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PROPHYLACTIC EFFECT OF *BOSWELLIA CARTERII* EXTRACT ON EXPERIMENTAL MURINE *CANDIDIASIS*

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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* (1×10^8) 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* (1×10^8 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 while no fungal isolation observed in 2nd group also notice severe pathological findings in both kidney and uterus tissues of control positive animal while evidence of hyperplastic changes with MNCs infiltration mainly recorded in treated group.

***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 psuedohyphae 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 Alhadad, 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 (1×10^8) 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* (1×10^8 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).

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

(++++) 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 reveled normal identified characterization associated by smooth, creamy-white glistening colonies on sabouraud dextrose agar and having positive result with gram stain, also evidence of pseudohyphea in lactophenol cotton blue smear examined and confirmed by presence of extension from of 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 (2): Showed the minimum inhibitory concentration of *Boswellia carterii* extracts against *C. albicans* growth.

Concentration mg/dl / Plant extract	50	25	12.5	6.25	3.12
***Boswellia carterii* extract**	-	-	-	-	Heavy growth

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 intertubullar 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 eosinophilc 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).

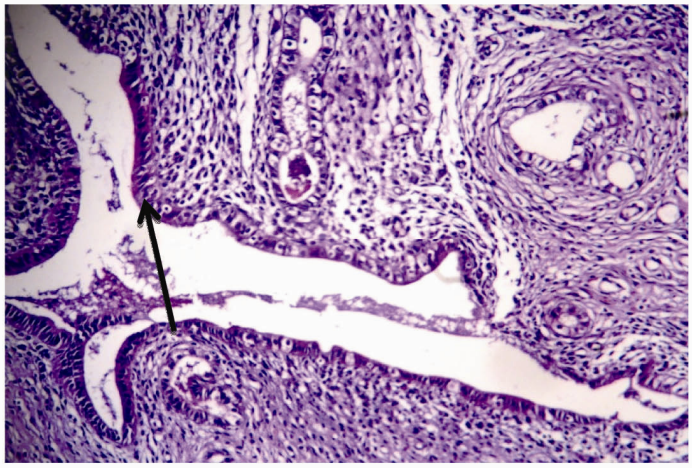

Fig. 1 : Histopatholigical section of uterus 1st group show endometrial epithelial hyperplasia with stromal MNCs infiltration → (H and E stain 20x).

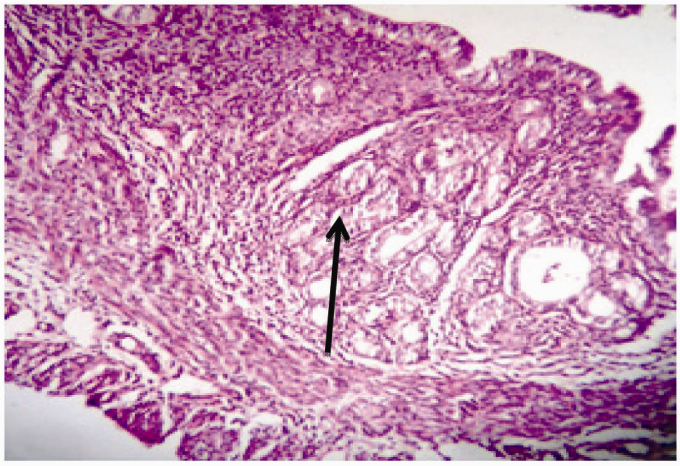

Fig. 2 : Histopathological section of uterus 1st group show endometrial epithelial hyperplasia together with evidence of presence inflammatory cells in their lumen with MNCs infiltration in sub mucosa → (H and E stain 40x).

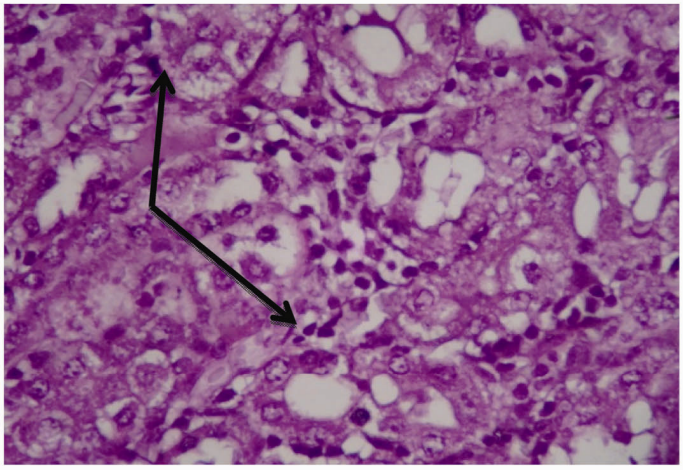

Fig. 3: Histopatholigical section of kidney 1st group show moderate degeneration of renal tubules accompanied with hyaline cast → formation in some dilated tubules (H and E stain 40x)

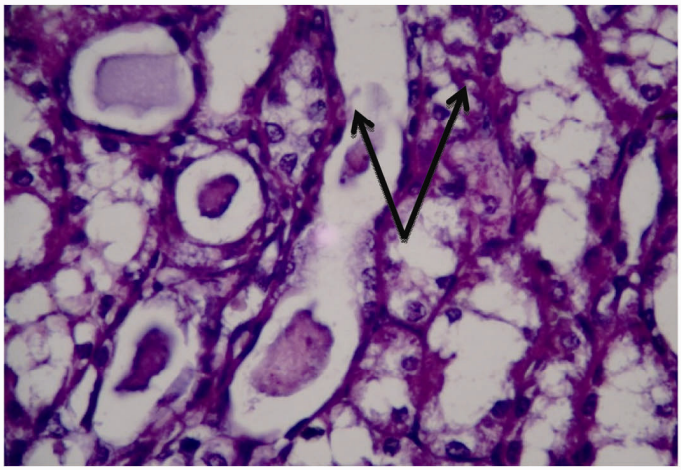

Fig. 4: Histopatholigical section of kidney 1st group show mild cellular infiltration between swollen tubules with evidence of intertubullar edema → (H and E stain 40x).

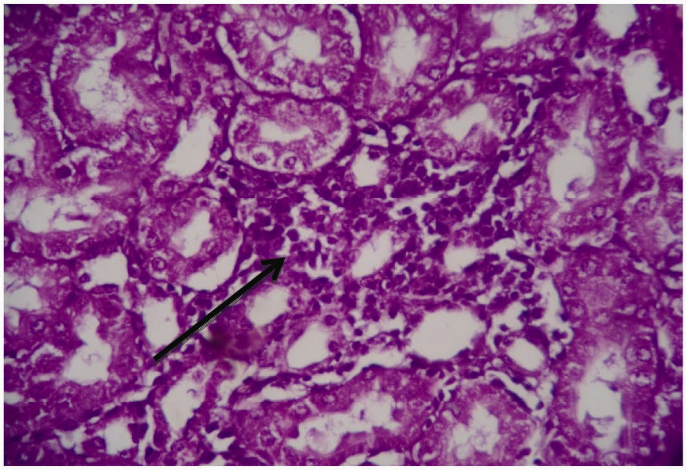

Fig. 5 : Histopatholigical section of kidney 2nd group show focal interstitial MNCs infiltration composed mainly lymphocyte with little change of renal tubules → (H and E stain 40x).

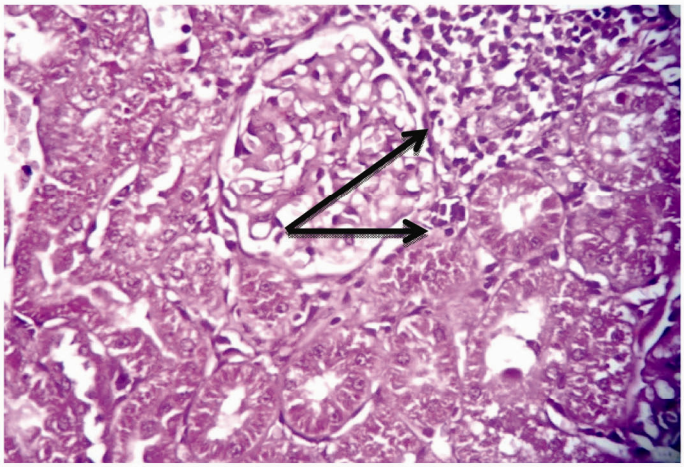

Fig. 6 : Histopatholigical section of kidney 2nd group show periglomerular MNCs aggregation with mild swelling of adjacent tubules → (H and E stain 40x).

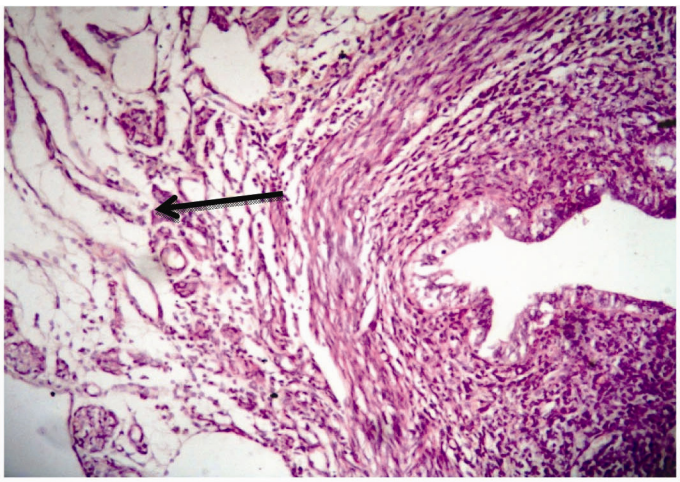

Fig. 7 : Histopatholigical section of uterus 2nd group show sever MNCs infiltration with slight stromal fibroplasia in uterus → (H and E stain 40x).

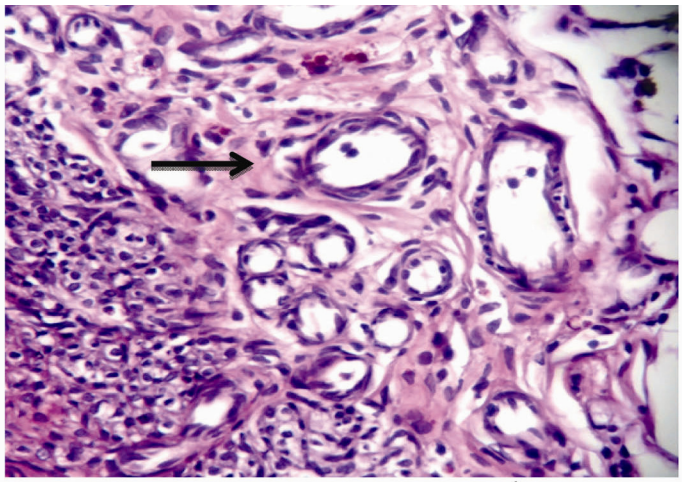

Fig. 8 : Histopatholigical section of uterus 2nd group show perivascular MNCs aggregation composed mainly of macrophage and lymphocyte with slight muscular degeneration → (H and E stain 40x).

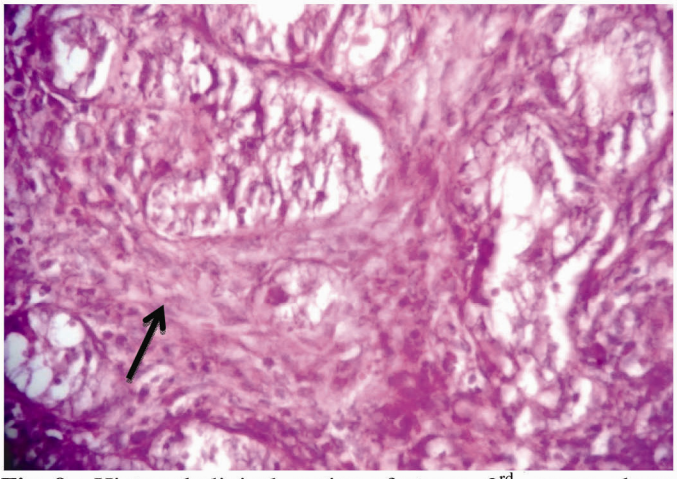

Fig. 9 : Histopatholigical section of uterus 3rd group show necrotic lesions in the endometrial gland with evidence of slight fibrosis of endometrial stroma → (H and E stain 40x).

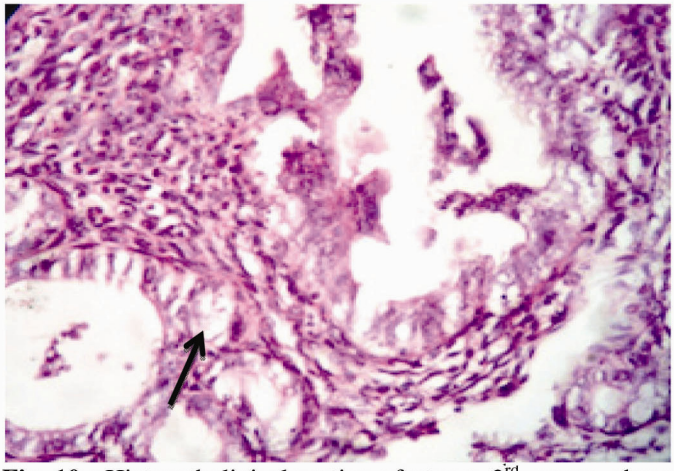

Fig. 10 : Histopatholigical section of uterus 3rd group show vaculation of endometrial mucosa with focal ulcerative together with neutrophilic infiltration in the adjacent parenchyma → (H and E stain 40x).

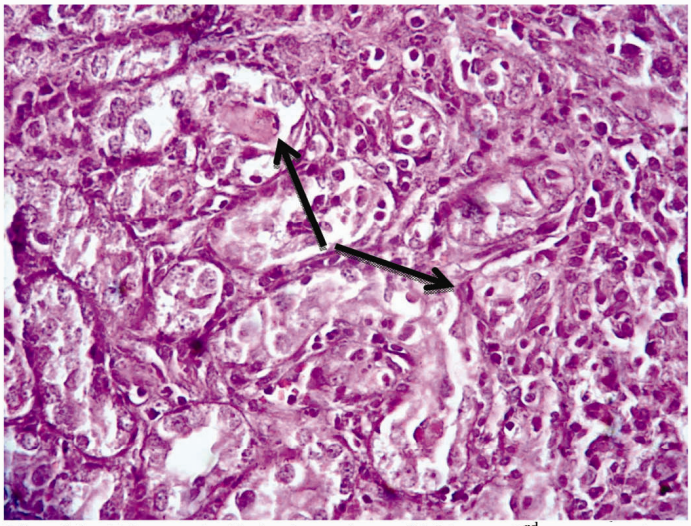

Fig. 11 : Histopatholigical section of kidney 3rd group show extensive destruction in the renal tissue with evidence of interstitial MNCs infiltration accompanied with oval yeast cell invasion together with eosinophilc pretentious substances → (H and E stain 40x).

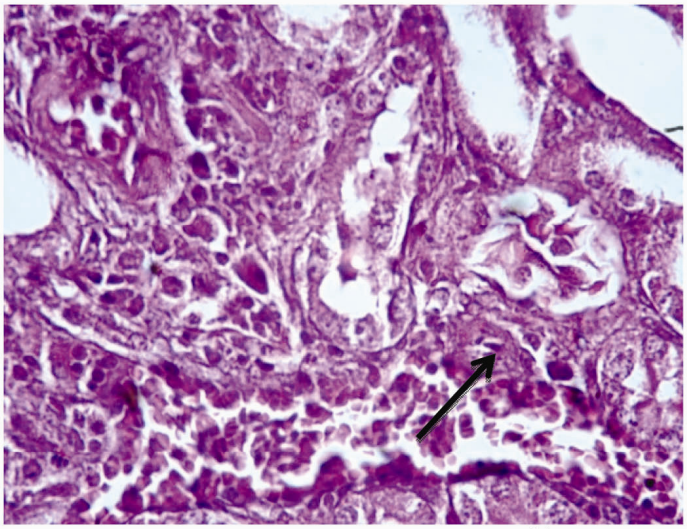

Fig. 12 : Histopatholigical section of kidney 3rd group show extensive destruction of glomerular tissue with vascular congestion and MNCs infiltration in adjacent necrotic tubules → (H and E stain 40x).

Microscopical variations in kidney of infected 3rd group indicated sever findings observed as reflect the ability of *Candida albican*s 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 oleguum 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).

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