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Role of *Matricaria recutita L.* and *Asparagus officinalis L.* against the neurotoxicity of diazinon in rats

Fahmy G. Elsaid [a,b,*], **Ali A. Shati** [a], **Mohammad A. Sarhan** [a]

[a] *Biology Department, Science College, King Khalid University, Saudi Arabia*
[b] *Zoology Department, Faculty of Science, Mansoura University, Egypt*

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Abstract Diazinon (DZN) is an organophosphorus insecticide widely used in agriculture. It has a variety of harmful effects on humans. Asparagus and chamomile have antioxidant properties and are used as antidotes of DZN in this study. Thirty-five adult male *Sprague Dawley* rats were divided into: control group; DZN group: subdivided into two subgroups which received ¼ LD_{50} and ½ LD_{50} dose of DZN for 30 days; DZN and asparagus extract group: subdivided into two subgroups which received ¼ LD_{50} and ½ LD_{50} dose of DZN respectively and treated with asparagus extract (300 mg/kg b. wt.) after 15 min of DZN administration; DZN and chamomile extract group: subdivided into two subgroups receiving DZN respectively and treated with chamomile extract (300 mg/kg b. wt.). The results herein showed that the antioxidant enzyme changes associated with the exposure to DZN are dose dependant in cerebrum, cerebellum and spinal cord tissues. The tumorigenicity of DZN was represented by the significant increase of arginase and the alpha-L-fucosidase in sera of all DZN groups. In addition, the molecular changes were investigated by the changes in Cu/Zn-dependent superoxide dismutase, glutathione-S-transferase and glutathione peroxidase enzymes that were altered after administration of DZN to rats. Present findings suggest that oral administration of aqueous extracts of asparagus or chamomile is able to restore the total antioxidant capacity, as demonstrated by the increase of superoxide dismutase activity, glutathione content and their relative enzymes in the investigated tissues. Due to their antioxidant activities, asparagus and chamomile are potential candidates as anti-neurotoxic agents.

Introduction

Diazinon (DZN) is an organophosphate (OP) insecticide used mainly in agriculture and in sheep dips (Bailey et al., 2000), and is designed as an irreversible acetylcholine esterase inhibitor (Davies and Holub, 1980). It is classified as moderately

* Corresponding author at: Zoology Department, Faculty of Science, Mansoura University, Egypt. Tel.: +966 536906036.
E-mail address: fahmygad@mans.edu.eg (F.G. Elsaid).

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hazardous class-II OP insecticide (Shah and Iqbal, 2010). Roegge et al. (2008) showed that neonatal DZN exposure, at doses below the threshold for cholinesterase inhibition has lasting effects on emotional responses, with preferential effects on males. Diazinon is oxidized by microsomal enzymes to cholinesterase inhibiting metabolites such as diazoxon, hydroxydiazoxon and hydroxydiazinon (WHO, 1998). Diazinon affects mitochondrial membrane transportation in rat liver (Nakagawa and Moore, 1999). Moreover, it interrupts cytochrome P450 system in human liver (Sams et al., 2003).

The higher oxidative stress induced by pesticide sprayers is initiated by overproduction of free radicals and alteration in antioxidant defense mechanisms, including detoxification and scavenging enzymes (Abdollahi et al., 2004). Oxidative stress has been reported to play an important role in the toxicity of various pesticides, including organochlorines (Lee et al., 2006), carbamates and pyrethroids (Kale et al., 1999). The higher oxidative stress in pesticide sprayers is evidenced by increased concentration of plasma and red blood cell thiobarbituric acid reactive substances (TBARS), changes in antioxidant status, and altered activities of cellular enzymes (Prakasam et al., 2001). Treatment of rats with DZN significantly enhances renal lipid peroxidation (LPO) which is accompanied by a decrease in the activities of renal antioxidant enzymes (e.g. catalase (CAT), glutathione peroxidase (GSH-Px), glutathione reductase (GSH-R), glucose-6-phosphate dehydrogenase, glutathione-S-transferase (GST) and depletion in the level of reduced glutathione (GSH) (Shah and Iqbal, 2010; El-Demerdash and Nasr, 2014). In blood, normal erythrocyte function depends on the intactness of cell membrane which is the target for many toxic factors including pesticides. Erythrocyte GSH together with GSH-Px, GSH-R, GST, gamma-glutamyl transferase (γ-GT), SOD and CAT efficiently scavenge toxic free radicals and are partly responsible for protection against LPO due to acute/chronic OP pesticide exposure (Shah and Iqbal, 2010).

There is a relationship between pesticide exposure and the decrease of antioxidant enzymes (Ĺopez et al., 2007). Oxidative stress and genotoxic effects of DZN were documented through the changes in total antioxidant capacity (TAC), reduced GSH and oxidative DNA damage (Tsitsimpikou et al., 2013). Exposure to low-level of pesticides is known to produce a variety of biochemical and molecular changes, some of which may be responsible for the adverse biological effects reported in humans and animal models. Many studies have reported that DZN can induce molecular changes and alter gene expression. Jamshidi et al. (2009) have found that administration of DZN to rats at doses of 60 mg/kg significantly decreased expression of glutamate dehydrogenase gene, whose product is the key enzyme of Langerhans islet for the secretion of insulin, 18 h post-administration. Other studies showed that DZN had affected the expression of neurotrophic factors that coordinate neuronal cell differentiation and brain assembly (Slotkin and Seidler, 2007). In addition, Timofeeva et al. (2008) found persisting effects of DZN cholinergic and serotonergic neurotransmitter systems and gene expression as well as behavioral function.

Matricaria recutita L. (family *Asteraceae*, commonly known as German chamomile) is one of the most widely used and well-documented medicinal plants in the world (Salaman, 1992). Chamomile is also extensively consumed as a tea or tonic. Chamomile is used to treat anxiety, hysteria, nightmares, insomnia and other sleep problems, convulsions and even delirium tremens (Martens, 1995). The main chemical constituents of the German chamomile are terpenoids like α-bisabolol, chamazulene, sesquiterpenes and flavonoids like apigenin, luteolin and quercetin (Newall et al., 1996). A potent neuroprotective activity of the methanol extract of German chamomile against aluminum fluoride induced oxidative stress was reported in rats (Ranpariya et al., 2011). The potential neuroprotective activity of German chamomile was evident from its ability to reduce the elevated LPO and severity of oxidative damage in brain tissue by increasing the levels of antioxidant enzymes SOD, CAT and non-enzymatic GSH and total thiols during ischemia/reperfusion-induced oxidative stress (Chandrashekhar et al., 2010).

Green asparagus (*Asparagus officinalis L.)* is a healthy and nutritious vegetable, containing antioxidants, such as rutin, ascorbic acid, tocopherol, ferulic acid and GSH. Among 23 commonly consumed vegetables, antioxidant activity of asparagus, based on dry weight, has been ranked as the greatest (Vinson et al., 1998). The major flavonoid antioxidant in asparagus has been reported to be rutin (Tsushida et al., 1994), with the content of 286.5 ± 6.0 mg/kg fresh weight (Makris and Rossiter, 2001). Rodriguez et al. (2005) and Sun et al. (2007) emphasized the antioxidant activity of asparagus as it contains flavonoids and phenolic components. Sakurai et al. (2013) showed that asparagus extract possesses a neuroprotective effect and attenuates cognitive impairment in senescence-accelerated mice.

Based on these reports, the present study aimed to investigate the role of aqueous extracts of chamomile and asparagus in ameliorating the biochemical and molecular changes resulting from the administration of DZN.

Materials and methods

Rats

Thirty-five adult male *Sprague Dawley* rats, weighing 180–200 g, were obtained from the Experimental Animal Unit, College of Science, King Khalid University, Saudi Arabia. All rats received food and water *ad libitum* and were kept in a room with the temperature regulated to 22 ± 1 °C. The experiment was approved by the Animal Ethics Committee, College of Science, King Khalid University.

Methods

Asparagus and chamomile extracts

Crude extracts were prepared from asparagus (*M. recutita L.*, family *Asteraceae*) and chamomile (*M. recutita L.*, family *Asteraceae*). 5 g of each herb was separately ground in a blender and then incubated in 100 ml of boiling distilled water for 30 min. Once at room temperature, the extracts were filtered once by four layers of cheese-cloth and once by a single layer of Whatmann 1 filter paper. Finally, each extract was centrifuged at 6000*g* for 10 min, decanted, divided into 10 ml aliquots and stored at −20 °C. Just prior to each experiment, an aliquot of the extract was thawed. Both extracts were orally administered at 300 mg/kg b. wt. using stomach tube.