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THERAPEUTIC POTENTIAL OF TWO POTENT HONEY BEE PRODUCTS AGAINST THE TOXICITY CAUSED BY *SALMONELLA ENTERICA* SEROVAR TYPHIMURIUM IN MURINE MODEL: A BIOCHEMICAL STUDY

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ABSTRACT
The free radicals leads to oxidative stress and are produced by virus, bacteria and parasite which may be present in the food, air and water. Oxidative stress results in the production of chronic diseases and finally organ damages. To remove this stress, intake of sufficient amount of antioxidants is needed. The present study is a comparative analysis of antioxidant potential of the two "P" of Apitherapy *i.e.* Pollen and Propolis. The whole *in vivo* experiment was divided into eight groups. *Salmonella enterica* serovar Typhimurium was given to BALB/c mice to induce the oxidative stress. And followed by treatment with selected doses of bee pollen and propolis in different groups. Various biochemical parameters were studied and compared like lipid peroxidation, superoxide dismutase, catalase, glutathione transferase etc. By examining the results carefully the effect of bee pollen as an antioxidant is quite strong as compared to that of propolis. The possible reason for that could be presence of more and strong metabolites in case of bee pollen.

KEYWORDS: Bee products, pollen, propolis, *Salmonella*, Oxidative stress, toxicity.

INTRODUCTION
Oxidative stress is a condition in which there is disturbance in the equilibrium condition of pro-oxidant/antioxidant system of the cell. Reactive oxygen species (ROS) are those which have reactive free radicals that can react with other molecules and initiate a series of reactions that produce oxidative stress. The healthy cell, in which there is balance between the pro-oxidant/antioxidant system, detoxifies the free radicals produced during normal aerobic metabolism. ROS act as a double edged sword. They are involved in promotion as well as prevention of the development of disease. ROS are involved in many processes such as differentiation, cell growth, progression and death. At low concentrations, they may be beneficial or even indispensable in processes such as defense against microorganisms and intracellular signaling. But at higher concentrations, they play a role in aging and also produce a number of human diseases including ischemia, cancer and failures in immunity and endocrine functions.[1] In the absence of adequate antioxidant reserves, cellular machinery is damaged by the reactive oxygen species resulting in the reduction of effectiveness of immunity, establishing infection and finally reducing lifespan of the cell.[2]

Like several micro-organisms, *Salmonella* is known to produce oxidative stress in human body. *Salmonella enterica* serovar Typhi causes one of the major health problem i.e. Typhoid worldwide. The World Health Organization[3] has reported 16 million new cases and 600,000 deaths due to typhoid fever each year. Emergence of antimicrobial resistant form of *S. Typhi* has further complicated the issue. The oxidative stress results in the production of a number of pathological conditions such as ageing, asthma, arthritis, AIDS, autoimmune diseases, atherosclerosis, cataract, carcinogensis, diabetes, liver disorders, pulmonary fibrosis, Parkinson's disease, Alzheimer's disease, neurogenerative diseases, inflammatory diseases.[4] A number of drugs are used against *Salmonella* but most of these often result in the emergence of resistant forms of bacteria. The increase in antimicrobial resistance, virulence, prevalence and adaptability are several challenges faced worldwide. *Salmonella enterica* serovar Typhimurium is resistant to a variety of antibiotics such as streptomycin, ampicillin, sulfonamides, chloramphenicol and tetracycline.[5] The main mechanisms by which bacteria exhibit resistance to antibiotics can be due to reduced drug accumulation, drug inactivation, alteration of metabolic pathway and target site.[6] Increasing multidrug resistance of *Salmonella* reduces effect of the treatment and increases

the treatment costs which results in serious complications and sometimes death.[7] The increased use of antibiotics produces side effects on different organs in the human body and leads to generation of newer diseases. In order to counteract these side effects, there is need to explore the curative antioxidant properties of herbal products which alone or in conjunction with antibiotics may prove useful for human body at specific concentration. The traditional knowledge system is replete with information of herbs that have been used as medicinal plants. It is now known that plants contain polyphenolic compounds which are effective ROS scavengers and offer good potential for exploitation as natural antioxidants. Many toxic effects are produced by the microorganism inside the human body treated with synthetic drugs and these drugs have many side effects on different organs of the body. So, the need of the hour is to shift towards some natural products and alternative therapies among which Apitherapy is lately drawing much attention

Recent times are witnessing a rise in the use of honey bee products for the formulation of medicines and supplements, due to their beneficial activities and lack of side effects. Bee pollen is collected by honey bees to feed their larvae. This product is important for the proper development of the brood. Honey bees collect pollen from different flowers in their pollen basket present on their hind legs. Bee pollen is a rich source of bioactive ingredients such as carotenoids, enzymes, free amino acids, vitamins, phenols and polyphenols which contribute antioxidant defence.[8,9] Bee pollen has also health promoting effects[10] as well as helpful in the prevention of a number of diseases such as diabetes, cancer and cardiovascular disorders.[11] Bee pollen contains a rich amount of proteins, carbohydrates, lipids, amino acids, vitamins, minerals and trace elements[12] as well as phenolic compounds mainly flavonoids.[13] Phytochemical compounds such as phenolic compounds decrease the risk of harmful diseases by inhibiting the macromolecular oxidation and oxidative stress.[14]

Another useful honey bee product is propolis. Propolis is a generic name for the complex resinous material produced by honey bees from plant exudates, bees wax and bee secretions.[15] It is used as a sealant for unwanted open spaces in the hive and is used by honey bees for scaffolding, reinforcing and protecting the hive from intruders and various pathogens. Propolis is used as an antiseptic in the breeder cells and is mixed with wax to varnish the hive. Chemically, propolis comprises flavonoids, phenolics, various aromatic compounds vitamins and minerals. Most of these compounds are not water soluble. Propolis is used as an emollient, immunomodulator, antioxidant, anti tumor growth agent, a dental antiplaque agent and has anti inflammatory, antibacterial and pain-killing (analgesic) properties. Certain bacteria have built resistance to antibacterial dressings. So, several groups of researchers have focused their attention on the biological activity of propolis and its active principles.[16-20]

In view of the potential medicinal properties of bee pollen and propolis, these bee product were selected for testing against oxidative stress produced by *Salmonella* infection in murine model.

MATERIAL AND METHODS

Collection and extraction of bee pollen

For the present study, bee pollen was collected by installing a pollen trap at the entrance of honey bee colonies (*Apis mellifera*), which were placed in the field of *Helianthus annus*. Water extract of bee pollen was prepared by using standard protocol.[21-23]

Collection and extraction of propolis

Propolis was collected from honey bee hives kept in an apiary maintained by Department of Zoology, Panjab University, Chandigarh. Propolis is harvested by scrapping it off with the help of hive tool from the top bars of comb frames where it is deposited by bees in relatively large amount. Propolis so collected is crude or raw and cannot be used as such. It was stored in refrigerator until extraction. In the present study, ethanol was taken as a solvent for propolis extraction by the standard method.[24,25]

Phytochemical studies

Chemical tests were carried out on extracts of bee pollen and propolis using standard procedures to identify the constituents.[26- 28]

Microorganisms

Bacterial strain *Salmonella enterica* serovar Typhimurium (MTCC 98) which was purchased from Institute of Microbial Technology (IMTECH), Chandigarh was used for the present study.

Animal model

For experimental investigations, five to six weeks old BALB/c mice of either sex, weighing 25 – 30 g were used. Ethical clearance was approved by Institutional Animal Ethics Committee (IAEC/411 dated 11/9/2013) of Panjab University, Chandigarh. And the animals were kept in Central Animal House facility of the University. Animals were housed in polypropylene cages bedded with clean rice husk in well aerated rooms. They were fed with standard feed (Ashirwaad Industries, Kharar, Punjab) and water *ad-libitum*.

Experimental groups

The dose of *Salmonella* enterica serovar Typhimurium used in present studies is $2X10^4$ CFU/ml.[17] The dose of bee pollen and propolis after infection was adjusted to 250 mg/kg body weight of mice (bw) in distilled water and given orally for 21 days.

Experimental animals were divided into different groups. Each group consisted of 6-8 mice as described below:
Gp 1: Normal mice - administered with normal saline orally.

Gp 2: Infected mice - administered intraperitonelly 0.2 ml of 2 ×104 CFU/ml of *Salmonella* enterica serovar Typhimurium.
Gp 3: Normal mice administrated Vitamin C only.
Gp 4: Treatment with Vitamin C in *Salmonella* enterica serovar Typhimurium infected mice.
Gp 5: Normal mice administrated bee collected pollen from *Helianthus annus* orally.
Gp 6: Treatment with bee collected pollen from H. annus (orally) in *Salmonella* enterica serovar Typhimurium infected mice.
Gp 7: Normal mice administrated propolis only.
Gp 8: Treatment with propolis in *Salmonella* enterica serovar Typhimurium infected mice.

Animal sacrifice
Bacterial infection ($2X10^4$ CFU/ml) was administrated intraperitonally and animals of untreated group were sacrificed on 5th day. In treated groups, bee pollen, propolis and Vitamin C were given orally at 250mg/kg body weight for 21 days to the mice after inducing bacterial infection. Animals were sacrificed after last day of treatment. Liver samples were rapidly excised after dissection, washed with ice-cold saline and 10% liver homogenates were prepared in ice cold phosphate buffer saline (PBS, pH 7.4) using mechanically driven Teflon fitted Potter- Elvehjem homogenizer for 2 mins at 3000 rpm in ice till total disruption of cells. Post mitochondrial supernatant was prepared by centrifugation at 10,000 g for 20 min at 4°C.

Preparation of tissue homogenate and PMS
All mice were anesthetized with diethyl ether. Gp 2 was sacrificed on 5th day as this was the peak day of infection while other groups were sacrificed immediately after 21st day of treatment. Liver was removed and perfused immediately with ice cold saline (0.9% NaCl) solution. The tissue was homogenized in 100 mM potassium phosphate buffer (pH 7.4) containing 150 mM KCl to obtain 10% homogenate (w/v). This homogenate was used for Lipid peroxidation (LPO) and Glutathione (GSH) estimation. Then, homogenates of different tissues were subjected to cold centrifugation at 10,000 × g for 30 minutes. The pellets were discarded and supernatants (Post-Mitochondrial Supernatant: PMS) were used for further estimation of Glutathione-S-transferase (GST), Glutathione peroxidise (GPx), Glutathine reductase (GR), Superoxide dismutase (SOD) and Catalase (CAT).

Biochemical estimations
Lipid peroxidation was determined by measuring malondialdehyde (MDA) content in homogenates according to the standard method[29] spectrophotometrically at 532 nm. The level of reduced glutathione (GSH) was assessed by the method of Moron *et al.*[30] at 412 nm. Superoxide dismutase (SOD) activity was measured by following the method of Kon[31] at 560 nm. Catalase (CAT) activity was determined according to method of Luck[32] based on determination of the H_2O_2 decomposition rate at 240 nm. Glutathione-S-transferase (GST) activity was determined by using the protocol of Habig *et al.*[33] at 340 nm. Glutathione reductase (GR) activity was determined according to the method of Carlberg and Mannervick.[34] Glutathione peroxidase (GSH-Px) was determined by the method of Pagila and Valentine[35] at 340 nm.

Statistical Analysis
All values were expressed as mean ± standard deviation (S.D.). Data was analysed using student's t-test and ANOVA. Values with $p < 0.05$ were considered as statistically significant, $p \leq 0.001$ very significant and $p \leq 0.0001$ extremely significant.

RESULTS

Phytochemical analysis
Both the bee products have complex composition and it cannot be used directly in the raw form. The preliminary ":phytochemical" screening of pollen and propolis extract showed the presence of some bioactive components like terpenoids, flavonoids, alkaloids, phenols, tannins and saponins and results are summarized in Table 1. The presence of these phytochemicals could be responsible for their biological activity.

Table 1: Phytochemical analysis of bee pollen and propolis.

S.No.	Tests	Pollen	Propolis
1	Tannins	+++	+++
2	Flavonoids	+++	++
3	Carbohydrates	+	+
4	Terpenoids	++	++
5	Alkaloids	+++	++
6	Coumarins	++	++
7	Quinones	++	+

++ abundant; + denotes average; - absent

Assessment of antioxidants
The levels of GST, SOD, GP, GR, CAT, LPO were significantly different in the infected group as compared to that of the control group ($p<0.05$). After treating the mice with pollen and propolis in the respective groups sign of improvement were recorded as shown in Table 2.

Table 2: Antioxidant potential of bee pollen and propolis in liver of BALB/c mice.

S. No	Tests	Gp 1	Gp 2	Gp3	Gp4	Gp5	Gp 6	Gp 7	Gp8
1.	LPO	0.21 ± 0.02	0.49 ± 0.03@	0.17 ± 0.02^	0.26 ± 0.03^	0.18± 0.02^	0.29± 0.01^	0.16 ± 0.01^	0.35±0.09
2.	GSH	1.6 ± 0.35	0.62 ± 0.11@	1.9 ± 0.3^	1.88 ± 0.08^	1.8 ± 0.38^	1.43± 0.06*	1.88 ± 0.31^	1.29±0.29
3.	SOD	9.6 ± 2.18	3.67 ± 2.40@	13.2 ± 1.75^	10.03± 2.61^	12.77±1.29^	7.62± 1.41^	13.03± 1.65^	5.85±2.50
4.	CAT	74.81± 2.09	46.03± 1.99@	76.07 ± 3.5^	71.87± 1.94^	75.77± 1.42^	61.37±4.03^	76 ± 3.0^	56.03±2.19
5.	GST	0.86 ± 0.04	0.43±0.03@	1.04 ± 0.14^	0.91 ± 0.02^	0.97 ± 0.03^	0.8 ± 0.02^	1.03 ± 0.06^	0.62±0.05
6.	GR	49.35± 1.32	32.8 ± 1.86@	53.1 ± 2.83^	49.58± 3.39^	52.5 ± 1.22^	43.99±1.33^	52.97± 1.13^	39.68±1.59
7.	GP	13.28± 0.89	6.43 ± 0.54@	15.33± 0.75^	12.77± 0.87^	14.92± 0.82^	10.83± 0.87^	15.21± 1.1^	7.72±0.92

Data is expressed as mean± S.D. (@: $p \leq 0.0001$: Gp 1 vs Gp 2, *: $p < 0.05$: Gp2 vs all treated groups, ^: $p \leq 0.0001$: Gp2 vs all treated groups).

DISCUSSION

In the present study, by comparing the phytochemicals in both bee pollen and propolis qualitatively, bee pollen showed higher concentration of the phytochemicals as compared to propolis. New and effective anti-microbials are discovered on the basis of the ethnopharmacological information gathered by phytochemical analysis of plants.[36] While comparing the phytochemicals in pollen and propolis it was concluded that of bee pollen had maximum amount of saponins, flavonoids[22] whereas propolis showed greater amount of tannins and glycosides.[37] The presence of these phytochemicals are responsible for the biological efficiency of pollen and propolis.

In the present study, ameliorative effect of bee products was tested against oxidative stress produced by bacteria. The entrance of harmful microorganisms produces changes in the body which build up the oxidative stress in the body. Entry of *Salmonella* into the body stimulates the production of reactive oxygen species. It stimulates the production of peroxynitrite which acts as a strong biological oxidant produced by the reaction of nitric oxide and superoxide. Peroxynitrite initiates lipid peroxidation and oxidizes thiol groups, modifies amino acetyl groups on protein and damages DNA. Increase in oxygen reactive species produces oxidative stress and reduces the antioxidant defense system of the body. LPO initiate a series of chain reactions of free radicals which in turn associated with the altered cell membrane functions. This results in a number of human diseases as cancer, cardiovascular diseases, diabeties, atherosclerosis, neurological diseases such as Parkinson's diseases, Alzheimer's disease etc. as well as in the ageing process.[38] But after treatment with bee pollen and propolis, decrease in LPO level was observed to near normal which showed their protective effect against oxidative stress. The results were supported by the earlier findings who reported the antioxidative potential of both bee pollen as well as propolis.[39-41]

Glutathione is synthesized by the body from three amino acids namely cystein, glutamic acid and glycine. GSH is known to reduce the level of LPO in the cell membrane.[42] In the present study the level of GSH, GST, GR and GP was found to decrease in case of bacteria infected group. But after administration of bee pollen and propolis, the increase of these enzymes to near normal was noticed. The antioxidant effect of the bee pollen was observed by various researchers.[43-45]

GST conjugates electrophilic substrates to GSH which contains thiol group so it act as detoxifying enzymes but decreased amount of GST after infection, decrease its ability to detoxification.[46] The increase in GST after tested bee products to near normal showed its effectiveness against oxidative stress.

Increase in superoxide radicals indicates the production of oxidative stress during infection. These radicals are dismutated by intracellular antioxidant enzyme SOD. SOD converts superoxide radicals into hydrogen peroxide (H2O2) and later detoxified by CAT.[47] Superoxide anion acted as a precursor to active free radicals that had potential of reacting with biological macromolecules resulting in tissue damage. Both enzymes decrease in *Salmonella* enterica serovar Typhimurium infection at day 5 but after treatment with bee pollen and propolis for 21 days, their levels were increased to near normal indicating their protective effects against *Salmonella*.

Both propolis and bee pollen showed strong antioxidative properties. And these could be due the the various phytochemicals which are present in both pollen and propolis like flavonoids, terpenoids, alkaloids and so on.[16, 22, 23] Flavonoids and phenolic compounds play a key role in the antioxidative properties of propolis. Antioxidants are capable of scavenging free radicals, chelate metal ions and hence protect the lipids, Vitamin C and other beneficial molecules from being oxidized.[48, 49] Earlier reports suggested that bee products like honey, propolis and royal jelly had antioxidant properties.[50, 51] This could be due to the fact that in honey and royal jelly a good amount of pollen was present which acted as a strong antioxidant as it terminated the free radical chains. The redox properties of polyphenol compounds, especially flavonoids, played an important role in absorbing and neutralizing free radicals, quenching oxygen and decomposing peroxides.[52]

CONCLUSION

In the present study, it was concluded that both extracts were effective against oxidative stress produced by

Salmonella enterica serovar Typhimurium. But by examining the results carefully the effect of bee pollen as an antioxidant is quite strong as compared to that of propolis. The possible reason for that could be presence of more and strong metabolites in case of bee pollen. The present findings favored the effectiveness of both the bee products as a prospective candidate for treating infections due to *Salmonella* and also open new avenues for research towards determining and isolating the active components of bee pollen and propolis involved in this action as an alternative treatment for increasing MDR typhoid.

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Author contributions

RK and PK did the practical research work and manuscript writing. NRK and KH helped during experimental work and in writing the manuscript.

Conflict of interest disclosure

The authors declare that there are no conflicts of interest.

REFERENCES

1. Mates JM, Perez-Gomez C, Castro IN. Antioxidant enzymes and human diseases. Clin Biochem, 1999; 32(8): 595–603.
2. Weiss G. Modification of iron regulation by the inflammatory response. Best Pract Res Cli Haematol, 2005; 18(2): 183-201.
3. World Health Organization. (2003). Manual for the laboratory identification and antimicrobial susceptibility testing of bacterial pathogens of public health importance in the developing world. Geneva: WHO, 382-381.
4. Gupta VK, Sharma SK. Plant as natural antioxidants. Nat Prod Radiance, 2006; 5(4): 326-34.
5. Montville TJ, Matthews KR. Food Microbiology: An Introduction. Washington, DC: ASM Press, 2005; 207.
6. Gordon MA. *Salmonella* infections in immunocompromised adults. J Infect, 2008; 56: 413-22.
7. Khan SM. Protective effect of black tea extract on the levels of lipid peroxidation and antioxidant enzymes in liver of mice with pesticide-induced liver injury. Cell Biochem. Funct, 2006; 24(4): 327-32.
8. Hurd L. Bee pollen: Top rank antioxidant. New studies show bee pollen has the highest antioxidant activity as whole foods. Total Health, 2003; 24(5): 40-42.
9. Kaur R, Kumar NR, Harjai K. Feeding bee pollen and bee bread to mice: effect and antioxidant status. Int J Therap App, 2014; 18: 26-9.
10. Kroyer G, Hegedus N. Evaluation of bioactive properties of pollen extracts as functional dietary food supplement. Innov Food Sci Emer Technol, 2001; 2(3): 171-4.
11. Nagai T, Inoue R, InoueH, SuzukiaN. Scavenging capacities of pollen extracts from *Cistus ladaniferus* on autoxidation, superoxide radicals, hydroxyl radicals, and DPPH radicals. Nut Res, 2002; 22(4): 519-26.
12. Isidorov VA, Isidorova AG, Sczczepaniak L, Czyzewska U. Gas chromatographic-mass spectrometric investigation of the chemical composition of bee bread. Food Chem, 2009; 115: 1056-63.
13. Villanueva MTO, Marquina AD, Serrano RB, Abellan GB. The importance of bee-collected pollen in the diet: A study of its composition. Int J Food Sci Nutr, 2002; 53: 217–24.
14. Silva BM, Andrade PB, Valentao P, Ferreres F, Seabra RM, Ferreira MA. Quince (*Cydonia oblonga* Miller) fruit (pulp, peel and Seed) and jam: antioxidant activity. J Agric Food Chem, 2004; 52: 705-12.
15. Drago L, de Vecchi E, Nicola L, Gismondo M. *In vitro* antimicrobial activity of a novel propolis formulation actichelated propolis. J Appl Microbiol, 2007; 103: 1914-21.
16. Kalia P, Kumar NR, Harjai K. Phytochemical screening and antibacterial activity of different extracts of propolis. Int J Pharma Biol Res, 2013; 3(6): 219-222.
17. Kalia P, Kumar NR, Harjai K. The therapeutic potential of propolis against damage caused by *Salmonella typhimurium* in mice liver: A biochemical and histological study. Arch Biol Sci, 2015; 67(3): 807-816.
18. Kalia P, Kumar NR, Harjai K. Efficacy of different extracts of propolis against *Salmonella enterica* serovar Typhimurium*:* An *in vitro* and *in vivo* study. J Appl Nat Sci, 2016a; 9(1): 144-49.
19. Kalia P, Kumar NR, Harjai K. Studies on the therapeutic effect of propolis along with standard antibacterial drug in *Salmonella* enterica serovar Typhimurium infected BALB/c mice. BMC Complement Altern Med, 2016b; 16: 485.
20. Kalia P, Kumar NR, Harjai K. Efficacy of different extracts of propolis against *Salmonella enterica* serovar Typhimurium*:* An *in vitro* and *in vivo* study. J Appl Nat Sci, 2017; 10(1).
21. Nagai T, Inoue R. Preparation and the functional properties of water and alkaline extract of royal jelly. Food Chem, 2004; 84: 181–6.
22. Kaur R, Kumar NR, Harjai K. Phytochemical analysis of different extracts of bee pollen. Int J Pharma Biol Res, 2013b; 4(3): 65-8.

23. Kaur R, Kalia P, Kumar NR, Harjai K. Preliminary studies on different extracts of some honey bee products. J Appl Nat Sci, 2013a; 5(2): 420-22.
24. Orsi RO, Sforcin JM, Funari SRC, Fernandes JRA, Rodrigues P, Bankova, V. Effects of propolis from Brazil and Bulgaria on *Salmonella* serovars. J Venom Anim Toxins Incl Trop Dis, 2007; 13(4): 748-57.
25. Kumar N, Ahmad MKK, Dang R, Husain A. Antioxidant and antimicrobial activity of propolis from Tamil Nadu zone. J Med Plants Res, 2008; 2(12): 361-4.
26. Harborne JB. Phytochemical Methods, 3th Edn, Chapman and Hall Ltd., London, 1973; 135-203.
27. Sofowora A. Medicinal Plants and Traditional Medicine in Africa. John Wiley and son Ltd, 1993; 150-153.
28. Trease, GE, Evans WC. Pharmacognosy. 11th Edn. Brailliar Tiridel and Macmillian Publishers, London, 1989.
29. Begue JA, Aust SD. Microsomal lipid peroxidation. Methods Enzymol, 1978; 52: 302-10.
30. Moron MS, Dipierre JW, Mannerwik B. Levels of glutathione reductase and glutathione-S-transferase activities in rat lung and liver. Biochemica et Biophysica Acta, 1979; 582: 67-78.
31. Kono Y, Takahashi M, Asada K. Superoxide dismutase from kidney bean leaves. Plant Cell Physiol, 1978; 20: 1229-35.
32. Luck H. Estimation of catalase activity: Methods of Enzymology Bergmeyer U (Ed), New York: Academic Press, 1971; 885.
33. Habig WH, Pabst MJ, Jakoby WB. Glutathione-S-transferases. The first enzymatic step in mercapturic acid formation. J Biol Chem, 1974; 249: 7130-39.
34. Carlberg I, Mannervik B. Purification and characterization of the flavoenzyme glutathione reductase from rat liver. J Biol Chem, 1985; 250(14): 5475-80.
35. Pagila DE, Velentine WN. Studies on the quantitative characterization of erythrocyte glutathione peroxidase. J Lab Clon Med, 1967; 70: 158-69.
36. Kumar RS, Moorthy K, Vinodhini R, Punitha T. Antimicrobial efficacy and phytochemical analysis of *Indigofera Trita* Linn. African J Trad Comp Alt Med, 2013; 10(3): 518–25.
37. Banskota AH, Tezuka Y, Adnyana IK, Ishii E, Midorikawa K, Matsushige K, Kadota S. Hepatoprotective and anti *Helicobacter pylori* activities of constituents from Brazilian propolis. Phytomed, 2001; 8: 16-23.
38. Kunwar A, Priyadarsini KI. Free radicals, oxidative stress and importance of antioxidants in human health. J Med Alli Sci, 2011; 1(2): 53-60.
39. Orsolic N, Sirovina D, Koncic MZ, Lackovic G, Gregorovic G. Effect of Croatian propolis on diabetic nephropathy and liver toxicity in mice. BMC Complement Altern Med, 2012; 12: 117-31.
40. Singla S, Kumar NR, Kaur J. *In vivo* studies on the protective effect of propolis on doxorubicin induced toxicity in liver of male rats. Toxicol Int, 2014; 21(2): 191-5.
41. Shukla G, Singh D, Sharma L, Koul A, Rishi P. Effect of *Plasmodium* and *Salmonella* co-infection in a murine model. Cent Eur J Med, 2009; 4(3): 340-7.
42. Kerksick C, Willoughby D. The antioxidant role of glutathione and n-acetylcysteine supplements and exercise-induced oxidative stress. J Int Soc Spor Nutr, 2005; 2(2): 38-44.
43. Eraslan G, Kanbur M, Silici S. Effect of carbaryl on some biochemical changes in rats: The ameliorative effect of bee pollen. Food Chem Toxico, 2009a; 47: 86–91.
44. Eraslan G, Kanbur M, Silici S, Liman BC, Altmordulu S, Sarica ZS. Evaluation of protective effect of bee pollen against propoxure toxicity in rat. Ecotoxicol Environ Saf, 2009b; 72: 931-37.
45. Khalil FA, El-Sheikh NM. The effect of dietary Egyptian propolis and bee pollen supplementation against toxicity of sodium fluoride in rats. J Am Sci, 2010; 6(11): 310-16.
46. Salinas AE, Wong MG. Glutathione- S-Transferases- A Review. Curr Med Chem, 1999; 6: 279-309.
47. Gulcin I, Huyut Z, Elmastas M, Aboul-Enein HY. Radical scavenging and antioxidant activity of tannic acid. Arabian J Chem, 2010; 3: 43–53.
48. Ahn MR, Kumazawa S, Hamasaka T, Bang KS, Nakayama T. Antioxidant activity and constituents of propolis collected in various areas of Korea. J Agric Food Chem, 2004; 52: 7286-92.
49. Kumazawa S, Hamasaka T, Nakayama T. Antioxidant activity of propolis of various geographic origins. Food Chem, 2004; 84: 329-39.
50. Nagai T, Sakai M, Inoue R, Inoue H, Suzuki N. Antioxidative activities of some commercial honeys, royal jelly, and propolis. Food Chem, 2001; 75: 237-40.
51. Nagai T, Inoue R, Suzuki N, Tanoue Y, Kai N, Nagashima T. Antihypertensive activities of enzymatic hydrolysates from honeybee-collected pollen of *Cistus ladaniferus*. J Food Agri Environ, 2007; 5(3-4): 86-9.
52. Stanciu OG, Marghitas LA, Dezmirean D. A comparison of methods used to define the antioxidant capacity of bee pollen and bee bread from Romania. Proceedings. 43rd Croatian and 3rd International Symposium on Agriculture. Opatija. Croatia, 2008; 751- 54.