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In vitro and in vivo studies on the anticandidal activity of *Carica papaya* seed extract

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ABSTRACT: Ten isolates, from 25 skin scraping of cutaneous infected site were diagnosed as *Candida albicans*. Infection with *C. albicans* was the highest among females than males with a rate of 80% and 20% respectively, and among patients with age 21-30 years(40%). *In vitro* antifungal susceptibility of ketoconazole, fluconazole, Nystatin, Amphotericin–B and Clotrimazole against clinical isolates of *C. albicans* indicate that the yeast isolates were susceptible (100%) to four antifungal, while 3 out of isolates were resistance to fluconazole with inhalation zone 20.4mm. The in vitro study using the seed extracts of *Carica papaya* at concentrations 10%, 25% and 50% inhibited the growth of *C. albicans* with rate 10mm, 9mm, 8mm, respectively compatible with control(0%) 15mm. *In vivo* antifungal activity studies on candidiasis in mice treated with the seed extract (2.5g/kg body weight), liver showed infiltration in lymphocyte and hydropic degeneration, while spleen and kidney showed in filtration of lymphocyte, compatible with negative control (not treatment) and positive control treated with ketoconazole.

KEYWORDS: *Candida albicans*, *Carica papaya*, antifungal activity, anticandida activity.

1 INTRODUCTION

Candida albicans is an important opportunistic fungal pathogen and the major cause of orophoryngeal candidiasis, gastrointestinal and female genital flora. Opportunistic pathogens are accounted for a substantial morbidity rat and can result in hospitalization and expensive therapies and they also reduce the survival rate of people with HLV infection [1]. Althorgh *C. albicans* is a common cause of nosocomial infections nosocomial candidiasis is a worldwide problem in immunocompetent as well as immune compromised individuals*, C.albicans* causes cutaneous or subcutaneous infections such as vaginitis or oral thrush or infections of the nails and skin[2]. *C.albicans* can enter the blood stream to cause serious systemic invasive disease. Unfortunately, long-term therapy of candidiasis might lead to resistance development to antifungal of fluconazole especially during long – term treatment of oropharyngeal candidiasis [3], [4]. The management of candida infections faces a number of problems including limited number of effective antifungal drugs, resistance of candida to commonly use antifungal drags, toxicity of antifungal drug and high cost of antifungal drugs [5], [6], [7]. According to the literature the investigation for natural products to be used against candida infections increased significantly in the last 10 years examining approximately 258 plant species from 94 families[8],[9].

Papaya (*Carica papaya*) an herbaceous fruit crop belonging to the family caricaceas, mostly grown in tropical and subtropical regions it is a single stemmed plant with an erect and branches. The ripe fruit of papaya usually eaten raw, without the skin or seeds but the unripe green fruit can be eaten cooked, usually in curries, salads and stews[10].

Literature has studies show that, papaya is not only known for its nutritional benefits but also considered to possess medicinal properties. it is low in calories and rich in natural vitamin and minerals like vitamin C, vitamin AM thiamine, iron and fiber. many biologically active phytochemical have been isolated from papaya and studied for application almost all parts of the papaya (leaves, latex, seed, fruit, bank, peel, roots)have important biologically active substances that can be isolated for application predominantly in the pharmaceutical industry[11]. Different parts of the plant are attributed with different medicinal values ,for example, the seed are effective as a vermifuge and in the treatment of hypertension, diabetes mellitus and hyper cholesterolemia [12].The fresh leaves of it are also efficacious in the treatment of gonorrhea syphilis and amoebic dysentery[12].

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Results from studies on biological activities non biological activities of *C.papaya* pants extracts and isolated compounds showed that the latex and root extracts inhibited *C. albicans,* while extracts of pulp and seeds showed bacteriostatic properties against *Staphylococcus aureus, Escherichia coli, Salmonella typhi, bacillus subtilis* and *Entamoeba histolytica, in vitro* [13]. The aim of this study were to isolate and identify *C.albicans* and studies on *anticandida albicans* activity of *Carica papaya* seeds in vitro and in vivo

2 MATERIALS AND METHODS

2.1 SPECIMEN COLLECTION

Samples including skin war collected from attending out patient deramatology department of Al-amam ali hospital. we started to collect samples in august and September, the collected samples were diagnosed by direct examination and laboratory

2.2 FUNGAL CULTURE

The collected samples were cultured on sabouraud dextrose agar (SDA) medium plates containing antibiotic 0.4mg chloramphenicol to prevent the growth of bacteria, then they were incubated at35ºC for 2 days. Laboratory diagnosis, sugar fermentation and assimilation tests microscopic observation of long germ tubes, hyphae blastoconidia and chlamydoconidia production was performed before the start of examination [14],[15],[16].

2.3 SUSPENSION PREPARATION

C. albicans colonies were suspended in 5ml of 0.85%normal saline suspension was mixed for 15 second with a vortex , then its concentration was adjusted to1.5x108 cfu/ml based on a standard 0.5 mcfarland [8],[17],[18].

2.4 PREPARATION OF AQUEOUS EXTRACT OF *CARICA PAPAYA*

The mature fruits of *Carica papaya* were collected and cut into pieces and the wet seeds were separated out these were then gently but thoroughly rinsed in top water and completely air –dried at room temperature for 4 weeks. The dried seed were pulverized in to fine powder using mixer grinder. 40g of the powdered *Carica papaya* seeds was boiled in 500ml of distilled water for 30 minute after which it was filtered using a piece of cotton gauze. The filtrate was evaporated to complete dryness at 40ºC.After filtration, the aliquot was centrifuged at 5000rpm for 30 minute. The supernatants were filtered through whatmann no.1 filter paper. The filtrate was evaporated to complete dryness at 40ºC. The extract was diluted into different concentration 25%, 50% and 75% then sterilization by passing through 0.2 m Millipore filter[19],[20].

2.5 DETERMINING ANTIFUNGAL ACTIVITIES OF *CARCIA PAPAYA* EXTRACT

The *anti C. albicans* activities of papaya was determining by using the well diffusion method, SDA medium was inoculated with *C. albicans* suspension by swap, Then 5 mm wells were caved in it by Pasteur pipette. Then 50µl of each concentration were added to wells the plates were incubated at 35ºC for 48h. After incubation inhibition zone was observed and it was determined in millimeters [1].

2.6 ANTIFUNGAL SUSCEPTIBILITY TESTING

The anti *C.albicans* of antifungal drug was determining by using the disc diffusion method. Suspension of *C.albicans* (1.5x108 CFU/ml)was plated on SDA plates by swap, standard discs of different types of antifungal agiant [ketoconazole (KT) 10 mcg, Fluconazole (FLC) 25 mcg, Nystatin (NS) 100 unite, Amphotericin-B (AP) 20 unite and Clotrmazole (CC) 10 mcg, were put on agar media with sterile forceps that inoculated with C. albicans, The platees were incubated at 35C for 48h. After incubation, the diameters of the zone recorded to the nearest millimeter [21].

2.7 *IN VIVO* ANTIFUNGAL ACTIVATES OF PAPAYA

LABORATORY ANIMALS

Young male swiss albino mice weighing between 25 and 35were used for this study. The mice were obtained from the animal house in college of medicine Baghdad university. The cages with the mice were placed in room (temperature 26 +2ºC), water and food were provided to animals.

ANTIFUNGAL ASSAY

The intravenous (i.v.) inoculation of *C. albicans* used in this study was $1x10^{7}$viable cell/ml PBS. 0.1 ml of which was injected into lateral vein of mice [22]. Animal were divided into three groups of 10 mice each and received in treatment as described in fallowing:

Group1 (negative control): 0.1ml of *C. albicans* intravenous injected [i.v.], 48h gap followed by treatment with phosphate buffer solution (PBS) intraperitoneal, i.p , once daily for 6 days.

Group2 (treatment): 0.1ml of *C.albicans*, i.v., injected,48h gap, followed by treatment with Carica papaya seed extract 2.5g/kg body weight, i.p.,once daily for 6 days [22].

Group3 (positive control): 0.1ml of *C. albicans*, i.v., injected 48h gap, followed by treatment with ketoconazole 10 mg/kg body weight ,i.p. once daily for 6 days.

All mice were killed by cervical dislocation on day 8 after i.v. *C.albicans* inoculation, the liver, kidneys and spleen of each animal were removed aseptically and placed in 10% formo –saline, dehydrated with 100% ethanol solution and embedded in paraffin. They were then processed in to 5mm thick sections stained with hematoxylin-eosin and observed under a photomicroscope.

2.8 STATISTICAL ANALYSIS:

Data are reported as mean ± standard deviation.

3 RESULTS AND DISCUSSION

Specimens (25 skin scraping) of this study were examined by direct microscopically examination and macroscopically on sabouraud dextrose agar and then all isolate submete to physiological testes to identification.

Ten isolates were diagnosed as the *Candida albicans* from the cutaneous infected site which represent 40% of the total cases. The relationship between *C. albicans* infection and gender was studies, infection with *C. albicans* was the highest among females than males with a rates of 80%and20%repecteviliy.In addition to gender age with *C. albicans* infection was also investigated in this study the high infection was among patients with age 21-30 years (4cases; 40%). *C. albicans* isolates affected two age groups of patients with equal percent (11-20;31-40;2%),infants and children (1-10years) and in patients age 41-50years showed equal percent10%.

The reason for high rate of cutaneous candidiasis infections in women especially the fingers could be due to the excessive and continued use of detergents in addition to exposure to moisture and water heavily at home business practices.

Table [1] the effect of seed aqueous extracts concentrations of *Carica papaya* on the growth of *C.albicans* , result shows that the growth is inhibited by aqueous extracts of *C.papaya* and this inhibition rate increases with the increase of concentrations. Thus, rate growth of *C.albicans* in 10%, 25% and 50% seed extract concentration is10mm, 8mm and 6mm respectively compactable with control(0%) 15mm.

To find suitable drug for the management of fungal diseases is difficult because fungi, like human beings, are eukaryotes. medicinal plant are cheap and renewable sources of pharma cologically –active substances and are known to produce certain chemicals that are naturally toxic to fungi, each part of the papaya plant has seen usefulness in one way or another, from its fruit to its stem, seed and leaves. This study agreed with the numerous studies which indicate to the effectiveness of the papaya plant in the treatment of many pathological cases and uses antibacterial and antifungal

The inhibitory effect of aqueous and crude extracts of *C.papaya* on some human and plant pathogenic fungi indicate its therapeutic potential as antifungal agents. Qualitative phytochemical analyses of organic and aqueous extract of dried seed of *C.*

papaya shown that they contain such phytochemicals as alkaloids, sapponins, tannins, flavonoids, phenols and glycosides [23],[24];and also okoyel(25) found that crude ethanolic and aqueous extracts of seed of *C. papaya* have antibacterial and antifungal activity against different test bacteria *Salamonella typhi Staph. aureus Pseuclomonas aeruginosa and E. coli, and fungi Aspergillus niger, Penicillium notatium, fusarium solan, Candida albicans* and dermatophytes. Also this study agreed with Satyapal [19] when showed that in 10% shoot and seed extract concentration the radial growth of *C.albicuns* was 96.6% and 93.5% of control respectively, while,these value in75% shoot and seed concentration are 51.7% and 54.8% of the control respectively. Antioxidant activities of the petroleum ether, ethanol, ethylacetate, n-butanol and aqueous extract of seed of papaya tested by Zhou *et al* [26], it is observed that high amount of total phenolies and total flavonoids contribute their antioxidant potential·

Table 1. Effect of different concentration of aqueous extract of Carica papaya on the growth of Candida albicans

C.papaya concentration(%)	Diameter of growth (mm)
0	15
10	10
25	8
50	6.5

The *in vitro* antifungal activities of ketoconazole (KT) 10 mcg, fluconazole (FLC) 25 mcg, Nystatin (NS) 100 units, Amphotericin–B(AP) 20 units and Clotrimazole (CC) 10mcg against 10 clinical isolates of C. albicans are given in table [2]. antifungal test result indicate that the yeast isolate were susceptible (100%) to KT, NS, AP, CC with inhibition zone 28.9 mm, 28.3 mm, 14.9 mm, 36.5 mm respectively, while 3 out of isolates of *C. albicans* were resistance to fluconazole with inhibition zone 20.4 mm. Recently antifungal susceptibility testing has become more important because of the increasing of both fungal infections and antifungal drug resistance several researches have shown that some isolates of *C.albicans* were resistant to different types of antifungal [21],[27],[28],[29].

Table 2. Antifungal susceptibility of the isolates of C.albicans

Drugs	ketoconazole	fluconazole	Nystatin	Amphotericin–B	Clotrimazole
Zone diameter(mm)	28.9± 0.18	20.4 ±0.15	28.3 ±0.12	14.9± 0.21	36.5± 0.22

The histomorpholgical study in G1(negative control),in which mice infected with C.albicans, showed that the liver had showing granuloma hydropic degeneration and hemorrhage, spleen had showing necrosis in spleen cells, while kidney showed shrinking in glomerula hemorrhage and necrosis. while after the mice treatment with ketoconazole (10mg /kg body weight) ,G3,liver showed hemorrhage in hepatic artery and sinusoidal, while spleen showed hydropic degeneration and increase in number of megakaryocyte. In kidney showed hemorrhage and hydropic degeneration. In G2, which was given seed aqueous extract of *C.papaya*, liver showed infiltration in lymphocyte and hydropic degeneration, while spleen and kidney showed in filtration of lymphocyte. Cutaneous candidiasis is an opportunistic infection that arises in most cases from endogenous saprophytic candidal blastospores that selectively colnize oral gastrointestinal vaginal and cutaneous epithelium Lossinsky *et al*.[30] showed that rats inoculated intraperetonial with the hyphae –producing strain showed pathology in the kidneys, liver, spleen and other tissues associated with inoculation tracks of the nose and muscle and connective tissue of the abdominal wall.

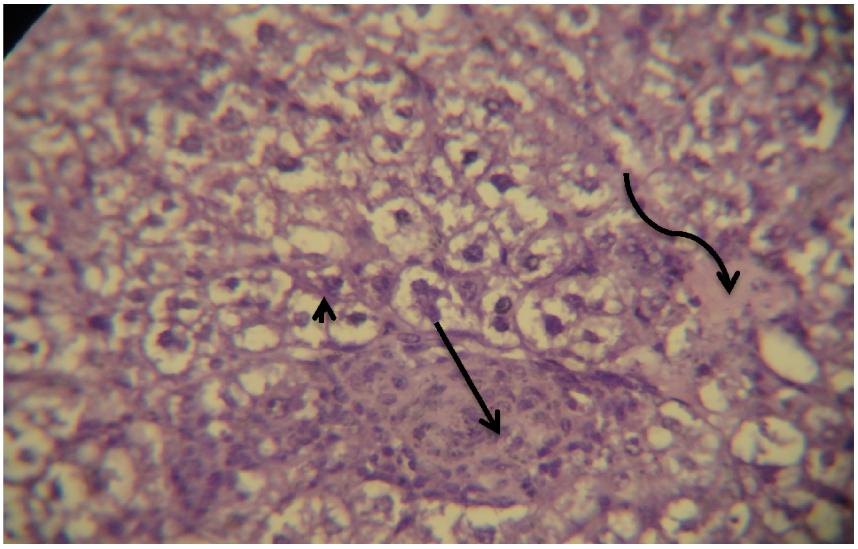

Fig. 1. Group1, showing granuloma and hydropic degeneration,hemorrhage in section of liver, X250.

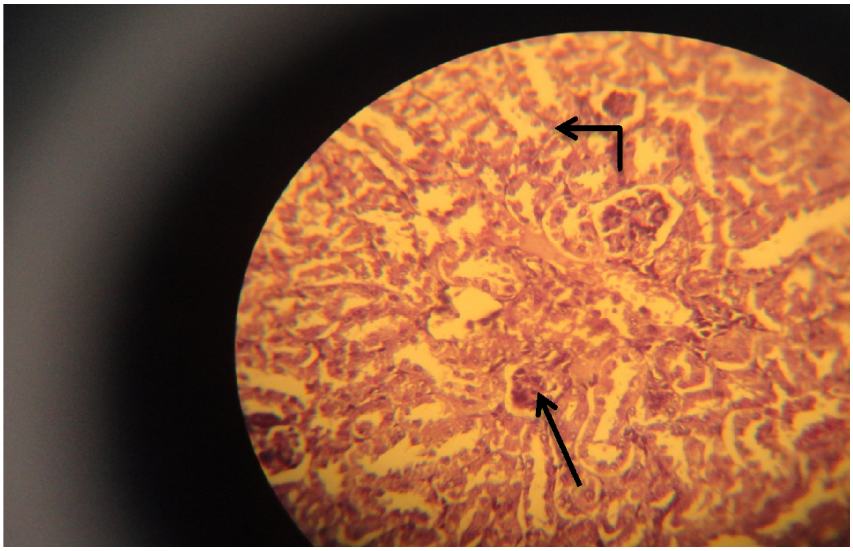

Fig. 2. Group 1, showing shrinking in glomerula, hemorhage and necrosis in section of kidney X250

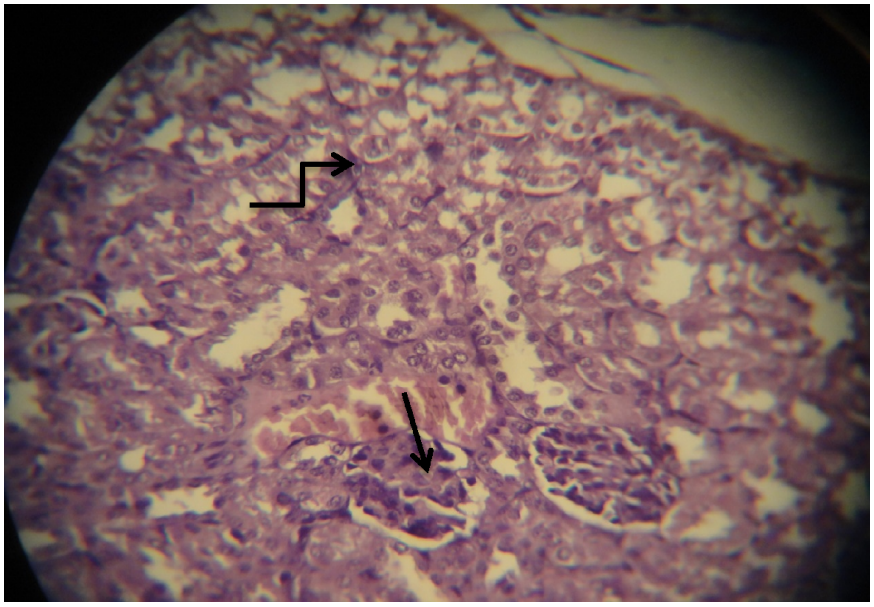

Fig. 3. Group 3, showing hemorrhage and hydropic degeneration in section of kidney X250

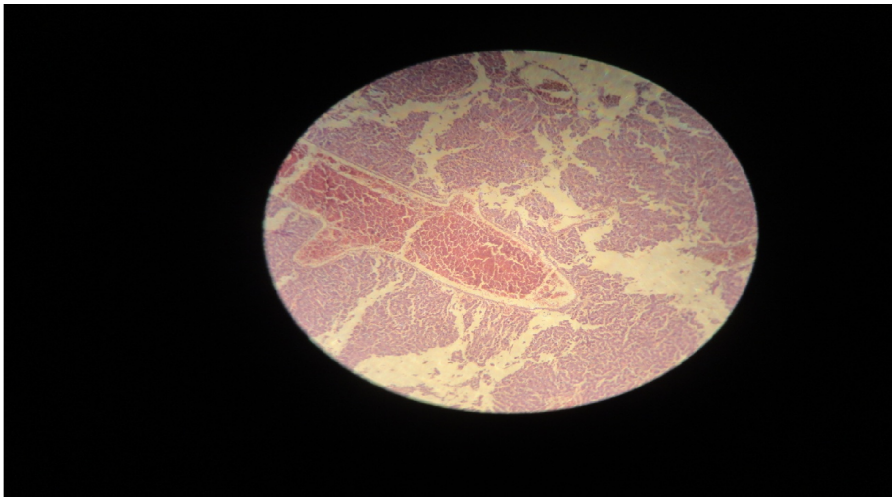

Fig. 4. Group 3, showing hemorrhage in Hepatic artery, necrosis and space in sinusoidal sinuses in section of liver

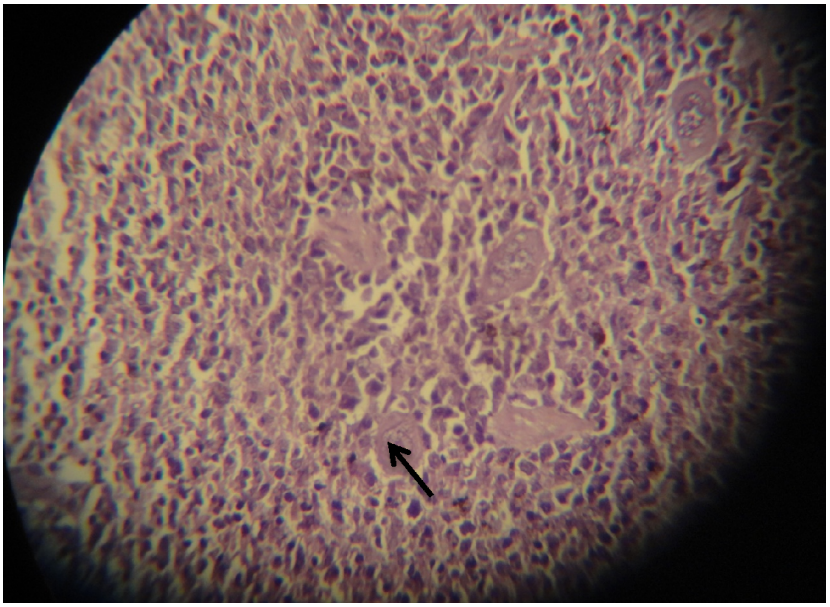

Fig. 5. Group 3, showing hydropic degeneration, hemorrahge, increase in number of megakaryocyte in section of spleen X250

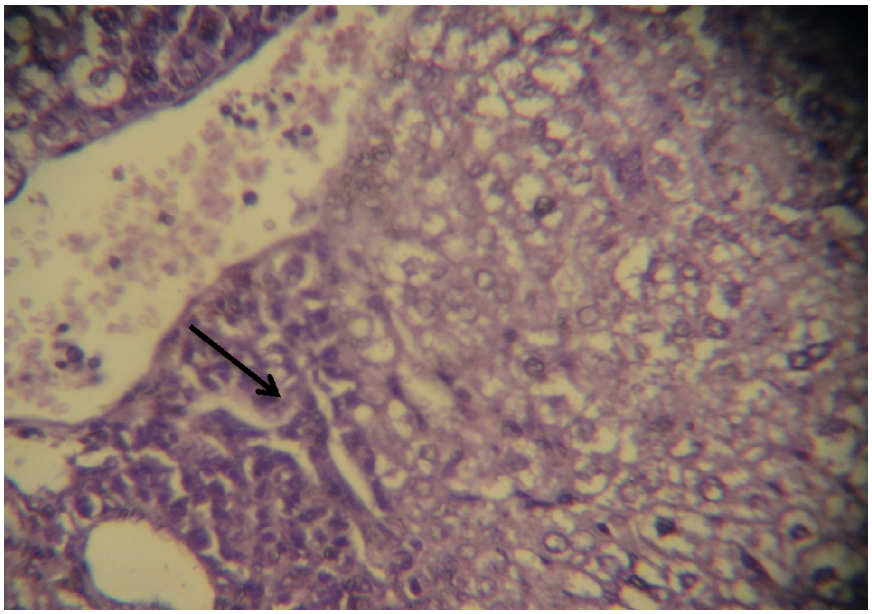

Fig. 6. Group 2, showing infiltration in lymphocyte and hydropic degeneration in section of liver X250

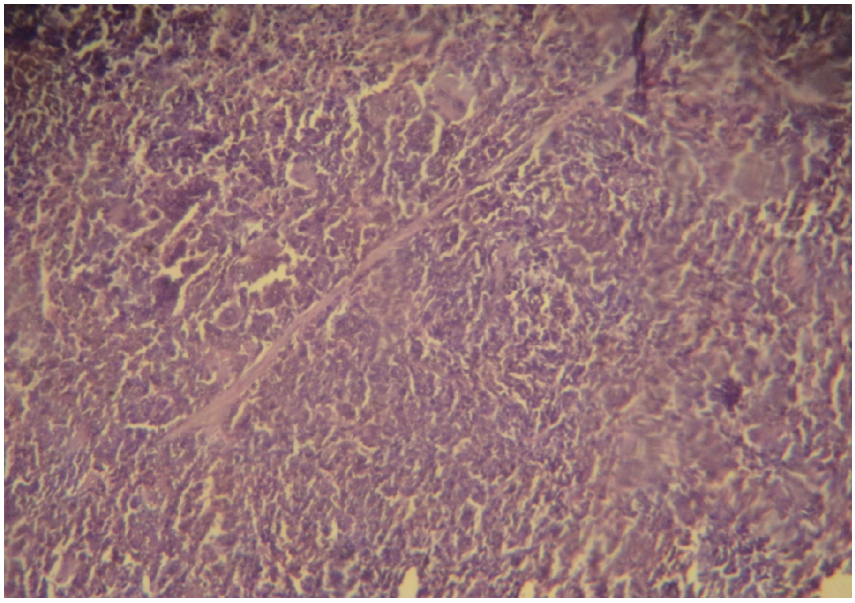

Fig. 7. Group 2, showing only simple infiltration of lymphocyte in section of spleen X250

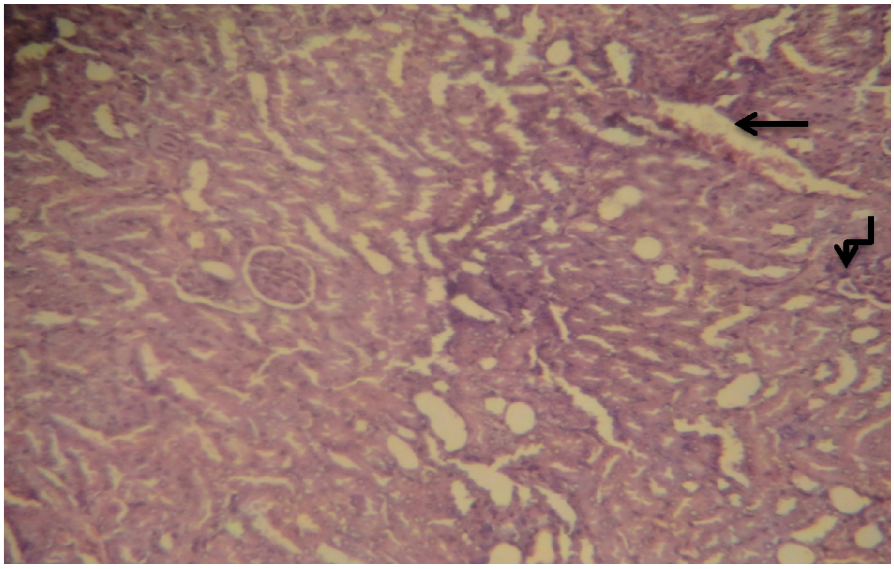

Fig. 8. Group 2, showing hemorrhage and infiltration of lymphocyte in section of kidney X250

The heapto protective activity of methanol extract of C. papaya Linn. leaves against paracetamol induced liver damage in rats was investigated in few studies [31], the results suggest that the heapto protective effect of C.papaya leaves might be contributed to its modulation on detoxification enzymes and its antioxidant and free radical scavenger effects. The extract of C. papaya leaves was investigated for its toxicity. the given doses even at the higher level (2000mg/kg) did not produce mortality or significant changes in body weight or food and water consumption. The investigated rats showed normal relative weights of the internal organs were observed and significant increases in hemoglobin, hematocrit, red blood cell [32]. Abdelgadir *et al*[33] showed that *C.papaya* is one of the most effective sources of natural medicine and widely used in pharmacological application it is used to treat several diseases such as tumors, nervous pain, asthma and wounds.

In this study the aqueous extract of the papaya was shown to have a significant anticandidal activity in vitro and in a mice model inoculated with *C.albicans* (*in vivo*) followed by the i.p of the aqueous extract of papaya indicating a strong antifungal activity. the extract gave a good effect on the reduction of the histomorphological changed in liver spleen and kidney.

4 CONCLUSION

The seed aqueous extract of papaya possesses *significant in vitro* and *in vivo* antifungal activity again *Candida albicans* and therefor it could be used in the treatment of *C.albicans* infection.

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