Possible action of grape seed oil on brain toxicity induced by methomyl or imidacloprid of male rats.

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Abstract

The present study was planned to investigate the effect of methomyl or imidacloprid on the brain of male rats. The effect grape seeds oil as an antioxidant was also evaluated. Animals were administered orally with 1/10 and 1/20 of LD_{50} methomyl and imidacloprid with 17 and 450 mg /kg bw for four and eight weeks. Grape seeds oil with 4 ml/kg b.wt. was used for protection from methomyl and imidacloprid toxicity. Brain cortex and hippocampus oxidative stress (glutathione S transferase GST, glutathione peroxidase GPX, superoxide dismutase SOD, malondialdhyde MDA and nitric oxide NO), Na^+,K^+, ATPase and acetyl-cholinesterase AChE were determined. The result showed that GST, GPX, MDA, and NO_2 were increased significantly. But SOD, Na^+K^+ATPase and AChE were significantly reduced in comparison with the control. Grape seed oil induced a significant improvement for the pesticides brain toxicity but not to the level of control. The study suggested that the oil antioxidants not improve the brain toxicity induced by methomyl or imidacloprid.

Key words: brain toxicity, methomyl, imidacloprid, Na^+K^+ATPase and AchE**.**

Introduction

Pesticides are widely used allover the world. they protect the plants from the harmful pests. Behavioral and neuro-chemical changes were reported after chronic exposure to low concentrations of carbamates (Boyd *et al*., 1990). Pesticide exposure at relatively low doses may affect brain cells producing a loss of neuron in particular regions of the brain that results in subsequent cognitive decline impaired memory and attention, and motor function (Baldi *et al.*, 2003 and Hayden *et al.*, 2010). Methomyl is a carbamate insecticide that acts by inhibiting the activity of acetyl cholinesterase (Mahgoub and El-Medany 2001). As a broad spectrum insecticide, it is one of the most frequently used pesticides in agriculture. Numerous incidents of acute carbamate poisoning have resulted from inhalation of sprays or contamination of crop or food (Sinhaseni *et al*., 1995 and Mahgoub and El-Medany 2001).

While, Imidacloprid is used as an acaricide in agriculture and as ectoparasiticide in pet animal practice (Gatne *et al*., 2006). Imidacloprid cause inflammation in central nervous system. (Duzguner and Erdogan 2010). Imidacloprid are selective agonists of the insect nicotinic acetylcholine receptors (nAchRs) and extensively used in area of crop protection and animal health to control a variety of insect pest species (Dick *et al*., 2005; Gatne *et al*., 2006 and Xu *et al*., 2010). Animal toxicities of imidacloprid are similar to that of toxicities in the

parent compound, nicotine. Such toxicities as fatigue, twitching, cramps and weakness leading to asphyxia are seen (Avery *et al*., 1994).

Pesticides lead to the generation of oxygenated and/or nitrogenated reactive species (ROS/RNS) which affect both the antioxidant levels in mammalian cells and the activity of the scavenging enzyme system (Banerjee *et al.,* 1999; Barlow *et al.,* 2005). AchE enzyme is responsible for the rapid hydrolysis of the neurotransmitter acetylcholine (ACh) to choline and acetate during neurotransmission at cholinergic synapses (Kwong, 2002; Wang *et al.,* 2009). Since the neurotransmitters, such as acetylcholine, play multiple developmental roles, the developing brain is extremely vulnerable to neuro-active chemicals that elicit or block neurotransmitter responses during periods of rapid brain growth, from the early embryonic stage through adolescence (Yanai, 1984). $Na^{+}K^{+}$ATPase is the enzyme responsible for active transport of Na and K across the plasma membrane. The enzyme plays a central role in mediating electrical activity in the nerve cells (Stekhoven and Bonting, 1981). It provides the efflux of three Na ions and the influx of two K ions per spliced molecule of ATP in several cell types, including neurons and glia (Grisar *et al.,* 1992). Due to its high importance in the maintenance of resting membrane potential and the propagation of neuronal impulse, the malfunction of this enzyme can negatively affect the developing brain.

Grape seed extract is free radical scavenger activity (Tebib *et al.,* 1996). The activity of grape seed extract is approximately 50 times greater than that of vitamin E and C in term of antioxidant action. (Shi *et al*., 2003).

Methomyl and imidacloprid insecticides may a very general or quite selective in their toxicity and extensive use in agriculture. In the present study methomyl and imidacloprid were taken into consideration because widely used in local environment against insect and pests on crops and considered as main causes of environmental pollution. This investigation primarily concerns the use of acute oral lethal dose LD50 for methomyl and imidacloprid. A dose of 17 mg/kg body weight proved to be LD50 for methomyl in male rats while the LD50 for imidacloprid was 450 mg / kg b.w. (Thomson 1992). The aim of the present work is to investigate the effects of methomyl, imidacloprid and grape seed oil on selected parameters in the brain. To explore the efficacy of a herb (grape seed oil), as antioxidant on brain toxicity of methomyl and imidacloprid.

Material and Methods

Experimental animals:

This study was carried out on adult male albino rats (Wister strain). One hundred animals were obtained from the National Research center, Cairo, Egypt. The animals weighting about 120±5g. The housing, the physiological and the histological studies were conducted in the Department of Zoology, Women College for Arts, Science and Education, Ain Shames University. Male rats were housed in iron metal cages, each cage contained six rats. Clean sawdust was used. It was changed daily to keep the animals dry and clean throughout the period of the study. Animals were kept under controlled temperature of

24±2ºC and 12 hours light/12 hours dark cycle throughout the experiment. A commercial pelleted diet and fresh vegetables were used and tap water *ad libitum.* The animals were allowed to adapt to the laboratory conditions for one week before the beginning of the experiment.

Chemicals:

Methomyl (A carbamate compound used as systemic insecticide – hilling by contact and stomach poisoning. It was supplied from Agriculture Ministry, Dokki, Egypt as powder. Purchased from Chandog Huyang Co. China. Assay was **90%. Imidacloprid (**A nitromethylene compound used as a contact stomach poison and systemic insecticide supplied from Agriculture Ministry, Dokki, Egypt. It was purchased from Bayer A.G. and Nihon Toyaku Seizo, Japan. Assay was 35 g/L.

The Grape seeds oil was obtained from Tact Company for oils extraction. WWW.Tactworld.com.

Experimental design:

Animals were divided into ten groups (n=10) as follows: Group 1: Control group. These received a daily oral administration of tap water by oral tube (1ml/100 g b wt). Group 2: Antioxidant group. These rats were received a daily oral dose of 4ml/kg b wt of grape seeds oil (Jiarong *et al.,* 1992). Group 3: Methomyl group 1/10 LD_{50}. Rats of this group were given a daily oral dose of 1/10 of LD_{50}. (LD_{50}=17mg/kg). Group 4: Methomyl group 1/20 LD_{50}. These rats were received a daily oral dose of 1/20 of LD_{50}. Group 5: These rat were protected with grape seeds oil then treated with methomyl 1/10 of LD_{50} after 2 hours of grape seed oil dose. Group 6: Rats were protected with grape seeds oil then treated with methomyl 1/20 of LD_{50} after 2 hours of grape seed oil dose. Group 7: Imidacloprid group 1/10 of LD_{50}. These rats were received a daily oral dose 1/10 of LD_{50}.(LD_{50}=450mg/kg). Group 8: Imidacloprid group 1/20 of LD_{50}. These rats were received a daily oral dose 1/20 of LD_{50}. Group 9: (Oil + Imidacloprid). Rats were protected with 4ml/kg.bw of grape seeds oil then treated with Imidacloprid 1/10 of LD_{50} after 2 hours of grape seed oil dose. Group 10: (Oil + Imidacloprid). Rats were protected with 4ml/kg.bw of grape seeds oil then treated with Imidacloprid 1/20 of LD_{50} after 2hours of grape seed oil dose. All the groups were treated daily for one and two months before decapitation.

Preparation of samples for biochemical investigations:

The rats were sacrificed by rapid decapitation after 4 and 8 weeks. Brain was excised on ice and the brain was dissected to prepare cortex and hippocampus and was frozen for biochemical assays. A portion of fresh tissue was homogenized in volumes of saline with 10% dilution until a uniform suspension was obtained. The homogenate was centrifuged at 3000 r.p.m for 10 minute. The clear supernatant was used for biochemical determination.

Biochemical analysis:

The GST activity was determined spectrophotometrically by the method of Habig *et al.* (*1974*). The GPX activity was determined colormetrically according to Gross *et al.*

(1967). The determination of (SOD) activity in brain tissues were performed according to the method described by Niskikmi *et al.* (1972). Malondialdhyde measured in the tissue using thiobarbituric acid (TBA) assay modified according to the suggestions of Draper and Hadley (1990). NO was determined as nitrite by the method of Green *et al*. (1982) and Moshage *et al*. (1995) using modified Griss reagent. The activity of Na^+K^+ATPases was measured according to Üner *et al*. (2005). The colorimetric determination of acetyl- cholinesterase (AchE) activity was carried out according to Gorun *et al.* (1978).

Statistical analysis :

Reported data represented means ± SE. Statistical analysis was performed by one way ANOVA. Once a significant F test was obtained, Duncan comparisons were performed to assess the significant differences among various treatment groups. The two way ANOVA was performed to assess time versus treatment difference. Statistical Package for Social Science ((SPSS)) for windows software, Release ((12)) (SPSS,Chicago IL) was used, $P \leq 0.05$ was considered statistically.

Results

The data presented in table 1 & 2 showed a significant increase in cortex and hippocampus GST, GPX activities and MDA contents in all treated groups in comparison with the corresponding control or grape seed oil rats at four and eight weeks of treatments at $P \leq 0.05$. while the cortex and hippocampus SOD activities were decreased significantly in all treated groups in comparison with the corresponding control or grape seed oil rats at four and eight weeks of treatments at $P \leq 0.05$. Co-adminstration of G with methomyl or imidacloprid induced a significant increase in cortex and hippocampus GST, GPX, MDA and NO and a significant decrease in cortex and hippocampus SOD in comparison with the control at four and eight weeks of treatments at $P \leq 0.05$. Grape seed oil did not decrease the oxidative stress effect of methomyl or imidacloprid on brain tissue.

Table (1): Effect of Methomyl or Imidacloprid with or without grape seed oil G on GST activity (umole Conj.CDNB/min/mg tissue), GPX (mg/g), SOD (U/g wt tissue) and MDA (nmole/g) in brain cortex of rats.

Group/parameter	Time	GST	GPX	SOD	MDA
Control	4	2.4 ± 0.48^{a}	275.7 ± 14.92^{a}	93.2 ± 1.03^{h}	272.88 ± 7.43^{a}
	8	2.9 ± 0.12^{a}	301.5 ± 11.23^{ab}	90.15 ± 1.79^{g}	284.92 ± 17.65^{a}
G	4	3.2 ± 0.47^{a}	344.6 ± 11.23^{bc}	87.6 ± 1.18^{fg}	288.94 ± 21.07^{a}
	8	3.1 ± 0.2^{a}	346.3 ± 12.6^{bc}	81.8 ± 1.49^{d}	283.95 ± 7.85^{a}
Methomyl 1/10	4	4.06 ± 0.65^{b}	456.63 ± 21.97^{d}	81.23 ± 0.64^{d}	413.44 ± 20.53^{bcde}
	8	4.27 ± 0.19^{b}	370.47 ± 116.12^{c}	76.92 ± 1.03^{c}	361.81 ± 34.95^{b}
Methomyl 1/20	4	7.81 ± 0.17^{fgh}	646.17 ± 17.87^{fg}	77.54 ± 0.52^{c}	476.56 ± 23.16^{fg}
	8	6.46 ± 0.18^{cd}	542.78 ± 34.57^{e}	72.62 ± 1.32^{ab}	396.24 ± 11.33^{bc}
Methomyl 1/10+G	4	6.04 ± 0.27^{c}	689.25 ± 10.55^{gh}	75.08 ± 0.55^{bc}	510.99 ± 15.71^{gh}
	8	6.35 ± 0.12^{c}	508.32 ± 20.21^{e}	74.77 ± 0.97^{bc}	407.71 ± 14.29^{bcd}

Methomyl	4	6.25±0.27^{c}	706.48±20.93^{h}	75.69±0.83^{bc}	556.89±18.77^{h}
1/20+G	8	8.65±0.32^{h}	611.71±12.18^{f}	70.77±0.89^{a}	430.66±9.77^{cdef}
Imidacloprid 1/10	4	6.35±0.28^{c}	594.48±10.9^{f}	85.23±0.66^{ef}	396.24±10.42^{bc}
	8	7.4±0.11^{efg}	344.62±9.44^{bc}	84±0.7^{de}	396.24±21.39^{bc}
Imidacloprid 1/20	4	6.98±0.32^{cdef}	628.94±24.37^{f}	77.23±1.57^{c}	424.92±18.5^{cdef}
	8	8.13±0.17^{gh}	508.32±15.41^{e}	76.92±1.43^{c}	413.45±14.63^{bcde}
Imidacloprid	4	6.56±0.26^{cde}	706.48±21.62^{h}	80.92±1.99^{d}	396.24±23.66^{bc}
1/10+G	8	8.54±0.15^{h}	518.66±16.21^{e}	75.08±0.78^{bc}	413.45±9.94^{bcde}
Imidacloprid	4	7.29±0.23^{defg}	740.94±10.9^{h}	77.85±0.44^{c}	465.09±10.42^{efg}
1/20+G	8	9.58±0.18^{i}	736.55±13.07^{f}	75.69±0.62^{bc}	459.35±8.21^{defg}

The results are presented as means ± SE of 5 rats.

Two way ANOVA (treatment * time) GST, F =8.158 at P≤0.001,GPX, F =11.483 at P≤0.001, SOD, F =2.019 at P≤0.05, and MDA, F =4.38 at P≤0.001.

Means in a column with a common superscript are not significantly different (P ≤0.05).

Table (2): Effect of Methomyl or Imidacloprid with or without grape seed oil G on GST activity (umole Conj.CDNB/min/mg tissue), GPX (mg/g), SOD (U/g wt tissue),and MDA (nmole/g) in brain hippocampus of rats.

Group/parameter	Time	GST	GPX	SOD	MDA
Control	4	2.9±0.25^{a}	301.5±18.68^{a}	89.5±0.89^{g}	292.96±13.7^{ab}
	8	2.8±0.27^{a}	292.9±15.41^{a}	92±0.57^{h}	281.48±19.46^{a}
G	4	3.3±0.14^{ab}	370±10.55^{bc}	84.6±0.4^{d}	304.43±19.96^{abc}
	8	3.05±0.22^{a}	361.9±12.78^{bc}	87.4±0.79^{efg}	292.96±9.43^{ab}
Methomyl 1/10	4	4.38±0.29^{cdef}	422.16±14.67^{cde}	84±0.65^{cd}	413.45±22.59^{efg}
	8	4.17±0.21^{cd}	327.39±30.94^{ab}	85.85±0.53^{def}	367.5±18.5^{de}
Methomyl 1/20	4	5.52±0.22^{gh}	534.17±12.78^{gh}	81.85±0.66^{bc}	442.14±22.59^{gh}
	8	5±0.11^{defg}	439.4±11.88^{def}	83.69±0.82^{cd}	356.07±11.48^{cd}
Methomyl	4	5.21±0.24^{fgh}	482.47±16.35^{efg}	77.85±0.54^{a}	436.4±22.66^{gh}
1/10+G	8	4.69±0.19^{cdefg}	404.93±23.12^{cd}	81.23±0.8^{b}	344.6±18.94^{bcd}
Methomyl	4	5.94±0.23^{h}	594.48±11.23^{i}	76.62±0.77^{a}	482.3±12.44^{h}
1/20+G	8	5.52±0.32^{gh}	499.7±11.88^{fgh}	80±0.59^{b}	413.45±13.03^{efg}
Imidacloprid	4	3.96±0.44^{bc}	448.01±12.49^{ef}	88±0.52^{fg}	356.07±25.21^{cd}
1/10	8	4.27±0.4^{cde}	396.32±45.59^{cd}	89.23±0.89^{g}	321.65±12.7^{def}
Imidacloprid	4	5.1±0.16^{efgh}	525.55±14.42^{gh}	83.69±0.51^{cd}	396.24±10.42^{defg}
1/20	8	5.21±0.19^{fgh}	448.01±11.56^{def}	85.84±0.47^{de}	367.55±15.71^{de}
Imidacloprid	4	4.9±0.31^{defg}	560.01±19.83^{hi}	85.23±0.92^{de}	390.49±16.02^{defg}
1/10+G	8	4.79±0.2^{defg}	456.63±14.67^{def}	87.38±0.59^{efg}	379.02±17.68^{def}
Imidacloprid	4	5.1±0.27^{efgh}	594.48±30.09^{i}	80.62±0.87^{b}	430.66±16.53^{fgh}
1/20+G	8	5.42±0.32^{gh}	525.55±9.82^{gh}	84±1.27^{cd}	413.45±20.61^{efg}

The results are presented as means ± SE of 5 rats.

Two way ANOVA (treatment * time) GST, F = 0.701 at P≤0.706 (not significantly different),GPX, F 1.596 at P≤0.131 (not significantly different), SOD, F = 0.56 at P≤0.825 (not significantly different), and MDA, F = 2.399 at P≤0.05.

Means in a column with a common superscript are not significantly different (P ≤0.05).

Table 3 & 4 revealed that a significant increase in cortex and hippocampus NO contents in all treated groups in comparison with the corresponding control or grape seed oil rats at four and eight weeks of treatments at P≤0.05. while the cortex and hippocampus AchE and Na^+K^+ ATPase activities were decreased significantly in all treated groups in comparison with the corresponding control or grape seed oil rats at four and eight weeks of treatments at P≤0.05. Co-adminstration of G with methomyl or imidacloprid induced a significant increase in cortex and hippocampus NO contents and a significant decrease in cortex and hippocampus AchE and Na^+K^+ ATPase activities in comparison with the control at four and eight weeks of treatments at P≤0.05. Grape seed oil treatment did not increase the AchE and Na^+K^+ ATPase activities of methomyl or imidacloprid treated rats in brain tissues.

Table (3): Effect of Methomyl or Imidacloprid with or without grape seed oil G on NO (mMole NaNO2/g wet tissue), AchE (mMole/g wet tissue) and Na^+K^+ATPase (uMole Pi/g wet tissue/h) in brain cortex of rats.

Group/parameter	Time	NO	AchE	Na^+k^+ATPase
Control	4	37.81 ± 1.63^{a}	7.7 ± 0.16^{ij}	25.14 ± 0.6^{m}
	8	36.2 ± 3.07^{a}	7.9 ± 0.08^{j}	25.05 ± 1.01^{m}
G	4	45.87 ± 1.52^{b}	7.2 ± 0.08^{h}	18.91 ± 0.45^{i}
	8	40.5 ± 3.51^{ab}	7.4 ± 0.1^{hi}	21.06 ± 0.31^{kl}
Methomyl 1/10	4	89.67 ± 2.15^{g}	3.42 ± 0.19^{cde}	20.32 ± 0.39^{j}
	8	113.05 ± 1.63^{h}	4.15 ± 0.12^{fg}	21.45 ± 0.14^{l}
Methomyl 1/20	4	64.41 ± 1.95^{d}	3.03 ± 0.11^{abc}	16.81 ± 0.37^{gh}
	8	88.06 ± 1.77^{g}	3.54 ± 0.12^{de}	17.57 ± 0.44^{h}
Methomyl 1/10+G	4	74.62 ± 3.98^{e}	2.98 ± 0.1^{abc}	15.41 ± 0.27^{ef}
	8	89.4 ± 3.71^{g}	3.42 ± 0.14^{cde}	19.51 ± 0.22^{ij}
Methomyl 1/20+G	4	56.35 ± 2.87^{c}	2.74 ± 0.09^{a}	14.74 ± 0.16^{de}
	8	82.95 ± 2.07^{fg}	3.08 ± 0.2^{abc}	14.71 ± 0.39^{de}
Imidacloprid 1/10	4	80 ± 1.79^{ef}	3.81 ± 0.18^{ef}	14.2 ± 0.30^{d}
	8	86.18 ± 2.69^{fg}	4.3 ± 0.14^{g}	16.34 ± 0.26^{fg}
Imidacloprid 1/20	4	46.68 ± 1.43^{b}	3.23 ± 0.12^{bcd}	11.56 ± 0.16^{bc}
	8	63.87 ± 4.14^{d}	3.76 ± 0.12^{ef}	12.68 ± 0.18^{c}
Imidacloprid 1/10+G	4	58.5 ± 1.94^{cd}	3.13 ± 0.15^{abcd}	11.69 ± 0.14^{bc}
	8	84.84 ± 1.84^{fg}	3.32 ± 0.07^{bcd}	14.43 ± 0.13^{de}
Imidacloprid 1/20+G	4	32.7 ± 2.41^{a}	2.93 ± 0.2^{ab}	8.6 ± 0.11^{a}
	8	35.39 ± 1.34^{a}	3.18 ± 0.1^{bcd}	11.46 ± 0.27^{b}

The results are presented as means ± SE of 5 rats.

Two way ANOVA (treatment * time) NO, F=11.404 at P≤0.001, AchE, F =0.867 at P≤0.558 (not significantly different) and Na^+K^+ATPase F =6.425 at P≤0.001.

Means in a column with a common superscript are not significantly different (P ≤0.05).

Table (4): Effect of Methomyl or Imidacloprid with or without grape seed oil G on NO (mMole NaNO2/g wet tissue), AchE (mMole/g wet tissue) and Na^+K^+ATPase (uMole Pi/g wet tissue/h) and in brain hippocampus of rats.

Group/parameter	Time	NO	AchE	Na^+k^+ATPase
Control	4	29.48 ± 1.6^{ab}	7.2 ± 0.23^{g}	25 ± 0.6^{l}
	8	28.94 ± 2.16^{ab}	7.2 ± 0.17^{g}	24.7 ± 0.7^{l}
G	4	28.14 ± 3.13^{ab}	6.98 ± 0.12^{g}	20.5 ± 0.4^{j}
	8	26.52 ± 3.28^{a}	7.1 ± 0.2^{g}	22.4 ± 0.1^{k}
Methomyl 1/10	4	64.68 ± 2.14^{f}	3.08 ± 0.14^{abcd}	12.6 ± 0.04^{efg}
	8	99.61 ± 5.29^{h}	3.91 ± 0.28^{f}	16.6 ± 0.7^{i}
Methomyl 1/20	4	58.5 ± 3.19^{ef}	2.64 ± 0.16^{ab}	11 ± 0.26^{def}
	8	85.37 ± 2.02^{g}	3.18 ± 0.19^{abcde}	14.9 ± 0.3^{h}
Methomyl 1/10+G	4	55.81 ± 1.31^{e}	3.13 ± 0.25^{abcde}	11.6 ± 0.16^{ef}
	8	63.34 ± 2.47^{ef}	3.32 ± 0.18^{cdef}	14.2 ± 0.2^{gh}
Methomyl 1/20+G	4	39.15 ± 3.31^{cd}	2.59 ± 0.14^{ab}	11.1 ± 2.1^{def}
	8	41.57 ± 3^{cd}	2.74 ± 0.2^{abc}	12 ± 0.2^{ef}
Imidacloprid 1/10	4	63.34 ± 2.62^{ef}	3.37 ± 0.27^{cdef}	10.8 ± 0.15^{de}
	8	78.12 ± 2.36^{g}	3.76 ± 0.13^{ef}	12.7 ± 0.5^{fg}
Imidacloprid 1/20	4	35.12 ± 1.55^{bc}	3.03 ± 0.32^{abcd}	8.3 ± 0.1^{bc}
	8	44.53 ± 1.97^{d}	3.23 ± 0.26^{bcde}	11.8 ± 0.3^{ef}
Imidacloprid 1/10+G	4	45.06 ± 2.43^{d}	2.93 ± 0.14^{abcd}	9.7 ± 0.2^{cd}
	8	59.04 ± 2.24^{ef}	3.52 ± 0.19^{def}	10.8 ± 0.2^{de}
Imidacloprid 1/20+G	4	29.47 ± 2.06^{ab}	2.54 ± 0.17^{a}	6.5 ± 0.11^{a}
	8	29.21 ± 1.35^{ab}	2.93 ± 0.15^{abcd}	8 ± 0.2^{ab}

The results are presented as means ± SE of 5 rats.

Two way ANOVA (treatment * time) NO, F =10.902 at P≤0.001, AchE, F =0.771 at P≤0.643 (not significantly different) and Na^+K^+ATPase, F =3.022 at P≤0.01.

Means in a column with a common superscript are not significantly different (P ≤0.05).

Discussion

Pesticides toxicity has been clearly demonstrated to alter neurological functions through different mechanisms. These mechanisms including: 1) redox cycling 2) mitochondrial inhibition 3) activation of NADPH oxidases have been proposed as a potential source of ROS formation, particularly for accumulation of $O^{2\cdot}$ radicals (Franco *et al.*, 2010).

Mostafalou and Abdollahi (2013) revealed that pesticides can disturb oxidative homeostasis through direct or indirect pathways, including mitochondrial or extra-mitochondrial production of free radicals, thiol oxidation, and depletion of cellular antioxidant reservoirs (Abdollahi *et al.*, 2004 and Mostafalou *et al.*, 2012a). Considering the oxidative stress as a powerful promoter of other cellular pathways involved in disease process and as a unique attendant in inflammatory response, it has been put in the spotlight of the most mechanistic studies regarding the association of pesticide's exposure with chronic disorders. Oxidative stress has been implicated in the onset and progression of pesticide

induced Parkinson disease (Singh *et al.*, 2007). In this regard, organochlorine pesticides have been reported to cause degeneration of dopaminergic neurons by an oxidative dependent pathway in Parkinson model (Kanthasamy *et al.*, 2002; Sharma *et al.*, 2010). Additionally, disrupting effects of organophosphates on glucose homeostasis have been reportedly linked to oxidative damages and inflammatory cytokines and thought to be compensatory responses accompanied with reduced insulin signaling in insulin sensitive organs such as liver, muscle, and adipose tissue (Mostafalou *et al.*, 2012b; Teimouri *et al.*, 2006). As such further disruption of glucose homeostasis in diabetic models of laboratory animals exposed to organophosphate insecticides has been associated with enhanced lipid peroxidation and decreased activity of antioxidant enzymes (Begum and Rajini, 2011).

In the present study, a significant increase was reported in GST, GPX and MDA in methomyl treated rats in comparison with the control, while a decrease was shown in SOD level in brain after 4&8 weeks of treatment of 1.7 mg /kg and 0.85 mg /kg methomyl. In the same trend, Garg *et al*. (2009) found changes in erythrocyte SOD, GSH, GST, GPX activities after methomyl treatment. The first line of defense against oxidative stress were SOD and CAT which convert superoxide anion 0^2- to hydrogen peroxide and then to H_2O and reduced glutathione GSSG. This reaction, catalyzed by glutathione peroxidase Panfili *et al.*, (1991) reported that SOD is responsible for decomposition of peroxides using reduced glutathione as the electron donating substrate. The two SOD isoenzymes (Mn –SOD) and zinc / copper isoenzymes both catalyze the dismutation of O^2- to yield H_2O_2 and O_2.

In the present study, the decrease in SOD activities could be explained by Karthikeyen *et al*. (2007). They found that the decreased SOD is insufficient to scavenge the superoxide anion produced during the normal metabolic process (Badary *et al*., 2004). The reduction in SOD may be due to the increased generation of ROS such as super oxide and hydrogen peroxide, which in turn leads to the inhibition in activities of this enzyme. Also, the increase in GST activity probably has been due to deficiency in GSH level (El-Gendy *et al.*, 2010). Similar observation was obtained by Duzguner and Erdogan, (2010). SOD plays a key in detoxifying superoxide anion, which otherwise damages the cell membranes and macromolecules (Olanow, 1993). There is relationship between increased ROS production and decreased SOD activity in brain regions. GST is a detoxifying enzyme that catalyzes the conjugation of a variety of electrophillic substrates to the thiol group of GSH, producing less toxic forms (Hayes *et al.*, 2005). Many studies reported a significant increase in GST after using different insecticides (El-Gendy *et al.*, 1990; Aly, 2005 and Singh *et al.*, 2006). They explained this increase in GST activity due to GSH system to provide a major protection against toxic agents. While, the GSTs offer protection in pesticide-induced brain and lung toxicities (Ahmad *et al.*, 2010). Another study showed that methomyl treatment resulted in a significant increase in SOD in erythrocytes of rats. This increase indicates elevation in formation of oxy free radicals which stimulated SOD activity to prevent the toxicity of free radicals during oxidative stress (Coursin *et al.*, 1985). Moreover, increase in the SOD activity can also be contributed to physiological response of animal in mitigating the toxic stress on

the body as SOD plays a vital role in detoxification of reactive oxygen species.

The effect of 1/10 & 1/20 LD_{50} of either imidacloprid or imidacloprid in cortex and hippocampus led to increase in MDA and GPX in male rats. Superoxide dismutase (SOD) activity was decreased in cortex and hippocampus of brain rat treated with imidacloprid when compared to the control. In agreement with the present study, Kapoor *et al*. (2010) showed that female rats treated with 20 mg /kg day has produce a significant changes in SOD, GPX, GSH, LPO in liver; SOD and GPX in brain and LPO in kidney. In the same trend, the result of Muthukumaran *et al*. (2008) found that nicotine treated rat decrease GSH level and the activities of SOD, and GPX in liver and brain. Imidacloprid is a member of relatively new class of insecticides, the chloronicotinyl neonicotinoid compounds (Elbert *et al.,* 1990). The stimulation of lipid peroxidation observed as a result of imidacloprid treatment can be due to the formation of free radicals through an exhaustion of antioxidants leading to oxidative stress. The observed difference in the levels of lipid peroxidation products in distinct brain regions may be due to the differences in their oxygen consumption rate which influence the generation of reactive oxygen species (ROS) (Floyed and Carney, 1991). In the same trend, Duzguner and Erdogan (2012) reported that Xanthine oxidase activity and superoxide anion generation by xanthine oxidase have been shown to increase in the presence of nicotine, an agonist of nAchR like imidacloprid. It is suggests that the agonists of nAchR such as nicotine or imidacloprid similarly induce in oxidative events in mammals. The previous results have demonstrated that the superoxide anion and H_2O_2 are the major source of nicotine – induced free radical production depleting the cellular antioxidants (Kalpana and Menon 2004). In the contrary Duzguner and Erdogn (2010) found that imidacloprid treatment caused 3-fold increase in activity of GPX in brain but significantly decreased in liver, they found the insecticide probably induced ROS production (Debnath and Mandal , 2000) indicated that chronic pesticide exposure may result in long-lasting oxidative stress and that polymorphic genes encoding PON1, GSTs and BChE are relevant genetic determinants of pesticide toxicity that significantly interact with exposure to modify antioxidant enzyme activities. These gene–pesticide interactions may play a key role in the development of numerous chronic and degenerative diseases, including cancer and the neurodegenerative ones.

In the present study, a significant increase in cortex and hippocampus NO contents was shown in all treated groups in comparison with the control. Evidence has accumulated for NO subserving an increasing number of functions in the mammalian central nervous system, as anticipated from the wide distribution of its synthetic and signal transduction machinery within it. With any transmitter, understanding its receptors is vital for decoding the language of communication. The receptor proteins specialized to detect NO are coupled to cGMP formation and provide an astonishing degree of amplification of even brief, low amplitude NO signals (Garthwaite, 2008).

It is suggested that imidacloprid elevated NO and the oxygen substances. NO is a free radical signaling molecule in neurosystem of most animals and human. This molecule reacts

with superoxide anion to generate peroxynitrite which is selective and strong oxidant and nitrating agents that interact with numerous biological molecules involved DNA fragmentation and oxidation of macromolecules such as protein and lipids (Choi *et al*., 2006). The present study showed that increase in NO in cortex and hippocampus tissue in all treated groups in comparison with the control. Duzguner and Erdogan (2010) demonstrated that imidacloprid elevated the production of NO levels due to the induction of endothelial nitric oxide synthase (iNOS) in liver, but neither neuronal nitric oxide synthase nNOS nor iNOS were induced in the brain. The oxidant generating enzymes xanthine oxidase and myeloperoxidase activities in both tissues were elevated. Furthermore, Astiz *et al.* (2013) explained the increase in serum NO levels in they work. The pesticide induced oxidative damage to proteins and lipids, also, a highest concentration of NO as the end metabolic product directly related to peroxynitrite overproduction. Astiz *et al.* (2013) observed increases in the levels of NOx could be the consequence of iNOS activation, which is induced specially under high OS conditions. The production of NO is enhanced in AchE inhibitor-induced seizer's activity (Bagetta *et al.,* 1993 and Kim *et al.,* 1997).

AchE is a specific cholinergic marker protein for the functional state of cholinergic neuron, which can play a key role in the maintenance of acetylcholine level at cholinergic neuron (Eckenstein and Sofroniew, 1983), being responsible for degradation of acetylcholine to acetate and choline in synaptic cleft. Carbamates AchE inhibitors lethal and convulsive effect may be due to the participation of glutamergic mechanisms in its neurotoxicity (Dekundya *et al.,* 2007). The carbamates mimic the action of acetylcholine (Ach) and enter the enzyme instead of Ach (Wille *et al.*, 2013 and Baltazar *et al.*, 2014). The hydroxyl attacks the carbonyl moiety of the carbamate. Although the normal acetylated serine is rapidly hydrolyzed and the enzyme is ready for another reaction, the carbamylated enzyme cannot readily undergo hydrolysis and is not reactivated because of steric hindrance or some other electronic factors (Rosman *et al.,* 2009). Moreover, this inhibition of AchE leads to the accumulation of Ach in gap junction causing hyperstimulation of cholinergic receptors (Novotny *et al.,* 2011). Overdose with Ach is followed by overstimulation of the receptors and finally it ends with a cholinergic crisis followed by muscarine, nicotine and central nervous signs, i.e.: miosis, hypersecretion of exocrine glands, bradycardia, tonic and clonic convulsions (O'Malley, 1997).

Decrease in brain AchE activity in the methomyl treated rats were observed at 4 & 8 weeks post treatment. Grape seed oil prevent the loss of AchE activity. These findings were in accordance with Mor and Ozmen (2009) which proved that treatment with endosulfan caused neurotoxicity and AchE inhibition in rabbit and prevent this inhibition by vitamin C. Vitamin C is an antioxidant that effectively scavenges ROS, thus preventing tissue damage. Vitamin C can reduce the lipid peroxidation induced by toxic methidathion (Altuntas *et al.,* 2002).

Imidacloprid like the other insecticide compounds had a specific AchE inhibition effect (Karlin, 2002 and Tomizawa and Casida, 2005). Nagata *et al*. (1996) early shown that

imidacloprid generates subconductance – state currents at the nAchR in rats PC12 cells , it was shown that imidacloprid generates excessive amounts of reactive free radical as consequence of induction of cytochrome CYP2A6 in development of lipid peroxidation. Cholinergic neurons express nAchRs are highly sensitive to imidacloprid and demonstrated a role in voltage gated calcium channels in amplifying imidacloprid induced increases in Ca^{+2} (Jepson *et al.,* 2006). Due to Ca^{+2} requirements for NO production, gating Ca^{+2} is a direct coupling between eNOS and nNOS and nicotinic stimulation (Zayas *et al.,* 2002). Evidence has shown that NO is involved in calcium mediated cellular toxicity in other systems (Kobzik *et al.,* 1994).

AchE prevents the accumulation of the acetylcholine that over-stimulate nicotinic and / or muscarinic receptors (Massoulie *et al.,* 1993 and Taylor and Radic 1994). The present study showed that decreased significantly in AchE in cortex and hippocampus after 4&8 weeks treatment of imidacloprid. This result was significantly improved after rat treated with Grape seed oil. In agreement with this view Rodrigues *et al.,* (2009) proved that supplementation with neonicotinoid thiomethoxan insecticide (50 or 100 mg /kg) decreased AchE activity in three analyzed region (hippocampus, cortex ,and striatum) in rats after 7 days administered. The results of Rodrigues *et al*. (2009) demonstrate a decrease in AchE activity induced by thiomethoxan is due to a direct action of this insecticide of AchE or a indirect effect through an action on nAchRs. The thiomethoxan or its metabolities could be acting on the central nAchRs , producing an imbalance in the pattern in compensatory mechanism e.g changes in AchE activity and high affinity choline uptake in synaptosomes from the rat brain. Gupta *et al.* (2001) suggested that the AchE inhibition followed by depletion in energy metabolism, and this inhibition could induce status epilipticus. Fukushima *et al.* (1997) also reported that organophosphorus pesticides via inhibiting AchE cause inhibition of oxidative phosphorylation in the rat brain. In the same trend with the present results, Kazi and Oommen (2012) reported that the neurotoxicity of organophosphate pesticide "monocrotophos" poisoning is characterized by the oxidative damage in the higher AchE activities in the brain and severe AchE inhibition. They found a persistent AchE inhibition in the striatum and hippocampus is correlated with increased lipid peroxidation.

In the present study Na^+K^+ATPase activity in homogenate of both cortex and hippocampus brain of rat treated with methomyl or imidacloprid was decreased after 4 and 8 weeks compared with control. These results could explain through the inhibition of AchE activity. Gupta *et al.* (2001) suggested that the AchE inhibition followed by depletion in energy metabolism. Grycova *et al.* (2009) found that ATP and magnesium drive conformational changes of the Na^+/K^+-ATPase cytoplasmic headpiece. Though the decrease in ATP content could simply clear the alevation of the Na^+K^+ATPase activities. Na^+K^+ATPase is the enzyme responsible for the active transport of sodium and potassium ions in the nervous system, maintaining the ionic gradient necessary for neuronal excitability and regulation of neuronal cell volume. It is present in high concentration in brain cellular membranes, consuming about 40–50% of the ATP generate din this tissue (Erecinska and

Silver, 1994). The inhibition of this enzyme activity could produce membrane depolarization, leading to the suppression of neuronal and excitatory transmission (Albrecht and Hilgier, 1994; Balestrino *et al.,* 1999). Moreover, Acker *et al.* (2009) have shown that malathion inhibited Na+ K+ ATPase activity in cerebral cortices of adult rats, an event that is involved in the neurotoxicity elicited by this compound. Na^+K^+ATPase is a component of sodium pump and it is involved in cellular contractility and growth and differentiation (Vasilets and Schwarz, 1993). Thus, activation of different PKC isoforms may either activate or inhibit Na^+K^+activation (Xia *et al.,* 1995). In the same trend, Liapi *et al.* (2009) observed that the differentially affected activities of AchE and Na^+K^+ATPase could result in modulation of cholinergic neurotransmission, neural excitability, metabolic energy production, Mg^{+2} homeostasis and protein synthesis in crucial rat brain regions.

In the present study the effect of grape seed oil on the investigated parameters (GST, GPX, SOD, MDA, NO, AchE, Na^+K^+ATPase) on cortex and hippocampus brain tissue was very weak. The coadminstration with methomyl or imidacloprid did not induce a significant improvement. Shaker (2006) research found that the peel extracts had a higher activity in preventing CSF oxidation than the seed did. On the other side, the seed extract showed higher pro-oxidative effect for sunflower. The composition of the phenolic compounds is likely to be more important than the total phenol concentration. Biologically, grape seed extract increases the antioxidant levels in plasma and enhances the resistance to LDL oxidation (Natella *et al.,* 2002). In contrast to our results, Ahn *et al.* (2002) concluded that grape seed extract have strong radical scavenging activity in *vitro,* and inhibited lipid peroxidation in *vivo*.

Maier *et al.* (2009) confirmed that the press residues of grape seed oil production still to be a rich source of polyphenolics with strong antioxidant activity. Data in the literature yields contradictory results on the effect of dietary poly phenols (PP) and other natural antioxidants on the activity of antioxidant enzymes. Young *et al.* (2000) showed and increased activity of erythrocyte GR and GPX in human volunteers consuming a PP-rich grape skin extract for one week, while SOD and CAT activities were not modified (Young *et al.,* 2000). These same authors also showed that GPX activity in red blood cells significantly increased with dose after fruit juice intake in humans (Young *et al.,* 2000). Green tea leaves caused an increase in SOD and CAT activity in rats (Lin *et al.,* 1998). An increased activity of GPX, GR and SOD was also observed in red blood cells after feeding young female rats with lycopene (Breinholt *et al.,* 2000). On the other hand, Breinholt *et al.* (1999) observed a decreased activity of GPX, GR and CAT after gavage administration of natural flavonoids to rats, whereas other authors found no effect on the activity of erythrocyte antioxidant enzymes in human intervention studies after administration of diets enriched in PP from red wine, beer and spirits (Van der Gaag *et al.,* 2000) from vegetable and fruit (Van der Berg *et al.,* 2001) or green tea (Young *et al.,* 2002).

Physiological explanations have been given in both cases, increased and decreased enzyme activities evoked by administration of PP-rich materials. An induction of the

antioxidant enzymes has been suggested to reflect an enhancement in cellular protection, ensuring that potential oxidants are metabolized and eliminated more rapidly (Duthie *et al.*, 2000). Conversely, decreased enzyme activity would result in an increased steady-state level of oxidants, contributing to cell injury (Lores-Arnaiz *et al.*, 1995). On the contrary, it has been speculated that a decreased activity of antioxidant enzymes may be a consequence of the sparing effect of dietary antioxidants, reducing the requirement for enzymatic antioxidant function when elevated concentrations of exogenous antioxidants are present in the circulatory system (Breinholt *et al.*, 1999). These contradictory theories put forward the lack of knowledge regarding the mechanism of action of PP and other natural antioxidants and the physiological implication of a variation in the activity of the antioxidant defense system in response to an increased consumption of dietary antioxidants. In the same trend, Alía *et al.* (2003) found that no protective effect of poly phenol-rich grape dietary fiber was seen, since it did not change the response of the hepatic antioxidant system against acetaminophen induced oxidative stress.

In contrast with the present study, Feng *et al.* (2005) demonstrates that treatment with grape seed extract significantly reduces the severity of brain injury in the focal ischemia model of the neonatal rat pups. Grape seed extract also significantly reduces a hypoxia-induced increase in brain 8-isoprostaglandin F2 and thiobarbituric acid reactive substances concentrations compared with the vehicle group. Also, Pallarès *et al.* (2013) demonstrated that several doses of GSPE can alleviate acute inflammation triggered by Lipid Peroxidation Stress LPS in rats at the systemic and local levels when administered for as few as 15 days before the injection of endotoxin. They assessed the preventive effects of GSPE on acute inflammateion, they focused on the NOx(nitrate and nitrite) species. In dissimilarity to our conclusion, Lakshmi and Aparna (2013) concluded that black grapes can be given as a dietary supplement to human populations exposed to environmental toxicants and can provide protection against toxic effects without being appreciably harmful itself.

Conclusion: Exposure to methomyl or imidacloprid induced significant change in the selected parameters in both cortex and hippocampus tissues which revealed brain damage. The study also suggested that the grape seed oil antioxidants not improve the brain toxicity induced by methomyl or imidacloprid.

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الملخص باللغة العربية

التأثير المحتمل لزيت بذر العنب على سمية المخ المحدثة بكلٍ من الميثوميل أو الإميداكلوبراید في ذكور الجرذان

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خططت هذه الدراسة لتعيين تأثير الميثوميل أو الإميداكلوبراید على المخ لذكور الجرذان. كما تم أيضاً دراسة تقييم تأثير زيت بذر العنب كمضاد للأكسدة. تم إعطاء الجر ذان 10/1، 20/1 من نصف الجرعة المميتة لكلٍ من الميثوميل أو الإميداكلوبراید عن طريق الفم. الجرعة 17، 450مجم لكل كجم من وزن الجسم لمدة 4، 8 أسبوع. وزيت بذر العنب 4مجم لكل كجم من وزن الجسم تم استخدامه للوقاية من سمية كلٍ من الميثوميل أو الإميداكلوبراید. تم قياس الجهد التأكسدي لكلٍ من القشرة المخية وقرن آمون (جلوتاثيون إس ترانسفيريز، جلوتاثيون بيرأكسيديز، سوبر أكسيد ديزميوتيز، مالوندالدهيد، وأكسيد النيتريك) كما تم قياس الصوديوم بوتاسيوم إيه تيبيز، والأسيتيل كولينستريز. و أوضحت الدراسة أن الجلوتاثيون إس ترانسفريز، والجلوتاثيون بيرأكسيديز، والمالونألدهيد، وأكسيد النيتريك يزداد زيادة معنوية ،كما أن السوبر أكسيد ديزميوتيز، والصوديوم بوتاسيوم إيه تيبيز، والأستيل كولينستريز ينقص نقصاناً معنوياً. كما تسببت المعالجة بزيت بذر العنب في تحسين معنوي للسمية لكلا من الميثوميل أو الإميداكلوبراید على المخ، ولكنها لم تمنع السمية إلى مستوى المجموعات الضابطة. وتقترح الدراسة أن زيت بذر العنب كمضاد للأكسدة لم يحسن السمية الناتجة عن الميثوميل أو الإميداكلوبراید في المخ.