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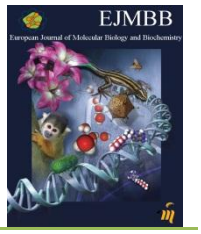

IN-VIVO ANTIFUNGAL ACTIVITY OF ETHANOLIC EXTRACT OF PROPOLIS AGAINST *ASPERGILLUS FLAVUS* IN RATS

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ABSTRACT

Propolis and its extracts have long been used for the prevention and treatment of a variety of diseases due to its antibacterial, antiviral, antifungal, antioxidant, anesthetizing, cytostatic, anti-inflammatory, and immune strengthening, hepatoprotective effect, etc. The present study was conducted to evaluate the *in vivo* antifungal activity of ethanolic extract of Propolis against *Aspergillus flavus* exposed excision wound in rats**.** Female Wistar rats were divided in to 3 groups of 6 animals each. Group I served control, group II served as reference control applied with Nystatin and group III topically applied with 5% Propolis ointment. Excision wound was induced and *Aspergillus flavus* was spread over the wound. After 14 days of topical application of test drugs, the percentage wound contraction and fungal hyphae were observed. 5% of Propolis ointment showed significant ($P<0.001$) wound contraction and absence of fungal hyphae on staining techniques. From the result it was concluded that, ethanolic extract of Propolis showed antifungal activity against *Aspergillus flavus* exposed excision wound in rats.

INTRODUCTION

Propolis is a natural product derived from plant resins collected by *Apis mellifera* bee, which uses as a building insulating material in the beehive as well as for keeping it in good health [1]. Propolis is a lipophilic in nature, hard and brittle material and it becomes soft, pliable, gummy, and very sticky when heated [2]. It possesses a characteristic and pleasant aromatic smell and varies in color from yellow green to red and to dark brown depending on its source and age [3].

Depending on the origin of the resins, it also ranges from yellow to dark brown. But even transparent Propolis has been reported. Its fairly complex chemical composition includes phenols, tannins, polysaccharides, terpenes, aromatic acids and aldehydes, among other compounds [4].

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The Propolis was found to contain β–amylase [5], many polyphenolic compounds, flavones, flavonones, phenolic acid and esters [6] and fatty acids [7]. Twelve different flavonoids, two phenolic acids, one stilbene derivative [8] and diterpenes [9] in Propolis extracts were determined. Propolis and its extracts have long been used for the prevention and treatment of a variety of diseases. Antimicrobial properties such as, antibacterial, antifungal, antiprotozoan activity of Propolis were reported [10-12]. The pharmacological potentials like antioxidant, antitumor, anti-inflammatory, hepatoprotective, antidiabetic, and immunomodulatory activities of Propolis were well established [13-18]. Propolis was also found to be effective in dental disorders such as dental caries [19].

Propolis was found to be valuable in the treatment of allergic and contact dermatitis and also used in the treatment of asthma [20]. Wound healing property of Propolis was quantified and its reepithelialisation, penetration rate were justified during cutaneous wounds healing [21].

Propolis has wide range of antimicrobial activity which was proved by in-vitro techniques. The present study was designed to establish the in vivo antifungal activity of Propolis using animal model.

MATERIALS & METHODS

Propolis Collection & Extraction

Propolis samples were collected from Bee farm in Kodumudi near Erode, in the month of September, stored in a plastic container. The collected Propolis were shade dried, grounded by mechanical grinder and 50 g of the obtained powder was dissolved in 500 ml ethanol solution (V/V) in a dark glass container and incubated at 37°C for 14 days. The solution was shaken twice a day throughout the incubation period. After 14th day, the obtained extract was filtered by Whatman filter paper (No. 4). To remove waxes and less soluble substances, the suspensions were subsequently frozen at -20°C for 24 hours, then filtered with Whatman (No.4) filter paper. The solutions were evaporated to near dryness on a rotary evaporator under reduced pressure. The dried ethanolic Propolis extract was stored in refrigerator [22].

Animals

Female Wistar albino rats of weighing 180 – 200 gm were used in the study. The animals were obtained from animal house of Sri Lakshmi Narayana Institute of Medical Sciences, Pondicherry, India. On arrival the animals were placed at random and allocated to treatment groups in polypropylene cages with paddy husk as bedding. Animals were housed at a temperature of 24 ± 2^{o}C and relative humidity of 30–70 %. A 12:12 light: dark cycle was followed. All animals were allowed free access to water and fed with standard commercial pelleted rat chaw (Hindustan Lever Ltd, Mumbai). All the experimental procedures and protocols used in this study were reviewed by the Institutional Animal Ethics Committee and were in accordance with the guidelines of the IAEC.

Source of the Organism

The pure culture of the pathogenic fungal strain of *Aspergillus flavus* was obtained from Department of Opthamology, Sri Lakshmi Narayana Institute of Medical Sciences, Pondicherry , for the present study.

Experimental Design [23]

The animals were dividend in to three groups of six animals each. All the animals were rats were immune suppressed by administration of 2mg/kg body weight of hydrocartizone for 3 consecutive days. Group I served as control and group II served as reference control treated with nystatin. Group III was served as test control treated with Propolis.

Induction of Wound and Inoculation of Spores

After 24 hrs the last dose of hydrocartizone, an external, skin depth round wound of approximately 50mm^2 was created using sterile surgical blades on the posterior mid dorsal side of all the rats under pentobarbitone (45mg/kg., i.p) anesthesia. Fresh spores of *Aspergillus flavus* were harvested in saline and the suspension was prepared 10^4 CFU/ ml count. 0.5 ml of this suspension was spread superficially on the wound. Group I animals were topically applied wih simple ointment, group II were topically applied with Nystatin cream and group III animals were topically with 5% Propolis ointment. All the test drugs were applied twice daily for 15 days. The wound area was measured and the percentage of wound closure was calculated with respect to the initial wound area at the end of the study.

Test to Recover Pathogen

On 16th day using a sterile swab the material recovered from the dry wound was stained with Gram's Stain and Lacto Phenol Cotton Blue (LPCB) staining method was observed under direct microscopy.

Statistical Analysis

Results were expressed as mean ± SEM. The data were analyzed by using one way analysis of variance (ANOVA) followed by Dunnet's '*t*' test using GraphPad version 3. P values < 0.05 were considered as significant.

RESULT

The wound healing effect of Propolis ointment on fungus *(Aspergillus flavus)* exposed excision wound model was shown in table 1. In this model, 5% of Propolis ointment showed significant wound healing property in the rats. 45.87% of wound contraction was took place on 8th day of treatment with Propolis ointment. On 8th day there was significant (P<0.01) reduction in wound area as compared to the control group. On 15th day of treatment nearly 83.45% of wound contraction was observed and there was significant (P<0.001) decrease in wound area as compared to control groups. The reference control Nystatin ointment treated group showed 92.64% of wound contraction and significant (P<0.001) decrease in wound area was observed on 14th day of treatment.

Antifungal effect of 5% Propolis ointment on fungus *(Aspergillus flavus)* exposed excision wound in rats was shown on table 2. The wound exposed to *Aspergillus flavus* in control group showed the presence of fungal hyphae in both the stains (Gram's and LPCB). The wound exposed to *Aspergillus flavus* in both reference control treated with Nystatin and 5% Propolis showed the absence of fungal hyphae in both the stains, which indicate the antifungal activity of ethanolic extract of Propolis.

Table 1. Wound Healing Activity of 5% Propolis ointment on fungus *(Aspergillus flavus)* exposed Excision Wound in Rats

Post Wound Days	Wound Area (mm^2)		
	Control (Ointment Base)	Reference Control (Nystatin)	Propolis Ointment (5%)
0	50.33±3.87	49.93±2.90	51.51±2.97
2	48.62±2.89 (3.40%)	45.72±3.24 (8.43)	48.31±2.16 (6.21)
4	42.22±2.41 (16.40)	39.66±3.20 (20.57)	40.65±2.78 (21.08)
8	39.66±3.10 (21.47)	16.32±0.07*** (67.31)	27.88±2.05** (45.87)
14	35.66±2.03 (29.39)	3.67±0.19*** (92.64)	8.52±0.06*** (83.45)

Values are in mean ± SEM (n=6); *P<0.05 , **P<0.01, ***P<0.001 Vs Control
Figures in parenthesis indicate the percentage of wound contraction.

Table 2. Antifungal effect of 5% Propolis ointment on fungus *(Aspergillus flavus)* exposed Excision Wound in Rats

Groups	Fungal Hyphae	
	Gram's Stain	LPCB
I Control (Ointment Base)	Present	Present
II Reference Control (Nystatin)	Absent	Absent
III Propolis Ointment (5%)	Absent	Absent

CONCLUSION

Propolis is the bee product noted for multiple biological effects, and therefore it is widely used for the prevention and treatment of a variety of diseases. Ethanolic extract of Propolis was evaluated for its antifungal activity in rats. The results showed that 5% of Propolis ointment exhibited antifungal activity against *Aspergillus flavus* exposed in excision wound in rats.

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CONFLICT OF INTEREST:
The authors declare that they have no conflict of interest.

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