The protective role of *Echium humile* extract against toxicity induced by insecticide (malathion) in male albino rats

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Abstract: The present study was designed to evaluate the influence of *Echium humile* extract treatment in rats exposed to malathion. Twenty eight adult male rats were used in this study and distributed into four groups. Animals of group 1 were treated with 1 ml of distilled water and served as control. Rats of group 2 were orally given malathion at a dose level of 75 mg/kg/b.wt for a period of 28 days. Rats of group 3 were supplemented with *Echium humile* extract at a dose level of (250 mg/kg/b.wt). Experimental animals of group 4 were orally given *Echium humile* extract at the same dose given to group 3 and after 1 hours exposed to malathion at the same dose given to group 2. The groups treated with Malathion showed elevation in serum glucose, creatinine,Cholesterol, Tri glyceride, aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) level while, cholinesterase(ChE) decreased significantly. Moreover, Administration of *Echium humile* extract after malathion exposure to rat can prevent severe alterations of hematobiochemical parameters. In conclusion, this study obviously demonstrated that pretreatment with *Echium humile* extract significantly attenuated the physiological alterations induced by malathion. Also, the present study identifies new areas of research for development of better therapeutic agents for liver, kidney, and other organs' dysfunctions and diseases.
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1. Introduction

The widespread use of organophosphate (OP) pesticides by public health and agricultural programs has led to severe environmental pollution that constitutes a significant potential health hazard because of the possibility of the acute or chronic poisoning of humans ***(Lasram et al., 2009)***. Indeed, residual amounts of organophosphate pesticides have been detected in the soil, water bodies, vegetables, grains, and other food products ***(Poet et al., 2004)***. OP pesticides compounds are known to induce toxicity in mammals by inhibiting acetylcholinesterase (AChE), which leads to the accumulation of acetylcholine and the subsequent activation of cholinergic muscarinic and nicotinic receptors ***(Bayir et al., 2013)***. OP pesticides are also known to inhibit pseudocholinesterase activity ***(Ogutcu et al., 2008)***. Other systems that may be affected by OP pesticide exposure include the immune system ***(Handy et al., 2002)***, the pancreas ***(Gokcimen et al., 2007)***, the liver ***(Kalender et al., 2005a)***, the kidney ***(Kalender et al., 2006)***, the hematological system ***(Omyma.,2011)***, and the reproductive system ***(Uzun et al., 2009)***.

Malathion [O,O-dimethyl-S-(1,2-dicarcethoxyethyl) phosphorodithioate] is an OP pesticide in agricultural and household products that is widely used to control pests. It is extensively used over the whole world, especially by developing countries, to control or eradicate disease-inducing arthropods targeted by public health programs; it is also used to eliminate animal ectoparasites, human head and body lice, and household insects, and to protect grain in storage ***(Rezg et al., 2008b)***. Malathion is known to inhibit acetylcholinesterase activity in target tissues ***(Rezg et al., 2008a,b)*** and has been linked to the dysfunction of several organ systems, including the liver ***(Lasram et al., 2009)***, the pancreas ***(Vasough-Ghanbari et al., 2007)***, and the reproductive system ***(Gonzales, 2003)***. For example, acute exposure to malathion has been shown to disrupt lipid metabolism, thereby elevating LDL and triglyceride levels ***(Lasram et al., 2009)***.

The lipophilic nature of OP facilitates their interaction with the cell membrane and leads to perturbation of the phospholipids bilayer structure ***(Videira et al., 2001)***. Therefore, they may enhance lipidperoxidation (LPO) by directly interacting with the cellular membrane and generated ROS ***(Aslan et al., 1997)***. LPO may impair antioxidant defenses, leading to cellular oxidative damage by changing the balance between oxidants and antioxidants ***(Torres et al., 2004)***. Some studies showed that LPO has been suggested as one of the molecular mechanisms involved in OP compounds induced toxicity ***(Yamano and Morita, 1992)***. Hence, treatment with antioxidants and free radical scavengers can decrease

the oxidative stress related to OP-induced toxicity***(Yurumez et al., 2007)***.

Echium humile is a small hispid biennial to perennial herb growing naturally in north Africa and southern Europe, the *Echium* genus contains several endemic frutescent species dispersed across Europe, the Mediterranean region, Macaronesia and North Africa ***(Croda Chemicals Europe Ltd, 2006)***.

Adel et al., 2007, reported that antidepressant effects of *Echium* species such as *E. vulgare* have been studied. The low doses of the aqueous extract of the aerial parts of *E. vulgare* and high doses of the alcoholic extract have a clear antidepressant activity that is comparable to imipramine.

Seeds, leaves and stems of two *Echium* species (Family *Boraginaceae*) from Iran surveyed for the first time in a search for new sources of γ-linolenic acid (GLA), stearidonic acid (SDA) and other unsaturated fatty acids, ***(Abbaszadeh et al., 2011)***,The n-3 and n-6 polyunsaturated fatty acids (PUFA) are essential for normal human growth. GLA is described as a fatty acid with anti-inflammatory properties. However, its therapeutic value is controversially discussed (atopic dermatitis, rheumatoid arthritis) ***(Gunstone, 1992)***.

Rosmarinic acid (RA) (á-o-caffeoyl-3,4-dihydroxyphenyllactic acid) is a diphenolic compound common to many species of herbs and spices, particularly in the families of *Boraginaceae* and *Lamiaceae* ***(Harborne, 1966)***. RA is regarded as a potential pharmaceutical plant product and is noted for its potent antioxidant properties. Herbs, many of which that contain RA as the dominant phenolic constituent, have long been used in traditional medicines in Southern Europe, Japan, and India for treatment of numerous maladies, from stomachache, headache, and diabetes mellitus to insect bites and acne.***(Malencic et al., 2000)***

2. Material and Methods.

2.1. Animals

Sexually mature male Albino rats (weighing approximately 180–200 g) obtained from the Cairo University Laboratory Animals Growing and Experimental Research Center (GUDAM) were used. The animals were housed in plastic cages, fed a standard laboratory diet and water, exposed to a 12 h light/dark cycle, and maintained at a laboratory temperature of 20 ± 2 ^{o}C The animals were quarantined for 10 days before beginning of the experiments. All rats were handled in accordance with the standard guide for the care and use of laboratory animals.

2.2. Chemicals

Malathion, commercial formula (lathion 57 %) was obtained from the Agricultural Center, Cairo, Egypt.

2.3. plant extract.

The *Echium humile* plant was obtained from "6 October, Alexandria, Egypt",where it grow then it was left in the air to be dried away from direct sun heat. After drying of the plant it was grinded then it was macerated in 70 % ethyl alcohol for 48h After that, the aqueous extract was filtered, concentrated at room temperature ***(Benhaddou–Andaloussi et al., 2008)*** it yield 50gm of extract then the dried extract was stored at 4^{o}C until use.

2.4. Animal treatment schedule

Animals were divided into four groups (n = 7), each group received orally daily dose for 28 days using metallic stomach tube as the following:

2.3.1. The 1st group (control group):

Animals received 1ml of distilled water.

2.3.2. The 2nd group(Malathion treated group):

Received malathion (75 mg/kg b.wt) (15 mg is equivalent to 1.0 ml of prepared solution) that represent 1/20 LD $_{50}$.

2.3.3. The 3rd group (Echium humile treated group):

Received *Echium humile* extract (250 mg/kg/b.wt) dissolved in distilled water (50 mg is equivalent to 1 ml of prepared solution).

2.3.4. The 4th group. (Malathion + Echium humile extract*):*

Received *Echium humile* extract (250 mg/kg / b.wt), after 2 minute of administation with malathion (75 mg/kg b.wt).

2.5. Blood sampling

At the end of 4th week, the blood specimens were drawn by orbital puncture (from eye plexus) using heparinzed microhematocrit capillary tubes and the Serum was harvested from blood without EDTA and subsequently used for the determination of biochemical parameters.

2..6. Evaluation of Some Biochemical parameters.

Carried by spectrophotometric determination of aspartate transaminase (AST), and alanin transaminase (ALT) according to **(*Reitman and Frankel., 1957)***, alkaline phosphatase (ALP) ***(Esakova and Ivanov., 1992)***, serum creatinine according to ***(Trinder, 1969b)***, Serum glucose level according to method described by ***(Trinder, 1969a)***, total cholesterol according to ***(Richmond, 1973)***, triglyceride according to ***(Fossati and Prencipe, 1990)*** Using Diamond kit. Cholinesterase test was done by biostic kinetic kits according to ***(Szasz., 1968)***.

2.7. Statistical Analysis

The experimental data are expressed as mean ± S.D. The data were analyzed by the student t-test. The differences were considered to be statistically significant when $P < 0.05$.

3. Results

3.1. Changes in hepatorenal functions

Results in **(Table 1)** showed that, When the *Echium humile* extract treated animals were compared to the control group at the end of the 4th week, they did not differ significantly in terms of any of the hepatic/renal parameters. Compared to the control group, the malathion treated group had a very high significant elevated ALP, ALT, AST and creatinine levels ($P < 0.001$). The malathion plus *Echium humile* extract-treated group also had a very high significant increase in ALP, ALT, AST and creatinine levels than the control group ($P < 0.001$). When the malathion plus *Echium humile* extract - treated group was compared to the malathion-treated group, they had a very high significant decrease in ALP, ALT, AST and creatinine levels ($P < 0.001$).

3.2. Changes in serum glucose level

Results in **(Table 2)** illustrated that, Compared to the control group, *Echium humile* extract treated group did not showed any significant change ($p > 0.05$) in serum glucose level. While, malathion and malathion plus *Echium humile* treated groups showed a very high significant ($P < 0.001$) increase in serum glucose level when compared with control group. On the other hand, malathion plus *Echium humile* treated group showed a very high significant ($P < 0.001$) decrease in serum glucose level when compared with malathion treated group.

3.3. Changes in some lipid profile parameters.

Results in **(Table 2)** demonestated that cholesterol and triglyceride parameters did not significantly ($p \rangle 0.05$) affected by the effect of *Echium humile* extract treatment.While, malathion treatment caused very high significant ($p \langle 0.001$) increase in the level of Tg and Cho when compared with control group. malathion – *Echium* treatment did not caused any significant ($p \rangle 0.05$) change in Tg level, While, caused a high significant ($p \langle 0.01$) increase in Cho level,when compared with control group. On the other hand, malathion – *Echium* treatment caused a very high significant ($p \langle 0.001$) decrease in Tg level and high significant ($p \langle 0.01$) decrease in the levels of Cho, when compared with malathion treated group.

3.4. Changes in serum Cholinesterase (ChE)

Results in **(Table 2)** showed that, Compared to the control group, *Echium humile* extract treated group showed a very high significant ($p < 0.001$) increase in serum ChE level. While, malathion plus *Echium humile* treated group did not showed any significant ($p \rangle 0.05$) change in serum ChE level when compared with control group. On the other hand, malathion treated group showed a very high significant ($p < 0.001$) decrease in serum ChE level when compared with control group. While, malathion plus *Echium humile* treated group showed a very high significant ($p < 0.001$) decrease in serum ChE level when compared with malathion treated group.

Table (1): Effect each of Malathion, *Echium humile* and Malathion plus *Echium humile* on ALT, AST, ALP and creatinine in the serum of male albino rats compared with control group. (N = 7)

Groups	Statistical parameter	ALT U/L	AST U/L	ALP U/L	Creatinine mg\dl
Control group	**Mean**	73	71.8	47.8	0.9
	SE	4.79	2.58	2.03	0.07 ±
Mal	**Mean**	138.8	145	107.9	1.87
	SE	12.8 ±	9.52 ±	7.9 ±	0.11 ±
	% of change	**+ 90.1**	**+ 102**	**+ 125.7**	**+ 107.8**
	P	**0.001*** <**	**0.001*** <**	**0.001*** <**	**0.001*** <**
Ech	**Mean**	74.8	74.6	46	0.94
	SE	2.12 ±	1.94 ±	2.92 ±	± 0.08
	% of change	**+ 2.5**	**+ 3.6**	**- 4.1**	**+ 4.4**
	P	**0.05 NS>**	**0.05 NS>**	**0.05 NS>**	**0.05 NS>**
Mal + *Ech*	**Mean**	93.2	85	82.5	1.39
	SE	3.03 ±	2.54 ±	± 4.11	0.06 ±
	% of change	**+ 27.7**	**+ 18.4**	**+ 72.6**	**+ 54.4**
	P	**0.001*** <**	**0.001*** <**	**0.001*** <**	**0.001*** <**
Mal + *Ech* Vs Mal	**% of change**	**- 32.8**	**- 41.38**	**- 23.54**	**- 25.66**
	P	**0.001*** <**	**0.001*** <**	**0.001*** <**	**0.001*** <**

Mal: Malathion; NS: : not significant; Sily: Silymarin; ***: very high significant; *Ech: Echium humile*

Table (2): Effect each of Malathion, *Echium humile* and Malathion plus *Echium humile* on glucose, Tg, Cho and Cholinesterase (ChE) levels in the serum of male albino rats compared with control group. (N = 7)

Groups	Statistical parameter	Glucose mg \ dl	Tg mg\dl	Cho mg\dl	ChE U/L
Control group	**Mean**	64	123	100.6	354.6
	SE	1.41	3.46 ±	3.5 ±	17.84 ±
Mal	**Mean**	139.5	191.4	126.6	212.4
	SE	8.5 ±	9.33 ±	2.07 ±	12.75 ±
	% of change	**+ 117.9**	**+ 55**	**+ 25.4**	**- 40**
	P	**0.001 *** <**	**0.001 *** <**	**0.001 *** <**	**0.001 *** <**
Ech	**Mean**	61.4	119	98	431.8
	SE	2.4 ±	2.7 ±	2.68 ±	19.8 ±
	% of change	**- 4.06**	**- 3.3**	**- 1.4**	**+ 21.8**
	P	**0.05 NS >**	**0.05 NS >**	**0.05 NS >**	**0.001 *** <**
Mal + *Ech*	**Mean**	85.8	126.9	112	354.4
	SE	1.48 ±	5.5 ±	5.2 ±	18.62 ±
	% of change	**+ 34**	**+ 3.2**	**+ 11.1**	**- 0.05**
	P	**0.001 *** <**	**0.05 NS >**	**0.01 ** <**	**0.05 NS >**
Mal + *Ech* Vs Mal	**% of change**	**- 38.49**	**- 33.7**	**- 11.5**	**+ 66.8**
	P	**0.001 *** <**	**0.001 *** <**	**0.01 ** <**	**0.001 *** <**

Mal: Malathion; NS not significan; Sily: Silymarin; ***: very high significant; *Ech: Echium humile*

4. Discussion

4.1. Effect on some liver function parameters:

In the current investigation, the daily treatment of rats by orally dose (75 mg / kg/ b.wt) of malathion for 28 days caused very high significant ($p < 0.001$) increase in the serum levels of ALT, AST and ALP by percent change reach to + 90.1, + 102, and + 125.7 respectively.

These results were in full agreement with ***(El – Fazaa et al., 2013 and Nighat et al., 2013)*** they reported that, malathion induced liver dysfunctions demonstrated by considerable increase in ALT, AST,and ALP activities.

In addition, ***(Nashwah et al., 2011)*** reported that an intermittent gavage malathion doses 30 mg/kg/b.wt for 4 weeks showed a significant increase in the serum levels of ALT and AST that may result from leakage of enzymes across damaged plasma membrane or the increase synthesis of enzyme by the liver.

Our results indicate that *Echium humile* treatment when given alone did not caused any significant ($p > 0.05$) effect when compared with control group. But when the rats were treated by malathion with *Echium*, the results showed protective effect against malathion hepatotoxicity appeared in a significant decrease in liver enzymes and increase in serum albumin when compared with malathion treated group.

The *Echium humile* treatment give protective effect against malathion hepatotoxicity as the Silymarin effect. *Echium* contains valuable amounts of gamma- linolenic acid (GLA), alpha - linolenic acid (ALA), stearidonic acid (SDA) in addithion to rosmarinic acid (RA), all these substances have a potent antioxidant properties, and thus restored the toxic effect of malathion ***(and lopez martinez et al., 2004, Guil-Guerrero et al., 2006 a,b, Ozcan., 2008, Alhazzaa et al., 2011).***

4.2. Effect on Kidney functions:

In the current study, the daily treatment of rats by orally dose (75 mg/kg/b.wt) of malathion for 28 days caused very high significant ($p < 0.001$) increase in the serum levels of creatinine by percent change reach to + 107.8 when compered with control group. these results coincides with ***(Fatma and Yusuf., 2011)*** they reported that the orally dose (27 mg/kg/b.wt) of malathion for 28 days to rats caused a significant increase in biochemical parameters of the kidney includig creatinine, urea and uric acid and these results were supported by the generative changes in the kidney.

The creatinine execretion is dependent almost on the process of glomerular filtration, thus, the significant rise in the serum creatinine level may due to the impairment of the glomerular function and tubular damage in the kidney ***(Mansour,. and Mossa., 2010).***Creatinin level is a good risk marker for chronic renal insufficiency ***(Karahan, et al., 2005)*** and ***(Yearout, et al., 2008)***. Increased creatinine level shows that damage of the glomerular

function and tubular damage in the kidneys ***(Mohnsen., 2001)*** and ***(Mora, et al., 2003)***.

The results in this investigation revealed that *Echium humile* treatment when given did not caused any significant ($P > 0.05$) effect on kidney function parameters when the rats were treated by malathion with *Echium*,the results cleared a protective effect against malathion a renal toxicity they showed a high significant ($p < 0.001$) decrease in kidney function parameters when compared with malathion treated group.

The protective action of *Echium* is explicable in terms of its capacity for trapping free radicals and their stabilizing effect on the cytoplasmic membranes, they promotes protein sysnthesis, helps in regenerating kidney tissues ***(Naglaa et al., 2012)***.

4.3. Effect on Glucose level:

Our results, indicate that malathion treated group showed a very high significant ($p < 0.001$) increase in serum glucose level when compared with control group, while malathion treatment with *Echium*, have protective effect against the diabetic effect of malathion when compared with malathion treated group.

these results are supported with many studies that were carried to find out the mechanisms involved in malathion – induced hyper glycemia ***(Abdollahi et al., 2004 and Pournourmohammadi et al., 2005)***. in this regard the liver plays major role in blood glucose homeostasis by maintaining a balance between the uptake and storage of glucose as glycogen and the release of glucose via glycogenolysis and glyconeogenesis ***(Abdollahi et al., 2003)***.

Abdollahi et al., 2004 reported that malathion induced hyper glycemia and increase the activities of the enzyme glycogen phosphorylase (GP) and phosphoenol pyuruvate carboxy kinase (PEPCK), which are the key enzyme of the hepatic glycogenolysis and gluconeogenesis respectively. in addition, it has been reported that malathion increase the rat skeletal muscle glycogenolysis and glycolysis as well as blood glucose and insulin. it was found that activities of skeletal muscle key enzymes of glucose metabolism including GP and phosphorfructo kinase (PFK) are increased by malathion exposure ***(Pounourmohammadi et al., 2005)***.

The hypoglycemic effect of *Echium humile* observed in this study, it may be promotes protein synthesis, helps in regenerating liver tissues, enhances glucuronidation and protect against glutathione depletion ***(Naglaa et al., 2012 and Rahimi et al., 2012).***

4.4. *Effect on some lipogram parameters:*

In this study, the orally treatment of rats by daily dose (75 mg/kg/b.wt) of malathion for 28 days caused very high significant ($p < 0.001$) increase in the serum level of (Tg and Cho) by percent change reach to + 55 and + 25.4 respectively, when compared with control group. Where as, these lipogram parameters did not significantly affected by *Echium humile* treatment when given to the rats by the same treated program, while *Echium humile* when given to the rats in the same treated program with next malathion dose, succeeded to modulate the malathion toxic effect and returned the lipograme parameter levels to about the normal value.

The malthion toxcicity responsible for production of oxidative stress, which is the key contributor in hepatic injury and it known to produce reactive oxygen species (ROS) that is responsible for significant change in lipid profile and hepatic dysfunction and more critical consequences. These suggestions were in full agreement with ***(Huang et al., 2011, Lebda et al., 2012 and Chtourou et al., 2013)*** they also reported that, Silymarin has been shown to be a potent scavenger of a variety of ROS including superoxide anion, hydroxyl radical and nitrogen dioxide radical.

These results were in full agreement with ***(El-Fazaa et al., 2013)*** they decided that an increase in Tg, Cho, LDL – Cho and a decrease in HDL – Cho in the plasma of malathion treated rats. Moreover, our results were supported by ***(Kalender et al., 2010)*** they reported that in an intermediated study for 28 days with malathion dose (27 mg/kg/b.wt) in rats, the results showed a significant increased in Tg, Cho and LDL – Cho in male rats. also, ***(Lasram et al., 2009)*** desided that a cute exposure to malathion has been shown to disrupt lipid metabolism, there by elevating LDL – Cho and Tg levels, these findings evidenced that malathion exposure induced liver oxidative stress and lipogram disorder.

4.5. Effect on Cholinesterase:

Concerning the Acetylcholine esterase (AChE), the obtained results in this study eliciated that, the daily treatment of rats by orally dose, (75 mg/kg/b.wt) of malathion for 28 days caused very high significant decrease ($p < 0.001$) in the serum level of AChE by percent change reach to – 40 when compared with control group, while given *Echium humile* alone to normal rats afforded a significant increase in serum level of AChE by percent change reach to + 21.8. On the other hand given of *Echium* with malathion afforded a protective effect against malathion toxicity and were able to ameliorate the toxic effect of malathion on AChE.

These results were compatable with ***(Bayir et al., 2013)*** and ***(Vijayakumar et al., 2011)***, they decided that Ops pestisides are alarge group of compounds that irreversibly inhibit cholinesterase (ChE), AChE and neuropathy target esterase in human and animals. Normally, AChE rapidly

hydrolyzes the neurotransmitter Ach into inactive fragments of choline and acetic acid after completion of neurochemical transmission. The phosphate radicals of Op compounds covalently bind to active sites of ChEs, transforming them into enzymatically inert proteins, thus acting as irreversible ChE inhibitors. The inhibition of Ach at synapses causes over stimulations and subsequent disruption of transmission in the central and peripheral nervous system. They also reported that the Atropine sulphate and Silymarin blocks the development of Op - induced cholinesterase inhibition.

Abdollahi et al., 2012, Evliyaoglu et al., 2012 and El – Fazaa et al., 2013 recorded the same subject, they concluded that the malathion exposure induced a high inhibitory effect of the brain, plasma and erythrocyte AChE activities and induced a cerebral alterations and oxidative stress in rat pups. Also, our results were in full agreement with ***(Karanth et al., 2006, Gokcimen et al., 2007, Elhalwagy and Zaki 2009 and Nashwah et al., 2011)*** they decided that the primary mechanism of action and most acutely like threaten effect of Ops insecticides are related to accumulation of acetylcholine within the cholinergic synapses due to inhibition of AchE by active oxon metabolites.

Our results, about the protective effect of *Echium humile* extract were in full agreement with ***(El-Shazly et al., 2004)*** as they described the isolation of three new pyrrolizidine alkaloids (PAs), echihumiline, pycnanthine, and echihumiline N-oxide from *E. humile*. Their structures were determined by spectroscopic methods. As **(Atal., 1978)** reported that some derivative are used pharmaceutically, platynecine has been employed as anti muscarinic agent and semisynthetic derivatives of PAs exhibit hypotensive, local anesthetic, ganglionic blocking, neuromuscular blocking and antispasmodic activities.

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