Histopathological study of Cinnamomum zeylanicum equeous extract and Cladosporium sp. Extract on different albino male mice organs

Mays T. Abdallah*  Hadeel M. Khalaf*  Athraa H. Muhsin **
* College of Biotechnology, Al-Nahrain University, Baghdad-Iraq.
** College of Science, University of Kufa, Najaf-Iraq.
E-mail: Mays_talip@yahoo.com.

Abstract
The objective of this study was to considered as an explorer for In vivo studies on the production of some secondary metabolites from local medical plants named Cinnamomum zeylanicum to study their effect on mice organs that treated with Cladosporium sp. extract (In vivo). Preparation of water extract of Cinnamomum zeylanicum. different doses were prepared (20,40,60,80%) of plant which (9) male mice were used and divided into three control group and dosed with distilled water mice were administrated with first dose 1ml/kg of Cladosporium extract for two weeks and mice were administrated Cladosporium with 80% of the aqueous extract of Cinnamomum zeylanicum at a dose of 1 ml / kg daily for two weeks. Mice treated with Cladosporium sp. fungal filtrate caused vascular congestion in the tissues of the liver resulting in cell bleeding, in addition to many changes in chromotin in and nuclei sized increased while the results indicate the ability of the mixture between fungal filtrate and Cinnamomum to counteract these adverse effect in mice and return its appearance looks like normal also the kidney of animals treated with Cladosporium fungal filtrate caused retention of congested glomeruli with a large pool of fluids while the result of interaction caused inhibition in radial growth of the fungus and reduced its effectiveness on kidney function and the synthetic form of the tissue.

Introduction
In a new therapeutic system, antibiotics have a big job in controlling the infectious diseases [1]. However, the drug resistance adopted by most of the pathogens certainly needs a suitable replacement of the presently available antibiotics"[2, 3]. Besides, many antimicrobial agents are known to exhibit serious inconvenient effects on host tissues which lead to the system toxicity [4]. In third-world countries, it is the Time to use phytochemicals for healthcare especially in remote areas [5]. "Nearly 30% of drugs across the globe are derived from plants and 252 drugs are in WHO essential medicine list"[6]. Cinnamon is a spice obtained from the inner bark of several tree species from the genus Cinnamomum. Cinnamon is used mainly as an aromatic
condiment and flavouring additive in a wide variety of cuisines, sweet and savoury dishes, breakfast cereals, snack foods, tea and traditional foods. The aroma and flavour of cinnamon derive from its essential oil and principal component, cinnamaldehyde, as well as numerous other constituents, including eugenol (1).

**Materials and Methods**

**Plant collection and identification**
The barks of the plant were collected from the local markets during September (2018), which had been identified previously by National Herbarium of Iraq.

**Plant extract Preparation**
10 grams of *Cinnamomum zeylanicum* were washed out and soaked in 200ml of distill water and mixed by magnetic stirrer for 15 min. Then after 1hr the suspension was filtered through three layers of gauze cloth a cheese cloth in order to remove insoluble fragment, then, Centrifuge for 15min. different doses were prepared (20,40,60,80%) from the stock solution then stored in the refrigerator at 4°C for further studies.

**Isolation and diagnosis of fungi isolated from yellow corn grains**
Yellow corn beans were sterilized superficially with 2% sodium hypochlorite solution for 3 minutes, washed with sterile water, dried and planted in petri dishes containing medium potato extract (PDA) with 40 mg/L chloramphenicol to prevent bacterial growth. In each dish, four of them were peripheral, and the fifth in the middle of the dish, the dishes were incubated at 25 °C for 7 days (7-8). After incubation, the Aspergillus flavus and Aspergillus niger isolates were purified by transferring a tablet from each colony and transplanting it into a plate containing a new P.D.A medium. Fusarium was isolated depending on the taxonomic qualities while Trichothicium sp. (9). Depending on the taxonomic qualities (10) and the isolates of the fungus Cladosporium sp. Depending on the taxonomic qualities(11).

**Test the efficiency of the aqueous extract of the bark of Cinnamomum zeylanicum in inhibiting the growth of fungi isolated from yellow corn grains:**
In this experiment, aqueous extract of the bark was obtained by three doses per extract (20, 40, 60 and 80%) and mixed with sterile PDA. After cooling, all dishes were inoculated with 0.5 cm tablets of each mushroom colony in the center of the dish. The dishes were incubated at 25 °C for 7 days and four replicates per treatment with comparative treatment for each fungus. After the colonies reached the edge of the dish, the inhibition rate of the radiative growth of the fungi was calculated by calculating the average of two perpendicular diameters and the amount of inhibition according to the equation (12):

\[
\text{Inhibition} = \frac{R1 - R2}{R1} \times 100
\]

R1 Maximum radial growth of the pathogenic colony (comparative treatment).
R2 Maximum radial growth of the pathogenic fungus colony in the extracted container dishes.

1. Preparation of Cladosporium leaches
   The medium of potato and dextrose extract dissolve in 500 mL and sterilize in an autoclave at a temperature of 121 °C and pressurize (1) atmosphere for 20 minutes, then inoculated with tablets of 0.5 mL PDA medium grown with 7-day-old Cladosporium mushrooms and incubated at 25 ± 1 °C for 21 days, the extract was filtered through Whatman No.4 filter paper in the Buchner funnel and kept in a sealed glass bottle and kept in the refrigerator until use (13).

2. Preparation of laboratory animals:
In order to assess the histopath activity of *Cinnamomum zeylanicum* (9) male mice were used and divided into (3) groups:

- **group (1)** included (3 mice were considered control group and dosed with distilled water).
- **Group 2**: mice were administrated with first dose 1ml/kg of *Cladosporium* extract for tow weeks (3 animals)
- **Group3**: mice were administrated *Cladosporium* with 80% of the aqueous extract of *Cinnamomum zeylanicum* at a dose of 1 ml / kg daily for two weeks. (3 animals)

The dose was performed every 24 hours and for 14 days and after two days after the last dose, the animals were sacrificed after being numbed with chloroform and explained by opening the abdominal cavity. Until you perform the following steps (14).

In order to repair these doses, the plant extract dissolved at few drops of DMSO and then completed by distilled water to reach the required doses which injected intraperitoneally (0.1 ml) for seven days as single dose/day. Finally mice were sacrificed at day 8 for laboratory assessments.

**Histopathological study**

Preparation the histology sections in the Najaf Teaching Hospital of the Department of Histology and followed the method (15), which included:

1. **Dehydration**: The models were passed in upward concentrations of ethyl alcohol (100%, 95, 90, 80, 70) for 2-1.5 hours at each concentration to remove water from them.
2. **Clearing**: Time the samples with xylene twice (1.5-1 hours) at a time to remove the fluid from the tissue.
3. **Infiltration**: Samples were drinking with molten paraffin wax at a temperature of (58-56) C by placing samples in it twice and for a period (1.5-1) hours each time.
4. **Embedding**: Samples are buried in special molds containing molten paraffin wax and left to harden.
5. **Cutting**: (Sectioning: prepared sections of tissue series (5) micrometer using rotary micrometer) and the models were fixed on glass slides using Meyers albumin adhesive (Meyers albumin) and then placed the slides in the oven at a temperature (58-56) Pile vertically for 20 minutes to remove excess wax.
6. **Staining**: the sections were dyed with the hematoxylin-eosin dye. The slides were passed in (Zelol - Absolute Ethyl Alcohol 100% Alcohol - 95% - 90% - 70% Hyatoxyl Dye) for two minutes each and then the sections were washed with tap water and immersed in Dye eosin for 2 minutes and then washed with tap water and passed upward concentrations in both ethyl alcohol (100% - 95% - 90 - 70%) and xylene for 2 minutes for each concentration.
7. **Mounting**: Place the slide cover using Canada Balm.

**Diagnosis of pathological changes in histological sections**

The samples were diagnosed by Dr. Asaad Al-Janabi, Head of Pathology Department, Faculty of Medicine, University of Kufa.

**Statistical Analysis**

Fully randomized (CRD) design was designed and averages were compared by testing the least significant difference L.S.D and the probability level of 0.05. (16).

**RESULTS AND DISCUSSION**

Many fungi have been isolated from yellow corn, *cladosporium sp*

Was selected being the most affected by cinnamon water extract and by measuring the efficiency of cinnamon water extract for inhibiting the radial growth of studied fungi
Table -1 showing the frequency of fungi isolated from yellow corn.

| Fungi                | Frequency% |
|----------------------|------------|
| Cladosporium sp.     | 80.5       |
| Fusarium sp.         | 77.9       |
| Trichothecium sp.    | 76.4       |
| penicillium spp      | 50         |
| Aspergillus ruber    | 43.6       |
| Aspergillus flavus   | 43.4       |
| Aspergillus oryzae   | 31.2       |

Table -2 showing measuring the efficiency of cinnamon water extract for inhibiting the radial growth of studied fungi

| Rate of inhibition | concentration % |
|-------------------|-----------------|
| 90                | 0               |
| 45                | 20              |
| 31                | 40              |
| 12                | 60              |
| 6                 | 80              |
| 92                | 0               |
| 52                | 20              |
| 41                | 40              |
| 22                | 60              |
| 15                | 80              |
| 90                | 0               |
| 54                | 20              |
| 47                | 40              |
| 26                | 60              |
| 19                | 80              |

Mice treated with *Cladosporium* sp. fungal filtrate caused vascular congestion in the tissues of the liver resulting in cell bleeding, in addition to many changes in chromatin in and nuclei sizes increased (fig2) while the results indicate the ability of the mixture between fungal filtrate and cinnamon to counteract these adverse effect in mice and return its appearance looks like normal (fig3).
Fig 1. Section showing normal appearance of liver in control groups (40×).

Fig 2. Section showing bleeding and increasing in liver tissue of mice treated with *cladosporium* fungal filtrate (40×).

Fig 3. Section showing look like normal histological structure appearance of cells of liver tissue after treated with cinnamon (40×).

Also, the kidney of animals treated with *cladosporium* fungal filtrate caused retention of congested glomeruli with a large pool of fluids (fig 5.) while the result of interaction caused inhibition in radial growth of the fungus and reduced its effectiveness on kidney function and the synthetic form of the tissue. (fig 6.) and return the Section look like normal histological appearance of kidney in control groups (X40).

Fig 4. Section showing normal appearance of kidney in control groups (X40).

Fig 5. Section of kidney tissue in mouse treated with *cladosporium* fungal filtrate showing slight retention of congested glomeruli with a large pool of fluids (400).
Fig 6. Section showing look like normal histological structure appearance of kidney tissues (X40).

Fig 7. Section that showing a normal appearance of small intestine in the control groups (X40)

Fig 8. Section of intestine tissue in mouse treated with *cladosporium* filtrated fungal

Fig 9. Section showing look like normal histological structure appearance of small intestinal after treated with interaction of cinnamon and cladosporim filtrated fungal

Medicinal plants, synthesise different chemical compounds that had defence against bacteria, fungi, due to its diversity of phytochemical (17). Cinnamon, contains many derivatives, such as, cinnamic acid, cinnamaldehyde, and other components such as polyphenols which act as antioxidant, anti-inflammatory, antimicrobial, anticancer effects (18). There are several reports that dealt with the properties of cinnamon in the forms of bark, essential oils, and the phenolic compounds, and each of these properties can play a important role as anti-microbial agent. The result showed the antifungal activity of the cinnamon extract due to the main
component (phenols) that have the ability to reduce the radial growth of the cladosporium in the mous and prevent it affecting on organs functions (19), the recent study cladosporium caused reported that degeneration renal tubules in the epithelial cells of the kidney and caused inflammatory cells infiltrated in the liver and inside the blood vessels showed a necrosis of hepatocytes (20). the occurrence of granuloma in the chronic infections of fungal diseases (21) thus inhibition the growth of this fungi by cinnamon and the activity of phenolic compound which are the major component as antimicrobial (22)

Reference :

1. Iqbal, Mohammed (1993). "International trade in non-wood forest products: An overview". FO: Misc/93/Y11 – Working Paper. Food and Agriculture Organization of the United Nations. Retrieved 12 November 2012.
2. Khan A, Safdar M, Ali Khan MM, Khattak KN, Anderson RA (2003). "Cinnamon improves glucose and lipids of people with type 2 diabetes". Diabetes Care. 26 (12): 3215–3218. PMID 14633804.
3. Maierean SM, Serban MC, Sahebkar A, Ursoniu S, Serban A, Penson P, Banach M (2017). "The effects of cinnamon supplementation on blood lipid concentrations: A systematic review and meta-analysis" (PDF). J Clin Lipidol. 11 (6): 1393–1406. doi:10.1016/j.jacl.2017.08.004. PMID 28887086.
4. "Cinnamon". National Center for Complementary and Integrative Health, U.S. National Institutes of Health. 2016. Retrieved 28 February 2017.
5. Wilson, J.E ;Wild’s, S (1995).Mycotoxins in human nutrition and healthDirectorate .General XII.Science. Research and development Evr.,V:186: (55)
6. El-Ghaoth, A. , Wilson, C. , Wisniewski, M. , Droby, S. , Smilanick, J. andKorsten, L. (2001) . Bioactive coating for the control of postharvest diseases of fruits . Phytopathology 91:S 155.
7. Tian, S. , Fan, Q. , Xu, Y. and Liu, H. B.( 2002) . Biocontrol efficacy of antagonist yeasts to gray mold and blue mold on apples and pears in controlled atmospheres .Plant Dis. 86:848-853 .
8. Janisiewicz, W.J., Conway,W.S. , Glenn,D.M. , and Sans, C. E. (1997) . Integratingbiological control and calcium treatment for controlling postharvest decay of apples . against decay , Agricultural Research Service.
9. Zhang, H. , Fu, C. , Zheng, X. , He, D. , Shan, L. and Zhan, X.( 2004) . Effect of Cryptococcus laurentii (Kufferath) Skinner in combination with sodium bicarbonate on biocontrol of postharvest green mold decay of citrus fruit . Bot. Bull. Acad. Sin. 45:159-164.
10. Jones, A. L. and Aldwinckle, H. S. (1990) . Compendium of apple and pears diseases . APS Press , St . Paul, M. N. , 125 pp.
11. Wilson, E. E. &amp; Ogawa, J. M.( 1979) . Fungal , bacterial and certain nonparasitic diseases of fruit and nut crops in California . Division of Agricultural Sciences, University of California , Berkeley .
12. Spadaro, D. , Vola, R. , Piano, S. and Gullino , M. L.( 2002) . Mechanisms of action and efficacy of four isolates of the yeast Metschnikowia oulcherrima active against postharvest pathogens on apples . Postharvest Biol. Technol. 24: 12-134 .
13. Scherm, B., Ortu, G., Muzzu, A., Budroni, M., Arras, G. and Migheli, Q. (2003) Biocontrol activity of antagonistic yeasts against Penicillium expansum on apple. J. Plant Pathol. 85: 205-213.
14. Ciegler, A.B.; Vesconder, H.R. and Hesseeltine, C.W. (1981). Mycotoxins and N-nitrosocompound: Environmental risks. (Shank, R.C. Ed). CRC press Vol 1:1-51.
15. Wood, J.B. (1998). Microorganism in foods. Academic and Professional, An imprint of Champan & Hallpress. pp. 1018.
16. Cocker, R.D.; Jones, B.D.; Nagler, M.J.; Gillman, G.A.; Walbridge, G.A. and Pangrahi, A.J. (1984). Mycotoxin training manual. Tropical development and research institute overseas development administration.
17. Tulunay, M., Aypak, C., Yikilkan, H., Gorpelioglu, S. (2015) Herbal medicine use among patients with chronic diseases. J Intercult Ethnopharmacol 4(3):217–220. PubMed PMID: 26401410. Pubmed Central PMCID: PMC4579486. Epub 2015/09/25. engGoogle Scholar
18. Shan, B., Cai, Y.Z., Brooks, J.D., Corke, H. (2007) Antibacterial properties and major bioactive components of cinnamon stick (Cinnamomum burmannii): activity against foodborne pathogenic bacteria. J Agric Food Chem 55(14):5484–5490. PubMed PMID: 17567030. Epub 2007/06/15. engGoogle Scholar
19. Singh, G., Maurya, S., DeLampasona, M.P., Catalan, C.A. (2007) A comparison of chemical, antioxidant and antimicrobial studies of cinnamon leaf and bark volatile oils, oleoresins and their constituents. Food Chem Toxicol Int J Published Br Ind Biol Res Assoc 45(9):1650–1661. PubMed PMID: 17408833. Epub 2007/04/06. engGoogle Scholar
20. Sandoval-Denis, M., Sutton, D.A., Martin-Vicente, A., Cano-Lira, J.F., Wiederhold, N., Guarro, J. and Gené, J. Cladosporium Species Recovered from Clinical Samples in the United States. Journal of Clinical Microbiology. 2015; 53 (9):2990-3000.
21. Plomer-Niezgoda, E., Baran, E. and Maj, J. Pathogenicity of fungi from the genus of Alternaria, Cladosporium and Chrysosporium. Mikol Lek. 1998; 5:187-190.
22. Schoene, N.W., Kelly, M.A., Polansky, M.M., Anderson, R.A. (2009) A polyphenol mixture from cinnamon targets p38 MAP kinase-regulated signaling pathways to produce G2/M arrest. J Nutr Biochem 20(8):614–620. PubMed PMID: 18835704. Epub 2008/10/07. engGoogle Scholar