5. SUMMARY AND CONCLUSION

The present work has been done to scientifically evaluate the protective potential of bee pollen and propolis against the oxidative stress induced by toxicity caused by anti-tuberculosis drugs (rifampicin and isoniazid) in liver, kidney and testis of male SD rats.

Bee pollen was collected by the installation of specially designed pollen trap at the entrance of Langstroth movable frame bee hive. Propolis was collected by scrapping from the comb frames with the help of hive tool.

Aquous extract of bee pollen was prepared by centrifugation and filtration. Propolis was used as ethanolic extract.

Male Sprague Dawley (SD) rats of body weight in the range of 160-200 g were obtained from the Central Animal House of Panjab University, Chandigarh. Animals were kept in polypropylene cages bedded with sterilized rice husk in a 12 hours light/dark cycle under hygienic conditions. Male SD rats were segregated randomly into twelve groups each for fifteen days treatment (n=4) and for 30 days treatment (n=6).

Group 1 (C) was assigned as control where rats were given distilled water orally. Group 2 (O) was bee pollen treated and rats were given 100mg/kg body weight of bee pollen extract orally. Group 3 (D1) was rifampicin treated (100mg/kg body weight) orally. Group 4 (D2) was isoniazid treated (50mg/kg body weight). Group 5 (D12) was given both rifampicin+ isoniazid (rifampicin 100mg/kg body weight and isoniazid 50mg/kg body weight).

Group 6 (D1O) was bee pollen + rifampicin treated and the dose was the same as given in group 2 and group 3. Group 7 (D2O) was bee pollen+isoniazid treated with the same dose of bee pollen and isoniazid as in group 2 and 4. Group 8 (D12O) was bee pollen+rifampicin +isoniazid treated. Group 9 (R) was propolis treated (200mg/kg body weight). Group 10 (D1R) was given propolis + rifampicin. Group 11 (D2R) was propolis+ Isoniazid treated and the dose was the same as given in previous groups. Group12 (D12R) was propolis+ rifampicin + isoniazid treated.
The body weights of these rats were taken daily for fifteen and thirty days using weighing balance. The findings indicated that anti-tuberculosis drugs (rifampicin, isoniazid) decreased the body weight indicating the deterioration of general metabolism as compared to control. Administration of bee pollen significantly increased the weight of the animal as compared to the control and drug treated groups. Both bee pollen and propolis were effective when co administered with anti-tuberculosis drugs. The animals were sacrificed at the end of 15 and 30 days.

Blood sample was collected for serum parameters studies and hematological analysis after 15 and 30 days treatment. Liver, kidney and testis tissue samples were taken after 15th and 30th day after sacrificing the rats.10% tissue homogenates were prepared in ice cold phosphate buffer saline. Homogenates were centrifuged at 10,000 rpm for 30 minutes at 4°C to obtain post mitochondrial supernatants. For GSH tissue homogenates were used as such. MDA, GR, GST, GPx, CAT, GSH and SOD in liver, kidney and testis were used as parameters of oxidative stress. ALT, AST and total bilirubin were studied as serum parameters and hemoglobin, total leucocyte count and red blood cell count were studied as hematological parameters.

Histopathology of liver, kidney and testis was performed to support biochemical observations. Tissues were fixed in neutral buffered formalin. Blocks were prepared in wax and section cutting was done at 5 µm using microtome. slides were stained in hematoxylin –eosin and observed under Nikon phase contrast microscope.

It was observed that anti-tuberculosis drugs (rifampicin and isoniazid) alone as well as in combination caused significant (p≤0.001) increase in the level of MDA in liver, kidney and testis indicating enhanced oxidative stress due to administration of these drugs. Bee pollen and propolis extract ameliorated toxic level of lipid peroxidation in liver, kidney and testis of SD rats thereby indicating their antioxidant potential (Tables5.1-5.6).
The activity of GSH, SOD, GR, GST, GPX and CAT in rifampicin treated, isoniazid treated and both the drugs administered in combination, was reduced in the liver, kidney and testis tissues as compared to control. Treatment with bee pollen and propolis extract significantly restored the activity to near normal level (Tables 5.1-5.6).

The activity of liver serum markers ALT, AST and total bilirubin was also enhanced in drug treated groups. Propolis and bee pollen when given along with anti-tuberculosis drugs significantly decreased the elevated levels of ALT, AST and total bilirubin (Table 5.7 and 5.8).

The haematopoietic system is the first and foremost target of toxic compounds and acts as an important parameter of physiological status in animals. In the present study the protective effect of dietary bee pollen and propolis supplementation was analysed against toxicity induced by anti-tuberculosis drugs rifampicin and isoniazid in Sprague Dawley rats and it was found that bee pollen and propolis extract showed great potential to increase the haemoglobin amount, total leucocyte count and RBC count and attenuate the toxicity induced by rifampicin and isoniazid alone and in combination in SD rats (Table 5.7 and 5.8).

Histopathological examination of liver, kidney and testis revealed that administration of anti-tuberculosis drugs, rifampicin and isoniazid, alone or in combination damaged the normal histo-architecture of these tissues. The protective potential of bee pollen and propolis extract against anti-tuberculosis drug induced toxicity was evidenced by effective restoration of the normal details on co-administration of either bee pollen or propolis (Plate 1-12).

On the basis of the present findings it is suggested that bee pollen and propolis extract act as excellent antioxidant agents by lowering the oxidative stress induced by anti-tuberculosis drugs at the biochemical, hematological and cellular level in liver, kidney and testis of SD rats. These can be recommended as wonderful supplements along with tuberculosis therapy.
Conclusion

Tuberculosis is one among the topmost causes of death worldwide. It is curable but the treatment period is very long and the side effect of anti-tuberculosis drugs compels the patients to withdraw treatment. The results of present study indicate that therapy with bee pollen or propolis if given along with anti-tuberculosis drug can certainly ameliorate the noxious toxicity generated due to the drug treatment. It was observed that bee pollen performed better than propolis for some parameters while propolis was seen to give better results in certain other parameters. Hence, both are almost equally effective as modulatory agents. Phenolic compounds like kaempferol-3-O-neohesperidoside, quercetin-3-O-neohesperidoside, quercetin-3-O-rutinoside and kaempferol-3-O-rutinoside were responsible for beneficial health effects of bee pollen and propolis. This has been validated by evaluation of biochemical parameters of enzyme activity, liver function test, haematological and histopathological observations. On the basis of data generated, the antioxidant potential of bee pollen and propolis has been confirmed as protective in tuberculosis drug therapy.