R .A.Sahib /J. Pharm. Sci. & Res. Vol. 11(3), 2019,1136-1139

ISSN:0975-1459
Journal of Pharmaceutical Sciences and Research
www.jpsr.pharmainfo.in

The Effects of Toxic Compounds of *Cladosporium herbarum* on Hormones of Female Rats and Ability of Ascorbic Acid to Decrease Growth of *C. herbarum*

R .A.Sahib[1*]

[1*] *Department of Biology, Faculty of Science /University of Kufa, 21-Najaf- Iraq*

Abstract

This study is conducted to reveal the effects of oral administration of *C.herbarium* of two compounds has been toxic effects on the reproductive hormonal, and the ability of ascorbic acid to decrease growth of *C.herbarum*. Fungi species which were isolated from indoor buildings were *Cladosporium herbarum*, *Aspergillus niger, Alterneria* sp. and *Fusarium* sp. *C. Herbarium* was founded more visible and frequent in indoor buildings and reached to about (70,60)% respectively .It is observed that *C.herbarium* is able to produce two compounds has been significant toxic effects on biochemical blood parameters which are causing decreased in the level of luteinizing hormone (LH) and follicle stimulating hormone (FSH) which is causing significant effects, as compared with the control group. *C.herbarium* produced two compounds which have been toxic effects on the reproductive hormones, causing decreased in the (LH), (FSH) and ability of ascorbic acid to decrease growth of *C.herbarum*.

Key words *Cladosporium herbarum*, LH, FSH, Ascorbic acid

Summary : Ascorbic acid has proven high efficiency to decrease growth of *C. herbarum* as compared with a control group which is untreated with ascorbic acid.

1.INTRODUCTION

Fungal spore concentrations in damp buildings are higher than normal buildings, the level of *Cladosporium* has been reported to be significantly higher in wooden buildings than in concrete/brick buildings *(1)*. The majority of the invasive fungal infections are due to *Aspergillus*, *Candida*, *Cladosporium*, *Mucor* and *Rhizopus* species *(2)*. Important species of this group, sometimes called indoor fungi, include *Alternaria alternata*, *Stachybotrys chartarum*, *Cladosporium cladosporioides* and *Aspergillus niger (3)*. *Cladosporium* spp. produce a variety of secondary metabolites like mycotoxins; most of mycotoxins are not susceptible to heat, they could be entered into the body not just through the gastrointestinal systems, but also by inhalation and through the skin *(4)*. Intranasal administration of *C. herbarum* spores for 7 weeks increased total serum IgE and the appearance of specific IgE. The intranasal exposure also resulted in sensitization of the previously unsensitized mice, as evidenced by the appearance of airway hyper-reactivity in response to methacholine challenge. The hyperactivity appeared within 3 weeks and continued for the entire 10-12 week period of treatment with the *C. herbarum* spores *(5)*.

Mycotoxins in feed consumed by animals and their serum cause disturbances in the hormonal profile related to fertility, including follicle stimulating hormone (FSH), luetinizing hormone (LH), testosterone (TES) and can cause abnormal fetal development in farm animals which affect the normal function of reproductive organs and elsewhere the productivity of animals*(5)*. Mycotoxins reduced progesterone synthesis by inhibition the follicle stimulating hormone secretion (FSH) *(6)*. Ascorbic acid (vitamin C) is an essential nutrient in aqua feeds and is an indispensable nutrient required to maintain the physiological processes of different animals, Vitamin E and vitamin C playimportant roles in animal health as antioxidants by inactivating damaging free radicals produced through normal cellular activity and from various stresses *(7)*.Moreover, it was reported that high levels of ascorbic acid are efficient in reduction of toxicity, preventing disease and enhancing fish tolerance to environmental stress *(8)*. AA is a small, water soluble, reduction sugar, acid with antioxidant properties and acts as a primary substrate in the cyclic pathway for enzymatic detoxification of a number of reactive oxygen species (ROS) such as H_2O_2, and many other, harmful to normal functioning of plant metabolism *(9)*.

2.MATERIALS AND METHODS

2.1 Indoor Samples Collection

Samples were collected from different areas of the school buildings,these samples were selected from different areas in each location that containing bathrooms, refrigerators, walls, carpets etc.

2.2 Isolation and Diagnosis of *C.herbarum*

Samples were taken by scraping and placed in petri dishes which containing P.D.A width 9cm which was added chloramphenicol,then put 5 pieces on each plate and incubated at temperature 25±2°C for 3 days. Fungi were sub cultured, in which were isolated by transporting the disk from each colony and cultured in a new petri dish. This process was repeated to obtain a pure culture.

The following criteria were taken into consideration to identify isolates:-

A-Morphological features of growth colony which including (color, texture, margin, colony reverse and pigments) were produced.

B-Microscopic examination, observing fungi shapes , conidia and mycelium using light microscope and scanning electron microscope (SEM).

Fungal isolation, visibility was calculated using the formulas (1) and (2) below

$$Visibility\ (\%) = \frac{The\ number\ of\ samples\ that\ appeared\ genus\ or\ species}{The\ number\ of\ samples} x\ 100 \quad (1)$$

$$Frequency\ (\%) \frac{Number\ of\ genus\ or\ species\ fungal\ isolates}{Totally\ of\ all\ fungal\ isolates} x\ 100 \quad (2)$$

2.3 Ability of the isolated *C. herbarum* to produce secondary metabolites toxin

A- Extraction

Pure isolated of *C. herbarum* was grown on PDA and incubated at 25C° for 14 days, one disk was taken from each pure culture of petri dishes containing PDA,then taking pure culture to detect produce toxic secondary metabolites (four repeated of each one) then the pure cutting by sterile knife and mixing it with 20 ml methanol, mixture was shaken for 15 minutes by a shaker apparatus,after that, mixture was filtered through a filter paper whatman No1. and filtrate by separate funnel finally dried by reflex condenser.

B- Detection

Thin layer chromatography (TLC) technique was used to detect secondary metabolites of *C. herbarum* by making a straight light line on the TLC plate by adjusting 1.5 cm from the base plate then put 15 µl of extract of each *C. herbarum* which isolated on the plate with a distance of 2 cm between two spots of each other,and leaving spot to dry and placed in the tank containing mixture of chloroform:methanol 95:5 v/v and observed until the arrival of solution at a distance of about 2cm from upper edge of the plate,then exiting the TLC plates for air drying for 5 minutes,follow that examining TLC plate under UV light with wavelength 360 nanometers to detect the presence of secondary metabolites by corresponding Rf and color for each compound appear on the plate.

C- Separation of secondary metabolites

Each compound determined by sterilize needle, then scrape off the silica gel by blade and putting it in sterilized tube, the process was repeated until obtained suitable quantities of silica gel content for each compound and adding 2 ml methanol each one gram silica gel and centrifuging at 3500 rpm for 10 min,finally the chloroform was vaporized, each compound was kept in the freezer.

2.4 Preparation of the laboratory animals for testing the toxic effects compounds of *C. herbarum*

Female albino rats (*Rattus rats*) with age 12-14 weeks, and weights 180-240 g, were used in laboratory experiments placed in plastic cages for animals divided into groups by three replicates per treatment. These animals were placed under control conditions in terms of light, ventilation, nutrition and temperature ranging between 25-30^{0}C.

Fifteen female rats with similar ages were prepared. Then it was divided into five groups; each group contains three animals. Each group of animals was treated with concentrations of toxic metabolic compounds which produced by *C. herbarum* as the following:-

1-First group of animals given 500µl /kg of body weight from the first toxic compound *Cl.*1.

2- Second group given 50 µl /kg from first toxic compound *Cl.*1.

3- Third group of animals given 500 µl /kg of body weight from the second toxic compound *Cl.*2.

4-Fourth group of animals given 50 µl /kg of body weight from the second toxic compound *Cl.*2.

5- Fifth group of animals given distilled water as a control group.

The dosages of all animals were arranged as single one day oral given and one day without for a period of thirty day, later on, each animal was prepared to dope with ketamine and xylizine depend on body weight of animals. After that sacrificing animals and opening the abdominal cavity.

2.5 Biochemical Parameter

Assess the effectiveness of the level of luteinizing hormone (LH) and follicle stimulating hormone (FSH) by instrument Reflotron Chemistry Analyzer (RCA).

2.6 Statistical Analysis

Statistical analysis was carried out for all samples which design completes random design (CRD), according to least significant difference (LSD).

3. RESULTS AND DISCUSSION

3.1 Isolation and Diagnosis of *Cladosporium herbarum*

Cladosporium herbarum was found more visible and frequent in indoorbuildings and reach to about (80,70)% respectively than other fungi. This result is an agreement *(10)* .It's appeared olive-green to darkly colonies on potato dextrose agar at the temperature (25±2) °C after 7 days of growth as shown in Fig. 1 which is closed results to that obtained by *(11)*.

3.2 Separation and Diagnosis of Secondary Metabolites

Thin layer chromatography (TLC) shows *C. herbarum* was produced two compounds with the relative flow (Rf) 29%, 35%. TLC is the most important method used in the separation and specific diagnosis of a large number of compounds, including toxins, enzymes, and alkaloid compounds to measure the values. This study agrees with *(4)* who approved that mycotoxins are produced by *Cladosporium* spp. Using this technique and produced toxins such as emotion, cladosporin and epicladosporic acid**.**

3.3 Biochemical Blood Parameters

Biochemical blood parameters are affected by the metabolic products which are causing a decrease in the level of luteinizing hormone (LH) and follicle stimulating hormone (FSH) which is causing significant effects. The compound1 in the concentration 500 µl/kg and Rf= 29 % decreased the levels of LH and FSH into (0.02 and 0.15) U /L, respectively, while the compound2 with Rf=35 %, in the same concentration, decreased these levels to (0.13 and 0.12) U/L respectively, the level of this parameter in control group reached (1.16 and 0.57) U/L respectively

Table 1 and Figure 3 show the results due to a reduction of the inhibitory effect of the two compounds on the LH and production and secretion of FSH according to the results of a study in female rats, which decreased in the level of the FSH in the follicular phase of toxins (12, 13). These changes in ovary tissue resulted reduced LH and FSH level lead to infertility of rats at different degrees and decrease the chance of normal reproductive activity agree with *(14)*.

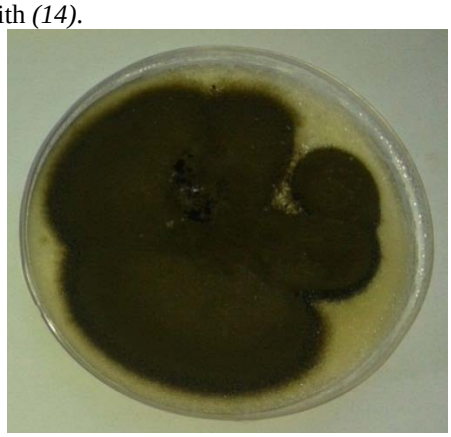
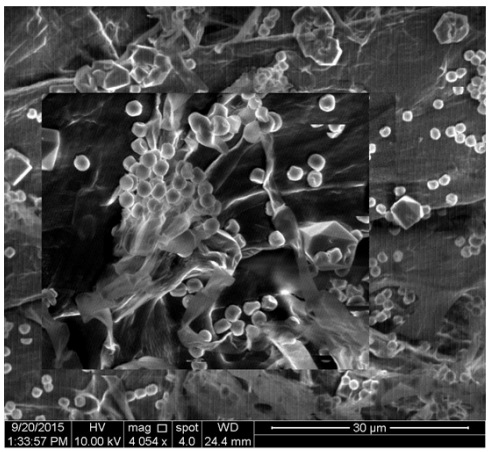
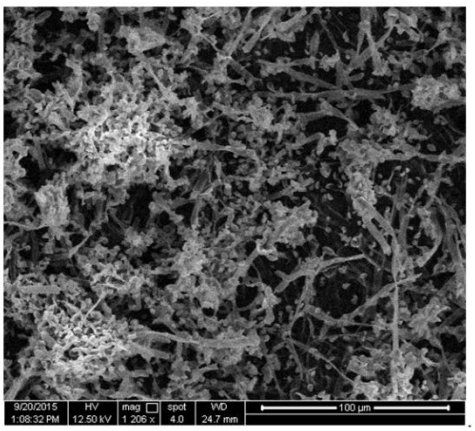


Fig. 1 : Colony of *C. herbarum*growing on the potato dextrose agar (25±2)^{0}C after 7 days .



A



B

Fig.2 : Scanning electron microscope of *C. herbarum* A: Arangment of conidia (30µm); B: mycelium and conidia (100 µm).

Figures 2 show the SEM images of *C. herbarum* with different magnification, A: 30µm, B: 100 µm. *C. herbarum* observation hypha which is septate and branched only in the apical region, conidia are 1 to 4 celled, round to oval, and produced in chains

Table 1 : The Effect of separated compounds of *C. herbarum* on the some biochemical blood parameters.

Compounds (µl /kg)	LH (U/L)	FSH (U/L)
Compound1 500	0.02	0.15
Compound1 50	0.15	0.10
Compound2 500	0.13	0.12
Compound2 50	0.11	0.13
Control	1.16	0.57
L.S.D. (0.05)	0.62	0.33

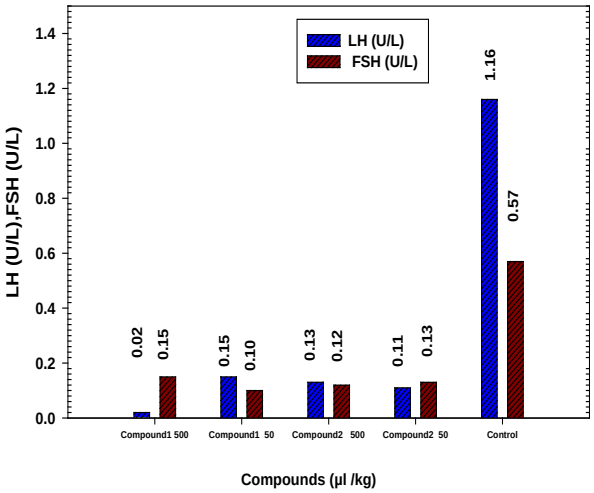


Fig. 3 : The Effect of separated compounds of *C. herbarum* on the LH and FSH hormones.

3.4 Using Ascorbic acid as fungus inhibition

Ascorbic acid has proven high efficiency to decrease *C. herbarum* growth by a percentage of 95.33% in the concentration of 300 ppm of ascorbic acid as shown in table 2 and Fig.4. The concentrations of ascorbic acid are causing more effect in averages of inhibition percentage when the concentration of ascorbic acid increases. It is observed that the concentration 400 ppm will cause a complete inhibition in the growth of *C. herbarum* as compared to a control group which is untreated with ascorbic acid.Ascorbic acid caused toxicity and inhibition fungi growth. AA is a small, water soluble, reduction sugar, acid with antioxidant properties and acts as a primary substrate in the cyclic pathway for enzymatic detoxification of a number of reactive oxygen species simply by acting as a secondary antioxidant during reductive recycling of the oxidized form of α-tocophero *(15)*.

Table 2 : The Effect of Ascorbic acid concentrations in averages of the inhibition percentage of *C. herbarum* by mixing with the medium (25±2)^{o}C after 7 Days.

Concentration (ppm)	Average of inhibition percentage (%)
0	77
100	77.70
200	86.77
300	95.33
400	100
500	100
L.S.D (0.05)	1.10

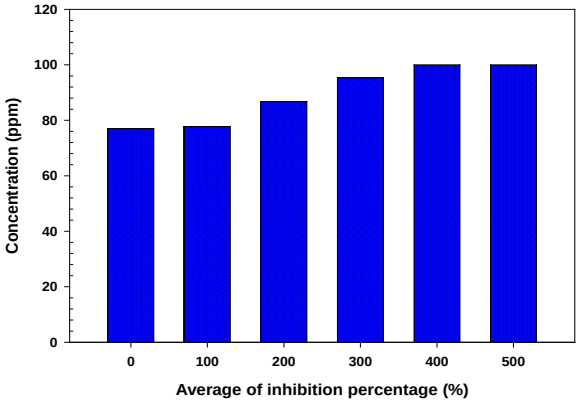


Fig. 4: The Effect of Ascorbic acid concentrations in averages of the inhibition percentage of *C. herbarum*.

4. CONCLUSIONS

It was observed that the fungi had been appearing in indoor buildings are *Aspergillus niger*, *Alterneria* sp., *Fusarium* sp. and *Cladosporium herbarum*. *C.herbarum* showed more visibility and frequency than other fungi which were being isolated from indoor buildings.It is observed that *C.herbarium* is able to produce two compounds have been significant toxic effects on biochemical blood parameters which are causing decreased in the level of luteinizing hormone (LH) and follicle stimulating hormone (FSH) which is causing significant effects, as compared with the control group. Ascorbic acid has proven high efficiency to decrease *C. herbarum* growth as compared to a control group which is untreated with ascorbic acid.

Acknowledgments

I would like to thank Faculty of Science/University of Kufa /IRAQ is highly acknowledged.

REFERENCES

1. T. Meklin, T.Husman, A.Vepsalainen, M.Vahteristo, J. Koivisto, J.Halla-Aho, A.Hyvarinen, D.Moschandreas, and A.Nevalainen, "Indoor air microbes and respiratory symptoms of children in moisture damaged and reference schools," *Indoor Air 12*,2002, 175-183.
2. N.Singh "Trends in the epidemiology of opportunistic fungal infections: predisposing factors and the impact of antimicrobial use practices," *Clin Infect Dis* 33, 2001,p.1692-1696.
3. L.Prester, " Indoor exposure to mould, allergens,", *Arh.Hig.Rada.Toksikol* 62(4), 2011, p.371–380.
4. P.Pokrzywa, E. Cieślik K. Topolska, " *Żywność Nauka Technologia Jakość,* 3 ,2007,p.139-146.
5. O. Denis, S.Van den Brûle, J. Heymans, X. Havaux, C. Rochard, F. Huaux, K.Huygen, "Chronic intranasal administration of mould spores or extracts to unsensitized mice leads to lung allergic inflammation, hyper-reactivity and remodeling," *Immunology*, 2007, p.122, 268-278.
6. U.S Tiemann, and J. Vanselow," Effect of the mycotoxin and beta zearalenol on regulation of progesterone synthesis in cultured granulose cells from procine ovaries," *Reproductive Toxicology*, Vol.,17, Issue 6, November-Desember 2003, pp.673-681.
7. B. P. Chew, ,"Antioxidant vitamins affect food animal immunity and health. *J. Nutri* 125,1995,p.18045- 18085.
8. M. Abdel-Tawwab, A. M. Shalaby, M. H Ahmed, and Y. A. Khattab," Effect of supplement dietary L-ascorbic acid (vitamin C) on mercury intoxication and growth performance of Nile tilapia (Orechromisniloticus L.).Annals of Agric,"*Sci. Moshtoher*. 39 (2),2001,p.961- 973.
9. G. Noctor and C.H. Foyer,"Ascorbate and glutathione: keeping active oxygen under control,".Annual Review of *Plant Physiology and Plant Molecular Biology* 49,1998 ,p.249–279.
10. B. G. Shelton, K. H. Kirkland, D. W. Flanders, and G. K. Morris,"Profiles of airborne fungi in buildings and outdoor environments in the United States," *Appl Environ Microbiol*, 68(4), 2002, p.1743-1753.
11. S.Tasic, N. Miladinovic, D.Tasic Zdravkovic, M.Avramovic, and M. Misic,"Fungal peritonitis caused by fungi of the *Cladosporium* genus in patients on peritoneal dialysis," *5th Congres of medical microbiology, Belgrad* ,2006,p. 139.
12. G.F Erickson, and S. Shimasaki, "The physiology of folliculogenesis: the role of novel growth factors", *Fertil.Steril*,76, 2001,p.943- 949.
13. N. Padhy, M.L Sathya, and T.R Varma, "Antra follicular size in the down regulated cycle and its relation to *in vitro* fertilization outcomes" *J. Hum. Reprod. Sci*, 2.2009, p.68-71.
14. Y.J Yang, X.G. Wang, L.J Liu,.and C.b Sheng," Toxic effects of zearalenone and alpha zearalenol on the regulation of steroidogenesis and testosterone production ion mouse leydig cells,*Toxicology in vitro* ," Vol.,21, Issues 4, June 2007 p. 558-565.
15. G.Kiddle ,"The role of ascorbate in plant defence and development," *Bristol*,2004, UK: University of Bristol.