

Mustard: the Great Gift of Danvantri for Vitiligo

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Abstract

The present study deals with mustard and its therapeutic potential for Vitiligo. We have created a Vitiligo like situation using mustard, *Pityrosporum ovale* and *Cryptococcus neoformans*. Our innovative experiment has not only opened door for the above simulation to be used for screening drugs for Vitiligo but also highlighted the enormous drug value of mustard.

Keywords: Vitiligo; Mustard; Cryptococcus; Pityrosporum

Introduction

Vitiligo is an autoimmune disorder of the skin occurs as a result of defective melanogenesis. The trigger for the disease is although unknown however the role of autoimmune factors responsible for the disease progression is well established. As on date no effective treatment is available for Vitiligo and use of steroids is often sought out medicament for Vitiligo.

Indian traditional system of medicine collectively called AYUSH (Ayurveda, Yoga, Unani Siddha and Homeopathy) has enormous claims on having effective treatment for Vitiligo. However such claims warrant scientific evidence. Several proprietary siddha products of Dr.JRK's Siddha Research and Pharmaceuticals are proven to be effective for the treatment of Vitiligo. Mustard (*Brassica nigra*) is highly venerated plant in Ayurveda and which is reported to have enormous medicinal values [1,2]. Shvitrhara kashaya is one of the famous Ayurveda drugs used for the treatment of Vitiligo [3]. Although several clinical evidences are available for mustard for increasing pigmentation of the skin still a credible scientific proof is lacking. A reliable scientific proof may help in the better exploitation of mustard for treating Vitiligo.

We have devised a novel tool in which we have mimicked melanocytes ex-situ as well as an auto-immune like trigger to evaluate the efficacy of the mustard.

For the above experiment we have used two different species of eukaryotic microbe viz., *Cryptococcus neoformans* and *Pityrosporum ovale*. *C. neoformans* is known to produce melanin like pigment in mustard medium [4,5]. Similarly *P. ovale* is known to produce Azelaic acid [6] that suppress tyrosinase and melanogenesis [7].

Finding of our research has shown a new dimension and promise for screening and developing novel drugs for the treatment of Vitiligo besides establishing the possible therapeutic benefits of mustard for Vitiligo.

Materials and Methods

Preparation of mustard medium

Mustard seeds were procured and subjected to quality check and then the seeds were powdered. Aqueous extract of mustard was prepared by adding 40 g of mustard powder to 200 ml of distilled water. It was boiled for 30 min. Then the mixture was filtered and the final filtrate was made up to 100 ml. To the mustard extract, 2 g of dextrose and 2 g of Agar agar were added and then autoclaved at 121°C for 15 minutes. The medium after autoclaving was then poured onto sterilized petriplates and was allowed to solidify. Similarly, Sabourauds dextrose Agar (Hi media laboratories) medium plates were also prepared and used.

Details of the fungal culture used

The clinical isolates of *Cryptococcus neoformans* (procured from Dr.P. Balakrishnan, YRG care, Voluntary Health Service) and the human isolate of *Pityrosporum ovale* were used for the present experiment. In brief, 7 day old culture of *C. neoformans* and *P. ovale* were used. The organisms were inoculated separately onto SDA medium and mustard agar medium. Similarly, both the organisms were inoculated together onto both the media. All the above plates in quadruplicate were incubated at room temperature for 14 days with every alternative day observation. The presence or absence of melanoid pigmentation over the culture of *C. neoformans* with reference to the extent of pigmentation and the period of incubation were recorded. Similarly, the presence or absence of pigmentation over *C. neoformans* grown along with *P. ovale* was also noted.

Statistical Analysis

Simple average value was calculated and the data is presented accordingly.

Study Design

In the present study, we have grown the culture of pigment producing organism *C. neoformans* and *P. ovale* separately and together (mixed culture) in two media viz., mustard medium and Sabouraud's dextrose Agar. Further both cultures were streaked adjacent to each other in the above media plates. The extent of pigment production/inhibition was read and scored.

Results

In mustard medium, *C. neoformans* produced intense melanoid pigment on day 14 and the pigment initiation was observed on day 10. On the contrary, no such pigmentation was observed in the above organism that was grown in SDA medium. *P. ovale* did not produce any pigment in either of the two media used. In co-culture environment i.e., *C. neoformans* grown along with the *P. ovale* in mustard medium, the

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melanoid pigmentation was observed over *C. neoformans* culture on day 5. The pigment initiation was observed on day 3 (Table 1 and Figure 1).

Discussion

Present study has opened vista of hope and promise for the treatment of Vitiligo from the Siddha system of medicine. The Siddha system of medicine is very vibrant, brilliant, scientific and time tested. The medicines of Siddha system are holistic and all inclusive it means they could prevent the disease (preventive), promote health (promotive) and cure diseases (curative). Siddha drugs not just work at the symptom level but work at the root of the disease Tridosha i.e., Vata, Pita, Kapha.

This is the first study to the best of our knowledge, where we have simulated Vitiligo like situation by using the isolates of *C. neoformans*, *P. ovale* and mustard medium. It is known that *C. neoformans* produce melanin like pigment in mustard medium [4] and the duration of pigmentation may range from 3 to 14 days. The ecosystem of mustard medium and the property of *C. neoformans* producing melanin like pigment we have contemplated for a mammalian system. The *P. ovale* is known to produce azelaic acid that suppresses melanogenesis [7]. Therefore, we used *P. ovale* as constant trigger for suppressing the pigmentation in *C. neoformans* when grown in mustard medium. We have co-cultured both the organisms in SDA as well as mustard medium with the hypothesis that the pigmentation in *C. neoformans* will be suppressed by the azelaic acid produced by *P. ovale*.

Interestingly, we found an intense pigmentation over *C. neoformans* and was unaffected by *P. ovale*. We presume that the azelaic acid may be getting inactivated in mustard medium and therefore no suppression of pigmentation has occurred. Another possibility may be that *P. ovale* may not be producing azelaic acid in mustard medium.

Our earlier experiment on the role of azelaic acid prepared from *Pityrosporum ovale* in affecting the process of phenol oxidation has clearly shown that the phenol oxidase enzyme gets downregulated thus resulting in poor coloration [8].

The model used in the present experiment can be considered as a microcosm of Vitiligo wherein *C. neoformans* plays the role of melanocytes and *Pityrosporum* the likely spoilsport of melanogenesis (the likely auto-immune cause). When we mimicked the above situation in mustard medium we found that mustard extract could overpower the effect of azelaic acid of *P. ovale* on melanin pigmentation by *C. neoformans*.

The mustard has been used for the treatment of Vitiligo in Ayurveda [2,9]. Shvitrahara kashaya is one of the famous Ayurvedic products with mustard oil used for the treatment of Vitiligo [3]. In Siddha system of medicine also we can find several corollaries for mustard.

Finding of the present study has clearly shown that mustard has enormous therapeutic potential for Vitiligo which was already discovered and used by the great scholars of Ayurveda and Siddha. Further the present study also has revealed the unimaginable science and uncommon wisdom of our ancient Ayurveda and Siddha scholars. If the world passionately and prudently follows Siddha and Ayurveda they can not only live 'disease free life' but also can possibly attain eternity and salvation.

| Details of media | Days | Organisms/Pigmentation | | |
|--------------------------|------|------------------------|-----------------|--|
| | | <i>C. neoformans</i> | <i>P. ovale</i> | <i>C. neoformans</i> + <i>P. ovale</i> |
| Saboraud's dextrose Agar | 7 | - | - | - |
| | 14 | - | - | - |
| Mustard medium agar | 3 | + | - | + |
| | 5 | + | - | ++ |
| | 14 | +++ | - | +++ |

+ = pigment initiation
 ++ = moderate pigmentation
 +++ = Intense pigmentation
 - = no pigmentation

Table 1: Pigmentation of *C. neoformans* in mustard medium.

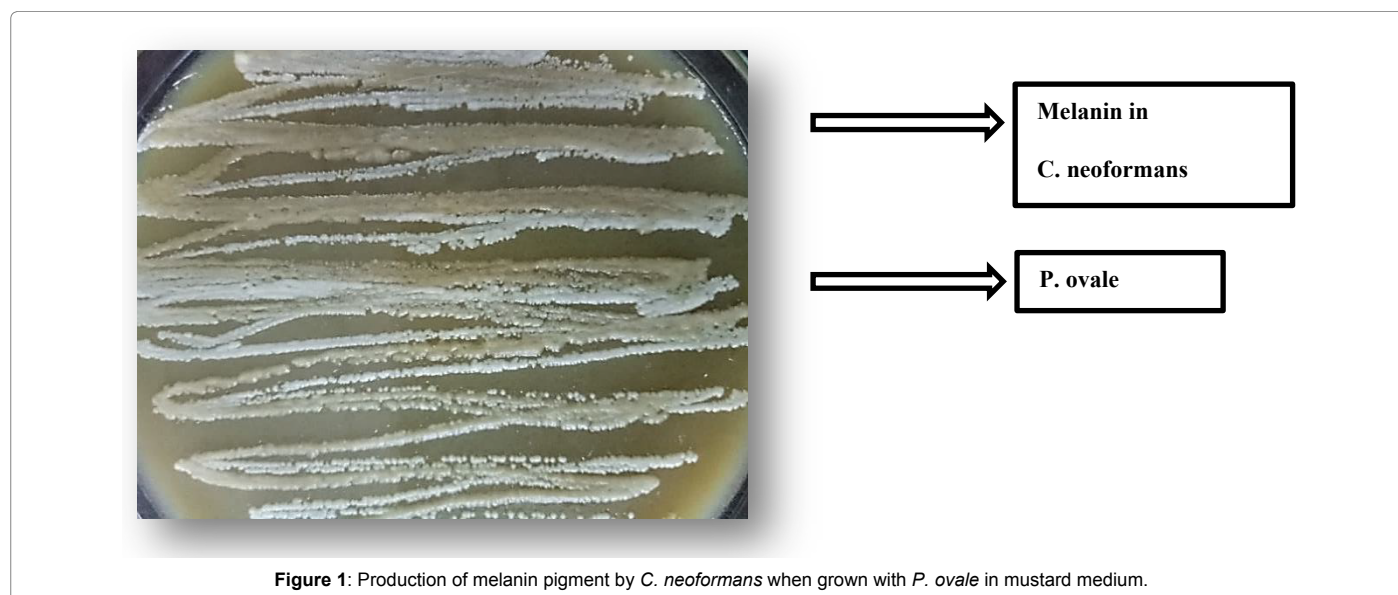


Figure 1: Production of melanin pigment by *C. neoformans* when grown with *P. ovale* in mustard medium.

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