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Effects of Aspergillus spp. Phospholipase to Induce Histological and Biochemical Changes in Male Rats

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Abstract--- *This study was achieved to exhibit effects of Aspergillus spp. phospholipase on rats (in vivo). In this study, 18 adult albino male rats were used which divided to three groups including the control group (received ad libidium), second group (injected with 0.05 ml/per animal of phospholaipase) and the third group (injected with 0.1 ml/per animal of phospholaipase) for 3 weeks. The results showed high significant increase ($p<0.05$) in levels of IL-1α, IL-2, IL-6 and IL-8 in second and third groups compared with the control group. Oxidative stress factor in second and third groups exhibited a significant increase ($p<0.05$) in levels of MDA (malonedialdehyied) and glutathione (GSH) compared with the control group. About the histological changes, the liver sections that prepared from the second and third groups showed the degeneration of hepatocytes, thickening wall of central veins with inflammatory cells infiltration and different changes in nuclei in many hepatocytes. It was concluded that phospholipase that extracted from Aspergillus spp. led to remarkable histological and biochemical changes in rats.*

Keywords--- *Aspergillus spp, Liver, Interleukins, Malonedialdehyied, Glutathione.*

I. INTRODUCTION

In the Roman Catholic formal custom, an aspergillum (pl. aspergilla, aspergillums or aspergills) is a brush or punctured compartment for sprinkling heavenly water. In mycology, an *Aspergillus* (pl. aspergilli or consistently however inaccurately 'Aspergilli') is any of a gigantic assortment of molds that replicates through making abiogenetic spores on a morphological structure that takes after the heavenly water sprinkler. The variety was once named by means of Antonio Micheli (1679-1736) in his 1727 booklet entitled Nova plantarum in which 1900 greenery had been portrayed, 1400 just because, of which 900 were organisms [1-2]. aspergilli cause an expansive range of contaminations which incorporate cutaneous indications, otomycosis, and obtrusive diseases, for example, aspiratory aspergillosis and endocarditis [3]. Clinical microbiology research facilities matter vigorously on morphology-based distinguishing proof methodologies for *Aspergillus* species whereby demonstrative norms incorporate the awareness of agamic or sexual structures and their qualities, for example, shape, size, shading, ornamentation as well as method of connection [4]. The most continuous species are *A. niger* and *A. flavus*, joined by methods for *A. parasiticus*, *A. ochraceus*, *A. carbonarius*, and *A. alliaceus*. They can pollute farming items at various degrees which incorporate pre-collect, reap, handling and overseeing [5]. Phospholipases are a heterogeneous gathering of compounds that hydrolyse one or additional ester linkages in glycerophospholipids [6]. Phospholipases are ordered into four major kind's phospholipase A (PLA1and PLA2), phospholipase B (PLB), phospholipase C (PLC), and phospholipase D (PLD) on the foundation of cleavage of ester linkage inside a

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phospholipid particle [7]. The PLA and PLB has a place with acyl hydrolase classification of phospholipids, while PLC and PLD are givers of the phosphodiesterase class of phospholipids [8].

II. MATERIALS & METHODS

Animal model

In current the work, 18 adult male albino rats, (weights ranged 200-250 g with age from 5-8 months) obtained from the North Technical University (Technical College),, and kept on standard pellet diet for 2 weeks for insuring their normal and there are not any type of infections.

Aspergillus spp isolates

Aspergillus spp isolates The isolates of *Aspergillus* spp. remoted from Rice, *Aspergillus* spp. isolates isolated from maize, had been got from Department of Biology, College of Science, University of Tikrit. All fungal isolates have been cultivated at 28 ˚C on potato dextrose agar (PDA) and stored at 4˚C [9] until use.

Phospholipase detection

Method of [10] was using to detect phospholipase sold media.

Phospholipase extraction

The extraction of phospholipase from *Aspergillus spp* was done according to method [11]

Partial purification of enzyme

Enzyme was once participated by means of add (NH4)2SO4 (60%) to enzyme extract, the mixture put on magnetic stirrer, the the use of centrifuge (6000rpm) for 30 Min. the deposit was once dissolved in buffer answer (0.01 sodium citrate and pH 6.7). then sample used to be storage (-20 0C) till used.

III. EXPERIMENTAL DESIGN

Male rats have been used and grouped to three groups as in the following groups (6 rats in each group):

A. Control: rats received normal diet and used as control.
B. Second group: rats injected (intraperitonelly) with (0.05ml/day per rat) phospholipase enzyme for 3 weeks and then killed.
C. Third group: injected (intraperitonelly) with (0.1ml/day per rat) phospholipase for 3 weeks and then killed.

Measurements

Detection of Interleukins

The ELISA (Enzyme-Linked-ImmunoSorbent Assays) method used to be used for the interleukins detection according to the manufacturer's directions furnished through BioSource Company. Interleukin concentrations have been detecting after two and four weeks from infections to all corporations of study.

Oxidative stress factors

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MDA (malonedialdehyied) was assessed by thiobarbituric acid (TBA) according to method of [12], and Glutathione (GSH) was assessed by using DTNB, with estimate catalase according to method of [13].

Samples collection

The blood samples were collected by cardiac puncture under anesthesia and put in test tubes. After clotting, the blood sample tubes 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 by deep freezing until use.

Statistical analysis

Data of this work were analyzed by the statistical Minitab software, using ANOVA test (Analysis of Variance) to estimate the significance of variability among the control group and treated groups.

IV. RESULTS

Interleukin concentrations

IL-1α, IL-2, IL-6 and IL-8 in rats of second and third groups that injected with *Aspergillus* spp phospholipase show significant increased (P<0.05) compared with control rats as shown in figures (1-4).

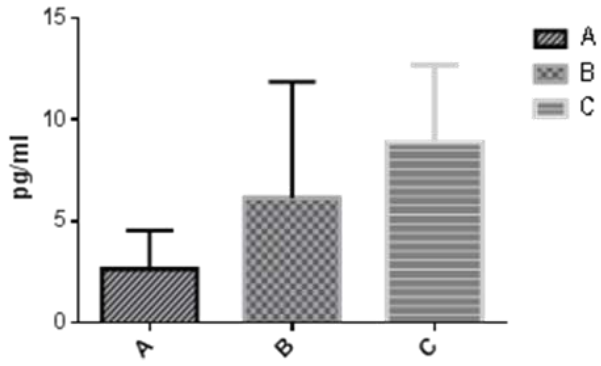

Figure (1): Levels of IL-1α in all groups

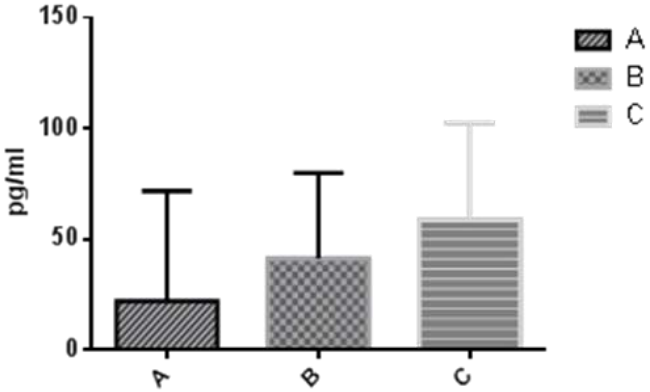

Figure (2): Levels of IL-2 in all groups

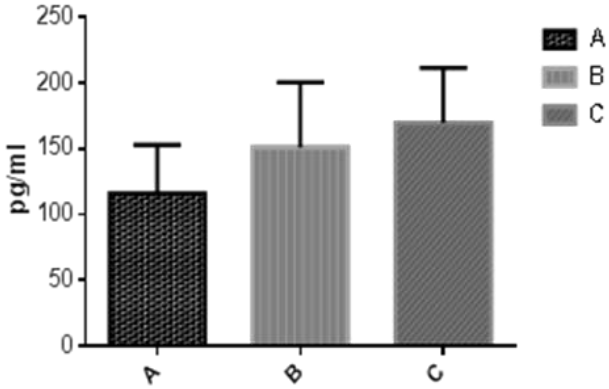

Figure (3): Levels of IL-6 in all groups

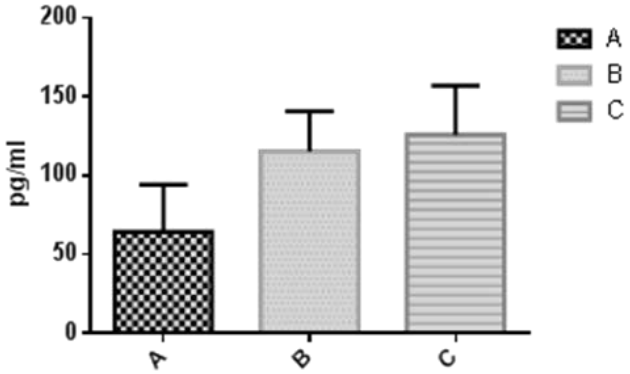

Figure (4): Levels of IL-8 in all groups

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with *Aspergillus spp* phospholipase show significant increased (P<0.05) compared with control rats as shown in figures (5-6).

MDA and GSH level

MDA and GSH levels in rats of second and third groups that injected.

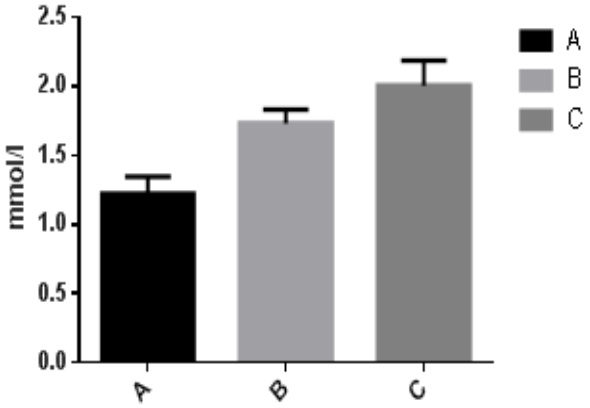

Figure (5): Levels of MDA in all groups

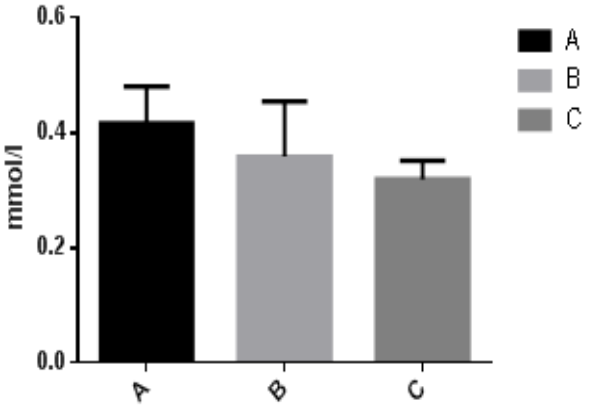

Figure (6): Levels of GSH in all groups

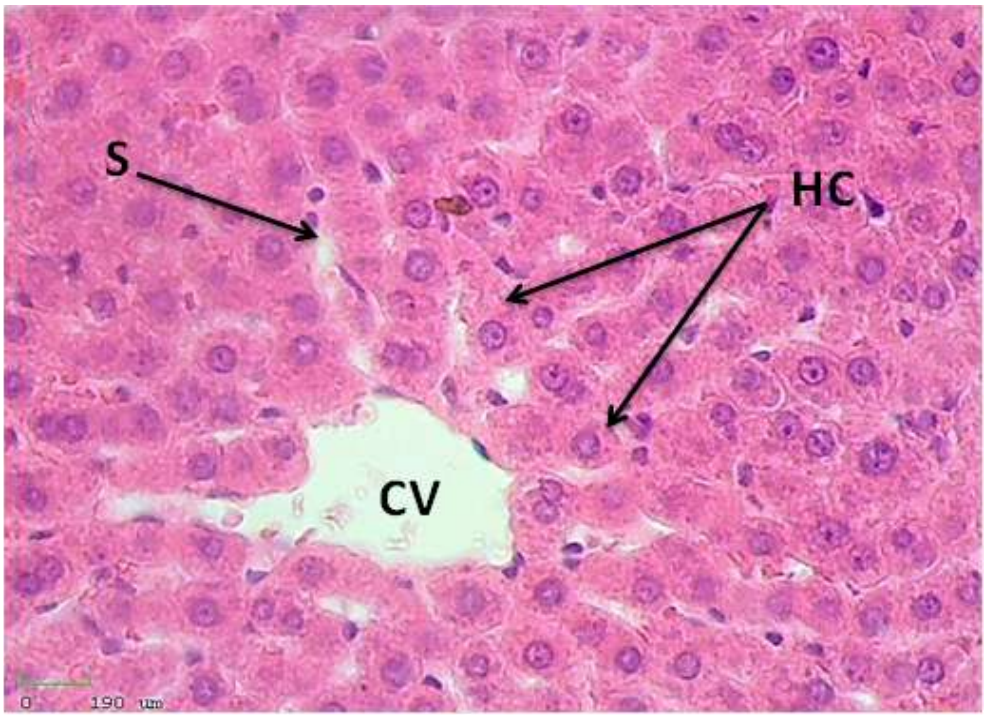

Figure (7): liver of control group show normal of central vein (CV), hepatocytes (HC) and sinusoids (S) H&E X400.

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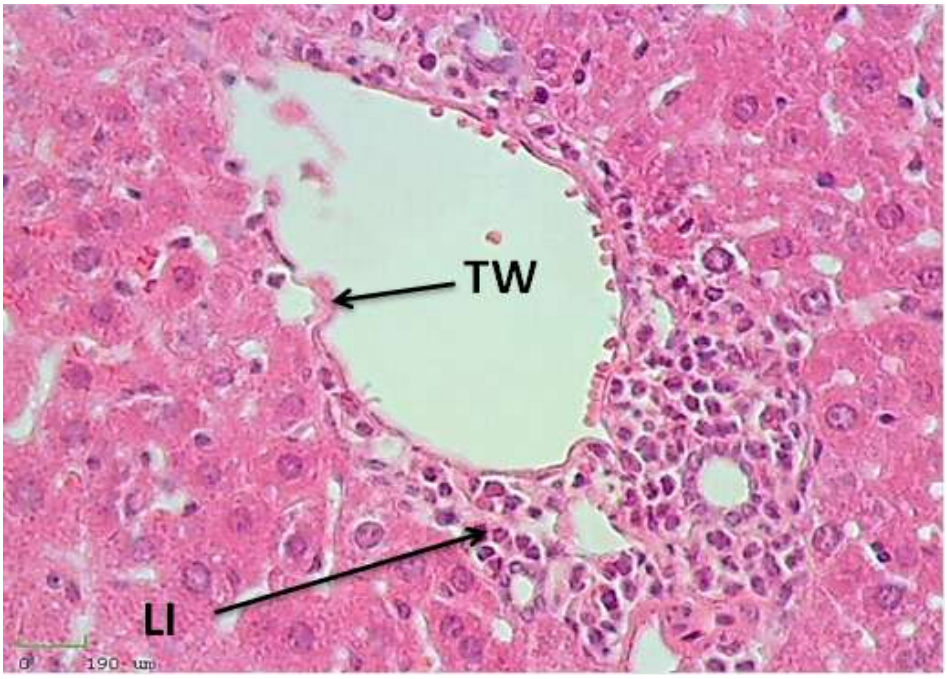

Figure (8): liver of second group show thickening wall (TW) of central vein and lymphocytes infiltration (LI) H&E X400.

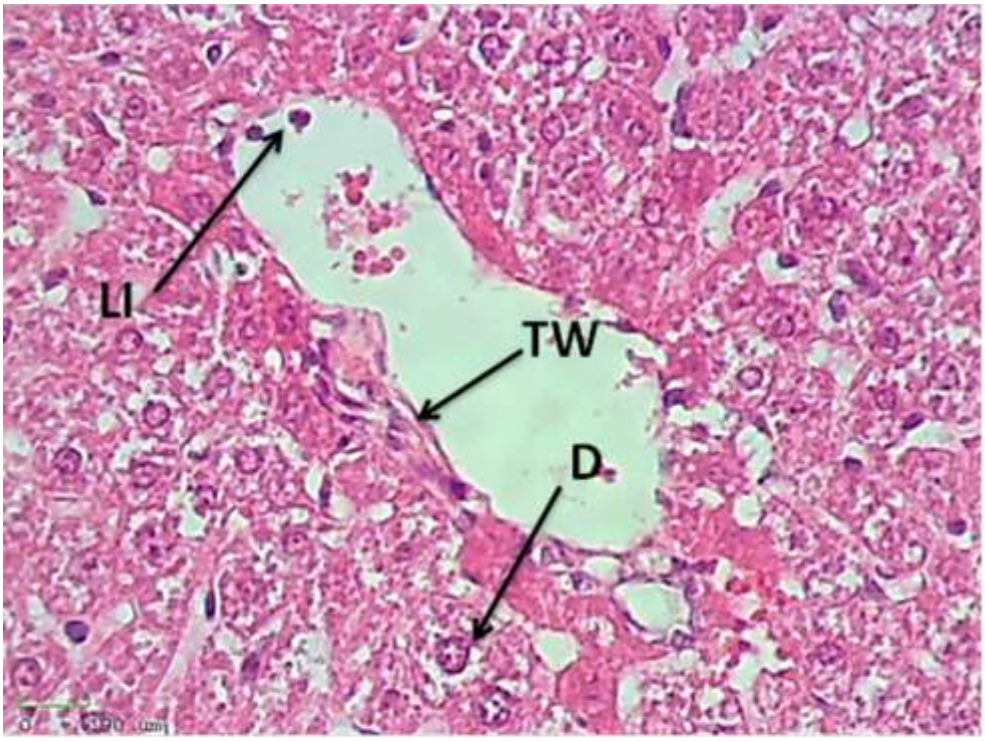

Figure (9): liver of third group show thickening wall of central vein, degeneration (D) of hepatocytes and lymphocytes infiltration H&E X400.

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Histological study

Sections of liver that prepared from the control group showed the normal structure form of central veins with the radical arrangement of hepatocytes around central veins. Also the sinusoids diameters that located between hepatocytes its normal in size and shape as exhibited in figure (7). however, the sections of liver that prepared from second and 3rd group showed degeneration of hepatocytes with thickening wall of central veins and infiltration of mono-nucleated inflammatory cells as shown in figures (8-9).

V. DISCUSSION

In this study, interleukins exhibit vast expanded examine with manipulate group, the cytokines that are produced at some stage in inflammatory processes, and that take part in them, are stimulators of the production of acute part proteins. These inflammations associated cytokines consist of IL-1α, IL-2, IL-6[14] and, possibly, IL-8 [15]. They are produced by using means of a range of cellular phone types, however the most indispensable sources are macrophages and monocytes at inflammatory websites [16]. Also, the properly identified domestically generated markers of irritation are TNF -α and IL-6 [17]. The effects of existing locate out about exhibit a excessive impact of phospholipase of *Aspergillus* spp. on antioxidant. Where, the effects are in settlement with Devendran et al, [18] referred Intraperitoneal route of aflatoxin (that extracted from *Aspergillus*) for 8 days triggered massive enlarge in lipid peroxidation in liver of aflatoxin treated rats, as in contrast to controls, lipid peroxidation is considered as one of the major key events in mobile damage. Therefore, an expand in lipid peroxidation ought to be due to sizable bargain in the activities of enzymatic antioxidant such as catalase, superoxide dimutase and glutathione peroxidase as well as non-enzymatic antioxidant such as entire ascorbic acid and α-tocopherol contents in the liver and kidney of aflatoxin, handled rat; as in contrast to the controls [19]. About the histological adjustments that caused by means of the usage of phospholipase that back to the capability of phospholipase to result in Cytoplasmic hepatocyte vacuolation, megalocytosis, nuclear vacuolation, inflammatory infiltrate, and necrosis have been present in mice liver [20].

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