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Toxic effect of Thiamethoxam and Lambda cyhalothrin on Nephrotoxicity and Hematological in rats

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Abstract

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.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 3weeks, 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 lambda cyhalothrin for 3 weeks. thiamethoxam and/or lambda cyhalothrin significantly decreased the activity of glutathione S-transferase, catalase, superoxide dismutase, glutathione peroxidase, glutathione reductase, and reduced glutathione content in the kidneys of rats. The protein content in the kidney tissues of rats treated with thiamethoxam and/or lambda cyhalothrin decreased. An increase in the level of urea and creatinine was also observed in the blood serum of rats treated with thiamethoxam and/or lambda cyhalothrin . The results showed clear changes in red blood cells in the liver and kidney tissues of rats treated with thiamethoxam and/or lambda cyhalothrin .

Keywords: thiamethoxam, lambda cyhalothrin, TBARS, blood pictures, Urea.

1. 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 **(1)**. Approximately $1 billion worth of chemicals in this class are sold annually, accounting for 11–15% of the pesticide industry**(2).** 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 **(3, 4)**. Neonicotinoids exhibit a much greater affinity as agonists at postsynaptic insect nAChRs **(5).** 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 **(4).**Over thirty percent of insecticides used globally in veterinary, home, and agricultural uses are synthetic pyrethroids **(6).** Because of their minimal toxicity to birds and animals, great effectiveness, and ease of biodegradability **(7).** Pesticides containing carbamates, organochlorines, and phosphorus are preferred over synthetic pyrethroids. Animal-produced symptoms categorize pyrethroids into two separate classes: types I and II **(8).** 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 **(9).** 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 **(10).** There are few studies explaining the mechanisms of Oxidative Stress in toxicity brought on by pyrethroids ,Not many recent Reports **(11)**. 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 **(12).** Therefore, the present study investigated the toxic combined effect of thiamethoxam and lambda cyhalothrin in rats.

2. material And method

2.1. Materials

thiamethoxam was purchased from Sigma-Aldrich (UK)., lambda cyhalothrin (LC) was obtained from Danyang Agrochemicals (Jiangsu, China).

2.2. 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 ± 2oC. Following two weeks of acclimation, rats were divided into four groups of ten each at random.

group I (contro l): control Rats were orally administered distelled water for a period of 3 weeks , group II (Thiamethoxam; TMX): Rats were orally given thiamethoxam (1/10 $LD_{50;}$ 156 mg/kg BW) for a period of three weeks (oral rat LD_{50} is 1563 mg/kg) **(13) .**group III (Lambda cyhalothrin; LC): Rats were orally given lambda cyhalothrin ((8 mg/kg; 1/10 LD_{50} orally) Throughout the course of three weeks (oral rat LD_{50} is 80 mg/kg BW) **(14).** 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

2.3. Blood and Tissue 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 °C.
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°C at 10,000 xg. The resulting supernatants were utilized to analyze several biochemical parameters, free radicals, and enzyme activity.

2.4. 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 **(15).**

2.5. 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 enzymwere measured in liver homogenate using the method of **(16,17,18)** .

2.6. 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 **(19 , 20,21,22,23).**

2.7. Determination of urea and creatinine concentration:

Urea and Creatinine level in serum was assayed by using commercial kit that was supplied by Diamond, Egypt. Urea was estimated according to the method according to **(24 , 25 ,26).**

3.8. Determination of blood pictures concentration:

Blood samples were collected in anticoagulant tubes to analyzed thefollowing parameters: white blood cells (WBCs), red blood cells (RBC), hemoglobin (HGB), hemoglobin (HB), , platelets (PLT) counts by using HA-VET CLINDIAG (Alfa Swelab, Sweden) using quality control reagent according to the manufacturing instructions to assess the validity of the assays according to **(27).**

2.9. Statistical analysis

The Statistical Package for the Social Sciences (SPSS software version 16) was used to analyze the findings. The data were displayed as mean ± standard error of mean (SEM) and subjected to one-way analysis of variance (ANOVA) and Dunnett test statistical analysis. Comparisons using the Dunnett test were used to determine how significant the differences between the groups were. To compare the significant difference between groups, an unpaired T-test was used. P < 0 05 was established as the threshold for statistical significance.

3. RESULTS

3.1. Effect of thiamethoxam (TMX), lambda-cyhalothrin and their combination on the concentration of urea and creatinine, enzyme activities and protein content in rats.

Table(1): Effect of thiamethoxam, lambda cyhalothrin and their combination on the concentration of urea and creatinine, enzyme activities and protein content in rats.

Parameters	Groups			
	Cont.	TMX	LC	TMX+LC
Serum Urea (mg/dl)	37.28±1.31^{b}	49.02±1.39^{a}	49.98±1.73^{a}	53.04±2.09^{a}
Creatinine (mg/dl)	0.696±0.020^{c}	0.936±0.026^{b}	0.955±0.026^{b}	1.03±0.029^{a}
Kidney LDH (U/mg protein)	845±21.48^{c}	1063±26.54^{b}	1105±35.73^{b}	1202±20.41^{a}
ALP (U/mg protein)	215±7.73^{a}	163±5.48^{b}	153±6.00^{b}	125±4.93^{c}

Protein (mg/g tissue)	64.41±1.74[a]	50.89±1.47[b]	48.42±1.91[b]	41.22±1.12[c]

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

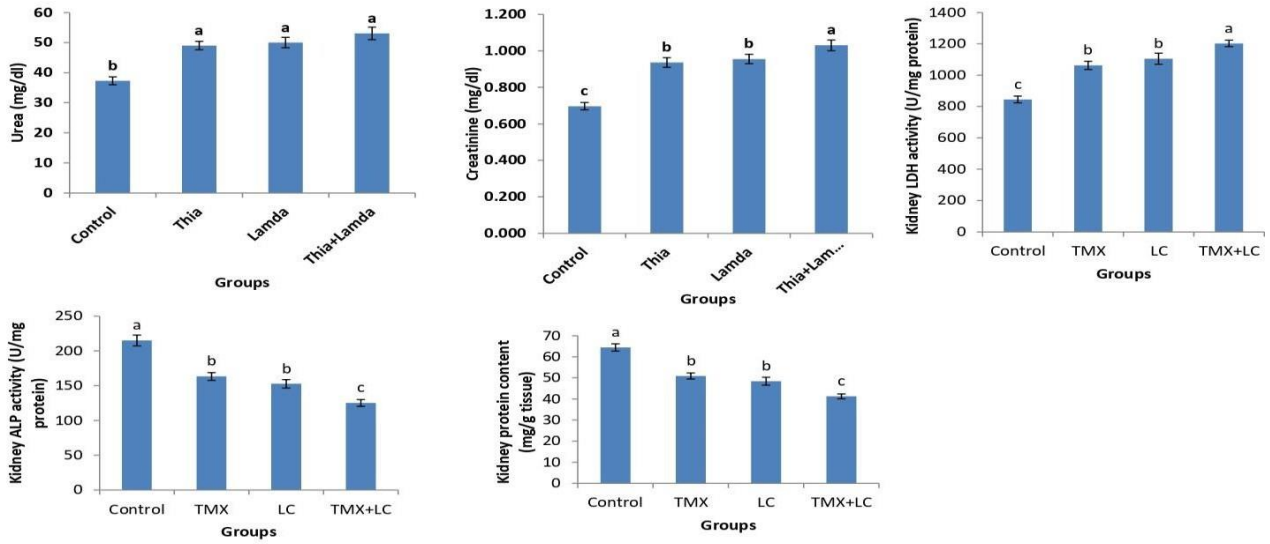

Figure (1) :.Changes in urea concentration and creatinine concentration ,lactate dehydrogenase (LDH) activity , alkaline phosphatase (ALP) activity , protein content in kidney of male rats treated with thiamethoxam (TMX), lambda cyhalothrin (LC) and their combination. Values are expressed as means±SE of seven rats per group. Means with different letters are significantly different (P<0.05).

3.2. Effect of thiamethoxam (TMX), lambda cyhalothrin (LC) and their combination on oxidative stress markers in rat kidney

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 kidney

Parameters	Groups			
	Cont.	TMX	LC	TMX+LC
TBARS (nmol/g tissue)	21.25±0.621[c]	28.02±0.614[b]	28.74±0.704[b]	30.40±0.575[a]
H_2O_2 (µmol/g tissue)	53.50±2.00[b]	68.51±2.05[a]	70.27±2.29[a]	73.04±2.32[a]
GSH (mmol/mg protein)	2.45±0.073[a]	1.66±0.053[b]	1.55±0.054[bc]	1.41±0.043[c]

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

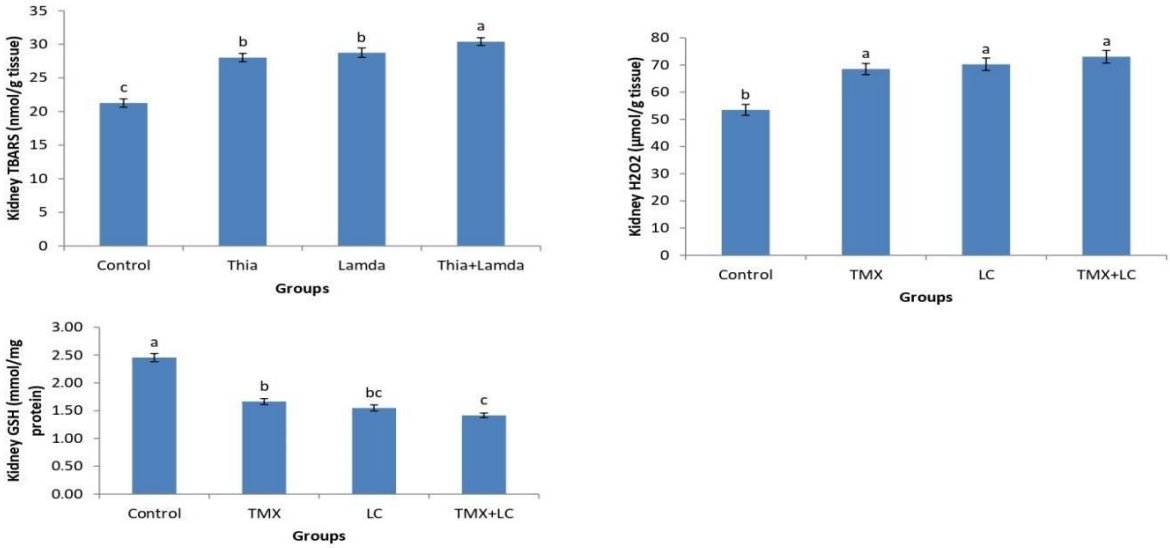

Figure (2) :.Changes in thiobarbituric acid reactive substances (TBARS) and hydrogen peroxide (H2O2),reduced glutathione (GSH) concentrations in kidney of male rats treated with thiamethoxam (TMX), lambda cyhalothrin (LC) and their combination. Values are expressed as means±SE of seven rats per group. Means with different letters are significantly different (P<0.05).

3.3. Effect of thiamethoxam (TMX), lambda-cyhalothrin (LC) and their combination on antioxidant enzymes activity in rat kidney

Table(3): Effect of thiamethoxam, lambda cyhalothrin and their combination on the activities of antioxidant enzymes in rat kidney

Parameters	Groups			
	Cont.	TMX	LC	TMX+LC
SOD (U/mg protein)	73.00±2.68^{a}	46.89±1.83^{b}	44.66±1.06^{b}	37.69±1.33^{c}
CAT (µmol/hr/mg protein)	51.26±1.34^{a}	32.39±0.75^{b}	30.29±0.75^{b}	25.94±0.88^{c}
GPx (U/mg protein)	25.54±0.752^{a}	16.59±0.490^{b}	15.81±0.597^{b}	13.76±0.472^{c}
GR (nmol/min/mg protein)	12.94±0.461^{a}	8.14±0.266^{b}	8.41±0.315^{b}	6.61±0.144^{b}
GST (µmol/hr/mg protein)	0.547±0.021^{a}	0.355±0.009^{b}	0.341±0.006^{b}	0.278±0.010^{c}

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

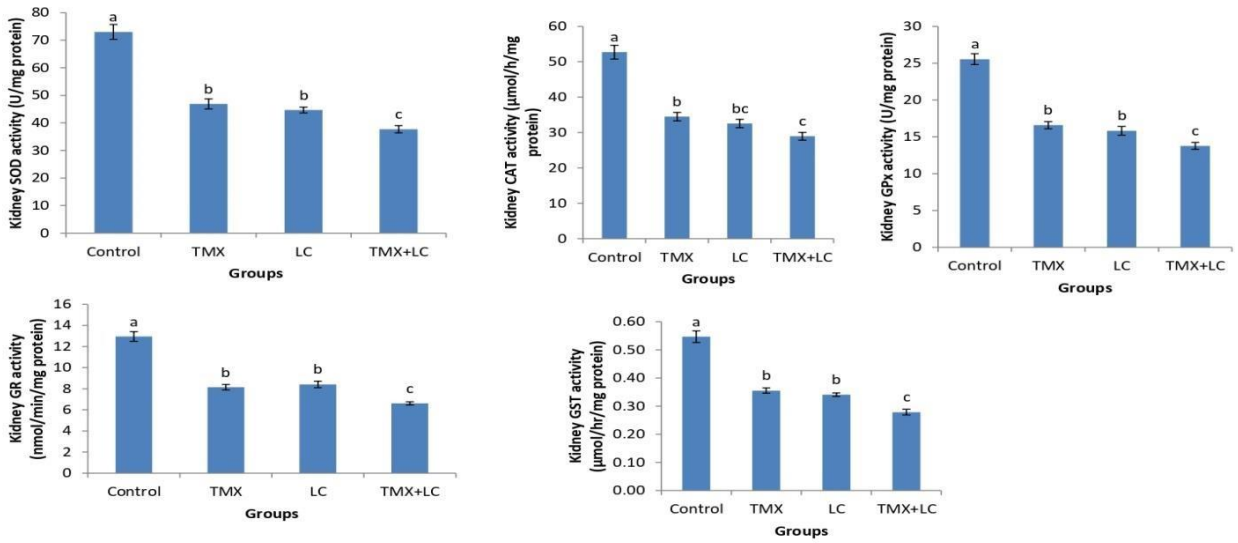

Figure (3) :.Changes in superoxide dismutase (SOD) and catalase (CAT) ,glutathione peroxidase (GPx) , glutathione reductase (GR) , glutathione S-transferase (GST) activity in kidney of male rats treated with thiamethoxam (TMX), lambda cyhalothrin (LC) and their combination. Values are expressed as means±SE of seven rats per group. Means with different letters are significantly different (P<0.05).

3.4. Effect of thiamethoxam (TMX), lambda cyhalothrin and their combination on some hematological parameters of rats

Table(4): Effect of thiamethoxam, lambda cyhalothrin and their combination on blood pictures in rats

Experimental groups	Parameters			
	RBCs (10^6/µl)	WBCs (10^3/mm^3)	Platelets (10^3 /mm^3)	Hb (g/dl)
Control	5.02±0.035^a	6514±156^a	312±9.00^a	13.98±0.295^a
TMX	3.71±0.110^b	8043±443^b	244±6.39^b	10.44±0.321^b
LC	3.95±0.073^b	8571±528^b	247±8.42^b	10.62±0.320^b
TMX+LC	3.38±0.080^c	14571±443^c	224±4.55^c	9.24±0.207^c

Values are expressed as means ± SE; n=6 for each treatment group. ^{abc}Mean values within a row not sharing a common superscript letters were significantly different, $p< 0.05$.

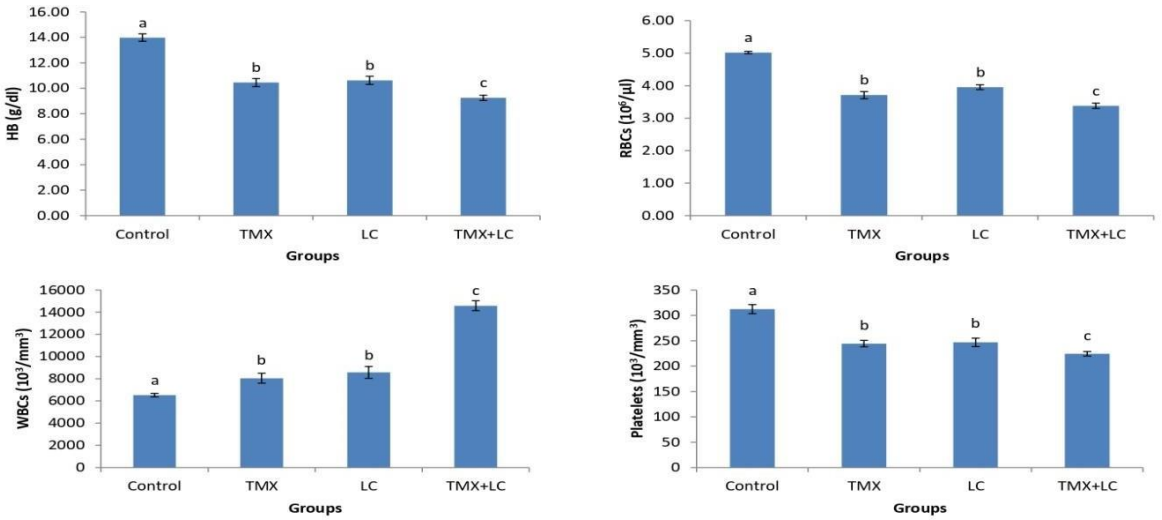

Figure (4) :.Changes in hemoglobin (HB) and red blood cells (RBCS) , white blood cells (WBCs),platelets (Plts) level in blood of male rats treated with thiamethoxam (TMX), lambda cyhalothrin (LC) and their combination. Values are expressed as means±SE of seven rats per group. Means with different letters are significantly different (P<0.05).

4. Discussion

Kidney is the critical target organ for xenobiotic compounds which produce a variety of renal toxic effects involving tubular cells and glomerulus (**28**). These compounds inhibit the incorporation of amino acid into protein causing an increase in urea levels which is the major nitrogen-containing metabolic product of protein metabolism (**29**). In the present study, increased plasma creatinine and urea levels reflect the diagnosis of renal failure (**30**). Moreover, elevated blood urea is known to be correlated with an increased protein catabolism in mammals and/or the conversion of ammonia to urea as a result of increased synthesis of arginase enzyme involved in urea production. The elevation in serum urea and creatinine levels in TMX and/or LC- treated rats are considered as significant markers, of renal dysfunction and it may be related to metabolic disturbances in liver function, as urea is the end-product of protein catabolism. Furthermore, xenobiotics intensify the acid-secretory function of kidney and change the transport of sodium (**31**). Creatinine is derived mainly from the catabolism of creatine found in muscle tissue and its catabolism to creatinine occurs at a steady rate. Severe kidney damage will lead to increased creatinine levels. In the present study, serum creatinine showed marginal increase in the thiamethoxam treated group in comparison to control animals and increase relates to renal failure. Serum creatinine and urea determine the glomerular filtration rate (GFR) improperly in

renal failure. Serum creatinine and BUN have the potential to be a more precise marker for GFR. Similar results were reported in earlier studies in rats (**32, 33**).

a significant ($P < 0.05$) increase in kidney TBARS and H2O2 concentrations, the indicator of LPO, after the administration of TMX and/or LC to rats as compared to control. On the other hand, TMX and/or LC- treated rats showed a significant decrease in glutathione content (GSH). In addition, administration of TMX and/or LC together significantly raised the levels of TBARS and H2O2 and decreased GSH content leading to more toxic effect in rat's kidney as compared to control. Reactive oxygen species (ROS) are constantly produced inside the mammalian body due to the exposure to many chemicals, drugs and xenobiotics in our ecosystem and/or many endogenous metabolic processes involving electron transport mechanism **(34).** Oxidative stress is a deleterious condition which induces cell injury, and subsequent cell death, due to oxidation of cardinal cellular components, such as lipids, proteins and DNA (**35**).

Therefore, SOD and CAT are thought to limit the ROS accumulation. ROS can be produced in cells as by-products of normal cellular metabolism and also under stress situations. Various pesticides may induce oxidative stress that causes generation of free radicals and alters antioxidants **(36**).

Toxicological and safety assessment studies in laboratory animals consists of well characterized hematological, biochemical, and histopathological analyses that inform on general body homeostasis and organ function and/or injury. Blood and hematopoetic tissues rank with liver and kidney as target organs for toxic effects of environmental chemicals, pharmacological agents and biologicals owing to the high mitotic rate of hematopoietic tissues, exposure of blood cells to agents absorbed or injected into the blood stream and the consequences of blood cells damage and bone marrow impairment. Blood being a potential target organ of toxic insult; hematotoxicity can result in altered number and/ or function of circulating blood cells. Complete blood count (CBC) was the core hematological test for assessment of xenobiotic induced hematotoxicity as it contained a variety of variables, providing quantitative (cell numbers) and qualitative (morphology and production) information concerning the functional status of the hematopoietic system (**37**). Hematological investigation of rats treated with TMX, LC and their combination was performed. Blood cell counts attained and related parameters are demonstrated. Rats administered TMX, LC and their combination orally showed significant decrease effect in RBCs, Hb and Platelets while WBCs are significantly increased specially in the combination

group that administrate both TMX and LC.

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