

Study Protocols

Protective effect of *Dorema glabrum* on diazinon-induced oxidative stress in rat kidney

Abstract:

Introduction: A wide number of pesticides such as diazinon (DZN) have deteriorating effect on fauna and flora by inducing oxidative stress and cell damage. The aim of current study was to investigate the protective effect of *D.glabrum* against the subchronic effect of DZN on serum urea and creatinine, oxidative damage markers such as lipid peroxidation (LPO) and the antioxidant defense system (catalase (CAT), glutathione peroxidase (GPx), superoxide dismutase (SOD)) in the kidney of male Wistar rats.

Methods: Twenty-four adult male Wistar 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 levels of LPO, urea, creatinine, and the decreased antioxidant defenses, like free radical scavenging enzymes viz., catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPx) significantly increased in DZN-treated group as compared to control group. Distinctly increased LPO levels, with alterations in endogenous antioxidant enzyme were evident in nephrotoxicity of DZN. 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.

Conclusion: The obtained results revealed that the oxidative stress of rats treated via DZN can be decreased if it is co-treated by *D.glabrum* with DZN. In addition, this co-treatment might serve a putative protective agent against kidney damage generated by DZN.

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

Introduction:

One of the chemical compounds widely utilized in agriculture is Organophosphorous (OP) pesticides which are believed to harness the agricultural plagues. Among the most frequently used OP insecticides, diazinon is used worldwide to eliminate crop and cattle plagues, as well as in household pest control (Sarabia *et al.* 2009). Considering the significant role of DZN and its potential impacts on the biochemical and hematological parameters of rats, rabbits, and mice, relevant findings have been reported in the field (Kalender *et al.* 2005 and 2006; Yehia *et al.* 2007). During inhalation, digestive system, the skin, or the

respiratory tract are more likely to absorb DZN. It is largely argued that the kidney is able to remove the absorbed DZN; however, DZN tends to create such potent acetylcholinesterase inhibitors as diazoxon, hydroxydiazoxon, and hydroxydiazinon when it is oxidized by microsomal enzymes in the liver (Quistad *et al.* 2001). Mammals can also experience DZN when lipid metabolism is negatively affected by the presence of DZN. Proposed insights from in vivo animal studies, additionally, revealed that oxidative stress is likely to be induced through organophosphorus insecticides (OPIs). The resulted oxidative stress is more likely to play a pivotal role in its induced poisoning (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). 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 pro-oxidant condition which pesticide toxicity brings about. To convert reactive oxygen species (ROS), Endogenous enzymatic and non-enzymatic antioxidants are considered indispensable since they are capable of making the metabolites harmless. In addition, they have the potential to protect and revitalize the metabolism and functions of normal cellular (Bebe *et al.* 2003). To detoxify ROS, an enzyme defense system including superoxide dismutase (SOD), catalase (CAT), and selenium-dependent glutathione peroxidase (GPx), or non-enzymatic systems via reduced glutathione is required. To detoxify organic peroxides, however, the activity of glutathione S-transferase (GST) is required. (GST) (Halliwell *et al.* 1999).

Native to Nakhchevan and Armenia zone (Transcaucasia) and Northwest of Iran is a species called *D. glabrum*. Regarding Iranian flora, there exist seven species which are believed to represent the genus *Dorema* from Apiaceae family (Mozaffarian, 1996). 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, mild diuretic, stimulant, vasodilator (Mozaffarian, 2007), and hepatoprotective (Govind *et al.* 2011) properties and are intensively used 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 diseases especially different kinds of cancer. In their seminal work, Dehghan *et al.* (2009) demonstrated that the crude extract of the plant can bring about antioxidant activity and anti-lipidemic effects. Therefore, the present study was aimed at evaluating the toxic effect of diazinon in rats with special reference to its possible reactive oxygen species (ROS) generating potential in kidney, which caused biochemical, hematological and histopathological alterations.

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. During the experiment, every attempt was made to continuously maintain the rats under temperature 20-22°C and 12/12h cycle of light and darkness. The rats, throughout the experiments, were provided with sufficient nutrition and water as well. 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).

Sample collection and processing:

All animals were anesthetized under ether after 21 days of treatment; then blood samples were taken out to measure the level of serum urea via cardiac puncture. Following this, we removed creatinine and kidneys from the animals and washed with cold saline buffer, then were 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 (Salehi, et al., 2010). Employing global units per mg protein (IU/mg protein), an attempt was made to discriminate and utilize the supernatants for enzyme activity of SOD, CAT and GPx. 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:

Pars Azmun kit and the method of Satho were utilized to determine and measure the concentration of urea and creatinine and thiobarbituric acid reactive substances (TBARS) as an index of lipid peroxidation respectively (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). To measure protein and total thiol content (TSH), Lowry (Lowry *et al.* 1951) and Hu (Hu *et al.* 1994) methods were employed respectively along with bovine serum albumin as standard.

Statistical analysis:

To statistically analyze the collected data, one-way analysis of variance (ANOVA) followed by Tukey's post hoc test (level of significance $P < 0.05$) was run using SPSS version 16, statistical program. The results are expressed as mean $\pm$ SEM. 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:

In the kidney increased lipid peroxidation was observed as measured by a significant increase in MDA (malondialdehyd) that expressed as nano moles of TBARS/g of protein in the rats treated with DZN when compared to control rats ($p=0.001, 48.9\%$) (Fig1). The group which received DZN and DGE 40 as cotreatment showed decreased levels of MDA when compared with DZN treated group ($P=0.003, 22.5\%$) and the group, which received DZN and DGE 80 showed decreased levels of MDA when compared with DZN treated group ($p=0.007, 20.44\%$) (Fig1).

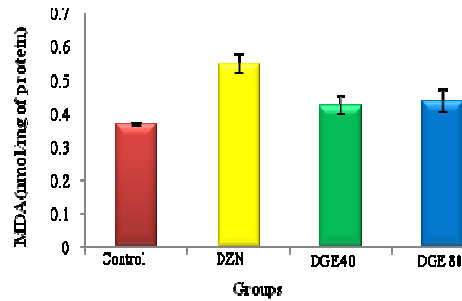
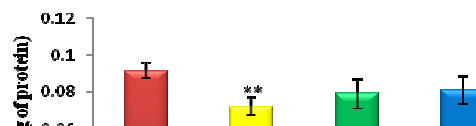


Fig 1: Effect of DGE 40 and DGE 80 on MDA level of DZN treated rats and effect of DZN (100 mg/Kg) on MDA level of normal rats in the kidney. Values are mean±SEM (n=6). ** P<0.01, compared to control group, ##P<0.01, compared to DZN group.

Antioxidant enzymes:

The activities of CAT (U/mg of protein) in kidney were reduced significantly in DZN treated animals compared to controls (P=0.008, 21.14%). Whereas in DGE 40 group a significant increase in the activity of this enzyme was observed compared to rats treated with DZN alone (P>0.05, 9.7%). In the DGE 80 group a significant increase in the activity of CAT was observed compared to rats treated with DZN alone (P>0.05, 12.5%) (Fig 2). As presented in Fig 3, SOD activities were diminished by 20.53% by treatment with DZN (p=0.006). Treatment with DGE 40 and DGE 80 showed a significant increase of SOD activities by 4.7%, 15.9% respectively (p>0.05). In GPx activities, there was no significant difference between the groups (Fig 4).



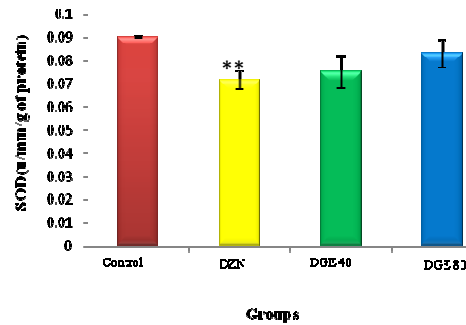


Fig 3: Effect of DGE 40 and DGE 80 on SOD activity of DZN treated rats and effect of DZN (100 mg/Kg) on SOD activity of normal rats. Values are mean±SEM, (n=6) ** P<0.01, compared to control group, P>0.05, compared to DZN group.

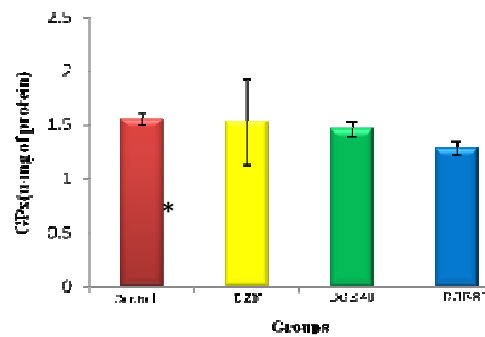


Fig 4: Effect of DZN (100 mg/Kg) on GPx activity of normal rats (*p<0.05) and effect of DGE 40 and DGE 80 on GPx activity of DZN treated rats (p>0.05).

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).

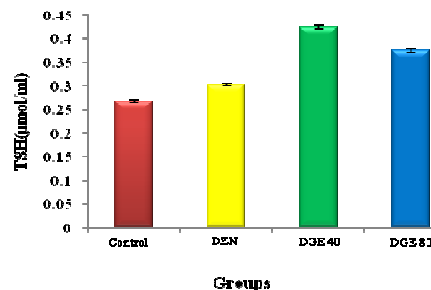


Fig 5: Values of total thiol in control group and treated rats with DZN, DGE 40 and DGE 80. There was not significant difference between the groups

Concentration of serum creatinine :

Data showed that DZN significantly increased level of creatinine ($p < 0.0001$) compare to control group. DGE 40 and DGE 80 significantly decreased level of creatinine ($p < 0.001$) compare to DZN group (Fig 6).

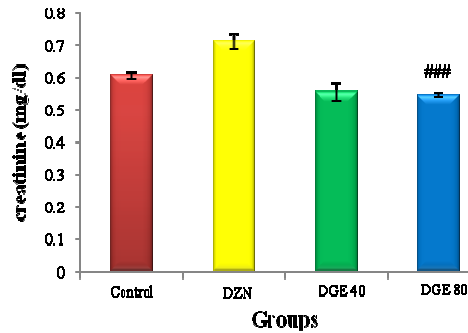
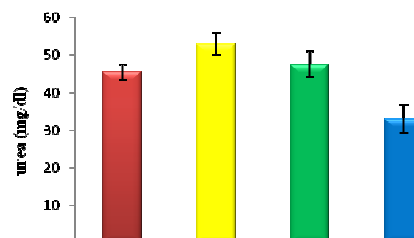


Fig 6: Effect of DZN (100 mg/Kg) on creatinine activity of normal rats (**** $p < 0.0001$) and effect of DGE 40 and DGE 80 on GPx activity of DZN treated rats (### $p < 0.001$, ### $p < 0.001$ respectively).

Concentration of serum urea :

Data showed that DZN significantly increased level of urea ($p < 0.01$) compare to control group. DGE 40 and DGE 80 significantly decreased level of urea ($p < 0.05$, $p = 0.001$) compare to DZN group (Fig 7).



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