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FEEDING BEE POLLEN AND BEE BREAD TO MICE: EFFECT AND ANTIOXIDANT STATUS

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ABSTRACT

The aim of present study was to determine the effect of bee pollen and bee bread given as feed additive to mice. The influence on parameters of antioxidant status was taken into consideration. Mice were randomly divided into 3 groups. The control group (C). The experimental group E1 received orally bee collected pollen @ 250 mg/kg and the group E2 received orally bee bread @ 250 mg/kg for 21 days. Antioxidant efficacy was evaluated by determination of Lipid peroxidation, Glutathione, Superoxide dismutase, catalase, Glutathione-S-transferase, Glutathione peroxidase, Glutathione reductase levels with spectrophotometer. Supplementation of the diet with bee pollen and bee bread at the tested dose did not show any negative influence on mice.

INTRODUCTION

Bee pollen is collected by honey bees directly from flowers, mixed with saliva and stored in the comb as bee bread. It is the fermented mixture of plant pollen, bee saliva and small amount of honey. Pollen is collected by the bees from different plant sources required for proper nourishment and development of their larvae (Kaur and Kumar 2013a). It is richest source of proteins and has several useful pharmacological properties, such as antibiotic, antineoplasic, antidiarrhoeatic and as an antioxidant agent (Campos, 1997). It was therefore considered important to test it for positive effects when given as a supplement in the feed to normal mice.

MATERIAL AND METHODS

1. **Collection of bee pollen:**
   Honey bee colonies were placed in *Helianthus annus* field in order to collect pollen. Bee pollen was collected by installing a pollen trap at the entrance of bee hive.

2. **Collection of Beebread:**
   Beebread was collected with the help of spatula from pollen cells in the comb.

3. **Preparation of extract:**
   Water was used as a solvent to prepare the aqueous extract of pollen. 3g of fresh bee pollen and bee bread was suspended and extracted by shaking with 10 volumes of water at 20^{o}C for 1 day and the extracts were centrifuged at 5000 rpm for 1h. The supernatants were collected, filled up to 30 ml with solvent for further studies (Kaur *et al.,* 2013b).

4. **Animal:**
   The present studies were carried out in Balb/c mice, weighing between 25-30 g. These animals were procured from the Central Animal House of Panjab University, Chandigarh and housed in polypropylene cages. They were maintained on a 12 h light/12 h dark cycle, under stable conditions, at a temperature 22 ± 4 ºC. The protocols and experiments carried out strictly followed the principals as given by the Institutional Animal Ethical Committee (IAEC), Panjab University, Chandigarh and also by Government of India. Animals were fed drinking water and standard laboratory pellet diet provided *ad libtium* throughout the study. The laboratory pellet diet was purchased from

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Ashirwaad Industries, Kharar, Punjab. The experimented animals were divided in three groups as follows-

C1: Normal mice that were given standard diet.
E1: Bee collected pollen given orally at a dose of 250 mg/kg bw for 21 days in addition to standard diet.
E2: Bee bread given orally at a dose of 250 mg/kg bw for 21 days in addition to standard diet.
The dose of bee pollen and bee bread was decided after experimentation using different concentration.

5. **Biochemical parameters:**
After 21 days mice were anaesthetized and dissected. Liver was carefully removed and homogenized to measure the LPO and GSH. The homogenate was centrifuged at 10,000 rpm and supernatant was used to determine SOD, CAT, GST, GR, GPx. Protein level was estimated by following the protocol of Lowry *et al.,* (1951). LPO level was estimated by using the method of Beuge and Aust (1978). GSH assay was determined by the protocol described by Moron *et al.* (1979). Estimation of GST was done by the protocol described by Habig *et al.* (1974). The protocol given by Kono (1978) was used to estimate the activity of SOD in liver. CAT activity was determined by the method of Luck (1971). GR assay was performed in different organs by the procedure of Carlberg and Mannervick (1985). GPx assay was done by the method of Pagila *et al.* (1967).

RESULTS

The antioxidants status in liver was assessed in mice after treatment with dietary bee pollen and bee bread. The level of LPO was decreased in bee pollen and bee bread treated group as compared to control. The more decrease was reported in bee bread treated group but this is not statistically significant as compared to control. The activity of GSH, SOD, CAT, GST, GR and GP in liver increased in bee pollen and bee bread treated groups as compared to control. In case of SOD statistically significant ($p < 0.05$) difference in bee collected pollen and very statistically significant ($p \leq 0.0001$) difference in bee bread was noticed. Bee bread in GST and GR also showed significant ($p < 0.05$) difference. This showed that bee bread treated groups showed more antioxidant potential.

DISCUSSION

Antioxidant activities of an agent depend upon their hydrogen donating ability which intercepts the free radical chain during oxidation. They donate hydrogen from phenolic hydroxyl groups and forms stable end products which stop further oxidation of lipids (Silva *et. al.* 2006). Honey bee hive products contains a number of bioactive compounds such as enzymes, cartotenoids, free amino acids, essential fatty acids, lipids, minerals, whole vitamin complex and trace elements, as well as phenols and polyphenols, which are responsible for its antioxidants capacity in the human body (Hurd, 2003; Kaur *et al.,* 2013b; Kaur *et al.,* 2013c; Kalia *et al.,* 2013). Bee collected pollen has health promoting effects and is also regarded as natural dietary food

Table 1. Antioxidant activity of bee collected pollen and bee bread of *H. annus*.

S. No.	Biochemical tests	Normal	*Helianthus annus*	
			Bee pollen	Bee bread
1.	LPO (nmoles/mg protein)	0.21 ± 0.02	0.18 ± 0.02	0.17 ± 0.01
2.	GSH (µmoles/mg protein)	1.6 ± 0.35	1.8 ± 0.38	1.88 ± 0.31
3.	SOD (Units/min./mg protein)	9.6 ± 0.46	12.77 ± 0.5*****	13.03±1.65**#**
4.	CAT (µmoles H_2O_2 decomposed/min,/mg protein)	74.81 ± 0.93	75.77± 1.42	76 ± 0.7
5.	GST (µmoles GSH adduct formed/min./mg protein)	0.86 ± 0.04	0.97 ± 0.03	1.03 ± 0.06*****
6.	GR (µmoles NADPH oxidized/min./mg protein)	49.35 ± 1.32	52.5 ± 0.69	52.97±1.13*****
7.	GP (nmoles NADPH consumed/min./mg protein)	13.28 ± 0.89	14.92± 0.82	15.21± 1.1

($p < 0.05$ = significant, $p \leq 0.001$ = very significant, $p \leq 0.0001$ = extremely significant)

supplement because of its radical scavenging capacity and presence of polyphenol substances (Kroyer and Hegedus, 2001). Bee bread possesses antioxidant and free radical scavenging abilities and can be use as a healthy food and in medicine (Nagais *et al.,* 2004). Beebread possess higher antioxidant activity than honey (Baltrusaityte *et al.,* 2007). In the present study bee pollen showed the antioxidant activities, similar potential was also reported by bee pollen against different toxic compounds such as propoxure and sodium fluoride (Eraslan *et al.,* 2009; Khalil and Sheikh 2010). Addition of Bee pollen at a dose of 500mg/kg was found to be a promising source for improving antioxidant status in animals and human (Capcarova *et al.,* 2013). Its use is recommended as everyday food or natural dietary supplement because it is a rich source of essential amino and fatty acids which are required for healthy and normal development of an organism (Aličić *et. al.* 2014). It has also been reported to be a good source of energy and a nourishing substance as it possess antioxidant, antibacterial, anti-inflammatory, antimutagenic properties (Pascoal *et al.* 2014).

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