Inhibitory effect of Cinnamon cassia against Candida albicans growth in vitro and in vivo

Bushra J. Mohamed       Rasha Abdilhussain
bbushra71@yahoo.com

Abstract
The inhibitory effect of Cinnamon cassia against Candida albicans which isolated from cases of sore mouth in vitro and in vivo was studied. The results of determining inhibitory effect of alcohol and aqueous extract of Cinnamon against C. albicans according to well diffusion method showed that Cinnamon extract had the best effect at 50% concentration with inhibition zone reached to (23) millimeter in aqueous extract and (20) millimeter in alcohol extract while the less effect was observe at 10% concentration which reached to (11) millimeter in aqueous extract and (9) millimeter in alcohol extract. The outcome of in vivo study (histological examination) clarified that C. albicans caused clinical pathological effect in mice tissue organs ( liver, intestine, and kidney) when administrated orally by 1.5x 10^8 cfu/ml C. albicans that effect decrease by orally inoculated with 5 mg/ml of the aqueous Cinnamon extract. The results reflect the ability of cinnamon to reduce certain clinical pathological change in mice organs, with promising encourage to use the Cinnamon as biotherapeutic agents against C. albicans infections.

Keywords: Cinnamon, Candida albicans
contain several active components, *Cinnamon* is native to India and Sri Lanka (Ceylon) (Rastogi and Mehrotra, 2002). The use of *Cinnamon* for health is not new. In fact, *Cinnamon* bark has been used for several thousand years in traditional eastern and western systems of medicine (Srinivasan et al., 2001), for such purposes as anorexia, dyspepsia with nausea, flatulent colic, treatment for diarrhea, and gastric ulcers, against respiratory ailments for treatment of bronchitis, coughs and externally as a skin antiseptic as a poultice to treat minor bacterial and fungal infections of the skin (Wijesekera, 1978). Thus, the aim of this study was the inhibitory effect of *Cinnamon* on *Candida albicans* which isolated from cases of sore mouth in vitro and in vivo.

**Materials and Methods**

*C. albicans*: isolated from patients whose suffering from sore mouth and identified by using standard microbiology techniques. The isolates were culture in Sabourauds dextrose broth (Difco, USA) and routine susceptibility testing of some antifungal agents (Clotrimazole, Griseofulavin, Ketoconazol, Miconazol and Nystatin Oxoid.-England) was performed by modified of disk diffusion method described by.

**Performed of Cinnamon cassia extracts**

Alcohol and aqueous extract of *C.cassia* was performed according to (Allagelly, 2009) and the dilutions of extract performed by adding autoclaved distil water to prepare the concentrations: 10%, 20%, 30%, 40%, 50%.

**Determining inhibitory effect of C.cassia against C. albicans**

The antifungal activity of Alcohol and aqueous extracts of *Cinnamon* was evaluated by agar well diffusion method according to (Anderson and Doane, 1972)

**In vivo study**

This was performed according to (Fang et al., 2003) as following:-

**Laboratory animals**

Twenty mice were obtained from biotechnology research center were used for in vivo experiments, their age between (8-12) Weekes and weighting (23-25) gm, the mice were divided into (4) groups and each group contain 5 mice as following:-

- **Negative control**: The animals of this group not given any things.
- **Positive control A**: The animals of this group administrated orally by 5 mg/ml of the aqueous *Cinnamon* extract daily.
- **Positive control B**: The animals of this group administrated orally by 0.1 ml (100 microliter) *C. albicans*: (1.5x 10⁸ cfu/ml) daily, suspensions diluted in 20% sucrose.
- **Test group** (Challenge dose): The animals of this group administrated orally with 0.1 ml *C. albicans*: (1.5x 10¹⁸ cfu/ml) suspensions diluted in 20% sucrose daily and one hour later, they were orally administered by 5 mg/ml of the *Cinnamon* extract daily.

Throughout each experiment mice were given water containing streptomycin (5 mg/ml) to reduce the level of facultative anaerobic bacteria that normally colonize the mouse intestine (Myhal et al., 1982). At 7 days post-infection, mice were sacrificed, and tissue specimens of kidney, liver and intestine, organs were transferred to histological examination.

**Histological Examination.**

Histological Examination was diagnosed under supervision of histopathologist in Biotechnology Research Center/AL-Nahrain university. The samples which was fixed in (10%) formalin solution then was washed by tap water for few minutes and left in ethanol (50%) for (30 minutes) while (70%) ethanol was used to keep the samples for long time. The samples was transferred to (2.5% absolute ethanol +75%
butanol) and left for (2 hr). Paraffin wax sectioned in (4µm) thickness to be easier to use, then samples was stained with hematoxyline–eosin stain.

**Results and discussion**

**In vitro**

The susceptibility tests of isolates to different antifungal agents were found that all tested isolates were sensitive to Miconazol, Clotrimazole and Ketoconazole, while resistant to Nystatin, Griseofulavin. When we compared present results with others it was found close similarity with (Gordon et al., 2002).

The results of this study revealed that alcohol and aqueous extracts of cinnamon cassia were effective against *C. albicans* in different concentration and had the best effect at 50% concentration which reach to (23) millimeter in aqueous extract and (20) millimeter in alcohol extract while the less effect was observe at 10% concentration which reached to(11) millimeter in aqueous extracts and (9) millimeter in alcohol extract and as shown in tables (1,2).

<table>
<thead>
<tr>
<th>Concentration of Cinnamon extract</th>
<th>Inhibition zone of Cinnamon against <em>C. albicans</em> measured by millimeter</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>11</td>
</tr>
<tr>
<td>20</td>
<td>15</td>
</tr>
<tr>
<td>30</td>
<td>18</td>
</tr>
<tr>
<td>40</td>
<td>19</td>
</tr>
<tr>
<td>50</td>
<td>23</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Concentration of Cinnamon extract</th>
<th>Inhibition zone of Cinnamon against <em>C. albicans</em> measured by millimeter</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>9</td>
</tr>
<tr>
<td>20</td>
<td>13</td>
</tr>
<tr>
<td>30</td>
<td>16</td>
</tr>
<tr>
<td>40</td>
<td>17</td>
</tr>
<tr>
<td>50</td>
<td>20</td>
</tr>
</tbody>
</table>

The *Cinnamon* extract was found to be effective against *C. albicans* but this activity strongly dependent on the kind of extract and this may due to concentrated the active compounds such as volatile oil (cinnamaldehyde, eugenol, cinnamic acid, weitherhin) and mucilage, diterpenes, proanthocyanidins, as claimed by (Matan et al., 2006).

This effect is in agreement with other researchers regarding the antifungal effect against *C. albicans* (Quale et al., 1996), however there is a difference in the concentration of extract of *Cinnamon* at which we found antifungal activity, this concentration was found much higher than that mentioned by the above researchers.

From the results proved that *Cinnamon* could offer as alternatives treatment and the studies should be aimed at opening new possibilities of *Cinnamon* antimicrobials synthesis to increase their production on an industrial scale.
Histological Diagnosis:

Histopathological changes of the organs by *C. albicans* with or without *Cinnamon* extracts were detected in different organs in administrated mice as following:-

- From positive control A which administrated with cinnamon extracts only liver sections shows normal appearance in hypatocyte as illustrated in figure (1).

![Fig(1) Normal liver tissue from control group( A) of mice show central vein.](image)

Liver sections from positive control( B) which administrated with *C. albicans* only, which showed an irregular conditions in arrangement in liver cells in addition to increase kuffer cell and there are areas of congestion distributed within the degenerative cells, filtration of inflammatory cell such as monocytes and netrophils, the presence of necrotic foci surrounded by inflammatory cell in great number and present of heamoidrin in liver sinuses, congestion and edematous changes with polymorphonuclear leukocyte infiltration within central and portal veins were observed as shown in figure (2).

![Fig(2)Histopathological sectioning of mice liver tissue from control( B) showed congestion(A) and necrosis(B) in liver cells](image)
In liver sections from animals of challenge dose showed normal appearance in hepatocyte and decreased in the lesion it was noticed as a slight congestion in central vein and a few filtration of inflammatory cell around it and in the portal area, but the rest the liver tissue looks healthy as illustrated in figure (3).

Kidney sections were taken from positive control (A) looks like normal organ as shown in figure (4).

while in positive control( B )sections revealed marked congestion with distributed area of degenerative tubules of kidney beside rare glomeruli degenerative changes and bleeding as appeared in figure (5) acute renal tubular necrosis and congestion within renal blood vessels were observed in these mice.
Fig(5) Histopathological section of kidney tissue from mice of control (B) showed degenerative changes (A) and bleeding (B) (hematoxyline–eosin stain 40x).

Mild degenerative changes in the renal tubules appeared in kidney sections taken from animals administration with challenge dose this may be due to the effect of cinnamon which protect the kidney from *C. albicans* as figure (6) illustrated.

Fig(6) Histopathological section of kidney tissue from animals of challenge dose showed mild degenerative changes (A) (hematoxyline–eosin stain 40x).

Intestine sections from positive control (A) looks like normal organ as shown in figure (7).
Fig(7) Histopathological section showed normal mice intestine tissue from control (A) (hematoxyline–eosin stain 40x).

By studying intestine sections from positive control (B) we found that there is reformation or shortness of intestinal villi and with inflammatory cell infiltration, edema in the muscular layer and increase in the numbers of Goblets cell and disintegration in mucus layer of the intestine as shown in figure (8). In addition, destruction and atrophy with ischemic necrosis within the mucosal layers of the small intestine were observed.

Fig(8) Histopathological section of mice intestine tissue from control (B) showed reformation or shortness of intestinal villi (A) and with inflammatory cell infiltration (B), (hematoxyline–eosin stain 40x).

Intestinal sections appeared no pathological lesions from animals of challenge dose, but a slight infiltration well be noticed in inflammatory cell of Lamina propira as figure (9) illustrated this is back to the cinnamon extract exert antagonistic action against intestinal Candidiasis which are capable of preventing the adherence, establishment, replication pathogenic action of specific enteropathogens (Delespaual et al., 2010).
The *in vivo* antifungal assay also revealed that the extract showed the effective inhibition of *C. albicans* growth and significantly reduced mouse mortality. In addition, clinical signs and histological damage were rarely observed in test mice, whereas untreated control mice showed severe clinical signs and histological damage in the tested organs. This result was agreed with (Lee et al., 2003) when they study the effect of *Cinnamon* to reduce Candidiasis infection in rat, found that the prolonged survival of rat, decreased severity of mucosal and systemic Candidiasis, modulation of immune responses and decreased number of *C. albicans* in the alimentary tract, and also agreed with (Ip et al., 1995) when they study antimicrobial activity of *Cinnamon* in mice model. *Cinnamon* has been reported to show antibacterial activity against *Helicobacter pylori* and several gram negative bacteria (Li et al., 2005; Burt et al., 2003). Friedman et al., 2002 found the remarkably inhibit of *Salmonella* growth, *Campylobacter jejuni*, *Escherichia coli* and *Listeria monocytogenes*. The antimicrobial activity has been attributed to the presence of some active constituents in the extracts (Hsieh, 2000). The major chemical constituents of *Cinnamon* are volatile oil (cinnamaldehyde, eugenol, cinnamic acid, weitherhin) and mucilage, diterpenes, proanthocyanidins. Studies suggested that the antimicrobial activity of *Cinnamon* was probably due to their major component, cinnamaldehyde and their properties could be multiple (Youn et al., 2008; Ziegenfguss et al., 2006). Cinnamaldehyde is a natural antioxidant and the animal studies suggested that an extract of *Cinnamon* bark taken orally may help prevent stomach ulcer (Nir et al., 2010). Cinnamaldehyde was completely inhibiting both sensitive and resistant strains of *Helicobacter pylori* which cause gastric ulcer. (Mahady et al., 2003). An important characteristic of plant extracts and their components is their hydrophobicity, which enable them to partition the lipids of the microbial cell membrane and mitochondria, disturbing the cell structures and rendering them more permeable. Extensive leakage from microbial cells or the exit of critical molecules and ions will lead to death. (Mi-hyang et al., 2006) From previous outcomes we conclude that *Cinnamon* extracts have the high inhibitory effect on *C. albicans* with promising inhibitory spectrum and can be used as therapeutic treatment of *C. albicans*. *Cinnamon* may survive in the human gut were significant role of intestinal microflora to resist the disease. We believe that *Cinnamon* is likely to become a novel antimicrobial treatment for Candidiasis. This antimicrobial study of the plant extracts demonstrated that folk medicine can be as effective as modern medicine to combat pathogenic microorganisms. The millenarian use of these plants in folk
medicine suggests that they represent an economic and safe alternative to treat infectious diseases. Moreover, further investigation is needed to ascertain more precisely the antimicrobial effect of this herb extract.

References:


