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Antifungal and Hepatoprotective effects of Lactoferrin purified from camel milk against *Candida albicans: in vitro* and *in vivo* studies

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Abstract

Candida albicans is the most common human fungal pathogen that causes significant morbidity and mortality worldwide. This work aims to purify the lactoferrin (LF) from camel milk, then evaluating its antifungal and hepatoprotective effects of the lactoferrin (LF) against *Candida albicans*. LF was purified from camel milk by CM-Sephadex and Sephadex G-200, respectively. The purity of LF to homogeneity was examined using denatured polyacrylamide gel electrophoresis with single band at molecular weight 78 kDa. *In vitro* study demonstrated notable growth inhibition of LF against a *C. albicans* isolate as indicated by measuring the mean diameter of the inhibition zone. *In vivo* study in Swiss mice received purified LF for 7 days before infection with *C. albicans* presented normal liver enzymes and returns liver function and histology to normal levels. Furthermore, pretreatment with LF significantly increased serum total antioxidant activity and decreased liver tissue malondialdehyde level as compared with the infected untreated group. In conclusion, LF pretreatment can alleviate oxidative stress and liver injury associated with *Candida albicans* infection in mice. Thus, the results suggest that LF is able to induce a protective immune response against *Candida albicans* infection.

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Introduction

Candida albicans (C. albicans) is a dimorphic fungus that grows both as yeast and filamentous cells and one of the few species of the candida genus that cause the infection. *Candida albicans* species belong to candida genus, Saccharomycetaceae family, Saccharomycetes order, Saccharomycetes class, Ascomycota division and Fungi kingdom, it is an opportunistic pathogen that causes superficial and systemic infections (Selvaraj *et al.*, 2014). *C. albicans* is a pathogenic yeast, which forms a range of polarized and expanded cell shapes (Canonico *et al.*, 2014). It is the most common human fungal pathogen and causes significant morbidity and mortality worldwide. (Noble and Johnson, 2005; Kaufman *et al.*, 2014). It exists as a commensal organism in healthy individuals by colonizing several niches of the human body which includes skin, oral cavity, mucosal surfaces, vagina, and gastrointestinal tract (Larriba *et al.*, 2000).

An altered balance between the host immunity and *C. albicans* fungus, as in the case of immune compromised patients, is one of the leading causes of candidiasis in humans (Bodey, 1993). After entering the blood stream, the yeast cells can infect all internal organs and may cause life-threatening septicemia (Karkowska-Kuleta *et al.*, 2009).

Camel milk generally has white opaque color and a sweet sharp taste, but sometimes, depending on the eaten plant in the desert by the camel, it may be prone to salinity. Camel milk has antimicrobial, anti-cancer, anti-diabetic, and hypoallergic properties for which LF, immunoglobulins, lysozyme or vitamin C play a major role among its compounds (Keykanlu *et al.*, 2016).

Lactoferrin (LF) is an iron-binding glycoprotein that is present in saliva, milk and other exocrine secretions as well as in neutrophil granules. LF has a number of biological functions, including antimicrobial and immunomodulatory effects *in vitro* and *in vivo* (Tomita *et al.*, 2002). It has the ability to combat bacteria (Gram+ and Gram−), fungi, yeast, viruses, and parasites. This protein is a multi-functional protein due to the wide distribution of LF in various tissues and the positive charge on its network (Farnaud *et al.*, 2003).

Therefore, this study aims to isolate and purify Lf from camel milk, *in vitro* study of its antifugal activity and *in vivo* evaluation of its hepatoprotective effect against a pathogenic *C. albicans* in mice.

Materials and methods

Candida albicans culture preparation and growth condition

A registered isolate of lyophilized *C. albicans* (NCPF 8154) was used as the test organism. The species were identified by molecular and traditional methods (National Committee for Clinical Laboratory Standards (2002). They were deposited at Assuit University Mycological Center (AUMC), Assuit, Egypt. The growth is indicated by growth of white, soft, cream-colored colonies with yeasty odor, which were confirmed by gram staining reaction and germ tube test (Doughari and Peter, 2009).

In vitro suscptibitity testing using the agar diffusion method

A standard *in vitro* agar diffusion growth inhibition assay was used to evaluate the anticandidal activity of LF against *C. Albicans* comparing with amphotericin B as a reference drug. *C. albicans* was grown on sabarouaud agar medium 24 hr before testing. A suspension of isolates was prepared by inoculating sufficient inoculums were added until the turbidity was equal to 0.5 McFarland (10^6 colony-forming units ml^{-1}) standard (bioMerieux, Marcy Petoile, France). The yeast suspension was then streaked evenly with a cotton swab on Mueller-Hinton agar plates (9cm in diameter). Following inoculation and swabbing, a cork borer of 6mm diameters was used to create wells in each plate. 100μl LF and amphotericin B were pipetted into the wells. Plates were placed in an incubator at 28°C for 24hr. After the incubation period, zones of growth inhibition are measured as the diameter of clear zone appeared around each well (Doughari and Peter, 2009).

Determination of MIC using Broth microdilution assay

The CLSI M27-A3 (National Committee for Clinical Laboratory Standards, 2002) guidelines were followed.

A sterile microdilution plate (96-flat bottomed wells) was used for each strain so that 100µl of RPMI media was added to each well then another 100µl of LF (700µg/ml) was or Amphotricin B (100µg/ml) added to the first well in. Two-fold serial dilutions were made so that rows 1-10 will contain the series of in 100µl LF. 100µl of yeast suspension was added to each well. The 11th row, which served as the positive growth control contained 100µl of inoculum suspension and 100µl of free medium, whereas the 12th row, which served as negative control contained 200µl of RPMI broth only. Microdilution trays were incubated at 35ºC. Minimum inhibitory concentrations (MICs) were read after 4-7 days. MIC was defined as the point at which there was 100% inhibition of growth as compared with the growth control when read visually in the microtitre plates (Hammer *et al.,* 2003).

Purification lactoferin from camel milk

Camel milk samples were collected from a Camel farm in Ras Seder, Egypt. All lactating camels consumed the same type of food. The milk was collected in the morning in sterile screw bottles and kept on ice during transportation to the laboratory where milk bottles were stored at 4°C.

At first the cream was separated by centrifugation (4000×g, 30min at 4ºC). Then casein removed from skim milk in acidic condition with gradual addition of 1N HCl until pH reached at 4.6, then incubated at 40ºC for 30min, the precipitated casein was removed by centrifugation at 10000×g, 20min at 4ºC. The acid colostrum whey (supernatant) was neutralized to pH 6.8 with 1N NaOH and dialyzed against 20mM phosphate buffer (pH 7.7) for 18hr, and then stored at -18 C until use (Al- MashakhiA and Nakai, 1987).

Isolation and purification of LF involved cation exchange carboxymethy Sephadex (CM-Sephadex C-50) and gel filtration chromatography by using Sephadex G-200. (Yoshida *et al.*, 2000). LF has a cationic nature according to its amino acid composition thus it can be purified by cation using 0.05 M Tris HCl (pH 7.5) and linear gradient NaCl from 0.0 to 0.5 M.

During chromatography, protein in the eluents was monitored by ultraviolet absorption at 280nm with the instrument and at a wavelength of 465nm which specially detects for protein lactoferrin (Shimazaki *et al.,* 1992).

Purity and Molecular weight determination by electrophoresis were done by using polyacrylamide gel electrophoresis under denatured condition (SDS-PAGE) method. (Laemmli, 1970). Standard proteins with known molecular weights were used and LF molecular weight was calculated from drawing the relation between the logarithms of the molecular weight for standard proteins compared to its relative mobility (Weber and Osborn, 1969).

Animals And Experiment design

Specific pathogen-free, male Swiss mice, each weighing 28-32 gm obtained from a closed random-bred colony at the animal house, National Research Center. Faculty of medicine, Ain Shams university. Animals were housed in polycarbonate boxes with steel-wire tops (not more than five animals per cage). Ambient temperature was controlled at 22±3ºC with a relative humidity of 50±15% and a 12-hr light/dark photoperiod. Food and water were provided ad libitum.

Experimental protocols and procedures used in this study were approved by the Ain Shams University and Institutional Animal Care and Use Committee (IACUC) (Egypt) (CUFS/S/08/13). All the experimental procedures were carried out in accordance with international guidelines for the care and use of laboratory animals.

The standard inoculation of *Candida albicans* was cultured on Sabouraud agar and washed three times in sterile normal saline and adjusted to a concentration of $1x10^7$ viable cells/ml on a hemocytometer. On day 8 of *in vivo* study, the mice were infected intravenously with *C. albicans via* the lateral tail vein (Jothy *et al.,* 2012). Each inoculum consisted of 0.1ml of fungal suspension.

Forty mice were randomly assigned into four main groups (10 mice/group). The 1st group was treated with saline (N group), the 2nd group was infected with

C. albicans (IN group) the 3rd group was treated orally with LF (0.5g/kg/day) (LF group), (Takakura *et al.* 2003), 4th group treated 7 days with LF then on day 8 the mice were infected with *C. albicans* (LFI group). Animals were euthanized after being anesthetized with sodium pentobarbital and sacrificed after 24h of infection after being fasted overnight; blood was collected in EDTA and centrifuge tubes for haematological and biochemical parameters, respectively. Part of the liver was removed and immediately blotted using a filter paper to remove traces of blood and stored at -80°C for biochemical studies. However, the other part was suspended in 10 % formal saline for fixation preparatory to histopathological processing.

Hematological and Biochemical analyzes differential counting

Leukocytes were counted in all groups. For differential counting, smears were fixed for 5min in methanol and stained with May-Grümwald Giemsa for 15min.

Liver function enzymes

The degree of damage induced by *C. albicans* was quantified by determination of the activity of the hepatocyte-specific enzymes. Serum alanine transaminase (ALT) and aspartate transaminase (AST) activities were determined according to the method described by Reitman and Frankel (1957).

Oxidative stress marker assessment

The liver was homogenized (10% w/v) in ice-cold 0.1M phosphate buffer (pH 7.6). The homogenate was centrifuged at 3000 rpm for 15min at 4°C and the resultant supernatant was used for oxidative stress markers.

Lipid peroxidation was estimated as the amount of thiobarbituric acid reactive substances (TBARS) determined by the reaction of thiobarbituric acid (TBA) with malondialdehyde (MDA) as described by Ohkawa *et al.* (1979). Total antioxidant activity (TAA) was determined according to the method of Koracevic *et al.* (2001).

Histopathological preparation

The liver specimens were cleared in xylene and embedded in paraffin at 56°C in hot air oven for 24hr. Paraffin bees wax tissue blocks were prepared for sectioning at 4 microns by sledge microtome. The obtained tissue sections were collected on glass slides, deparaffinized and stained by hematoxylin and eosin (H&E) stains for histopathological examination using the electric light microscope (Bancroft *et al.,* 1996).

Statistical analysis

The statistical analyses in this study were performed using the Statistical Program for Social Sciences (SPSS) software 16.0 (SPSS Inc., Chicago, USA). All results were expressed as mean value ± standard error (S.E.). Statistical significance was tested among and between groups using the one-way analysis of variance (ANOVA) test.

Results

Isolation and purification of lactoferrin

The prepared colostrum whey was passed through CM-Sephadex C-50 column and eluted by 0.5M NaCl in 0.05M Tris HCl (pH 7.5) elution buffer as indicated by a single peak at the wavelength of 465nm which specially detects for protein lactoferrin (Fig. 1). The fractions of the peak were pooled and desalted by dialysis against 0.2M phosphate buffer (pH 7.7) and then concentrated by sucrose.

The concentrated solution containing LF was passed through Sephadex gel filteration G-200 and eluted as a single peak, according to its molecular weight at 280nm and 465nm (Fig. 2). The purity of purified LF was confirmed by PAGE as it gave a single protein band. The MW of the purified LF was calculated from calibration curve of standard proteins to be 78 kDa. (Fig. 3).

In vitro susceptibility test and MIC determination

Fig. (4), shows that lactoferrin (LF) exhibited a potent anti-candidal activity against *C. albicans* with inhibition zone diameter 25mm and MIC 125µg/m compared with the reference drug; Amphotericin B (AMB); (30 mm inhibition zone diameter and MIC 0.5µg/ml

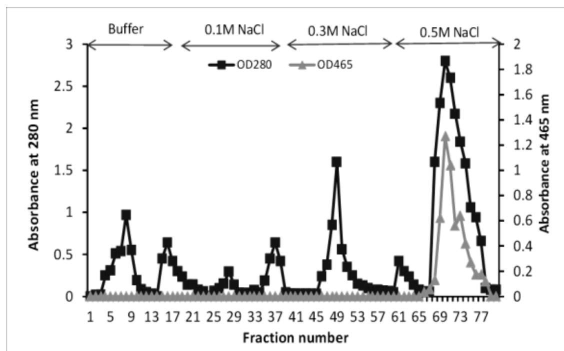

Fig. 1. Ion exchange chromatography for purification of lactoferrin using CM-Sephadex C50 (C1.6/20) column equilibrated with Tris-HCL buffer (50 mM, pH 7.5) eluted with Tris-HCL buffer with NaCl gradient (0-0.5M) in flow rate 30 ml/ hr, 2 ml for each fraction.

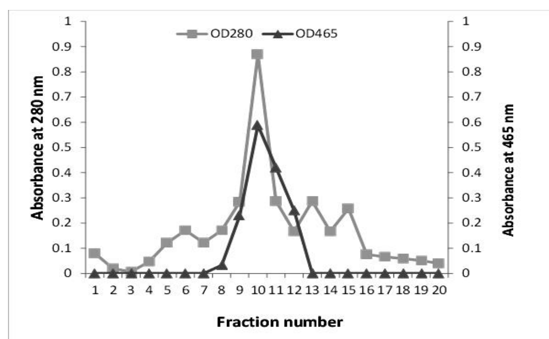

Fig. 2. Gel filteration chromatography for purification of lactoferrin using G-00 (C1.6/20) column equilibrated with phosphate buffer (50 mM, pH7.4) containing 0.01M NaCl in flow rate 30 ml/ hr, 2 ml for each fraction.

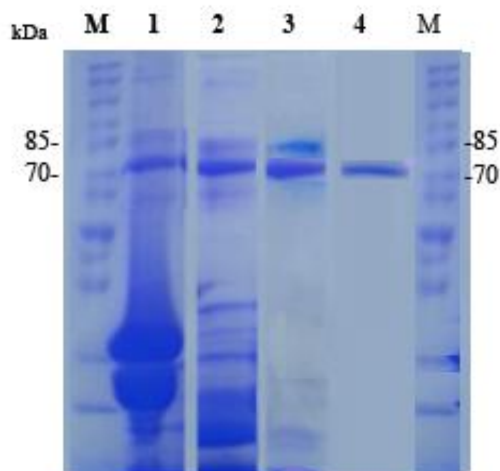

Fig. 3. Polyacrylamide gel electrophoresis of lactoferrin. Lane 1: protein marker, lane 2, crude milk, lane 3: colostum whey, lane 3: LF after purification on CM-Sephadex C-50, lane 4: LF after purification on Sephadex G-200.

Fig. 4. Anticandidal activity of lactoferrin using the well diffusion assay, a: Amphotericin B and b: Lactoferrin.

Hematological parameters

We evaluate the variation of hematological parameters. The leukocytes and platelets counting showed a significant decrease in an infected mice (IN group), compared to normal healthy controls (N group). Pretreatment with LF significantly increased the total leukocyte and platelet counting in both normal and LFI groups.

On the other hand, WBC differential count shows a formula inversion with an increase of neutrophils and the concomitant decrease of lymphocytes on LFI group, compared with infected control (Table 2). LF also increased eiosinophils and monocytes counting in healthy animals ($p<0.05$) as compared with the normal control group. There was no difference seen in the cell counts of basophils among any group of mice (Table 1). Values are expressed as means ± the standard error of the mean of 10 mice in each group. Values are statistically significant at * $P < 0.05$, compared to the control group (N). # $P < 0.05$, compared to the infected group (IN).

Liver enzymes

In this work, the protective effect of LF was investigated. Administration of LF 7 days before infection with *C. albicans* (1×10^7 yeast cells) prevented liver injury, as verified by normal AST and ALT activities as compared to a normal control group. In contrast to the infected control group there was a significant increase in AST and ALT activities as compared to a normal control group (Fig. 5). The lone administration of LF didn't generally causes any significant change in the activity levels.

Table 1. Effect of LF on total, differential leukocytes and platelet count.

Parameters	N	IN	LF	LFI
Total leukocytes (mm^3x10^3)	7.5±0.4	5.4±0.37*	11.7± 0.67*#	12.6±0.77*#
Neutrophils (%)	18.6±0.49	14±0.29*	10.25 ± .59*#	50.3±0.62*#
Eosinophils (%)	0	0	2.35 ± 0.07*#	0.64±0.037*#
Basophiles (%)	0	0	0	0
Lymphocytes (%)	70.7±4.3	83±2.18*	72±4.33#	38±3.9*#
Monocytes (%)	6.16±1.17	5.1±0.69	11.75±1.17*#	15±1.21*#
Platelet (mm^3x10^5)	3.45±0.198	2.06±0.15*	6.64±0.38*#	7.8±0.45*#

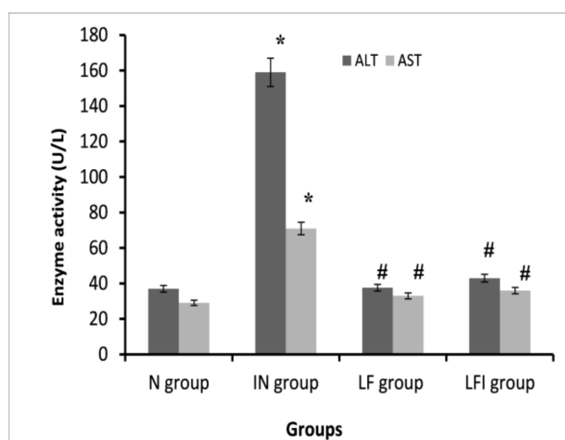

Fig. 5. Effect of lactoferrin *on C. albicans* -induced elevations in serum liver enzyme activities in mice. Values are expressed as means ± the standard error of the mean of 10 mice in each group. *P < 0.05, compared to the control group (N)# P < 0.05, compared to the infected group (IN). ALT, alanine transaminase; AST, aspartate transaminase; N, normal control group; IN, infected group; LF, lactoferrin group; LFI, lactoferrin plus *C. albicans* group.

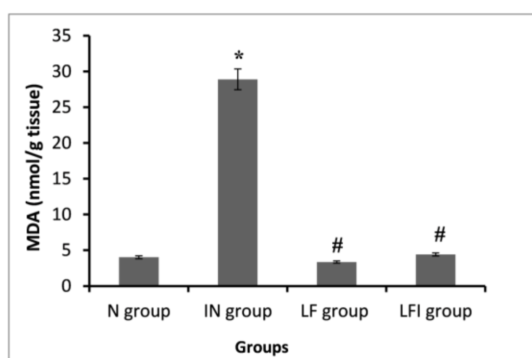

Fig. 6. Effect of lactoferrin on *C. albicans*-induced elevation of serum malondialdehyde (MDA) in mice. Values are expressed as means ± the standard error of the mean of 10 mice in each group. * P < 0.05, compared to the control group (N). # P < 0.05, compared to the infected group (IN). N, normal control group; IN, infected group; LF, lactoferrin group; LFI, lactoferrin plus *C. albicans* group.

Effect of the LF on oxidative stress markers

Oxidative stress resulting from the *C. Albicans* infection in the liver plays a critical role in damaging the liver. In order to evaluate the protective effect of LF against *c. albicans* induced oxidative stress in mice, the levels of both MDA and TAA were investigated. As shown in Fig.6, there was a significant increase (P < 0.05) in the MDA level of the infected mice as compared to normal control mice. Notably, there was no significant change in MDA levels of LF groups (LF&LFI) as compared to a normal control group (P >0.05).

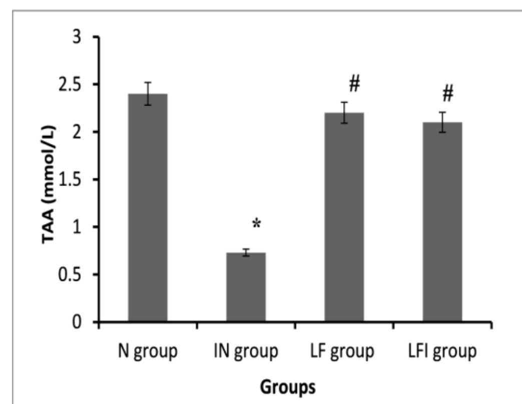

Fig. 7. Effect of lactoferrin on *C. albicans*-induced reduction in serum total antioxidant activity (TAA) in mice. Values are expressed as means ± the standard error of the mean of 10 mice in each group. * *P* < 0.05, compared to the control group (N). # *P* < 0.05, compared to the infected group (IN). N, normal control group; IN, infected group; LF, lactoferrin group; LFI, lactoferrin plus *C. albicans* group.

Fig. 7 shows that Candida infection in mice caused a significant decrease (P <0.05) in the TAA level, as compared to control mice. On the other hand, pretreatment with LF significantly (P< 0.05) increased the TAA level and restored near control level,

as compared with the infected untreated group. In contrast, no significant change in the levels of MDA or TAA was observed among mice receiving LF, alone. These results demonstrate the protective effect of the LF against *C. albicans*-induced oxidative stress and its ability to augment cellular antioxidant defenses.

Histopathological study

Liver sections of normal control and LF groups show normal histological structure of the central vein and surrounding hepatocytes (Fig.8 A, B & D, respectively). in the portal vein and inflammatory cell infiltration were observed between the degenerated hepatocytes (Fig. 8C)

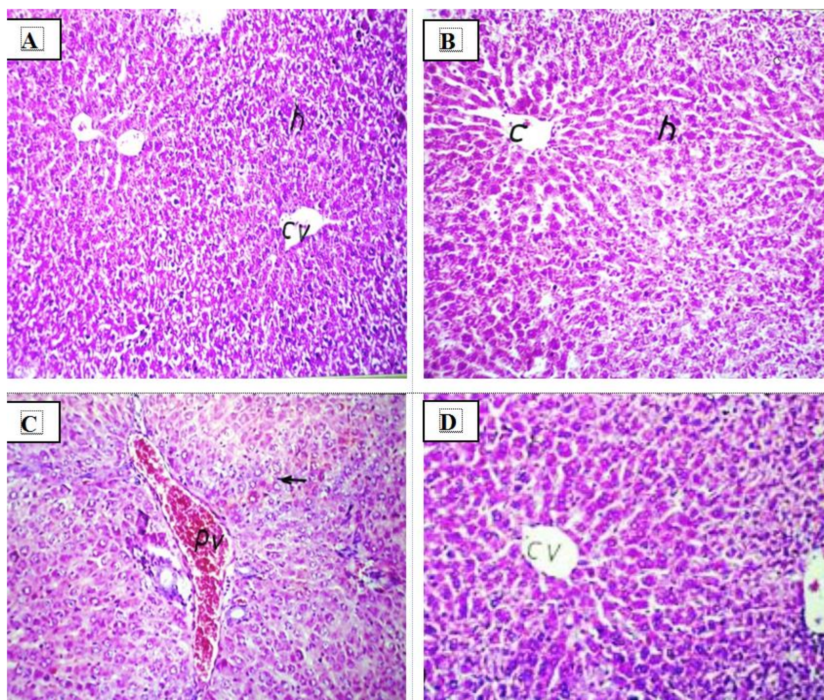

Fig. 8. Effect lactoferrin on *C. albicans*-induced histopathological changes in mice liver. Liver sections were stained with hematoxylin and eosin (H & E) and examined with a light microscope by × 400 magnification. (A) liver section of a mouse from; (B) represents liver section of a mouse from; (C) represents liver section of a mouse from the *C. albicans* group; (D) represents liver sections of rats from the lactoferrin plus *C. albicans* group. H-Hepatocytes; cv-central vein; pv-portal vein.

Discussion

Candida species are responsible for about 8% of all hospital-acquired bloodstream infections, and among these species is *Candida albicans*. Infection with pathogenic fungi increased dramatically over the past two decades. *C. albicans* causes >80% of all fungal infections and account for >20% of the fatal infections in patients with leukemia and for 13% of those in patients with lymphoma.

These infections range from thrush in immunocompetent colonized hosts to life-threatening systemic infections in immunocompromised individuals such as patients with cancer (Martchenko *et al.,* 2004; Kupfahl *et al.,* 2007).

The search for new antimicrobial agents is of great concern today, because of the increasing development of drug resistance to human pathogens and the appearance of undesirable effects of certain antifungal agents (Phongpaichit *et al.,* 2005). A multidisciplinary approach to drug discovery, involving the generation of truly novel molecular diversity from natural product sources, providing the best solution to the current productivity problems in the scientific society involved in drug discovery and development (Newman and Cragg, 2007).

The virulence of *C. albicans* seems to be multifactorial (Chauhan *et al.,* 2003), but the ability of this fungus to mount stress responses is an

important aspect, as this promotes survival in the host during systemic infections (d'Enfert and Hube, 2007). It was demonstrated that a large proportion of *C. albicans* cell surface antigens related to acute candidemia are involved in oxidative stress (Mochon *et al.,* 2010).

Our hematological analysis suggests that LF has immunostimulatory effects which are characterized by an increase in leukocyte, and platelet counts in healthy mice. Similar results by Shabbir *et al.* (2016), they reported that treatment with Guava extract showed a significant increase in the counts of WBC and platelet, as compared with the control group. It has been reported that oral administration of human LF enhances murine peyer's patches to secrete IgA and IgG (Debbabi *et al.* 1998).

Moreover, previous study demonstrated that oral administration of LF and LF-derived peptides, one day before the infection reduced the severity of oral candidiasis in a mouse model. (Takakura *et al.* 2003). Also It has been reported that LF up-regulates the anti-inflammatory cytokines and down-regulates the proinflammatory cytokines. (Sorimachi *et al.* 1997). It is well known that macrophages and neutrophils are stimulated by TNF-α and IFN- γ that directly kill *C. albicans* (Crouch *et al.* 1992; Tansho *et al.* 1994).

Neutrophils are the components of the immune system and help in the killing of microorganisms. Severe and prolonged neutropenia increases the susceptibility to fungal and bacterial infections. Monocytes and macrophages are important in commencement and resolution of inflammation through the release of cytokines and activation of the acquired immune system. They play a vital role in wound healing, cancer progression, tissue homeostasis, atherosclerosis and arthritis (Parihar *et al.*, 2010). Lymphocytes are a crucial player of immune response and are subdivided into T cells, B cells and NK cells. Among these cells, NK cells are responsible for mounting an innate immune response, while T cells and B cells are involved in the adaptive immune response (Slifka *et al.*, 2000).

Platelets are also immune cells that have the potential to start and speed up many vascular inflammatory disorders like transplant rejection, atherosclerosis, rheumatoid arthritis, and malaria infection (Morrell *et al.*, 2014).

In the present study there are significant decreased in leukocyte, and platelets counts, which due to *C. albicans* induce immunosuppression through Toll-like receptors (TLR2)-mediated IL-10 release, and this leads to generation of CD4+CD25+T-regulatory cells with immunosuppressive potential. (Blanco *et al.,* 2008). Moreover, an increase of neutrophils and the concomitant decrease of lymphocytes in LFI group is in agreement with previous data that neutrophils are the primary effector cells in preventing infection by *Candida albicans* and *Aspergillus fumigatus* (Aerts *et al.,* 2008).

At early stages of the infection, the liver constitutes the first barrier for the control of fungal spreading. The ability of this organ to limit the growth of the yeast and to mount an efficient inflammatory reaction is crucial in determining the outcome of the fungal infection (Correa *et al.*, 2004; Fahmy *et al.,* 2014). *C. albicans* was disseminated to liver following intravenous injection causing an intense inflammation. The organisms reach the liver through different pathways, including ascending infection in the biliary tract, vascular seeding, either arterial or portal, invasion of the liver from a nearby source and a penetrating injury (Kumar *et al.*, 2008).

In the assessment of liver damage, the determination of enzyme levels, such as serum AST and ALT is largely used. High levels of serum AST indicate liver damage, such as that due to viral hepatitis as well as cardiac infraction and muscle injury. Serum ALT is more specific to the liver, and is thus a better parameter for detecting liver injury (Williamson *et al.*, 1996). Results from this study demonstrate that *C. albicans* induces severe hepatic damage as represented by the markedly elevated levels of the hepatic enzymes and by the marked histopathological alterations, in agreement with previous reports

demonstrating that *C. albicans* causes acute hepatocyte injuries with a consequent increase of ALT in the plasma occurred. (Martino *et al.*, 2011). Pretreatment with LF significantly maintain the normal levels of the hepatic enzymes and the histopathology of the liver.

Phagocytic cells are the first line of defense against fungal infections. These cells generate reactive oxygen species (ROS), including superoxide, hydrogen peroxide (H_2O_2), and hydroxyl radicals, that can damage all biomolecules and destroy phagocytosed pathogens. ROS are also byproducts of normal aerobic metabolism, and all aerobic organisms possess mechanisms to maintain very low levels of these species (Soliman *et al.*, 2015). The superoxide radicals are known to inactivate [4Fe-4S] cluster-containing enzymes by oxidizing one iron and releasing it from the cluster. (Liochev and Fridovich, 1994; Fridovich, 1995). Free iron can react with hydrogen peroxide to generate toxic hydroxyl radicals (OH^-) by Fenton chemistry. (Meneghini, 1997). The hydroxyl and superoxide radicals react with cellular components, resulting in oxidation of proteins and nucleic acids as well as lipid peroxidation. These effects can lead to inactivation of enzymes, disruption of membranes, mutations, and ultimately cell death. The lipid peroxidation was assessed on the basis of malondialdehyde (MDA) estimation. It has been reported that the increased MDA level suggests enhanced lipid peroxidation leading to tissue damage and failure of antioxidant defense mechanisms to prevent formation of excessive free radicals. (Park *et al.,* 2010). MDA level of infected mice with *C. albicans* increased significantly. The results of Zgai and Chhibber (2010) and Mahmoud *et al.* (2011) are consistent with the present finding. The decreased level of MDA of pretreated mice with LF may be due to the binding of LF to free iron, preventing formation of hydroxyl radicals, which inhibits lipid peroxidation of phosphatidyl choline and linoleate liposomes (Ozcelik *et al.*, 2014).

In the present study pretreatment with LF significantly decreased MDA levels, suggesting that the hepatic curative effect of it against *C. albicans* oxidative stress-induced injury might be involved in decreasing lipid peroxide generation and stimulating antioxidant enzyme.

Measuring serum TAA may help in the evaluation of physiological, environmental, and nutritional factors of the redox status in humans. Determining serum TAA may help to identify conditions affecting oxidative status *in vivo*. The measure of TAA considers the cumulative action of all the antioxidants present in serum and body fluids, thus providing an integrated parameter rather than the simple sum of measurable antioxidants. The capacity of known and unknown antioxidants and their synergistic interaction is therefore assessed, thus giving an insight into the delicate balance *in vivo* between oxidants and antioxidants. (Bahrami *et al.*, 2016)

It was reported that great loss of SOD activity, which is the first defense line against oxidative stress, occurs with enhanced production of oxygen radicals by inflammatory cells. (Comhair *et al.*, 2000). Superfluous free radicals may damage protein and nucleic acids, and induces lipid peroxidation to produce large amounts of MDA that injures cells and results in disequilibrium of the internal environment and diseases. Consequently, SOD activity and MDA levels can reflect the antioxidant ability of the body (Liu *et al.*, 2011).

These are consistent with the results presented in this study that showed a significant elevation in the level of MDA with a concomitant decrease in the level of TAA in infected untreated mice, implying a down-regulation for numerous enzymatic oxidation reactions in the cell. Notably, pre-administration of LF could maintain the normal levels of MDA and TAA, indicating that the hepatoprotective effects of LF might be due to their ability to protect biomembranes against lipid peroxidation and to augment cellular antioxidant defenses.

During both superficial and systemic infections, *C. albicans* relies on a battery of virulence factors and fitness attributes.

The capacity of *C. albicans* to form biofilms on host cells is an important virulence factor. Biofilms form in a sequential process, including adherence of yeast cells to the substrate, proliferation of these yeast cells, formation of hyphal cells in the upper part of the biofilm, accumulation of extracellular matrix material and, finally, dispersion of yeast cells from the biofilm complex. Mature biofilms are much more resistant to antimicrobial agents and host immune factors in comparison to planktonic cells. Additionally, fitness attributes include powerful nutrient acquisition systems like trace metals which metals are essential for the growth and survival of all living organisms, including humans, animals, plants, bacteria and fungi. The most widely investigated transition metal with regard to pathogenesis *C. albicans* is iron (Mayer *et al.*, 2013).

It was reported that human lactoferrin plays a role in the host defense by (1) releasing lactoferricin during pepsinolysis and various smaller N-terminal peptides with potent antimicrobial activities and (2) modulating immune responses, affecting the production of reactive oxygen intermediates and cytokines by phagocytes and T lymphocytes. Furthermore, human LF triggers the production of antimicrobial peptides by mucosal epithelial cells, and it is able to prevent biofilm formation (Lupetti *et al.,* 2007).

Conclusion

The present study demonstrates good *in vitro* and *in vivo* anticandidal activity of camel LF. The pretreatment of LF to mice for 7 days before infection could protect mice liver from the severity of infection when compared to infected untreated mice. LF plays an important role in combating the infection, LF has immunostimulatory effects on immune system which characterized by increase in total and differential leukocyte counts, and platlet count, The lack of toxicity in addition to the immunomodulatory action becomes LF a potential safe treatment of disseminated candidiasis.

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