Biodistribution of sodium pertechnetate and biochemical parameters in experimental osteoporosis in rats treated with extract of *Chenopodium ambrosioides L* (mastruz)

Biodistribuição do pertecnetato de sódio e parâmetros bioquímicos em modelo experimental de osteoporose em ratas tratadas com extrato de Chenopodium ambrosioides L (mastruz)

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ABSTRACT

Objective: The aim of this study was to evaluate the effect of treatment with *Chenopodium ambrosioides L* (mastruz) hydroalcoholic extract in rats with osteoporosis on the biodistribution of sodium pertechnetate (Na99mTcO4) and biochemical parameters. **Methods:** Ten rats underwent ovariectomy to induce osteoporosis. The first group (n = 5) received no treatment, the second group (n = 5) received 50mg HaME for thirty days. After finishing the treatment the animals had been anesthetized and injected 0.1 mL (0.66 MBq) of Na^{99}mTcO4 the femural vein. After 30 minutes, blood was collected for dosages, and then the animals were killed by an overdose of anesthetic (thiopental 100mg/kg) and removed the heart, lungs, stomach, femur, kidneys, liver, intestine and thyroid to calculate the percentage of radioactivity per gram of tissue (% ATI/ g) of each organ. **Results:** There was no significant difference in any of the organs analyzed, but in all of them, except in the intestine, the uptake of Na^{99}mTcO4 was higher in the group treated with mastruz. Biochemical parameters showed a significant increase in lactate dehydrogenase, total cholesterol, and aspartate aminotransferase. These results allow us to state that mastruz may have hepatotoxic effect. **Conclusion:** Treatment with mastruz in rats with osteoporosis does not alter the biodistribution of the radiopharmaceutical $Na^{99m}TcO_4$ and showed some hepatotoxicity

Key words: Osteoporosis. *Chenopodium ambrosioides L*. Bioavailability. ^{99m} Tc. Pertechnetate. Rats.

RESUMO

Objetivo: O objetivo deste estudo foi avaliar o efeito do tratamento com extrato hidroalcoólico de *Chenopodium ambrosioides L* (mastruz) em ratas com osteoporose na biodistribuição do pertecnetato de sódio ($Na^{99m}TcO_4$) e em parâmetros bioquímicos. **Métodos:** Dez ratas foram submetidas à ooforectomia para indução de osteoporose. O primeiro grupo (n=5) não recebeu tratamento, e o segundo grupo (n=5) recebeu diariamente 50 mg de EhaM por trinta dias. Depois de finalizado o tratamento, os animais foram anestesiados e injetado 0,1 mL (0,66MBq) de $Na^{99m}TcO_4$ através da veia femural. Após 30 minutos foi coletado sangue e em seguida os animais foram mortos com superdose de anestésico (tiopental 100mg/Kg) e removidos coração, pulmões, estômago, fêmur, rins, fígado, intestino e tireóide para cálculo do percentual de radioatividade por grama de tecido (%ATI/g) de cada órgão. **Resultados:** Não houve diferença significativa em nenhum dos órgãos analisados, porém em todos eles, exceto no intestino, a captação do $Na^{99m}TcO_4$ foi maior no grupo tratado com mastruz. Dos parâmetros bioquímicos analisados houve aumento significativo de Lactato desidrogenase, colesterol total e aspartato aminotransferase. Esses resultados permitem afirmar que as provas de função hepática sinalizam que o mastruz pode apresentar efeito hepatotóxico. **Conclusão:** O tratamento com mastruz em ratas com osteoporose não altera a biodistribuição de $Na^{99m}TcO_4$, mas pode apresentar hepatotoxicidade nas doses empregadas.

Descritores: Osteoporose. *Chenopodium ambrosioides L*. Biodisponibilidade. Pertecnetato. Tecnécio Tc^{99m}. Ratos.

INTRODUCTION

Diagnostic techniques have evolved in recent decades and among the advances are the advent of bone scintigraphy, a relevant technique for detection of bone metastases, primary tumors and benign bone diseases[1-3]. However, several pathological conditions can alter the biodistribution of radiopharmaceuticals used in these exams[4,5]. We did not find studies assessing the biodistribution of radiopharmaceuticals in rats treated with mastruz. The sodium pertechnetate ($Na^{99m}TcO_4$) is one of the most widely used isotope in nuclear medicine and research on their biodistribution in various physiological and pathological situations have been done[2,3].

Osteoporosis (OP) is a metabolic bone disease, considered a public health problem as it affects at least 30% of women in post-menopause. It is a chronic disease caused by increased osteoclastic activity and suppression of osteoblastic activity. Clinically various effects of this disease, including increased risk of bone fractures,[4-6] are observed. The mastruz has osteogenic effect, evidenced by Souza et al (2010) and may be a promising therapy for osteoporosis and other bone diseases[7].

The *Chenopodium ambrosioides L* (mastruz) is a medicinal plant widely used in the world. Studies have proved their anthelmintic, anti-inflammatory and analgesic

activities. It has osteogenic effect and hence its effect has been studied in osteoporosis. The main advantages of using herbal medicines are easy accessibility (cost and availability) and reduction of adverse effects compared with convencional drugs[7-9].

Systemic effects of herbal medicines are essential for indicating or not for the treatment of diseases. Some drugs (biphosphonates) used to treat osteoporosis are associated with alterations in biodistribution of radiopharmaceuticals, and may result in misinterpretation of images in nuclear meedicine[6,10]. Capriles *et al*. evaluated the influence of eggplant extract on the biodistribution of technetium in blood of rats[11].

The aim of this study was to evaluate the biodistribution of sodium pertechnetate ($Na^{99m}TcO_4$) and biochemical parameters in rats with induced osteoporosis by bilateral oophorectomy, treated with mastruz hydroalcoholic extract (MaHaE).

METHODS

The experimental protocol was submitted to the Ethics Committee (CEP/UNP) and approved under number 007/2013. This protocol followed the recommendations of the Animal Experimentation Code of Ethics and Brazilian College of Animal Experimentation.

Extract

Fresh plant *Chenopodium ambrosioides L* was collected and placed in drying oven for three days. Then ethanol was added and homogenized in 1:3 ratio for percolation process. The material was filtered and concentrated in rotoevaporator at constant temperature of 60ºC. The concentrated extract was weighed and diluted in distilled water, obtaining hydroalcoholic mastruz extract (MaHaE) at a concentration of 10%, maintained under refrigeration (5°C). All experiments were carried out before the expiry date of the product.

Surgery and sample collection

Ten female Wistar rats 90 days old, 200g ± 50g, provided by the Animal Facility of Potiguar University were kept in an environment with lighting (cycles of 12 h light / dark), Ventilation and appropriate temperature (21ºC), getting balanced diet (Labina® Purina) and water *ad libitum.*

Bilateral ovariectomy was performed for induction of osteoporosis in all animals. After surgery the rats were divided into two groups: Osteoporosis (G1, n = 5) received 0.5 ml distilled water by gavage for 30 days (G2, n = 5) and osteoporosis+ MaHaE group rtas (n = 5), which received daily MaHaE 10% (50 mg) 0.5 mL by gavage at by the same route and period.

To perform bilateral ovariectomy, animals were anesthetized using solution of Zoletil 50 at a dose of 0.3 ml/100 mg intramuscular in the quadriceps region. After anesthesia, the anterior abdominal region was shaved, and antisepsis with 2%

chlorhexidine, followed by longitudinal incision with a length of approximately 3 cm in the abdominal wall with exposure of uterus and ovaries. After identification of ovaries, bilateral ligation of mesovaries were done with 4-0 silk sutures (Ethicon ® / Johnson & Johnson) and subsequent bilateral oophorectomy with hemostasis review. At the end of the procedure, abdominal wall was closed with Polydioxanone - 4-0 PDS (Ethicon® /Johnson & Johnson) suture and the skin with 4-0 nylon (Ethicon ® / Johnson & Johnson). Postoperative pain was treated with tenoxicam 0.5 mg/kg (IM), (Roche® Pharm Brazil) once a day for 3 days. Both groups remained in postoperative observation for 10 days, during which, the rats were daily weighted.

Treatment with distilled water (G1) and matruz extract (MaHaE) (G2) was restarted 24 hours after bilateral oophorectomy.

Biodistribution analysis

Thirty days after the begining of treatments, animals were anaesthetized again and 0.1 ml of $Na^{99m}TcO_4$ was injected through the femoral vein, corresponding to a radioactivity of 0.66MBq. Technetium-99m was eluted from a 99 Mo/99m Tc generator produced by the Institute of Nuclear Energy Research, São Paulo/Brazil and kindly donated by Norteriograndense League against Cancer, Natal / RN. After 30 minutes, blood was collected, then the rats were killed with anesthesia overdose (Tiopental 100mg/Kg i.p.) and organs samples were collected from heart, right lung, stomach, right femur, right kidney, liver, duodenum and thyroid. They were washed in 0.9% saline solution and weighed in a digital precision balance (160-Bel -Mark Itália®-II). The radioactivity uptake of each organ was determined by an automated gamma counter (1470 Wizard, Perkin Elmer®, Finland) with automatic correction for decay and efficiency of 86%, at the Nucleus for Experimental Surgery-UFRN. The counts per minute (CPM) in each organ alone were performed in standard time of 1min. We calculated the percentage of radioactivity per gram of tissue (%ATI/g) in the organs, by dividing the radioactivity present in each organ by the total radioactivity injected into the animal.

Laboratory analysis

Biochemical parameters were measured using the auto-analyzer spectrophotometer (Konelab 60i®) in the Laboratory of Clinical Analysis of Federal University of Rio Grande do Norte, Brazil. Serum lactate dehydrogenase (LDH), aspartate aminotransferase (AST), cholesterol, glucose, triglycerides and globulin were measured using test kits Weiner® São Paulo, Brazil. All data are presented as mean±standard deviation.

Statistical analysis

The data of %ATI/g were analysed in GraphPad PRISM ® 5.0 software and statistically processed using the Mann-Whitney test and Student t test. Data were considered significant at a level of 5% ($p<0.05$).

RESULTS

Biodistribution of $Na^{99m}TcO_4$

The results of the $Na^{99m}TcO_4$ uptake in different organs selected for this study are summarized in Table 1.

Table 1 – Percent of radioactivity per gram (%ATI/g) of tissue in each organ.

	%ATI/g		
Organs	**Osteoporosis**	**Osteoporosis+** MaHaE	***p*-value**
Liver	0.18 ± 0.0112	0.25 ± 0.0443	0.1447
Intestine	0.44 ± 0.0861	0.31 ± 0.0506	0.2427
Heart	0.12 ± 0.0111	0.14 ± 0.0139	0.2946
Kidney	0.33 ± 0.0321	0.34 ± 0.0241	0.8099
Thyroid	4.81 ± 0.786	5.51 ± 1.252	0.6495
Lung	0.26 ± 0.0212	0.31 ± 0.0312	0.2391
Femur	0.12 ± 0.0096	0.17 ± 0.0566	0.4093
Estomach	2.73 ± 0.3559	3.55 ± 0.2636	0.1026

*Mean±standard deviation. No significant difference comparing the groups ($p<0.05$).

The values of the percentage of the $Na^{99m}TcO_4$ activity in all organs showed no statistically significant differences when the two groups were compared ($p>0.05$).

Biochemical parameters

Serum levels of AST, LDH, total cholesterol, triglycerides and globulin were significantly higher in the group treated with mastruz than in the control group ($p<0.03$). The levels of glucose in the blood of animals treated with mastruz were lower than in controls, but the diference was not statistically significant ($p=0.0727$). These data are summarized in table 2.

Table 2 – Effect of 30 days mastruz (HaME) treatment on biochemical parameters.

Parameters	Osteoporosis	Osteoporosis + HaME	p-value
Glucose	147.0 ± 13.63	110.2 ± 12.31	0.072
AST	132.2 ± 15.61	246.2 ± 33.19	0.011*
LDH	2539 ± 754.3	602.7 ± 36.42	0.028*
Cholesterol	67.50 ± 5.340	84.67 ± 4.088	0.028*
Triglycerides	24.33 ± 1.764	57.17 ± 2.455	< 0.0001*
Globulin	4.470 ± 0.2720	1.783 ± 0.1869	< 0.0001*

Mean±standard deviation. * Statisticaly significant diferences comparing the groups ($p<0,05$); HaME, Hidro-alcoholic mastruz extract.

DISCUSSION

The mastruz is one of the most widely used herbal medicines in the world. Several medicinal properties have been attributed to mastruz and its use is common in

many regions of Brazil. Recently, mastruz was included by the Brazilian government in the list of priorities for study on the National Program on Medicinal Plants and Herbal Medicines[14]. Amounts of flavonoids, tannins and phenols are found in phytochemical analysis of the plant. The main component of the essential oil that can also be found in the crude extract of the plant is ascaridol. The ascaridol and their isomeric forms are organic components of the monoterpenes group, with antioxidant, analgesic, anthelmintic and sedative properties[9,12-14]. Souza et al. observed osteogenesis inducing activity promoted by mastruz andso it may be a promising candidate for treatment of bone disorders including osteoporosis[15]. However, despite the beneficial pharmacological effects of monoterpenes present in the mastruz extract, its chronic use may be toxic to certain organs. Further research are needed in this area of knowledge[13,15].

In this study there was a significant increase in AST, LDH and cholesterol, suggesting a possible hepatotoxic effect. Other studies evaluated the effects of chronic administration of mastruz. In the present essay, to evaluate the therapeutic effect of mastruz in osteoporosis, we used 50mg daily for 30 days. It should be noted that the animals in this study were treated with mastruz hydroalcoholic extract, then the amount of alcohol may have induced these alterations. Extracts obtained with the freeze-dried or aired in emulsions plant material can answer these questions and optimize the use of the compounds of mastruz.

Pereira et al. studied administration of mastruz at concentrations of 5, 50 and 500mg/kg by 15 days and concluded that the treatment induced specific changes in the groups treated with high doses, but they were not letal[13]. They did not use hydroalcoholic extract. Mastruz was manipulated after lyophilization of the plant material, supporting the hypothesis that the hepatotoxic effect may have been from alcohol and not mastruz.

Similar results were described by Holland et al., who treated rats for 10 days with propolis extract, at a dose of 100mg/Kg. They concluded that the treatment altered liver enzymes and alkaline phosphatase, causing a possible hepatotoxic effect[16]. The hepatotoxic effects of medicinal plants has been widely estudied[17]. People need to be alerted that the indiscriminate use of such substances may cause adverse effects. Studies comparing different doses and periods of administration are needed to clarify these questions.

The biodistribution of $Na^{99m}TcO_4$ has an important clinical significance in scintigraphic examinations, in which tissues capture the radiopharmaceutical to produce images. Several physiological, pathological and therapeutic conditions have been associated with changes in the activity $Na^{99m}TcO_4$ such as bariatric surgery, regular administration of herbal medicines, intestinal resection, inflammation, etc[11,18,19]. The mastruz was not able to alter the biodistribution in organs studied. So, we can infer that in animals and probably in humans, the chronic use of mastruz do not alter the results of scintigraphy with $Na^{99m}TcO_4$, avoiding false negative results. Further studies must be conducted on this line of research to elucidate the unclear questions.

CONCLUSION

According to the experimental model used in this study, it is concluded that administration of mastruz in rats did not alter the biodistribution of sodium pertechtenate. However, elevated levels of liver enzymes, suggest a possible hepatotoxic effect in the period and dose used.

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