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Pathological Effects of *Candida Auris* Yeast on Liver of White Male Mice Pre-Treated with Alcoholic Extract of *Syzygium Aromaticum*

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Abstract | *Candida auris* (*C. auris*) is a yeast which is mainly identified in gastrointestinal, skin, mucous membrane and female genital tract. The *Syzygium aromaticum* (cloves) are extensively being used as food preservative and carry therapeutic, cosmetic and agricultural application. In order to understand the impact of *Syzygium aromaticum* against *C. auris*, 30 white male mice (aged 2-3 month and 35-40 gram of bodyweight) were divided into 3 groups. The first group (n=10) acted as untreated control group; the second group of mice (n=10) received a single intraperitoneal administration of 2×10^8 cell of *C. auris*. In another group of 10 mice, animals were subjected to a single intraperitoneal of 2×10^8 cell of *Candida auris* followed by 1000 mg/kg alcoholic extract orally of *Syzygium aromaticum* for 90 days. Blood samples were collected from heart for assay catalase enzyme activity and lymphocyte count at 90 days of experiment whereas tissue sample from liver were taken for pathological examination. Comprehensive analysis of the biochemical analysis indicated a decrease in catalase enzyme and lymphocyte count in *C. auris* treated groups. Pathological changes were characterized by fibrosis, necrosis, hemorrhage, infiltration of inflammatory cells and liver cirrhosis in *C. auris* treated group compared to *Syzygium aromaticum* treated and *C. auris* infection mice. Taken together, it is clearly attributed that alcoholic extract of *Syzygium aromaticum* protect liver damage against *C. auris* potentially by increasing in catalase enzyme and lymphocyte count in treated mice.

Keywords | *Candida auris*, Pathological changes, Biochemical parameters, *Syzygium aromaticum***Received** | July 12, 2024; **Accepted** | October 01, 2024; **Published** | October 31, 2024***Correspondence** | Anas A. Humadi, Department of Pathology and Poultry Diseases, College of Veterinary Medicine, University of Diyala, Iraq; **Email:** anashumady@yahoo.com**Citation** | Humadi AA, Sabeeh SI, Aldossary ATY, Al-Kaisei BI (2024). Pathological effects of *Candida auris* yeast on liver of white male mice pre-treated with alcoholic extract of *Syzygium aromaticum*. J. Anim. Health Prod. 12(s1): 61-66.**DOI** | <http://dx.doi.org/10.17582/journal.jahp/2024/12.s1.61.66>**ISSN (Online)** | 2308-2801**Copyright:** 2024 by the authors. Licensee ResearchersLinks Ltd, England, UK.This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

INTRODUCTION

Candida auris (*C. auris*) is a yeast and is commercially found in the gastrointestinal tract, skin, mucous membrane and female genital tract (Ahmad and Alfouzan, 2021). *C. auris* is a worldwide health threat (Steinmann et al., 2021), and its nosocomial transmission reported in all over the world (Schelenz et al., 2016). More than a decade ago, since the discovery of *C. auris*, there has been a dearth

of worldwide identification strategies, particularly in developing nations. Consequently, there is a pressing need for efficient treatment to combat this multidrug-resistant infection (Alvarado et al., 2021; Cernakova et al., 2021).

Several factors including older age, diabetes, indwelling medical devices, immunosuppression, hemodialysis, chronic renal illness, and use of broad-spectrum antibiotics and/or antifungals predispose its colonization (Du et al.,

2020). This colonization culminates in the septicemia in tow wet neonate and infants. It has been estimated that Candidemia cause 15-35% mortality in adult and in neonatal the mortality ranges from 10-15% (Gudlaugsson et al., 2003; Hornik et al., 2012; Greenberg et al., 2017). *C. auris* has recently been reported to establish a multi-drug resistance which results in difficulties to control, treatment and resistance cleaning and other decontamination route (Khan and Ahmad, 2017).

The *Syzygium aromaticum* plant (clove) belong to myrtaceae family and is used as food preservative and therapeutics, cosmetic, food and agricultural application due to phenolic chemical including eugonal. The plant is also used in mouth washes, dental lotion and toothpastes, and is anti-inflammatory and anti-arthritis (Devi et al., 2010; Milid and Deepa, 2011; Mbaveng and Kuete, 2017; Haro-Gonzalez et al., 2021). Cloves carry antioxidant activity by increased prostaglandine E2 synthesis which cause reduction inflammatory cells infiltration in living organs (Jin et al., 2016).

Given the promising medicinal potential of *Syzygium aromaticum*, this study aims to investigate the pathological effects of *Candida auris* yeast on liver of white male mice pre-treated with alcoholic extract of *Syzygium aromaticum*. The study also aims to explore the potential of white mice as therapeutic investigational animal model against yeast infections.

MATERIALS AND METHODS

Yeast was screened and cultivated from swabs of rectum, groin, nose, urine and ear on chromogenic yeast medium (Chrom ID, Biomerieux, Nurtingens, Candida, Brilliances, ThermoFisher Scientific, Wesets Germany). The cultures were monitored for forty-eight hours at 37°C, and the presence of *C. auris* confirmed by MALDITOF Msc Bruker, Bremen, Vitek biomerieux-Marcy-I-Etoiles, France (Steinmann et al., 2011). Samples were collected from Al-Kindi Hospital Laboratory/Baghdad, Iraq.

Alcoholic extraction of *Syzygium aromaticum* (clove) was prepared from dried clove which were prepared locally from Baghdad market. The clove was pre-identified in the Department of Biology Science, College of Science, University of Baghdad. The cloves were grinded to fine powder by grinder and dissolved in 80% methanol (2 gm/20 ml) percentage (Dua et al., 2014).

LABORATORY ANIMALS

The animals were randomized across the study and thirty white male mice with age of 2 to 3 months and 35-40 gram in body weight were housed in plastic cages with a dimension

of 60x60x10 cm³ as approved by the Veterinary Medicine College Animal House, Baghdad, Iraq. The animals were divided into 3 groups with identical management protocol. The first 1group (n=10) acted as control group, second group (n=10) was given intraperitoneal 2x10⁸ cells of *C. auris*, whereas the third group was injected intraperitoneally with 2x10⁸ cells of *C. auris* with orally alcoholic extract of clove with 1000 mg/kg for 100 days (Xin et al., 2019).

SAMPLES COLLECTION

Heart blood samples (2 ml) were collected for lymphocytes counting and separated into EDTA for biochemical analysis at end of experiment. The tissue samples from liver were taken for histopathological examination and kept in 10% formalin-fixed solution. Thereafter, samples were dehydrated at room temperature in graded alcohol (70%, 80%, 90%, and 100%) for two hours, then submerged into xylene for two additional hours and melted paraffin wax for three hours. After that, the samples were placed in an orientation and embedded in newly made paraffin blocks. Using a 5-µm microtome, blocks were sectioned and inspected on microscope slides, then stained by routine stain (hematoxyline and Eosin stain) and special stain (masson trichome stain) for detection of fibrosis (Bancroft and Gamble, 2008). Biochemical assay including catalase activity (U/mg) performed according to Hadwan and Kadhum (2018).

ISOLATION AND COUNTING OF LYMPHOCYTES

Viability from heparinized whole blood was performed according to Porkinsville et al. (2004) for total lymphocytes assessment.

STATISTICAL ASSAY

Using SAS (Statistical Analysis System- version 9.1), data statistical analysis was carried out. The significance of differences between means was evaluated using One-way ANOVA and the Least Significant Differences (LSD) post hoc test. Statistical significance is indicated by P > 0.05 (SAS, 2018).

RESULTS AND DISCUSSION

The catalase activity and lymphocytes counting in mice groups are outlined in Table 1.

Table 1: Catalase activity and lymphocytes counting of mice groups.

Groups	Catalase activity (U/mg)	Lymphocytes (%)
1 st group	6.91 ± 0.23 a	57.18 ± 4.00 b
2 nd group	4.11 ± 0.20 c	36.12 ± 3.20 c
3 rd group	6.00 ± 0.22 b	65.21 ± 4.20 a

Values are reveled as means±SE with different are significantly different (P<0.05).

Based on the catalase and lymphocyte counts, it is clear that 2nd group showed significantly decreased ($P<0.05$) catalase and lymphocytes count, while 3rd group showed significantly increased ($P<0.05$) catalase and lymphocytes when compared to 2nd and 1st groups. Immunologically competent cells (lymphocytes) help phagocytes and protect the body from infection by releasing antibodies (humoral immunity) against acute and chronic infections.

PATHOLOGICAL ASSAY

As expected, no noticeable histological changes were observed in liver of control group (Figure 1). The histopathological investigation of second group showed irregular edges, enlarged size with pale in color. Additionally, the histopathological changes in liver showed multiple irregular granuloma characterized by centrolobular necrosis surrounded by mononuclear cells (macrophage and foreign body giant cells) with fibrous connective tissue, all hepatocytes necrotic (Figure 2A), liver cirrhosis characterized by severe fibrosis, newly bile ducts, newly central veins and bile duct with irregular necrotic hepatic cells (Figure 2B).

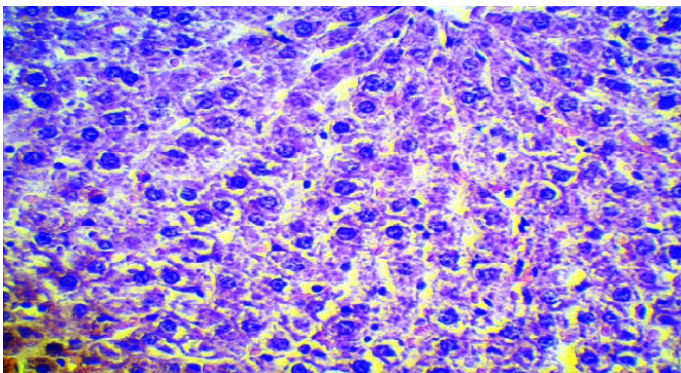


Figure 1: Photomicrograph of liver in control group depicting normal liver tissue (H and E stain; 20x).

In some areas showed fibrosis, mononuclear cells infiltration with hemorrhage, necrotic hepatocyte with mononuclear and polymorphic cells infiltration, severe apoptotic cells were observed (Figure 2C). All hepatic tissue heavily fibrotic and cirrhotic with newly blood vessels and bile duct (Figure 2D, E).

Liver cirrhosis characterized by newly lobules without central vein and bile ducts heavily surrounded by blue colored fibrous tissue (Figure 2F), and newly bile duct (Figure 2G) with multiple irregular new lobules characterized by center of necrosis, severe blood vessels, totting and sinusoids hemorrhage (Figure 2H).

Vis-a-vis third group, grossly the liver showed slightly congestion while the histopathological changes of liver tissue showed acute cellular swelling, increased width of sinusoids, heavily infiltration of mononuclear cells (lymphocytes) with eosinophils, newly bile ducts and few

fibrous connective tissues (Figure 3A, B).

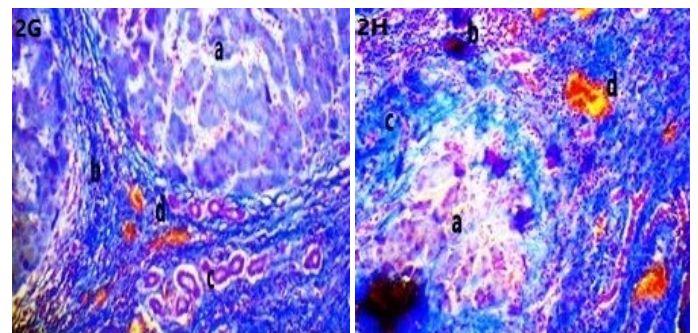
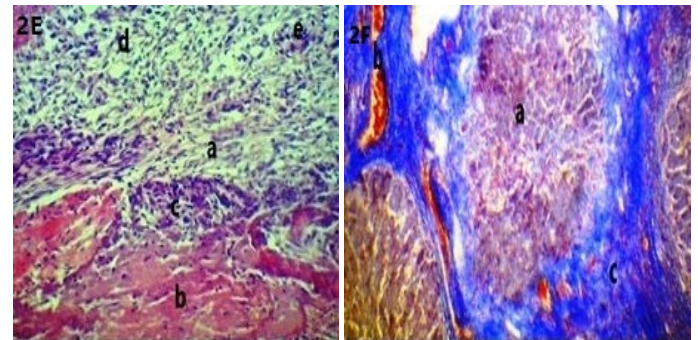
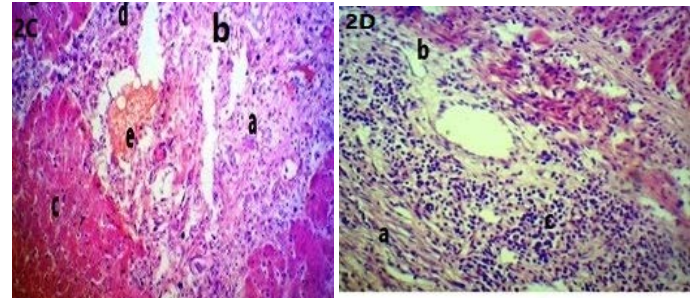
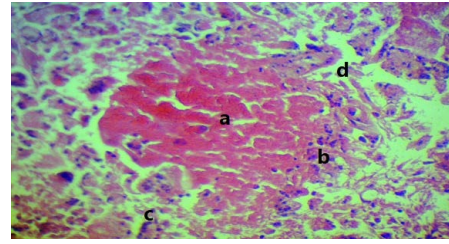


Figure 2: A: Photomicrograph of the liver in 2nd group showed: (a) center of necrosis, (b) mononuclear cells, (c) foreign body giant cell, (d) fibrosis (H and E stain; X40). B: (a) fibrosis, (b) newly central vein, (c) necrotic hepatocytes (H and E stain; X20). C: (a) fibrosis, (b) newly central vein, (c) necrotic hepatocytes, (d) mononuclear cells, (e) hemorrhage (H and E stain; X20). D: (a) fibrosis, (b) newly blood vessels, (c) severe infiltration of inflammatory cells (H and E stain; X20). E: (a) liver fibrosis, (b) necrosis, (c) mononuclear cells infiltration, (d) newly blood vessels, (e) newly bile duct (H and E stain; X20). F: (a) cirrhotic liver, (b) newly blood vessels, (c) fibrosis (Masson trichrome stain; X20). G: (a) pseudolobulated liver, (b) fibrosis, (c) newly bile duct, (d) congested blood vessels (Masson trichrome stain; X40). H: (a) pseudonecrotic lobules, (b) clotted blood vessels, (c) mononuclear cells infiltration, (d) newly bile duct (Masson trichrome stain; X20).

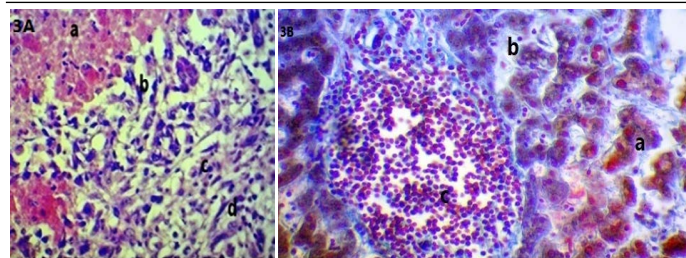


Figure 3: A: Photomicrograph of the liver in 3rd group showed: (a) acute cellular swelling, (b) increase width of sinusoid, (c) infiltration of mononuclear cells, (d) newly bile duct (H and E stain; X40). **B:** Photomicrograph of the liver in 3rd group showed: (a) acute cellular swelling, (b) width sinusoid, (c) mononuclear cells infiltration and eosinophil (Masson trichrome stain; X40).

Exposure to different toxic substances and infections cause adverse effect on hematological profile due to interface of the metabolites of these causes with blood component (Howard and Hamilton, 2013; Orun and Erdogan, 2014).

Several previous studies have reported that cloves cause rise in monocytes which enhance nonspecific immunity, and enhance granulocytes, the phytoconstituents food in many plants such as flavonoids, alkaloids, glycosides and saponins, (AlSharif and Hassoun, 2004; Ita and Udofia, 2011; Malaguarnera et al., 2012). Therefore, clove carry phenolic acid such as salicylic acid, ellagic, caffeine and play as anti-inflammatory, antipyretic and antistress responses. The clove has remedy values against diarrhea, vomiting and gastrointestinal disease, anticancer activity and aqueous extraction of cloves increased humoral immune by suppressing IF-N release and inducing IL-4 and shift in distribution of cytokines (Ali et al., 2014).

Significant decreased in percentage lymphocytes counting was observed in second group when compared with first and third groups. Catalase activity was increased, highlighting oxidative stress in condition when there is an imbalance between free radical and antioxidant defense system which led to lipid peroxidation and inactivation enzyme due to candida auris effect (Xin et al., 2019). The primary responsible for drug metabolism is the liver. The toxic substance, infection, and the primary pathogenic process accountable for liver damage include inflammation, cytochrome, P450 dysfunction, mitochondrial malfunction, and oxidative stress. Therefore, we observed that the liver cirrhosis in second group is due to C auris which is first fungal pathogen to cause a global public health threat through blood stream wound and ear infection. The necrosis, inflammation of giant cells and granuloma with liver cirrhosis due to the virulence of C albicans, and neutrophils elastase (NE) is an important component of neutrophils extracellular traps which is important immunity against C albicans infection (Johnson

et al., 2018; Xin et al., 2019).

The effectiveness of *S. aromatic* against pathogenesis of *C. auris* by reducing oxidative stress and inflammatory alteration which is supported by biochemical assay and increased lymphocytes infiltration (Jose et al., 2017). It is identified that cloves containing eugenol molecules which can boost macrophage formation and proliferation which cause increased activities of mice macrophage (Mandey, 2022) indicated that cloves increase liver lymphocytes. Additionally, cloves contain a significant amount of eugenol and anti-inflammatory and antioxidant substances which can preserve the liver from damage by prevent liver cirrhosis because cirrhosis puts the liver as a risk for cancer which characterized by cell proliferation. Cloves reduce oxidative stress by inhibiting the cellular growth which may be consider as the mechanism of against liver cirrhosis (Ali et al., 2014).

CONCLUSIONS AND RECOMMENDATIONS

The infection by *Candida auris* which lead to pathological damage in liver and biochemical changes, while using of alcoholic extract of *syzygium aromaticum* leads to strong elimination in liver tissue and biochemical parameters. These finding suggest the need of future studies to investigate the pathological and pharmacological changes ameliorated by the *syzygium aromaticum*.

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NOVELTY STATEMENT

The novelty of this study is explaining the role of alcoholic extract of *syzygium aromaticum* on *Candida auris* in liver tissue which lead to decrease or remove effects of *Candida* potentially by increasing in catalase enzyme and lymphocyte count.

AUTHOR'S CONTRIBUTION

These authors each contributed equally.

CONFLICT OF INTEREST

The authors have declared no conflict of interest.

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