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Exposure to Pyrethroid Insecticides Initiates Time-course Hematotoxicity that may be ameliorated with Prophylactic Combined Vitamin C and E Therapy

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ABSTRACT

Objectives: To determine the impact of pyrethroids exposure on hematological parameters and to evaluate the effect of prophylactic combined vitamin C and E therapy (PVT).

Experimental Protocol: 60 albino rats were divided into 6 equal groups: IA received no medications, IB, IIA and IIB received 2-wk PVT, IIIA and IIIB did not receive PVT. Animals of groups IIA and IIIA received short-term exposure (4 weeks) and animals of groups IIB and IIIB received long-term exposure (12 weeks) at 2-bouts/min for 240 min/day. At the end of the exposure period, blood samples were obtained for estimation of hemoglobin concentration (HBC), packed cell volume (PCV), mean corpuscular volume (MCV), and total and differential leucocytic counts (TLC & DLC).

Results: Pyrethroid exposure significantly reduced RBCs indices and increased TLC and neutrophil count. These changes were more significant after long-term than short-term exposure. Further, these changes were significant in animals without than animals received PVT. Animals that received PVT followed by short-term exposure showed insignificant estimates compared to controls and showed significantly lower estimates than animals without PVT. Also, animals that received long-term exposure without PVT showed significant changes to animals that received PVT. Low MCV was defined as screening and high neutrophil count as a specific variate to identify samples of exposed animals. A high monocyte count was defined as positive and a high HBC was a negative indicator for long-term exposure. High PCV was defined as specific, while low TLC was a screening predictor for receiving PVT.

Conclusion: Pyrethroid exposure induced hematotoxicity, which is proportional to the duration and time of exposure. Estimated HBC, PCV% and TLC could discriminate long-term exposure. Prophylactic combined vitamins C and E could ameliorate or decrease the pyrethroid-exposure effects.

INTRODUCTION

Pesticides are a family of chemicals that induced worldwide water pollution and comprised a huge number of subfamilies of which pyrethroids are insecticides that adversely affect the human nervous system (Dara & Drabovich, 2023). The potential occupational exposure to, misuse of pesticides and improper application within homes, apartments, and other buildings may require remediation prior to reoccupation (Willison *et al.*, 2023).

Pyrethrum is extracted from the Dalmatian pyrethrum (Tanacetum cinerariifolium), which is an outcrossing plant species endemic to the Eastern Adriatic coast and is a source of the natural insecticide (Yamashiro *et al.,* 2022). Pyrethrum is characterized by being rapidly broken by UV light and changes in pH, so it is safe for repeated use as a spray for fruit and vegetables and for quick knock-down (Varga *et al.*, 2022).

Pyrethrum is a composite material of pyrethrins which are effective on a broad range of insects and have very low risk to humans and pets when used around the home (Zeng *et al.,* 2021). Pyrethroids are synthetic insecticide with similar activity to pyrethrins but are advantageous as an insecticide for being more persistent and resistant to the decomposing effects of UV and pH, thus it is characterized by their longer duration of activity, to be used for long-term insect control (Yan *et al.,* 2021).

Pyrethroids exposure significantly increases the production rate of reactive oxygen species and induced cellular apoptosis with activation of the inflammasome complex (Castillo *et al.,* 2022). Another study reported upregulation of the production rate of interferon-gamma, interleukin (IL)-13, and keratinocyte chemo-attractant/human growth-regulated oncogene in the developing hippocampus (Pitzer *et al.,* 2022).

Subchronic and chronic exposure to pyrethroids induces hematotoxicity, hepatotoxicity and immunotoxicity with a decrease in the numbers of natural killer cells and splenic proliferative response to lipopolysaccharide with increased serum IgG levels (Aroonvilairat *et al.,* 2018).

This study tried to determine the effects of pyrethroids exposure on hematological parameters and to evaluate if there is a possible prophylactic role for combined vitamin C and E therapy against the effects.

MATERIALS AND METHODS

Design:

A prospective comparative experimental animal study.

Setting:

Departments of Forensic & Clinical Toxicology and Clinical Pathology, Faculty of Medicine, in conjunction with Physiology Department, Faculty of Agriculture, Benha University

Ethical Considerations:

This study followed the guidelines previously described by Clark *et al.*, (1997) for the Care and Use of Laboratory Animals and the protocol was approved by the Local Ethical Committee in the Faculty of Medicine, Benha University by RC: 4.2.2023.

Experimental Protocol:

The studied animals were purchased from The Animal Farm and grown in the animal house, Faculty of Veterinary Medicine, Benha University. Sixty adult albino rats of the weight range of 250-300 g and aged 8-10 weeks were provided with a standard diet and free water supply and kept under a temperature of 20^{o}C, humidity rate of 60%, and 12-hs day/night cycle for two weeks to be acclimatized to the new housing. After the period of

acclimatization, animals were divided into three groups but all were maintained on the same growing standard conditions till the end of the study duration.

Grouping

1. Animals of Group-I were divided into two subgroups: group-IA did not receive prophylactic therapy or exposure to pyrethroids and group-IB received ration fortified by a combination of vitamins C and E.
2. Animals of Group-II received the prophylactic therapy for 2 weeks and then were divided into two subgroups; Group-IIA and Group-IIB according to the duration of pyrethroid exposure.
3. Animals of Group-III were divided into Group-IIIA and Group-IIIB according to the duration of pyrethroid exposure

Prophylactic Vitamin C & E regimen:

The prophylactic vitamin therapy (PVT) was prepared as 250 mg of vitamin C (Assayed *et al.,* 2010) and 250 mg of vitamin E (Lamfon *et al.,* 2013) mixed with one kilogram of ration and freshly prepared according to requirements for the 2-wks of PVT.

Exposure Procedure:

The pyrethroids-exposed animals were divided between four special transparent fiberglass cages to allow easier observation during the exposure. The pyrethroid spray was provided using Bosharon Wall Mounted LCD Automatic Air Freshener Dispenser with spray intervals of 1-60 minutes (Guangdong, China, Model Number: B-301). Bosharon air freshener is characterized by a capacity of 300 ml can which could accommodate the container of pyrethroid spray. The pyrethroid was provided as New Pyrosol Plus spray (El Nasr Co. for Intermediate Chemicals, Reg. No. 1793 at the Egyptian Ministry of Health, 300 ml). The dispenser was adjusted to provide two bouts of pyrethroid spray and 1-min spray-off for 240 minutes.

Exposure Duration:

1. Short-term exposure: Animals of groups IIA and IIIA were exposed to pyrethroids for 4h/day for four continuous weeks
2. Long-term exposure: Animals of groups IIB and IIIB were exposed to pyrethroids for 4h/day for twelve continuous weeks

Blood Sampling:

Blood samples were obtained as previously described (Kumar *et al.,* 2017) from the saphenous vein in a shaved hind limb after the rat was restrained for a very short time. The hind limb was immobilized in the extended position and using a fine needle, the saphenous vein was punctured and the blood sample was collected and the puncture site was pressed on to guard against open or subcutaneous bleeding. Blood was collected into a dry clean tube containing ethylene diamine tetra-acetic acid (EDTA) about 1.8 mg trik EDTA/ 1 ml blood to prevent blood coagulation and kept at -20^{o}C till being assayed.

Investigations:

1. Hemoglobin concentration (HBC) was measured by cyanmethemoglobin method (International committee for standardization in Hematology, 1967).
2. Packed cell volume (PCV) is measured as the ratio between the volume occupied by the red blood cells and the volume of the whole used blood sample and was expressed as a fraction (Bull *et al.,* 2000).
3. Mean corpuscular volume (MCV) is calculated as PCV divided by the number of RBCs per liter to be expressed in femtoliter (10^{-15} L). MCV measures the average volume of RBC in a given blood sample and routinely was expressed in μm^3 (Bull *et al.,* 2000).
4. Total and differential leucocytic counts (TLC & DLC) were determined.

Statistical Analysis:

Results were analyzed using the t-test for independent means by SPSS software package (IBM, USA). The predictive value of the estimated variate was evaluated using the Regression analysis, Receiver operating characteristic curve, and the predictive cutoff points were determined and assessed using Kaplan-Meyer analysis. P-values were illustrated at the bottom of the tables.

RESULTS

All estimated RBCs indices showed non-significant differences between groups IA and IB. Estimated HBC and PCV% in samples of group-IIA were non-significantly lower, while MCV were significantly lower in comparison to estimates of samples of groups IA and IB (P=0.040 & 0.026, respectively). On contrary, RBCs estimates in samples of group-IIB were significantly lower than that of groups IA and IB, and HBC was significantly (P=0.0179) lower, while PCV% and MCH were non-significantly lower than estimates in samples of group-IIA. Estimated HBC, PCV% and MCV in samples of group-IIIB were significantly lower compared to levels estimated in samples of groups IA, IB, IIA and IIIA. Further, estimated RBCs indices in samples of group-IIIA were significantly lower in comparison to estimates in samples of group-IIA. As for the estimated 1st-h ESR, the differences between samples of the exposed and unexposed animals were non-significant (Table 1).

Table 1: Hematological variate estimated in blood samples obtained from the studied animals categorized according to receiving PVT and duration of pyrethroid exposure.

Variate	Group		Group I (No Exposure)		Group II (2-wk PVT)		Group III (No PVT)	
			IA (No PVT)	IB (2-wk PVT)	IIA (4-wk exposure)	IIB (12-wk exposure)	IIIA (4-wk exposure)	IIIB (12-wk exposure)
HBC (g/dl)	Value		12.2±0.53	12.25±0.45	12±0.49	11.44±0.7	11.26±0.62	10.77±0.36
	Significance of difference versus	Group-IA		0.411	0.245	0.0067†	0.0009†	<0.001‡
		Group-IB			0.166	0.0033†	0.0004†	<0.001‡
		Group-IIA				0.0179*	0.0029†	
		Group-IIIB				0.0075†	0.0222*	
PCV (%)	Value		42.75±0.84	42.8±0.77	42.23±0.78	41.83±0.95	40.6±1	39.5±0.91
	Significance of difference versus	Group-IA		0.446	0.085	0.017*	0.00003†	<0.001‡
		Group-IB			0.059	0.011*	0.00001†	<0.001‡
		Group-IIA				0.159	0.00034†	
		Group-IIIB				0.0093†	<0.001‡	
MCV (μ^3)	Value		64.21±2.22	64.35±2.1	62.52±1.86	61.33±2.2	61±0.73	59.51±1.4
	Significance of difference versus	Group-IA		0.443	0.040*	0.0045†	0.0002†	<0.001‡
		Group-IB			0.026*	0.0026†	<0.001‡	<0.001‡
		Group-IIA				0.104	0.0136*	
		Group-IIIB				0.0039†	0.020*	
1-h ESR	Value		2.27±0.59	2.29±0.64	2.25±0.21	2.23±0.37	2.18±0.42	2.15±0.28
	Significance of difference versus	Group-IA		0.471	0.461	0.429	0.350	0.284
		Group-IB			0.427	0.400	0.327	0.266
		Group-IIA				0.442	0.391	
		Group-IIIB				0.323	0.297	

HBC: Hemoglobin concentration; PCV: Packed corpuscular volume; MCV: mean corpuscular volume; ESR: Erythrocyte sedimentation rate; PVT: Prophylactic vitamin therapy; Statistical analysis was performed using t-test for two independent means; * indicates significant, † indicates very significant and ‡ indicates highly significant difference

Estimated TLC and DLCs showed non-significant differences between samples of groups IA and IB. Samples of group-IIA showed significantly (P=0.027) higher neutrophil count than samples of group-IA, while TLC and other estimated variate of DLC were non-

significantly higher than in samples of both groups I. Samples of group-IIB and IIIA showed significantly higher TLC and neutrophil counts than samples of groups IA, IB and IIA with significantly higher counts in samples of group-IIIA than samples of group-IIB. TLC and neutrophil count were significantly higher in samples of group-IIIB than in samples of groups I and II, while the difference was non-significant in comparison to samples of group-IIIA. On contrary, lymphocyte, eosinophil and basophil counts showed non-significant differences between all samples (Table 2)

Table 2: Total and differential leucocytic counts estimated in blood samples obtained from the studied animals categorized according to receiving PVT and duration of pyrethroid exposure.

Variate	Group		Group I (No Exposure)		Group II (2-wk PVT)		Group III (No PVT)	
			IA (No PVT)	IB (2-wk PVT)	IIA (4-wk exposure)	IIB (12-wk exposure)	IIIA (4-wk exposure)	IIIB (12-wk exposure)
TLC (cells/ml)	Value		7159±431	7260±341	7398±223	8009±617	8564±968	8895±730
	Significance of difference versus	Group-IA		0.284	0.069	0.0011†	0.0003†	<0.001‡
		Group-IB			0.149	0.0018†	0.0004†	<0.001‡
		Group-IIA				0.0044†	0.0008†	
		Group-IIIB				0.0045†	0.199	
Neutrophil count (cells/ml)	Value		5266±374	5337±644	5553±233	6089±669	6792±899	6932±726
	Significance of difference versus	Group-IA		0.383	0.027*	0.0017†	0.0001†	<0.001‡
		Group-IB			0.166	0.01†	0.0003†	<0.001‡
		Group-IIA				0.014*	0.00025†	
		Group-IIIB				0.007†	0.353	
Lymphocytes (cells/ml)	Value		1230±161	1263±134	1285±167	1257±194	1227±158	1247±85
	Significance of difference versus	Group-IA		0.312	0.233	0.367	0.482	0.385
		Group-IB			0.377	0.471	0.294	0.377
		Group-IIA				0.370	0.219	
		Group-IIIB				0.439	0.363	
Monocyte count (cells/ml)	Value		291±58	284±65	262±49	327±60	241±48	424±43
	Significance of difference versus	Group-IA		0.402	0.121	0.094	0.025	<0.001‡
		Group-IB			0.202	0.071	0.056	<0.001‡
		Group-IIA				0.008†	0.172	
		Group-IIIB				0.0003†	<0.001‡	
Basophil count (cells/ml)	Value		189±56.7	178±30.8	152±28.7	146±23	140±31	128±23
	Significance of difference versus	Group-IA		0.298	0.124	0.111	0.096	0.119
		Group-IB			0.176	0.154	0.131	0.170
		Group-IIA				0.307	0.188	
		Group-IIIB				0.051	0.167	
Eosinophil count (cells/ml)	Value		180±55	177±31.6	162±27	177±51.6	165±45	192±52.7
	Significance of difference versus	Group-IA		0.439	0.181	0.342	0.254	0.141
		Group-IB			0.133	0.235	0.248	0.057
		Group-IIA				0.212	0.112	
		Group-IIIB				0.209	0.264	

TLC Total leucocytic count; PVT: Prophylactic vitamin therapy; Statistical analysis was performed using t-test for two independent means; * indicates significance, † indicates the very significance and ‡ indicates highly significant difference

The studied variate was evaluated as discriminators for samples of exposed animals using the Multivariate Regression analysis which defined high MCV as a negative (β=-0.400, P=0.005), while high neutrophil count as a positive predictor (β=0.287, P=0.042) for pyrethroid exposure, irrespective of the duration of exposure. ROC curve analysis of the studied variate assured these findings and defined low MCV as a sensitive screening variate for suggesting exposure with AUC of 0.121±0.05 (95%CI: 0.023-0.220; P<0.001) and high neutrophil count as specific variate to identify samples of exposed animals with AUC of 0.907±0.044 (95%CI: 0.821-0.994) as shown in Figure 1.

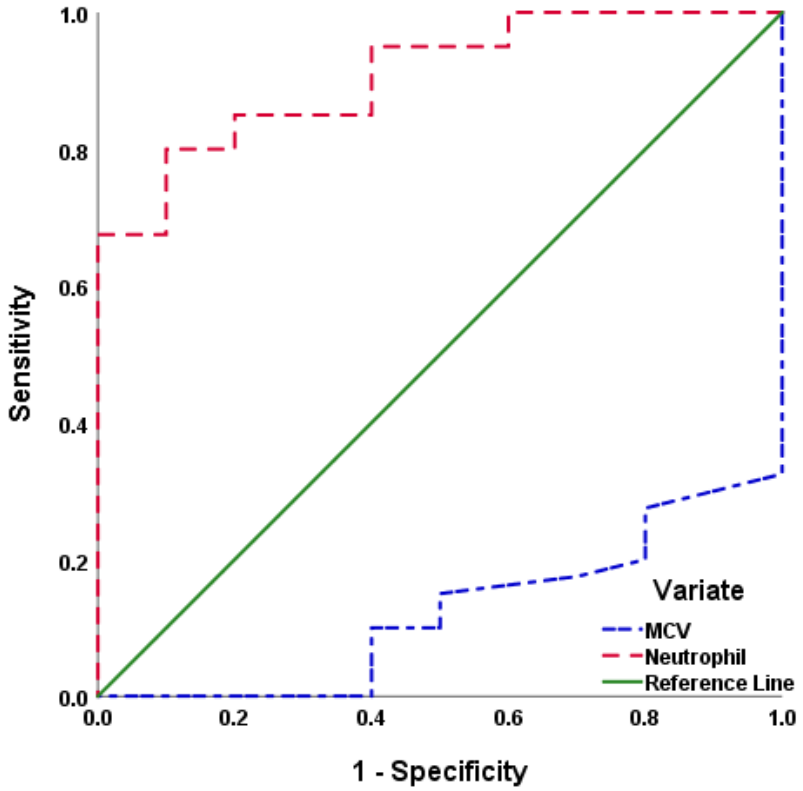

Fig. 1: ROC curve analysis of the estimated variate for discriminating samples of exposed animals

Kaplan-Meyer analysis defined MCV of ≤56 (μ^3) as indicative of the risk of exposure by 100% and MCV at 62 (μ^3) as indicative of a 50% risk of exposure (Fig. 2), while the neutrophil count of 6000 (cells/ml) indicates a 50% risk of exposure and this risk is doubled to 100% if the neutrophil count was about 6500 (cells/ml), (Fig. 3).

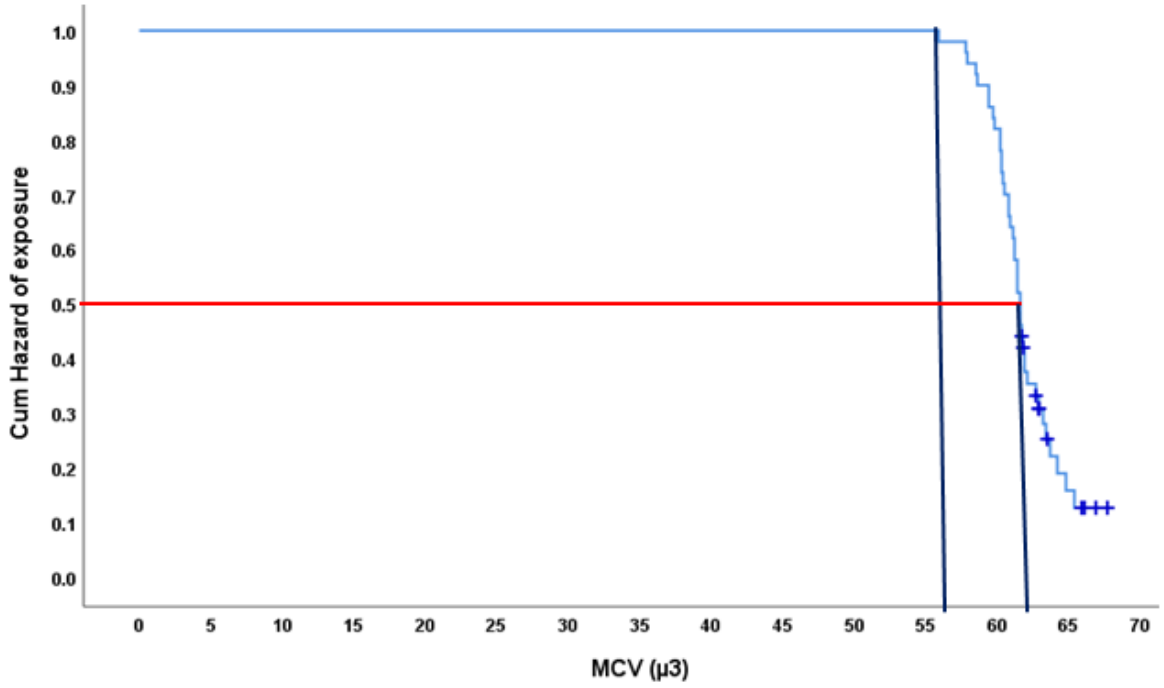

Fig. 2: The cumulative hazard curve of Kaplan-Meyer analysis for pyrethroid exposure according to MCV

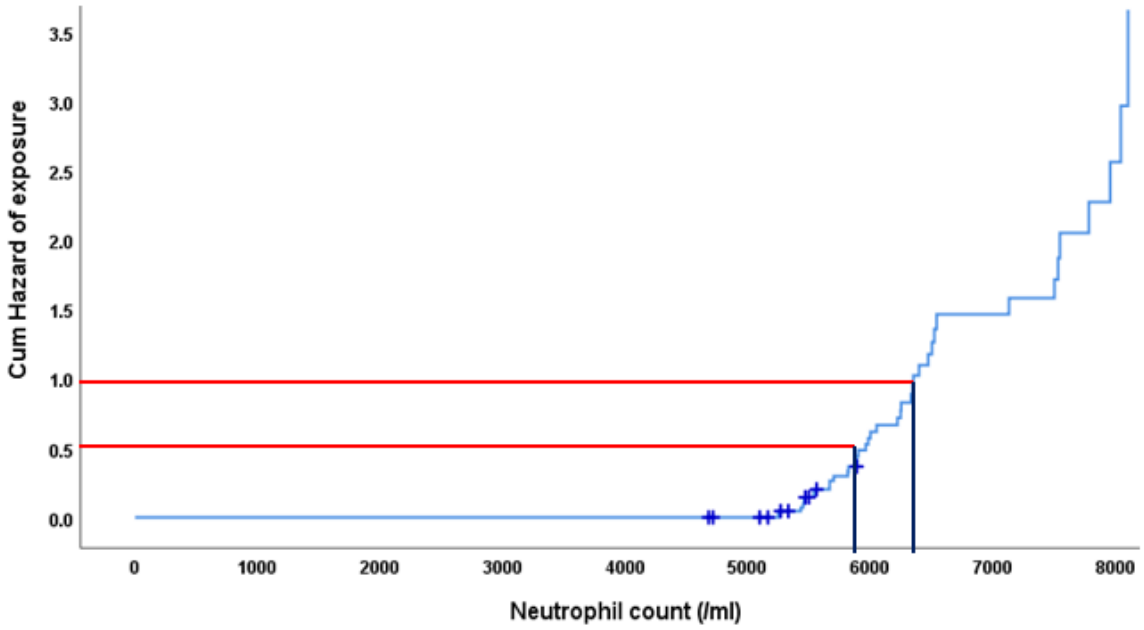

Fig. 3: The cumulative hazard curve of Kaplan-Meyer analysis for pyrethroid exposure according to neutrophil count

Regression analysis of the estimated variate defined high monocyte count as a positive predictor (β=0.588, P<0.001) and high HBC as a negative indicator (β=-0.266, P=0.034) for chronic pyrethroid exposure. ROC curve analysis also identified high monocyte count as a specific predictor for chronic exposure with AUC of 0.868±0.059 (95%CI: 0.752-0.983; P<0.001) and low HBC as a significant screening variate for chronic exposure with AUC of 0.253±0.078 (95%CI: 0.100-0.405; P=0.007) as shown in Figure 4.

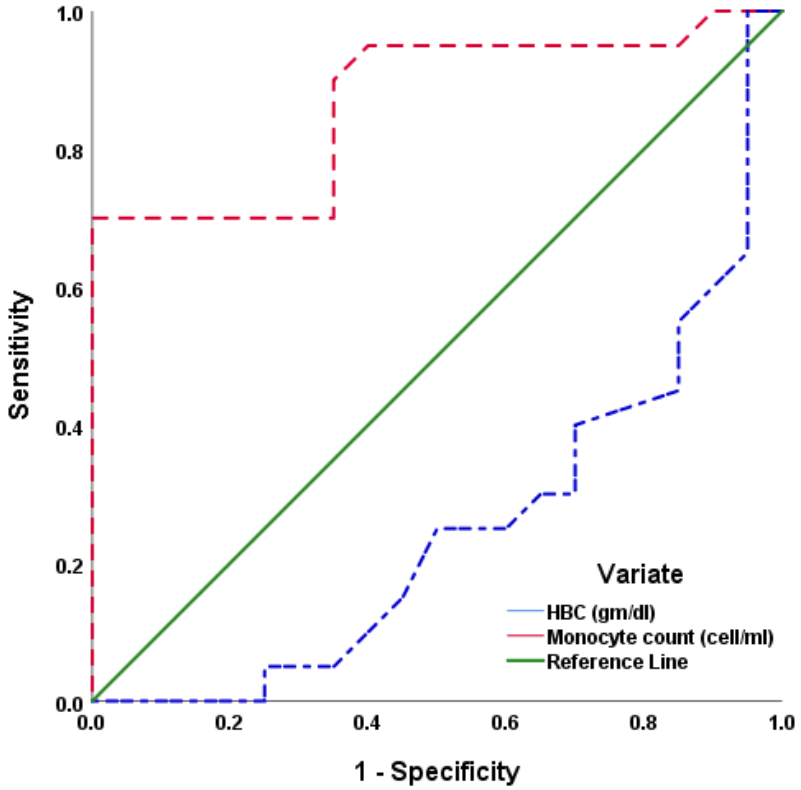

Fig. 4: ROC curve analysis of the estimated variate for discriminating samples of chronically exposed animals.

Kaplan-Meyer analysis defined monocyte count of about 400 (cells/ml) and HBC at 12 g/dl as cutoff points suggestive of a risk of chronic exposure of 50%, but this risk is 100% at HBC of ≤10.3 g/dl (Figs. 5 & 6, respectively).

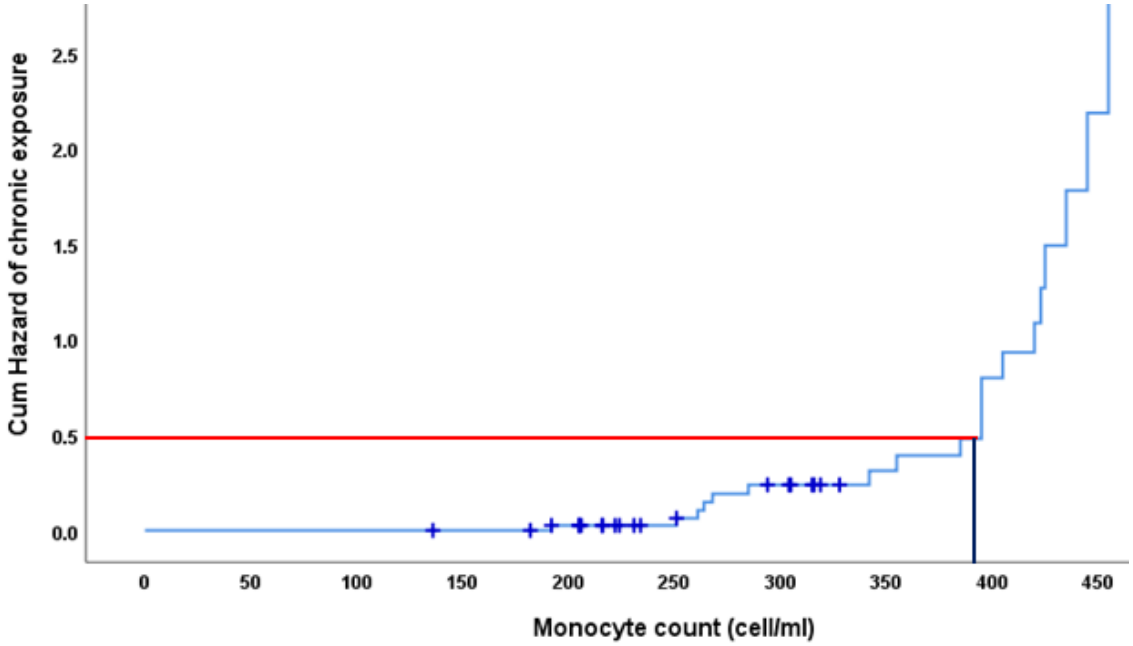

Fig. 5: The cumulative hazard curve of Kaplan-Meyer analysis for chronic pyrethroid exposure according to monocyte count

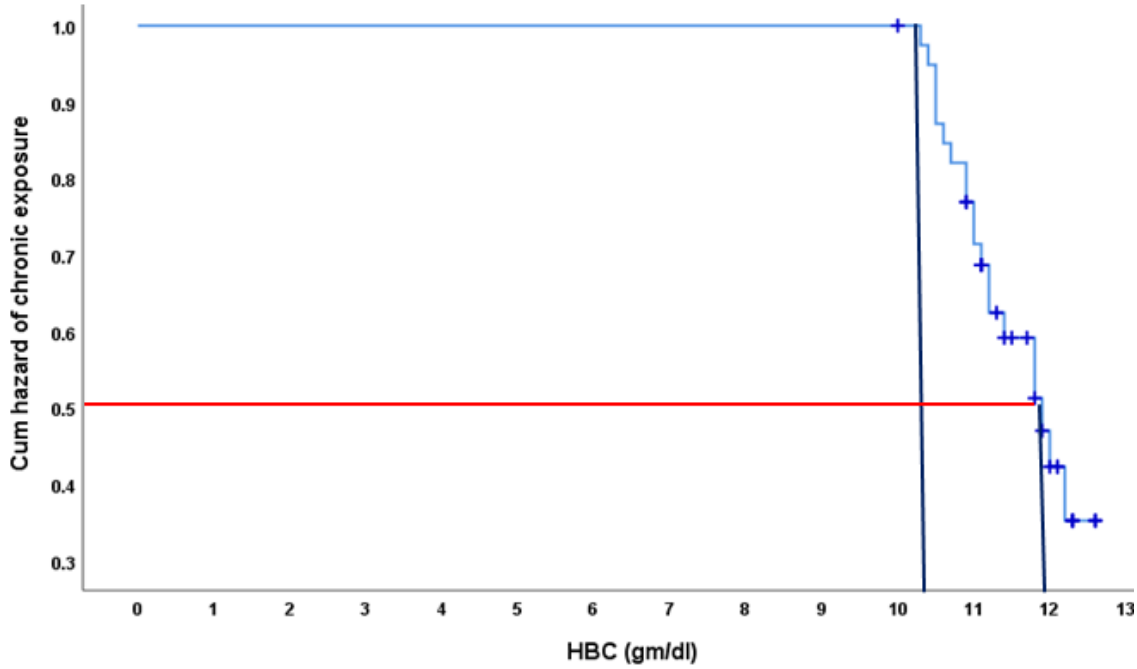

Fig. 6: The cumulative hazard curve of Kaplan-Meyer analysis for chronic pyrethroid exposure according to HBC.

Evaluation of the estimated variables to identify samples of animals that received PVT using ROC curve analysis identified high PCV as a specific variate that can predict receiving PVT with AUC of 0.893±0.05 (95% CI: 0.795-0.990; P<0.001), while high TLC

as a screening variate to predict the absence of PVT with AUC of 0.170 (95% CI: 0.042-0.298; P<0.001) as shown in Figure 7.

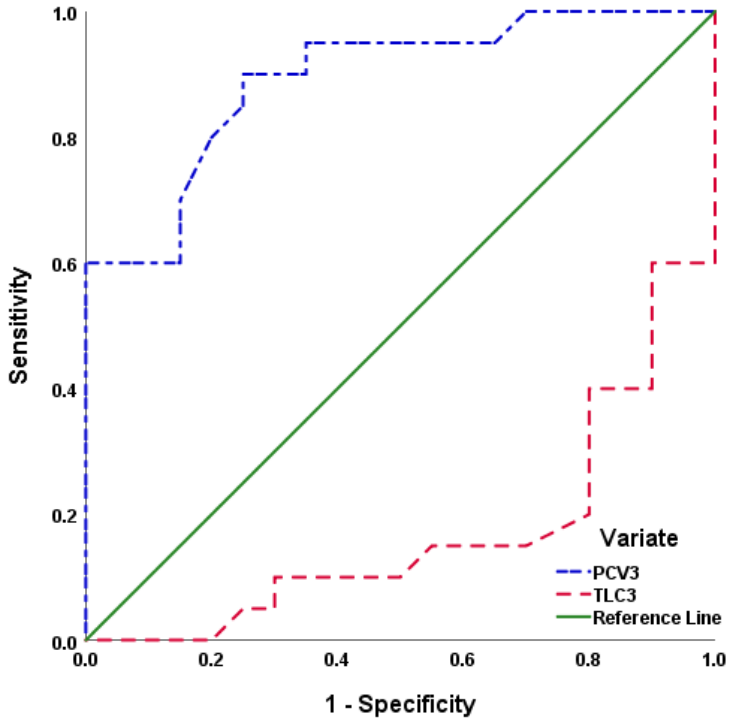

Fig. 7: ROC curve analysis of the estimated variate for identifying samples of animals that received PVT

Regression analysis defined high PCV as the predictor for receiving PVT (β=0.548, P<0.001) with higher significance than high TLC (β=-0.274, P=0.039). Kaplan-Meyer analysis defined PCV at ≤40% to define the risk of absence of PVT with a risk ratio of 100% and PCV at 42.5% to predict this risk by >70% (Fig. 8), while TLC of >7000 cell/ml indicates this risk by about 22% and >8000 cell/ml by about 60% (Figs.8& 9).

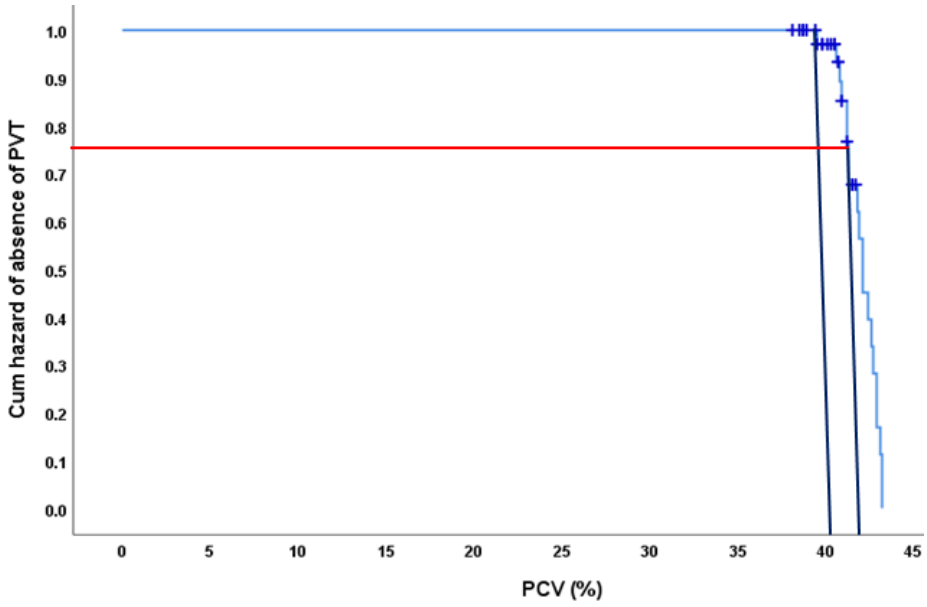

Fig. 8: The cumulative hazard curve of Kaplan-Meyer analysis for the absence of PVT according to PCV

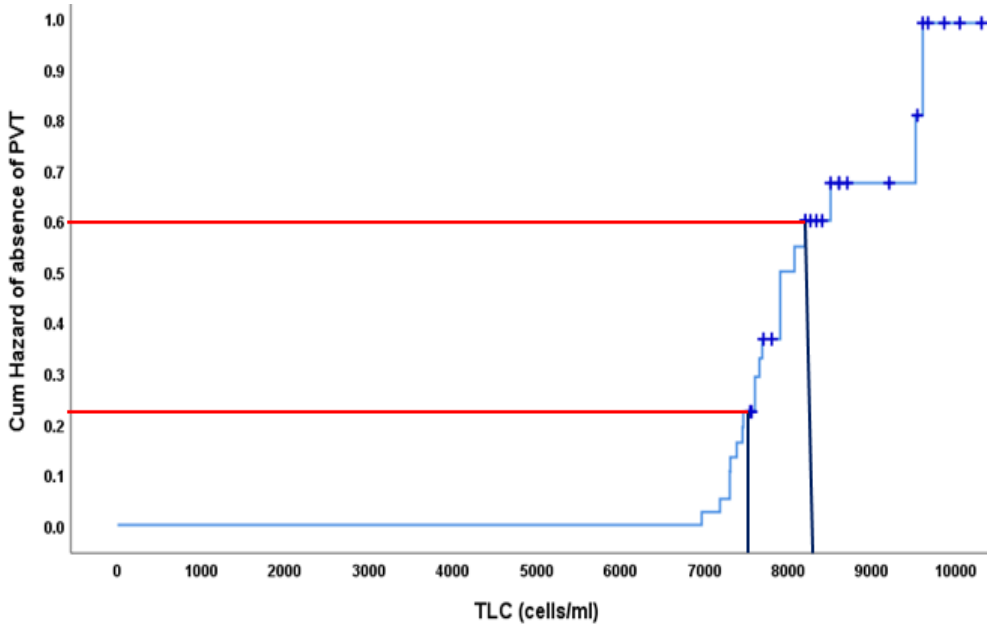

Fig. 9: The cumulative hazard curve of Kaplan-Meyer analysis for the absence of PVT according to TLC.

DISCUSSION

Pyrethroids' exposure showed manifest hematotoxicity that was exaggerated with long-term exposure. Pyrethroids seriously affected all cellular blood components, a finding indicating the possibility of deregulation of the synthetic ability of bone marrow or shift of progeny cells to differentiate in leucocyte direction. In support of this assumption, all RBC-related measures were significantly lower, while TLC and DLCs were significantly higher in exposed than unexposed animals and the changes were related to the duration of exposure with inverse and paralleled relation, respectively.

These findings supported the results of Pande *et al.* (2014) and Dar & Kaur (2014) who detected a significant decrease in the total RBCs count, hemoglobin concentration, PCV, MCV, and MCH, while TLC and DLCs were significantly increased on cutaneous exposure of cypermethrin, a type II synthetic pyrethroid insecticide. Thereafter, Narra (2016) reported that pyrethroid exposure causes dwindling of blood respiratory burst activity, RBCs count, hematocrit value and hemoglobin concentration with elevated white blood cell count, lipid peroxidation levels and reduction of glutathione levels and glutathione peroxidase activity

Also, Aroonvilairat *et al.*, (2018) observed significant changes in various biological parameters including hematotoxicity that was manifested as increased leukocyte counts and percent neutrophil, with decreased RBC count. Moreover, in an observational study, Mestre *et al.* (2019) detected an immunosuppressant effect of pyrethroid manifested as decreased heterophils/ lymphocytes index, lobularity index, natural antibodies titers and complement system activity in exposed than unexposed Salvator merianae. Recently, Cestonaro *et al.*, (2022) suggested that pyrethroids interfere with innate and adaptive immune functions on both cellular and humoral levels and induce changes in specific immune cells leading to initiation of apoptosis, upregulation of pro-inflammatory cytokines and changes in factor nuclear kappa B expression.

Multiple previous studies tried to explore the underlying mechanisms for decreased RBCs indices; Khan *et al.*, (2009) attributed this effect to decreased RBCs synthesis and/or reduced heme biosynthesis in the bone marrow. In line with this explanation, Abbassy &

Mossa (2012) suggested that the reduction in RBCs number is due to erythrocytopenia with impaired hemoglobin synthesis in the erythroblasts secondary to the concomitant bone marrow hypoplasia and dilutional anemia. Thereafter, Lamfon *et al.,* (2013) attributed these changes to the capability of pyrethroids to induce alterations in blood-forming organs and Bhushan *et al.,* (2013) reported that pyrethroids induce interference of flowing of mature components in peripheral blood or induction of altered development of such integral blood components.

Another explanation was provided by Dar & Kaur, (2014) who detected renal affection by pyrethroid exposure with reduced production of erythropoietin and suggested that this may be the cause for the decreased viability of cells of the erythropoietic tissue because erythropoietin is required for competent erythropoiesis. In support of this suggestion, Kašuba *et al.,* (2022) detected decreased cholinesterase activities on pyrethroid exposure that also induced DNA damage in the studied cell types at almost all of the applied doses.

Interestingly, combined vitamin C and E pretreatment of animals exposed to pyrethroid nearly ameliorated the hematotoxic effect of short-term pyrethroid exposure and significantly decreased its effects on long-term exposure. These ameliorative effects of vitamin C, a water-phase antioxidant and vitamin E, lipid-(membrane) phase antioxidant could be attributed to their antioxidant effects and the nearly abolishing of hematotoxicity of short-term exposure to pyrethroids may be due to the previously documented synergistic antioxidant action (Kaźmierczak-Barańska *et al.,* 2020; Polutchko *et al.,* 2021).

Multiple previous studies documented a similar protective effect of vitamin C and/or E against noxious effects of exposure to materials inducing oxidative stress that is characterized by increased oxidative products and consumption of antioxidants or reduction of their activities (Kumari *et al.,* 2013; Hongsibsong *et al.,* 2014; Kandpal *et al.,* 2019; Paduraru *et al.,* 2021).

Further, Ghasemi *et al.,* (2021) found the treatment of mesenchymal stem cells with vitamin E enhanced its ability to inhibit dendritic cells and improved its immunomodulatory effects with decreased expression levels of inflammatory and upregulated expression of anti-inflammatory cytokines.

Concerning pyrethroid toxicity, the protective effect of vitamins C and E was previously assured for various forms of pyrethroid-induced organ toxicity, wherein, Manzoor *et al.,* (2016) reported that vitamin C neutralized pyrethroid renal toxicity in mice and Bhardwaj *et al.,* (2018) found co-administration of vitamins C and E significantly increased antioxidant enzymes' activity, decreased lipid peroxidation and ameliorated pyrethroid-induced testicular germ cell apoptosis. Moreover, using an animal model of pyrethroid-induced organ toxicity, Al-Omar *et al.,* (2020) found supplemental vitamins C and E allowed organ recovery and suggested a prophylactic effect of the concurrent use of this vitamin combination for the subjects under the exposure of pyrethroids.

The reported ameliorating effect of vitamin combination on pyrethroid-induced hematotoxicity might be due to their antioxidant properties that increased membranes' reactive oxygen species (ROS) scavenging causing their reduction into hydroperoxides and restoring antioxidant enzyme activities (Cinar *et al.,* 2010), decreased levels of lipid peroxidation and hydrogen peroxide production (Azeez *et al.,* 2020), and protecting RBCs cell membranes against oxidative damages (Hesham *et al.,* 2021).

Moreover, Feriani *et al.,* (2020) found vitamin E pre-treatment had prevented the pro-atherogenic effect of pyrethroids with decreased plasma and aortic tissue levels of oxidized low-density lipoprotein and the plasma levels of the pro-inflammatory cytokines. Recently, using in-vitro exposure of human glioblastoma cells to pyrethroid, Lin *et al.,* (2022) found pyrethroid-induced cytotoxicity by increasing ROS productions, decreasing

reduced glutathione content and regulation of apoptosis-related protein expression levels and detected partial reversal of these effects in cells pretreated with vitamin E.

In support of the possibility of oxidative stress as the mechanism of toxicity of pyrethroids, Odetti *et al.,* (2022) detected downregulation in the expression of genes of superoxide dismutase and catalase in experimental than control animals and thereafter detected significantly lower expression levels of heat-shock protein-70 with altered growth parameters and DNA damage in exposed than unexposed animals (Odetti *et al.,* 2023).

Conclusion

Exposure to pyrethroid spray induced hematotoxicity in the form of reduced RBCs indices and leukocytosis and this effect is proportional to duration and time of exposure. Estimated HBC, PCV% and TLC could discriminate chronically exposed subjects. Prophylactic combined vitamins C and E could ameliorate or decrease the pyrethroid-exposure effects.

Recommendations

Agricultural workers and persons exposed to pyrethroids must be continuously checked for their hematological variate. Unexposed persons have to receive prophylactic vitamins C and E before being exposed to pyrethroids. Even persons who did not receive prophylaxis have to receive this combination to compensate for the pyrethroid effect. In subjects presenting with any inflammatory condition requiring estimation of TLC and DLC, a history of pyrethroid exposure must be inquired for. Further trials to evaluate the effects of stoppage of exposure are mandatory to determine the effective duration-off exposure for the exposed persons.

REFERENCES

Abbassy MA, Mossa ATH (2012): Haemato-biochemical effects of formulated and technical cypermethrin and deltamethrin insecticides in male rats. *Journal of Pharmacology and Toxicology;* 7:1–10.

Al-Omar MS, Naz M, Mohammed S, Mansha M, Ansari M, Rehman N, Kamal M, Mohammed H, Yusuf M, Hamad A, Akhtar N, Khan R (2020): Pyrethroid-Induced Organ Toxicity and Anti-Oxidant-Supplemented Amelioration of Toxicity and Organ Damage: The Protective Roles of Ascorbic Acid and α-Tocopherol. *International Journal of Environmental Research Public Health*, Aug 25;17(17):6177. Doi: 10.3390/ijerph17176177.

Aroonvilairat S, Tangjarukij C, Sornprachum T, Chaisuriya P, Siwadune T, Ratanabanangkoon K (2018): Effects of topical exposure to a mixture of chlorpyrifos, cypermethrin and captan on the hematological and immunological systems in male Wistar rats. *Environmental Toxicology and Pharmacology,* Apr; 59:53-60. Doi: 10.1016/j.etap.2018.02.010.

Assayed ME, Khalaf AA, Salem HA (2010): Protective effects of garlic extract and vitamin C against in vivo cypermethrin-induced cytogenetic damage in rat bone-marrow. *Mutation Research;* 702:1–7.

Bhardwaj JK, Kumari P, Saraf P, Yadav A (2018): Antiapoptotic effects of vitamins C and E against cypermethrin-induced oxidative stress and spermatogonial germ cell apoptosis. *Journal* of *Biochemical* and *Molecular Toxicology,* Aug;32(8): e22174. Doi: 10.1002/jbt.22174.

Bhushan B, Pande S, Saxena N, Saxena PN (2013): Serum biochemical responses under stress of cypermethrin in albino rat. *Environmental and Experimental Biology;* 11:81–89.

Bull BS, Koepke JA, Simson E, van Assendelft OW (2000): Procedure for determining packed cell volume by Microhematocrit Method: Approved Standard. NCCLS document H7-A3, the 3rd ed. NCCLS, Wayne, Pennsylvania.

Castillo G, Barrios-Arpi L, Ramos-Gonzalez M, Vidal P, Gonzales-Irribarren A, Ramos-Cevallos N, Rodríguez J (2022): Neurotoxicity associated with oxidative stress and inflammasome gene expression induced by allethrin in SH-SY5Y cells. *Toxicology and Industrial Health,* Dec;38(12):777-788. Doi: 10.1177/ 07482337221089585.

Cestonaro LV, Macedo S, Piton Y, Garcia S, Arbo M (2022): Toxic effects of pesticides on cellular and humoral immunity: an overview. *Immunopharmacology and Immunotoxicology,* 44(6):816-831. Doi:10.1080/08923973.2022. 2096466.

Chao B, Ju X, Zhang L, Xu X, Zhao Y (2020): A Novel Prognostic Marker Systemic Inflammation Response Index (SIRI) for Operable Cervical Cancer Patients. *Frontiers in Oncology,* 10:766. 10.3389/fonc.2020.00766

Cinar M, Yigit A, Eraslan G (2010): Effects of vitamin C or vitamin E supplementation on cadmium induced oxidative stress and anaemia in broilers. *The Revue de Médecine Vétérinaire;* 161:449–454.

Clark JD, Gebhart GF, Gonder JC, Keeling ME, Kohn DF (1997): Special Report: The 1996 Guide for the Care and Use of Laboratory Animals. *Journal of Institute of Laboratory Animal Resources;* 38(1):41-48. Doi: 10.1093/ilar.38.1.41. 1997.

Dar S, Kaur R (2014): Hematobiochemical evaluation of dermal subacute cypermethrin toxicity in buffalo calves. *Toxicology International,* Sep-Dec;21(3):283-7. doi: 10.4103/0971-6580.155364.

Dara & Drabovich (2023): Assessment of risks, implications, and opportunities of waterborne neurotoxic pesticides. *Journal of Environmental Sciences (China), Mar;* 125:735-741. Doi: 10.1016/j.jes.2022.03.033.

Feriani A, Hachani R, Tir M, Ghazouani L, Mufti A, Borgi M, Allagui M (2020): Bifenthrin exerts proatherogenic effects via arterial accumulation of native and oxidized LDL in rats: the beneficial role of vitamin E and selenium. *Environmental Science and Pollution Research,* Feb;27(6):5651-5660. Doi: 10.1007/s11356-018-3771-7.

Ghasemi F, Khoshmirsafa M, Safari E, Asgari M, Alemrajabi M, Nojehdehi S, Khorrami S (2021): Vitamin E and selenium improve mesenchymal stem cell conditioned media immunomodulatory effects. *Stem Cell Investigations,* May 7; 8:9. Doi: 10.21037/sci-2020-008.

Hashem M, Abd El Hamied S, Ahmed E, Amer S, Hassan A (2021): Alleviating Effects of Vitamins C and E Supplementation on Oxidative Stress, Hematobiochemical, and Histopathological Alterations Caused by Copper Toxicity in Broiler Chickens. *Animals,* 11, 1739

Hongsibsong S, Stuetz W, Sus N, Prapamontol T, Grune T, Frank J (2014): Dietary exposure to continuous small doses of α-cypermethrin in the presence or absence of dietary curcumin does not induce oxidative stress in male Wistar rats. *Toxicology Reports,* Nov 5; 1:1106-1114. doi: 10.1016/j.toxrep.2014.10.025.

International committee for standardization in Hematology (1967): Recommendations for hemoglobinometry in human blood. *British Journal of Haematology;* 13(Suppl.1): 71.

Kandpal V, Kumar D, Bisht R (2019): Protective effect of vitamin E on haematological parameters in chronic toxicity of hexavalent chromium in laboratory chicks. *Journal of Drug Delivery and Therapeutics,* 9, 388–392. 56.

Kašuba V, Lovaković B, Vrdoljak A, Katić A, Kopjar N, Micek V, Milić M, Pizent A, Želježić D, Žunec S (2022): Evaluation of Toxic Effects Induced by Sub-Acute Exposure to Low Doses of α-Cypermethrin in Adult Male Rats. *Toxics,* Nov 23;10(12):717. doi: 10.3390/toxics10120717.

Kaźmierczak-Barańska J, Boguszewska K, Karwowski BT (2020): Nutrition Can Help DNA Repair in the Case of Aging. *Nutrients,* Nov 1;12(11):3364. Doi: 10.3390/nu12113364.

Khan A, Faridi H, Ali M, Khan M, Siddique M, Hussain I, Ahmad M (2009): Effects of cypermethrin on some clinico-hemato-biochemical and pathological parameters in male dwarf goats (Capra hircus). *Experimental and Toxicologic Pathology,* Mar;61(2):151-60. Doi: 10.1016/j.etp.2008.07.001.

Kumar M, Dandapat S, Sinha MP, Kumar A, Raipat BS (2017): Different blood collection methods from rats: A review. *Balneo Research Journal,* 8(2): 46-50.

Kumari RR, Kumar P, Mondal TK (2013): Effect of vitamin E and selenium on haematological parameters in sub-acute toxicity of hexavalent chromium in broiler chick. *National Journal of Physiology, Pharmacy & Pharmacology,* 3, 158–161.

Lamfon HA (2013): Modulatory effect of vitamin E against fenvalerate induced immunotoxicity in albino mice. *Journal of Immunology Research,* 3(1):21–24.

Lin Y, Chen W, Liang W (2022): Investigation of Cytotoxicity and Oxidative Stress Induced by the Pyrethroid Bioallethrin in Human Glioblastoma Cells: The Protective Effect of Vitamin E (VE) and Its Underlying Mechanism. *Chemical Research in Toxicology,* May 16;35(5):880-889. Doi: 10.1021/acs.chemrestox. 2c 00033.

Manzoor S, Mehboob K, Naveed A (2016): Comparison Of Protective Effect Of Green Tea And Vitamin C Against Cypermethrin Induce Nephrotoxicity In Mice. *Journal of Ayub Medical College Abbottabad,* Apr-Jun;28(2):241-244.

McDowell LR (2008): Vitamins in Animal and Human Nutrition. John Wiley & Sons; Hoboken, NJ, USA.

Mestre A, Amavet P, Vanzetti A, Moleón M, Marcó M, Poletta G, Siroski P (2019): Effects of cypermethrin (pyrethroid), glyphosate and chlorpyrifos (organophosphorus) on the endocrine and immune system of Salvator merianae (Argentine tegu). *Ecotoxicology and Environmental Safety,* Mar; 169:61-67. Doi: 10.1016/j. ecoenv.2018.10.057.

Narra MR (2016): Single and cartel effect of pesticides on biochemical and haematological status of Clarias batrachus: A long-term monitoring. *Chemosphere.,* Feb; 144:966-74. doi: 10.1016/j.chemosphere.2015.09.065.

Odetti L, González E, Siroski P, Simoniello M, Poletta G (2023): How the exposure to environmentally relevant pesticide formulations affects the expression of stress response genes and its relation to oxidative damage and genotoxicity in Caiman latirostris. *Environmental Toxicology and Pharmacology,* Jan; 97:104014. Doi: 10.1016/j.etap.2022.104014.

Odetti L, Simoniello M, Poletta G (2022): Alterations in the Expression of Antioxidant Enzyme Genes in Response to Pesticide Exposure During Embryonic Development in the Native Reptile Species Caiman latirostris. *Bulletin of Environmental Contamination and Toxicology,* 9;110(1):3. Doi: 10.1007/s00128-022-03652-x.

Paduraru E, Flocea E, Lazado C, Simionov I, Nicoara M, Ciobica A, Faggio C, Jijie R (2021): Vitamin C Mitigates Oxidative Stress and Behavioral Impairments Induced by Deltamethrin and Lead Toxicity in Zebrafish. *International Journal of Molecular Sciences,* Nov 24;22(23):12714. Doi: 10.3390/ijms222312714.

Pande S, Saxena P, Bhushan B, Saxena N (2014): Peripheral blood and bone marrow responses under stress of cypermethrin in albino rats. *Interdisciplinary Toxicology,* Mar;7(1):33-40. Doi: 10.2478/intox-2014-0006.

Pitzer EM, Sugimoto C, Regan SL, Gudelsky GA, Williams MT, Vorhees CV (2022): Developmental deltamethrin: Sex-specific hippocampal effects in Sprague Dawley rats. *Current Research in Toxicology,* Nov 2; 3:100093. Doi: 10.1016/j.crtox. 2022.100093.

Polutchko SK, Glime G, Demmig-Adams B (2021): Synergistic Action of Membrane-Bound and Water-Soluble Antioxidants in Neuroprotection. *Molecules,* Sep 4;26(17):5385. Doi: 10.3390/molecules26175385.

Song M, Graubard BI, Rabkin CS, Engels EA (2021): Neutrophil-to-lymphocyte ratio and mortality in the United States general population. *Scientific Reports*, 11:464. doi: 10.1038/s41598-020-79431-7.

Turner P, Brabb T, Pekow C, Vasbinder M (2011): Administration of Substances to Laboratory Animals: Routes of Administration and Factors to Consider. *Journal of the American Association for Laboratory Animal Science,* Sep; 50(5): 600–613.

Varga F, Liber Z, Jakše J, Turudić A, Šatović Z, Radosavljević I, Jeran N, Grdiša M (2022): Development of Microsatellite Markers for Tanacetum cinerariifolium (Trevis.) Sch. Bip., a Plant with a Large and Highly Repetitive Genome. *Plants (Basel),* Jul 5;11(13):1778. Doi: 10.3390/plants11131778.

Willison SW, Stout 2nd D, Mysz A, Starr J, Tabor D, Wyrzykowska-Ceradini B, Nardin J, Morris E, Snyder EB (2023): Evaluating wipe sampling parameters to assess method performance and data confidence during remediation of hazardous pesticide misuse chemicals on indoor materials. *Science of the Total Environment,* Jan 15;856(Pt 1):159053. Doi: 10.1016/j.scitotenv.2022.159053.

Yamashiro T, Shiraishi A, Nakayama K, Satake H (2022): Draft Genome of Tanacetum Coccineum: Genomic Comparison of Closely Related Tanacetum-Family Plants. *International Journal of Molecular Sciences,* Jun 24;23(13):7039. Doi: 10.3390/ijms23137039.

Yan R, Zhou Q, Xu Z, Wu Y, Zhu G, Wang M, Guo Y, Dong K, Chen M (2021): Pyrethrins elicit olfactory response and spatial repellency in Aedes albopictus. *Pest Management Science,* Aug;77(8):3706-3712. Doi: 10.1002/ps.6390.

Zeng T, Li J, Zhou L, Xu Z, Li J, Hu H, Luo J, Zheng R, Wang Y, Wang C (2021): Transcriptional Responses and GCMS Analysis for the Biosynthesis of Pyrethrins and Volatile Terpenes in Tanacetum coccineum. *International Journal of Molecular Sciences,* Nov 30;22(23):13005. Doi: 10.3390/ijms222313005.