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EVALUATION OF ANTIFUNGUS ACTIVITY IN OLIVE (*OLEA EUROPAEA*) LEAF EXTRACT AGAINTS *CANDIDA ALBICANS* IN MICE

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Abstract

In present study were used Twenty four mice, were divided into three groups (8 in each group) as follows :-

Group (1) control rats. 0.1 ml of distilled water was administered orally with by gastric intubation daily for a month.

Group (2) was infected by a fungus *Candida albicans.*

Group (3) was infected by a fungus *Candida albicans*, and then treat with methanolic extract olive leaf were fed extract (0.1ml) the needle inserting carefully by gastric-intubation tube into the oral cavity of mice daily for one month. to observe effects therapeutic extract in organs (liver, kidney and stomach), has been showed histological changes when fungal invasion and after treatment compared with the control group, where it was noted therapeutic response of structures of the organs. The aim of this study was to evaluate the antifungal activity of Methanolic extract of olive leaf.

Key words : *Candida albicans,* Olive*,* Antifungal.

Introduction

candidiasis are a major cause a number of risk factors. The infections with *Candida albicans* are increasing in frequency. (Eksie *et al.*, 2013) An increase in serious human infections caused by fungi and a progression of drug resistance to conventional therapeutics triggered a need for more effective treatment. Several studies have reported that olive leaf extract and its constitutes, particularly oleuropein and hydroxytyrosol, have health benefits, including antioxidant and antimicrobial properties (Zoric *et al.*, 2016) The olive tree, botanically-classified as *Olea europaea* L., have been extensively used in medicine against microbial diseases. And they contain several pharmacological activities. Because Olive leaf compounds like oleuropein and its derivatives including hydroxytyrosol and tyrosol exhibit antimicrobial activities, which can reduce the risk of microbial infections. (Benavente *et al.*, 2000) These compounds can inhibit, bacteria, yeasts, fungi, molds, mycoplasmas, and other parasites, particularly in the gastrointestinal and respiratory tracts (Khanmm *et al.*, 2012) it is important to employ safe and effective antifungal agents (Citarasut *et al.*, 2010).

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Materials and method

Animals :-twenty four mice weighing between (25-30g) grams were used in this study. the animals were maintained and acclimatized in the college of veterinary medicine-Tikrit University under laboratory conditions in group cages .The mice were collected randomly into three groups, group (1) was kept as control 4 mice. was administered orally with 0.1 ml of distilled water by gastric intubation daily for a month, group (2) 10 mice was infected of fungus (*Candida albicans*) group (3) 10 mice was infected by a fungus *Candida albicans* treat with methanolic extract olive leaf were fed extract (0.1ml) by carefully inserting the needle of the gastric-intubation tube into the oral cavity of mice daily for one month.. Treatment was last for thirty days.

Preparation of olive leaf aqueous extracts

In this study, the olive leaves were collected in spring from a farmer Tikrit city in Iraq. The leaves extracts under The methanolic extract was prepared by using 10 g of leaf powder extracting with 200 ml of methanol. The methanolic extract was concentrated using Rotatory evaporator according to (Khan *et al.*, 2012).

Samples:-Isolated from laboratory of veterinary medicine collage of Tikrit university.

Histology:-at the day after the last dose killed the animals under intensive dose of chloroform. Liver, kidney and stomach of the animals were rapidly removed and micro dissected to obtain tissue samples for histological examination. immediately the tissues were fixed in 10% neutral buffered formalin, dehydrated with graded series of ethyl alcohol and embedded in paraffin. Sections of 5 microns were cut and stained with eosin and hemotoxylin according to (Yigit *et al.*, 2001). By digital camera attached to light microscope were taking Photomicrographs of the slides .

Results

The whole photomicrographs were compared with those of parts of liver, stomach & kidney of control group (1) with other groups, were showed the following:

Liver control

The parenchyma of the liver was containing a polygonal shape of the liver cell which had one or two spherical nuclei, the live cells were present in the form of cords extended in the liver lobules toward the central vein. In between the liver cell, her was a channels of blood sinusoid with RBC and kupffer cells (fig. 1). The central veins and veins of portal areas were engorged with blood.

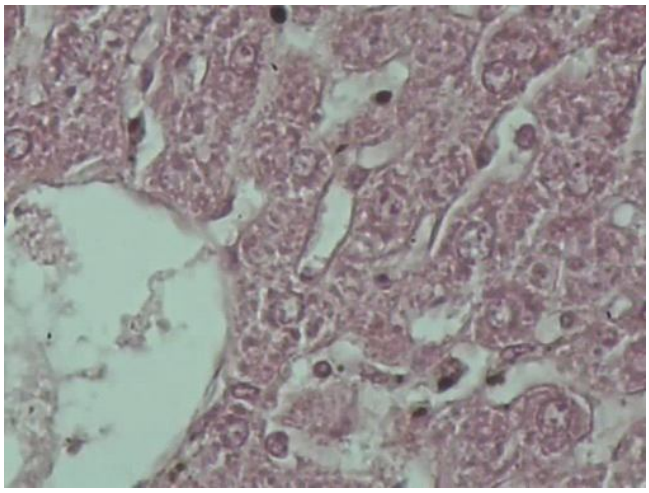

Fig. 1: Liver control show a-playground cells, b-blood sinusoids with kuffer cells, c-central vein (H & EX10)

Liver infection

There was degenerative change in the liver cell, most of the liver cell revealed dissolution of the cytoplasm or presence an eosinophil granules in its cytoplasm (fig. 2A) and the most of these cells had karyolitic nucli .there was an thickening of the cell membrane and other had breaking down of its cell membrane there was certain number of the central vein with hemolysis blood (fig.2B).

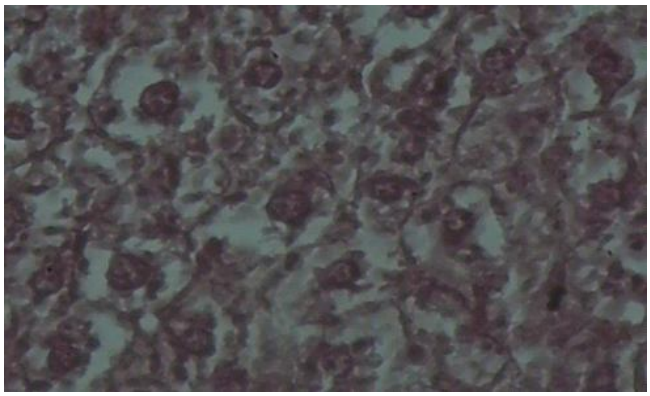

Fig. 2A: Liver infected show extensive degeneration of liver cells (H & EX10)

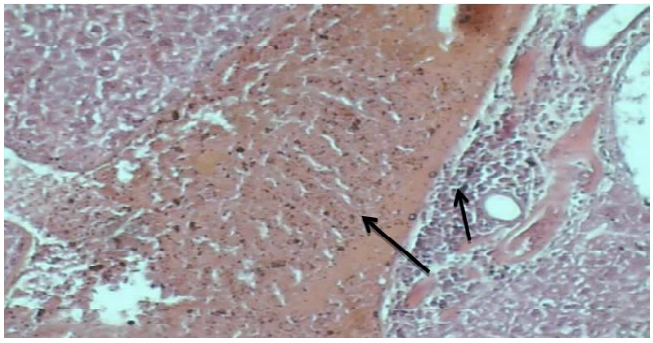

Fig. 2B: Liver infected show,lymphocyte aggregation,in portal area congested portal vein

Liver treatment

The parenchyma of the liver had an hyperatrophy of the liver cells with granulated cytoplasm (fig. 3A) and

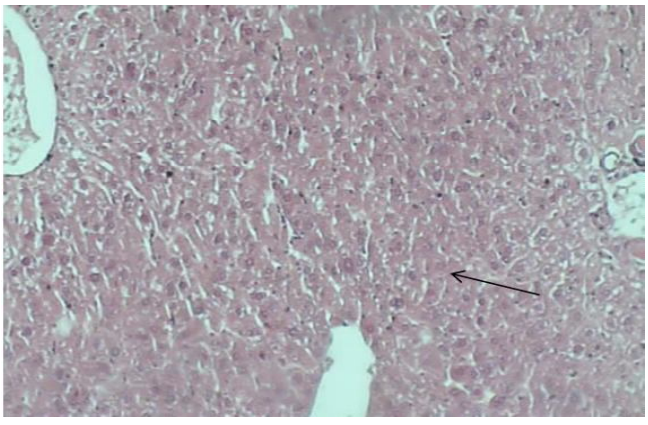

Fig. 3A: Show liver mice treated by 0.5ml of olive leaf extract group(3), radical of liver cells around the central vein.

around the central veins there was a cords of liver cells of polyhedral shepe, normal pattern with 1-2 nuclei. the blood sinusoid were containing cupffer cells and there was lymphocytic aggregation around portal vein in the area (fig. 3B).

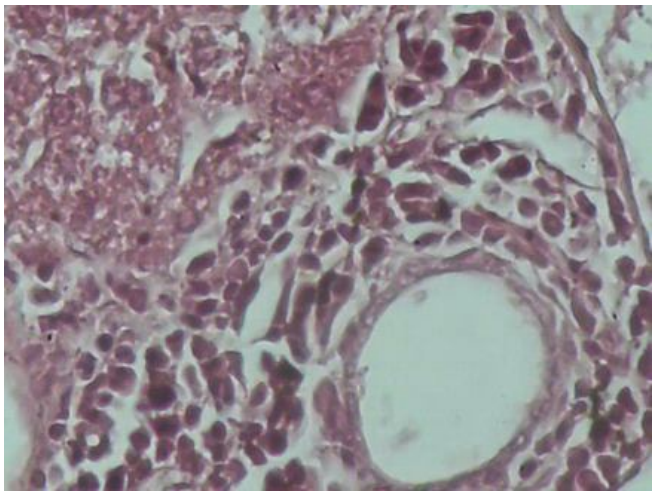

Fig. 3B: show liver mice treated by 0.5ml of olive leaf extract group (3), lymphocytic aggregation. around a branch of portal vein of the portal area of liver

Stomach Control :

The mucosa of the stomach was formed by gastric pits and the surface of the mucosa which was lined by simple column epithelium and mucosa nucleic cell, the gastric pits were lined by the mucus cell, parietal cell (fig. 4A) and chief cell, the submucosa of the stomach was containing mainly the blood vessel (vein and arteries) and surrounded this layer by the muscular coat of smooth muscles fibers (fig. 4B).

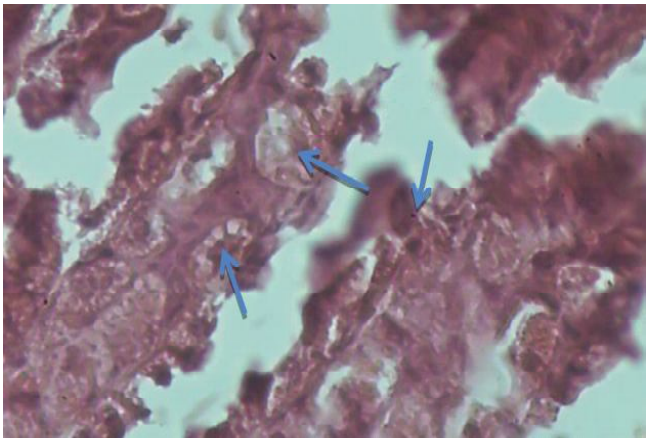

Fig.4A: Showed stomach normal histological changes of mice received distilled water (H & EX40).mucus neck cells, parietal cells, simple columnar epithelial

Stomach infection

The mucosa of the stomach was containing different

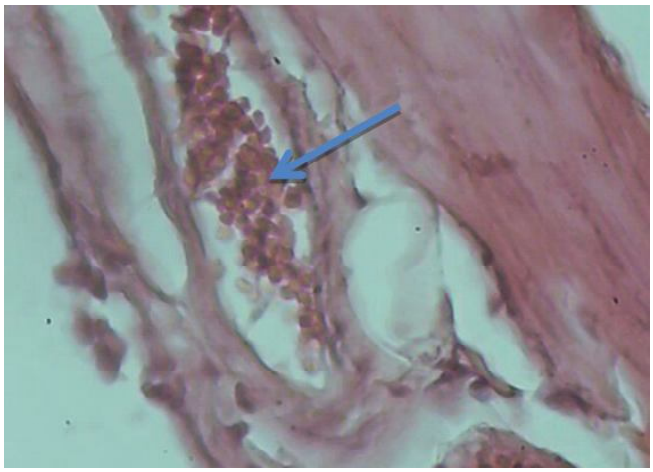

Fig. 4B: Showed stomach normal histological changes of mice received distilled water, sub mucous blood vessel (H & EX40)

layer of cell which one -simple column epithelial, mucus nuclei cell and partial cell .In the neck of gastric pits and main or chief cells in the gastric gland, the predominant

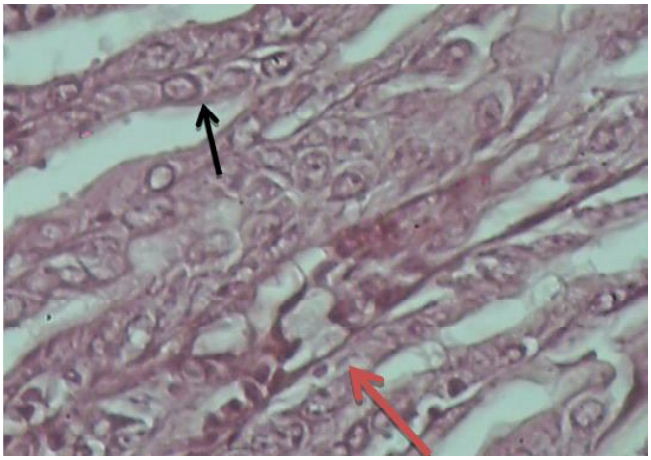

Fig. 5A: Sstomach mice group (2)infected,showed mucus of stomach Degeneration and atrophied of cells gastric gland (arrow black),degeneration the parietal cells (arrow red) (H & EX40)

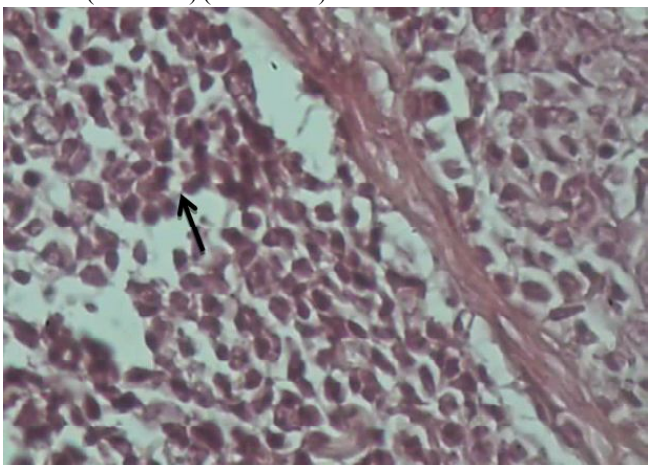

Fig.5B: Stomach mice group (II) infected, showed lymphocytes infiltration (arrow black) (H & EX40)

percentage of theirs cells were degenerated and atrophied (fig. 5A) the bases of the gastric gland were infiltrated with lymphocyte which extended to the sub mucosa (fig. 5B). associated this layer with congestion of blood vessel with blood.

Stomach treatment

The surface epithelium of the mucosa of the stomach had degenerated column epithelial cell (fig. 6A) and most cells of gastric gland were also degenerated like the main or chief cell, mucus cells and partial cell (fig. 6B) and the blood vessels below these gland were congested with blood.

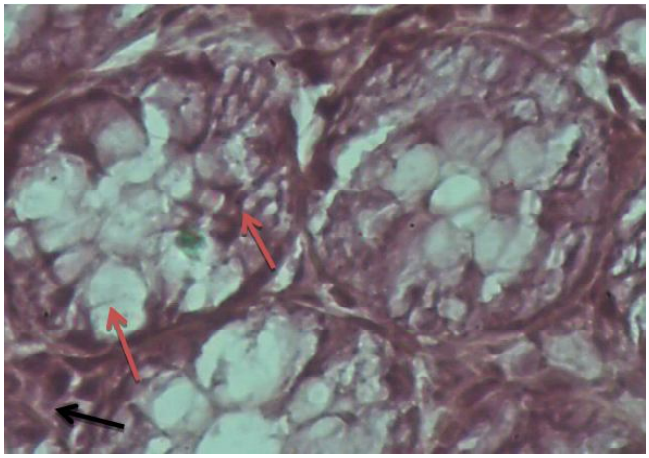

Fig. 6A: Stomach mice treated by 0.5ml of olive leaf extract group (3), showed (arrow red) degenerated gastric gland cells (mucus neck, parietal cells). (arrow black) blood vessels congested, (H & EX40)

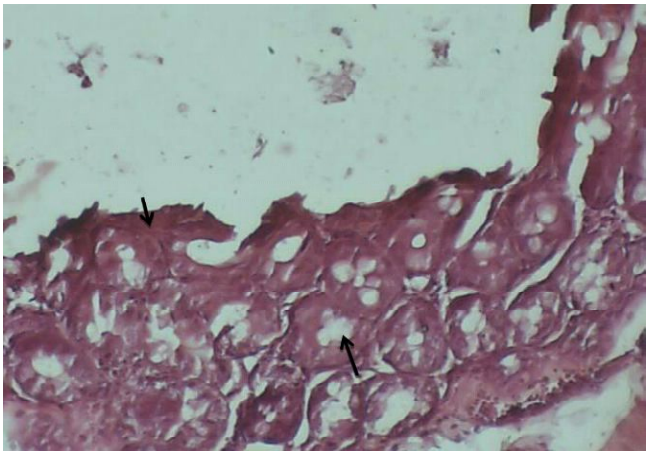

Fig. 6B: stomach mice treated by 0.5ml of olive leaf extract group(3), showed (arrow black) degenerated of columnar epithelial cells (H & EX40)

Kidney

Kidney control

The cortex of the kidney was containing a renal corpuses which formed by the glomeruli surrounded by the Bowman's capsule (fig. 7A). These are surrounded by a great number of proximal convoluted tubule and distal convoluted the medulla was containing the collection ducts and renal tubule also Henal loops (thin and thick sequntats) (fig. 7B) in the cross section of blood capillaries with RBC and the interstitial C.T and blood enriched with the cells of C.T and blood capillaries.

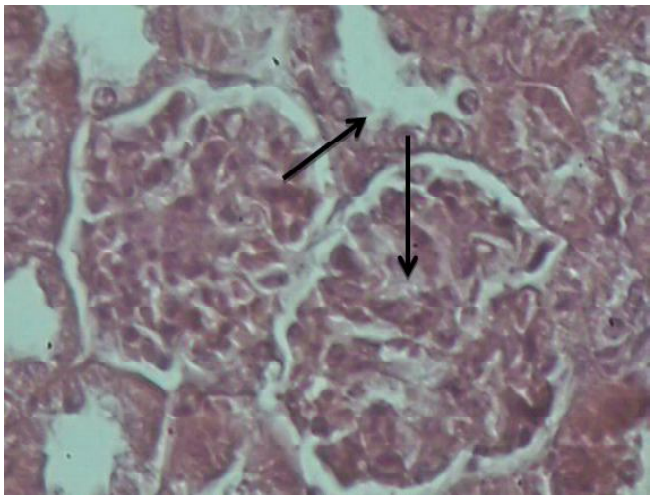

Fig. 7A: Showed normal histological changes of mice kidney received distilled water, cortex of kidney normal view of glomerular, proximal convoluted tubule (H & EX40).

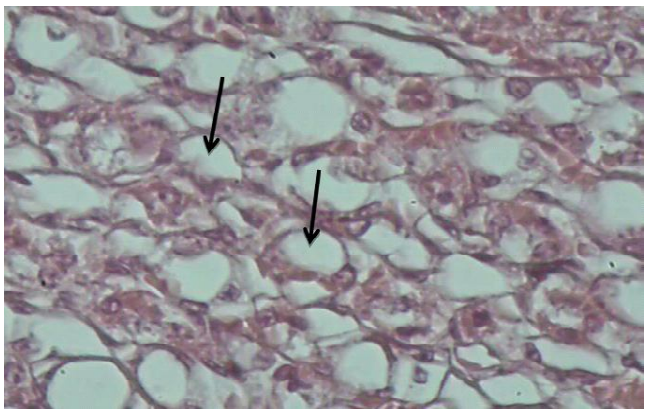

Fig. 7B: Showed normal histological changes of mice kidney received distilled water, medulla of kidney normal view of Henleloops (H & EX40).

Kidney infection

The most glomeruli of the renal cortex were appeared normal in the size and structure, however still there was a number of these glomeruli with atrophic pattern (fig. 8A) and the most of the proximal and distal convoluted tubule were normal (fig. 8B) but there was a certain number of degenerated epithelial cells in lumen mostly under capsule of kidney. The tubules of the renal medulla appeared normal.

Kidney treatment

The cortex of the kidney was containing predominant

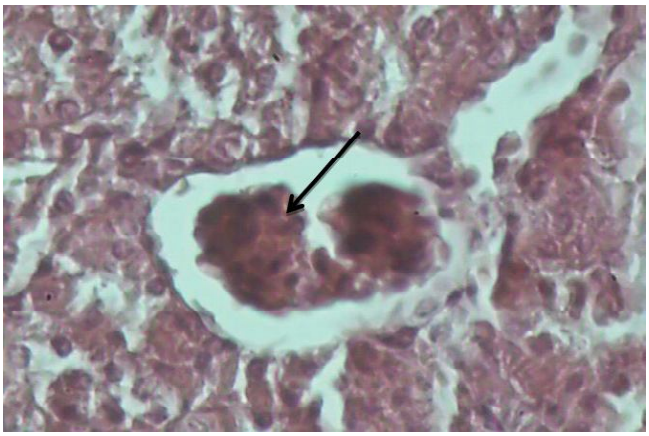

Fig. 8A: Showed kidney infected, atrophic glomerulus of the renal cortex (H & EX40).

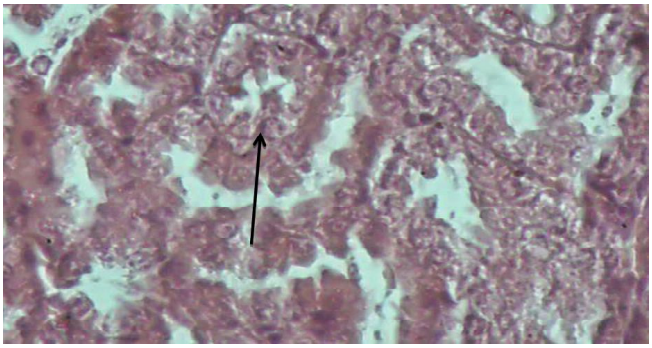

Fig. 8B: Showed kidney infected, atrophic proximal convoluted tubule (H & EX40).

number of the normal shape and structure of glomeruli (fig. 9A) but also still there was individual glomerular which appeared atrophic and displaced to the peripheral of Bowman's capsule and the capsular wide. The proximal and distal convoluted tubular of epithelial cells in the form of degenerative process. The medulla had extensive capillaries congested with blood in between the renal tubular (fig. 9B).

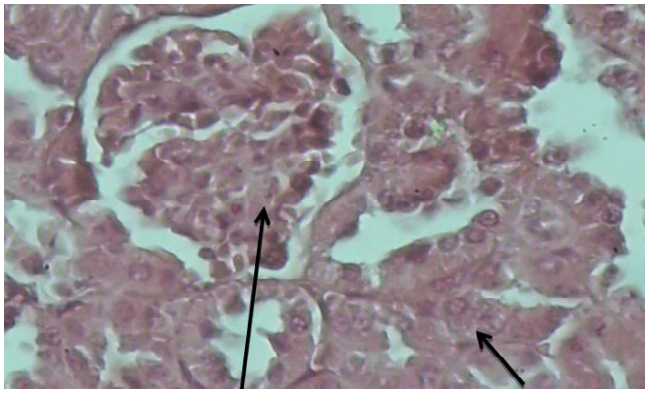

Fig. 9A: kidney mice treated by 0.5ml of olive leaf extract group (3) showed, normal glomerular of renal cortex, proximal convoluted tubule (H & EX40)

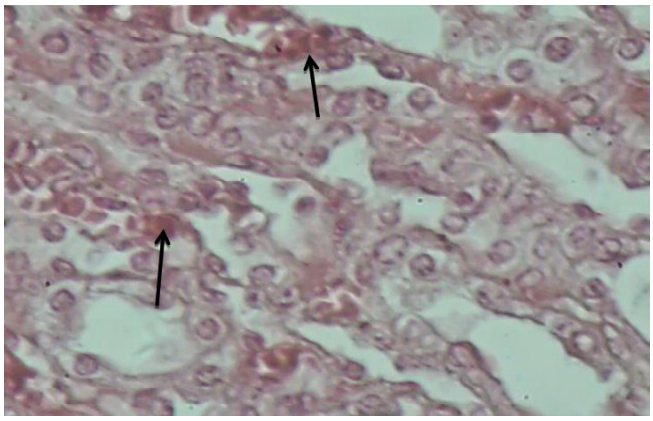

Fig. 9B: kidney mice treated by 0.5ml of olive leaf extract group (3) showed, congested blood capillaries (H & EX40)

The antimicrobial activities of olive leaf extracts against tested yeasts and their potency .Phenolic compounds isolated from olive fruit have been shown to inhibit the growth of *Escherichia coli*, *Klebsiella pneumoniae* and *Staphylococcus aureus* (Markind *et al.,* 2003). The results of the present study were in accordance with previous research. According to a study by Markin *et al.,* (2003)*;* Soler *et al.,* (2000), olive leaf aqueous extracts destroyed 15% of *C. albicans* within 24 h. Previous studies suggest that phenols in olive have antimicrobial properties (Juven and Henis, 1970). The effect of oleuropein, present in high amounts in olive leaves, was investigated on its antifungal activity against Rhodotorula ssp., C. albicans and S. cere- visiae by Juven and Henis, (1970); Faiza, (2011) and no inhibitory effect against these yeasts were observed. In study Faiza *et al.,* (2011). The olive extracts showed an unusual combined antibacterial and antifungal action and ethyl acetate and acetone extracts revealed a wide range of antimicrobial activity (Soler, 2000). The products of olive tree that can live for many years with their beneficial effects on health (Mastres, 2007). Its reported by some researchers that the oleuropein which is included in these products has a lot of pharmacological properties including antioxidant, antimicrobial, antiinflammatory, antiatherogenic anticarcinogenic and antiviral activities.

In this study demonstrate the antifungal effect of olive leaf extracts growing in Iraq against *C. albicans* strain.

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