

# Evaluation of the antioxidative potential of Bee Products: Pollen and Bee Bread against *Staphylococcus aureus* Infected Balb/c mice

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## ABSTRACT

Pollen is the male plant reproductive part and is a rich source of proteins, collected by worker honey bees for the rearing of their larvae. Before feeding pollen to its larvae honey bees add some enzymatic secretions and store it in the cells of the comb as 'bee bread'. It was investigated whether water extract of pollen and bee bread exerts antioxidative effect against *Staphylococcus aureus* infected BALB/c mice. The *in vivo* liver damage of BALB/c mice weighing between 25 to 30 g was induced by intraperitoneal injection of *S. aureus* ( $5 \times 10^6$  CFU/ml bacterial solutions). All the animals were sacrificed on the 16th day by decapitation. *S. aureus* induced toxicity produced oxidative stress in mice which caused increase in *Lipid peroxidase* (LPO) and decrease in *Glutathione* (GSH), *Catalase* (CAT), *Superoxide dismutase* (SOD), *Glutathione S transferase* (GST), *Glutathione peroxidase* and *glutathione reductase* levels. The treatment with pollen and bee bread during the present study caused decrease in the level of LPO and increase in other antioxidant enzymes which showed the positive effect of both bee products pollen and bee bread against bacteria and compared with antibiotics Ampicillin and Amoxicillin as positive control. The study concluded that the water extract of pollen and bee bread had protective effect against *Staphylococcus aureus* induced toxicity in mice liver.

**KEY WORDS:** antioxidative potential, pollen, bee bread, intraperitoneal, *Staphylococcus aureus*.

## 1. INTRODUCTION

Honey bees collected pollen is called life giving dust as it is primary food source for all the developmental stages in the hive (Almeida-Muradian, 2005). It is rich in secondary metabolites and Phytochemical. The phenolic composition of pollen principally consists of flavonol glycosides and hydroxycinnamic acids and it is species-specific depends upon the vegetation, season and climatic conditions. (Almaraz-Abarca, 2004). From ancient times it is used as therapeutic agent and is characterized as a functional food with varied enhancing effects in human health due to its nutritional properties (Bogdanov, 2004), and used to cure conditions such as cold, flu, ulcers, premature ageing, anemia and colitis. (Hanssen, 1979).

This powder-like material is produced by flowering plants pollen, mixed with nectar and bee secretions (Leblanc, 2009) and is collected by the worker honey-bees. After gathering honey bees knead the collected pollen with honey and saliva and process this mixture for fermentation. By the help of salivary enzymes (catalase, amylase etc), microorganisms, moisture and temperature of the hive, bee pollen gets ripened in to bee bread in two weeks. Due to fermentation its stabilization and therapeutic potentiality increases as compared to the bee pollen.

The present study focused on the antioxidative effects of water extract of the bee product "Pollen and Bee Bread" against *Staphylococcus aureus* infected balb/c mice.

## 2. MATERIAL AND METHODS

**Collection of the pollen:** The bee product pollen from pollen baskets of worker honey bees was collected by installing pollen trap at the entrance of Langstroth hive placed in the field of *Brassica campestris* at an apiary at village Tierra near Chandigarh.

**Collection of bee bread:** Bee bread is a fermented mixture of plant nectar, plant pollen and bee saliva. It is stored by worker bees in the center of the combs as a food for the developing larvae's and was collected from the pollen cells present inside the hive on the comb with the help of forceps and spatula.

**Preparation of the extracts:** Bee pollen and bee bread water extracts were prepared by following the methods of Nagai (2004), with some modifications. For this 3g of fresh bee pollen/bee bread was suspended and extracted by shaking with 10 volumes of solvent at 20°C for one day. And the extracts were centrifuged at 5000 rpm for 1h. The supernatants were collected, filled up to 30ml with solvent for biochemical analysis.

**Microorganisms:** *S. aureus* (MTCC-1144) was procured from IMTECH (Institute of Microbial Technology) Sector-39, Chandigarh, India. The organism was maintained in suitable media (agar plates at 4°C). The organism was checked biochemically prior to storage at -30°C. *S. aureus* was grown in BHI broth for further experiments.

**Animal model:** BALB/c strain of mice of either sex was used. Mice were obtained from Central Animal House, Panjab University, Chandigarh, India and fed with a standard pellet diet purchased from Ashirwad Industries, Kharar (Punjab) and water. The mice were kept in animal house and the treatment was according to the guidelines of institutional ethical committee for the purpose of control and supervision of experiments on animals. Following groups were taken for the present study: Group 1 Normal mice, Group 2 Mice infected with *S. aureus* (intraperitoneal injection of  $5 \times 10^6$  CFU/mL) on day first of treatment, Group 3 Mice infected with *S. aureus* were given pollen

extract (250 mg/kg body weight) every day for 15 days, Group 4 Mice infected with *S.aureus* were given bee bread extract (250 mg/kg body weight) every day for 15 days, Group 5 Mice infected with *S.aureus* were given antibiotic Ampicillin and Group 6 Mice infected with *S.aureus* were given antibiotic Amoxycillin.

**Separation, homogenization of liver tissue and Biochemical studies:** On 16<sup>th</sup> day mice were sacrificed by decapitation. Liver tissue was excised from mice of different experimental groups, washed with cold normal saline, homogenized in the ice-cold buffer containing 0.25 M sucrose, 1mM EDTA, and 1mM Tris-HCl, pH 7.4. The homogenate was first centrifuged at 1000 rpm for 10 min at 4°C and the supernatant was used for the biochemical estimation.

For the biochemical estimation, Protein level was estimated with the method Lowry (1951). Lipid peroxidation was assayed by the method of Beuge and Aust (1978). Estimation of glutathione was done by the procedure described by Sedlak and Lindsay (1968). Enzyme activity of Glutathione -S-transferase (GST) was determined by the method of Habig (1974). Activities of Superoxide dismutase (SOD) enzyme were determined by the procedure described by Kono (1978). Catalase activity was determined by the method of Luck (1971). Glutathione peroxidase assay was done by the method of Pagila and Valentine (1967). Glutathione reductase assay was done by the procedure of Carlberg and Mannervik (1985).

### 3. RESULTS AND DISCUSSION

**Body Weight:** There was decrease in the body weight of *S.aureus* infected mice as compared to the normal mice. The administration of bee pollen and bee bread to the *S.aureus* infected mice restored the values to near about normal and the results obtained were comparable with the positive control (standard antibiotics used for the said studies i.e. Ampicillin and Amoxycillin).

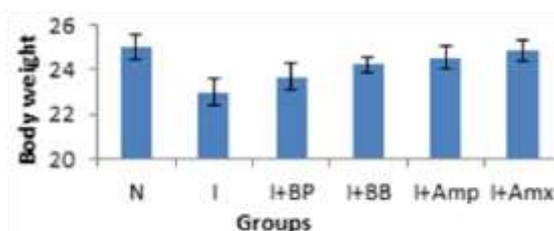


Fig.1. Effect on body weight of *S.aureus* infected mice after treatment with Bee Pollen (BP), Bee Bread (BB) and positive controls: Ampicillin (Amp) and Amoxycillin (Amx)

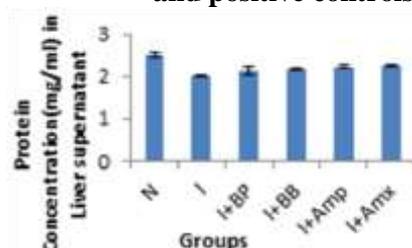


Fig.2. Protein concentration (mg/ml) in liver supernatant of *S.aureus* infected mice after treatment with Bee Pollen (BP), Bee Bread (BB) and positive controls: Ampicillin (Amp) and Amoxycillin (Amx)

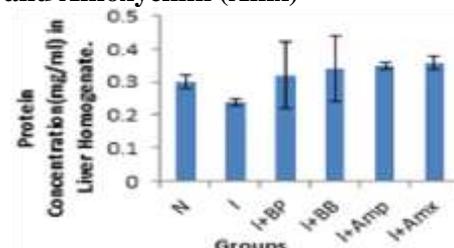


Fig.3. Protein concentration (mg/ml) in liver homogenate of *S. aureus* infected mice after treatment with Bee Pollen (BP), Bee Bread (BB) and positive controls: Ampicillin (Amp) and Amoxycillin (Amx)

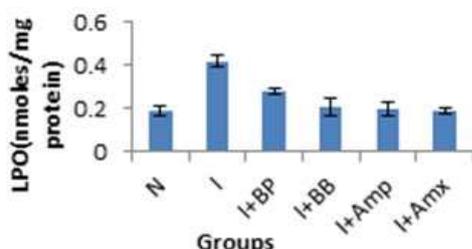


Fig.4. LPO (Lipid peroxidation) activity in liver of *S. aureus* infected mice after treatment with Bee Pollen (BP), Bee Bread (BB) and positive controls: Ampicillin (Amp) and Amoxycillin (Amx)

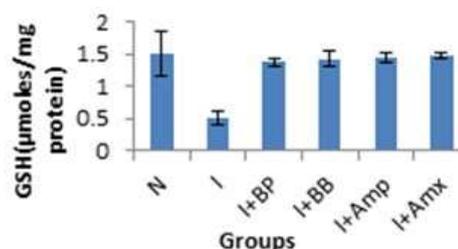
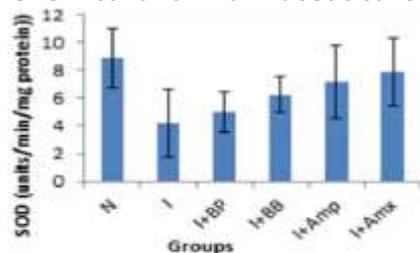
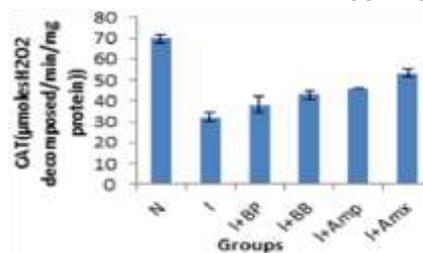


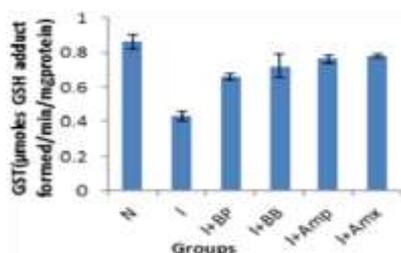
Fig.5. GSH (Reduced Glutathione) activity in liver of *S.aureus* infected mice after treatment with Bee Pollen (BP), Bee Bread (BB) and positive controls: Ampicillin (Amp) and Amoxycillin (Amx)



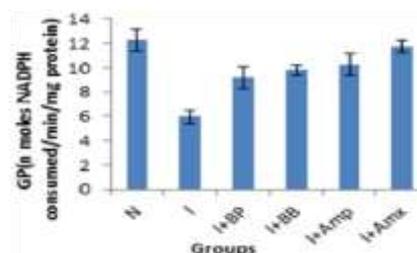
**Fig.6. SOD (Superoxide dismutase) activity in liver of *S.aureus* infected mice after treatment with Bee Pollen (BP), Bee Bread (BB) and positive controls: Ampicillin (Amp) and Amoxycillin (Amx)**



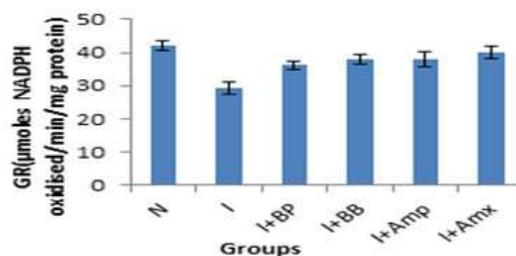
**Fig.7. CAT (Catalase) activity in liver of *S. aureus* infected mice after treatment with Bee Pollen (BP), Bee Bread (BB) and positive controls: Ampicillin (Amp) and Amoxycillin (Amx)**



**Fig.8. GST (Glutathione-S-transferase) activity in liver of *S. aureus* infected mice after treatment with Bee Pollen (BP), Bee Bread (BB) and positive controls: Ampicillin (Amp) and Amoxycillin (Amx)**



**Fig.9. GP (Glutathione peroxidase) activity in liver of *S. aureus* infected mice after treatment with Bee Pollen (BP), Bee Bread (BB) and positive controls: Ampicillin (Amp) and Amoxycillin (Amx)**



**Fig.10. GR (Glutathione reductase) activity in liver of *S. aureus* infected mice after treatment with Bee Pollen (BP), Bee Bread (BB) and positive controls: Ampicillin (Amp) and Amoxycillin (Amx)**

The phytochemicals such as phenolic compounds are found to be beneficial for the human health as they decrease the risk of degenerative diseases produced by oxidative stress (Silva, 2004). This neutralization of free radicals activity of phenolic compounds is associated with their structure such as multiple bonds and the presence of hydroxyl groups in their aromatic rings as observed by Leja (2007). The antibacterial, anticancer, anti-allergic, antiviral, anti-inflammatory, antioxidative and vasodilatory activities are all associated with their flavonoids content (Abdella, 2009).

The flavonoids also inhibit the lipid peroxidation, platelets aggregation, capillary permeability and fragility and also the activity of enzyme systems including cyclo oxygenase and lipoxygenase (Estevinho, 2008; Viuda Martos, 2008).

The present study focused on the antioxidative effect of Pollen and Bee Bread against *Staphylococcus aureus* infected balb/c mice and the results obtained from *in vivo* studies done on different groups were comparable with those of the standard antibiotics used as positive control. Results showed that the LPO level in the infected group was increased where levels of GSH, Catalase (CAT), Superoxide dismutase (SOD) and Glutathione S transferase (GST), Glutathione peroxidase (GP) and Glutathione reductase (GR) levels were decreased as compared to the control group.

The treatment with pollen and bee bread during the present study caused decrease in the level of LPO and increase in other antioxidant enzymes which showed the positive effect of both bee products pollen and bee bread against bacteria.

The level of LPO was  $0.42 \pm 0.02$  n moles/mg of protein in infected as compared to  $0.19 \pm 0.01$  n moles/mg of protein in normal. As shown in the Fig. 4. after treatment with pollen and bee bread the level came to near about normal  $0.28 \pm 0.03$  n moles/mg of protein and  $0.21 \pm 0.02$  n moles/mg of protein respectively. The level of GSH Fig.5 was decreased from  $1.52 \pm 0.2$  µ moles/mg proteins to  $0.52 \pm 0.1$  µ moles/mg protein in the liver of infected mice and administration of pollen and bee bread led to the restoration of the GSH level  $1.38 \pm 0.1$  µ moles/mg protein and  $1.43 \pm 0.2$  µ moles/mg protein respectively. Catalase was found to be decreased from  $69.80 \pm 0.29$  µmoles H<sub>2</sub>O<sub>2</sub>

decomposed/min/mg to  $32.10 \pm 0.9$   $\mu\text{moles H}_2\text{O}_2$  decomposed/min/mg in liver of infected mice Fig.7 and administration of pollen and bee bread led to the restoration of its level  $42.90 \pm 0.88$   $\mu\text{moles H}_2\text{O}_2$  decomposed/min/mg and  $46 \pm 0.66$   $\mu\text{moles H}_2\text{O}_2$  decomposed/min/mg respectively.

The level of Superoxide dismutase Fig.6 also decreased from  $8.90 \pm 0.55$  units/min/mg protein to  $4.20 \pm 0.7$  units/min/mg protein in the liver of infected mice and pollen and bee bread administration leads to its restoration  $4.99 \pm 0.24$  units/min/mg protein and  $6.02 \pm 0.68$  units/min/mg protein respectively. The level of Glutathione-S transferase (Fig.8) was also decreased from  $0.86 \pm 0.46$   $\mu\text{moles GSH adduct formed/min/mg protein}$  to  $0.43 \pm 0.11$   $\mu\text{moles GSH adduct formed/min/mg protein}$  in the liver of infected mice and administration of pollen and bee bread led to its restoration  $0.66 \pm 0.19$   $\mu\text{moles GSH adduct formed/min/mg protein}$  and  $0.72 \pm 0.46$   $\mu\text{moles GSH adduct formed/min/mg protein}$  respectively. The level of Glutathione peroxidase decreased from  $12.28 \pm 0.16$  n moles NADPH consumed/min/mg protein to  $5.98 \pm 0.10$  n moles NADPH consumed/min/mg protein in the liver of infected mice Fig.9 and administration of pollen and bee bread led to the restoration  $9.2 \pm 0.10$  n moles NADPH consumed/min/mg protein and  $9.8 \pm 0.12$  n moles NADPH consumed/min/mg protein respectively. Glutathione reductase level Fig.10 was also decreased from  $42.15 \pm 0.19$   $\mu\text{moles NADPH oxidized/min./mg protein}$  to  $29.18 \pm 0.22$   $\mu\text{moles NADPH oxidized/min./mg protein}$  in the *S. aureus* infected mice and administration of pollen and bee bread led to the restoration  $36.2 \pm 0.55$   $\mu\text{moles NADPH oxidized/min./mg protein}$  and  $37.92 \pm 0.88$   $\mu\text{moles NADPH oxidized/min./mg protein}$  respectively.

All the above mentioned results were comparable with the positive control taken i.e. standard antibiotics Ampicillin and Amoxycillin.

Hence it is clear from the results that the natural honey bee product "Pollen and bee bread" is having antioxidative properties and can be used further in therapeutic applications.

#### 4. CONCLUSION

The study concluded that the water extract of pollen and bee bread possess good antioxidative activity against *Staphylococcus aureus* induced toxicity in mice liver suggesting that it could be useful in preventing diseases where free radicals are implicated. Bee bread in comparison with bee pollen is more effective and is because of the fermentation and the salivary enzymatic action which increases its stabilization and therapeutic potentiality as compared to the bee pollen. Further the studies also showed that Amoxicillin is more effective as compared to Ampicillin and the results were comparable with that of the natural honey bee products pollen and bee bread. Hence both bee products pollen and bee bread can be used further in clinical application in the emerging world of drug resistance.

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