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The Role Of Bee-Propolis As Antioxidants On Antioxidant Enzymes Inmale Rabbits

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ABSTRACT

Propolis has been reported to be important antioxidantThe biological effects exhibited by propolis could be related to an overall effect of the phenolic compounds present in propolis (flavonol galangin; hydroxycinnamic acids, caffeic acid, p-cumaric acid, ferulic acid and caffeic acid phenethyl ester Rabbits were orally given propolis (50 mg/kg bw) was given alone. The tested doses were given to rabbits every day for 12 weeks. The effects of propolis on plasma and testes homogenates thiobarbituric acid-reactive substances (TBARS), antioxidant enzyme glutathione (GSH), glutathione peroxidase (GPx), glutathione S- transferase (GST), catalase (CAT) and superoxide dismutase (SOD) activities Treatment with propolis caused significant ($P<0.05$) increase in the activity of antioxidant enzyme GSH, GPx, GST, SOD and CAT in plasma and testes homogenates compared to control. While, propolis caused a significant ($P<0.05$) decrease in blood plasma and testes homogenates TBARS as compared with control.

دور صمغ النحل كمضاد للأكسدة علي الإنزيمات المضادة للأكسدة في ذكور الأرانب

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الكلمات المفتاحية:
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الخصيه
الجذور الحره

الملخص

تم الإبلاغ عن أن صمغ النحل هو أحد مضادات الأكسدة المهمة. يمكن أن تكون التأثيرات البيولوجية التي يظهرها صمغ النحل مرتبطة بالتأثير العام للمركبات الفينولية الموجودة في صمغ النحل (الفلافونول جالانجين، وأحماض الهيدروكسيسينناميك، وحمض الكافيك، وحمض الكوماريك، وحمض الفيروليك، وحمض الكافيين فينيثيل إستر). تم إعطاء صمغ النحل عن طريق الفم (50 ملجم/كجم من وزن الجسم) بمفرده. تم إعطاء الجرعات المختبرة للأرانب يوميًا لمدة 12 أسبوعًا. تعمل تأثيرات صمغ النحل على البلازما والخصيتين على تجانس المواد المتفاعلة مع حمض الثيوباربيتوريك (TBARS)، وإنزيم الجلوتاثيون المضاد للأكسدة (أنشطة GSH)، الجلوتاثيون بيروكسيديز (GPx)، الجلوتاثيون S- ترانسفيراز (GST)، الكاتلاز (CAT) وفوق أكسيد ديسموتاز (SOD) تسببت المعالجة بصمغ النحل في زيادة كبيرة ($P<0.05$) في نشاط الإنزيم المضاد للأكسدة GSH، GPx، GST تجانس SOD وCAT في البلازما والخصيتين مقارنة بالسيطرة، بينما تسبب صمغ النحل في انخفاض معنوي ($P<0.05$) في بلازما الدم وتجانس الخصيتين TBARS مقارنة مع السيطرة.

Introduction

Propolis is a sticky, resinous substance produced by bees by mixing saliva and beeswax with substances obtained from plant parts, buds, and exudates [1]. Propolis is a strong adhesive made of a resinous substance produced by bees. Its name was derived from the Greek pro (“in front of” or “at the entrance of”) and polis (“community” or “city”) [2]. Propolis rich with polyphenols, flavonoid aglycones, phenolic acid and their esters, and phenolic aldehydes and ketones, terpenes, sterols, vitamins, amino acids[3]. Propolis has also been reported to have diverse pharmacological activities, such as antidiabetic, anti-inflammatory, antioxidant, anticancer, immunomodulatory, antibacterial, antiviral, antifungal, and anticaries. [4]. It can scavenging free radical and inhibits the membrane lipid peroxidation and free radical formation [5]. Propolis is one of the bee products that exhibits numerous biological activities such as antioxidant, anti-inflammatory, antitumor, antiviral, antibacterial, antifungal, antidiabetic activities and is also listed in the London Pharmacopoeias and Chinese Pharmacopoeias[6] and is a lipophilic substantial that is firm and fragile when cold however

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elastic, soft, and very gummy when it heated. Possesses an agreeable aromatic odor and 3 diverse color, including brown, green, and red, among others [7]. The chemical composition of it, generally composed of 50% resin, 30% wax, 10% essential oils, 5% pollen, and 5% other elements which contain flavonoids, organic compounds like phenolic acids (cinammic and caffeic acid), minerals, fatty acids and esters of phenolic acids terpenes, and alcohols [8]. The composition of propolis varies according to the plant species available in each region [9]. Recent researchers reported that propolis has a cytoprotective role due to its antioxidant property, which attributed to its ingredients from phenol compounds [10].Propolis widely began to attract the attention of scientists. The results of many animal researches showed that propolis may relieve the negative effects of oxidative stress on the body's defense system[11-14]. propolis supplementation improve antioxidant status in rabbit blood serum [15].

Materials and methods

In this study propolis was used Propolis was supplied from California Health Products, Inc. 11577W. Olympic Bivd. Los Angeles and CA90064.

2.1Animals and management

Ten adult male rabbits weighting (2.917 ± 28.9 kg)were obtained from shahat city. Animals were housed (5 rabbits in each group) at the animal facility at chemistry Department, Faculty of Science, *Omar Al-Mokhtar University, El -Beida-Libya*.In clean and cages and kept under standard situation. The rabbits were fed with rodent pellets and tap water ad libitum.

2.2Administration schedule of propolis

The animals were equally divided into two groups (n = 5). The first group served as control (G1) and received distilled water, Rabbits of the second group were received propolis (G2) at the dose of 50 mg/kg /day [16] by gavage for 12-week days. At the end of the experimental period animals were left night fasted and at the next day they were euthanized following protocols and ethical procedures.

Sample collection and biochemical assays

Blood samples were collected from the ear vein of all animals every other week throughout the 12-week experimental period. Blood samples were obtained in the morning before accesses to feed and water and placed immediately on ice. The blood samples were collected in two tubes, one containing heparin to obtain plasma and the other one without anticoagulants to obtain serum. Plasma was obtained by centrifugation of samples at 860×g C) until used for analysis. Stored°for 20 min, and was stored at (–80 plasma samples were analyzed for glutathione S-transferase (GST; EC 2.5.1.18) activity was determined according ***to*** [17]. Catalase (CAT; EC 1.11.1.6) activity was determined using the Luck method involving the decomposition of hydrogen peroxide [18]. Superoxide dismutase (SOD; EC 1.15.1.1) activity was measured according to [19]. Plasma 40 thiobarbituric acid-reactive substances (TBARS) were measured by the method of [20]. The testes was quickly removed and weighed. One testis from each rabbits was frozen at -20ºC, homogenized and assayed for Catalase (CAT), Glutathione *S*-transferase (GST), Superoxide dismutase (SOD). Also, Reduced glutathione (GSH), Malondialdhyde (MDA) ons were assayed in testicular homogenate.

Statistical analysis

In the present study, all results were expressed as Mean ± S.E of the mean. One-way analysis of variance (ANOVA) was used to assess significant differences among treated groups and controls using Graph Pad Prism 7 (La Jolla, CA, USA). The Tukey Test was used to compare all groups with each other and to show the significant effect of treatment. Values were considered statistically significant when $p<0.05$.

RESULTS

The effects of propolis on plasma and testes homogenates glutathione (GSH), glutathione peroxidase (GPx), glutathione S- transferase (GST), catalase (CAT) and superoxide dismutase (SOD) activities during the 12-week experimental period are shown in Tables (1) represents the biweekly mean values of these parameter expressed as absolute values. Treatment with propolis caused significant ($P<0.05$) increase in the activity of GSH, GPx, GST, SOD and CAT in plasma compared to control. While, decrease in the level of TBARS in blood plasma and testes homogenates.

Table (1). Average of plasma glutathione (GSH; U/ml), glutathione peroxidase (GPx; U/ml), glutathione S-transferase (GST; µmol/hr), glutathione catalase (CAT; U/min/ml), superoxide dismutase (SOD; U/ml) and thiobarbituric acid-reactive substances (TBARS) of male rabbits treated with propolis(means ± SE).

Parameters	Animal Groups	
	G1	G2
Glutathione (GSH; U/ml)	4.98± 0.104[a]	5.55± 0.065[b]
Glutathione peroxidase (GPx; U/ml)	9.95± 0.171[a]	10.82± 0.230[b]
Glutathione S-transferase (GST; µmol/hr)	1.010± 0.017[a]	1.310 ± 0.068[b]
Catalase (CAT; U/min/ml)	0.990 ± 0.018[a]	1.120 ± 0.020[b]
Superoxide dismutase (SOD; U/ml)	0.902 ± 0.022[a]	1.054 ± 0.025[b]
Thiobarbituric acid-reactive substances (TBARS)	1.223 ± 0.036[b]	1.139 ± 0.016[b]

Values are means ± SE of 5 rabbits in each group. Mean with different letters **(a and b)** are significantly difference ($p \leq 0.05$). Mean with the same letters (a and b) are non significantly difference ($p \geq 0.05$).

Table (2). Average of testes homogenates glutathione (GSH; U/ml), glutathione peroxidase (GPx; U/ml), glutathione S-transferase (GST; µmol/hr), glutathione catalase (CAT; U/min/ml), superoxide dismutase (SOD; U/ml) and thiobarbituric acid-reactive substances (TBARS) of male rabbits treated with propolis(means ± SE).

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Discussion

Increase in GST, SOD, GSH, GPx and CAT activities in plasma and testes of rabbits treated with propolis (50 mg/kg) are in agreement with the finding of [21] who reported that propolis increased the antioxidant SOD and CAT enzyme activities. Also, [22] showed that propolis has an antioxidant property. [23]reported that the antioxidant effect of propolis by decreasing lipid peroxidation,

increasing GSH level and maintaining normal levels of antioxidant enzymes. They found that propolis (at a dose of 50 mg/kg) restored the hepatic levels of total and reduced GSH, GPx and SOD to control values of rats treated with CCl_4, the same dose of propolis as used in the present study. This direct free radical scavenging activity of propolis might also be involved in the exhibited hepatoprotective activity due to the presence of antioxidant compounds in propolis extract. [24] reported that treatment of propolis in isoproterenol-treated rats increased the levels of endogenous myocardial antioxidants (catalase, superoxide dismutases and tissue glutathione. Thiobarbituric acid reactive substances (TBARS) are produced by lipid peroxidation (LPO) and are considered as indicators of oxidative stress. LPO was assessed by measuring the concentrations of thiobarbituric acid-reactive substances (TBARS) in plasma and organs [25]. his result is in accordance with results indicating that propolis is able to exert hepatoprotective effects on paracetamol-induced liver damage in mice, which is dependent on GSH depletion provoked by paracetamol overdoses and is reversed by propolis administration [26]**.** It is well known that GSH is one of the most important antioxidant molecules and at physiological concentrations contribute to maintain the normal redox state of the cells [27]**.**Taken together, these findings constitute evidence that the antioxidative properties of the propolis contribute to the prevention of damage induced by $AlCl_3$ in rats. The present work also showed that treatment with propolis alone caused reduction in TBARS levels and increased the activities of GST and CAT, and the levels of GSH in testes. These data are in agreement with the results obtained by **[28]**who reported that propolis caused reduction in malondialdehyde (MDA) levels and increase in the activities of the antioxidant enzymes (SOD, GSH-Px, and CAT). Furthermore, [29]reported that propolis increased the activity of CAT. The first mechanism of this effect of propolis may involve the scavenging of free radicals that causes lipid peroxidation. The second mechanism can be its ability to prevent the xanthine oxidase activity, which is known to cause generation of free radicals. The reason for increase in CAT activity and decrease in MDA level in the present study may be explained by potent free radical scavenging activity of flavonoid content of propolis[30].

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