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Research Article

The Potential Role of Alkaloid Extract against Phospholipase Extracted from Aspergillus Flavus in Male Rats

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Abstract

This article aims to evaluate the role of alkaloid extract against phospholipase toxicity. The study sample contained 20 adult albino male rats divided into four groups, where each group consists of 5 rats. The groups are categorized as follows:

- The group of control which received ad libidium.
- The infected group, which was injected with (0.05ml/per animal) phospholipase.
- A group injected with (0.05ml/per animal) phospholipase and treated with (0.05ug/per animal) alkaloid extract.
- A group injected with (0.05ml/per animal) phospholipase and treated with (0.05ug/per animal) alkaloid extract.

The results show a highly significant increase ($P < 0.05$) in the levels of Alanine Aminotransferase (ALT) and Aspartate Aminotransferase (AST) in the infected group compared with the control group. Oxidative stress factor in the infected group demonstrates a significant increase ($P < 0.05$) in the MDA (malonedialdehyied) levels. Nevertheless, it reported a significant decrease ($P < 0.05$) in the glutathione (GSH) levels compared with the control group. After using alkaloid extract in the treatment, non-significant changes ($P < 0.05$) are found in liver functions. Furthermore, MDA and GSH report non-significant changes ($P < 0.05$) compared with the group of control. The study demonstrates that alkaloid extract has a potential role against phospholipase that is extracted from A. flavus in rats.

Keywords: *Alkaloid extract; A. flavus; liver function; MDA (malonedialdehyied); glutathione (GSH).*

Introduction

Aspergillus flavus, a saprophytic soil fungus, is the most crucial producer of mycotoxins including the potent aflatoxins. Consequently, this fungus is involved in crop colonization and contamination with aflatoxins, both during pre- and post-harvest stages of crops. Previous studies have suggested that the dynamics of A. flavus growth and its potential to produce toxins are influenced by factors of global climate changes (e.g., temperature, drought stress and CO2 concentration) [1-2].

A. flavus mainly infects peanuts, maize, figs, cotton, tree nuts [3] and spices [4]. Species of aflatoxigenic Aspergillus can invade food and feeds, particularly in hot climates. Foods can be deteriorated by species of Aspergillus. This includes stored rice, corn, wheat, flour bran, soy- bean and peanut. Secondary metabolites are produced by some classes of this genus in food as aflatoxins (AFs). Mainly, A.flavus and A.parasiticus produce aflatoxins [5]. Ruta graveolens (family Rutaceae) is another medicinal plant that has been used since time immemorial [6]. Medicinal properties like anti-inflammatory, analgesic, antibacterial, antidiabetic and antifertility effects of R. graveolens extracts have been reported in various studies [7, 8].

Materials and Methods

Model of Animal

In this study, twenty adult male albino rats (weighted 200-250 gm, aged 5-8 months)

were obtained from Technical College, University of North Technical, Iraq. Standard pellet diet was applied to them for two weeks to ensure it is normal, and there is no infection.

The Alkaloids Extract Preparation

The extraction of dry defatted seeds of Peganum harmala (30 gm) was performed with (250 ml) of (5%) acetic acid in (60%) ethanol. A hot plate was used to heat the mixture for (3 hr) at (50 ℃). After that, the extract was centrifuged. In addition, the filtrate was collected and concentrated to (20 ml). The solution was alkaline by adding conc. NaOH. Then, it was filtered. The crude alkaloids were precipitated, washed with (1%) NH4OH and then dried [9].

A. flavus Isolates

A. flavus isolates isolated from rice and maize were obtained from the Biology Department, College of Sciences, Tikrit University, Iraq. The cultivation of all fungal isolates was performed at 28 ˚C on potato dextrose agar (PDA) and then stored at 4˚C [10].

Phospholipase Detection

We used the method proposed in [11] to detect phospholipase sold media.

Phospholipase Extraction

The extraction of phospholipase from A. flavus was performed according to the method [12].

Partial Purification of the Enzyme

The enzyme was precipitated by adding (NH4)2SO4 (60%) to enzyme extract. The mixture was put on a magnetic stirrer, using centrifuge (6000rpm) for 30 Min.

The deposit was dissolved in buffer solution (0.01 sodium citrate and pH 6.7). Then, the sample was stored (-20 ℃) until used.

Experimental Design

Male rats were used and divided into four groups, where each group consisted of five rats. The groups are categorized as follows:

- Control group: rats received normal diet and used as control.
- Infected group: injected (intraperitonelly) with (0.05ml/day per rat) phospholipase enzyme. After infection, they were killed.
- Treated group: injected (intraperitonelly) with (0.05ml/day per rat) phospholipase and treated with (50ug/day per rat) for 3 weeks. After that, they were killed.
- Treated group: injected with (0.05ml/day per rat) phospholipase and treated with (100ug/day per rat) for 3 weeks. After that, they were killed.

Oxidative Stress Factors

MDA (malonedialdehyied) by thiobarbituric acid (TBA) according to the method [13], and Glutathione (GSH) by using DTNB, with estimate catalase according to method [14].

Liver Function Enzymes

Serum ALT and AST were measured following the instructions of the manufacturer company kit (Randox).

Samples Collection

The blood samples were collected by cardiac puncture under anesthesia. Then, the samples were kept in test tubes. After clotting, the tubes of blood sample were centrifuged (5000 cycle/min for 10 min) to isolate blood serum. The lung was removed immediately and homogenized with NaCl2. After that, supernatant and serum were taken and stored at deep freezing until use.

Statistical Analysis

A statistical Minitab software through employing the test of Analysis of Variance (ANOVA) was used to analyze research data for evaluating the importance of variability between control and treated groups.

Results and Discussion

Oxidative stress (MDA) & Antioxidant Parameter (GSH)

The MDA (2.31 ± 0.17) and GSH (0.309 ± 0.021) levels in second group showed high significant changes ($P < 0.05$) in comparison to the control group of rats (1.39 ± 0.21; 0.438 ± 0.027, respectively). In the third and fourth groups, the levels of MDA (1.53 ± 0.28; 1.26 ± 0.12) and GSH (0.42 ± 0.026; 0.453 ± 0.021) demonstrated non-significant changes ($P < 0.05$) in comparison to the control group of rats, as illustrated in Figure 1.

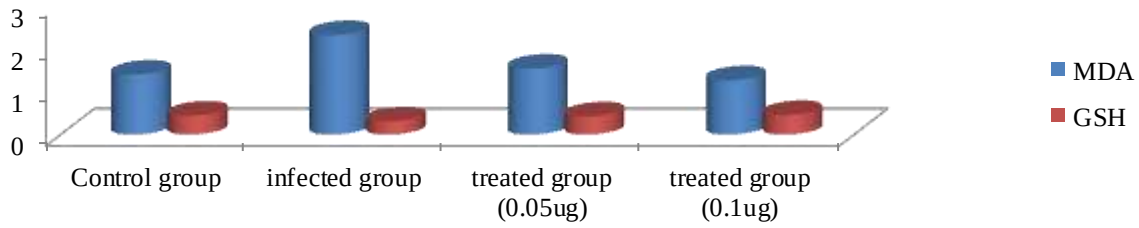

Figure 1: The levels of MDA and GSH in the liver extract

The results of the present study showed a high effect of phospholipase of A. flavus on antioxidant. The findings were consistent with those of the study of Devendran et al. [15] who referred to that Intraperitoneal route of aflatoxin (that extracted from A. flavus) for 8 days caused a significant increase in lipid peroxidation in liver of aflatoxin treated rats as compared to controls. It has been found that lipid peroxidation is among the key symptoms of cellular damage.

Therefore, an increase in lipid peroxidation could be due to a significant reduction in the enzymatic antioxidant activities like catalase, superoxide dimutase and glutathione peroxidase. Additionally, a non-enzymatic antioxidant such as total ascorbic acid and α-tocopherol contents in the liver and kidney of aflatoxin treated rat as compared to the control group [16]. On the other hand, the use of *Peganum harmala* alkaloid extract revealed a positive effect and improvement in the antioxidant state. In a study carried out by Bourogaa et al. [17], they demonstrated the protective effect of *Peganum harmala* extract in mice liver. They also found that *Peganum harmala* extract leads to improve the levels of hepatic superoxide dismutase, catalase and glutathione peroxidase (GPx) activities.

Liver Function Tests

ALT levels (82.34 ± 6.02) and AST (93.14 ± 5.19) in the infected group presented a highly significant increase ($P < 0.05$) in comparison to the control group of rats (11.14 ± 1.93 and 10.83 ± 1.72, respectively). In the third and fourth groups, the levels of ALT (12.02 ± 3.91 and 9.3 ± 1.14, respectively) and AST (11.83 ± 2.41 and 9.53 ± 1.66, respectively) showed a non-significant decrease ($P < 0.05$) in comparison to the control group of rats, as illustrated in Figure 2.

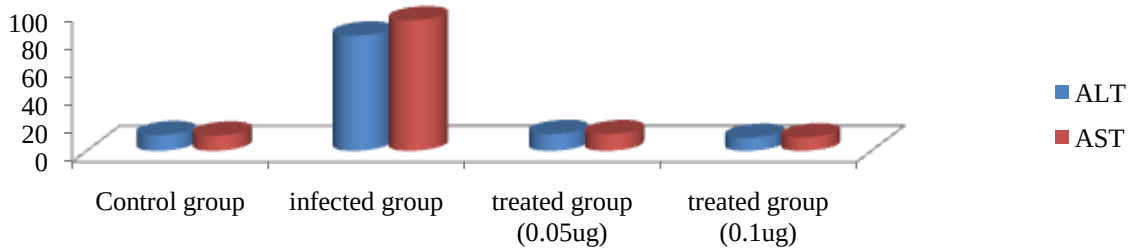

Figure 2: The levels of ALT and AST in serum

The results showed a high effect of phospholipase of A. flavus on liver enzymes. The ability of phospholipase to induce cytoplasmic hepatocyte vacuolation, megalocytosis, nuclear vacuolation, inflammatory infiltrate, and necrosis was detected in mice liver [18]. On the other hand, the use of *Peganum harmala* alkaloid extract showed a positive effect and improvement in liver enzymes. Concerning the protective effect of *Peganum harmala* extract in mice liver, it is found that *Peganum harmala* extract leads to improve the levels of ALT and AST [19]. Also, Mahajan et al. [20] reported that Alkaloids impaired kidney and liver functions.

Conclusions

The results of this study show the alkaloids that extracted from seeds of peganum harmala has a potential activity against the phospholipase of A. flavus in rats and this is activity its back to the ability of alkaloid extract as antioxidant role.

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