EVALUATION of SUBCHRONIC TRIAZOPHOS EXPOSURE on BIOCHEMICAL and HISTOLOGICAL CHANGES in MALE ALBINO RATS

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ABSTRACT: The present study aimed to evaluate the effect following subchronic exposure to the organophosphorus Pesticides, triazophos (TAP) on plasma bio-chemical markers and architecture of some organs in male albino. Two equal groups (10 each) of adult male albino rats were orally administered TAP at doses of 0.82 and 1.64 mg/kg body weight. Doses represented 1/100 and 1/50 LD_{50} of TAP respectively. The administration of TAP was carried out in a rate of five days /week for 90 days; the third group was kept as control.

The present results indicated a dose dependent induction of oxidative stress, as evident by highly significant increase of malondialdehyde ((MDA) level. While, total antioxidant capacity (TAC) was decreased in lowest and highest doses treated groups. As well as, ATPase activity was decreased in highest dose (1.64 mg / kg b.w.) when compared with lowest dose and control group.

Highly significant increase in triiodothyronine (T_3) level and total protein (T. Protein) level in lowest and highest dose of TAP treated groups were observed while,a marked drop in thyroxin (T_4) level was noticed in both lowest and highest doses treated groups when compared with control group. On the other hand, highly significant reduce of acetylcholinesterase (AChE) activity was noticed after administeration with TAP at in the . lowest and highest doses treated groups.

The present results revealed that treatments with both lowest and highest doses of TAP induced a significant rise in plasma alanine amihotransferases activity (ALT) ($p \leq 0.05$) ($p \leq 0.001$) respectively. Meanwhile; AST activity showed significant increased in highst dose treated group and albumine (ALB) level did not exhibite significant changes in the lowest and highest dose treated groups in comparing with control group. In addition, urea level was significantly decreased in the highst dose treated group when compared with the lowest and control group.

As well as, histopathological examination showed some lesions in liver; necrosis of some hepatocytes, kidney; lobulation and congetion of some glomerular tuft and thyriod; necrosis of thyroid aciner epithelium.

In conclusion, subchronic exposure to TAP induced oxidative stress associated with biochemical alterations in male albino rats.

Keywords: Subchronic Toxicity, Triazophos. AST; ALT,ALB, Urea, Creatinine, MDA, TAC, ATPase, T_3, T_4, Male Rats.

1 - INTRODUCTION:

Triazophos, TAP, (O,O-diethyl-O-1-phenyl-1H-1, 2, 4 – triazol -3 - yl phosphorothioate,), is one of the broad-spectrum, moderately toxic, nonsystemic contact organophosphorus pesticides (OPs). It has been extensively used in agriculture for controlling pests as a stomach and contact poison against a broad spectrum of pest insects, acarids, flies and some nematodes that damage agricultural, horticultural and forest crops since the late 1970s (**Aungpradit *et al.* 2007; Lin and Yuan 2005**). The main fields of application of TAP are as acaricide, insecticide and nematicide on various crops, vegetables, fruits, coffee and ornamentals (**Kumar and Kumar 2007; Ma *et al.*, 2004**).

The residual levels of triazophos (TAP) in crops, vegetables and water may pose risks to the health of humans and other animals because the severity of TAP intoxication may vary with dose, route and extent of exposure (**Dharmender, *et al.*, 2014**).

Due to the persistent nature of triazophos in the environment, it undergoes biomagnifications through food-chain thereby causing serious concern to the human health (**Abdul Naveed *et al.*, 2011**). Exposure to TAP for 30 days caused dose dependent induction of oxidative stress in blood and significant histopathological alterations in liver (**Jain *et al.*, 2010**). Organophosphorus insecticides disrupt the endocrine system and they are suspected as triggers for harmful effects on the reproductive system (**Oruç, 2010**).

The objective of this study was to clarify the effects of subchronic daily exposure to TAP; at two dosage levels for 90 days, in male albino rats via evaluation of liver, kidney and thyroid gland function. As well as ChE activity was estimated, furthermore, the effect on some oxidative parameters was studied.

2 - MATERIALS and METHODS:

2.1. Tested Substance:

Triazophos (TAP) formulated form Hostathion 20% EC was used in this study. The formulation was obtained from Syngenta Ltd., Egypt.

2.2. Animals and treatment:

Twelve week - old Wistar male rats weighing 120 – 150 g were placed in polypropylene cages (56 × 32 × 20 cm) with 5 rats per cage and acclimatized for at least 2 weeks and kept under standard laboratory conditions of temperature (25 ± 2 ºC) and relative humidity of 50 ± 10%. They were provided with a nutritionally adequate standard laboratory diet. The rats were randomly divided into three groups of ten animals each either control or treatments. TAP was administered orally by gavage in two sublethal doses viz., 0.82 and 1.64 mg / kg body weight (b.w). All treatments were daily given (5 days / week) for 3 successive months , these doses represent, 1/50 and 1/100 of oral LD_{50} of TAP. The median lethal dose of TAP was carried out according to the guidelines of **OECD, 2007** and calculated according to **Weil, 1952.** The calculated LD_{50} of TAP was 82 mg/Kg b.w. Animals were given food and water *ad libitum*.

2.3. Samples:

At the end of 90 days, all the rats were put on overnight fasting and the blood samples were collected through retero - orbital plexus vein according to **Schalm (1986)** in heparinized vials. Plasma samples were separated by centrifugation of the blood samples at 3600 rpm for 15 min in a refrigerated centrifuge at 4 ◦C. Plasma samples were kept at - 40 ◦C till biochemical investigations were carried.

Rats were sacrificed by cervical dislocation. The tested organs; liver, kidney, and thyroid were excised and fixed in 10% formaline for histopathological examinations.

2.4. Biochemical Analysis:

Lipid peroxidation was estimated in plasma as malondialdehyde (MDA) level by method of **Ohkawa, *et al.* (1979)**. Also the total antioxidant capacity (TAC) is determined according to method **Koracevic, *et al.* (2001)**. The activity of total ATPase was determined as the rate of release of inorganic phosphate by method of **Samson and Quinn, (1967).** Standard and quantitative assay for determination total protein (T. Protein) was done by method of **Bradford (1976)**. Tri - iodothyronine (T_3) and Thyroxin (T_4) determination were performed in plasma using method of **Britton *et al.* (1975)**. Acetylcholinesterase (AChE) activity was determined by the method of **Ellman *et al.* (1961)**, also both of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities were measured colorimetrically according to the method adopted by **Reitman and Frankel (1957).** Albumin content was measured by calorimetrically method of **Doumas *et al.* (1971).** Urea level was measured by calorimetrically method of **Fawcett and Soctt (1960)**. Creatinine was determined by calorimetrically method of **Schirmeister *(*1964)**.

2.5. Histopathological Examination.

Liver, kidney and thyroid gland tissues were dissected out and examined grossly. Subsequently, the tissue specimens were taken and fixed in 15 % formalin saline for the histopathological alternations. The fixed tissues were processed by dehydration in a series of graded ethanol concentrations, cleared with xylol and embedded in paraffin blocks. Sections at 4 - 6µ thickness were obtained and stained by Hematoxilen - Eosin stain (H & E) to further analysis in a light microscope for histopathological changes (**Humason, 1979**). The sections were examined and photographed (X 400) on an Olympus light microscope (Olympus BX51, Tokyo, Japan) with attachment photograph machine (Olympus C-5050, Olympus Optical Co. Ltd., Japan).

2.6. Statistical Analysis Procedures:

All values presented in the text are mean ± standard error (M±SE). In additional, all data from biochemical analysis were subjected to

statistical analysis by Analysis of variance (ANOVA) one – way test (**Gad 1999 & 2001**) by SPSS software for Windows version 17 was used to run a Least Significant Differences (LSD) test to identify all biochemical parameters that differed between each treatment and the control at an overall significance level of $p \leq 0.05$, 0.01, and 0.001.

3 - RESULTS

3.1. Oxidative Stress Biomarkers:

Oxidative stress in plasma of male rats after subchronic treatment (90 days) by triazophos (TAP) tabulated in table (1). The data indicated that the levels of MDA were highly significant increase in both TAP-treated groups versus control at $p \leq 0.001$. In contrast the total antioxidant capacity (TAC) and ATPase activity were decreased in both treated groups (the lowest and highest dose) when compared with control group.

Table (1): Oxidative stress in plasma of male rats after subchronic treatment (90 days) by triazophos (TAP).

Treatments / Parameters	Control	Lowest Dose (0.82 mg/ kg b.w.)	Highest Dose (1.64 mg/ kg b.w.)
MDA (mmol/dl)	6.211 ± 0.4326	11.068 ± 0.79440^{***a}	11.683 ± 0.839^{***a}
TAC mmol/l	2.520 ± 0.037	1.961 ± 0.144^{**a}	1.786 ± 0.077^{***a}
ATPase (µmol P_i/mg protein/hr)	45.461 ± 3.162	39.517 ± 2.657	20.249 ± 1.495^{***ab}

TAC = Total Antioxidant Capacity. MDA= Malondialdehyde. ATPase = Adenosine Triphosphatases.
All data were expressed as mean ± SE
a Significant differences versus control at $p \leq 0.05$. b Significant differences versus low conc. at $p \leq 0.05$.
* Significant differences versus control at $p \leq 0.05$. ** Significant differences versus control at $p \leq 0.01$.
*** Significant differences versus control at $p \leq 0.001$.

3. 2. Stress Biomarkers:

Table (2) showed that the total protein levels were increased in rats exposed to TAP at lowest dose. Also, T_3 level exhibited significant increase in highest dose-treated group in comparing with control group. On the other hand T_4 level was decreased significantly in both lowest and highest treated groups at $p \leq 0.001$ and ≤ 0.01 respectively.

3. 3. Acetylcholinesterase Activity:

Data in table (3) showed marked inhibition of acetylcholinesterase activity (AChE) .it is clear that TAP at lowest and highest doses inhibited AchE activity (-23.65%, -28.32%) when compared with control group. It means that; there is a dose-dependent manner between treated groups

Table (2): Stress biomarkers in plasma of male rats after subchronic treatment (90 days) by triazophos (TAP).

Treatments / Parameters	Control	Lowest Dose (0.82 mg/ kg b.w.)	Highest Dose (1.64 mg/ kg b.w.)
T. Protein (g/dl)	4.875 ± 0.307	6.475 ± 0.403^{**a}	5.176 ± 0.335^{b}
T_3 (ng/dl)	34.021 ± 2.623	32.245 ± 2.580	45.805 ± 3.985^{*ab}
T_4 (µg/dl)	5.953 ± 0.518	3.268 ± 0.280^{***a}	3.938 ± 0.135^{**a}

T. Protein = Total Protein. T_3 = Triiodothyronine. T_4 = Thyroxin.
All data were expressed as mean ± SE
a Significant differences versus control at $p \leq 0.05$. b Significant differences versus low conc. at $p \leq 0.05$.
* Significant differences versus control at $p \leq 0.05$. ** Significant differences versus control at $p \leq 0.01$.
*** Significant differences versus control at $p \leq 0.001$.

Table (3): Acetylcholinesterase (AChE) activity in plasma of male rats after subchronic treatment (90 days) by triazophos (TAP).

Treatments / Parameters	Control	Lowest Dose (0.82 mg/ kg b.w.)	Highest Dose (1.64 mg/ kg b.w.)
AChE (U / L)	888.700 ± 50.597	678.547 ± 55.079**a	636.994 ± 23.712**a
Inhibition (%)	-	23.65	28.32

$$\text{The percentage of inhibition in AChE} = \frac{\text{The activity in control samples - The activity in samples}}{\text{The activity of control}} \times 100$$

All data were expressed as mean ± SE

a Significant differences versus control at $p \leq 0.05$. b Significant differences versus low conc. at $p \leq 0.05$.

* Significant differences versus control at $p \leq 0.05$. ** Significant differences versus control at $p \leq 0.01$.

*** Significant differences versus control at $p \leq 0.001$.

3. 4. Liver and Kidney Functions:

Data Tabulated in Table (4) indicated that the activity of ALT and AST were significantly increased in TAP-treated groups (lowest and highest doses) when compared with control group. While, the albumin level was not change significantly in both doses. Urea level exhibited Significant decrease in group treated with the highest dose (1.64 mg/ kg b.w) at $p \leq 0.01$ but the level of creatinine was not altered significantly in both treated groups with respective to control group.

3. 5. Histopathological Examination.

Liver of control group showed normal histological structure of hepatic lobule (**Fig.1**). Meanwhile, rat liver of highest dose treated group showed sinusoidal leucocytosis, also kupffer cells activation (broken arrow) and portal infiltration with leucocytes (arrow) (**Fig. 2**).Kupffer cells activation (broken arrow) and small focal necrosis of hepatocytes associated with leucocytic cells infiltration (arrow) (**Fig. 3**). In addition, liver of rats treated with lowest dose showed portal oedema (broken arrow) and congestion of hepatoportal blood vessel (arrow) (**Fig. 4**) and necrosis of sporadic hepatocytes (arrow) (**Fig. 5**). Meanwhile, kidneys of control rat revealed normal histological structure of renal parenchyma (**Fig. 6**). Whereas, some examined kidney sections of the highest dose treated group showed lobulation and congestion of glomeruler tuft (broken arrow) and congestion of intertubular blood capillaries (arrow) (**Fig. 7**), and congestion of intertubular blood vessels and atrophy of some glomeruler tuft as well as congestion of intertubular blood capillaries. Moreover, other examined kidney sections of lowest dose treated group revealed congestion of renal blood vessels (arrow) (**Fig. 8**). On the other hand, thyroid gland of control group revealed normal histopathological structure (**Fig. 9**). However, thyroid gland of rat of highest dose treated group showed necrosis of thyroid aciner epithelium (arrow) (**Fig. 10**), in addition cystic dilation of acini (broken arrow) and interacinar oedema (arrow) was noticed in group treated with high concentration (**Fig. 11**). Examined thyroid sections of lowest dose treated group revealed interacinar leucocytic cells infiltration (arrow) (**Fig. 12**), and interacinar oedema.

Table (4): Liver and kidney functions of male rats after subchronic treatment (90 days) by triazophos (TAP).

Treatments / Parameters	Control	Lowest Dose (0.82 mg/ kg b.w.)	Highest Dose (1.64 mg/ kg b.w.)
Liver Function			
ALT (U / l)	13.4 ± 0.872	17.72 ± 1.536 *a	22.08 ± 1.33656 ***ab
AST (U / l)	127.086 ± 5.496	151.194 ± 14.767	171.735 ± 9.591 *a
Albumin (g / dl)	3.670 ± 0.083	3.683 ± 0.269	4.316 ± 0.335
Kidney Function			
Urea (mg/dl)	18.337 ± 1.102	15.752 ± 0.799	13.830 ± 1.083 **a
Creatinine (mg/dl)	0.982 ± 0.074	0.942 ± 0.061	0.844 ± 0.067

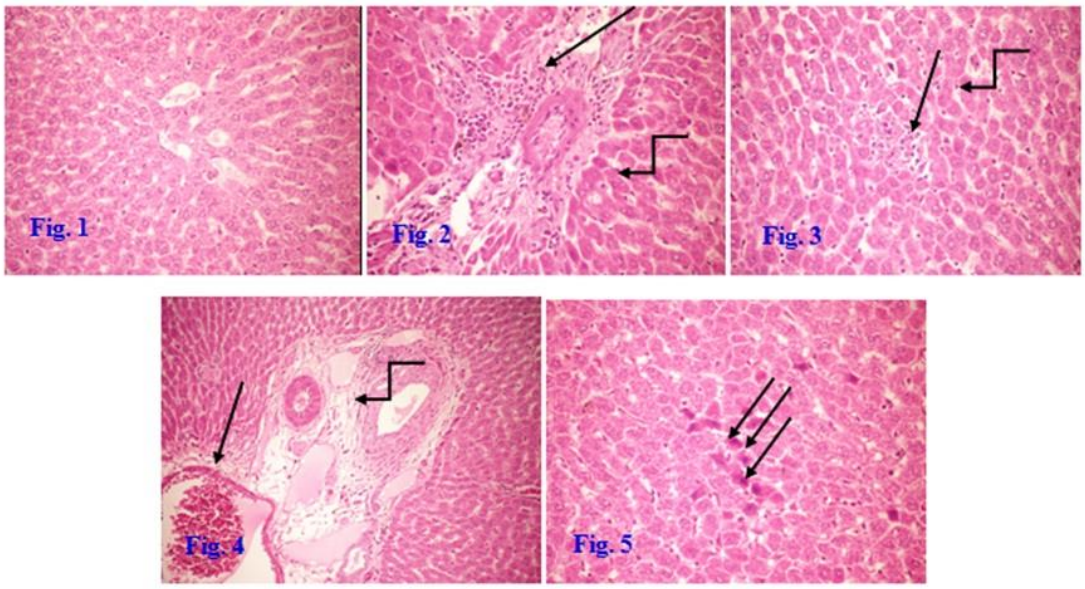
ALT = Alanine Aminotransferase. AST = Aspartate Aminotransferase.

All data were expressed as mean ± SE

a Significant differences versus control at $p \leq 0.05$. b Significant differences versus low conc. at $p \leq 0.05$.

* Significant differences versus control at $p \leq 0.05$. ** Significant differences versus control at $p \leq 0.01$.

*** Significant differences versus control at $p \leq 0.001$.



Liver of rat (H & E X 400) from:

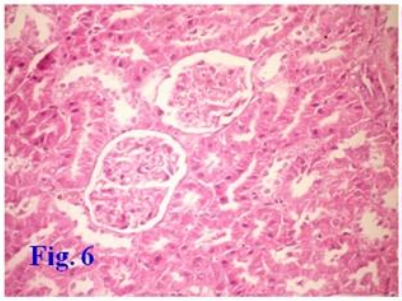
Fig. (1): Control group, showing the normal histological structure of hepatic lobule.

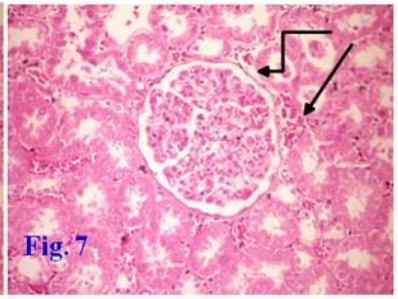
Fig. (2): Treated group with triazophos at high concentration showing kuffer cells activation (zigzagged arrow) and portal infiltration with leucocytes (striated arrow).

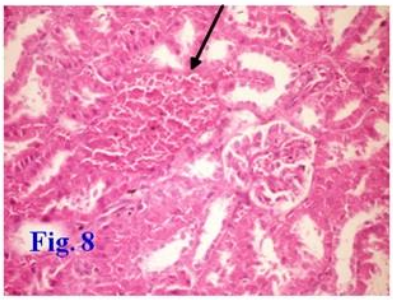
Fig. (3): Group - high concentration triazophos showing kuffer cells activation (zigzagged arrow) and small focal necrosis of hepatocytes associated with leucocytic cells infiltration (striated arrow).

Fig. (4): low concentration of triazophos group showing, portal oedema (zigzagged arrow) and congestion of hepatoportal blood vessel (striated arrow).

Fig. (5): Group treated with low concentration of triazophos showing, necrosis of sporadic hepatocytes.





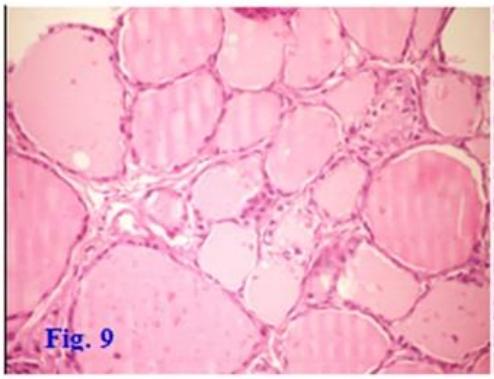


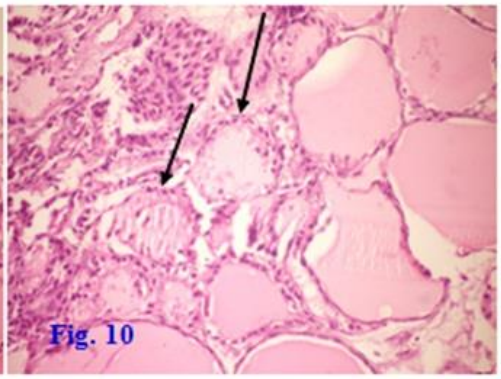
Kidney of rat (H & E X 400) from:

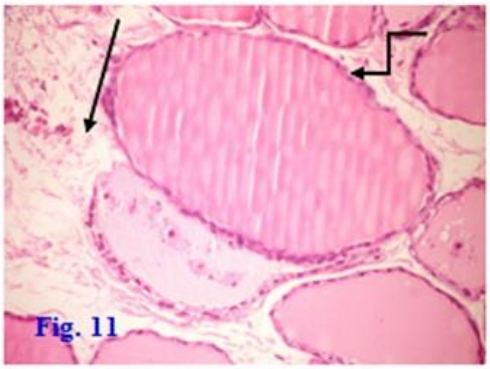
Fig. (6): Untreated group showing, the normal histological structure of renal parenchyma.

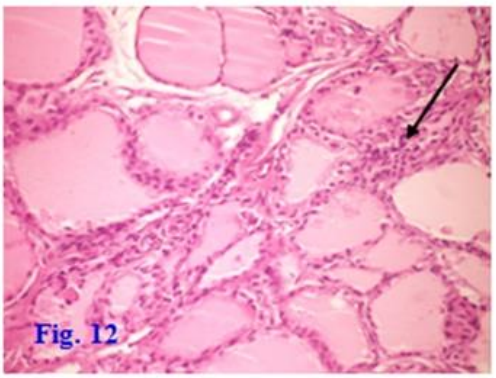
Fig. (7): High concentration group of triazophos showing, hypertrophy and congestion of glomerular tuft (zigzagged arrow) and congestion of intertubular blood capillaries (striated arrow).

Fig. (8): Low concentration group of triazophos showing, congestion of renal blood vessel.









Thyroid gland of rat (H & E X 400) from:

Fig. (9): Control group showing no histopathological changes.

Fig. (10): High concentration of triazophos group showing necrosis of thyroid acini epithelium.

Fig. (11): Group treated with high concentration of triazophos showing cystic dilation acini (zigzagged arrow) and interacinar oedema (striated arrow).

Fig. (12): Low concentration group of triazophos showing interacinar leucocytic cells infiltration.

4 – DISCUSSION:

4. 1. Biochemical markers:

OPs, apart from neurotoxicity and neurobehavioral changes in animals, have been shown to induce oxidative stress (OS) by generating elevated levels of reactive oxygen species, ROS, (**Dharmender *et al.*, 2014**). Oxidative stress (OS) occur when production of reactive oxygen species (ROS) overrides antioxidant capacity in target cells, resulting in the damage of macromolecules such as nucleic acids, lipids and proteins (**Agrawal and Sharma, 2010**).

Regarding oxidant and antioxidant analysis in the present study, the obtained data revealed gradual significant increase in plasma

MDA (as an index of in vivo lipid peroxidation) level in dose dependent manner.. These observations substantiated that TAP might exhibit its toxic effects through induction of oxidative stress as suggested by **Milatovic, *et al.*, (2006)** who showed that organophosphates exhibit their toxic effect through the generation of free radicals. In addition the increase of MDA level in the present study may be is an indicator to formation of free radicals and lipid peroxidation induced nephrotoxicity caused by organophosphorus compounds (TAP) in kidney tissue of rats (**Kalender**, ***et al.*, 2004** and **Kalender**, ***et al.*, 2005**).

In the present study significant decrease in plasma total antioxidant capacity (TAC) that accompanied with significant increase of MDA in both lowest and highest doses of TAP. These findings are in agreement with **Ranjbar, *et al.* (2005)** who reported significant increase in lipid peroxidation accompanied with decreased TAC and ChE activity (**Hundekari, *et al.*, 2013)**.

Total antioxidant capacity group (TAC) considers the cumulative effect of all antioxidants present in blood and body fluids (**Nagy, *et al.*, 2006**). The decrease of plasma TAC level reflects reduction in total antioxidant capacity. This is probably due to the depletion of the antioxidant molecules as they are consumed in the process of protecting cells against ROS generated by triazophos (**Ogunro, *et al.*, 2005**).

The obtained data revealed significant inhibition in plasma ATPase in TAP- highest dose –treated group as compared to control group.

Na^+, K^+ - ATPase is an enzyme present in all animal cell membranes and plays essential roles for the maintenance of neuronal excitability. Although the mechanism of the toxic effect of various Na^+, K^+ - ATPase activity modulators has not been completely understood yet, so this enzyme can be taken as meaningful index of cellular activity and represents a useful toxicological tool (**Duchnowicz, *et al.*, 2005)**. Recent studies show that in addition to pumping ions, Na^+, K^+ - ATPase interacts with neighboring membrane proteins and organized cytosolic cascades of signaling proteins to send messages to the intracellular organelles (**Xie, and Askari, 2002;Xie, and Cai, 2003)**. **Brown, *et. al.*, 1983 & 1987)** suggested that the Na^+, K^+ - ATPase had a role in the secretion of parathyroid hormone (PTH). Also the authors observed that ouabain, a specific inhibitor of the Na^+, K^+ - ATPase, and low extracellular potassium inhibited PTH secretion in vitro from bovine parathyroid cells.

Inhibition of Na^+, K^+ - ATPase by an insecticide may be due to its binding with the ATPase molecule at or near the ouabain site (**Desaiah, 1980)** or may be a result of its interaction with the dephosphorylation step of the phosphoryl intermediate of the enzyme (**Bansal, and Desaiah, 1982)**. As well as inhibition of ATPase implicates that the utilization of energy derived from hydrolysis of ATP, by the enzyme, is less in hypertension as in normotensive conditions. This phenomenon might be of physiological importance especially in conditions of increased intracellular ATP requirements under conditions when the heart is working against the increased systemic resistance (**Vrbjar *et al.*, 2002**).

The present results revealed that, both doses induce significant increase in plasma total protein level in treated groups which is an indication of toxic and perhaps a stimulatory effect on liver function; as the liver is the site of intense metabolism, biotransformation, detoxification, deamination, and storage of proteins and other compounds in the system of animals (**Hodgson, 2004**).

Endocrine disrupting chemicals (EDCs) interfere with the body's endocrine system and produce adverse developmental, reproductive, neurological, cardiovascular, metabolic and immune effects in humans (**Thaddeus *et al.*, 2011).** The hormonal disruption was noticed in the present study; as depletion in T_3 and T_4 levels (stress biomarker). Thyroid is vulnerable to some pesticides; endocrine disrupting chemicals (**Nicolle-Mir, 2010**); this effect may be considered as hypothyroidism (**Lidia and Bruno 2011**).

Reduction of AChE activity may occur in certain cases of liver diseases (**Plla and Charbonneau 1994**). On other hand, lipid peroxidation is one of the main processes induced by oxidative stress and the first step of cellular damage (cell membrane damage) caused by OP insecticides (**Hazarika *et al.*, 2003**), and thereby induce the formation of free radicals and may be the initiator of the AChE

inhibitor-induced cell injury (**Yang and Dettbarn, 1996; Yang *et al.*, 1996**).

Rats administered TAP showed a significant elevation of ALT and AST activities except in lowest dose-treated group; AST activity did not exhibit any changes. Histopathological examination in liver showed congestion of central veins and hepatic sinusoids as well as necrosis of hepatocytes. Elevation in the enzyme activities may be due to permeability alteration and leakage of lysosomal enzymes (**Choudhary *et al.*, 2003; Ksheerasagar *et al.*, 2011)**.

Concerning the kidney function; rats treated with TAP; at the highest dose, exhibited a significant decrease in plasma urea. On contrary, no alteration in plasma creatinine level was noticed when compared with control group. Reduction of blood urea nitrogen (BUN) could either be as a result of an enhanced efficiency in the clearance function of the kidney or a reduced output of these metabolites by the liver due to a suppressed metabolic rate, ornithine cycle, **(Ashade and Joseph; 2014)**

4. 2. Histopathological Changes:

Oxidative stress in cells ocurr due to chemical exposure such as pesticides could result in either apoptosis or necrosis depending on the experimental conditions, degree and duration of toxic insult and chemical structure of pesticides (**Olgun *et al.* 2004)**. It is well documented that ALT activity increase in good correlation with the severity of hepatic necrosis; thus considered as a test of choice in liver necrosis. The acute tubular necrosis which accompanies OP toxicity is related to reactive oxygen species and lipid peroxidation (**Poovala, *et al.*, 1999)**. The acute effects may resulted as an accumulation of lipids and the appearance of degenerate ve processes leading to cell death (**Plaa 1986**).

Onset of such biochemical alterations is one of the early adaptive responses to TAP exposure which leads to histopathological alterations in terms of diffuse fatty changes expanding from mid-zonal area to whole lobule in liver. The present study concludes that induction of oxidative stress, leading to subsequent histopathological alterations in liver, is an important mechanism underlying the TAP induced subchronic toxicity.

5 – CONCLUSION:

In conclusion, results of the present investigation suggest that subchronic exposure to TAP produces oxidative injury and tissue damage especially in liver, kidney and thyroid gland via exerting a dose dependent effect. It is suggested that oxidative stress is involved, at least partly in the mechanism of TAP induced during subchronic and chronic toxicity.

Accordingly, further studies are recommended to determine the exact mechanism of toxicity of TAP and assess the reversibility of the described changes on cessation of experiments.

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