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Toxic Effect of Methylation and Chlorpyrifos on Liver of Male Rabbits

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Abstract

The irrational use of organ phosphorus insecticides in Yemen play a crucial role in the occurrence of many diseases affecting plants, animals, and man. Methidathion (MD) and Chlorpyrifos (CF) are two of widely used organ phosphorus insecticides in agriculture. The purpose of the present study was to investigate the in vivo effect of (MD) and (CF) on lipid peroxidation (LPO), antioxidant defense system in the liver cells and liver function in male rabbits after orally administration a single dose of 1/8 of LD50 of (MD) and (CF) period of 20 days. Thirty healthy male rabbits weighting 1400-1600g., were divided into 3 groups with 10 animals in each, first group served as control animals, they received 3ml.of corn oil, while animals in second and third groups received 1/8 of LD50 of (MD) and (CF) respectively. The level of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), and gamma glutamyl transferase (γ-GT) in serum were analyzed, the level of (LPO), and methylglyoxal (MG), in liver homogenate were also studied. Results showed a significant ($P<0.01$) increase in all studied parameters in animals treated with (MD) and (CF) as compared to control animals. Our results indicated to the high toxicity of (MD) and (CF).

Keywords: Methidathion; Chlorpyrifos; Lipid peroxidation; Liver

Abbreviations: MD: Methidathion; CF: Chloropyrifos; LPO: Lipid peroxidation; AST: Aspartate aminotransferase; ALP: Alkaline phosphatase; LDH: Lactate dehydrogenase; MG: Methylgyoxal; TBARS: Thiobarbituric acid reactive substances; ROS: Reactive oxygen species.

Introduction

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 organ phosphorus insecticides [1]. Many alterations have been observed in organs of animals due to the organ phosphorus insecticides. The organ phosphorus insecticides have been implicated as playing a certain role in etiology of tumors [2], blood diseases [3], liver injury [4-6], heart and vascular muscles atrophy [7,8], digestive system diseases [2], renal disorders [2,9], and metabolism disorders [9]. Methylation (MD) and Chlorpyrifos (CF) are an organ phosphorus insecticides with anticholinesterase mode of action [7,10], (MD) and (CF) are two of the most widely and irrationally used insecticides in agriculture in Yemen, especially in khat cultivation [1]. It has been found that the sub chronic orally administration of (CF) significantly increased the oxidative stress in rats erythrocytes caused their hemolysis [10]. The orally administration of (CF) resulted in significant increase in the level of thiobarbituric acid reactive substances (TBARS), and significant decrease in superoxide dismutase and catalase in rats lung tissues [11]. White blood cells, red blood cells, hemoglobin, and total protein significantly decreased, while cretonne and uric acid significantly increased in blood of mice orally treated with (CF) [9]. The orally administration of (MD) increased the oxidative stress and decreased the antioxidant enzymes activities in rats erythrocytes [12]. Histopathological change like infiltration with mononuclear

cells at parenchymal tissue, sinusoidal dilatation, focal necrotic areas, granular degeneration and panoptic nuclei in the hepatocytes were observed in rats liver after orally administration of (MD) [5]. The level of cardiac troponin in serum and MDA in cardiac tissues significantly increased in rats after orally administration of (MD), this indicates to the diffuse loss of striation and myocytolysis of the cardiomyocytes caused by (MD) [7].

The purpose of the present study was to investigate the in vivo effect of (MD) and (CF) on lipid peroxidation (LPO) and antioxidant defense system in the liver cells and on liver function in male rabbits.

Materials and Methods

Animal's Treatment and Blood Collection

Thirty healthy male rabbits local breed (1400-1600g) were divided into 2 treated groups and control, as follows:
Control group: 10 animals treated with a single daily dose of 3ml.corn oil orally period of 20 days.
(MD) group: 10 animals treated with a single daily dose of 1/8 of LD_{50} of (MD) (5mg/kg) in 3ml. corn oil orally period of 20 days.
(CF) group: 10 animals treated with a single daily dose of 1/8 of LD_{50} of (CF) (12mg/kg) in 3ml. 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.

The experiment was administrated in the Animal Physiology Laboratory, Department of Biology, Faculty of Sciences and Education, and Central Researches Laboratory Aden University.

After 20 days the animals were fasted overnight for 12hrs, then they were sacrificed, the blood was immediately collected and centrifuged, serum was discarded and kept at - 21 º C for the biochemical analyze. Livers were removed immediately after death, perfused with normal saline containing heparin, homogenized with phosphate buffer saline (pH 7.2) using homogenizer, centrifuged at 3000g for 30 min. The supernatant was removed and stored at -20ºC.

Analysis

Alanine-Aminotransferase and Asparatate-Aminotransferase Assay

The level of ALT and AST in serum was determined according to the procedure described by Gehringer, et al. [13].

Gamma Glutamyl Trransferase Assay

Determination of γ-GT activity in serum was based on the method of Szasz [14].

Lactate Dehydrogenase Assay

Serum LDH was measured according to Doyle and Griffiths [15].

Alkaline Phosphatase Assay

ALP was determined using a colorimetric method as described by Kind and King [16].

Lipid Peroxidation Assay

The lipid peroxidation (LPO) level in hepatocytes homogenate was measured via the measuring of malondialdehyde MDA according to method described by Husseinzadeh, et al. [17].

Methylglyoxal Assay

The MG was determined in the liver homogenate according to the method described by Ratliff, et al. [18].

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.

Results

Enzymes	Treatments Control	MD	CF
ALT IU/L	41.35±1.32	141.25±4.43	120.10±2.11
AST IU/L	32.20±1.65	141.25±4.43	161.20±5.12
AST IU/L	59.65±2.73	139.60±6.34	161.20±5.12
LDH IU/L	210.25±4.12	360.34±4.11	302.61±6.54
γ-GT IU/L	6.13±0.12	36.11±1.14	22.16±1.09

Table 1: The level of enzymes in serum after 20 days of orally administration of (MD) and (CF) in dose 5mg/kg and 12mg/kg respectively.
Values are expressed as mean of 10 animals±S.E, P<0.01 vs. control.

Parameters	Treatments Conttrol	MD	CF
MDA µM	0.03±0.01	18.23±2.11	13.21±3.43
MDA µM	1.12±0.33	59.18±4.87	41.02±4.86

Table 2: The level of MDA and MG in liver homogenate after 20 days of orally administration of (MD) and (CF) in dose 5mg/kg and 12mg/kg respectively.
Values are expressed as mean of 10 animals±S.E, P<0.01 vs. control.

Data in table1 show that, the administration 1/8 of LD_{50} (MD) and (CF) leads to the critical damage in liver function. The results in table1 show high significant (P<0.01) increase in the level of aminotransferases ALT and AST, as well as in ALP,LDH and γ-GT, in the serum of animals treated with (MD) and (CF) as compared to control animals, this increase was higher in the serum of animals treated with (MD).

Data in table2 show high significant (P<0.01) increase in the level of MDA a product of lipid peroxidation (LPO) and in MG a toxic substance in the liver homogenate of treated with (MD) and (CF) animals as compared to control animals, this increase was higher in the serum of animals treated with (MD).

Discussion

According to the literatures the increase in AST and ALT indicates to acute liver diseases, cirrhosis liver and toxic lever cells necrosis [19,20], while the increase in LDH indicates to acute hepatitis, renal tubular necrosis and acute myocardial infraction [19]. The increase in ALP is due to the lesions of liver, ALP also increased in acute liver diseases [19]. The γ-GT increases in the most of hepatobiliary [19]. These facts suggested that the exposure to (MD) and (CF) in our experiment leaded to serious damage in liver cells.

One important consequence of exposure to (MD) and (CF) is the excessive of free radicals production, which attack many organic molecules in cells membrane including polyunsaturated fatty acid leading to increase in LPO [21]. Several studies reported that reactive oxygen species (ROS) initiate LPO through the action of hydroxyl radicals [22]. Exposure to the toxic compounds has been linked with increase of ROS production not only in domestic and wildlife animals but also in human [23]. Formation of reactive oxygen species (ROS) and oxidative stress is associated with development of many pathological statuses [24]. Oxidative stress may occur either due to the decrease of cellular antioxidant level, or due to the over production of ROS [22].

Our results clearly showed that (MD) and (CF) in the negligible dose have the ability to free radicals formation, decrease of cellular antioxidant level, therefore initiate of the LPO which leading to damage of cells membrane and death of cells [24]. Our results are close to that found in previous studies [4,5,12].

Methylglyoxal (MG), (also called Pyruvaldehyde), formed as a side product of dihydroxyacetone phosphate in glycolysis, MG must converted into lactic acid and/or pyruvic acid in presence of glyoxalaseI [19]. MG is considering being toxic for mammals, the MG cytotoxicity is due to it accumulation in cells [17]. The animals exposure to (MD) and (CF) in our experiment showed increase in the MG level in liver cells homogenate. The accumulation of MG is due to the inhibition of glyoxalase I [18,25], the accumulation of MG in liver caused depletion of antioxidant defense system found in liver, leading to significant generation of free radicals which might in further strengthen the damage and affect the hepatocytes function [25-27]. Our results clearly showed that the exposure to the organ phosphorus insecticides (MD) and (CF) in negligible dose inhibited the glyoxalaseI, therefore, accumulation of MG in liver cells, that leads to depletion the antioxidant defense system, caused significant increase in free radicals generation; which leads to increase in LPO, thus damage of hepatocytes function, and so increase in the level of enzymes concerning liver dysfunction. The high toxicity of organ phosphorus insecticides may due to their ability to free radicals formation. The free radicals have been implicated as playing a major role in induce oxidative stress, therefore, the etiology of many diseases and alterations [24].

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