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Studies on Effect of Analogs of Microbial Iron Chelators on *Candida albican*

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Abstract: The aim of the present research was to check the effect of iron chelators, namely Gallic acid and Salicylhydroxamate on the growth of *Candida albican in vitro*. *C. albican* is the opportunistic paltogen present as the normal flora inside human body. *In vivo* the growth of *C. albican* is disturbed by the use of antibiotics and immuno suppressers.

Key words: Iron chelator, *Candida albican*, iron overload

INTRODUCTION

Candida albican is the most pathogenic fungi. It is a thin walled non-capsulated oval yeast having 2.5-4.0 um diameter, nucleus is eukaryotic. It has mitochondria and endoplasmic reticulum. Cell membrane of *candida albican* contains a sterol with chitin (Homo polymer of N-Ac Glu) is major content of cell wall. It is gram positive[1]. It is the part of normal flora of mucous membrane of the upper respiratory tract, gastrointestinal tract and female genital, tract impaired diseases may result. Another factor is the disturbance of normal flora due to use of antibiotics, steroids and immuno suppressors or cytotoxic drugs[2,3]. *Candida* may be carried by the blood stream to any organs. Dissemination and infection may occur in patients with compromised cellular immunity e.g. those undergoing cancer chemotherapy, lymphoma, AIDS and other conditions. The opportunistic *Candida* infection may occur in pregnancy, neonatal abnormality, senility, minor trauma, continued exposure of skin to moisture or when the patient is debilitated by diabetes or alcoholism[4]. The over growth of *Candida albican* in mouth causes thrush or white patches especially in neonates at the times of birth from their infected mother. *Vulvo vaginitis* is an other common problem faced by 20% of female population[5]. Iron is essential for the life of all microbe cells. It generally exists in the oxidized form Fe (III). Even under anaerobic reducing condition the metal appear to be taken up as Fe (III)[6,7]. Thus free-living microorganisms require specific and effective ferric iron transport system to cope with the low availability of the metal. In the iron deficient environment they produce a low molecular weight specific chelators called siderophores or microbial iron chelators[8]. Siderophores compete for limited supplied of iron. These siderophores come out of the cell but can not re-enter with out iron due to high affinity of these siderophores to Fe (III).The siderophores often have more than one catechol/hydroxmate functions and are multi dentate (usually hexa dentate ligands)[9,10]. The Toxcity of metal compounds has important implication in human and animal health, agriculture ecological processes and biotechnology. Siderophores are best chelators for iron but they also disturb the equilibrium of other metal ions in living system. They also form complexes with other trace metals present in the body. Co-ordination of metal ions by siderophores may be desirable in view of the potential clinical significance. When a host is challenged by siderophores either as a drug for iron over dose or as a result of microbial invasion, balance metals may be disturbed[11]. The aim of present research was to study the effect of analogs of microbial iron chelators (siderophores) on the growth of *Candida albican*. Gallic acid and salicylhydroxamate were used as analogs of microbial iron chelators, catechol type and hydroxamate type, respectively.

MATERIAL AND METHODS

Isolation and culture of *C. albican*

Selection of Media: In order to culture and isolate *C. albican* 2% glucose sabouraud is chosen that contain the following ingredients are Peptone 20 g, D(+) Glucose 40 g, Agar 15 g, Distilled H_2O 1000 mL and 2% glucose Sabourad 75 g/1000 mL.

Preparation of media: 4.5 of 2% Sabouraud is weighed dissolved is 60 mL distilled water, in order to prepare three media streak plates containing 20 mL 2% Glu Sabouraud

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each. The solution is made soluble by medium heating and constant shaking. Then the pH of the media is determined that is 5.6. After which the media is autoclaved for 1 h at 180°C. Twenty milliliter of 2% Glu Sabouraud is measured and then streaked in to 3 petri dishes. The streaked plates are left in ultra violet ray for 1 h[12].

Germ tube test: This test is particular for *C. albican* that helps in differentiating *C. albican* from other species. This test is proceeded in the following steps:

1. The human blood is centrifuged to obtain serum.
2. The serum is sterilized and then inoculated with the few budding yeast.
3. The inoculated serum is placed in the incubator at 37°C for 3 h.
4. After 3 h, a drop of serum is placed on the slide to be examined under the microscope.
5. A few chlamydospores and germ tubes appeared on the slide, which confirms that the species cultured so far is *Candida albican*

Growth with analogs of microbial iron chelators gallic acid/salicylhydroxamic acid

Preparation of 1% stock sol of GA and SHA: One percent of stock solution of gallic acid or salicylhydroxamic acid is prepared by dissolving 0.1 g of gallic acid or salicylhydroxamic acid in 10 mL of distilled water.

Preparation of 2% glucose sabouroud media with different concentration of gallic acid or salicylhydroxamic acid: Glucose Sabouroud agar is weighed out 7.5 g and dissolved in 100 mL of distilled water in order to prepare 5 streak plates each containing 20 mL of 2% sabouroud having the pH 5.6. Now 20 mL of media is measured out separately and poured into 5 universal bottles. Each bottle is marked as 1, 2, 3, 4, 5 and then different concentration of 1% gallic acid or salicylhydroxamic acid is added to each bottle in the following manner.

To the first bottle containing 20 mL of 2% glucose Sabouraud media, the gallic acid/salicylhydroxamic acid is not added and is marked as the control. To the rest of the bottle 1% gallic acid/salicylhydroxamic acid is added in the increasing order. All of these five bottles are autoclaved for 1 h at 180°C. After autoclaved each of the bottle is emptied into the respective petri dishes marked as 1, 2, 3, 4 and 5 these streaked plates are then kept in the ultra violet rays of the laminar of or another hour.

Table 1: Two percent glucose sabouroud media with different concentration of gallic acid or salicylhydroxamic acid

Bottle no.	Media taken (mL)	Con. of gallic acid or salicylhydroxamate (μg μL^{-1})	Vol. of 1% GA or SHA (μL^{-1})
1	20	0	0
2	20	200	400
3	20	400	800
4	20	800	1600
5	20	1000	2000

Growth Identification: After three days the culture strain grew on the media agar in all plates the procedures for the identification of the organism are carried out separately for each plate, microscopic examination, Gram staining and germ tube test. In order to check the effect of gallic acid/salicylhydroxamic acid on the growth of *Candida albican*. It is checked by the simple method of hyphae counting, that is carried out under the micro scope. The procedure is as follows; -First of all the control plate is placed under the micro scope and its hyphae are counted. Then the bottle no. 2, 3, 4 and 5 are examined and the hyphae of each plate are counted in the same way[13].

RESULTS AND DISCUSSION

The present research has made an attempt to study the effect of analogs of iron chelators on the growth of microbial flora that is *Candida albicans in vitro*. The organisms has been cultured and isolated under favourable conditions, which includes nutrient filled media, appropriate pH values, fixed temperature and the most important chances of contamination has been tried to reduce to some extent. This made possibly by the use of autoclave, laminar and ultra violet light. In the first part of the experiment to isolate the required organism that is *Candida albican* from pathologically samples of urine, that has been inoculated in to 2% glucose sabouraud agar, having pH value 5.6 left for incubation at 25°C for 3 days. In this way the organism was obtained. In the other half of this experiment several tests were performed to verify whether the obtained species was a fungus and if so, for this purpose the organism was gram stained which prove to be gram positive. It showed that the cultured species was fungi then another confirmatory and specified test was performed for *Candida albican*, known as germ tube test. In this test the organisms were inoculated at 37°C for 3 h. After 3 h the formation of various germ tubes was observed under the microscope. Hence this test confirmed the presence of *C. albicans*. In the last part of the experiment, the growth of *C. albican* was to be checked against microbial iron chelators such as gallic acid and salicylhydroxamic acid, with various concentrations. Table 2 showed the effect of gallic acid while Table 3 indicated the effect of salicylhydroxamic

Table 2: Growth of *C. albican* with Gallic acid an different concentration

Plate no.	Media taken (mL)	Con. of GA ($\mu g\ \mu L^{-1}$)	Vol. of GA (μL)	No. of hyphae
1	20	0	0	12
2	20	200	400	38
3	20	400	800	76
4	20	800	1000	104
5	20	1000	2000	145

Table 3: Growth of *C. albican* with salicylhydroxamate on different concentration

Plate no.	Media taken (mL)	Con. of SH ($\mu g\ \mu L^{-1}$)	Vol. of 1% SH (μL)	No. of hyphae
1	20	0	0	15
2	20	200	400	42
3	20	400	800	87
4	20	800	1600	120
5	20	1000	2000	166

acid on the growth of *C. albican*, for these experiments again fine streak plates of 2%. Glucose Sabouraud agar were prepared each containing 20 mL of the media having pH 5.5 and marked as 1, 2, 3, 4 and 5. The plate number 1 was control while in the rest of the plates had 1% gallic acid/ salicylhydroxamic acid solutions were added in the quantity as 400, 800, 1600 and 2000, respectively then autoclaved. After autoclave the organism was inoculated in to each plate and then they were placed in the incubator at 25°C for 3 days. When the growth was observed in all plate again gram staining and germ tube test were performed with organisms of each and every plate, both of these tests were positive confirming that the cultured species were fungi and particularly *C. albican*. Now in the next half of the experiment the effect of gallic acid/salicyhydroxamic acid were studied on the growth of *C. albican*. Its effect was observed microscopically by the simple counting of hyphae or mycelium, that were long thin filament like structure, greater the number of the *C. albican* greater the number of hyphae. During the microscopic examination of each plate it was observed that as the concentration of the analogs of microbial iron chelators were increased, the growth of *C. albican* has also increased in the respective manner. It is also proved by the literature[14]. The hyphae count of each plate is listed in the Table 2 and 3. The Fig. 1 showed the comparative effect of the two metal ion chelators that was salicyhydroxamic acid and gallic acid from this it was observed that both had almost similar effects.

It can be concluded from above-performed experiments that iron chelators are responsible to enhance the growth of the organism *in vitro*. This relationship is directly proportional to each other, as the concentration of the iron chelators are increased it has resulted in the increased growth of *C. albican*. One hypothesis can be drawn out from these experiments is that the use of iron chelators as a drug, in the cases of sever iron over dosage

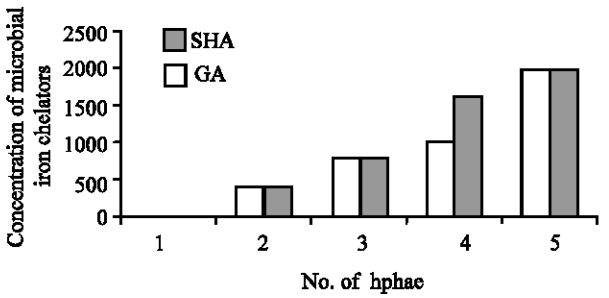

Fig. 1: Comparative growth of graph of *C. albican* with microbial iron chelators

should be limited. While in the case of mild iron over dosage, the iron chelators therapy should be avoided particularly in female. As the female especially pregnant women are at a greater risk of disturbance of the normal growth of *C. albican* in the various sites of the body. If the iron chelators are administrated to the pregnant women, they are at the higher risk of neonatal disability which may lead to *C. albican* septicemia in the neonate. The diabetic and immuno suppressed patients should also avoid the use of iron chelators.

Microbial iron chelators or siderophores are the compounds secreted by fungi in iron deficient environment, iron is very important for the performance of vital functions of fungi[8]. Present results showed that as the gallic acid and salicylhydroxamic acids were given to fungi their growth increases because these compounds made transportation of iron easier from the host to the fungi, as the reported data showed that iron chelators come out of the cell but cannot reenter without iron due to high affinity of these compounds to Fe (III)[9,10].

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