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In vitro and *in vivo* Investigations of a Sudanese Honey in the Management of Dermatological Fungal Infections

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Authors' contributions

This work was carried out in collaboration among all authors. Author RSS designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors HSA and AAH managed the analyses of the study. Author BMAE managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Superficial fungal infections represented 17% of skin diseases in patients attending the outpatient clinic of Khartoum Hospital of Skin and Venereal Diseases.
The treatment of these infections usually involves the use of systemic drugs and/or topical well-tried preparations. All of the drugs carry a potential of adverse reactions, besides their relatively

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high cost. Honey, which is a natural product of the honeybee, is believed to be active against fungi. In this study, honey was used as topical treatment for six different clinical syndromes of superficial fungal infections.
The study, which was carried out at the outpatient clinic of Khartoum Hospital of Skin and Venereal Diseases, included 360 patients (210 males and 150 females) with skin, hair and nail infections. Patients, diagnosed by clinical examination and direct microscopy (KOH), were given honey to be applied on their lesions twice daily. Follow-up was made weekly by both clinical and laboratory evaluations. A complementary follow-up visit after 4 weeks of completion of treatment was needed in order to trace any clinical or mycological changes. Clinical and mycological cure showed some difference. Two hundred and sixty patients (72.4%) showed clinical cure associated with hyphal clearance on direct microscopy. Spores were not affected by honey, a direct cause of high relapse rate (42.3%). It is concluded that honey had a therapeutic effect on skin and scalp fungal infections but nail infection were not affected.

Keywords: Natural products; superficial fungal infections; Sudanese honey.

1. INTRODUCTION

Fungi are non-photosynthetic entities that do not contain chlorophyll. They exist as saprophytes or parasites [1]. In addition of being common causes of damage to crops and foodstuffs, fungi cause diseases in man. Fungi diseases are primarily infections and allergies but they may also be caused by eating foodstuffs contaminated by fungal toxins (mycotoxins).

The number of cases of fungal diseases cannot be categorically stated as it varies from one part of the world to another [1,2]. The WHO acknowledged that mycoses are a serious medical and social problem throughout the world. The widest range of fungal infections are found in the tropical and developing countries. In the Sudan, superficial fungal infections represent 17% of skin diseases [3].

The treatment of fungal infections (dermatophytes) can be effected systemically or topically. The drugs used for both systemic and topical treatment include Terbinafine, Iitraconazole, Ketoconazole, Fluconazole and Griseofulvin (see Fig. 1). The topical antifungals are mainly the Imidazoles (Clotrimazole and Miconazole) because of their broad spectrum efficacy against both yeast and dermatophyte infections of the skin (See Fig. 2) demonstrate the chemical structures of the available antifungals used in the treatment of fungal infections.

It is reported that honey is used as a medicine for thousands of years, and has been found to be an effective antimicrobial and antibacterial agent [3]. This antimicrobial activity stems primarily from the production of hydrogen peroxide from glucose and oxygen by glucose oxidase, a bee-derived enzyme [4]. Honey from certain species of *Leptospermum* flora native to Australia and New Zealand contains additional phytochemical components that further enhance its antibacterial activity [5].

These agents are applied topically for the treatment of candidiasis, tinea infections and pityriasis versicolor and can be used for vaginal candidiasis as well. Tolnaftate, a thiocarbamate derivative, is also used for the local treatment of skin fungal infections.

The undesired effects caused by these antifungal drugs vary greatly and have been reviewed by Breathnach [6]. The dermatological adverse effects include drug eruptions, toxic epidermal necrosis, urticarial, thrombocytopenia, exfoliative dermatitis. Systemic side effects include oligospermia in males, impotence and decreased libido. Mild adverse reaction include nausea, headache and abdominal discomfort [7].

Natural products are now being largely resorted to for alternatives of pure chemical substances in different fields of medicine under the motto “back to nature” and alternative medicine”. According to a study [8] the chemical compositions of honey is as in Fig. 3.

For the treatment of fungal infections, honey from honeybee, has been chosen as the natural product to be investigated in this work since its use as a remedy for skin infections caused by both bacteria and fungi as documented in ancient history. Although honey contains proteins, starches, vitamins and minerals, substances

needed for the viability of cells, fungi cultured in honey did not survive; a prove that honey doesn't contain substances with antibacterial action alone, but contains substances with antifungal action [9]. The antimicrobial effects of honey are cited in a comprehensive review by Brady et al. [10]. The chemistry of honey is depicted in Fig. 4.

The aim of this study is twofold: (a) to investigate the effect of honey on superficial fungal infections affecting man, by both clinical assessment and laboratory tests, and (b) to establish a therapeutic protocol for superficial fungal infections using honey; this includes dose, mode of administration and duration of use.

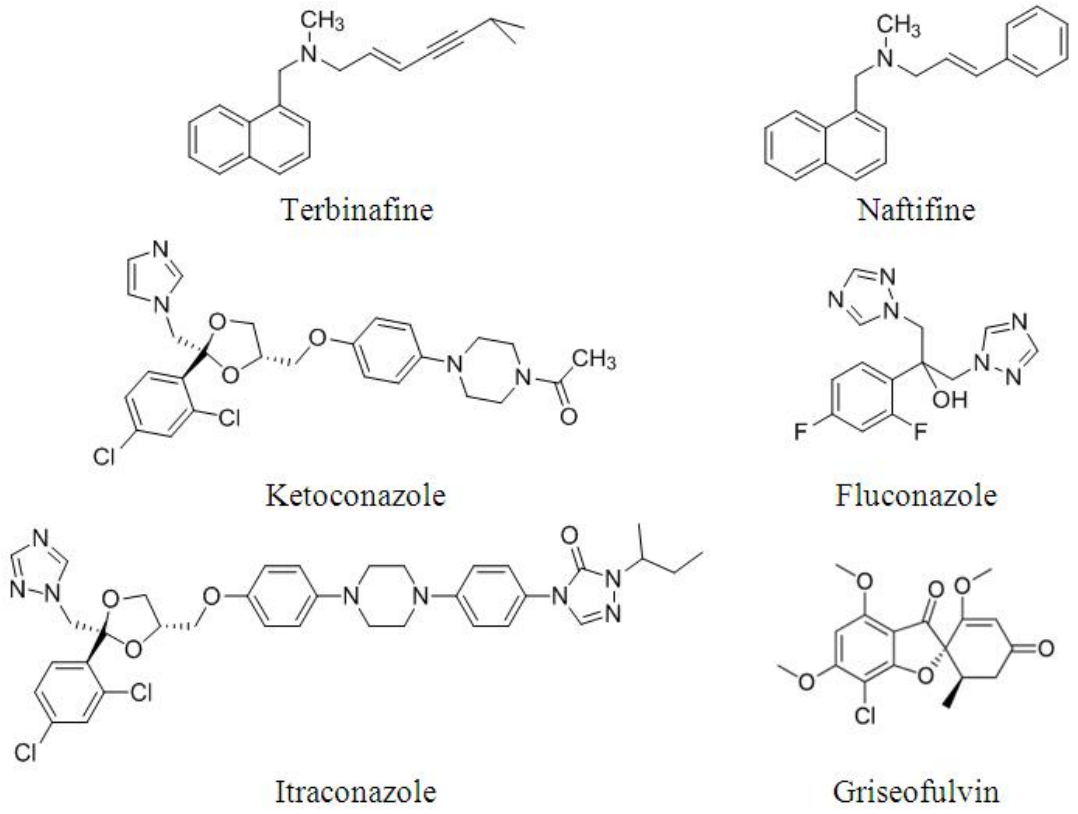


Fig. 1. The chemical structures of the drugs used for both systemic and topical fungal infections treatment

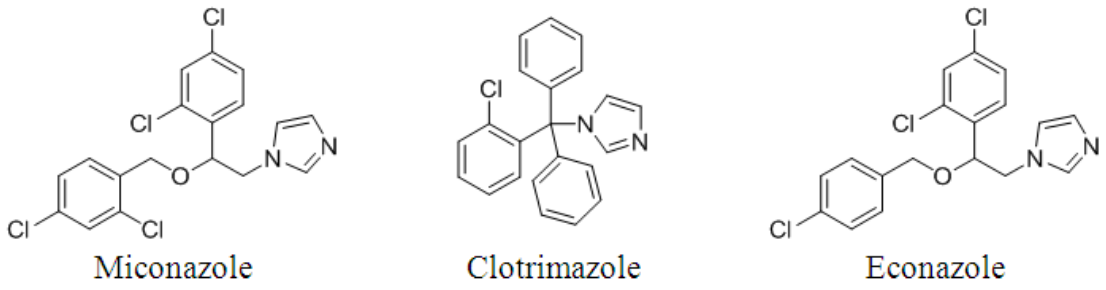


Fig. 2. The chemical structures of the drugs used for only topical fungal infections treatment

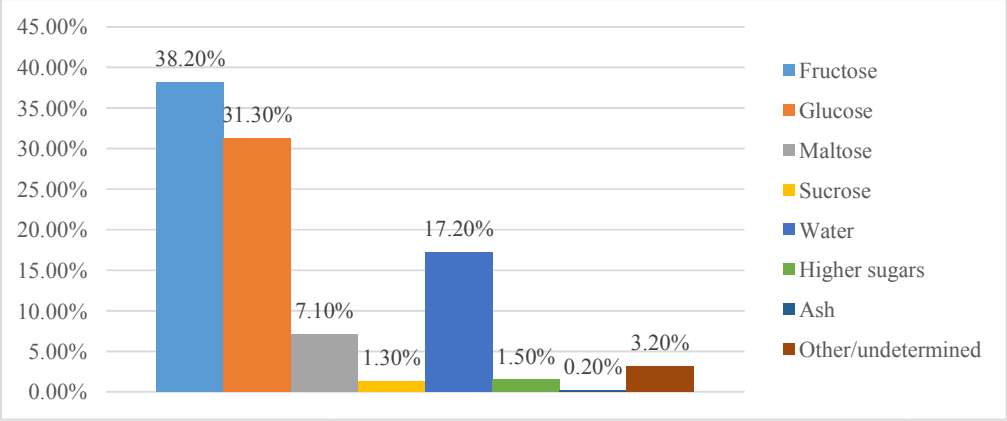


Fig. 3. Chemical compositions of honey

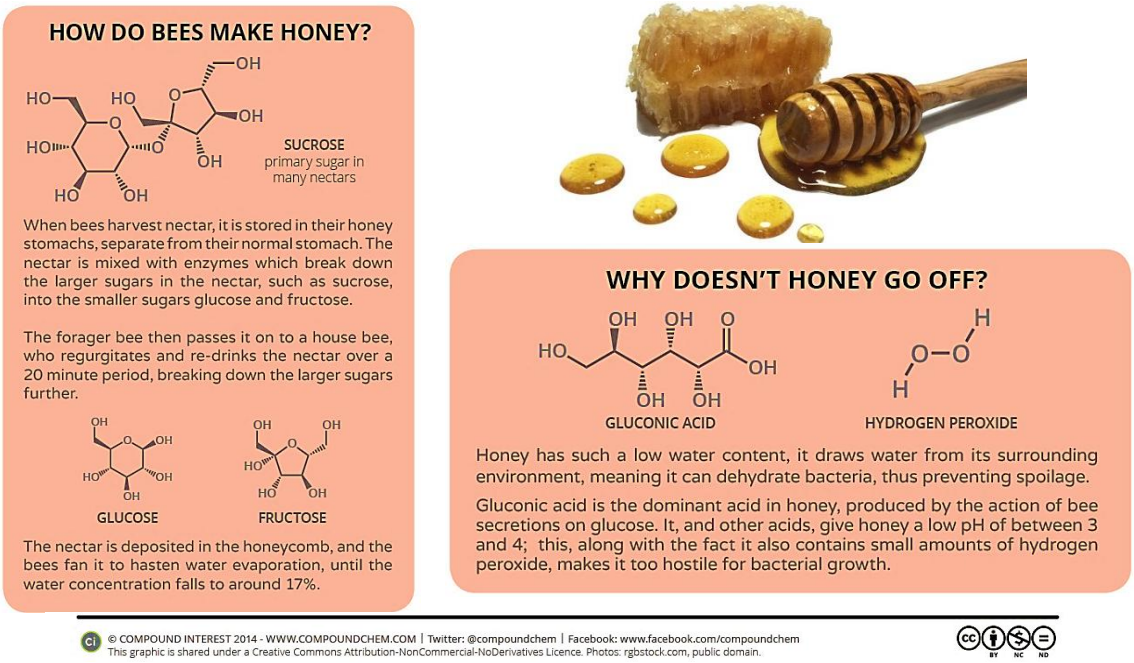


Fig. 4. Chemistry of honey

2. PATIENTS AND METHODOLOGY

All cases investigated presented themselves to Khartoum Teaching Hospital for Dermatological and Venereal Diseases during the period December 2016 to February 2017. The study approved from the ethical committee of the hospital ETHD/765/16. The number of cases was 360. Diagnosis of superficial fungal infections was made on clinical bases and laboratory investigations. Patients with different syndromes of superficial fungal infections (*Tinea corporis*, *Tinea capitis*, *Tinea cruris*, *Tinea pedis*, *Tinea versicolor* and Tinea *unguium*) were treated with honey. Follow-up of cases was continued up to the fourth week by clinical evaluation and laboratory tests. Complementary visits were made after four weeks of stopping treatment. The patients were classified according to their ages and clinical syndromes [11].

The total number of cases studied was 360, 210 males and 150 females. The experimental nature of the study was explained to the patients and they all gave their written consent to participate in the honey treatment of fungal infections.

2.1 Study Design

As for an open perspective study, honey (*Apis mallifera* L.) is collected from different areas of Khartoum, honey is given to all 360 patients, as this is not a double blind study.

Beside clinical examination, a potassium hydroxide (KOH) preparation was necessary for the microscopic confirmation of the diagnosis and for follow-up evaluation. In the KOH preparation, two parameters were considered separately: the spore index (presence or absence of spores in the specimens examined) and the hyphae index (the presence or absence of hyphae in the specimens examined) [12].

2.2 Register Book

Each patient was listed in the register book with the following information: name, age, sex, signs and symptoms, duration of disease and laboratory examination results [13].

2.3 Methodology

Honey was dispatched to patients in glass bottles each containing 6 ounces, for home use. The patients were asked to apply honey on the affected areas of the skin twice daily with gentle rubbing. Patients with nail fungal infections (*Tinea unguium*) were asked to cover their nails with wax prepared for this purpose, after the night application, so as to keep honey on the nail as long as possible [14].

In a study by Mercan et al., honey exhibited high anticandidal activity on *C. albicans, P. aeruginosa, E. coli, and S. aureus.* The honey samples that were obtained from Izmir proved more effective as inhibitors against *P. aeruginosa*, *E. coli*, and *S. aureus*. The honey that was obtained from Muğla exhibited high anticandidal activity on *C. albicans* [15].

Laboratory investigation was done using 25%w/v solution of KOH. Two different figures were used in the diagnosis and follow-up of mycological cure: hyphae and spores (which represent vegetative and sporulation phases of the fungus, respectively). Patients were asked to return to the clinic weekly for a period of one month. The follow-up criteria included clinical examination and KOH preparation. After completion of treatment, the patients were seen after another 4 weeks for detection of relapses.

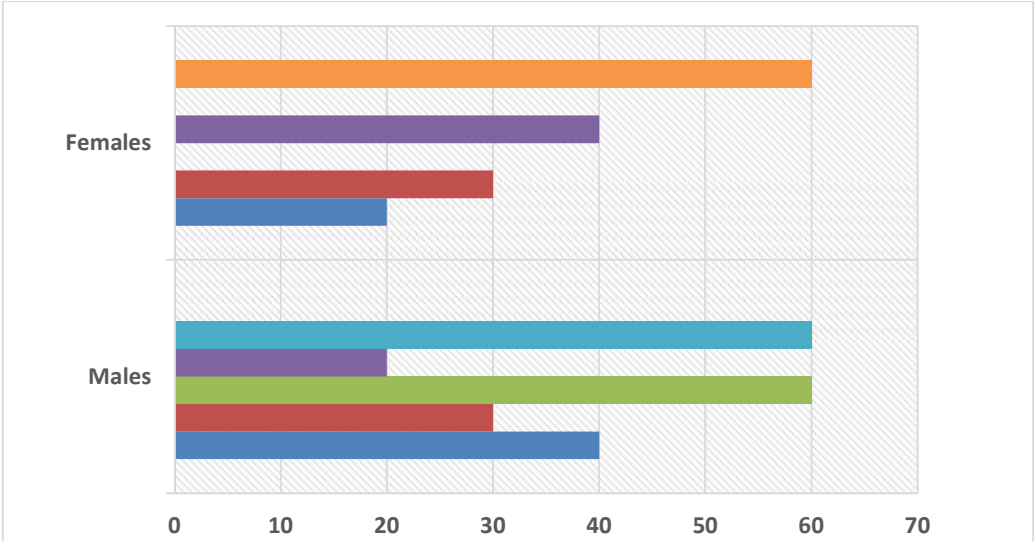
Analyses of the results were done by manual tabulation. Graphic presentations were done using Harvard graphic [16].

3. RESULTS

The distribution of the clinical syndromes according to age groups is shown in Table 1. and Fig. (1) It can be seen that *Tinea capitis* (scalp ringworm) is high (100%) in the low age group (5 – 15)years, while *Tinea* (pityriasis) *versicolor* is high on the age gp.(26- 35)years; the same is also observed for *Tinea corporis* (100%) in this age group. *Tinea pedis* affects the age groups (26–35) and (36+) in 100% rate, while *Tinea unguium* affects the same age groups in 83.4% rate.

Table 1 and Fig. 5. It shows that prevalence of infection was relatively high among males (58%) with some variations in clinical entities occurrence between males and females. *Tinea versicolor* (pityriasis) was higher in males (66.7%); *Tinea corporis* affects males and females equally. *Tinea capitis* was found exclusively in males (100%) while *Tinea unguium* exclusively affected females (100%).
Evaluation of the effect of treatment with honey on different clinical syndromes for superficial fungal infections are shown in Table 2. At different follow-up visits, thirty patients out of sixty (50%) having *Tinea pitryriasis* versicolor responded to treatment starting from week II. *Tinea capitis* patients responded to treatment at weeks III and IV with cure rate of forty out of sixty (66.70) and one relapse (25%). Patients with *Tinea pedis* all responded to therapy in the first two weeks (100% cure rate), but forty out of the sixty patients (66.70%) relapsed by week VIII. Forty out of sixty patients (66.70%) with *Tinea cruris* had their lesions cleared between week III and week IV, while twenty of them (50%) relapsed by week VIII. Ten patients out of sixty (16.70%) with *Tinea unguium* (nail infection) was showing signs of improvement at week IV of treatment, but all signs of nail involvement reappeared by week VIII.

The microscopic changes in the number of organisms upon KOH treatment are shown in Table 3 (hyphae index) and Table 4 (spore index).



	Males	Females
T.ungium	0	60
T.curis	60	0
T.pedis	20	40
T.capitis	60	0
T.coporis	30	30
T.vercicolor	40	20

Fig. 5. Sex distribution of fungal infection

Because counting the number of these elements was impractical, a change in their numbers was estimated by using the mark (+). The initial laboratory investigation, which was done before starting treatment with honey, is represented by four plus signs (++++). In the follow-up laboratory tests, a mold reduction in the number of the hyphae and spores is represented by three (+++), a moderate reduction by two (++) and a large reduction by one (+). Table 3 shows that honey application on lesions produced by superficial fungal infections led to a large reduction of the hyphae elements in five types of the six clinical entities studied. The only exception was *Tinea unguium*. Table 4 shows that honey has almost no effect on the spores of fungi. Table 2 shows that 110 cases out of the 260 which fully responded to treatment have their clinical signs relapsed.

4. DISCUSSION

It is obvious from mycological tests that honey reduced the hyphae, which are the thread-like structures by which active uptake of nourishment from the host environment is performed [6, 15]. Death of the fungi thus resulted by deprivation from nourishment. In contrast, honey doesn't seem to affect the spore form of the organism. This might be the cause of the high relapse rate (42.30%).

It has been observed from the clinical follow-up that response to treatment in the majority of cases started after the second or even the third week of treatment. Infection with *Tinea unguium* hardly started to respond at the last week (week IV) of the experiment. This observation suggests that an arbitrary treatment period of 4 weeks, as indicated in the study protocol, is inadequate and that treatment should be continued much longer and adjusted to different types of clinical syndromes individually, not by guidance of clinical clearance but rather by mycological clearance.

Table 1. Age distribution of superficial fungal infections

Type of tinea infection	*T. versicolor*	*T. corporis*	*T. capitis*	*T. pedis*	*T. cruris*	*T. unguium*	Total
Age group							
5 – 15 years	0	0	60 (100%)	0	0	0	60 (100%)
16 – 25	40(66%)	30 (50%)	0	0	0	10 (16.7%)	80 (22.2%)
26 – 35	20 (33.3%)	30 (50%)	0	0	10 (16.7%)	10 (16.7%)	70 (19.4%)
36 +	0	0	0	60 (100%)	50 (83.3%)	40 (66.7%)	150 (41.7%)
Total	60	60	60	60	60	60	360

Table 2. Evaluation of treatment by clinical findings

Time (weeks)	Week 0	Week 1 R/T* (%)	Week 2 R/T (%)	Week 3 R/T (%)	Week 4 R/T (%)	Total	Week 8 S/T** (%)
Type of infection							
T. versicolor	60 (100%)		30/60 (50)	20/60 (33.3)	10/60 (16.7)	60/60 (100)	10/60 (16.7)
T. corporis	60 (100%)	30/60 (50)	20/60 (33.3)			50/60 (83.4)	20/50 (40)
T. capitis	60 (100%)			10/60 (16.7)	30/60 (50)	40/60 (66.7)	10/40 (25)
T. Pedis	60 (100%)	40/60 (66.7)	20/60 (33.3)			60/60(100)	40/60 (66.7)
T. cruris	60 (100%)			20/60 (33.3)	20/60 (33.3)	40/60 (66.7)	20/40 (50)
T. unguium	60 (100%)				10/60 (16.7)	10/60 (16.7)	10/10 (100)
Total	360 (100%)	70	70	50	70	260 (72.2)	110/260 (42.3)

**R/T: Responsors/Total*
*** S/T: Relapses/Total*

Table 3. KOH preparation: Hyphae index; reduction of hyphae with treatment as seen at follow-up visits

Time (weeks)	Week 0	Week 1	Week 2	Week 3	Week 4
Type of infection					
T. versicolor	+ + + +	+ + +	+ +	+	+
T. corporis	+ + + +	+ +	+	+	+
T. Capitis	+ + + +	+ + + +	+ + +	+ +	+
T. pedis	+ + + +	+	+		
T. cruris	+ + + +	+ + +	+ +	+ +	+ +
T. unguium	+ + + +	+ + + +	+ + + +	+ + +	+ ++

Table 4. KOH preparation: Spore index; reduction of spore with treatment as seen at follow-up visits

Time (weeks)	Week 0	Week 1	Week 2	Week 3	Week 4
Type of infection					
T. versicolor	+ + + +	+ + +	+ +	+	+
T. corporis	+ + + +	+ +	+	+	+
T. Capitis	+ + + +	+ + + +	+ + +	+ +	+ + +
T. pedis	+ + + +	+	+		+ + +
T. cruris	+ + + +	+ + +	+ +	+ +	+ + +
T. unguium	+ + + +	+ + + +	+ + + +	+ + + +	+

5. CONCLUSIONS

This study indicates that honeybee is effective against superficial fungal infections provided that it is used for long periods of time under the guidance of mycological clearance and not the apparent clinical clearance. The fact that honey did not affect the spore forms of the fungi, suggests modification of the therapeutic protocol by either prolonging the duration of treatment or adding to honey some agents that are known to affect fungal spores in order to prevent relapses.

It is recommended that honey should be used for skin and scalp fungal infections in twice daily applications. It is also recommended to be used as topical adjuvant therapy when systemic treatment is needed, beside incorporation of the honey in a suitable formulation. A further study is recommended to enhance alternative ways for eradicating problems of relapse and resistance.

CONSENT

The experimental nature of the study was explained to the patients and they all gave their written consent to participate in the honey treatment of fungal infections.

ETHICAL APPROVAL

All cases investigated presented themselves to Khartoum Teaching Hospital for Dermatological and Venereal Diseases during the period December 2016 to February 2017. The study approved from the ethical committee of the hospital ETHD/765/16.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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