In-Vivo Antifungal Activity Of Some Indian Medicinal Plants Extracts

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Abstract
The aim of the study was to evaluate the *in-vivo* antifungal activity of extracts of some plant species used in traditional medicine against various fungus spp. The plants were selected on the basis of their reported ethnobotanical uses and Minimal inhibitory concentration (MIC) and minimum fungicidal concentration (MFC). We conclude from this that these extracts exhibit amazing fungicidal properties that support their traditional use as antiseptics. *Plant extract* was efficient at inhibiting the growth of *C. albicans*, *in vivo*. The results of fungal burden determination in the kidney at different time points, indicated a significant reduction in CFU/g in the tissue ($p < 0.05$) starting from day 2 post-infection. Cell viability was significantly higher in mice kidney tissues treated with *plant extract*28 days post-infection. Histopathological analysis showed the presence of *C. albicans* between the tubules, as well as a few scattered lymphocytes and moderate congestion in the infected kidney 10 days post-infection. While in kidney section from mice treated with *plant extract*, mild congestion within the interstitial tissue was observed compared to Fluconazole treated groups.

Keywords: Plant Extracts, Fluconazole , Antifungal activity, *C. albicans*, *Invivo* study

INTRODUCTION

Man and perhaps some of his closer relatives have always made use of plants to treat illness. The relationship between man and plants has been very close throughout the development of human cultures. Ethnobotany is the study of plant human interrelationship embedded in dynamic ecosystem of natural and social components. An ethnobotanical text revolves around a human community's use and management of vegetation. Plants have been a rich source of medicines because they produce a host of bioactive molecule, most of which probably evolved as chemical defence against predation or infection [1].

Fungi grow as yeasts, molds or a combination of both. They reproduce through very tiny spores. These spores can exist in soil or become airborne. Most fungi cause no problems or the infections are easily treatable. People who have compromised immune systems are more likely to develop serious fungal infections. Antifungal agents are pharmaceutical fungiscides or fungistatics used to treat fungal infections or mycosis, which most commonly affect skin, hair and nails. They are also known as antimycotic agents [2, 3].

Antifungal drugs can treat fungal infections. There are a wide number of different drugs, as there are many different fungi that can cause infections. Most of the time, healthcare professionals can treat fungal infections easily, but occasionally some may be more serious. People with weakened immune systems should seek medical attention if they suspect they may have a fungal infection. Anyone with a fungal infection should speak with their doctor if it does not resolve with OTC treatments [4-6]

MATERIALS AND METHODS

Plant Materials Some plants viz. Allium sativum (leaves), Zingiber officinale (rhizomes), Glycyrrhiza glabra (roots), Curcuma longa (rhizomes), Mentha piperita (leaves), Azadirachta indica (leaves), Withaniasomnifera(leaves), Acorus calamus (leaves), Piper betle(leaves), Solanum xanthocarpum(leaves), Aloe vera (leaves) and Ocimum sanctum (leaves) were selected for the present study, based on their utility as antifungal agent, also these form common ingredients in many polyherbal formulations available as antifungal agents. All the plants parts were collected in the month of July from local market of Indore (M.P.). All these plants parts were identified and authenticated.

Preparation of Plant Extracts

The selected medicinal plants parts have been dried and powdered separately. Plants parts were mixed together in equal ratio at first than hot extraction process has been done. 50 grams of dried shade powder was exhaustively extracted with chloroform, ethyl acetate, ethanol and water using soxhlet extraction apparatus. The extracts were evaporated above their boiling points. [7]

***In vivo* antifungal activity**

Animals

Animals were procured from Local shop in Nagar Nigam market area, Indore, Madhya Pradesh. Female Albino mice (20-25gm) of almost the similar age were used for the activity. Polypropylene cages were used for housing of animals, standard rodent nutrition along with water ad libitum and an alternate cycle of twelve hours of darkness and light were provided for their nourishment. Drug was given orally by means of orogastric cannula. Animals were subjected to fasting for minimum 12hours, before any of the experiment perform; measures for test were presented for the assessment of the Institutional Animals Ethical Committee and were passed by the correspondent. Giving to CPCSEA rules for care of research creatures and the moral rule for examinations of exploratory torment in cognizant creatures, tests were acted in morning.

Acute toxicity studies

For LD_{50} determination, an oral acute toxicity study is to perform for which guidelines were regulated by OECD. OECD is an international organization named as Organization for Economic co-operation and Development, working for animal welfare. The objective is to reduce number of animals and the extent of pain accompanying with acute toxicity study. [8]

Guideline 423

Test samples were used at a pre-specified fixed dose of 5mg/kg, 50mg/kg, 300mg/kg, 2000mg/kg which is given orally to fasted healthy young adults and detect their mortality by the effect of these doses. After giving dose to animals, they were keep under observation for upto 15 days and determine body weight and necropsy.

Procedure

For the acute oral toxicity studies overnight fasted mice was selected and weighed.
All three plants extract were dosed in a stepwise procedure separately. The selection of initial dose was aimed to cause some indications of toxicity which required a two weeks' observation.
According to Guideline 423, toxic dose was selected.

***In-vivo* activity**

Survival study

C. albicans was used for infection. This isolate was maintained between studies at 4°C on Sabouraud dextrose agar. Before these studies, the isolate was transferred to brain heart infusion broth and grown at 37°C overnight. The inoculum was washed three times in saline, and an aliquot was used for the hemacytometer counting. The inoculum was adjusted to 0.2 ml/mouse, and the count of viable organisms was determined by colony count dilutions. Male ICR mice (30 g, 10 mice/group) were infected through tail vein (intravenously [i.v.]) inoculation of 5 × 106 CFU/mouse (0.2 ml of 2.5 × 107 CFU/ml). At 24 h postinfection, the animals were treated with Plant extract at concentration of 50mg/kg(p.o), 0.2 ml/mouse as a once-daily dose for 5 days. Comparison was achieved with groups of mice receiving Fluconazole 3mg/kgp.o. The survival of animals was monitored and compared to controls for 28 days post infection.

Tissue burden study

For determination of tissue burden, mice were infected and treated as described above and the tissue burden was assessed on days 2, 4, 7, 10, 14 and 28 post-infection. The kidneys were aseptically removed and homogenized in 1 ml of sterile normal saline and cultured on SDA plates and assessed by determination of fungal colonization.

Histopathological analyses

Tissues were fixed in 10% neutral buffered formalin (Merck, Germany) for at least 2 days and blocked by paraffin wax using a Shandon Automated Tissue Processor (ThermoShandon, PA, USA). The blocked tissues were cut using a Leica microtome (Leica, model RM2025, Germany) in 4-µm thickness. The sections were stained by hematoxylin and eosin (H&E) and periodic acid shiff's (PAS) and viewed under light microscopy. [9]

RESULTS AND DISCUSSION

Acute toxicity studies (Determination of LD_{50})

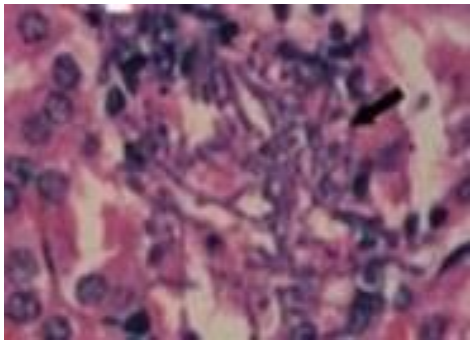
No.	No. of Animals	Dose mg/kg	Results
1	3	5	No death
2	3	50	No death
3	3	300	Death
4	3	2000	-
5	3	5000	-

Survival study

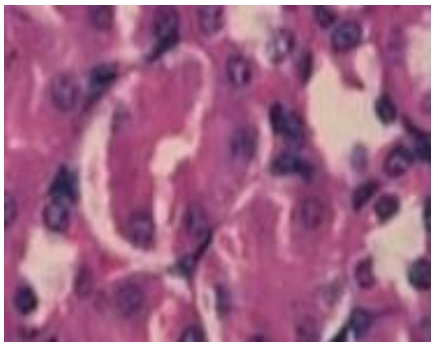
S No.	Group	Dose mg/kg	Mean survival time (In days)
1	Group I	Control Treated with saline	-
2	Group II	Injected with C. Albicans	15.54±2.83
3	Group III	Injected with C. Albicans, treated with 50mg/kg Plant extract	19.82±2.38
4	Group IV	Injected with C. Albicans, treated with 3mg/kg Fluconazole	24.28±2.65

Tissue burden study

Days post-infection	Plant extract	Fluconazole	Untreated Control
2	4.21±0.35	4.22±0.44	4.29±0.41
4	3.98±0.27	3.82±0.32	4.51±0.42
7	3.89±0.25	3.42±0.38	5.21±0.52
14	3.21±0.58	2.89±0.42	5.39±0.46
28	3.08±0.39	2.34±0.36	6.54±0.34

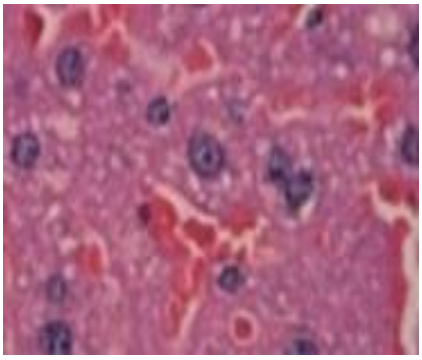


a. Normal kidney section of mice



b. Untreated Positive control

c. Treated with plant extract



b. Treated with Fluconazole

Fig. No. 1 Histological structure of normal, treated and untreated mice kidney sections

CONCLUSION

Plant extract was efficient at inhibiting the growth of *C. albicans*, *in vivo*. The results of fungal burden determination in the kidney at different time points, indicated a significant reduction in CFU/g in the tissue ($p<0.05$) starting from day 2 post-infection. Cell viability was significantly higher in mice kidney tissues treated with *plant extract*28 days post-infection. Histopathological analysis showed the presence of *C. albicans*between the tubules, as well as a few scattered lymphocytes and moderate congestion in the infected kidney 10 days post-infection. While in kidney section from mice treated with *plant extract*, mild congestion within the interstitial tissue was observed. On the other hand, kidney section from mice treated with fluconazole, showed a normal appearance of closely-packed tubules without any congestion.

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