

Original research Articles

Amelioratory effect of *Dorema glabrum* on diazinon-induced oxidative stress in rat liver

Abstract:

Introduction: A wide number of pesticides, including highly persistent organophosphorous compounds, such as diazinon (DZN) have deteriorating effect on fauna and flora by inducing oxidative stress. DZN induces cell damage by producing free radicals and reactive oxygen species. Considering that the involvement of reactive oxygen species (ROS) has been implicated in the toxicity of organophosphate insecticides (OPIs), the level of lipid peroxidation, were analysed in addition to the antioxidant enzymes such as catalase (CAT), glutathione peroxidase (GPx), superoxide dismutase (SOD) activities in the liver tissue. The aim of this study was to investigate the ameliorative properties of *D. glabrum* against the subchronic effect of DZN on oxidative damage markers such as lipid peroxidation (LPO) and the antioxidant defense system (ADS) in the liver of male Wistar rats.

Methods: Twenty-four adult male Wistar rats were used in this study. The rats randomly divided into four groups including a control group, and three experimental groups. Two of three experimental groups received different doses of *D. glabrum* (40 and 80 mg/kg) as pre-treatment for 21 days along with DZN (100 mg/kg) that injected intraperitoneally in the last day of *D. glabrum* usage, and one group received only DZN (100 mg/kg).

Results: The significantly increased levels of LPO and the decreased antioxidant defenses, like free radical scavenging enzymes viz., catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPx) were noted in DZN-treated group as compared to control group. Distinctly increased LPO levels, with alterations in endogenous antioxidant enzyme were evident in hepatic toxicity of DZN. Hepatic specific marker enzymes were restored to normalcy in rats supplemented with *D. glabrum* following treatment with DZN which otherwise was decreased in the DZN-treated rats. The results show that co-treatment of *D. glabrum* with DZN prevents or diminishes the oxidative stress of DZN-treated rats and may act as a putative protective agent against DZN-induced liver tissue injury.

Key words: Diazinon, Oxidative stress, *Dorema glabrum*, liver

Introduction:

Organophosphorous (OP) pesticides are among the chemical compounds most commonly used to control agricultural plagues. Their mechanism of action is based on the inhibition of acetylcholinesterase activity through covalent binding to its serine residues, thus producing a

detention of the nerve impulses that leads to death (Singh *et al.* 2002; Abdollahi *et al.* 2004). Among the most frequently used OP insecticides, O,O-diethyl O-2-isopropyl- 6-methyl pyrimidinyl-4-g-1-phosphorothioate (diazinon), a chemical synthetic substance, is used worldwide to eliminate crop and cattle plagues, as well as in household pest control (Grafft *et al.* 2002; Sarabia *et al.* 2009). Some reports have been published, with respect to DZN and its effects on biochemical and hematological parameters of rats, rabbits, and mice (Quistad *et al.* 2001; Kalender *et al.* 2005 and 2006; Yehia *et al.* 2007; El-Shenawy *et al.* 2009). DZN can be absorbed through the digestive system, the skin, or via the respiratory tract when inhaled. Although it is mainly eliminated by the kidney, microsomal enzymes in the liver oxidize DZN produce more potent acetylcholinesterase inhibitors, such as diazoxon, hydroxydiazoxon and hydroxydiazinon (Quistad *et al.* 2001). DZN may interfere with lipid metabolism in mammalian animals. Furthermore, organophosphorus insecticides (OPIs) could induce oxidative stress, which might play a crucial role in its induced poisoning, suggested from in vivo animal studies (Monteiro *et al.* 2006), observations in humans (Zhou *et al.* 2002) and in vitro studies (Gultekin *et al.* 2000) over the past several years. Acute effects of DZN as inducers of oxidative stress on certain biomarkers in various tissues have been studied (Isika *et al.* 2008). Estimation of lipid peroxidation has been found to have predictive importance of a number of studies as a biomarker for oxidative stress (Ogutcu *et al.* 2006; Amirkabirian *et al.* 2007). It can also occur as a consequence of an imbalance between antioxidant system and pro-oxidant state generated by pesticide toxicity. Endogenous enzymatic and non-enzymatic antioxidants are essential for the conversion of reactive oxygen species (ROS) to harmless metabolites as well as to protect and restore normal cellular metabolism and functions (Bebe *et al.* 2003). ROS can be detoxified by an enzyme defense system, comprising superoxide dismutase (SOD), catalase (CAT), and selenium-dependent glutathione peroxidase (GPx), or non-enzymatic systems by the scavenging action of reduced glutathione, while organic peroxides can be detoxified by the activity of glutathione S-transferase (GST) (Halliwell *et al.* 1999). Many insecticides have hydrophobic molecules, that bind extensively to biological membranes, especially to the phospholipids bilayers (Lee *et al.* 1991). Numerous studies showed that antioxidant substances protect cells against deleterious effects of several environmental agents (Almeida *et al.* 1998). Approximately half of the drugs currently in clinical use are of natural origin (Yano *et al.* 1994; Oubre *et al.* 1997; Gao *et al.* 2011). Although herbal therapies are becoming increasingly popular worldwide, we know little about active ingredients and the molecular mechanisms in many of those therapeutic herbs (Kumar *et al.* 2006; Gao *et al.* 2011). Some of them tend to possess functional groups (providing hydrogen bond acceptor/donors, etc) (Gao *et al.* 2011).

D. glabrum is a species that grows in Transcaucasia (Nakhchevan and Armenia zone) and north west of Iran. The genus *Dorema* from Apiaceae family is represented by seven species in Iranian flora, among them *D. glabrum* Fisch., *D. aucheri* Boiss and *D. ammonicum* D. Don are endemic

(Mozaffarian, 1996). *D. glabrum* which grows in loamy or rocky slopes is a perennial herb. It is useful as an food additive or herbal remedy in mentioned regions (Asnaashari *et al.* 2011). Members of this genus possess expectorant, antispasmodic, carminative, diaphoretic, mild diuretic, emmenagogue, stimulant, vasodilator (Mozaffarian, 2007), antimicrobial and antifungal (Shahidi *et al.* 2002; Kumar *et al.* 2006; Yousefzadi *et al.* 2011) and hepatoprotective (Govind *et al.* 2011) properties and are intensively used as a green vegetable or as a folk medicine for treatment of many diseases (Ibadullayeva *et al.* 2011). According to the common folk beliefs of Armenian and Azeri people, *D. glabrum* can cure many deseases especially different kinds of cancer. In a preliminary work, the crude extract of the plant demonstrated antioxidant activity and anti-lipidemic effects (Dehghan *et al.* 2009). It is in our interest to investigate amelioratory effect of *D. glabrum* organic extract (DGE) on organophosphorus insecticide DZN-induced oxidative stress in rat liver.

Materials and methods:

Animals:

Twenty-four adult male Wistar rats (weighting approximately 250-350 g) used in the present study. Animals were obtained from the animal house of the veterinary department of Tabriz University. The rats were kept under constant temperature 20-22°C and 12/12h cycle of light and darkness and access to enough food and water throughout the experiments. Ethics of working with laboratory animals have been followed during all procedures.

Animal treatment schedule:

The rats randomly divided into four groups including group I (C): normal control rats, group II (DZN): received DZN in single dose (100 mg/kg), group III (DGE 40): received (40 mg/kg) of *D.glabrum* and (100 mg/kg) of DZN, group IV (DGE 80): received *D.glabrum* (80 mg/kg) and DZN (100 mg/kg). This two experimental groups received different doses of *D.glabrum* (40 and 80 mg/kg) as pre-treatment for 21 days with DZN (100 mg/kg) that injected intraperitonealy on the last day of *D.glabrum* usage.

Plant Material :

Seeds of *D. glabrum* were collected during the fruiting stage from the slopes of Aras river; Jolfa, Eastern Azerbaijan, Iran. Air dried and then powdered seeds were subjected to extraction by refluxing ethanol using a soxhlet. Then the extract was dried by a rotary evaporator (Heidolph, Germany).

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Chemicals:

DZN was applied from Aria chemistry Co. (Iran) containing 96% active ingredies. It was diluted with corn oil as DZN solvent. Thiobarbituric acid (TBA), trichloroacetic acid (TCA), H₂O₂ (30%), ethylenediaminetetraacetic acid (EDTA), Tris-HCl, 2,2'-dinitro-5,5'dithio-dibenzoic acid (DTNB), ethanol of technical grade and the other chemicals used in this study were procured from Merck Co. (Germany).

Tissue preparation:

Livers were removed from the animals under ether anesthesia after 21 days of treatment and washed with cold saline buffer. Then washed tissues were immediately stored at -80°C. To obtain the enzyme extract, tissues were homogenized in ice-cold KCl 1.15% to yield 10% (W/V) homogenate. Then the homogenates were centrifuged at 1000 rpm for 10 min at 4°C. The supernatants were separated and used for enzyme activity of SOD, CAT and GPx which was expressed in international units per mg protein (IU/mg protein). Biomarkers for tissue damage were measured using the UV kinetics methodology and total protein was determined using bovine serum albumin (BSA) as standard and the values were expressed as mg/dl.

Analytical procedures:

Thiobarbituric acid reactive substances (TBARS) were measured as an index of lipid peroxidation by the method of Satho (Satho, 1978). SOD was determined according to the method described by Ukeda (Ukeda *et al.* 1997). CAT was measured by monitoring the decomposition of hydrogen peroxide, as described by Aebi (Aebi, 1984). GPx was evaluated by the method of Paglia and Valentine (Paglia *et al.* 1967). Protein was measured by the method of Lowry (Lowry *et al.* 1951) using bovine serum albumin as standard. Total thiol content (TSH) was measured in homogenate by the method of Hu (Hu *et al.* 1994).

Statistical analysis:

Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test (level of significance P < 0.05) using SPSS version 16, statistical program. The results are expressed as mean ± SEM, and were obtained from at least 6 rats in each group. Statistical analysis was based on comparing the values between the DZN and control group, while DZN-treated groups concomitantly with extract of *D. glabrum* (DGE) were compared with their corresponding group of DZN-treated rats.

Results:

Lipid peroxidation:

Lipid peroxidation (LPO) is refers to the oxidation of lipids by free radicals and it is one of the main manifestations of oxidative damage in tissues and cells. In the liver increased lipid peroxidation was observed as measured by a significant increase in MDA (malondialdehyd) that expressed as nano moles of TBARS/g of protein ($P<0.001$) in the rats treated with DZN when compared to control rats (90.37% Fig1). The group, which received DZN and DGE 40 and DGE 80 as cotreatment showed decreased levels of MDA when compared with DZN treated group ($P<0.001$) (23.4%, 26.4% respectively) (Fig1).

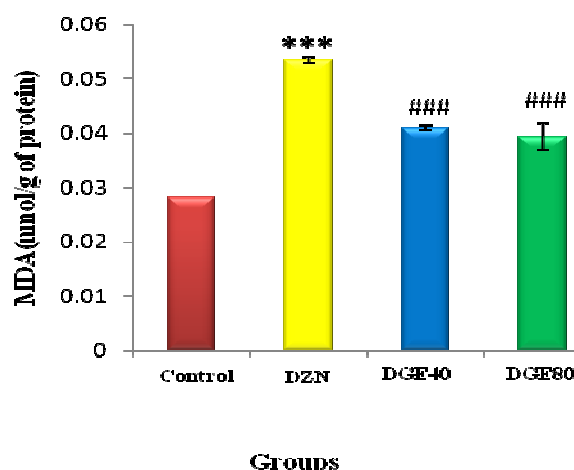


Fig 1: Effect of DGE 40 and DGE 80 on MDA level of DZN treated rats and effect of DZN (100 mg/Kg) on MDA level of normal rats in the liver. Values are mean $\pm$ SEM (n=6). *** $P<0.001$, compared to control group, ### $P<0.001$, compared to DZN group.

Antioxidant enzymes:

SOD, CAT and GPx play vital role in the cellular antioxidant defense mechanism. The activities of these enzymes (U/mg of protein) in liver were reduced significantly in DZN treated animals compared to controls ($P<0.001$). Whereas in DGE 40 and DGE 80 groups a significant increase in the activity of these enzymes was observed compared to rats treated with DZN alone ($P<0.001$). As presented in Fig 2, SOD activities were diminished by 60.35% by treatment with DZN. Treatment with DGE 40 and DGE 80 showed a significant increase of SOD activities by 142.47%, 121.68% respectively. In rats' liver treated with DZN, CAT activity was decreased by 6.06%. Nevertheless, a significant increase in CAT activity was observed in rats treated with DGE 40 and DGE 80. CAT increase activity was of about 33.77% and 53.5% respectively (Fig3). GPx activities were diminished by 22.26% by treatment with DZN. In rats supplemented with DGE 40 and DGE 80 we observed an increase in GPx activities about 12.26% and 27.27% respectively (Fig 4).

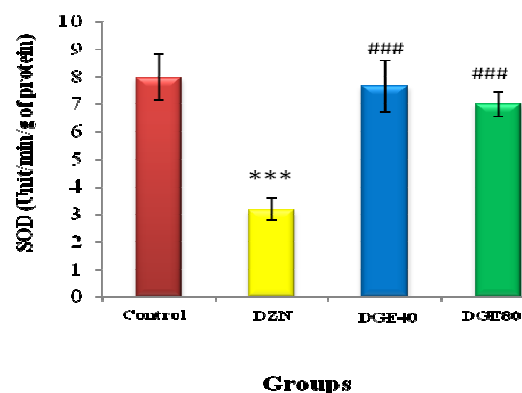


Fig 2: Effect of DGE 40 and DGE 80 on SOD activity of DZN treated rats and effect of DZN (100 mg/Kg) on SOD activity of normal rats. Values are mean±SEM, (n=6) *** P<0.001, compared to control group, ### P<0.001, compared to DZN group.

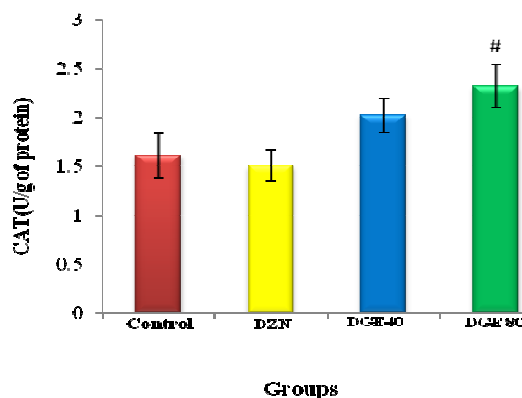


Fig 3: Effect of DZN (100mg/Kg) on CAT activity of normal rats ($p>0.05$) and the effect of DGE 40 and DGE 80 on CAT activity of DZN treated rats ($p>0.05$, # $p<0.05$ respectively).

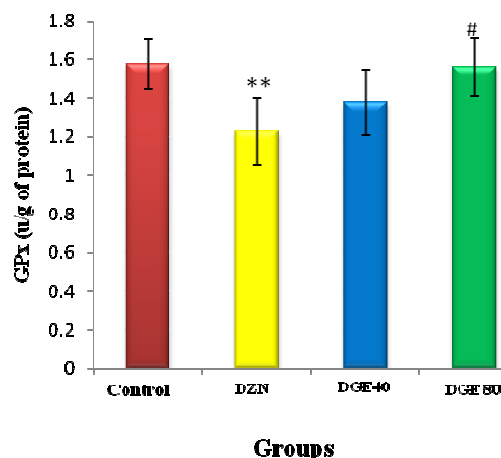


Fig 4: Effect of DZN (100 mg/Kg) on GPx activity of normal rats (**p<0.01) and effect of DGE 40 and DGE 80 on GPx activity of DZN treated rats (p>0.05, #p<0.05 respectively).

Total thiol content:

There was not significant difference in values of total thiol in rats which received DZN, DGE 40 and DGE 80 when compared with control rats (Fig 5).

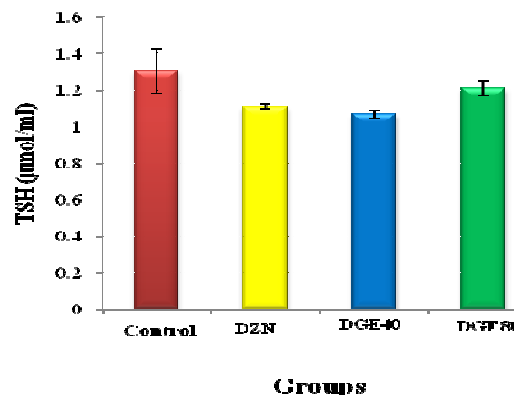


Fig 5: Values of total thiol in control group and treated rats with DZN, DGE 40 and DGE 80. There was not significant difference between the groups

Discussion:

Since years ago, considerable attention has been directed towards free radical mediated damage in biological systems due to pesticide exposure (Banerjee *et al.* 1999). The search for herbal drugs with antioxidant activity has gained importance as the dietary intake of antioxidants obtained from natural sources is considered to be relatively safe and without undesirable side effects (Dehghan *et al.* 2012). *D. glabrum* has extensive biological properties but most of the biological action of *D. glabrum* seems to be associated with its antioxidant potential. In the present study, the protective effect of *D. glabrum* has been evaluated against DZN-induced toxicity in the liver of rats. According to our results, DZN treatment promotes MDA content in the liver of rats. The increase of MDA observed in the liver following DZN exposure, was probably ascribed to the excessive production of ROS, which could be related to hepatocyte enzyme leakage. Mitochondria are the main source of ROS production (Wang *et al.* 2006 a ; Wang *et al.* 2006 a). Moussa and Hafez (Moussa *et al.* 1995) showed that the organophosphate and dimethoate inhibit mitochondrial enzyme activity. Moreover, DZN caused vacuoles and swelling of mitochondria in rat hepatocytes (Kalender *et al.* 2006; Ogutcu *et al.* 2006). The induction of an excess of ROS production can lead to oxidative injuries of important cellular macromolecules such as lipids, proteins, and nucleic acids (Bergamini *et al.* 2004). The present

study indicated that DZN treatment induced oxidative stress, as demonstrated by compromised antioxidant defenses and increased LPO in the liver.

Our results are in accordance with the studies of (El-Shenawy *et al.* 2010; and Abdulaziz *et al.* 2011) who demonstrated a significant increase in LPO level and a significant decrease in SOD, CAT and GPx activities in liver of rats exposed to DZN.

GSH redox cycles serve as a crucial component of cellular antioxidant defenses and are essential for the tissues to protect themselves against the ROS damage. They participate in the elimination of ROS, acting both as a non-enzymatic oxygen radical scavenger and as a substrate for various enzymes such as GPx (Tsukamoto *et al.* 2002). GPx is an important selenocysteine-containing enzyme for cellular antioxidant defense (Arthur, 2000). In this study, a significant decrease in GPx activity was observed in DZN treated animals. This may be due to enhanced free radical production, as evidenced by increased MDA levels. The decrease of GPx activity induced by DZN may be attributable to a direct inhibitory oxidative effect on the enzyme. Antioxidant enzymes such as SOD and CAT are considered to be a primary defense that prevents biological macromolecules from oxidative damage. SODs rapidly dismutate superoxide anion ($O_2^{\cdot-}$) to a less reactive molecule (H_2O_2), which is further degraded by CAT and GPx to water and oxygen (Wetscher *et al.* 1995). The results of the present study showed a significant decrease in SOD activity by DZN treatment. It is known that xenobiotics can induce mitochondria superoxide radical production (Peixoto *et al.* 2004) and if additionally SOD is inhibited the amount of ($O_2^{\cdot-}$) formed in a cell can reach dangerous levels. Superoxide radical is a potent inhibitor of CAT (Ashakumari *et al.* 1996). Depletion of antioxidant enzyme activity could be caused by a direct effect on the enzyme by DZN-induced ROS generation, depletion of the enzyme substrates and down-regulation of transcription and translation processes. DZN can affect both animals and human, although by a single exposure (Sarabia *et al.* 2009). Whereas its first toxicological effects are attributed to acetylcholinesterase inhibition. Oxidative stress is also attributed to DZN toxicity. From our results we can assume that hepatotoxic effects of DZN are mainly attributed to oxidative stress increase since the effects were prevented by *D. glabrum* supplementation. The antioxidant activity promoted by *D. glabrum* is essentially performed in the lipid membrane environment. *D. glabrum* allows free radicals to abstract a hydrogen atom from the antioxidant molecule, thus breaking the chain of free radical reactions (Pascoe *et al.* 1987). We have concluded that DZN toxicity in the liver could be attributed to the oxidative stress on the hepatic cells, which was increased leading to the depletion of the antioxidant enzymes that scavenge the toxic superoxide and hydrogen peroxide radicals, leading to an increase of LPO. Nonetheless, *D. glabrum* treatment could be useful to decrease DZN toxicity by quenching oxidative stress imposed by DZN.

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257 <http://profdoc.um.ac.ir/articles/a/1042855.pdf> ” date 26-29 August 2014

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