[HP#176]

Halal Alternative Formulation to Enhance Sertraline/ Fluconazole Antifungal Activity and Minimize Drug Side Effect in *Cryptococcus Meningitis*

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ABSTRACT

Development of drug resistance in *Cryptococcus meningitis* CM or (*C. neoformans*), which causes around 1 million symptomatic infections yearly, have an overwhelming impact on human health. Up to 60% of meningitis patients experienced relapse due to drug resistance that were linked mostly to Fluconazole. The limited array of antifungal drugs and the global resistance in *C. neoformans* vindicates the need to develop new therapeutic approaches. The objective of this research is to utilize a halal nanoemulsion formulation that maximizes the drug bioavailability, minimizes drug toxicity and resistance to *C. neoformans*. Four nanoemulsions (NEs) of clove oil, sertraline, fluconazole and sertraline/fluconazole were prepared. Particle size and polydispersity index determination (PDI) of NEs were measured utilizing Dynamic light scattering technique and Zetatrac Particle size analyzer. In vitro antifungal evaluation was applied to assess the sensitivity of the NEs using agar diffusion technique. The tested NEs showed nanometric size with PDI ranging from 0.281 to 0.493 indicating homogenously dispersed systems and elicited stronger zone of inhibition compared to same drugs suspended in water. As NEs formulation showed greater antifungal inhibition zone compared to traditional formulation, these findings can be utilized to produce a Halal potent antifungal drug using minimal inhibitory concentration that generate maximum effect.

Keywords: Nanoemulsion; Antifungal drugs; Halal alternative; Sertraline; Fluconazole; Clove oil; Particle size

1 Introduction

According to World Health Organization's 2023 guidelines recommendations, the treatment of choice for CM is based on induction therapy of liposomal amphotericin B plus flucytosine, followed by lifelong maintenance therapy with Fluconazole [1]. Fluconazole works by inhibiting ergosterol synthesis and its main attributes are high absorption and good tolerability, however, alone is prone to resistance. Thus, combination therapies are recommended to generate maximum fungicidal effect, minimize drug resistance and to decrease rate of recurrence or relapsed *Cryptococcal meningitis* [2].

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Multiple studies reported a synergistic antifungal effect between Fluconazole and Sertraline. Sertraline ability to cross blood brain barrier (BBB) and concentrate in the nervous system makes it a potential treatment for *Cryptococcus meningitis* [6,7]. Studies proposed that sertraline interacts with P-glycoprotein (P-gp) drug efflux transporter and causes its inhibition[5,6]. This Sertraline inhibitory effect of P-gp would increase Fluconazole gut absorption, central nervous system (CNS) penetration, produce higher brain concentrations, and reduce renal clearance of Fluconazole [7]. Although drug combination of Fluconazole and Sertraline showed promising antifungal effect, the common oral rout of administration for this regimen is limited by drug interactions and side effect [3,4]. To overcome these challenges, this research demonstrates a halal nanoemulsion (NE) that can be administered directly through the nasal route, which effectively bypass the BBB, gives maximum antifungal effect with minimum drug concentration and adverse reaction.

Halal NEs refers to a type of NE that avoid the usage of any non-halal ingredients. As clove oil was found to be extremely successful in the treatment of *C. neoformans*, specifically in Fluconazole resistant strains. This study approaches clove oil as halal oil phase in the NE formulation in order to investigate the efficacy of combinational therapy of clove oil, Fluconazole and Sertraline against *C. neoformans*. As NEs formulation increases drug loading and enhances bioavailability, the aim is to maximizes the therapeutic effectiveness by formulating a potent fungicidal NEs with minimal drug toxicity and resistance.

2 Materials and Methods

Sertraline, Fluconazole and clove oil was obtained from King Abdulaziz University, Pharmaceutics Department. *C. neoformans* in blood agar was obtained from King Abdulaziz Hospital. All reagents and chemicals used were of analytical grade. NE was prepared by a spontaneous emulsification method with modification. Accurately weighed amount of clove oil (0.5 g) was mixed with 400 µl of surfactant/cosurfactant mixture (Smix) (Tween: ethanol, 4:1 w/w), with magnetic stirring for 30 min. The resultant mixture was then vortexed for 60sec. Distilled water 500 µl was then added slowly to the mixture for the formation of NE. For preparation of either sertraline, fluconazole, or mixture of both loaded NE, 50 mg of the drug was initially dissolved in the clove oil prior to mixing with the Smix.

2.1 Particle size and polydispersity index determination:

Dynamic light scattering technique were employed to measure the size (z-average) and polydispersity index **(PDI)** of the prepared NE utilizing Zetatrac Particle size analyzer (Microtrac ® Inc., Montgomery, PA, US). Three replicated were done and the results were calculated as mean ± standard deviation. The size was recorded after appropriate dilution with distilled water.

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2.2 In vitro antifungal assay

Antifungal evaluation was applied to assess the sensitivity of Sertraline, Fluconazole, Sertraline/Fluconazole mixture and clove oil against *C. neoformans*. The Preliminary screening of the antifungal activity conducted using agar diffusion technique as described previously. Briefly, Petri dishes (150 mm) were filled with 50-mL Muller-Hinton agar containing 1 mL of fungal culture (1×10^6 CFU/mL). *C. neoformans* were inoculated separately and the visible fungal growth on medium were assessed. Wells (4 mm in diameter) were made in the seeded agar plates. The wells were then filled with 50-μL of each sample, clove oil (blank), Fluconazole NE/suspension (as +ve), Sertraline NE/ suspension, Sertraline/Fluconazole NE/suspension. Dishes were then incubated for 48-72 h at 37 ^{o}C. Minimal Inhibitory Concentration *MIC* was defined as the absence of fungal growth in the area surrounding the holes. The diameter of zone of inhibition is used a parameter to assess the antifungal activity and measured (mm) using a caliber. All experiments were performed in triplicate and standard deviation was calculated accordingly.

3 Results

Based on the results of our solubility trials, the mixture of surfactant and cosurfactant (Smix) in ration 4:1 has shown extensive miscibility with clove oil. The results of droplet size and polydispersity index (PDI are presented in Table 1. All the formulations showed nanometric size with PDI ranging from 0.281 to 0.493 indicating homogenously dispersed systems.

Table1: *Droplet size of the prepared nanoemulsions.*

Formulation	Droplet size (nm) ± SD
Blank NE	14 ± 4.10
Sertraline loaded NE	10 ± 2.60
Fluconazole NE	95.83 ± 1.60
Sertraline/Fluconazole NE	190 ± 30.60

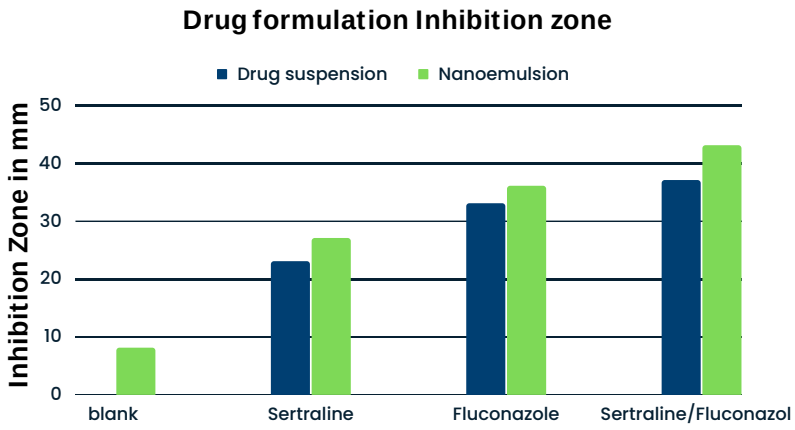

Figure 1: *Antifungal activity of clove oil, sertraline, fluconazole, and their mixture loaded nanoemulsions against C. neoformans.*

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4 Discussion

The fungistatic effect of Fluconazole, Sertraline, Clove oil, Sertraline/Fluconazole mix either suspended in water or loaded in NEs were tested using MIC. The result of *In vitro* antifungal activities against *C. neoformans* are shown in table 1 and Figure 1. The blank vehicle water showed no clear zone of inhibition (zero) compared to NE vehicle Clove oil that yielded an inhibition zone of (8 mm ±0.3). This result is in agreement with earlier reports that showed antifungal activity of clove oil in terms of zone of inhibition assay. It is also supported by previous report that illustrated a medium to strong antifungal activity of eugenol extracted from clove oil against *C. neoformans*. Although fluconazole is known to be the first line treatment of *C. neoformans* along with amphotericin B, numerous reports demonstrated cases of resistance in treatment of cryptococcosis, most of them were linked to Fluconazole. Thus, the combination of clove oil as a vehicle for Fluconazole NE may provide an additional synergistic antifungal effect against *C. neoformans* and lower the chances for fluconazole resistance. Results from this research elicited a well-marked clear zone of inhibition with Fluconazole NE (36 mm ±0.2) compared to water suspension formulation (33 mm ±0.29). This improvement in Fluconazole antifungal efficacy was attainable due to NE composition that contains clove oil.

Sertraline is the most antidepressant that demonstrated a potent antifungal activity against *C. neoformans*, the main causative behind fungus-related meningitis. Several previous studies showed the effects of sertraline alone are statistically significant in reducing fungal burden in the brain ($P < 0.05$) [7]. However, this result was not supported by clinical investigations because it has been shown that experiments requiring higher doses may be toxic. Hence, in this experiment sertraline formulated as NE, which can minimize toxicity and enhance antifungal activity. Previous study tested different drug concentration of sertraline and showed that at dose of (15mg/kg) sertraline yielded an excellent *In vivo* and *In vitro* activity against *C. neoformans* with efficacy profile comparable to the fluconazole. Our *In vitro* results showed that sertraline as NE is more susceptible to *C. neoformans* with an inhibition zone (27 mm ±0.2) as compared to sertraline in a water suspension (23 mm ±0.26).

Fluconazole reported to be a substrate for the active P-gp transporter that restrict the brain uptake of Fluconazole, which makes it susceptible to resistance mediated by the effect of P-gp efflux transporter [6]. To antagonize this effect, combining sertraline with meningitis regimen may increases fluconazole tolerance of *C. neoformans* by inhibiting the efflux effect of P-gp where sertraline acts as a potent inhibitor of P-gp [6]. An earlier study demonstrated that Sertraline inhibitory effect of P-gp may increase the brain permeation and absorption of drugs that are substrates of P-gp, which may increase their therapeutic effect [7]. Following Sertraline/Fluconazole NE treatment, our analysis demonstrated a remarkable zone of inhibition (43 mm ±0.1) against *C. neoformans*. Whereas the water suspension form of the same

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drug mixture exhibited a 10 mm difference in the inhibition zone (37mm ±0.25). This apparent difference in the inhibition zone between the NE formulation and the water suspension form could be attributed to the antifungal properties of the blank added in both formulations.

5 Conclusion

This research illustrated that halal NE formulation generated greater antifungal activity than traditional formulation, which can be clinically utilized to produce a potent loaded antifungal drug using minimal MIC that yielded the maximum effect. Using NE drug combination of sertraline/fluconazole could be also preferable in clinical setting, as patients will receive the most attainable plasma concentration in the brain. this study was employed to further enhances drug bioavailability, increases efficacy, minimizes toxicity and resistance by developing a non-conventional halal nano-formulation of sertraline/fluconazole combination and compare its fungicidal activity to the traditional drug combination form.

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