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RESEARCH ARTICLE

Age-Related Diazinon Toxicity Impact on Blood Glucose, Lipid Profile and Selected Biochemical Indices in Male Rats

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ARTICLE HISTORY

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Abstract: ***Background*:** Diabetes and its complications are age-related diseases. Low-grade inflammation plays the main role in the aging processes. Diazinon (DZN), an organophosphate pesticide, has been found to induce metabolic disturbances.

***Objective*:** The present study was designed to investigate the impact of DZN on age-related changes on inflammatory cells, blood glucose concentration, lipid profile, and liver and kidney function indices in adult and aged rats.

***Methods*:** Male rats (2 and 16 month old) were orally administrated with DZN (15 mg/kg) for 4 weeks. Then the blood was obtained for measuring inflammatory cells, lipid profile, glucose and serum biochemical indices such as liver enzymes, albumin, total protein, creatinine (Cr), urea, and uric acid in the serum of adult and aged male rats.

***Results*:** DZN increased the blood levels of glucose and the percentage of lymphocytes and also serum levels of TChol, TG, LDL-c, AST, ALT, ALP, LDH, Cr, urea, and uric acid in the adult and aged rats versus the aged matched control rats ($p< 0.001$). A marked reduction in HDL-c levels, total protein, albumin, and in the percentage of neutrophils were seen in the adult and aged animals exposed to DZN versus the aged matched control rats. DZN also increased the levels of LDL-c and ALT in the aged rats versus adult animals.

***Conclusion*:** The present study indicated that DZN can cause metabolic disturbance. However, the age-dependent effects of DZN on metabolic indices were not be confirmed by the present data.

Keywords: Organophosphate, diazinon, liver, kidney, glucose, lipid profile, aging.

1. INTRODUCTION

Aging is a normal physiological process accompanied by a gradual functional reduction of all body organs with a concurrent elevation in oxidative stress and low grade inflammation [1]. Generally, reduction in enzymes activities in very old population can affect the biotransformation processes that are involved in the metabolism and detoxification of toxic agents [2]. Aging reduces the body water, blood flow rate of the liver and kidneys and also decreases renal excretion and elimination of metabolites, which can lead to a decrease in the rates of metabolism [3, 4]. During aging, the acceleration of inflammatory responses may lead to the initiation and progression of diabetes and its complications. Indeed, aging increases the sensitivity of various tissues to toxic agents [5]. It was proposed that environmental toxicants may deteriorate the age-related diseases and accelerate the decline in cellular function [6]. Diazinon (DZN) is an Organophosphate (OPs) insecticide that has been used to the control of animal and plant pests for a year [7]. Although, the inhibitory effect of DZN on Acetylcholine Esterase (AChE) is the main mechanism of DZN toxicity, quite a few evidence indicated the role of cellular and molecular events such as oxidative stress and inflammation in the toxicity of DZN [8, 9]. Inflammation has been recognized as an important mechanism underlying the toxic effects of OPs on hyperglycemia and its complications [10]. There is strong evidence on the relation between OPs exposure and various chronic disorders including neurodegenerative diseases, diabetes, cardiovascular, kidney and liver diseases, *etc* [11-13]. The detoxification rate of OPs plays major role in the induction and progression of diseases [14]. With advancing age, a decrease in the activity of cytochromesP450 (CYP), the responsible enzymes for biotransformation of OPs, may increase the toxic effects of OPs [15]. However, the aged-dependent toxic effect of OPs is not clear [16]. Thus, this study was designed to investigate the effects of DZN on age-related changes in inflammatory cells, glucose, and lipid profile and also serum biochemical indices in rats. For this reason, inflammatory cells [total white blood cells (WBCs), neutrophils, lymphocytes), lipid profile [cholesterol (TChol), Triglyceride (TG), Low Density Lipoprotein (LDL-c), high-density lipoprotein (HDL-c)], glucose and serum biochemical indices such as Aspartate Aminotransferase (AST),

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Alanine Aminotransferase (ALT), Alkaline Phosphatase (ALP), Lactate Dehydrogenase (LDH), albumin, total protein, Creatinine (Cr), urea, and uric acid were assessed in the serum of adult and aged male rats.

2. MATERIALS AND METHODS

2.1. Chemicals

All the biochemical parameters were measured using diagnostic kits [Pars Azmoon kit, Tehran, IRI, Glucose (LOT: 97006), TChol (LOT: 96006), TG (LOT: 97003), LDL-c (LOT: 97002), HDL-c (LOT: 96002), Cr (LOT: 97009), urea (LOT: 97004), uric acid (LOT: 97002), uric acid (LOT: 97002), albumin (LOT: 97001), total protein (LOT: 96002), ALT (LOT: 97003), AST (LOT: 97002), ALP (LOT: 97004) and LDH (LOT: 97001)]. DZN was provided from Jiangsu Co., Ltd, China.

2.2. Animals

Male Wistar rats (2 and 16 month-old (n=40)) were utilized for this study. The rats were maintained in an animal house at the standard condition in Ilam University, Ilam, Iran. During the experimental period, the animals were fed with standard foods at the animal lab (Behparvar Karaj Co. Iran). The procedures were performed according to the "Institutional Animal Ethical Committee" Ilam University ethical committee. The rats were randomly allocated into 4 groups as follows: Adult control group (C2), adult DZN-treated group (DZN2), aged control group (C16) and aged DZN-treated group (DZN16).

2.3. Experimental Procedure

DZN (15 mg/kg, orally, 4 weeks) was administered to the adult and aged rats [17, 18]. Olive oil was used as a vehicle for DZN and administrated to the control groups. At the end of the study, the fasted animals were anesthetized and their blood samples were obtained from the retro-orbital sinus. Blood samples were used to analyze the total counts of WBCs, and the percentage of lymphocytes and neutrophils. The hematological analysis was performed by Blood Cell Counter (Exigo, Sweden). The experimental tests were conducted in duplicate. Additionally, for other biochemical measurements, blood and sera were separated by centrifuging at 3000 rpm for 15 minutes. Glucose, TC, TG, LDL-C, HDL-C, Cr, urea, uric acid, albumin, total protein, ALT, AST, ALP and LDH levels were measured by using Pars Azmon kits (Tehran, Iran) and an auto-analyzer (BT 1500, Italy).

2.4. Statistical Analysis

Two way ANOVA with post-hoc Tukey was used for analyzing data by the InStat 3.0 program. The data were indicated as means ± SEM. $P<0.05$ was shown as significant.

3. RESULTS

3.1. Effect of Diazinon on Glucose and Lipid Profile

In this study, the aged control group was compared with the adult control group. DZN-treated animals were compared with aged-matched control rats. Additionally, the aged DZN-treated group was compared with the adult DZN-treated group. The blood glucose level significantly elevated with increasing age ($p< 0.05$). DZN administration for four weeks increased the levels of blood glucose in the adult and aged groups versus the aged-matched control rats ($p < 0.05$; $p < 0.01$, respectively). The findings also indicated that DZN did not cause a significant change in the blood glucose level in the aged rats versus adult animals (*F=0.03, p=0.87*). A significant elevation was observed in the serum levels of TC, TG and LDL-c in the aged animals versus the adult rats ($p < 0.001$, $p < 0.001$, $p < 0.05$, respectively). DZN administration significantly increased the serum levels of TC ($p < 0.05$, $p < 0.001$, respectively), TG ($p < 0.05$, $p < 0.01$, respectively) and LDL-c ($p < 0.05$, $p < 0.01$, respectively) in adult and aged rats versus the age-matched control animals. DZN dramatically elevated the serum levels of LDL-c in the aged animals versus the adult rats (F=23.92, $p < 0.001$). A significant decrease in HDL-c levels was seen in aged rats versus adult animals ($p < 0.001$). DZN administration also significantly reduced the serum levels of HDL-c in the adult and aged rats versus the non-treated 2 and 16-months old animals ($p < 0.05$, $p < 0.01$, respectively). DZN could not deteriorate the serum levels of TG, TC and HDL-c in the aged rat versus adult animals (*F=0.28, p=0.60; F=0.07, p=0.78; F=2.43, p=0.13, respectively*) (Table **1**).

3.2. Effect of Diazinon on Inflammatory Cells

The total number of WBCs did not dramatically alter between all the groups. The percentage of lymphocytes significantly increased in the aged rats versus adult animals ($p< 0.05$). The percentage of neutrophils significantly reduced in the aged group versus the adult group ($p< 0.05$).DZN administration significantly decreased the percentage of neutrophils and also increased the percentage of lymphocytes in adults and aged rats (for neutrophils: $p< 0.05$; for lymphocytes: $p< 0.001$) versus the aged-matched control animals. The findings also indicated that DZN could not increase the percentage of lymphocytes (*F=0.47, p=0.49*) and also decrease the percentage of neutrophils (*F=0.05, p=0.83*) in aged rats *versus* adult animals (Table **2**).

3.3. Effect of Diazinon on Kidney Function

The serum levels of Cr, urea and uric acid significantly increased in aged rats versus adult animals ($p < 0.05$, $p < 0.05$, $p < 0.001$, respectively). The findings indicated that DZN caused a significant elevation in the serum levels of Cr, urea and uric acid in adult ($p < 0.05$) and aged ($p < 0.01$) rats versus the aged-matched control animals. The effect of DZN on the serum levels of Cr, urea and uric acid in aged animals was similar to the adult rats (*F=0.33, p=0.57*; *F=0.05, p=0.85*; *F=0.47, p=0.50*, respectively) (Table **2**).

3.4. Effect of Diazinon on Liver Function

The serum levels of AST, ALT, ALP, and LDH significantly decreased in aged rats versus adult animals ($p < 0.05$, $p < 0.05$, $p < 0.05$, $p < 0.01$, respectively). DZN administration increased the serum levels of ALT ($p < 0.05$, $p < 0.001$, respectively), AST ($p < 0.05$, $p < 0.01$, respectively), ALP ($p < 0.05$, $p < 0.001$, respectively) and LDH ($p < 0.01$, $p < 0.001$, respectively) in adult and aged rats versus the age-matched

Table 1. Blood glucose and lipid profile in the C2: control 2 months old, C16: control 16 months old, DZN2: Diazinon-treated 2 months old, DZN16: Diazinon-treated 16 months old.

Parameters	Control (C2)	Diazinon-Treated 2 Months Old (DZN2)	Control 16 Months Old (C16)	Diazinon-Treated 16 Months Old (DZN16)
Glucose (mg/dl)	87.8 ± 4.58	121.1 ± 6.49 *	118.9 ± 8.67 +	154.5 ± 8.04 **
TC (mg/dl)	54.60 ± 2.08	71.70 ± 2.45 *	92.00 ± 4.89 +++	122.00 ± 5.83 ***
TG (mg/dl)	45.60 ± 2.84	60.66 ± 4.56 *	67.80 ± 2.38 +++	84.61 ± 3.19 **
LDL-c (mg/dl)	29.60 ± 2.14	43.64 ± 2.36 *	55.22 ± 3.83 +	70.41 ± 3.43 **, ###
HDL-c (mg/dl)	34.4 ± 1.87	27.8 ± 1.46 *	28.33 ± 1.52 +	20.11 ± 1.04 **,

: Significant differences between C2 and DZN2 and C16 and DZN16 (: p<0.05; **: p<0.01; ***: p<0.001).
+: Significant differences between C2 and C16groups (+: p<0.05; +++: p<0.001).
#: Significant differences between DZN2 and DZN16 groups (###: p<0.001).

Table 2. Total WBCs count and the percentage of neutrophil and lymphocyte and kidney function tests in the C2: control 2 months old, C16: control 16 months old, DZN2: Diazinon-treated 2 months old, DZN16: Diazinon-treated 16 months old.

Parameters	Control (C2)	Diazinon-Treated 2 Months Old (DZN2)	Control 16 Months Old (C16)	Diazinon-Treated 16 Months Old (DZN16)
WBC (%)	6.91 ± 0.89	7.34 ± 2.77	8.03 ± 1.07	8.68 ± 0.76
Neutrophil (%)	24.94 ± 1.53	19.59 ± 1.26 *	19.77 ± 1.33 +	13.86 ± 0.81 *
Lymphocyte (%)	57.91 ± 2.20	73.20 ± 2.96 ***	67.81 ± 1.60 +	86.32 ± 2.42 ***
Total protein (mg/dl)	6.38 ± 0.29	5.34 ± 0.10 *	6.82 ± 038	5.41 ± 0.16 **
Albumin (mg/dl)	33.02 ± 2.70	21.04 ± 1.15 **	39.81 ± 3.31	22.5 ± 1.33 ***
Creatinine (mg/dl)	0.30 ± 0.04	0.45 ± 0.02 *	0.47 ± 0.03 +	0.66 ± 0.05 **
Urea (mg/dl)	34.20 ± 0.75	39.91 ± 1.45 *	39.70 ± 0.81 +	45.92 ± 1.77 **
Uric acid (mg/dl)	2.37 ± 0.11	3.06 ± 0.12 *	3.44 ± 0.19 +++	4.36 ± 0.21 **

: Significant differences between C2 and DZN2 and C16 and DZN16 (: p<0.05; **: p<0.01; ***: p<0.001).
+: Significant differences between C2 and C16 groups (+: p<0.05; +++: p<0.001).

control groups. DZN also increased the serum levels of ALT in aged rats versus adult rats (F=18.28, $p < 0.001$). No significant difference was observed in the serum levels of total protein and albumin between aged and adult animals. DZN administration caused a reduction in the serum levels of total protein ($p < 0.05$, $p < 0.01$, respectively) and albumin ($p < 0.01$, $p < 0.001$, respectively) of adult and aged rats versus the aged-matched control animals (Fig. **1**).

4. DISCUSSION

Aging is associated with "chronic low-grade inflammation" in several tissues resulting in hyperglycemia and dyslipidemia [19, 20]. It has been suggested that toxic agents can deteriorate hyperglycemia and dyslipidemia during aging [21]. Recently, the association between elevation in inflammatory cells and metabolic disorder has been reported.

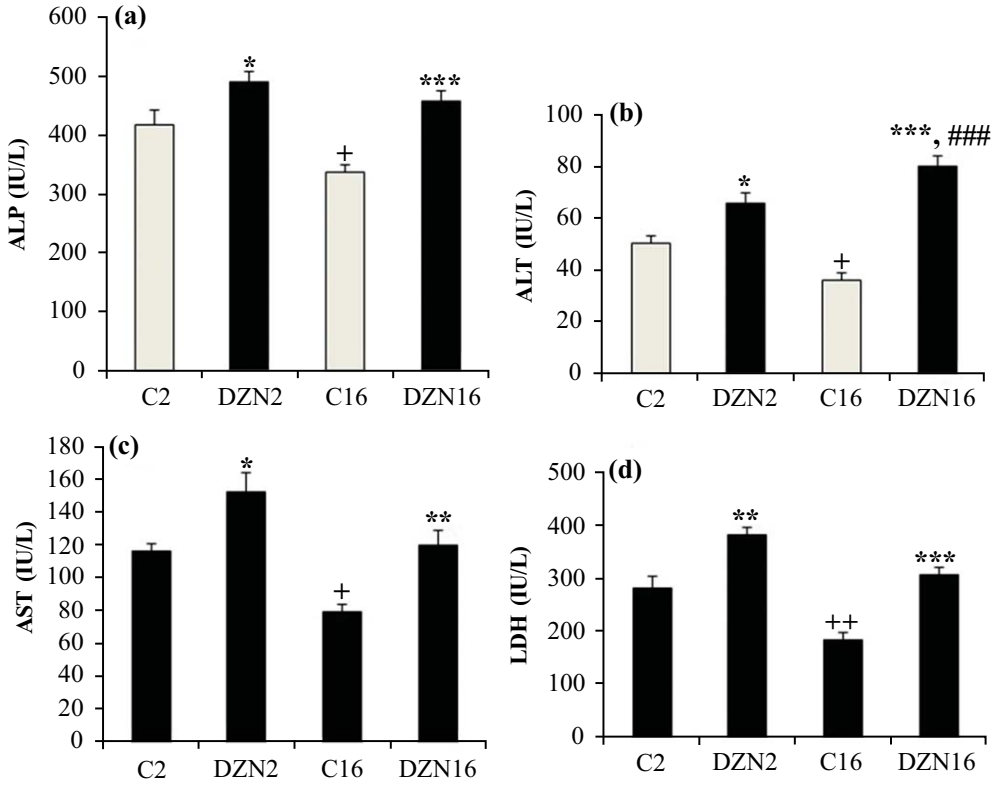

Fig. (1). The levels of liver enzymes [(ALP (**a**), ALT (**b**), AST (**c**) and LDH (**d**)] in the C2: control 2 months old, C16: control 16 months old, DZN2: Diazinon-treated 2 months old, DZN16: Diazinon-treated 16 months old.
: Significant differences between C2 and DZN2 and C16 and DZN16 (: $p<0.05$; **: $p<0.01$; ***: $p<0.001$).
+: Significant differences between C2 and C16 groups (+: $p<0.05$; ++: $p<0.01$).
#: Significant differences between DZN2 and DZN16 groups (###: $p<0.001$).

Additionally, it was suggested that the induction of an inflammatory response by toxic agents have an adverse impact in aging [22, 23]. In this study, we indicated that aging can increase the percentage of inflammatory cells and glucose levels. These findings indicated that inflammation may have a correlation with the pathogenesis of dyslipidemia and hyperglycemia induced by aging. This study indicated that the serum levels of LDL-c, TC, and TG increased with aging. We also observed that DZN increased the percentage of lymphocyte and also the serum levels of glucose, LDL-c, TC and TG in adult and aged animals versus the aged-matched controls. Induction of inflammation with DZN appears to be responsible for the deterioration of metabolic abnormalities induced by OP in animals. However, the exact mechanisms of such abnormalities are not clear. We also observed that the serum levels of liver enzymes (ALT, AST, ALP, and LDH) decreased with advancing age. However, DZN exposure significantly increased the serum levels of the liver enzyme in adult and aged rats versus the aged matched control. Similar to the previous investigations, our findings indicated that DZN stimulated a significant increase in the serum levels of ALP ALT, AST, and LDH enzymes [24-26]. AST, ALT, and ALP are the most sensitive markers related to liver toxicity [27]. Thus, the elevated serum levels of these enzymes may be due to the liver necrosis [27]. Generally, aging is accompanied by a decrease in protein expression especially enzymes such as liver enzymes [28]. Age-associated alteration in kidney function has been also observed in this study. The aged dependent effects of DZN were observed on the levels of ALT and LDL-c. The findings indicated that DZN administration also significantly increased the serum levels of Cr, urea and uric acid in adult and aged rats *versus* aged-matched control rats. The adverse effects of aging on kidney and liver function in aged rats may be related to the decline in the organ function due to an increase in oxidative stress and inflammatory responses during aging [29].

Our results also indicated DZN did not alter the total WBCs but it modified the differential count. Neutropenia is caused following the immigration of neutrophils to the injured site induced by the DZN [30]. DZN can cause lymphocyte proliferation for compensation of neutropenia [31]. As has been indicated in DZN-administrated rats, a considerable decrease in total protein may be related to the decrease in albumin [26]. Pesticides may decrease amino acids and protein metabolism [32]. Thus, the decrease in the total protein particularly albumin could be related to the decreased protein synthesis in the liver which is induced by DZN. Persistent inflammation or "inflamm-aging" increases the aging process and the progression of age-related diseases such as metabolic syndrome. Inflammatory mediators play the main role in "inflamm-aging" induced by chronic inflammation [33]. It was also indicated that lymphocytes play an important role in the induction of cytokine production resulting in "inflamm-aging" [34]. "Inflamm-aging" is also an important risk factor for the progression of hyperglycemia and hyperlipidemia [27].

CONCLUSION

Although several studies have indicated hyperglycemia and hyperlipidemia following exposure to OPs, but this effect of OPs is controversial [34, 35]. The present study indicated the hyperglycemic and hyperlipidemic effects of DZN due to its stimulatory effect on inflammatory responses. However, the present findings do not surely confirm the age-associated DZN toxicity in animal models. More experimental and clinical studies should be designed to clarify aged-related OPs toxicity.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This study was approved by the Ilam University Ethical Committee, Iran.

HUMAN AND ANIMAL RIGHTS

No humans were involved in this study, the reported experiments on animals were 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 Dr. Saeed Samarghandian (samarghandians1@nums.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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Declared none.

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