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RESEARCH ARTICLE

A Comparative Study for The Alcoholic Extract Effect of Ajwa Dates (*Phoenix Dactylifera L) in* Protection of the Hepatic and Renal Tissues from Toxicity Induced by *Aspergillus Niger* in Albino Rats

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Abstract

The plant extracts and their chemical components have a long history as antifungal agents to control the growth of isolations that released toxins and for this reason, they were used in the bio techniques as a preservative due to increased fungal resistance to the fungal antibiotics. The study aimed to evaluate the efficiency of the alcoholic and aqueous extracts for Ajwa dates in inhibiting the production of Ochratoxin A from *Aspergillus Niger*, and study the possible protective effect of these extracts on the tissues of vital organs such as liver and kidneys against the toxicity that induced by Ochratoxin A. The analysis results of the chromatography methods (HPLC, TLC) showed an influence of these two extracts on the production of Ochratoxin A, and the full inhibitory impact for the production of this type of fungal toxin was in the two concentrations (25, 20 mg / mL) by using the medium of Yeast Extract Sucrose (YES). The present study did not find any significant differences ($P>0.05$) between the activity of aqueous and alcoholic extract in Ochratoxin A inhibition. The present study performed in the animal house of Biology department/Faculty of Education for Girls, twenty laboratory male rats of Sprague-Dawley strain were divided into four equal groups, the first group of animals (control) was orally administrated with normal saline solution (0,9%),the second group was orally given alcoholic extract of Ajwa dates at the concentration of (1) mg/kg, while the third group was orally treated with Ochratoxin A at the concentration of (289) µg /kg ,with regard to the last group was orally received alcoholic extract of Ajwa dates (1) mg/kg + Ochratoxin A (289) µg /kg respectively, the treatment of all animals was once daily for five days a week. During this study the body weight as well as the relative weights of liver and kidneys for rats were recorded, also the effectiveness of some hepatic enzymes (Aspartate amino transferase AST, Alanine amino transferase ALT and Alkaline phosphatase ALP) was estimated, in addition to the other biochemical parameters such as urea and creatinine levels, also the study was comprised the preparation of histological sections for liver and kidneys. The statistical analysis results of the current study were revealed ,Ochratoxin A caused a significant decrease ($P<0.05$) in the body weight at the group of rats that treated with it when compared with the other experimental groups ,contrary to that, the Ochratoxin A showed a significant increase ($P<0.05$) in the relative weights of liver and kidneys ,effectiveness of hepatic enzymes AST,ALT , ALP, levels of urea and creatinine at the male rats were submitted to this type of mycotoxin compared to the other groups of study. In relation with the microscopic examination of liver and kidney sections for the laboratory animals that treated with Ochratoxin A, several patho-histological changes were noted as compared with the other groups. From the results of this study can be concluded that the alcoholic extract of Ajwa dates was ameliorated the functional performance of both liver and kidneys through its preservative properties to protect the tissues of those organs from the nephrotoxicity and hepatotoxicity that induced by Ochratoxin A.

Keywords: *Ochratoxin A, Ajwa dates, Patho-histological effects, Biochemical properties.*

Introduction

Fungal toxins are toxins produced by some fungus such as Mushroom, Molds and Yeast. Some molds are capable of producing toxins and some are non-productive of toxins .These non-toxins-producing molds do not cause a danger to food and foodstuffs. The food containing the molds does not necessarily contain mold poison. There can be toxins in the food, although the food does not contain any mold. Therefore, we cannot confirm the risk of poisoning unless the tests are carried out on the sample. With the help of these tests, it is possible to distinguish between rotten food and food contaminated

with fungal toxins [1,2] .Ochratoxin A is one of the most important groups of fungal toxins, a secondary metabolic material produced by species of the genus Aspergillus and the genus Penicillium, the production of Ochratoxin A is dependent on various factors such as temperature, water activity and the composition of the food medium, these factors physiologically affect the fungal production of the toxin, in tropical and subtropical regions, Ochratoxin A is first produced by Aspergillus ochraceus ,while in the cold and temperate area, Ochratoxin A is produced by Penicillium verrucosum [3].

Another species is A. niger, secondary source of Ochratoxin A, which is produced in infected cocoa beans and dried fruits. Ochratoxin is classified as nephrotoxic, teratogenic, genotoxic and immunologic [4, 5]. In addition, it may be the cause of Balkan Endemic Nephropathy (BEN) and urinary tract tumor in humans [6]. Ochratoxin A was classified as a group 2B a carcinogen for humans by the International Agency for Research on Cancer (IARC) [4].

All date varieties are a good source of natural antioxidants and have anti-mutagenic properties, the selenium present in dates has the potential to prevent the formation of cancer cells. It also has immune function, as well as the dates containing 23 kinds of amino acids ,and at least 6 types of vitamins include a small amount of vitamin A, vitamin B1 (Thiamine), vitamin B2 (Riboflavin), vitamin C and Nicotine acid (niacin) [7,8].

The history of Ajwa dates back to 5000 years BC, It is a fruit and a kind of lithely dates and has a soft texture, cultivated only in Saudi Arabia / Al-Medina. This type of dates contains large amounts of natural fiber that helps digestion. A good source of potassium, as well as contain essential elements to protect muscle contractions in the body, stimulates large intestine and relieves constipation [9].

The Current Study Axes Were as Follows

- Study the possible inhibitory effects of the alcoholic and aqueous extracts of Ajwa dates on the production of Ochratoxin A by *A.niger* fungus in the broth culture media.
- Diagnosis of the toxic impacts of Ochratoxin extracted from *A. niger* on the body weight and relative weights of some organs such as liver and kidneys.
- Detection of the side-physiological influences associated with OTA on the functional performance of some organs, such as liver and kidney by evaluating the effectiveness of hepatic enzymes, levels of creatinine and urea, as well as the preparation of histological sections for the tissues of those organs (liver, right and left kidneys).
- Determination of whether the Ajwa dates extract has a preventive action in the protection against the adverse effects that may be shown by Ochratoxin A on the liver and kidneys tissues, in addition to some of the blood biochemical parameters. The study also aimed to try to use the aqueous and alcoholic extracts of Ajwa dates to reduce or lower the levels of Ochratoxin A in the blood stream and tissues of the body that exposed to these types of fungal toxins.

Materials and Methods

Date Fruits

The fruits of date were obtained from Saudi Arabia /AL-Madinah AL-Munawwarah, and then this type of date was identified and classified by the taxonomist in the department of Biology/Faculty of Education for Gils-Kufa University.

Aspergillums Niger Isolation

The isolation of *Aspergillums Niger* was equipped by advance laboratory of fungi, department of Plant Protection/ Faculty of Agriculture -Kufa University.

Preparation of Culture Media

Potato Dextrose Agar Media (PDA)
According to the method of (10), the PDA media was prepare and use for the *Aspergillus niger* growing.

Yeast Extract Sucrose Broth Media (YES)
To evaluate the effectiveness of plant extracts in the inhibition of mycotoxins production, [11] described the YES media prepared and used according to the method.

Preservation of the *A. Niger* isolation

Aspergillums Niger isolation was kept on (PDA) slant media, then put in the

incubator at 25±2°C for seven days, after that the fungal isolation was preserve in the refrigerator until used.

Preparation of Ajwa Dates Alcoholic Extract

In relation to the alcoholic extract of Ajwa dates, was preparing by using the method of [12].

Preparation of Ajwa Dates Aqueous Extract

On the other hand, for preparation the aqueous extract of Ajwa dates, the method of [12] was performed.

The Inhibition Effect of Ajwa Dates Alcoholic Extract on Ochratoxin a Production from *A. Niger* by YES Media

The method described by [13], was used to study the inhibition influence of Ajwa dates alcoholic extract, by preparation the Yeast Extract Sucrose broth media.

Extraction of Ochratoxin A from the Broth Media

In order to extract Ochratoxin A from the (YST) media, the method of [14] was carried out.

Quantitative Estimation of Ochratoxin A by the High Performance Liquid Chromatography (HPLC)

To evaluate Ochratoxin A quantitatively in the samples that extracted from the YES media ,the system of high performance liquid chromatography, type (LC-2010A HT) was used, equipped by Shimadzu company/Japan, the methods of [15] as well as [16] were dependent at (445) nm.

Detection of Ochratoxin A by Using Thin Liquid Chromatography Technique (TLC)

According to [17] method, Ochratoxin A was detected and the plate's technique of thin liquid chromatography was performed**.**

Evaluation of Ajwa Dates Efficiency in the Determining of Ochratoxin A Toxic Effects on the Biochemical Parameters of Albino Male Rats

Preparation of Laboratory Animals

Twenty-albino male rats return to Sprague-Dawley strain were used in this study, the body weight ranging between (180-220) g, less than three months in age, equipped from the National center for Control and Pharmaceutical Research-Baghdad, put in the animal house of Biology department in the Faculty of Education for Girls-Kufa university. The floor of animal cages was covered with sawdust, which displaced twice or three times weekly, the feeding of rats was specialized and enriched with proteins. When the age of male rats became three months (sexually matured), the experiment was carried out.

Ajwa Dates

Ajwa dates were purchased from Saudi Arabia /AL-Madinah AL-Munawwarah, identified and classified by the taxonomist in the department of Biology/Faculty of Education for Gils-Kufa University.

Preparation of Ajwa Dates Alcoholic Extract

According to [12] the alcoholic extract of Ajwa date was prepared for performing the current study.

Ochratoxin A preparation

Ochratoxin A was prepared according to the method of [18].

The Experimental Groups

- The first group was the control group orally given normal saline solution (0.9%).
- The second group was orally administered with Ajwa dates alcoholic extract (1mg/kg).
- The third group was orally subjected to Ochratoxin A only (289 µg /kg).
- The forth group, was orally submitted to Ajwa dates alcoholic extract (1mg/kg) +Ochratoxin A (289 µg /kg). All the animal treatments were one time at the day and for five days weekly.

Animals Sacrificing and the Blood Samples Collection

The body weight of all male rats were measured, diethyl ether was used, then (5ml) of the blood collected by the heart puncture and transferred into a gel tube, by cutting the abdominal cavity the liver, right and left kidneys were extracted and weighted, then kept in the formalin (10%) containing tubes to use for the histological study .

Preparation of the Serum Samples

Gel tubes that containing the blood were centrifuged for ten minutes at (3000) rPm to separate the serum, then the serum was

transferred into a serum tube to keep in (-4°C) for the biochemical study.

The Biochemical Study

- Estimation of aspartate amino transferase (AST) effectiveness in the serum:In order to evaluate the AST activity, the kits from Biolabo-France Company were used; the colored derivative of hydrazine was formed at (546) nm in the basic media [19].
- Estimation of alanine amino transferase (ALT) effectiveness in the serum: The activity of ALT enzyme estimated by using the kits of Biolabo-France Company, the formation of colored hydrazine derivative was in a basic medium at (340) nm, according to [20].
- Estimation of alkaline amino transferase (ALP) effectiveness in the serum:With regard to ALP activity, the kits were purchased from Biolabo-France Company and the effectiveness of this enzyme was determined by using the spectrophotometer at (510) nm [20].
- Estimation of creatinine level in the serum:Concerning the level of creatinine, the method of [21] was used, the kits were equipped by Biolabo-France Company and Jaffe reaction occurred at (490) nm.
- Estimation of urea level in the serum

As for urea level evaluating, the kits were purchased from Biomerieux-France Company and the absorbance was measured at (550) nm [21].

The Histological Study

According to the method was described by [22], the histological sections of liver, right and left kidneys were prepared.

The Statistical Analysis

The findings of current this study were analyzed by using SPSS (Statistical Package Social Sciences, Version [21] and (ANOVA) test, then the test of least significant difference (L.S.D) at the level of ($P<0.05$) was used according to [23].

Results and Discussion

Test of *A.niger* Isolation Ability to Produce Ochratoxin A by Using the Thin Layer Chromatography Plates Method (TLC)

The chemical examination by using the thin layer chromatography (TLC) has indicated that *A.niger* has the ability to produce Ochratoxin A as shown in figure (1). This has agreed with most of the other studies [18, 24], who pointed that the isolation of *A.niger* has the ability to produce Ochratoxin A.

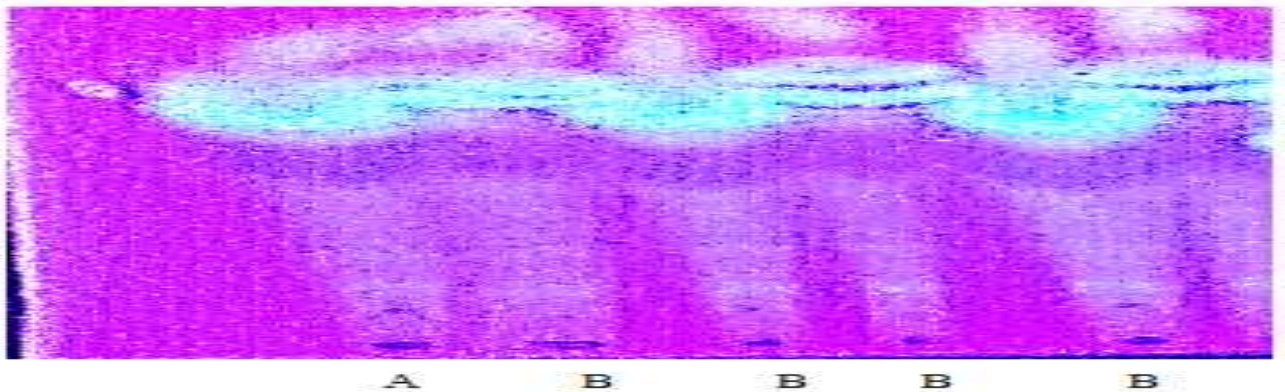


Figure.1:The ability of *A. niger* isolation to produce Ochratoxin A by thin layer chromatography method (TLC) (A) toxin standard,(B) extract of sample

Effect of the Aqueous and Alcoholic Extracts of Ajwa Dates on the Inhibition of Ochratoxin a Produced by *A. Niger*

The results of high performance chromatographic technology (HPLC) have shown that both the aqueous and alcoholic extracts of Ajwa dates had an effect in the inhibition of Ochratoxin A productivity level by A. Niger.

Figure (2) shows the standard curve of Ochratoxin A, the highest reduction percentage of Ochratoxin A was at the concentrations [20, 25] mg/ml. The percentage was less than the sensitivity level. However, the percentages were (50, 90, 95) % at the concentrations (5, 10, 15) mg/ml, as illustrated in the Figures (3-12). It possible that the similarity of both activities

of the Ajwa dates aqueous and alcoholic extracts may be referred to the fact that both extracts have the same chemical ingredients, such as (Phenols, Carotenoids, and Flavonoids).

A study by [25] found that the Ajwa dates extract contains phenolic compounds, glycoside and flavonoids. As for the high efficiency of Ajwa dates alcoholic extract to reduce the productivity of Ochratoxin A, it may be attributed to the active phenols that work as antioxidants, which includes flavonoids and procyanidins [26, 27].

It is notable that the study performed by [28] in which he examined Foeniculum vulgare and Sesamum indicum seeds extract, and another study conducted by [29] on the seeds extract of Citrullus colocynthis, which also concluded that the extracts of these plants have a high efficacy in the inhibition of Ochratoxin A productivity rate.

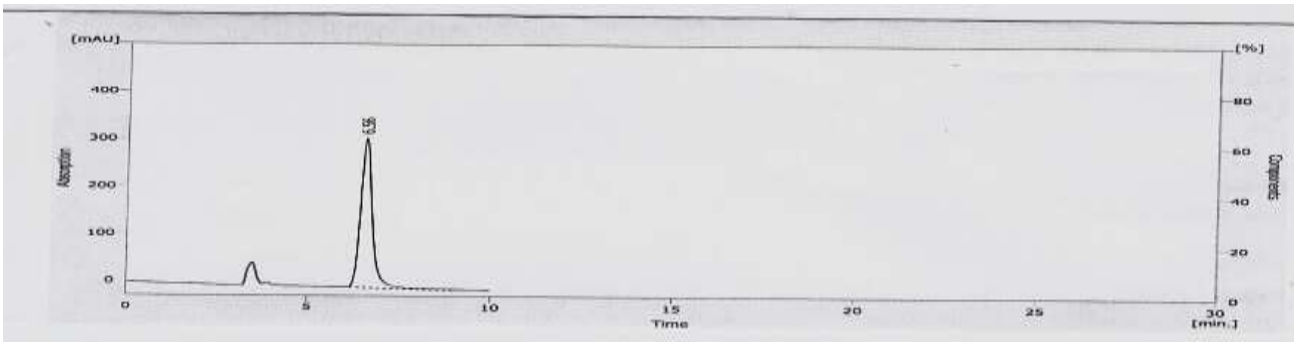


Figure 2:The standard curve of Ochratoxin A by HPLC technique

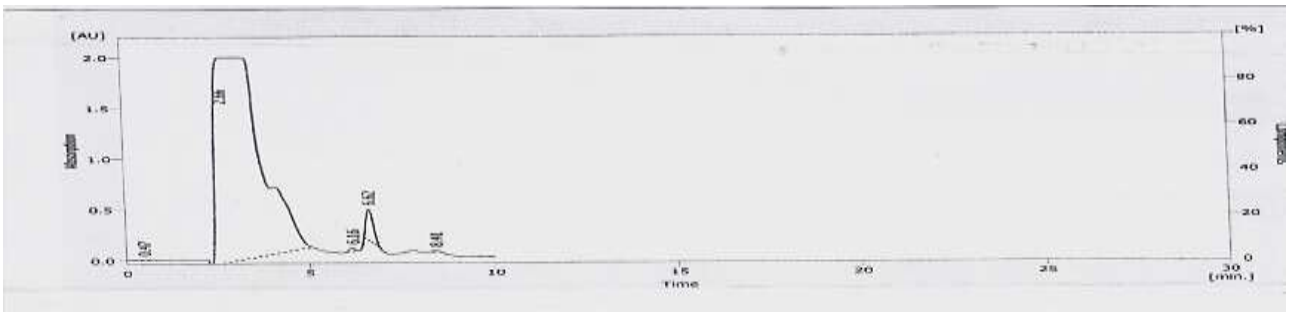


Figure 3:Effect of Ajwa dates alcoholic extract on Ochratoxin A production by A.niger at the concentration of (5) mg/ml by HPLC technique

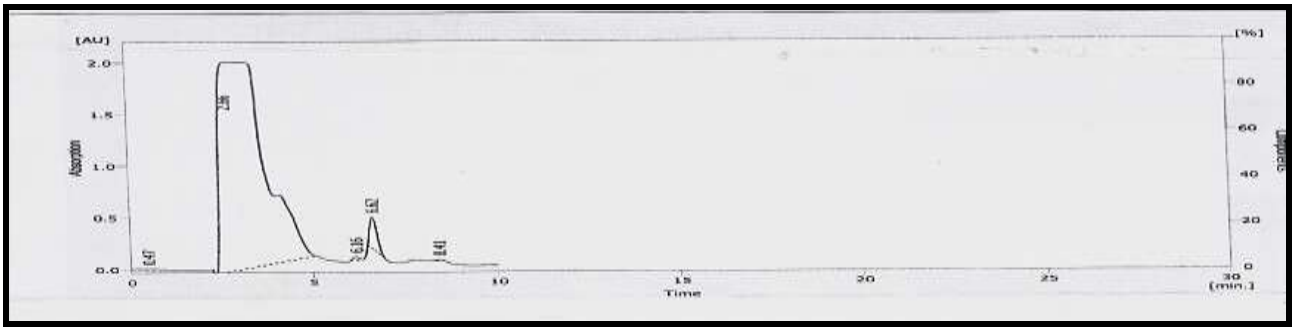


Figure 4:Effect of Ajwa dates aqueous extract on Ochratoxin A production by *A.niger* at the concentration of (5) mg/ml by HPLC technique

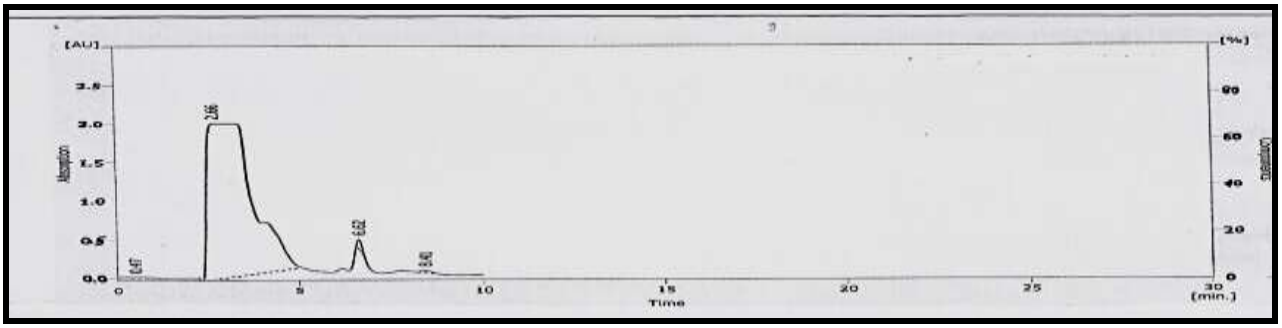


Figure 5:Effect of Ajwa dates alcoholic extract on Ochratoxin A production by *A .niger* at the concentration of (10) mg/ml by HPLC technique

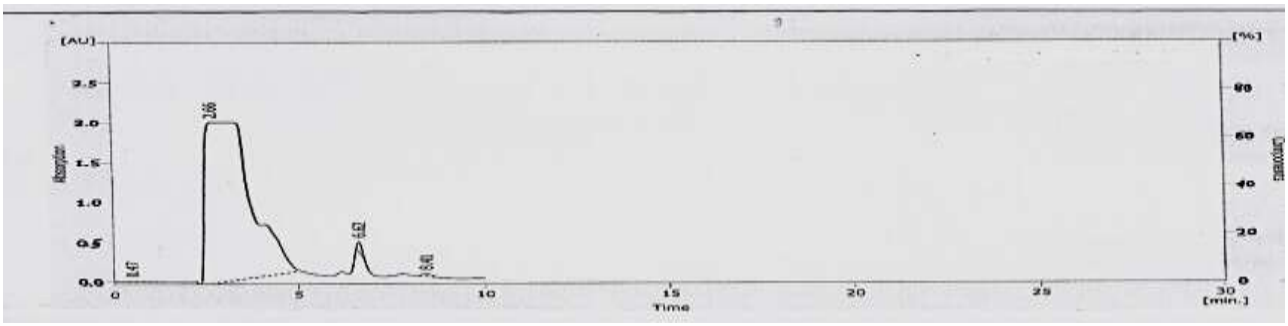


Figure 6: Effect of Ajwa dates aqueous extract on Ochratoxin A production by *A.niger* at the concentration of (10) mg/ml by HPLC technique

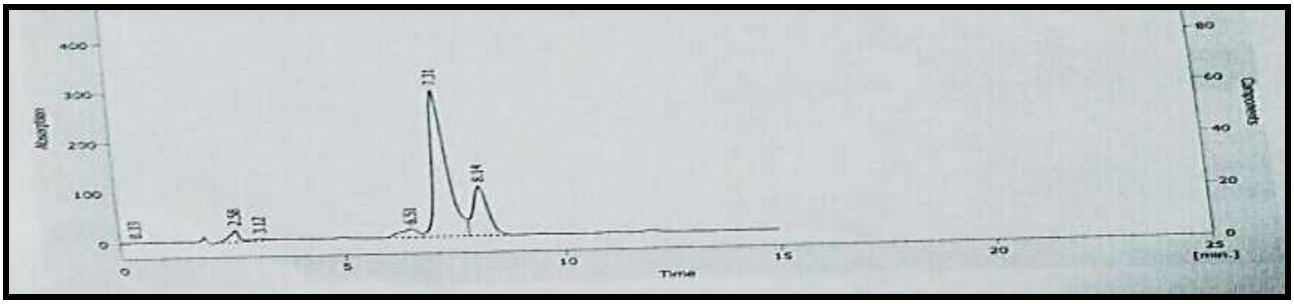


Figure 7: Effect of Ajwa dates alcoholic extract on Ochratoxin A production by *A.niger* at the concentration of (15) mg/ml by HPLC technique

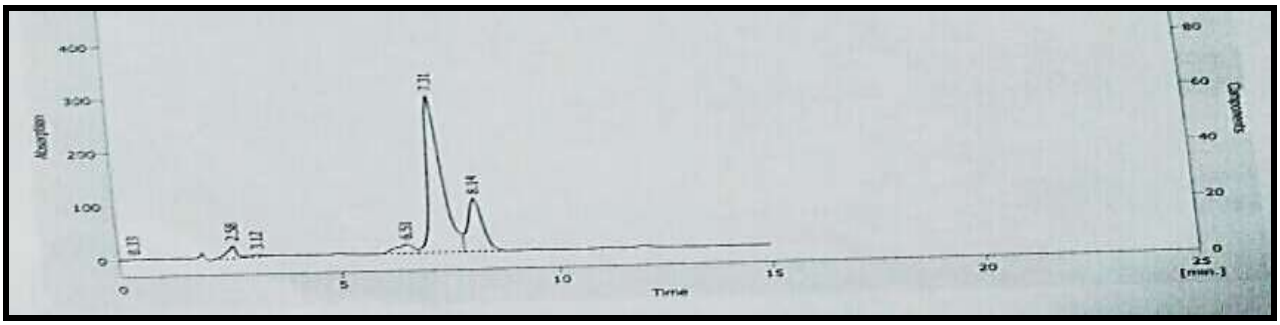


Figure 8:Effect of Ajwa dates aqueous extract on Ochratoxin A production by *A.niger* at the concentration of (15) mg/ml by HPLC technique

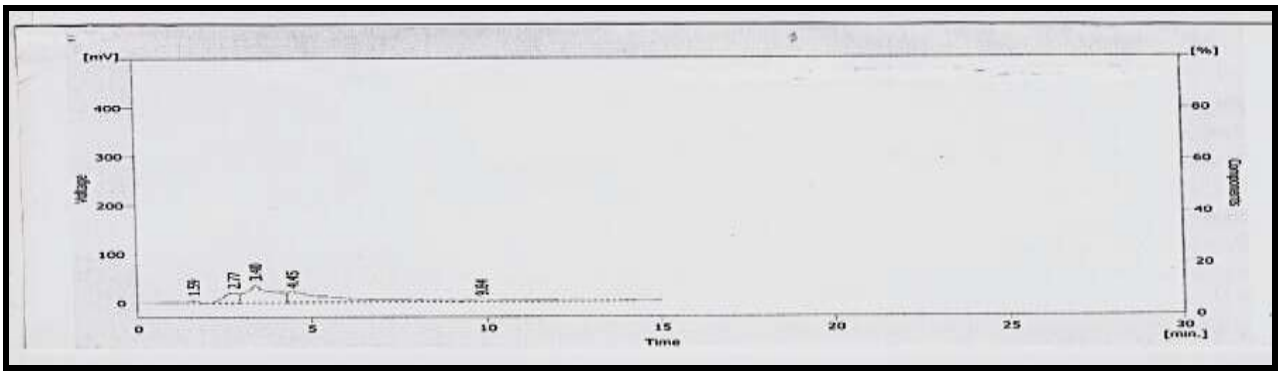


Figure 9: effect of Ajwa dates alcoholic extract on Ochratoxin A production by *A.niger* at the concentration of (20) mg/ml by HPLC technique

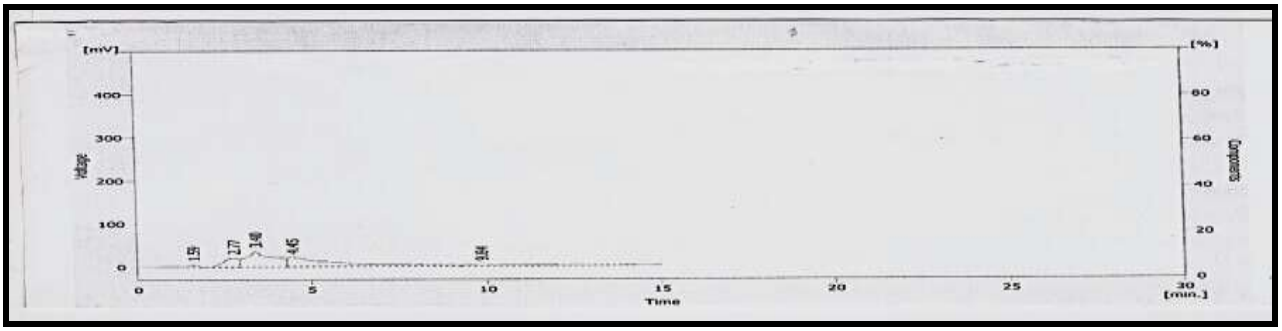


Figure 10:Effect of Ajwa dates aqueous extract on Ochratoxin A production by *A.niger* at the concentration of (20) mg/ml by HPLC technique

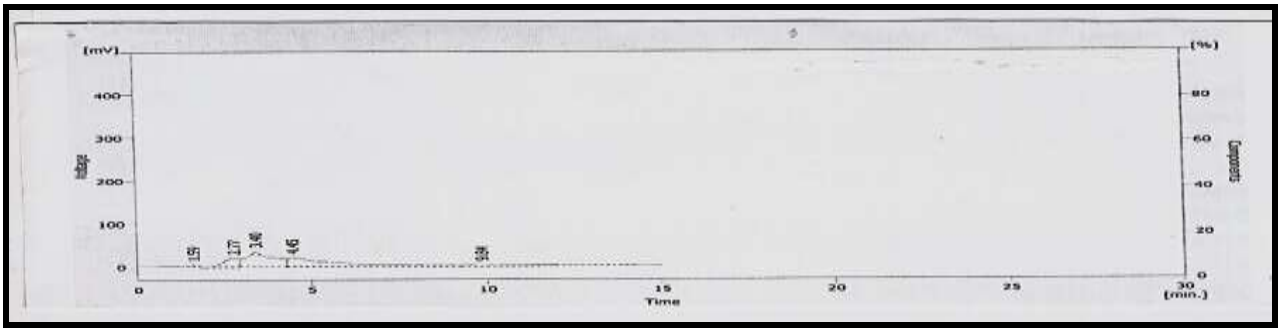


Figure11:Effect of Ajwa dates alcoholic extract on Ochratoxin A production by *A.niger* at the concentration of (25) mg/ml by HPLC technique

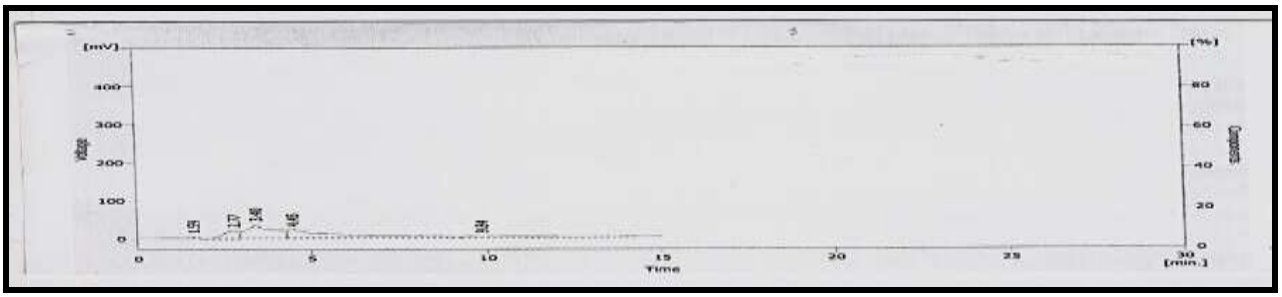


Figure 12:Effect of Ajwa dates aqueous extract on Ochratoxin A production by *A.niger* at the concentration of (25) mg/ml by HPLC technique

Effect of Ochratoxin A and Ajwa Dates on the Body Weight

Figure (13) shows that the consumption of Ochratoxin A by lab rats have led to a significant decrease (P<0.05)in the body weight of these rats, when compared with the other groups, but no significant differences(P>0.05)revealed in this parameter when the other groups of treatment were compared with each other. The weight gain rates have recorded a significant increase ,for the control group (24.94±1.67), for the alcoholic extract of Ajwa dates group (25.58±6.34),the alcoholic extract of Ajwa dates and Ochratoxin A group was (25.8±7.48) respectively compared to the group of rats that consumed Ochratoxin A (-44.4 ± 4.63), as illustrated in Figure (14).

Figure.13: Effect of Ochratoxin A and Ajwa dates alcoholic extract on the body weight. The values represented as (M±SE). Similar letters mean no significant differences between groups at the level (P<0.05) Different letters mean significant differences between groups at the level (P<0.05)

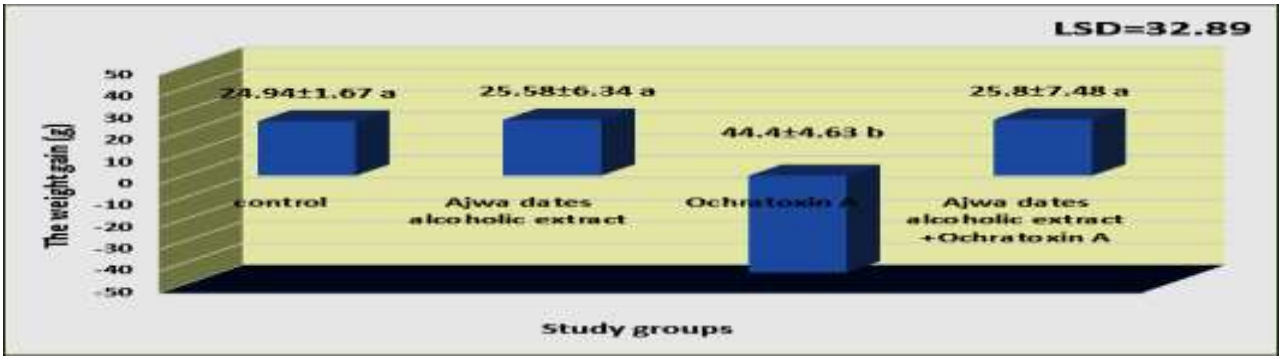


Figure.14:Effect of Ochratoxin A and Ajwa dates alcoholic extract on the body weight

The results of this study have agreed with many other studies [30, 31, 32].This body weight loss may be explained to Ochratoxin A, which is one of the fungal toxins that causes many toxic symptoms such as loss of appetite, diarrhea, decrease in the rate of water and food consumption. It is also probable that the bio-manufacturing process of all body proteins may be decreased, which may negatively affect the body weight and lead to a significant decrease, as confirmed by many other scientific researches [30,33].

This weight loss may also be due to a great decrease in the anabolism and, in contrast increase in the catabolism rates, this deplete the fats that stored in the cells. It is possible that the Ochratoxin A also affects the gastrointestinal tract, and may lead to an internal bleeding, which generally causes the animal to wither away. Many behavioral changes were noticed in the animals throughout the period in which they were being treated with Ochratoxin A ,have mentioned that not eating their diet, loss of appetite, and the latter inability to move when compared with the status they were before consuming the Ochratoxin A. [34] ,have indicated that treating lab animals with Ochratoxin A have decreased the absorption rate of food because of the ulceration, inflammation, and irritation of the gastrointestinal tract in general, which may cause a loss of appetite and the will to intake food anymore, and ultimately a loss of body weight. This was also confirmed by [35].

The decrease perhaps explain to the fact that the animals were administered with Ochratoxin A which may have caused a deficiency in the kidney functions leading to a decrease in diet consumption, affecting the growth rate of these animals, and decreasing their body weight. [36] Have mentioned that treating rats with Ochratoxin A have caused an unbalanced acidity in the kidneys, inhibiting the absorption rate in the renal tubules, and altering the whole kidney functions in general. The diagnosed damage to the renal tubules in the male rats as a result of treating them with 289 µg / kg of the Ochratoxin A may be caused adverse effects to the body weight, because the function of these tubules is reabsorbed the water and re-filtered it, therefore, the rats treated with this fungal toxin were dehydrated, which significantly decreased their weights subsequently. [37] Have confirmed that treating rats with Ochratoxin A severely damaged the renal tissues as well as inflecting numerous other negative effects for this type of toxin that affect the functional performance of these tubules.

This decrease in the body weight is probable referred to the poisonous impacts of Ochratoxin A on the hepatic tissues which has been documented in the current study, such as necrosis, degeneration, hemorrhage in the hepatic cells, severe damage and hemorrhage in the central vein, as well as release the hepatic enzymes, and raising their levels in the blood stream, which may have also affected the carbohydrates metabolism disturbance, an inhibition in the glycogenolysis in the liver, and lowering the energy production rate that the body needs for its different vital processes, accordingly, the body weight significantly decreased. [34], postulated that Ochratoxin A worked on decreasing the glycogenolysis and accumulated it in the hepatic cells, thus these negative metabolic responses have contributed to inhibit Krebs cycle, which is responsible for saving the necessary energy for different biological processes in the body cells.

Furthermore, the hepatic tissues are considered the main store for iron ions (Fe^{+2}) and many other fat-soluble vitamins such as (A, B2, B12, E), as well as these tissues are responsible for the biological synthesis of plasma proteins such as albumin and fibrinogen from amino acids, in addition to the effective role of liver in regulating the level of total cholesterol in the blood through conversion of some of them to the bile salts to be dealt with directly [38]. Therefore, the damage of liver tissues by Ochratoxin A may have adversely affected liver performance of its various functions resulting in a significant decrease in the body weight. lastly, the decline in body weight may be explained by the fact that Ochratoxin A has negatively affected the centers of satiety and hunger which located in the hypothalamus, or may have caused a disturbance in the effectiveness of certain glands responsible for metabolism and metabolic processes (anabolism and catabolism) in the body, especially the thyroid and adrenal glands through the direct impact of this type of mycotoxins on the anterior lobe of the

pituitary gland, causing significant loss of the body weight. As for the Ajwa dates alcoholic extract + Ochratoxin A group and the group of Ajwa dates alcoholic extract only, there was no significant change in the body weight compared to the control group, and it is likely that the phenolic chemical compositions of the alcoholic extract ,including phenolic acids, flavonoids, and anthocyanin[7]. However, that might also be referred to the date content of many other compounds that improved the histological structure and the functional performance of the digestive tract, which kept the body weight without notable change and these ingredients: minerals, antioxidants, fats, vitamins (B and C) and carotenoids, as confirmed by many studies [7, 8]

Effect of Ajwa Dates and Ochratoxin A on the Relative Weight of Liver

The statistical analysis of the current study results showed a significant increase ($P<0.05$) in liver weight for the group that treated with Oxitoxin A as compared with the other groups included in the study ,whereas no significant difference ($P>0.05$)was revealed in this standard when the other experimental groups were compared with each other ,Figure (15).

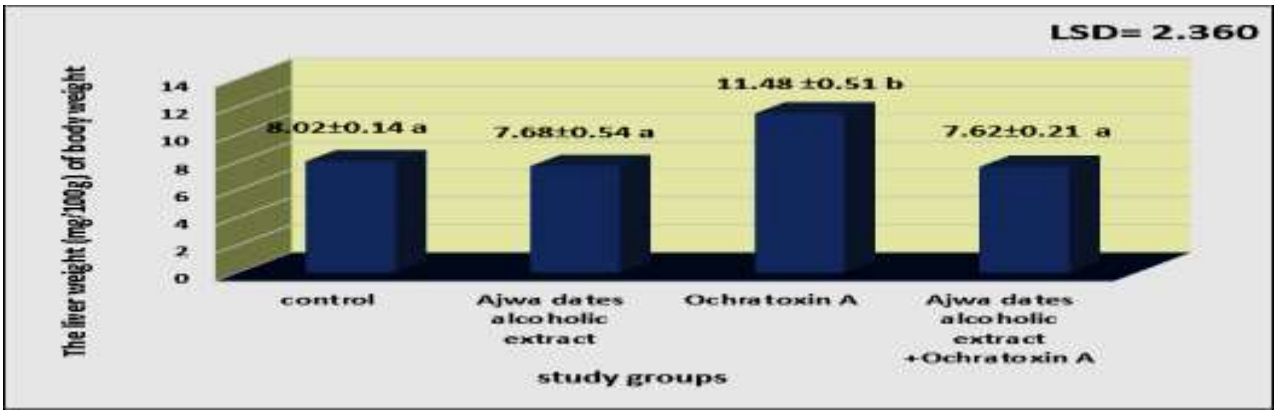


Figure 15 :Effect of Ochratoxin A and Ajwa dates alcoholic extract on the relative weight of liver

The results of this study did not agree with the study conducted by [39], but agreed with many other studies [31,40,
41]. This increase may be explained by the toxic effects of Ochratoxin A on the hepatic tissues of male rats, after autopsy and organ extraction, a significant hypertrophy was observed, as well as congestion and discoloration. In addition to, the histological examination of the liver revealed several pathological changes that may have a significant impact on the weight of the organ. [42] Mentioned that Ochratoxin A stimulates many hepatic tissue alterations such as congestion of central veins, portal veins and hepatic sinusoids, with a severe aggregation of mononucleated inflammatory cells around the portal regions of the degenerated parenchyma tissue of the liver and degeneration of the blood vessels surrounding the hepatic cells.

Moreover, [43] found the same result. The increase in the relative weight of liver may be because the process of lipid peroxidation and resulting in the increased production of free radicals, who have destroyed the hepatic tissues, causing the enlargement of the organ and increase its size subsequently. The increase in liver weight is likely to be explained by a significant rise in the level of urea at male rats that treated with Ochratoxin A, which was recorded during the current study. The liver is responsible for removing toxins from the blood and extracting them by metabolizing them into less toxicity smaller molecules, and as a result of the direct treatment of liver tissues with the high toxicity chemical compounds such as urea, thus these tissues have been exposed to many of the changes, which have the effect of the organ enlargement and significantly increasing its weight.

As for the other groups of experiment, that treated with alcoholic extract of Ajwa dates + Ochratoxin A and the alcoholic extract of Ajwa dates only, the study did not find any remarkable alter in relative weight of the liver compared to the control group, and likely to explain that the Ajwa dates have shown high efficiency in the protection of hepatocytes membranes from the lipid peroxidation which considered the most possibly mechanisms of Ochratoxin A, so as to possess the dates of special dietary fibers of a therapeutic and preventive effect as an anti-inflammatory. In addition to the high

activity in preserving the hepatic tissues, as confirmed by many other studies [25, 44, 45].

Effect of Ajwa Dates and Ochratoxin A on the Relative Weight of Kidneys

Figures 16 and 17 revealed that Ochratoxin A notably increased (P <0.05) the right and left kidney weights compared to other groups, while no significant differences (P>0.05) were observed between these weights when the other groups of treatment compared with each other.

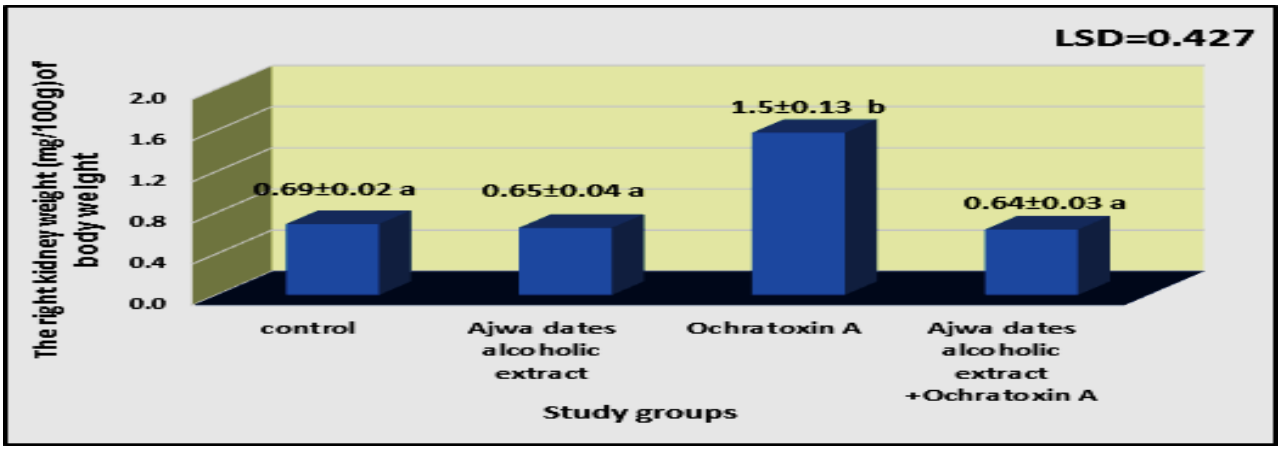


Figure 16:Effect of Ochratoxin A and Ajwa dates alcoholic extract on the relative weight of right kidney

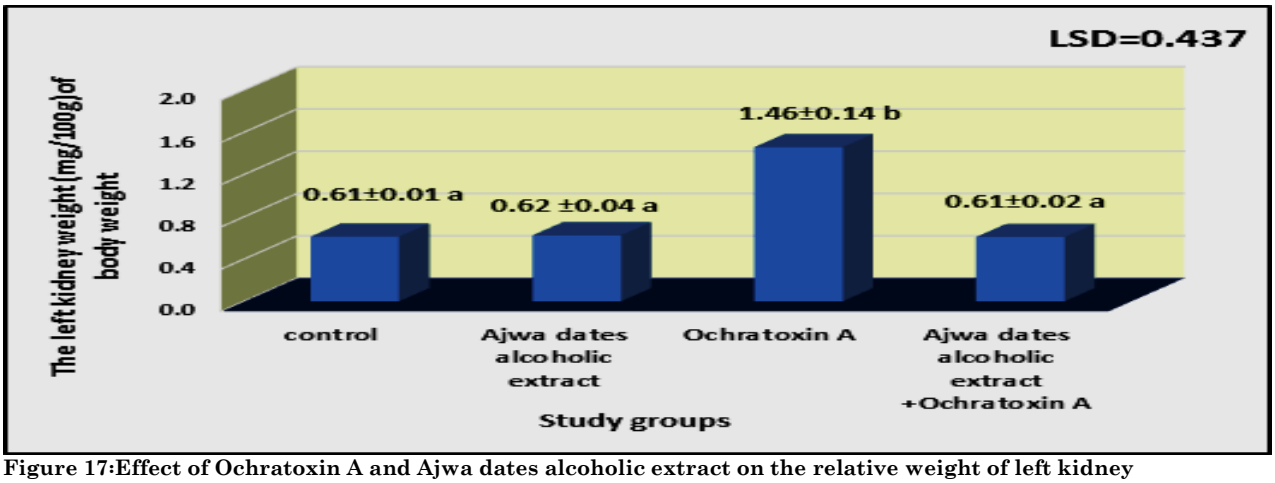


Figure 17:Effect of Ochratoxin A and Ajwa dates alcoholic extract on the relative weight of left kidney

The statistical analysis results of the current study were contradictory to some studies [39], but agreed with several studies [41, 46]. Increased weight may be due to the pathological changes in the renal tissues that caused by Ochratoxin A, including atrophy of some renal glomeruli and hypertrophy of the others, as well as hemorrhage in some glomeruli, interstitial tissues of the cortex and medulla, with sever infiltration of multinucleated inflammatory cells, in addition to the tubular necrosis, which had the influences on the kidneys enlargement or hypertrophy and subsequent rise in the organs weight (right and left kidneys) as confirmed by [47]. In another study conducted by [48], the treatment of animals with Ochratoxin A showed many of the tissue effects, comprising congestion of the renal tubule blood vessels, degeneration of the renal tubule cells and the cytoplasmic vacuolation, as well as the accumulation of neutrophil cells. [18] Proved that Ochratoxin A has led to expansion or dilatation of the convoluted renal tubules. For the other groups, that treated with the alcoholic extract of Ajwa dates + Ochratoxin A and the group was only received alcoholic extract of Ajwa dates, no noticeable change in the weight of the kidneys were appeared as compared with the control group, due to the possibility that the date is rich with carbohydrates, proteins, minerals and glycosides which may have a positive effect on optimizing renal tissues protection, as many studies have previously shown [49, 50]. Another study have added that Ajwa dates increase the efficacy of antioxidant systems and ,in contrast decrease the lipid peroxidation since it reveals a scavenger

effect for the free radicals because it contains tannins, steroids, flavonoids, and vitamin C [51], in addition to possessing many anti-oxidation factors as it works on preventing the kidneys from being damaged by the toxicity of the Ochratoxin A and protecting their tissues from any further deterioration caused by this kind of fungal toxins, as [52] has indicated.

Study of Biochemical Parameters of the Blood

Effect of Ochratoxin A and Ajwa dates on the Effectiveness of AST, ALT and ALP

The treatment by Ochratoxin A made increase ($P < 0.05$) in the efficiency of hepatic enzymes AST, ALT and ALP compared to the other study groups. Moreover, no significant differences ($P > 0.05$) were observed in the effectiveness of these enzymes when the other experimental groups were compared with each other Figure (18, 19 and 20).

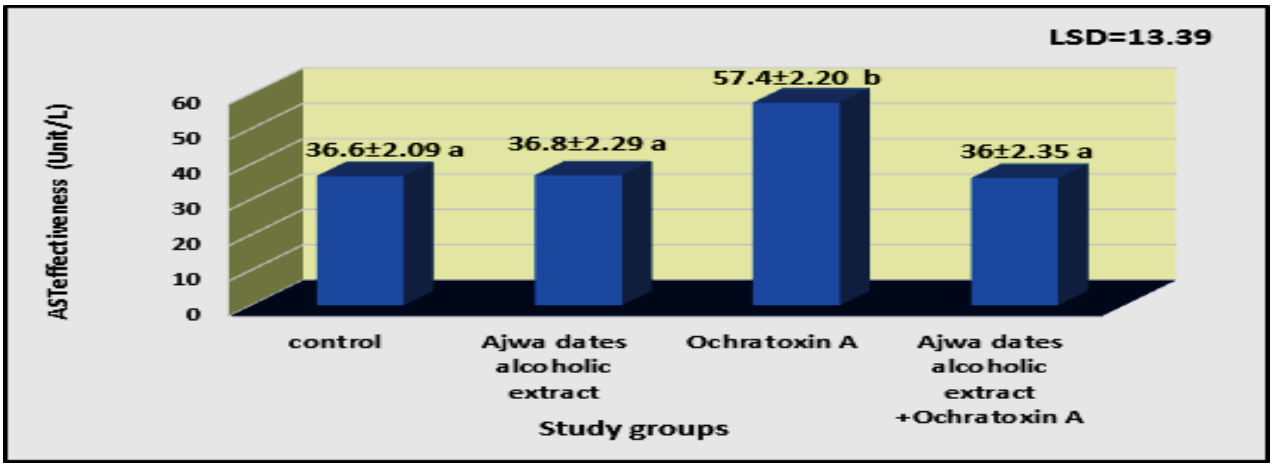


Figure 18:Effect of Ochratoxin A and Ajwa dates alcoholic extract on the AST effectiveness

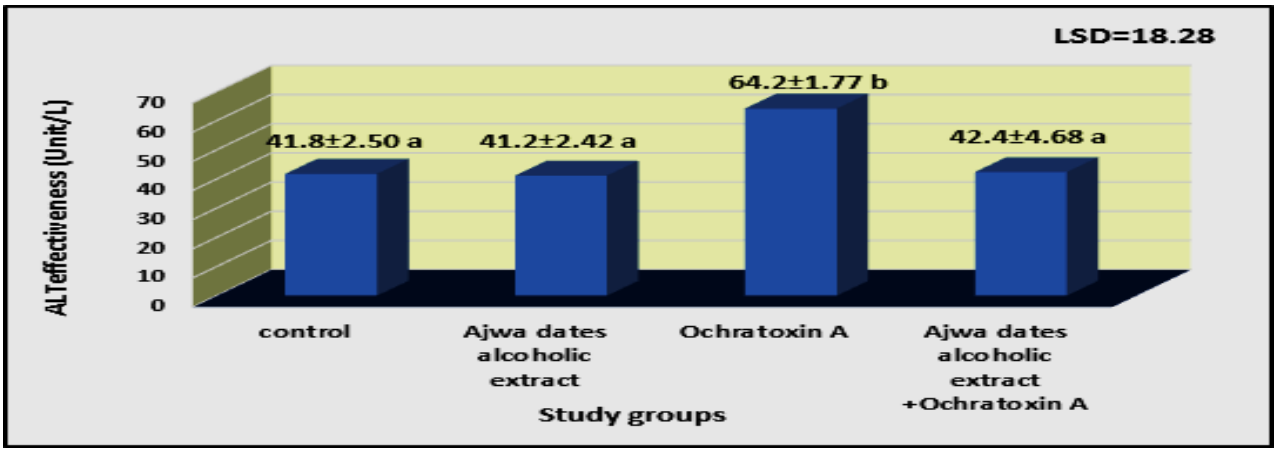


Figure 19: Effect of Ochratoxin A and Ajwa dates alcoholic extract on the ALT effectiveness

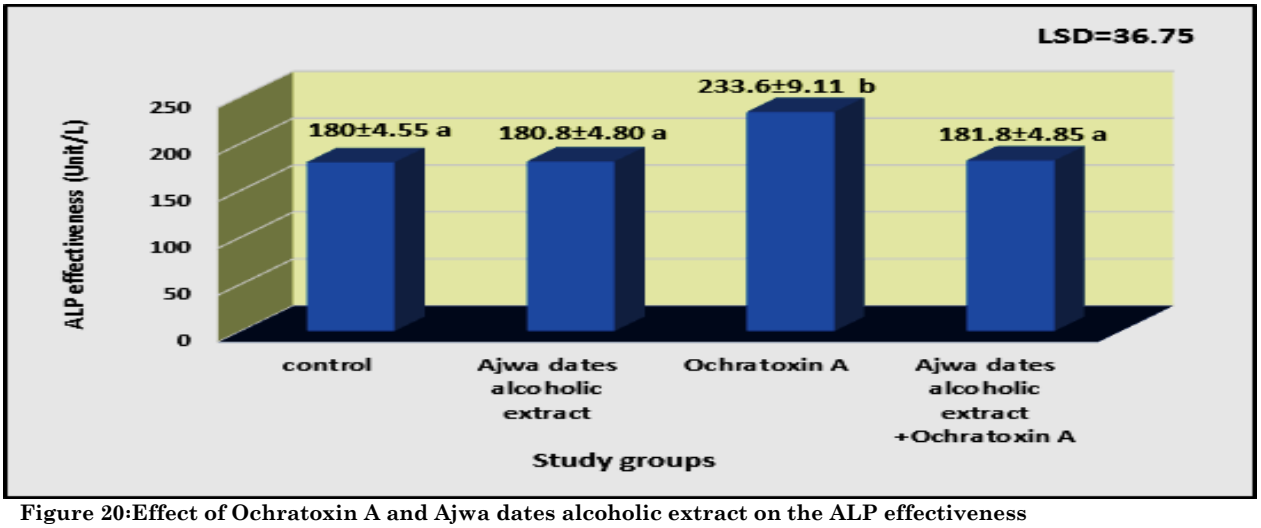


Figure 20:Effect of Ochratoxin A and Ajwa dates alcoholic extract on the ALP effectiveness

This findings compatible with several studies [39,53,54], the increment in the effectiveness of hepatic enzymes may attribute to the toxic effects of Ochratoxin A on the liver tissues which cause damage to the hepatocytes membranes, the cytoplasmic and nuclear contents of these cells, and thus the membranes became more permeable, that leads to liberate of enzymes into the bloodstream and increase their levels in the blood subsequently, [55] proposed that the rise in levels of the hepatic enzymes indicates that there is a liver injury. The raise in the activity of these enzymes may be explained by the lipid peroxidation and subsequent increased production of different reactive oxygen species (ROS), as a result, there is an imbalance in the levels of natural antioxidants such as glutathione, which is one of the essential cellular components, as many studies have shown, [56,57].

Furthermore, the increment in the hepatic enzymes effectiveness probably due to inflammation of hepatic cells, [49] demonstrated that Ochratoxin A has the high potential of infecting the liver cells with inflammation, causing a significant leakage of many cellular components, especially liver enzymes into the bloodstream, in addition to the lipid peroxidation, which causes cellular toxicity. It is worth noting that ALP enzyme is a highly sensitive liver enzyme for various types of hepatic infections, so it is released into the bloodstream in very large quantities when the hepatic tissue is exposed to any oxidative stress [58]. In the opposite, there was no notable variations in the efficacy of hepatic enzymes AST, ALT, and ALP at the group of male rats that were given Ajwa dates only, and the Ajwa dates + Ochratoxin A group compared to the control group.

The results of present study possibly because of Ajwa dates contain many highly effective anti-oxidants that act as free radical scavengers, thus providing super protection of the plasma membranes surrounding hepatic cells, leaving the permeability of these cells as normal and thus the survival of cytoplasmic and nuclear components including hepatic enzymes inside the cells without emancipated into the bloodstream, as it explained by many scientific studies [59, 60].

As well, the dates of the Ajwa have preventive properties or potentialities that provide preservation to the liver tissues and reduce the occurrence of hepatotoxicity [61] due to its strong anti-oxidants, such as the phenolic compounds containing p- coumaric and sinapic acids [26,27].

B-Effect of Ochratoxin a and Ajwa Dates on the Levels of Creatinine and Urea in the Serum

As regard with creatinine and urea levels, the Figures (21 and 22) show that Ochratoxin A exerted a remarkable rise ($P < 0.05$) in these levels as compared with the other groups were included in the present study. On the other hand, the statistical analysis of the results appeared no significant differences ($P > 0.05$) in those parameters when the remaining groups were compared with each other.

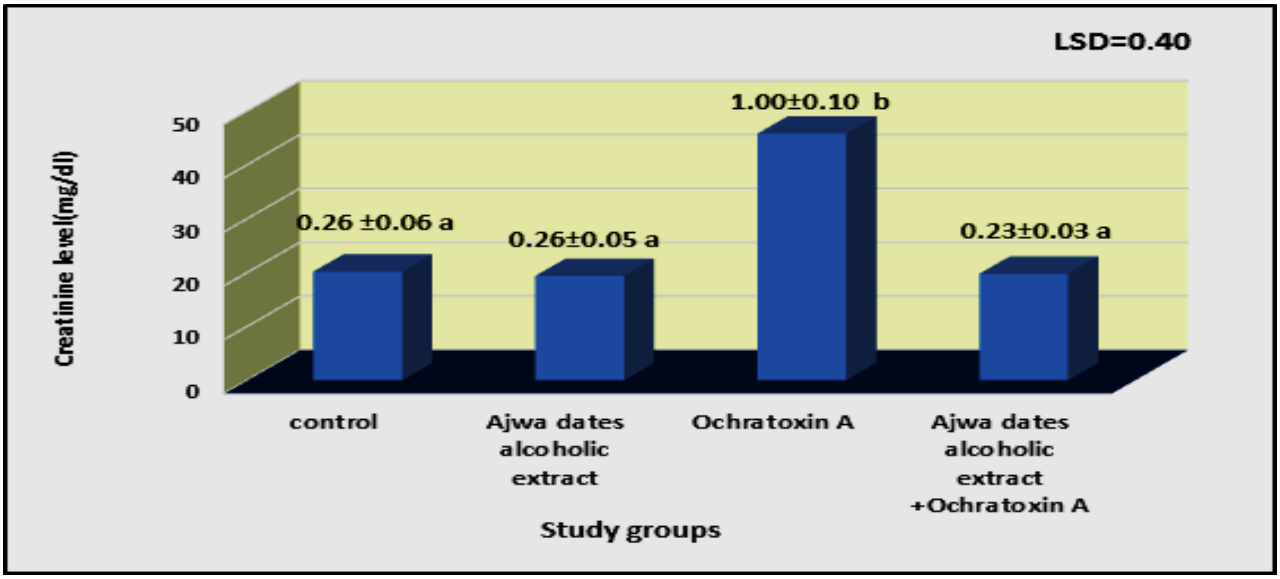


Figure.21:Effect of Ochratoxin A and Ajwa dates alcoholic extract on the creatinine level

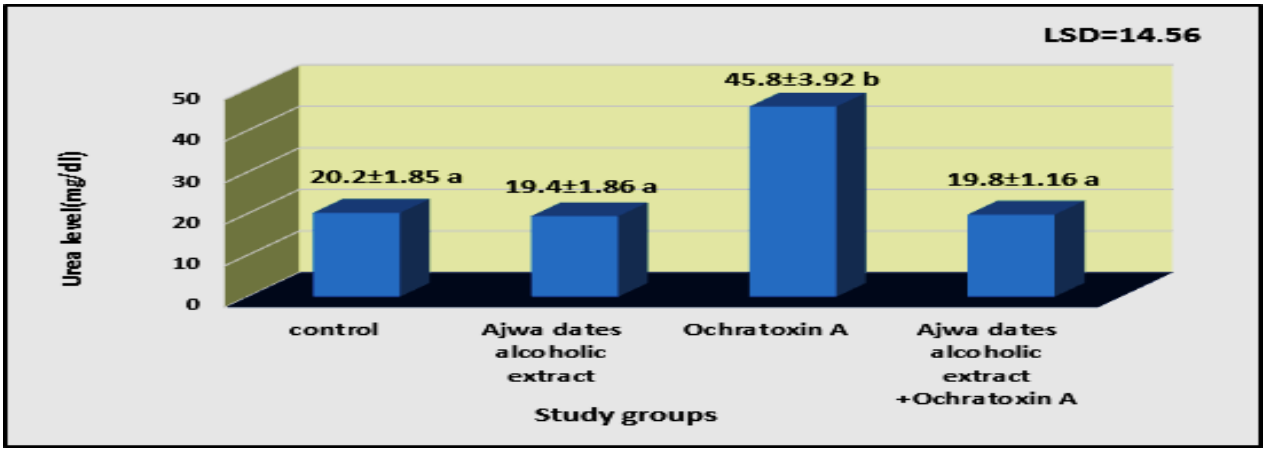


Figure.22:Effect of Ochratoxin A and Ajwa dates alcoholic extract on the urea level

The findings of this study have agreed with many other studies (62,63,64),these results possibly due to the fact that Ochratoxin A has negatively affected the renal tissues and caused a disturbance in the performance of various functions especially the disposal of particles with high toxicity such as creatinine and urea through the process of glomerular filtration, the current study was diagnosed numerous patho-histological alterations in the sections taken from the cortex and the medulla of the kidneys of male rats that orally given Ochratoxin A [37].

Confirmed that exposure to this type of fungal toxin reduces the blood supply of the kidneys, causing a low glomerular filtration rate. A further study suggested that Ochratoxin A caused a significant increment in the levels of these two biomarkers (creatinine and urea) in the blood, resulting in a cellular necrosis of renal tubules and death of some of them, as well as atrophy of some renal glomeruli , hemorrhage in others and enlargement of renal glomeruli capsules [47].

The rise in creatinine and urea levels may be explained to ulcers in the internal tissues of the kidney that caused by Ochratoxin A, or a blockage in the lower urinary tract or shrinkage in glomerular mesangail cells, which may have adversely affected the functional performance of the proximal tubules, and excessively increase sodium chloride output, in contrast to a decline in the process of secretion of other ions such as potassium and hydrogen, as confirmed by some studies [37].

It is likely that the increment attribute to the stimulation of lipid peroxidation and the resulting increase in the production rate of the oxygen reactive types such as the radicals of hydroxide, hydrogen peroxide, alkoxy, peroxynitrite and other species which have high-capacity destruction to the cellular construction of renal tissues, resulting in depletion of the enzymatic systems of anti-oxidant specially the glutathione molecules that is represented the first defense line in the body cells, it is responsible for the detoxifying of numerous deteriorative radicals, as demonstrated by several studies [65,66], which indicated that these free radicals caused ulcers in the cellular membranes, in addition to their devastating impacts to the biological molecules, especially proteins, reducing the efficiency of cellular defenses.

A study by [67] showed that the treatment of rats with Ochratoxin A caused the failure of the glomerular filtration process, damaged the filtration barrier, and inhibited protein synthesis and energy production (ATP).

In contrast, it stimulated the excessive oxidation of phospholipids in the cellular membranes and a negative change in the permeability of membranes became more permeable because hypertrophy of the renal cells, degeneration in the cellular membranes and organelles, especially the nucleus and mitochondria.

In a previous study used the rabbits, it was observed that Ochratoxin A caused programmed cell death events (apoptosis), as well as damage to the renal tubules tissues and adverse variations in the mitochondria similar to those reported above [68].

Concerning the groups that treated with the alcoholic extract of Ajwa dates only and alcoholic extract of Ajwa dates + Ochratoxin A, the levels of creatinine and urea did not have any noticeable alter when compared with the control group, and it may explain that this type of dates contains many antioxidants and vital essential dyes represented by carotenoids, flavonoids and anthocyanins [69], as well as it possesses high efficacy in resisting tumors [49].

It also has an effective role in the preservation of kidney tissues from oxidative damage caused by free radicals that produced by Ochratoxin [70].

In addition, other varieties of Ajwa dates are a rich source of dietary fibers that act as anti-oxidants [71].

Effect of Ochratoxin A on the Hepatic and Renal Tissues

The liver histological examination of the males rats that were treated with Ochratoxin A has shown a number of pathological alterations including, hepatocytes hypertrophy with a marked increase in the volume of nuclei, the appearance of some bleeding hepatocytes, as well as the cytoplasmic granulation of other cells, severe destruction and hemorrhage of the liver lobule central vein, intensive infiltration of inflammatory cells, irregularity of hepatic strands due to the fatty degeneration and dilatation of hepatic sinusiods of the liver lobules as shown in Figures (25,26,27 and 28) compared to the control group,whereas the group that treated with the alcoholic extract of the Ajwa dates only, and the group administered with the alcoholic extract of the Ajwa dates + Ochratoxin A,were revealed no abnormal changes, Figures (23,24 and 29), respectively.

Regarding the microscopic examination of the kideny tissues belonging to the male rats that were treated with the Ochratoxin A , many of the negative effects of this mycotoxin have been identified, these detrimental influences include: the destruction of the capsules of some renal glomeruli, renal glomeruli hyppertrophy and appearance of hemorrhage within them, degeneration and necrosis of the squamous epithelial cells of the glomeruli with egression of nuclei, the emergence of edema in the interstitial tissue of the kidney cortex, dissociation of the simple cubiodal cells lining of the renal tubules from the basement membranes, hemorrhage within some renal tubules, and stricture their diameters, in addition to the hemorrhage and edema were diagnosed inside the interstitial tissue of medulla, as shown in the Figures (32,33,34,38,39 and 40).

In contrast to what have been mentioned above, the medulla and cortex of the male rats kidneys of the control group, the group which orally given the alcoholic extract of Ajwa dates, as well as the group of alcoholic extract of Ajwa dates + Ochratoxin A, all were normal and the histological sections of these experimental groups did not reveal any pathological variation, as it is shown in the Figures (30, 31, 35, 37 and 41), respectively.

In relation to the patho-histological effects of Ochratoxin A on the hepatic tissues, have been agreed with several studies [42,43,48], which reached that the treatment with this type of fungal toxin causes many adverse changes, comprising: cytoplasmic vacuolation of hepatocytes with the emergence of necrosis in some of them , infiltration of mononucleated inflammatory cells around the portal regions of the liver degenerated paranchyma tissue ,as well as in the central veins, with emergence of small hemorrhage areas and congestion in the central veins.

In addition to,a congestion of the central veins, portal veins and hepatic sinusiods, dilatation of the central vein and hepatic sinusiods of liver lobules, severe congestion of the liver's interstitial tissue, irregularity of the hepatocytes, the proliferation of Kupffer's cells [18,43,48].

Other study has been added,a slight hypertrophy and vascular degeneration of the hepatic cells, finally proliferation of the connective tissue around the portal area [40].

With regard to the renal tissues, the impacts of Ochratoxin A on these tissues have compatible with several other studies [36,48], which postulated that this fungal toxin has caused a notable congestion in the

renal tissues, other studies [40,42], recorded a congestion in the blood vessels of the kidney cortex , capillaries surrounding the renal tubules, as well as the blood vessels circulating among the renal tubules,severe invasion of the inflammatory cells especially neutrophils with accumulation of red blood corpuscles in the cavities of those tubules ,emergence of a focal aggregation of lymphocytes and few plasma cells, renal glomeruli hypertrophy, cellular hyperplasia of the capillaries of renal glomeruli as a result of the epithelial cells proliferation of the capillaries of those glomeruli, accumulation of the inflammatory cells in the interstitial tissues, thickening of the wall of the Bowmanś capsule by the significant proliferation of fibrous connective tissue, severe cellular degeneration of the epithelial linning of renal tubules due to the cytoplasmic vaculation. A subsequent study also indicated occurrence of necrosis in the epithelial cells of proximal convoluted tubules [62].

Moreover, [47], have stated that Ochratoxin A has other pathological and histological effects, such as renal glomeruli atrophy and hemorrhage, as well as necrosis and death of renal tubule cells.

A further study suggests that treatment with the Ochratoxin A may cause severe damage to the renal tissues, comprising: cellular necrosis of the renal tubules, swelling of the proximal convoluted tubules with a dense accumulation of acidophils within the cavities of these tubules, the separation of simple cubiodal cells lining of the convoluted tubules from the basement membranes, congestion and fibrosis in the interstitial tissue of the kidney medulla as well as the loss of the characteristic brushing edges of those tubules and the decay of nuclei [52]. It is worth mentioning that the study of [30] have proposed that the treatment with this toxin caused swelling of epithelial cells of renal tubules due to the emergence of dense granules within the cytoplasm of these cells, narrowing Bowmanś space, the proliferation of the mesangial cells of the renal glomerulus.

The toxic influences of Ochratoxin A on the hepatic and renal tissues may be suggested to the harmful free radicals were generated due to the lipid peroxidation which caused by this toxin and the consequent remarkable damage to the biological molecules such as proteins, fats, carbohydrates, nucleic acids, especially DNA bases, as well as rupture of the plasma membranes of the cells and exit of nuclear and cytoplasmic components.

Contrary, the other experimental groups that were received the alcoholic extract of Ajwa dates + Ochratoxin A and the alcoholic extract of Ajwa dates only, have exerted a notable improvement in the functional performance, as well as the cellular construction of those tissues did not show any disturbance with the survival of the nuclear and cytoplasmic contents of cells in its normal condition when compared with the control group.

This is likely to be explained by the fact that the alcoholic extract of the Ajwa dates contains many food components such as carbohydrates, proteins, fats, dietary fiber [72], antioxidants such as carotenoids and phenols [73], vitamins A, B_2 and C [74], mineral salts (potassium, calcium and magnesium) [75], so it is a sweeping of toxic free radicals through its ability to attack them and put them outside of living cells.

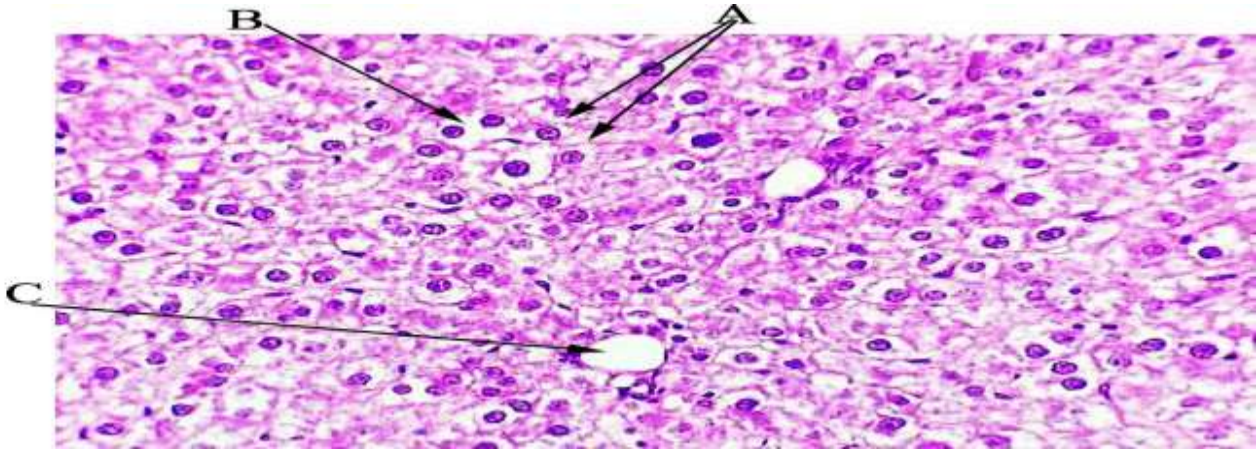


Figure 23 sections in the liver at the control group: normal hepatocytes (A) binucleated hepatocytes (B) normal central vein of liver lobule (C) Staining :hematoxylin-eosin (400)

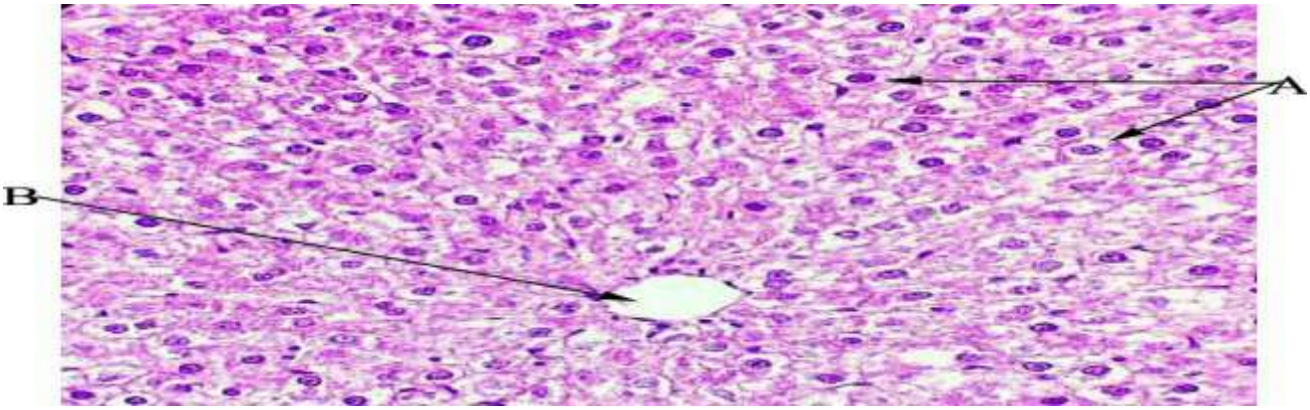


Figure 24: section in the liver at the group that treated with alcoholic extract of Ajwa dates: normal hepatocytes (A) normal central vein of liver lobule (B) Staining :hematoxylin-eosin (400)

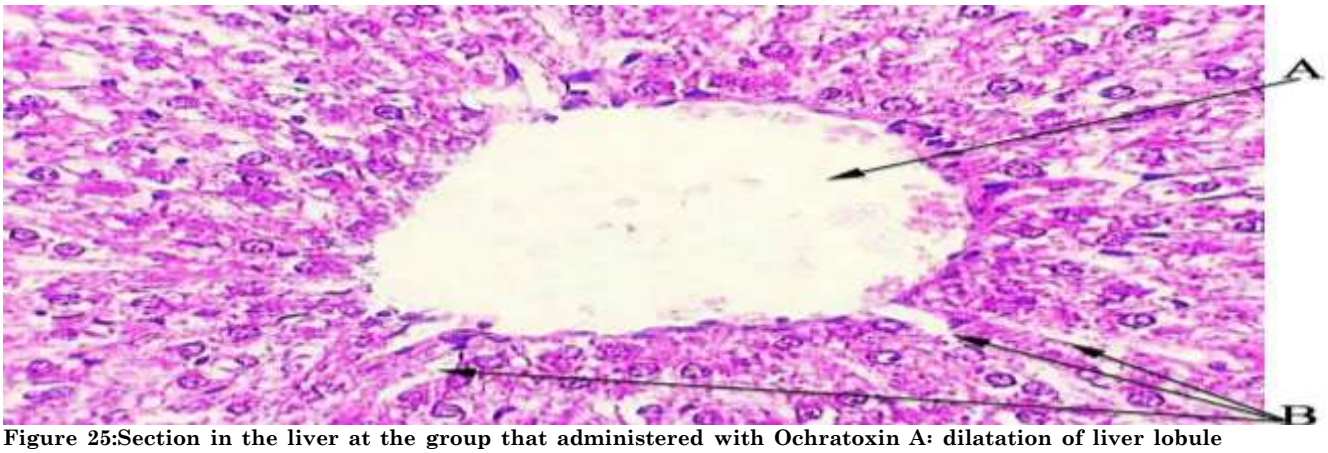


Figure 25:Section in the liver at the group that administered with Ochratoxin A: dilatation of liver lobule central vein (A) dilatation of liver lobule hepatic sinusoids (B). Staining :hematoxylin-eosin (400)

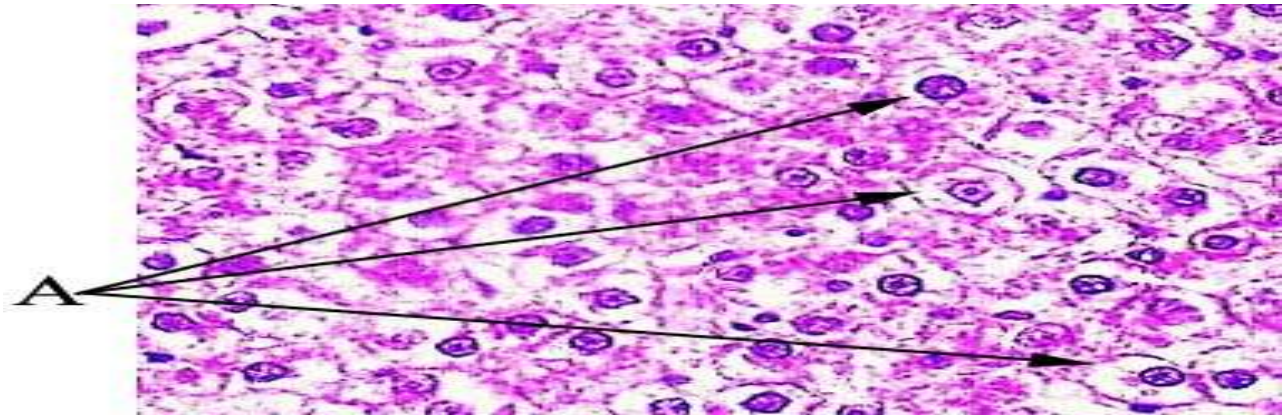


Figure.26: section in the liver at the group that administered with Ochratoxin A: hepatocytes hypertrophies with increase the nuclei volume (A). Staining: hematoxylin-eosin (400)

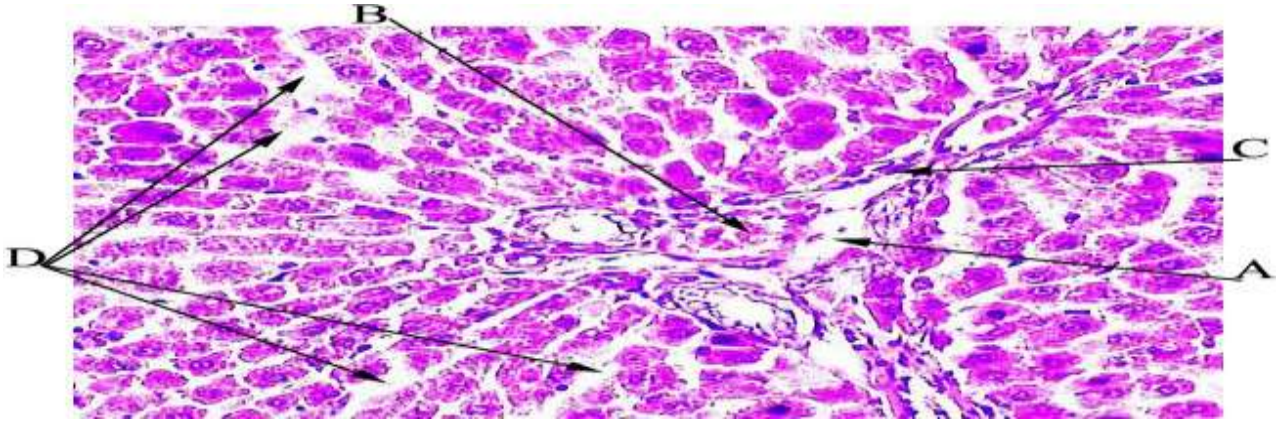


Figure 27: section in the liver at the group that administered with Ochratoxin A: destruction of liver lobule central vein (A) aggregation of red blood corpuscles within the central vein (B) infiltration of inflammatory cells inside the central vein(C) disarrangement of the hepatic strands due to fatty degeneration(D). Staining :hematoxylin-eosin (400)

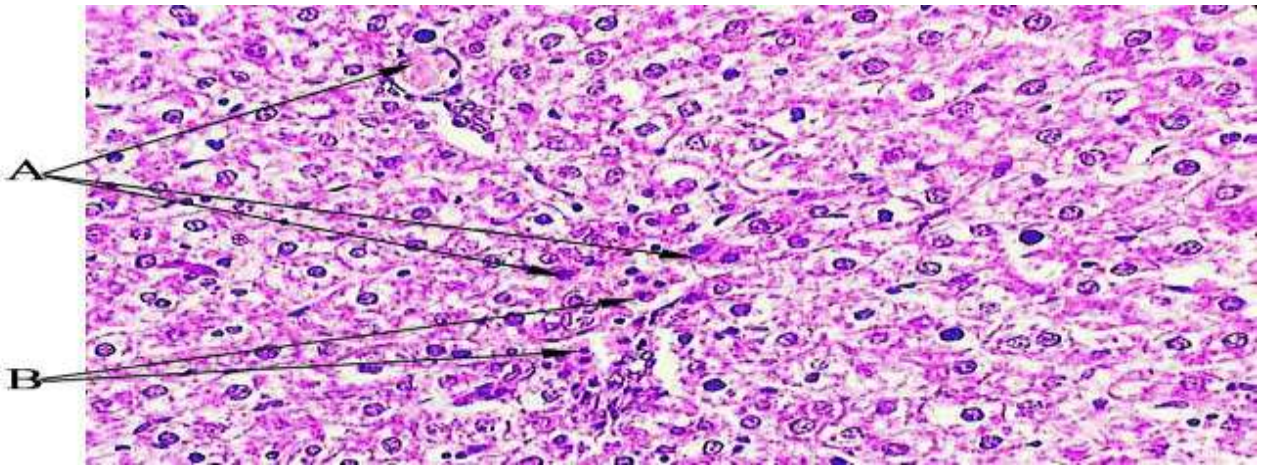


Figure 28: section in the liver at the group that administered with Ochratoxin A : bleeding hepatocytes (A) necrosis of nuclei (B).Staining :hematoxylin-eosin (400)

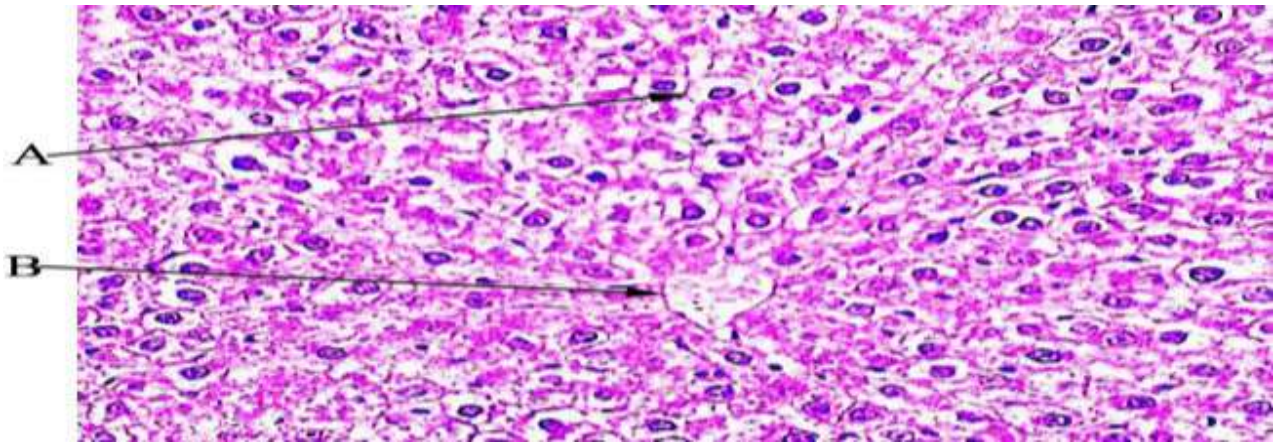
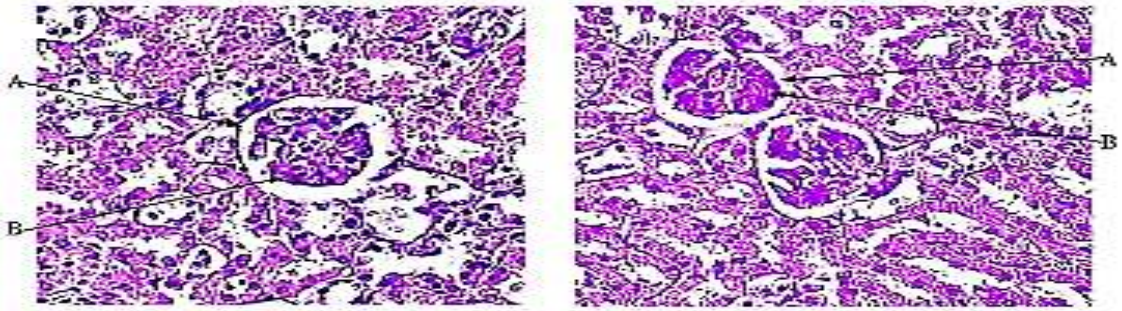


Figure 29: section in the liver at the group that orally given Ajwa dates alcoholic extract + Ochratoxin A: normal hepatocytes (A) normal liver lobule central vein (B).Staining :hematoxylin-eosin (400).



30 31

Figure (30) section in the kidney cortex at the control group ?normal renal glomerulus capsule (A) normal renal glomerulus (B).

Figure (31) section in the kidney cortex at the group that received Ajwa dates alcoholic extract ?normal renal glomerulus capsule (A) normal renal glomerulus (B).Staining ?hematoxylin-eosin (400).

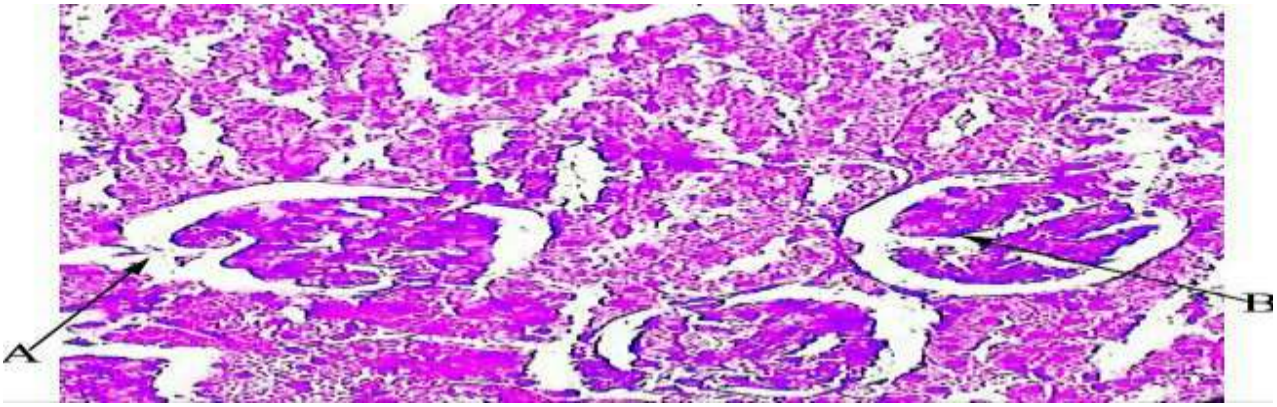


Figure 32: section in the kidney at the group that administered with Ochratoxin A : destruction of renal glomerulus capsule (A) necrosis of squamous epithelial cells of renal glomerulus (B). Staining :hematoxylin-eosin (400)

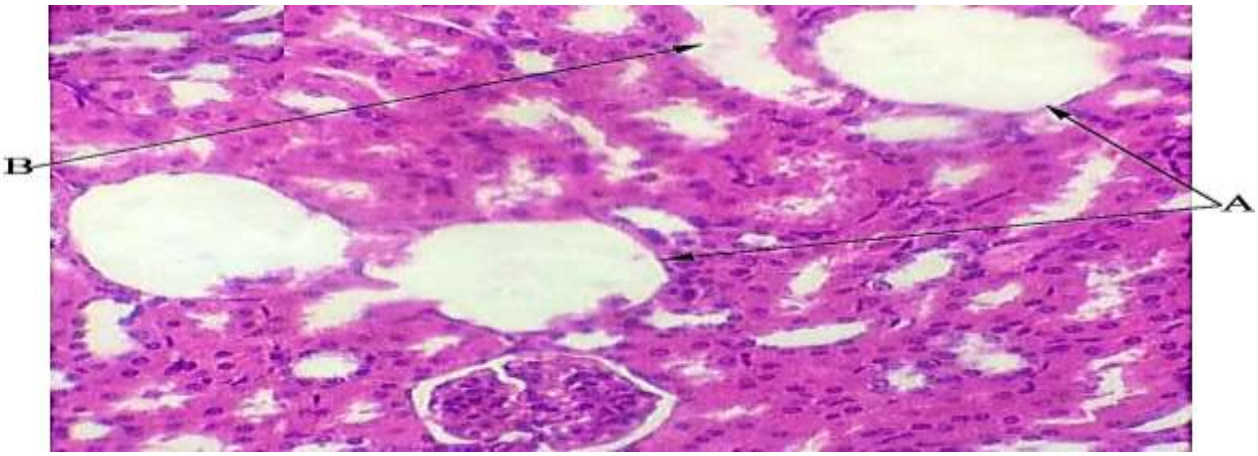


Figure 33: section in the kidney at the group that orally given Ochratoxin A : severe destruction of renal glomeruli capsule(A) edema in the interstitial tissue of cortex(B). Staining :hematoxylin-eosin (400)

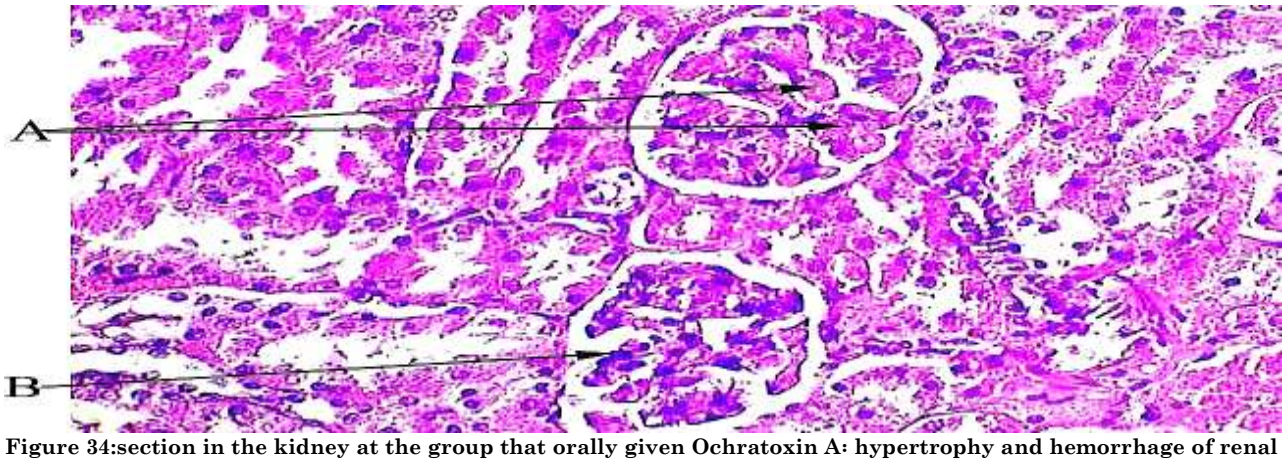


Figure 34:section in the kidney at the group that orally given Ochratoxin A: hypertrophy and hemorrhage of renal glomerulus (A) sever necrosis of squamous epithelial cells of renal glomerulus with nuclei egression(B). Staining: hematoxylin-eosin (400)

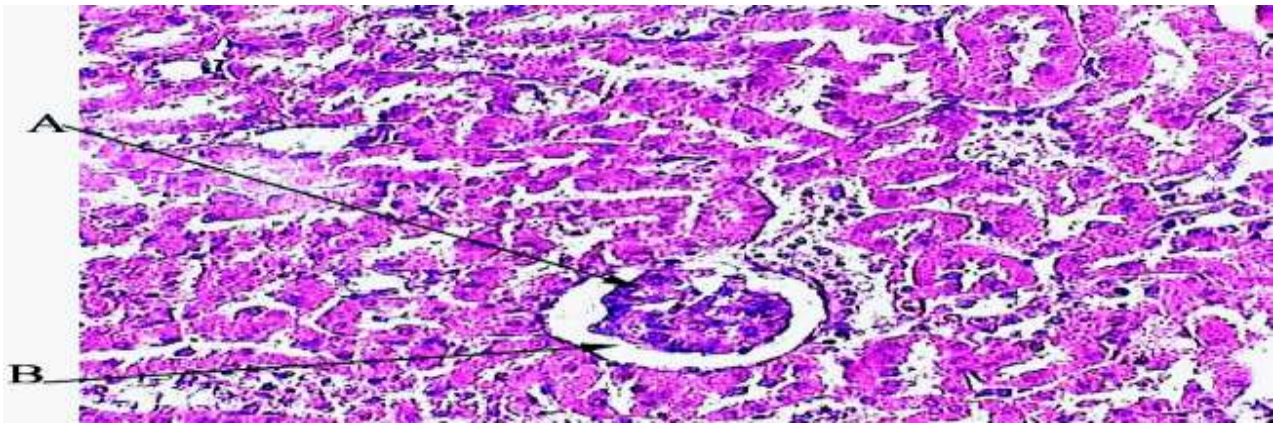
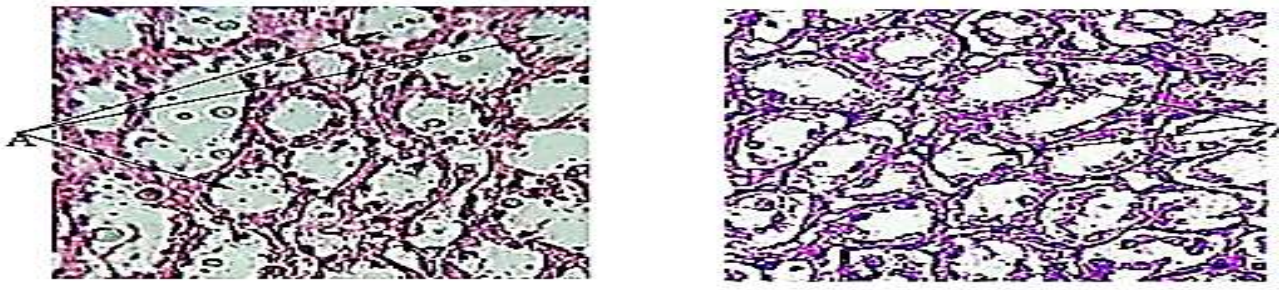


Figure.35: section in the kidney at the group that treated with Ajwa dates alcoholic extract + Ochratoxin A : normal renal glomerulus (A) normal renal glomerulus capsule (B). Staining :hematoxylin-eosin (400)



36 37

Figure (36) section in the kidney medulla at the control group ? normal renal tubules (A).

Figure (37) section in the kidney medulla at the group that received Ajwa dates alcoholic extract ? normal renal tubules (A). Staining :hematoxylin-eosin (400).

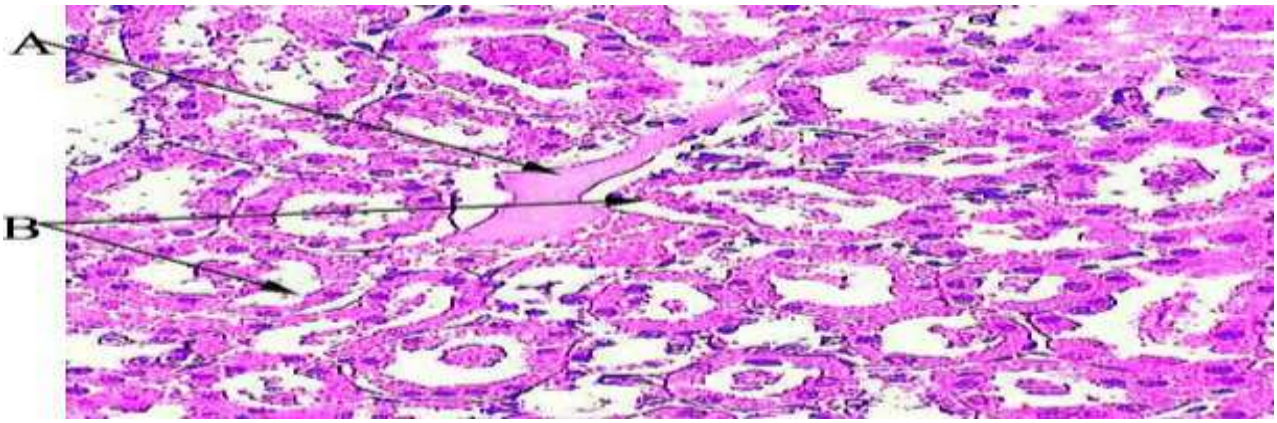


Figure 38: section in the kidney at the group that administered with Ochratoxin A :edema and hemorrhage within the interstitial tissue of kidney medulla (A) dissociation of the simple cuboidal cells lining of the renal tubules from the basement membranes(B). Staining :hematoxylin-eosin (400)

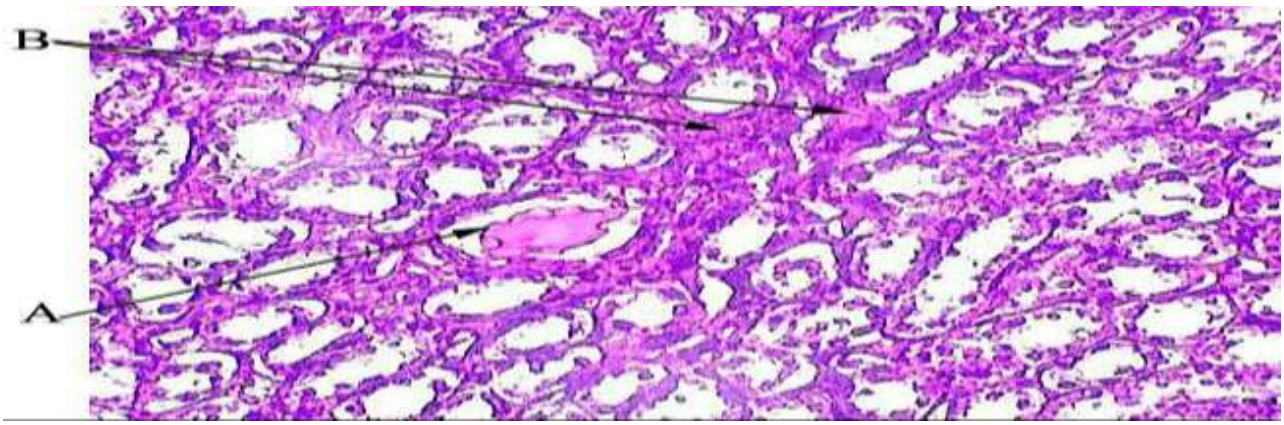


Figure.39: section in the kidney medulla at the group that orally given Ochratoxin A: bleeding renal tubules (A) hemorrhage inside the interstitial tissue of kidney medulla (B).Staining :hematoxylin-eosin (400)

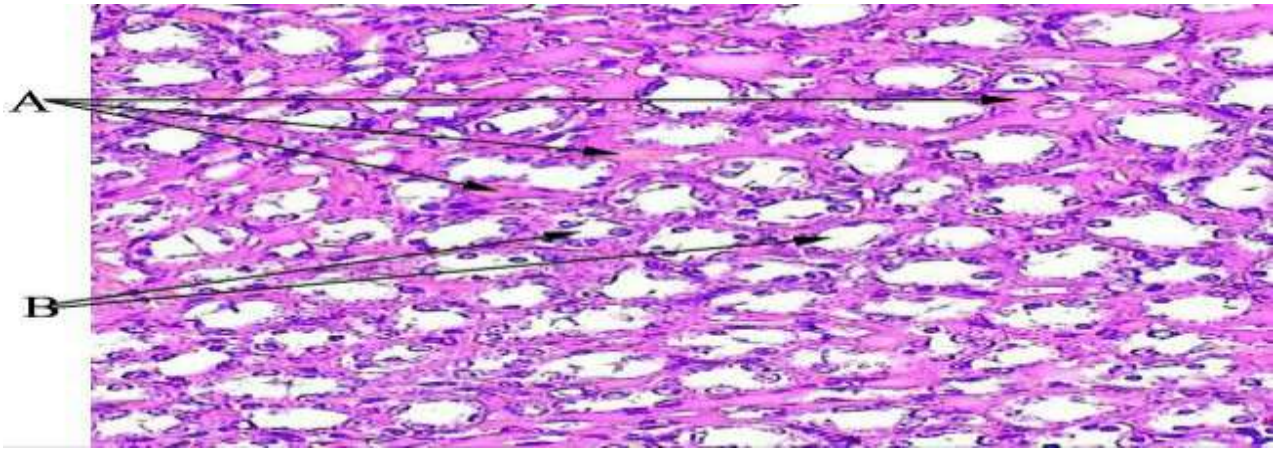


Figure.40: section in the kidney at the group that treated with Ochratoxin A: severe hemorrhage within the interstitial tissue of kidney medulla (A) stricture of some renal tubules diameters (B). Staining :hematoxylin-eosin (400)

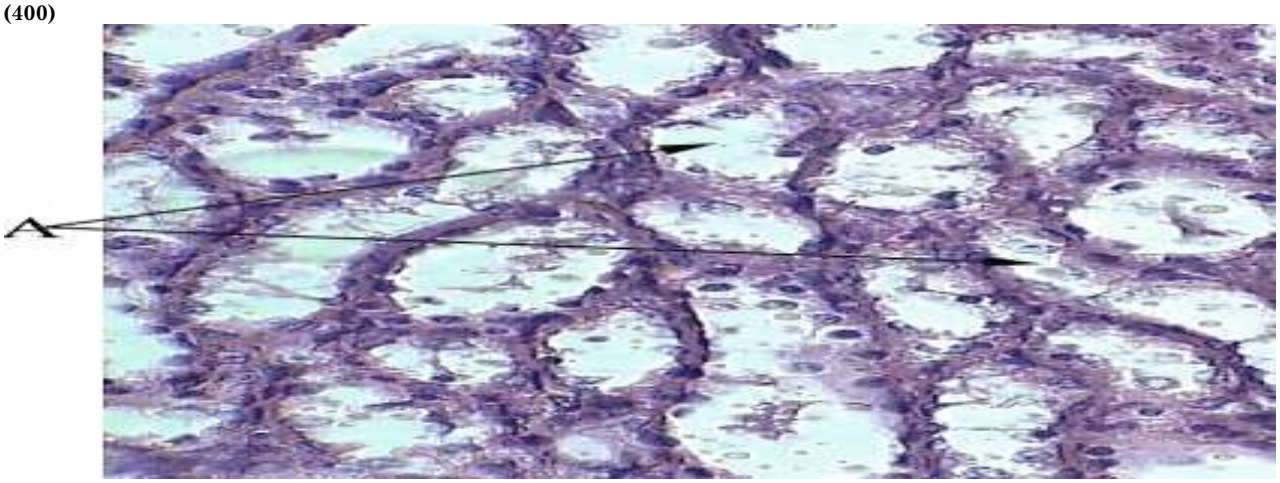


Figure 41: section in the kidney at the group that received with Ajwa dates alcoholic extract+ Ochratoxin A: normal renal tubules (A).Staining: hematoxylin-eosin (400)

Conclusion

- The study found that both alcoholic and aqueous extracts of the Ajwa dates had the same effect in inhibiting the formation of Ochratoxin A by *A.niger*.
- Ochratoxin A injection caused reduction in the body weight, possibly due to the impact of this type of toxins in the effectiveness of certain glands responsible for the metabolism in the body, or due to disturbances in the gastrointestinal tract. Meanwhile, the toxin caused significant increase in the liver and kidney relative weights due to the patho-histological changes in the cellular components of the tissues of those organs.
- OchratoxinA has been exerted many adverse effects on both liver and kidney functions for the high efficiency of hepatic enzymes AST, ALT and ALP, as well as the increased levels of creatinine and urea.
- The alcoholic extract of Ajwa dates has been shown to be highly effective in the protection of weight criteria and some biochemical blood parameters, in addition to the liver and kidney tissues from the toxic influences of Ochratoxin A.

Recommendations

- The study recommends the use of Ajwa dates to reduce the levels of toxins in the blood and body tissues that have been exposed to poisoning such toxins.
- Conducting future studies to diagnose the side effects associated with other types of fungal toxins on the physiological and biochemical blood indicators, as well as the hepatic and renal tissues .
- Performing further studies to determine the adverse impacts of Ochratoxin A on the weight parameters and various blood components, as well as the cellular structures of both liver and kidneys in female rats and compare the results with findings of the current study to detect which sex is more affected by this fungal toxin.
- Identifying the possible pathological changes of Ochratoxin A in other organs such as spleen, pancreas, lungs and heart, in addition to the diagnosis of the influences of this type of toxins on the immune system by measuring the levels of some interleukins in the serum.
- Testing the probable toxic effects of Ochratoxin A on male reproductive efficiency through tissue sections preparation in the testes, epididymis and accessory sexual glands, as well as the evaluating of the levels of some male hormones, especially the testosterone after injecting male laboratory animals with this type of mycotoxins.
- Isolation of the chemical components of the alcoholic extract of Ajwa dates to determine which is the most efficient in the preservation against the negative effects associated with Ochratoxin A.
- Studying the possible preventive actions of the organic extracts of Ajwa dates in protecting the weight and blood standards, as well as the histological components of different body organs, to compare the results with the data of the present study and determine the most efficient ones.
- Investigation about the preservative and curative efficacy for the extracts of other types of dates against the detrimental effects of Ochratoxin A on different blood properties.

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