Physiological study on the influence of some plant oils in rats exposed to a sublethal concentration of diazinon

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Abstract The present study was aimed to evaluate the influence of olive, sesame and black seed oils on levels of some physiological parameters in male rats exposed to diazinon (DZN). Body weight changes, and levels of serum total protein, albumin, glucose, triglycerides, cholesterol, high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C), very low density lipoprotein cholesterol (VLDL-C), atherogenic index (AI), atherogenic coefficient (AC), cardiac risk ratio (CRR), glutathione (GSH), superoxide dismutase (SOD) and malondialdehyde (MDA) were selected as physiological parameters. The experimental animals were distributed into nine groups. Rats group exposed to DZN and fed with normal diet resulted in pronounced severe changes including reduced body weight gain rate, significantly increase in levels of serum albumin, glucose, cholesterol, LDL-C, AI, AC, CRR and MDA while levels of HDL-C, GSH and SOD were decreased. In rats treated with DZN, the supplementation of the olive, sesame and black seed oils showed remarkable lowering influences of physiological alterations. Moreover, the present results confirmed that these oils possess antioxidative effects against DZN toxicity. Finally, the present findings suggest that these oils are safe and promising agents for the treatment of physiological disturbances induced by DZN and may be also by other pollutants, and toxic and pathogenic factors.

1. Introduction

Currently, the environment and people are continually exposed to numerous environmental pollutants. The majority of pollutants are potentially toxic for organisms, some being connected to disease development. In this context, the increase of chronic degenerative disease including cancer in humans, is of considerable concern (Gupta, 2006). Pesticides are a very important group of environmental pollutants used in intensive agriculture for protection against diseases and pests. The esti-
mated annual application is more than 4 million tons, but only 1% of this reaches the target pests (Gavrilescu, 2005). Organophosphorus compounds make up about 70% of the pesticides used worldwide. High-level of exposure to these neurotoxins can result in death, while repeated or prolonged exposure can cause delayed cholinergic toxicity and neurotoxicity (Tuovinen et al., 1994). According to World Health Organization (WHO), 3 million cases of pesticide (mainly organophosphorus compounds) poisoning occur every year, resulting in an excess of 250,000 deaths (Banerjee et al., 2014). Of these, about 1 million are accidental, and 2 million are suicidal poisonings (Joshi et al., 2006). Organophosphate insecticides are widely used for the control of agricultural, industrial and domestic pests. However, the uncontrolled use of insecticides has diverse effects on ecological system and public health (Tuna et al., 2011). Diazinon (DZN) is an organophosphorous insecticide widely used in agriculture and pest control in the environment, which can be highly toxic (Poet et al., 2004; Sarabia et al., 2009). DZN is bioactivated by cytochrome P450 enzymes through desulphurization to its corresponding oxon derivative (Sams et al., 2004). Additionally, experimental studies showed that the exposures to DZN induced physiological and histopathological changes in rats, mice and rabbits (Kalender et al., 2006; Al-Attar, 2009, 2015; Al-Attar and Al-Taisan, 2010; Sarhan and Al-Sâhâf, 2011; Al-Attar and Abu Zeid, 2013; Boroushaki et al., 2013; Abdel-Daim, 2016; Razavi et al., 2016).

Recently, more attention has been paid to the natural antioxidants owing to its protective effects against the toxicity of various pollutants and pathogenic factors, especially whenever reactive oxygen species (ROS) are involved. Currently, there is a strong interest in developing new therapeutic agents from natural products (Fujimori et al., 2010). Olive oil is an integral ingredient in the Mediterranean diet. Olive oil appears to be a functional food with various components such as monounsaturated fatty acids (MUFA) which may have nutritional benefits. It is also a good source of phytochemicals, including polyphenolic compounds (Visioli and Galli, 1998; Lavelli, 2002). The increasing popularity of olive oil is mainly attributed to its antioxidant and antiinflammatory effects which may help prevent disease in humans (Tuck et al., 2001; Tuck and Hayball, 2002; Covas, 2007). Sesame oil has been employed in the food and pharmaceutical industries due to the high lipids and protein content and its distinctive flavor (Abou-Gharbia et al., 1997, 2000). Sesame oil contains sesamin, sesamolin and sesaminol lignan fractions, which are known to play an important role in its oxidative stability and antioxidative activity (Elleuch et al., 2007). Sesame seeds and sesame oil have long been used as health foods and display multiple physiological functions against different pathological factors and symptoms (Matsumura et al., 1995; Namiki, 1995; Sacco et al., 2008; Philip, 2010; Sharif et al., 2013; Hsu et al., 2016). Black seed has been used in many Middle Eastern countries as a natural remedy (Swamy and Tan, 2000). Black seed is most extensively investigated for therapeutic purposes (Aggarwal et al., 2008). Recently, clinical and animal studies have shown that extract of the black seeds have many therapeutic effects (Al-Attar and Al-Taisan, 2010; Boskabady et al., 2011; Parhizkar et al., 2011; Babazadeh et al., 2012; Hamed et al., 2013; Imam et al., 2016). Therefore, the present study was undertaken to evaluate the effectiveness of olive, sesame and black seed oils as protective factors against DZN-induced physiological alterations in male rats.

2. Materials and methods

2.1. Animals

The experiments were done using male rats of the Wistar strain, weighing 92.8–133.3 g. Rats were obtained from the Experimental Animal Unit of King Fahd Medical Research Center, King Abdulaziz University, Jeddah, Saudi Arabia. The experimental animals were allowed to acclimatize for one week before starting the experimentations. Rats were maintained in controlled temperature (20 ± 1°C), humidity (65%) and a 12 h dark-light cycle, with balanced food and free access to water. The principles of laboratory animal care were followed throughout the duration of experiment and instruction given by the King Abdul Aziz University ethics committee was followed regarding experimental treatments.

2.2. Experimental protocol

Rats were randomly distributed into nine groups of ten each. The first group was untreated and served as control. The second group was orally treated with 50 mg/kg body weight of DZN in corn oil, daily for 6 weeks. The third group was orally supplemented with olive oil at a dose of 600 mg/kg body weight and after 4 h exposed to DZN at the same dose given to the second group, daily for 6 weeks. The fourth group was orally supplemented with sesame oil at a dose of 600 mg/kg body weight and after 4 h subjected to DZN at the same dose given to the second group, daily for 6 weeks. The fifth group was orally supplemented with black seed oil at a dose of 600 mg/kg body weight and after 4 h treated with DZN at the same dose given to the second group, daily for 6 weeks. The sixth, seventh and eighth groups were orally supplemented with olive, sesame and black seed oils respectively at a dose of 600 mg/kg body weight and after 4 h subjected to DZN at the same dose given to the second group, daily for 6 weeks. The body weights of rats were determined at the start of the experimental period and after six weeks using a digital balance. These weights were measured at the same time during the morning (Al-Attar and Zari, 2010). Moreover, the experimental animals were observed for signs of abnormalities throughout the period of study.

At the end of the experimental period, rats were fasted for 12 h, water was not restricted, and then anaesthetized with diethyl ether. Blood samples were collected from orbital venous plexus in non-heparinized tubes, centrifuged at 2500 rpm for 15 min and blood sera were then collected and stored at −80°C. The level of serum total protein was measured according to the method of Peters (1968). The level of albumin was estimated using the method of Doumas et al. (1973). The level of glucose was determined using the method of Trinder (1969). To estimate the triglycerides level, Fossati and Prinicip method (1982) was used. The method of Richmond (1973) was used to determine the level of cholesterol. The method of Warnick et al. (1983) was used to measure the level of high density lipoprotein cholesterol (HDL-C). The level of serum low density lipoprotein cholesterol...
Fig. 1. A gradual increase in the body weight of normal control after 6 weeks of all experimental groups are represented in ity of a one-way analysis of variance (ANOVA). Statistical probabil-
mean ± standard deviation (S.D.). Data were analyzed using the Statistical Package for Social Sciences (SPSS) for Windows in the values of body weight gain were observed in rats treated compared with their initial body weights. Significant decreases black seed oil (151.8%) and corn oil (195.4%) was recorded (175.8%), DZN plus sesame oil (194.6%), DZN plus black rats (173.2%) and those exposed to DZN plus olive oil (39.4%) compared with control rats. The minimum body weight gain was noted in DZN-intoxicated rats (130.2%).
Levels of serum total protein, albumin, glucose, triglycer-
erides, cholesterol, HDL-C, LDL-C, VLDL-C, AI, AC and CRR in control, DZN, olive oil plus DZN, sesame oil plus DZN, black seed oil plus DZN, olive oil, sesame oil, black seed oil and corn oil treated rats are represented in Fig. 2(A–K). Specifically, there were no significant differences in levels of serum total protein in DZN, olive oil plus DZN, sesame oil plus DZN, black seed oil plus DZN, olive oil, sesame oil, black seed oil and corn oil treated rats as compared with control rats (Fig. 1A). Notable increases in the level of serum albumin were observed in rats treated with DZN (9.6%), olive oil plus DZN (5.5%) and sesame oil plus DZN (4.5%) compared with control rats (Fig. 2B). DZN administration to normal rats significantly increased the level of serum glucose (27.2%) compared with control rats (Fig. 2C). Serum triglycerides level was markedly decreased in mice treated black seed oil plus DZN (32.1%) compared to control rats (Fig. 2D). In comparison with control rats (Fig. 2E), the level of serum cholesterol was significantly elevated in rats treated with DZN (29.8%). Statistically decline in the level of serum cholesterol was detected in rats treated with olive oil (27.0%). As shown in Fig. 2F, a signif-

ductance in the level of serum HDL-C was observed in DZN-intoxicated rats (26.2%) as compared with control rats. Rats treated with black seed oil plus DZN showed a significant increase in the level of serum HDL-C (29.1%) compared with control rats. In comparison with control data (Fig. 2G), the level of serum LDL-C was significantly raised in rats treated with DZN (116.1%). Noticeably declines of serum VLDL-C were observed in rats subjected to black seed oil plus DZN (29.2%) and black seed oil (33.3%) compared with control rats (Fig. 2H). In comparison with control rats, the administration of DZN alone significantly elevated the level of serum AI (141.0%). Treatment with black seed oil plus DZN induced a significant decrease (39.3%) in the level of AI (Fig. 2I). As it is shown in Fig. 1J, the level of serum AC was statistically increased in rats exposed to DZN (219.7%). Remarkable declines in the level of serum AC were noted in rats treated with black seed oil plus DZN (47.2%), olive oil (80.8%), sesame oil (44.3%) compared with control rats. The level of serum CRR was statistically enhanced in rats treated with DZN (75.9%). The level of serum CRR was statistically decreased in rats supplemented with olive oil (32.1%) compared with control rats (Fig. 2K).
Levels of serum GSH, SOD and MDA are represented in Table 1. Serum GSH was declined in rats exposed to DZN (43.7%) and sesame oil plus DZN (24.2%) compared with control rats. Levels of serum SOD (Fig. 11) were significantly decreased in rats exposed to DZN (31.1%) and sesame oil plus DZN (9.7%). Additionally, levels of serum MDA were significantly increased in rats treated with DZN (39.4%) and sesame oil plus DZN (21.1%) compared to control rats.
4. Discussion
The exposure to organophosphorus pesticides has been associ-
ated with many physiological, biochemical and histopatholog-
ical alterations. The exposure of pesticides and other toxicants to humans and to a variety of forms of life has become an overall health problem. The present study showed that the
maximum increases of body weight were observed in rats treated with corn oil (195.4%) followed by DZN plus sesame oil (194.6%), olive oil (183.8%), DZN plus olive oil (175.8%), DZN plus black seed oil (173.8%), control (173.2%), sesame oil (153.8%) and black seed oil (151.8%) treated rats. The significant increase in body weight in the control rats may be representative of the normal pattern of growth in rodents. Contrary, the minimum increase of body weight was observed in DZN treated rats (130.2%). These observations were also noted in rats exposed to DZN and other pesticides (Mansour et al., 2007; Johari et al., 2010; Mossa et al., 2011; Heikal et al., 2012; Adjrah et al., 2013; El-Sheikh and Galal, 2015). Additionally, the decreased body weight could be due to reduced diet consumption, anorexia, reduced absorption of nutrients from the intestine, increase of catabolic processes, increased degradation of lipids and proteins, and excessive loss of water, salts and proteins as a result of kidney dysfunction.

The present increase in levels of serum albumin, glucose, triglycerides, cholesterol, HDL-C, LDL-C, VLDL-C (H), AI (I), AC (J) and CRR (K) in control, DZN, olive oil plus DZN, sesame oil plus DZN, black seed oil plus DZN, olive oil, sesame oil, black seed oil and corn oil treated rats.
such, it represents a major synthetic protein and is a marker of the ability of the liver to synthesize proteins (Johnston, 1999). The changing levels of serum albumin, thus, provide valuable indices of severity, progress, and prognosis in hepatic disease (Saxena and Saxena, 2010). Hyperalbuminemia is the medical terminology used to describe elevated levels of serum albumin. Chief factors responsible for hyperalbuminemia are severe infections, congenital disorders, severe dehydration, hepatitis, malnourishment, chronic inflammatory diseases, tuberculosis, an overdose of cortisone drugs, excessive synthesis of cortisol by the adrenals, tumor that manufactures cortisol-like substances, congestive cardiac failure, kidney diseases, HIV and cancer. An increase in serum albumin indicates poor liver function or impaired synthesis and it may be either in liver cells damage or diminished protein intake (Kalaiselvi et al., 2015). Blood glucose concentration is known to depend on the ability of the liver to absorb or produce glucose. The liver performs its glucostatic function owing to its ability to synthesize or degrade glycogen according to the needs of the organism, as well as via gluconeogenesis (Ahmed et al., 2006). Several investigations showed that levels of serum glucose were significantly enhanced in rats, mice and rabbits exposed to DZN and other pesticides (Das et al., 2010; Salih, 2010; Zari and Al-Attar, 2011; Al-Attar and Abu Zeid, 2013; Lukaszewicz-Hussain,
Table 1  Levels of serum GSH, SOD and MDA of control, DZN, olive oil plus DZN, sesame oil plus DZN, black seed oil plus DZN, olive oil, sesame oil, black seed oil and corn oil treated rats. Percentage changes are included in parentheses.

| Treatments          | Parameters | GSH (mg/ml) | SOD (U/ml) | MDA (nmol/ml) |
|---------------------|------------|-------------|------------|---------------|
| Control             |            | 39.94 ± 4.00 | 228.67 ± 18.01 | 135.79 ± 11.15 |
| DZN                 |            | 22.49 ± 4.37 | 15.04 ± 23.22 | 31.26 ± 20.1 (± 39.4) |
| Olive oil + DZN     |            | 35.94 ± 5.31 | 214.80 ± 13.65 | 132.01 ± 8.00 (± 2.8) |
| Sesame oil + DZN    |            | 30.37 ± 5.75 | 206.34 ± 16.06 | 164.37 ± 21.7 (± 21.1) |
| Black seed oil + DZN|            | 37.14 ± 5.91 | 208.71 ± 22.02 | 142.64 ± 18.32 (± 5.1) |
| Olive oil           |            | 38.42 ± 4.34 | 221.26 ± 27.24 | 135.64 ± 18.31 (± 0.1) |
| Sesame oil          |            | 39.17 ± 4.41 | 220.26 ± 17.23 | 139.30 ± 14.62 (± 2.6) |
| Black seed oil      |            | 40.40 ± 7.62 | 238.29 ± 27.18 | 133.66 ± 16.02 (± 1.6) |
| Corn oil            |            | 38.83 ± 3.59 | 231.06 ± 30.83 | 137.13 ± 14.31 (± 1.0) |

2013; Abd Elmonem, 2014). Additionally, Al-Attar (2015) reported that the progressive accumulation of blood glucose revealed that rats became hyperglycemic due to DZN intoxication. This case may be due to the enhancement of the activities of the enzymes involved in gluconeogenesis leading to formation of glucose from non-carbohydrate sources coupled with inhibition of liver glycogenolysis or stimulating glycogenolysis processes to increase the level of blood glucose from the liver as a main source of carbohydrates in the body.

Dyslipidemia, characterized by abnormally elevated plasma triglyceride and cholesterol concentrations, is an established risk factor in the development of coronary heart disease (Al-Attar, 2010). Therefore, it is reasonable to expect an abnormal lipid profile in those with severe liver dysfunction (Ghadir et al., 2007). Hyperlipidemia is one of the established major risk factors of coronary heart disease and cerebrovascular disease. The most common risk factors associated with increased risk of atherosclerotic heart disease or stroke are abnormalities of lipids, hypertension, smoking and some coagulation and hemostatic factors (Sarraf-Zadegan et al., 1999). Hypertriglyceridemia could be attributed to the reduction of lipase activity, which could lead to a decrease in triglyceride hydrolysis (John et al., 1985). Hypercholesterolemia could be attributed to damage of hepatic parenchymal cells that lead to disturbance of lipid metabolism in the liver (Havel, 1986). The liver injury of different etiologies is often accompanied by secondary lipoproteinemia, which may lead to the development of atherosclerosis, particularly when associated with hypercholesterolemia characterized by an increase in LDL-C and decrease in HDL-C (Miller and Miller, 1975; Steinberg et al., 1989). Atherogenic indices (AI, AC and CRR) have been described as powerful indicators of the risk of cardiovascular disease; the higher the value, the higher the risk of developing the disease and vice versa (Dobiasová, 2004; Rang et al., 2005; Martirosyan et al., 2007; Ikewuchi and Ikewuchi, 2009; Ikewuchi, 2012). Traditionally, the atherogenic lipid profile is made up of increased triglycerides, cholesterol and LDL-C, and decreased HDL-C (Popa et al., 2012). AI indicates the deposition of foam cells or plaque or fatty infiltration or lipids in heart, coronaries, aorta, liver and kidney. The higher the AI, the higher is the risk of above organs for oxidative damage (Basu et al., 2007). However, the present increase of serum AI, AC and CRR indicates that DZN caused the risk of cardiovascular injury. In addition, several studies showed that levels of blood triglycerides, cholesterol, LDL-C and VLDL-C were significantly increased with the decrease in levels of serum HDL-C in experimental animals exposed to DZN and other pesticides (Attiia and Nasr, 2009; Rai et al., 2009; Al-Attar and Abu Zeid, 2013; Bhushan et al., 2013; Abd Elmonem, 2014; El-Demerdash and Nasr, 2014; Al-Attar, 2015). The present study showed that the supplementation of olive oil significantly decreased levels of serum cholesterol and CRR in normal rats. Previous studies showed that olive oil reduced levels of serum cholesterol in mice (Rezq et al., 2010), rats (Ayad et al., 2013) and guineapig (Kalita et al., 2014). Olive oil is regarded as healthy dietary oil because of its high concentration of MUFA and phenolic compounds, which have tangible health benefits (Owen et al., 2000; Covas et al., 2006). Olive oil improves the lipid profile because of high concentration of MUFA and phenolic compounds both having a lipid lowering action and prevents LDL-C oxidation. Epidemiological studies also suggest that Mediterranean diet rich in olive oil decreases the risk of cardiovascular disease and improves its major risk factors (Gorinstein et al., 2002; Perez-Jimenez et al., 2005; Cicerale et al., 2010).

In the present study, the administration of DZN induced oxidative stress which was confirmed by the increases of serum GSH and SOD levels and a decrease of MDA level. However, several experimental studies showed similar observations in rats and mice exposed to DZN and other pesticides (Salehi et al., 2012; Abbasy et al., 2014; Mohamed and Ali, 2014; Arafa et al., 2015; Beydilli et al., 2015; Eraslan et al., 2015; Liu et al., 2016). Recently, data indicate that the toxic action of pesticides may include the induction of oxidative stress and accumulation of free radicals in the cell. Many reports showed that the exposures to pesticides caused oxidative damages in humans and animals (Gaikwad et al., 2015). The effects of DZN as inducers of oxidative stress on certain biomarkers in various tissues have been studied (Isika and Celik, 2008). One of the biomarkers for oxidative stress is lipid peroxidation which is claimed to possess predictive significance in quite a number of investigations (Ogutcu et al., 2006; Amirkabirian et al., 2007). Oxidative stress, moreover, is triggered as an absence of balance between antioxidant system and oxidant condition which pesticide toxicity brings about. In fact, reactive oxygen species (ROS) are produced by univalent reduction of dioxygen to superoxide anion (O2·−), which in turn is disproportionate to H2O2 and O2 spontaneously or through a reaction catalyzed by SOD. Endogenous H2O2 may be converted to H2O either by catalase or glutathione peroxidase (GSH-Px). Otherwise, it may generate a highly reactive free hydroxyl radical (·OH) via a Fenton reaction, which is responsible for...
oxidative damage. GSH-Px converts H$_2$O$_2$ or other lipid peroxides to water or hydroxyl lipids, and during this process GSH is converted to oxidized glutathione (Bachowshi et al., 1997). Antioxidants are defense against free radical and oxidative attacks. They act as free radical scavengers and slow down not only radical oxidation but also the accompanying damaging effects in the body (Nice, 1997).

In the present study, the pretreatment of the studied oils inhibited the hematobiochemical alterations induced by DZN toxicity. The effect of these oils was confirmed by an inhibition of oxidative stress which indicated by the enhancement of GSH and SOD levels as well as the decline of MDA level compared to DZN intoxication alone. Scientists have focused on the preventive effects of phenols against degenerative diseases mediated by the ROS. It has been reported that the phenolic compounds are able to interact with the biological systems and act as bioactive molecules. They are particularly important inhibitors of lipid peroxidation, and are believed to be effective through their free radical scavenging and metal-chelating properties (Kandaswami and Middleton, 1994; Salah et al., 1995; Rice-Evans et al., 1996). In experimental studies, olive oil phenolic compounds showed strong antioxidant properties (Kandaswami and Middleton, 1994; Salah et al., 1995; Rice-Evans et al., 1996). In experimental studies, olive oil phenolic compounds showed strong antioxidant properties against lipids, DNA and LDL oxidation (Covas et al., 2006). Hydroxytyrosol, one of the phenolic compounds present in olive oil, has been suggested to be a potent antioxidant, thus contributing to the beneficial properties of olive oil (Detana et al., 1999). Hydroxytyrosol administration has been shown to reduce the concentrations of passive smoking-induced oxidative stress, prevent LDL oxidation, platelet aggregation and inhibit leukocyte 5-lipoxygenases (Petroni et al., 1995; Wiseman et al., 1996; De la Puerta et al., 1999; Visioli et al., 2000). Hydroxytyrosol has shown efficacy in preventing oxidative stress in the liver of rats intoxicated by cadmium (Casalino et al., 2002). In addition, when human hepatoma HepG2 cells were pre-treated with hydroxytyrosol prior to submission to tert-butyl hydroperoxide-induced oxidative stress, cell toxicity was completely prevented, indicating that the antioxidant-treated cells were totally protected against the oxidative insult (Goya et al., 2007). Nakbi et al. (2010) assessed the effects of olive oil, hydrophilic and lipophilic fractions on 2,4-dichlorophenoxyacetic acid-induced oxidative damage in the liver of rats. They showed that olive oil and its extracts protect against oxidative damage of hepatic tissue by preventing excessive lipid peroxidation to increase MUFA composition and by maintaining serum marker enzymes and hepatic antioxidant enzyme activities at near normal concentrations. It is the hydrophilic fraction of olive oil which seems to be the effective one in reducing 2,4-dichlorophenoxyacetic-induced oxidative stress, indicating that hydrophilic extract may exert a direct antioxidant effect on hepatic cells. Abd El-Fattah and Barakat (2013) evaluated the effect of dietary olive oil on liver damage and oxidative stress induced by 2,4-dichlorophenoxyacetic acid in rats. Olive oil administration during 2,4-dichlorophenoxyacetic acid treatment induced an improvement in hepatic antioxidant enzymes, serum transaminase activities and liver free fatty acids levels. Biochemical results were confirmed by the liver histopathological examination. They concluded that olive oil has protective effect against oxidative stress and liver damage induced by 2,4-dichlorophenoxyacetic acid. Amamou et al. (2015) examined the possible protective effect of olive oil consumption against cadmium-induced damage on plasma lipids and stress biochemical parameters of male rats. Co-treatment with olive oil significantly improved the oxidative damage induced by cadmium. The antioxidant potential in the plasma and liver was markedly restored with a significant decline in MDA levels and activity of transaminases. They suggested that olive oil consumption could protect the rat liver against cadmium-induced injury by increasing the activities of antioxidant enzymes and reducing oxidative stress.

Previous studies showed that the sesame oil ameliorated the influences of several chemical factors such as acetaminophen, aminoglycoside, iodinated contrast, cypermethrin, acetic acid, formalin and deoxycorticosterone acetate. Generally, these studies suggested that the protective effect of sesame oil against the influences of chemical toxicities associated with inhibition of the oxidative stress in experimental animals (Chandrasekaran et al., 2010; Hsu et al., 2011; Hussien et al., 2013; Monteiro et al., 2014; Liu et al., 2015; Soliman et al., 2015). Danladi et al. (2013) examined the effect of black seed oil on carbon tetrachloride (CCl$_4$)-induced liver damage in rats. The results showed that the increase and decrease in liver enzymes were almost restored to normal in animals treated with black seed oil and CCl$_4$ has that of the control. CCl$_4$ caused elevated levels of thiobarbituric acid reactive substances (TBARS) and a marked depletion of liver endogenous antioxidant enzymes. Black seed oil treatment positively protects the alterations in these biochemical variables in the CCl$_4$ plus black seed oil-treated rats. Black seed oil markedly reduced elevated TBARS and significantly increased levels of antioxidant enzymes. Black seed oil showed hepatoprotection on liver structures of rats exposed to CCl$_4$. They demonstrated that that black seed oil through its antioxidant activity effectively protects CCl$_4$-induced liver toxicity. Hamed et al. (2013) investigated whether black seed oil could attenuate hepatoportal injury induced by bromobenzene exposure. Treatment with black seed oil alleviated the elevation of GSH, succinate dehydrogenase (SDH), lactate dehydrogenase (LDH), glucose-6-phosphatase, serum protein, nitric oxide, Na-K-adenosine triphosphatase, phospholipids levels and attenuated MDA, SOD, alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP). Diminution of collagen content and improvement in liver and kidney architectures were observed. They concluded that black seed oil enhanced the hepatorenal protection mechanism, reduced disease complications and delayed its progression. El-Sheikh et al. (2015) investigated the mechanisms by which thymoquinone, the primary bioactive component of black seed, can prevent methotrexate-induced hepatorenal toxicity in rats. They demonstrated that the thymoquinone reversed oxidative and nitrosative stress, as well as inflammatory and apoptotic signs caused by methotrexate alone. Thus, thymoquinone may be a beneficial adjuvant that confers hepatorenal protection to methotrexate toxicity via antioxidant, antinitrosative, antiinflammatory, and antiapoptotic mechanisms. Samarghandian et al. (2015) studied the protective effect of thymoquinone on gentamicin-induced acute renal failure in rats. They suggested that thymoquinone may ameliorate acute renal failure through modulation of the oxidative stress and inflammatory responses. Collectively, the obtained results provided evidence indicating that the supplementation of the olive, sesame and black seed oils inhibited the physiological alterations induced by DZN intoxication. The influences of these oils were attributed to its ability to reduce the oxidative...
stress. Therefore, these oils could be beneficial additional therapeutic agents in the treatment of physiological disturbances induced by DZN. Longer duration investigations with different doses of these oils are required to develop potent therapeutic agents against the toxicity of DZN and may be also against other pollutants, and toxic and pathogenic factors.

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