ISSN NO. 2320-5407

Journal homepage: http://www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH

RESEARCH ARTICLE

Study the effectiveness of Artemisia herba-alba leaves extract on the experimental infection with Candida albicans isolated from urogenital tract in cows

Osama F.A. Atshan and Zainab A.A. Al-Haddad
Zoonotic diseases unit /Veterinary Medicine College/Baghdad University

Manuscript Info

Manuscript History:

Received: 12 February 2014
Final Accepted: 22 March 2014
Published Online: April 2014

Key words:

Corresponding Author

Osama F.A. Atshan

Abstract

This research aimed to study the effectiveness of *Artemsiaherba-alba* as a medicinal plant against *Candida albicans* isolated from urogenital tract in cows and used in the treatment of experimental infection in mice (*in vivo*) as well as determining the effect of alcoholic extract of this plant (*in vitro*) against same isolated pathogenic yeast by Minimum inhibitory concentration test.

Artemisia herba-alba alcoholic extract was used in a dose 1mg/Kg.B.wt and 2mg/Kg.B.wt for one time when inducing the infection by *Candida albicans* in mice, extract of *Artemisia herba-alba* was able to cause significant decrease in the clinical signs as well as in reducing the post mortem changes that appear in very clear picture in the mice infected with *Candida albicans* alone, also *Artemisia herba alba* extract participated in the significant reduction in the numbers of *Candida albicans* colonies isolated from kidney and uterus of mice treated with *Artemisia herba alba* extract, the results of (fungal isolation) was supported by examining the ability of *Artemisia herba-alba* extract to inhibit *Candida albicans* growth *in vitro* by determining minimum inhibitory concentration (MIC).

Keywords: *Candida albicans, Artmesia herba alba,* Uorogenital tract, cows, MIC test

Introduction:

Candida albicans is an opportunistic fungal pathogen that exists as a harmless commensal in the gastrointestinal and genitourinary tracts in animals and humans (1).

The yeast and yeast like fungi have been implicated as causes of bovine reproductive problems including abortion in cows and infertility in bulls (2).

Artemesia herba-alba, a medicinal and aromatic dwarf shrub that grows wild in arid areas of the Mediterranean basin, extending in to north western Himalayas. 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* (3).

Materials and methods

Isolation and identification of *Candida albicans*:-

Candidaalbicans had been isolated from cows with urogenital infection and diagnosed according to clinical signs; the specimens from infected area were taken by sterile swabs and inoculated into sabauroud dextrose broth in the universal bottles for 18 hrs. then cultured on sabauroud dextrose agar at 35℃ for 24-48 hrs. and examined macroscopically and microscopically by making Gram's stain smears and lacto phenol cotton blue smears (4), and this confirmed by examining for the ability of the isolated yeast to produce germ tube in the human serum according to (5) as well as ability to produce chlamydospores and blastoconidia in addition to psuedohyphae and true hyphae when propagated on corn meal agar according to (6).

Preparing of plant extracts:

The plant material *Artemisia herba-alba* was dried in shade at room temperature and grinded by using a blender. Two hundred and fifty grams of plant powder was soaked in 1.25- 1.5 L of 95% ethanol for 5 days at room temperature. The mixture was mixed daily for regular infusion. After a five days period , the extract was filtered by using Whatman filter paper No .1. The filtrate was dried by using a rotary evaporator at 50℃. The dried extract was stored in sterile glass bottles at 20℃ until using (7,8).

Minimum inhibitory concentration test (MIC):

Artemisia herba-alba alcoholic extracts used in this experiment by dissolving 1 gm. from the plant extract in 10 ml from DMSO solvent so that each 1 ml from DMSO contain 100mg from plant extract, serial (two fold dilution) was made from these primary concentration for the plant extract, for the preparation of (50, 25, 12.5, 6.25, 3.12, 1.56) mg/ml respectively, 10 µl from *Candida albicans* suspension was added to each dilution from the stock one which contain 1×10^8 live cell/ ml, incubated for 24 hrs. in 35°C, then culturing were done on SDA and incubated for 24-48 hrs. in 35°C to examined for *C. albicans* survival in these dilutions (9,2).

Experimental design:-

Forty BALB/C female mice were used to perform the experiment.

They were divided in to 4 groups each group consist of (10) mice

The infected dose of *Candida albicans* given for each group in this experiment was 1×10^8 live cell/ ml.

First group: -was given *Candida albicans* alone 1×10^8 live cell/ ml without any additives (positive control).

Second group: -keeping them without any treatments (negative control).

Third group: -infected with *Candida albicans* according to the dose determined previously and treated with *Artemisia herba-alba* extract in a dose 1mg/Kg.B.wt once/ day for 14 days.

Fourth group: -infected with *Candida albicans* according to the dose determined previously and treated with *Artemisia herba alba* extract in a dose 2mg/Kg.B.wt once/ day for 14 days.

All animals had been observed during the experiment period, and then all animals were sacrificed after treatment with *Artemisia herba alba* extract had been done, post mortum changes achieved on all animals where kidneys and uterus were collected from animals in all groups for isolation of *Candida albicans* from both kidney and uterus on SDA.

Results and Discussion:-

Isolation of *Candida albicans* from cows

The selected strain appeared as smooth, creamy white, glistening colonies and having Gram positive staining microscopically, and the appearance of pseudohyphea in lacto phenol cotton blue smear confirmed by presence of extension from yeast cells as germ tube when propagated in human serum figure (1) which is agree with(10), who established that the observation of germ tube production as a method for presumptive identification of *Candida albicans* has been in use for many years.

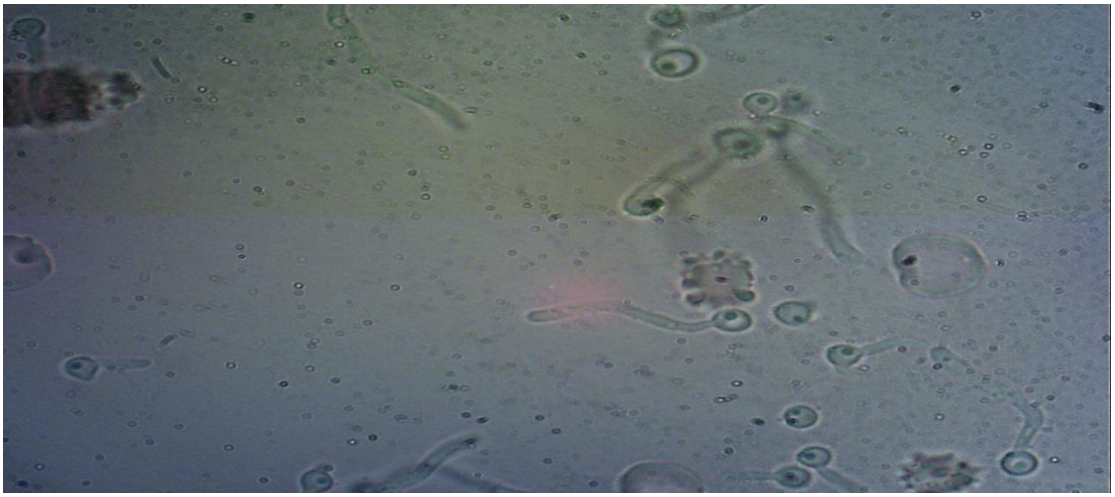

Figure (1) Germ tube of Candida albicans (100x).

Production of chlamydospores and hyphae in addition to blastoconidia appear on corn meal agar figure (2), which is agree with (11)who proved that chlamydosporses were spherical, thick-wall, and usually produced on suppurating cells that occur along pseudohyphae or at the tip of hyphae (12).

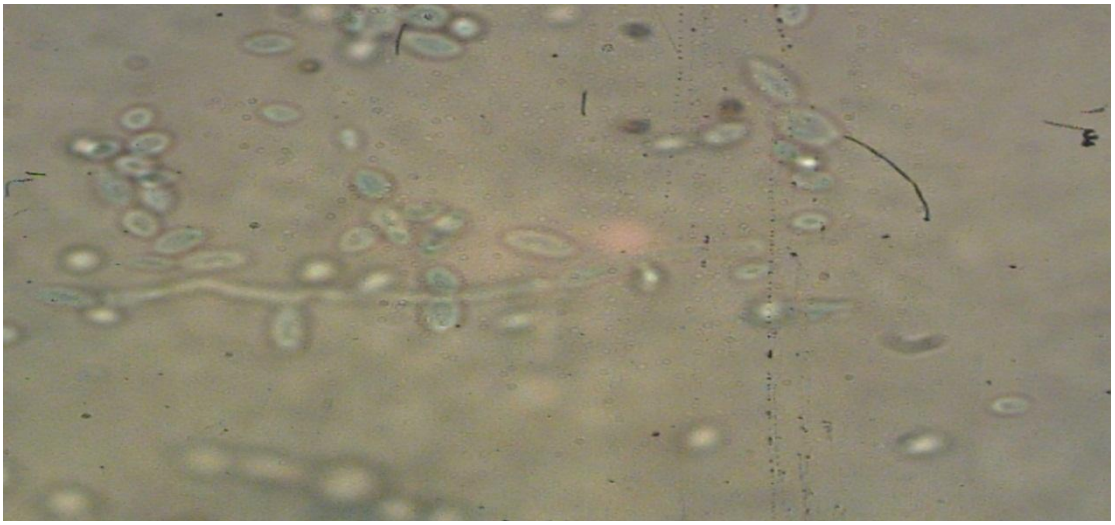

Figure (2): Chlamydospore formation of *Candida albicans* (100X).

Minimum Inhibitory concentration results:

The Minimum Inhibitory Concentration (MIC)of alcoholic extracts of *Artemisia herba-alba* against *Candida albicans* isolated from pathogenic cases showed that the (MIC) for *Artemisia herba-alba* reached to 3.12 mg/ml as final concentration, beyond it the pathogenic *Candida albicans* could grow and showed heavy growth on the plate which represent 1.56 mg/ml.(fig. 3, tab. 1).

Table 1: Showed the minimum inhibitory concentration of *Artemisia herba-alba* extracts against *C.albicans* growth.

Concentration mg/ml / **Plant Extract**	**50**	**25**	**12.5**	**6.25**	**3.12**	**1.56**
Artemesia hreba-alba	-	-	-	-	-	**Heavy growth**

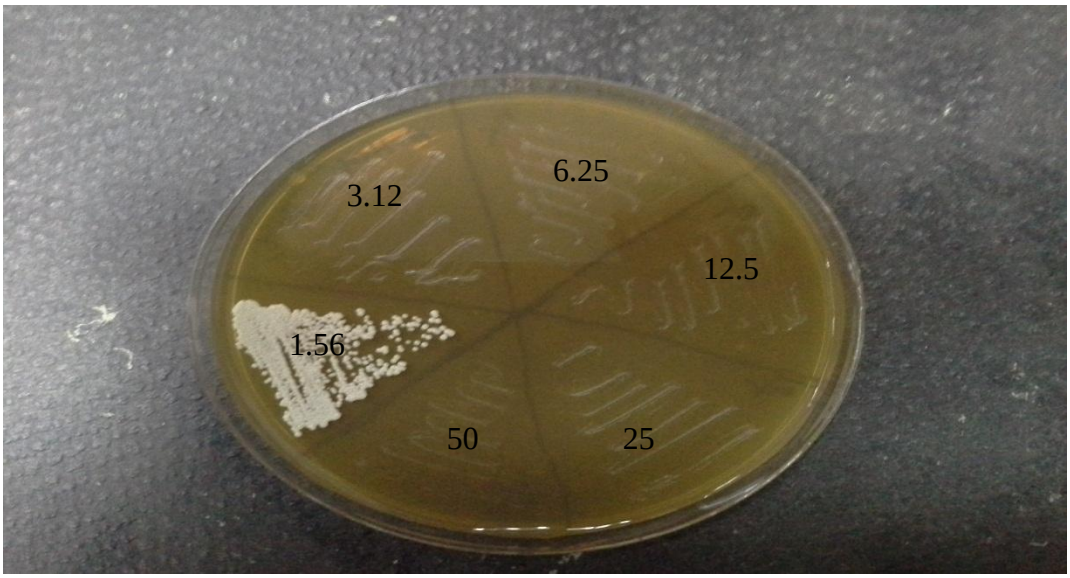

Figure (3): show results of minimum inhibitory concentration of *Artemesia herba-alba* alcoholic extract.

The results in this research indicate that *Artemesia herba-alba* extract have higher potency and effectivity against growth of *Candida albicans* ,the current result is in agreement with (2) who worked on the anti candidal activity of nineteen Jordanian plant extracts among them was *Artemesia herba-alba* which show 6.3±0.8 mg /ml(MIC) against *Candida albicans*.

In Jordan (1) suggested that the *Artemisia herba-alba* was considerably inhibited the growth of *Candida albicans* in a dose 4000 ppm.in MIC test.

Results of this research of MIC test were in agreement with all the forthcoming authors' results about the effectivity of *Artemesia herba-alba* in controlling pathogenic *Candida albicans* growth (3, 4).

Isolation of *Candida albicans* from experimental animals

Yeast isolation results (tab. 2) showed that positive group (first group) showed heavy isolation of *Candida albicans* from uterus and kidney compared to the isolation results from (3rd and 4th groups) which revealed the ability and effectiveness of *Artemisia herba-alba* used in different concentrations in (3rdand 4th groups), so that the

internal organs from mice of these groups do not revealed any isolation percent which agree with (13)who referred that herbal formulations are gradually taking a very important place due to their efficacy against a large repertoire of diseases and ailments without any notable derogatory effects and this is established by (1,9) who found that *Artemisia hrba-alba* has stronger growth inhibitory effect on many fungi among them was *Candida albicans*, also the antimicrobial activity of *Artemisia hrba-alba* in some yeast strains of Candida (*C.albicans, C.glabrata, C.tropicalis*, and *C. sake*) has been confirmed by (14).

Table 2: Revealed the isolation of *Candida albicans* from uterus and kidney of experimental mice**.**

Groups	Appearance of *Candida albicans* in the uterus	Appearance of *Candida albicans* in the kidney
First	++++	+++++
Second	- Ve	- Ve
Third	-	- Ve
Fourth	-	- Ve

The results in table (2) of *Candida albicans* culture from the kidney and uterus of all mice in all groups after scarifying them, in which(+++++) refer to heavy growth, (++++) refer to 100-150 colonies,– (-ve) refer to no growth appeared after culturing samples on the Sabauroud dextrose agar and incubated in 35℃ for 24-48 hrs.

Post mortem changes:

The P.M changes appeared in mice (tab. 3) in the first group reflect the ability of *Candida albicans* isolated from urogenital tract in cows as pathogenic yeast to produce the urogenital tract infection in mice which agree with (15) who recorded that the kidneys of animals were the organ that bore the heaviest foci of infections throughout the experiment (fig. 4).

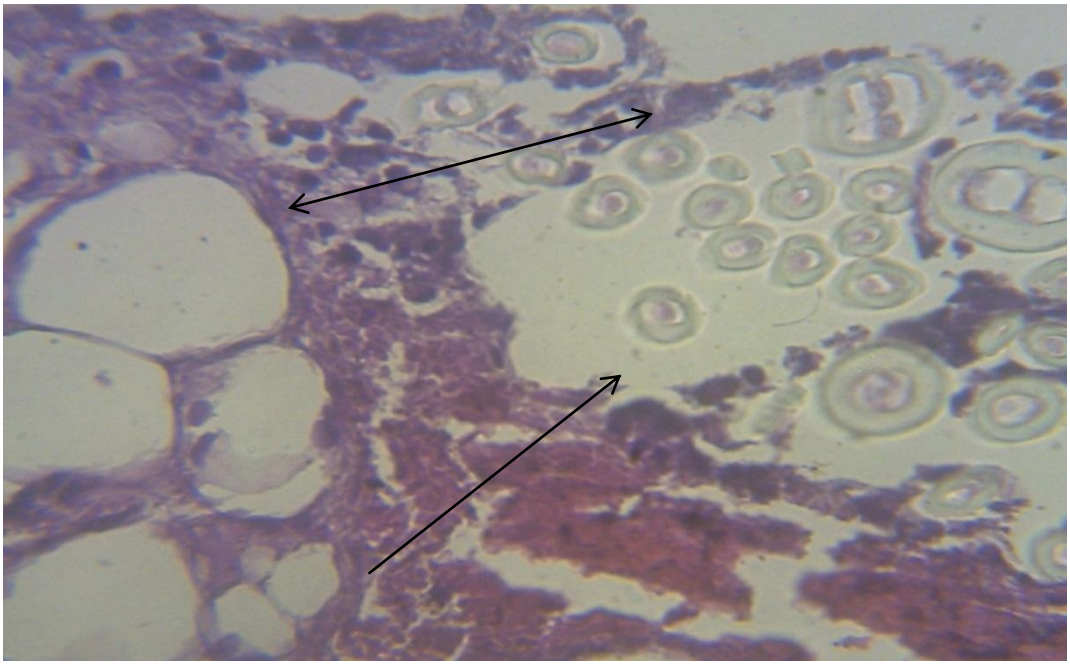

Figure (4): Histopatological section in the adipose tissue of the kidney of animal at 14 days post infection shows hemorrhagic area ⟷ and large number of yeast surround by neutrophil ⟶ (H and E stain 40×).

C. albicans multiplied to a greater extent in the kidneys of mice than in their spleens, lungs, or livers, so that infection in mice was chronic; and this is agree with (16) who showed that increasing numbers of *C. albicans* were observed in their kidneys until about 17 to 24 days post challenge, hence the data reported here for mice show that the kidney is the most susceptible organ to *C. albicans* infection figure (5).

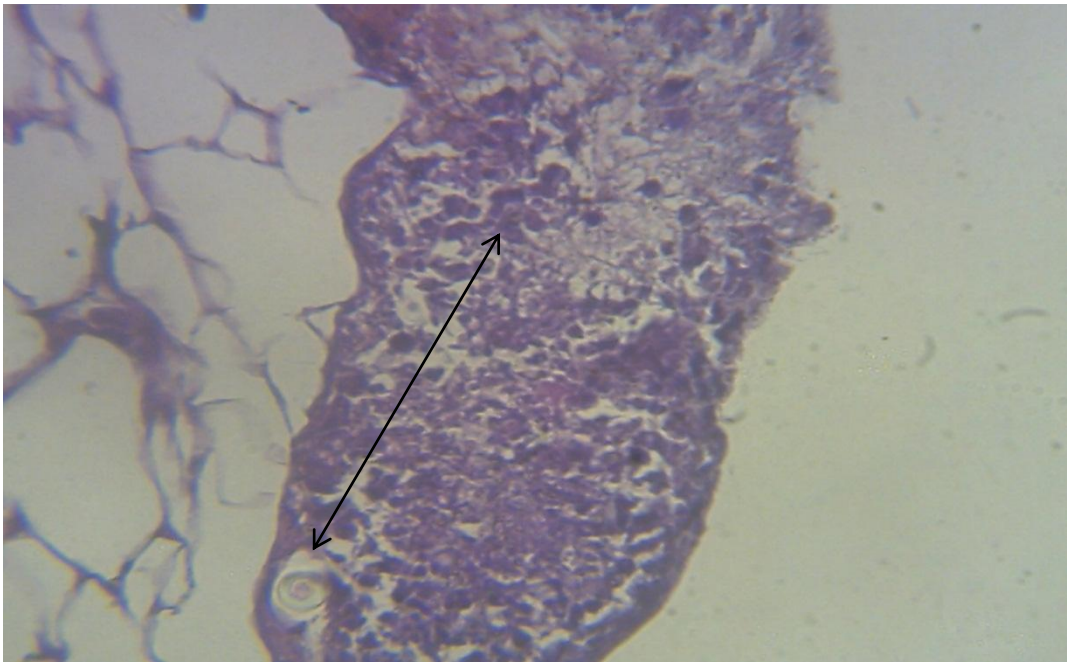

Figure (5): Histopatological section in adipose tissue around the kidney of animal at 14 days post infection shows yeast cells and hyphae of the fungi surround by sever aggregation of neutrophil ⟷ (H and E stain 40×).

According to the post mortem changes appear on the mice in first group in this research which suggested that the renal burden of *C. albicans* during systemically-induced candida infections in mice remained high which is comparable to results (16).

Table 3: Showed the most important P.M changes that were noticed after killing and dissecting the mice.

Groups	Post mortem changes
First	Emaciation, odema in the kidney, swelling and enlargement of uterus, presence of whitish color around the kidney and genital tract which represent the accumulation of *Candida albicans* growth.
Second	Healthy mice, without any changes.
Third	Normal mice, without any changes.
Fourth	Normal mice, without any changes.

This research revealed that the physiological conditions and the poor phagocytic system of kidneys are contributing factors for predisposition of kidneys to the long-term systemic candida infections which is recorded by (15).

Anatomical architecture and physiological conditions of kidneys contribute to their susceptibility to candida infections; so that a few yeasts may escape the renal phagocytic system and reside in regions such as the renal medulla or tubular area which is agree with (16). where, due to increased osmolarity and high content of urea and ammonia, the phagocytic and chemotactic activities of recruited poly morphonuclear leukocytes and monocytes are reduced and this is may be the cause in the delay in inflammatory response certainly helps the yeasts develop

pseudohyphae, penetrate the tubular area and produce foci of infection as appeared in present results and agree with (17).

Whereas the results of 3rd and 4th groups shows the ability of *Artemisia herba-alba* as medicinal plant used in the treatment and resist the infection of the same pathogenic strain used in the first group (fig.6) and this agree with(1).

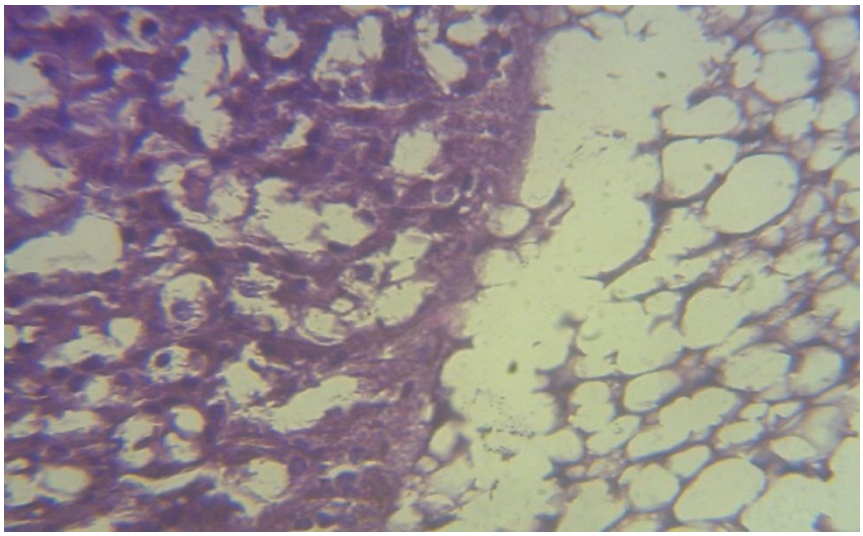

Figure (6): Histopatological section of the kidney in animal treated with *Artemisia herba alba* in a dose2 mg/Kg.B.wt. shows no clear lesion in the adipose tissue and in the epithelial lining cell of renal tubule ⟷ (H and E stain 40×).

Treatment of mice with *Artemisia herba-alba* or its analogues as in the 3rd and 4th groups induces activation of macrophages which enhanced clearing capabilities of the organs significantly as established by (14).

Hence Candidacidal activities of the organs by *Artemesia herba-alba* treated animals within short period of time are due to activation of phagocytic systems (fig.7).

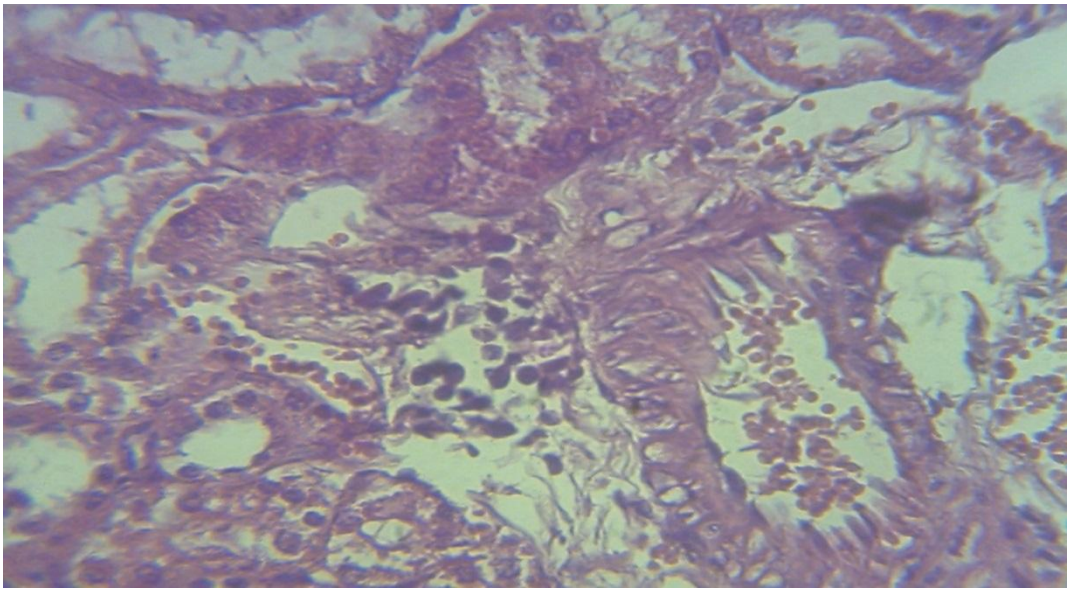

Figure (7): Histopatological section of the kidney in animal treated with Artemesiaherbaalbain a dose 2 mg/Kg.B.wt. appears mononuclear cells aggregations around the congested blood vessels and in the renal tubules (H and E stain 40×).

So, current research demonstrated that, kidneys possess a strong phagocytic system and *Artemesia herba-alba* treatment of mice potentiates this system (18).

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