CHAPTER VI

ANIMAL STUDIES ON THE PREPARED IRON AND SILVER NANOPARTICLES

6.1 INTRODUCTION

Nanotechnology deals with materials below 100 nm to exploit their phenomena and novel properties (Ross et al., 2004). When a material or element is reduced to a nanoscale from micro scale, it exhibits a variety of properties with unique functions and has a large surface to volume ratio which results in more surface activity than the bulk materials. Nanotechnology is currently employed as a tool to explore the darkest avenues of medical sciences in several ways like targeted delivery of substances, antibacterial as well as enhanced bioavailability and increased retention (Bogunia and Sugisaka, 2002, Emerich and Thanos, 2003). Hence, there are several fields in which nanotechnology could be applied to study the science and engineering of agriculture and animal systems. Despite its imminent importance, little research has been done to explore the efficacy of the nanominerals on animals.

6.1.1 Animal Studies of Fe Nanoparticles

Iron being a vital element to the body, its absorption into the biological system occurs at the small intestine. But its absorption rate is poor compared to other elements. There are numerous factors which affect iron absorption and bioavailability such as age, species, status of iron, dose, and form of iron and other nutrient component of diet.

Particle size can be an important determinant of iron absorption from poorly soluble iron compounds in foods. Decreasing the particle size of elemental iron powders by 50–60%, to a mean particle size of 7–10 µm, increases iron absorption by 50% in rats (Motzok et al., 1975, Verma et al., 1977). Hematological parameters decreased in parallel with blood iron levels in ewes (Kozat et al.,
Among all kinds of animals, pigs are very sensitive to iron deficiency and exhibit iron deficiency anemia. Hence, much of the studies on iron supplementation are carried out on pigs.

Suckling piglets with no extra iron supplementation develop iron deficiency after 14 days post-partum (Zimmermann et al., (1959)). Supplementation of iron to piglets with Fe$^{2+}$ fumarate was efficient in preventing anaemia (Svoboda and Drabek (2002)). Nano-disperse forms of iron supplementation to piglets showed hematological values were within physiological norms (Prochorov et al., (2002)).

Keeping the above facts into consideration, the present study was formulated to test the utilization of iron nanoparticles in preventing iron deficiency anemia in piglets as model.

6.1.2 Animal Studies of Ag Nanoparticles

From ancient days silver compounds are widely used for both hygienic and healing purposes, due to their antimicrobial effect. With the introduction of antibiotics in the early part of the 20th century, the use of silver salts as wound healing agents decreased. Owing to the emergence and increase of microbial organisms resistant to multiple antibiotics, and the continuing emphasis on healthcare costs, many researchers have tried to develop new, effective antimicrobial reagents free of resistance and cost. Such problems and needs have led to a resurgence in the use of silver based antiseptics that may be linked to a broad-spectrum activity and a far lower propensity to induce microbial resistance than antibiotics (Jones et al., (2004)). Currently silver based salts have gained importance in a variety of biomedical applications like dental work, catheters etc. Silver is applied in the treatment of wound either in the form of impregnated bandages or as a cream containing silver sulfadiazine.

In past few decades, the mechanism of action of silver has been investigated. It seems that silver shows a multilevel antibacterial effect, due to the blockage of respiratory enzyme pathways, as well as alteration of microbial DNA and the cell
wall (Melayie and Youngs (2005)). Silver has also been demonstrated to be effective against multidrug-resistant organisms (Silvestry et al., (2007) and Atiyeh et al., (2007)), whilst maintaining a low systemic toxicity (Neal (2008)). Clinically, several studies have confirmed their safety for patients (Okan et al., (2007) and Cutting et al., (2007)).

Silver nanoparticles gained application in consumer products, food technology (e.g., food processing equipments, packaging materials, food storage), textiles/fabrics (e.g., antimicrobial clothing), and medical field (e.g., wound care products, implantable medical devices). Nano-silver is an effective killing agent against a broad spectrum of Gram-negative and Gram-positive bacteria (Burrell et al., (1999) and Yin et al., (1999)) including antibiotic-resistant strains (Wright et al., (1998) and Percival et al., (2007)).

In veterinary surgery the chances of introducing infection is relatively very high than human surgery. At farm level, animals living in less hygienic area would have a greater number and type of bacteria on their skin, which could lead to post-surgical infections. Wound healing is delayed as many topical antibiotics used at present are slowly losing their bactericidal property owing to the development of bacterial resistance.

A bactericidal agent with a greater potency and lesser likelihood of developing bacterial resistance is an urgent need in the therapeutic management of wounds. Recently, silver nanoparticles have been suggested as having high bactericidal property with lesser development of resistance to silver nanoparticles in pathogenic microbes. This would be especially beneficial in veterinary medicine to keep the wound area clean and hygienic. Incorporation of silver nanoparticles in surgical dressings may yield great benefits as many of the surgical procedures are carried out at the farmer’s site with sterile conditions.

Keeping the above facts into consideration, the present study was formulated to test the utilization of silver nanoparticles in wound dressings in rabbits as model.
6.2 APPLICATION OF Fe NANOPARTICLES ON ANIMAL (PIGLET) MODEL

Iron deficiency anemia is a global public health issue affecting human beings. Among domestic animals, the effect of iron deficiency is more pronounced in piglets. Anemia in baby pigs occurs, almost without exception, if no supplemental iron is provided during the first few days after farrowing (Lipinski et al., 2010). This can be attributed to the factors that piglets are born with limited reserves of iron, rapid growth rate of the piglets, the iron content of the sow’s milk is inadequate for suckling piglets to meet the demand and limited access to soil as the pigs are reared on solid floors under modern scientific rearing (Kegley et al., 2002). Hence piglets are more prone to develop iron deficiency anemia than any other animal species.

Attempts to increase the body reserves of iron in piglets at birth or the iron concentration in milk by iron supplementation in the sow’s diet have been largely unsuccessful (Pond et al., 1965, Egeli et al., 1998, Peters and Mahan, 2008). Therefore, it is necessary to give iron supplementation to the piglets through oral route or by injection. A single injection of iron-dextran (200mg) is effective against iron deficiency anemia. But this approach is painful as well as stressful, laborious and time consuming to the piglets. Supplementation of iron through oral route would be less stressful to the piglets. However, oral form of iron needs to be given in multiple doses as the bioavailability of iron is less.

Absorption and bioavailability of minerals in the biological system can be enhanced through nanotechnology approach (Hilty et al., 2010). Nowadays, nanotechnology approach is gaining importance in the mineral nutrition of animals. Bioavailability of iron compounds was enhanced by reduction of particle size and this causes fewer sensory changes (Rohner et al., 2007, Hilty et al., 2010, Zimmermann and Hilty, 2011) thus showing promise as food supplement. Therefore, iron nanoparticles may be utilized to enhance the bioavailability of iron and it has a marked effect in the prevention of anemia due to iron deficiency in
piglets. Besides, very limited studies are available on the use of iron nanoparticles in piglets to prevent iron deficiency anemia.

With these views, the present study was framed to determine the effect of nano-iron on the performance of piglets and certain hematological parameters.

6.2.1 Function of Fe in Biological cells

Iron is needed for a number of highly complex processes that are continuously taking place on a molecular level and indispensable in the life of an individual. Iron is required for the production of red blood cells, but it is also a part of haemoglobin (pigment of the red blood cells) binding to the oxygen and thus facilitating its transport from the lungs via the arteries to all the cells throughout the body. Iron is also involved in the conversion of blood sugar to energy. Metabolic energy is crucial for athletes since it allows muscles to work at their optimum during exercise or heavy work. Iron is a fundamental element for the normal development of the immune system. Iron is essential for proper cell differentiation and cell growth. It is an important component of peroxide-generating enzymes and nitrous oxide-generating enzymes that are critical for proper enzymatic functioning of immune cells (Beard (2001)). Besides, iron is necessary for the activity of several enzymes and catalytic pathways at cellular level (Toledano et al., (2010)).

6.2.2 Materials and methods (Fe on Piglet)

The biological experiment was carried out at Department of Veterinary Physiology, Veterinary College and Research Institute, Namakkal, Tamil Nadu.

Experimental animals

The study was conducted on piglets born to large White Yorkshire Sows. Individual piglets born in each litter were assigned to one of the five groups as:

<table>
<thead>
<tr>
<th>Group</th>
<th>Number</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>7</td>
<td>No supplementation of iron</td>
</tr>
<tr>
<td>II</td>
<td>8</td>
<td>Iron injection</td>
</tr>
<tr>
<td>III</td>
<td>6</td>
<td>Oral supplementation of ferric ammonium citrate</td>
</tr>
<tr>
<td>IV</td>
<td>9</td>
<td>Oral supplementation of nano iron (3mg/day)</td>
</tr>
<tr>
<td>V</td>
<td>6</td>
<td>Oral supplementation of nano iron (6mg/day)</td>
</tr>
</tbody>
</table>
Piglets of Group II were injected with a single injection (i/m) of iron-dextran on day-3 after birth. Piglets of Group III were fed orally ferric ammonium citrate equivalent to elemental iron (10 mg)/day given on alternate days from the 3rd day to 28 days of age. Group IV and Group V piglets were administered orally nano-iron from the 3rd day to 28 days of age on alternate days @ 3mg/day and 6mg/day respectively. While piglets of Group I were not given any iron supplementation and served as control.

The experimental animals were housed in concrete floor pens. The pens were washed during morning and evening daily. Exogenous iron contamination from the surroundings was avoided with adequate care. On the third day of age, teeth clipping was done to all the piglets as a standard management practice. The piglets of each litter were allowed to stay with their respective dam. The piglets were fed 200 and 250 g of creep feed at 2-3 and 3-4 week of age respectively.

Blood samples were collected using EDTA as anticoagulant from all the piglets in each group through ear vein at 1st, 2nd, 3rd and 4th week of age. The hematological parameters were analyzed immediately after the collection of the sample. Total erythrocyte count was determined by haemocytometric method. Hematocrit was measured by capillary tube method. The Hemoglobin concentration of blood was measured by cyanmethemoglobin method.

Statistical analysis of the data collected was subjected to completely randomized design method as per (Snedecor and Cochran (1994)).

6.2.3 RESULTS AND DISCUSSION

6.2.3.1 Body weight measurement

Piglets of all the groups were weighed at 1st, 2nd, 3rd and 4th week of age individually on electronic scale. The mean body weight (kg) of the piglets supplemented with different iron preparations is presented in Table 6.1 and illustrated in Figure 6.1.
Table 6.1 Mean (±SE) body weight (kg) of the piglets supplemented with different iron preparations

<table>
<thead>
<tr>
<th>Age (Weeks)</th>
<th>Body weight</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Group I</td>
</tr>
<tr>
<td>First</td>
<td>2.29±0.04</td>
</tr>
<tr>
<td>Second</td>
<td>3.59±0.18</td>
</tr>
<tr>
<td>Third</td>
<td>4.79±0.15</td>
</tr>
<tr>
<td>Fourth</td>
<td>6.73±0.26</td>
</tr>
</tbody>
</table>

[Means within the same row bearing different superscripts differ significantly (P<0.01)]

Figure 6.1 Bar Chart for Mean (±SE) body weight (kg) of the piglets supplemented with different iron preparations

It was observed from the results that at the end of 2nd week of age, body weight of the piglets Group V supplemented with nano-iron (6mg/day) differed
significantly (P<0.01) with all other groups. At 3rd week of age, supplementation of nano-iron at both levels (3 mg and 6mg/day) improved weight gain (P<0.01) over control, iron injection and ferric ammonium citrate fed group. However, the body weight did not differ significantly at 1st as well as 4th week of age. Iron dextran injection and oral ferric ammonium citrate did not influence the body weight gain in piglets compared to unsupplemented group.

The results of iron dextran injection were in agreement with Bruininx et al., (2000) and Rincker et al., (2005), who did not find improved weight gain by iron dextran injection over control. However, Furugouri et al., (1983) and Pollmann et al., (1983) reported that iron dextran injection lead to higher weight gain over the untreated piglets.

The results of oral iron supplementation concurred with Svoboda and Drabek (2002) and Maes et al., (2011), who also did not observe any difference in weight gain by supplementation of iron through both oral and injection route. The oral supplementation of ferrous sulphate and iron methionine did not improve weight gain in piglets (Kegley et al., (2002)).

Improvement in weight gain by supplementation of iron nanoparticles in this study was in agreement with Rohner et al., (2007), who reported that rats fed ferric pyrophosphate nanoparticles had improved weight gain over control group. However, there was no difference in the weight gain between ferric pyrophosphate nanoparticles fed rats and ferrous sulphate fed rats.

6.2.3.2 Hemoglobin

The mean hemoglobin (g%) of the piglets supplemented with different iron preparations is presented in Table 6.2 and illustrated in Figure 6.2.
Table 6.2 Mean (±SE) hemoglobin (g %) level of the piglets supplemented with different iron preparations

<table>
<thead>
<tr>
<th>Age (Weeks)</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
<th>Group V</th>
</tr>
</thead>
<tbody>
<tr>
<td>First</td>
<td>9.62±0.21</td>
<td>11.09±0.31</td>
<td>10.43bc±0.19</td>
<td>11.72d±0.19</td>
<td>11.83d±0.30</td>
</tr>
<tr>
<td>Second</td>
<td>7.51a±0.24</td>
<td>11.74bc±0.36</td>
<td>11.21b±0.33</td>
<td>12.65c±0.21</td>
<td>12.72c±0.19</td>
</tr>
<tr>
<td>Third</td>
<td>6.27a±0.22</td>
<td>11.83c±0.40</td>
<td>11.21b±0.23</td>
<td>13.19d±0.19</td>
<td>13.33d±0.27</td>
</tr>
<tr>
<td>Fourth</td>
<td>5.98a±0.29</td>
<td>12.59c±0.28</td>
<td>11.57b±0.27</td>
<td>13.86d±0.23</td>
<td>14.26d±0.30</td>
</tr>
</tbody>
</table>

[Means within the same row bearing different superscripts differ significantly (P<0.01)]

Iron supplementation increased the hemoglobin content from the first week of age onwards in all the groups. The hemoglobin content of iron nanoparticle supplemented group was higher (P<0.01) than oral ferric ammonium citrate fed group. At 3rd and 4th week of age, the iron supplementation effect was superior in
Group IV and V followed by Group II and III. However, hemoglobin level was similar at both the levels of iron nanoparticle supplementation.

The hemoglobin concentration is commonly used as an indicator of a pig’s iron status (Rincker et al., 2005). Piglets are considered to be anemic when the hemoglobin concentration is lower than 8 g% (Kegley et al., 2002) whereas piglets with hemoglobin level of 10 g% are considered to be normal (Hill et al., 1999).

The result of iron dextran injection was in agreement with Kernkamp et al., 1962, and Wang and Kim (2012), who also reported an increased hemoglobin level compared to untreated piglets. Jiang et al., 2009, who recorded an increase in hemoglobin concentrations of piglets received iron dextran on days of age 7, 14, and 21.

Kegley et al., 2002 observed that oral supplementation of iron resulted in higher hemoglobin than iron injection on 7 days of age, whereas it was comparable on days 14 and 21 of age in piglets. However, Zimmerman et al., 1959 viewed that oral treatment did not prevent the development of an iron deficiency as evidenced by hemoglobin levels in piglets. Rydberg et al., 1959 viewed that the oral method of iron administration was beneficial although it was slightly inferior to the injection method.

The rats that were fed ferric pyrophosphate had higher hemoglobin level than control and ferrous sulphate supplemented groups (Rohner et al., 2007). Svoboda and Drabek (2002) also noticed the iron micro-emulsion supplemented piglets had increased hemoglobin level from 7-14 days of age onwards.

6.2.3.3 Hematocrit

The mean hematocrit (%) level of the piglets supplemented with different iron preparations is presented in Table 6.3 and illustrated in Figure 6.3.
Table 6.3 Mean (±SE) hematocrit (%) level of the piglets supplemented with different iron preparations

<table>
<thead>
<tr>
<th>Age (Weeks)</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
<th>Group V</th>
</tr>
</thead>
<tbody>
<tr>
<td>First</td>
<td>29.39±2.12</td>
<td>34.94±1.44</td>
<td>31.97±0.76</td>
<td>32.66±1.25</td>
<td>34.18±0.85</td>
</tr>
<tr>
<td>Second</td>
<td>22.43±1.14</td>
<td>35.18bc±1.17</td>
<td>34.27bc±0.73</td>
<td>38.24bc±1.27</td>
<td>38.92±1.38</td>
</tr>
<tr>
<td>Third</td>
<td>19.27a±0.83</td>
<td>37.88bc±1.12</td>
<td>36.15b±1.41</td>
<td>41.26d±1.28</td>
<td>40.98cd±1.07</td>
</tr>
<tr>
<td>Fourth</td>
<td>16.47a±1.29</td>
<td>40.70bc±1.28</td>
<td>39.16b±0.99</td>
<td>42.83c±1.72</td>
<td>43.71c±1.26</td>
</tr>
</tbody>
</table>

[Means within the same row bearing different superscripts differ significantly (P<0.01)]

Figure 6.3 Bar Chart for Mean (±SE) hematocrit (%) level of the piglets supplemented with different iron preparations

Iron supplementation increased hematocrit level (P<0.01) over un-supplemented group from the second week of age onwards. At the first week of age, there was no difference in hematocrit level among all the groups. In subsequent weeks, Group IV and V had higher hematocrit level than Group III. However, the hematocrit level of Group II was comparable with that of Group IV and V.
Svoboda and Drabek (2002) observed that iron injection increased hematocrit level at days 14 and 21 of age, but it was not significant at days of 35 compared to control piglets. Furugouri et al, (1983) and Lipinski et al., (2010) also noted that iron dextran injection was able to restore the hematocrit level to normal level in piglets.

Kegley et al., (2002) reported oral supplementation of iron caused an increase in hematocrit level compared to un supplemented piglets from days 7 to 21. Rincker et al., (2005) and Toledano et al., (2010) also reported similar findings by oral supplementation of iron to the piglets.

Iron nanoparticles supplementation favorably improved the hematocrit level which was supported by Prochorov et al., (2002), who viewed that erythropoiesis was activated by feeding of iron nanoparticles to the piglets. Svoboda and Drabek (2002) also reported oral application of iron micro-emulsion to suckling piglets caused increase in hematocrit level from day 7 onwards and the effect was comparable with iron dextran injection from day 14 onwards.

6.2.3.4 Total erythrocytic count

The mean total erythrocytic count ($10^6/\mu l$) level of the piglets supplemented with different iron preparations is presented in Table 6.4 and illustrated in Figure 6.4.

**Table 6.4 Mean ($\pm$SE) total erythrocyte count ($10^6/\mu l$) of the piglets supplemented with different iron preparations**

<table>
<thead>
<tr>
<th>Age (Weeks)</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
<th>Group V</th>
</tr>
</thead>
<tbody>
<tr>
<td>First</td>
<td>6.38±0.31</td>
<td>6.41±0.19</td>
<td>6.35±0.09</td>
<td>6.39±0.10</td>
<td>6.50±0.08</td>
</tr>
<tr>
<td>Second</td>
<td>5.19±0.22</td>
<td>6.59±0.28</td>
<td>6.32±0.21</td>
<td>6.49±0.18</td>
<td>6.57±0.22</td>
</tr>
<tr>
<td>Third</td>
<td>4.51±0.18</td>
<td>6.77±0.11</td>
<td>6.69±0.20</td>
<td>7.01±0.25</td>
<td>6.97±0.13</td>
</tr>
<tr>
<td>Fourth</td>
<td>4.04±0.09</td>
<td>6.72±0.14</td>
<td>6.63±0.31</td>
<td>7.27±0.21</td>
<td>7.29±0.17</td>
</tr>
</tbody>
</table>

Means within the same row bearing different superscripts differ significantly (P<0.01)
Figure 6.4 Bar Chart for Mean (±SE) total erythrocyte count (10⁶/µl) of the piglets supplemented with different iron preparations

From the second week of age onwards, iron supplementation to the piglets increased (P<0.01) the total erythrocyte count compared with the control group. At the first week of age, there was no difference in total erythrocyte count among all the groups. From second to fourth week of age, iron nanoparticle fed groups had higher (P<0.01) total erythrocyte count than ferric ammonium citrate fed groups, whereas, the total erythrocyte count of the iron the dextran injected group was comparable with that of iron nanoparticle fed groups. At the second and fourth week of age, significant difference was noticed between iron dextran injected and ferric ammonium citrate fed groups. It was observed that iron nanoparticle administration produced effects similar to parental administration of iron.

The results of total erythrocyte count was in agreement with Pollmann et al., (1983), who found that at day 0 no differences were observed by iron injection, whereas at day 10 and 21, the total erythrocyte count of the treated piglets was higher than that of the control piglets. At day 50, no difference in total erythrocyte
count was observed between the groups. Furugouri et al., (1983) and Lipinski et al., (2010), also observed similar finding by iron injection in piglets.

Kotrbaaek (2001) and Svoboda and Drabek (2002) did not find any difference in TEC in piglets by oral iron supplementation and iron injection. However, Iben (1998) found that the erythrocytic count was significantly lower in piglets with oral iron supplementation than in piglets that were given iron injections.

The results of nano-iron supplementation were in agreement with Prochorov et al., (2002), who observed that by the supplementation of nano-iron in piglets, the erythrocyte counts returned to normal level and relieved the anemic state. Svoboda and Drabek (2002) reported similar findings by oral application of iron micro-emulsion to suckling piglets.

6.3 APPLICATION OF Ag NANOPARTICLES ON ANIMAL (RABBIT) MODEL

Silver nanoparticles were demonstrated to induce immune response in rabbits (Abd (2014)). Wound healing is a complex process involving coordinated interactions between diverse immunological and biological systems. Silver nanoparticles significantly accelerated wound healing and reduced scar appearance through suppression of immune system as indicated by decreasing levels of all inflammatory factors (Heydarnejad et al., (2014)). Silver nanoparticles exert positive effects through their antimicrobial properties, reduction in wound inflammation, and modulation in some of the liver and kidney functions during skin wound healing (Hendi (2011)).

Silver nanoparticles have been recently known to be promising antimicrobial agents that act on a broad range of target sites both extra-cellularly as well as intra-cellularly. Silver nanoparticles show very strong bactericidal activity against gram positive as well as gram negative bacteria including multi-resistant strains (Shrivastava et al., (2007), Zhang et al., (2007), Roe et al., (2008)). Recent evidence suggests that Silver nanoparticles have potent anti-
inflammatory effects (Tian et al. (2007), Nadworny et al., (2008)) and accelerate wound healing (Wright et al., (2002) and Huang et al., (2007)). Skin wound healing proceeds through an overlapping pattern of events including coagulation, inflammation, proliferation, matrix and tissue remodeling. The use of silver nano-crystalline chitosan wound dressings in rabbits promotes wound healing and combat infection, and also decrease the risk of silver absorption in comparison with silver sulfadiazine dressings (Lu and Gu (2008)).

6.3.1 Function of Ag in Biological cells

Silver nanoparticles are highly antimicrobial to several species of bacteria, including the common kitchen microbe, E. coli. According to the mechanism reported, silver nanoparticles interact with the outer membrane of bacteria, and arrest the respiration and some other metabolic pathways that lead to the death of the bacteria.

6.3.2 Materials and methods

The biological experiment was carried out at Department of Veterinary Physiology, Veterinary College and Research Institute, Namakkal, Tamil Nadu.

6.3.2.1 Preparation of wound dressing and ointment

Preparation of wound dressing

Hospital grade sterilized cotton wound gauze was soaked overnight in silver nanoparticles solution and dried under air in a sterile environment. The cotton gauze pieces thus prepared were stored in a sterile container for later use.

Preparation of ointment

To explore the possibility of adding silver nanoparticles in the paraffin ointment base for topical use, a 10% silver nanoparticles loaded ointment was prepared for applying on the wound area as an alternative to the antibiotic ointment.
6.3.2.2 Biological experiment

Five numbers of 6 months old healthy rabbits (Soviet chinchilla breed) were obtained from the Rabbit section and kept separately in cages. Water and feed were offered ad libitum. Under local anesthesia (Lignocaine Hcl, 2%), about 1.5 cm wound was created following standard surgical procedure, and wound was dressed with silver nanoparticles loaded gauze, commercial antibiotic ointment (gentamicin with betamethasone), silver nitrate solution soaked gauze and ointment prepared with silver nanoparticles. Dressing was changed on alternate days. One rabbit was not dressed and left as control. Wound healing was assessed at alternate day interval for nine days and the healing of wound was recorded as reduction in size of the wound area as given in Table 6.5 and illustrated in Figure 6.5.

<table>
<thead>
<tr>
<th>Table 6.5</th>
<th>Wound size (in cm) treated with silver nitrate, silver nanoparticles and antibiotic in rabbits.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>Day 1</td>
</tr>
<tr>
<td>Control</td>
<td>1.7</td>
</tr>
<tr>
<td>Silver nitrate solution soaked gauze</td>
<td>II</td>
</tr>
<tr>
<td>Nanosilver solution soaked gauze</td>
<td>III</td>
</tr>
<tr>
<td>Nanosilver ointment</td>
<td>IV</td>
</tr>
<tr>
<td>Commercial antibiotic ointment</td>
<td>V</td>
</tr>
</tbody>
</table>

6.3.3 RESULTS AND DISCUSSION

The wound healing effect of antibiotics and silver treatment in rabbit is presented in the Table 6.5. Treatment of wound with silver nitrate solution soaked gauze resulted in 0.2, 0.4 and 0.5 cm reduction in wound area on day 3, 5 and 7 respectively. The wound was healed on the 9\textsuperscript{th} day. Treatment of wound with silver nanoparticles loaded gauze resulted in 0.2, 0.4 and 0.7 cm reduction in wound area on day 3, 5 and 7 respectively and total wound healing was observed on day 9.
Treatment of wound with ointment containing silver nanoparticles in rabbit yielded about reduction of 0.4, 0.5 and 0.5 cm in wound area on day 3, 5 and 7 respectively. The wound healing was observed on 9th day. Treatment of wound with commercial antibiotic ointment resulted in 0.5, 0.4 and 0.5 cm reduction in wound area on day 3, 5 and 7 respectively and wound healing was observed on day 9. In control animal as well, reduction in the size of wound area by 0.2, 0.4 and 0.4 cm on day 3, 5 and 7 was respectively observed. On day 9, there was 82% of the wound area that was healed and 18% of the wound area that was yet to be healed.

The findings of this experiment is supported by Rigo et al., (2013), who observed that the application of silver nanoparticles based dressings allow wound healing and recovery. Tian et al., (2007) also reported that the wound healing was better in silver nanoparticles treated mice compared with silver sulfadiazine treated mice. Significant decrease in wound adhesion time was observed by Chowdhury et al., (2014) following daily silver nanoparticle treatment in rabbits which may be attributed to the increased collagen deposition in the wound area. Nano-crystalline silver promoted healing by reducing microbial burden and reducing inflammation in
pigs, Wright et al., (2002), Margaret et al., (2006) and Madhumathi et al., (2009) also supported the observations of this study.

From the study it is observed that the rate of wound healing by treatment with commercial antibiotic ointment was higher followed by ointment containing silver nanoparticles and surgical gauze loaded with silver nanoparticles. The silver nitrate was also equally better.

6.4 CONCLUSION

From the studies conducted on piglets, iron nanoparticles supplementation improved the weight gain and hematological characteristics. Iron nanoparticles supplementation both at 3 mg/day and 6 mg/day yielded similar effects in many aspects. Efficacy of iron nanoparticles in preventing iron deficiency anemia in pigs was superior followed by iron dextran injection and oral ferric ammonium citrate. However, extensive studies involving a large number of animals may be conducted before this method is advocated to field applications. Further, studies on bioavailability, organoleptic changes in meat and safety aspects including tissue residues would be valuable before recommending iron nanoparticles for use at field level.

Studies on the application of silver nanoparticles as wound healing agents, concluded that both silver nanoparticle ointment and solution may be used as an alternate to antibiotic cream when antibiotic resistance is suspected. However, extensive trials are to be conducted before widespread application.