CHAPTER 8

WOUND HEALING STUDIES OF CH-CN PS2 SUTURE IN NORMAL AND DIABETIC GROUPS

8.1 INTRODUCTION

This chapter deals with the *in vitro* drug release and cytotoxicity studies of CH-CN PS2 suture. *In vivo* wound healing efficacy of CH-CN PS2 is compared with commercial suture in normal and diabetic animals.

8.2 *In vitro* DRUG RELEASE STUDY

The drug release of *Cynodon dactylon* was determined by *in vitro* drug release studies using dialysis membrane method. Percentage release of drug was observed for 4 days (96 hrs) to analyse the antibacterial effect of the CH-CN PS2 suture in sutured wound. Drug release after 4 days was found to be 93% (Figure 8.1). The remaining drug present as residue in the suture which acts against the bacteria in the wound site after 4 days. The observation was well correlated with the previous study (Shanmugasundaram et al. 2011).
8.3 **In vitro CYTOTOXICITY STUDY**

As per ISO 10993 part 5, direct contact test, the cytotoxicity grades greater than 2 or more than 30% toxic effect is considered as cytotoxic effect. It was inferred from the Table 8.1 and Figure 8.2 that the test sample had 9% cytotoxicity as compared to the negative control and was not effective on L929 fibroblast cells which was graded as 0 without toxic effect. So the CH-CN PS2 suture was taken for further *in vivo* studies.

**Figure 8.1** Drug release of CH-CN loaded suture

**Figure 8.2** Biocompatibility of CH-CN suture in L929 cells culture (a) control cell culture (b) CH-CN PS2
Table 8.1 Reactivity and cytotoxicity grading of CH-CN treated suture

<table>
<thead>
<tr>
<th>Sample</th>
<th>Grade</th>
<th>Reactivity</th>
<th>% Cytotoxicity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative control</td>
<td>0</td>
<td>None</td>
<td>0</td>
</tr>
<tr>
<td>CH-CN suture</td>
<td>0</td>
<td>None</td>
<td>9</td>
</tr>
</tbody>
</table>

8.4 **In vivo WOUND HEALING STUDIES**

8.4.1 Incision Wound Healing Study of Normal Groups

The animals were divided into 3 groups (n=3) with 3 animals in each group. Group I was sutured with commercial silk suture which served as negative control. Group II were sutured with commercial suture and applied with antiseptic ointment cipladin which served as positive control and group III were sutured with the CH-CN PS2 silk suture. From observation, it was noticed that the fluid was oozing from the control groups for 4 days and to some extent in groups applied with antiseptic. In group III, the herbal drug prevented the discharges from the wound.

Tensile strength of wound tissues taken on day 10 and day 15 is shown in the Table 8.2. It was found that the tensile strength increased from day 0 to day 15, due to increased collagen formation in the extracellular matrices which was contributing to wound strength (Muralidharet al. 2011). Tensile strength depends upon the Vander Waals force of interaction among the hydrogen ion bonds of the triple helix collagen, leading to twisting of the collagen fibres. The more the twisting of fibres the greater the tensile strength and better the healing of wound (Neerajkumar et al. 2010).

The increase in tensile strength may be due to various effects of phytoconstituents present in *Cynodon dactylon* and natural healing capacity of chitosan. Firstly, bioactivity and collagenation of flavonoids and phenolics
due to their high level of antioxidant property which protected from reactive oxygen species and decomposed super oxide and peroxide radicals. Flavonoids formed a complex with extra cellular soluble proteins and with bacterial cell walls (Zsuzsanna et al. 2010; Sovanpattanaik et al. 2012). Secondly, due to increased angiogenesis, the drug provided the nutrients demanded by the healing tissue and helped in the formation of granulation tissues (Baura et al. 2009; Diegelmann et al. 2004).

### Table 8.2 Tissue strength of normal animal groups

<table>
<thead>
<tr>
<th>Animal groups</th>
<th>Tensile strength (g)</th>
<th>Day 10</th>
<th>Day 15</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group I</td>
<td>160</td>
<td>220</td>
<td></td>
</tr>
<tr>
<td>Group II</td>
<td>289</td>
<td>300</td>
<td></td>
</tr>
<tr>
<td>Group III</td>
<td>290</td>
<td>315</td>
<td></td>
</tr>
</tbody>
</table>

Thirdly, tannins with astringent property stopped bleeding in the initial stage. It promoted wound healing through chelation of free radicals and reactive species of oxygen, promoted contraction in wound, supported the formation of capillary vessels and fibroblasts (Choudhary 2011). Fourthly, non-enzymatic antioxidants such as Vitamin A, C and E helped in early synthesis of collagen fibres by mimicking fibroblastic activity, increased the scar tissue formation and enhanced the formation of new capillaries (Kumarasamyraja et al. 2012).

At the same time, chitosan acted as a lubricant on suture and prevented the tissue rupture (Hoekstra et al. 1998). Chitosan enhanced the function of polymorphonuclear cells, macrophages, fibroblastic proliferation and migration (Esam et al. 2010). Notable difference was found in wound
healing of all the animal groups. On day 18 the healing of groups sutured with CH-CN PS2 suture was comparable with positive control groups.

Photographs of incision wounds of three animal groups taken on day 0, day 6, day 12 and day 18 is shown in Figure 8.3. The healing of positive control group may be due to the effect of antiseptic and was comparable with CH-CN PS2 groups. Negative control group showed delayed wound healing and the healing was due to the presence of cytokines (growth factors) such as platelet derived growth factor (PDGF) and transforming growth factor beta (TGF-β) which was naturally present in the wound site (Mckay & Leigh 1991).

Figure 8.3 Photographs of incision wound healing in normal groups: (a) negative control group (b) positive control group (c) CH-CN PS2 group

8.4.2 Histopathological Study

Photomicrographs shown in Figure 8.4 represents the histopathological analysis of tissues conducted on day15 and one animal
from each group was sacrificed. Wound tissue sample of negative control clearly exhibits the inflammation and was prone to bacterial adherence with lot of blood clots in the wound site. The presence of intermediary spaces reveals that there was a delay in epithelisation.

Figure 8.4  Histopathological photographs of normal groups: (a) CH-CN PS2 (b) Positive control and (c) Negative control; i-intermediary spaces, bc-blood clots, bv-blood vessels, se-squamous epithelium

Tissue sample of positive control showed growth of squamous epithelium with granulation tissue with neutrophils and numerous proliferating blood vessels. In CH-CN PS2 suture, the sample showed the presence of squamous epithelium, neutrophils and new tiny blood vessels. Histopathological study confirms that the wound healing activity of positive control and CH-CN PS2 suture were comparable but higher than negative control without any bacterial colonies.
8.4.3 Incision Wound Healing Study of Diabetic Groups

The animals were divided into 3 groups (n=3) with 3 animals in each group. Group I was sutured with commercial silk suture which served as negative control. Group II were sutured with commercial suture and applied with antiseptic ointment cipladin which acts as positive control and group III were sutured with the CH-CN PS2 silk suture.

Figure 8.5 Photographs of incision wound healing in diabetic induced groups: (a) negative control group (b) positive control group (c) CH-CN PS2 group

In diabetic groups the wound healing was impaired which can be seen from the tissue tensile strength values taken on day10 and day15 as shown in Table 8.3. It was found that tensile strength increased from day 0 to day 15. On day 15, the tensile strength was in the range of 125 g, 187 g and 256 g for group I, group II and group III respectively. Delay in healing may be due to increased blood glucose level.
Group III animals sutured with CH-CN PS2 showed better healing compared to group I and group II which may be due to the ability of alkaloids and flavanoids to regenerate β cells which increases the insulin secretion. It was reported that saponins, alkaloids, phenolic compounds, flavonoids and terpenoids are responsible for anti-diabetic activity. Tannins have the ability to reduce the blood sugar level and was reported that LDL and VLDL cholesterol which delays the healing process gets elevated in diabetic rat was reduced by *Cynodon dactylon* (Karthik & Ravikumar 2011).

### Table 8.3 Tissue strength of diabetic animal groups

<table>
<thead>
<tr>
<th>Animal groups</th>
<th>Tensile strength (g)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 10</td>
<td>Day 15</td>
</tr>
<tr>
<td>Group I</td>
<td>120</td>
<td>146</td>
</tr>
<tr>
<td>Group II</td>
<td>153</td>
<td>187</td>
</tr>
<tr>
<td>Group III</td>
<td>197</td>
<td>256</td>
</tr>
</tbody>
</table>

8.4.4 **Comparison of Wound Healing in Normal Group and Diabetic Group**

Wound healing was enhanced by fibroblasts which is responsible for the secretion of growth factors and deposition of collagen. In diabetic rats, function of fibroblasts was impaired due to the delay in maturity of granulation tissues. This result matched with the previous study conducted by Tkalcevic et al. (2009).

In normal groups, wound healing was completed in day 23, day 18 and day 18 in group I group II and group III respectively. In diabetic animals, healing was completed in 28, 25 and 22 days in group I, group II and group III respectively.
Transformation of immature granulation tissues into matured granulation tissues was characterized by elongation of fibroblasts, early deposition of collagen fibers and angiogenesis. When CH-CN PS2 groups were compared after day 6, whole wound bed was covered with granulation tissues in normal groups but there was five days delay in diabetic groups. Collagen tissues were deposited earlier between day 6 and day 9 in the wound bed of normal groups, whereas the deposition of collagen in the extracellular matrix had just begun at the wound edges in diabetic groups.

8.4.5 Histopathological Study

Photomicrographs shown in Figure 8.6 represent the histopathological analysis of tissues conducted on day 15. One animal from each group was sacrificed.

Figure 8.6 Histopathological photographs of diabetic groups: (a) CH-CNPS2(b) Positive control and (c) Negative control; i-intermediary spaces, bc-blood clots, bv-blood vessels, se-squamous epithelium
Wound tissue sample of negative control clearly exhibits the inflammation and was prone to more bacterial adherence with blood clots and cholesterol content in the wound site. The presence of intermediary spaces revealed that there was a delay in epithelisation. Tissue sample of positive control showed necrosis and exhibited hemorrhage. It can be seen the presence of blood clots along with inflammatory exudates. In CH-CN PS2 suture, the sample showed the appearance of squamous epithelium with granulation tissues composed of neutrophils and new tiny blood vessels. Very few spaces can be noticed due to the increased blood sugar level which delayed wound healing. Histopathological study confirms that the wound healing activity of CH-CN PS2 suture was higher than positive and negative control groups.

8.5 CONCLUSIONS

The results of the above study is summarised below.

- Drug release of chitosan-*Cynodon dactylon* loaded silk suture after 4 days (96 hrs) was 93%. The remaining drug present as residue acts against the bacteria present in the wound site.

- Cytotoxicity test showed 9% toxicity in CH-CN PS2 sample against control sample, which can be used for *in vivo* studies as it was graded as 0.

- In normal groups *in vivo* incision wound healing studies showed that the tensile strength measurement on day 15 in group I, group II and group III was 224 g, 310 g and 345 g respectively. The healing in group III was enhanced by the presence of flavanoids, tannins, saponins and other secondary metabolites.
In diabetic animals tensile strength on day 15 was 146 g, 190 g, and 286 g for group I, II and III respectively. The healing was impaired due to increased level of blood glucose level and in group III the healing was enhanced due to the presence of phytoconstituents which activated the beta cell generation, increased the secretion of insulin and reduced the cholesterol level.

In normal groups, wound healing was completed on day 23 in group I and day 18 in group II and group III. In diabetic animals, healing was completed in 28, 25 and 22 days in group I, group II and group III respectively.