EFFECT OF ORAL ADMINISTRATION OF SUBLETHAL CONCENTRATION OF ATRAZINE ON THE HAEMATOLOGICAL PROFILE OF ALBINO RAT

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ABSTRACT

The study investigated the effects of 28 day exposure of albino rats to graded sublethal concentrations of atrazine on the haematological profile. Thirty six (36) male albino rats used for the study were divided into four groups of nine rats each. Group I served as the untreated control while groups II, III and IV were treated with atrazine at 150, 200 and 300 mg/kg body weight orally administered for 28 days. Packed cell volume (PCV), red blood cell (RBC) count, haemoglobin concentration (Hb), total white blood cell (WBC) count and differential WBC count were assessed on day 14, 21, and 28. The exposure to atrazine led to significant reductions in PCV, Hb and RBC of the treated groups especially on day 21 and 28. The treated groups also had significantly higher (p<0.05) total WBC counts all through the study, and group IV rats had significantly higher (p<0.05) lymphocytes and significantly lower (p<0.05) neutrophil on day 28 when compared with the control.

Keywords: Albino rat, *Rattus norvegicus,* Atrazine, Haematology

INTRODUCTION

The impact of man - made compounds on wild life has been critically assessed in recent times. The chemicals originated from a variety of compounds of anthropogenic origin such as pesticides, detergents and plasticizers. Atrazine (2-chloro-4 (ethylamino) 6-isopropylamino)-S-triazine) was a selective chloroatrazine herbicides used on agricultural crops. It was available as dry flowable liquid, water dispersible granular and wettable powder formulations. Atrazine and its primary metabolite, diethylatrazine, are the most commonly encountered groundwater contaminants, mixed use, undeveloped and urban - use areas, the primary justification for most immunotoxicological studies on environmentally relevant xenobiotics was to determine the potential risk of xenobiotic to the human beings (Kristinia *et al.*, 2012). Toxicological manifestations in the immune system following xenobiotic exposure in experimental animals may appear as: changes in lymphoid organ weights and/or histology; quantitative or qualitative changes in cellularity of lymphoid tissue, bone marrow or numbers of peripheral leukocytes; impairment of immune cell function; and increased susceptibility to infectious agents. Most laboratory animal - based immunotoxicology studies stimulate the animal's immune system so that the ability of its immune system to respond to an antigenic challenge can be measured. Mice and rats are the primary mammalian laboratory animals used in immunotoxicology studies because of the availability of a large number of antibodies for assessing the immune system. The existence of

inbred and knockout mouse strains provide additional resources not available in other animal models. The most common pathway for atrazine degradation involves the intermediate cyanuric acid, in which carbon was fully oxidized, thus, the ring was primarily a nitrogen source for aerobic micro - organisms. Atrazine was moderately to highly mobile in soils, especially where soils have low clay or organic content, because, it does not adhere strongly to soil particles and has a lengthy soil half-life (13 to 261 days) (Cai *et al.,* 2003). Atrazine was also subject to photodecomposition and volatilization, when high temperature and prolonged sunlight occur after precipitation, but these effects are not significant under formal field conditions. Atrazine can persist for longer than one year under conditions which are not conducive to chemical or biological activity, such as dry or cold climate. Low concentration of atrazine in water, food and air causes health problems that have endocrine disruptor, possible carcinogenic, immunotoxic effects and low sperm count in men (Birnbatum and Fenton, 2003). According to extension toxicology network, the oral median lethal dose or LD_{50} for atrazine was 3090 mg/kg in rats, 1750 mg/kg in mice, 750 mg/kg in rabbits and 1000 mg/kg in hamsters. The one hour inhalation LC_{50} was greater than 0.7 mg/l in rats (Ciba-Geigy, 1987). There was a growing awareness of the role, which changes in the blood indices of rat could play in the evaluation of the pollution status in the environment. Donna *et al.* (1989) noted that changes in the haematological parameters were useful tools in assessment of the physiological status of rat. Atrazine was absorbed from the gastrointestinal tract based on recovery of orally administered radio labeled material in rats (Bakke *et al.,* 1972). The measurement of haematological changes in blood and tissue of rat under exposure to toxicant may be used to predict the toxic effects of herbicides; detection of specific physiological abnormalities and provide an indication of any gross damage. Highly effective herbicides used on entering the environment may bring about multiple changes in the organism by altering the nutritional values, growth rate and behavioural patterns. Changes in haematological parameters (RBC count, Hb and haematocrit) were reported in Sprague-Dawley rats chemically exposed to 0, 10, 70, 500 or 1000 part per million (ppm) of atrazine for two years (Donna *et al.,* 1989). Haematological studies are essential because the blood has been regarded by man as the essence of life, the seat of the soul and progenitor of psychic and physical strength. The purpose of the study was to investigate the effects of atrazine administered orally on the haematological profile of albino rat (*Rattus norvegicus*) with particular reference to concentration and duration of exposure.

MATERIALS AND METHODS

The present study was done with 36 male *Rattus norvegicus* with mean body weight of 168.89 ± 22.61 purchased from Faculty of Veterinary Medicine, University of Nigeria, Nsukka. It was transported to Animal Genetics and Breeding Laboratory, Department of Zoology and Environmental Biology, University of Nigeria, Nsukka. They were acclimatized for two weeks before the commencement of the study. During the acclimatization and experiment phases of the study, the rats were fed (Growers' Marsh, Grand Cereals Limited, Nigeria) and watered *ad libitum*. The rats were randomly divided into four groups of three rats each replicated thrice. The rats in group I were not treated with atrazine and served as the control. Group II, III and IV rats were treated with 150, 200 and 300 mg/kg of atrazine per body weight (bw), respectively for 28 days. Atrazine was administered based on the maximum tolerated dose of a pilot study conducted with various doses of atrazine (Narotsky *et al.,* 2001). The weekly weights of the rats were measured electronically. Blood samples were collected from the rats from each group on days 14, 21 and 28 post treatment and haematological parameters assayed.

Blood: Blood sample for haematological analysis were collected from the retro - bulbar plexus of the medial canthus of the eye of the rats. A microcapillary tube was carefully inserted into the medial canthus of the eye to puncture

the retro - bulbar plexus and thus enable outflow of blood into a labeled sample bottle containing ethylene diamine tetra - acetic acid (EDTA). The sample bottle was shaken gently to mix up the blood with EDTA and prevent blood clotting (Stone, 1954).

Haematology: Standard procedures were followed in all the haematological procedures. The haemoglobin concentration of the blood samples was determined by the cyano-menthaemoglobin method (Kachmer, 1970). The PCV was determined by the microhaematocrit method (Cole, 1986). A microcapillary tube nearly filled with the blood sample and sealed at one end with plasticine was centrifuged at 10,000 rpm for 5 minutes using a microhaematocrit centrifuge. After centrifugation, the PCV was read using a microhaematocrit reader.

Red blood cells were counted using an improved Neubaur haemocytometer chamber (Schalm *et al.,* 1975). Erythrocytes were counted in the loaded Neubaur chamber and total numbers were reported as 10^6 mm^{-3} (Wintrobe, 1967). Total WBCs were counted using an improved Neubaur haemocytometer (Schalm *et al.,* 1975). The differential leucocyte count was done using the Leishman technique. The blood sample was shaken and a drop of blood was smeared on the slide using a cover slip to make a thin smear. The smear was air dried and thereafter stained using Leishman stain. The stained slides were examined with an immersion objective. 200 cells were counted by the longitudinal counting method and each cell type was identified and scored using the differential cell counter. The results for each type of WBC was expressed as a percentage of the total count and converted to the absolute value per microlitre of blood.

The data were analyzed using one way analysis of variance (ANOVA) and variant means were separated using least significant difference (LSD) post hoc test. Means were accepted as significant at $p < 0.05$.

RESULTS

After 14 days of treatment, the PCV of the control group (Group 1) was 39.00 ± 0.57% while that of the groups given atrazine (Group II to IV) were 37.66 ± 1.45, 36.33 ± 1.20, and 37.16 ± 0.60% for the groups II, III and IV, respectively (Figure 1). The PCV of the rat groups given atrazine were significantly lower ($p< 0.05$) than the control rats on day 14 post treatment. After 21 days of exposure to atrazine, the mean PCV of the groups (Groups 1, II, III and IV) were 41.00 ± 0.57; 38.66 ± 0.88, 37.66 ± 0.33 and 35.25 ± 0.73%, respectively. At 21 days of exposure, only the PCV of group IV rats was significantly lower ($P < 0.05$) than that of the control (Figure 1). The PCV results after 28 days of treatment for the groups were 44.66 ± 0.88, 36.66 ± 0.88; 35.66 ± 0.88, 35.33 ± 0.33% for group I, 11, III, and IV (Figure 1). The effects of the PCV were observed to be dose dependent and the PCV of all the rat groups exposed to atrazine was found to be significantly lower ($p<0.05$) than that of the control.

There were no significant variations ($p > 0.05$) in the haemoglobin concentration of all, the rat groups after 14 days of exposure of the treated groups to atrazine, but after 21 and 28 days of exposure the haemoglobin concentration of the groups exposed to atrazine was found to be significantly lower ($p<0.05$) than that of the control (Figure 2).

On all the days of assessment (day 14, 21 and 28), the total WBC count of the groups exposed to atrazine were significantly higher ($p < 0.05$) than that of the control group (Figure 3).

There were no significant variations ($p > 0.05$) in RBC counts between all the groups after 14 days of exposure of the treatment groups to atrazine (Figure 4). However, after 21 and 28 days of exposure to atrazine groups, their RBC counts of the treated groups were found to be significantly lower ($p < 0.05$) than that of the control (Figure 4).

Though the lymphocyte counts of the group IV rats was higher than that of other groups after 14 days of exposure to atrazine, there was however no significantly ($P > 0.05$)

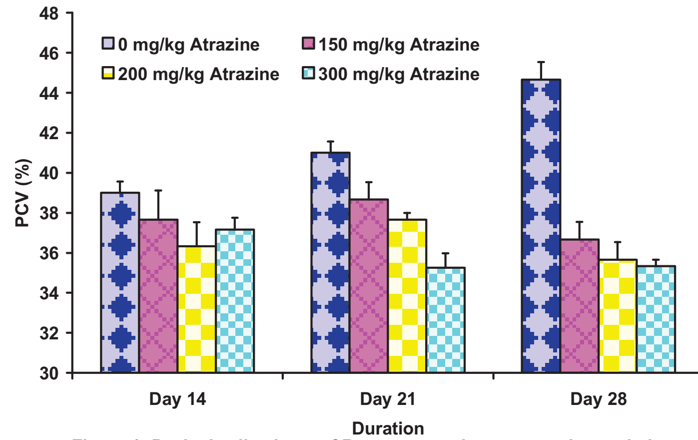

Figure 1: Packed cell volume of Rattus norvegicus exposed to varied concentrations of atrazine

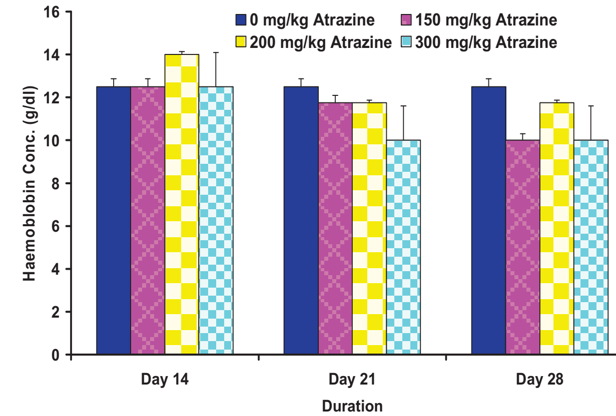

Figure 2; Haemoglobin concentration of *Rattus norvegicus* exposed to varied concentrations of atrazine

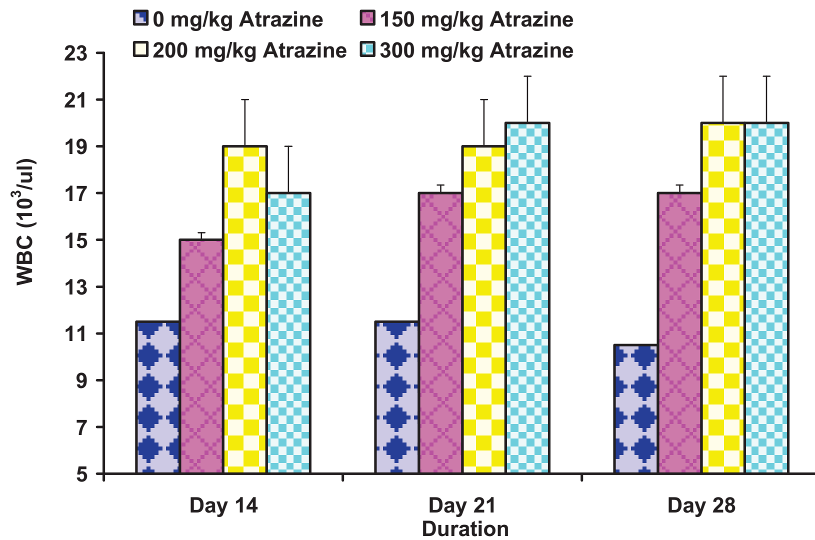

Figure 3: White blood cell counts of *Rattus norvegicus* exposed to varied concentrations of atrazine

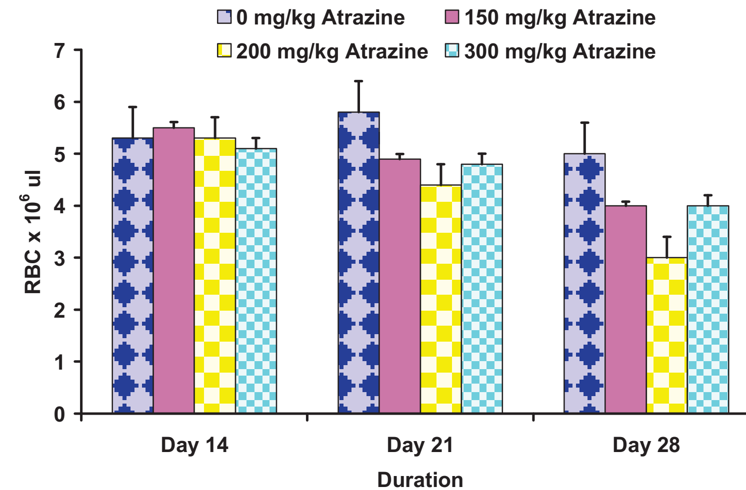

Figure 4: Red blood cell counts of *Rattus norvegicus* exposed to varied concentrations of atrazine

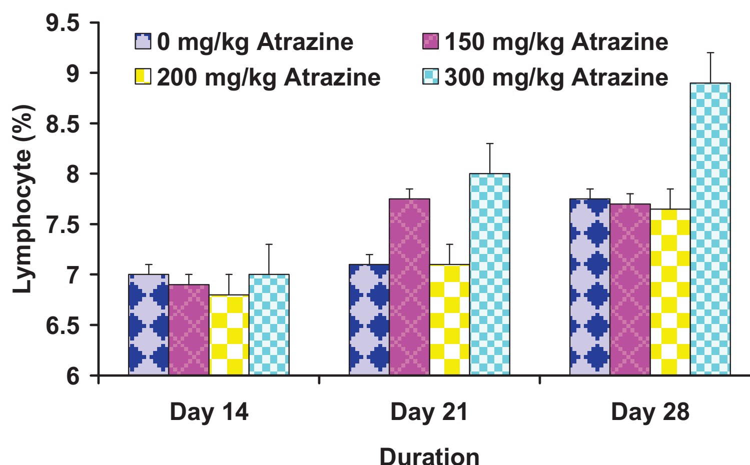

Figure 5: Lymphocyte cell counts of *Rattus norvegicus* exposed to varied concentrations of atrazine

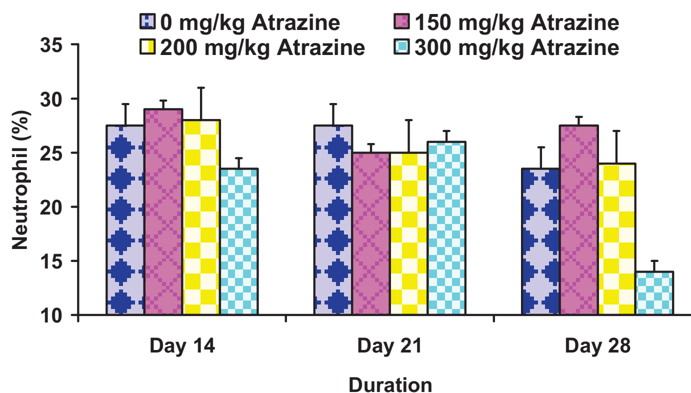

Figure 6: Neutrophil cell counts of *Rattus norvegicus* exposed to varied concentrations of atrazine

variation between all the groups (Figure 5).However the lymphocyte counts of group iv rats was significantly higher ($p < 0.05$) than that of all other rat groups on day 21 and 28 (Figure 5). After 14 days of exposure to atrazine, the neutrophil count of the group 4 rats was significantly lower ($p < 0.05$) than that of all other groups, but at day 21 of assessment, there were no significant variations ($p > 0.05$) between the groups (Figure 6). The day 28 assessment showed that the neutrophil count of the group IV was far lower and significantly lower ($p < 0.05$) than that of all the other groups, and that of group 2 was significantly higher ($p < 0.05$) than that of the control (Figure 6).

DISCUSSION

In recent years, haematological variables have been used more to determine the sublethal concentration of pollutants. In the present study, the results of the present investigation show that atrazine exposure as used in this study led to significant reductions in PCV, haemoglobin concentration and RBC counts. The recorded reduction in erythrocytic parameters in this study was in accordance with the findings of Ciba-Giegy (1987) in CF-1 mice and beagle dogs exposed to atrazine. This implied that exposure to atrazine at the level used in this study could lead to anaemia, probably due to decrease in rate of RBC production or outright impairment of erythropoiesis (Ihedioha and Chineme, 2004). Baroni and Sahai (1992) also reported that

haematological changes resulted in reduction of haemoglobin, RBC and WBC counts after administration of benzene hexachloride on *Rattus rattus.* The disturbances in the antioxidative capacity reported previously in rats' RBCs after sodium metavanadate (SMV; 0.125mgV/ml) intoxication (Ścibior *et al*., 2012) may suggest that oxidative stress could also be, in part, involved in the mechanism responsible for the development of anaemia.

In the present study there was increase in WBC counts in the atrazine exposed rats. This finding was in agreement with the reports of Alexander *et al.* (2008). WBCs play a major role in the defense mechanism of the body. The observed leukocytosis was an indication that the body defense mechanisms of the rats recognized and responded appropriately to atrazine as a foreign chemical agent. The observed leucocytosis in this study represented a physiological response and attempt of protection against damage by a chemical agent. Similar leucocytosis had been reported in fish exposed to pesticides (Allen, 1994). The recorded high lymphocyte counts of group IV rats implies that exposure of rats to atrazine of 300mg/kg bw may be immunogenic. The immunogenicity may be part of protective response or a stimulatory action (Ihedioha and Chineme, 2004). The finding of a significant reduction in neutrophil count of group IV rats was noteworthy. It suggests either a specific use - up of neutrophils or decreased production of these specific cells in rats given 300mg/kg atrazine. Based on the results of this study, it was concluded that exposure of rats to atrazine led to significant reduction in PCV, RBC counts and haemoglobin concentration of exposed rats, significant elevation of total WBC counts of exposed rats and significant increase in neutrophil counts of the rats exposed to 300 mg/kg bw. Some investigators are of the opinion that any immune alteration observed in rodents following xenobiotic exposure was of potential consequence for man (Bourdeau *et al.,* 1990; Kristinia *et al*., 2012). An alternative opinion was that only those immune alterations in rodents which are associated with hypersensitivity or altered host resistance to infectious agents or neoplastic cells are of major concern. The use of the immune system as a sensitive parameter for detecting sub clinical toxic injury was justified for several reasons: functionally immunocompetent cells are required for host resistance (Rooney *et al*., 2004).

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