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Effects of Aqueous Extract of *Withania somnifera* on Some Liver Biochemical and Histopathological Parameters in Male Guinea Pigs

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Abstract: Organophosphorus (OP) insecticides constitute one of the most widely used classes of pesticides being employed for both agricultural and landscape pest control. The purpose of this study was to investigate the effect of dimethoate (DM), an organophosphorus insecticide, on some biochemical and histopathological parameters in liver of adult male guinea pigs as well as the possible role of *Withania somnifera* extract in attenuation of DM-induced hepatotoxicity. The animals were divided randomly into 5 groups and kept at 5 animals per group in an environmentally controlled condition with free access to food and water *ad libitum*. The first group was served as a control group and administered with olive oil orally; the group II received aqueous extract of *W. somnifera* (100 mg kg^{-1} b.wt.) orally, group III, IV and V was administered with DM (14 mg kg^{-1}; 1/25LD50) for 21 days orally. Group IV and V received 100 mg kg^{-1} of *W. somnifera* extract and silymarin, respectively half hour before DM administration for 21 days. DM caused a statistically significant increase in the serum level of liver enzymes (AST, ALT, ALP) when compared to control animals, whereas, *W. somnifera* and silymarin pre-treatment to the DM-intoxicated animals resulted in a significant normalization of the enzymes activities. On the other hand *W. somnifera* extract reduced the incidence of histopathological changes such as cytoplasmic vacuolization and degeneration in nuclei, rupture of epithelia lining the central vein, widened sinusoidal space and lymphocyte infiltration induced by DM treatment in guinea pigs. In conclusion, the results of this study suggest that *W. somnifera* aqueous extract could protect the liver against DM-induced oxidative damage.

Key words: *Withania somnifera*, dimethoate, hepatotoxicity, protection, guinea pig

INTRODUCTION

Organophosphate (OP) compounds are used worldwide and include the most toxic chemical agents. Recently, thousands different OP compounds have been synthesized and are used in agriculture for crop protection and pest control, in medicine as ectoparasiticides, in public health programs as insecticides, acaricides and nematicides and in commerce as lubricants, plasticizers and flame-retardants (Buyukokuroglu *et al*., 2008). Many studies have been carried to evaluate the residual amounts of OP pesticides. Some of these studies reported a detectable amount in water, soil and foods (IARC, 1983; Poet *et al*., 2004). The inhibition of acetylcholinesterase (AChE) activity is one of the primary effects of OP pesticides (Jayaratnam and Maroni, 1994) leading to acetylcholine accumulation in nerve ending and preventing the normal impulse transmission causing repeated stimulation of muscles which finally result in fatal consequences. Previous reports indicated that the toxicity of OP insecticides caused devastating effects on many organs and systems and caused oxidative stress involved by generation of free radicals and lipid peroxidation (Aly and El-Gendy, 2000; Sharma *et al*., 2005; Sayim, 2007). Dimethoate (DM), which is considered as one of the most important OP insecticide, is frequently used in agriculture against a wide range of insects especially in Khat farms in Yemen. Recent study showed that DM was one of the more contamination sources (Yarsan and Cakir, 2006) and was detected at high concentration in water samples (Al-Wabel *et al*., 2011) in Saudi Arabia. The main groups at risk of high rates of DM exposure are pesticide producers, pesticide workers and farm owners (Sharma *et al*., 2005).

Previous studies showed that DM intoxication cause oxidative stress by generation of free radicals and induce hepatic lipid peroxidation in chicken (El-Saeid *et al*., 2011) and in mice (Maiti *et al*., 1996). The mechanism of the toxic action of DM (similar to other OP compounds) is one of inhibiting the AChE activity. The liver is the primary organ involved in xenobiotic metabolism and is a major target organ for chemicals and drugs. As a result, liver toxicity is one of the most used indicators in the evaluation of a given xenobiotic effects. Different

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biochemical and histopathological parameters are used worldwide to detect tissue-specific effects related to chemical intoxication (Maiti and Kar, 1997).

Natural antioxidants from plants origin are reported to provide a good protection that slows down the process of oxidative damage caused by Reactive Oxygen Species (ROS) (Travlos *et al*., 1996; Jacob and Burri, 1996). ROS has been found to be involved in the toxicity of various OP pesticides (Bagchi *et al*., 1995; Mansour and Mossa, 2010). *Withania somnifera* (*W. somnifera*) (L.) Dunal (Solanaceae) is an evergreen perennial tomentose herb, grown wild in tropical and moderate areas of the world and commonly known as "Obab" in Yemen (Al-Dubaie and Al-Khulaidi, 1996; Aqlan, 2008). It is a medicinal plant commonly used in folk medicine for centuries to treat variety of diseases and ailments as anti-inflammatory agent, aphrodisiac, liver tonic, astringent and more recently to treat bronchitis, asthma, ulcers, hypotensive, anti-tumour, antiperoxidative and cardiotonic (Sivarajan and Balachandran, 1994). Many pharmacological studies have been carried out to describe multiple biological properties of *W. somnifera* (Mishra *et al*., 2000). These studies have shown that the plant preparation had anti-inflammatory (Bhatnagar *et al*., 2005), anticancer (Devi *et al*., 1992; Mohan *et al*., 2004) and reduced the lipid peroxidation (Dhuley, 1998). The main constituents of *W. somnifera* are steroidal alkaloids and steroidal lactones (withanolides) (Elsakka *et al*., 1990). In view of the preceding background, it is of vital importance to study the effect of plant products on oxidative stress induced by pesticide intoxication. Hence, it prompted us to investigate the sub-chronic DM exposure on oxidative stress by assessing liver. Simultaneously, protective action of *W. somnifera* extract on DM-intoxicated guinea pigs was investigated.

MATERIALS AND METHODS

The whole plant of *W. somnifera* was collected in Oct-Nov 2011 from Ibb city, Yemen. The plant was authenticated by Mr. Esam Aqlan, Biology Department, Ibb University-Yemen. Voucher specimens were kept in the Herbarium for future references.

Preparation of the aqueous extract: The plants were washed, cut into small pieces, shade dried for 5 days and then dried overnight in an oven. The dried plants (200 g) were boiled for 30 min with distilled water (2000 mL). The resulting water extract was filtered and subsequently concentrated with a water bath (90°C) until it became creamy and was then dried in an oven (60°C) that finally gave 20 g (10% of initial amount) of powder. The dried extracts were dissolved in saline and administrated orally when experiments were performed.

Chemicals: Dimethoate 40 EC was applied as a commercial emulsifiable concentrate formulation containing 40% active ingredient. It was diluted in olive oil for the final concentration.

Animals and treatments: Adult male guinea pigs (500±100 g) were obtained from the animal house of Biology department, Faculty of Science, Ibb University, Yemen and kept for 1 week on a commercial diet in environmentally controlled conditions with free access to diet and water *ad libitum*. Animals were divided into five groups of five each. Animals of group I received oral administration of olive oil daily for 21 days. The animals of group II were given oral administration of DM (14 mg kg^{-1}; 1/25 of the LD_{50}) dissolved in olive oil daily for 21 days. The animals of group III were given the aqueous extracts of *W. somnifera* (100 mg kg^{-1}) orally dissolved in normal saline daily for 21 days. The animals of group IV were given the aqueous extracts of *W. somnifera* (100 mg kg^{-1}) orally dissolved in normal saline followed by oral DM administration (14 mg kg^{-1}; 1/25 of the LD_{50}) daily for 21 days. The animals of group V were orally given silymarin (100 mg kg^{-1}) suspended in normal saline daily followed by oral administration of DM (14 mg kg^{-1}; 1/25 of the LD_{50}) daily for 21 days. At the end of the experiment, the animals of each group were anaesthetized with ether and blood was collected directly from the portal vein. The blood sample of each animal was divided in two tubes, one of them mixed with heparin to prevent coagulation and the other was allowed to clot at room temperature for 1 h and then centrifuged at 3000 rpm and 4°C for 15 min to obtain sera. The separated serum was sampled into clean tubes and kept in a deep-freezer at -24°C for further analysis.

Biochemical indicators of liver function: Serum Aspartate Aminotransferase (AST) and Serum Alanine Aminotransferase (ALT) were determined using Medichem Middle East Diagnostics kits (Syria) according to the method of Tietz (1982). The activity of serum alkaline phosphatase (ALP) was determined using Reactivos GPL Diagnostics kits (Spain) according to the method of King (1965).The enzyme activity was expressed as U L^{-1}.

Histopathological examination: Control and experimental animals were put under light ether anaesthesia, dissected

as quickly as possible and then livers were removed. Small pieces were fixed in 10% neutral formalin for 24 h, then washed by the running tap water and stored in 70% ethyl alcohol, until further processing. Blocks of about 5×5 mm size were dehydrated, cleared and embedded in paraffin wax. Paraffin sections of 5 microns thickness were cut using rotary microtome (Leica, Germany) and stained with haematoxylin and eosin.

Statistical analysis: Results of the biochemical estimations are reported as Mean±S.D. Total variation, present in a set of data was estimated by one-way Analysis of Variance (ANOVA). Differences with a p-value of <0.05 were considered as statistically significant.

RESULTS

Results of liver enzymes (AST, ALT and ALP): DM treatment (14 mg kg^{-1}) for 21 days in guinea pigs caused a statistically significant increase ($p<0.005$) in the level of AST, ALT and ALP (89.4±10.6, 75.4±8.2 and 84.6±6.6 U L^{-1}, respectively) when compared to control animals, whereas *W. somnifera* (100 mg kg^{-1}) and silymarin (100 mg kg^{-1}) pre-treatment to the DM-intoxicated animals resulted in a significant decrease ($p<0.005$) in the enzymes activities. The activities of AST, ALT and ALP in the *W. somnifera* plus DM treated group were 55.6±8.9, 57.0±6.2 and 61.6±5.9 U L^{-1}, respectively (Table 1).

Results of liver morphological changes: After 21 days of DM administration, the liver sections of the guinea pigs showed many histopathological changes (Fig. 1) compared with those of controls (Fig. 1a). The parenchymatous cells showed cytoplasmic vacuolization. Moreover, an increase in the number of Kupffer cells was observed. Also, rupture of epithelia lining the central vein, widened sinusoidal space, lymphocyte infiltration in the parenchymatous tissue and portal area, enlargement of the central and the portal veins, remarkable dilated veins and disruption of hepatic architecture. Changes in the nucleus appeared in the form of condensed chromatin and fragmented nuclei. However, pre-treatment of DM treated animals with *W. somnifera* extract and/or silymarin showed little pathological alterations when compared with those of DM alone (Fig. 1d, 1f). Moreover, administration of *W. somnifera* extract alone to the animals did not induce any pathological changes and the liver tissue appears like the control (Fig. 1b).

Table 1: Effects of *W. somnifera* aqueous leaves extract on serum marker enzymes in DM intoxicated guinea pigs

Groups	AST (U L^{-1})	ALT (U L^{-1})	ALP (U L^{-1})
Control	32.6 ± 6.2^{a}	35.6 ± 6.4^{a}	40.0 ± 5.9^{a}
W. Somnifera (100 mg kg^{-1})	41.0 ± 7.2^{a}	45.8 ± 5.6^{b}**	48.6 ± 7.2^{a}
DM (14 mg kg^{-1})	89.4 ± 10.6^{b}***	75.4 ± 8.2^{b}***	84.6 ± 6.6^{b}***
DM+100 mg kg^{-1} *W. Somnifera*	55.6 ± 8.9^{c}***	57.0 ± 6.2^{c}**	61.6 ± 5.9^{c}**
DM+100 mg kg^{-1} Silymarin	52.2 ± 6.1^{c}***	53.8 ± 8.6^{c}***	57.6 ± 7.7^{c}***

Values are expressed as Mean±SD; n = 5 for each treatment group. *p<0.05, **p<0.01, ***p<0.001 compared with control, respectively

DISCUSSION

Insecticide poisoning is a major cause of morbidity and mortality especially in developing countries (WHO/PCS, 1996). It has been estimated that 3000,000 cases of severe poisoning and about 220,000 deaths are caused worldwide every year due to insecticides exposure (WHO, 1997). Large numbers of xenobiotics have been identified to have potential to generate free radicals in biological system (Sivapiriya *et al*., 2006; Ahmed *et al*., 2000). Free radicals have become an attractive means to explain the toxicity of numerous xenobiotics. Some of these free radicals interact with various tissue components, resulting in dysfunction. So, OP insecticides have been claimed to have harmful effects on hepatic tissue and other biochemical functions of the liver (Sayim, 2007; Mansour and Mossa, 2010). Also, lipid peroxidation has been suggested as one of the molecular mechanisms involved in OP pesticide (Sharma *et al*., 2005).

The current study was performed to investigate the hepatotoxicity of a commonly used OP insecticide, DM, in guinea pigs following subchronic exposure. In addition, to evaluate the hepatoprotective activity of aqueous leaves extract of *W. somnifera*. The oral administration of DM to guinea pigs caused a significant hepatic damage, as observed from the elevation of hepatospecific enzyme activities. In fact available data on the hepatotoxicity action of DM were limited for adult mice (Sivapiriya *et al*., 2006) and rat (Sharma *et al*., 2005) but there is little data on the effect of DM on guinea pigs (Al-Awthan *et al*., 2012). The liver seemed to be mostly affected by DM treatment alone. The changes were hepatocytic vacuolation, infiltration of lymphocytes around the central veins, rupture of epithelia lining the central vein and widened sinusoidal space. To a lesser extent were nuclear death, hepatocytic rupture, as the liver is the most active mammalian organ in xenobiotic metabolism and contains a larger variety of enzymes for this action. Accordingly, its role in metabolic conversions is its susceptibility to chemical injury (Shakoori *et al*., 1990). Our results were in consistence with previous reports where, DM intoxication

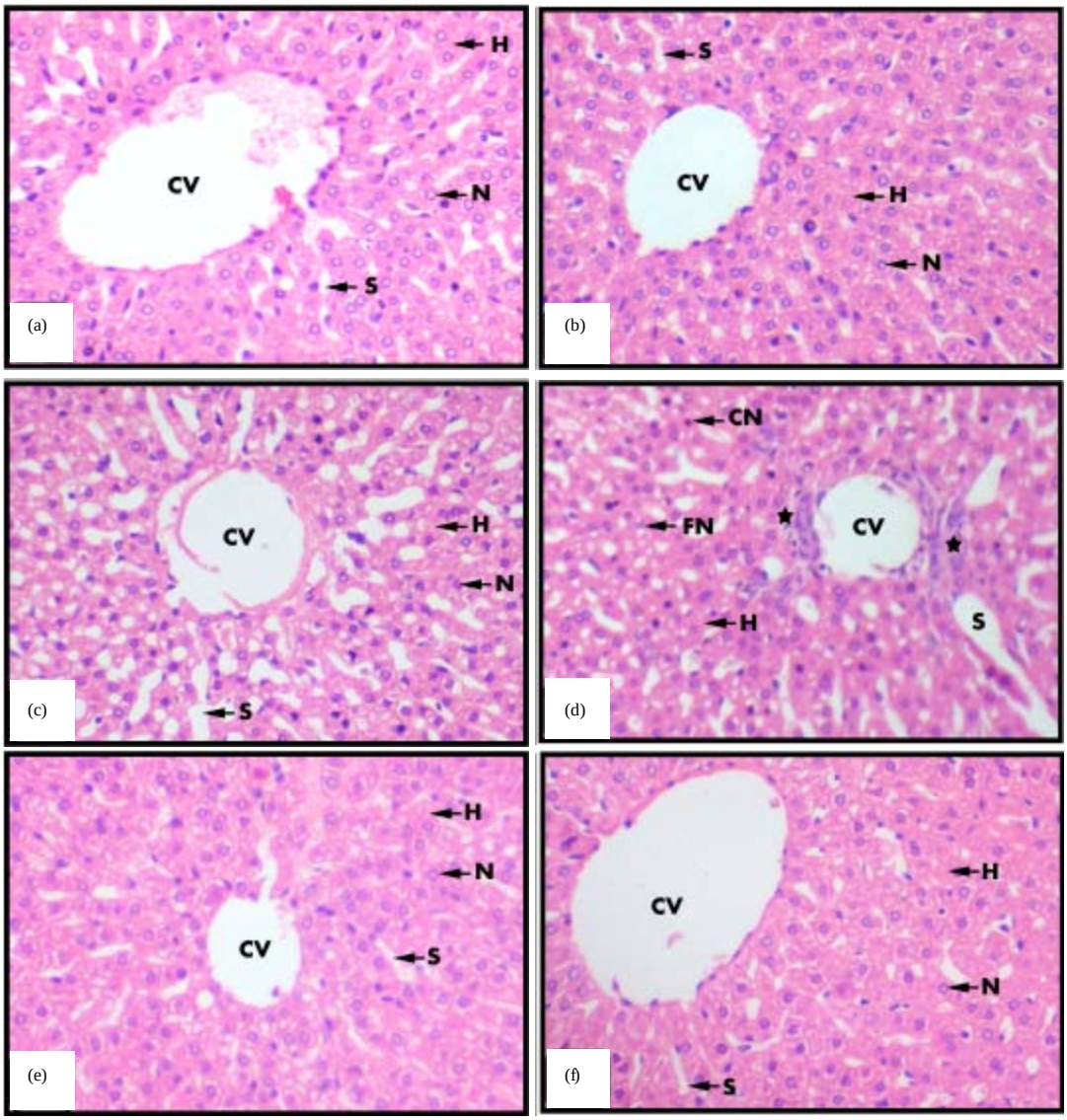

Fig. 1(a-f): The effect of *W. somnifera* against DM-induced hepatotoxicity in guinea pigs. Liver sections were stained with H and E, (a) Normal, (b) *Withania somnifera* (c, d): DM-treated animals, (e) *Withania somnifera*+DM, (f) Silymarin+DM, magnification 400 X, H: Hepatocytes, CV: Central vein, N: Nucleus, S: Sinusodial space, H: Hepatocytes, FN: Fragmented nucleus, CN: Condensed nucleus, *Lymphocytic infiltration

in experimental animals caused an increase in liver marker enzymes (Sharma *et al*., 2005; Sayim, 2007; Ahmed *et al*., 2000). In addition, our previous finding (Al-Awthan *et al*., 2012) add supporting to the present results which showed that DM administration to guinea pigs caused an increase in liver marker enzymes and these changes was normalized by co-administration with vitamins C and E.

A study of some commonly used plant products as antioxidants against xenobiotic-induced oxidative stress therefore appeared to be of interest (Badary, 1999; Ahmed *et al*., 2000). In this study we have investigated the effects of administration of *W. somnifera* extract in comparing with well-known antioxidant, silymarin on liver intoxication induced by DM in guinea pigs. The co-administration of *W. somnifera* extract and silymarin

with DM to guinea pigs resulted in marked improvement of the liver enzymes activities when compared to that which received DM alone. A significant rise in ALT and AST might be taken as an indicator of liver damage. The increased transaminase activity was reversed to normal level following *W. somnifera* extract-supplementation with healing of hepatic parenchyma and regeneration of hepatocytes. One of the possible explanations for the observed recovery of various enzyme activities involved in the detoxification following *W. somnifera* or silymarin treatment could be because these materials exert their hepatoprotective influence by acting as antioxidants (Nagababu *et al*., 1995; Ramadan *et al*., 2002). Previous study reveal that gamma irradiation caused a marked increase in serum levels of AST and ALT, indicating liver injury and these changes were ameliorated by using *W. somnifera* extract (Mansour and Hafez, 2012). Another study also shows that *W. somnifera* extract protected liver cells against oxidative stress induced by lead intoxication (Chaurasia *et al*., 2000). The excessive production of free radicals and lipid peroxides might have caused the leakage of cytosolic enzymes such as (AST and ALT) (Cromheecke *et al*., 2000). The restoration of AST and ALT to their respective normal level was observed in *W. somnifera* treated group. This is consistent with previous report of Ju *et al*. (2008). AST, ALT and ALP levels act as an indicator of liver function hence restoration of normal level of these enzymes indicates that the normal functioning liver. *W. somnifera* has been reported to produce anabolic effects, enhancing the synthesis of certain modulator proteins in rat liver and increasing the body weight in humans (Anbalagan and Sadique, 1981). We have now demonstrated that administration of *W. somnifera* aqueous extract to guinea pigs modulates the liver marker enzymes and suggests a possible adaptive mechanism to counteract oxidative stress. Hence, *W. somnifera*, given to guinea pigs, may prevent the formation of unwanted free radicals and protected them against DM intoxication. In addition, there are other several reports supported the role of antioxidant in attenuating the histopathology of some pesticides and toxins in experimental animals, for example, ascorbic acid supplementation prevents the testicular damage induced by DM intoxication (Salem, 2005) also, Al-Awthan *et al*. (2012) revealed a hsistopathological changes in liver tissue of guinea pigs treated with DM and the severity of these lesions was reduced by administration of a combination of vitamin C and E. In support of our finding DM produced enzymatic changes in liver of dams associated with mild pathomorphological changes in liver and brain (El-Elaimy and Gabr, 1990; Srivastava and Raizada, 1996). The present findings clearly suggest that *W. somnifera* extract is capable of scavenging DM-induced free radical generation. It thus appears that the plant extract may prove useful in treating or preventing DM toxicity to some extent.

CONCLUSION

DM treatment to male guinea pigs caused an increase in the liver marker enzymes (AST, ALT and ALP) levels and caused many histopathological changes to liver of guinea pigs. The administration of either *W. somnifera* extract and/or silymarin to guinea pigs modulates the toxicity of DM and suggests a possible adaptive mechanism to counteract oxidative stress. But, further *in vitro* and *in vivo* studies are needed to assess the effects of this plant on antioxidant system of the body.

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