



EFFICACY OF CINNAMON (*CINNAMOMUM ZEYLANICUM*) EXTRACT IN REDUCING TOXICITY OF *ASPERGILLUS NIGER* ISOLATED FROM DRIED FIGS IN THE RAT BIO-SYSTEM

Buthina Fahim Nasir and Sadeq Mohammad Ali*

Plant Protection Department, Faculty of Agriculture, University of Kufa, Najaf, Iraq

*Correspondence author email: sadeq.almusailmawi@uokufa.edu.iq

Abstract

The current study carried out to isolate and identify fungal species producing mycotoxins from dried figs and the possibility of reducing their presence in fruits and reducing their toxic effects using Cinnamon extract. The isolation results showed contamination of local and imported dried figs with stored fungi, especially *A. niger* and *A. flavus*. It was found that the frequency of appearance rate of *A. niger* on the local (Iraqi) and imported (Iranian, Syrian, Turkish) dried figs was 60.72.72.16.66 and 75%, while the appearance of occurrences rate was 100, 100, 20 and 60%, respectively. The results of chemical analysis of TLC showed that 19 out of the 26 tested isolates of *A. niger* were produced Ochratoxin A with a production rate of 73.07%. The results also showed that different concentrations of Cinnamon extract had high efficacy in inhibition of *A. niger* where the inhibition of fungal growth was increased with increasing Cinnamon extract concentration, with the highest inhibition (97.84%) at a concentration of 3%. These results showed that Cinnamon extract was highly effective in protecting some bio-systems of white rats (urea levels, protein ratio, haemoglobin, haematocrit and WBC rate) from the toxic effects of Ochratoxin A. The tissue study showed that Cinnamon extract provided high protection for organs (liver, kidney, spleen).

Keywords: *Cinnamomum zeylanicum*, *Aspergillus niger*, dried figs, white rat females.

Introduction

Figs are semi-tropical trees that often have more than one stem, their branches are not intertwined, and are distinguished by milky liquid with a distinct smell. The fig fruit is false and it is called botanically (Syconium) where it is a fleshy pink holder (Receptacle) encapsulates a cavity and this is attached to the outside with a small hole called the eye (Ostiolum). Dried fruits production is the major marketable figs after being dried using special dryers or sun-dried. Drying fig fruits is as old traditional method as fourth millennium BC for preserving and storing fruits for a long period. Many fruits, such as peaches, apricots, raisins, and figs are sold as fresh fruits or dried with respect to their nutritional values (Wu *et al.*, 2004; Yabe *et al.*, 1987; Kim *et al.*, 2000).

Dried fruits are exposed to be infected with bacteria and or fungi at different stages pre and post-harvest and during the storage. Many studies were confirmed contamination of dried fruits with several fungal genera such as *Apergillus*, *Fusarium*, *Mucor*, *Rhizopus*, *Alternaria*, *Penicillium* and *Ulocladium*. However, *Apergillus*, *Fusarium* and *Alternaria* are major contaminants of many dried fruits (Almeida *et al.*, 2006; Barnett and Hunter, 1972). These fungi produce mycotoxins, a secondary metabolites that have a harmful effects on the public health. Mycotoxins cause chronic severe effects on the liver, kidneys and immune system, causing kidney failure, liver cancer and weak immune system when they are exposed to high doses (Kuiper *et al.*, 2004; Richard, 2007). Aflatoxins and Ochratoxins, Strigmatocystin and Cyclopiazonic are the most important groups of acutely toxic compounds which produced by *A. ochraceus*, *A. parasiticus* and *A. flavus* on dried fruits. Various traditional methods were used to control and reduce infection by these fungi on dry fruits including the use of herbs and spices.

Cinnamon *Cinnamomum zeylanicum* (belongs to Lauraceae plant family) has been utilized as a potential therapeutic agent in various cultures for centuries.

Historically, cinnamon bark is among the oldest known spices used against gastrointestinal complaints, chronic bronchitis, and inflammation of eyes in Ayurvedic medicine for over 6000 years (Al-Zubaidi *et al.*, 1996). Cinnamic aldehyde is diagnosed in Cinnamon oil as an effective and toxic substance against respiratory fungi such as *A. fumigatus*, *A. niger*, *A. flavus*, *A. indulans*, *Candida albicans*, *C. tropicalis*, and *Histoplasma capsulatum*. The vapour of this oil was effective against these fungi. It was conclude that the fumes are a new trend for typical chemotherapy for fungal respiratory diseases (Singh *et al.*, 1995). It was found that among the 13 medicinal plants and 7 commercial dry drinks that the Cinnamon bark completely suppressed the growth of fungi *A. ochraceus*, *A. flavus*, *A. parasiticus* and *A. versicolor*. Adding of Cinnamon to bread and its effect on the growth of *A. parasiticus* and its production of Aflatoxin was also studied by (Abdel Hamid, 2000) who found that all concentrations of Cinnamon (0.02-20%) were inhibited the growth of fungi and the production of Aflatoxin. He recommended to use an average concentration such as 2% to inhibit the production of aflatoxin by 97-99%. The objective of this study, therefore, is to isolate and identify fungal species producing mycotoxins from dried figs and the possibility of reducing their presence and toxic effects using Cinnamon extract.

Materials and Methods

Samples

Different types of the dried figs from various sources including Iraqi, Syrian Iran and Turkey were collected from retail markets in provinces of Najaf, Karbala, Baghdad and Diwanayah. The samples were transferred to the laboratory of Fungi at the Faculty of Agriculture-University of Kufa for the purpose of isolating accompanying fungi and bacteria. For isolation and diagnosis of fungi associated with dried figs, five samples of each type of dried fig were cut into small pieces and sterilized with sodium hypochlorite solution

(2%) for 2 minutes. Then, they were washed with distilled water and placed on filter papers to dried, placed in petri-dishes with PSA (five piece in each dish). The Petri dishes were incubated at 25°C for 7 days. After the of incubation period, growing fungal isolates were purified and identified according to the taxonomic characteristics described by Koching and Pitt (1997). Fungal isolates of *Aspergillus* were purified and preserved (Evens *et al.*, 1993) in the refrigerator at 4 °C.

Detection of Ochratoxin A produced by *A. niger* isolates

The ability of *A. niger* isolates to produce Ochratoxin A was assessed using thin layer chromatography (TLC) following the procedure described by Balzer *et al.* (4). The same method was also used to collect Ochratoxin A by scratching the detected layers containing the toxin to be gathered and forming the final stock.

The effect of Cinnamon extract on *A. niger* growth on PDA medium

Three concentrations of Cinnamon (1, 2, 3 g / 100 ml) were selected to evaluate the efficacy of using the Poisoned Food technique (Dixit *et al.*, 1976). Where they placed into sterile 9 cm diameter Petri dishes, 3 replicates were used for each treatment, as well as control treatment (without Cinnamon extract). Then, 5mm disk of *A. niger* was placed in the central of each dish, then the dishes were incubated at 30 ± 2 °C for 7 days. The results were calculated using the mean of two orthogonal diagonals from each colony at the time of full growth in the control dish, and the percentage of inhibition was calculated using the following equation:

$$\% \text{ inhibition} = \frac{\text{Mean diagonal colony of control} - \text{Mean diagonal colony of Treatment}}{\text{Mean diagonal colony of control}} \times 100$$

Efficacy of Cinnamon extract in protecting the bio-systems of white rat from Ochratoxin A effects

General Procedure

Twelve white female rats (Albino Rats), type *Rattus rattus* (strain: Sprague Dawley), 8-10 week-old were

supplied from the Faculty of Science - University of Kufa and placed in the cages at 23-28 °C and appropriate lighting. As for treatments, 30 mg of Ochratoxin A was prepared by growing *A. niger* isolate on the centre petri dishes containing PDA media. Petri-dishes were incubated at 30 °C for 10 days and then Ochratoxin A was extracted using (TLC) method. For separating Ochratoxin A on the TLC plates depending on the location of the presence of the standard Ochratoxin A and then aspirating the silica gel as the poison was presented using a sharp blade. The same method was repeated several times for obtaining appropriate amount of silica containing poisons. Then, an equal amount of chloroform was added to the sterile test tubes and the mixture was well discarded and then abandoned and left and then placed at 45 °C to be completely dried and so repeated the process until you get 30 mg of Ochratoxin A. A concentration of 500 mg/ ml of the Ochratoxin A was prepared by melting the with the Ochratoxin A with substance Dimethyl sulfoxide (DMSO) which was added at a rate of 500 mg/1kg of animal weight (2011). Cinnamon extract was obtained by adding 400 ml of sterilised water to 100 g of cinnamon powder in a 1000 ml beakers and mixed together using the electrical mixture for 10 min. The mixture was then kept in the refrigerator until use.

Experimental treatments

Twelve white female rats were divided into four groups of three rats each. Rats were continuously dosed for three weeks (Table1), during this time the clinical symptoms on rates were recorded. By the end of the experiment period, the rats were drugged with chloroform and then dissected by opening the abdominal cavity and pulling the blood in a stab of heart (heart puncture). Partial amount of the blood was placed into test tubes free of EDTA and the other amount was placed into tubes containing EDTA for physiological and chemical blood tests. Parts of the liver, kidneys, intestines and spleen were taken from the rats and kept in formalin at a concentration of 10% to study the tissue changes.

Table 1 : Treatment applied to female white rat

Treatment	Treatment Describe
Dimethyl sulfoxide	Treating rats with dimethyl sulfoxide 1 ml/Kg animal weight.
A poison of Ochratoxin A	Treating rats with Ochratoxin A at a rate of 500µg / kg animal weight every 48 hours.
<i>Cinnamomum zeylanicum</i> extract	Treating rats with <i>C. zeylanicum</i> extract at rate of 1 ml/kg animal weight every 48 hours.
A poison of Ochratoxin A+ <i>Cinnamomum zeylanicum</i> extract	Treating rats with <i>C. zeylanicum</i> extract at rate of 1ml/kg animal weight followed by Ochratoxin A at 500 µg/kg animal weight every 24 hours.

Measurements and Data analysis

Physiological blood criteria were studied including Calculation the total haemoglobin concentration (Hb total), mean number of White Blood Cell (WBC), packed cell volume (P.C.V) haematocrit, as well as the Biochemical Blood criteria in terms of T.S. protein ratio G/dl and Urea mg/dl. Histopathological Study was also performed on prepared body tissue sections at the Department of Tissue Diseases/Najaf Teaching Hospital using the method described by Bancroft and Stevens (1982). Analysis of variance ANOVA was performed after data being analysed using computing GenStat 12th edn program. Means were

compared among treatments based on least significant difference L.S.D. ($P \leq 0.05$).

Results and Discussion

Isolation and diagnosis of fungi associated with dried figs

The results of isolation and diagnosis of fungi associated with dried figs showed that two species belong to *Aspergillus* genus were appeared, *A. niger* and *A. flavus*. The appearance rate of *A. niger* in the types of studied figs (Iraqi, Iranian, Syrian, Turkish) was (100%, 100%, 20%, 60%) respectively. While the rate of frequency in all

studied types of figs were 60%, 72.2%, 16.6%, 75%, respectively (Table 2). The results obtained in the present study are similar to those conducted by Hadrawi (2011), who proved that the fungal species belong to *Aspergillus* and *Penicillium* were infected important crop seeds under bad conditions. The dominance of *Aspergillus* spp. was due to its wide spread in the environment, which comes from its ability to produce a large number of spores that resistant to adverse environmental conditions, which form plankton in the air because its diameter is less than 15 nm and thus it can reach many places where it can enter the stores through the windows and other openings, as well as its growth in wide ranges of temperature and humidity, as some species of *Aspergillus* species are growing in ranges of temperatures ranging from 5-45 °C or higher and relative humidity between 15% to 18 (Agarwal and sinclair, 1996; 1997, Rustum).

Table 2 : Percentages of frequency and onset of fungi isolated from dried fig samples.

Dried fig type	<i>A. niger</i>	
	Appearing%	Frequency%
Local fig (Iraqi)	100%	60%
Iranian fig	100%	72.72%
Syrian fig	20%	16.66%
Turkish fig	60%	75%

Detection of *A. niger* isolates producing Ochratoxin A using thin layer chromatography (TLC)

Chemical analysis of TLC showed that 19 isolates out of 26 isolates of *A. niger* were able to produce Ochratoxin A (Table 3). However these isolates were varied in their ability production. An2 and An5 were the most productive of Ochratoxin A. The results obtained in the present study are consistent with those of Gherbawy *et al.* (13) who indicated that 25% of *A. niger* isolates which isolated from apple were able to produce Ochratoxin A. Ali (2015) found that 20% isolates of *A. niger* which isolated from dates were able to produce Ochratoxin A. Differences in the ability of *A. niger* isolates to produce Ochratoxin A may be related to their genetic differences.

Effect of Cinnamon extract on growth of *A. niger* on PDA medium

The extract of cinnamon was inhibited the growth of *A. niger*, where the rate of inhibition was increased with increasing the concentration, with the highest inhibition rate at a concentration of 3% which reached 97.84% (Table 4). These results are in agreement with many studies that have proven the effect of cinnamon oil in inhibiting many fungi such as *A. ochraceus* and *A. flavus*, *A. parasiticus* and *A. versicolor* and *F. moniliforme* (Abdul Hamid, 2000; Soliman and Badeaa, 2002).

Table 3 : TLC test for *A. niger* isolates to producing Ochratoxin A isolated from infected dates

Innate isolation	Ochratoxin A producing Ability	Innate isolation	Ochratoxin A producing Ability
An1	+	An14	+
An2	+++	An15	-
An3	++	An16	++
An4	-	An17	++
An5	+++	An18	+
An6	++	An19	-
An7	+	An20	++
An8	-	An21	-
An9	+	An22	-
An10	-	An23	++
An11	++	An24	++
An12	+	An25	++
An13	++	An26	++

Table 4 : Effect of different concentrations of Cinnamon *C. zeylanicum* extract powder on growth of *A. niger* on PDA medium.

Treatment	Radial growth diameter (cm)	Rate of inhibition %
PDA comparison only	6.23	00.00
1g <i>C. zeylanicum</i>	2.73	55.91
2g <i>C. zeylanicum</i>	1.23	80.10
3g <i>C. zeylanicum</i>	0.13	97.84
L.S.D (P<0.05)	0.4449	6.073

Efficacy of Cinnamon extract of I in reducing the toxic effects of Ochratoxin A on the biological systems of female white rat.

Effect on blood biochemical characteristics

Estimating the level of urea in serum: The results of the level of urea in the blood showed significant increase (P<0.05) in the level of urea when rats were treated with Ochratoxin A, which reached (31.47 mg/100ml) compared

to the control treatment (17.50 mg/100ml) (Figure 1A). This finding is in agreement with Baily *et al.* (1989) who reported increasing of uric acid in the blood plasma of birds exposed to Ochratoxin A. In addition, Abdul Hamid (2000) recorded significant increase in uric acid in the blood plasma of birds exposed to medium and high doses of Ochratoxin A. Al-Ghazali and Ali (2014) found that the level of urea significantly increased in white rats treated with Ochratoxin A compared to the control treatment. The

increase level of urea in the blood of animals treated with Ochratoxin A can be related to the kidneys which are the primary organ targeted by Ochratoxin A as it negatively affects the effectiveness of kidney enzymes such as alkaline, phosphate, leucine amino, peptidases and Transferase-r-glutamyl. In addition, exposure to Ochratoxin A leads to the destruction of some genes in the DNA of kidney cells such as GADD153 and GADD45, which negatively affects the functional efficiency of the kidneys and causes the decomposition of renal nephrology and kidney fibrosis (Guyton, 1986). While, the interaction treatment between cinnamon extract and Ochratoxin A had a significant effect in reducing the level of urea to normal ratios (18.87 mg/100 ml), compared to the Ochratoxin A treatment (31.47 mg/100).

Estimating the total protein level in the serum:

Figure 1B showed the results of the chemical analysis of the percentage of total protein in the serum. Where, the rate of protein was significantly decreased when treated with Ochratoxin (6.13 g /100 ml) compared to the control treatment (8.73 g/100 ml). This is consistent with several studies indicating that Ochratoxin causes a reduction in the synthesis of total protein in treated birds (Baily *et al.*, 1989; McMasters and Vedani, 1999; Verma Et, 2002; Khatoun, 2004). While, the interaction treatment between cinnamon extract and Ochratoxin A had a significant effect in increasing the level of protein (7.40 g /100 ml), compared to the Ochratoxin A treatment (6.13 g/100ml).

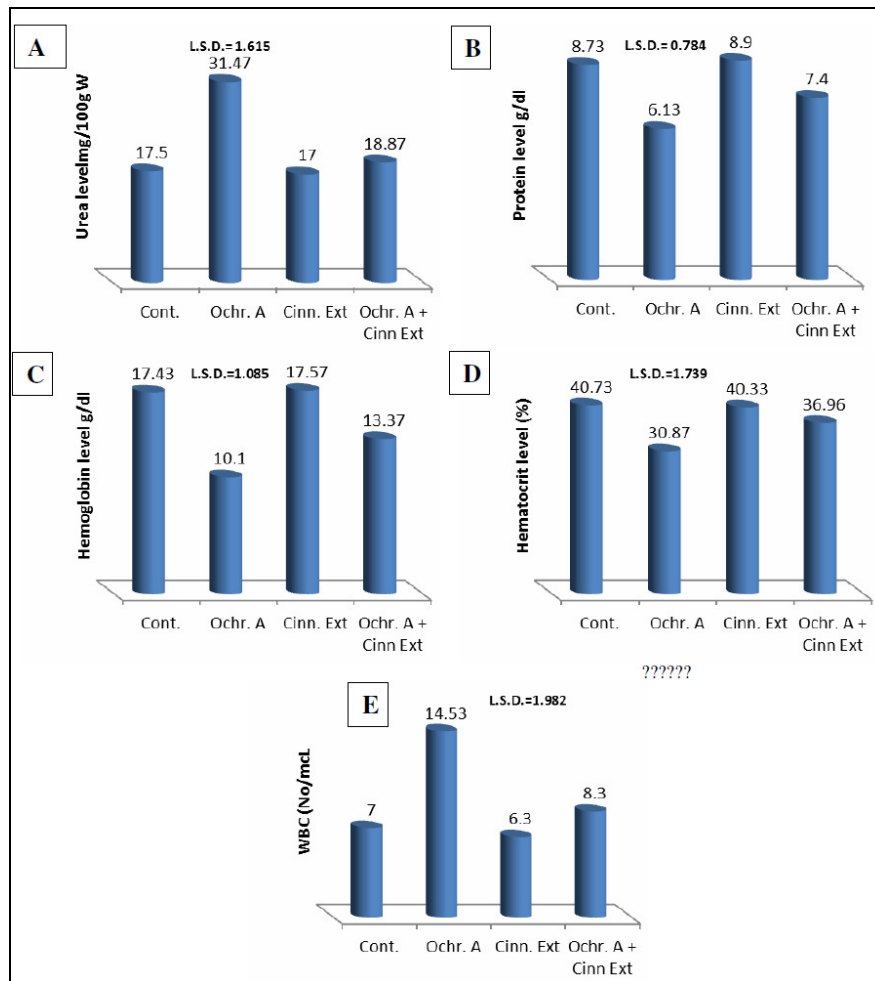


Fig. 1 : Effect of cinnamon extract and Ochratoxin A, individually or in combination, blood characteristics including urea level (A), protein content (B), hemoglobin level (C), hematocrit level (D) and No. of WBC (E) in the treated white female rats.

Blood Physiological parameters

Hb concentration (g/100ml): The results showed that Ochratoxin A had a significant effects in decreasing the Hb concentration in the blood of treated animals, which was (10.10g/100ml), compared with control treatment (17.43 g/100 ml) (Figure 1C). The results proved that cinnamon extract had a positive effect in raising the Hb concentration to normal proportions in the blood of treated animals. This result is similar to (Back, 2011) who reported a significant decreasing in the Hb concentration when white rate males treated with aflatoxins B1 and B2 (10.7 g/100ml and 9.2

g/100ml) compared to the control treatment. The low Hb concentration in the blood of animals treated with B1 and B2 may be caused by increasing the production of immune response factors, including cytokines that exposed to the toxins which may be increased the oxidation in the cell, which increases the process of oxidation in the cell. This can cause attack in red blood cells causing their destruction, then Hb concentration. Or, aflatoxins have the potential to be highly correlated to blood proteins, which include albumin, cholesterol and transphrine, which are

responsible for producing of red blood cell (Kubena and others, 1997).

PCV blood test: Results (Figure 1D) showed that PCV rate was significantly decreased in the blood of animals treated with Ochratoxin A (30.87%), compared to control treatment control (40.73%). These results are similar to (2012) who found that Ochratoxin A reducing the size of PCV cells for quail birds compared to the control treatment. While treatments (Ochratoxin A + cinnamon extract and cinnamon extract only) have a positive effect in raising the rate of PCV for treated animals (40.33%, 36.96%) respectively. These results are in agreement with the findings of Khudhair (2007), where he found a significant reduction in the rate of PCV blood of white rat (29.2%) when treating with *P. chrysogenum* compared to control treatment (35%). The cause of this decline is that Ochratoxin A negatively affect the sources of red blood cell production where there is a relationship between the total number of red blood cells and the rate of PCV (Groopman and others, 2003).

WBC count: The results shown in Figure 1E indicated a significant high rate of white blood cell numbers in the blood of animals treated with Ochratoxin A, where it reached (14.53×10^3 cell / mm^3) compared to control treatment (7.00×10^3 cell/ mm^3). Using the cinnamon extract was led to back the rate of white blood cells Powder studied with poison to the return of the rate of egg blood cell number to the normal level (8.60×10^3 cells / mm^3). These results are very similar to the findings of Al-Khalidi (2011) who found reported that the rate of white cells in the blood of animals treated with tomato juice infected with *Geotrichum candidium* and *G. penicillium* was significantly increased compared to control treatment. The reason for the high rate of white blood cell in the blood of animals treated with Aflatoxin B1 and B2 may

due to the fact that some toxins induce the immune response in laboratory animals by increasing the number of lymphocytes to be equal you foreign substances in the body while acting as inhibitors competitive of enzymes responsible for synthesis of red blood cells (Peraica *et al.*, 1999). Sakhara (2007), found that Aflatoxins were increased the number of white blood cells in the treated laboratory animals.

Tissue study

The results of microscopic examination of the tissue sections of liver, kidneys, spleen for white rat females treated with of Ochratoxin A showed the presence of vascular mild congestion and filtration of mononuclear cells around the central vein mononuclear cells in the hereditary sections of the liver of treated animals. Kidneys of animals treated with Ochratoxin A showed infection of the glomerulus, enlarged the glomerulus wall, a total or partial decomposition of the glomerulus, congestion and haemorrhage. Ochratoxin A also resulted in spleen tissue changes (Figure 2) Congestion and hypertrophy of Red bulb hyperplasia and hypertrophy of White bulb hyperplasia. Richardson *et al.* (1987) pointed the degradation of Parenchyma tissue for liver as a result of the exposure for fungal toxins. This may be due to the effect of these toxins on the metabolism of carbohydrates in the liver which cause increases the Glycogen in the liver, which in turn disrupts the function of the liver (Chinoy *et al.*, 1991) The cause of haemorrhage in the tissues may be related to bleeding blood vessels, and the discharge of hematopoietic cells into the tissue with exudation, and the exit of these cells is the dominant characteristic of inflammation (MacSween and Whaley, 1992).

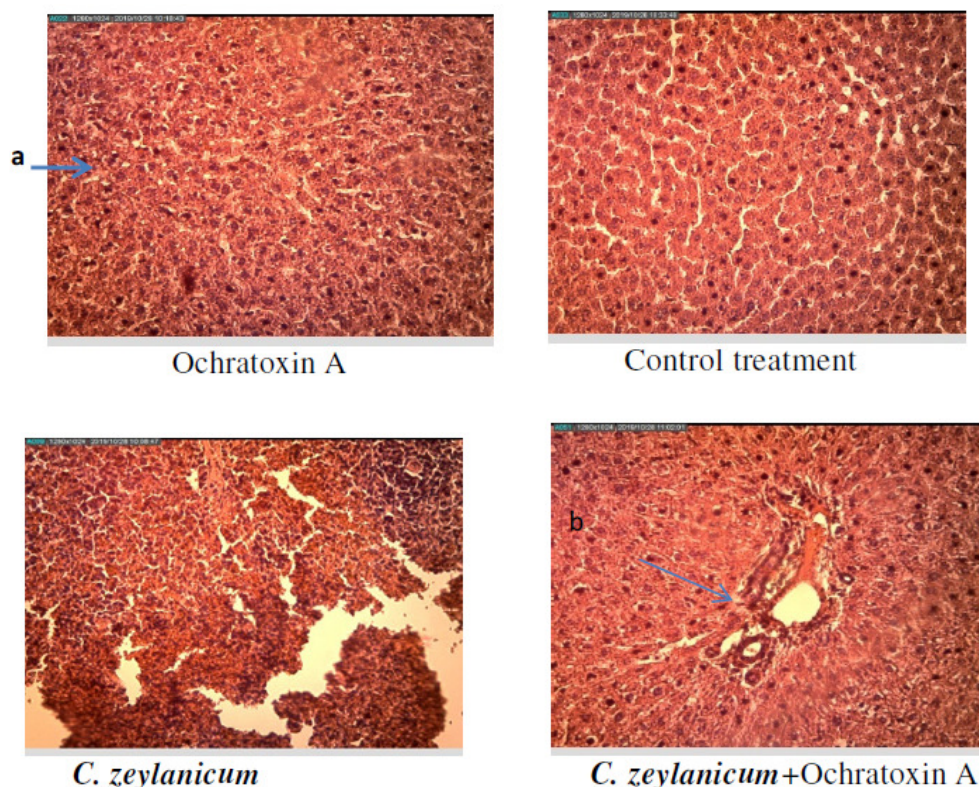


Fig. 6 : Histological section of the female liver of the white rat, a) Bloody congestion, b) Gnaw

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