The Ameliorative Effect of Vitamin C against Sub-chronic Thiamethoxam Toxicity in Male Rats

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ABSTRACT  
Thiamethoxam (THM), a neonicotinoid insecticide, controls various insect pests that attack fruits, vegetables, and field crops. However, improper handling and prolonged exposure to thiamethoxam can lead to adverse health effects. The objective of this study was to eliminate the toxic effects of THM in liver, kidney and brain of male rats by incorporating vitamin C into their diets. Rats were divided into six groups (8 males per group): the control group (which supplied distilled water orally), the vitamin C group (which injected 200 mg/kg BW of vitamin C), three THM groups received two graded levels as follows, 1/10 LD₅₀ (156.3) and 1/20 LD₅₀ (78.15) mg/kg BW orally. The results showed that exposure to THM at the two levels (1/10 and 1/20) induces food intake, which reduces body weight gain, as well as carcass weights. It also reduces red blood cells and hemoglobin. It raises blood glucose and liver enzymes (ALT, AST, and ALP), as well as MDA, which indicates a high level of oxidative stress leading to decrease in immune-markers (IgM and IgA). Therefore, adding vitamin C in the rats’ diet reflects all the above-mentioned parameters near to the control levels. The simultaneous application of vitamin C along with THM eliminated the harmful impacts of the THM toxicity on the enzymatic and non-enzymatic antioxidant system, enhancing the liver, kidney, and immunoglobulin markers. The side effects of THM were also confirmed in histopathological examinations, where major alterations in liver, kidney, and brain tissues’ structure were recorded. It concluded that vitamin C can mitigate adverse effects of the THM toxicity in non-target species.


INTRODUCTION  
Neonicotinoid insecticides are almost potential systemic insecticides worldwide, accounting for more than 25% of the international pesticide trade (Craddock et al., 2019). Neurotoxins are classified because nicotinic acetylcholine receptors (nAChRs) obstruct and inhibit acetylcholine transmission via nerve impulses. This results in the paralysis and eventual demise of the insect (Tariba et al., 2021). Thiamethoxam (THM) is the most widely used and the first commercially available neonicotinoid insecticide.  

Toxicity studies are available on THM, and there are few reports regarding this insecticide’s sub-chronic toxicity and its elimination by antioxidant substances (Cooper and Dobson, 2007). THM is quickly absorbed after oral doses in rats (Beyaz et al., 2016). It is widely distributed in the body and mainly concentrated in the liver. The time to reach maximum concentrations in blood is 1–4 hours. The absorbed material is rapidly excreted from the body of rats, predominantly in urine (Bednarska et al., 2013) and their residues were detected in different food samples (El-Sheikh et al., 2022; El-Sheikh et al., 2023). THM was found to act as a hepatotoxicity
Biomarker, possibly with histopathological alterations, cancer development, and cell necrosis in mice (Arfat et al., 2014). In recent studies, hematobiochemical investigations have validated the association between anemia and alterations in hepatic and renal biomarkers in rats (Khaldoun-Oulabri et al., 2017), cockerels (Gul et al., 2017) birds (Gul et al., 2020), and fish (Ghaffar et al., 2020) that received THM in their diet. Additionally, numerous studies have authenticated the mutagenic effects of THM (Sinha and Thaker, 2013).

Oxidative stress can be induced by an overabundance of reactive oxygen species (ROS) produced by various pesticides, which are widely used in agriculture to enhance food production and public health to control nuisance animals. A discrepancy between the production of cellular antioxidants and free radicals gives rise to this imbalance (Milatovic et al., 2006). Reactive oxygen species (ROS) are by-products of normal cell activity. They are produced in many cellular compartments and play a major role in signaling pathways. Overproduction of ROS is associated with the development of various human diseases (Snezhkina et al., 2019). Extensive research has been conducted on the toxic potential and induction of oxidative stress by pesticides, demonstrating that oxidative stress (OS) can occur in animals and people when subjected to different poisonous substances (Uchendu et al., 2012). Jameel et al. (2020) demonstrated that thiamethoxam directly interacts with DNA, generating ROS, and has a major impact on the many biological and biochemical parameters of the organisms exposed to it. Significant alterations were noted in stress-related enzymes, including GST, CAT, and SOD. The oxidative markers for DNA damage, 8-OHdG, and lipid damage, MDA, were also found to exhibit similar patterns. Numerous mechanisms of antioxidant agents are used to mitigate the detrimental effects of free radicals (Abdollahi et al., 2004; El-Sheikh and Galal, 2015; Hassan et al., 2021). These substances eliminate, impede, or postpone oxidative stress to a specific molecule (Pruchniak et al., 2016). The principal natural antioxidants, such as vitamins A, C and E, and carotenoids, are derived from dietary sources (Xu et al., 2017).

Ascorbic acid (vitamin C) protects against xenobiotic intoxication. Furthermore, vitamin C is recognized for its efficacy as an antioxidant agent that protects cells against OS (Aitken and Roman, 2008; Zhong et al., 2017). Its function, as an antioxidant agent prevents the detrimental effects of free radicals on vital tissues and is critical in safeguarding against insecticide toxicity, particularly liver toxicity (Djurasevic et al., 2008).

Vitamin C has been observed to ameliorate biochemical and hematological alterations induced by organophosphate pesticides in humans and animals (Saoudi et al., 2021). A relatively low-cost, readily available, and relatively safe antioxidant has exhibited exceptional effectiveness in reducing the deleterious effects of most pesticides (Hamed et al., 2023). Therefore, this study was planned to investigate the positive effect of vitamin C on THM toxicity in rats’ model along with biochemical and histopathological changes in the renal, cerebral, and hepatic tissues of albino male rats.

MATERIALS AND METHODS

Chemicals: An imported 25 WG formulation of thiamethoxam (THM, CAS 153719-23-4) from Greensboro, USA, was commercially acquired from the local Syngenta crop protection agrochemicals distributor. The insecticide formulation was diluted with distilled water to the specified concentrations; Thiamethoxam was produced individually in distilled water with a concentration of 1000 mg/L and kept at -20°C until it was used. The water comprising all the stated pesticides was created by placing the correct amount of each stock solution in a 50 ml volumetric flask and then filling it up to the desired volume with the appropriate solvent. Subsequently, a diluted standard solution of 10 mg/L in an appropriate solvent was made from the original standard solution of 50 mg/L and the different concentrations. The source of vitamin C (CAS 50-81-7) was Sigma Aldrich, located in St. Louis, MO, USA.

Animal and experimental design: A total of 48 male Wister Albino Rats (Rattus norvegicus Bork) weighted (180-190g), and kept under full hygienic conditions. The plastic boxes were subjected to a 12-hour dark-light cycle, 40–60% relative humidity, and a temperature of 23.2°C. They delivered water at their discretion and a rodent diet throughout the experiment (NRC, 1996). The rats were given two weeks to acclimate to the experimental animal laboratory setting. The accommodation and administration of the animals and the experimental protocols were conducted as per the principles delineated in the Guide for the Care & Use of Lab Animals. The experimental period was 28 days. After the accommodation period, the animals were weighed. Randomly, eight males were allocated to Six groups: the control group (which was given distilled water, orally), the vitamin C group (injected 200 mg/kg BW of vitamin C), two THM groups received two graded levels as follows, 1/10 LD50 (156.3) and 1/20 LD50 (78.15) mg/kg BW orally, and two groups received Vitamin C then received two graded levels of THM (U. S. EPA, 2011; Hamed et al., 2023).

Determination of weight gain and organ weight: At the end of the experiment (after 28 days), the animals were anesthetized with ether and then decapitated from the cervical region. Following the dissection of the heart, brain, liver, kidney, lung, and spleen, excess fat was eliminated to evaluate the relative weight of these organs (Zhou et al., 2023).

Blood biochemistry
Sample collection and preparation: At the end of 28 days, the rats were fasted overnight, then were slaughtered via jugular vein severance, and two blood samples were obtained. The first 0.5 ml sample was collected in an EDTA tube for hematological examination. The other sample (2 ml was collected in EDTA-free tubes, centrifugated at 3000 rpm for 10 minutes to collect the serum, and then kept at -20°C until they were utilized for biochemical tests within two weeks (Saad et al., 2022).

Hematology: The blood sample with EDTA was subjected to the evaluation of the total count of red blood cells (RBCs), white blood cells (WBCs), and hemoglobin
(Hb). The WBCs, RBCs lymphocytes, & platelets were determined using an automated cell counter (HOSPITEX analyzer, Italy (Lynch et al., 1969).

Serum biochemical parameters: The levels of aspartate transaminase (AST), alanine transaminase (ALT), & alkaline phosphatase (ALP) in serum were measured using a colorimetric technique (Reitman and Frankle, 1957), while ALP was determined following Belfield and Goldberg (1971). Total protein concentrations in serum were estimated through Grant and Het (1987), whereas the quantities of albumin were tested using the technique developed by Westgard and Poquette (1972). Serum globulin was determined by subtracting the albumin value from the total protein. The concentration of glucose was measured using the Trinder (1969) method. Following Bartels et al. (1972), the creatinine content was colorimetrically measured. The colorimetric approach of Fawcett and Scott (1960) was employed to estimate the urea area. The microplate reader (Infinite M Nano, manufactured by TECAN, Austria) was used in colorimetric measurements.

Antioxidant enzymes: Malondialdehyde (MDA), the lipid peroxidation marker, was determined following the instructions given by Ohkawa et al. (1979) using BioDignostic kits (Cat No. 23225). The total non-enzymatic antioxidant capacity (TAC) was assessed using the BioDignostic kits following the method of Koracevic et al. (2001). The measurements were conducted via a microplate reader at the respected wavelength (Infinite M Nano, TECAN, Austria).

Immunity markers: The levels of serum immunoglobulin M (IgM, Catalog Number: 6208010) & immunoglobulin G (IgG, Cat No. E17200-1) were determined by applying the techniques outlined in the commercially available IgM & IgG ELISA kits (BioSource, San Diego, CA, USA) (Zhou et al., 2023).

Histopathological examination: Carcass tissues (brain, liver, and kidney) were collected, preserved in formalin, and processed by an automated processor. An initial phase was fixed and then dehydrated. The fixation was conducted by immersing the tissue for 48 hrs in 10% formalin, after which the fixation solution was removed using distilled water for 30 min. The tissues were subsequently dehydrated by immersing in ascending levels of alcohol (70%, 90%, & 100%). The dehydration was subsequently cleared using multiple cycles of xylene. The procedure involved submerging the tissue for one hour in a solution of 50% xylene & 50% alcohol and then for an additional 1.5 hrs in pure xylene. The specimens were then infiltrated with melted paraffin wax, encased, and sealed. Hematoxylin & eosin were used for 4-5µm paraffin cut sections (Suvarna et al., 2013). Blood circulation disruptions, irritation, degenerations, apoptosis, necrosis, & additional histopathological alterations in the liver and kidney tissues were recorded. Also, shows degeneration & shrinkage of neurons, with the formation of clumps of amorphous pink material in the cerebral cortex, necrosis of neurons, creation of localized regions of malacia in the cerebrum, neuritic plaques and neurofibrillary tangles in brain tissues.

Statistical analysis: Results were expressed as mean ± SD. The data means were analyzed via one-way analysis of variance (ANOVA) using Statistical Package for the Social Sciences SPSS (Version 20.0, Chicago, IL) for Windows followed by LSD Test at P<0.05 significance value to determine statistical differences between groups.

RESULTS

Performance properties: Table 1 illustrates the changes in the rats’ final body weight (FBW), weight gain (BWG), & organ weight across all experimental groups. Compared to the 1/20 THM LD50 + vitamin C group, rats in the 1/10 and 1/20 THM LD50 treated groups experienced a significant decrease (P<0.05) in FBW and WG. The co-administration of vitamin C prevented rats’ ultimate BW loss & BWG when treated with THM LD50 at concentrations of 1/10 & 1/20. BW and BWG did not differ statistically among the control & vitamin C-treated groups (Table 1). The brain (1.5±0.58), liver (6.3±0.81), and kidney (1.75±0.74) weights of animals received 1/10 of the THM LD50, which resulted in significant increases (P<0.05) compared to the control group (2.12±0.31, 9.2±0.52, and 2.98±0.44, respectively). When vitamin C was administered in conjunction with 1/20 of the THM LD50, the relative weights of the brain (1.87±0.61 g), liver (7.62±0.47 g), & kidney (2.18±0.65) comparable to values of the control group (Table 1). This restoration was statistically significant (P<0.05). Mortality was not monitored throughout the investigation. A change in activity and reduced food intake were among the toxicity indicators generally observed in rats administered 1/10 and 1/20 of THM LD50. Additionally, the animals exposed to a dose of vitamin C (200 mg/kg) exhibited no adverse effects.

Hematology: Table 2 displays the impacts of THM exposure and the effect of vitamin C on the hematology indices. In comparison to control animals, administration of THM LD50 at concentrations of 1/10 and 1/20 resulted in substantial decreases (P<0.05) in red blood cells (RBCs) (5.59±0.58 and 6.85±0.06), hemoglobin levels (Hb) (11.85±1.7 and 14.2±0.2), white blood cells (7.63±0.59 and 8.23±0.89), and platelet count (273.5±10.92 and 286.2±13.56). The alterations in hematological measurements (RBCs: 8.11±0.44, Hb: 15.75±0.41, WBC: 9.37±1.01; Plts: 453.0±11.15; Lymphocyte: 79.5±1.12) were markedly reversed (P<0.05) in groups received the combination of vitamin C and 1/20 of THM LD50.

Serum biochemical parameters: A substantial rise in blood glucose levels (274.25±0.31 and 229.34±0.04) was observed in the groups subjected to 1/10 & 1/20 of THM LD50, respectively, compared to the control group (137.26±0.01). The findings are presented in Table 3. Results in the group supplied with 1/20 of THM LD50 with vitamin C showed no significant changes (P>0.05) in total protein, albumin, and globulin (6.88±0.54, 4.61±0.13 and 2.27±0.41, respectively) compared with vitamin C group (7.31±0.42, 4.81±0.32 and 2.27±0.41, respectively). The ratio of albumin to globulin did not differ significantly among control & vitamin C-supplied groups.
The changes in ALP, ALT, & AST activities were significantly elevated after being subjected to 1/10 of THM LD<sub>50</sub> (476.97±1.62, 74.5±1.77 and 131.4±1.25, respectively) and 1/20 of THM LD<sub>50</sub> (410.99±1.74, 59.3±1.51 and 120.7±1.07, respectively) when compared with control (Table 4). ALP, ALT, and AST activities in the group supplied with 1/20 of THM LD<sub>50</sub> + vit C decreased significantly compared with the control group. The changes in creatinine and urea concentrations in male rats exposed to THM has been presented in Table 4. In contrast to the control group and the 1/10 and 1/20 of THM LD<sub>50</sub> + vitamin C groups, the creatinine & urea levels in the 1/20 & 1/10 of THM LD<sub>50</sub> groups increased significantly (P<0.05).

**Impact on IgG & IgM levels:** After 28 consecutive days of THM administration, there was a significant decrease (P<0.05) in serum IgG and IgM levels contrasted to the control (Fig. 1). No substantial disparities were observed (P>0.05) between the control and vitamin C groups. The concurrent supply of vit C with 1/20 of THM LD<sub>50</sub> facilitated the reduction in serum IgG and IgM levels induced in rodents treated with 1/10 of THM LD<sub>50</sub>.

**Impacts on the antioxidant status:** The findings of the study indicated that rodents subjected to 1/10 & 1/20 of THM LD<sub>50</sub> exhibited a substantially elevated concentration of Malondialdehyde (MDA) in terms of oxidative stress parameters (P<0.05) contrasted to control. In contrast, the total antioxidant capacity (TAC) is substantially reduced due to the administered quantities. On the contrary, the combined application of vitamin C and THM amplified the insecticide’s weakened effects, as no substantial alterations were detected when contrasted to control (Fig. 2).

**Histological observations:** Section tissue from the brain showed no changes in the cerebral and cerebellar tissues of vit C and control groups (normal neurons and normal blood vessels). At the same time, moderate meningeo-cerebra-vascular dilatation, congestion, and edema perineurial edema, and focal white matter neuronal degeneration (and demyelination are seen in the cerebral tissue of 1/10 of THM LD<sub>50</sub> plus vit C & 1/20 of THM LD<sub>50</sub> plus vit C, additionally cerebellar medullary and molecular nerve fibers focal demyelination, partial loss of the granular cell layer & focal deterioration of Purkinje cell. The groups that received doses as 1/10 and 1/20 of the lethal dose (LD50) of THM exhibited dilatation of the blood vessels in the brain associated with meningioma, as well as congestion, swelling, and occasional bleeding. These effects were accompanied by extensive degeneration of neurons and vacuolation of the myelin sheath surrounding degenerated oligodendroglia cells in the white matter. Where the co-administration of Vitamin C and THM showed a reduction in oligodendroglia cells, necrosis, total damage of Focal Purkinje cells, & disruption of the granular cell layer, and the brain structure recovered to be close to control. Pathognomonic hippocampus neuronal degeneration, vacuolation (VN, light blue star), neurotoxic axonal degeneration, and demyelination are seen. The cerebellum exhibits visible degeneration, necrosis, total damage of Focal Purkinje cells, & disruption of the granular cell layer (Fig. 3).

Liver sections tissue in control and vit C groups showed maintained liver cords, portal triad structures, biliary system, vascular tributaries, sinusoids, Von Kuepfer’s cells, & supporting stroma (Fig. 4). The liver section of 1/10 and 1/20 THM LD<sub>50</sub> treated groups showed moderate portal biliary proliferative reactions. The portal blood vessels appear moderately to markedly dilate with occasional portal edema and infiltration of
Table 4: Effect of thiamethoxam, vitamin C and their combination on liver and kidney functions of male rats.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>ALP (IU/l)</th>
<th>ALT (IU/l)</th>
<th>AST (IU/l)</th>
<th>Creatinine (mg/dl)</th>
<th>Urea (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>256.27±1.23</td>
<td>44.2±1.11</td>
<td>94.3±1.02</td>
<td>0.88±0.06</td>
<td>38±1.31</td>
</tr>
<tr>
<td>Vit C</td>
<td>256.47±1.87</td>
<td>45.4±0.87</td>
<td>95.8±1.17</td>
<td>0.93±0.06</td>
<td>41.3±1.63</td>
</tr>
<tr>
<td>1/10 of THM LD50</td>
<td>476.97±1.62</td>
<td>74.5±1.77</td>
<td>131.4±1.25</td>
<td>2.7±0.05</td>
<td>69.6±1.75</td>
</tr>
<tr>
<td>1/20 of THM LD50</td>
<td>410.9±1.74</td>
<td>59.3±1.56</td>
<td>120.7±1.07</td>
<td>2.1±0.14</td>
<td>62.8±1.08</td>
</tr>
<tr>
<td>1/10 of THM LD50 + vit C</td>
<td>309.2±1.03</td>
<td>57.02±1.09</td>
<td>110.2±1.21</td>
<td>2.0±0.13</td>
<td>58.4±1.22</td>
</tr>
<tr>
<td>1/20 of THM LD50 + vit C</td>
<td>292.47±2.01</td>
<td>51.2±1.32</td>
<td>108.3±1.43</td>
<td>1.8±1.07</td>
<td>44±1.4</td>
</tr>
</tbody>
</table>

Note: Data are represented as mean± SD (n=8). Means within the same column carrying different letters are significant at (p<0.05).

Fig. 1: The mean value (±SD) of the effect of thiamethoxam, vitamin C, and their combination on immunity parameters (IgG and IgM) of male rats (n=8). There is no significant difference between bars that contain the same lowercase letter (P=0.05).

Fig. 2: The mean value (±SD) of the effect of thiamethoxam, vitamin C, and their mix on MDA and TAC in male rats (mean± SD). There were no significant differences in bars with the same letters (P<0.05).

Fig. 3: Photomicrographs of brain sections were stained using H&E to examine histopathological alterations. The images show brain sections from the following groups: control (A), vitamin C (B) at a dosage of 1/10 of the lethal dose 50 (LD50) of THM, vitamin C (C) at a dosage of 1/20 of the LD50 of THM, a combination of vitamin C and 1/10 of the LD50 of THM (D), and a combination of vitamin C and 1/20 of the LD50 of THM (E). (H&E 200X).

Fig. 4: Histopathological changes in liver sections were observed by H&E staining. The photomicrographs depict the following: control (A), vitamin C (B), 1/10 of THM LD50 (C), 1/20 of THM LD50 (D), 1/10 of THM LD50 + vitamin C (E), and 1/20 of THM LD50 + vitamin C (F). (H&E 200X).

round cells (lymph-plasmacytes). The hepatic sinusoids are mild to moderately dilated, sometimes with atrophy of the surrounding hepatocytes. Hepatic Sections from 1/10 & 1/20 THM LD50 co-treated with vitamin C revealed mild to moderate vascular dilatations, round cell aggregations, and biliary proliferation: mild sinusoidal dilation and atrophy of the surrounding hepatocytes in (Fig. 4).

Renal parenchyma and stroma in the control and vitamin C-treated groups maintained the integrity of nephron units, collecting tubules, papillary and pelvic structures, and other characteristic features. Renal sections
from the LD₅₀ groups of THM (1/10) and (1/20) exhibited moderate renal blood vessel and capillary congestion, occasionally accompanied by perivascular edema. In certain instances, focal interstitial lymphoplasmacytic nephritis was identified.

Diverse levels of degenerative alterations were observed, such as clouded edema, hydropic and vacuolar degeneration, moderate dilatation of collecting and distal convoluted tubules accompanied by partial atrophy of their lining epithelium, and the sporadic formation of hyaline casts within the tubules. There was evidence of partial contraction atrophy and lobulation of specific glomeruli. In addition to dilatations in certain distal convoluted and collecting tubules, kidney sections from subjects co-treated with vitamin C and 1/10 and 1/20 of the THM LD₅₀ exhibited degenerative changes (including hydropic degeneration and cloudy swelling) and focal tubular atrophy. Additionally, there was mild, moderate to severe congestion of renal blood vessels. In a limited number of instances, focal atrophy of the renal pelvic epithelium of transition was evident (Fig. 5).

**DISCUSSION**

Different biomarkers were evaluated in this study, involving hematological index, liver & kidney functions, and oxidative stress/antioxidant biomarkers, and serum IgM and IgG are considered suitable biomarkers for some physiological & histopathological alterations of animal health (Vasyliova et al., 2017). Bailey et al. (2004) established that alterations in body weight served as highly responsive indicators in the identification of potentially hazardous substances. Contrasted to the control, significantly lower body and organ weights and BWG were observed in the 1/10 and 1/20 THM LD₅₀-treated groups. The potential causes for this include reduced food consumption, unappealing tastes in food, or heightened breakdown of proteins and lipids because of the treatment-induced toxicity (Mansour and Mossa, 2010). Male rats reduced body weight after receiving methyl amino-aboramectin (Emamectin), a finding consistent with ours (Xing et al., 2000). Vitamin C co-administered to THM-treated rats increased BW and WG. Similarly, co-administration of fenitrothion and Vit C exhibited a protective role, which was confirmed by the reduction of oxidative stress levels and restoration in the values of examined parameters. Because of their beneficial effects, Vit C may be used to reduce injuries caused by pesticides (Milosevic et al., 2018).

In THM-treated groups, RBC count, Hb concentration and WBC decreased significantly, according to the findings. RBCs are prone to oxidative stress due to different factors: direct exposure to molecular oxygen, the abundance of metal ions that facilitate OS, & the presence of substantial quantities of polyunsaturated fatty acids (PUFAs), which are vulnerable to lipid peroxidation (Edik and Tomur, 2006). Hence, the reduction in RBCs, WBCs, and Hb concentration can be ascribed to the breakdown of RBCs induced by ROS, causing oxidative injury to the cell membrane (El Okle et al., 2018). Further, co-treatment with vitamin C resulted in a notable reduction in lymphocytes and platelets (1/10 and 1/20 of THM LD₅₀, respectively). This may be attributed to the lysis of RBCs & the consequent decrease in Hb concentration.

THM-processed the rats had hypochromic microcytic anemia as a result of being treated with THM for 28 consecutive days, as shown by the results above. The findings presented in this study are consistent with the authors’ assertion that avermectins decreased the concentrations of erythrocytes, leukocytes, and hemoglobin in rodents and rabbits (Anubama et al., 2001). Similarly, our findings are consistent with those of Eissa and Zidan, (2009), who established that administering a high abamectin dosage (1/10 LD₅₀) to treated rats substantially decreased RBCs, WBCs, and hemoglobin concentrations. Co-administering vitamin C with 1/10 & 1/20 of the THM LD₅₀ significantly restored treated rats’ RBCs, Hb, and lymphocytes to normal levels. The beneficial impact of vitamin C on erythrocytes may be mediated through the existence of D-limonene and Myrcene, which have the potential to function as antioxidants or reduce free radical production (Taher et al., 2007). This would enable vitamin C to safeguard RBCs against THM-induced oxidative stress.

Oxidative stress can affect the cellular components of the blood due to the elevated concentration of PUFAs in serum (Chew and Park, 2004). Thus, it is hypothesized that the substantially reduced platelet count observed in THM rats can be attributed to oxidative injury in the platelet membranes. The significant increase in platelet count observed in rodents who received vitamin C in conjunction with THM provides evidence that vitamin C can inhibit the oxidative stress induced by THM on blood cells. The lymphocyte totals of the rats were significantly reduced following THM administration. Multiple prior studies have reported data consistent with the present investigation’s findings (Eissa and Zidan, 2009). Co-administration of vitamin C and THM eliminated these total and differential lymphocytes count alterations. Consistent with prior research (Abbassy et al., 2014), the data of this work indicate that sprayers’ serum total protein (TP), albumin (alb), and globulin (glob) levels

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**Fig. 5:** Histopathological changes in kidney sections were observed using H&E staining: control (A), vitamin C (B), 1/10 of THM LD₅₀ (C), 1/20 of THM LD₅₀ (D), 1/10 of THM LD₅₀ + vitamin C (E), and 1/20 of THM LD₅₀ + vitamin C (F). (H&E 100X, 200X, 400X).
decreased significantly upon exposure to various pesticides. The decline in their concentrations could be ascribed to the addiction between protein synthesis & degradation rates.

Similarly, these alterations could be attributed to reduced caloric consumption. When ALb is at a low level, it allows for the observation of liver injury caused by the harmful effects of high doses of THM. Since the liver is responsible for the extensive synthesis of albumin, adverse impacts on the liver can impair hepatocyte function and production capacity. Vitamin C co-administered to rodents treated with THM elevated serum albumin concentrations, globulin, & TP, resulting in levels close to control. The augmentation of these parameters could be ascribed to the appetite-stimulating and hepatoprotective properties of vitamin C. Biomarkers such as IgG and IgM were employed to evaluate humoral immunity. The findings of the present investigation demonstrated that THM-treated rodents exhibited a substantial reduction in serum immune levels. The observed outcomes suggest that THM inhibits humoral immunity. This effect can be ascribed to lymphopenia, white pulp lymphoid cell depletion, and necrosis in the spleen. According to the present study, the observed reductions in TP and globulin may also contribute to these results. Constant with the data recorded by Sakin et al. (2012), which demonstrated that avermectin reduces immunoglobulin and total leukocyte counts in rainbow trout, the present study's findings support this notion.

The alterations in immunoglobulin concentration were substantially reversed by co-administering vitamin C and THM, which corroborated a nearly average lymphocyte count. As a pivotal organ, the liver performs various critical functions, including xenobiotic detoxification, metabolic processes, and synthesis of functional macromolecules (Djordjevic et al., 2011).

Hepatotoxicity serves as a critical endpoint when assessing the impact of specific xenobiotics. Histopathological and clinical chemistry assessments are frequently employed to identify the organ-specific effects associated with chemical exposure (Mossa et al., 2012). Enzymes ALT, AST, and ALP are markers for the oxidative status of the liver (Harper, 1979; Mossa et al., 2015) because they are secreted into the blood, and their levels rise in response to hepatocellular injury. (Hernández et al., 2013) Rats treated with THM for 28 days exhibited significantly elevated ALT, AST, and ALP levels, supporting the hypothesis that pesticide exposure induces biochemical hepatic toxicity. Additionally, experimental studies have documented an elevation in the activity of ALT, AST, & ALP, which serve as biomarkers for liver damage after subchronic or continuous exposure to organic phosphorus compounds (Binukumar et al., 2010; Rezg et al., 2008).

Alterations in the activity of liver marker enzymes may be attributable to histopathologically identified deleterious changes in hepatic tissue. Furthermore, the detrimental consequences can be ascribed to the toxic impacts of THM, predominantly through the formation of ROS, which in turn induce harm to the diverse cellular membrane constituents & result in the discharge of intracellular enzymes (Bagchi et al., 1995; Toghan et al., 2022). A concurrent administration of vitamin C and THM was found to alleviate the adverse effects of THM on liver marker enzymes. Consistent with the results of Mansour et al. (2011), our findings indicate that co-treatment of FEO and chlorpyrifos decreased hepatic lipid peroxidation and ALT activities while increasing albumin levels in chlorpyrifos-treated rats. The liver protective effect of vitamin C may be attributable to its ability to inhibit oxidative stress induced by THM treatment (Tripathi et al., 2013).

TAC is an indispensable antioxidant defense component in virtually all oxygen-exposed living cells. In this work, rats treated with THM exhibited TAC activity inhibition, potentially producing ROS, specifically superoxide anion. The accumulation of superoxide anion can trigger multiple signaling pathways, resulting in OS (Djordjevic et al., 2011). THM, conversely, significantly increased MDA concentration after 28 days of exposure. Mammalian oxidative stress is quantified by utilizing MDA production as a biomarker (Kurutas, 2016).

MDA is produced when the peroxidation of polyunsaturated fatty acids occurs due to ROS degradation, which supports cellular toxic stress. Furthermore, it is worth noting that an increased level of MDA has the potential to induce mutagenic and thermogenic effects on DNA and proteins through interactions (Del Rio et al., 2005; Ibrahim et al., 2019). The data presented here are comparable to those reported by Zhu et al. (2013), wherein it was demonstrated that avermectin induced hepatic impairment, impeded SOD activity, and elevated MDA levels. Vitamin C co-administration mitigated the adverse consequences of THM on TAC and MDA by boosting TAC activity and decreasing MDA concentrations, thereby enhancing antioxidant defense and reducing oxidative stress.

Vitamin C’s ability to mitigate the toxic effects of THM is likely attributable to its capacity to scavenge free radicals, thereby potentially restricting the detrimental effects of free radicals within the body (Shahat et al., 2011). This indicates that FEO may have health-promoting properties. Moreover, in diabetic rodents, D-limonene can decrease MDA concentrations and enhance the activity of antioxidant enzymes, according to research (Murali et al., 2013). Contingent to the current findings, the hepatoprotective mechanism of vitamin C might be associated with its capacity to augment the lipid peroxidation process in the liver and impede TAC activity, thereby causing a reduction in serum AST, ALT, & ALP levels. The findings presented here align with those of Taher et al. (2007), which suggest that D-limonene & Myrcene might function as antioxidants or inhibit the generation of free radicals, thereby promoting hepatocyte membrane stabilization and minimizing enzyme secretion into the bloodstream.

According to the study's findings, male rats subjected to sub-chronic THM treatment are less susceptible to hemotoxicity, immunotoxicity, and hepatotoxicity when pretreated with vitamin C. Brain and liver histological examinations demonstrate focal hepatic hemorrhage, infiltration, and inflammation, as well as severe degeneration. The kidney exhibited focal hemorrhage, necrosis, inflammation, & atrophy of the glomerular tuft. Additionally, there was vacuolization. These observations suggested that THM significantly altered the histological
composition of the brain, liver, and kidney. These alterations may result from THM’s cytotoxic effects, predominantly attributed to the release of ROS, which damage the cell’s membrane components.

Comparable histological findings were observed in the hepatic & renal tissues of animals treated with THM and chlorpyrifos (Mansour and Mossa, 2010) and fenitrothion (Kalender et al., 2005), respectively. It is recognized that insecticides can cause various histopathological changes in the hepatic & renal tissues. The findings of prior research validate and bolster our conclusions. This may result from the anti-inflammatory, antioxidant, and hepatoprotective properties of vitamin C. Hence, based on the study’s findings, vitamin C supplementation may mitigate toxic effects for those susceptible to prolonged THM exposure. 

Conclusion: Research on rat toxicity has provided evidence that vitamin C exhibits anti-toxic characteristics towards commonly used pesticides in the agricultural sector (THM). The continuous exposure of THM to rats significantly affected the biomarkers. The findings of this study illustrated the importance of co-treatment of vitamin C and THM in eliminating oxidative stress induced by exposure to THM. Administering vitamin C supplements to individuals in regular contact with this insecticide may eliminate the adverse effects on multiple organs.

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