

Effect of Garden Cress (*Lepidium sativum*) Extracts on Aflatoxin B₁ and Blood Parameters in Rats

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ABSTRACT

The crude chloroform and methanol extracts of *Lepidium sativum* seeds belong to family Brassicaceae, were used in this work to study their efficiency to reduce Aflatoxin B₁ in rats. The more effective of chloroformic extract which decreased Aflatoxin B₁ level from 8.7 to 1.0 ppb at a dose of 300 mg/kg body weight comparing with liganon (Silymarin) which reduced Aflatoxin B₁ to 0.97 ppb in concentration of 100 mg/kg after 5 weeks, followed by methanolic extract at a dose of 300 mg/kg body weight which showed the mean value of 1.2 ppb. Also, the more effective treatments may be Silymarin in concentration of 100 mg/kg followed by chloroformic extract, while methanolic extract of *Lepidium sativum* seeds was represented middle effect for blood tests of rats injured with Aflatoxin B₁ e.g. Serum marker enzyme parameters (MDA, SOD, ALP and Bilirubin), kidney functions (Creatinine and Urea), liver functions (ALT, AST, Total proteins, Albumin and Globulins), lipid profile (triglycerides, total cholesterol, HDL-c, LDL-c and vLDL-c) and haematological parameters (HB, RBCs, Plt and WBCs), were determined in injured rats comparing with control rats. It is quite clear that Silymarin is considered synthetic product, so the natural extracts of garden cress are more suitable for usage due to, they considered more safety and economic products, so these extracts can be additive to foodstuffs and therapeutic drugs owing to their multi medicinal benefits.

Keywords: *Lepidium sativum* seeds extracts, kidney functions, liver functions, Silymarin and Aflatoxin B₁.

INTRODUCTION

Aflatoxins (AFs) are secondary metabolites produced mainly by three species of *Aspergillus* including *A. flavus*, *A. parasiticus* and *A. nomius*. The most known AFs which can be found as contaminants of food and feed are B₁, B₂, G₁ and G₂ and their two metabolic products M₁ and M₂. They are probably the most known and most intensively researched mycotoxins in the world. Aflatoxins have been associated with various diseases, such as aflatoxicosis in livestock, domestic animals and humans throughout the world. (Jovana *et al.*, 2013).

Liganon (Silymarin), as a standard drug is used in humans for the treatment of liver diseases of different etiologies. So, it has been used to compare the results of obtained extracts garden cress seeds. (Tedesco *et al.*, 2004).

Medicinal plants are widely used as home remedies and raw materials for the pharmaceutical industries. During harvesting, handling, storage and distribution, medicinal plants are subjected to contamination by various fungi, which may be responsible for spoilage and production of mycotoxins. The increasing consumption of medicinal plants has made their use a public health problem due to the lack of efficiency surveillance of the use, efficiency, toxicity and quality of these natural products. (Kostik, 2015).

Lepidium sativum (Garden cress) is an annual herb, belonging to Brassicaceae family. It is a fast-growing, edible plant botanically related to watercress and mustard and sharing their peppery, tangy flavor and aroma. Seeds, leaves and roots are economically important, however, the crop is mainly cultivated for seeds. In some regions garden cress is known as garden pepper cress, pepper grass or pepperwort. It is also known as Asalio or chandrasur in India and it is an important medicinal crop in India. Garden cress is a perennial plant, and an important green vegetable consumed by human beings, most typically as a garnish or as a leaf vegetable. (Tiwari and Kulmi, 2004).

Divanji *et al.* (2012), mentioned that *Lepidium sativum*, mainly contains alkaloids, saponins, anthracene glycosides, carbohydrates, proteins, amino acids, flavonoids, sterols as chief phytochemical constituents. Glutamic acid is the most abundant amino acid. Leucine and methionine were represented the high and low

concentrations essential amino acids respectively. The extracts of this plant have been found to possess various pharmacological activities.

A Comprehensive review of its medical uses, chemical constituents and pharmacological profile as a medicinal plant, mainly focused on its anti-inflammatory, antipyretic, analgesic, coagulant, antihypertensive, diuretic, anti-diabetic, hepatoprotective, anti-asthmatic and antioxidant activity for better evaluation in various therapeutic applications.

The aim of this study is to examine the effect of *Lepidium sativum* seeds chloroform and methanol extracts at doses of 100, 200 and 300 mg/kg body weight on Aflatoxin B₁ in rats after 5 weeks through the determination of Aflatoxin B₁ in blood of rats. Also, seed extracts and its effects on serum marker enzyme parameters, kidney functions, liver functions, lipid profile and haematological parameters comparing with liganon (Silymarin) as a synthetic standarder drug (100 mg/kg) were studied.

MATERIALS AND METHODS

Sampling:

The present investigation was carried out using *Lepidium sativum* seeds belong to family Brassicaceae. Samples were kindly obtained from Agricultural Research Center, Giza, Egypt. Seeds samples were air dried at room temperature and ground into a fine powder. Seeds powder (2Kg) was extracted by soaking at room temperature for six times with methanol (30 L), then the successive extraction was carried out by using pet.ether, chloroform and ethyl acetate. Four extracts were obtained and then concentrated to dryness under vacuum using rotary evaporator at 45°C to achieve the dried methanol, pet.ether, chloroform and ethyl acetate extracts which kept at 4°C till further use.

Experimental animals:

A number of 63 male albino rats (100-150g) were obtained from the animal house of Faculty of Pharmacy, Mansoura University, Egypt. All rats were housed in microlon boxes in a controlled environment (temperature 25±2°C and 12 hr dark/light cycle) with standard laboratory diet and water. (Saluja *et al.*, 2010).

Aflatoxigenic organism:

Aspergillus flavus was used for the production of Aflatoxin B₁ in this study. The strain was inoculated into Potato Dextrose Agar (PDA) plate and incubated at 28 ± 2°C for 3-5 days. After incubation, the pure produced fungal Aflatoxin B₁ was used for injection in experimental animals. (Devendran and Balasubramanian, 2011).

Experimental design:

Male albino rats (63 animals) were divided into 9 groups each group contains 7 animals, as follows: Group 1 (normal): represents normal rats by meaning without Afltoxins. Group 2 (control): Aflatoxin B₁ through single intraperitoneal injection (1.0 mg AFB₁/kg body weight).

Group 3: Aflatoxin B₁-bearing rats treated with ligalon (Silymarin) which was administered in traperitoneally injections (100 mg/kg body weight), was used as a standard hepatoprotective drug for comparison according to Banu *et al.* (2009).

Group 4, 5 and 6: Aflatoxin B₁-bearing rats treated orally with chloroformic extract of *Lepidium sativum* seeds (100, 200 and 300 mg/kg), respectively.

Group 7, 8 and 9: Aflatoxin B₁-bearing rats treated with methanolic extract of *Lepidium sativum* seeds (100, 200 and 300 mg/kg), respectively. Single dose of the extracts were administrated orally to each animal. (Ramamurthy and Rajeswari, 2015).

Rats were subjected to natural photoperiod of 12hr light: dark cycle throughout the experimental period (5 weeks). Blood samples were collected from the eye canthus by heparinized tubes after 3 and 5 weeks from the beginning of the experiment. Then, all blood samples were divided into two portions. First portion was centrifugation to obtain the blood serum.

Serum samples were kept at refrigerator under freezing conditions for the determination of the serum marker enzyme parameters (MDA, SOD, ALP and Bilirubin), liver functions (ALT, AST, proteins, albumin and Globulins), kidney functions (creatinine and urea) and lipid profile (Triglycerides, total cholesterol, HDL-c, LDL-c and vLDL-c).

Second portion was treated with 10% of ethylene diamine tetracetic acid (EDTA) with a good shaking to determine complete blood count (CBC) as a haematological analysis which contain (HB, RBCs, PCV, MCV, MCHC and other parameters).

Determination of Aflatoxin B₁:

Afla-V Strip Tests utilize the proven sensitivity and selectivity of VICAM's monoclonal antibodies to accurately detect and measure Aflatoxin B₁ at levels as low as 2 ppb and as high as 100 ppb. Sample preparation is easy and Afla-V's simplified procedure saves time and materials. After just 5 minutes development time, the Afla-V Strip Test is ready for quantitation using the Vertu® Lateral Flow Reader. Results are displayed on the digital screen and may also be printed or transferred to Excel for storage and use as a vital quality assurance tool.

Chemical analysis of blood:

Determination of serum marker enzyme parameters (MDA, SOD, ALP and Bilirubin). Determination of malondialdehyde (MDA) and superoxide dismutase (SOD) activity were assayed by the method of Habig *et al.* (1974) and Nishikimi *et al.* (1972), respectively. On the other hand

ALP was determined according to Kind and King (1954), while Bilirubin was estimated by Mally and Evelyn (1937). Liver functions (ALT and AST) were determined as described by Randox (United Kingdom) according to the method of Reitman and Frankel (1957). Also, protein and albumin were determined according to a method mentioned by George (1939). Kidney functions (creatinine and blood urea) were determined by a colorimetric method according to Patton and Crouch (1977) as described in a commercial kits by Human (Germany).

Lipid profile (triglycerides, total cholesterol, HDL-c, LDL-c and vLDL-c) were determined by enzymatic colorimetric method of Richmond (1973) described in a commercial kits by Human (Germany).

Haematological analysis (HB, RBCs, PCV, MCV, MCHC, Plt, MPV, PCT, PDW, WBCs, LYM, Mono and GRA) were through using apparatus namely ABX Micros 60 which a fully automated Haematological analyzer from Sysmex Corporation International Company according to Nakul *et al.* (2003).

Statistical analysis of obtained data were done using the statistical software package CoStat (2005). All comparisons were first subjected to one way ANOVA and significant differences between treatment means were determined using Duncan's multiple rang test at p<0.05 as the level of the significance (Duncan, 1955). The experimental animals and determination of Aflatoxins and blood parameters were achieved in Sciences Academy for Experimental Researches.

RESULTS AND DISCUSSION

I : Effect of crude chloroform and methanol extracts of *Lepidium sativum* seeds on Aflatoxin B₁ in rats:

The yield of investigated chloroformic and methanolic extracts of *Lepidium sativum* seeds were 19.8% and 18.3%, respectively. Data in Table (1) revealed that the Aflatoxin B₁ in zero time for all rats was ranged from 7.2 and 8.7 ppb. From the same table, it was clear that the Aflatoxin B₁ for negative control rats (Group 2) was raised from 8.7 at zero time to 10.0 and 13.6 ppb after 3 and 5 weeks, respectively. While, the treatment of legalon (Silymarin) in concentration of 100 mg/kg inhibit Aflatoxin B₁ to 2.91ppb after 3weeks and 0.97 ppb after 5weeks. Also, the chloroformic and methanolic extracts at doses of 100 mg/kg reduced the Aflatoxin B₁ to 3.19 and 3.25 ppb after 3 weeks. While these values reduced to 2.1 and 2.5 ppb after 5 weeks, respectively.

Although, at doses of 200 mg/kg of the same extracts, reduced the Aflatoxin B₁ to 3.08 and 3.16 ppb, after 3 weeks while the same doses of these extracts reduced Aflatoxin B₁ to 1.2 and 1.36 ppb after 5 weeks, respectively. On the other hand, at doses of 300 mg/kg of the same extracts, reduced the Aflatoxin B₁ to 2.77 and 3.00 ppb, after 3 weeks while the same doses of these extracts reduced Aflatoxin B₁ to 1 and 1.2 ppb after 5 weeks at the end of the expermental period.

The present data showed the highest impact on Aflatoxin B₁ was related to chloroformic extract at dose of 300 mg/kg, followed by methanolic extract at dose of 300 mg/kg, of *Lepidium sativum* seeds, respectively. In spite of, Silymarin may be appeared low level of Aflatoxin in group 3, but using the natural extracts of *Lepidium sativum*

seeds for reducing Aflatoxins in diet is favourite due to it considered more safety, available and cheap source.

Fapohunda *et al.* (2014), found that the mycotoxins level (ppb) in melon seeds treated with ginger reduced from 16.8 to 7.2ppb. while, the melon seeds treated with pepper reduced mycotoxins from 16.8 to 7.8ppb. Moreover, the highest effect of cinnamon reduced mycotoxins from 16.8 to 5.8ppb in melon seeds.

Table 1. Determination of Aflatoxin B₁ in infected rats.

Groups	No. of rats	AflatoxinB ₁ (ppb)		
		Zero time	3 Weeks	5 Weeks
Group 1	7	0 ^f	0 ^f	0 ^f
Group 2	7	8.7 ^h ±0.75	10.0 ^g ±1.19	13.6 ^a ±1.46
Group 3	7	7.5 ^l ±0.56	2.91 ^f ±0.35	0.97 ^d ±0.37
Group 4	7	7.8 ^p ±0.39	3.19 ^c ±0.65	2.1 ^b ±0.65
Group 5	7	7.3 ^m ±0.67	3.08 ^c ±0.504	1.2 ^c ±0.74
Group 6	7	7.2 ^m ±0.67	2.77 ^c ±0.504	1.0 ^c ±0.74
Group 7	7	8.1 ^{op} ±0.42	3.25 ^b ±0.301	2.5 ^b ±0.42
Group 8	7	7.8 ^{oo} ±0.58	3.16 ^d ±0.25	1.36 ^c ±0.41
Group 9	7	7.5 ^{oo} ±0.58	3.00 ^d ±0.25	1.2 ^c ±0.41
LSD=0.05		1.63		

Group 1: normal rats, Group 2: negative control, Aflatoxin B₁ through single intraperitoneal injection (1.0mg AFB₁/kg) body weight, Group 3: legalon (Silymarin, 100 mg/kg), Groups 4,5,6: chloroformic extract (100,200 and 300 mg/kg), Group 7,8,9: methanolic extract (100, 200 and 300 mg/kg) body weight, respectively.

The application of chemicals compounds has led to a number of environmental and health problems due to their residual toxicity, carcinogenesis, hormonal imbalance and spermatotoxicity. There is a need to design new and environmentally safe methods of reducing infection by aflatoxigenic *aspergilli* and to inhibit Aflatoxin biosynthesis. Plants are considered as sources of useful metabolites. Plants contain a wide variety of secondary metabolites such as tannins, terpenoids, alkaloids, and flavonoids, reported to have in vitro antifungal properties. From the previous determination , it can be evaluate the fungitoxic effect of various plant extracts against *Aspergillus flavus* production and to test the selected extracts on reducing of Aflatoxin B₁ levels in rats.

II: Effect of *Lepidium sativum* extracts on some serum marker enzyme parameters:

Data in Table (2) cleared that the malondialdehyde (MDA) of normal and control rats were 3.7 and 7.2µmol/ml. While, the most effective treatments with Silymarin in concentration of 100mg/kg, chloroformic and methanolic extracts at doses of 300mg/kg which decreased MDA levels to 3.9, 4.01 and 4.3µmol/ml, respectively. Also, the effect of chloroformic and methanolic extracts for *Lepidium Sativum seeds* at a dose of 200mg/kg reduced MDA to 4.33 and 4.4µmol/ml, respectively. While, the lowest effect of chloroformic and methanolic extracts for *Lepidium sativum seeds* at a dose of 100mg/kg reduced MDA to 4.6 and 4.7µmol/ml, respectively.

From Table (2), it was noticed that the total superoxide dismutase (SOD) of normal rats was 1.67 IU/g which decreased to 1.23 IU/g in control rats. Though, the effect of Silymarin in concentration of 100mg/kg, chloroformic and methanolic extracts at doses of 300mg/kg which decreased SOD levels to 1.59, 1.58 and 1.55 IU/g, respectively. Also, the effect of chloroformic and methanolic extracts for *Lepidium sativum seeds* at a dose of 200mg/kg reduced SOD to 1.57 and 1.54 IU/g, respectively. While, the

lowest effect of chloroformic and methanolic extracts for *Lepidium sativum seeds* at a dose of 100 mg/kg reduced SOD to 1.45 and 1.42 IU/g, respectively.

Table (2) cleared also that the Alkaline Phosphatase (ALP) of normal and control rats were 115 and 258 (IU/L). While, the most effective treatments were Silymarin in concentration of 100 mg/kg, chloroformic and methanolic extracts at doses of 300 mg/kg which were 127, 137 and 142 (IU/L), respectively. Also, the effect of chloroformic and methanolic extracts of *Lepidium sativum seeds* at a dose of 200 mg/kg were 140 and 148 (IU/L), respectively. While, the effect of chloroformic and methanolic extracts of *Lepidium sativum seeds* at a dose of 100 mg/kg were 183.7 and 186.7 (IU/L), respectively.

Results exhibited that Bilirubin raised from 0.85 mg/dL in normal rats to reach 2.89 (mg/dL) after bearing Aflatoxin B₁. The most effective treatments were Silymarin (100mg/kg), chloroformic and methanolic extracts at doses of 300 mg/kg which were 0.98, 1.00 and 1.12(mg/dL), respectively. Furthermore, the effect of chloroformic and methanolic extracts of *Lepidium sativum seeds* at a dose of 200 mg/kg decreased the Bilirubin values to 1.02 and 1.17(mg/dL), respectively. On the other hand, the effect of chloroformic and methanolic extracts of *Lepidium sativum seeds* at a dose of 100 mg/kg decreased the Bilirubin values to 1.76 and 1.81(mg/dL), respectively. Normal values of Bilirubin are about 0.1 to 1.0 mg/dL according to <https://www.medicinenet.com/liver>

Table 2. Effect of *Lepidium sativum* extracts on some serum marker enzyme parameters in infected rats.

Groups	MDA (µmol/ml)	SOD (IU/g)	ALP (IU/L)	Bilirubin (mg/dL)
Group 1	3.7 ^a ±1.40	1.67 ^a ±0.05	115 ^f ± 2.2	0.85 ^e ± 0.15
Group 2	7.2 ^a ±2.03	1.23 ^a ±0.08	258 ^a ±2.9	2.89 ^a ±0.53
Group 3	3.9 ^a ±0.95	1.59 ^a ±0.54	127 ^a ±2.3	0.98 ^a ±0.18
Group 4	4.6 ^b ±1.56	1.45 ^a ±0.41	183.7 ^b ±5.7	1.76 ^b ±0.05
Group 5	4.33 ^b ±1.87	1.57 ^a ±0.26	140 ^c ±1.91	1.02 ^b ±0.07
Group 6	4.01 ^b ±1.66	1.58 ^a ±0.12	137 ^a ±1.44	1.00 ^b ±0.02
Group 7	4.7 ^b ±1.33	1.42 ^a ±0.36	186.7 ^b ±0.9	1.81 ^b ±0.15
Group 8	4.4 ^b ±1.72	1.54 ^a ±0.29	148 ^a ±1.08	1.17 ^b ±0.11
Group 9	4.3 ^b ±1.54	1.55 ^a ±0.44	142 ^a ±1.02	1.12 ^b ±0.03
LSD=0.05	2.2	3.02	4.07	1.02

Group 1: normal rats, Group 2: Aflatoxin B₁ through single intraperitoneal injection (1.0mg AFB₁/kg) body weigh, Group 3: Silymarin (100 mg/kg), Groups 4,5,6: chloroformic extract (100,200 and 300 mg/kg), Group 7,8,9: methanolic extract (100, 200 and 300 mg/kg), respectively. Where MDA : Malondialdehyde, SOD : Superoxide dismutase, ALP: Alkaline phosphatase

These results are not agreed with those reported by Mohamed and Metwally (2009), who studied the effect of various plants i.e cinnamomum, trigonella, camellia and salvia on MDA enzyme. They found that MDA control was 17.50 µmol/g decreased to 15.60, 15.90, 16.00 and 16.50 µmol/g after treatment with these samples, respectively. In the present investigation the effect of Aflatoxin on MDA in experimental rats was 35.7 µmol/g as compared with normal control for MDA (17.50 µmol/g).

III: Effect of *Lepidium sativum* extracts on liver functions:

Alanine amino transferase (ALT) and aspartate amino transferase (AST) activities are known as cytosolic marker enzymes reflecting hepatocellular necrosis as they

are released into the blood after damaging of the cell membrane. Therefore both enzymes are used as indicators for hepatic damage (Andallu and Vardacharyulu, 2001).

Data in Table (3) and Fig.(1) showed that the alanine amino transferase (ALT) was raised from 38.36 for normal rats to reach 117.78 U/L after bearing Aflatoxin B₁. While, the most effective treatments were Silymarin in concentration of 100mg/kg, chloroformic and methanolic extracts at doses of 300mg/kg on ALT, which were 42.84, 44.12 and 49.37 U/L, respectively. Similarly, the effect of chloroformic and methanolic extracts of *Lepidium sativum* seeds at a dose of 200 mg/kg which were 46.09 and 51.11 U/L, respectively. Likewise, the effect of chloroformic and methanolic extracts of *Lepidium sativum* seeds at a dose of 100 mg/kg which were 81.94 and 83.14 U/L, respectively.

Table (3) and Fig.(2) showed that the aspartate amino transferase AST of normal rats was 36.25U/L which raised to 106.02 U/ L in control rats. Though, the effect of Silymarin in concentration of 100 mg/kg, chloroformic and methanolic extracts at doses of 300mg/kg on AST, were 41.12, 42.28 and 45.00 U/L, respectively. Furthermore, the effect of chloroformic and methanolic extracts of *Lepidium sativum* seeds at a dose of 200 mg/kg were 44.54 and 48.54 U/ L, respectively. Moreover, the effect of chloroformic and methanolic extracts of *Lepidium sativum* seeds at a dose of 100 mg/kg were 77.92 and 79.12 U/L, respectively. It is quite clear, that the infected rats showed higher level in ALT and AST, which were 117.78 and 106.02 (U/L) compared with normal rats which recorded 38.36 and 36.25 (U/L), respectively. The treated rats with 300 mg/ kg of chloroformic and methanolic extracts of *Lepidium sativum* reduced the level of ALT and AST to be 44.12 and 49.37 (U/L) respectively. The results showed the importance of these extracts for lowering ALT and AST levels.

Shyamal *et al.* (2010), studied the effects of Aflatoxin B₁ on serum enzyme levels in rats such as AST (GOT) and ALT (GPT), which were 144 and 62.4 U/L, as compared with normal rats, which were 72 and 26.3 U/L, respectively. While, the effect on (AST) of *Ixora coccinea*, *Spilanthes ciliate* and *Rhinacanthus nasuta* extracts were 78.8, 95.3 and 76.3 U/ L, at a dose of 200 mg/kg, respectively. Also, they studied the effect on (ALT) of *Ixora coccinea*, *Spilanthes ciliate* and *Rhinacanthus nasuta* extracts which were 30.1, 40.3 and 32.3 U/ L, at a dose of 200 mg/kg, respectively. Compared with the effects of Silymarin (100 mg/kg) on AST and ALT, were 70.1 and 28.3 U/ L, respectively.

The percentage of decreasing ALT level which calculated from Table (3) was recorded 60.86 % for the chloroformic extract of 200 mg/ kg while the methanolic extract achieved 56.60 %. On the other hand, the percentage of decreasing in the level of AST was recorded 57.99% for chloroformic extract of 200 mg/kg, while the methanolic extract achieved 54.21% . These results agreed with those obtained by Shyamal *et al.* (2010) who recorded 47.01 % for the extract of *Rhinacanthus nasuta* on AST while ALT recorded 51.76 % for the extract of *Ixora coccinea* . The percentage of decreasing AST level obtained from the present results (57.99 % and 54.21% for chloroformic and methanolic extracts, respectively.) were more efficiency than the obtained results by Shyamal *et al.* (2010) which was 47.01%.

The obtained data were agreed with El-Bahr *et al.* (2015), who found that the effect of oral administration of AFB₁ on ALT and AST, were 28.00 and 150.33 U/L, compared with normal rats which were 18.33 and 110.33 U/L, respectively. While the effect of *curcumin* on ALT and AST, were 22.00 and 120 U/L, respectively.

IV:Effect of *Lepidium sativum* extracts on total proteins, albumins and globulins activity:

Data in Table (3) cleared that the total proteins in normal rats was 7.5 g/dL which reduced to 4.5 g/dL in treated rats, while it showed that the effect of Silymarin in concentration of 100mg/kg, chloroformic and methanolic extracts of *Lepidium sativum* seeds at a dose of 300 mg/kg on the total proteins, were 6.9, 6.7 and 6.4 g/dL, respectively. Chloroformic and methanolic extracts of *Lepidium sativum* seeds at a dose of 200 mg/kg, were 6.5 and 6.3 g/dL, respectively. The treatment with chloroformic and methanolic extracts of *Lepidium sativum* seeds at a dose of 100 mg/kg, raised the total protein to 5.5 and 5.6 g/dL, respectively. The previous results were compared with normal and control rats, which were 7.5 and 4.5 g/dL, respectively.

It is quite clear from the results in Table (3) that the protein level of treated rats lowered from 7.5 in normal rats to 4.5 g/dL in contaminated rats . But when infected rats treated with 300 mg/kg of chloroformic and methanolic extracts, protein levels improved to be 6.7 and 6.4 g/dL, respectively. These results show the efficiency of these extracts in raising the protein levels in contaminated rats with Aflatoxin.

The same table revealed that the total albumins in normal rats was 2.77g/dL. The effect of Silymarin in concentration of 100 mg/kg, chloroformic and methanolic extracts of *Lepidium sativum* seeds at a dose of 300 mg/kg for total albumins, were 2.49, 2.41 and 2.32 g/dL, respectively. The effect of chloroformic and methanolic extract of *Lepidium sativum* seeds at a dose of 200 mg/kg for total albumins, were 2.33 and 2.23 g/dL, respectively. These results were followed by the effect of chloroformic and methanolic extracts of *Lepidium sativum* seeds at a dose of 100 mg/kg, which were 1.85 and 1.78 g/dL, respectively. These results were compared with control rats, which was 1.39 g/dL.

From data in Table (3) it is quite clear that the level of Albumin reduced in infected rats which was recorded 1.39 g/dL compared with normal rats (2.77 g/dL). But by treated of contaminated rats with chloroformic and methanolic extracts increased the Albumin levels to 2.41 g/dL and 2.32 g/dL at a dose of 300 mg/kg, respectively. Albumine is a very common protein found in the blood with a variety of functions. It is also produced only in the liver and if its levels are lower than normal (3.5 to 5g/dL) it can be suggestive of chronic liver disease or liver cirrhosis.

From Table (3), it was noticed that the total Globulins was reduced from 4.73 in normal rats to reach 3.11g/dL after bearing Aflatoxin B₁. While, the most effective treatments were Silymarin in concentration of 100 mg/kg, chloroformic and methanolic extracts of *Lepidium sativum* seeds at a dose of 300 mg/kg on globulins, were 4.41, 4.29 and 4.08 g/dL, respectively. Likewise, the effect of chloroformic and methanolic extracts for *Lepidium*

sativum seeds, were 4.17 and 4.07 g/dL, at a dose of 200 mg/kg, respectively.

Also, the effect of chloroformic and methanolic extracts for *Lepidium sativum* seeds, were 3.65 and 3.82 g/dL, at a dose of 100 mg/kg, respectively.

Results in Table (3) clear that the infected rats were lower in its level of globulins (3.11 g/ dl) compared with normal rats (4.73 g/dL). The treated rats with a dose of 300 mg/kg of both chloroformic and methanolic extracts of *Lepidium sativum* increased the globulin levels to 4.29 and 4.08 g/dL, respectively.

Table 3. Effect of *Lepidium sativum* extracts on liver functions in contaminated rats.

Groups	ALT (U/L)	AST (U/L)	Protein (g/dL)	Albumin (g/dL)	Globulins (g/dL)
Group 1	38.36 ^c ±0.97	36.25 ^d ±0.12	7.5 ^a ±1.22	2.77 ^a ±0.08	4.73 ^a ±0.14
Group 2	117.78 ^a ±0.22	106.02 ^a ±0.16	4.5 ^d ±1.18	1.39 ^b ±0.28	3.11 ^b ±0.9
Group 3	42.84 ^d ±0.73	41.12 ^c ±0.09	6.9 ^b ±0.41	2.49 ^a ±0.05	4.41 ^a ±0.36
Group 4	81.94 ^b ±0.33	77.92 ^b ±0.34	5.5 ^c ±1.32	1.85 ^b ±0.09	3.65 ^b ±1.23
Group 5	46.09 ^d ±0.02	44.54 ^c ±0.11	6.5 ^b ±1.09	2.33 ^a ±0.43	4.17 ^a ±0.79
Group 6	44.12 ^d ±0.11	42.28 ^c ±0.09	6.7 ^b ±0.12	2.41 ^a ±0.22	4.29 ^a ±0.79
Group 7	83.14 ^b ±0.80	79.12 ^b ±0.23	5.6 ^c ±1.42	1.78 ^b ±0.15	3.82 ^b ±1.27
Group 8	51.11 ^c ±0.32	48.54 ^c ±0.51	6.3 ^b ±1.31	2.23 ^a ±0.37	4.07 ^a ±0.94
Group 9	49.37 ^d ±0.21	45.00 ^c ±0.01	6.4 ^b ±1.55	2.32 ^a ±0.55	4.08 ^a ±0.94
LSD=0.05	1.9	4.29	2.34	1.43	1.09

Group 1: normal rats, Group 2: Aflatoxin B₁ through single intraperitoneal injection (1.0mg AFB₁/kg) body weight, Group 3: Silymarin (100 mg/kg), Groups 4,5,6: chloroformic ex. (100,200 and 300 mg/kg), Group 7,8,9: methanolic (100, 200 and 300 mg/kg), respectively. where : ALT: Alanine amino transferase, AST: Aspartate amino transferase

The obtained data agreed with those results obtained by (El-Bahr *et al.* 2015), who found that effect of oral administration of AFB₁ on total proteins, Albumin and Globulin, which were 5.1, 3 and 2.1 g/dL, in control rats compared with normal rats, which were 6.56, 4.40 and 3.1 g/dL, respectively. While, the effect of *curcumin* extract on total proteins, Albumin and Globulin, were 6.80, 4.33 and 3.50 g/dL, respectively.

Some results in Table (2and3) agreed with those obtained by Ramamurthy and Rajeswari (2015), who found that effect of *Phyllanthus niruri* extracts at a dose of 300 mg/kg, on Protein, GOT, GPT, ALP and Bilirubin were 6.5 g/dL, 131 IU/L, 46.1 IU/L, 145 IU/L and 1.22 mg/dL, respectively. While, the effect of Silymarin in concentration of 25 mg/kg, on Protein, GOT, GPT, ALP and Bilirubin, were 6.9 g/dL, 135 IU/L, 43.5 IU/L, 137 IU/L and 0.98 mg/dL, respectively. Compared with control rats, which were 4.5 g/dL, 198 U/L, 99.5 U/L, 258 U/L and 2.89 mg/dL, respectively. On the other hand, the normal rats recorded 7.5 g/dL, 121 IU/L, 38.2 IU/L, 115 IU/L and 0.85 mg/dL, for Protein, GOT, GPT, ALP and Bilirubin, respectively.

AST (GOT) and ALT (GPT) normal values ranged from 5 to 40 and 7 to 56 units / liter serum, respectively. These values may be differ slightly depending on the technique and protocols used by different laboratories world-wide . ([https:// www.medicinenet.com/liver](https://www.medicinenet.com/liver))

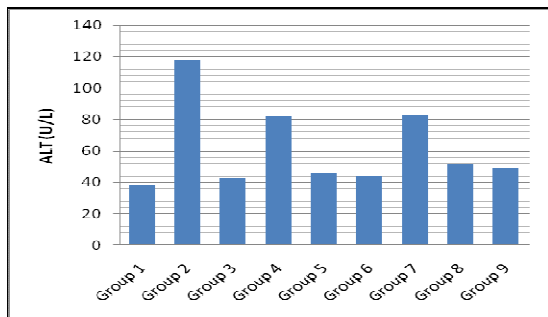


Fig. 1. Effect of *Lepidium sativum* extracts on ALT

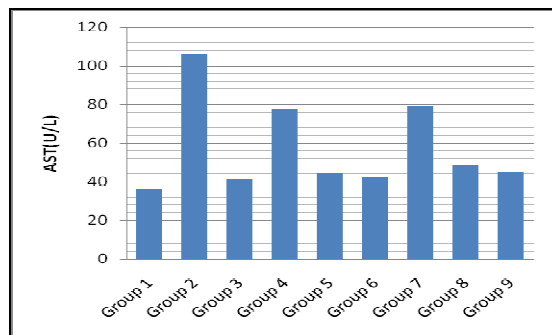


Fig. 2. Effect of *Lepidium sativum* extracts on AST

V: Effect of *Lepidium Sativum* seeds extracts on kidney functions:

Determination of serum creatinine and urea were used as indicators for kidney functions. The effect of *Lepidium sativum* extracts of investigated seeds on serum creatinine and urea levels on contaminated rats with Aflatoxin B₁ during the experimental period after 5 weeks only are tabulated in Table (4).

Table (4) and Fig. (3) cleared that the serum creatinine in normal rats was 1.08 mg/dL, although the chloroformic and methanolic extracts of *Lepidium sativum* seeds at a dose of 300 mg/kg, reduced serum creatinine to 1.22 and 1.25 mg/dL, respectively. While the chloroformic and methanolic extracts of *Lepidium sativum* seeds at a dose of 200 mg/kg, reduced serum creatinine to 1.24 and 1.29 mg/dL, respectively. Also, the effects of chloroformic and methanolic extracts of investigated seeds were 1.61 and 1.67 mg/dL at a dose of 100 mg/kg, respectively. Silymarin in concentration of 100 mg/kg reduced serum creatinine to 1.21 mg/dL after 5 weeks, as compared with control rats treated with Aflatoxin B₁ which recorded at 2.14 mg/dL.

Also, the same Table (4) and Fig.(4) revealed that urea level of normal rats was 44.45 g/dL, while the effect of Silymarin (100 mg/kg), chloroformic and methanolic extracts of *Lepidium sativum* seeds, at a dose of 300 mg/kg

were 51.81, 52.12 and 54.47 mg/dL. Also, the effects of chloroformic and methanolic extracts of investigated seeds were 54.43 and 56.15 mg/dL at a dose of 200 mg/kg respectively. Likewise, the effects of chloroformic and methanolic extracts of investigated seeds were 63.64 and 67.64 mg/dL, at a dose of 100 mg/kg, respectively as compared to control rats contaminated with Aflatoxin B₁ which was 75.91 mg/dL.

Table 4. Effect of *Lepidium Sativum* extracts on kidney functions in AflatoxinB₁ intoxicated rats.

Groups	Creatinine (mg/dL)	Urea (mg/dL)
Group 1	1.08 ^b ± 0.14	44.45 ^d ± 0.34
Group 2	2.14 ^a ± 0.78	75.91 ^a ± 5.36
Group 3	1.21 ^b ± 0.46	51.81 ^c ± 0.26
Group 4	1.61 ^b ± 0.92	63.64 ^b ± 2.66
Group 5	1.24 ^b ± 0.32	54.43 ^c ± 0.09
Group 6	1.22 ^b ± 0.12	52.12 ^c ± 0.22
Group 7	1.67 ^b ± 0.15	67.64 ^d ± 0.66
Group 8	1.29 ^b ± 0.08	56.15 ^b ± 2.13
Group 9	1.25 ^b ± 0.21	54.47 ^b ± 0.09
LSD=0.05	1.87	4.18

Group 1: normal rats, Group 2: Aflatoxin B₁ through single intraperitoneal injection (1.0mg AFB₁/kg) body weigh, Group 3: Silymarin (100 mg/kg), Groups 4,5,6: chloroformic ex. (100,200 and 300 mg/kg), Group 7,8,9: methanolic ex. (100, 200 and 300 mg/kg), respectively.

This finding was in the same level of Soliman *et al.* (2012), who studied that the effect of *Curcuma longa* or *Nigella sativa* on kidney function tests (Creatinine and Urea), which were 1.56 and 47.34 mg/dL, respectively. On the other hand, the normal rats were 1.00 and 37.01 mg/dL, respectively. While the contaminated sample with Aflatoxin B₁ (control rats) were 2.74 and 60.65 mg/dL, respectively.

Creatinine is the waste product of creatine, which used in muscles to produce energy. Typically, creatinine travels in the blood to the kidneys and it excreted out side. High levels in the blood might indicate that the kidneys are not working correctly. The typical reference for serum creatinine is 0.7 to 1.2 mg/dL for men and 0.5 to 1 mg/dL for women. (<https://www.medicalnewstoday.com/articles/321750.php/sar>.)

The blood urea nitrogen test are measured in milligrams per deciliter (mg/dL) in general, round 7 to 20 mg/dL is considered normal. Blood urea concentration may be raised to 40-50 mg/dL even without any apparent loss of renal functions. (<https://acute-care-testing.org/en/articles/urea-and/the-clinical-value-of-measuring-blood-urea-concentration>.)

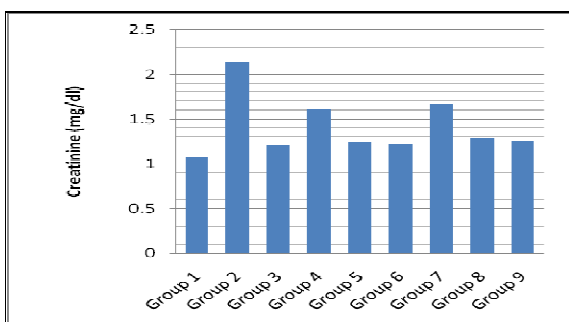


Fig. 3. Effect of *Lepidium sativum* extracts on creatinine

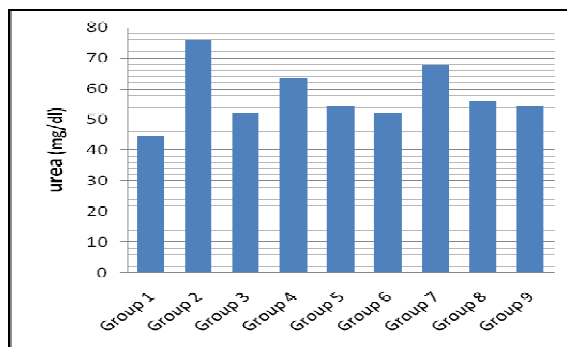


Fig. 4. Effect of *Lepidium sativum* extracts on urea
VI: Effect of extracts on lipid profile:

Serum total cholesterol, triglycerides, HDL-c, LDL-c and vLDL-c were evaluated by measuring the effect of chloroformic and methanolic extracts on lipid profile as follows:

a- Effect of extracts on total cholesterol and triglycerides:

Table (5) revealed that total cholesterol and triglycerides values increased from 69.43 mg/ dl to 99.01 and from 98.5 to 157.6 mg/dL in normal and control rats contaminated with Aflatoxin B₁, respectively.

From the same table, it could be noticed that T-cholesterol decreased by using the concentration of Silymarin 100 mg/kg, chloroformic and methanolic extracts of investigated seeds at a dose of 300 mg/kg have the most effective, which were 70.65, 70.88 and 77.11 mg/dL, respectively. Though chloroformic and methanolic extracts of investigated seeds at a dose of 200 mg/kg have middle effective which were 71.89 and 79.14 mg/dL of *Lepidium sativum* seeds extracts, respectively. Whereas, chloroformic and methanolic extracts of investigated seeds at a dose of 100 mg/kg have the lowest effect, which were 75.42 and 81.44 mg/dL of *Lepidium sativum* seeds extracts, respectively comparing with control rats which was 99.01 mg/dL.

From Table (5), it could be noticed that triglycerides decreased by increasing the concentration of chloroformic and methanolic extracts of investigated seeds after 5 weeks for all samples under investigation. Accordingly, the treatment of control rats with silymarin 100 mg/kg, chloroformic and methanolic extracts of investigated seeds at a dose of 300 mg/kg have high values for reducing triglycerides levels 109.7, 110.4 and 122.7 mg/dL, respectively.

While, chloroformic and methanolic extracts of investigated seeds at a dose of 200 mg/kg have a moderate values for reducing triglycerides levels which were 113.2 and 127.2 mg/dL, of *Lepidium sativum* seeds extracts. On the other hand, chloroformic and methanolic extract of investigated seeds at a dose of 100 mg/kg have lower value for reducing triglycerides levels 121.9 and 132.6 mg/dL, of *Lepidium sativum* seeds extracts respectively, as compared with control rats which was 157.6 mg/dL.

It is quite clear that the effect of the concentration 300 mg/kg chloroformic extract was more effective in reducing both triglycerides and total cholesterol levels than methnolic extract at the same concentration.

b- Effect of extracts on HDL, LDL and vLDL - cholesterol:

From Table (5), It is quite clear that LDL-c and vLDL-c cholesterol increased from 13.36 to 45.35 and from 19.7 to 31.32 mg/dL in normal and control rats, respectively. On the other hand, Table (5) declare that there is highly significant decrease in serum HDL-c level which was 36.38 mg/dL for normal rats comparing with 21.35 mg/dL for contaminated control rats.

Data in Table (5) showed that oral administration of extracts led to a gradual increase of serum HDL-c . Raising concentration of extracts in the experiment caused an increase in serum HDL-c, which reached 35.11 and 32.19 mg/dL, for chloroformic and methanolic extracts of investigated seeds at a dose of 300mg/kg, respectively. However, the effect of chloroformic and methanolic extracts of investigated seeds at a dose of 200mg/kg, were 34.71 and 30.66mg/dL, respectively.

On the other hand, the effect of chloroformic and methanolic extracts of investigated seeds at a dose of 100 mg/kg, were 31.42 and 28.43 mg/dL, respectively. Compared with normal and control rats which were 36.38 and 21.35mg/dL respectively. Silymarin at concentration of 100 mg/kg, increased the level of HDL-c to 35.41 mg/dL.

Data in Table (5) showed also the effect on serum LDL-c Raising the concentration of extracts in the experiment caused a decrease in serum LDL-c which were 13.88 and 15.24mg/dL of chloroformic and methanolic

extracts of investigated seeds at a dose of 300mg/kg, respectively.

The effect of chloroformic and methanolic extracts of investigated seeds at a dose of 200 mg/kg, were 14.16 and 16.84mg/dL, respectively. On the other hand, the effect of chloroformic and methanolic extracts of investigated seeds at a dose of 100 mg/kg, were 18.72 and 20.31 mg/dL, respectively. Compared with normal and control rats, which were 13.36 and 45.35 mg/dL, respectively. Silymarin 100 mg/kg, decreased the level of LDL-c from 45.35 to 13.9 mg/dL.

It is quite clear that the effect of the concentration 300 mg/kg chloroformic extract was more effective than methnolic extract for reducing the levels of LDL-c and vLDL-c in blood serum of infected rats .

Data for vLDL-c values reduced the levels of vLDL-c as a result of treatment with Silymarin 100mg/kg, chloroformic and methanolic extracts of investigated seeds at a dose of 300 mg/kg in rats, were 21.94, 22.00 and 24.07 mg/dL, respectively. While, the effect of chloroformic and methanolic extracts of investigated seeds at a dose of 200 mg/kg, were 22.64 and 25.73 mg/dL, respectively.

On the other hand, the effect of chloroformic and methanolic extracts of investigated seeds at a dose of 100 mg/kg, were 24.32 and 26.72 mg/dL, as compared with normal and control rats contaminated with Aflatoxin B₁, which were 19.7 and 31.32mg/dL, respectively.

Table 5. Effect of *Lepidium sativum* extracts on lipid profile in contaminated rats.

Groups	T-Cholesterol (mg/dL)	Triglycerides (mg/dL)	HDL-c (mg/dL)	LDL-c (mg/dL)	vLDL-c (mg/dL)
Group 1	69.43 ^d ± 0.22	98.5 ^f ± 01.55	36.38 ^a ± 3.53	13.36 ^c ± 0.12	19.7 ^e ± 1.18
Group 2	99.01 ^a ± 0.06	157.6 ^a ± 9.18	21.35 ^b ± 4.49	45.35 ^a ± 1.06	31.32 ^a ± 0.48
Group 3	70.65 ^c ± 2.33	109.7 ^c ± 0.22	35.41 ^a ± 3.35	13.9 ^c ± 0.01	21.94 ^b ± 0.21
Group 4	75.42 ^c ± 0.43	121.9 ^c ± 4.19	31.42 ^a ± 3.53	18.72 ^b ± 5.46	24.32 ^b ± 1.08
Group 5	71.89 ^c ± 0.03	113.2 ^d ± 0.03	34.71 ^a ± 6.44	14.16 ^c ± 9.07	22.64 ^b ± 0.36
Group 6	70.88 ^c ± 0.22	110.4 ^d ± 0.11	35.11 ^a ± 3.72	13.88 ^c ± 4.22	22.00 ^b ± 0.04
Group 7	81.44 ^b ± 3.16	132.6 ^b ± 5.07	28.43 ^b ± 3.56	20.31 ^b ± 4.05	26.72 ^b ± 2.09
Group 8	79.14 ^b ± 9.07	127.2 ^c ± 5.04	30.66 ^a ± 8.17	16.84 ^b ± 0.92	25.73 ^b ± 1.32
Group 9	77.11 ^b ± 1.04	122.7 ^c ± 4.01	32.19 ^a ± 4.09	15.24 ^b ± 0.33	24.07 ^b ± 0.54
LSD=0.05	3.13	6.05	2.37	2.16	1.68

Group 1: normal rats, Group 2: Aflatoxin B₁ through single intraperitoneal injection (1.0mg AFB₁/kg) body weigh, Group 3: Silymarin (100 mg/kg), Groups 4,5,6: chloroformic ex. (100,200 and 300 mg/kg), Group 7,8,9: methanolic ex. (100, 200 and 300 mg/kg), respectively. Where: HDL-c: high density lipoprotein, LDL-c: light density lipoprotein, vLDL-c : very light density lipoprotein

Results in Table (5) agreed with those obtained by El-Bahr *et al.* (2015), who found that effect of oral administration of curcumin for five weeks on total cholesterol and triglycerides of rats, were 2.17 and 2.10 m mol/l, respectively. While, the levels of total cholesterol and triglycerides on normal rats, were 2.10 and 2.0 m mol/l, respectively. Compared to control rats, which were 3.87 and 2.3 m mol/l, for total cholesterol and triglycerides, respectively.

Al-Hamedan (2010), mentioned the effect of Garden cress (*Lepidium sativum* L.) seeds extracts on some serum lipid (cholesterol and triglyceride), which were 110.01 and 98.01mg/dL, respectively. While, the level of (cholesterol and triglyceride), in control rats, were 199.77 and 155.14mg/dL, respectively. Compared with normal rats, which were 80.34 and 70.31mg/dL, respectively.

Also, the present results in Table (5) were in agreement with those reported by Al-Hamedan (2010), who studied the effect of Garden cress (*Lepidium sativum* L.) seeds extracts on some serum lipid (HDL-c, LDL-c and vLDL-c). Their values were 28.88, 61.53 and 19.60 mg/dL, respectively. While, the levels of (HDL-c, LDL-c and vLDL-c), in control rats, were 20.11, 104.01 and 31.02 mg/dL, respectively. Compared with normal rats, which were 32.32, 33.06 and 14.01 mg/dL, respectively.

VII: Effect of *Lepidium sativum* extracts on haematological parameters:

The haematological parameters were used as a broad screening test to check such disorders as anemia, infection and many other diseases. It is actually main of tests that examines different parts of the blood, which play an important role in metabolism and important indicators of health in both human or animals (Bain, 2006).

The haematological parameters includes the following tests:

a- The Effect of extracts on HB, RBCs, PCV, MCV and MCHC:

Data in Table (6) showed that the haemoglobin level (HB) in normal rats, was 12.79 g/dL and decreased to 8.29 g/dL, in control rats. While, Silymarin in concentration of 100 mg/kg in chloroform and methanol extracts at a dose of 300mg/kg were the most effective treatments for increasing haemoglobin levels to 12.75, 12.74 and 12.00 g/dL, respectively.

Likewise, the effect of *Lepidium sativum* seeds chloroform and methanol extracts at a dose of 200 mg/kg, were 12.70 and 11.88 g/dL, respectively. while, the effect of chloroform and methanol extracts at a dose of 100 mg/kg of the same plant extracts were 11.95 and 10.39 g/dL, respectively.

From the same Table, it was clear that the total red blood cells (RBCs), were reduced from $6.18 \times 10^6/\mu\text{l}$ for normal rats to reach $3.12 \times 10^6/\mu\text{l}$ after bearing Aflatoxin B₁. Whereas, the effect of Silymarin in concentration of 100 mg/kg, chloroformic and methanolic extracts at a dose of 300mg/kg were 4.81, 4.75 and $4.26 \times 10^6/\mu\text{l}$ on control rats, respectively. Also, the effect of chloroformic and methanolic extracts at a dose of 200 mg/kg, were 4.69 and $3.96 \times 10^6/\mu\text{l}$, respectively.

The same Table showed that the total packed cell volume (PCV) of normal rats was 41.14% which decreased to 29.01% for control rats. While, the effect of Silymarin in concentration of 100mg/kg, chloroformic and methanolic

extracts at a dose of 300mg/kg, raised PCV to 40.34, 40.11 and 37.22%, respectively.

Also, the effect of chloroformic and methanolic extracts at a dose of 200 mg/kg, were 39.89 and 35.02%, respectively. Similarly, the effect of chloroformic and methanolic extracts at a dose of 100 mg/kg, were 37.69 and 34.15%, respectively.

Previous data revealed that the total mean corpuscular volume (MCV) of normal and control rats were 59.96 and $33.94 \mu\text{m}^3$, respectively. Although, the effect of Silymarin in concentration of 100mg/kg, chloroformic and methanolic extracts at a dose of 300 mg/kg on MCV values were 58.83, 58.80 and $48.12 \mu\text{m}^3$, respectively. Furthermore, the effect of chloroformic and methanolic extracts at a dose of 200 mg/kg, were 58.77 and $45.32 \mu\text{m}^3$, respectively. Moreover, the effect of chloroformic and methanolic extracts at a dose of 100 mg/kg, were 55.18 and $43.49 \mu\text{m}^3$, respectively.

On the other hand, Table (6) declare that the total mean corpuscular hemoglobin concentration (MCHC) was reduced from 34.12 g/dL for normal rats to reach 19.29 g/dL after bearing Aflatoxin B₁. Whereas, the effect of Silymarin in concentration of 100 mg/kg, chloroformic and methanolic extracts at a dose of 300 mg/kg on MCHC values, were 33.92, 33.90 and 30.71 g/dL, respectively. Likewise, the effect of chloroformic and methanolic extracts at a dose of 200 mg/kg, were 33.86 and 29.45 g/dL, respectively. Moreover, the effect of chloroformic and methanolic extracts at a dose of 100 mg/kg, were 31.89 and 27.97 g/dL, respectively.

Table 6. Effect of *Lepidium sativum* extracts on haematological parameters, which include HB (g/dL) RBCs ($10^6/\mu\text{l}$), PCV (%), MCV (μm^3) and MCHC (g/dL) in contaminated rats.

Groups	HB (g/dL)	RBCs ($10^6/\mu\text{l}$)	PCV (%)	MCV(μm^3)	MCHC (g/dL)
Group 1	12.79 ^a ± 0.23	6.18 ^a ± 0.29	41.14 ^a ± 1.49	59.96 ^a ± 0.11	34.12 ^a ± 0.59
Group 2	8.29 ^c ± 0.29	3.12 ^c ± 0.20	29.01 ^c ± 0.84	33.94 ^c ± 0.08	19.29 ^c ± 0.17
Group 3	12.75 ^a ± 0.43	4.81 ^b ± 0.77	40.34 ^a ± 0.49	58.83 ^a ± 0.47	33.92 ^a ± 0.57
Group 4	11.95 ^b ± 0.77	4.22 ^b ± 0.86	37.69 ^b ± 1.16	55.18 ^a ± 0.45	31.89 ^a ± 0.36
Group 5	12.70 ^a ± 0.45	4.69 ^b ± 0.67	39.89 ^b ± 0.63	58.77 ^a ± 0.41	33.86 ^a ± 0.25
Group 6	12.74 ^a ± 0.22	4.75 ^b ± 0.67	40.11 ^a ± 0.44	58.80 ^a ± 0.33	33.90 ^a ± 0.05
Group 7	10.39 ^d ± 0.52	3.41 ^c ± 0.96	34.15 ^b ± 1.97	43.49 ^b ± 1.11	27.97 ^b ± 0.62
Group 8	11.88 ^c ± 0.33	3.96 ^c ± 0.82	35.02 ^b ± 0.57	45.32 ^b ± 0.28	29.45 ^b ± 0.52
Group 9	12.00 ^a ± 0.42	4.26 ^b ± 0.82	37.22 ^b ± 0.09	48.12 ^b ± 0.32	30.71 ^a ± 0.22
LSD=0.05	2.28	3.09	1.13	1.43	5.79

Group 1: normal rats, Group 2: Aflatoxin B₁ through single intraperitoneal injection (1.0mg AFB₁/kg) body weigh, Group 3: Silymarin (100 mg/kg), Groups 4,5,6: chloroformic extract (100,200 and 300 mg/kg), Group 7,8,9: methanolic extract (100, 200 and 300 mg/kg), respectively. Where: HB: Haemoglobin blood , RBCs: Red blood cells, PCV: Packed cell volume, MCV: Mean corpuscular volume, MCHC: Mean corpuscular haemoglobin concentration.

This finding was in the same line with Ramamurthy and Rajeswari (2015), who described the effect of Aflatoxin on haematological variables RBCs, HB, PCV, MCV and MCHC, which were 4.54($10^6/\mu\text{l}$), 9.2(g/dL), 21.8(%), 44.2(fL), [fL: femtoliter = 10^{-15} liter] and 27.5(g/dL), respectively. While, the highest effect of Silymarin at concentration 25 mg/kg, increased the value to 7.02 ($10^6/\mu\text{l}$), 12.2(g/dL), 41.1(%), 51.8(fL) and 34.2(g/dL), respectively. Moreover, the effect of *Cynodon dactylon* extracts, which were, 6.62($10^6/\mu\text{l}$), 11.9(g/dL), 39.2(%), 51.1(fL) and 33.4(g/dL), respectively. Compared with normal rats, which were 7.48($10^6/\mu\text{l}$), 13.8(g/dL), 45.5(%), 53.1(fL) and 35.4(g/dL), respectively.

The obtained data were agreed with those reported by Fapohunda *et al.* (2014) who found that the effect of Ginger (*Zingiber officinale*), on PCV and HB, were 38.33% and 12.77g/dL, respectively. While, the effect of crushed red pepper (*Capsicum annum*) on PCV and HB, were 38.67% and 12.90 g/dL, respectively. Compared with normal rats, which were 39% and 13g/dL, respectively. While the control rats, were, 35.1% and 11g/dL, on PCV and HB, respectively.

b- Effect of extracts on Plt, MPV, PCT and PDW:

Data in Table (7) declare that the platelet blood (Plt) level in normal rats was $1277 \times 10^3/\mu\text{l}$ and decreased to $175 \times 10^3/\mu\text{l}$ in control rats. While, the effect of Silymarin in concentration of 100 mg/kg, chloroformic and methanolic

extracts at a dose of 300 mg/kg on Plt increased to $1243 \times 10^3/\mu\text{l}$, $1231 \times 10^3/\mu\text{l}$ and $1218 \times 10^3/\mu\text{l}$, respectively. Likewise, the effect of chloroformic and methanolic extracts of *Lepidium sativum* seeds at a dose of 200 mg/kg on Plt increased to $1221 \times 10^3/\mu\text{l}$ and $1199 \times 10^3/\mu\text{l}$, respectively. Similarly, the effect of chloroformic and methanolic extracts of *Lepidium sativum* seeds at a dose of 100 mg/kg on Plt increased to $1211 \times 10^3/\mu\text{l}$ and $1181 \times 10^3/\mu\text{l}$, respectively.

Also, Table (7) showed that the mean platelet volume (MPV) was reduced from $9.9 \mu\text{m}^3$ for normal rats to reach $5.2 \mu\text{m}^3$ after bearing Aflatoxin B₁. Though, the effect of Silymarin in concentration of 100 mg/kg, chloroformic and methanolic extracts at a dose of 300 mg/kg on (MPV) increased to 8.5, 8.3 and $8.1 \mu\text{m}^3$, respectively. Similarly, the effect of chloroformic and methanolic extracts of *Lepidium sativum* seeds at a dose of 200mg/kg on (MPV) increased to 8.1 and $7.9 \mu\text{m}^3$, respectively. Likewise, the effect of chloroformic and methanolic extracts of *Lepidium sativum* seeds at a dose of 100mg/kg on (MPV) increased to 7.7 and $7.3 \mu\text{m}^3$, respectively.

The same table showed that the platelets hematocrit value (PCT) in normal rats was 8.4 which reduced to 4.6% after bearing Aflatoxin B₁. While, the effect of Silymarin in concentration of 100 mg/kg, chloroformic and methanolic extracts at a dose of 300 mg/kg on (PCT) increased to 8.01, 8.00 and 7.14 %, respectively. Likewise, the effect of chloroformic and methanolic extracts of *Lepidium sativum* seeds at a dose of 200 mg/kg on (PCT) increased to 7.97 and 7.06 %, respectively. Equally, the effect of chloroformic and methanolic extracts of *Lepidium sativum* seeds at a dose of 100mg/kg on (PCT) increased to 6.68 and 6.16 %, respectively.

Table 7. Effect of *Lepidium sativum* extracts on haematological parameters, which contain Plt ($10^3/\mu\text{l}$), MPV(μm^3), PCT(%) and PDW(%) in contaminated rats.

Groups	Plt ($10^3/\mu\text{l}$)	MPV (μm^3)	PCT (%)	PDW (%)
Group 1	$1277^a \pm 93.31$	$9.9^a \pm 0.23$	$8.4^a \pm 0.08$	$12.9^a \pm 0.44$
Group 2	$175^c \pm 17.55$	$5.2^c \pm 0.36$	$4.6^c \pm 0.02$	$6.2^c \pm 0.13$
Group 3	$1243^a \pm 16.29$	$8.5^b \pm 0.24$	$8.01^a \pm 0.04$	$12.0^b \pm 0.06$
Group 4	$1211^b \pm 77.09$	$7.7^c \pm 0.33$	$6.68^c \pm 0.06$	$10.7^c \pm 0.65$
Group 5	$1221^a \pm 90.88$	$8.1^b \pm 0.97$	$7.97^b \pm 0.06$	$11.8^b \pm 0.89$
Group 6	$1231^a \pm 12.04$	$8.3^b \pm 0.44$	$8.00^a \pm 0.01$	$11.9^b \pm 0.99$
Group 7	$1181^d \pm 12.17$	$7.3^d \pm 0.31$	$6.16^c \pm 0.05$	$9.3^d \pm 0.18$
Group 8	$1199^c \pm 19.43$	$7.9^c \pm 0.39$	$7.06^c \pm 0.04$	$10.9^c \pm 0.37$
Group 9	$1218^a \pm 19.43$	$8.1^b \pm 0.74$	$7.14^c \pm 0.22$	$11.02^c \pm 0.19$
LSD=0.05	1.57	4.83	4.62	1.21

Group 1: normal rats, Group 2: Aflatoxin B₁ through single intraperitoneal injection (1.0mg AFB₁/kg) body weigh, Group 3: Silymarin (100 mg/kg), Groups 4,5,6: chloroformic (100,200 and 300 mg/kg), Group 7,8,9: methanolic (100, 200 and 300 mg/kg), respectively. Where: Plt: Platelet blood, MPV: Mean platelet volume, PCT: Platelet hematocrit, PDW: Platelet distribution width.

On the other hand, Table (7) also declared that the total platelet distribution width (PDW) of normal and control rats were represent 12.9 and 6.2%, respectively. Although, the treatment with Silymarin achieved 12%, which was slightly similar for normal rats value (12.9 %). As well, the effect of chloroformic and methanolic extracts of *Lepidium sativum* seeds (300 mg/kg), were 11.9 and 11.02 %, respectively. Equally, the effect of chloroformic and

methanolic extracts of *Lepidium sativum* seeds at a dose of 200 mg/kg on (PDW) increased to 11.8 and 10.9%, respectively. Similarly, the effect of chloroformic and methanolic extracts of *Lepidium sativum* seeds at a dose of 100 mg/kg on (PDW) increased to 10.7 and 9.3%, respectively.

This finding was in the same line with Ramamurthy and Rajeswari (2015), who described the effect of Aflatoxin on haematological variables Platelet which increased to $1315 \times 10^3/\mu\text{l}$, while, the highest effect of *Cynodon dactylon* extracts, decreased the value to $1195 \times 10^3/\mu\text{l}$, moreover, the effect of Silymarin at concentration 25mg/kg, was $1225 \times 10^3/\mu\text{l}$, compared with normal rats, which was $894 \times 10^3/\mu\text{l}$, respectively.

Results in Table (7) were agreed also with those reported by Fapohunda *et al.* (2014), who found that the effect of Ginger (*Zingiber officinale*), on Platelets, was $>1000 \times 10^9/\text{L}$, while, the effect of crushed red pepper (*Capsicum annum*) on Platelets, was $360 \times 10^9/\text{L}$, compared with control rats, which was $630 \times 10^9/\text{L}$, while the normal rats, was $592 \times 10^9/\text{L}$.

C- The effect of extracts on WBCs, Lym, Mono and GRA:

Data in Table (8) cleared that white blood cells (WBCs) level in normal rats was $11.24 \times 10^3/\mu\text{l}$, which raised to $24.25 \times 10^3/\mu\text{l}$ in control rats. While, the effect of Silymarin in concentration of 100 mg/kg, chloroformic and methanolic extracts at a dose of 300 mg/kg, decreased the values to 12.47, 12.88 and $13.66 \times 10^3/\mu\text{l}$, respectively. Also, the effect of chloroformic and methanolic extracts of *Lepidium sativum* seeds at a dose of 200 mg/kg on WBCs decreased to 13.17 and $14.83 \times 10^3/\mu\text{l}$, respectively. Furthermore, the effect of chloroformic and methanolic extracts of *Lepidium sativum* seeds at a dose of 100 mg/kg on WBCs decreased to 15.38 and $16.89 \times 10^3/\mu\text{l}$, respectively.

Table 8. Effect of *Lepidium sativum* extracts on haematological parameters, which contain WBCs($10^3/\mu\text{l}$), Lym($10^3/\mu\text{l}$), Mono(%) and GRA(%) in contaminated rats.

Groups	WBCs ($10^3/\mu\text{l}$)	Lym ($10^3/\mu\text{l}$)	Mono (%)	GRA (%)
Group 1	$11.24^a \pm 0.25$	$5.36^a \pm 0.29$	$0.45^a \pm 0.01$	$38.3^a \pm 0.25$
Group 2	$24.25^a \pm 0.37$	$7.11^a \pm 0.01$	$0.77^a \pm 0.03$	$25.1^b \pm 0.31$
Group 3	$12.47^f \pm 0.54$	$5.52^c \pm 0.20$	$0.48^a \pm 0.01$	$38.2^a \pm 0.17$
Group 4	$15.38^e \pm 0.93$	$5.98^c \pm 0.31$	$0.54^a \pm 0.15$	$35.5^a \pm 0.49$
Group 5	$13.17^e \pm 0.34$	$5.64^c \pm 0.07$	$0.52^a \pm 0.00$	$37.7^a \pm 0.21$
Group 6	$12.88^f \pm 0.34$	$5.55^c \pm 0.11$	$0.50^a \pm 0.02$	$38.0^a \pm 0.33$
Group 7	$16.89^b \pm 0.89$	$6.51^b \pm 0.46$	$0.63^a \pm 0.02$	$32.7^a \pm 0.19$
Group 8	$14.83^d \pm 0.75$	$6.11^b \pm 0.21$	$0.60^a \pm 0.03$	$35.4^a \pm 0.21$
Group 9	$13.66^c \pm 0.75$	$6.00^b \pm 0.08$	$0.58^a \pm 0.01$	$37.2^a \pm 0.01$
LSD=0.05	1.17	1.06	1.01	1.13

Group 1: normal rats, Group 2: Aflatoxin B₁ through single intraperitoneal injection (1.0mg AFB₁/kg) body weigh, Group 3: Silymarin (100 mg/kg), Groups 4,5,6: chloroformic (100,200 and 300 mg/kg), Group 7,8,9: methanolic (100, 200 and 300 mg/kg), respectively. where: WBCs: White blood cells, Lym: Lymphocytes, GRA: Granulocytes, Mono: Monocytes.

From Table (8), it was indicated that the level of Lymphocytes (Lym) increased from $5.36 \times 10^3/\mu\text{l}$ for normal rats to reach $7.11 \times 10^3/\mu\text{l}$ after bearing Aflatoxin B₁. Although, the effect of Silymarin in concentration of 100 mg/kg, chloroformic and methanolic extracts at a dose of 300 mg/kg, on (Lym) decreased to 5.52, 5.55 and $6.00 \times 10^3/\mu\text{l}$, respectively. Equally, the effect of

chloroformic and methanolic extracts of *Lepidium sativum* seeds at a dose of 200 mg/kg, on (Lym) decreased to 5.64 and $6.11 \times 10^3/\mu\text{l}$, respectively. Likewise, the effect of chloroformic and methanolic extracts of *Lepidium sativum* seeds at a dose of 100 mg/kg, on (Lym) decreased to 5.98 and $6.51 \times 10^3/\mu\text{l}$, respectively.

Previous data in Table (8) revealed that the Monocytes (Mono) values of normal rats was 0.45% which raised to 0.77% for control rats. Whereas, the effect of Silymarin in concentration of 100 mg/kg, chloroformic and methanolic extracts at a dose of 300 mg/kg, on (Mono) decreased to 0.48, 0.50 and 0.58%, respectively. Similarly, the effect of chloroformic and methanolic extracts of *Lepidium sativum* seeds at a dose of 200 mg/kg, on (Mono) decreased to 0.52 and 0.60%, respectively. Also, the effect of chloroformic and methanolic extracts of *Lepidium sativum* seeds at a dose of 100 mg/kg, on (Mono) decreased to 0.54 and 0.63%, respectively.

On the other hand, Table (8) declared that the Granulocytes (GRA) of normal rats was 38.3%, decreased to 25.1% for control rats. While, the effect of Silymarin in concentration of 100 mg/kg, chloroformic and methanolic extracts at a dose of 300 mg/kg, on (GRA) were increased to 38.2, 38.0 and 37.2%, respectively. Also, the effect of chloroformic and methanolic extracts of *Lepidium sativum* seeds at a dose of 200 mg/kg, on (GRA) increased to 37.7 and 35.4%, respectively. Similarly, the effect of chloroformic and methanolic extracts of *Lepidium sativum* seeds at a dose of 100 mg/kg, on (GRA) increased to 35.5 and 32.7%, respectively.

This results were in the same line with those reported by Ramamurthy and Rajeswari (2015), who described the effect of Aflatoxin with concentration of 500 $\mu\text{g}/\text{kg}$, on haematological variables such as WBCs, which was $15.5 \times 10^3/\mu\text{l}$, while the effect of *Cynodon dactylon* extract with concentration of 500 mg/kg, was $9.01 \times 10^3/\mu\text{l}$. Furthermore, the effect of Silymarin at concentration 25 mg/kg, recorded $8.87 \times 10^3/\mu\text{l}$.

CONCLUSION

The effect of Silymarin (100mg/kg), chloroformic and methanolic extracts of *Lepidium sativum* seeds at a dose of 300mg/kg showed the highest positive values for decreasing of Aflatoxin B₁ levels in blood rats. Moreover, the data showed the highest optimum parameters of serum marker enzyme, liver functions, kidney functions, lipid profile and complete blood count (CBC) as a haematological analysis were appeared by the effect of chloroformic and methanolic extracts at a dose of 300 mg/kg followed by 200 and 100 mg/kg , respectively. It is quite clear that, may be Silymarin in concentration of 100 mg/kg was more effective than some of these extracts on some parameters, but Silymarin is considered synthetic product, so the natural extracts of *Lepidium sativum* is more suitable and more safety . For these reasons, the obtained extracts can be used as dietary additional in foodstuffs and therapeutic drugs.

REFERENCES

Al Hamedan, W. A. (2010). Protective Effect of *Lepidium sativum* L. Seeds Powder and Extract on Hypercholesterolemic Rats. *Journal of American Science*;6(11).

- Andallu, B. and Vardacharyulu, N. (2001). Effect of mulberry leaves on diabetes. *Int. J. Diab. Dev. Countries*, 21, 147-151.
- Bain, B. J. (2006). *Blood Cells (A Practical Guide)*, 4th edition. Blackwell. ISBN: 9781405142656 / 1405142650 Hardcover; Revised.: Wiley-blackwell,
- Banu, G. S; Kumar, G. and Murugesan, A. G. (2009). Effect of Ethanolic Leaf Extract of *Trianthema Portulacastrum* L. on Aflatoxin Induced Hepatic Damage In Rats. *Indian Journal of Clinical Biochemistry*, V 24 (4) 414-418.
- CoStat program, Version 6.311 (2005). Cohort Software, 798 Lighthouse Ave. PMB 320, Monterey, CA, 3940, USA. <http://www.cohort.com>.
- Devendran, G. and Balasubramanian, U. (2011). Biochemical and histopathological analysis of Aflatoxin induced toxicity in liver and kidney of rat. *Asian J. of Plant Scie and Research*, 1(4):61-69.
- Divanji, M; Viswanatha, G. L. Nagesh, S. Vishal, J. and Shivaprasad, H. N. (2012). Ethnopharmacology of *Lepidium Sativum* Linn (Brassicaceae). *International Journal Of Phytotherapy Research*, Volume (2) Issue (1), 1-7.
- Duncan, D. (1955). Multiply range and multiple F test. *Biometrics*, 11, 1-42.
- El-Bahr, S. M; Embaby, M. A; Al-Azraqi, A. A; Abdelghany, A. M; Hussein, Y. A; ALHizab, F. A. and Althnaian, T. A. (2015). Effect of Curcumin on Aflatoxin B₁-Induced Toxicity in Rats: A Biochemical and Histopathological Study. *International Journal of Biochemistry Research*, 5(1): 63-72.
- Fapohunda, S. O; Esan, A; Alabi, O. A; Adebote, O; Kolawole, O; Olayinka, O; Atehnkeng, J. and Kuhlmann, J. (2014). Treated Melon Seeds and Aflatoxin Profile in Relation to Blood Parameters in Exposed Mice. *Journal of Microbiology Research and Reviews*, Vol. 2(5): 40-47.
- George, R. K. (1939). Reaction Globulin By The Biuret Total Protein, Albumin, And The Determination Of Serum. *Article J. Biol. Chem.* 1939, 131:197-200.
- Habig, W. H; Pabst, M. J. and Jakoby, W. B. (1974). Glutathione S-transferases. The first enzymatic step in mercapturic acid formation. *J. Biol. Chem.*, 249(22): 7130-9.
- [https:// acute care testing org/en/articles/urea-and/the-clinical-value-of-measuring - blood - urea - concentration.](https://acutecaretesting.org/en/articles/urea-and/the-clinical-value-of-measuring-blood-urea-concentration)
- http://www.medicinenet.com/liver_blood_tests/article.htm#what_blood_tests_are_done_to_detect_liver_function
- [https://www.medicalnewstoday.com/articles/321750.php/sar.](https://www.medicalnewstoday.com/articles/321750.php/sar)
- Jovana, J. Kos; Elizabet, P. J. H; Jasna, S. M; Ivan, L. M. and Bojana, M. K. (2013). The Influence of Drought on The occurrence of Aflatoxins In Maize. *Jour. Nat. Sci, Matica Srpska Novi Sad*, No 124, 59—65.
- Kind, P.R.N and King, E.J.(1954). Estimation of plasma phosphatase by determination of hydrolysed phenol with amino antipyrine. *J. Clin. Pathol.*, Vol. 7, Pp. 322.

- Kostik, V. (2015). Occurrence of Aflatoxins in some medicinal plants stored under different conditions. IOSR Journal Of Pharmacy, Volume 5, Issue 2, Pp. 38-44.
- Mallay, H.T and Evelyn, K.A.(1937). The determination of bilirubin with the photoelectric colorimeter. *J.Biol. Chem.*, Vol. 119, Pp. 481-484.
- Mohamed, A. M. and Metwaly, N. S. (2009). Antiaflatoxic Activities of some plant aqueous extracts against Aflatoxin-B1 induced renal and cardiac damage. *Journal of pharm and toxic.* 4 (1) :1-6.
- Nakul, A. D; Sudaka, I; Ferrero, C; Starck, B. and Bayle, J. (2003). Evaluation of the Sysmex Xe-2100® hematology analyzer in hospital use. *J Clin Lab Anal*;17:113-123.
- Nishikimi, M; N. Appaji. and Yagi, K. (1972). The occurrence of superoxide anion in the reaction of reduced phenazine methosulfate and molecular oxygen. *Biochem. Biophys. Res. Commun.* 46(2): 849-54.
- Patton, C. J. and Crouch, S. R. (1977). Enzymatic determination of urea. *Anal. Chem.*, 49, 464-469.
- Ramamurthy, V. and Rajeswari, D. M. (2015). Evaluation Of Ethanolic Leaf Extract of *Cynodon Dactylon* and its effect on Aflatoxin intoxicated male albino rats. In *Journal of Innovative Res in Science, Eng and Technology*, Vol. 4, Issue 12, 12460-12467.
- Reitman, S. and Frankel, S. (1957). Colorimetric methods for determining GOT and GPT. *Amer. J. Clin. Path*, 28, 56-63.
- Richmond, W. (1973). Preparation and properties of cholesterol oxidase from *Nocardia* sp. And its application to the enzymatic assay of total cholesterol in serum. *Clin. Chem.*, 19 (12), 1350-1356.
- Saluja, M. S; Sangameswaran, B. and Sharma, A. (2010). Cytotoxic Activity of *Vitex negundo* against Ehrlich Ascites Carcinoma (EAC) in mice. *Inter, J. of Pharm. Research*, 1369-1375.
- Shyamal, S; Latha, P. G; Suja, S. R; Shine, V. J; Anuja, G. I; Sini, S; Pradeep, S; Shikha, P. and Rajasekharan, S. (2010). Hepatoprotective effect of three herbal extracts on Aflatoxin B1-intoxicated rat liver. *Singapore Med J.* 51(4) : 326.
- Soliman, G. Z.A; Alia, M. H. and Mohamed, A. (2012). Protective Effect of *Curcuma longa* or *Nigella sativa* on Aflatoxin B1-Induced Hepato-Toxicity in Rats in Relation to Food Safety on Public Health. *Med. J. Cairo Univ.*, Vol. 80, No. 2, 191-203.
- Tedesco, D.; Tava, A; Galletti, S; Tameni, M; Varisco, G; Costa, A. and Steidler, S. (2004). Effects of Silymarin, a Natural Hepatoprotector, in Periparturient Dairy Cows. *J. Dairy Sci.* 87:2239-2247.
- Tiwari, P. N. and Kulmi G. S. (2004). Performance of Chandrasur (*Lepidium sativum*) under different levels of nitrogen and phosphorus. *J. Med. Arom. Plant Sci* ; 26: 479-481.

تأثير مستخلصات بذور حب الرشاد على الأفلاتوكسين B₁ ومكونات الدم في فئران التجارب مصطفى ابراهيم سند ، سامي طلعت ابو طالب ، احمد محمد يوسف و محمد شبل عباس قسم الكيمياء الزراعية – كلية الزراعة – جامعة المنصورة

تم في هذه الدراسة إستخلاص المركبات الفعالة لبذور حب الرشاد عن طريق الاستخلاص المتتابع بمذيبات الميثانول و الاثير البترولي والكلوروفورم وخالات الايثايل ثم التركيز تحت تفريغ بغرض الحصول على المستخلص الخام للبذور تحت الدراسة ومقارنتها بعقار ليجالون (السليمارين). ثم دراسة تأثير اثنين من هذه المستخلصات وهما المستخلص الكلوروفورمي والميثانولي على بعض مكونات دم فئران التجارب المصابة بالأفلاتوكسين ب₁. وكان مستخلص الكلوروفورم اكثر نشاطا في التأثير على نسبة الأفلاتوكسين وتخفيضها من 8.7 الى 1.00 ppb (جزء في البليون) بالحقن بتركيز 300 مليجرام / كيلوجرام من وزن الجسم مقارنة بالسليمارين بتركيز 100 مليجرام/كيلوجرام الذي خفضه الى 0.97 ppb بعد 5 أسابيع من المعاملة. يليهم في التأثير بنسبة متوسطة المستخلص الميثانولي والذي سجل 1.2 ppb. كذلك فان التأثير الأعلى نشاطا يكون للمركب النقي السليمارين بليه مستخلص الكلوروفورم ويكون نشاط مستخلص الميثانول متوسط التأثير في اختبارات الدم المختلفة في الفئران المصابة بالأفلاتوكسين مثل مضادات الأوكسدة والانزيمات ، وظائف الكبد ، وظائف الكلى ، مكونات الليبيدات ، وصورة الدم من الهيموجلوبين وكرات الدم الحمراء والبيضاء والصفائح الدموية ، مقارنة بالفئران المصابة بالأفلاتوكسين B₁ لمدة 5 أسابيع. ومن الجدير بالذكر ان الليجالون (السليمارين) يعتبر منتج مخلوق ولذا فان مستخلصات بذور حب الرشاد الطبيعية المذكورة تكون اكثر مناسبة للاستخدام واكثر امانا وأقل تكلفة ولذا يمكن اضافتها للمواد الغذائية والعقاقير العلاجية لتعدد الفوائد الطبية لها .