

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. In addition to the antioxidant enzymes such as catalase (CAT), glutathione peroxidase (GPx), superoxide dismutase (SOD) activities in the liver tissue, reactive oxygen species (ROS) have been employed in the toxicity of organophosphate insecticides (OPIs) and the level of lipid peroxidation was analyzed. The present study was designed to explore the ameliorative characteristics of *D.glabrum* against the subchronic impact of DZN on such oxidative damage markers as lipid peroxidation (LPO) and the antioxidant defense system (ADS) existing 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: Compared with the control group, we noticed significantly high levels of LPO and the low antioxidant defenses, like free radical scavenging enzymes viz., catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPx) in DZN-treated group. Considering the hepatic toxicity of DZN, evident changes were also noticed in endogenous antioxidant enzyme along with high LPO levels. In rats supplemented with *D.glabrum* as well as treated with DZN, hepatic specific marker enzymes were restored to normalcy which otherwise was lowered in the DZN-treated rats. The obtained results revealed that the oxidative stress of DZN-treated rats is diminished when *D.glabrum* is co-treated with DZN. This co-treatment may also 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 [1,2]. 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 [3,4]. Some reports have been published, with respect to DZN and its effects on biochemical and hematological parameters of rats, rabbits, and mice [5, 6, 7, 8, 9]. 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 [10]. 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 [11], observations in humans [12] and in vitro studies [13] over the past several years. Acute effects of DZN as inducers of oxidative stress on certain biomarkers in various tissues have been studied [14]. As a biomarker for oxidative stress, estimation of lipid peroxidation has the potential to predict the importance of a number of studies [15,16]. Owing to the absence of balance between antioxidant system and pro-oxidant state engendered by pesticide toxicity, lipid peroxidation can take place as well. To convert reactive oxygen species (ROS) to harmless metabolites, endogenous enzymatic and non-enzymatic antioxidants are required as indispensable elements because they also have the capability to protect and restore normal cellular metabolism and functions [17]. To detoxify ROS, an enzyme defense system is required composed of superoxide dismutase (SOD), catalase (CAT), and selenium-dependent glutathione peroxidase (GPx), or non-enzymatic systems by the scavenging action of reduced glutathione; however, to detoxify organic peroxides, the activity of glutathione S-transferase (GST) is essential [18]. Numerous insecticides possess hydrophobic molecules, that bind extensively to biological membranes, particularly to the phospholipids bilayers [19]. Quite a number of studies demonstrated that antioxidant substances protect cells against pernicious effects of some environmental agents [20]. Approximately half of the drugs currently in clinical use are of natural origin [21, 22, 23]. Despite the increasing popularity of herbal therapies worldwide, there is insufficient insight about active ingredients and the molecular mechanisms in a large number of those therapeutic herbs. Some of them tend to possess functional groups (providing hydrogen bond acceptor/donors, etc) [24].

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 [25]. *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 [26]. Members of this genus possess expectorant, antispasmodic, carminative, diaphoretic, mild diuretic, emmenagogue, stimulant,

vasodilator [27], antimicrobial and antifungal [28, 29] and hepatoprotective [30] properties and are intensively used as a green vegetable or as a folk medicine for treatment of many diseases [31]. Drawing on Armenian and Azeri's common folk beliefs, a number of illnesses especially various types of cancer can be cured by *D. glabrum*. Dehghan *et al.* (2009) in their seminal work demonstrated antioxidant activity and anti-lipidemic effects in the crude extract of the plant [32]. 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 intraperitoneally 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).

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 [33]. SOD was determined according to the method described by Ukeda [34]. CAT was measured by monitoring the decomposition of hydrogen peroxide, as described by Aebi [35]. GPx was evaluated by the method of Paglia and Valentine [36]. Protein was measured by the method of Lowry [37] using bovine serum albumin as standard. Total thiol content (TSH) was measured in homogenate by the method of Hu [38].

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 $\pm$ 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).

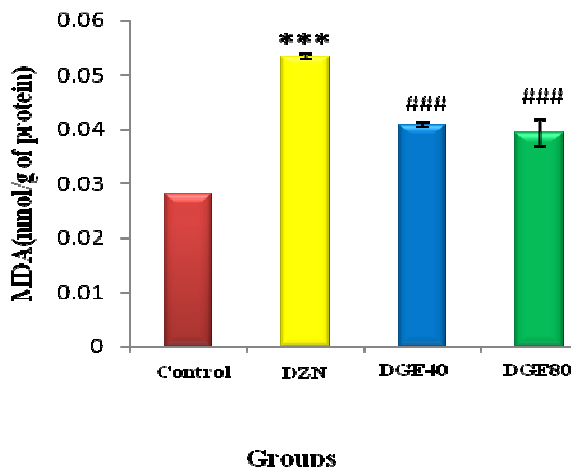


Fig 1: Effect of DGE 40 and DGE 80 on MDA level of DZN treated rats and effect of DZN (100 mg/Kg) on MDA level of normal rats in the liver. Values are mean $\pm$ SEM (n=6). *** $P<0.001$, compared to control group, ### $P<0.001$, compared to DZN group.

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).

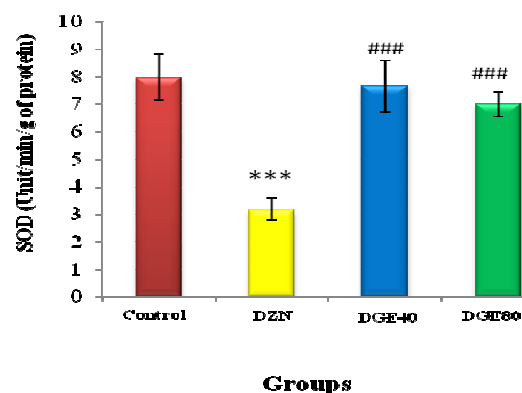


Fig 2: Effect of DGE 40 and DGE 80 on SOD activity of DZN treated rats and effect of DZN (100 mg/Kg) on SOD activity of normal rats. Values are mean $\pm$ SEM, (n=6) *** P<0.001, compared to control group, ### P<0.001, compared to DZN group.

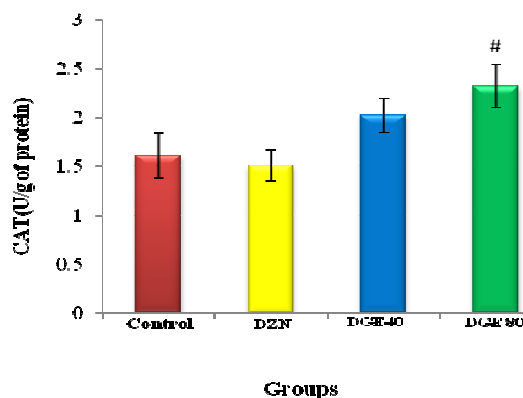


Fig 3: Effect of DZN (100mg/Kg) on CAT activity of normal rats ($p>0.05$) and the effect of DGE 40 and DGE 80 on CAT activity of DZN treated rats ($p>0.05$, # $p<0.05$ respectively).

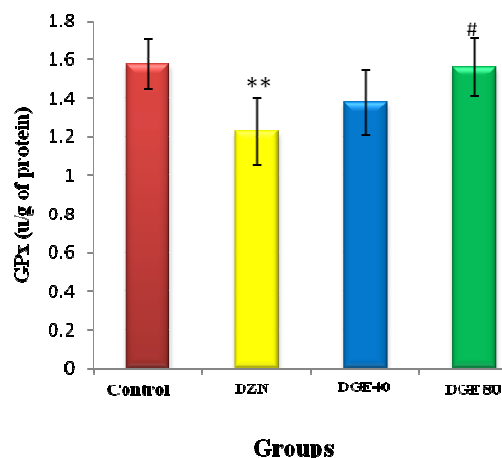


Fig 4: Effect of DZN (100 mg/Kg) on GPx activity of normal rats (**p<0.01) and effect of DGE 40 and DGE 80 on GPx activity of DZN treated rats (p>0.05, #p<0.05 respectively).

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).

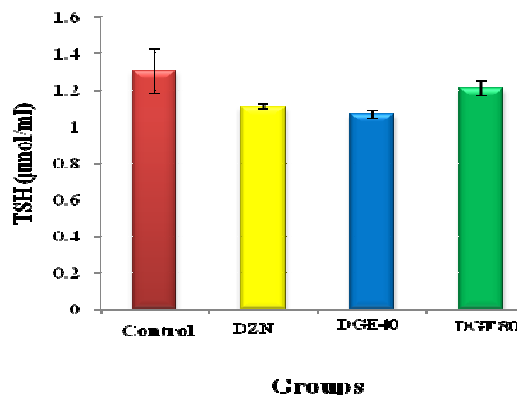


Fig 5: Values of total thiol in control group and treated rats with DZN, DGE 40 and DGE 80. There was not significant difference between the groups

Discussion:

Since years ago, considerable attention has been directed towards free radical mediated damage in biological systems due to pesticide exposure [39]. 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 [40]. *D. glabrum* has extensive biological properties but most of the biological action of *D. glabrum* seems to be associated with its antioxidant potential. The protective effect of *D. glabrum*, in the current study, has been estimated against toxicity induced by DZN in the liver of rats. Building on the obtained results, MDA content can be promoted via DZN treatment in the liver of rats. The increased MDA content in the liver following DZN exposure was likely attributed to the inordinate generation of ROS, which could be explained in terms of hepatocyte enzyme leakage. It is argued that mitochondria are the primary source of ROS production [41- 42]. Moussa and Hafez [43] indicated that mitochondrial enzyme activity is inhibited due to the organophosphate and dimethoate. DZN, furthermore, brought about vacuoles and swelling of mitochondria in rat hepatocytes [17 -15]. Bergamini *et al.* (2004) concluded that exceedingly induced ROS tends to result in oxidative injuries of significant cellular macromolecules including lipids, proteins, and nucleic acids [44]. The present investigation revealed that oxidative stress is induced by DZN

treatment, since compromised antioxidant defenses and increased LPO in the liver provide relevant evidence for this finding.

Our results are in accordance with the studies of [45] 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.

One of the indispensable components of cellular antioxidant defenses are GSH redox cycles which play an essential role for the tissues to protect themselves against the ROS damage. Serving as a non-enzymatic oxygen radical scavenger and as a substrate for differing enzymes like GPX, they take part in the removal of ROS [46]. GPX is claimed to be an essential selenocysteine-carrying enzyme for cellular antioxidant defense [47]. In this study, we observed a significant decrease in animals' GPX activity treated by DZN. This can be explained owing to increased free radical production, as documented by enhanced MDA levels. The decrease of GPx activity induced by DZN may be attributable to a direct inhibitory oxidative effect on the enzyme. Biological macromolecules can be prevented from oxidative damage through antioxidant enzymes like SOD and CAT which are regarded as a primary defense. 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 [48]. The obtained results demonstrated a significant decrease in SOD activity followed by DZN treatment. Needless to mention, xenobiotics have the capacity to induce mitochondria superoxide radical production [49] and the amount of ($O_2^{\cdot-}$) formed in a cell can reach hazardous levels if SOD is inhibited. Superoxide radical often tends to be a potent inhibitor of CAT [50]. Moreover, several processes are involved in the depletion of antioxidant enzyme activity which could be attributed to a direct impact on the enzyme. These processes can include DZN-induced ROS generation, depletion of the enzyme substrates and down-regulation of transcription and translation. Even a single exposure to DZN can affect both animals and human [4]. However, its initial toxicological effects are characterized by acetylcholinesterase inhibition. DZN toxicity can also account for oxidative stress. According to the results, it can be assumed that oxidative stress increase mainly give rise to hepatotoxic effects of DZN since the effects were prevented by *D. glabrum* supplementation. The promoted antioxidant activity via *D. glabrum* is necessarily performed in the lipid membrane environment. Free radicals enabled by *D. glabrum* abstract a hydrogen atom from the antioxidant molecule, hence breaking the chain of free radical reactions [51].

We have concluded that the increased oxidative stress on the hepatic cells can account for the DZN toxicity in the liver, resulting in processes such as the depletion of the antioxidant enzymes that scavenge the toxic superoxide and hydrogen peroxide radicals leading to an increased LPO. Nonetheless, *D. glabrum* treatment could be useful to decrease DZN toxicity by quenching oxidative stress imposed by DZN.

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