Summary

The present study embodies results of investigations on the antibacterial potential of honey bee propolis against the damage caused by *S. typhimurium* infection in BALB/c mice.

- **Phytochemical analysis**

Phytochemical analysis was done using three different extracts of propolis viz. ethanolic, methanolic and water. In total 13 phytochemicals were tested. It was observed that maximum intensity of the phytochemical components was obtained in the ethanolic extract. This was therefore used for further experimentation. The prominent phytochemical present were alkaloids, coumarins, quinines, tannins.

- **GC-MS**

GC-MS analysis of the ethanolic extract of propolis revealed the presence of about 20 compounds. The components were identified with the help of NIST library. The major components were 4,5,7 –trihydroxy flavanone , 4H-1-benzopyran-4-one, isoquinoline, propenone, cinnamic acid.

- **In vitro antibacterial activity**

The MIC and MBC were calculated for all the three extracts and were 160 mg/mL and 250 mg/mL, 200 mg/mL and 260 mg/mL, 220 mg/mL and 310 mg/mL respectively for EEP, MEP and WEP.

- **In vitro combination/synergistic effect of propolis**

The effectiveness of propolis in combination with standard antibiotic (cefixime) was also studied. The results showed that 0.25 MIC of cefixime (0.02µg/mL) and 0.25 MIC of propolis (40mg/mL) when used in combination increased each other’s efficacy and decreased the effective dosage. The reduction in the dose of cefixime could be a great achievement as this could act as a barrier for increasing cases of multi drug resistant against typhoid bacteria.

- **Experimental design**

The whole study was divided into 3 experiments

**First experiment** focused on analyzing the therapeutic potential of different doses of propolis after different days of treatment. Three different doses of propolis *i.e.* 100 mg/kg b.w., 300 mg/kg b.w. and 500 mg/kg b.w (P100, P300 and P500 groups) were
taken. Mice were divided into 7 groups including normal control, *S. typhimurium* infected group, infected and standard antibiotic cefixime treated, infected and propolis treated groups (P100, P300 and P500) and only propolis treated group without infection. The effect of treatments was studied after 5<sup>th</sup>, 15<sup>th</sup> and 30<sup>th</sup> days of treatment.

**Second experiment** was designed to study the effect of different combinations of cefixime and propolis *i.e.* 3 mg/kg b.w. of cefixime+ 220 mg/kg b.w. of propolis (CP1 group), 3 mg/kg b.w. of cefixime+ 150 mg/kg b.w. of propolis (CP2), 2 mg/kg b.w. of cefixime+ 220 mg/kg b.w. of propolis (CP3) and 2 mg/kg b.w. of cefixime+ 150 mg/kg b.w. of propolis (CP4). The above doses were selected by taking different combinations of ½ and ¾ of the effective dose of propolis and cefixime alone as in experiment 1. Effect was studied after completion of treatment regimen *i.e.* after 5<sup>th</sup> day. CP1 and CP2 gave the best results.

**Third experiment** was designed to study the prophylactic/ preventive effect of propolis. Animals were given 300 mg and 500 mg/kg b.w. of propolis for 10 and 20 days before infecting the mice with *S. typhimurium* (Groups P1, P2, P3 and P4 respectively). This study helped to understand the effect of regular intake of propolis on the intensity of occurrence of the disease.

- **Percentage survival**

The animals of infected group did not survive after 5<sup>th</sup> or 6<sup>th</sup> day of infection. The survival percentage after treatment was least in P100, CP4 groups in their respective experiments. 100% survival was observed in CP1, CP2, CP3, P3 and P4 groups respectively.

- **Body weight**

*S. typhimurium* infection caused decrease in the body weight of mice from 25.4±0.40g in normal to 21.33 ± 0.28g in mice having infection. The body weight was restored to normal levels in all experimental groups except in P100, CP4, P1 and P2.

- **Bacterial load in blood and different organs**

Bacterial load was found to be 6.57± 0.15 log CFU/mL in blood of infected mice. In liver, spleen and kidney the bacterial count was observed to be 8.21± 0.50 log CFU/g, 8.14± 0.05 log CFU/g and 7.78±0.10 log CFU/g respectively. In case of treated groups the bacterial load was reduced to almost negligible levels in all experimental groups as compared to infected group except in P100, CP4, P1 and P2.

- **Biochemical studies**
The induction of biochemical responses by the propolis treatment in mice having typhoid infection was studied by evaluating liver and kidney function tests in serum of different groups of animals and after different days of treatment.

**Liver function tests:** The activity of SGPT, SGOT, ALP and bilirubin in normal mice was observed to be 21.4±0.87 IU/L, 25.97±1.04 IU/L, 8.75±0.73 KA units and 0.85±0.12 mg/dL respectively. The levels increased to 140.57±1.27 IU/L, 94.03±3.5 IU/L, 28.33±1.52 KA units and 2.2±0.17 mg/dL respectively in case of infected group which were significantly higher than normal mice. On treating the mice with propolis in different experiments the levels of different liver enzymes approached near normal range except in P100, CP4, P1 and P2 groups.

**Kidney function tests:** In case of *Salmonella* infected group alteration in all the renal function parameters was observed as compared to normal group. There was an increase in urea, uric acid and creatinine level to 82.76±5.68 mg/dL, 38.07±2.6 mg/dL, 6.26±0.25 mg/dL and 0.78±0.02 mg/dL respectively. The normal levels of urea, BUN, uric acid and creatinine were 43.26±0.9 mg/dL, 19.89±0.41 mg/dL, 3.03±0.2 mg/dL and 0.38±0.05 mg/dL respectively. On treating the mice in different experiments the levels of all enzymes were restored to near normal values except in P100, CP4, P1 and P2 groups.

* Haematological studies

The induction of haematological response was studied by estimating RBC count, Hb, PCV, MCH, MCV, MCHC, TLC and DLC in different groups of experimental animals after different days of treatments.

Anemia was observed in *S. typhimurium* infected mice with Hb level 9.06±0.11 g/dL as compared to 12.48±0.66 g/dL in normal mice. Decrease in RBC count, Hb levels was observed in infected group. However in animals treated with propolis especially in group P300, P500, CP1, CP2, CP3, P3 and P4 significant revival of red blood cell count was observed as compared infected group. The PCV, MCV, MCH and MCHC levels were altered as compared to normal level. The percentage of PCV was recorded to be 42±1% and 28±1% in normal and infected group respectively. In case of treatments with propolis in all the three experiments values of these haematological indices were restored to normal range except in P100, CP4, P1 groups.

While calculating the white blood cells, leucopenia was observed in infected group. The count was 5766.66±28.86/mm$^3$ in infected group as compared to 7730±170.88/mm$^3$ in normal mice. Even in differential leucocyte count (DLC), lymphocytosis and neutropenia were observed in *Salmonella* infected mice as the values were 83.33±1.44% and 14.33±0.57% which were altered significantly as
compared to normal control with 69.16±1.44% and 20.83±1.14 % respectively. In all the propolis treated groups the percentage came to near normal value.

- **Histological studies**

The histopathological studies of different organs of all groups was done using light microscopy.

**Liver:** The HE stained sections of infected liver showed typical typhoidal nodules formed by infiltrations of lymphocytes and increasing sinusoidal spaces. Kupffer cell hyperplasia was observed in infected group. However, mice treated with propolis especially in P300, P500, CP1, CP2, CP3, P3 and P4 groups showed remarkable recovery with almost normal architecture of liver.

**Spleen:** Liver and spleen are major organs of reticuloendothelial system and histology of these organs was altered the most in case of infected mice. Non follicular lymphoid hyperplasia was prominent in infected mice. Expansion of white pulp into the red pulp was observed in infected group. Treatment with propolis helped to restore the spleen histology to near normal with clear distinction between red pulp and white pulp.

**Kidney:** Infection did not much influence the histo architecture of the kidney and it was quite similar to normal mice. However, abnormal infiltration of lymphocytes was pronounced in the cortex region of infected kidney. Treatment with propolis in all the experimental groups showed signs of recovery except in P100 group in experiment 1, CP4 group in experiment 2 and P1 and P3 groups in experiment 3 respectively.

- **Conclusion**

The mice treated with P300 and P500 in experiment 1 showed considerable therapeutic efficacy against *S. typhimurium*. This was revealed by significant reduction in bacterial log count after 15 days of propolis treatment and by the restoration of normal values in various biochemical, haematological and histopathological parameters used for testing. In case of combination of cefixime and propolis in experiment 2, maximum efficacy was observed in CP1 and CP2 groups on the basis of parameters used in the investigations. In experiment 3, P500 dose given for 10 (P3) or 20 (P4) days gave better results as compared to P1 and P2 groups.

With these results, the effectiveness of propolis as a prospective candidate for treating infections due to *Salmonella* cannot be ignored rather it opens new avenues for research towards determining and isolating the active principles of propolis involved in this action as an alternative treatment for MDR typhoid or at least in reducing the dose of standard antibiotics.