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RESEARCH ARTICLE

Antioxadative and antigenotoxic effects against cytotoxicity of thiamethoxam on mice .

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Abstract

In order to determine the effect of thiamethoxam on cytotoxicity on mice,20 white female mice 9-10 weeks aged were divided randomly into two groups equally and treated as the following: the 1st group was administrated orally with 83.7 mg\kg BW of thiamethoxam daily for 8 weeks, 2nd group was administrated orally with 0.4 ml of normal saline daily for 8 weeks and served as control negative group. All animals were sacrified at 8 weeks post-treatment, blood samples were taken for hormonal and biochemical examination, in addition bone marrow samples were taken for cytogenetic examination. The result showed that the ratio of mitotic index,blastic index and micronuclei higher (3.50 ± 0.15, 38.90 ± 1.21 and3.27± 0.31) respectively than those values in control negative group (2.18±0.6 , 24.30 ± 0.2,0.55±0.15) respectively. The current result expressed high levels of serum peroxynitrite and malondialdhyde radical concentration (M\L) on thiamethoxam treated group at 4 and 8 weeks post-treatment (2.40 ± 0.006, 3.49 ± 0.002; 2.50 ± 0.007, 3.59 ± 0.0073 respectively) were higher than those values (0.24 ± 0.005, 0.25 ± 0.0036; 0.30 ± 0.0051,. 0.35 ± 0.0036 respectively) in control negative group ..In addition the result showed that thiamethoxam lead to significant decrease values of Estradal (E2) followed by ,Follicular stimulating hormone (FSH) ,luteinizing hormone(LH), and progesterone (20.20 ± 0.25, 9.05± 0.089, 3.66 ± 0.027, 1.25 ± 0.077respectively).as compared with control group (25.54 ± 0.18. 12.83 ± 0.47**,** 6.10 ± 0.036, 2.80± 0.025respectively), at 8weeks post-treatment with thiamethoxam. It was concluded that the thiamethoxam had oxidative, genotoxic and hormonal effects .

Introduction

Insects had extensive economic effects on the agricultural products ,therefore many synthetic pesticides were used for control these insects **(Vicky, 2009)**Insecticides may be natural (e.g.nicotine) or man-made(e.g. neonicotinoids) are applied to target pests in a myriad of formulations and delivery systems such as sprays, baits, slow-release diffusion **(Casida and Quistad,1998),** however, the application of these pesticides was based on selective ,toxicity for certain organisms but it has serious effects on many non-target organisms and the use of pesticides were considered a harmful to other living organisms

The genotoxic effects of pesticides occur by reacting with certain sites of DNA and modifying it through a number ways such as cleavage of phosphodiester bonds, insertion, deletions and substitutions. chronic feeding with agrochemicals associated with increasing incidence of mouse liver tumors **(Carmichael,1997).** Thiamethoxam is a

neonicotinoidinsecticide widely used in the world **(Shalaby, 2010)** and actively used against a broad range of commercially important sucking and chewing pests that causes deposition in plant due to used repeated **(Green,2005).**

The major metabolites of thiamethoxam that responsible for the hepatic changes is CGA322704 **(Federal Register, 2003). A**lso the thiamethoxam, metabolite CGA265307 was found to be structurally similar to known inhibitors of inducible nitric oxide synthese and it play important role in the development of liver toxicity **(Brennan and Moncada2002). A**lso the toxic effects of thiamethoxam was interference with the nicotinic acetylcholine receptors of central and peripheral nervous system of insects and animals as well as induce tumor after long fed diet contaminated with these agent **(Green,2005).**

The repeated use of thiamethoxam against insects causes deposition in the plants and environmental contamination water and the fish may be exposed to great range of these agents during the course of their life cycle **(Banaee et al.,2011; Banaee 2012),** Thiamethoxam induced formation of free radical which lead to damage of cellular constituents such as lipid, nucleic acid, protein and carbohydrate **(Ramzi et al.,1994).**Malondialdehyde (MDA) is one of marker of Lipid peroxidation of lipid membranes damage by ROI **(Linden et al.,2008., Del Rio et al.,2005).** MDA reacts with thiobarbituric acid (TBA) under strong acidic condition and heating. This reaction is actually the formation of a pink-color product (TBA-MDA) which can consequently be measured by the colorimetric method **(Lykkesfeldt ,2007)**.

Antioxidants such as vitamin C, vitamin E, glutathione (GSH), superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), catalase and beta-glucan normally inactivates free radicals **(Klaunig et al.,1998).**In Iraq, there are limited researches about the pesticides, therefore the aim of the present study is to determine the influence of the cytotoxic effects of thiamethoxamin in mice.

Materials and methods

Source of thiamethoxam**;-Thiamethoxam 25 %Syngenta (Switzerland).Determine the effective dose** of thiamethoxam according to "up-and-down" method **(Dixon, 1980)** was used for determination of median lethal dose **(LD50)** of thiamethoxam given orally to mice.

Experimental design

Twenty female white mice, 9-10 weeks in aged were divided randomly into (2) groups equally and treated as following:-

1- 1st group (n=10) was administration orally with 873.35 mg/kg BW of thiamethoxam daily for 8 weeks.
2- 2rd group was administrated orally with o.4ml of sterile normal saline for 8 weeks and served as control negative group.

At the end of the experiment, animals were sacrificed and blood samples were collected directly from their hearts in aseptic condition for hormonal and oxidative marker study according to **(Schulster et al.,1984**; **Günzler et al.,1974; Wysockaet al.,1995)** , in addition bone marrow samples were collected for cytogenetic examination {mitotic index (MI), blast index (BI) and micronuclei rate (Mn)} according to **(Evans and Lake 1998).**

Statistical Analysis : Statistical analysis were conducted to determine the statistical differences among different groups using ready – made statistical design statistical package for social science (SPSS).

Ethics Approval: This study was approved by the ethical and research committee of Veterinary Medicine College/University of Baghdad

Results and discussion:-

Cytogenetic examination

The results showed that the ratio of mitotic index (MI), blast index (BI) and micronuclei rate (Mn) in animals administrated with thiamethoxam were higher (3.50 ± 0.15, 38.90 ± 1.21 and3.27± 0.31) respectively than those values in control negative group (2.18±0.6 , 24.30 ± 0.2,0.55±0.15) respectively (Table:1).

Table (1) Effects of thiamethoxam on mitotic index (MI), blast index (BI), micronuclei rate (Mn) in bone marrow cells of mice after 8 weeks.

Groups	8 weeks		
	MI	BI	Mn
G1	3.50 ± 0.15 A	38.90 ± 1.21 **A**	3.27± 0.31 **A**
G2	2.18±0.6 B	24.30 ± 0.2 D	0.55±0.15 C

Differences small letters mean significant between groups

G1: animals treated with 837.35 mg/kg.bw thiomethoxam.

G2: Contol animals treated with o.4ml of sterile normal saline and served as control negative group.

The current study showed that thiamethoxam induced significantly increasing the ratio of mitotic index (MI) and blast index (BI) in bone marrow cells as compared with control negative group, these result may be indicated that these agent stimulated cell division

These results was in consistence with **(Evaluation report, *C*anada.2007)** who showed increased values of mitotic index in an animal treated with thiamethoxam. also the current finding revealed that Mn significantly increased in control positive group as compared to those values in control negative group, these result may indicated that thiamethoxam induced damage DNA since Mn formed as a result of losses of whole or portion of chromosomes from daughter nuclei during cell division **(Tucker et al.,1986)** .However, the present finding may indicated that thiamethoxam has genotoxicity effects and it may be induce tumor in animal fed diet contaminated with these agents, these evidence was supported idea that mentioned by **(Noriaki,2010)** who explained that micronuclei are generated during an abnormal mitosis and because their presence reflect the damage of genomic materials, the micronuclei may be used as a biomarker for the presence of genotoxic stress induced by drugs or environmental. The result of mitotic index was agreement with the result of Mn ratio ,these observation may indicated that the thiamethoxam induced abnormal cells division and these idea was in consistence with **(Hoffelder, 2004)** who found that most of the chromosome-type micronuclei are generated after an abnormal mitosis

Biochemical analysis:-

The mean values in table (2) revealed that of serum peroxynitrite and malondialdhyde radical concentration (M\L) on thiamethoxam treated group at 4 and 8 weeks post-treatment (2.40 ± 0.006, 3.49 ± 0.002 2.50 ± 0.007 , 3.59 ± 0.0073 respectively) were higher than those values (0.24 ± 0.005 , 0.25 ± 0.0036 ; 0.30 ± 0.0051,. 0.35 ± 0.0036 respectively) in control negative group .

Table (2): Effects of thiamethoxam on serum peroxynitrite radical and malondialdhyde concentration (M\L) of mice after 4,8weeks.

	Groups	4weeks	8weeks
Peroxynitrite	**G1**	2.40 ± 0.006 **B**	3.49 ± 0.002 **B**
	G2	0.24 ± 0.005 **D**	0.25 ± 0.0036 **D**
Malondialdhyde	**G1**	2.50 ± 0.007 **B**	3.59 ± 0.0073 **B**
	G2	0.30 ± 0.0051 **D**	0.35 ± 0.0036 **D**

Differences small letters mean significant between groups.

G1: animals treated with 873.35 mg/kg.bw thiomethoxam.

G2: Contol animals treated with o.4ml of sterile normal saline and served as control negative group.

The present finding indicated that the thiamethoxam act as oxidative agent and induced damage of the cell membrane via lipid peroxidation due to malondialdhyde and peroxynitrite radical are the main lipid per oxidation products ,these idea was agreement with **(John et al.,2010)** who explained that the peroxidation of polyunsaturated lipid generates a range of substances that possess DNA damaging potential such as malondialdehyde, alkoxyl and peroxynitrite radical, however, the result of free radical examination was supported idea that thiathemoxam have genotoxic effects these demonstration was in consistence **(Esterbauer et al.,1991).**who showed that oxidative stress induced lipid peroxidation of unsaturated fatty acid and subsequently form reactive aldehyde species that have cytotoxic and genotoxic effects through their ability to convalently modify proteins and DNA.

Hormonal changes:-

The results shows that the animals treated with thiamethoxam expressed significant decrease values of Estradal (E2) followed by ,Follicular stimulating hormone (FSH) ,luteinizing hormone(LH), and progesterone (20.20 ± 0.25, 9.05± 0.089 , 3.66 ± 0.027, 1.25 ± 0.077respectively).as compared with control group (25.54 ± 0.18. 12.83 ± 0.47**,** 6.10 ± 0.036, 2.80± 0.025respectively), at 8weeks post-treatment with thiamethoxam(Table:3).

Table (3): percentage of serum Esterodial, FSH, LH and Progesteron concentration (ng|ml) of mice after 8weeks post treatment with thiamethoxam.

groups	Esteroidal	FSH	LH	Progesterone
G1	20.20 ± 0.25 **D**	9.05± 0.089 **C**	3.66 ± 0.027 **C**	1.25 ± 0.077 **C**
G2	25.54 ± 0.18 **B**	12.83 ± 0.47**A**	6.10 ± 0.036 **B**	2.80± 0.025 **A**

Differences small letters mean significant between groups.
G1: Animals treated with 873.35 mg/kg.bw thiamethoxam.
G2: Control (-ve) animals treated with distal water

The present finding may be indicated that the thiamethoxamcauses a significant (P≤0.05) decrease in estrogen and progesterone concentration compared with control group and influence on the fertility of the animals these evidence was agreed with **(Buchanan et al.,2000)** Through AhR is essential for the anti-estrogenicity effect, the precise mechanism by which activation of AhR leads to anti-estrogenic effects is unknown. It has been suggested that the ligandedAhR may induced anti-estrogeniceffects by binding to dioxin response elements in estrogen responsive genes andphysically interfering with the ability of the liganded ER to bind to the DNA and initiate transcription **(Safe and Krishnan 1995).**

In addition, thiamethoxamcan also inhibit lanosterol 14α-demethylase in exposed animals. This enzyme in mammals has inhibitor effects on cholesterol biosynthesis pathway **(Stromstedt et al.,1996),** Since cholesterol is a substrate for subsequent steps in the production of other sterols, e.g., sex steroid hormones, the disruption of this pathway can lead to endocrine changes and abnormalities in reproduction, development, and fertility **(Georgopapadakou and Walsh 1996; Zarn et al.,2003**). Direct effects may depend on the mode of action of the compound **(Barata and Baird 2000),** the concentrations **(Naddy,2000),** and duration of the exposure (**Reynaldi and Liess2005).**

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