



Evaluation of Antifungal Activity of Olive Oil Based Nanoemulsions

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ABSTRACT

*Addition of skin penetration enhancer in the formulation is the simplest and most common technique to improve transdermal permeation. However chemical penetration enhancers are known to cause skin irritation. In this research project we are proposing natural penetration enhancer (olive oil) based nano-emulsion. The aim of the present study was to investigate the olive oil based nano-emulsion for poorly water soluble drug, fluconazole. Surfactant Tween 80 and co-surfactant n-butanol were selected on the basis of solubility and emulsification ability. Nanoemulsions were prepared by aqueous titration of 1:3.5 mixtures of olive oil and surfactants. Anti-fungal activities of fluconazole nano-emulsion formulations were compared with equivalent amount of fluconazole dissolved in vehicle only. Observed zone of inhibition by all treatment were statistically analyzed to achieve a valid conclusion by one-way analysis of variance with $p < 0.05$ considered as significant. All tested samples were found effective against *Aspergillus niger* except blank nanoemulsion. The statistical analysis revealed that there is significant difference ($p < 0.05$) between pure fluconazole and its formulations such as fluconazole nanoemulsions and gels and nanosuspension however there was no significant difference between the formulations.*

KEYWORDS: *Fluconazole, Antifungal activity, Sabouraud agar, diffusion method.*

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INTRODUCTION

Topical delivery as an option to oral delivery of drugs has several advantages such as reduced adverse effects, improved efficacy and patient compliance especially for the drugs that having extensive first effect metabolism [1]. However, a very few number of drugs with low dose, low molecular weight and high octanol-water partition coefficients can be successfully delivered, because of the anatomical structure of the barrier layer of skin. Topical delivery is better than painful hypodermic injections that produce hazardous biological waste and causes disease transmission because of needle contaminations [2]. To achieve successful transdermal drug delivery, enhancement of skin permeability is of prime concern. Recently several physical, electrical, chemical and biochemical techniques have been proposed to increase the permeability of the skin. Among these, modification of permeability by chemical method is most widely used owing to economical, simple and rapid. Chemical permeation enhancers either improve the solubility or partition coefficient or increase the diffusion of drugs across the skin. However these are always toxic and irritant to the skin. This led to quest of natural skin penetration enhancers. Oleic acid, an unsaturated fatty acid in olive oil has demonstrated skin penetration potential in various studies [3,4] However, there is very limited permeation enhancement study of olive oil on human skin [5]. Olive oil as such may be used as permeation enhance especially for lipophilic drugs. In one study on the use of olive oil in blood-vessel suturing, olive oil was found to be non-irritating when compared to Vaseline Paraffin oil [6]. Olive oil or other parts of olive are known to possess antifungal activity as well [7-10]. Fluconazole

is one of the most common antifungal agents. It is based on bistriazole hence has significantly different pharmacokinetic properties from other imidazole based antifungal agents. Due to halogenated phenyl ring it has more antifungal activity than other counterpart however, it is less lipophilic and more hydrophilic and therefore it is supposed to have less skin penetration potential as compared to other antifungal agents [11]. This study has been proposed to evaluate the potential of olive oil based nanoemulsion of fluconazole as transdermal antifungal formulation.

We hypothesize that olive oil, with unsaturated fatty acid, oleic acid as primary constituent, could have permeation enhancer potential and could prove as natural, non-irritating, non-toxic permeation enhancer, thus substituting the toxic and irritant synthetic chemical permeation enhancers. We assume that when olive oil based fluconazole nano-emulsion will be formulated utilizing non irritating GRAS ingredients; transdermal penetration would be further improved as nano-emulsions promote penetration, and moreover antifungal action of olive oil would be an added advantage.

MATERIALS AND METHODS

Fluconazole was obtained as gift from Riyadh Pharma. (Riyadh, Saudi Arabia). Agar, Tween 20, Tween 80, span 80, Polyethylene glycols, poly propylene glycol, isopropyl alcohol, n-butanol were purchased from Sigma-Aldrich (St Louis, MA, USA).

Screening of Excipients

Various Surfactants and co-surfactants were screened based on their capability to solubilise the drug carried out by Higuchi and Connors method [13].

Nano emulsion formulation

Based on solubility studies, different ratio of screened surfactant and co-surfactant mixtures were optimized. Varying proportions of surfactant mixture and oil were mixed together by gentle vortexing and titrated with aqueous phase to prepare nanoemulsions. Selected mixtures giving clear nano emulsions by gentle mixing only were taken for further evaluations.

Antifungal activity

Antifungal activity of blank nano-emulsion and fluconazole nano-emulsion (2% w/v) was evaluated by agar well diffusion method. Fluconazole solution 2% w/v in a mixture of surfactant and co-surfactant were used as positive control. Standard cultures of *Candida* and or *Aspergillus* species were prepared in Sabouraud Dextrose Agar. 100 μ l of the all treatments were added into wells. Each sample was used in triplicate for the determination of antifungal activity. The diameters of zone of inhibition produced by the treatments were observed after 48 h of incubation at 28°C.

STATISTICAL ANALYSIS:

Observed zone of inhibition by all treatment were statistically analyzed to achieve a valid conclusion. Statistical significance was determined by one-way analysis of variance with $p < 0.05$ considered as significant.

RESULTS AND DISCUSSION

The study was conducted to compare the antifungal activities of fluconazole with fluconazole preparations such as nanoemulsion, gel or plain vehicle using Sabouraud agar diffusion test. Besides, additional arguments are in favor of the preferential use of the first variant for the screening of natural products with antibacterial activity. The agar well plate method is the only suitable diffusion technique as the presence of suspended particulate matter in the sample being tested is much less likely to interfere with the diffusion of the drug into the agar than in the filter paper disc. Precipitation of water-insoluble substances in the disc will indeed prevent any diffusion of drug into the agar. Moreover disks are somewhat more expensive and time consuming. Nevertheless, the goal the study was to make a qualitative comparison between different treatments such as pure ciprofloxacin and inclusion products. The results of the study in terms of inhibition potency measured as zone diameters in mm are given in table 1. All tested treatments were found effective against tested strain except treatment 2 which was introduced as a negative control. The statistical analysis revealed that there is significant difference between pure fluconazole and its formulations such as fluconazole gel and nanoemulsions however there was no significant difference between the formulations. Olive oil based nanoemulsions were found to be most effective of all tested formulations.

Table 1: Zone of inhibition of different treatments (F-NE: Fluconazole Nanoemulsions, NE: Blank Nanoemulsions; F-V: Fluconazole in vehicle F-G: Fluconazole gel.) against *Aspergillus niger*.

S.N	F-NE	NE	F-V	F-G
1	35.0	0.0	25.0	28.0
2	34.0	0.0	24.0	29.0
3	35.0	0.0	24.0	30.0
MEAN	34.7	0.0	24.3	29.0
SD	0.6	0.0	0.6	1.0

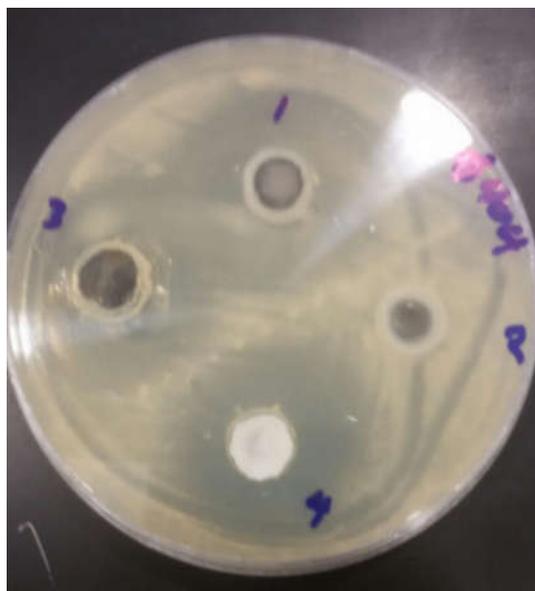


Fig.1: Zone of inhibition of different treatments (1: Fluconazole Nanoemulsions, 2: Blank Nanoemulsions; 3: Fluconazole in vehicle 4: Fluconazole gel.) against *Aspergillus niger*

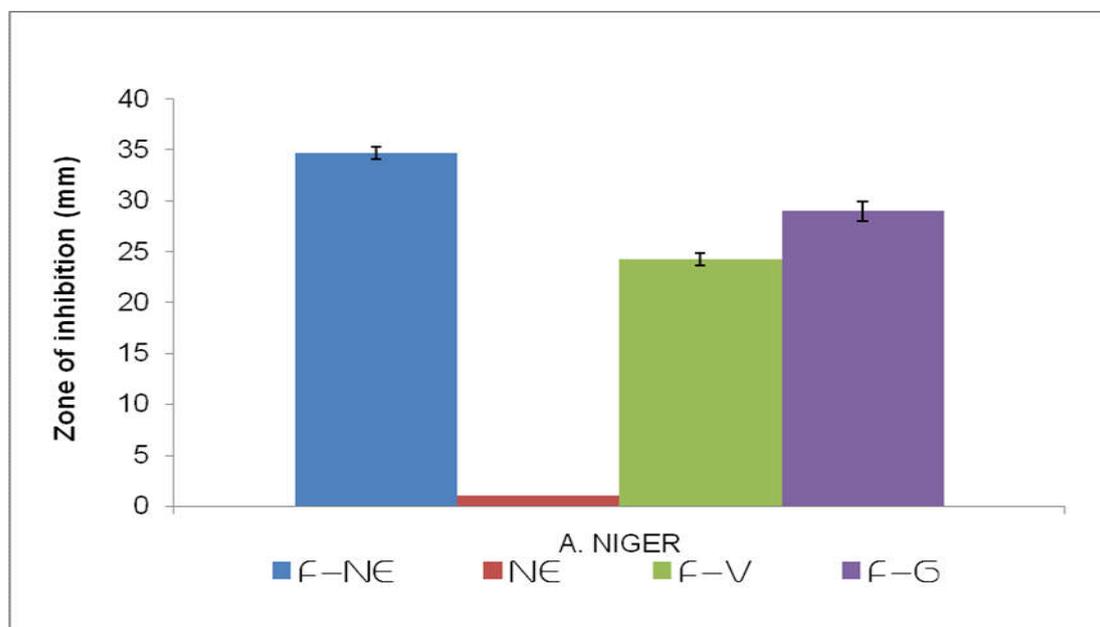


Fig.2: Histograms showing mean zone of inhibition (n=3, \pm SD) of different treatments. F-NE: Fluconazole Nanoemulsions, NE: Blank Nanoemulsions; F-V: Fluconazole in vehicle F-G: Fluconazole gel.

CONCLUSION

Antifungal activities of different formulations of fluconazole were investigated by agar diffusion method. Formulations demonstrated better antifungal activities as compared to pure fluconazole which is due to

the fact that nanoemulsions enhance the efficacy of drugs by improving drug properties like solubility and permeability.

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