

# Toxic Effect of Dimethoate and Diazinon on the Biochemical and Hematological Parameters in Male Rabbits

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## Abstract

Dimethoate and Diazinon are two of widely used organophosphorus insecticides in agriculture. The irrational use of Dimethoate and Diazinon in Yemen play a crucial role in the occurrence of many diseases affecting plants, animals and man. The present work was conducted to investigate the alterations in biochemical and hematological factors in male rabbits after orally administration a single dose of 1/4 LD<sub>50</sub> of Dimethoate and Diazinon for 20 days. 30 Male Rabbits weighting 1500-1700g., were divided into 3 groups with 10 animals in each, first group served as control animals, they received 5 ml. of corn oil, while animals in second group received 1/4 LD<sub>50</sub> of Dimethoate, animals in third group received 1/4 of LD<sub>50</sub> of Diazinon. The concept of this study was to evaluate the hepatotoxic, and nephrotoxic effects of Dimethoate and Diazinon, therefore, the followings Biochemical parameters in serum were studied: aminotransferases (ALT and AST), alkaline phosphatase (ALP), total proteins, albumin, uric Acid, creatinin, and blood glucose. The followings hematological parameters were studied in blood: red blood cells (RBC), hemoglobin (Hb), and erythrocytes sedimentation rate (ESR). The Biochemical analysis showed that the levels of the ALT and AST as well as ALP, uric acid, creatinin, and blood glucose in the serum of treated rabbits significantly ( $P < 0.01$ ) increased compared to control animals, whereas either, total protein, and albumin, significantly decreased ( $P < 0.01$ ). Hematological factors were reduced in the treated groups.

## المخلص

يعتبر المبيدات الحشرية الديمثوات والديازينون من أكثر المبيدات الحشرية ذات التركيب الفسفوري استخداماً في الزراعة. ويلعب الاستخدام العشوائي لهذين المبيدين دوراً كبيراً في إصابة النباتات والحيوانات والانسان بالعديد من الأمراض في اليمن. أجريت هذه الدراسة لمعرفة التأثير السمي الذي يحدثه التعاطي عن طريق الفم لمادته ربع الجرعة المميته لهذين المبيدين على الدلائل الدموية والكيموحيوية في دم ذكور الأرانب. ولهذا الغرض تم استخدام 30 أرنباً تراوحت أوزانهم بين 1500-1700 جرام، قسمت إلى ثلاث مجموعات تحتوي كل مجموعة على 10 حيوانات. عولمت المجموعة الأولى على أنها مجموعة ضابطة وأعطيت جرعة يومية مقدارها 5 مل من زيت الذرة لمدة عشرة يوماً وأعطيت المجموعة الثانية ربع الجرعة المميته من الديمثوات مرة في اليوم لمدة عشرة يوماً، وأعطيت المجموعة الثالثة ربع الجرعة المميته من مبيد الديازينون مرة في اليوم لمدة عشرة يوماً. وكان الهدف الأساس للدراسة هو معرفة الأثر السمي لهذا المبيد على خلايا الكبد والكلى، وعلية وفي نهاية التجربة فحصت الدلائل الكيموحيوية التالية في مصل الأرانب: الأنزيمات الناقلة للأمين (الالانين والاسبريتيت)، وإنزيم الفوسفاتاز القلوي، والبروتين الكلي، و الألبومين، وحمض اليوريك، والكرياتينين وسكر الدم. كما تم فحص المؤشرات الدموية التالية: عدد خلايا الدم الحمراء، والهيموجلوبين ومعدل ترسيب خلايا الدم الحمراء. أشارت التحاليل الكيموحيوية إلى ارتفاع ذو معنوية عالية في معدل الأنزيمات الناقلة للأمين وكذا إنزيم الفوسفاتاز القلوي، اليوريك، الكرياتينين، وسكر الدم، بينما أنخفض معدل كلا من البروتين الكلي والألبومين في مصل الحيوانات التي تعاطت المبيدات مقارنة بحيوانات المجموعة الضابطة. المؤشرات الدموية (خلايا الدم الحمراء والهيموجلوبين) انخفضت معدلاتها في دم الحيوانات التي تعاطت المبيدات مقارنة بحيوانات المجموعة الضابطة، بينما ارتفع معدل ترسيب خلايا الدم الحمراء بمعنوية عالية.

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## 1. Introduction

The control of insect pests relies heavily on the use of synthetic insecticides. But, their widespread use has led to some serious problems including toxic residues on grass and toxicity to non-target organisms such as mammals, birds and fishes (Zettler and Cuperus 1990; White 1995; and Riebeiro *et al.*, 2003).

The pollution of the environment plays a crucial role in the occurrence of many diseases affecting plants, animals and man. One of the main factors causing pollution of the environment is the irrational use of organophosphorus insecticides (Al-Haj *et al.*, 2005).

Many alterations have been observed in organs of animals due to the organophosphorus insecticides (Betrosian *et al.*, 1995; and Senanayke 1998), specially CNS, (Desi *et al.*, 1998; and Lengyl *et al.*, 2005), liver (Gomes *et al.*, 1999), and kidney (Kossmann *et al.*, 1997).

Dimethoate is an organophosphorus insecticide widely used in agriculture (Sharma *et al.*, 2005), Diazinon, also is an organophosphorus insecticide extensively used in

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agriculture (Alahyary *et al.*, 2008). Both Dimethoate and Diazinon, are two of the most widely and irrationally used insecticides in agriculture in Yemen. (Al-Haj *et al.*, 2005).

Dimethoate is an insecticide with anticholinesterase mode of action (De-Bleecker *et al.*, 1993; and Dongren *et al.*, 1999).

Begum and Vijavaraghaven (1995) observed that, the exposure of Dimethoate to the fresh water fish *claries batrachus* reduced the carbohydrates and proteins metabolism, and affect the aminotransferase activity in the liver. An increase in blood glucose in experimental rats was reported after Dimethoate orally administration period of 2 months in dose 21mg/kg. (Hagar and Fahmy 2009). An increase in lactate dehydrogenase, serum transaminase, and a decrease in the serum total protein, albumin, and globulin was observed in experimental rats after Dimethoate orally administration in dose 75mg/kg (Attia and Nasr 2009). Diazinon is an organophosphorus insecticide with anticholinesterase mode of action (Alahyary *et al.*, 2008).

Mild structural and functional change in liver as well as in tests of experimental mice was observed after a single intraperitoneal administration of Diazinon (Dikshith *et al.*, 1975).

Matin *et al* (1990) showed that the administration of Diazinon to experimental rats resulted in carbohydrate metabolism changes that were abolished by adrenalectomy, suggesting a possible involvement the adrenals in the induced changes in Diazinon-treated animals. The exposure of zebra fish to the Diazinon for up to 168 hours, a significantly reduced DNA, RNA and the total protein in the liver (Ansari and Kumar 1988).

Jyostana *et al* (2003), observed a significant biochemical and hematological alterations due to the exposure to the various pesticides. Significant damage in the hepatic cells and glucose metabolism in liver was observed as the result of Diazinon administration (Fatima *et al.*, 2006).

At the last 5 years in Yemen, we have noted a critical increase in number of people suffering from various liver and kidney diseases, as well as diabetic mellitus. Therefore, the purpose of the present study was to evaluate the hepatotoxic and nephrotoxic effects of the Dimethoate and Diazinon on male rabbits, also their effects on some blood factors and blood glucose level will be determined.

## 2. Materials and Methods:

### 2.1. Chemicals:

All chemicals used in this experiment were obtained from Sigma, USA, including Dimethoate and Diazinon.

### 2.2. Animals treatment and blood collection:

Thirty healthy male rabbits (1500-1700g) were divided into 2 treated groups and control, as follows:

-Control group: 10 animals treated with a single daily dose of 5ml.corn oil orally period of 20 days.

-Dimethoate group: 10 animals treated with a single daily dose of 1/4 of LD<sub>50</sub> of Dimethoate (20mg/kg) in 5ml. corn oil orally period of 20 days.

-Diazinon group: 10 animals treated with a single daily dose of 1/4 of LD<sub>50</sub> of Diazinon (25mg/kg) in 5ml. corn oil orally period of 20 days.

All animals were maintained in standard environmental conditions and kept a standard commercial diet with water *ad libitum*.

All experiment was administrated in the Animal Physiology Laboratory, Department of Biology, Faculty of Science and Education, Aden University.

After 20 days the animals were fasted over night for 12h. Then they were sacrificed, the blood was immediately collected. Blood samples were divided in two parts, one was maintained in EDTA bulb and plain tube for assay of blood factors, other was centrifuged, and serum was discarded and kept at - 21 ° C for the biochemical testes.

### 2.3. Alanine- aminotransferase (ALT) and Aspartate- aminotransferase (AST) Assay:

The estimation was carried out according to the method originally developed by (Reitman and Frankel 1957).

### 2.4. Alkaline phosphatase Assay:

ALP was determined using a colorimetric method as described by (Kind and King 1954).

### 2.5. Total Protein Assay:

The total protein was determined by Biuret method explained by (Tietz 1976)

### 2.6. Albumin Assay:

Serum albumin was determined according to the method of (Doumas *et al.*, 1971).

### 2.7. Glucose Assay:

Glucose was determined according to method of (Trinder *et al.*, 1969).

### 2.8. Creatinine and Uric acid Assay:

Creatinine and uric acid was estimated according to method explained by (Houot 1985).

### 2.9. R.B.C., Hb and E.S.R. Assay:

The R.B.C. count, Hb level and E.S.R.time, were determined using method described by (Sood 1990).

### 2.10. Statistical analysis:

The statistical analysis was performed by SPSS; continuous data are expressed as mean ±S.E. Data were compared using one – way ANOVA. P value <0.01 was considered to be statistically significant.

## 3. Results:

Data in table1 show that the treatment with 1/4 of LD<sub>50</sub> of Dimethoate and Diazinon resulted in a statistically high significant increase in the level of alanine-aminotransferase (ALT) and aspartate-aminotransferase (AST) in the serum of both treated groups, as compared to the control, this increase was higher in the Diazinon treated rabbits.

As shown in the table 1 the level of alkaline phosphatase (ALP) in the serum of rabbits treated with Dimethoate and Diazinon statistically high significant

increased compared to control rabbits, this increase was higher in Diazinon treated rabbits.

Table1: The biochemical parameters after 20 days of orally administration of Dimethoate in dose 20mg/kg. and Diazinon in dose 25mg/kg.

Biochemical Parameters	Treatments		
	Control	Dimethoate	Diazinon
AST IU/L	35.37±3.7	85.47±2.32**	93.31±2.11**
ALT IU/L	44.50±2.70	168.27±4.86**	176.41±6.75**
ALP IU/L	65.52±2.3	160.25±3.7**	174.41±4.8**
T.Protein g/dL	7.67±0.12	5.16±0.13*	5.17±0.17*
Albumin g/dL	3.58±0.40	2.01±0.19*	2.11±0.22*
Glucose mg/dL	108.83±6.91	210.17±4.31**	245.20±5.40**
Uric acid mg/dL	4.02±0.74	7.85±1.34*	7.65±1.75*
Creatinine mg/dL	0.98±0.21	3.03±1.16*	3.9

Values are expressed as means of 10 animals ± S.E.\* Significance;\*\* High significance at (P<0.01) vs. control.

Table2: The hematological parameters after 20 days of orally administration of Dimethoate in dose 20mg/kg. and diazinon in dose 25mg/kg.

Hematological Parameters	Treatments		
	Control	Dimethoate	Diazinon
R.B.C.	4.6±0.05	2.9±0.10*	2.6±0.25*
Mill/cu.mm			
Hb. g/dL	12.6±1.01	8.7±0.23*	8.1±0.34*
E.S.R. Mm/hr	5.0±1.04	13.0±1.09**	12.0±0.85**

Values are expressed as mean of 10 animals ± S.E.\* Significance \*\* High significance at (P<0.01) vs. control

Total protein and albumin levels significantly decreased in the serum of treated with Dimethoate and Diazinon rabbits, as compared to control.

Blood sugar level highly significant increased in the serum of Dimethoate and Diazinon treated rabbits, as compared to control, the increase in blood glucose was higher in the serum of Diazinon treated rabbits than in the Dimethoate treated group.

Uric acid and creatinine levels significantly increased in the serum of Dimethoate and Diazinon treated rabbits, compared to control.

Results in table 2 showed, that the R.B.C. count and Hb level significantly decreased in the blood of Dimethoate and Diazinon treated rabbits compared to control.

The erythrocytes sedimentation rate highly significant increased in the blood of Dimethoate and Diazinon treated rabbits as compared to control.

#### 4. Discussion:

The noticed increase in the levels of aminotransferase (ALT and AST) and the level of ALP as well as the decrease in the in the levels of total protein and albumin in the serum, are the major diagnostic symptoms of liver diseases (Chatterjea and Shinde 2005).

The decrease in the serum albumin may also indicate to the renal inability keeps it in; therefore it excreted with urine (Albumiurea) (Vasilenko and Grebenev1990). The increase in the uric acid and creatinine in the serum are the major symptoms of glomerular filtration damage (Chatterjea and Shinde 2005).

Blood glucose increasing in many diseases such as Diabetes mellitus, and damage of the hepatic glycogenesis pathway (Guyton and Hall 2006).

Our results clearly showed the hepatotoxic and nephrotoxic effects of Dimethoate and Diazinon. The orally administration of 1/4 of LD<sub>50</sub> of Dimethoate and Diazinon for 20 days seriously affected the hepatocytes and renal functions, and may also the pancreas β-cells function. Our results are in agreement with (Ansari and Kumar 1988), who found that, Diazinon reduced the total protein level in Zebrafish, (Matin *et al.*, 1990), who observed that Diazinon administration to rats reduced the carbohydrate and protein metabolism, (Begum and Vijavaraghaven 1995), who showed that Dimethoate inhibited the carbohydrates and proteins metabolism, and affected the aminotransferase activity in rats, (Fatima *et al.*, 2006), who indicated an increase in blood glucose level in Diazinon administrated rats, (Attia and Nasr 2009), who noticed increase in serum aminotransferase,alkaline phosphatase and decrease in total protein and albumin in rats serum after orally administration of Dimethoate, (Hagar and Fahmy 2009), who showed that, Dimethoate orally administration resulted the increase in blood glucose level, and (kossmann *et al.*, 1997), who assured the nephrotoxic effect of pesticides.

The results of this study showed that, the hematological parameters RBC and Hb were significantly decreased in Dimethoate and Diazinon treated rabbits when the erythrocytes sedimentation rate was highly significant increased as compared to control. The effect of organophosphorus pesticides on the Hb of several workers has been studied by (Bhatnagar 1980; and Ray 1992). The decrease in the Hb along with the decrease in the RBC might be due to the effect of pesticides on blood forming organ (bone marrow and liver), and inhibition of many steps of heme biosynthesis in rabbits, as the result of pesticides exposure (Ray 1992). The poisoning by

pesticide residues leads to the development of anemia due to interference of Hb biosynthesis and shortening of the life span of circulating erythrocytes (Betrosian 1995; and Jyotsana *et al.*, 2003). The increase of E.S.R. indicates to inflammation caused by organophosphorus pesticides (Elias and Saif 2009). Our finding is in agreement with (Jyotsana *et al.*, 2003), that showed that pesticides decrease R.B.C. and Hb levels, and (Elias and Saif 2009), who noticed the reduce of R.B.C., Hb, and increase in erythrocytes sedimentation rate in rabbits exposure to orally dose of 10mg/kg. of the organophosphorus pesticide Methidathion.

The above mentioned effects of organophosphorus pesticides could be due to their ability to form free radicals (Hazarika *et al.*, 2003; and vidyasagar *et al.*, 2004). The results of our previous work on Methidathion showed that the using of antioxidants vitamins A, C, and E, reduces the toxicity of Methidathion (Elias and Saif 2009). This fact may ensure the hypothesis of the ability of organophosphorus pesticides to form free radicals, which have been implicated as playing a role in the etiology of many alterations (Halliwell and Gutteridge 1995).

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