

**Biochemical determinations of propolis-treated rats: effects of different concentrations, extracts and intake period**

**Determinações bioquímicas de ratos tratados com própolis: efeitos de diferentes concentrações, extratos e período de administração**

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**Abstract**

Propolis is a resinous hive product, with several biological and pharmacological properties, such as immunomodulatory, antimicrobial, anti-inflammatory, among others. Our group has been investigating the possible side effects after propolis administration to rats. Thus, this work was carried out in order to analyze the specific activity of some enzymes (aminotransferases, 3-glutamyl transferase, lactate dehydrogenase, amylase), as well as the serum concentration of urea, creatinine and total proteins after propolis administration in some conditions: a) different concentrations of propolis (1, 3 and 6 mg/kg/day); b) different extracts (water and ethanol); c) different intake periods (30, 90 and 150 days). No alterations were seen in the specific activity of such enzymes, or in the serum components. However, urea and creatinine were elevated after treatment with ethanolic extract of propolis (6 mg/kg/day), suggesting possible kidney damage, in these assay conditions. Since humans have used propolis for different

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proposals, further studies will help to a better understanding of relation propolis-biochemical variables of clinical significance.

**Keywords:** Propolis, biochemical analysis, side effects.

### **Resumo**

A própolis é um produto apícola, apresentando inúmeras propriedades biológicas e farmacológicas, como imunomoduladora, antimicrobiana, anti-inflamatória, entre outras. Nosso grupo tem investigado possíveis efeitos colaterais após administração de própolis a ratos. Assim, este trabalho foi realizado com a finalidade de analisar a atividade específica de algumas enzimas (aminotransferases, g $\alpha$ -glutamil transferase, desidrogenase láctica, amilase), bem como a concentração sérica de uréia, creatinina e proteínas totais após administração de própolis em algumas condições: a) diferentes concentrações de própolis (1, 3 e 6 mg/kg/dia); b) diferentes extratos (aquoso e etanólico); c) diferentes períodos de administração (30, 90 e 150 dias). Não foram observadas alterações na atividade específica de tais enzimas, nem nos componentes séricos avaliados. Entretanto, a concentração de uréia e creatinina apresentou-se elevada após tratamento com extrato etanólico de própolis (6 mg/kg/dia), sugerindo um possível comprometimento renal, nestas condições. Considerando que a população tem utilizado a própolis para os mais diferentes fins, novos estudos auxiliarão na melhor compreensão da relação própolis-variáveis bioquímicas de significado clínico.

**Palavras-chave:** Própolis, análises bioquímicas, efeitos colaterais.

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## INTRODUCTION

Propolis is one of the hive products that have been used extensively in folk medicine. It is a resinous material collected by bees from bud and exudates of the plants, which is mixed with products of their salivary glands and wax. Its color varies from green, red to dark brown, and its composition is dependent upon the source plant and local flora. Propolis has a characteristic smell and shows adhesive properties, because it strongly interacts with oils and proteins of the skin <sup>(1)</sup>.

Propolis has attracted researchers' interest in the last decades in order to investigate its constituents and biological

properties, because of its several pharmacological activities, such as immunomodulatory, antitumor, antimicrobial, anti-inflammatory, antioxidant, among others <sup>(2; 3; 4; 5; 6)</sup>. Besides, propolis-containing products have been intensely marketed by the pharmaceutical industry and health-food stores <sup>(7)</sup>.

Propolis samples, collected in the Beekeeping Section of the University, UNESP, Campus of Botucatu, SP, Brazil, were chemically analyzed, revealing that its main components are phenolic compounds, di- and triterpenes, essential oils, among others. Seasonal differences in propolis composition were not significant and were predominantly quantitative <sup>(8,9)</sup>.

As to side effects, some cases of propolis allergy and contact dermatitis have been reported <sup>(10)</sup>, differently from the common allergy to honey, which contains allergens derived from flowers. Beekeepers usually show sensitivity to propolis <sup>(11)</sup>. Ethanol and water extracts of propolis possess anti-allergic action, inhibiting histamine release in rat peritoneal mast cells <sup>(12)</sup>. However, in higher concentrations (300 mg/ml), propolis directly activates mast cells, promoting inflammatory mediators release, what could be linked to allergic processes in propolis-sensitive individuals <sup>(13)</sup>.

Since propolis composition is complex and its mechanism of action is still unclear, alterations in biochemical variables of clinical significance must be analyzed in dose-effect relation studies. Thus, the aim of this work was to evaluate the specific activity of aminotransferases (AST and ALT),  $\beta$ -glutamyl transferase ( $\beta$ -GT), lactate dehydrogenase (LDH) and amylase, as well as the concentration of urea, creatinine and total proteins, after propolis treatment with different concentrations (1, 3 and 6 mg/kg/day), extracts (water or ethanol) and periods of administration (30, 90 and 150 days).

## MATERIAL AND METHODS

### *Propolis Sample*

Propolis was collected in the Beekeeping Section, UNESP, Campus of Botucatu, Brazil. Propolis was ground and 30% ethanolic extracts of propolis (EEP) were prepared (30 g of propolis, completing the volume to 100 ml with 70% ethanol), in the absence of bright light, at room temperature, with moderate shaking. Water extracts of propolis were prepared in the same way (30 g of propolis, completing the volume to 100 ml with water) in the absence of bright light, at room temperature, with moderate shaking. After a week, extracts were filtered and the dry weight of the extracts was calculated <sup>(14)</sup>.

### *Animals and Treatment*

This work agrees with Ethical Principles in Animal Research adopted by Brazilian College of Animal Experimentation (nº. 76/01), and male Wistar rats weighing 200g were used in 3 different assays, as follows:

#### *First assay: Different Concentrations of Propolis*

Rats were divided into 5 groups (G1, G2, G3, G4 and G5) of 5 rats each. G1 and G2 were considered as controls, and received distilled water and 30% hydroalcoholic solution, respectively. G3, G4 and G5 received 1, 3 and 6 mg/kg/day of EEP, respectively, for 30 days. Propolis was added to drink water, after preliminary studies to calculate propolis intake for each group.

#### *Second assay: Different Extracts of Propolis*

Rats were divided into 4 groups (G1, G2, G3 and G4; n=5). G1 and G2 received water and hydroalcoholic solution,

respectively. G3 and G4 received 1 mg/kg/day of water or ethanolic extracts of propolis in the drink water, respectively, for 30 days.

*Third assay: Propolis Administration for Different Intake Periods*

In this assay, rats were divided in the 5 groups (G1, G2, G3, and G4; n=5). G1 and G2 received water and hydroalcoholic solution, respectively. G3 and G4 received 1 mg/kg/day of EEP for 90 and 150 days, respectively <sup>(15)</sup>.

*Serum Samples and Biochemical Determinations*

After propolis treatments, animals were sacrificed by decapitation, and blood samples were collected and centrifuged at 200 x g for 15 minutes. Serum was used for urea, creatinine and total proteins determination, as well for AST, ALT, 3-GT, LDH and amylase specific activities determination (test Kit CELM, Modern Laboratory Equipment Company, São Paulo, Brazil) <sup>(16)</sup>.

*Statistical analysis*

Analysis of variance was used to examine the treatment effect, and comparison between the means was performed by Tukey test, with 0.05 as the significant level <sup>(17)</sup>.

## RESULTS

Propolis administration in different concentrations for 30 days did not affect enzymes specific activities or biochemical variables, but increased creatinine levels

In the first assay, EEP administration (1, 3 and 6 mg/kg/day) for 30 days did not affect the specific activity of AST, ALT, 3-GT, amylase and LDH. Normal levels of total proteins

and urea were seen. However, creatinine concentration was significantly different between G5 (6 mg/Kg/day) and control (G1) (table 1).

**Table 1.** Enzymes specific activities and serum levels of total proteins, urea and creatinine of propolis-treated rats (1, 3 and 6 mg/kg/day) after 30 days. Data are shown as means  $\pm$  standard deviation (sd) of 5 animals. \* significantly different from G1 ( $P < 0.05$ ).

Biochemical determinations	Control (G1)	Solvent (G2)	1 mg/kg/day (G3)	3 mg/kg/day (G4)	6 mg/kg/day (G5)
<b>Amylase</b> (U/L)	775.84 $\pm$ 92.80	647.11 $\pm$ 125.18	741.65 $\pm$ 65.66	805.57 $\pm$ 19.19	723.89 $\pm$ 63.77
<b><math>\gamma</math>-GT</b> (U/L)	34.89 $\pm$ 4.85	32.26 $\pm$ 4.76	42.37 $\pm$ 3.22	38.62 $\pm$ 4.27	43.196 $\pm$ 2.20
<b>AST</b> (U/L)	189.96 $\pm$ 12.76	152.94 $\pm$ 9.60	164.67 $\pm$ 13.54	152.94 $\pm$ 12.40	145.82 $\pm$ 24.89
<b>ALT</b> (U/L)	62.50 $\pm$ 5.53	70.53 $\pm$ 6.64	68.30 $\pm$ 7.80	66.70 $\pm$ 4.63	65.30 $\pm$ 11.10
<b>Total proteins</b> (mg/dL)	9.01 $\pm$ 2.87	8.21 $\pm$ 1.65	9.46 $\pm$ 1.45	9.83 $\pm$ 2.87	9.01 $\pm$ 3.24
<b>Urea</b> (mg/dL)	39.50 $\pm$ 9.57	36.44 $\pm$ 7.57	40.75 $\pm$ 8.30	46.68 $\pm$ 10.17	67.87 $\pm$ 6.53*
<b>Creatinine</b> (mg/dL)	0.55 $\pm$ 0.11	0.68 $\pm$ 0.15	0.71 $\pm$ 0.17	1.50 $\pm$ 0.38	3.05 $\pm$ 0.55*
<b>IDH</b> (U/L)	366.82 $\pm$ 75.79	420.99 $\pm$ 56.71	369.61 $\pm$ 54.33	343.48 $\pm$ 42.68	285.95 $\pm$ 26.65

*Different extracts showed no alterations in enzymes specific activities neither in biochemical variables, but increased urea levels non significantly*

In the second assay, water or ethanolic extracts of propolis (1 mg/kg/day) for 30 days showed no alterations in the biochemical parameters. Urea concentration was elevated in G4 (6 mg/Kg/day), although not significantly (table 2).

**Table 2.** Enzymes specific activities and serum levels of total proteins, urea and creatinine of propolis-treated rats (1 mg/kg/day of ethanolic or water extracts) after 30 days. Data are shown as means  $\pm$  standard deviation (sd) of 5 animals.

<b>Biochemical determinations</b>	<b>Control (G1)</b>	<b>Solvent (G2)</b>	<b>Water (G3)</b>	<b>Ethanol (G4)</b>
<b>Amylase</b> (U/L)	416.96 $\pm$ 32.83	407.61 $\pm$ 43.95	365.58 $\pm$ 21.04	511.85 $\pm$ 44.95
<b><math>\gamma</math>GT</b> (U/L)	33.65 $\pm$ 2.21	32.40 $\pm$ 3.69	37.38 $\pm$ 3.15	28.66 $\pm$ 2.31
<b>AST</b> (U/L)	183.33 $\pm$ 9.87	178.79 $\pm$ 15.85	172.85 $\pm$ 5.12	174.95 $\pm$ 8.70
<b>ALT</b> (U/L)	64.42 $\pm$ 3.92	77.52 $\pm$ 4.76	61.81 $\pm$ 1.95	68.79 $\pm$ 5.42
<b>Total proteins</b> (mg/dL)	11.10 $\pm$ 0.97	9.25 $\pm$ 1.69	8.57 $\pm$ 1.37	15.23 $\pm$ 0.94
<b>Urea</b> (mg/dL)	5.23 $\pm$ 0.14	4.59 $\pm$ 0.36	6.28 $\pm$ 0.75	5.96 $\pm$ 0.25
<b>Creatinine</b> (mg/dL)	1.87 $\pm$ 0.06	1.81 $\pm$ 0.31	2.86 $\pm$ 0.55	1.71 $\pm$ 0.43
<b>LDH</b> (U/L)	1562.50 $\pm$ 331.63	1538.00 $\pm$ 74.17	1353.40 $\pm$ 362.99	711.40 $\pm$ 121.17

*No alterations in biochemical variables were seen after administration of EEP in different period patterns*

In the third assay, rats were treated with EEP (1 mg/kg/day) for 90 and 150 days. No significantly differences were seen in the evaluated variables (table 3).

**Table 3.** Enzymes specific activities and serum level of total proteins, urea and creatinine of propolis-treated rats for 90 or 150 days. Data are shown as means  $\pm$  standard deviation (sd) of 5 animals.

Biochemical determinations	90 days			150 days		
	Control (G1)	Solvent (G2)	Propolis (G3)	control (G1)	Solvent (G2)	Propolis (G4)
<b>Amylase</b> (U/L)	894.32 $\pm$ 24.20	885.63 $\pm$ 24.77	909.78 $\pm$ 13.79	898.14 $\pm$ 51.30	918.24 $\pm$ 52.46	790.87 $\pm$ 53.02
<b><math>\gamma</math>-GT</b> (U/L)	38.25 $\pm$ 4.58	49.40 $\pm$ 5.19	44.04 $\pm$ 2.88	38.89 $\pm$ 5.41	41.96 $\pm$ 6.27	39.05 $\pm$ 7.27
<b>AST</b> (U/L)	146.32 $\pm$ 5.30	144.05 $\pm$ 6.20	142.32 $\pm$ 8.34	180.54 $\pm$ 7.80	170.06 $\pm$ 9.80	177.04 $\pm$ 11.70
<b>ALT</b> (U/L)	53.60 $\pm$ 6.35	54.12 $\pm$ 8.16	55.52 $\pm$ 6.77	56.32 $\pm$ 6.54	56.57 $\pm$ 1.98	61.45 $\pm$ 8.47
<b>Total proteins</b> (mg/dL)	5.96 $\pm$ 0.20	5.82 $\pm$ 0.19	5.84 $\pm$ 0.14	5.80 $\pm$ 0.39	5.23 $\pm$ 0.25	6.12 $\pm$ 0.23
<b>Urea</b> (mg/dL)	43.97 $\pm$ 2.03	46.99 $\pm$ 2.56	42.80 $\pm$ 1.79	41.37 $\pm$ 6.32	40.25 $\pm$ 6.57	43.73 $\pm$ 4.74
<b>Creatinine</b> (mg/dL)	1.35 $\pm$ 0.06	1.34 $\pm$ 0.05	1.67 $\pm$ 0.02	1.27 $\pm$ 0.03	0.976 $\pm$ 0.02	1.05 $\pm$ 0.03
<b>LDH</b> (U/L)	785.23 $\pm$ 63.89	731.00 $\pm$ 63.20	753.70 $\pm$ 64.06	802.31 $\pm$ 58.90	774.21 $\pm$ 57.27	797.35 $\pm$ 56.24

## DISCUSSION

Propolis has been used empirically for centuries, and it has attracted researchers interest in the last decades, because of several biological and pharmacological properties <sup>(2; 18)</sup>.

Propolis is non-toxic, its DL50 ranges from 2.0 to 7.3 g/kg in mice, and it was suggested that the safe concentration for humans could be 1.4 mg/kg and day, or approximately 70 mg/day <sup>(19)</sup>.

In acute or chronic pathologic conditions, cell damage leads to cytoplasmatic enzyme release to blood, which can



be a tool to clinical diagnosis of cell viability, changes in cell membrane permeability and tissue injury <sup>(20)</sup>.

Our group has investigated possible side effects after propolis administration to rats. In a previous work, we showed that 24 h after propolis administration to rats, ALT and amylase had no alterations in their specific activities, suggesting no damage in the liver and pancreas, respectively <sup>(21)</sup>. Evaluation of total protein, glucose, urea, creatinine, triglycerides, cholesterol and HDL-cholesterol was performed after propolis administration for 72 h to rats, evidencing no changes in these parameters, or in AST, ALT and LDH specific activities. The lack of clinically important changes in biochemical variables was probably because propolis showed no side effects under these conditions <sup>(14)</sup>. After treatment of rats with different concentrations of propolis (1, 3 and 6 mg/kg/day), different extracts (water or ethanol) and varying the time of administration (30, 90 and 150 days) no significant alterations in total lipids, triglycerides, cholesterol and HDL-cholesterol concentrations were observed, nor in AST and LDH specific activities. The body weight of rats was measured in all these protocols, and propolis administration did not induce alterations in their weight <sup>(15)</sup>.

In the continuity of these works, this project was designed in order to investigate propolis effect on AST, ALT,  $\gamma$ -GT, LDH and amylase specific activities, as well on the concentration of urea, creatinine and total proteins, in different protocols.

Propolis administration to rats (1, 3 and 6 mg/kg/day) did not induce alterations in ALT, AST,  $\gamma$ -GT and LDH activities. These enzymes are widely distributed in tissues: AST is predominantly found in the heart, liver, skeletal muscle, kidney and pancreas; ALT is in the liver, kidney and heart. LDH is also found in the cells of many body tissues, including the

heart, liver, kidneys, skeletal muscle, brain, red blood cells, and lungs, and cellular damage causes an elevation of the total serum LDH. 3-GT determination helps to detect liver and bile duct injury.

Propolis protective effects against alcohol-induced liver injury were reported <sup>(22)</sup>. Its hepatoprotective effect on econazole-induced liver injury was also observed, evaluating AST and ALT activity <sup>(23)</sup>. Protective effects of caffeic acid phenethyl ester (CAPE) – an active component of propolis, have been evaluated on carbon tetrachloride (CCl<sub>4</sub>)-induced hepatotoxicity in rats. AST, ALT and other biochemical parameters were evaluated, indicating that CAPE treatment prevented CCl<sub>4</sub>-induced liver damage <sup>(24)</sup>.

No alterations were seen on amylase specific activity. Since this enzyme is related to pancreas damage, it might be hypothesized that propolis did not affect this tissue. Water and ethanolic extracts of propolis may control blood glucose and modulate the metabolism of glucose and blood lipid, leading to decreased output of lipid peroxidation and scavenge the free radicals in rats with diabetes mellitus <sup>(25)</sup>.

Blood protein concentration may change according to the nutritional status, insufficient kidney or liver function, multiple myeloma, or metabolic disorders. In our work, no alterations were found in total proteins, in all protocols. Urea concentration in blood is a consequence of its production rate during amino acids catabolism and its excretion by the kidney, whereas creatinine concentration is a result of the balance between creatinine production by the muscle and excretion by the kidney. In the present work, urea and creatinine concentrations were elevated in EEP-treated rats (6 mg/Kg/day), suggesting a possible kidney compromising after its administration. Brazilian propolis and one of its active component (artepillin C) prevented oxidative renal damage

and the carcinogenesis induced by Fe-NTA in mice <sup>(26)</sup>. CAPE treatment prevented lipid peroxidation and protein oxidation in doxorubicin-induced nephrotoxicity in rats <sup>(27)</sup>. CAPE was also efficient to protect the kidney against CCl<sub>4</sub> <sup>(28)</sup> and gentamicin-induced toxicity in rats <sup>(29)</sup>.

On the basis of these data, one may suggest that the intake of 1 mg/kg/day of EEP does not lead to side effects over a short- or long-term in rats, although it may not be discarded a possible renal damage. Nevertheless, with respect to the immune system, the best results were observed when propolis was administered over a short-term to animals (2), probably leading to no side effects. Although the published evidence to date supports propolis safety and effectiveness, its importance to human health is not known with sufficient detail, what opens a new perspective for further studies.

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