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Evaluation of the Antifungal Effect of Fluconazole and Silymarin against *Candida Albicans* in Vitro and in Vivo"

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Abstract: **Background:** Cutaneous candidiasis has been considering to be a major public health problem and opportunistic infection affect skin and mucus membrane. if left untreated it leads to serious dermal, mucus, systemic complications. Fluconazole is effective in the treatment of cutaneous and systemic candidiasis. Resistance of candida albicans to fluconazole has led to choose an alternative therapy. Dietary supplement silymarin have antifungal activity against candida albicans.

Objective: This study was carried out to evaluation of the antifungal effect of Fluconazole and Silymarin alone against drug resistant Candida albicans isolates and evaluation combination therapy between silymarin and fluconazole to overcome problem of resistance. Material and Method: A cutaneous candidiasis in adult male rats model was used to assess Fluconazole and Silymarin efficacy in vivo in treating Candida albicans infections. in vitro minimum inhibitory concentrations (MICs) determined by broth micro dilution. the antifungal efficacy was determined by measuring the zone of inhibition in a well diffusion experiment.

Results: The current study included performing a series of dilutions of 100µg to a concentration of 0.4µg for the determining the minimum inhibition concentration of drugs on Candida albicans the MIC was 33.3 µg for Silymarin and 11.1 µg for Fluconazole. Antifungal assay alone of silymarin (60µg) show best and large inhibition zone (15.8mm) compare to (33.3µg) show inhibition zone (12.7 mm) and (15 µg) show small inhibition zone. Antifungal assay of silymarin in combination with fluconazole (60 - 11µg) show large inhibition zone (37.9mm) compare to (33.3- 11µg) show less inhibition zone (26.9 mm) and last combination (15- 11µg) show small inhibition zone (17.2mm). Animal study of silymarin show the best combination with fluconazole give faster healing time and reduce size of infection from 2 cm to 0.4 cm and disappear redness of skin and return growth of hair is 60- 11µg. Effectiveness of silymarin against Candida albicans increases when the antibiotic is mixed with fluconazole and the effectiveness also increase with an increase in the concentration of silymarin.

Conclusion. The effect of silymarin was increased proportionally with increasing the concentration. The mean inhibition zone of silymarin was the highest with higher doses in a statistically significant relation. A synergistic effect of fluconazole and silymarin with the most effective concentration being statistically significant. The rats treated with a combination of fluconazole and the silymarin 60-11µg concentration reached 100% healing within 15 days in a statistically significant relation.

Key points: Candida albicans; fluconazole; silymarin; synergistic effect.

INTRODUCTION.

Fungal pathogens have a major global impact upon human health over a quarter of the world's population have a fungal skin infection that 75% of women suffer at least one episode of vulvovaginal candidiasis during their lifetime and that over a million people die each year from an invasive fungal infection(1). This is because fungal infections are often difficult to diagnose and are particularly challenging to treat (2). The mortality rate associated with fungal infections exceeds that of many diseases including malaria (3). wide range of factors including an ever-increasing high-risk population (patients with immune suppression, immune dysfunction, diabetes mellitus and patients carrying indwelling catheters or undergoing surgery and organ transplantation), indiscriminate usage of antibiotics, a limited antifungal arsenal and emerging resistance to current antifungal drugs, contribute to the increasing incidence of fungal infections (2).

Species belonging to the *Candida albicans* genus are the most prevalent agents of hospital-acquired invasive fungal infections (4).. *Candida albicans* is the best studied and most prevalent of the human fungal pathogens and Candida species are fungi that grow as yeasts and that are 'imperfect', meaning they apparently lack a complete sexual cycle (5).*Candida albicans* remains the most common cause of life-threatening systemic candidiasis *C. albicans* thought to be an obligate diploid can form true filamentous hyphae in addition to the budding yeast and pseudo hyphal (elongated yeast) cells seen in other Candida species and in the model yeast Saccharomyces cerevisiae(6)**.** *Candida albicans* is an opportunistic pathogen that resides as a harmless commensal in the gut, genital urinary tract and skin and it becomes an opportunistic pathogen under a number of different host conditions usually involving reduced immune competence or an imbalance of the competing bacterial microflora(7)**.**

although the frequent prophylactic use of azole antifungal drugs has led to the emergence of other Candida species with intrinsic resistance to these drugs (6).

Major developments in research into the azole class of antifungal agents during the 1990 have provided expanded options for the treatment of many opportunistic and endemic fungal infections (7). Fluconazole and Iitraconazole have proved to be safer than both amphotericin B and ketoconazole (8). Despite these advances serious fungal infections remain difficult to treat and resistance to the available drugs is emerging (8). Fungal infections affect the lives of at least 12 million people each year killing more than 1.5 million (9). The widespread use of fungicides and prophylactic antifungal therapy has increased resistance in many serious fungal pathogens and there is an urgent need to expand the current antifungal arsenal (10). Dietary supplements are very popular and commonly used throughout the world and their goal is to supplement the normal diet with vitamins and minerals or other nutrients (single or complex) that have a nutritional or other physiological effect so number of them are obtained from plants(11). Uses of flavonoids, such as Quercetin, in medicine recently expanded largely depending findings from in vitro and in vivo studies. Where findings from these studies showed these chemical substances have capability to fight many diseases including diabetes, cancers, cardiovascular, osteoporosis and other human diseases(12). Bioactive products of plants as antibiotic adjuvants can also enhance the effect of antimicrobials drugs(13). Milk thistle (*Silybum marianum*, family: *Asteraceae*) is a medical plant that has been used for at least 2000 years in ancient Greece, India, and China, *Silybum marianum* was administered to cure liver and gallbladder diseases in addition to the detoxification of organisms the bioactive extract of milk thistle seeds and fruits, silymarin was classified by the World Health Organization as an official drug with hepatoprotective properties in the 1970s currently silymarin is also commonly used as a dietary supplement, particularly in supporting liver function but also as a medicine employed in many liver diseases and metabolic syndromes including obesity, diabetes, hypertension, and dyslipidemia(14). Silymarin (SM) is a flavonoid mixture, extracted from the *Silybum marianum* (milk thistle) plant and SM extract contains approximately 65% to 80% flavonolignans (silibinin A and silibinin B, isosilybin A, isosilybin B, silychristin and silydianin), with small amounts of flavonoids, and approximately 20% to 35% of

fatty acids and polyphenolic compounds possessing a range of metabolic regulatory effects(15). Natural silymarin is a unique mixture containing flavonolignans, especially silybin, isosilybin, silychristin, isosilychristin, silydianin, and silimonin in addition to flavonolignans (16). The hepatoprotective effect of silymarin results from its numerous biological activities (e.g., antioxidative, antifibrotic, anti-lipid peroxidative, anti-inflammatory, and immunomodulatory activities) as well as involvement in liver regeneration mechanisms(17). In recent years many studies have demonstrated that besides hepatoprotection flavonolignans possess various healthy properties so new derivatives or new drug combinations of silymarin may result in the achievement of antifungal , antiviral, antimalarial, anticancer, antidiabetic, neuroprotective, and neurotropic activities(18). The flavonoid silymarin and one of its structural components, silibinin, are substances with documented hepatoprotective(15). Silibinin was recently reported to have antifungal effect related to fungal apoptosis against *Candida albicans* and antibiofilm activity also silibinin inhibited the dimorphic transition of *C. albicans* and resulted in the inhibition of biofilm development at an early stage so the interest in the therapeutic effect is increasing(19).Recent research has focused on improving the most successful azole class of antifungals by searching for synergistic interactions with secondary compounds (10). Synergists may cooperate with azoles by targeting steps in the relevant ergo sterol synthesis pathway pathways or may act on resistance-associated mechanisms such as activating efflux or on completely dissimilar pathways or processes (20). a variety of potential synergistic sources have been explored including pre-existing antimicrobials, pharmaceuticals approved for other uses, natural bioactive compounds and phytochemicals and new synthetic compounds (21). Synergy can successfully expand the spectrum of antifungals, reduce inhibitory doses, reduce toxicity and prevent the development of resistance (10). this study describes the current and future uses of currently available azole antifungal agents in the treatment of systemic and superficial fungal infections and provides a brief overview of the current status of in vitro susceptibility testing and the growing problem of clinical resistance to the use of azoles. azoles currently available with supplements with different mechanisms of action are likely to provide enhanced efficacy (22).

MATERIALS AND METHODS.

Methodology.

This prospective case-control study was conducted from October 2024 to April 2024. A total number of 25 clinical *candida albicans* isolates resistance to fluconazole were gathered for the study from Basrah university/ collage of science/ Microbiology Department. According to microbiological guidelines, various clinical specimens were taken from patients admitted to alfyhal outpatient clinics or those hospitalized in alfyhal hospital from various departments, including urology, nephrology, internal medicine, surgery, and intensive care units (ICUs). These specimens were rapidly delivered to the Microbiology laboratory of collage of science for further processing. Identification and speciation of isolates were done by Vitek-2 system, which was taken as the gold standard method.

This Research was approved by the Ethics Committee of the Institutional Review Board (Code: MS-72-2021).

Isolation and identification of *Candida* spp:

Sabouraud dextrose agar (SDA) (Oxoid, UK) was used for inoculation of clinical specimens and then incubated at 37 °C for 24-72 h. The colonies were examined microscopically after Gram staining. Identified *Candida* isolates were further categorized to the species level by the standard protocol that includes germ tube test (GTT) and Vitek 2 compact system.

Primary identification of isolates to species level:

GTT was used to categorize *Candida* isolates into *C. albicans* and *NAC* spp. It is positive for *C. albicans* and *C. dubliniensis* and negative for other species(23).

Primary identification by chromogenic agar medium:

Further species identification to *C. albicans, C. tropicalis, C. krusei*, and other species was done using chromogenic media HiCrome™ *Candida* Differential agar (HiMedia, Mumbai, India) and incubated aerobically at 30 °C for 48 hours. The colony color was recorded and interpreted following the manufacturer's instructions. Confirmation by broth micro dilution:

All Isolates were further tested by the broth micro dilution for their identification and testing of their antifungal susceptibility(23).

Antifungal susceptibility test:

Identified candida albicans isolates were tested for their antifungal susceptibility by agar well diffusion method against 1 antifungal agent namely: fluconazole, and dietary supplement silymarin. Broth micro dilution method was used to determine the minimal inhibitory concentration (MIC) for clinically relevant *Candida* spp. using the following antifungal agent; fluconazole and dietary supplement silymarin(24) .

In Vitro Susceptibility of Candida albicans to Fluconazole and Ivermectin Combination

Broth Micro Dilution Assay

The inhibitory effects of fluconazole alone and in combination with ivermectin against Candida albicans were evaluated using broth microdilution. Stock solutions of fluconazole (33 mg/mL) and ivermectin (96 mg/mL) were prepared in DMSO. Serial three-fold dilutions yielded six concentrations of fluconazole (100, 33.3, 11.1, 3.7, 1.2, and 0.4 µg/mL) and ivermectin. The minimum inhibitory concentration (MIC) was determined as the lowest concentration inhibiting visible growth(25) with slightly modification.

Agar Well Diffusion Assay

The agar well diffusion method assessed the antifungal activity of fluconazole and ivermectin combinations. Mueller-Hinton agar plates were inoculated with Candida albicans. Wells received 50 µL of fluconazole (11 µg/mL) and ivermectin (1, 3.7, and 8 µg/mL) solutions. After overnight incubation at 37°C, antifungal activity was measured by inhibitory zone diameter(26) with slightly modification.

Animal Study

The study consisted of eight groups (A-H), each receiving a different treatment regimen. The groups were monitored for 21 days, with wound area measurements and weight recordings taken at regular intervals.

Eight groups of rats (n = 4) were used. Rats were housed in separate cages at 30% humidity, 22°C, and a 12-hour light-dark cycle. Standard provender and water were provided(27)with slightly modification. Animal handling protocols were approved by Basra University's animal ethics committee.

Induction of cutaneous candidiasis and treatment protocol.

Cutaneous candidiasis was induced in rats by injecting a yeast stock suspension containing 1-5 × 10^6 Candida albicans cells/ml (OK631832) into groups A-H. The wound area was measured using the rule technique (length × width), and rat weights were recorded on days 0, 3, 6, 9, 12, 15, 18, and 21.

Preparation of Skin for Transdermal Application

Rats' back hairs were shaved using a hair removal shaving machine, and a 2 cm^2 region was marked for application of the formulations. The following day, a Derma roller was used to create micropores, facilitating transdermal delivery of the medication.

Treatment Protocol.

The rats received a single treatment once daily for 8 days. The control group (positive) received fluconazole, while the untreated group received no treatment. After 8 days, the responses of the treated groups were compared to those of the control group.

RESULTS.

Morphology identification of *Candida albicans*

The isolated colonies appear creamy white this represent growth of *Candida albicans,* shown in (Figure 1).

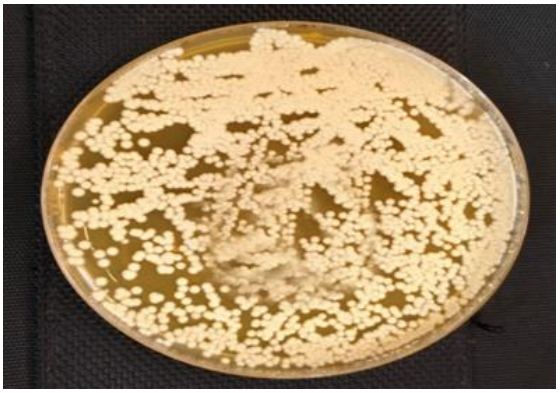


Figure 1: morphology of *Candida albicans* on Sabouraud dextrose agar

Growth on to Chrome Agar

The isolated colonies appear creamy white and *Candida* differential agar appeared of different colors on the chrome according to the *Candida* species. Light green colonies were identified as *C. albicans*.as shown in (Figure 2).

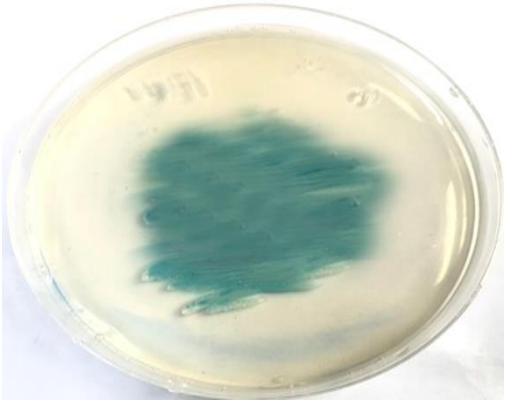


Figure 2: Growth of *Candida albicans* on chrom agar. HiCrome

Candida differential Agar is a selective and differential medium, which facilitates rapid isolation of yeasts from mixed cultures, used in the qualitative direct detection, differentiation, and presumptive recognition of Candida species namely *C. albicans*, C. *krusei, C. tropicalis and C. glabrata* based on coloration and colony morphology .CHROM agar is a rapid plate-based test containing chromogenic material which is functioned upon by distinct enzymes secreted by different *Candida spp*. to yield a characteristic colony color. The composition of the substance includes glucose, peptone, a chromogenic mixture, and agar, with a pH adjustment to 6.1. Peptone special and yeast extract supply nitrogenous, carbonaceous chemicals, and other vital growth ingredients. Phosphate effectively stabilizes the media. Sample must be inoculated and then incubated at a temperature range of 25-37°C for 24-48 hours. This medium yield results within 48 hours, making it valuable for swiftly and tentatively identifying prevalent yeasts in the fields of Mycology and Clinical Microbiology Laboratory. documented that *C. albicans* produce an enzyme called b-N-acetyl-glycos aminidase. further stated that the addition of chromogenic or flu orogenic hexosaminidase

substrates to the growth medium aids in the direct identification of *C. albicans* isolates during primary isolation.

Microscopic identification Germ tube

The isolates were tested on their ability to form the germ tube to identify them *C. albicans* and other candida species that cannot create the germ tube. The isolates obtained were able to form a germ tube identified as *C. albicans.*as shown in (Figure 3).

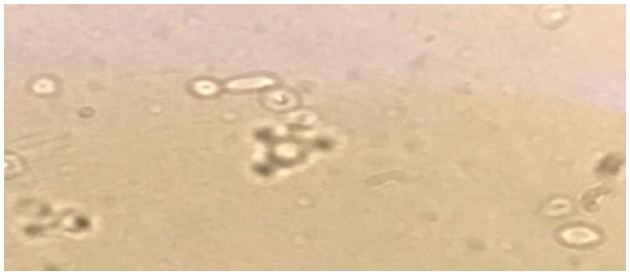


Figure 3: Germ tube formation of *Candida albicans*

Germ Tube test, also known as REYNOLDS BRAUDE phenomenon, proves yeast germination. Assumed colonies are inoculating 0.5 ml of human serum with a small quantity of young test fungus at 37°C for (2-3) hours. A small amount of suspension is applied on a slide and examined using a microscope. Germ tubes are observed as elongated tube-like extensions that arise from the yeast cells. There is no narrowing or tightening at the place where pseudo hyphae are attached .

Candida albicans is identified by the production of germ tubes or chlamydospores within three hours. However, not all strains of *C. albicans* produce germ tubes, particularly if the strain has been isolated from a cancer patient or those on anti-candida medications . Other Candida isolates are speciated with a battery of biochemical reactions.

Serial Dilution for MIC Detection for Fluconazole and Silymarin.

The current study included performing a series of dilutions, starting from a concentration of 100µg to a concentration of 0.4µg for the determining the minimum inhibition concentration of drugs on *Candida albicans* this study was showed the concentration MIC.

Table (3): Serial dilution for detection MIC of Fluconazole and Silymarin**.**

Substance	Threefold dilution					
	100 µg	33.3 µg	11.1 µg	3.7 µg	1.2 µg	0.4 µg
Silymarin	+	+	-	-	-	-
Fluconazole	+	+	+	-	-	-

In vitro evaluation antifungal Activity of Silymarin Alone against *C. albicans* by agar well method.

The current study was showed that the MIC concentration of Silymarin against *C. albicans,* was noted that the MIC concentration was 12.7 ± 0.72, double MIC (60µg) concentration was15.8 ± 0.65, and Half MIC concentration was 9.13 ± 0.85 with significant differences between three concentrations as in the table below.

Table (4): Activity of Silymarin alone against *C. albicans.*

Concentration µg	**Silymarin Inhibition Zone mm**			
	Frequencies			**Mean ± S. D**
60	15.2	16.5	15.8	15.8 ± 0.65^{a}
30	12.3	12.4	13.6	12.7 ± 0.72^{b}
15	9.1	10	8.3	9.13 ± 0.85^{c}
p. value and LSD				< 0.001; 1.49

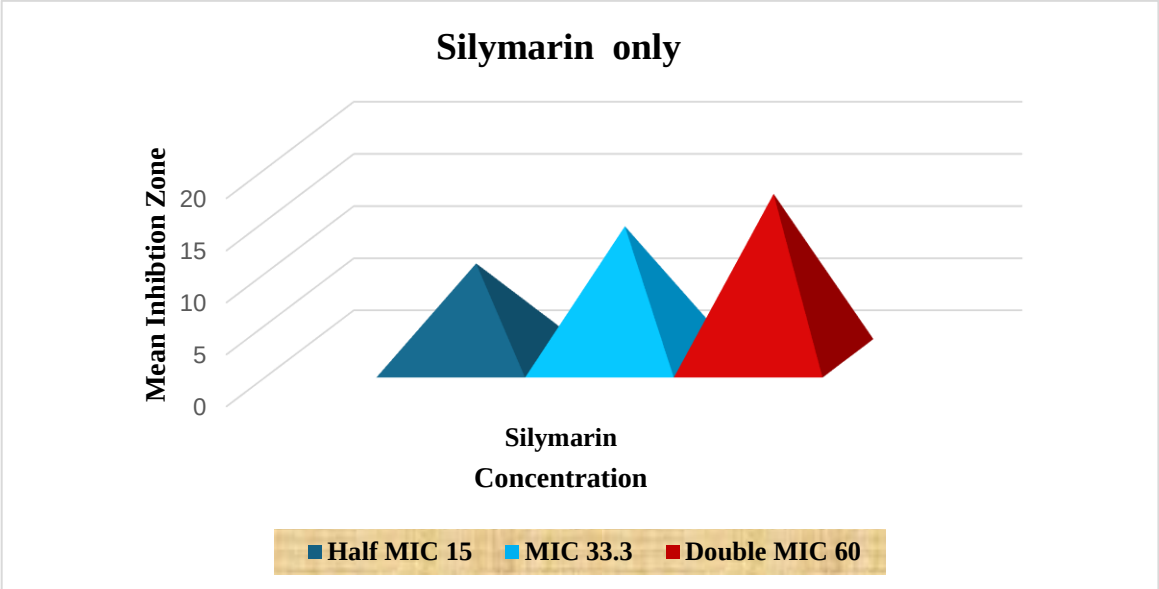
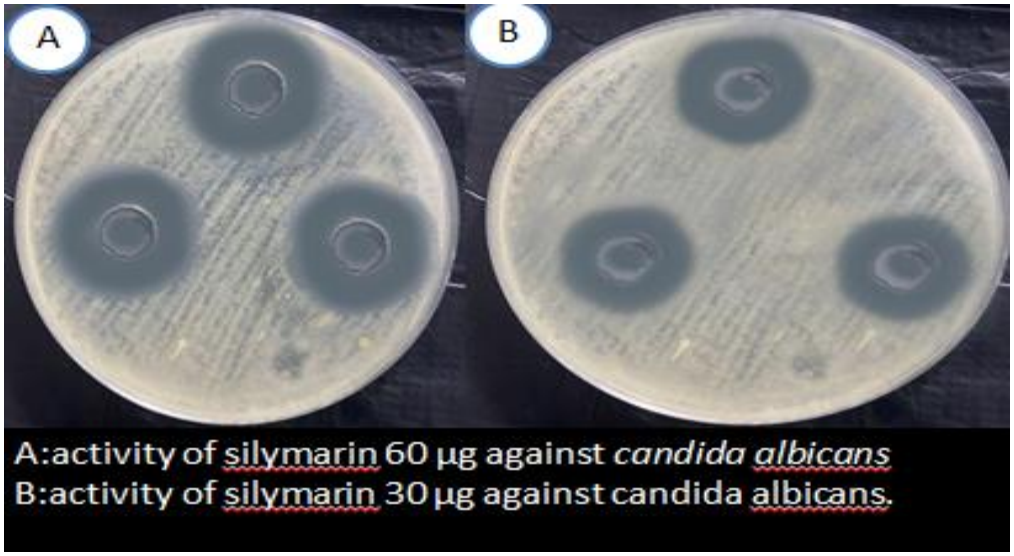


Figure (2): Activity of Silymarin alone against *C. albicans.*

Figure (2) show the effect of silymarin alone against *candida albicans*. The impact of silymarin increased by increasing the concentration of silymarin. Activity of silymarin alone against *C. albicans*. The table 3 and figure 2 show antifungal activity of silymarin against *candida albicans*. silymarin (60µg) give large inhibition zone (15.8mm) while (15µg) give small inhibition zone (9.13mm) this indicate the antifungal activity of ivermectin increase with increase its concentration and increase size of inhibition.

In vitro Activity of Silymarin only Against *C. albicans* by agar well method.

The results of the current study showed that the effectiveness of silymarin against *Candida albicans* increases with increasing concentration was the experiment repeated three times, as shown in the figure 3 and 4.



Figure(3): Activity of Silymarin (60 and 30mg) only Against *C. albicans.*

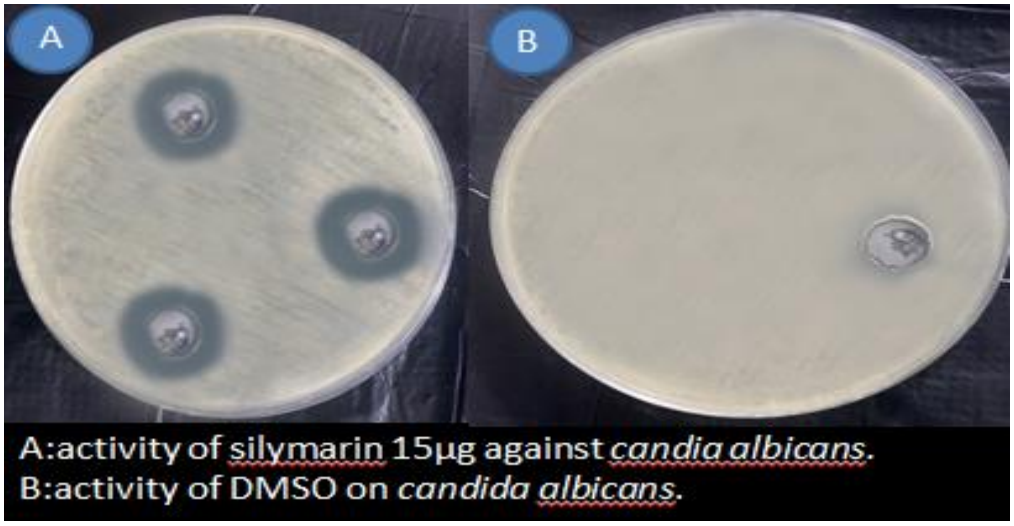


Figure (4): Activity of Silymarin (15mg) only and DMSO Against C. albicans.

The agar well method results demonstrate a clear concentration - dependent relationship between ivermectin and the inhibition zone diameter against *candida albicans*. A- 60µg/ml largest inhibition zone (15.8 mm) indicating highest antifungal activity. B - 30 µg/ml moderate inhibition zone (11.9 mm), indicating moderate antifungal activity. C- 15µg/ml, smallest inhibition zone (9.13mm), indicting lowest antifungal activity. compared with D: DMSO control.

In vitro Activity of Silymarin Combination with fluconazole Against *C. albicans* by agar well method.

The current study was showed that the activity of Silymarin combination against *C. albicans,* was increased significantly compared with their activity alone was noted that the 60-11µg concentration was 37.9 ± 1.60 and 30-11µg concentration was 26.9 ± 1.33 and 15-11µg concentration was 17.2 ± 1.05 with significant differences between three concentrations as in the table below.

Table (5): Activity of silymarin combination against *C. albicans*. The combination of silymarin and fluconazole exhibits synergistic effect, resulting in larger inhibition zone compared to individual compounds. The inhibition zone diameter increases with increasing silymarin concentration, while fluconazole concentration remain constant (11µg). The combination of 60µg silymarin and 11µg fluconazole shows the largest inhibition zone (37.9 mm), indicating enhanced antifungal activity.

Concentration	Silymarin Inhibition Zone mm			
	Frequencies			Mean ± S. D
60-11	36.2	38.1	39.4	37.9 ± 1.60[a]
30-11	28.1	27.3	25.5	26.9 ± 1.33[b]
15-11	18.2	17.4	16.1	17.2 ± 1.05[c]
p. value and LSD				< 0.001; 2.70

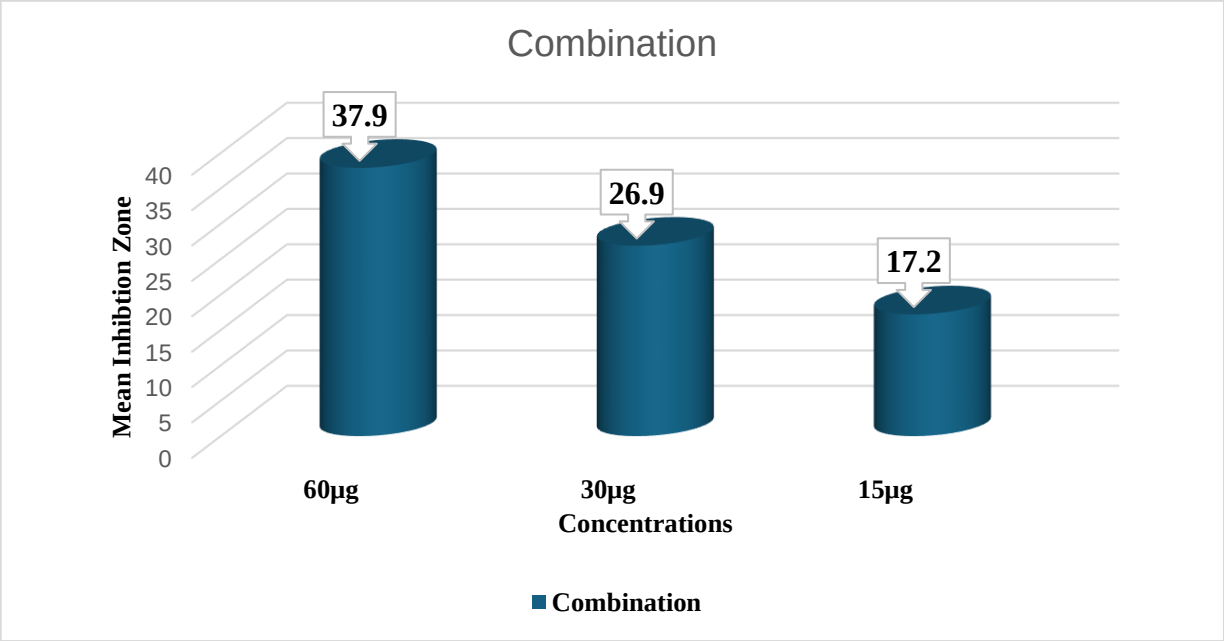


Figure (5): Activity of Ivermectin combination against *C. albicans*. The highest bar (37.9 mm) represents the largest inhibition zone, indicating the most effective combination (60+11µg). The moderate bar (26.9 mm) represents a moderate inhibition zone, indicating a moderately effective combination (30+11µg). The shortest bar (17.2 mm) represents the smallest inhibition zone, indicating the least effective combination (15+11µg).

In vitro Activity of Silymarin Combination with Fluconazole Against *C. albicans* by agar well method.

The results of the current study showed that the effectiveness of silymarin against *Candida albicans* increases when the antibiotic is mixed with fluconazole and the effectiveness also increase with an increase in the concentration of silymarin as shown in the figure 6.

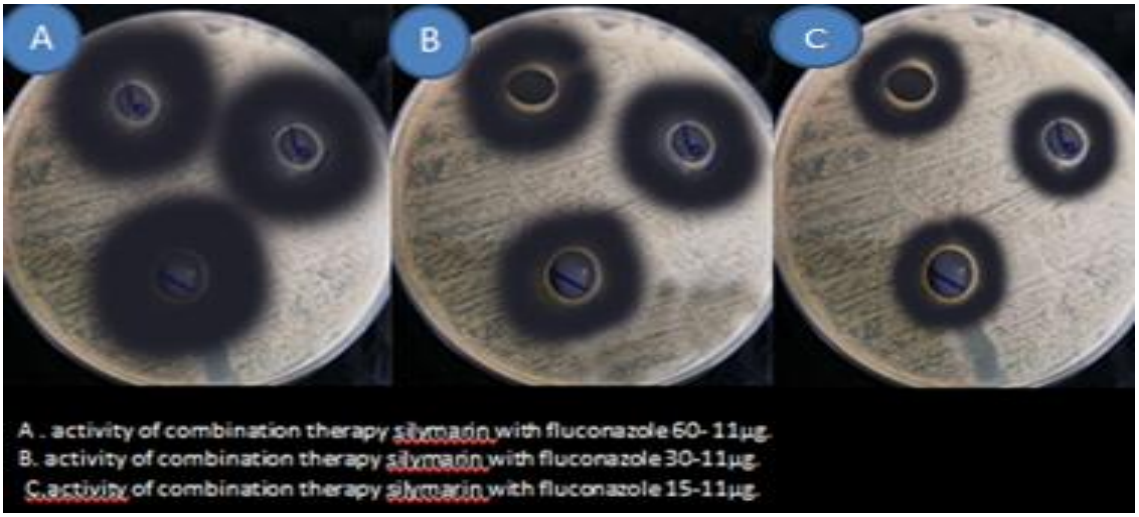


Figure (6): Activity of silymarin combination with Fluconazole against *C. albicans*. A: 60-11µg concentration. B: 30-11µg concentration. C: 15-11µg concentration compared with DMSO control (D).

Treatment of rate Infection with Silymarin Alone and Silymarin Combination with fluconazole.

The results of the animal experiment after rate were infected with *Candida albicans* and treated once with Silymarin alone and combination with fluconazole showed that the speed of response to treatment was higher with the combination compared to using the Silymarin alone, as shown in the Figure (7) below.

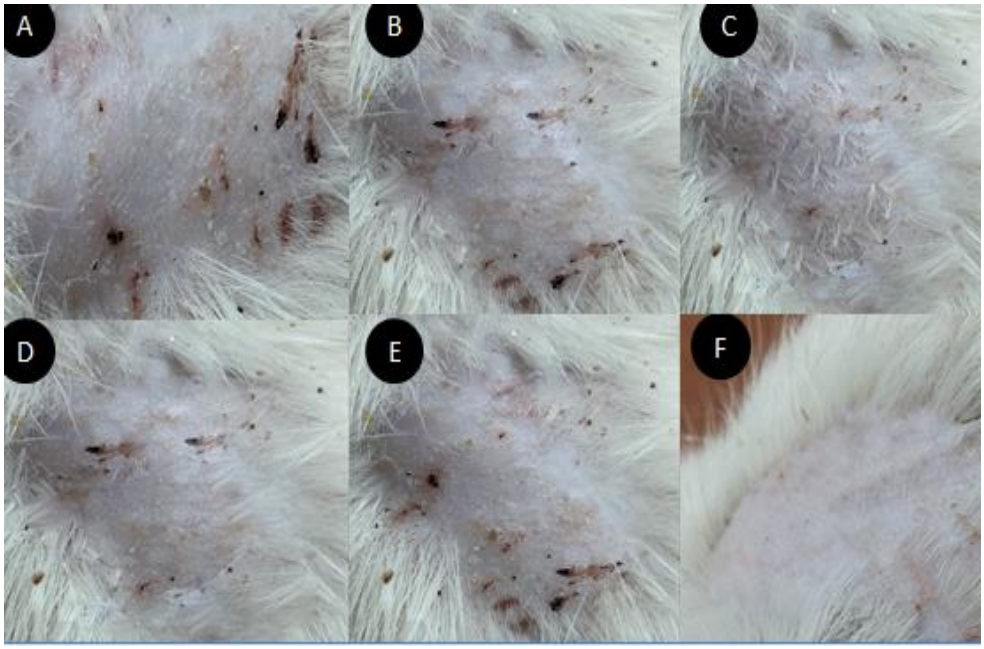


Figure (7): Mouse skin after fungal infection and treatment A: mice infection with *C. albicans* B: control sever redness, scarring, and hair loss. C: treatment with silymarin alone (15µg) mild redness some scaring and partial hair loss, D: treatment with silymarin combination 15-11minmal redness reduced scaring and significant hair regrowth. E: treatment with silymarin combination 30-11 almost complete hair re growth minimal scaring and no redness. F: treatment with silymarin combination 60-11complet hair re growth, no scaring and healthy skin appearance

Assessment of rat Skin Infection and Time after Treatment with Antifungal Silymarin Combination.

After causing a fungal infection with *Candida albicans* with a diameter of 2 cm it was treated with silymarin combination fluconazole for 21 days the fungal infection was taking 7 days to occur after which the infection was treated and the diameter of the infection was recorded during the eighth day until it fully recovered on the 21st day as in the following figure (8).

Figure (8): Assessment of Mice Skin Infection and Time after Treatment with Antifungal Silymarin Combination.

Association between fungal infection and treatment with silymarin only, and fluconazole only according to time.

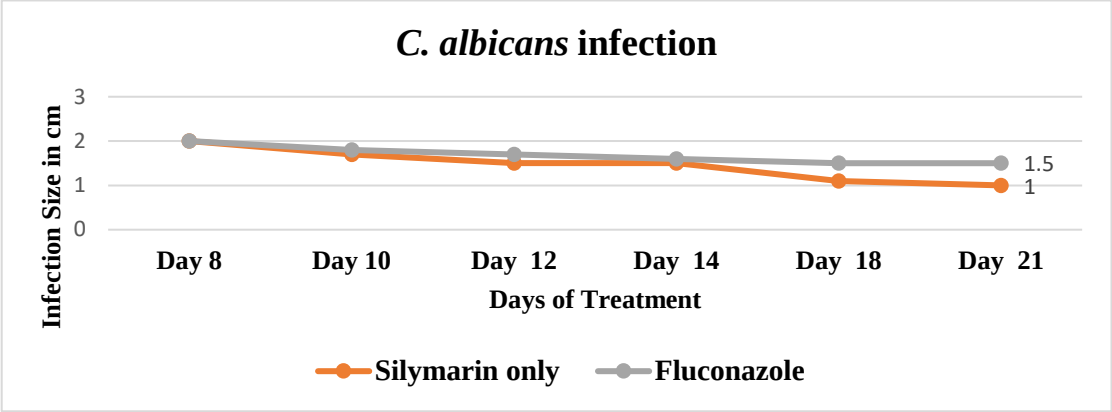


Figure (9): Association between fungal infection and treatment with silymarin only, and fluconazole only according to time.

Association between fungal infection and treatment with silymarin combination with fluconazole according to time.

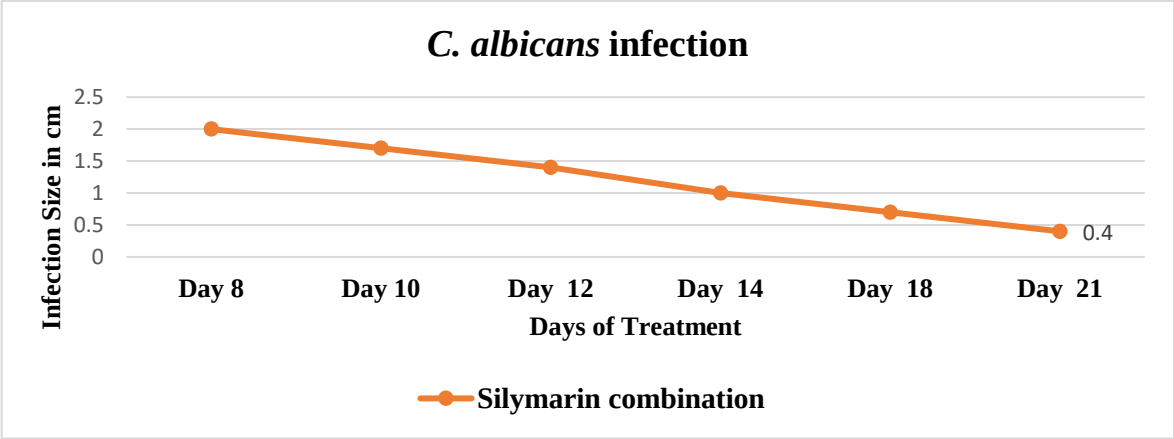


Figure (10): Association between fungal infection and treatment silymarin combination with fluconazole according to time.

DISCUSSION.

Silymarin derived from the seeds of Silybum marianum has been widely used to prevent and treat liver disorders. it is also consumed as a dietary supplement to improve liver function as it does not exhibit any toxic effects in humans. recently silymarin has been reported to show antimicrobial effects against various pathogenic microorganisms (28).

The current study included a series of dilutions starting from a concentration of 100µg to a concentration of 0.4µg to determine the minimum inhibition concentration (MIC) of silymarin on *Candida albicans*. the results showed that the MIC concentration(30µg) was 12.7 ± 0.72 the double MIC concentration(60µg) was 15.8 ± 0.65 and the half MIC concentration (15µg) was 9.13 ± 0.85 with significant differences between the three concentrations (as shown in Table 3).

This suggests that the effectiveness of silymarin against *Candida albicans* increases with increasing concentration the experiment was repeated three times as demonstrated in Figures 3 and 4.

Previous studies have shown that natural compounds such as phenols, flavonoids, and lignans have antimicrobial potential (29). silymarin which is composed mainly of flavonolignans has also been reported to exhibit antimicrobial activities against various microorganisms (18–24). recent studies have demonstrated the antifungal effects of silymarin against clinical strains of C. albicans, C. krusei, and C. tropicalis with MIC values more than 100 µg/mL for all the tested isolates (30).

When silymarin was combined with an antifungal agent the activity against *C. albicans* was significantly increased compared to their individual activities. the data showed significant differences between the three concentrations (as presented in Table 4) suggesting a synergistic effect. this is consistent with studies by Janeczko and Kochanowicz, Fozouni and Palang (31) and Sharma et al(32).

The in vivo experiment involved infecting rate with *Candida albicans* and treating them with either silymarin alone or a combination of silymarin and fluconazole. The results showed that the speed of response to treatment was higher with the combination compared to using silymarin alone (Figure 7). This is similar to the findings of Qasim and Hashim (33), Casalinuovo et al. (34), and Heng et al. (35) which indicated a better healing tendency when silymarin was used in combination with fluconazole compared to silymarin alone.

The antifungal activity of silymarin and its synergistic effect with fluconazole may be attributed to its ability to alter the permeability of the cell membrane favoring the penetration of antifungal agents (36),(37). Several studies have shown that natural compounds including silymarin can disrupt the plasma membrane leading to antimicrobial activity ((38)–(14)). Silymarin has been reported to induce significant cell membrane damage and reactive oxygen species (ROS) generation at concentrations above the MIC eventually leading to cell death (39).Antioxidants such as flavonoids and polyphenols have been exploited for their beneficial effects against oxidative stress (15). New derivatives or drug combinations of silymarin may result in the achievement of various therapeutic activities including antiviral, antimalarial, anticancer, antidiabetic, antifungal, neuroprotective, and neurotropic effects (17).

CONCLUSION

The current study demonstrates the antimicrobial potential of silymarin against *Candida albicans* both alone and in combination with the antifungal agent fluconazole. The effect of silymarin was increased proportionally with increasing the concentration. The mean inhibition zone of silymarin was the highest with higher doses in a statistically significant relation. the study revealed a synergistic effect of fluconzole and silymarin with the most effective concentration being statistically significant. the rats treated with a combination of fluconzole and the silymarin 60-11µg concentration reached 100% healing within 15 days in a statistically significant relation. in vivo the efficacy evaluation showed significant differences in wound healing between candida-inoculated groups of rats. the groups with different silymarin concentrations combined with fluconzole underwent an outstanding healing process while the group treated with fluconzole only healed marginally and experienced moderate weight loss. the synergistic effect observed suggests that silymarin may be a promising adjuvant therapy for the treatment of Candida infections.

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