Protective Effects of *Origanum majorana L.* against Hepatotoxicity and Nephrotoxicity in Albino Rats Intoxicated with Abamectin

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Abstract: The present study was undertaken to examine the protective effect of leaf marjoram extract against abamectin (ABA) which induced hepatic toxicity and lipid profile disturbance in male albino rats. Twenty four male albino rats were divided into four groups of six rats each. One of them is negative control which, received distilled water, the positive control group received marjoram extract (Mar) (100 mg/kg), the ABA group intubated with ABA (1.44 mg/kg=1/20 LD_{50}) and the last groups were treated sequentially with marjoram extract (100 mg/kg) and ABA (1.44 mg/kg=1/20 LD_{50}). The serum samples were analyzed for proteins, enzymes, urea, creatinine levels and lipid profile. Oral administration of ABA (1.44 mg/kg b.w.) for four weeks led to elevated significant (P>0.05) in the serum hepatic markers such as serum aspartate transaminase (AST), serum alanine transaminase (ALT), alkaline phosphatase (ALP), γ-glutamyl transpeptidase (γGT). The levels of urea and creatinine were significantly elevated in ABA group when compared to the control. In addition, the selected biopesticide led to disturbance in lipid profile. It is proposed that marjoram extract improved the alterations in the biochemical parameters partly through its antioxidant contents, hepatoprotective and nephroprotective properties. It is concluded that marjoram extract is a useful against biochemical toxicity in individuals that are constantly co-exposed to ABA in the environment.

Keywords: Abamectin, *Origanum majorana*, liver function, kidney function and lipid profile.

1. Introduction

Environmental contamination effects on human health are one of the most problems that face the world today. The growing world economy and movement towards global marketing have led a competition in industrial and technological development towards the improvement of mankind. However, in nearly all countries such as developments have focused on increased production and economic gains before realizing their impact on the environment and human health **(Deivanayagam *et. al*., 2014).**

Many compounds that used for human welfare are at the same time injurious from safety point view. Some compounds such pesticides that are used against various pests for human welfare enter human through food chain and induce health injuries (**Uthman *et al.,* 2013)**.

Avermectin derivatives are a family of macrocycliclactons (MLs) that applied as veterinary drugs for food-producing animals, especially in aquaculture, and as plant protection agents in the agricultural sector, to control insects and mites on a wide range of agricultural products such as fruit, vegetable and ornamental crops (**Hernando *et. al.,* 2007**). Abamectin (ABA) is a mixture of avermectin B1a (>90%) and avermectin B1b (<10%) which, have very similar biological and toxicological properties (**Kita *et. al.,* 2007,** and **Horvat *et. al.,* 2012**). ABA has been used extensively all over the world and is still one of the most commonly used pesticides. It affects inhibitory synapses *via* a mode of action involving glutamate-sensitive chloride channels (**Kita *et. al.,* 2007**). ABA intoxication can impair the function of hepatocytes and perturbs the mitochondrial bioenergetics (**Castanha *et al.,* 2012)**. The target for abamectin involves the γ-aminobutyric acid (GABA) receptor in the peripheral nervous system. It appears that, the glutamate-gated chloride channel (GluCl), along with the γ-aminobutyric acid (GABA)-gated chloride channel (GABR) and the histamine-gated chloride channel (HisCl), is a target site of avermectin and ivermectin in insects and nematodes (**Mc-Caver *et al.,* 2007).**

Liver is a target organ and primary site of detoxification and is generally the major site of intense metabolism and is therefore prone to various disorders as a consequence of exposure to the toxins of extrinsic as well as intrinsic forms. Liver plays important role in metabolism to maintain energy level and structural stability of body **(Guyton and Hall, 1996**). It is also site of biotransformation by which a toxic compound has been transformed to less harmful form to reduce toxicity **(Hodgson, 2004**). All the major functions of the liver can be detrimentally altered by acute or chronic exposure to toxicants .When toxicants inhibit hepatic transport and synthetic processes, dysfunction can occur without appreciable cell damage. Loss of function also occurs when toxicants kill an appreciable number of cells and when chronic insult leads to replacement of cell mass by nonfunctional scar tissue

Liver is one of the organs that are mostly affected by pesticides responsible for lipid metabolism (**Aly *et al.*, 2010; and Tripathi and Srivastav, 2010**) and detoxification of xenobiotics. Therefore, the liver, due to these functional roles is vulnerable to injury, which may alter its ability to metabolize lipids. Studies have shown alterations in lipid profiles following pesticides exposure (**El-Mazoudy *et al.*, 2011**). Lipids plays important role in cells such as, homeostasis, reproductive, organ physiology and numerous aspects of cellular biology. Many pathological alterations are also linked with disturbance in lipid metabolism, such as obesity, diabetes, heart disease and inflammation **(Lee *et. al.*, 2003**). Alterations in lipoproteins transport system which deliver cholesterol and fatty acids to the periphery affect lipid metabolism with resultant dyslipidemia, a risk factor in atherosclerosis (**Ambali *et al.*, 2011**).

The *O. majorana* (OM), a member of the mint family, Lamiaceae, is a widely used plant in folk medicine. Its aromatic leaves are used as flavoring and other culinary purposes. It is widely used as antihyperglycemic, anti-inflammatory **(Kelp *et al.*, 2000, and Islander *et al.*, 2013**), cytotoxic (**Al-Harbi, 2011**), antioxidant (**Koukoulitsa *et al.*, .2006**), antifungal, antibacterial (**Viuda *et al.*, 2008**) where, *O. marjorana* are rich in phenolic and volatile active compounds known for their antimicrobial, antioxidant. Phenolic compounds of oregano represent 71% of the total oil, thymol and carvacrol, are responsible for many of the properties of the essential oil (**Bozin *et al.*, 2006**).

Thereby, the present investigation aims to evaluate the protective effect of aqueous extract of *Origanum majorana* against abamectin-induced hepatotoxicity, nephrotoxicity and dyslipidemia in male albino rats.

2.Materials and Methods

2.1.Abamectin:

(1.8% EC) was supplied by Syngenta Company. The chemical name is 5-O-demethyl avermectinA_{1a}(i) mixture with 5-O-demethyl-25-de (1-methylpropyl)-25-(1-methylethyl) avermectin A_{1a}(ii).

2.2.Marjoram: (*Organum majorana L.*)

Is an aromatic herbal spice; plant family Laminaceae (mint family).the sensooric quality is aromatic & slightly bitter. The plant leaves used in this study obtained from Mepaco Co., Egypt for pharmaceutical & medicinal plants.

2.3.Animals:

Twenty four male albino rats (130±10g) were obtained from the breeding unit in national research center. Animals were maintained at the animal care facilities of Central Agricultural Pesticides Laboratory (CAPL) in plastic cages under controlled temperature (23±2^{o}C), 12-h light/dark cycle and relative humidity (50±5%). Water and food were available *ad libitum*. Rats were acclimatized to the laboratory environment for two weeks prior to the start of experiments.

2.4.Experimental Design:

After the acclimation period, animals were divided into four groups with six animals each. The first group was used as control (Cont) (*-ve* control); hence these animals were orally given distilled water .The second group was orally treated with the aqueous extract of marjoram (Mar) at the concentration level 100 mg/Kg b.w. (*+ve* control). The third group was intubated orally with selected pesticide, abamectin (ABA) at a dose level of 1.44 mg/Kg b.w. (1/20 LD_{50}) and fourth group was treated with combination of ABA (1.44 mg/kg b.w.) and aqueous extract of marjoram (Mar) at the level 100 mg/Kg, 2-hr prior ABA exposure pesticide intoxication. The daily oral administration of the experiments lasts for 28-days according to Organization for Economic Co-operation and Development (**OECD, 407**).

2.5.Sampling

At the end of experiment, the animals were fasted overnight and blood samples were collected from retro orbital plexus vein into cleaned tubes. Blood samples were centrifuged at 3000 r.p.m for 15-min to obtain serum. Serum were separated and kept in deep-freezer at -20°C till the assays were carried out.

2.6.Biochemical markers of liver function:

The activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were estimated in blood serum by the method described by **Reitman and Frankel (1957)**. The activity of alkaline phosphatase (ALP) was measured according to **Bowers, and McComb (1966)**the activity of γ-glutamyl transpeptidase (γ-GT) was estimated according to the method of **Szasz (1969).** Total protein (TP) level in serum was quantified by the procedure of **Lowry *et al.* (1951)**. Albumin level (ALB) in serum was determined by the method of **Dumas and Biggs (1972).**

2.7.Biochemical markers of lipid profile:

Triglycerides were measured according to the method of **Bergmeyer (1983).**Cholesterol was measured according to the method of **Allain *et al. al.*, (1974**).High density lipoprotein cholesterol (HDL) was measured according to the method of **Burstein et al. (1984),** and Low-Density Cholesterol (LDL) was calculated using the formula of **Friedewald *et al.* (1972):LDL=Total Cholesterol–(HDL+VLDL)**

2.8.Biochemical markers of Kidney function:

Serum urea was estimated by the method of **Fawcett and Scott (1960).** Creatinine in the serum was estimated using the diagnostic kit based on the methods of **Teitz (1978).**

2.9.Statistical analysis:-

Statistical analysis was performed on a PC using SPSS, V.13, (special package for social sciences). Data are presented as arithmetic mean ± S.E. The difference among means has been analyzed by one way ANOVA value of (P<0.05) was considered as statistically significant **Snedecor and Cochran, (1986).**

3.Results

3.1.Hepatotoxicity Biomarkers:

Data presented in Table (1) show the impact of ABA on biomarkers of liver function in male albino rats treated with 1.44 mg/Kg b.w. of ABA. The results indicated that, ALT, AST, ALP and γ-glutamyl transferees were significantly increased (P<0.05) in male rats exposed to ABA for 28-days comparing with negative and positive control. In addition, in the same table the results revealed that total protein was reduced significantly in rats exposed to ABA while ALB insignificantly changed. Administration of Mar water extract improved the effect of ABA where the ALT, AST, γ-glutamyl transpeptidase, total protein but not returned to control level. Also, Mar water extract led to increase in albumin and total protein levels in comparing with the pesticide exposed group. Also, marjoram water extract improved the level of ALT activity compared with ABA treated group.

3.2.Lipid Profile:-

Results depicted in Table (2) indicated that, trigleceride (TG), total cholesterol, low density lipoprotein (LDL) were significantly increased (P<0.05) in ABA exposed group comparing with negative and positive control group. Whereas, high density lipoprotein was significantly reduced (P<0.05) in male rats exposed to ABA. Regarding, male albino rats intubated with Mar water extract before the pesticide exhibited an improvement in lipid profile where, TG, total cholesterol and LDL were significantly decreased in comparing with pesticide group.

3.3.Nephrotoxicity biomarkers:

Regarding, the kidney function (urea and creatinine) of male rats exposed to ABA, the data revealed a significant increase (P<0.05) in urea and creatinine compared with negative and positive control. Mar water extract led to an improvement in creatinine level with comparing with pesticide group **Table** (**3**).

4.Discussion

Due to its role in the transformation of environmental xenobiotics, the liver is at great risk of injury. Where, the liver is the main organ of detoxification of pollutants **(Sefi *et al.*, 2011).** A variety of biochemical changes, which may be responsible for the adverse effects on humans, may be occurred after Exposure to low level of pesticides (**El-halwagy *et al.*, 2009; and Ibrahim *et al.*, 2011**). ALT is a cytoplasmic enzyme present in liver and other cells. It is particularly useful in measuring hepatic necrosis, especially in small animals. The determination of serum levels of AST, ALT, ALP and other biochemical parameters is of great significance in the evaluation of toxic effects of pesticides on the liver (**Kallender *et al.*, 2005**). Results of the present study revealed that abamectin treatment caused a significant increase in the activities of ALT, AST and ALP in serum of male albino rats in comparison with control group. These results are concomitant with **Abd-Elhady and Abou-Elghar (2013).**These enzymes were present in hepatocyte and their level increased in circulation may be due to hepatocytes dysfunction with alteration in the permeability of liver membrane takes place (**Khan *et. al.*, 2005**). Alanine transaminase (ALT) is an enzyme that helps metabolize protein. When the liver is damaged, ALT is increased in liver and released in the bloodstream. Aspartate transaminase (AST) is an enzyme plays a role in the metabolism of the amino acid alanine. An increase in AST levels may indicate liver damage or disease. Aspartate transaminase is the mitochondrial enzyme, predominantly found in the liver, skeletal muscles and kidneys. Alanine transaminase is a cytosolic enzyme, which is more specific for the liver than aspartate transaminase (**Paliwal *et. al.*, 2009).** Alkaline phosphotase (ALP) is an ectoenzyme and a marker enzyme for the plasma membrane and endoplasmic reticulum, (**Bandyopadhyay *et al.*, 1999**). ALP is produced in the canaliculi and sinusoids membrane of the liver. The significant rise in ALP observed in the ABA treated group suggests the increased permeability of plasma membrane or cellular necrosis, and this showed the stress condition of the treated animals (**Rahman *et al.*, 2000**). γ- GT is a microsomal brush border enzyme found notably in liver. It is involved in the transfer of amino acids across the cellular membrane. It is also involved in GSH metabolism by transferring the glutamyl moiety to a variety of acceptors leaving the cysteine product to preserve the intracelluluar homeostasis of oxidative stress (**Muthuviveganandavel *et al.*,**

2011). Serum γ-GT level is considered as a better index and to be highly sensitive to biliary tract damage (**Ozer *et al.*, 2008**). The protein depression may be due to reduced protein synthesis or increased

Table (1): Effect of Aqueous marjoram extract (Mar) and/or abamectin pesticide (ABA) on hepatotoxicity biomarkers of treated male rats for 28-days.

Treatment	ALT	AST	ALP	γ-Gt	TP	ALB
Control (1ml distilled water)	**37.58** ±0.505	**30.26** ±0.84	**240.47** ±12.23	**3.56** ±0.22	**14.12** ±0.65	**3.27** ±0.21
Mar (100mg/Kg b.w.)	**37.38** ±0.442	**30.96** ±0.64	**246.04** ±18.75	**3.50** ±0.17	**14.18** ±0.95	**3.82** ±0.29
ABA (1.44mg/Kg b.w.)	**43.68** ±0.755 a,b	**35.50** ±1.00 a,b	**295.81** ±10.65 a, b	4.69 ±0.21 a,b	**8.25** ±0.6 a,b	**3.83** ±0.35
ABA&Mar (1.44mg/Kg b.w.) &(100mg/Kg b.w.)	**39.84** ±0.47 a,b,c	**35.25** ±0.85 a, b	**267.58** ±11.12	**3.16** ±0.33 c	**10.29** ±0.18 a,b,c	4.44 ±0.16 a

Data expressed as mean±S.E. (a) Significant different from corresponding control group by one-way ANOVA at $P \leq 0.05$, (b) Significant different from corresponding Mar group by one-way ANOVA at $P \leq 0.05$, and (c) Significant different from corresponding ABA group by one-way ANOVA at $P \leq 0.05$.

Table (2): Effect of Aqueous marjoram extract (Mar) and/or abamectin pesticide (ABA) on lipid profile of treated male rats for 28-days.

Treatment	TG	Cholesterol	HDL	LDL
Control (1ml distilled water)	**148.7** ±2.887	**67.93** ±1.82	**46.6** ±0.55	**22.86** ±1.124
Mar (100mg/Kg b.w.)	**148.7** ±1.802	**68.202** ±3.11	**47.94** ±2.79	**20.26** ±1.052
ABA (1.44mg/Kg b.w.)	**259.8** ±10.6 a,b	**88.73** ±1.78 a,b	**37.35** ±0.69 a,b	**51.38** ±1.09 a,b
ABA&Mar (1.44mg/Kg b.w.) &(100mg/Kg b.w.)	**144.3** ±5.913 c	**49.903** ±3.73 a,b,c	**38.36** ±0.96 a,b	**13.21** ±0.86 a,b,c

Data expressed as mean±S.E. (a) Significant different from corresponding control group by one-way ANOVA at $P \leq 0.05$, (b) Significant different from corresponding Mar group by one-way ANOVA at $P \leq 0.05$, and (c) Significant different from corresponding ABA group by one-way ANOVA at $P \leq 0.05$.

Table (3): Effect of Aqueous marjoram extract (Mar) and/or abamectin pesticide (ABA) on nephrotoxicity biomarkers of treated male rats for 28-days.

Treatment	Urea	Creatinine
Control (1ml distilled water)	**36.68** ±2.26	**0.62** ±0.01
Mar (100mg/Kg b.w.)	**37.85** ±1.40	**0.74** ±0.01
ABA (1.44mg/Kg b.w.)	**48.76** ±0.80 a,b	**1.12** ±0.04 a,b
ABA&Mar (1.44mg/Kg b.w.) &(100mg/Kg b.w.)	**47.91** ±0.87 a,b	**0.52** ±0.04 b,c

Data expressed as mean±S.E. (a) Significant different from corresponding control group by one-way ANOVA at $P \leq 0.05$, (b) Significant different from corresponding Mar group by one-way ANOVA at $P \leq 0.05$, and (c) Significant different from corresponding ABA group by one-way ANOVA at $P \leq 0.05$.

proteolytic activity. The observed decrease in proteins could be also attributed in part to the damaging effect of the pesticide on liver cells as confirmed by the increase in the activities of plasma AST, ALT, and ALP (**Uboh *et al.*, 2009).**

Lipids are essential for energy homeostasis, reproductive and organ physiology and numerous aspects of cellular biology. They are also linked with many pathological processes, such as obesity, diabetes, heart disease and inflammation. To meet the different demands from a variety of tissues, the body has evolved a sophisticated lipoprotein transport system to deliver cholesterol and fatty acids to the periphery **(Lee *et. al.*, 2003).**Free fatty acids enter the liver cell and most are esterified to triglyceride in order to be secreted by liver, intercellular triglyceride must be complexed with specific apoprotein molecules called "lipid acceptor protein" to form lipoprotein **(Kumar *et. al.*, 2006)**.

Alterations in the concentrations of these lipoproteins affect lipid metabolism resulting dyslipidemia, a risk factor in atherosclerosis. Hypercholestrolemia is a sign of liver damage (**Abdou and El-Mazoudy, 2007).**The elevation in serum total cholesterol level observed in the ABA group could be due to blockage of liver bile ducts causing reduction of its secretion to the duodenum (**Aldana *et al.*, 2001**). Similarly, the ability of the pesticide to interfere with the permeability of the liver cell membrane may have been partly responsible for the high total cholesterol (**Kallender *et al.*, 2005**).

HDL mainly synthesized in the liver and intestinal cells plays an important role in cholesterol efflux from tissues and carries it back to the liver for removal as bile acids. It has been established that the elevated serum or plasma HDLs levels have anti-atherogenic effect. Whereas, the reduced levels of HDL are associated with an increased risk for coronary artery disease. HDLs have been proposed to act in concert to remove excess cholesterol from arterial tissue (**Ambali, *et. al.*, 2011**).

The kidney is a vital organ, which plays an essential role in health, disease and overall development and growth. The main function of kidney is to maintain total body fluid volume, its composition and acid base balance. A number of environmental xenobiotic influence these functions (**Fatima *et. al.*, 2004**). It has been postulated that increased levels of serum urea and creatinine is linked to kidney disease**(Chawla, 2003).** Urea is the main nitrogenous end product of protein catabolism. Increased urea concentrations in the serum indicating reduced glomerular filtration rate in exposed rats**. (Satarug and Moore, 2004).** Elevated creatinine is correlated with an increased protein catabolism, as creatinine is the end product of protein catabolism. This is supported by the result of **Abd-Elhady and Abou-Elghar (2013)** who noticed a decrease in total protein leveldue toits enhanced catabolism in ABA-exposed rats.

Plant extracts, as herbal medicine have been utilized as a treatment for a wide variety of clinical disease. Whereas, these herbs that have the protective effect as natural antioxidants against chemicals-induced toxicities (**Frei and Higdon, 2003**). The decreased levels of these hepatic enzymes, ALT, γ-GT and improvement in total protein in groups administered Mar aqueous extract indicate the curative and therapeutic effect against ABA-induced hepatotoxicity. The water extract of Mar had a therapeutic effect against kidney damage induced by xenobiotics. Where, the creatinine level was improved in rats treated with water extract of Mar plus ABA pesticides. These results are in agreement with (**Shati, 2011).** Aqueous extracts of Mar showed antihyperlipidemic (**Pimple *et al.*, 2012**). Where, lipid profile markers have been decreased in rats administered the aqueous extract of Mar and intoxicated with ABA The possible mechanisms of Mar hepatoprotective by activation the enzyme defense systems which act as a scavengers against the free radical that produced by the toxicants and improvement of the antioxidant/detoxification system in liver. Furthermore, the free radical scavenger effect of Mar has been reported by many authors **(Singh *et al.*, 2005)**. Marjoram (*O. majorana L*.) has strong antioxidant activity, mainly because of its high content of phenolic acids and flavonoids. Marjoram is rich in phenolic content (**Vagi *et al.*, 2005**).

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