AMELIORATIVE EFFECT OF M. FORSSKALEI GRAINS EXTRACT ON CCL_4 – INDUCED OXIDATIVE STRESS AND HEPATOTOXICITY IN RATS

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Abstract

Purpose: The aim of the present study is to evaluate the hepatoprotective effects of M. *forsskalei* (traditional use in Saudi Arabia). Many herbs have been used as natural remedies for the prevention and/or treatment of liver diseases. Various herbs and herbal products are believed to have liver protective functions and are widely used in clinical practice.

Design/methodology/approach: CCl_4 continues to provide an important model substance to elucidate the mechanisms of action of hepatotoxic and oxidative stress effects. Rats were pretreated with aqueous extracts of the grains of M. *forsskalei*.

Findings: Pretreatment of rats with aqueous extract of the grains of M. *forsskalei* in multiple doses (50 and 100 mg/kg b.w. for 60 days) significantly prevented the CCl_4 induced hepatic damage as indicated by the serum marker enzymes (AST, ALT and LDH). It also prevented CCl_4-induced oxidative stress in the rats' liver by inhibiting lipid peroxidation (MDA) and restoring the levels of antioxidant enzyme (SOD) and glutathione.

Originality/value: Our results suggest that M. *forsskalei* effectively prevents liver injury, mainly through down regulation of oxidative stress.

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Paper type: Research paper

INTRODUCTION

Oriental herbal medicines, widely used for the treatment of various diseases for over 2000 years, have recently attracted the interest of modern scientific communities as alternative therapies. There has been a sharp upward trend in the use of phytomedicines over the last decade in Europe and the US (Ernst, 2000; Kessler *et al.,* 2001). Between 1990 and 1997, the use of alternative medicines in the US experienced unprecedented growth (approximately 380 per cent), and Americans spent $4.2 billion on herbal and other botanical remedies in 2001 alone (Eisenberg *et al.*, 1998; Kelly 2005). Since the metabolic function of the liver is primarily responsible for detoxification of diverse therapeutic agents, toxins and carcinogens, drug-induced liver injury may manifest as acute hepatitis, cholestasis, and become further developed as cirrhosis.

Liver damage is a widespread pathology, which in most cases involves oxidative stress and is characterized by a progressive evolution from steatosis to chronic hepatitis, fibrosis, cirrhosis, and hepatocellular carcinoma. Various xenobiotics are known to cause hepatotoxicity, and one amongst them is carbon tetrachloride (Srivastava and Shivanandappa, 2010).

Steroids, vaccines and antiviral drugs have been used as therapies for liver pathologies and have potential adverse side-effects, especially if administered chronically or sub-chronically. Therefore, herbal products and traditional medicines with better effectiveness and safe profiles are needed as a substitute for chemical therapeutics. As oxidative stress plays a central role in liver pathologies and their progression, antioxidants have been proposed as therapeutic agents, as well as drug co-adjuvants, to counteract liver damage. A number of studies have shown that those plant extracts with antioxidant activity protect against CCl_4 hepatotoxicity by inhibiting lipid peroxidation and enhancing antioxidant enzyme activity (Shahjahan *et al.,* 2004; Sheweita *et al.,* 2001).

Several environmental toxins and carcinogens are also converted into reactive intermediates during metabolism, resulting in tissue damage. Carbon tetrachloride (CCl_4), a potent hepatotoxic chemical, is widely

used to induce acute hepatic damage in experimental animal models. Reductive metabolism of the hepatotoxin, CCl_4, by CYP450 involves the production of the trichloromethyl free radical (CCl_3), which interacts with oxygen to form the highly toxic CCl_3O_2 (Szymonik-Lesiuk *et al.*, 2003; Weber *et al.*, 2003). Reactive oxygen species (ROS) generated by metabolic intermediates of xenobiotics via induction of CYP450 families as well as activated inflammatory cells through NADPH oxidases promote the accumulation of lipid-derived oxidation products that cause liver injury, resulting in cell necrosis (Lee *et al.*, 2004).

A number of plants are traditionally used to treat liver diseases. *Mesembryanthemum forsskalei (M. forsskalei)*, which is traditionally known as samha grains in Saudi Arabia, is commonly used for this purpose. It is an annual vegetation plant, and according to Bhattacharya (1987), samha, considered food grains, contain 21 per cent protein, which is twice as much as that found in common cereal grains, such as corn, wheat, etc. The energy content of *M. forsskalei* is similar to that of cereal grains.

The present study is intended to explore whether the grains of this herb could have a protective effect on hepatocytes, including hepatic oxidative stress and inflammation, in an experimental model CCl_4-induced hepatotoxicity.

MATERIALS AND METHODS

PLANT MATERIAL

The plant was authenticated by Dr A.N. Bhattacharya, an expert in food and agriculture organization for the United Nations. Grains of *M. forsskalei* were washed with water, then crushed with a roller to separate the inner woody core from the outer fleshy layer. The fleshy portions were pooled, dried at 40°c in a hot air oven and finely powdered. The powder was used for extraction. The aqueous extract was prepared by homogenizing the grain powder in warm water (50°c) and allowed to stand for 24 h, filtered with Whitman paper No. 1 and the filtrate was lyophilized (Srivastava *et al.*, 2006).

The aqueous extract of *M. forsskalei* was chosen for this study as it is traditionally used by Saudi Arabian people, including some elderly citizens, who use it in the morning with dates as a custom. They dry the grains and mince them before use.

ANIMALS

Thirty two adult male Wister rats (180–200 g) were divided into groups of eight each. Appropriate guidelines of the local animal ethics committee were followed for the animal experiments. During a 60 day dietary study on rats it was established that the grains extract of M. *forsskalei* is safe for the mammalian system at the highest dose. This study was based on preliminary experiments on the hepatoprotective dose of the aqueous extract of M. *forsskalei*. In single dose pretreatment (oral) experiment, aqueous extract of the grains at 50,100 and 200 mg M. *forsskalei* mg/kg b.w. was administered with the help of a gastric canula and the control group was maintained on saline followed, after 1 h, by oral administration of CCl_4 (1/2 LD50-1 ml/kg b.w.). In a multiple dose pretreatment experiment, the aqueous extract of the grains of M. *forsskalei* was administered for 60 days (day after day) at 50 and 100 mg/kg b.w. followed by a single oral dose of CCl_4 (1 ml/kg b.w.) on the last day. Animals were sacrificed by anaesthesia 24 h after CCl_4 administration. The livers were perfused with saline, dissected out and processed immediately for biochemical assays.

Multiple doses: Group I – control; Group II – CCl_4 (sunflower oil was used as the vehicle); Group III aqueous extract of M. *forsskalei* 50 mg/kg b.w. + CCl_4; Group IV–M. *forsskalei*100 mg/kgb.w. + CCl_4.

SERUM ENZYMES AND LIPID PROFILE

Blood samples were collected in tubes, allowed to clot and serum was collected by centrifugation at 2000g for 10 min and stored at 4°c for biochemical analysis. Serum alanine transaminase (ALT) and aspartate transaminase (AST) were determined by Reitman and Frankel (1957). The reaction mixture containing the substrates (L-alanine (200 mM) or L-aspartate (200 mM) with a-ketoglutarate) and enzyme in phosphate buffer (0.1 M, pH 7.4) was incubated for 30 min and 60 min for ALT and AST, respectively. After incubation, DNPH (1 mM) was added and kept for another 30 min at room temperature. The colour was developed by the addition of NaOH (0.4 M) and read at 505 nm in a spectrophotometer.

Lactate dehydrogenase (LDH) activity was assayed according to Kornberg (1955). The reaction mixture consisted of NADH (0.02 M), sodium pyruvate (0.01 M) in sodium phosphate buffer (0.1 M, pH 7.4). The change in the absorbance was recorded at 340 nm at 30s interval for 3 min.

LIPIDPEROXIDATION

Liver tissue was homogenized (10% w/v) in ice-cold 50 mM Phosphate buffer (pH 7.4), centrifuged at 10,000g for 20 min at 4°c and the supernatant was used to assay the following parameters:

Malondialdhyde (MDA) as end product of lipidperoxidation Malondialdehyde was determined by the method of Dousset *et al*. (1983).

Superoxide dismutase (SOD) activity was measured using pyrogallol (2 mM) autoxidation in Tris buffer (Marklund and Marklund, 1974).

Glutathione, 10% (w/v) liver homogenate was prepared in 5% (w/v) trichloroacetic acid, centrifuged at 2000g for 10 min and the glutathione (GSH) content in the deproteinised supernatant was estimated by Ellman's reagent with a standard curve (Ellman, 1959).

Sera were subjected to the following investigation:

Quantitative determination of triglycerides (TG) by Young and Pestaner (1975), quantitative determination of serum total cholesterol (TC) by Trinder (1969) and quantitative determination of serum high density lipoprotein cholesterol (HDL-c) and low density lipoprotein cholesterol (LDL-c) by Friedewald (1972).

STATISTICS

Data were expressed as mean ± SE (n = 8) and significant difference between groups was statistically analyzed and compared using the *t*- test. A difference was considered significant at $p < 0.05$.

RESULTS

SERUM ENZYMES

Levels of serum enzymes AST, ALT and LDH were measured after 24h of CCL_4 administration (Fig. 1). Their activities were highly significantly increased (202%, 71% and 130% respectively) compared to control rats. Treatment of rats with various concentration of samha grains extract (50,100mg/kg b.w.) significantly decreased enzyme

activity. Multiple pretreatment of aqueous extract at dose (100mg/kg b.w.) was more effective.

LIPID PROFILE

In this study, administration of animals with CCl_4, highly significantly elevated the concentration of TG, TC and LDL-c (52%, 135% and 77% respectively) and significantly decreased HDL-c ($P<0.001$) compared with control animals (Fig. 2). Administration of samha grains extract after CCl_4, showed a highly significant decrease ($P<0.001$) in TG, TC and LDL-c, and a highly significant increase ($P<0.001$) in HDL-c at 50mg/kg b.w.dose. This hypolipidimic effect increased at 100mg/kg b.w.

LIPID PEROXIDATION

Administration of CCl_4 induced lipidperoxidation (Fig. 3), where CCl_4 significantly increased MDA concentration as the end product of lipidperoxidation ($P<0.001$). The increase in MDA was reversed by pretreatment of rats with samha grains extract (100mg/kg b.w.).

ANTIOXIDANT ENZYMES

The hepatic antioxidant enzymes were highly significantly decreased in the liver of CCl_4 administrated rats (Fig. 3). Activities of SOD and GSH were restored after pretreatment of samha grains extract.

A negative correlation between MDA and serum ALT is represented in Fig. 4, while Fig. 5) shows a positive correlation between ALT and LDL-c. Fig. 6 represents a positive correlation between serum TG and serum LDL-c.

DISCUSSION

In the present study, hepatotoxicity was induced by CCl_4 in animals commonly used for screening the hepatoprotective activity of plant extracts. Mechanistic studies provide evidence that metabolism of carbon tetrachloride via CYP2E1 to highly reactive free radical metabolites plays a critical role in the postulated mode of action. The primary metabolites, trichloromethyl and trichloromethyl peroxy free radicals, are highly reactive and are capable of covalently binding locally to

cellular macromolecules, with preference for fatty acids from membrane phospholipids. The free radicals initiate lipid peroxidation by attacking polyunsaturated fatty acids in membranes, setting off a free radical chain reaction sequence. Lipid peroxidation is known to cause membrane disruption, resulting in the loss of membrane integrity and leakage of microsomal enzymes. By-products of lipid peroxidation include reactive aldehydes that can form protein and DNA adducts and may contribute to hepatotoxicity and carcinogenicity, respectively (Manibusan *et al.*, 2007; Weber *et* al., 2003).

Our results show that serum liver enzymes ALT, AST and LDH levels, which reflect the severity of hepatotoxicity, are markedly increased.

Increased serum liver enzymes may be attributed to damage of the structural integrity of liver cells, since these enzymes are located in cell cytoplasm and released into the circulation after cell damage (Ji young Shim, 2010).

This elevation in the liver enzymes was significantly decreased after pretreatment with samha grains aqueous extract. These results imply that the extract acts in preventing liver damage.

Our results are in agreement with those reported by Srivastava and Shivanandappa (2010), who reported elevation in serum hepatic enzymes after CCl_4 treatments.

Mukazayire *et al.* (2009) also reported that administration of CCl_4 induces hepatotoxicity in terms of a marked increase in AST, ALT and LDH activities where CCl_4 is metabolized by CYP2E1 in an extremely reactive radical, and CCl_3 induces hepatic lesions via lipid peroxidation.

LIPID PROFILE

In this study, administration of animals with CCl_4 elevated total cholesterol (TC), LDL-c and TG concentrations and decreased HDL-c concentration due to toxicity induced by CCl_4 administration, which is in agreement with the results of Sumitha and Thirunalasundari (2011), and Al-Said *et al.* (2011), who reported that CCl_4 induced hepatoxicity and found that the concentrations of serum lipids including total cholesterol, triglyceride and LDL-c were elevated, and this indicated deterioration in hepatic function due to the damage caused by CCl_4 administration. In

our results, treatment with M. *forsskalei* extract significantly decreased the effect of CCl_4 induced damage, which was evidenced by the decreased level of total cholesterol, triglycerides, LDL-c and increased levels of HDL-c in M. *forsskalei* treated groups.

Lipid peroxidation is an autocatalytic process, which is a common consequence of cell death. This process may cause peroxidative tissue damage in inflammation, cancer, ageing and toxicity of xenobiotics. MDA is one of the end products in the lipidperoxidation process (Kurata *et al*, 2003).

Glutathione peroxidase (GSH-Px) plays an important role in the elimination of H_2O_2, while super oxide dismutase (SOD), removes the super oxide radical (O_2^-) by converting it to H_2O_2. This H_2O_2 can be rapidly converted to a water molecule by glutathione per-oxide (GPx).

These enzymes can be induced by reactive oxygen species and are therefore considered indicators of oxidative stress (Pavlovic *et al.*, 2004).

The present study revealed that administration of CCl_4 increases the rate of lipid peroxidation of the cell membrane, which in turn leads to significant elevation in levels of MDA. It also produced a remarkable decrease of hepatic antioxidant parameters (GSH content and SOD activity). Pretreatment of animals with samha grains aqueous extract prevented lipid peroxidation, which could be attributed to the radical-scavenging antioxidant ability of this extract.

These results are explained by Srivastava and Shivanandappa (2010) and Lee (2008), who concluded that CCl_4 administration leads to a significant decrease in GSH levels, which act as an important factor in CCl_4 toxicity.GSH is the major non-enzymatic antioxidant and plays a critical role in eliminating the toxic metabolites that cause liver diseases.

Kuriakose and Muraleedhara (2011) reported statistically significant losses in the activities of antioxidant enzymes and an increase in MDA and liver function marker enzymes in the serum of the CCl_4-treated group compared with the control group.

Chen *et al.* (2002) also reported that such decreases in both GSH and SOD were related to a decreased antioxidant defense system and elevated concentration of free radicals as a result of oxidative stress.

The focus of public health intervention is to improve health and quality of life through the prevention and treatment of disease. It is well established that traditional medicine (TM) plays a crucial role in healthcare for a large part of the population living in developing countries. In fact, for centuries, TM was the only healthcare system available for the prevention and treatment of diseases in different cultures. Samha grains aqueous extract acts as hepatoprotective via suppression of oxidative stress, reduction of lipid peroxidation and retaining liver enzyme markers in the normal range.

Our study supports the traditional use of samha grains aqueous extract as hepatoprotective, where this extract inhibits the toxic effects of CCl_4. It decreases MDA levels in stressed rats and restores intracellular antioxidants GSH and SOD levels when compared to control animals. The extract also protected the animals significantly from the hepatotoxicity induced by CCl_4, as revealed by a decrease in AST, ALT and LDH concentrations.

Further work will have to be done to separate and identify the active ingredients of this aqueous extract to identify its chemical composition.

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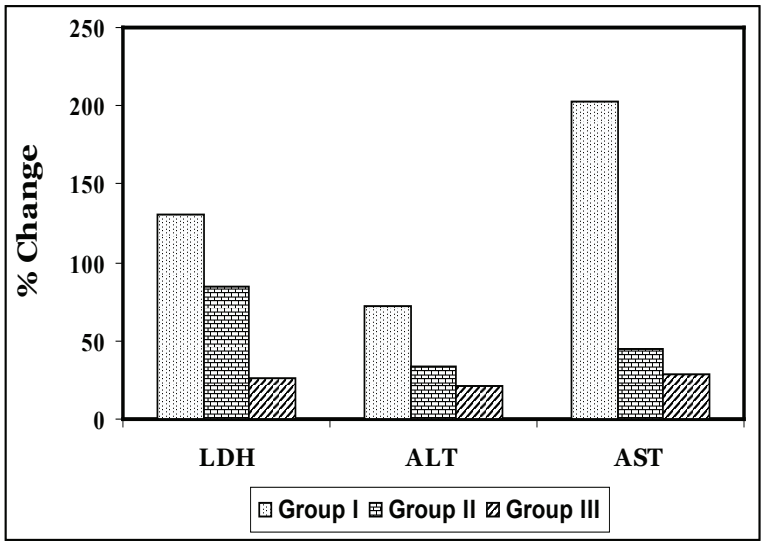

Fig. 1. Effect of *M. forsskalei* on some liver enzymes

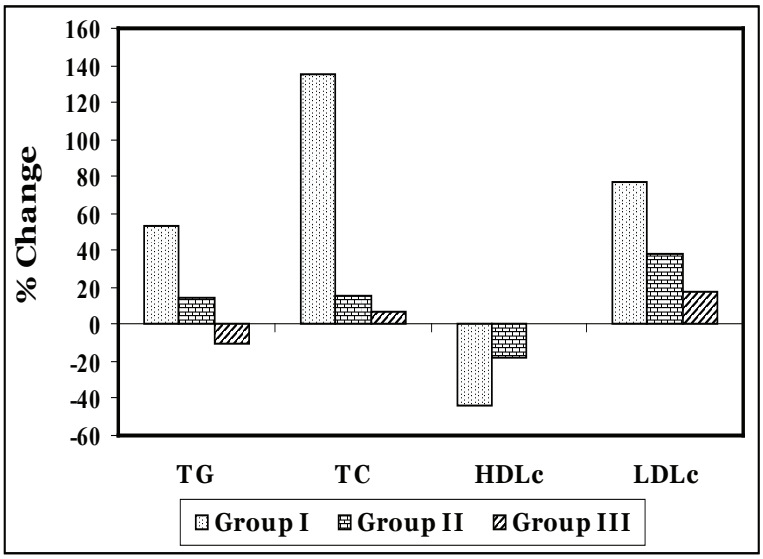

Fig. 2. Effect of *M. forsskalei* on lipid profile

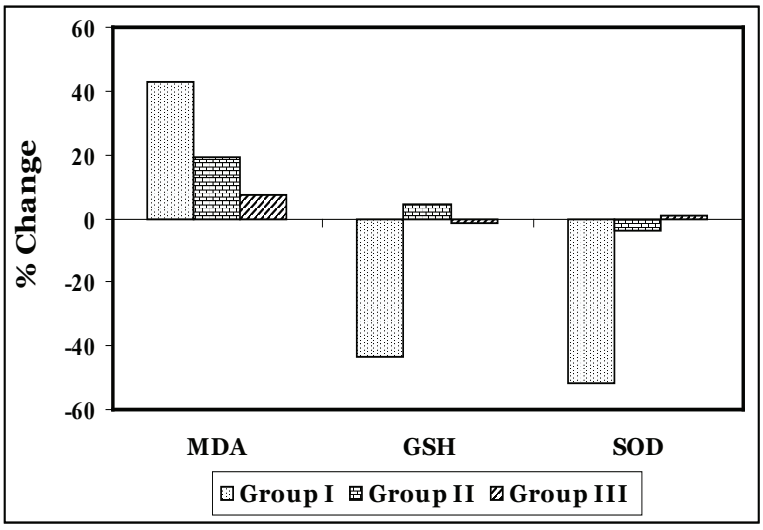

Fig. 3. Effect of *M. forsskalei* on some antioxidant and lipid peroxidation parameters

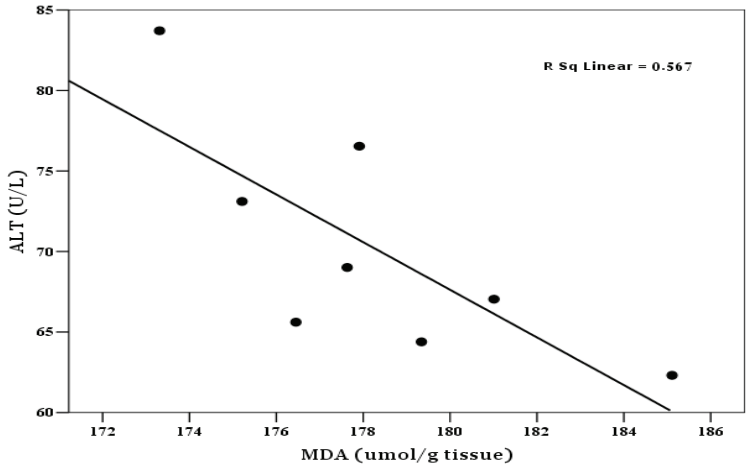

Fig. 4. Correlation between serum ALT and MDA A negative correlation between MDA and serum ALT

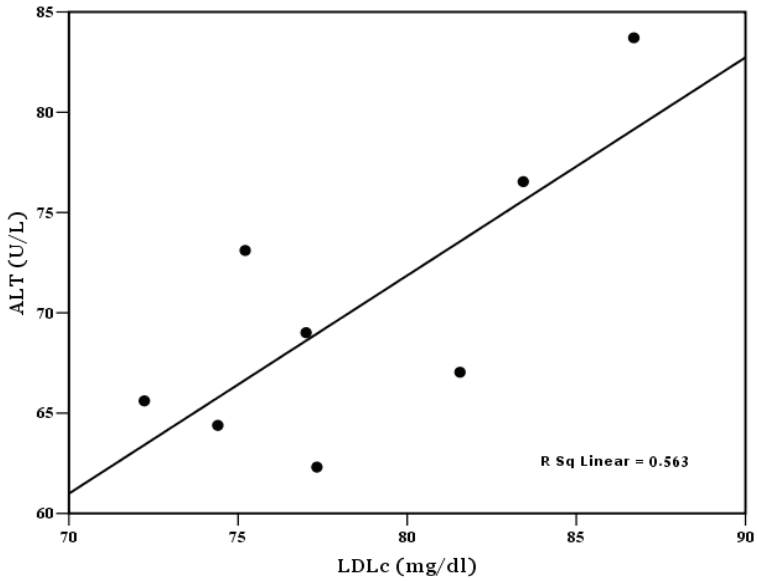

Fig. 5. Correlation between serum ALT and LDL-c A positive correlation between ALT and LDL-c

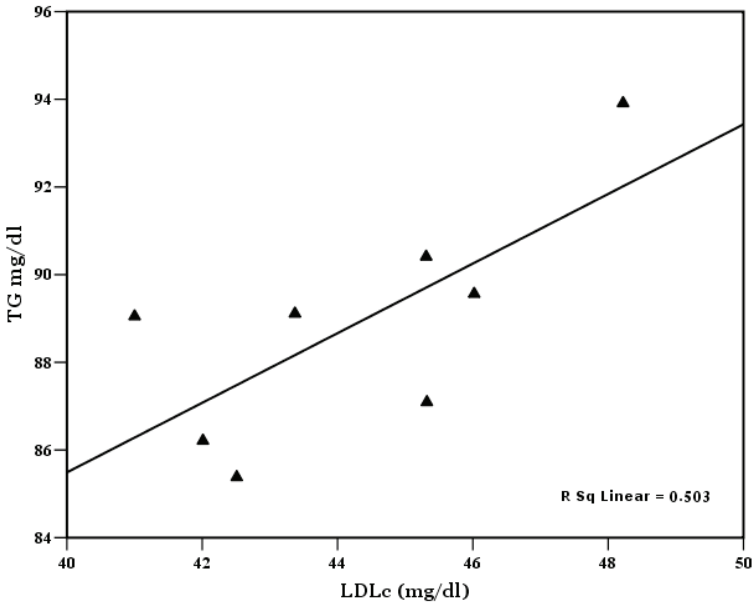

Fig. 6. Correlation between serum TG and serum LDL-c A positive correlation between serum TG and serum LDL-c

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