Original Research Article

Protective Effect of Dorema glabrum on induced Oxidative Stress by diazinon in Hippocampus of Rat

Abstract

Introduction: Diazinon (DZN) administration produces lipid peroxidation as an indicator of oxidative stress in the brain. Some medicinal plants like *Dorema glabrum* has antioxidant properties, so can be used as an antioxidant that may protect neurons from oxidative stress. The aim of present study was to investigate the effect of *D.glabrum* against DZN-induced oxidative stress in hippocampus.

Methods: Twenty-four adult male Wistar rats were used in this study. The rats randomly divided into four groups including a control group, and two groups received different doses of *D.glabrum* (40 and 80 mg/kg) as pre-treatment for 21 days with DZN (100 mg/Kg) that was injected intraperitoneally (ip) in last day of *D. glabrum* usage, and one group received only DZN. Thiobarbituric acid reactive substances (TBARS), which are the indicators of lipid peroxidation, and the activities of antioxidant enzymes (glutathione peroxidase, superoxide dismutase and catalase) were determined in the rats' hippocampus.

Results: Administration of DZN significantly increased TBARS levels and superoxide dismutase activity and decreased glutathione peroxidase activity but there were no significant changes in catalase activity in the hippocampus. Combined *D.glabrum* and DZN treatment, caused a significant increase in glutathione peroxidase, a significant decrease of TBARS and a significant decrease in superoxide dismutase and again no significant changes in catalase activity in the rats' hippocampus when compared to the rats treated with DZN.

Conclusion: Our study demonstrated that *D.glabrum* had an amelioratory effect on oxidative stress induced by DZN.

Key words: Diazinon, Oxidative stress, *Dorema glabrum*, Hippocampus, male rat

Introduction:

Diazinon is a thiophosphorus organophosphate pesticide (OPs) which is used commonly as insecticide, nematicide and acaricide in soil, fruit , plants and vegetable crops in agriculture and urban pest control and veterinary medicine [1]. DZN inhibit acetylcholinesterase activity, which is an important brain neurotransmitter, and Causing accumulation of acetylcholine in the synapses [2]. Symptoms of acute DZN poisoning include headache, dizziness, nausea, tearing, and sweating. Some symptoms, including headaches, blurred vision, and memory problems, can last for months or years [3]. Low level repeated exposure to OPs cause inflammatory responses in cultured astrocytes [4] and upregulate

inflammatory cytokines in vivo [5] and inflammatory cytokines are induce significant impairment in spatial memory [6]. DZN at high doses induces oxidative stress and the production of free radicals in rats by alteration of antioxidant enzyme activity, depletion of glutation S-transferas (GST), and lipid peroxidation [7]. Endogenous enzymatic and non-enzymatic antioxidants are so important for the conversion of reactive oxygen species (ROS) to harmless metabolites as well as to protect and restore normal cellular metabolism and functions [8].The brain is particularly vulnerable to oxidative stress [2] because it metabolizes about 20% of total body oxygen and has a limited amount of antioxidant capacity [9]. The hippocampus, which is a part of the limbic system, is a critical center for memory and learning processes and plays an important role in forming and saving spatial memory. Reactive oxygen species (ROS) are involved in several diseases including ischemic injury, aluminum toxicity, Parkinson's disease, Alzheimer's disease and Down's syndrome all of which affect cognitive processes [11,12,13].

ROS can be detoxified by an enzyme defense system, containing 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 glutathione S-transferase (GST) [14].

Many insecticides have hydrophobic molecules that can bind extensively to biological membranes, especially to the phospholipids bilayers [15].

Dorema glabrum belongs to the family of Umbelliferae that distributed throughout the Mediterranean to Central Asiaaltitudes [16]. The most of the active constituents of this plant are polar phenolic components, which shows antioxidant activity and has many beneficial pharmacological effects [17]. In a previously work, the crude extract of the plant demonstrated antioxidant activity and anti-lipidemic effects [18].

Members of the genus Dorema (Apiaceae) possess antispasmodic, carminative, expectorant, diaphoretic, mild diuretic, emmenagogue, stimulant, vasodilator [19], antimicrobial and antifungal [20, 21,22] and hepatoprotector [23] properties and are extensively used as a green vegetable or as a folk medicine for treatment of many diseases [24]. According to the common folk believes of Azeri and Armenian people, D.glabrum can suppress different kinds of cancer.

The present study was designed to investigate the effect of DZN on enzymes of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) activities, total thiol (TSH) and thiobarbituric acid reactive substances (TBARS) as an indicator of lipid peroxidation in the hippocampal areas of the rats brain as well as on antioxidant enzymes activities and TBARS levels.

Materials and Methods:

Plant Material:

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

Animals:

Twenty-four adult male Wistar rats (weighting approximately 250-350 g) used in present study. Animals were obtained from the animal house of the veterinary department University of Tabriz. The rats were kept under constant temperature 20-22ºC 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 DGE and (100 mg/kg) of DZN, group IV (DGE 80): received DGE (80 mg/kg) and DZN (100 mg/kg). This two experimental groups received different doses of DGE (40 and 80 mg/kg) as pre-treatment for 21 days with DZN (100 mg/kg) that injected intraperitonealy in last day of DGE usage.

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), H_2O_2 (30%), ethylenediaminotetraacetic 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:

Brains (hippocampus) 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 enzymatic 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 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 [25]. SOD was determined according to the method described by Ukeda [26]. CAT was measured by monitoring the decomposition of hydrogen peroxide, as described by Aebi [27]. GPx was evaluated by the method of Paglia and Valentine (21). Protein was measured by the method of Lowry [28] using bovine serum albumin as standard. Total thiol content (TSH) was measured in homogenate by the method of Hu [22].

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 groups, while DZN-treated groups concomitantly with DGE were compared with their corresponding group of DZN-treated rats.

Results:

Lipid peroxidation:

Lipid peroxidation (LPO) is refers to oxidation of lipids by free radicals and it is one of the main manifestations of oxidative damage in tissues and cells. In the hippocampus, increased lipid peroxidation was observed by a significant increase in MDA (malondialdehyd) levels by 30.64% (expressed as nano moles of TBARS/g of protein) in the DZN group when compared to control group ($P<0.01$) (Fig1). The DGE 40 group, showed decreased levels of MDA by 31.11% when compared with DZN group ($P<0.0001$) and the DGE 80 group showed decreased levels of MDA by 29.87% when compared with DZN group ($P<0.01$) (Fig1).

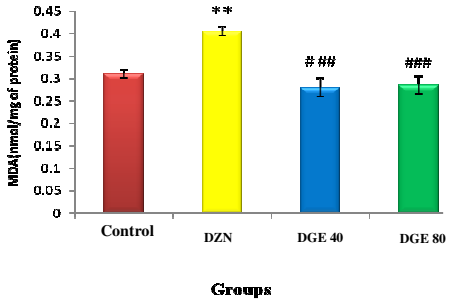

Fig 1: Effect of DGE 40 and DGE 80 on MDA level of DZN treated rats and effect of DZN (100 mg/Kg) on MDA level of normal rats in the hippocampus. Values are mean±SEM (n=6). ** P<0.01, compared to control group, ### P<0.001, compared to DZN group.

Antioxidant enzymes:

SOD, CAT and GPx play crucial role in the cellular antioxidant defense mechanism. The activities of SOD (U/mg of protein) in hippocampus were increased significantly by 11.56% in DZN treated animals compared to control group (P<0.001). Whereas in the DGE 40 group, a significant decrease by 27.4% were observed in the activities of SOD compared to DZN group (P<0.05). In the DGE 80 group, a significant decrease by 21.61% was observed (p<0.01). In rats' hippocampus non-significant differences were observed between the groups in CAT activity (p>0.05) (Fig3). GPx activity was diminished by 39% by DZN (p<0.05). In rats supplemented with DGE 40, 80 we observed an increase about 64.3%, (p<0.05) and 92.8%, (p<0.05) respectively (Fig4).

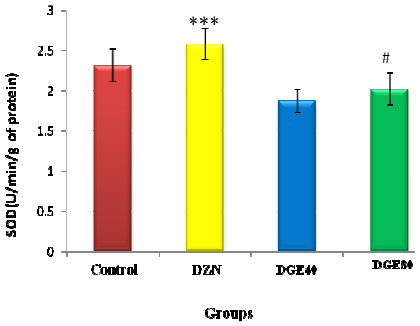

Fig2: Effect of DGE 40 and DGE 80 on SOD level of DZN treated rats and effect of DZN (100 mg/Kg) on SOD level of normal rats. Values are mean±SEM (n=6). *** P<0.001, compared to control group, * p<0.05, compared to DZN group.

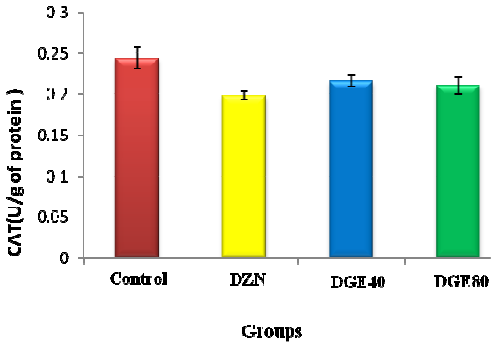

Fig3: Activities of CAT in control group and treated rats with DZN, DGE40 and DGE 80.

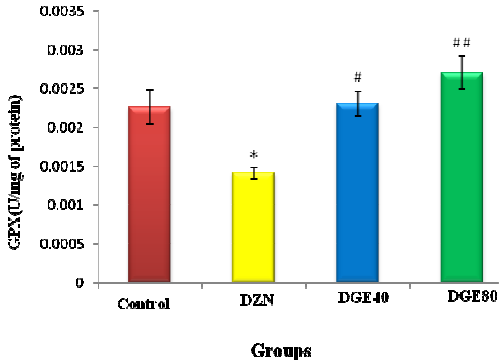

Fig4: Effect of DGE 40 and DGE 80 on GPx activities of DZN treated rats and effect of DZN (100 mg/Kg) on GPx activities of normal rats in the hippocampus. * $p<0.05$, compared to control group, $^{\#}p<0.05$ and $^{\#\#}p<0.01$, compared to DZN group.

Total thiol content:

Rats which received DZN showed non-significant values of total thiol (TSH) when compared with control rats ($p>0.05$). Rats in DGE 40 group showed non-significant values of total thiol when compared with DZN group ($p>0.05$). The DGE 80 group showed a significant increase by 85.6% when compared with DZN group ($p<0.01$) (Fig5).

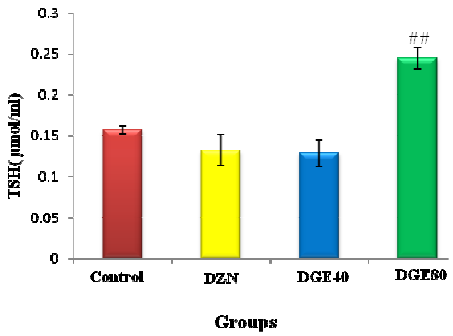

Fig5: Values of total thiol in control group and treated rats with DZN, DGE40 and DGE 80. ## p<0.01, compared to DZN group.

Discussion:

Recently, an increasing amount of attention has been concentrated towards free radical mediated damage triggered by pesticide exposure in biological systems [29]. Some herbal drugs with antioxidant activity have gained importance as the dietary intake of antioxidants obtained from natural sources is considered to be relatively safe and without undesirable side effects [18]. The aim of present study was to investigate the effect of *D.glabrum* against DZN-induced oxidative stress in hippocampus.

The current study indicated that DZN treatment induced oxidative stress, and increased MDA levels and SOD activities in the hippocampus but decreased GPx activities and no significant changes showed in CAT activities. Salehi et al., showed that intraperitoneal injection of DZN leads to an increase in SOD activity and MDA levels in DZN doses higher than 50 mg/kg and no-significant differences in CAT activity. The increase of MDA levels observed in the hippocampus following DZN exposure may be attributable to the excessive production of ROS. In a study by Salehi et al. it was identified that DZN increased SOD activities at doses of 50 and 100 mg/kg in compared with the control group but there were no significant changes observed in brain CAT activity. Also MDA concentration was significantly increased at 100 mg/kg dose in comparison with the control group. Abbasnejad et al. indicated that intraperitoneal injection of DZN leads to an increase in CAT and SOD activities in doses higher than 30 mg/kg and increase in MDA levels in 100 mg/kg doses in compared to control group. Our results are in agreement with the studies of [30, 31, and 32]. Under the oxidative stress conditions, SOD acts as the first line of defense against superoxide as it converts the superoxide anion to H_2O_2 which is converted to H_2O by CAT and GPx. Therefore the increased lipid peroxidation may be interpreted here by an inhibition of SOD, CAT, GPx activities and other antioxidants in the brain tissue leading to membrane injury and neuron death [33,34].

In our investigation, ethanolic extract of *D.glabrum* showed an amelioratory effect on DZN-induced changes in MDA levels and antioxidant enzymes (SOD, CAT and GPx). Ghollassi Mood, Yousefzadi et al., Shahidi et al. and Kumar et al. showed that members of the genus Dorema (Apiaceae), has a lot of biological properties such as antioxidant, antispasmodic, expectorant, carminative, diaphoretic, mild diuretic, emmenagogue, stimulant, vasodilator [35,36] antimicrobial, antifungal [37,38] and hepatoprotector [39] properties. Most of the biological action of *D. glabrum* is probably ascribed to its antioxidant potential. In the present study, DGE as pre-treatment with DZN that injected intraperitonealy in last day of DGE usage, caused a significant increase in glutathione peroxidase, a significant decrease of TBARS and a significant decrease in superoxide dismutase and no significant changes in catalase activity in the rats' hippocampus when compared to the rats treated with DZN. Our study demonstrated that dorema had an amelioratory effect on oxidative stress induced by DZN.

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