Saudi Pharmaceutical Journal 26 (2018) 349–357

Contents lists available at ScienceDirect

Saudi Pharmaceutical Journal

journal homepage: www.sciencedirect.com

Original article

Modulation of Cyp450, ALS1 and COX-2 signaling pathways induced by *Candida albicans* infection via novel antifungal agents

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ARTICLE INFO

Article history:
Received 26 November 2017
Accepted 22 January 2018
Available online 1 February 2018

Keywords:
Candida albicans
Fluconazole
Cyp450
ALS1
COX-2

ABSTRACT

Although, fluconazole is widely used in clinical treatment as an antifungal drug, it recorded potential problems as resistance and intracellular accumulation. Female albino mice were injected with single *ip* dose of *Candida albicans* (1.5×10^6 CFU). Three weeks post treatment with fluconazole and two novel synthesized compounds [(2-(4-(Pyridin-2-yl) aminosulfonylphenylamino)-6-(naphthalen-2-yl)-4-(pyridin-2-yl) pyridine-3carbonitrile) and (2-(4-(Pyrimidin-2-yl) aminosulfonylphenylamino)-6-(naphthalen-2-yl)-4-(pyridine-2-yl)pyridine-3-carbonitrile) (13b & 14b, respectively)] in both low and high doses (50 mg/kg & 200 mg/kg), liver function and vaginal inflammation were assessed. *Candida albicans* significantly elevated serum alanine aminotransferase (ALT) and butrylcholinesterase (BCHE) as well as hepatic malondialdehyde (MDA). Molecular analysis confirmed a significant up-regulation in mRNA gene expression of Agglutinin-like sequence (ALS1), hepatic cytochrome p450 (Cyp450). Vaginal COX-2 gene expression was also elevated. Nevertheless, a significant down-regulation was apparent in mice treated with the aforementioned compounds. Meanwhile, administration of 14b in a high dose noticeably down-regulated the altered parameters expression showing a significant effect in comparison to animals treated with the variable doses of the tested compounds. Histopathological finding confirmed the obtained results. The current work investigated the efficiency of new synthetic pyrimidine derivatives 14bas anti-microbial agents and recommended to be improved and evaluated as a novel antifungal drug to overcome the emergence of resistance problem.

1. Introduction

Candida albicans (*C. albicans*) exist as a normal constituent of the human mucosal microbial ecology. However, inherited or acquired immunodeficiency syndromes in addition to other factors, such as antibiotics, may cause perturbations in the local mucosal immune environment then develop mucosal candida infections and systemic candidiasis due to translocation of yeast from mucosal surfaces into the systemic circulation (Vazquez and Sobel, 2002). The most common forms of mucosal candidiasis are oropharyngeal candidiasis (OPC) and vulvovaginal candidiasis (VVC) (Smeekens et al., 2013). Human mucosal candidiasis has a substantial global disease burden where, the majority of HIV-infected patients develop oral mucosal candidiasis (de Repentigny et al., 2004). Candidiasis reports declared that approximately 75% of healthy reproductive age women develop at least one episode of VVC during their life time (Sobel, 1997).

The largest family of antifungal drugs is azole family that disrupts cell membrane of fungi through inhibiting lanosterol 14-α-demethylase activity (Hof, 2006). Previously, fluconazole has been used extensively for chemoprophylaxis and treatment of systemic fungal infections (Hoffman et al., 2000). Meanwhile, fluconazole resistance has been declared in most of the patients (Redding et al., 2003). Furthermore, some of azole compounds are

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Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

https://doi.org/10.1016/j.jsps.2018.01.007
1319-0164/

hepatotoxic, however the specific mechanisms of azole-induced toxicity are not known. Many N-substituted azoles induce and/or inhibit mammalian hepatic cytochrome P450s (Cyps) (Sun et al., 2006). Cyps are responsible for the metabolism of endogenous (steroids) as well as exogenous compounds including drugs and xenobiotic chemicals. This metabolism can lead to metabolic activation to toxic or carcinogenic metabolites. Generally, inhibition of Cyp isoforms can decrease metabolism and hepatic clearance of substrates metabolized by that specific isoform (Kobayashi et al., 2002).

Agglutinin-like sequence (ALS) gene is well-characterized gene family in candidiasis due to its importance in pathogenicity of *C albicans*. Previously, *C. albicans* ALS1 gene product was reported as cell surface protein that mediates adherence to endothelial and epithelial cells in vitro and during bio-film development (Fu et al., 2002). Evaluation of *C. albicans* ALS proteins in human oral epithelial cell interactions was previously detected (Fu et al., 2002; Hoyer et al., 2008). Moreover, previous correlation between *C. albicans* and COX-2 was also investigated (Athikomkulchai et al., 2006).

Regarding high cost associated with mucosal candidiasis and emerging resistance of Candida spp. to available antifungal agents that limit therapeutic options novel antifungal approaches have attracted the interest of many researchers in this field (Marchaim et al., 2012)

Recently, two novel compounds 2-(4-(Pyridin-2-yl) aminosulfo nylphenylamino)-6-(naphthalen-2-yl)-4-(pyridin-2-yl) pyridine-3 carbonitrile and 2-(4-(Pyrimidin-2-yl) aminosulfonylphenyla mino)-6-(naphthalen-2-yl)-4-(pyridine-2-yl) pyridine-3-carbonitrile (13b & 14b respectively) were synthesized (Kotb et al., 2015). These compounds have been characterized and demonstrated antimicrobial evaluation in vitro, especially against *C. albicans* (Kotb et al., 2015).

The main aim of the current study is to investigate more efficient and less toxic synthetic compounds for *C. albicans* treatment via two target new synthesized compounds (13b & 14b) were estimated as anti-fungal agents in vivo.

The current study extends to demonstrate the safety margin in new synthetic compounds for different organs and comparing toxicity percentage of these compounds with fluconazole which is the most common reference drug in marketing.

2. Material and methods

2.1. Chemicals

Fluconazole was provided from Pfizer Company, Egypt. Kits used for the determination of anti-inflammatory and oxidative stress biomarkers were obtained from Randox Company (UK). Kits for RT-PCR determination of cyp450, ALS1and COX-2were provided from R & D systems (MN, USA). Primers used in real time-PCR were purchased from Shine Gene (China). All other chemicals were of the highest analytical grade.

2.2. Characterization of new synthetic compounds

13b and 14b compounds were synthesized and characterized in a previously published article by Kotb et al. (2015). In addition to significant antifungal effect in vitro study was also observed against *C. albicans.*

2.3. Isolation of C. albicans

C. albicans ATCC 36082 was purchased from the Egyptian Type Culture Collection, Microbiology Department, Cairo University, Egypt), then used throughout the study. The organism was maintained on Sabouraud dextrose agar (BBL Microbiology Systems, Cockeysville, Md.) at 4 °C until use. For each study, two to three colonies of the fungus were sub cultured onto fresh potato dextrose agar (BBL), and the plates were incubated at 35 °C for 48 h. A fungal suspension was prepared by transferring three to four colonies of *C. albicans* to 10 ml of sterile, pyrogen-free normal saline "0.9% sodium chloride" (Baxter Inc., Chicago, Ill.) and was quantified by hemocytometry. The suspension was diluted with normal saline to a final concentration of 1.5×10^6organisms per ml. Morphologic examination revealed that >95% of the organisms were blastoconidia. The viability of the yeast was >90% by trypan blue exclusion analysis (Louie et al., 1998).

2.4. Animals

Female albino mice, weighing 18–22 gm, obtained from the animal house of National Research Center were used in this study. Animals were housed in cages kept at standardized conditions (2 2 ± 5 °C, 55 ± 5% humidity, and 12 h light/dark cycle). They were allowed free access to water and pelleted standard chow diet. All procedures relating to animal care and treatments strictly adhered to the ethical procedures and policies approved by Animal Care and Use Committee of National Research Center (12-038), and complied with the Guide for Care and Use of Laboratory published by the US National Institute of Health.

2.5. Experimental design

After one week of acclimatization, animals were randomly divided into 8 groups (10 mice each)

Group 1: Received DMSO and served as negative control.
Groups 2 to 8: Animals were infected with *C. albicans,* intraperitoneally 0.5 ml of the previously prepared concentration (1.5×10^6 organisms per ml). After 2 days post – infection, the following was applied (Louie et al., 1998)
Group 2 (Candida group): +ve control *C. albicans* infected animals were left untreated.
Group 3: *C. albicans* infected animals were treated with low dose (50 mg/kg/day; as previously described by Sun et al. (2006) of reference antifungal drug (fluconazole) for consecutive three weeks.
Group 4: *C. albicans* infected animals were treated with low dose (50 mg/kg/day) of new synthesized chemical compound (13b) for consecutive three weeks (Sun et al., 2006)
Group 5: *C. albicans* infected animals were treated with low dose (50 mg/kg/day) of new synthesized chemical compound (14b) for consecutive three weeks (Kotb et al., 2015).
Group 6: *C. albicans* infected animals were treated with high dose (200 mg/kg/day) of fluconazole for consecutive three weeks (Sun et al., 2006).
Group 7: *C. albicans* infected animals were treated with high dose (200 mg/kg/day) of (13b) for consecutive three weeks (Kotb et al., 2015).
Group 8: *C. albicans* infected animals were treated with high dose (200 mg/kg/day) of (14b) for consecutive three weeks (Kotb et al., 2015).
Note: All utilized drugs (fluconazole, 13b and 14b) were dissolved in DMSO as a solvent.

2.6. Sample preparation

At the end of the experiment all groups were sacrificed and blood samples were taken from each animal by puncture of the sublingual vein into sterilized tubes and let stand for 10 min to

clot. Serum was separated by centrifugation at 3000 rpm for 10 min and kept at −80 °C for further terminations. In the same time, vagina and liver were removed carefully then rinsed with cold saline (0.9% sodium chloride), and homogenized in 50 mM phosphate buffer, pH 7.4 (1:5 w/v). The homogenate was centrifuged at 3000 rpm for 10 min at 4 °C to separate cell debris. The supernatant was stored at −80 °C for subsequent biochemical determinations.

2.7. Microbiological examination

Sections from liver and vaginal tissue were weighed, suspended in 2.0 ml of saline solution and homogenized by a motor driven homogenizer. Undiluted and 1000-fold diluted 0.1-ml samples of organ homogenates were streaked onto plates of Sabouraud dextrose agar and incubated at 35 °C for 48 h. The number of colonies was counted, and the number of viable organisms per ml was determined. The homogenates were also applied to the surface of hemocytometer glass slides and examined microscopically (Louie et al., 1998).

2.8. Biochemical analysis

2.8.1. Serum aspartate aminotransferases (AST) and alanine transaminase (ALT) activity

AST and ALT activity were estimated spectrophotometrically using commercially available kits provided from Randox Company (United Kingdom, Antrim; AS2359). In brief, in presence of AST, L-aspartate forms oxaloacetate and L-glutamate. In alkaline solution, the formed oxaloacetate reacts with 2,4-dinitrophenyl hydrazine to give the hydrazone derivative that can be measured at 540 nm (Reitman and Frankel, 1957).

2.8.2. Hepatic malondialdehyde (MDA) level

MDA, as an index of lipid peroxidation, was measured using kit provided by Randox Company (Antrim, UK). MDA reacts with thiobarbituric acid (TBA) in acid medium giving a pink-colored complex that can be measured spectrophotometrically at 520 nm and 535 nm, using 1, 1, 3, 3-tetramethoxy propane as standard (Ohkawa et al., 1979)

2.8.3. Hepatic butyrylcholinesterase (BCHE) activity

BCHE was estimated spectrophotometrically in homogenate using commercially available kits provided from Randox Company.

2.9. Molecular analysis (real time PCR)

Target genes expression analyses were detected using real-time PCR according to specific forward and reverse primers for Cyp 450, ALS1 and COX-2 (all primers sequence are listed in Table 1). Firstly total RNA was extracted from liver and vagina samples using SV total RNA isolation system (Promega, Madison, WI), then Extracted RNA reverse transcribed into cDNA and amplified by PCR using RT-PCR kit (Stratagene, USA). Reactions were performed in a 50 μL final volume (25 μL SYBR Green Mix (2x), 0.5 μL cDNA, 2 μL primer pair mix (5 pmol/μLeach primer), 22.5 μL H_2O). PCR reaction was: 50 °C for 2 min (1 cycle), 95 °C for 10 min (1 cycle) and 95 °C for 15 s, 45 to 60 °C for 30 s (according to optimum annealing temperature for each gene) and 72°C for 30 s (40 cycles) then 72°C for 10 min (1 cycle). Data from the real-time assays were calculated by Sequence Detection Software version 1.7 (PE Biosystems, Foster City, CA, USA) (Tago et al., 2001; Sun et al., 2006; Murciano et al., 2012).

2.10. Histopathlogical examination

Paraffin embedded samples were prepared for sectioning at 4-μm thickness. Slides were stained with hematoxylin and eosin and examined by light microscope (Bancroft and Stevens, 1996).

2.11. Statistical analysis

Data were expressed as means ± SEM. Statistical analysis was performed using Instat-3 computer program (Graph Pad Software Inc., San Diego, CA). One-way analysis of variance was performed by SPSS 12program followed by post hoc test. The level of significance was set at $p < .05$ using Tukey's test.

3. Results

3.1. C. albicans viability in target organs

As demonstrated in Fig. 1, *C. albicans* count was significantly high in the candida group. After the treatment with the three drugs, the progression of the fungi was mitigated. In liver tissues it was clear that 14b treated group displayed the most pronounced effect in reducing candida count. On the other hand, the reference drug fluconazole declared the most significant improvement in the vaginal tissue treatment as compared to the other groups. Moreover, 14b treated group high dose nearly approached the value of the fluconazole treated group.

3.2. Inhibition of Candida-induced liver injury

As shown in Fig. 2, candida infection significantly increased serum ALT level by 180.8% as compared to the control value. On the other hand, in groups treated with fluconazole, 13b and 14b either low or high doses the level of liver enzyme was comparatively lower than that of Candida intoxicated group, implying the possible therapeutic effects of these agents on liver injury. Interestingly, the tested parameter was reverted back to near normal when 14b compound was administrated in a high dose. Data represented in Fig. 2 declared significant elevation in serum AST level by almost 234%. However, non significant modulation was declared within all groups.

3.3. Modulation of oxidative stress biomarkers

C. albicans infection induced a state of oxidative stress evidenced by an elevation in hepatic MDA along with an increment of butyrlcholinesterase level reaching 216.8%and 520.5%, respectively as compared to the control value (Fig. 3). Administration offluconazole, 13b and 14b at low dose significantly reduced MDA values as compared to Candida group. Obviously, fluconazole high dose and 14b regimens reduced MDA and butyrlcholinesterase level by almost 3-fold relative to candida group, displaying thus the most pronounced effect.

3.4. Modulation of liver inflammation

Data recorded in Fig. 4 declared that *C. albicans* infection caused a significant up-regulation in mRNA gene expression of ALS1 by almost 8 folds as compared with the −ve control group. On the other hand, a significant down regulation was demonstrated in all treated groups. Besides, 14b high dose regimen recorded the most significant reduction reaching the normal value (see Fig. 4).

The data in Fig. 4 indicated that *C. albicans* infection caused a significant up-regulation in mRNA gene expression of Cyp450 by almost 14 folds as compared to the control value. Nevertheless, a

Table 1
Oligonucleotide primer sequence of Cyp450, ALS1, COX-2 and β-actin (ACT1).

Gene name	Forward primer	Reverse primer
Cyp450	5′AAGCGCTTCGGGCCAG3′	5′TAGCCATGCAGGACCACGA3′
ALS1	5′CCTATCTGACTAAGACTGCACC3′	5′ACAGTTGGATTTGGCAGTGGA3′
COX-2	5′CAAGGGAGTCTGGAACATTG3′	5′ACCCAGGTCCTCGCTTATGA3′
β-actin (ACT1)	5′CGTTGTTCCAATTTACGCTGGT3′	5′TGTTCGAAATCCAAAGCAACG′

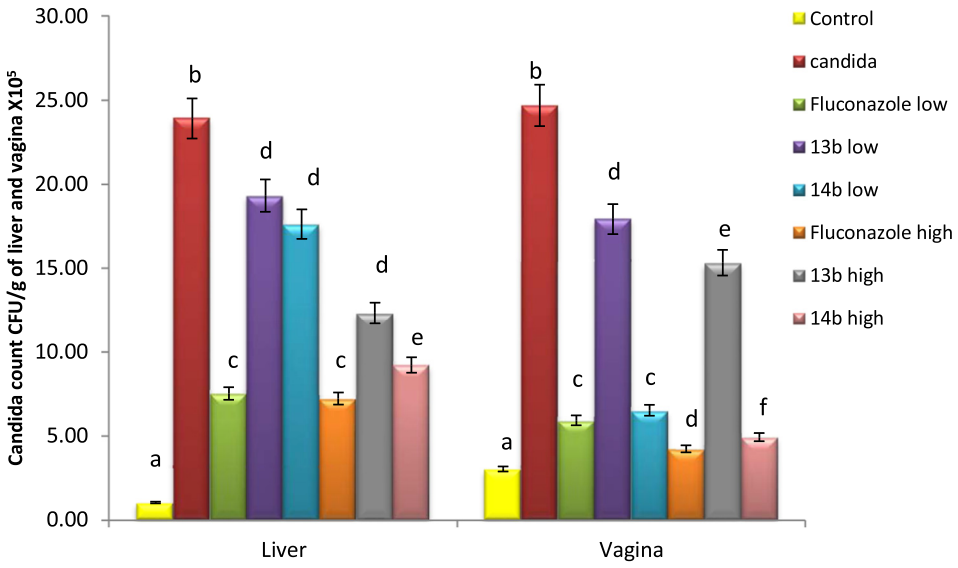

Fig. 1. Effect of new synthetic compounds and fluconazole on *C. albicans* counting in liver and vaginal tissues. Data are expressed as means ± SEM (n = 10). $p < .05$ is considered significant. Groups having the same letter are not significantly different from each other, while those having different letters are significantly different from each other.

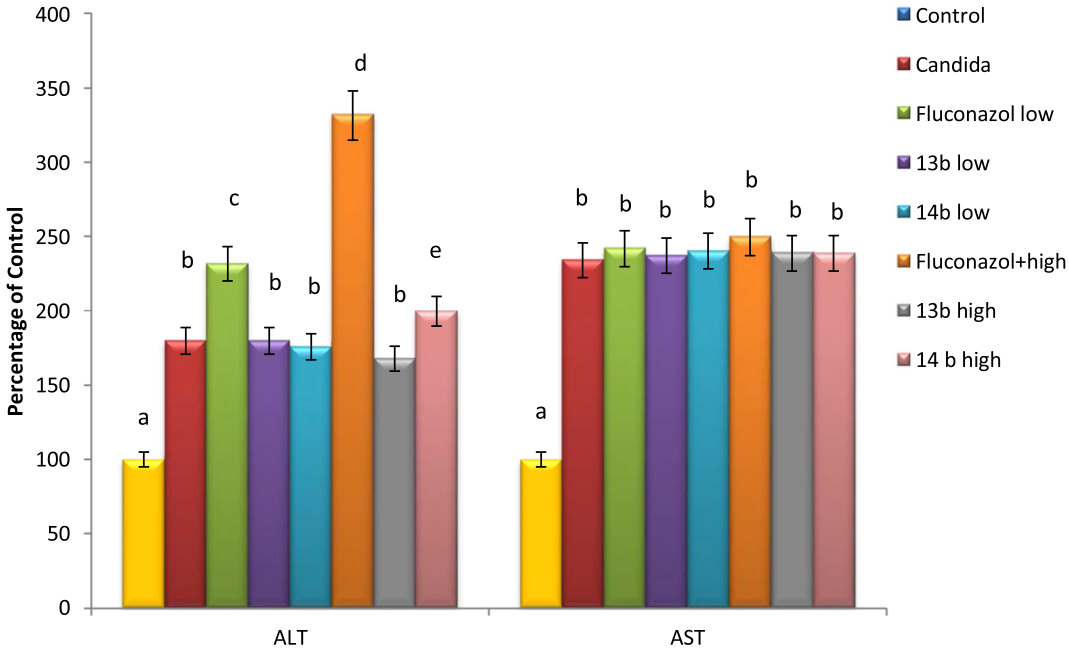

Fig. 2. The effect of new synthetic compounds and fluconazole on ALT and AST enzymes activity in *C. albicans* infected groups. Data are expressed as means ± SEM (n = 10). $p < .05$ is considered significant. Groups having the same letter are not significantly different from each other, while those having different letters are significantly different from each other.

significant down-regulation was apparent in mice treated with fluconazole, 13band 14b. Besides, 13b and 14b high doses regimen considerably reversed the level of Cyp450 back near to the normal value.

3.5. Modulation of vaginal inflammation

A significant elevation in the vaginal inflammatory marker Cox-2 gene expression post *C. albicans* infection was recorded.

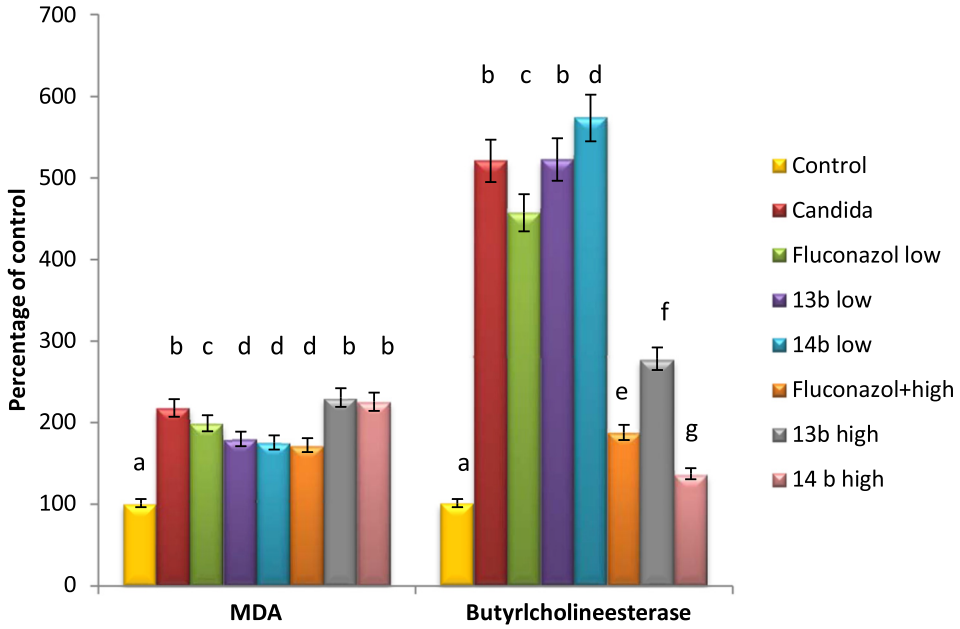

Fig. 3. Effect of new synthetic compounds and fluconazole on hepatic MDA and serum BCHE activities in *C. albicans* infected groups. Data are expressed as means ± SEM (n = 10). $p < .05$ is considered significant. Groups having the same letter are not significantly different from each other, while those having different letters are significantly different from each other.

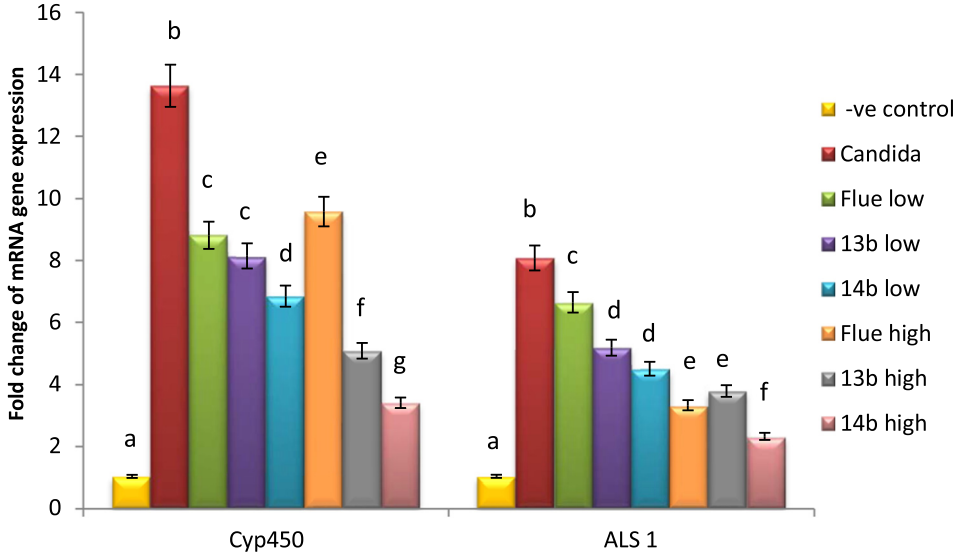

Fig. 4. Effect of fluconazole, 13b and 14b on hepatic ALS1 and Cyp450 genes expression in *C. albicans* infected groups. Data are expressed as means ± SEM (n = 10). $p < .05$ is considered significant. Groups having the same letter are not significantly different from each other, while those having different letters are significantly different from each other.

Administration offluconazole, 13b and 14b revealed a significant down-regulation in the gene expression of Cox-2 as compared to the control value. Meanwhile, administration of 14b in a high dose noticeably down-regulated its expression showing a significant effect in comparison to animals treated with the variable doses of the tested compounds (Fig. 5).

In general from data listed, it was obviously that 13b at low dose revealed an oxidative stress as compared with the other tested groups for both MDA and butyrlcholinesterase level .

3.6. Histopathlogical examination

Fig. 6 declared vaginal tissue Sections (2) showed candida group with atrophic epithelial layer in proximal and central part of vagina, candidiasis showing many hyphae in the lumen. (3) fluconazole treated group with healed almost normal epithelial layer in proximal and central part of vagina, candidiasis showing few hyphae in the lumen. (4) 13b treated group in a low dose with moderately healed epithelial layer in proximal and central part of vagina, no hyphae in the lumen. (5) 14b treated group in a low dose with almost normal epithelial layer in proximal and central part of vagina, candidiasis showing few hyphae in the lumen. (6) fluconazole high dose treated group with healed normal epithelial layer in proximal and central part of vagina, candidiasis showing few hyphae in the lumen. (7) 13b high dose declared normal epithelial layer in proximal and central part of vagina, candidiasis showing few hyphae in the lumen. (8) 14b in a high dose declared normal epithelial layer in proximal and central part of vagina, candidiasis showing few hyphae in the lumen.

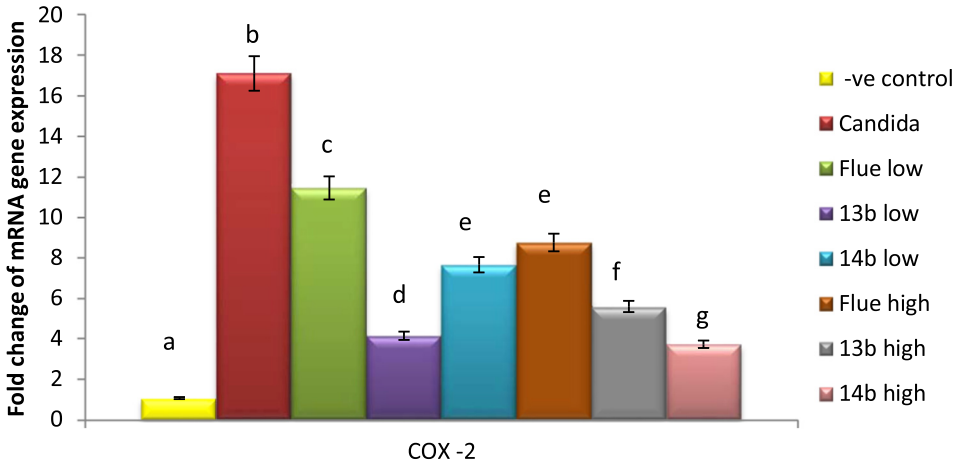

Fig. 5. Effect of fluconazole, 13b and 14b on vaginal COX-2 gene expression in *C .albicans* infected groups. Data are expressed as means ± SEM (n = 10). $p < .05$ is considered significant. Groups having the same letter are not significantly different from each other, while those having different letters are significantly different from each other.

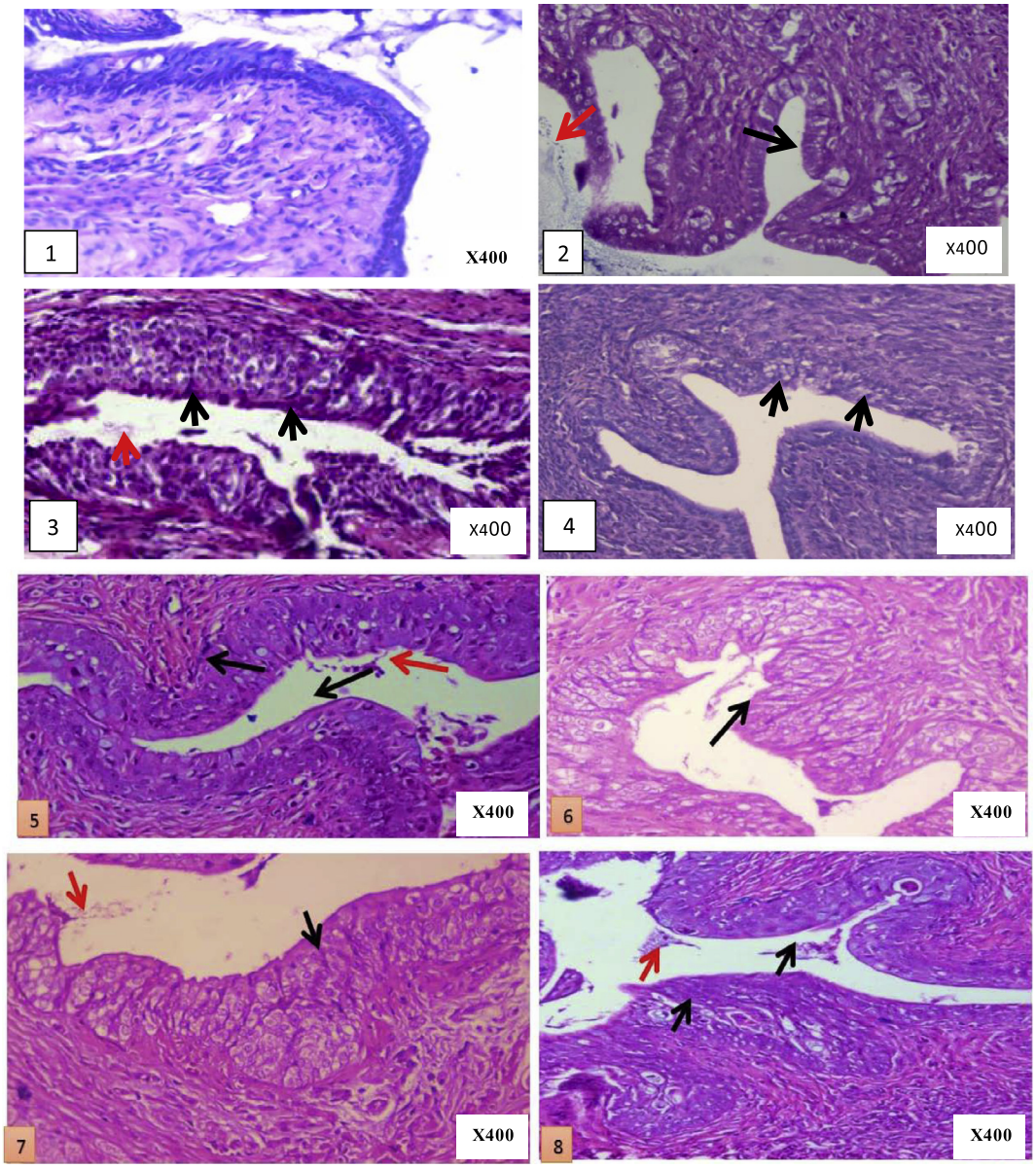

Fig. 6. Histopathlogical examination for vaginal female mice showing sections 1,2,3,4,5,6, 7 & 8 where (1) is -ve control (healthy group), (2) is candida (Candida group), (3, 4, & 5) are treated groups with low dose of fluconazole, 13b and 14b, respectively. (6, 7, & 8) are treated groups with high dose of fluconazole, 13b and 14b, respectively.

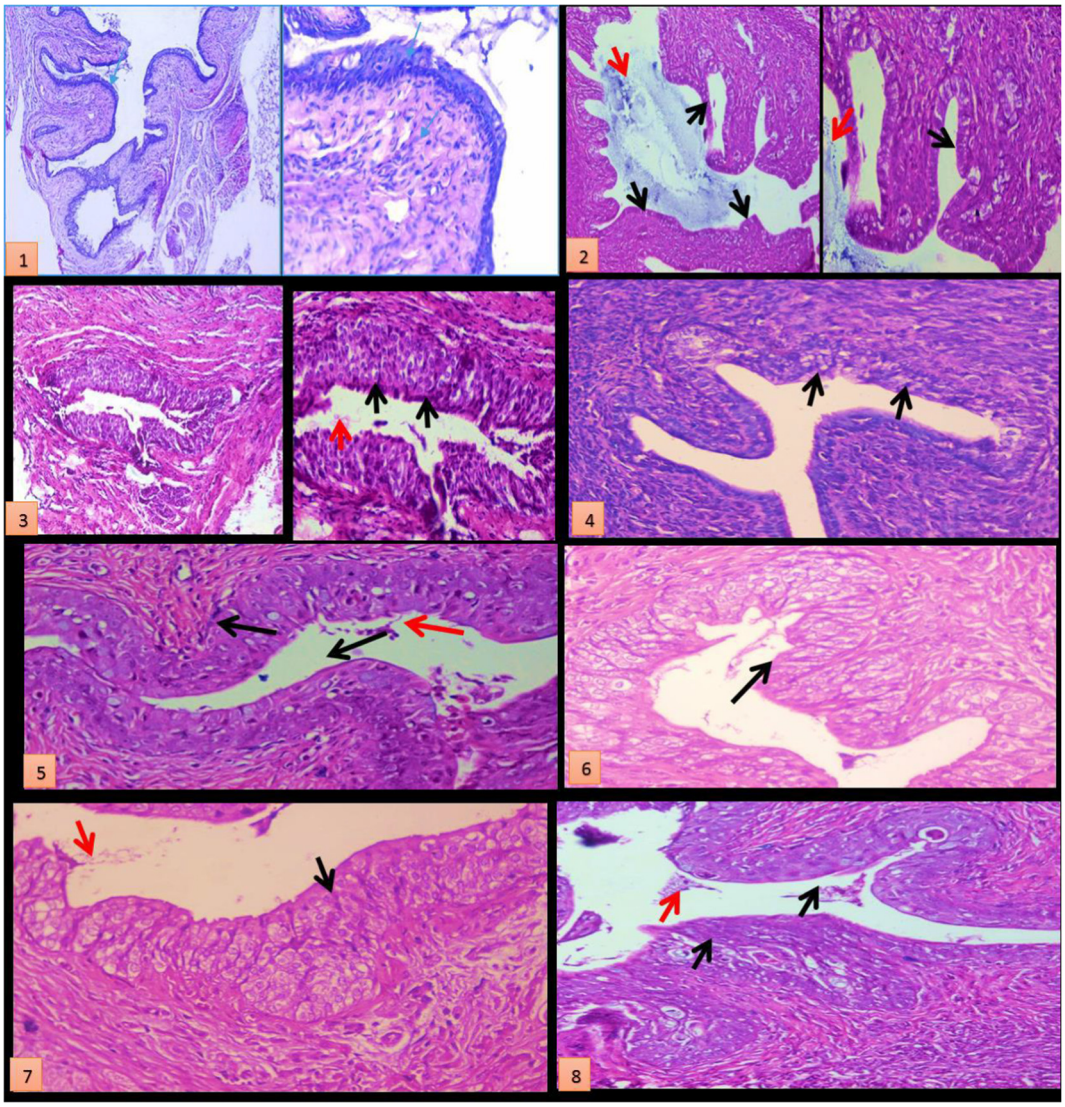

Fig. 6 (*continued*)

4. Discussion

Although fluconazole is the most commonly used drug for *C. albicans* treatment, lots of problems were associated with fluconazole administration as resistance and accumulation in the body that influence several inflammation reactions along with affecting function of different organs (Elizabeth and Shawn (2017); Popp et al. 2017). Previously, some studies elucidated the ability of triazoles to induce hepatic cytochromes (Cyps) which play a vital role in the metabolism of azole drugs in the liver (Somchit et al., 2004; Sun et al., 2006).

Different available antimicrobial drugs are composed of naphthalene nucleus such as naacillin, naftifine, tolnaftate and terbinafine (Rokade and Sayyed, 2009). These antifungal drugs are used for treatment of tinea pedis, tinea cruris and tinea corporis (Desai et al., 2012). It affects bactericidal activity via inhibition of bacterial cell wall synthesis by binding one or more of the penicillin binding proteins (PBPs) (Overington et al., 2006). Previously, pyridine was reported as antimicrobial agent. It inhibits folate synthesis which is responsible for DNA and RNA synthesis in bacteria. So it inhibits cell division. Furthermore it is a competitive inhibitor of the bacterial enzyme dihydropteroate synthase (Imming et al., 2006; McDonald et al., 2009). In the present study, assessment of the efficiency of the new synthetic compounds (13b and 14b) that are formed of a combination of both naphthalene and pyridine moieties in order to overcome the resistant problems associated with traditional antifungal drugs in marketing is carried out.

The current study revealed that *C. albicans* infection elevated serum ALT and AST activities as well as butyrylcholinesterase as compared to the control value. Increased liver enzymes were pointed to cellular leakage indicating liver damage. It has been previously demonstrated that numerous liver function tests were altered post *C. albicans* infection (Minemura et al., 2014). In addition to hepatic MDA elevation indicating oxidative stress induced by *C. albicans*.

Treatment with fluconazole exhibited a significant increment in ALT liver marker as compared to candida group specialy in high dose. This elevation suggested hepatic toxicity of fluconazole (Khoza et al., 2017). On the other hand, treatment with 13b and 14b exhibited a significant reduction in ALT as compared to Candida group. This reduction reflected the hepato-protective effect of these agents.

As previously reported, azole class antifungal drugs inhibit fungal CYP450 14α-demethylase, as this interrupts the conversion of lanosterol to ergosterol, a component of the fungal cell membrane (Hof, 2006). Furthermore, correlation between fluconazole dose,

the extent of hepatic hypertrophy, the levels of Cyp450 expression, and Cyp450 mediated enzymatic activities was also investigated (Lee et al., 2009). Here in, an elevation in Cyp450 in *C. albicans* intoxicated group was observed. Nevertheless, a significant down-regulation was apparent in mice treated with fluconazole, 13b and 14b. Besides, 13b and 14b high doses regimen considerably reversed the level of Cyp 450 back near to the normal value indicating hepatotoxic effect of fluconazole and demonstrated the efficiency of the new synthetic compounds 13b and 14b in regulation of Cyp 450 gene expression.

C. albicans has multiple factors that cause disease. These factors includes phenotypic switching, adherence and secreted hydrolyses. They are regulated by different genes, particularly the agglutinin-like sequence (ALS), secreted aspartyl proteinase, and lipase families which are involved in the pathogenesis and adhesion of *C. albicans* to mucosa and epithelial cells (Hoyer et al., 2008). In the current study *C. albicans* infection caused a significant up-regulation in mRNA gene expression of ALS1 as compared to the control value. This finding was previously reported by Murciano et al., 2012 as an elevation in *C. albicans* ALS1 proteins expression in human oral epithelial cells. Moreover, a significant down regulation was demonstrated in all treated groups with the superiority of 14b high dose regimen.

Oxidative stress has been linked to intracellular MAP kinase signaling pathways which lead to up-regulation of COX-2 gene expression (Tago et al., 2001). In this concept, we investigated an elevation in vaginal COX-2 gene expression post *C. albicans* infection. Previously, it has been reported that COX-2 induction occurs following *C. albicans* infection (Lee et al., 2009). Moreover, modulation of COX-2 gene expression in all treated groups with the superiority of 14b high dose was observed. This finding indicated the efficiency of 14b as antifungal agent. It is well known that *C. albicans* ability to establish a persistent infection depends on signals that regulate release of factors from target cells responsible for replication of pathogen. This cell signaling is designed to serve the purpose of cell survival for both host and pathogen. Infection by *C. albicans* of host tissue and cells is mediated through surface receptors, such as mannose, glucan, integrins, etc. (Castro et al., 1996) and has been found to release pro inflammatory cytokines and large amount of arachidonic acid (AA) from host cells. AA is subsequently converted by lipoxygenases and cyclooxygenases (COXs) to eicosanoids (Noverr et al., 2001).

The antiinflammatory effect of pyridine derivativates has been previously reported via prostaglandin-2 reduction (Mohamed et al., 2010).

Naphthalene is important aryl ring in many active compounds such as anti-inflammatory, anti-bacterial, anti-microbial and anti-cancer. In recent trends, heterocycles plays a major role in drug synthesis (Sharma and Singh, 2006). Pyrazole derivatives have been the subject of substantial attention by synthetic and medicinal chemists due to their role in many biological activities such as anticancer, antiviral, anti-inflammatory, antifungal, antimicrobial, antihistaminic, antiplatelet and analgesic activities. The mechanism of action for this compounds is linked to the non-selective or selective inhibition of two cyclooxygenase isoform, COX-1 & COX-2 (Feixas and Jimenez, 2011)

Animals given new synthetic compounds 13b, 14b showed histopathological findings in line with normal mucosal recovery from the induced infectious process thereby, confirming the therapeutic efficiency of these compounds as antifungal agents.

5. Conclusion

From the current investigation, it could be concluded that the combination of pyridine and Naphthalene derivatives (principally 14b in a high dose) could be used as a novel antifungal agents to treat important fungal infections caused by *C. albicans*. So it is recommended to be improved and evaluated as a novel antifungal drug with less effect on liver function and able to overcome the emergence of the resistance problem.

Acknowledgment

Acknowledgment is directed to prof. Dr. Laila Rashed, professor of Biochemistry Faculty of medicine, Cairo University for technical aid, and Dr. Yasmin Syam, researcher of Therapeutic Chemistry Department, National Research Center for providing new chemical antifungal agents.

Conflict of interest

The authors have no conflicts of interest to declare.

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