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Proximal composition of bee pollen and its functional effect on stress

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ABSTRACT

Bee pollen is an agglomerate of microscopic grains rich in proteins and phenolic compounds. Other products rich in flavonoids and vitamins, as well as bee pollen, have been shown a positive effect on stress, which is a physical condition that can lead to several somatic disorders. In this work the compositional characteristics of bee pollen were measured, and its effect in volunteers' adult men was evaluated by a cortisol salivary test, and by responding to Lip's Stress Symptom Inventory. The volunteers were given bee pollen for a month. After that, the salivary cortisol was measured and applied the questionnaire. Bee pollen shown an adequate nutritional composition. Its administration had a 23% reduction in salivary cortisol. The values obtained in the questionnaire were consistent with the results of the cortisol dosage, which relieved the symptoms reported by the volunteers. A phytochemical screening was also performed on this material showing the presence of flavonoids, which may be an active compound responsible for the functional effect in the stress relieving of the participants.

Keywords: Bee Pollen; Stress; Proximal Composition; Functional Food.

INTRODUCTION

Bee pollen (BP) is an agglomerate of microscopic grains which are the male reproductive cells of the plants. Such grains are collected by *Apis mellifera* and carried back to the hive, where they will serve as a protein source to feed the worker larvae until the third day of life and will be part of the composition of royal jelly^[1]. As it is a product rich in essential nutrients such as amino acids, vitamins, and minerals, it has been used in several countries as a diet supplement^[2]. Besides its nutritional value, there is evidence of its therapeutic effect on health. Some of its indications include an anticarcinogenic activity, antioxidant, and antibacterial activity^[3].

The therapeutic effect of several apicultural products in general is assigned to their phenolic compound contents, which have an antioxidant activity. Other products rich in phenolic compounds have also been indicated in the treatment of stress symptoms^[4].

Stress is defined traditionally as an organism's responses to physical, mental, or infectious aggressions which may be capable of disrupting homeostasis, characterized by negative effects, mainly anxiety. These processes can be associated with the hypothalamic pituitary adrenal axis (HPA), that stimulates the adrenal gland to release cortisol in response to stress^[5]. (Gerber *et al.*, 2018).

According to hypotheses of Meyerhof and Schmidt^[6], the stress can promote a cumulative effect, influenced by individual differences and environment, but these symptoms can possibly make the body more susceptible to illness, by suppressing the immune system^[7].

The loss of nutrients such as vitamin C, zinc and mineral salts can take place under stressful situations, worsening the organism's symptoms, and on other hand, lifestyle, specific nutrients, like antioxidants, omega-3 fatty acids, and vitamins, can modulate the cognitive function and mental health^[8]. In this sense, BP contains a high nutritional value due its composition of essential amino acids, fatty acids, vitamins, fibres and flavonoids, which may be an alternative in the stress treatment.

The objective of this work is to research if the intake of BP has an influence on the stress level of Fire Service Professionals, as individuals who are constantly encountering risk and to characterize the bee pollen composition.

MATERIALS AND METHODS

Bee pollen was purchased from local commerce in the Pedro de Toledo municipality (SP, Brazil), in the rainforest region.

To access the centesimal composition the sample was submitted to the following analysis: dry matter, protein and ash contents were determined according to the official AOAC methods [9]. Dry matter was obtained by desiccation at 105°C until constant weight, whereas the ash content was given by incineration at 550°C, crude protein was determined by the Kjeldhal procedure, using 6.25 as converting factor, and the Bligh and Dyer [10]. method was used for lipids quantification. The determination of the carbohydrates was carried out by difference.

The following pharmacological groups were researched in the phytochemical characterization of BP: alkaloids, anthraquinone glycosides, cardioactive glycosides, flavonoids, tannins and saponin glycosides, which followed the procedures used by Jacome *et al* [11].

The polyphenol content was obtained by adapting Augusto's Method [12], where an aliquot of bee pollen sample previously left in contact with water for 24 hours and kept out of the light, was added to a reagent solution of Folin-Ciocalteu (1:10, v/v) (Merck, Lot: HC894012) for 5 minutes, and then added to a solution of 2 mL of sodium carbonate (4%, p/v), followed by two-hours out of the light.

After that period, the sample was read by a spectrophotometer UV/VIS (Genesis 2), $\lambda=740$ nm. For the calculation of the results, a standard curve was made with gallic acid and was performed (Sigma Aldrich, Lot: 117K0057) in a concentration ranging from 10 to 60mg/L ($R^2=0,9999$, $y=0,0121x+0,0028$). The polyphenol content was expressed in mg GAE/100 g.

The subjects consisted of 52 males from local Fire Service, volunteers in the municipality of Santos, SP, Brazil. Aged between 20 and 49 years old, who were randomly assigned to a double-blind study. The volunteers received an Informed Consent Form, which was read by them, containing the aims of the research, responsible researches, mode of consumption, the length of the experiment and the possible adverse effects.

The subjects were randomly assigned into two groups, the experimental group (EG) and the control group (CG). The experimental group received flasks containing commercial dehydrated bee pollen, while the control group received flasks containing oat flakes as a placebo. A daily intake of five capsules was recommended to both groups, three in the morning and two in the afternoon, totalling 2.5 g per day for 30 days.

The bee pollen was encapsulated in the Pharmaceutical Laboratory of the Catholic University of Santos.

The ethical aspects were respected in accordance with Resolution 196/96 of the National Health Council referring to the recommendations on research involving humans.

Samples of the participants' saliva were collected in the morning period, before starting the treatment and at the end of the experiment for salivary cortisol dosage. The participants were asked to continue to fast and to rest for one hour prior to the collection.

A cotton cylinder previously moistened with saliva was used and stored in a plastic device (sarstedt salivette™). The samples were refrigerated and immediately delivered for cortisol analysis by the immunoassay technique using the DSL-10-67100 kit.

The samples were centrifuged for 2 min/1000 rpm. The aliquots of 25µL were transferred to the cavities of microtiter plates coated with IgG and 100 µL of enzyme conjugate diluent solution (1:50, v/v) was added to each plate. After being gently rotated, 100 µL of antiserum-

cortisol was added, and then the plate was incubated for 45 minutes at 25°C on an orbital shaker at 300 rpm.

After washing the cavities with a specific solution, a chromogen solution of tetramethylbenzidine was added and after a 30 min orbital agitation (300 rpm), 100 µL of stop solution was added and then the sample was read by a spectrophotometer Lab systems Multiclan MS at 450 nm.

This experiment consisted in the application of a validated questionnaire, the "Inventory of Stress Symptoms for adults," developed by LIPP [13]. in the professional Fire Service on the first and the last days of the experiment.

This questionnaire is composed of 37 items of a somatic approach and 19 of a psychological approach, applied and interpreted by a psychologist, in accordance with the Federal Council of Psychology, for the classification of a stress phase in which the volunteer was present for two trials of the study, before consumption bee pollen and after 28 days, taking the capsules of bee pollen or placebo (oat), daily.

The descriptive statistics and the graphs were performed using Microsoft Excel for Windows. The student's t-test was used to determine the differences between the mean values at 5% significance level using STATISTICA for Windows, version 5.0 [14].

RESULTS

Table 1 presents the centesimal composition of the pollen, the presence/absence of phytochemical groups and the total content of phenolic compounds.

Table 1: Proximal composition and phytochemical screening of bee pollen

Chemical composition (%)		Phytochemical groups
Moisture	17.5±0.48	Alkaloids and saponin glycosides -
Ash	2.3±0.33	Anthraquinone and cardioactive glycosides -
Protein	19.2±0.12	Flavonoids +
Lipids	6.3±0.24	Tannin -
Carbohydrates	54.7	Total polyphenols 84.3mg/g

The centesimal composition of the bee pollen has been shown in accordance with the requirements in Normative Instruction no. 3 of January 19, 2001 determining the identity and quality parameters of fresh and dehydrated bee pollen in Brazil [15].

The results were negative for the phytochemical groups with potential anti-nutritional activity such as tannins, alkaloids, saponin glycosides, anthraquinone glycosides, cardioactive glycosides, and positive for flavonoids due to their antioxidant activity. The total phenolic content was 843 mg/100 g, including flavonoids.

Cortisol is a hormone produced in stressful situations and since the volunteers are constantly exposed to high-risk activities, we opted for salivary cortisol research as it is a less invasive method of collection.

The mean salivary cortisol observed in the experimental group before the BP administration was 19.8±13.9 nmol/L with a minimum of 0 nmol/L and a maximum of 41.4 nmol/L values. For the control group, before the administration of the placebo, a mean of 16.8±11.3 nmol/L of salivary cortisol was observed; 2.8 nmol/L and 41.4 nmol/L were the respective minimum and maximum values.

After the experimental period, the group that consumed the Bee Pollen showed a significant reduction of salivary cortisol to 12.7±7.3 nmol/L ($p=0.0136$), while the control group increased to 19.2± 12.8 nmol/L (Figure 1).

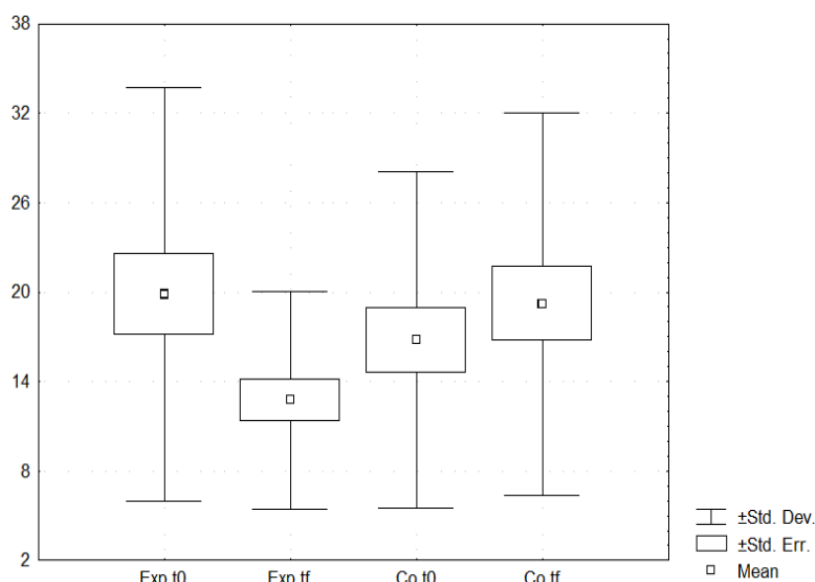


Figure 1: Salivary cortisol in nmol/L of male individuals before (t0) and after (ft) the consumption of bee pollen (Exp) or placebo (Co) for 30 days (different letters indicate the statistical differences in the significance level of 5% of probability by student's t-test).

The salivary cortisol from the experimental group that consumed the Bee Pollen was also statistically lower than the group that received the placebo after 30 days ($p= 0.454$).

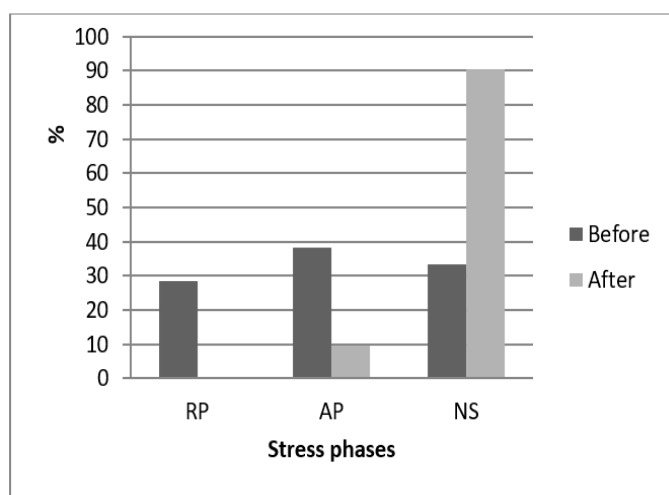
According to Lipp ^[13], the analysis of LSSI is divided into four phases: the alert phase (AP) is a positive diagnosis of stress where a human being adapts themselves to an adrenaline producing action; in the resistance phase (RP) the organism prevents the total energy waste and as the individual continues to produce cortisol, they are vulnerable to infections. In the exhaustion phase (EP) the tension exceeds manageable limits. The increased cortisol has a negative

effect and diseases may start to arise. The exhaustion phase (EP) is pathological, which can lead to a big imbalance resulting in depression, ulcers, hypertension, psoriasis among others somatic or auto-immune diseases.

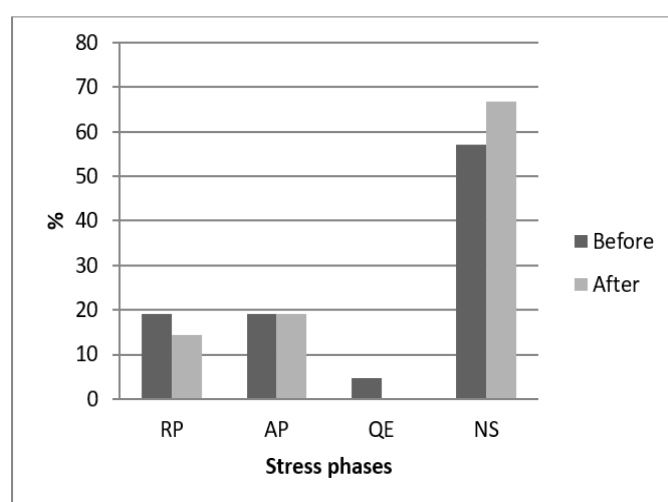
The results obtained with the LSSI application showed agreement with the salivary cortisol measurements.

Before the consumption of the BP, 31% of the individuals from the experimental group were in the resistance phase, 34% ($n=9$) were in the alert phase and 31% ($n=8$) had no symptoms. After 30 days of consumption nobody was in the quasi-exhaustion phase, 4% ($n=1$) were in the resistance phase, 8% ($n=2$) were in the alert phase while 88% ($n=23$) moved to a phase without symptoms (Figure 2).

For the control group, 12% of the individuals were in the quasi-exhaustion phase before receiving the placebo, 23% ($n=6$) were in the resistance phase, 15% ($n=4$) were in the alert phase and 50% ($n=13$) had no symptoms. 30 days after consuming the placebo, nobody was in the exhaustion phase, while 19% ($n=5$) were in the resistance phase, 19% ($n=5$) were in the alert phase and 62% ($n=16$) were classified as symptom free (Figure 2).



a



b

Figure 2: Classification of stress phases in volunteers according to LSSI in the experimental group (a) and control group (b) before and after 30 days of using BP, and placebo respectively. RP - resistance phase; AP - alert phase; QE - quasi-exhaustion phase; NS - no symptoms.

DISCUSSION

The bee pollen composition may vary according to the processing technique employed or the flora characteristic of the production location. In relation to the moisture content, bee pollen collected in the original form has to contain a maximum moisture reading of 30% when it comes to dehydrated bee pollen at a temperature higher to 42°C, the moisture content should not be higher than 4%.

The sample used showed adequacy established by the Brazilian Legislation, in the case of dehydrated bee pollen, commercialized by a traditional company in this branch; however, this is commonly sold by small producers where the standardization of the final product cannot always be achieved.

Melo, Este vinho and Almeida-Mouradian [16], analysed different bee pollen samples collected in several regions of Brazil observing the presence of many microbial groups, such as molds and yeasts, coliforms and total bacteria count in a magnitude of up to 10⁴ CFU g⁻¹. If the humidity of a product like this is above the values recommended by the current legislation, this contamination can draw attention to the risk of the development of mycotoxigenic molds.

The total phenolic content found in the bee pollen used was 84,3 mg GAE/g and this value was close to the interval observed by Altiner *et al* [17], in samples collected in the Turkey. These authors also demonstrated that hydrolysis, simulating gastric process, rose the antioxidant capacity, more than obtained by solvent extract.

As far as the benefits of polyphenols are concerned, Sakkara and Shimon [18], reviewed a potential anti-stress activity in experimental animals and human trials where products rich in these compounds has emerged as essential for prevent stress-related health problems.

The cortisol is a hormone essential to life because it is responsible for adequate responses to stressful conditions such as serious diseases, severe traumas and surgery. However, it is harmful to organisms when continuously released, being responsible for catabolism and neurophysiological changes [5].

As a strategy to develop a treatment without side effects, functional foods with anti-stress action have been investigated. Moreover, a milk protein hydrolysate (Lactium™) has already been patented as it demonstrated an anxiolytic effect in animals and human being volunteers [19].

According to Noah *et al* [20], the administration of high levels of vitamin B6 and magnesium may be expected to have a favourable impact on stress related with mood and anxiety. The recommended daily intake of pyridoxine is 1.3 mg for adults and the deficiency of this vitamin tends to selectively reduce the cerebral production of serotonin and gamma-aminobutyric acid (GABA) that are crucial for depression control, pain perception and anxiety.

Mean values of pyridoxine of 0.64 mg/100 g for pollen *in natura* and 0.55 mg/100 g for dehydrated pollen were found in the bee pollen produced in the state of São Paulo/Brasil [21].

The results obtained in this study show that the bee pollen reduced the salivary cortisol concentration after thirty days of intake in adults submitted to stressful situations. This data is in agreement with the evaluation obtained with the inventory application.

In addition to an increase in individuals in the classification of “without symptoms”, the symptoms reported by the volunteers during the period that they consumed BP were: an improvement in tightness in the mandible, insomnia, professional performance, sense of humour, sensation of well-being, greater physical and mental disposition, facility for thinking, relaxation, tranquillity, motivation, increase in libido, absence of dizziness and an increase in emotional control.

CONCLUSION

The outcomes of this study suggest that the consumption of BP results in a positive effect in the reduction of stress in males who are constantly submitted to tense situations. Further studies are necessary that will elucidate the mechanism of action, which may be attributed to the presence of specific amino acids, flavonoids or vitamins.

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Conflict of Interest

None declared.

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