Effect of hydroalcoholic leaves extract of *Datura stramonium* on pathogenic *Candida albicans*

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Summary

This research aimed at studying the effect of different concentration of hydroalcoholic leaves extract of Datura stramonium concentration against pathogenic Candida albicans isolated from clinical cases of diarrhea in cows and dogs in Baghdad province. Hydroalcoholic extract of the leaves of Datura stramonium were prepared in different concentrations for In vitro and In vivo study against Candida albicans. In vitro test includes the determination of minimum inhibitory concentration 50, 25, 12.5, 6.25, 3.12 and 1.75mg/1ml), and it was found that the minimum inhibitory concentration was 3.12 mg/1ml. Whereas the in vivo test was performed for the determination of sensitivity test of Candida albicans in concentration of plant 10, 15 and 20 mg/1ml which was compared with same concentration of Nystatin. The study was performed in seven groups of mice according to different concentrations. The infective dose of Candida albicans was 1X108, which was proved by histopathology changes after eight day. The result obtained from invivo study revealed that after the end of the therapeutic period that lasted for 8 days. Confirm the efficacy of *Datura stramonium* extract at 20% as a treatment for mice infected with *Candida albicans*.

Keywords: *Datura stramonium*, *Candida albicans*, Hydroalcoholic extract, White mice.

Introduction

The fungal diseases constituted a high percentage from the whole diseases in animals and humans, which are categorized in third order after the bacterial and viral diseases (1). Candidaiases is one of the fungal diseases worldwide; there are about 200 species of Candida occur saprophytically, but only *Candida albicans* is commonly associated with diseases in humans and animals (2 and 3). *Candida albicans* is found as a commensules of mucocutanous areas particularly of intestinal and genital tracts of humans and animals. Most infection are endogenous in origin, but predisposing causes such as immunosuppreion, prolong antibiotic or corticosteroids, cancer especially in human. In cattle, *Candida albicans* may introduce into the udder from the nosal of tubes of intramammary antibiotics (4). In dogs, Candidaiases is one of the diseases, which is regarded important to be studied, because the disease can be transmitted to human, especially the cutanous infection in dogs (5) which occurs after treatment of dogs with antibiotics orally. Most of the chemical treatment for fungi may have effect on the patient cells. Also the use of some synthetic antifungals may have an effect on the patient cells. In order to avoid the negative effects of chemicals and some of antimicrobial drugs, recent studies are directed towards the use of the medical plants, which contain active ingredients, and do not have side effects on patients. *Datura stramonium* is one of the medical plants which is important, the datura plant grows as woody, leafy stalk with spiny seed pods and large white or purple trumpet shaped flowers facing upwards (6), containing Scopolamine, atropine and hyoscyamine (7). It is known by many names, a few of which are Jimson weed, Davils Apple, Stinkweed and toalach (8). Scarce of studies on the use of *Datura stramonium* leaves extract on the pathogenic fungi which cause systemic and cutanous mycoses in animals. Therefore the aim of this research is to use the hydroalcoholic extract of *Datura stramonium* as antifungal *in vivo* and *in vitro* on *candida albicans* isolated from feces of cows and dogs.

Materials and Methods

Candida albicans, is isolated from clinical cases of diarrhea in cattle and dogs. The media used for cultivation of *Candida albicans* was Sabouraud dextrose agar, corn meal agar and use Rapid ^{tm} yeast plus system used for confirmation of *Candida albicans.* A handered

fecal samples were taken directly from feces of cows and thirty samples from dogs suffering from diarrhea during the period from May, 2013 till the end of November, 2013. The samples were obtained from different regions in Baghdad.

The plant *Datura stramonium* was collected from desert of Al_Anbar province in west Iraq. This plant is naturally grown in the desert. *Datura stramonium* leavess were dried in shade at room temperature and ground by using blender. Two hundred fifty grams of plant powder was soaked in 1.25 -1.5 L of 95% methanol for 5 days at room temperature , the mixture was mixed daily for regular infusion. After 5 days the extract was filtrated by using Whitman filter paper No.1, the filtrates were dried by using a rotary avaporter at 60ºC, the dried extract was stored in sterile glass bottles at 20ºC until use according to (9).

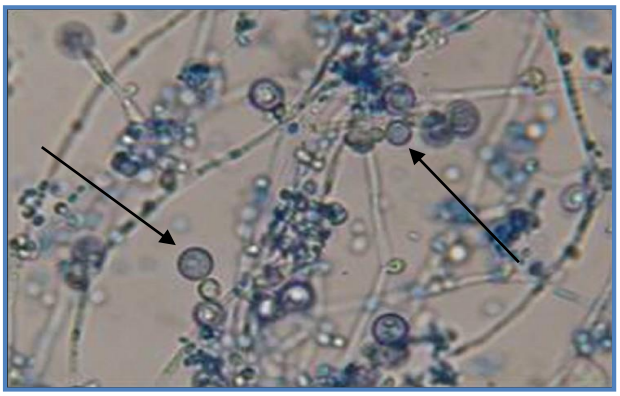
Different concentration of the plant were prepared (50, 25, 12.5, 6.25, 3.12, and 1.56 mg/1ml) at the same time. Also the same concentrations were prepared for standard antifungals Nystatin (50, 25, 12.5, 6.25, 3.12, and 1.56 mg/1ml) for the *in vitro* study for the minimum inhibitory concentration of Methanolic extract of *Datura stramonium* against pathogenic *C. albicans* isolated from fecal samples in cows and dogs suffered from diarrhea. The systemic experiment used the plant alcoholic extract for treatment of diarrhea in mice 56 white Swiss BALB/C mice, the age range 5-8 weeks, divided randomly into 7 equal groups, and treated as follows. G1: (control positive) administrated *Candida albicans* suspension orally containing (1ml) infection dose of $1x10^8$live cell/ml. G2: This was administered with suspension of infective dose of *Candida albicans* of $1x10^8$ live cell/ml and treated with DMSO. G3: administered suspension of *Candida albicans* $1x10^8$ live cell/ml and treated with standard antifungal Nystatine. G4: administered with suspension of *Candida albicans* $1x10^8$ live cell/ml and treated with dose 3g/kg of B.w with concentration of 100mg/ml of datura plant extract. G5: administered with suspension of infective dose of *Candida albicans* $1x10^8$ live cell/ml and treated with 3mg/kg b.w with concentration of 150mg/ml of datura plant. G6: administered with suspension of *Candida albicans* $1x10^8$ live cell/ml with dose 3 mg/kg b.w with 200mg/ml concentration of datura plant. G7: control negative Group not treated with anything.

Results and Discussion

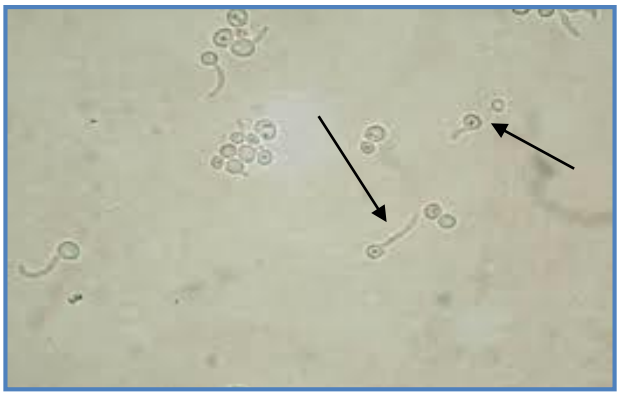
Candida albicans was cultivated on Sabouraud dextrose agar, for 48-72 hr and appeared as small, smoth white -cream, glistening round and curved colonies at 37ºC (Fig. 1). These result agreed with (10).

Figure, 1: *Candida albicans* on sabouruad dextrose agar.

The microscopical appearance of *Candida albicans* of tested mounts showed psedohyphae cluster of budding. In addition *Candida albicans* form germ tubes when grown on humen or rabbit serum for 3 hours at 37ºC, further more *Candida albicans* can form chlamydospores when grown on Corn meal agar, and incubated at 37ºC for 48 hours, as appeared in the present study which agrees with (11). The main confirmed tests for identification of *Candida albicans* was Rapid ^{tm} yeast plus test as established by (12), which confirms the biochemical tests and API yeast. Also Chlamydospores formation is another test which is used for the identification of *Candida albicans* when culturing it on corn meal agar with 1% of tween-80 and incubation at 30ºC for 48 hours. After that small part from the *Candida albicans* growth was examined for the formation of chlamydospores, as a thick-wall and usually produced on supporting cells at the end of the pseudomycelium. As stated by (13) illustrated in (Fig. 2). Germ tube is another diagnostic and conformation test for the pathogenic *Candida albicans* (14). In the present study *Candida albicans* isolate showed positive result as the formation of germ tubes seen as a long extending from the yeast cells like projection as in (Fig. 3).



Figure, 2: Chlamydospores of *Candida albicans.*



Figure, 3: Germ tube of *candida albicans*.

Germ tubes were formed within 2-3 hours when Candida albicans was inoculated in the serum and incubated at 37°C for 3 hours. The result of this study agrees with (15). Furthermore, up to 5% of the strains of Candida albicans may be germ tube negative (16). From another point, (17) found that other Candida spp may form germ tube, but rarely such as Candida tropicalis, which form psudogerm tube. The pathogenic Candida albicans isolated from clinical cases of diarrhea in cows and dogs were diagnosed confirmally by using Rapid^{tm} yeast plus system, and the result of this test is shown in (Fig. 4)**.**

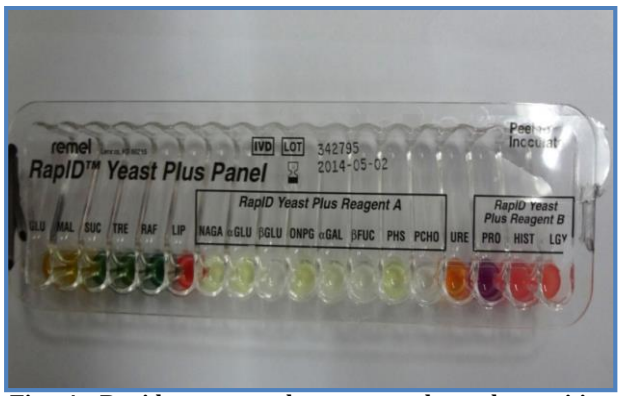


Fig. 4: Rapidtm yeast plus system show the positive reaction of Candida albicans.

The Rap^{tm} yeast plus system is a qualitative micro method that uses conventional and chromogenic substrates for the identification of medically important yeast, yeast _like fungi and similar organisms isolated from clinical speciments (18). The isolation percentage of Candida albicans from feces of clinical cases of diarrhea were 7% from in cow, whereas the isolation percentage of Candida albicans from feces of dogs suffured from diarrhea was 23% as illustrated in (Table, 1).

Table, 1: Source of fecal samples from cows and dogs.

No.	Region	No. of sample	Animals	No. of positive samples	Positive %
1	College of Veterinary medicine in Baghdad	15	Cow	0	7.0 % cows
2	College of Agriculture in Baghdad	45	Cow	3	
3	Abu-Gryab in Baghdad City	20	Cow	1	
4	Aden Veterinary Hospital	20	Cow	3	
5	Different Veterinary clinical in Baghdad city	30	Dog	7	23 %

The data obtained in this study showed that the percentages of isolation of *Candida albicans* from feces were 7% isolation in cows and 23% in dogs, which represented a high percent of infection with *Candida albicans* especially in dogs. The higher percentage of infection may related to the prolong antibiotic therapy, or the addition of antibiotics in animal feed, or due to immunosuppresion. *Candida albicans* is a commensal and occurs most frequently on the mucus membarane of digestive and gental tract. Candidiases involving the gastrointestin-al may result from prolong antibiotic therapy (15).

The minimum inhibitory concentration (MIC) of hydroalcoholic extract of *Datura stramonium* on pathogenic *Candida albicans* isolated from feces of infected cows were compared with MIC of standard antifungal (Nystatin), (Table, 2).

The result at this study showed that the MIC for hydroalcoholic extract of *Datura stramonium* against pathogenic *Candida albicans* was about 3.12 mg/ml, as afinal concentration, beyond this concentration 1.56 mg/ml. The pathogenic *Candida albicans* could grow and gave heavy growth. The result in this study revealed that the hydroalcoholic extract of *Datura stramonium* gave a high potency and effectivity against the pathogenic *Candida albicans*; this agrees with (19) who worked on the anticandidal activity of nineteenth Jordaniant plant extract. Other researchers (20) stated that ethanolic extract of *Datura setramunium* showed antimicrobial activities from other point (21) who worked on the effect of aqous extract of *Datura stramonium* found that the leaves extract of *Datura stramonium* at 20% concentration was more inhibitory activity against some pathologic plant fungi as *Fusarium oxosporum* and *Aspergillus soloni*.

Table, 2: Minimum inhibitory concentration (MIC) of *Datura stramonium* on pathogenic *Candida albicans*.

Concentration mg/ml	Plant extract	Nystatin
50	N.G	N.G
25	N.G	N.G
12.5	N.G	N.G
6.25	N.G	N.G
3.12	N.G	N.G
1.56	65_70 C.F.U	90_100 C.F.U

N.G: No Growth, C.F.U: Colony forming unit.

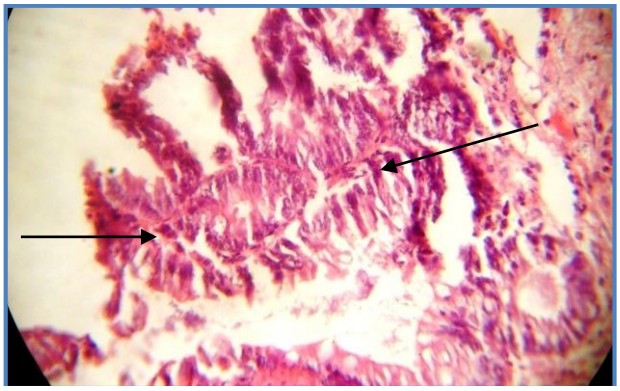
Table, 3: Sensitivity test of different concentration from leaves extract of *Datura stramonium*.

zone of inhibition (mm)	Concentration mg/ml		
	200mg/ml	150mg/ml	100mg/ml
Datura Stramonium	12.00	17.00	26.00
Nystatin	11.00	16.00	24.00
DMSO	0.00	0.00	0.00

The ability of hydroalcoholic leaves extract of *Datura stramonium* was examined and compared with the same concentration of Dim ethyl sulfooxid (DIMSO) as negative control and Nystatin as positive control. The result of hydroalcoholic leaves extract with 100, 150, 200 mg/ml showed the mean value of zone of inhibition ranging from 12.00, 17.00 and 26.00, respectively, (Table, 3).

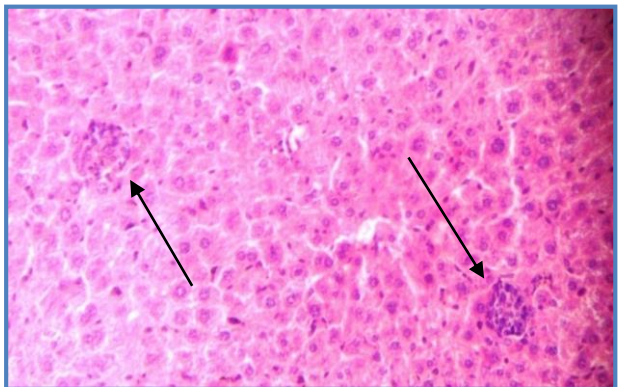
Pathological finding confirmed the fungal isolation, characterized by marked suppurative reaction, inflammatory cells particularly, mononuclear cells, macrophage and Neutrophils aggregation and acute cellular degeneration. The histopathological examination of intestine after 10 days post infection without treatment, showed necrosis in intestinal villi and in liver showed microabscesses in the liver parenchym (Fig. 5 and 6).

This study used three concentrations of plant extract *Datura stramonium* 100, 150 and 200 mg/ml respectively. The results of *Datura stramonium* extract show no clear pathological changes in the organs (intestine, liver) treated for 10 days. This referred to role of this extract in killing yeast cell and repairing of tissue because this extract contains active ingredient which may act as antifungal agent. The histopathological change of intestine of mice at 10 days post infection by *Candida albicans* and treated with *Datura stramonium* extract 10% and 15% revealed infiltration of inflammatory cell mainly neutrophils and MNCs in the submucosa and the muscularis mucosa (Fig. 7 and 8).

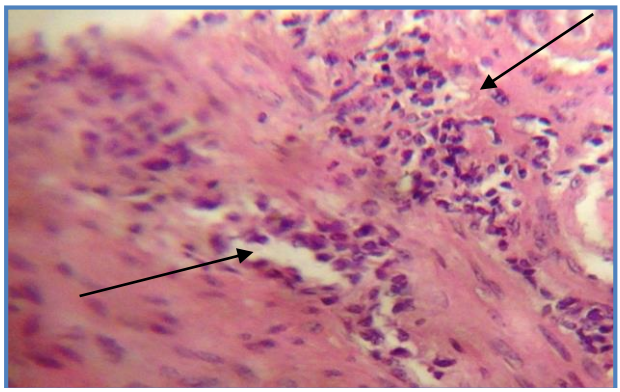


Figure, 5: Intestine of mice at 10 days Post infection by *Candida albicans* (⟶) shows necrosis of intestinal gland and villi. (Heamatoxcyline and Eosin stain 40X).

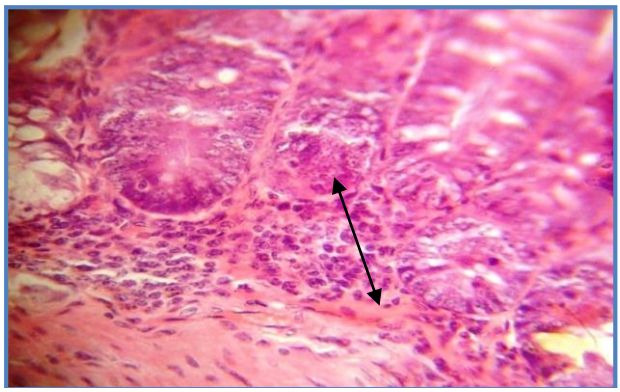
While in liver of mice at 10 days post infection by Candida albicans and treated with 10% and 15% of Datura stramonium extract for 8 days revealed aggregation of MNCs in the paranchyma with proliferation of Kupffer cells (Fig.9 and 10).



Figure, 6: Liver of mice at 10 days post infection by Candida albicans (⟶) shows microabscesses in the liver parenchyma (Heamatoxcyline and Eosin stain 40X).

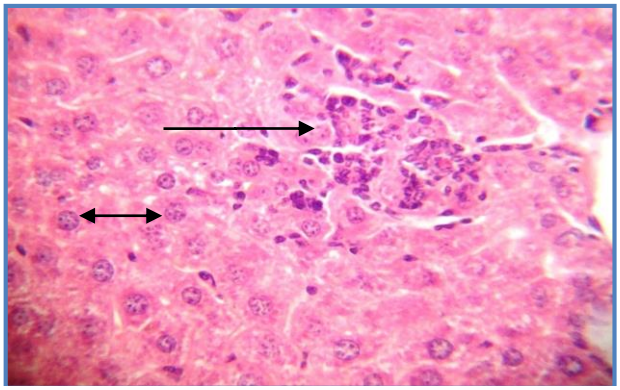


Figure, 7: Intestine of mice at 10 days post infecttion by *Candida albicans* and treated with Datura stramonium extract 10% (⟶) shows infiltration of inflammatory cell mainly neutrophils and MNCs in the submucosa and the muscularis mucosa (Heamatoxcyline and Eosin stain 40X).

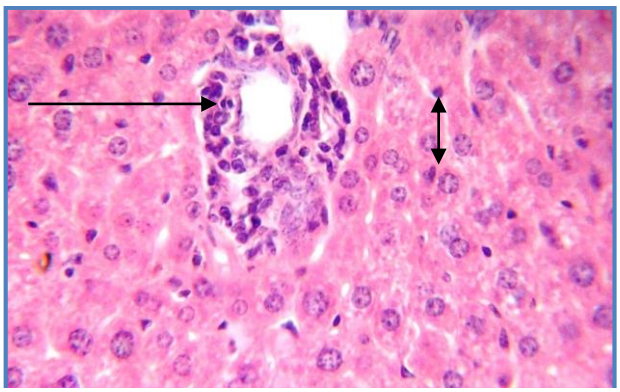


Figure, 8: Intestine of mice at 10 days post infection by *Candida albicans* and treated with 15% of *Datura stramonium* extract for 8 days (⟷) shows mononuclear cells infiltration between mucosal gland (H and Es 40X).

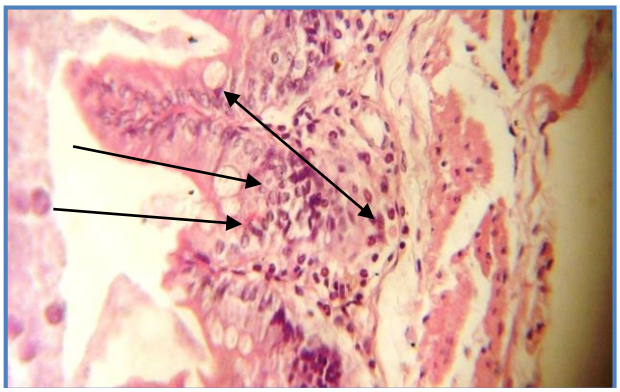
While histopathological change in intestine of mice at 10 days post infection by Candida albicans and treated with 20% of Datura stramonium extract after 8 days inflammation cells mainly esoinophelis and showed neutrophils in the sub epithelial layer with hyperplasia of goblet cells (Fig. 11) and pathological change in liver of mice at 10 days post infection by Candida albicans and treated with 20% of *Datura stramonium* extract showed Neutrophils in the liver sinasoid (Fig. 12).



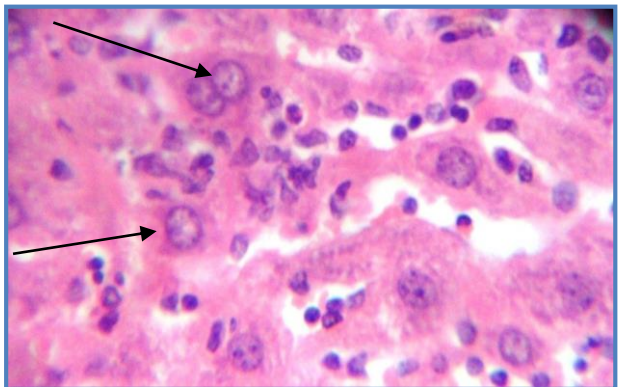
Figure, 9: Liver of mice at 10 days post infection by Candida albicans and treated with 10% of Datura stramonium extract for 8 day (⟶) shows aggregation of MNCs in the paranchyma with (⟷) proliferation of Kupffer cells (Heamatoxcyline and Eosin stain 40X).



Figure, 10: Liver of mice at 10 days post infection by *Candida albicans* and treated with Datura stramonium extract after 8 days (⟶) shows MNCs aggregation in the portal area with marked proliferation of Kupffer cells (⟷) (Heamatoxcyline and Eosin stain 40X).

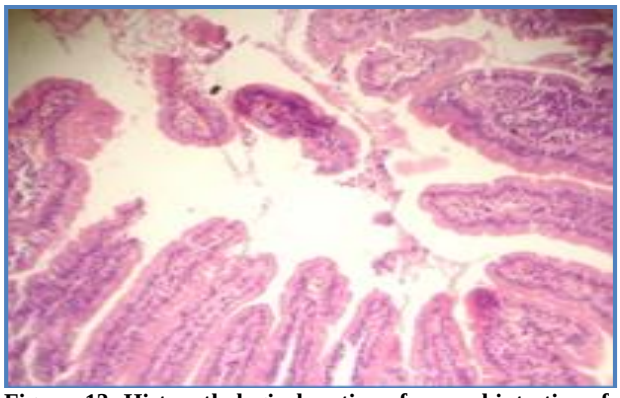


Figure, 11: Intestine of mice at 10 days post infection by *Candida albicans* and treated with 20% of *Datura stramonium* extract after 8 days (⟶) shows inflammation cells mainly esoinophelis and Neutrophils in the sub epithelial layer with hyperplasia of goblet cells (⟷) (Heamatoxcyline and Eosin stain 40X).

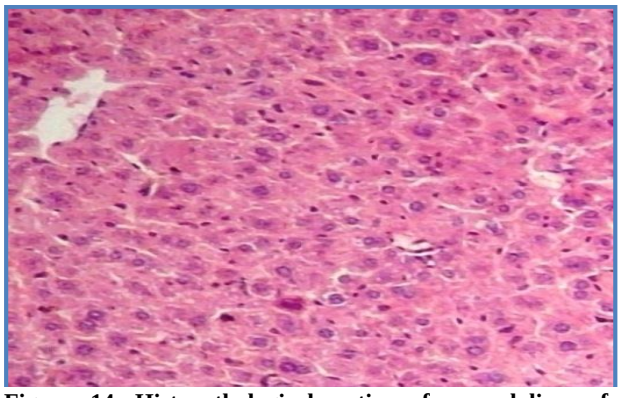


Figure, 12: Liver of mice at 10 days post infection by *Candida albicans* and treated with 20% of *Datura stramonium* extract (⟶) shows neutrophils in the liver sinasoid. (Heamatoxcyline and Eosin stain 40X).

The histopathological changes in the above sections where compared with the normal sections of intestine (Fig. 13) and liver (Fig. 14) to distinguish the effecting of *Datura stramonium* extract at 20% as a treatment for mice infected with *Candida albicans.*



Figure, 13: Histopathological section of normal intestine of mice. (H and E 40X).



Figure, 14: Histopathological section of normal liver of mice. (H and E, 40X).

References

1. Aniassie, E. J.; McGinnis, M. R. and Pfaller, M. A. (2003). Clinical Mycology. Churchill Livingstone, Phialadelphia. Pp: 89.
2. Manolakaki, D.; Velmahos, G.; Kourkoumpetis, T.; Chang, Y.; Alam, H. B.; De Moya, M. M. and Mylonakis, E. (2010). Candida infection and colonization among trauma patients. Virulence, 1(5): 367-375.
3. Fugelsang, K. and Edwards Wine, C. (2010). Microbiology Second Edition, Springer Science and Business Media, New York. Pp: 3–28.
4. Krasner, R. I. (2002). Concepts of microbial disease. In the Microbial Challenge: Human–Microbe Interactions, Washington, DC: Am. Soci. for Microb., chapter 6, Pp: 103–120.
5. Favrot, C.; Steffan, J.; Seewald, W. and Picco, F. (2010). "A prospective study on the clinical features of chronic canine atopic dermatitis and its diagnosis". Veterinary Dermatol., 21: 23–31.
6. Joshi, S. G. (2000). Medicinal Plants, Oxford and IBH Publishing Co. Pvt. Ltd., New Delhi, Pp: 370-371.
7. Eftekhar, F. Yousefzadi, M. and Tafakori, V. (2005). Antimicrobial Activity of *Datura innoxia* and *Datura stramonium*. *Fitoterapia.* 76: 118-120.
8. Kritikar, K. R. and Basu, B. D. (1999). Indian Meditional Plants (2nd Ed.), International Book Distributors, Dehradun, India, Volume III, Pp: 1783-1787.
9. Sharma, M. C.; Sharma, S. and Kohhi, D. V. (2010). Formulation and evaluation of analgesic activity, Antiinflammatory and ant-anxiety activity of using plant extracts. Dig. J. Nanomaterial and Biostrudures. 5(1):147-157.
10. Alhussaini, M. S.; Noha, F. EI-Tahtawi and Ahmad, M. M. (2013). Phenotypic and molecular characterization of Candida albicans species in urin samples from renal failure patients. Sc. J. Clin. Med. 2(1): 14-25.
11. Reeta, P. R.; Ally, H.; Olga, K. and Jennifer, N. (2010). Aberrant Synthesis of Indole-3-acetic acid in Saccharomyces cerevisiae Triggers Morphogenic transition, a Virulence Trait of pathogenic fungi. Genetics Society of America DOI:10.1534/genetics.109.112854.
12. Hossein, M.; Seied, H. M. and Laura R. (2011). Comparison of Enzymatic Method Rapid Yeast Plus System with RFLP-PCR for Identification of Isolated Yeast from Vulvovaginal Candidiasis. Soudian J. Basic Medical Sci., 14(5): 443-450.
13. Zavalza-stiker, A.; Ortiz-saldivar, B.; Garcia-Hernandez., M.; Castillocasanova, M. and Bonifaz, A. (2006). Rapid production of

candida albicans chlamydospores in liquid mediaunder various incubation conditions. Jpn. J. Med. Mycol., 47: 231-234.

14. Koneman, E. W.; Allen, S. D.; Janda, W. M. J.; Schrecken berger, P. C. Kuleta, J. K.; and KoziK, A. (2009). Fungi pathogenic to humans: Molecular basis of virulence of *Candida albicans*, *Cryptococcus Neoformans* and *Aspergillus Fumigatus*. Acta. Biochim. Pol., 56: 211-224.
15. Carter, G. R. and Darla. J. W. (2004). Essentials of veterinary Bacteriology and Mycology 6th edition Lowa state press. Blackwell publishing company. Pp: 105.
16. Salkin, I. F.; Land, G. A.; Hurd, NM. J.; Goldson, P. R. and McGinnis, M. R. (1987). Evaluation of the yeast Ident and uni-yeast identification systems. J. Clin. Microbiol., 25: 624-627.
17. Baron, E. J. and Finegold, S. M. (1990). Formulas and preparation of culture media and reagent. Bailey and Scotts Diagnostic Microbiology, 8thed. The C.V. Mosby Company, St. Louis, MO. Pp:60.
18. Tasan, T. K.; Michael, R. J.; Michael, R. M. and Peter, C. A. (1996). Ability of Rap ID Yeast plus System to Identify 304 clinically significal yeast within 5 hours. J. Clin. Microbiol., 34: 1067-1071.
19. Darwish, R. and Aburjia, T. (2010). Effect of Ethanomedicinal Plant Used in folkloric Medicine as Antibiotic resistant inhibitor on *Escherichia coli*. BMC. Complementary and Alternative Medicine, 10(9): 1-8.
20. Aqib S. and Mohib S. (2014). Phytochemstry, pharmacological and traditional uses of *Datura Stramonium* L. review. J. Pharmacognosy and Phytochemistry, 2(5): 123-125.
21. Gachande, B. D. and Khillare, E. M. (2013). In-vitro Evaluation of Datura species for potential Antimicrobial activity. Bioscience Discovery, 4(1): 78-81.

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تأثير المستخلص المائي الكحولي لأوراق نبات الداتورة في المبيضات البيضاء المرضية

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الخلاصة

أجريت دراسة تأثير التراكيز المائية الكحولية لاوراق نبات الداتورة ضد المبيضات البيضاء المرضية والتي عزلت من براز الابقار والكلاب المصابة سريرياً بالإسهال في محافظة بغداد. حُضرت تراكيز مائية-كحولية مختلفة من أوراق نبات الداتورة لدراسة تأثيراتها ضد المبيضات البيضاء المرضية للزجاج وداخل الجسم الحي. أظهرت النتائج التركيز الادنى المثبط للمستخلص النباتي بالتراكيزالمستعملة (50 و25 و12.5 و6.25 و3.12 و1.75) ملغم/1مل، حيث إن التركيز الأدنى المثبط للمبيضات البيضاء كان 3.12 ملغم/ 1مل. أما دراسة داخل الجسم وبعد تحضير المستخلص النباتي لأوراق الداتورة بالتراكيز (10 و15 و20) % ومقارنتها بنفس التراكيز من المضاد الفطري القياسي (Nystatin)، فقد أجريت الدراسة على سبعة مجاميع من الفئران وكانت الجرعة المصيبة $1X10^8$ وحسب التراكيز المختلفة وبعد انتهاء المدة العلاجية والتي استمرت لمدة ثمانية ايام، ظهر أن المعالجة بتراكيز20% قد أعطت كفاءة عالية لشفاء الفئران المصابة بالمبيضات البيضاء المرضية، وأثبتت كفاءة العلاج من الفحوصات النسجية المرضية للفئران.

الكلمات المفتاحية: نبات الداتورة، المبيضات البيضاء المرضية، التراكيز المائية الكحولية، الفئران البيضاء.