



# Hepatotoxicity of Oral Sub-chronic Exposure to Thiamethoxam and Lambda Cyhalothrin in Rats

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## ABSTRACT

**Background:** Thiamethoxam is a synthetic organic insecticide belong to The most significant new class of pesticides created in the last thirty years is neonicotinoids. This study's objective was to determine the effect of thiamethoxam, lambda cyhalothrin and their combination on biochemical parameters, the levels of free radicals and enzymes activities liver of male.

**Methods:** Forty rats (150-170 g) were used animals were separated into four groups, each with ten rats. The Gp1 was used as control, the Gp2 was used to study the effect of thiamethoxam for 3 weeks, the Gp3 was employed to examine the impact of lambda cyhalothrin for 3 weeks and the Gp4 was used to research the impact of thiamethoxam and lambdacyalothrin for 3 weeks.

**Result:** Treatment with thiamethoxam (TMX), lambda cyhalothrin (LC) and their combination (TMX+LC) lowered the activities of glutathione s-Transferase, Superoxide dismutase, Catalase, Glutathione peroxidase, Glutathione reductase and decreased glutathione concentration. Increased liver thiobarbituric acid reactive compounds and hydrogen peroxide of liver as compared to control. Rats treated with both TMX and LC showed more pronounced effect. Rats given the treatment showed a substantial drop in protein levels TMX, LC and their combination as compared to control. Treatment with TMX, LC and their combination generated a considerable rise in lactate dehydrogenase and a significant reduction in the activities of alkaline phosphatase, aspartate aminotransferase and alanine aminotransferase in rat liver homogenates. Histological examinations showed changes and this proved the Biochemical disruptions happened as a result of to TMX and/or LC toxicity in rat liver.

**Key words:** CAT, Lambda cyhalothrin, SOD, TBARS, Thiamethoxam.

## INTRODUCTION

Synthetic organic pesticide thiamethoxam is a member of the neonicotinoids class, which is the most significant new insecticide class created in the last thirty years. Neonicotinoids had been the pesticide class with the fastest growth rate since they were introduced to the market in 1991. This is likely because of their potential mild toxicity to mammals and their ability to battle insects that have developed resistance to other pesticide classes (Bingham *et al.*, 2008). Approximately \$1 billion worth of chemicals in this class are sold annually, accounting for 11-15% of the pesticide industry (Tomizawa *et al.*, 2003). Comparing well to the insecticides pyrethroid. Thiamethoxam, marketed under the names Actaras for foliar application and Cruisers for seed treatment, belongs to the thianicotinil family of pesticides. Nicotinic acetylcholine receptors (nAChRs), which belong to a class of ligand-gated ion channels that are responsible for fast excitatory cholinergic neurotransmission, are the target of neonicotinoids' insecticidal effect (Karlin *et al.*, 2002). Neonicotinoids exhibit a much greater affinity as agonists at postsynaptic insect nAChRs (Tomizawa *et al.*, 2003). as well as the fact that these drugs' toxicity is thought to be centrally mediated due to the fact that poisoning symptoms resemble those of nicotine (Tomizawa *et al.*, 2005). Over thirty percent of insecticides used globally in veterinary, home and agricultural uses are synthetic pyrethroids (Eisler, 2021). Because of their minimal toxicity to birds and animals, great effectiveness and ease of biodegradability (Kale *et al.*, 1999;

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Pavviya *et al.*, 2017). Pesticides containing carbamates, organochlorines and phosphorus are preferred over synthetic pyrethroids. Animal-produced symptoms categorize pyrethroids into two separate classes: types I and II (Ecobichon *et al.*, 1996). Type II pyrethroids cause a prolonged delay in the inactivation of the sodium channel, causing the neuronal membrane to remain depolarized without repeatedly firing. Type I pyrethroids alter sodium channels in nerve membranes, resulting in prolonged negative after-potential and recurrent neuronal firing. Their nature is more hydrophobic (Michelangeli *et al.*, 1990) and the cellular membrane is their intended target. Furthermore, type I syndrome affects the peripheral nerves, whereas

type II syndrome involves the nerve system in the center (Lawrence *et al.*, 1982; Mandal *et al.*, 2012). There are few studies explaining the mechanisms of Oxidative Stress in toxicity brought on by pyrethroids, Not many recent Reports (Giray *et al.*, 2001). Have shown how pyrethroids like fenvalerate and cypermethrin may induce oxidative stress. One of the most recent pyrethroid insecticides is lambda-cyhalothrin (type II), which effectively and persistently acts against a wide range of arthropods that are detrimental to the health of humans and animals as well as the production of vegetables (Giray *et al.*, 2001). Therefore, the present study investigated the toxic combined effect of thiamethoxam and lambda cyhalothrin in rats.

## MATERIALS AND METHODS

- 1-Thiamethoxam was purchased from Sigma-Aldrich (UK ).
- 2-Lambda cyhalothrin (LC) was obtained from Danyang Agrochemicals (Jiangsu, China ).

### Animals and experimental design

Forty Male albino Wistarr rats weighing (150-170 g) the Experimental methodology was authorized by the local Animals Research Committee and Ethics Committee and the animals were treated in compliance with the guidelines for laboratory animal welfare found in the NIH guidance for laboratory animal welfare. The rats were kept in wire cages with stainless steel bottoms and their temperature was maintained at  $22\pm 2^{\circ}\text{C}$ . Following two weeks of acclimation, rats were divided into four groups of ten each at random.

- **Group I (Contro I):** Control Rats were orally administered distilled water for a period of 3 weeks.
- **Group II (Thiamethoxam; TMX):** Rats were orally given thiamethoxam ( $1/10 \text{ LD}_{50}$ ; 156 mg/kg BW) for a period of three weeks (oral rat  $\text{LD}_{50}$  is 1563 mg/kg) (Shalaby *et al.*, 2010).
- **Group III (Lambda cyhalothrin; LC):** Rats were orally given lambda cyhalothrin (8 mg/kg;  $1/10 \text{ LD}_{50}$  orally) Throughout the course of three weeks (oral rat  $\text{LD}_{50}$  is 80 mg/kg BW) (Ramadhas *et al.*, 2014).
- **Group IV (Thiamethoxam + Lambda cyhalothrin):** Rats were orally given both thiamethoxam (150 mg/kg) and lambda cyhalothrin (8 mg/kg) with the same doses daily throughout the course of three weeks.

Rats were sacrificed via cervical decapitation after being deprived for the whole duration of the trial. Every rat was given a glass tube with a heparinized and non-heparinized blood sample taken from its aorta, This study began in July 2021 and the practical part was completed in April 2023, The theoretical part and writing of the research began and the research was finally completed in January 2024 and The present study was carried out at Biotechnology Research Center, Al-Nahrain University, Baghdad, Iraq.

### Blood samples

Individual blood samples were taken in non-heparinized glass tubes from each rat's aorta. Centrifugation was used to separate the serum for 15 minutes at 3000 rpm. Before analysis, the collected serum was kept in storage at  $-18^{\circ}\text{C}$ .

### Tissue samples

Rats were scarified and the liver were taken out right away and cleaned with cold saline. They were then weighed and cleaned with a 0.9% cooled saline solution. The homogenates were centrifuged for 20 minutes at  $4^{\circ}\text{C}$  at  $10,000 \times g$ . The resulting supernatants were utilized to analyze several biochemical parameters, free radicals and enzyme activity.

### Determination of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) and alkaline phosphatase and lactate dehydrogenase and protein concentration activity

The Alanine Aminotransferase (ALT; EC 2.6.1.2) and AST, alkaline phosphatase , lactate dehydrogenase and protein concentration Activity in the liver and serum was measured using the technique of (Reitman *et al.*, 1957).

### Determination of thiobarbituric acid-reactive substances and hydrogen peroxide and reduced glutathione and glutathione peroxidase enzyme and content

Thiobarbituric acid-reactive substances (TBARS) and hydrogen peroxide and reduced glutathione and glutathione peroxidase enzymewere measured in liver homogenate using the method of (Ohkawa *et al.*, 1979; Velikova *et al.*, 2000; Hafeman *et al.*, 1974).

### Determination of glutathione reductase enzyme and glutathione-S transferase and superoxide dismutase and catalase enzyme activity activity

Glutathione reductase and enzyme and glutathione-S transferase and superoxide dismutase and catalase enzyme activity (GR; EC 1.8.1.7) was measured according to (Habig *et al.*, 1974; Misra *et al.*, 1972; Aebi *et al.*, 1984; Ezz *et al.*, 2023; Alankooshi *et al.*, 2023).

### Histopathological examination

Liver were taken out after sacrificing the rats and fixed in 10% buffered neutral formalin solution for a period of at least 24 hours before histopathological study. Tissue was processed for the purpose of dehydration, clearing (dealcoholization) impregnation and embedding according to (Bancroft *et al.*, 1990; Hasan *et al.*, 2023; Hameed *et al.*, 2023; Hasan *et al.*, 2022; Hasan *et al.*, 2021).

## RESULTS AND DISCUSSION

Rats given TMX in the current investigation had altered ALT and AST activity in comparison to the control group (Table 1). Following hepatocellular damage, the blood is released with these enzymes (Gokcimen *et al.*, 2007). It is well recognized that lipid peroxidation compromises the integrity of cellular membranes, allowing cytoplasmic enzymes to seep out (Bagchi *et al.*, 1995). It has been demonstrated that xinobiotics affect a wide range of enzyme activity in different tissue organs. This disruption in liver enzymes might be brought on by liver injury, changes in cell membrane permeability, or an increase or reduction in

aminotransferase catabolism (El-Shenawy *et al.*, 2010; Kalender *et al.*, 2010). In agreement with the present study (Prasanthi *et al.*, 2005) reported that fenvalerat; a pyrethroid insecticide and diazinon, an organophosphate. Due to their hydrophobic properties, pesticides have been shown in earlier research to mostly collect in biological membranes, particularly in phospholipid bilayers (Alsahhaf *et al.*, 2006). in internal organs rich in lipids, such as body fat, skin, liver, ovaries and parts of the central and peripheral nerve systems (Gupta *et al.*, 1999; Soderlund *et al.*, 2002; Giri *et al.*, 2018). The primary location of pesticide metabolism, where a high concentration of its metabolites accumulates, is the liver. Their harmful effects most likely resulted from the production of ROS, which damaged the cell's numerous membrane components. In the current study, TMX and/or LC treatment might cause oxidative stress in the rat liver, as seen by increased amounts of TBARS and  $H_2O_2$ , as well as a reduction in GSH. This would cause more lipid peroxidation, release free radicals, damage to the hepatocellular membrane and ultimately result in liver damage. The results

and comparison between thiamethoxam (TMX), lambda cyhalothrin and their combination are represented in Table 2. Results indicated that TBARS and  $H_2O_2$  concentrations were significantly ( $P<0.05$ ) increased, while GSH were significantly decreased in liver homogenate of rats treated with TMX and/or LC. Rats treated with both TMX plus LC showed more significant increase in TBARS and  $H_2O_2$  levels, while GSH content was more significantly decreased as compared to control rat group. Table 3 showed the effect of TMX and/or LC on antioxidant enzymes in rat liver. The results showed significant inhibition in antioxidant enzyme activities in rat livers treated with TMX and LC in addition to more pronounced effect in the group treated with TMX+LC. After entering the environment, pesticides can cause oxidative stress by changing the body's entire system of antioxidant defenses (Banerjee *et al.*, 2001) and antioxidant protection via enzymes (Valavanidis *et al.*, 2006). In agreement with these results discovered that the liver's enzymatic defense mechanism was altered as a result of

**Table 1:** Effect of thiamethoxam, lambda cyhalothrin and their combination on the enzyme activities and protein content in rat liver.

| Parameters            | Groups                  |                         |                         |                         |
|-----------------------|-------------------------|-------------------------|-------------------------|-------------------------|
|                       | Cont.                   | TMX                     | LC                      | TMX+LC                  |
| AST (U/mg protein)    | 114±3.31 <sup>a</sup>   | 84±3.42 <sup>b</sup>    | 83±2.47 <sup>b</sup>    | 74±2.83 <sup>c</sup>    |
| ALT (U/mg protein)    | 171±4.32 <sup>a</sup>   | 134±3.78 <sup>b</sup>   | 124±4.48 <sup>b</sup>   | 109±3.69 <sup>c</sup>   |
| LDH (U/mg protein)    | 1139±35.41 <sup>b</sup> | 1463±48.84 <sup>a</sup> | 1502±32.13 <sup>a</sup> | 1577±76.47 <sup>a</sup> |
| ALP (U/mg protein)    | 362±11.78 <sup>a</sup>  | 269±10.58 <sup>b</sup>  | 240±7.43 <sup>c</sup>   | 216±7.85 <sup>c</sup>   |
| Protein (mg/g tissue) | 185±4.28 <sup>a</sup>   | 145±5.39 <sup>b</sup>   | 137±4.94 <sup>b</sup>   | 119±3.66 <sup>c</sup>   |

Values are expressed as means ± SE; n=7 for each treatment group. <sup>abcd</sup>Mean values within a row not sharing a common superscript letters were significantly different,  $p<0.05$ .

**Table 2:** Effect of thiamethoxam, lambda cyhalothrin and their combination on the level of thiobarbituric acid reactive substances, hydrogen peroxide and reduced glutathione content in rat liver.

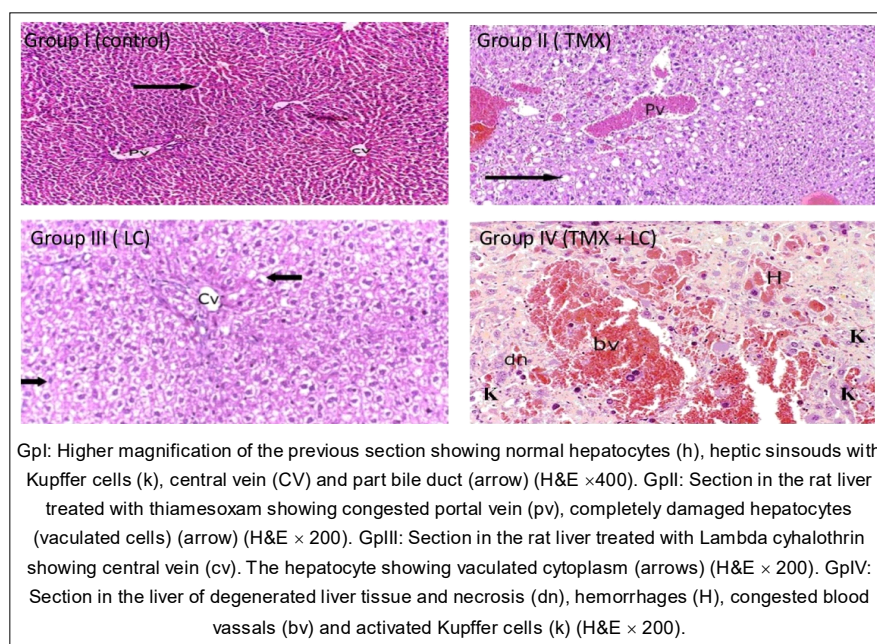
| Parameters               | Groups                  |                         |                          |                         |
|--------------------------|-------------------------|-------------------------|--------------------------|-------------------------|
|                          | Cont                    | TMX                     | LC                       | TMX+LC                  |
| TBARS (nmol/g tissue)    | 32.45±1.15 <sup>b</sup> | 43.45±0.79 <sup>a</sup> | 44.36±1.49 <sup>a</sup>  | 46.81±1.40 <sup>a</sup> |
| $H_2O_2$ (μmol/g tissue) | 64.84±1.98 <sup>b</sup> | 84.64±2.23 <sup>a</sup> | 87.95±3.09 <sup>a</sup>  | 91.91±3.09 <sup>a</sup> |
| GSH (mmol/mg protein)    | 2.70±0.089 <sup>a</sup> | 1.78±0.047 <sup>b</sup> | 1.64±0.064 <sup>bc</sup> | 1.50±0.048 <sup>c</sup> |

Values are expressed as means ± SE; n=7 for each treatment group. <sup>abcd</sup>Mean values within a row not sharing a common superscript letters were significantly different,  $p<0.05$ .

**Table 3:** Effect of thiamethoxam, lambda cyhalothrin and their combination on antioxidant enzymes activities in rat liver.

| Parameters               | Groups                  |                         |                         |                         |
|--------------------------|-------------------------|-------------------------|-------------------------|-------------------------|
|                          | Cont                    | TMX                     | LC                      | TMX+LC                  |
| SOD (U/mg protein)       | 81.77±2.67 <sup>a</sup> | 54.23±1.76 <sup>b</sup> | 56.08±1.90 <sup>b</sup> | 41.00±1.19 <sup>c</sup> |
| CAT (μmol/hr/mg protein) | 51.26±1.34 <sup>a</sup> | 32.39±0.75 <sup>b</sup> | 30.29±0.75 <sup>b</sup> | 25.94±0.88 <sup>c</sup> |
| GPx (U/mg protein)       | 1.22±0.043 <sup>a</sup> | 0.78±0.027 <sup>b</sup> | 0.73±0.026 <sup>b</sup> | 0.60±0.021 <sup>c</sup> |
| GR (U/mg protein)        | 1.37±0.040 <sup>a</sup> | 0.89±0.021 <sup>b</sup> | 0.70±0.024 <sup>c</sup> | 0.86±0.028 <sup>b</sup> |
| GST (μmol/hr/mg protein) | 1.36±0.051 <sup>a</sup> | 0.87±0.024 <sup>b</sup> | 0.90±0.047 <sup>b</sup> | 0.75±0.027 <sup>c</sup> |

Values are expressed as means ± SE; n=7 for each treatment group. <sup>abcd</sup>Mean values within a row not sharing a common superscript letters were significantly different,  $p<0.05$ .



**Fig 1:** Effect of thiamethoxam (TMX), lambda cyhalothrin (LC) and their combination on liver histological examination.

oxidative damage caused by fenvalerate (Yarsan *et al.*, 2002). The fluidity of the membrane is modulated by changes in phospholipids, fatty acids and cholesterol levels, which in turn affects the activity of the enzymes (Nasuti *et al.*, 2003). It has been demonstrated that brief exposure to sub-lethal doses of neonicotinoids in zebrafish livers affects GST's capacity to prevent DNA damage and reduces GST activity while raising CAT activity (Saraivaa *et al.*, 2017). shown how brief exposure to low doses of TMX affects antioxidant defenses as seen by a drop in antioxidant enzyme activity and CAT activity (Altuntas *et al.*, 2003). By conjugating with SH-groups, like glutathione, GST detoxifies a range of electrophilic chemicals into less hazardous forms and also lowers lipid peroxides (Mosialou *et al.*, 1993). The lowered activity of GST may be the cause of the reduced liver GSH contents in rats treated with TMX and/or LC. In keeping with the current findings, a substantial drop in GST activity following a single oral cypermethrin dosage. Additionally (Cervello *et al.*, 1992). Proposed that the thiol (SH) group of glutathione is used by the GST enzyme to catalyze the reaction, neutralizing and increasing the solubility of the products. The current study's findings of hepatorenal damage in rats exposed to TMX included abnormalities in the renal tubules and the loss of hepatic architecture. These findings concur with (Ecobichon *et al.*, 1996; Hasan *et al.*, 2024) (Fig 1), who reported that treatment of male albino mice with imidacloprid at a dose level of 15 mg/Kg/day/ for 15 days induced decrease in body weight and no mortality occurred during treatment period. High dose of 15 mg/Kg/day for 15 days Liver sections in rats treated with thiamethoxam, lambda cyhalothrin and their combination groups (Kaur *et al.*, 2006; Balani *et al.*, 2011). It could be caused by the hepatocytes' degeneration and necrosis,

which is linked to a higher permeability of the cell membrane that releases transaminases into the bloodstream and subsequently causes a reduction in the amount of liver tissue. These results were connected with the ultrastructural and histological alterations seen in the mice's liver by (Arfat *et al.*, 2014). Imidacloprid has been linked to oxidative stress and inflammation in rat liver and brain, according to prior studies (Duzguner *et al.*, 2010; Hasan *et al.*, 2024) (Fig 1). Our results complement who documented that rats exposed to imidacloprid for a subchronic period of time developed moderate localized necrosis of the liver and hepatocellular injur.

## CONCLUSION

It is clear from our results that thiamethoxam and lambda cyhalothrin encouraged it causes changes in liver functions and histological structure.

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## Conflict of interest

Authors have declared that no competing interests exist.

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