PROPHYLACTIC EFFECT OF BOSWELLIA CARTERII EXTRACT ON EXPERIMENTAL MURINE CANDIDIASIS

Osama Faid Allah Atshan¹; Inam Badr Faleh¹ and Sura Ayed Radam²

¹Department of Microbiology, College of Veterinary Medicine, University of Baghdad, Iraq
²Department of Pathology, College of Veterinary Medicine, University of Baghdad, Iraq

Abstract

This research aimed to study the effect of Boswellia carterii alcoholic extract that used in the treatment of mice experimentally infection by Candida albicans. Twenty female mice were divided into (4) groups. The first group (5) mice were septic with infected dosage of C. albicans (1×10⁸) living cell/ml. and cured with (1 mg/Kg. B.wt) of Boswellia carterii extract once/day for (14) days, the second group (5) mice were infected as previously and treated with (2 mg/Kg.B.wt) of Boswellia carterii extract once/day as above, the third group (5) mice was given Candida albicans (1×10⁸ live cell/ml) and served as control positive while the fourth group (5) mice were inoculated with (0.2) ml of sterile phosphate buffer saline and served as control negative.

Totally animals detected during the experiment period after treatment with Boswellia carterii alcoholic extract were sacrificed, specimens from kidneys and uterus were collected for fungal isolation and histopathological changes from treated and control groups.

Heavy fungal isolation from uterus in control group with evidence of mild isolation in 1st group treated with (1mg/Kg. B.wt) of alcoholic extract + + and cured with (1 mg/Kg. B.wt) of alcoholic extract once/day for (10) days, the 2nd group (5) mice were infected with infected dose of Candida albicans as previously and treated with Boswellia carterii extract in a dose (2 mg/Kg. B.wt) once/day for (10) days, the 3rd group (5) mice was given Candida albicans (1×10⁸ live cell/ml) and served as control positive while the 4th group (5) mice were inoculated with (0.2) ml of sterile phosphate buffer saline and served as negative control.

Keywords: Candida albicans, Boswellia carterii, urogenital tract, Mice, Human.

Introduction

Candida albicans is a profiteering fungal pathogen that subsists as a harmless commensally in the gastrointestinal and genitourinary tracts in animals and humans (Tropicos et al., 2012).

Boswellia carterii is a genus of trees in the order Sapindales, known for their fragrant resin. The biblical incense frankincense was an extract from the resin of the tree Boswellia sacra (Tropicos et al., 2012). This plant contains active ingredient and essential oil with fungicidal activity, so it's used in the treatment of urogenital tract infection caused by C. albicans (Milos et al., 2016).

Materials and Methods

Candida albicans specimens had been taken by sterile swabs from human with urogenital infection and according to clinical signs identified and inoculate in sabouraud dextrose broth in the universal decanters for eighteen hours then cultivated on sabouraud dextrose agar at (37)°C for (24-48) hrs. and examined macroscopically and microscopically by making Gram's stain and lacto phenol cotton blue smears (Chengappa et al., 1984), and this confirmed by examining for the ability of the isolated yeast to produce germ tube in the human serum according to (Gow et al., 1997) as well as ability to produce chlamydospores and blastoconidia in addition to pseudohyphae and true hyphae when propagated on corn meal agar according to (Zavalza-stiker et al., 2006).

The plant material Boswellia carterii was dry in gloom at hall heat and used a blender to grind these herbal, (250) grams of plant fine particles was drenched in (1.25-1.5) litter of (96%) ethanolic alcohol for (5) days at hall temperature, the medley was mixed every day, the extract was filtered by using What man filter paper No.1. after (5) days, then used a rotary evaporator at 50°C to dried the filtrate, the dehydrated abstract was stocked at 20°C until using sterilized pitcher (Chevrier et al., 2005) (Atshan and Alhadiad, 2014).

A total number (n=20) female mice with ages ranged from (2-3) months old obtained from the (National Center of Researches and Drugs Monitor in Baghdad); then separated into 4th groups. The 1st group (n=5) mice were infected with (1×10⁸) live cell/ml of Candida albicans and treated with (1 mg/Kg. B.wt) of alcoholic extract once/day for(10) days, the 2nd group (5) mice were infected with infected dose of Candida albicans as previously and treated with Boswellia carterii extract in a dose (2 mg/Kg. B.wt) once/day for (10) days, the 3rd group (5) mice was given Candida albicans (1×10⁸ live cell/ml) and served as control positive while the 4th group (5) mice were inoculated with (0.2) ml of sterile phosphate buffer saline and served as negative control.

Result and Discussion

Clinical sign and fungal isolation:

The present study showed that the animals treated with Boswellia carterii extract showed good healthy after injection with challenge dose of Candida albicans during the course of the experiment while non-treated infected mice characterized by depression, loss of appetite, heavy, mild to moderate or no fungal isolation from immolate animals was reported as in tables (1) below.

Table (1): Candida albicans isolation from uterus and kidney of experimental mice in (1st, 2nd, 3rd and 4th groups).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Growth isolation of uterus</th>
<th>Growth isolation of kidney</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st group treated with (1 mg/Kg. B. wt) of Boswellia carterii extract</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2nd group treated with (2 mg/Kg. B. wt) of Boswellia carterii extract</td>
<td>ve</td>
<td>ve</td>
</tr>
<tr>
<td>3rd group positive control</td>
<td>++++</td>
<td>++</td>
</tr>
<tr>
<td>4th group negative control</td>
<td>ve</td>
<td>ve</td>
</tr>
</tbody>
</table>

(++++) heavy growth, ( - ve ) no growth.
Heavy growth of *Candida albicans* isolation was recorded mainly in uterus followed by kidney of +ve control group while mild scanty growth notice in both kidney and uterus sample of first treated group, no evidence of growth of *Candida albicans* were recorded in 2nd and 4th groups.

According to above finding absence of *Candida albicans* growth in the treated group mainly at (2 mg/Kg. Bwt.) may attributed to the potential effect of *Boswellia carterii* extract in killed and induced growth inhibition of present isolate is correlate with (Bhanu et al., 2014) who find that the essential oils of *Boswellia* kinds significantly exhibited antifungal action against both *Candida albicans* and *Candida tropicalis*.

The general morphological appearance of present *Candida* isolate revealed normal identified characterization associated by smooth, creamy-white glistening colonies on sabouraud dextrose agar and having positive result with gram stain, also evidence of pseudohyphae in lactophenol cotton blue smear examined and confirmed by presence of extension of from yeast cells as germ tube when propagated in human serum, also production of chlamydospores and hyphae in addition to blasto conidia appear on corn meal agar (Parveen et al., 2013).

We showed kept in mind that the positive group (3rd group) showed heavy isolation of *Candida albicans* from uterus and kidney may indicate that infected group exposure to highly virulent dose *C. albicans* overcome the host innate immune system then proliferate and penetrate the tissue parenchyma by secretion hydrolytic enzymes like lipases or proteinases which were be a key virulence determinant of *C. albicans* (Schaller et al., 2005) and they circulated within blood and disseminated to the visceral organs mostly kidney and uterus associated with sever lesion in this organs, this observation is correlated with (Julian et al., 2014) who explain the host defense against systemic candidiasis reported due to ingestion and destruction of *C. albicans* by cell of innate immune system specifically macrophages, monocyte and neutrophils.

While the treated with *Boswellia carterii* extract (1st and 2nd group) show mild growth of *C. albicans* in kidney and uterus which revealed the ability and effectiveness of plant extract which agree with (Parveen et al., 2013) who referred that herbal formulations are gradually taking a very important effect due to their efficacy against several data of this elements without any notable mode-regulatory effects and this established by (Baghian et al., 2014) who mention that *Boswellia carterii* extract has potential inhibitory effect on many fungi among them was *Candida albicans*, also the antimicrobial activity of *Boswellia carterii* extract in some yeast strains of *Candida* (*C. albicans, C. glabrata, C. tropicalis* and *C. sake*) has been confirmed by (Milos et al., 2016).

**Minimum Inhibitory concentration results:**

The Minimum Inhibitory Concentration (MIC) of alcoholic extracts of *Boswellia carterii* against *Candida albicans* isolated from pathogenic cases showed that (MIC) for *Boswellia carterii* reached to (6.25 mg/ml) as final concentration, beyond it the pathogenic *Candida albicans* could grow and showed heavy growth on the plate which represent (3.12 mg/ml) (table2).

<table>
<thead>
<tr>
<th>Plant extract</th>
<th>Concentration mg/dl</th>
<th>50</th>
<th>25</th>
<th>12.5</th>
<th>6.25</th>
<th>3.12</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Boswellia carterii</em> extract</td>
<td>Heavy growth</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

The results in current research indicate that *Boswellia carterii* extract have higher potency and affectivity against growth of *Candida albicans*, so the result is in agreement with (Milos et al., 2016) who worked on the anti-candidal activity of nineteen Jordanian plant extracts among them was *Boswellia carterii* which show (6.3±0.8 mg /ml0) of MIC against *Candida albicans*, moreover, the present data of MIC test were in correlated with all the forthcoming authors' results about the affectivity of *Boswellia carterii* in controlling pathogenic *Candida albicans* growth(Tropicos et al., 2012).

**Histopathological examination:**

The main finding of uterus on 1st group showed slight endometrial epithelial desquamation with stromal MNCs infiltration (Fig. 1), another section of uterus show endometrial epithelial hyperplasia together with evidence of presence mucinous exudate in their lumen accompanied with MNCs infiltration in sub mucosa (Fig. 2), while kidney showed moderate degeneration of renal tubules accompanied with hyaline cast formation in some dilated tubules (Fig. 3), another section show mild cellular infiltration between swollen tubules with evidence of intertubular edema (Fig. 4).

The uterine manifestation of 2nd group showed focal interstitial MNCs infiltration composed mainly lymphocyte with little change of renal tubules (Fig. 5), another section show periglomerular MNCs aggregation with mild swelling of adjacent tubules (Fig. 6) the main uterine examination revel epithelial vaculation with degeneration of endometrial gland together with fragmentation of stromal tissue. Sever MNCs infiltration with slight stromal fibroplasia in uterus (Fig. 7) and cystic distention of endometrial gland with mild cellular infiltration (Fig. 8).

Various degree of necrotic lesions were recognized in the endometrial gland with evidence of slight fibrosis of endometrial stroma (Schaller et al., 2005), as well as marked vaculation of endometrial mucosa with focal ulcerative lesion together with neutrophilic infiltration in the adjacent parenchyma (Julian, and Naglik 2014), extensive destruction in the renal tissue also observed with evidence of interstitial MNCs infiltration accompanied with oval yeast cell invasion together with prescience of eosinophile pretentious substances (Baghian, and Lee, 1991), additional finding showed extensive destruction of glomerular tissue due to invasion by pseudo hyphae with vascular congestion and MNCs infiltration in adjacent necrotic tubules, Radam and Faleh (2015).
Prophylactic effect of *Boswellia carterii* extract on experimental murine candidiasis

**Fig. 1:** Histopathological section of uterus 1\textsuperscript{st} group show endometrial epithelial hyperplasia with stromal MNCs infiltration $\rightarrow$ (H and E stain 20x).

**Fig. 2:** Histopathological section of uterus 1\textsuperscript{st} group show endometrial epithelial hyperplasia together with evidence of presence inflammatory cells in their lumen with MNCs infiltration in sub mucosa $\rightarrow$ (H and E stain 40x).

**Fig. 3:** Histopathological section of kidney 1\textsuperscript{st} group show moderate degeneration of renal tubules accompanied with hyaline cast $\rightarrow$ formation in some dilated tubules (H and E stain 40x)

**Fig. 4:** Histopathological section of kidney 1\textsuperscript{st} group show mild cellular infiltration between swollen tubules with evidence of intertubular edema $\rightarrow$ (H and E stain 40x).

**Fig. 5:** Histopathological section of kidney 2\textsuperscript{nd} group show focal interstitial MNCs infiltration composed mainly lymphocyte with little change of renal tubules $\rightarrow$ (H and E stain 40x).

**Fig. 6:** Histopathological section of kidney 2\textsuperscript{nd} group show periglomerular MNCs aggregation with mild swelling of adjacent tubules $\rightarrow$ (H and E stain 40x).
**Fig. 7**: Histopathological section of uterus 2\textsuperscript{nd} group show severe MNCs infiltration with slight stromal fibroplasia in uterus → (H and E stain 40x).

**Fig. 8**: Histopathological section of uterus 2\textsuperscript{nd} group show perivascular MNCs aggregation composed mainly of macrophage and lymphocyte with slight muscular degeneration → (H and E stain 40x).

**Fig. 9**: Histopathological section of uterus 3\textsuperscript{rd} group show necrotic lesions in the endometrial gland with evidence of slight fibrosis of endometrial stroma → (H and E stain 40x).

**Fig. 10**: Histopathological section of uterus 3\textsuperscript{rd} group show vacuolation of endometrial mucosa with focal ulcerative together with neutrophilic infiltration in the adjacent parenchyma → (H and E stain 40x).

**Fig. 11**: Histopathological section of kidney 3\textsuperscript{rd} group show extensive destruction in the renal tissue with evidence of interstitial MNCs infiltration accompanied with oval yeast cell invasion together with eosinophile pretentious substances → (H and E stain 40x).

**Fig. 12**: Histopathological section of kidney 3\textsuperscript{rd} group show extensive destruction of glomerular tissue with vascular congestion and MNCs infiltration in adjacent necrotic tubules → (H and E stain 40x).
Microscopical variations in kidney of infected 3rd group indicated sever findings observed as reflect the ability of Candida albicans to produce the urogenital tract infection in mice which agree with (Baghian et al., 1991) who recorded that the kidneys of animals were the target organs that bore the heaviest foci of infections throughout C. albicans multiplication associated with evidence of chronic infection in mice injected with challenge dose that correlated with investigation by (Rogers et al., 1976) who showed that amassed quantities of C. albicans were detected in renal till (17 – 24) days post challenge, hence the data reported in murine the most volatile body part to C. albicans infection was the kidney.

Treatment of mice with Boswellia carterii or its analogues as in the 1st and 2nd groups induces activation of macrophages which enhanced clearing capabilities of the organs significantly as established by (Chevrier et al., 2005). also other research referred to use of Eos in contradiction of certain fungi leads to cytoplasmic retraction and the wall of hyphal fragmentation also its component can delay activity of enzyme within the hyphae and effect mycological growing and morphogenesis(Chelsea et al., 2018) in addition the immunomodulatory biological assay-directed fractionation of the oleogum dammar of frankincense (Boswellia carterii) result in the isolation and identification of nine combinations palmitic acid and eight triterpenoids belonging to lupine, ursan, oleanane and tirucallane skeletal were insulated from the resin (Badria et al., 2003).

Hence Candidacidal activities of the organs by Boswellia carterii treated animals within short interval of time is due to activation of phagocytic systems, so current research demonstrated that, kidneys own a strong phagocytic system attributed to Boswellia carterii therapy result in potential activity of this system (Jawetz et al., 2004).

References
Parveen, S.D. (2013). An approach to etiology, diagnosis and management of different types of candidiasis. Department of Microbiology, Kurukshetra University, Kurukshetra-136119, Haryana, India.