5. SUMMARY AND CONCLUSIONS

The present thesis embodies results of investigations undertaken to evaluate honey bee products; propolis, pollen, honey and bee venom for their biological activities. The antimicrobial and antihelminthic properties of all bee products were studied by \textit{in vitro} methods. Propolis was tested for its \textit{in vivo} activity by biochemical and histological methods.

\textbf{In vitro antimicrobial activity}

Determination of antimicrobial activity of honey bee products viz. propolis, pollen, honey and bee venom was done by disc diffusion method and broth dilution method against pathogenic and non pathogenic bacteria and yeasts.

In disc diffusion method, the organisms were screened for their susceptibility towards above mentioned honey bee products (extracted in ethanol, methanol and water), applied on the disc of agar plate at the concentration range of 1.562-300mg/disc.

\textbf{Propolis}

Zone of inhibition measurement revealed that \textit{Staphylococcus epidermidis} was most susceptible to ethanolic and methanolic extracts of propolis, followed by \textit{S. aureus} and \textit{Streptococcus pneumonia}. \textit{Staphylococcus epidermidis} was susceptible to water extract of propolis as well. Gram (–ve) bacteria were less susceptible to the extracts than Gram (+ve) except for \textit{Salmonella enterica}, where equivalent inhibition could be seen with methanolic extract of propolis.

In yeast, zone of inhibition was observed for \textit{Candida albicans} and \textit{Saccharomyces cerevisiae} with both ethanolic and methanolic extracts of propolis. Higher inhibitory activity was observed for \textit{Candida albicans}

\textbf{Pollen}

Zone of inhibition measurement done for the organisms studied showed that \textit{Staphylococcus aureus} was most susceptible to ethanolic and methanolic extracts of
pollen, followed by *S. epidermidis* and *Streptococcus pneumonia*. *Staphylococcus epidermidis* was also inhibited by water extract of pollen.

In yeast, higher inhibition was observed for *Saccharomyces cerevisiae* than *Candida albicans* with ethanolic extract of pollen while higher inhibition was observed with water extract of pollen against *Candida albicans*.

**Honey**

Application of honey for determination of antimicrobial activity revealed that antimicrobial activity was much higher in Gram (−ve) organisms and on pathogenic yeast *Candida albicans* with both methanolic as well as for ethanolic extract of honey as compared to Gram (+ve) organisms. With methanolic extract of honey, highest inhibitory activity was observed for *E. coli*. Water extract of honey was not much effective on organisms as compared to other extracts of honey.

**Venom**

Water extract of venom was used instead of alcoholic solvents because of its higher solubility in water and high protein content. *S. aureus* and yeast *Candida albicans* were observed to be more susceptible to water extract of venom than other organisms.

- **In vitro antihelminthic activity:**
  - Amphistomes (*Gastrothylax crumenifer*) obtained from the gut of sheep/goat were taken as test organism.
  - The entire range of bee products at all concentrations used in the present study did not show any effect different from the negative control on the mortality of amphistome.
  - The positive control using albendazole was very effective even at much lower concentrations.

- **In vivo antioxidative activity of propolis against *S. aureus* infected BALB/c mice**
• **Body weight:** *S. aureus* infection caused decrease in the body weight of mice from 26.88±0.46g in normal to 19.76±0.31g after infection. The body weight was restored to near normal in *S. aureus* infected+ propolis+ampicillin and *S. aureus* infected+ propolis+ amoxicillin treated group.

• **Biochemical studies:** Biochemical parameters were studied in liver, kidney and spleen. For this the whole experiment was divided into seven groups. G1: Normal mice-administered with normal saline only. G2: Mice infected with *S. aureus* (intra-peritoneal injection of 0.5 x 10^6 CFU/mL). G3: Mice infected with *S. aureus* and given propolis extract (250 mg/kg body weight) everyday for 15 days. G4: Mice infected with *S. aureus* and given antibiotic (ampicillin; 250 mg/kg body weight) everyday for 15 days. G5: Mice infected with *S. aureus* and given antibiotic (amoxicillin; 250 mg/kg body weight) everyday for 15 days. G6: Mice infected with *S. aureus* and given ampicillin and propolis extract at dosages as above with a difference of two hours, everyday for 15 days. G7: Mice infected with *S. aureus* and given amoxicillin and propolis extract at dosages as above with a difference of two hours, everyday for 15 days.

• Blood was collected for serum parameters studies.

• The oxidative stress parameters studied were LPO, GSH, CAT, SOD, GST, GR and GPx.

• Serum parameters for kidney function test included urea, uric acid and creatinine.

• Serum parameters for liver function test included SGPT, SGOT, ALP and Bilirubin.

• It was observed that in kidney, liver and spleen the level of LPO was increased and GSH, CAT, SOD, GST, GR and GPx were decreased in *S. aureus* infected mice. The values were restored near to normal after treating with propolis and antibiotics alone and normal after using the combination of propolis and
Summary and conclusions

- Antibiotics. Best results were obtained with *S. aureus* infected + amoxicillin + propolis treated group (Table 4.31-4.32)

- Kidney function test: With respect to the serum parameters in case of kidney function tests the level of urea, uric acid and creatinine, there was significant increase in case of infected group but a significant decline in G3, G4, G5 as well as highly significant decrease in G6 and G7 was observed. It was observed that infection with *S. aureus* caused significant increase in urea (46.32±1.58 to 85.81±3.37), uric acid (4.09±0.204 to 8.96±0.86) and creatinine (0.44±0.03 to 0.84±0.04) mg/dl. The observed disturbance in serum parameter levels indicated kidney damage caused by *S. aureus* infection. Treatment with propolis, ampicillin, amoxicillin and combination of propolis and antibiotics against infection of *S. aureus* in the present studies caused significant decrease in the levels of urea, uric acid and creatinine to near normal. Urea levels obtained in different groups were as shown- G3: 56.92±2.17, G4 and G5: 55.55±1.05 and 50.32±1.83 respectively, G6: 48.10±1.13 and G7: 44.56±1.28. While for uric acid it was G3: 5.88±0.36, G4: 5.13±0.18, G5: 4.63±0.44 while G6&7 were 3.95±0.80&3.38±0.44 mg/dl. For creatinine the values were G3: 0.55±0.02, G4: 0.49±0.02, G5: 0.45±0.04 and G6: 0.41±0.07, G7: 0.39±0.02 mg/dl (Table: 4.34).

- Liver function test: The activity of SGPT, SGOT, ALP and bilirubin in normal mice was observed to be 23.098±0.69 IU/L, 25.266±0.504 IU/L, 7.912±0.221 KA units and 0.670±0.009 mg/ml respectively. After *S. aureus* infection the levels were found to be increased significantly to 138.77±1.15 IU/L, 94.162±0.753 IU/L, 25.614±0.308 KA units and 1.494±0.028 mg/ml respectively. On treating the infected mice with propolis, ampicillin and amoxicillin the levels were found to be significantly increased in propolis treated group while there were highly significant increase to near normal in the combination treatment of propolis and antibiotics (Table: 4.35).
Histological studies: Histological studies were performed to observe the microarchitecture of liver, kidney and spleen in normal, infected and treated animals by using light microscopy.

- **Kidney:** Severe damage and disorganization of tubules was observed in *S. aureus* infected kidney. There was glomerular constriction and necrotic changes observed in the glomeruli with abnormal proliferation of mesangial cells which led to congestion. Shrinkage of renal corpuscles and vacuolation of tubules was observed. Mesangial space was also observed to be increased in sections of infected kidney under higher magnification. Treatment with propolis, ampicillin, amoxicillin alone and in combination showed remarkable recovery with almost normal architecture of kidney especially when propolis and amoxicillin were given in combination (Plate 4.1-4.7).

- **Liver:** The histology of normal liver revealed a huge network of hepatic cords with large polygonal cells called hepatocytes arranged in single-cell thick plates separated by vascular sinusoids. The portal tracts could be seen as triangular or round structures which enclosed portal veins, terminal branches of hepatic artery and bile ducts embedded in fibrous connective tissue. The *S. aureus* infected liver showed prominent alterations like presence of scattered, degenerated hepatocytes which were infiltrated with polymorphonuclear cells. Kupffer cell hyperplasia, acute liver necrosis, portal triaditis and enlargement or vacuolization of sinusoids was the main alteration found in the *S. aureus* infected liver. However after treatment with propolis, ampicillin, amoxicillin alone and in combination remarkable recovery with almost normal architecture of liver was observed especially when propolis and amoxicillin were given in combination (Plate 4.8-4.14).

- **Spleen:** Liver and spleen are two most important organs of reticuloendothelial system and histology of these organs was affected the most in case of *S. aureus* infected mice. The histological analysis of *S. aureus* infected spleen revealed that there were severe pathological changes and tissue injury with internal degeneration of red and white pulp. The marginal zones were enlarged and
number of follicles was also found increased. The most important damage caused by *S. aureus* administration was the ruptured capsular wall. All these ailments were treated after propolis and antibiotic administration very good results were observed with the combination of propolis and amoxicillin (Plate 4.15-4.21).