

Study Protocols

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

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 levels of urea and serum creatinine and lipid peroxidation, were analysed in addition to the antioxidant enzymes such as catalase (CAT), glutathione peroxidase (GPx), superoxide dismutase (SOD) activities in the kidney tissue. The aim of this 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 (ADS) in the kidney 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, urea, creatinine, 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 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 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 kidney damage.

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

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). Diazinon affects mitochondrial membrane transportation in rat liver (Nakagawa and Moore, 1999). Furthermore, it disturbs cytochrome P450 system in human liver (Sams *et al.*, 2003). Meanwhile, diazinon causes toxic effects on other organisms (Keizer *et al.*, 1995). Treatment of rats with diazinon also resulted in hyperglycemia, depletion of glycogen from the brain and peripheral tissues accompanied with increased activity of glycogen phosphorylase in the brain and liver and increased activity of the hepatic gluconeogenic enzyme, phosphoenolpyruvate carboxykinase (Matin *et al.*, 1990). It has also been shown that 335 mg/kg body weight of diazinon caused an increase in lipid peroxidation in rat erythrocytes (Sutcu *et al.*, 2007). 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). 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. 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:

All animals were anesthetized under ether after 21 days of treatment then blood samples were taken out by cardiac puncture for measuring the level of serum urea and creatinine and kidneys were removed from the animals 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:

Urea and creatinine concentration was determined by Pars Azmun kit, 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 $\pm$ 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 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).

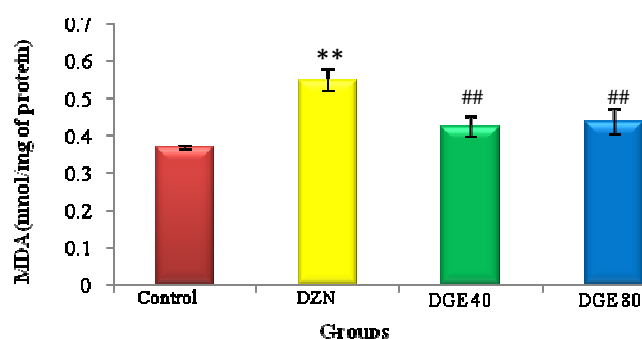


Fig 1: Effect of DGE 40 and DGE 80 on MDA level of DZN treated rats and effect of DZN (100 mg/Kg) on MDA level of normal rats in the kidney. Values are mean $\pm$ SEM (n=6). ** $P<0.01$, compared to control group, ## $P<0.01$, compared to DZN group.

Antioxidant enzymes:

SOD, CAT and GPx play vital role in the cellular antioxidant defense mechanism. 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).

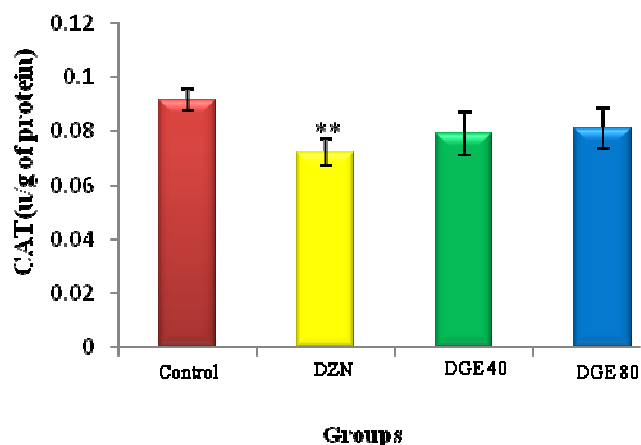


Fig 2: Effect of DZN (100mg/Kg) on CAT activity of normal rats (** $p<0.01$) and the effect of DGE 40 and DGE 80 on CAT activity of DZN treated rats ($p>0.05$).

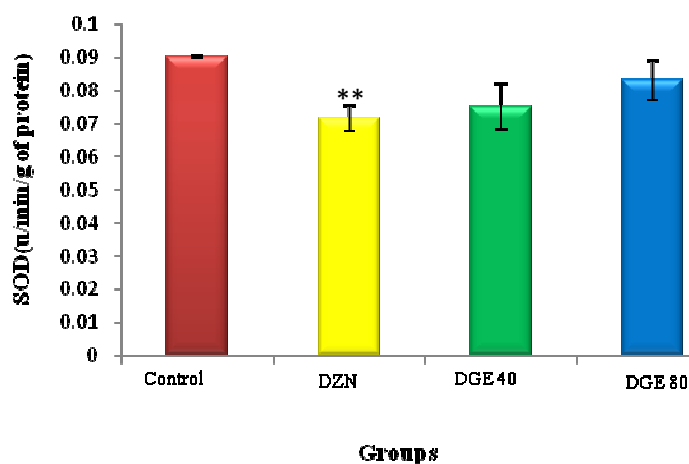


Fig 3: Effect of DGE 40 and DGE 80 on SOD activity of DZN treated rats and effect of DZN (100 mg/Kg) on SOD activity of normal rats. Values are mean $\pm$ SEM, (n=6) ** $P<0.01$, compared to control group, $P>0.05$, compared to DZN group.

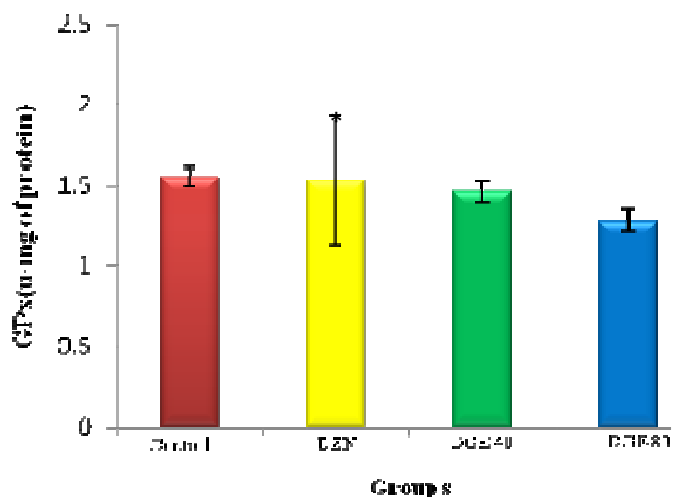


Fig 4: Effect of DZN (100 mg/Kg) on GPx activity of normal rats (* $p < 0.05$) and effect of DGE 40 and DGE 80 on GPx activity of DZN treated rats ($p > 0.05$).

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).

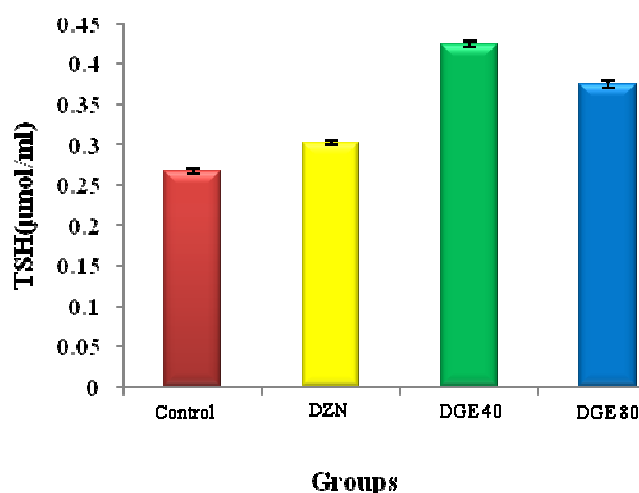


Fig 5: Values of total thiol in control group and treated rats with DZN, DGE 40 and DGE 80. There was not significant difference between the groups

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).

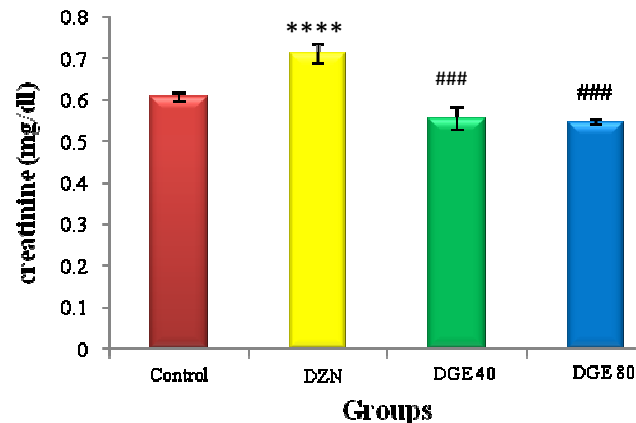


Fig 6: Effect of DZN (100 mg/Kg) on creatinine activity of normal rats (**** $p < 0.0001$) and effect of DGE 40 and DGE 80 on GPx activity of DZN treated rats (### $p < 0.001$, ### $p < 0.001$ respectively).

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).

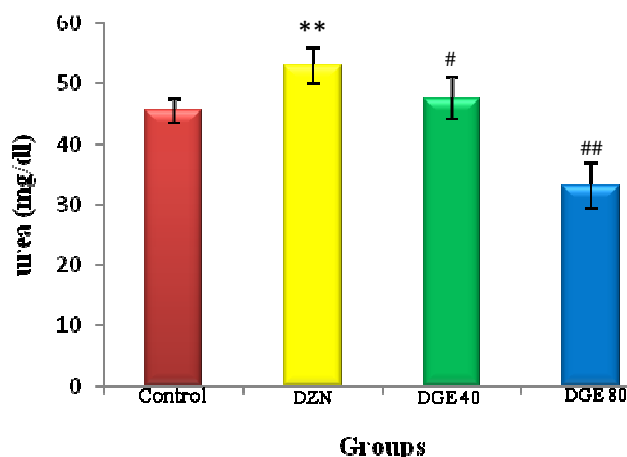


Fig 7: Effect of DZN (100 mg/Kg) on urea activity of normal rats (** $p < 0.01$) and effect of DGE 40 and DGE 80 on urea activity of DZN treated rats ([#] $p < 0.05$, ^{##} $p < 0.001$ respectively).

Discussion:

Considerable attention has been directed towards free radical mediated damage in biological systems due to pesticide exposure (Banerjee *et al.* 1999). It has been recognized that oxidative stress plays a role in the pathogenesis of kidney toxicity by many toxins and drugs (Sabolic I, 2006). 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. glabrom* 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 kidney of rats. The kidney is responsible for elimination of active metabolites. DZN metabolites are excreted via kidney. However, DZN and its metabolites elimination through kidney are associated with kidney and tubular damage. This study indicated that DZN has induced oxidative stress and kidney dysfunction, also acute toxicity of DZN increases serum creatinine and urea. Concentration of creatinine is a marker of kidney function and its elevation is associated with kidney injury. Serum creatinine also shows kidney clearance. Therefore, increasing of creatinine is resulting of DZN-induced kidney damage and clearance reduction. According to our results, DZN treatment promotes MDA content in the

kidney of rats. The increase of MDA observed in the kidney following DZN exposure, was probably ascribed to the excessive production of ROS, which could be related to nephrocyte enzyme leakage. Mitochondria are the main source of ROS production (Wang *et al.* 2006 a ; Wang *et al.* 2006 b). Moussa and Hafez (Moussa *et al.* 1995) showed that the organophosphate and dimethoate inhibit mitochondrial enzyme activity. 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 kidney.

Our results are in accordance with the studies of (Shah *et al.* 2010; and Boroushaki *et al.* 2013) who demonstrated a significant increase in LPO level in kidney tissue, serum urea and creatinine, and a significant decrease in SOD, CAT and GPx activities in kidney 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 nephrotoxic 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 kidney could be attributed to the oxidative stress on the nephrotic 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 have a protective effect against DZN-induced nephrotoxicity in rat and could be useful to decrease DZN toxicity by quenching oxidative stress imposed by DZN.

References:

- Abdollahi, M., S. Mostafalou, S. Pournourmohammadi, and S. Shadnia (2004). Oxidative stress and cholinesterase inhibition in salvia and plasma of rats following subchronic exposure to malathion. *Comp Biochem Physiol.* 137: 29-34.
- Aebi, H. (1984). Catalase *in vitro* assay. *Meth Enzymol.* 105: 121–6.
- Almeida, M.G., C.D. Ropke, S.C. Davino, F.A.F. Nobre, E. Santos, and S.B.M. Barros, (1998). Modulation of hexachlorobenzene induced liver oxidative stress by α -tocopherol. *Toxicol. Lett.* 95 (1): 57.
- Amirkabirian, N., F. Teimouri, and H. Esmaily (2007). Protection by pentoxifylline of diazinon-induced toxic stress in rat liver and muscle. *Toxicol. Lett.* 172 (1): S61–S62.
- Arthur, J.R. (2000). The glutathione peroxidises. *Cell. Mol. Life Sci.* 57: 1825–1835.
- Ashakumari, L., and P.L. Vijayammal (1994). Addictive effect of alcohol and nicotine on lipid peroxidation and antioxidant defense mechanism in rats. *J. Appl. Toxicol.* 16: 305–308.
- Asnaashari, S., E. Dadizadeh, A.H. Talebpour, M. Eskandani, and H. Nazemiyeh, (2001). Free radical scavenging potential and essential oil composition of the dorema glabrum fisch. C.A. mey roots from Iran. *BioImpacts.* 1(4): 241-4.

- 324 Banerjee, B.D., V. Seth, A. Bhattacharya, S.T. Pasha, and A.K. Chakraborty (1999).
325 Biochemical effects of some pesticides on lipid peroxidation and free-radical scavengers.
326 Toxicol Lett. 107(1–3): 33–47.
- 327 Bebe, F.N., and M. Panemangalore (2003). Exposure to low doses of endosulfan and
328 chlorpyrifos modifies endogenous antioxidants in tissue of rats. J. Environ. Sci. Health B. 38:
329 349–363.
- 330 Bergamini, C.M., S. Gambetti, A. Dondi, and C. Cervellati (2004). Oxygen, reactive oxygen
331 species and tissue damage. Curr. Pharm. Des. 10: 1611–1626.
- 332 Boroushaki MT, Sadeghnia HR and Banihasan M. Protective effect of pomegranate seed oil on
333 Hexachlorobutadiene -induced nephrotoxicity in rat. *J. Ren. Fail.* (2010) 32: 612-617.
- 334 Dehghan, G., G. Fatholahi, and N. Sheikhzadeh (2009). Hypocholesteremic and antioxidant
335 effects of Dorema glabrum extract in rats fed high cholesterol diet. Journal of the Iranian
336 Chemical Society. 6: 115-143.
- 337 Dehghan, G., and Z. Khoshkam (2012). Tin(II)-quercetin complex: Synthesis, spectral
338 characterization and antioxidant activity. Food Chemistry. 131: 422-427.
- 339 El-Shenawy, N.S., R.A. Al-Eisa, F. El-Salmy, and O.Salah, (2009). Prophylactic effect of
340 vitamin E against hepatotoxicity, nephrotoxicity, haematological indices and histopathology
341 induced by diazinon insecticide in mice. Curr. Zool. 55 (3): 219–226.
- 342 Gao, H., J. Lamusta, WF. Zhang, R. Salmonsens, Y. Lin, E. O’Connell, J.E. Evans, S. Burstein,
343 and J.J. Chen, (2011). Tumor Cell Selective Cytotoxicity and Apoptosis Induction by an Herbal
344 Preparation from Brucea javanica. N Am J Med Sci (Boston). 4(2): 62-6.
- 345 Govind, P. (2011). Medicinal plants against liver diseases. International Research Journal of
346 Pharmacy. 2(5): 115-121.
- 347 Grafft, S.J., K. Jones, H.J. Mason, and J. Cocker (2002). Exposure to the organophosphate
348 diazinon: data from a human volunteer study with oral and dermal doses.
349 Toxicol. Lett. 134 :105–113.
- 350 Gultekin, F., M. Ozturk, and M. Akdogan (2000). The effect of organophosphate insecticide
351 chlorpyrifos-ethyl on lipid peroxidation and antioxidant enzymes (in vitro). Arch. Toxicol.
352 74:533–538.
- 353 Halliwell, B., and J.M.C. Gutteridge (1999). Free Radicals in Biology and Medicine. Oxford
354 University Press. London. p. 936.

355 Hu, ML., and CJ. Dillared (1994). Plasma SH and GSH measurement. *Methods Enzymol.*
356 233: 385–7.

357 Ibadullayeva, S., N. Movsumova, H. Gasymov, and T. Mamedli, (2011). Protection of some rare
358 and endangered vegetable plants in the flora of the Nakhichevan AR. *International Journal of*
359 *Biodiversity and Conservation.* 3(6); 224-229.

360 Isika, I., and I. Celik (2008). Acute effects of methyl parathion and diazinon as inducers for
361 oxidative stress on certain biomarkers in various tissues of rainbow trout (*Oncorhynchus*
362 *mykiss*). *Pest. Biochem. Physiol.* 92 (1): 38–42.

363 Kalender, S., A. Ogutcu, M. Uzunhisarcikli, F.Acikgoz, D. Durak, Y. Ulusoy, and Y. Kalender,
364 (2005). Diazinon-induced hepatotoxicity and protective effect of vitamin E on some
365 biochemical indices and ultrastructural changes. *Toxicol.* 211: 197–206.

366 Kalender, S., A. Ogutcu, M. Uzunhisarcikli, F.Acikgoz, D. Durak, Y. Ulusoy, and Y. Kalender,
367 (2005). Diazinon-induced hepatotoxicity and protective effect of vitamin E on some
368 biochemical indices and ultrastructural changes. *Toxicol.* 211: 197–206.

369 Kalender, Y., M. Uzunhisarcikli, A. Ogutcu, F. Acikgoz, and S.Kalender, (2006). Effects of
370 diazinon on pseudocholinesteras activity and haematological indices in rats: the protective role of
371 vitamin E. *Environ. Toxicol. Pharmacol.* 22 (1): 46– 51.

372 Keizer, J.D., Agostino, G., Nagel, R., Volpe, T., Gnemi, P., Vittozzi, L., 1995.Enzymological
373 differences of AChE and diazinon hepatic metabolism: correlation of in vitro data with the
374 selective toxicity of diazinon to fishspecies. *Science of the Total Environment* 171, 213–220.

375 Kumar, V.P., N.S. Chauhan, H. Padh, and M. Rajani, (2006). Search for antibacterial and
376 antifungal agents from selected Indian medicinal plants. *Journal of Ethnopharmacology.*
377 107:182- 188.

378 Lee, A., J. East, and P. Balgaug (1991). Interactions of insecticides with biological membranes.
379 *Pest. Sci.* 32: 317–327.

380 Lowry, OH., NJ. Roserbrough, AL. Farr, and RJ. Randall, (1951). Protein measurement with the
381 folin phenol reagent. *J Biol Chem.* 193: 265–75.

382 Matin, M.A., Sattar, S., Husain, K., 1990. The role of adrenals in diazinon-induce changes in
383 carbohydrate metabolism in rats. *Archives of Industrial Hygiene and Toxicology* 41, 347–356.

384 Monteiro, D.A., J.A. de Almeida, F.T. Rantin, and AL. Kalinin, (2006). Oxidative stress
385 biomarkers in the freshwater characid fish, *Brycon cephalus*, exposed to organophosphorus

386 insecticide Folisuper 600 (methyl parathion). *Comp. Biochem. Physiol. C Toxicol. Pharmacol.*
387 143: 141–149.

388 Moussa, T.A., and M.M. Hafez (1995). The effect of dimethoate on the mitochondria of the
389 guinea pig. *Egypt. J. Histol.* 6: 101–106.

390 Mozaffarian, V. A. (1996). Dictionary of iranian plant names. Tehran: Farhang Moaser.

391 Mozaffarian, V. (2007). Tehran, Research Institute of Forests and Rangelands. *Flora of Iran,*
392 *Umbelliferae.* 54: 368- 374.

393 Nakagawa, Y., Moore, G., 1999. Role of mitochondrial membrane permeability transition in p-
394 hydroxybenzoate ester-induced cytotoxicity in rat hepatocytes. *Biochemical Pharmacology* 58,
395 811–816.

396 Ogutcu, A., M. Uzunhisarcikli, S. Kalender, D. Durak, F. Bayrakdar, and Y. Kalender, (2006).
397 The effects of organophosphate insecticide diazinon on malondialdehyde levels and myocardial
398 cells in rat heart tissue and protective role of vitamin E. *Pest. Biochem. Physiol.* 86 (2): 93–98.

399 Oubre, A.Y., T.J. Carlson, S.R. King, and G.M. Reaven, (1997). From plant to patient: an
400 ethnomedical approach to the identification of new drugs for the treatment of NIDDM.
401 *Diabetologia.* 40(5):614-7.

402 Paglia, DE., and WN. Valentine (1967). Studies on the quantitative and qualitative
403 characterization of glutathione peroxidase. *J Lab Med.* 70: 158–65.

404 Pascoe, G.,and F. Olafs, and D. Read (1987). Vitamin E protection against chemical induced cell
405 injury. Maintenance of cellular protein thiols as a cytoprotective mechanism *Arch. Biochem.*
406 *Biophys.* 256: 150–158.

407 Peixoto, F., J. Vicente, and V.M.C. Madeira. (2004). A comparative study of plant and animal
408 mitochondria exposed to paraquat reveals that hydrogen peroxide is not related to the observed
409 toxicity. *Toxicol. in Vitro.* 18: 733–739.

410 Quistad, GB., SE. Sparksand, and GE. Casida (2001). Fatty acid amide hydrolase inhibition by
411 neurotoxic organophosphorus pesticides. *Toxicol. Appl. Pharmacol.* 173: 48–55.

412 Sabolic I. Common mechanisms in nephropathy induced by toxic metals. *Nephron Physiol.*
413 (2006) 104: 107-14.

414 Sams, C., Cocker, J., Lennard, M.S., 2003. Metabolism of chlorpyrifos and diazinon by human
415 liver microsomes. *Toxicology Letters* 144, 146–153.

- 416 Sarabia, L., I. Maurer, and E. Bustos-Obrego (2009). Melatonin prevents damage elicited by the
417 organophosphorous pesticide diazinon on the mouse testis. *Ecotoxicol. Environ. Safety*. 72:
418 663–668.
- 419 Satho, K. (1978). Serum lipid peroxidation in cerebrovascular disorders determined by a new
420 colorimetric method. *Clin Chem Acta*. 90:37–43.
- 421 Shah MD and Iqbal M. Diazinon-induced oxidative stress and renal dysfunction in rats. *Food*
422 *Chem. Toxicol.* (2010) 48: 3345-53.
- 423 Shahidi, GH., MR. Moein, AR. Foroumadi, and F. Rokhbakhsh-Zamin, (2002). Cytotoxic
424 activity of medicinal plants used in Iranian traditional medicine on two strains of *Saccharomyces*
425 *cerevisiae*. *DARU*. 10(4): 162-164.
- 426 Singh, B., TD. Dogra, and CB. Tripathi (2002). A study of serum cholinesterase activity in
427 agricultural and industrial workers occupationally exposed to organophosphates insecticides. *Int*
428 *J Med Toxicol*. 5: 2–5.
- 429 Sutcu, I., Altuntas, B., Buyukvanli, O., Akturk, O., Ozturk, H., Koylu, 2007. The effects of
430 diazinon on lipid peroxidation and antioxidant enzymes in rat erythrocytes: role of vitamins E
431 and C. *Toxicology and Industrial Health* 23, 13–17.
- 432 Tsukamoto, M., Y. Tampo, M. Sawada, and M. Yonaha, (2002). Paraquatinduced oxidative
433 stress and dysfunction of the glutathione redox cycle in pulmonary microvascular endothelial
434 cells. *Toxicol. Appl. Pharm.* 178: 82–87.
- 435 Ukeda, H., S. Maeda, T. Ishii, and M. Sawamura, (1997). Spectrophotometric assay for
436 superoxide dismutase based on tetrazolium salt 3'–1–(phenylamino)-carbonyl–3, 4-tetrazolium]-
437 bis(4-methoxy-6-nitro)benzenesulfonic acid hydrate reduction by xanthine-xanthine oxidase.
438 *Anal Biochem*. 251: 206–9.
- 439 Wang, Y.M., S.Q. Peng, Q. Zhou, M.W. Wang, C.H. Yan, H.Y. Yang, and G.Q. wang, (2006) a.
440 Depletion of intracellular glutathione mediates butenolide-induced cytotoxicity in HepG2 cells,
441 *Toxicol. Lett*. 164: 231–238.
- 442 Wang, Y.M., S.Q. Peng, Q. Zhou, M.W. Wang, G. Han, C.H. Yan, H.Y. Yang, and G.Q. Wang,
443 (2006) b. Role of mitochondria in cellular oxidative injuries induced by butenolide. *Chin. J.*
444 *Pharmacol. Toxicol*. 20: 484–489.

445 Wetscher G.J., M. Bagchi, D.Bagchi, G. Perdakis, P.R.Hinder, k. Glaser, R.A.Hinder, (1995).
 446 Free radical production in nicotine treated pancreatic tissue. Free Radic Biol Med.
 447 18 (5): 877-82.

448 Yano, H., A. Mizoguchi, K. Fukuda, M. Haramaki, S. Ogasawara, S. Momosaki, and M. Kojiro,
 449 (1994). The herbal medicine sho-saiko-to inhibits proliferation of cancer cell lines by inducing
 450 apoptosis and arrest at the G0/G1 phase. Cancer Res. 54(2): 448-54.

451 Yehia, M.A.H., S.G. El-Banna, and A.B. Okab (2007). Diazinon toxicity affects
 452 histophysiological and biochemical parameters in rabbits. Exper. Toxicol. Pathol. 59: 215–225.

453 Yousefzadi, M., M. Heidari, M. Akbarpour, MH. Mirjalili, A. Zeinali, and M. Parsa, (2011). In
 454 vitro cytotoxic activity of the essentialoil of Dorema ammoniacum D. Don. Middle-East Journal
 455 of Scientific Research. 7(4): 511-514.

456 Zhou, J.F, G.B. Xu, and W.J. Fang (2002). Relationship between acute organophosphorus
 457 pesticide poisoning and damages induced by free radicals, Biomed. Environ. Sci. 15: 177–186.

458
 459
 460
 461
 462
 463
 464
 465
 466
 467
 468
 469
 470