Effect of *Propolis* Extract on Biodistribution of Sodium Pertechnetate ($Na^{99m}TcO_4$)

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ABSTRACT

Objective: *Propolis* is a bee product useful natural substance applied in medicine. We have investigated the effect of *propolis* extract on biodistribution of sodium pertechnetate ($Na^{99m}TcO_4$) and laboratorial parameters in rats. Methods: It was administered, by gavage, 1.0ml/day of *propolis* into 10 rats (treated group) during 10 days and 1.0 ml/day of saline solution into other 10 rats (control group), by the same via and period. On the 10^{th} day, after 60min, all the animals received 0.1ml of $Na^{99m}TcO_4$ by ocular via. 50% of each group of rats was killed after 10min and other 50%, after 30min. Samples of brain, stomach, small bowel, liver, kidneys, thyroid and lungs were isolated and the %ATI/g of each organ was calculated. Tissue samples were taken from all the organs and stained by HE and PAS. Data were compared by Mann–Whitney and Student's t-tests ($p<0.05$). Results: There was a statistically significant increase in the %ATI/g, from control to treated group, respectively, in small bowel (0.15±0.01 to 0.32±0.09, after 10min and 0.32±0.03 to 0.42±0.02, after 30min) and blood (0.22±0.01 to 0.48±0.02, after10min and 0.38±0.02 to 0.51±0.04, after 30min).Conclusion: *Propolis* extract facilitated the uptake of $Na^{99m}TcO_4$ in some organs of rats and increased levels of some laboratorial parameters.

Keywords: *Propolis*. Herbal Medicine. Sodium Pertechnetate Tc 99m. Radiopharmaceuticals. Rats

INTRODUCTION

Propolis (bee glue) is the generic name given to resinous substances collected by honeybees from various plant sources. Propolis has attracted much attention in

recent years as a useful natural substance applied in medicine, even it is known in folk medicine since ancient times. This natural substance contains more than 300 components, including phenolic aldehydes, polyphenols, sequiterpene quinines, coumarins, steroids, aminoacids and inorganic compounds[1]. Interest in *propolis* as harmless medicine has increased because of its broad spectrum of biological properties, such as: antibacterial, analgesic, anti-inflammatory, antioxidant and immunoenhancement, antiproliferative activity in human tumor cells, antitumor activity in mice and radioprotective effects in *in vitro* cultures[2,3,4].

Ionizing radiation in interaction with living cells causes a variety of changes depending on exposed and absorbed dose, duration of exposure and interval after exposure and susceptibility of tissues[5]. Efforts to reduce toxicity to normal tissue cells and organs have led into searching for cytoprotective agents. Unfortunately, most of chemical radioprotectors have toxic side effects, which limit their use in medical practice. Investigations for effective and nontoxic compounds with radioprotection capability led to increasing interest in naturally occurring antioxidant such as propolis and its polyphenolic compounds[5].

Technetium-99m (^{99m}Tc) is an artificial radionuclide obtained in the form of sodium pertechnetate ($Na^{99m}TcO_4$) directly from the generator of $^{99}Mo/^{99m}Tc$ on elution with physiologic saline (0.9% NaCl). The vital role of ^{99m}Tc in the field of diagnostic nuclear medicine is well established. The evolution of diagnostic nuclear medicine can be principally attributed to the existence and chemical versatility of ^{99m}Tc, the ideal radiotracer, which is used predominantly in one form or another in nuclear medicine[6]. Rapid growth in this field in the last few decades is attributable, apart from its ideal radionuclidic characteristics, to the conception and development of generators and lyophilized kits for ease of formulation of ^{99m}Tc compounds in hospital radiopharmacies[6].

Factors, such as synthetic drugs as glucantime[7], mefloquine[8], paclitaxel[9], rochagan[10], morphine[11], tamoxifen[12] or natural drugs such as *artemisinin*[8], *Punica granatum*[13], *Aloe vera*[14] and dietary conditions could affect the biodistribution of radiopharmaceuticals[8,14].

The radiopharmaceutical sodium pertechnetate is uptaken by stomach, intestinal tract, thyroid and salivary glands and several natural or synthetic drugs can change the biological effect of the radiopharmaceutical and their interaction can lead to hypo or hyper uptake of radiopharmaceuticals in a particular organ, causing incorrect diagnosis or misinterpretation of results[15]. In addition, repeated scintigraphic may result in unnecessary radiation for patients[15]. Although the *propolis* extract is widely used and useful natural substance applied in folk medicine, no data about diary consumption of this product on biodistribution of radiopharmaceuticals or effects upon the histopathology and laboratorial parameters in rats have been described. Thus, the aim of this work was to evaluate the chronic effect of the *propolis* extract on

histology, biochemical and hematological parameters and on the uptake of sodium pertechnetate in organs of *Wistar* rats.

METHODS

ANIMALS

Wistar rats (2-3 months, 150-250g) were housed in an environmentally-controlled room (23±2°C), with free access to water and food. Experimental procedures were conducted in accordance with the Ethical Committee of Animal Use of the *Centro de Biociências, Universidade Federal do Rio Grande do Norte* (protocol number CEUA 027/2011).

PROPOLIS EXTRACT

The *propolis* extract (prepared from a 10% dye-mother solution and diluted in saline solution, *Herbarium* Laboratory, Rio de Janeiro/Brazil) was administered orally (1.0 mL/day) into male *Wistar* rats (n=10), in single dose during 10 days (treated group). The control group (n=10) received 1.0 mL/day of saline solution by the same way and period.

BIODISTRIBUTION OF SODIUM PERTECHNETATE

Sixty minutes after the last dose of *propolis* extract or saline solution (on the 10^{th} day of the treatments) all the animals were injected with 0.1 mL of the $Na^{99m}TcO_4$ (0.66 MBq), via orbital plexus. The sodium pertechnetate was eluted from a $^{99}Mo/^{99m}Tc$ generator produced by the Institute of Energy and Nuclear Research, São Paulo/Brazil. After this, 50% of each group of animals were quickly killed by anesthetic overdose (thiopental sodium 50mg/kg, intraperitoneally) after 10 min, and 50%, after 30 min. Blood and organ samples (brain, stomach, small bowel, colon, liver, kidneys, thyroid and lungs) were isolated, cleaned with 0.9% saline solution and the radioactivity of each organ was determined by means of an automatic gamma counter (1470 Wizard, Perkin Elmer, Finland) with automatic correction for decay and efficiency of 86%. The percentage of radioactivity per gram of each tissue or organ (%ATI/g) was calculated as described elsewhere Bernardo-Filho et al. (2005)[15].

HISTOLOGICAL EXAMINATION

Frozen tissue samples were taken from all the organs studied. The specimens were fixed in 10% formalin, cut as 5 µm tissue sections and stained with Hematoxylin-Eosin (HE) and Periodic Acid-Schiff (PAS). All specimens were

examined by the same pathologist, who had no knowledge of the study groups. Morphometric measurements were made using light micrographs (100 X) of the stained sections.

LABORATORIAL PARAMETERS

Hematological blood parameters were measured by an Abbot CellDyn 3500R autoanalyzer. The biochemical levels were measured using the Konelab 60i spectrophotometer, (assay kit from Weiner, São Paulo, Brazil). All data were presented as mean ± standard deviation.

STATISTICAL ANALYSIS

Data is reported as means ± SD. The %ATI/g and histological analysis were compared by Mann–Whitney test and hematological analysis and biochemical parameters were compared by the Student's t-test, considering $p<0.05$ statistically significant in both tests. In Stat Graph Pad software was used to perform statistical analysis.

RESULTS

The Table 1 shows the biodistribution of $Na^{99m}TcO_4$ in organs of control group rats and treated group rats. There was a statistically significant increase ($p<0.05$) in the uptake of $^{99m}TcO_4^-$ in the small bowel and blood in both period (after 10 and 30 min) of treated animals, when compared with controls. The $^{99m}TcO_4^-$ had no altered biodistribution in the other organs studied in both periods.

Table 1 Effect of *propolis* extract on the uptake of $Na^{99m}TcO_4$ in blood and small bowel of rats.

Organs	Period (min)	% ATI/g		p-value
		Control group	Treated group	
Blood	10	0.22±0.01	0.48±0.02	0.0285*
Blood	30	0.38±0.02	0.51±0.04	0.0485*
Small bowel	10	0.15±0.01	0.32±0.09	0.0208*
Small bowel	30	0.32±0.03	0.42±0.02	0.0305*

Data are reported as means±SD.*$p<0.05$, compared with control.

The Table 2 shows a significant increase in the seric levels of glucose, albumin, alkaline phosphatase and alanine aminetransferase in the treated rats, compared with controls. Histological analysis of the organs by HE and PAS of both groups showed no histopathological alterations. There was no alteration on hematological blood parameters of the treated group when compared with control group (data no showed).

Table 2 - Effect of *propolis* extract in some biochemical parameters of rats.

Parameters	Control group	Treated group	p-value
Albumin (mg/dL)	2.27±0.17	3.77±0.15	0.0418*
Alkaline phosphatase (UI/L)	156.01±6.14	189.20±7.12	0.0250*
Alanine aminetransferase (UI/L)	40.90±3.30	56.70±3.91	0.0241*
Glucose (mg/dL)	96.60±6.59	111.40±7.30	0.0312*

Data are reported as means±SD.*p<0.05, compared with control.

DISCUSSION

Propolis is a bee product, made from plant exudates, used for the construction and repair of the hive, as well as protection against microorganisms. It is a complex mixture, with more than 300 compounds already identified in different samples[1]. Cumulative evidence suggests that *propolis* may have anti-inflammatory, antibiotic, antioxidant, antihepatotoxic, and antitumor properties. Such effects have been associated with the presence of phenolic compounds, such as flavonoids and aromatic acids[2]. In addition, to topical applications, products containing *propolis* have been used increasingly as dietary supplements. Although reports of allergic reactions are not uncommon, *propolis* is reputed to be relatively nontoxic. Its systemic toxicity is rarely reported and hence may be underestimated[16]. Since *propolis* extract has been widely used in traditional phytomedicine and no data about diary consumption of this product on biodistribution of radiopharmaceuticals or effects upon the histopathology and the laboratorial parameters in rats have been described, it was the main reason for its use in this study.

The composition of *propolis* depends on time, vegetation, and the collection area. Considerable variation exists in the chemical composition of *propolis*, even within such a country as Brazil[16]. Chemical analysis of *propolis* extracts indicated that *propolis* samples had high concentrations of aromatic acids, esters and other derivatives, such as benzyl cinnamate, methyl cinnamate, caffeic acid, cinnamyl cinnamate and cinnamoylglcine, so the composition of *propolis* is extremely complex. In patients with suspected drug induced organ damage, *propolis* often is regarded as a natural compound and an innocent bystander. Hence, the cause of systemic toxicity usually is unknown and rarely attributed to *propolis*[16].

Yi-Jung et al. (2005)[17] studied the first case report of *propolis*-induced acute renal failure. This case indicated that *propolis* can induce acute renal failure and emphasizes the need for vigilance and care when *propolis* is used as a medicine or dietary supplement. Moreover, it is very difficult to identify definitely the compound causing acute renal failure. Furthermore, contamination by other toxic agents in the process of extracting, manufacturing and storing *propolis* is another potential factor in *propolis*-induced acute renal failure[1,17]. As an herbal remedy, *propolis* is used commonly and reputed for its therapeutic properties and relatively few systemic adverse effects[1].

Scintigraphic techniques allow the measurement of physiological processes, as well as the determination of alterations related to various diseases. In general, a radiopharmaceutical used in scintigraphic techniques presents a normal biodistribution that might be altered due to diseases and many others factors, such as drugs or natural products[6,14]. This fact helps the physicians to define the diagnosis about a disease with the analysis of scintigraphic images[15].

Although the *propolis* extract is widely used and useful natural substance applied in folk medicine, no data about diary consumption of this product on the biodistribution of radiopharmaceuticals or effects upon the histology and biochemical parameters in rats have been described. However, others natural products also abundantly used in folk medicine were already studied being evaluated those aspects. For example, Holanda et al. (2009)[14] showed that the aqueous extract of *Aloe vera* increased the uptake of the sodium pertechnetate in femur, kidneys, liver, stomach, testis and thyroid of organs of *Wistar* rats and decreased the levels of some biochemical parameters. Santos-Filho et al. (2005)[18] demonstrated that *Hypericum perforatum* reduced significantly the radioactivity percentage in bone, muscle and thyroid and increased in the pancreas of animals. Moreno et al. (2004)[19] showed that *Ginkgo biloba* also altered the uptake of the radiopharmaceuticals in kidneys, liver and duodenum of rats and Braga et al. (2012)[6] showed that the aqueous carqueja extract decreased the radiolabeling of blood constituents with technetium-99m (^{99m}Tc).

The present study showed that *propolis* extract increased the uptake of the sodium pertechnetate in blood and small bowel of *Wistar* rats after 10 and 30 min of the injection of $Na^{99m}TcO_4$. This fact indicate that the *propolis* extract facilitated the uptake of the $Na^{99m}TcO_4$ in organism of *Wistar* rats treated with this natural drug and, probably, it not interfere in scintigraphic examinations of patients in use of this natural product. There was also increase in seric levels of glucose, albumin, alkaline phosphatase (AP) and alanine aminetransferase (ALT) in the treated rats, compared with controls. The increase of levels of serum AP, ALT and albumin can indicate deterioration in the functions of the liver and cellular hepatic damage. Unfortunately, hepatic toxicity is a potential complication of natural compounds that may lead to hepatic insufficiency[20].The alterations on hepatic enzymes (ALT and AP) in this

study, probably, occurred due to the alcohols, esters and aldehydes present in the studied concentration of *propolis* extract capable to damage hepatic cells[20].

CONCLUSION

In conclusion, the results of this study indicate that the propolis extract was responsible for the increased levels of liver enzymes alkaline phosphatase and alanine aminetransferase, demonstrating a small toxic effect on the liver, and it does not prevent uptake of the radiopharmaceutical $Na^{99m}TcO_4$ in the small bowel and blood of experimental animals.

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