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RESEARCH ARTICLE

Toxicity Evaluation of the Subacute Diazinon in Aged Male Rats: Hematological Aspects

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Abstract: ***Background & Objective*:** Age-dependent Organophosphates (OPs) toxicity is a controversial topic. The present study was designed to investigate the effect of the sub-acute exposure to diazinon (DZN), one of the main OPs insecticides, on the hematological alterations in adult and aged male rats.

***Methods*:** For the aim of this approach, the adult and aged rats were administered with DZN (15 mg/kg, orally) for 4 weeks. Then, the blood samples were collected from the retro-orbital sinus for measuring red blood cell (RBC), hemoglobin (Hb), hematocrit (Hct), platelets (PLT), MCV (mean corpuscular volume), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin (MCHC).

***Results*:** The obtained results indicated that DZN significantly decreased RBCs (4.93 ± 0.41), Htc (28.12 ± 1.21), Hb (10.31 ± 0.36), MCHC (30.51 ± 2.04), MCV (62.86 ± 2.58), and PLT (265.6 ± 34.81) values in the adult and aged rats versus the age-matched control rats. Moreover, RBC, Hb, and Htc levels decreased significantly in the aged rats versus adult rats. However, no significant differences were observed between MCHC, MCV, and PLT levels in adult and aged rats. Moreover, the MCH concentration did not change in any group. Additionally, DZN did not deteriorate the hematological alterations in the aged rats versus adult rats.

***Conclusion*:** the present study showed that the toxicity of DZN is not associated with age. However, more studies should be conducted to confirm this finding.

Keywords: Aging, diazinon, hematology, organophosphate, rat, diabetes.

1. INTRODUCTION

Organophosphates (OPs) are the main class of pesticides which are extensively applied in industry and agriculture [1]. These compounds are considered as the major environmental pollutants in air, water, soil, and vegetables [1]. According to the World Health Organization (WHO) toxicity classification, OPs belong to the toxic agents in class II that are highly toxic for humans and animals [2]. Several studies have indicated that OPs-induced oxidative stress is involved in the pathogenesis of various diseases such as diabetes, cardiovascular, neurodegenerative, kidney, liver disease, and cancer [3]. Diazinon (DZN, O, O-diethyl O-[4-methyl-6-(propan-2-yl) pyrimidin-2-yl] phosphorothioate) is one of the most common organophosphate pesticides that has been used globally for agriculture and veterinary purposes for years [4]. The maximum daily intake of DZN from the diet, estimated by the "US food and drug administration (FDA)", is 0.33 mg/day [5].

It has been indicated that the acute oral lethal dose of 50% (LD_{50}) of DZN is 1340 mg/kg for male rats [6]. The acute toxicity of DZN is mostly related to the irreversible acetylcholinesterase inhibition that may lead to animal death. Additionally, DZN is metabolized by liver enzymes to generate hydroxydiazoxon, diazoxon, and hydroxydiazinon, which are potent inhibitors of acetylcholinesterase [7]. However, there are several mechanisms such as oxidative stress and inflammation involving in the DZN toxicity. The toxicity occurs by disturbing the cellular and molecular functions of body organs [8]. Oxidative stress also leads to several age-associated diseases such as kidney, cardiovascular and hematological diseases, liver, and neurodegenerative diseases [9]. Aging is related to the disruption of cellular homeostatic mechanisms resulting in susceptibility to internal and environmental toxic materials with elevating rates and severity of various disorders [10]. In addition, it is reported that toxic agents accelerate the progression of aging processes in various tissues by inducing oxidative stress, inflammation, and

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Table 1. Hematological alterations in control (C) and diazinon (DZN)-treated rats.

Parameters	C (adult)	DZN (adult)	C (aged)	DZN (aged)
RBC (10^{12}/l)	7.99 ± 0.20	6.56 ± 0.44*	6.35 ± 0.32+	4.93 ± 0.41*
Hb(g/dl)	13.21 ± 0.25	11.91 ± 0.28 *	11.45 ± 0.68+++	10.31 ± 0.36*
Htc(%)	36. 18 ± 1.10	31.78 ± 0.58*	31.84 ± 0.71+	28.12 ± 1.21*
MCH(pg)	17.35 ± 0.19	21.58 ± 2.89	18.46 ± 0.45	18.06 ± 0.33
MCHC(g/dl)	38.35 ± 0.25	33.13 ± 1.22*	35.69 ± 1.12	30.51 ± 2.04*
MCV(fl)	57.32 ± 3.50	68.70 ± 1.30*	51.06 ± 3.47	62.86 ± 2.58*
PLT(10^9/l)	437.4 ± 22.54	337 ± 21.53*	375.7 ± 19.02	265.6 ± 34.81*

Note: *: Significant differences between control (C) and diazinon (DZN)-treated rats (*: $p<0.05$) +: Significant differences between adults and aged control (C) groups (+: $p<0.05$; +++: $p<0.001$).

apoptosis [11]. However, the age-related toxicity of OPs is controversial [12]. Therefore, the present study was designed to compare the impact of DZN on the hematological parameters including red blood cell (RBC), hemoglobin (Hb), hematocrit (Hct), platelets (PLT), MCV (mean corpuscular volume), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) in adult and aged male rats.

2. MATERIALS AND METHODS

2.1. Animals

Male Wistar adult (246 ± 4.12, n=20) and aged rats (520 ± 7.65, n=20) were provided for this investigation. Rats were kept under the standard animal house (temperature: 25°C, humidity: 50±10%, and 12:12-hour light/dark cycle) for 4 weeks in Ilam University, Ilam, Iran. The rats were fed ad libitum with standard chow (Behparvar Karaj Ltd., Iran) during the experimental period. All procedures were according to the "Institutional Animal Ethical Committee". The adult and aged rats were randomly allocated into DZN (Jiangsu, China) treated groups and non-treated (vehicle) group.

2.2. Experimental Procedure

DZN (15 mg/kg) was dissolved in olive oil and administered orally to the adult and aged rats daily for four weeks [13, 14]. The control groups were administrated with vehicle (olive oil). Then, the rats were anesthetized and their blood samples were collected from the retro-orbital sinus. Blood samples were taken from all animals after 10h fasting period. The blood was used to measure total counts of RBCs, PLT, Hct, Hb, MCV, MCH, and MCHC by Hematology Analyzers Blood Cell Counter (Exigo, Sweden). All the experiments were conducted in duplicate.

2.3. Statistical Analysis

One-way ANOVA and Dunnett's *post hoc* test were done by the InStat 3.0 program for comparing the analytical results. The data were indicated as means ± SEM. *P*-values <0.05 were shown as significant.

3. RESULTS

Table **1** indicates the hematological alterations in all groups. DZN administration significantly decreased RBCs, Htc, Hb, MCHC, MCV, and PLT values in the adult and aged rats versus the age-matched control rats ($p<0.05$). Moreover, RBC, Hb and, Htc levels significantly decreased in the blood of aged rats versus the adult rats ($p<0.05$, $p<0.001$, $p<0.05$, respectively). However, no significant differences were observed between MCHC, MCV and PLT values in the adult and aged rats. The MCH concentration did not change in any group. In addition, DZN could not deteriorate the hematological alterations in the aged rats versus the adult rats.

4. DISCUSSION

In this investigation, DZN modified some hematological parameters in the adult and aged animals. According to these findings, DZN decreased RBCs, Htc, Hb, MCHC, MCV and PLT levels in the adult and aged rats. However, the impact of DZN on the hematological indices was the same between the adult and aged animals. The findings indicated that DZN caused anemia as demonstrated by the considerable reduction in RBC count, hemoglobin concentration, and hematocrit percentage. The hematotoxic impacts of DZN have been confirmed by several previous investigations [14, 15]. It has been indicated that OPs stimulate the production of reactive oxygen species (ROS) that interfered with hemoglobin synthesis [16]. MCH is referred to as the average amount of hemoglobin in the erythrocytes [13]. The present findings indicated that RBC count and hemoglobin concentration decreased in the DZN-treated animals. Thus, MCH was not markedly altered. In this study, the significant difference was not observed between the effects of DZN on hematological changes in the adult and aged rats. However, previous studies reported that aging increased the susceptibility to toxic agents and deteriorated toxic damages in various tissues *via* inducing oxidative stress and inflammation responses [17, 18]. Scientific evidence is provided to confirm the association between OPs and the over-production of ROS [19]. In this regard, C. Moser *et al*. (1998) indicated that chlorpyrifos caused an aged-related change in behavioral function and

cholinesterase activity in an animal model [20]. They suggested that the sensitivity of the young animals to OPs toxicity is higher than the adult. Phosalone (PLN), other members of OPs, induced "senescence in rat embryonic fibroblast (REF) cells"; however, antioxidant administration decreased the PLN-caused toxic damages by modulating oxidative stress indices, inflammatory mediators, telomerase activity, and the expression of the genes associated with aging [21]. The kinetic mechanisms involved in the toxicity of cholinesterase inhibitors are responsible for age-associated differences in OPs toxicity [22]. Aging causes a reduction in the activity of detoxification enzymes, which leads to increasing the toxicity of xenobiotic [23]. During the developing age, the biotransformation capacity of the liver is not also enough for suitable detoxification [23, 24]. The present findings did not indicate age-related DZN toxicity. The observed age-related hematological impact of DZN in this study may differ from those of other studies on the hematological effects of OPs. This difference can be related to the dose and exposure duration to the DZN. In this context, Marty and co-workers (2012) indicated that acute exposure to a low dose of chlorpyrifos (2 mg/kg) had no significant age-dependent effects on cholinesterase inhibition in the pup and adult rats [20].

CONCLUSION

The present study shows that the toxicity of DZN is not associated with age. Therefore, it was suggested to perform further studies to identify the age-dependent toxicity of OPs.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This study was approved by the Research Ethics Committee of Ilam University of Medical Sciences, Iran, with approval number: IR.MEDILAM.REC.1398.130.

HUMAN AND ANIMAL RIGHTS

No humans were used for studies that are the base of this research. The reported experiments on animals were followed in accordance with the standards set forth in the 8th Edition of Guide for the Care and Use of Laboratory Animals (http://grants.nih.gov/grants/olaw/Guide-for-thecare-and-use-of-laboratory-animals.pdf) published by the National Academy of Sciences.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

The data that support the finding of this study are available from the corresponding author [Shahnaz Yousefizadeh] (sh.yousefizadeh@ilam.ac.ir), upon reasonable request.

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CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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