing hepatocytic necrosis (*N*) and evident vacuolation (*arrow*) of hepatocytes. **g**, **h** Liver group (CCl_4+50 mg/kg body weight; drug,) at low power (×100) showing moderate sinusoidal dilatation in the centrizonal area. No necrosis or hepatocytic vacuolation is seen. *PT* portal triad and *CV* central vein. Same section at high power (×400) showing moderate degree of sinusoidal dilatation. **i**, **j** Liver group (CCl_4+100 mg/kg body weight; drug), at low power (×100) showing moderate sinusoidal dilatation in the centrizonal area. *PT* portal triad and *CV* central vein. Same section at high (×400) power showing the same. **k**, **l** Liver group (CCl_4+150 mg/kg body weight; drug), at low power (×100) showing mild sinusoidal dilatation in the centrizonal area. *PT* portal triad and *CV* central vein. Same section at high power (×400) showing the same. *Scale bar* 100 μm at ×100 and 20 μm at ×400 magnifications

Fig. 5 (continued)



0.05) increased the activity of these enzymes in the CCl_4+OV3 groups as compared to the CCl_4 group.

OV leaves treatment restored GSH in the CCl_4-induced rat model of hepatotoxicity

Level of GSH did not affect by OV supplementation in the control+OV-treated group compared to the control group. However, a significant ($P<0.05$) depletion in GSH was observed in the CCl4-induced hepatotoxic group compared to the control group. OV supplementation significantly ($P<0.05$) restored GSH level in the CCl_4+OV3 (150 mg/kg body weight) group compared to the CCl4 group (Fig. 4).

Histopathological observation

H and E staining is used to visualize and differentiate between tissue components in normal and pathological conditions. The histological examination of the H and E-stained control liver tissues showed normal architecture of hepatocytes (Fig. 5a, b). Liver section of CCl_4-induced hepatotoxic group showed necrosis (N) and vacuolization (arrow) of hepatocytes (Fig. 5e, f). The CCl_4+OV (50 and 100 mg/kg body weight) groups that received OV leaves extract showed normal hepatic parenchyma except for only moderate sinusoidal dilatation in centrizonal area of liver section (Fig. 5g–j). Maximum protection was found in CCl_4+OV*3* (150 mg/kg body weight) groups with mild sinusoidal dilatation of hepatocytes (Fig. 5k, l). OV supplementation did not show any remarkable effects in the group treated with OV alone compared with the control group (Fig. 5c, d).

Discussion

The present study demonstrates the role of OV in protecting against CCl_4-induced hepatotoxicity and this study showed the effective hepatoprotection of aqueous extract of OV leaves against CCl_4-induced toxicity. It is a commonly used hepatotoxin (Lee et al. 2010) metabolized in the liver to excretable glucuronide and sulfide conjugates (Jollow et al. 1974). As we all know, the specificity of CCl_4 towards the liver damage by metabolic activation also maintains the semi-normal metabolic functions. The estimation of the serum enzyme is always a useful quantitative marker of the extent and class of liver damage (Sreelatha et al. 2009). The protective effect of *O. vulgare* in different doses was an indication of plasma membrane stabilization as well as repair and regeneration of damaged hepatic tissues caused by CCl_4. The increased serum level estimation of AST, ALT, and ALP, along with the decrease level of LPO, GST, catalase, SOD, GPx, and GR, point towards cellular leakage and loss of functional integrity of hepatocytes (Rajesh and Latha 2004).

Available research studies on the antioxidant potential of flavonoids and triterpinoids reveal their stimulatory actions on antioxidative enzymes (i.e., SOD, GPx, GR, GST, and CAT). Some flavonoids exert a stimulatory effect on protein synthesis and gene expression of specific antioxidant enzymes (Rohrdanz et al. 2002) which play a defensive role to damaged hepatic tissues. The present study favors the ameliorative effect of aqueous extract of *O. vulgare* on oxidative stress induced by CCl_4.

Significant elevation in the activities of serum hepatospecific enzymes was seen when hepatocellular damage leads to abnormalities of liver function (Malik et al. 2012). It has been reported that these enzymes (AST, ALT, and ALP) exhibit higher activity in abnormally functioning liver, thus establishing themselves as index of liver function recovery degree in liver transplant patients (Simonsen and Uirji 1984). A significant increase in ALT, ALP, and AST enzyme levels in serum has also been reported after inducing hepatocellular tumors by administering CCl_4 in rats (Kim et al. 1994). In the present study, the high levels of ALT and AST confirm the hepatocellular degeneration and decrease by the administration of OV extract. The most significant levels of ALT, ALP, and AST were seen in CCl*4*+OV*3* (150 mg/kg body weight) groups. Another sensitive indicator of hepatocyte injury is the release of intracellular enzyme ALP in the circulation (Jagan et al. 2008). The measurement of phosphatase activity is a useful indicator of liver function (Kim et al. 1994). Our results have shown the elevated levels of

ALP after CCl_4 administration, confirming the liver damage and this damaged significantly improved by the administration of OV in CCl_4+OV3 groups.

Oxidative stress is one of the key factors during carcinogenesis (Banaker et al. 2004). Lipid peroxidation, a destructive process of liver damage due to CCl_4 intoxication (Mondal et al. 2011) processed by biotransformation of CCl_4. It is one of the most studied biologically relevant free radical chain reactions and is initiated by the attack of a free radical on a fatty acid or fatty acyl side chain of any chemical species that has sufficient reactivity to extract a hydrogen atom from a methylene carbon side chain. Lipid peroxidation may lead to the formation of several toxic byproducts such as malondialdehyde and 4-hydroxynonenal, which can attack cellular targets including DNA, inducing mutagenecity and carcinogenicity (Zawart et al. 1999; Banaker et al. 2004). There was a significant increase in the levels of lipid peroxidation in CCl_4-induced hepatotoxic groups vis-à-vis the controls but the improved levels of MDA contents were seen after supplementation of OV extract and highest improvement was found in CCl4+ OV3 groups.

The changes in hepatic oxygen radical metabolism were demonstrated by measurement of antioxidant enzymes such as CAT, GST, SOD, GPx, and GR activity (Halliwell and Gutteridge 1989; Rathore et al. 2000). SOD catalyzes dismutation of O2·− to H_2O_2, which is then deactivated to H_2O by CAT (Aebi 1984; Kumuhekar and Katyane 1992). Abnormal liver cells show a decrease in the activities of SOD and CAT though the mechanism is still unclear. As CAT and SOD are the two major scavenging enzymes that remove radicals in vivo, a decrease in activity of these antioxidants can lead to an excess availability of superoxide anion (O2·−) and hydrogen peroxide (H_2O_2), which in turn generate hydroxyl radicals (·OH), resulting in initiation and propagation of lipid peroxidation. In the present study, we report that the levels of these antioxidative enzymes were also decreased in experimental groups vis a vis control groups. GSH plays an important role in the antioxidant defense system. It is suggested that the decrease in GSH level could be the result of decreased synthesis, or increased degradation of GSH caused by oxidative stress in hepatotoxicity (Shaarawy et al. 2009). GSH is a direct scavenger of free radicals and has a multifaceted role in antioxidant defense. Besides being a direct scavenger of free radicals, it is also substrate for GST. The depletion of GSH content also may lower GST activity due its role in GST activity (Rathore et al. 2000; Hwang et al. 2007). GPx catalyzes the reaction of hydroperoxides with GSH to form glutathione disulphide. GPx uses GSH as a proton donor, converts H_2O_2 to water and molecular oxygen; in this process GSH is oxidized to GSSG, which is reconverted to GSH by the action of enzyme GR, thus maintaining the pool of GSH. A significant decrease in GPx and GR activity could suggest inactivation by reactive oxygen species, which are increased in hepatotoxic rats (Gupta et al. 2006). The decrease may also be due to the decreased availability of its substrate, GSH, which has been shown to be depleted during toxicity (Gupta et al. 2006; Kumar et al. 2008). It has been demonstrated that the activity of these antioxidant enzymes (GST, CAT, SOD, GPx, and GR) decrease in hepatotoxic rats (Gupta et al. 2006).

In the present study, we report that the levels of these antioxidative enzymes were also decreased in CCl_4 groups as compared to control groups but the treatment with OV prevented lipid and protein oxidation by enhancing the level of GSH and the status of antioxidant enzymes in the CCl_4+ OV groups. These results are consistent with previous reports that OV has the ability to enhance the status of antioxidant enzymes and protect them from oxidative damage (Wei et al. 1997; Nelson et al. 1998; Packer et al. 1999; Milos et al. 2000). Studies have demonstrated that OV directly inhibits the activity of enzymes which are involved in reactive oxygen species generation, such as cyclooxygenase, protein kinase C, NADH oxidase, and xanthine oxidase (Goze et al. 2009).

In conclusion, the present finding showed that OV possesses several beneficial properties including control of hepatotoxicity, control the liver function enzyme levels, not only to normalize the antioxidative enzyme levels but also to provoke the benefits of regeneration and repairment of the insulted hepatocytes. Thus, OV may be implicated as a preventive agent against hepatotoxicity. However, more work is warranted to elucidate its myriad mechanisms of action.

Acknowledgments Dr. Mohammad Sikander is thankful to Dr. Ashok Mukherji (All India Institute of Medical Science) for the necessary helps to conduct this study.

Conflict of interest statement The authors declare that there are no conflicts of interest.

References

Aebi H (1984) Catalase in vitro. Methods Enzymol 105:121–126

Avadhani S, Komali ZZ, Shetty K (1999) A mathematical model for the growth kinetics and synthesis of phenolics in oregano (*Origanum vulgare*) shoot cultures inoculated with pseudomonas species. Process Biochem 35(3–4):227–235

Banaker MC, Paramacivan SK, Chattopadhyay MB, Datta S, Chakraborty P, Chatterjee M, Kannan K, Thygarajan E (2004) 1α, 25-dihydroxyvitamin D3 prevents DNA damage and restores antioxidant enzymes in rat hepatocarcinogenesis induced by diethylnitrosamine and promoted by phenobarbital. World J Gasteroenterol 10:1268–1275

Bayder H, Osman S, Ozkan G, Karadoan T (2004) Antibacterial activity and composition of essential oils from *Origanum, Thymbra* and *Satureja* species with commercial importance in Turkey. Food Control 15:169–172

Carlberg I, Mannerviek B (1975) Glutathione reductase levels in rat brain. J Biol Chem 250:5475–5480

Chatterjee TK (2000) Herbal options, 1st edn. Books and Allied (P) Ltd, Kolkata

Claiborne A (1985) Catalase activity. In: Green Wald RA (ed) Handbook of methods for oxygen radical research. CRC Press, Boca Raton, pp 283–284

Cosge B, Turker A, Ipek I et al (2009) Chemical compositions and antibacterial activities of the essential oils from aerial parts and corollas of *Origanum acutidens* (Hand.-Mazz.) Ietswaart, an endemic species to Turkey. Molecules 14:1702–1712

Eesha BR, Mohanbabu Amberkar V, Meena Kumari K, Sarath B, Vijay M, Lalit M, Rajput R (2011) Hepatoprotective activity of *Terminalia paniculata* against paracetamol induced hepatocellular damage in Wistar albino rats. Asi Pac J Trop Med 4(6):466–469

Ertas ON, Guler T, Ciftci M, Dalkilic B, Simsek UG (2005) The effect of an essential oil mix derived from oregano, clove and anise on broiler performance. Inter J Poultry Sci 49(11):879–884

Geesin JC, Gordon JS, Bergand RA (1990) Retinoids affect collagen synthesis through inhibition of ascorbate-induced lipid peroxidation in cultured human dermal fibroblasts. Arch Biochem Biophys 278:350–355

Goun E, Cunningham G, Solodnikov S, Krasnykch O, Miles H (2002) Antithrombin activity of some constituents from *Origanum vulgare*. Fitoter 73(7–8):692–694

Goze I, Alim A, Tepe AS, Sokmen M, Sevgi K, Tepe B (2009) J Med Plants 3(4):246–254

Gupta AK, Chitme H, Dass SK, Misra N (2006) Antioxidant activity of Chamomile recutita capitula methanolic extracts against CCl_4-induced liver injury in rats. J Pharmacol Toxicol 1:101–107

Habig WH, Pabst M, Jakoby WB (1974) Glutathion S-transferase: the first enzymatic step in mercapturic acid formation. J Biol Chem 249:7130–7139

Halliwell B, Gutteridge JMC (1989) Protection against oxidants in biological system: the superoxide theory of oxygen toxicity. In: Cheeseman KH, Slater TF (eds) Free radicals in biology and medicine. Clarendon, Oxford, pp 144–147

Hwang YP, Choi CY, Chung YC, Jeon SS, Jeong HG (2007) Protective effects of puerarin on carbon tetrachloride-induced hepatotoxicity. Arch Pharm Res 30(10):1309–1317

Jagan S, Ramakrishnan G, Anandakumar P, Kamaraj S, Devaki T (2008) Antiproliferative potential of gallic acid against diethylnitrosamine-induced rat hepatocellular carcinoma. Mol Cell Biochem 319:51–59

Jollow DJ, Thogerison SS, Potter WZ, Hashimoto M, Mitchell JR (1974) Acetaminophen induced hepatic necrosis. IV metabolic disposition of toxic and non toxic doses of acetaminophen. Pharmaco 12:251–271

Kelm MA, Nair MG, Strasburg GM (2000) Antioxidant and cyclooxygenase inhibitory phenolic compounds from *Ocimum sanctum* Linn. Phytomed 7(1):7–13

Kikuzaki H, Nakatani N (1989) Structure of a new antioxidative phenolic acid from oregano (*Origanum vulgare* L.). Agric Biol Chem 53:519–524

Kim DJ, Lee KK, Han BS, Ahn B, Bae JH, Jang JJ (1994) Biphasic modifying effect of indole-3-carbinol on diethylnitrosamineinduced preneoplastic glutathione-S-transferase placental formpositive liver cell foci in Sprague-Dawley rats. Jpn J Cancer Res 5:378–383

Kind PRN, King EJ (1954) Estimation of plasma phosphatase by determination of hydrolysed phenol with amino antipyrine. J Clin Path 7:322–326

Koukoulitsa C, Karioti A, Bergonzi C, Pescitelli G, Bari LD, Skaltsa H (2006) Polar constituents from the aerial parts of *Origanum vulgare* L. ssp *hirtum* growing wild in Greece. J Agric Food Chem 54:5388–5392

Kumar RS, Manivannan R, Balasubramaniam A, Rajkapoor B (2008) Antioxidant and hepatoprotective activity of ethanol extract of *Indigofera trita* Linn. On CCl_4 induced hepatotoxicity in rats. J Pharmacol Toxicol 3:344–350

Kumuhekar HM, Katyane SS (1992) Altered kinetic attributes of Na+, K+ ATPase activity in kidney, brain and erythrocyte membrane in alloxan diabetic rats. Indian J Exp Biol 30:26–32

Lee BJ, Senevirathne M, Kim JS, Kim YM, Lee MS, Jeong MH (2010) Protective effect of fermented sea tangle against ethanol and carbon tetra chloride-induced hepatic damage in Sprague-Dawley rats. Food and Chem Toxicol 48:1123–1128

Lemhadri A, Zeggwagh NA, Maghrani M, Jouad H, Eddouk M (2004) Anti hyperglycaemic activity of aqueous extract of *Origanum vulgare* growing wild in Tafilate region. J Ethanopharmacol 92 (2–3):251–256

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with the Folin phenol reagent. J Biol Chem 193:265–275

Lozano C, Arcila C, Loarca-Pina G, Lecona-Uribe S, Gonzalez De Mejıa E (2004) Oregano: properties, composition and biological activity. Archivos Latinoamericanos de Nutricion 54(1):100–111

Malik S, Bhatnagar S, Chaudhary N, Katare DP, Jain SK (2012) DEN +2-AAF-induced multistep hepatotumorigenesis in Wistar rats: supportive evidence and insights. Protoplasma. doi:10.1007/s00709-012-0392-8, Received: 2 January 2012/Accepted: 21 February 2012

Matsura H, Chiji H, Asakawa C, Amano M, Yoshihara T, Mizutani J (2003) DPPH radical scavengers from dried leaves of oregano (*Origanum vulgare*). Biosci Biotech Biochem 67:2311–2316

Milos M, Mastelic J, Jerkovic I (2000) Chemical composition and antioxidant effect of glycosidically bound volatile compounds from oregano (*Origanum vulgare* L. ssp. hirtum). Food Chem 71(1):79–83

Mondal A, Maity TK, Pal D, Sannigrahi S, Singh J (2011) Isolation and in vivo hepatoprotective activity of *Melothria heterophylla* (Lour.) Cogn. Against chemically induced liver injuries in rats. Asi Paci J Trop Med 4(8):619–623

Nelson AB, Lau BHS, Ide N, Rong Y (1998) Pycnogenol inhibits macrophage oxidative burst, lipoprotein oxidation, and hydroxyl radical-induced DNA damage. Drug Dev Indust Pharm 24:139–144

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

Packer L, Rimbach G, Virgili F (1999) Antioxidant activity and biologic properties of a procyanidin-rich extract from pine (*Pinus maritima*) bark, pycnogenol. Free Radic Biol Med 27:704–724

Rajesh MG, Latha MS (2004) Protective effects of *Glycyrrhiza glabra* Linn. on carbon tetra chloride induced peroxidative damage. Ind J Pharmaco 36:284–286

Rao GV, Mukhopadhyay T, Annamalai T, Radhakrishnan N, Sahoo MR (2011) Chemical constituent and biological studies of *Origanum vulgare* Linn. Pharmacog Res 3(2):143–145

Rathore N, Kale M, John S, Bhatnagar D (2000) Lipid peroxidation and antioxidant enzymes in isoproterenol induced oxidative stress in rat erythrocytes. Ind J Physiol Pharmacol 44:161–166

Reitman S, Frankel S (1957) A colorimetric method for the determination of serum oxaloacetic and glutamic pyruvic transaminases. Am J Clin Pathol 28:56–63

Rohrdanz E, Ohler S, Tran Thi QH, Kahl R (2002) The phytoestrogen diadzein affect the antioxidant enzyme system of rat hepatoma H4IIE cells. J Nutri 132(3):370–375

Sahin F, Gulluce M, Daferera D, Sokmen A, Polissiu M, Agar G, Ozer H (2004) Biological activities of the essential oils and methanol extract of *Origanum vulgare* ssp. vulgare in the Eastern Antolia region of Turkey. Food Control 15:549–557

Shaarawy SB, Tohamy AA, Elgendy SM, Abd Elmageed ZY, Bahnasy A, Mohamed MA, Kandil E, Matrougui K (2009) Protective effects of garlic and silymarin on NDEA-induced rats hepatotoxicity. Int J Biol Sci 5(6):549–557. doi:10.7150/ijbs.5.549

Simonsen R, Uirji MA (1984) Interpreting the profile of liver-function tests in pediatric liver transplants. Clin Chem 30:1607–1610

Sivropoulou A, Papanikolau E, Nikolaou C, Kokkini S, Lanaras T, Arsenakis M (1996) Antimicrobial and cytotoxic activities of *Origanum's* essential oils. J Agric Food Chem 44(5):1202–1205

Sreelatha S, Padma PR, Umadevi M (2009) Protective effects of *Coriandrum sativum* extracts on carbon tetrachloride-induced hepatotoxicity in rats. Food and Chem Toxico 47:702–708

Stevens MJ, Obrosova I, Cao X, Van HC, Greene DA (2000) Effects of DLα-lipoic acid on peripheral nerve conduction, blood flow, energy metabolism, and oxidative stress in experimental diabetic neuropathy. Diabetes 49:1006–1015

Tian H, Lai DM (2006) Analysis on the volatile oil in *Origanum vulgare*. Zhongyaocai 29(9):920–921

Viuda-martos M, Ruiz-Navajas Y, Fernandez-Lopez J, Angel Peraz-Alvarez J (2008) Antibacterial activity of different essential oils obtained from species widely used in Mediterranean diet. Inter J Food Sci and Techno 43(35):526–531

Wei ZH, Peng QL, Lau BHS (1997) Pycnogenol enhances endothelial cell antioxidant defenses. Redox Rep 3:219–224

Wheeler R, Jhaine AS, Elsayeed MN, Omaye TS, Korte JWD (1990) Automated assays for superoxide dismutase, catalase, glutathione peroxidase and glutathione reductase activity. Anal Biochem 184:193–199

Wolf PL (1999) Biochemical diagnosis of liver diseases. Ind J Clin Biochem 14:59–90

Zawart LL, Meerman JH, Commandeur JN, Vermeulen NP (1999) Biomarkers of free radical damage applications in experimental animals and humans. Free Radic Biol Med 26:202–226