Effect of oral ingestion of an *Arctium lappa* extract on the biodistribution of the radiopharmaceutical sodium pertechnetate in rats


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The aim of the present study was to assess the effect of the oral ingestion of an extract of the *Arctium lappa* (burdock) on the bio-distribution of the radio-pharmaceutical (radio-biocomplex) sodium pertechnetate (Na$^{99m}$TcO$_4^-$) in rats. Male *Wistar* rats (3 to 4 months of age, 329 ± 16 g) were treated with a burdock extract (1 ml, 20 mg/ml, n = 5) or 0.9% NaCl solution (control: n = 5) for 7 days. After this period of time, Na$^{99m}$TcO$_4^-$ (3.7 MBq, 0.3 ml) was injected through the ocular plexus. After 10 min, the rats were sacrificed, the organs isolated and counted in an automatic gamma counter. The percentage of radioactivity was calculated per gram of tissue (%ATI/g) or per whole organ (% ATl/organ). Alteration in Na$^{99m}$TcO$_4^-$ uptake was observed in liver from 1.72 ± 0.38 to 0.27 ± 0.07 (% ATl/organ, p < 0.05) and % ATI/g in lung (from 0.45 ± 0.40 to 1.02 ± 0.15 % ATI/g), in testis (from 0.12 ± 0.01 to 0.18 ± 0.02 % ATI/g), in tooth (from 0.24 ± 0.08 to 0.06 ± 0.13 % ATI/g), in tongue (from 0.38 ± 0.06 to 0.08 ± 0.16 % ATI/g) and in liver (from 1.07 ± 0.06 to 0.56 ± 0.15) after treatment with burdock. These findings could result from the interaction between components of the *A. lappa* extract and the radio-biocomplex which may influence the uptake of Na$^{99m}$TcO$_4^-$ in some organs of rats. Therefore, precautions are suggested in the interpretation of nuclear medicine results in patients using burdock.

Key words: *Arctium lappa* (Burdock), biodistribution, sodium pertechnetate, radiobiocomplex.

INTRODUCTION

Herbal products uses are increasing in most countries of the world, as part of a resurging belief in efficacy and safety of natural and traditional remedies (Simões et al., 2010). *Arctium lappa* L. (burdock) has been cultivated as...
a vegetable for a long time in orient, especially, Taiwan and Japan (Gentil et al., 2006). Its roots are widely used as food, whereas the seeds are used in traditional Korean medicine as a diuretic, anti-inflammatory or detoxifying agent (Predes et al., 2011), for hypertension and arteriosclerosis treatment (Neves et al., 2007; Liu et al., 2012). Its anti-diabetic property may be attributed to arctin fraction (Lu et al., 2012). Jian-Feng et al. (2012) have reported a study using an aqueous extract of A. lappa L. roots (1,200 mg/kg) administered for a duration of 3, 7 and 15 days and they observed an enhancement of the sexual behavior in male rats. Further, Huang et al. (2010) suggest that burdock extract (100 mg/kg) administration for 8 days can prevent intestinal damage and decrease inflammatory cytokines in mice with ulcerative colitis.

Some investigations have demonstrated that burdock extract possesses hepatoprotective action that could be attributed, at least in part, to its anti-oxidative activity (Cunha et al., 2003; Song-Chow et al., 2005; Predes et al., 2012). This study evaluated the anti-bacterial activity of a phytotherapeutic agent prepared from an ethyl acetate fraction (AcOEt) extracted from A. lappa (Gentil et al., 2006). An infusion of the leaves is useful to impart strength and tone to the stomach, for some forms of long-standing indigestion (Song-Chow et al., 2005).

Phytochemistry analysis carried out by some authors has demonstrated that the species A. lappa contains inulin (45 to 60%), sesqueripenical lactones, phenol acids, essential oils, policeticlenes, tannins (Simões et al., 2010), flavonoid (baicalin), lignans (arctigenin), vitamins B and C, calcium and phosphorus (Gomes et al., 2011). Lignans, tannins and flavonoids have properties of anti-tumor, anti-oxidant, anti-inflammatory, anti-hepatotoxic, anti-coagulant (Rotblat and Ziment, 2002). Although the toxicity of A. lappa extract is not known, cases of allergy due to burdock have been reported as contact dermatitis resulting in anaphylaxis (Rodríguez et al., 2006). Meanwhile, tannic acid, a specific substance found in certain tannin-containing herbs, can be a gastrointestinal irritant when taken in large amounts (Rotblat and Ziment, 2002). It has been related that plants of Asteraceae family as A. lappa possess anti- leukemic properties and induce cells death via apoptosis (Wegiera et al., 2012). Haghi et al. (2013) related the presence of chlorogenic acid (5-CQA) and 1,5-dicaffeoylquinic acid (1,5-DCQA) as main compounds, total phenolic, which are caffeoyl esters present in wild and cultivated A. lappa L. (Haghi et al., 2013).

Radiopharmaceuticals (radiobiocomplexes) (Moreno et al., 2005) are radioactive tracers employed in nuclear medicine for the investigation of several morphological and physiological conditions, such as blood flow and absorption, biodistribution and metabolism in target and non-target organs. These considerations are highly relevant in early detection of a disease and the images obtained are denominated metabolic images. This fact permits proper clinical action in the beginning of the disease increasing the possibility of a successful intervention (medication, surgical) (Saha, 2010). The incorporation of a radionuclide into a drug formulation permits the determination of the biodistribution kinetics and the release sites of the latter (Owunwanne et al., 1996). Technetium-99m ($^{99m}$Tc) has been widely used in nuclear medicine due to its optimal half-life 6.0 h and energy characteristics, providing images with high efficiency with the administration of low doses to the patient (Moreno et al., 2005). Radio-biocomplexes such as sodium pertechnetate (Na$^{99m}$TcO$_4^-$) are tracers widely employed in scintigraphic studies (single-photon emission computed tomography - SPECT) mainly of the thyroid but also of the brain and stomach (Saha, 2010).

Natural and synthetic products have been reported to affect the biodistribution of different radiobiocomplexes (Owunwanne et al., 1996; Bernardo-Filho, 2005; Saha, 2010). PubMed (www.ncbi.nlm.nih.gov/sites/entrez) is a service of National Library of Medicine US that includes over 22 million citations from MEDLINE and other health sciences, among others. Scielo (Scientific Electronic Library Online) is an important index (www.scielo.org) of scientific publications. Review of the literature available in this data base did not show any reference about of the effect of A. lappa extract on the bioavailability of sodium pertechnetate (Na$^{99m}$TcO$_4^-$). This finding, as well as the possibility of human beings that are undertaking burdock may need a nuclear medicine procedure, the aim of this investigation was to evaluate the effect of the oral ingestion of an extract of the burdock on the biodistribution of the radiobiocomplex sodium pertechnetate (Na$^{99m}$TcO$_4^-$) in rats.

MATERIALS AND METHODS

Preparation of extract

The burdock extract was prepared with 2 g of leaf, stem and flowers of A. lappa (Estrella da Terra Produtos Naturais LTDA, Brazil, lot 003) in 100 ml of NaCl 0.9% solution at room temperature. It was triturated with a domestic electric extractor. This mixture was filtered (Schleicher and Schull filter paper Lot number K 932, Size 11 cm) and the filtered solution was considered to be 20 mg/ml or 100%. The absorbance spectrum (Spectrophotometer, Analyzer Comércio e Indústria Ltda, Brazil) was determined, in the range of 400 to 700 nm as described by Neves et al. (2007). The value of the absorbance at 500 nm (0.754 ± 0.002) was considered as a marker of the reproducibility of the conditions of the extract at the highest concentration (Neves et al., 2007). As there is not a defined dosage of the extract (Huang et al., 2010; Jian-Feng et al., 2012) that is administered to the animals, as well as the time of the treatment, we decided to use in our investigation the dosage of 70 mg/kg during 7 consecutive days. The protocols of the experiments were performed without sacrificing of the animals and was approved by the Ethical Committee of the Instituto de Biologia Roberto Alcântara Gomes, Universidade do Estado do Rio de Janeiro with the protocol number CEA/141/2008.

Strategy adapted for survey of literature in the PubMed and Scielo data base

It was performed in PubMed (www.ncbi.nlm.nih.gov/sites/entrez)
and Scielo (www.scielo.org) a search (April 18th, 2013) using the keywords “Arctium lappa” and Na$^{99m}$TcO$_4$, “burdock” and “Na$^{99m}$TcO$_4$, “burdock” and “radiopharmaceutical”, “A. lappa” and “radiopharmaceutical”, “burdock” and “sodium pertechnetate”; “A. lappa” and “sodium pertechnetate”.

Treatment of animals

Adult male Wistar rats (n = 5), 3 to 4 months of age, 329 ± 16 g of weight following the Ethical Guidelines of the Institution were used in all the experiments. They were obtained from the Laboratório de Radiofarmácia Experimental (Departamento de Biofísica e Biometria, Universidade do Estado do Rio de Janeiro, UERJ, RJ, Brazil). The animals were used after an acclimatization period of 7 days and maintained under controlled room conditions corresponding to 22 ± 5°C, 12 h of light/dark cycle with water and a normal diet ad libitum during the experimental period. The A. lappa preparation (20 mg/ml, 70 mg/kg) was administered (1 ml) to the animals (n = 5) using a metal oropharyngeal cannula, daily doses for 7 days. The control group received 0.9% NaCl solution. Na$^{99m}$TcO$_4$ radiobiocomplex (0.3 ml, 3.7 MBq; Instituto de Pesquisas Energéticas e Nucleares, Comissão Nacional de Energia Nuclear, São Paulo, SP, Brazil) was administered (after 7 days) through the ocular plexus and the animals were sacrificed 10 minutes later.

Heparinized whole blood was rapidly obtained by cardiac puncture. The organs (brain, liver, duodenum, heart, kidney, lung, spleen, stomach, pancreas, testis, bone, muscle, thyroid, right upper incisor tooth and tongue) were isolated and weighed and the radioactivity was counted in a well counter (Automatic Gamma Counter, Packard Instrument Co, Illinois, USA). The samples were put in specific and appropriated tubes the conditions were always the same and the well counter was adjusted to the photonic energy of the $^{99m}$Tc (gamma emission, 140keV). After that, the % of radioactivity (%ATI) was calculated in relation to the total dose that was injected. As some authors have already published, we used in our investigation two ways to assess the %ATI (%ATI/organ) of the radiobiocomplex Na$^{99m}$TcO$_4$ in the treated animals with burdock extract and in the control group. Increase in the uptake of the Na$^{99m}$TcO$_4$ in the stomach, from 1.87 ± 0.56 (control) to 2.75 ± 0.76 (treated, p = 0.07, not statistically significant) was observed. Significant increase in the uptakes in liver from 1.07 ± 0.06 (control) to 0.56 ± 0.15 (treated, p = 0.0001), in tooth from 0.24 ± 0.08 (control) to 0.06 ± 0.13 (treated, p = 0.029) and in tongue from 0.38 ± 0.06 (control) to 0.08 ± 0.16 (treated, p = 0.029) were found.

DISCUSSION AND CONCLUSION

Much of the medical literature on medicines suggests that the safe or toxicity of medicinal plants is based on suboptimal evaluations of the available data (Rotblat and Ziment, 2002; Simões et al., 2010). The results obtained indicate that the burdock extract may affect the biodistribution of Na$^{99m}$TcO$_4$ in specific organs. Moreno et al. (2005) reported that Ginkgo biloba extract altered the uptake of Na$^{99m}$TcO$_4$ in rats. Nectandra membranacea extract altered the radioactivity uptake in heart, thyroid, kidney and muscle (Moreno et al., 2007a; Saha, 2010). The percentage of radioactivity per gram of tissue (%ATI/g) was calculated dividing the %ATI/organ by the mass of each organ (Moreno et al., 2007a; Saha, 2010).

Statistical analysis

Analysis involved one-way analysis of variance (ANOVA), followed by the Turkey-Kramer multiple comparisons test, with the significance level being P < 0.05. InStat Graphpad software was used to perform statistical analysis (GraphPad InStat version 3.01 for Windows 95/NT, GraphPad Software, San Diego Ca, USA).

RESULTS

Some publications were found in the PubMed and Scielo following the used strategy: “A. lappa” and/or burdock (153 in PubMed and 11 in Scielo). When the keywords were “burdock”, “A. lappa” and Na$^{99m}$TcO$_4$, “burdock” and “Na$^{99m}$TcO$_4$, “burdock” and “radiopharmaceutical”, “A. lappa” and “radiopharmaceutical”, “burdock” and “sodium pertechnetate” or “A. lappa” and “sodium pertechnetate” no item was found. The results in Table 1 show the relationship between the percentage of radioactivity per organ (% ATI/organ) of the radiobiocomplex Na$^{99m}$TcO$_4$ in the experimental group treated with A. lappa extract and the control group. Results indicate a significant increase in the uptake of the Na$^{99m}$TcO$_4$ in stomach, from 2.46 ± 0.70 (control) to 3.82 ± 0.54 (treated, p = 0.010) and tooth from 0.05 ± 0.02 (control) to 0.16 ± 0.08 (treated, p = 0.0001). A significant decrease in the uptake of the Na$^{99m}$TcO$_4$ in liver from 1.72 ± 0.38 (control) to 0.27 ± 0.07 (treated, p = 0.0011) was also found. No significant changes in the uptake of this radiobiocomplex in the brain, duodenum, heart, kidney, spleen, pancreas, lung, blood, thyroid, testis, muscle, tongue and bone (% ATI/organ) were found.

Table 2 shows the percentage of radioactivity per gram of tissue (% ATI/g) of the radiobiocomplex Na$^{99m}$TcO$_4$ in the treated animals with burdock extract and in the control group. Increase in the uptake of the Na$^{99m}$TcO$_4$ in the stomach, from 1.87 ± 0.56 (control) to 2.75 ± 0.76 (treated, p = 0.07, not statistically significant) was observed. Significant decrease in the uptakes in liver from 1.07 ± 0.06 (control) to 0.56 ± 0.15 (treated, p = 0.0001), in tooth from 0.24 ± 0.08 (control) to 0.06 ± 0.13 (treated, p = 0.029) and in tongue from 0.38 ± 0.06 (control) to 0.08 ± 0.16 (treated, p = 0.029) were found.

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Table 1. Shows the effect of the *Arctium lappa* extract on the biodistribution of $^{99m}$Tc (% ATI/organ) in the male Wistar rats which had received (20 mg/ml) or not (control group) the extract.

<table>
<thead>
<tr>
<th>Organ</th>
<th>Control (% ATI/organ)</th>
<th>Treated (% ATI/organ)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brain</td>
<td>0.07±0.01</td>
<td>0.19±0.27</td>
</tr>
<tr>
<td>Liver</td>
<td>1.72±0.38</td>
<td>0.27±0.07*</td>
</tr>
<tr>
<td>Duodenum</td>
<td>0.15±0.03</td>
<td>0.18±0.09</td>
</tr>
<tr>
<td>Heart</td>
<td>0.33±0.09</td>
<td>0.35±0.06</td>
</tr>
<tr>
<td>Kidney</td>
<td>0.57±0.16</td>
<td>0.59±0.06</td>
</tr>
<tr>
<td>Stomach</td>
<td>2.46±0.70</td>
<td>3.82±0.54*</td>
</tr>
<tr>
<td>Pancreas</td>
<td>0.18±0.08</td>
<td>0.22±0.54</td>
</tr>
<tr>
<td>Lung</td>
<td>0.05±0.02</td>
<td>0.06±0.02</td>
</tr>
<tr>
<td>Testis</td>
<td>0.17±0.02</td>
<td>0.20±0.01</td>
</tr>
<tr>
<td>Bone</td>
<td>0.19±0.36</td>
<td>0.12±0.07</td>
</tr>
<tr>
<td>Muscle</td>
<td>0.06±0.01</td>
<td>0.08±0.01</td>
</tr>
<tr>
<td>Thyroid</td>
<td>1.64±0.16</td>
<td>1.49±0.14</td>
</tr>
<tr>
<td>Spleen</td>
<td>0.23±0.02</td>
<td>0.21±0.53</td>
</tr>
<tr>
<td>Blood</td>
<td>1.12±0.17</td>
<td>1.17±0.16</td>
</tr>
<tr>
<td>Tooth</td>
<td>0.05±0.02</td>
<td>0.16±0.08*</td>
</tr>
<tr>
<td>Tongue</td>
<td>0.22±0.02</td>
<td>0.22±0.01</td>
</tr>
</tbody>
</table>

Data are reported as mean ± SD for 5 animals in each group.

After 7 days of treatment with extract of *Arctium lappa* (burdock) by intragastric via, once a day, (20 mg/mL), male Wistar rats received 0.3 mL Na$^{99m}$TcO$_4$ by the intravenous route. The animals were sacrificed, the organs isolated and %ATI/organ determined. Asterisks indicate significant differences (p<0.05).

examination, with an increase in the radiation dose administered to the patient (Bernardo-Filho, 2005). The knowledge about this phenomenon may contribute for proper clinical decisions and correct diagnosis.

Tsai et al. (2011) have enfaced that the protective effect on hepatocytes and the inhibition of interleukin-2 in primary human T lymphocytes might be attributed to the arctigenin bioactive component of *A. lappa*. It is possible to speculate that the alteration in the uptake of the studied radiobiocomplex (Tables 1 and 2) could be associated with the action described by Tsai et al. (2011). As tannins-containing herbs can be a gastrointestinal irritant (Rotblat and Ziment, 2002), the increase of radiobiocomplex uptake in stomach (Table 1) of animals treated with burdock extract could be associated with the presence of tannins in this extract. Moreover, the alteration of radiopharmaceutical uptake in liver (Tables 1 and 2) and stomach (Table 1), are in accordance with the literature, that also have described hepato-protective and gastro-protective action promoted by burdock extract (Song-Chow et al., 2002; Lima et al., 2006).

An interesting finding is related with the alteration of the uptake in the testis (Table 2) and this fact could be associated with the enhancement of the sexual behavior in male rats as reported by Jian-Feng et al. (2012). The aphrodisiac effects of the plant extract may be related to the presence of flavonoids, saponins, lignans and alkaloids, acting via a multitude of central and peripheral mechanisms. These results thus support the traditional use of *A. lappa* L. root extract for treating impotence and sterility. These considerations are described by Jian-Feng et al. (2012).

Meanwhile, precautions are suggested in the interpretation of nuclear medicine results in patients using the burdock since it alters the biodistribution of the sodium pertechnete radiopharmaceutical in some organs and this fact could influence proper actions related to the diagnosis and therapy of some diseases.

**Conclusion**

The metabolization of the *A. lappa* (*in vivo*) could generate active metabolites with properties that could influence the biodistribution of the Na$^{99m}$TcO$_4$ radiobiocomplex in the treated animals with this extract.

**ACKNOWLEDGEMENTS**

The present work was carried out with support of the CAPES, Institution of the Brazil Government for formation of human resources. We are also indebted to FAPERJ, CNPq, UERJ, UFRN.
Table 2. Shows the effect of the A. lappa extract on the biodistribution of $^{99m}$Tc (% ATI/g) in the male Wistar rats which had received (20 mg/ml) or not (control group) the extract.

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<thead>
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<th>Organ</th>
<th>Control (% ATI/organ)</th>
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<tbody>
<tr>
<td>Brain</td>
<td>0.04 ± 0.01</td>
<td>0.11 ± 0.05</td>
</tr>
<tr>
<td>Liver</td>
<td>1.07 ± 0.06</td>
<td>0.56 ± 0.15*</td>
</tr>
<tr>
<td>Duodenum</td>
<td>0.93 ± 0.57</td>
<td>0.89 ± 0.17</td>
</tr>
<tr>
<td>Heart</td>
<td>0.29 ± 0.05</td>
<td>0.25 ± 0.17</td>
</tr>
<tr>
<td>Kidney</td>
<td>0.53 ± 0.12</td>
<td>0.57± ± 0.09</td>
</tr>
<tr>
<td>Stomach</td>
<td>1.87 ± 0.56</td>
<td>2.75 ± 0.76</td>
</tr>
<tr>
<td>Pancreas</td>
<td>0.37± ± 0.01</td>
<td>0.36 ± 0.03</td>
</tr>
<tr>
<td>Lung</td>
<td>0.45 ± 0.40</td>
<td>1.02 ± 1.15*</td>
</tr>
<tr>
<td>Testis</td>
<td>0.12 ± 0.01</td>
<td>0.18 ± 0.02*</td>
</tr>
<tr>
<td>Bone</td>
<td>0.25 ± 0.36</td>
<td>0.15 ± 0.06</td>
</tr>
<tr>
<td>Muscle</td>
<td>0.11 ± 0.01</td>
<td>0.11 ± 0.15</td>
</tr>
<tr>
<td>Thyroid</td>
<td>5.71 ± 0.91</td>
<td>5.37 ± 0.93</td>
</tr>
<tr>
<td>Spleen</td>
<td>0.40 ± 0.01</td>
<td>0.39 ± 0.06</td>
</tr>
<tr>
<td>Blood</td>
<td>2.93 ± 0.17</td>
<td>2.92 ± 0.38</td>
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Abbreviations

AcOE, Ethyl acetate fraction; Na$^{99m}$TcO$_4$, sodium pertechnetate; MBq, mega becquerel; A. Lappa, Arctium lappa; Tc-99m, technetium-99m.

REFERENCES


