Chapter- IV

*In vivo* antifungal and wound healing activity of hexane extract of *Dillenia indica* in albino rats
4.1 Introduction

*Candida albicans*, yeast (Fig 4.1) that lives in the mouth, throat, intestine and genitourinary tract of most humans and is usually considered to be normal part of bowel flora (the organisms that coexists with us in our lower digestive tract known as fungi). *Candida* coexists in our bodies with many bacterial species in a competitive balance. Other bacteria act in part to keep *Candida* growth in check in our body ecology unless that balance is upset. When health is present, the immune system keeps *Candida* proliferation under control, but when immune response is weakened, *Candida* growth can precede unhindered. It is an “opportunistic organism” one which when given the opportunity, will attempt to colonize all body tissues. The uncontrolled growth of *Candida* is known as *Candida* overgrowth. The major culprits of *Candida* overgrowth are antibiotics and sulfa drugs; they kill the “good” flora, which normally keeps the *Candida* under control. This allows for the unchecked growth of *Candida* in the gastrointestinal tract. *Candida spp.* in particular is the predominant mycotic pathogen whose isolation from the blood is an independent risk factor for mortality (Pfaller et al., 1997). A cost of illness study has indicated that the annual cost of treating hospitalized patients suffering from Candidiasis is to the tune of $ 250 millions (Rentz et al., 1998).

![Image](www.doctorfungus.org)

Figure 4.1: Yeast in oral scraping (www.doctorfungus.org).

4.2 Candidiasis:

Candidiasis is caused by infection with species of the genus *Candida*, predominantly with *Candida albicans*. *Candida* species are ubiquitous fungi (yeasts) that represent the most common fungal pathogens that affect humans. The growing
problem of mucosal and systemic candidiasis reflects the enormous increase in the number of patients at risk and the increased opportunity that exists for Candida species to invade tissues normally resistant to invasion. Candida species are true opportunistic pathogens that surpass recent technological advances to gain access to the circulation and deep tissues.

**Fig 4.2: Percentage of Candida species infection** (Adapted from Pfaller et al., 1997).

### 4.2.1 Pathophysiology:

*Candida* species are yeast-like fungi that can form true hyphae and pseudohyphae. For the most part, *Candida* species are confined to human and animal reservoirs; however, they are frequently recovered from the hospital environment, including foods, countertops, air-conditioning vents, floors, respirators, and medical personnel (Fig 4.2). They are also normal commensals of diseased skin and mucosal membranes of the gastrointestinal, genitourinary and respiratory tracts.

*Candida* species also contain their own set of well-recognized but not well-characterized virulence factors that may contribute to their ability to cause infection (Yang, 2003).

The main virulence factors include the following:

- Surface molecules that permit adherence of the organism to other structures (e.g. human cells, extracellular matrix, prosthetic devices).
• Acid proteases and phospholipases that involve penetration and damage of cell envelopes.
• Ability to convert to a hyphal form (phenotypic switching).
• As with most fungal infections, host defects also play a significant role in the development of candidal infections.

Host defense mechanisms against *Candida* infection and their associated defects that allow infection are as follows:

• Intact mucocutaneous barriers - wounds, intravenous catheters, burns, ulcerations.
• Phagocytic cells - Granulocytopenia.
• Polymorphonuclear leukocytes - Chronic granulomatous disease.
• Monocytic cells - Myeloperoxidase deficiency.
• Complement - Hypocomplementemia.
• Immunoglobulins - Hypogammaglobulinemia.
• Cell-mediated immunity - Chronic mucocutaneous candidiasis, diabetes mellitus, cyclosporin A, corticosteroids, HIV infection.
• Mucocutaneous protective bacterial flora - Broad-spectrum antibiotics.
• The first step in the development of a candidal infection is colonization of the mucocutaneous surfaces. All of the factors outlined above are associated with increased colonization rates. The routes of candidal invasion include: (1) disruption of a colonized surface (skin or mucosa), allowing the organisms access to the bloodstream, and (2) persorption *via* the gastrointestinal wall, which may occur following massive colonization with large numbers of organisms that pass directly into the bloodstream. Candidiasis can cause a wide spectrum of clinical syndromes, as described below. The clinical presentation can vary depending on the type of infection and the degree of immunosuppression (Pappas, 2006).

### 4.2.2 Different forms of Candidiasis:

Cutaneous candidiasis (4.3a) syndromes, chronic mucocutaneous candidiasis, gastrointestinal tract candidiasis, respiratory tract candidiasis, genitourinary tract candidiasis, hepatosplenic candidiasis (chronic systemic candidiasis), systemic
4.2.3 Treatment:

Antifungal drugs such as amphotericin-B, fluconazole, micanozole, clotrimazole etc are used for treatment but these drugs are showing adverse side effects. In the present era, rapid occurrence of multi drug resistance (MDR) and extreme drug resistance (XDR) of fungi has prompted researchers to go for newer and effective drugs. In this quest, medicinal plants and their byproducts prove to be important therapeutics against these opportunistic pathogens with fewer side effects.

Many plants species are known to produce a variety of secondary metabolites with known therapeutic properties. Compounds that exhibit either fungistatic or fungicidal activity with low toxicity to host cells are considered good candidates for developing new antimicrobial drugs. Traditional preparations of some medicinal plants are used in wound healing. Wound healing is a well known physiological process that consists of cascade of events that re-establish the integrity of the damaged tissue. This process protects damaged tissues from infection with pathogens, especially with bacteria and fungi, and promotes the sealing of the damaged tissue (Sumitra et al., 2005). Wound healing is promoted by several plant products (Suguna et al., 1999), which contains several different active compounds (Sharama et al., 1990) and biomolecules (Chithra et al., 1995).

In the present chapter, cutaneous and oropharyngeal candidiasis models were taken to study the effect of efficacy of *Dillenia indica* on wistar albino rats, *in vivo*. Oropharyngeal candidiasis is the common opportunistic infection associated with oral injuries (Odds, 1988; Mac Phail et al., 1993; Samaranayake and Samaranayake,
2001). The expression of *C. albicans* virulence in the oral cavity is strongly correlated with impairment of the immune system; particularly in patients with human immunodeficiency virus infection (Lopez-Ribot et al., 1999). Cutaneous candidiasis is arguably the most common form of candidiasis. The infection involves the very outermost layers of the skin. Non hematogenous primary skin infections typically occur as intertrigo in skin folds, especially in obese and diabetic patients.

4.3 Dexamethasone:

Dexamethasone was used as potent synthetic member of the glucocorticoid class of steroid hormone, was used as an immunosuppressant to weaken the immune system of rats in the present study. It is normally acts as an anti-inflammatory and immunosuppressant. It therapeutically used as anti-inflammatory, in oncological use, endocrine, obstetrics, and high altitude illness.

![Structure of Dexamethasone](image)

**Contraindications**
- Existing gastrointestinal ulceration
- Cushing's syndrome
- Severe forms of heart insufficiency
- Severe hypertension
- Uncontrolled diabetes mellitus
- Systemic tuberculosis
- Severe systemic viral, bacterial, and fungal infections
- Preexisting wide angle glaucoma
- Osteoporosis

162
4.3.1 Side Effects

If dexamethasone is given orally or by injection (parenteral) over a period of more than a few days, side-effects common to systemic glucocorticoids may occur. These may include:

- Stomach upset, increased sensitivity to stomach acid to the point of ulceration of esophagus, stomach, and duodenum
- Increased appetite leading to significant weight gain
- A latent diabetes mellitus often becomes manifest. Glucose intolerance is worsened in patients with preexisting diabetes.
- Immunosuppressant action, particularly if given together with other immunosuppressant such as cyclosporine. Bacterial, viral, and fungal disease may progress more easily and can become life-threatening. Fever as a warning symptom is often suppressed.
- Psychiatric disturbances, including personality changes, irritability, euphoria, mania
- Osteoporosis under long term treatment, pathologic fractures (e.g., hip)
- Muscle atrophy, negative protein balance (catabolism)
- Elevated liver enzymes, fatty liver degeneration (usually reversible)
- Cushingoid (syndrome resembling hyperactive adrenal cortex with increase in adiposity, hypertension, bone demineralization, etc.)
- Depression of the adrenal gland is usually seen, if more than 1.5 mg daily is given for more than three weeks to a month.
- Hypertension, fluid and sodium retention, edema, worsening of heart insufficiency (due to mineral corticoid activity)
- Dependence with withdrawal syndrome is frequently seen.
- Increased intraocular pressure, certain types of glaucoma, cataract (serious clouding of eye lenses)
- Dermatologic: Acne, allergic dermatitis, dry scaly skin, ecchymoses and petechiae, erythema, impaired wound-healing, increased sweating, rash, striae, suppression of reactions to skin tests, thin fragile skin, thinning scalp hair, urticaria.
Allergic reactions (though infrequently): Anaphylactic reaction, anaphylaxis, angioedema. (Highly unlikely, since dexamethasone is given to prevent anaphylactoid reactions).

4.4 Materials and Methods

4.4.1 Animals

Male wistar rats (approximately 180-200g) were used in this study. They were randomized into groups of four animals, housed in large cages. The photoperiods were adjusted to 12 h of light and 12 h of darkness, daily. The environmental temperature was constantly maintained at 21°C. The rats were given ad libitum access to food and water. During the experiment, food composition was complete and equilibrated, free from antifungal agents.

4.4.2 Preparation of Animals:

The tests were conducted using a single gender as a way of reducing variability and to minimize the number required (OECD, 2000). Rats were kept in their cages for at least 2 weeks prior to the treatment to allow acclimatization to the laboratory conditions (Spielmann et al., 1999). They were also handled daily in this period. Immunosuppressed was done on the rats before one week prior to the experiment according to Martinez et al., (2001).

4.4.3 Cutaneous Candidiasis in Rats:

4.4.3.1 Inoculum Preparation:

*Candida albicans* was grown on potato dextrose agar slant at 28°C for 48h. Culture material was scraped aseptically from slants and pooled in 30ml of sterile water and briefly homogenized. Experimental protocol was represented in flowchart 4.1.

4.4.3.2 Induced Fungal Infections:

Volumes of 100μl of fungal suspension were introduced onto the test area. The area was covered with an occlusive wrapping (Transpore®) and left to incubate for 48h. After incubation the test products were introduced and the resultant inhibition of growth or healing quantified on the basis of erythema, exudates and physical size of the lesion. Infection by *Candida* was clinically detected by the presence or absence of the swelling, erythema, pain and ulceration of the inoculation sites. Not taking food properly and weight loss are signs of clinical infections.
4.4.3.3 Preparation of Ointment:

*Dillenia indica* was extracted with hexane as described in Chapter 1. Extracts were dried at room temperature and mixed with petroleum jelly and grind with mortar and pestle to a concentration of 10% (1g in 10 g of cream) and kept at 4°C until use (Neetu Jain and Meenakshi Sharma, 2003).

4.4.3.4 Preparation of Wound Sites:

The wound site was prepared following the excision wound model with slight modifications (Opara, 1999). The animals were anaesthetized with diethyl ether and the hairs on the skin of the back, shaved with sterilized razor blades. A circle of diameter 20 mm was marked on the skin and circular incisions were then made. The area was measured by using a transparent tracing paper.

4.4.3.5 Determination of Wound Healing Rate:

Treatment with ointment started after 48h of infection on the wound surface on alternate days. All the ointments were applied topically after dressing the wound. The wound areas were measured while the animals were under anesthesia on the 2nd, 4th, 6th and 8th day. Percentage of wound area was measured according to walker formula after measurement of the wound area (Walker, 1996). Percentage of wound healing was computed at the beginning of experiments. The resulting data were analyzed statistically using the One-way Analysis of Variance (ANOVA), and the significant means were separated using the Duncan multiple range test. The probability level was 5%.

\[
\text{Wound area in the day of X} = \frac{\text{Wound area in the first day}}{\text{Percentage of wound healing} = 100 - \text{percentage of wound area}} \times 100
\]

Sites were randomly selected when the treatment was repeated. Four rats were placed in each group. Rats were weighed and ointment was applied on alternative days from the day of getting infection. Lesions were measured. All rats were observed daily for any indication of interference with dress wounding. Severe irritation and enlargement of the wounds lead to termination of that specific treatment. If there are no signs of irritation, the experiment was terminated after the healing of wound completely. Experiment was repeated twice to determine the efficacy of hexane extract of *Dillenia indica*. 

165
4.4.3.6 Immunosuppression of Rats:

A model of immune suppressed rats was according to experiments Martinez et al., (2001) and repeated twice. To enhance the infection rate, rats were immunosuppressed with dexamethasone (Cortametason, vetoquinol) and treated with tetracycline (Liprophan). One week before infection, rats received drinking water with 0.5 mg/L of dexamethasone with tetracycline (0.1%). From the day of infection, dexamethasone was raised to 1 mg/L, while tetracycline was reduced to 0.01% and maintained throughout the experiment.

4.4.3.7 Experimental design for Cutaneous Candidiasis:

- Group I: Normal rats
  - Group II: Rats infected with Candida albicans
  - Group III: Rats infected with Candida albicans+ Treated with hexane extract of Dillenia indica (5mg on each rat).
  - Group IV: Rats infected with Candida albicans+ Treated with hexane extract of Dillenia indica (10mg on each rat).
  - Group V: Immuno suppressed rats infected with Candida albicans + Treated with hexane extract of Dillenia indica (10mg on each rat).

4.4.3.8 Observations:

After infection, daily observations were systematically recorded for each group of rats during the first 30 min and periodically. Observations were included changes in skin, fur, diarrhea, lethargy, weight loss and coma. The presence of factors such as erythema, exudate, swelling, ulceration, crust formation, healing and infection were checked.

4.4.3.9 Evaluation of Erythema and Exudate

These are clinical sign of infection erythema and exudates. Based on the severity, erythema and exudate results were scored and were recorded on alternate days using the Table 4.1.

| Table 4.1: Scores used to evaluate erythema and exudate. |
|----------------|------------------|
| Severity | Erythema | Exudate |
| - | No red colour at all | No exudate |
| + | Light red | Exudate |
| ++ | Clearly red | Easily visible |
| +++ | Dark area, not whole area | Substantial quantity |
| ++++ | Dark red wide spread | Large quantity. |
Male wistar albino rats of 180-200g grouped in to 5 groups

Shaving the rats on back of the skin

Creation of wound by excision model

*C. albicans* culture were introduced on the wound of rats

Allow it for 48 h to attain infection

Treatment with hexane extract of *D. indica* was given on alternate days

Treatment was given up to complete wound healing

Erythema, exudate, weight loss, irritation are observed regularly

After complete wound healing, sacrifice animals

Experiment was repeated twice

Infected area of skin of rat removed

Histopathological studies


Oral Candidiasis in the Rats

4.4.3.10 Inoculum Preparation

*Candida albicans* (ATCC10231) was grown on PDA. Mid log phase of *Candida albicans* was taken and it is grown in potato dextrose broth for 48h at 28°C. The culture was harvested by centrifugation at 2500g, and then cells were washed three times in phosphate buffer saline (PBS) and adjusted to a final concentration of $3 \times 10^8$ CFU/mL (using a haemocytometer chamber for counting cells).
4.4.3.11 Oropharyngeal Candidiasis:

A model of oral candidiasis in immune suppressed rats was according to experiments Martinez et al., (2001), and repeated twice. To enhance the infection rate, rats were immunosuppressed with dexamethasone (Cortametasone, vetoquinol) and treated with tetracycline (Laprophan). One week before infection, rats received drinking water with 0.5 mg/L of dexamethasone with tetracycline (0.1%). On the day of infection, dexamethasone was raised to 1 mg/L, while tetracycline was reduced to 0.01% and maintained throughout the experiment. The rats were orally infected three times at 48 h intervals with 0.1mL of saline suspension containing $3 \times 10^8$ viable cells of C. albicans. Oral infection was achieved by means of a cotton swab rolled twice over all parts of the mouth. Just before inoculation, the animals were sampled to confirm the absence of C. albicans in the oral cavity, and 48 h after the last inoculation all groups were sampled in the same manner to check for the presence of the fungi. Experimental protocol was represented in flowchart 4.2

![Flow chart 4.2: Oral candidiasis in rats.](image)

4.4.3.12 Experimental Design for Oropharyngeal Candidiasis:

Four rats in each group were placed and divided into three groups.
- Group I: Normal rats
- Group II: Rats infected with *Candida albicans*
- Group III: Immunosuppressed rats infected with *Candida albicans* + Treated with hexane extract of *Dillenia indica* (10mg on each rat).
- Group IV: Immunosuppressed rats alone
4.4.3.13 Antifungal Treatment:

At 48 h post-infection, the animals were randomly assigned to one antifungal effect of hexane extract of *Dillenia indica*. Group III rats were treated with a topical application in the oral cavity on alternate days for eight consecutive days (day 0 to day 7). The control, infected and untreated animals received an oral 0.5 ml sterile saline solution. An additional group of nonimmunosuppressed animals infected and untreated was added to the experiment to study the impact of dexamethasone/tetracycline treatment on the development of the infection. Animals immunosuppressed but none infected were used as a negative control group.

4.4.3.14 Determination of Infection level and Therapeutic efficacy of Hexane extract of *Dillenia indica*:

Microbiology and histopathology scores were used to assess the determination of infection level and therapeutic efficacy of hexane extract of *Dillenia indica* between the control and treated rats (Martinez *et al.*, 2001).

**Microbiology:**

Samples were collected after the last treatment by rolling a sterile cotton swab over the oral cavity, which was then suspended in 1 mL of sterile saline. 25µl samples from this suspension were dropped in duplicate, plated on PDA plates. All plates were incubated at 28°C for 48 h, and the colonies were counted.

**Histopathology:**

At day 8, i.e. 24 h after the administration of the last dose of antifungal agent or saline, the animals were sacrificed with an overdose of diethyl ether. The tongues were removed, fixed in toto by immersion in Bouin solution for at least 48h. Tongue sections were embedded in paraffin and 5µm thick serial transverse sections were stained with both hematoxylin-eosin stain and periodic acid Schiff (PAS), to assess the fungal infection. The results of histopathology were presented in next chapter.

4.5 Results and Discussion:

Experimental models of cutaneous and oral candidiasis in rats have been shown to be simple and highly reproducible *in vivo* method of studying antifungal efficacy of medicinal plant extracts. To achieve a good adhesion on skin and mouth parts, extract was mixed with petroleum jelly and was used as an excipient. The efficacy of plant extract on *C. albicans* infection on skin and tongue was compared by microbiological and histopathological studies.
4.5.1 Body Weight of Rats:

After attaining infection, daily observations were systematically recorded for each group of rats during the first 30 min and periodically. Observations were included changes in skin, fur, diarrhea, lethargy, weight loss and coma. Initial weight loss was observed in all groups of rats. Weight loss was observed in *C. albicans* infected rats. Compared with normal rats, the rats infected with *Candida albicans* did not regain its weight during the experiments because of not intake of food and irritation. On the 0th day the weight is 200 and on 8th day the weight was found to be 189g. Rats treated with hexane extract of *Dillenia indica* showed regain in body weight. The body weights of different groups of rats are represented in the Table 4.2.

<table>
<thead>
<tr>
<th>Groups</th>
<th>0th Day</th>
<th>2nd Day</th>
<th>4th Day</th>
<th>6th Day</th>
<th>8th Day</th>
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<tbody>
<tr>
<td>I</td>
<td>180</td>
<td>180</td>
<td>185</td>
<td>185</td>
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<tr>
<td>II</td>
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<tr>
<td>III</td>
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<tr>
<td>IV</td>
<td>210</td>
<td>193</td>
<td>195</td>
<td>197</td>
<td>207</td>
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</table>

4.5.2 Percentage of Wound Healing:

Wound healing is an important biological process involving tissue repair and regeneration. A wound is described as 'a break in the continuity of tissue, from violence or trauma' and is regarded as healed if there is a restoration of the wounded or inflamed tissue to normal condition (Taber, 1965). Certain factors that influence wound healing include bacterial infection, nutritional deficiency, drugs, sterility, obesity, movement of wound edges, site of wound, and wasting diseases. Several drug classes have been used in the management of wounds. Among these are the antibiotics. Penicillin and streptomycin have been widely employed in combating post-operative infections in man and animals (Gyang, 1986). Wound healing is a multifactorial process where microbial infections and the formation of free radicals may contribute to retard or inhibits its resolution. Free radicals can oxidize the endogenous inhibitors or proteases; this reduces their ability to inhibit elastase and the proteases responsible for the deterioration of the extracellular matrix (Kudi *et al.*, 1999). The wound healing activities of plants have since been explored in folklore.
Increased wound healing activity was observed in all the treated rats. At higher concentration (10 mg/rat), the % of wound healing was 100% whereas as 80% in lower concentration (5 mg/rat) was observed on 8th day (Fig 4.4 and 4.5).

![Graph showing wound healing activity in different groups of rats.]

**Fig 4.4:** Percentage of wound healing indifferent group of rats.

Healing process does not normally require much help but still wounds cause discomfort and are prone to infection and other complications. Therefore, use of agents expediting healing is indicated. Further, some diseases like diabetes, immunocompromised conditions, ischemic and conditions like malnourishment, aging, local infection, local tissue damage due to burn or gunshot wounds lead to delay in healing. Such conditions often require the use of agents which can facilitate the healing process (Mensah et al., 2001).

### 4.5.3 Erythema:

Erythema is redness of the skin caused by capillary congestion. Erythema is a common side effect of radiotherapy treatment due to patient exposure to ionizing radiation. In about 30-50% of cases, the cause of erythema is unknown. It is one of the fundamental properties of the skin, ability to respond to treatment. In rat populations these responses are clearly adaptive where the first response, erythema (redness) is a sign that the immune system is active and healing process has begun. The resulting healing was quantified on the basis of erythema, reported in the Table 4.3. They took long time to heal in all cases. The hexane extract of *Dillenia indica* tended to decrease the erythematic condition in practically in all cases (Table 4.3).
Table 4.3: The influence of hexane extract of *Dillenia indica* on the wound erythema of rats infected with *C. albicans* in different groups of rats.

<table>
<thead>
<tr>
<th>GROUP</th>
<th>2&lt;sup&gt;nd&lt;/sup&gt; Day</th>
<th>4&lt;sup&gt;th&lt;/sup&gt; Day</th>
<th>6&lt;sup&gt;th&lt;/sup&gt; Day</th>
<th>8&lt;sup&gt;th&lt;/sup&gt; Day</th>
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<tr>
<td>I</td>
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Fig 4.5: a. Wound created on the skin of rat; b. Wound formation and infected with *Candida albicans*; c. Complete wound healing.
4.5.4 Exudate:

An exudate is a fluid, cells or other substances that have been slowly exuded, or discharged, from cells or blood vessels through small pores or breaks in cell membranes. Its composition varies but generally includes water and the dissolved solutes of the main circulatory fluid such as sap or blood. In the case of blood: it will contain some or all plasma proteins, white blood cells, platelets and (in the case of local vascular damage) red blood cells. The basic concept behind moist wound healing is that the presence of exudate in a wound will provide an environment that stimulates healing. Exudate contains various components, including: lysosomal enzymes, WBC's, lymphokines and growth factors. Exudate formation was also one of the parameters used to quantify the healing process. Same scale was used as used in erythema. Exudate formation was observed up to 6th day in rats infected with Candida albicans. In treated rats and immunosuppressed treated rats the exudate formation was observed up to 4th day. From 6th day onwards no exudates were observed (Table 4.4).

Table 4.4: The influence of hexane extract of D. indica on the wound exudates of rats infected with C. albicans in different groups of rats.

<table>
<thead>
<tr>
<th>GROUP</th>
<th>2nd Day</th>
<th>4th Day</th>
<th>6th Day</th>
<th>8th Day</th>
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4.5.6 Crust Formation:

A solidified, hard outer layer formed by the drying of a body exudate, such as blood or pus, common in dermatologic conditions such as eczema, impetigo, seborrhea, and favus and during the healing of burns and lesions; a scab. It is also called as crusta. Wound healing process was also quantified by crust formation. The treated groups present a rigid dark and thick crust. It is probably due to proteins and wound exudates, interconnected with the extract constituents favouring the local homeostasis and protecting the new tissue by forming an external cover that furnished mechanic protection. The crust formation in all infected rats follow the same patterns i.e., crust start forming on after 2nd day until 8th day. There was no marked difference in crust formation of all the treatments.
4.5.7 Oropharyngeal Candidiasis:

Oropharyngeal candidiasis is the most common opportunistic infection associated with oral injuries (Odds, 1988; Samarnayke and Yacoub, 1990; Mac Phaik et al., 1993). The expression of Candida albicans virulence in the oral cavity is strongly correlated with impairment of the immune system, particularly in patients with human immunodeficiency virus infection (Lopez-Ribot et al., 1999). In addition, several conditions, such as hypo salivation, diabetes mellitus and prolonged antibiotic and corticoid therapy can predispose to oral candidiasis (Knight et al., 1997). Specific features of this fungus that contribute to the development of oral candidiasis include its ability to adhere and to colonize the oral mucosa (Kennedy, 1988) and to form germ tubes (Casanova et al., 1997).

Prior to initiating the study, oral cavity micro flora of each rat was examined, and no C. albicans was found. Before sacrificing the rats, a cotton swab rolled twice in both control and treated rats over all parts of the mouth and spread on plate containing PDA and incubated for 48h at 28°C to check for the presence of Candida albicans. A single colony was found in the plate after incubation in treated rats (Fig 4.6). Histopathological data showed the presence of Candida albicans in both skin and dorsum part of the tongue in infected rats and no Candida albicans growth in treated rats. The experimental model of oral candidiasis in rats has been shown to be a simple and highly reproducible in vivo method of studying the efficacy of antifungal agents (Jones et al., 1976).

Fig 4.6: Single colony was observed after treatment with hexane extract of D. indica.
A number of antifungal agents are available for the management of candidal infections (McGinnis and Rinaldi, 1996). The major agents that are currently used for oropharyngeal candidiasis belong to either the polyenes (amphotericin B and nystatin), the imidazoles (clotrimazole, econazole, ketoconazole, and miconazole), or the triazoles (fluconazole and itraconazole) (Ellepolla and Samaranayake, 2000). Nystatin is ideal for topical treatment of oral infections since it is not absorbed from the gastrointestinal tract and hence the adverse effects are minimal. Amphotericin B is less widely used for this purpose due to its treatment-limiting adverse effects such as nephrotoxicity.

The introduction of the imidazole and azole groups of antifungal during the last two decades has revolutionized the management of fungal infections. The approved azole antifungal agents for the treatment of oral candidiasis are miconazole, clotrimazole, ketoconazole, fluconazole, and itraconazole. Miconazole is effective for almost all oral manifestations of candidiasis including chronic mucocutaneous candidiasis. Until the introduction of the triazoles (itraconazole and fluconazole), ketoconazole (an imidazole) was widely used as an alternative to amphotericin B, but it suffered from the drawbacks of hepatotoxicity and endocrine toxicity. However the more recently introduced triazole agents, itraconazole and fluconazole, are far superior since they are orally active and water soluble and have a significantly lower toxicity than do the imidazoles (Kauffman, 1996). Indeed, fluconazole is the drug of choice in the treatment of candidiasis in HIV infection. The histopathological studies were discussed in next chapter.