In vitro and *in vivo* therapeutic activity of ibuprofen against dermatophytes

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ABSTRACT

الأهداف: لتقييم نشاط وفعالية الدوائيات في الأنبوب وفي الجسم الحي لايبوبروفين ضد الفطريات الجلدية Dermatophytes.

الطريقة: أجريت دراسة خلال الفترة مابين يونيو 2008م وحتى سبتمبر 2008م، اعتمد تصميم التجربة على تطبيق فعالية الايبوبروفين ضد الفطريات الجلدية في المختبر وعلى الحيوان المخبري وعلى مستوى التطبيق المخبري. تم استخدام طريقة قياس قطر مستعمرة الفطر النامي وقياس الوزن الجاف لأربعة عزلات من الفطريات الجلدية المعزولة من 46 مريض (30–43 سنة) مصابين بمرض الفطريات الجلدية خلال مراجعتهم لمستشفى مرجان – مرجان – العراق، خلال شهر يونيو 2008م. لتقييم مدى فعالية الايبوبروفين عند التطبيق على الحيوان، تم تحضير مرهم الايبوبروفين (15mg/gm) لمعالجة الأرانب المصابة بالفطريات الجلدية.

النتائج: أظهر الايبوبروفين في المختبر فعالية قاتلة للعزلات الفطرية وعند اقل تركيز بلغ (200µg/ml) وكذلك تم معالجة الحيوانات المصابة بنجاح بواسطة مرهم الايبوبروفين.

خاتمة: اظهر الايبوبروفين فعالية ممتازة كعلاج لأمراض الفطريات الجلدية وبفترة زمنية قصيرة اعتماداً على نتائج العمل المخبري وعلى الحيوان.

Objectives: To evaluate the *in vitro* and *in vivo* therapeutic activity of ibuprofen against dermatophytes.

Methods: The period of study ranged from June to September 2008. For *in vitro* investigation of ibuprofen activity, measurement of colony diameter, and dry weight were employed against 4 isolated strains of dermatophytes from 46 patients (30-43 years) suffering from dermatophytoses at Morgan Hospital, Hilla City, Iraq in June 2008. For the *in vivo* evaluation of ibuprofen, rabbits as the main subjects, were infected with dermatophytes and treated with prepared ibuprofen cream (15mg/gm).

Results: *In vitro* application of ibuprofen showed cidal activity on 4 strains of dermatophytes at minimum inhibitory concentrations of 200µg/ml. The infected rabbits were successfully cured of dermatophytoses after treatment with ibuprofen cream.

Conclusion: Based on *in vitro* and *in vivo* application, Ibuprofen can be used as a short-term cure for dermatophytoses.

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Ibuprofen, 2-(4-isobutyl-phenyl)-propionic acid, is an anti-inflammatory drug widely used all over the world. It has a long history of safe and effective use by consumers.[1] Ibuprofen has been reported to have antimycotic effects in a few studies.[2-3] In the medical field, most of these studies are focused on the activity of ibuprofen on the yeast form of fungi, especially *Candida* species. *Candida albicans* (*C. albicans)* as one of opportunistic pathogenic fungi in human beings exhibited susceptibility to 170.16 µg of ibuprofen.[2] This susceptibility to ibuprofen is dependent on its concentration. Ibuprofen showed rapid cidal activity against the exponential growth phase of *C. albicans* at 10 mg/ml, while this activity converted to inhibition action at 5 mg/ml.[3] The antimicrobial activity of ibuprofen on *Candida* is not restricted by direct action, but also increasing therapeutical efficiency of standard antifungal agents, such as fluconazole and voriconazole.[4] The minimum inhibitory concentration (MIC) of fluconazole for the fluconazole-resistant strains of *Candida* decreased 2-128 folds when the drug was associated with ibuprofen.[3] Moreover, ibuprofen

in combination with amphotericin B exhibited an additive action on *C. albicans* and synergistic action in combination with econazole.[2] The filamentous form of fungi is also affected by ibuprofen, such as plant pathogenic fungi, including brown and white-rot fungi. A total of 15 from 17 isolates of brown-rot fungi revealed susceptibility to the fungicidal action of ibuprofen at a MIC of 100 µg/ml, while 8 of the white-rot fungi exhibited resistance at the same concentration.[5] Dermatophytes are a special group of fungi that require keratin for their growth. These fungi cause infections on the superficial layers of the human body, including skin, hair, and nails.[6,7] Three main genera of dermatophytes are diagnosed at the present time; *Trichophyton, Microsporum, and Epidermophyton*.[7] The most important genus of these, *Trichophyton*, infects a wide range of animals and humans.[6] In a previous study, ibuprofen showed *in vitro* inhibitory action on some species of dermatophytes.[8] The aim of this study was to evaluate the *in vitro* and *in vivo* therapeutic activity of ibuprofen against dermatophytes.

Methods. Five strains of dermatophytes were recently clinically isolated from 46 patients (30-43 years) suffering from dermatophytoses at Morgan Hospital, Hilla City, Iraq in June 2008. The period of study ranged from June to September 2008. Scales of infected skin were examined directly by light microscope with 20% potassium hydroxide (KOH) for fungal hyphae and spores. Positive scales samples were cultured on Sabouraud's dextrose agar (HiMedia, Mumbai, India) containing chloramphenicol (0.05 gm/L) and cycloheximide (0.5 gm/L). Cultures were incubated at 28°C for 1-3 weeks. The grown strains were identified according to criteria recording by Rippon[9] and Emmons.[6] The isolated strains were: *Trichophyton mentagrophytes var. mentagrophytes, Trichophyton mentagrophytes var. interdigitale, Trichophyton rubrum,* and *Trichophyton simii.*

Chemical agents. Ibuprofen purchased from Sigma (Aldrich, Germany) was dissolved in methanol. Griseofulvin and clotrimazole supplied by Arabic Drug Industry Manufacture (Baghdad, Iraq) were dissolved in sterilized hot distilled water.

In vitro antimicrobial assay. Colony diameter test. Several concentrations of ibuprofen were mixed with melting Sabouraud's dextrose agar. Then, poured in sterile Petri dishes. A disk (9 mm) of old grown fungi (at 28°C for one week) was inoculated on the center of culture media. Plates were incubated at 28°C for one week. Perpendicular colony diameters (mm) of grown strains were measured.[5]

Dry weight measurement. The same previous concentrations of ibuprofen were singly maligned in Sabouraud's dextrose broth. Approximately $2.5x10^3$ cells/ml of isolates counting in a hematocytometer were inoculated in previous broth media. Cultures were incubated at 28°C for one week. Grown colonies were filtered through filter paper (Whatman No. 1) and dried in a dry oven at 140°C for one hour. Dried colonies were weighed (in grams) using Sartorius laboratory balance (Sartours, Germany).[10] Three controls were used in the above methods, including griseofulvin (250 µg/ml), clotrimazole (200 µg/ml), and untreated media.

Minimal inhibitory concentrations determination. The MIC was performed according to Santos and Hamdan.[11] Serial 2-fold serial dilutions of ibuprofen were prepared in Sabouraud's dextrose broth. The fungal colony grown at 28°C for one week was covered with 5 ml of sterile saline (0.9%), and the suspensions were made by gently probing the surface with the tip of a Pasteur pipette. Inoculum quantification was made by counting fungal cells in a hemocytometer to obtain $2x10^4$ cfu/ml. Microdilution plates (96 wells) were set up in accordance with the National Committee of Clinical Laboratory Standard (NCCLS), reference method.[11,12] Each well of the microdilution plate that contains 100 µl of ibuprofen concentration received 100 µl of inoculum suspension. For each test plate, 2 controls were included, one with the medium alone, and the other with 100 µl of medium plus 100 µl of inoculum suspension. The microdilution plates were incubated at 28°C and were read visually after 4-7 days of incubation. A fungicidal or fungistatic effect of ibuprofen was determined by reculturing of affected colonies on free compound media. The absence of growth was recorded as fungicidal and reverse result was fungistatic.

In vivo test. Twelve male rabbits of around 3 months old were randomly distributed into 4 groups with 3 rabbits in each group. The ethical approval was obtained from the University (College of Pharmacy) for the animal studies. During the study, animals were caged separately in the same room and were supplied with foods and water. The experimental protocol followed the ethical guidelines on the proper care and use of animals. Approximately $2.5x10^3$ cells/ml of *T. mentagrophytes var. mentagrophytes* from cultures of one week at 28°C were suspended in sterile distilled water. The animal's backs of all groups were sheaved with a clipper. The naked skin was scarified with sand paper. The fungal cell suspension was applied to the scarified area.[13] Very clear lesions were observed after 15-18 days from the beginning of inoculation.

Treatment. Ibuprofen was mixed with a cream base supplied from the factory of Samara Drug Industrial (SDI, Bagdad, Iraq) to form ibuprofen cream (15 mg/gm). The first group of infected animals was treated with prepared ibuprofen cream; the second group with clotrimazole cream (1%), and the third group with cream alone. The fourth animal group was left untreated (control). Lesions improvement was noted daily for 35 days.

Statistical analysis. The results were statistically analyzed using two-way variance of analysis (ANOVA) with less significant difference (LSD) at property level (0.01).

Results. The antidermatophytic activity of ibuprofen was evaluated according to *in vitro* and *in vivo* tests. All 4 isolates showed more susceptibility to ibuprofen with complete inhibition of colony growth at concentrations of 1 and 0.5 mg/ml (**Table 1**). The activity of ibuprofen to reduce grown colonies diameter was supported by decreasing the weight of these colonies after drying (**Table 2**). After statistical analysis, ibuprofen showed more significant effects (p<0.01) on colony growth compared with griseofulvin and clotrimazole, especially by the colony diameter method (**Table 1**). The MIC of ibuprofen on isolated strains was estimated at 200 µg/ml (**Table 3**). Additionally, the action of ibuprofen revealed a fungicidal effect when no growth was observed after reculturing of inhibited colonies. The ibuprofen cream exhibited greater capacity to treat infected rabbits over a short period than that of the clotrimazole cream (**Figure 1**). After 10-14 days, the infected rabbits were completely cured from dermatophytosis, supported by negative culture of hair from the treated lesion. Meanwhile, the clotrimazole cream required 17-19 days for the animals to show improvement (**Table 4**).

Discussion. Recently, great amounts of ibuprofen have been consumed in many countries due to its lower risk than other NSAIDs with respect to overdose.[1] In addition to activity on inflammation, ibuprofen has shown antimicrobial action on various species of

Table 1 - Colony diameter (mm) of grown strains on Sabouraud's dextrose agar containing ibuprofen with inoculum disk (9 mm).

Strains	Control	Ibuprofen concentrations (µg/ml)					Griseofulvin 250 µg/ml	Clotrimazole 200 µg/ml
		1	50	100	500	1000		
T. ment. var. mentagrophytes	58 ± 0.3	45 ± 0.2	33 ± 0.1	21 ± 0.09[a]	Zero[a,b,c]	Zero[a,b,c]	12 ± 0.08	19 ± 0.2
T. ment. var. interdigitale	52 ± 0.2	40 ± 0.1	38 ± 0.2	20 ± 0.3[a]	Zero[a,b,c]	Zero[a,b,c]	13 ± 0.3	22 ± 0.1
T. rubrum	30 ± 0.1	24 ± 0.3	18 ± 0.2	14 ± 0.1[a]	Zero[a,b,c]	Zero[a,b,c]	11 ± 0.1	Zero
T. simii	49 ± 0.2	39 ± 0.2	28 ± 0.1	19 ± 0.09[a]	Zero[a,b,c]	Zero[a,b,c]	11 ± 0.1	12 ± 0.3

Mean ± SE: a) significant difference between ibuprofen and control (p<0.01), b) significant difference between ibuprofen and griseofulvin (p<0.01), c) significant difference between ibuprofen and clotrimazole (p<0.01).
T. ment. - Trichophyton mentagrophytes, T. rubrum - Trichophyton rubrum, T. simii - Trichophyton simii

Table 2 - Dry weight (gm) of grown strains on Sabouraud's dextrose broth containing ibuprofen.

Strains	Control	Ibuprofen concentrations (µg/ml)					Griseofulvin 250 µg/ml	Clotrimazole 200 µg/ml
		1	50	100	500	1000		
T. ment. var. mentagrophytes	3.59 ± 0.3	2.91 ± 0.03	1.51 ± 0.4	1.09 ± 0.08[a]	0.042 ± 0.09[a,b]	0.041 ± 0.08[a,b]	0.08 ± 0.1	0.26 ± 0.08
T. ment. var. interdigitale	3.31 ± 0.2	2.33 ± 0.1	1.63 ± 0.3	1.21 ± 0.1[a]	0.042 ± 0.08[a,b]	0.040 ± 0.08[a,b]	0.085 ± 0.09	0.30 ± 0.1
T. rubrum	2.10 ± 0.1	1.75 ± 0.09	1.31 ± 0.3	0.81 ± 0.09[a]	0.043 ± 0.1[a]	0.040 ± 0.09[a]	0.060 ± 0.08	0.04 ± 0.2
T. simii	3.50 ± 0.2	2.52 ± 0.08	1.88 ± 0.1	0.94 ± 0.3[a]	0.044 ± 0.07[a]	0.041 ± 0.1[a]	0.060 ± 0.2	0.05 ± 0.1

Mean ± SE: a) significant difference between ibuprofen and control (p<0.01), b) significant difference between ibuprofen and griseofulvin (p<0.01).
T. ment. - Trichophyton mentagrophytes, T. rubrum - Trichophyton rubrum, T. simii - Trichophyton simii

Table 3 - Minimum inhibitory concentrations of ibuprofen for dermatophytes.

Strains	Ibuprofen concentrations (µg/ml)			
	50	150	200	250
Trichophyton mentagrophytes var. mentagrophytes	clear growth	clear growth	No growth	No growth
Trichophyton mentagrophytes var. interdigitale	clear growth	clear growth	No growth	No growth
Trichophyton rubrum	clear growth	clear growth	No growth	No growth
Trichophyton simii	clear growth	clear growth	No growth	No growth

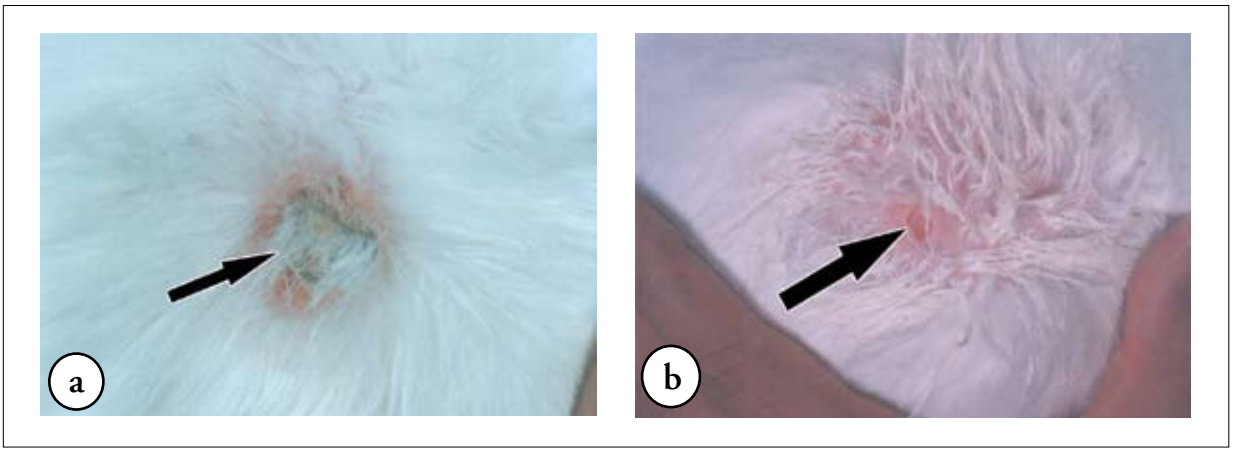

Figure 1 - Treatment of dermatophytoses in infected rabbits by ibuprofen cream (15 mg/gm), a) before treatment, b) After 14 das if treatment.

Table 4 - Improvement time (days) of infected rabbits with dermatophytes after treated with ibuprofen, clotrimazole and two controls.

Animals groups	Cream type	Improvement time (days)
1	Ibuprofen (15 mg/gm)	10-14*
2	Clotrimazole (1%)	17-19*
3	Cream alone	29-35
4	Control	28-35

* significant difference from control ($p< 0.01$)

dermatophytes. Four isolated strains of Trichophyton were susceptible *in vitro* to lower concentrations of ibuprofen. These effects of ibuprofen passed the activity of standard antifungal agents through inhibition of dermatophytic growth at lower levels compared with clotrimazole and griseofulvin. Sanyal et al1993[8] found the susceptibility of *Trichophyton rubrum* and *T. mentagrophytes* to ibuprofen at 5-20 µg/ml, while *T. tonsurance* and *Epidermophyton floccosum* at 20-40 µg/ml. Therefore, the successful action of ibuprofen on isolated dermatophytes encourages the beginning of a second therapeutic step by involving this compound in a cream formula to treat infected animals. From the results of the *in vivo* use of ibuprofen, treatment of infected rabbits with 15 mg/gm ibuprofen cream reduced improvement time, with good elimination of fungal cells from the lesion area. This therapeutic efficiency may be related to easy penetration of ibuprofen via the skin, and to its faster reach into the deep tissue of skin. Additionally, many compounds can facilitate ibuprofen penetration into the skin, such as 5% polyoxyethylene alkyl ethers.[14] In antifungal cream, ibuprofen will provide an additional advantage by virtue of its anti-inflammatory activity, which does not exist in other antifungal organic acids. Thus, ibuprofen may find efficient application as a topical skin cream for the treatment of fungal infections particularly in cutaneous mycoses. Its toxic concentrations for humans are estimated at >100 mg/L and has no life-threatening indication.[15] In topical preparations, any possible adverse and toxic effects of ibuprofen can be reduced. The mode of action of ibuprofen in the human body has been determined by inhibition of cyclooxygenase activity, which leads to inhibition of prostaglandin synthesis.[16]

In the present study, ibuprofen showed *in vitro* fungicidal activity against tested dermatophytes, and *in vivo* results confirmed this phenomenon through the absent of fungal growth from treated skin with prepared ibuprofen cream. Pina-Vaz et al[4] concluded that killing of *Candida* cells by ibuprofen is due to direct damage in the cytoplasm membrane.

In conclusion, ibuprofen is a beneficial cure for dermatophytoses and is a promising efficient drug for the treatment of many fungal skin infections after topical application.

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Ethical Consent

All manuscripts reporting the results of experimental investigations involving human subjects should include a statement confirming that informed consent was obtained from each subject or subject's guardian, after receiving approval of the experimental protocol by a local human ethics committee, or institutional review board. When reporting experiments on animals, authors should indicate whether the institutional and national guide for the care and use of laboratory animals was followed.