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EXPOSURE TO SUB-LETHAL DOSES OF CARBOFURAN INDUCES HEMATOLOGICAL ALTERATIONS IN WISTAR RATS

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ABSTRACT

Carbofuran is a widely used carbamate pesticide that has been detected in ground, surface and rain waters. To assess the toxic effect of carbofuran in this study, Wistar rats numbering twenty-eight, were orally exposed to sub-lethal doses of carbofuran at 1/40th, 1/20th, and 1/10th of its LD50. After 50 days of exposure certain hematological parameters were evaluated. A significant decrease ($p < 0.05$) in red blood cell, hemoglobin, hematocrit, mean corpuscular hemoglobin and mean corpuscular volume was observed in the carbofuran-exposed rats. Meanwhile, the exposure resulted in the elevation of the white blood cells, lymphocytes, eosinophils and neutrophils levels in the rats. However, a mixed trend in the thrombocytic indices (total platelet count, mean platelet volume, pro-calcitonin and platelet distribution width) was observed. The exposure to the pesticide, carbofuran produced microcytic anemia and leukocytosis in the rats, in a non-dose dependent manner.

Keywords: carbofuran, hematology, hematotoxicity, thrombocytic indices, leucocyte counts.

1. INTRODUCTION

The past decades have seen an increase in the use of pesticides for the improvement of agricultural yield(Jaiswal et al, 2013). Pesticides have also find use in the protection of household and industrial items (Milatovic et al, 2006). The application of these chemicals has toxicological implications for non-target organisms in the environment. The lower toxicities and lesser environmental persistence of some class of pesticides, such ascarbamateshave resulted in their higher application frequency than most other pesticides(Jaiswal et al, 2013).

Carbofuran (CF) is a broad spectrum insecticide, acaricide, and nematicide belonging to the carbamategroup of pesticides and its presence has been reported in nontarget mammalian systems including humans (Hossen et al, 2017). It accumulates in fat depots and exerts adverse effects on organs such as brain, liver, skeletal muscles and heart (de Siqueira et al, 2015; Ruiz-Suárez et al, 2015). It has also been reported to be toxic to the mammalian nervous system with earlier studies establishing that carbofuran could inhibit acetylcholine esterase activity(Gupta et al, 2016). Carbofuran exposure has been found to induce reactive oxygen and nitrogen species, resulting in oxidative stress through the enhancement of malondialdehyde generation and

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alteration of the activities of antioxidant enzymes such as superoxide dismutase, catalase, and glutathione transferase(Hossen et al, 2017). These reactive species and free radicals are responsible for the peroxidation of membrane phospholipids which disrupt the normal function of lipid bilayer, and leading to the development of several pathological conditions.

Detection of carbofuran in ground water and its ingestion through contaminated vegetables and fruits have spurred considerable interest and concern about its use.Since changes in hematological profile can indicate the toxicity of a toxic agent in the body, the present study therefore, investigated the effect of sub-lethal doses of carbofuran on some hematological parameters in Wistar rats.

2. MATERIALS AND METHODS

Chemicals

Commercial grade carbofuran marketed as Furadan® was obtained from IrorunAgbe Agrochemical Company (Ogbomoso, Nigeria).

Animals and treatment

Twenty eight (28) adult male rats, weighing between 120 and 150 g were used in this study. The animals were housed at 25 ± 2^{o}C under 12h cycles of dark and light. The animals were allowed access to standard laboratory food and water *ad libitum*. After a one-week acclimatization period, they were divided into four groups of seven rats each: a control group, CT 0, CT 1/40th, CT 1/20th, and CT 1/10th. The animals orally received0, 1/40th, 1/20th, and 1/10th LD50 of CF for rats, respectively. the oral LD50 for CF is 8 mg/kg (Steeger & Branch IV, 2003). The treatment was continued for 50 days. At the end of the experimental period, the rats were sacrificed by cervical dislocation after a light anesthesia with ether. Blood samples were collected through cardiac puncture in separate aliquots containing di-potassium salt of EDTA.

Hematological parameters including, red blood cell count (RBC), hemoglobin (Hb), hematocrit (HCT), red blood cell distribution width (RDW), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular hemoglobin (MCH), red cell distribution width-standard deviation (RDW–SD), red cell distribution width-coefficient of variation (RDW-CV), white blood cell count (WBC) and differential leucocytic count, thrombocytic indices [total platelet count (PLT), mean platelet volume (MPV), pro-calcitonin (PCT) and platelet distribution width (PDW)] were analyzed. The analyses were conducted with the use of an automated hematological assay analyzer (Medonic CA 620, Sweden).

Statistical Analysis

Data were analyzed by one-way analysis of variance, followed by Tukey's multiple comparisons test. Results were presented as mean ± standard error of means (SEM) and values were considered statistically significant at $p < 0.05$. Data were analyzed using GraphPad Prism for Windows, version 6.01 (GraphPad Software, Inc., San Diego, CA, USA.).

3. RESULTS

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Red blood cell indices

Results showing the effect of sub-lethal doses of carbofuran on the erythrocyte indices of rats are presented in Table 1. After 50 days, RBC count was non-significantly reduced in all the groups ($p > 0.05$).Hb and HCT on the other hand, were significantly decreased by the exposure to carbofuran, although not in a dose-dependent manner ($p < 0.05$).Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and the mean corpuscular hemoglobin concentration (MCHC) were also determined in this study. In all the CF-treated groups, MCVand MCH were lowered by as much as, 17 %, 15 %, 19 % and 17%, 7% 17% respectively. The exposure however, increased the MCHC values, though not significantly.

Leucocyte and differential leucocyte counts

Table 2 depicts the effect of CF exposure on the differential leucocytes counts in the rats. Compared to the control, CF significantly caused an increase in WBC, neutrophils, and lymphocytes counts in the animals ($p < 0.05$). WBC was increased by 61%, 35% 71% and the neutrophils by 209%, 181% 233% in the CF $1/40^{th}$, CF $1/20^{th}$, and CF $1/10^{th}$ groups, respectively. For the lymphocytes, only the CF $1/20^{th}$ and $1/10^{th}$ groups were increased by 32% and 39% respectively. Eosinophils were elevated by CF at the highest dose, $1/10^{th}$ LD_{50} by 200%. However, with the monocytes, CF modulated the concentrations differently with the low dose, i.e. CF $1/40^{th}$ causing an increase of 34%, while the other two doses, CF $1/20^{th}$ and CF $1/10^{th}$, decreased the levels by 83% and 84% respectively. Basophils were unaffected by exposure to the pesticide.

Thrombocytic indices

PLT, MPV, and PCT values were all significantly altered in the CF-treated group compared to the control, while PDW was left unchanged (Table 3). PLT in the exposed rats was increased by 83%, 47% and 38% in the CF $1/40^{th}$, CF $1/20^{th}$, and CF $1/10^{th}$ groups, respectively. Similarly, PCV was elevated by 76%, 21% and 21%, respectively. Meanwhile, the CF $1/40^{th}$ group displayed no adverse effect of CF on PCT but reduced this index by more than 10% in the other two groups.

4. DISCUSSION

The widespread use of pesticides results in a number of adverse health effects in both humans and animals. Derangement in hematological parameters is indicative of the toxic effects of foreign compounds such as pesticides, as well as, changes in physiological and pathological status of an organism (Stoytcheva, 2011). Several studies have utilized hematological changes as a biomarker of pesticide exposure and also for the evaluating the interaction between a toxic agent and biological system (Da Cuna et al, 2011). Generally, inducement of non-specific immunity by a pesticide leads to alterations in hematological parameters (Aroonvilairat et al, 2015). Carbofuran exposure in the present study resulted in remarkable hematological alterations.

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Carbofuran caused a reduction in RBC, HCT, and Hb in our study. These alterations may result from the disruptive action of the pesticide on the red cell membranes (Ramesh & Saravanan, 2008). Furthermore, the lysing or shrinkage of red blood cells caused by the action of the pesticide on the erythropoietic tissue and the suppression of erythropoiesis may lead to a reduction in red blood cell, hemoglobin and hematocrit (Saravanan et al, 2011). The decrease in Hb and Hct values in our study indicates a non-specific immune response of the rats to the insecticide. It is also reflective of the rats' defensive reaction against pesticide stress (Narra, 2016). The observed decrease in MCV and MCH in the treated groups suggested shrinkage of the RBCs. It may also be due to an increase in the level of immature RBCs in the circulation (Saravanan et al, 2011). The MCH reflects Hb content and Hb rate to RBC which were reduced following carbofuran exposure (Hossen et al, 2017). The decrease indicates microcytic normochromic anemia in the exposed rats (Adhikari et al, 2004). The observed increase in MCHC value, though non-significant, indicates an upsurge in Hb synthesis which could be a compensatory mechanism for the depleted Hb concentration in the carbofuran-exposed rats. The reduction in RDW-SD and RDW-CV, along with RBC, Hb, and Hct support the suggested of an up-regulation of the erythropoiesis in the bone marrow. This might have resulted in the production of impaired red cells or atrophied erythrocytes which would have been destroyed in circulation.

The deleterious effects of carbofuran on the total WBC, neutrophils, lymphocytes, and monocytes were evident in this study. The WBC count is a biomarker of systemic inflammation, as the cells are involved in the regulation of immunological function (Farhangi et al, 2013). A significant increase in total WBC, neutrophils, and lymphocytes levels, along with a significant increase in the platelet count were observed in the carbofuran-exposed rats. This indicates either a protective mechanism against the pesticide or the activation of the rat immune system against the carbofuran-induced stress. An increased WBC count can be adduced to an increased antibody production in order to ameliorate pesticide-induced toxicity (Joshp et al, 2002). Leukocytosis in this study may occur as a result of increased leukocyte mobilization, reflective of the severity of causative stress condition (Celik et al, 2009).Other pesticides have been reported to stimulate immune functions by increasing WBC levels, indicating the activation of defense and immune system where oedema and inflammation have been induced (Yousef et al, 2003). The increase in leucocytes was particularly evident in the case of lymphocytes which could have resulted from the stimulation of lymphopoietic process in the rats. Leukocytosis has been demonstrated in animals intoxicated with pesticides and has been suggested to be due to the mobilization of the immunological system and/or a shift in the leucocytic pool from the spleen to peripheral blood (Haratym-Maj, 2003). Non-specific irritation caused by the toxicant and/or its metabolites may induce the production and release of inflammatory mediators like prostaglandins thatproduceneutrophilia. Neutrophilia can lead to enhanced release of mature neutrophils from the bone marrow and the subsequent demargination, contributing to the circulating pool (Khan et al, 2013). A significant increase in PLT counts was found in all the CF-treated groups. Pesticides can induce histopathological damage (Afolabi et al, 2018) and such damage can increase the production of PLT, as well as WBC as seen in our study.

5. CONCLUSION

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It can thus, be concluded that sub-lethal doses of carbofuran elicited hematological alteration, which was characterized by microcytic normochromic anemia and leukocytosis. Also, the modulatedhematotoxic response induced by the pesticide in the rats was not dose-dependent. The data from this study further provide evidence of the toxic effect of carbofuran.

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Table 1. Effect of carbofuran on the hematological parameters of rats

	RBCs ($x10^{12}$/L)	Hb (g/dL)	HCT (%)	MCV (fL)	MCH (pg)	MCHC (g/dL)	RDW-CV	RDW-SD
Control	8.31 ± 0.17^{a}	16.87 ± 0.13^{a}	61.07 ± 1.66^{a}	71.63± 3.12^{a}	20.40 ± 1.37^{a}	27.70 ± 0.55^{a}	0.20 ± 0.01^{a}	64.53 ± 4.70^{a}
CF 1/40th	8.58 ± 0.23^{a}	14.57 ± 0.48^{b}	50.80 ± 1.57^{b}	59.17 ± 0.42^{b}	16.97 ± 1.61^{b}	28.67 ± 0.22^{a}	0.17 ± 0.00^{bc}	46.53 ± 0.17^{bc}
CF 1/20th	7.14 ± 0.35^{b}	15.52 ± 0.16^{b}	51.98 ± 0.40^{b}	61.00 ± 0.45^{b}	18.90 ± 0.28^{c}	28.75 ± 0.43^{a}	0.18 ± 0.00^{b}	53.15 ± 0.74^{b}
CF 1/10th	6.90 ± 0.17^{c}	15.57 ± 0.25^{b}	46.90 ± 0.75^{c}	58.23 ± 1.60^{b}	16.97 ± 0.29^{b}	29.20 ± 0.29^{a}	0.16 ± 0.00^{c}	41.97 ± 1.06^{c}

Each value represents the mean ± SEM of 7 rats. Values within a column with different alphabets are significantly different at $p<0.05$.

Table 2: Effect of carbofuran treatment on the total and differential leucocytes counts of rats

	WBCs	Lymphocytes	Neutrophils	Monocytes	Eosinophils	Basophils
Control	6.44 ± 0.12^{a}	4.61 ± 0.18^{a}	0.75 ± 0.14^{a}	1.29 ± 0.10^{a}	0.09 ± 0.01^{a}	0.06 ± 0.01^{a}
CF 1/40th	10.38 ± 0.38^{b}	4.31 ± 0.25^{b}	2.39 ± 0.13^{bc}	1.73 ± 0.04^{b}	0.13 ± 0.01^{a}	0.06 ± 0.01^{a}
CF 1/20th	8.67 ± 0.18^{c}	6.10 ± 0.10^{c}	2.10 ± 0.03^{b}	0.22 ± 0.04^{c}	0.17 ± 0.01^{ab}	0.08 ± 0.00^{a}
CF 1/10th	11.02 ± 0.36^{b}	6.42 ± 0.34^{c}	2.59 ± 0.11^{c}	0.21 ± 0.03^{c}	0.27 ± 0.05^{b}	0.05 ± 0.02^{a}

Each value represents the mean ± SEM of 7 rats. Values within a column with different alphabets are significantly different at $p<0.05$.

Table 3: Effect of carbofuran treatment on the thrombocytic indices of rats

	PLT (10^{9}/L)	MPV (fL)	PDW (µm)	PCT (%)
Control	405.70 ± 41.99^{a}	8.60 ± 0.07^{a}	15.78 ± 0.09^{a}	3.49 ± 0.36^{a}
CF 1/40th	742.30 ± 34.09^{b}	8.27 ± 0.08^{a}	15.67 ± 0.04^{a}	6.12 ± 0.21^{b}
CF 1/20th	596.70 ± 37.00^{c}	7.35 ± 0.16^{b}	15.75 ± 0.07^{a}	4.23 ± 0.30^{a}
CF 1/10th	560.00 ± 19.94^{c}	7.60 ± 0.13^{b}	15.83 ± 0.15^{a}	4.22 ± 0.33^{a}

Each value represents the mean ± SEM of 7 rats. Values within a column with different alphabets are significantly different at $p<0.05$.