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Table of Contents: Volume 8 Number 29 29 July, 2014

ARTICLES

Research Articles

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Full Length Research Paper

Biochemical alterations induced by phytotherapic tincture with antiophidic activity in male Wistar rats

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For more 30 years, phytotherapic tincture Específico-Pessôa has been used as supportive therapy in envenoming by snakebites in Brazilian folk medicine. However, little or no information is available in the literature about the safety/toxicity of this phytotherapic tincture. The present work was designed to investigate the effect of this tincture by maximum dose (0.75 ml kg^{-1} body weight) on acetyl- and butyrylcholinesterase and other biochemical parameters in male Wistar rats. Male rats were treated with 0.75 ml kg^{-1} body weight dose of phytotherapic tincture Específico-Pessôa, and biochemical parameters were evaluated in 24, 48 h and ten days after treatment. Clinical signs of toxicity, body weight gain and cholinesterasic activities in brain and liver were also observed. The phytotherapic tincture exhibited significant effect ($P < 0.05$) on weight body gain, organs weight ratio (brain, heart and lungs), aspartate transaminase (AST), acetyl- and butyrylcholinesterase activities, cholesterol and low dencity lypoproteins (LDL) levels. The results indicate that tincture is active on physiologic system. These findings suggest precaution in the use of this phytotherapic tincture at the dose utilized in this study.

Key words: Antivenom, cabenegrins, pterocarpans, snakebite, toxicity.

INTRODUCTION

It is well known that snakebites have an important role in morbidity and mortality in tropical and subtropical countries, particularly where agricultural activity is intense (Cruz et al., 2009). In Brazil, the highest incidence of ophidic accidents is related to different genres of the family Viperidae snakes (Silva et al., 2004). Effective treatment is administration of antivenom, which is determined according to the genre of the snake (Lallo and Theakston, 2003). However, there is a widespread practice, not only in Brazil but worldwide, of using extracts of local plants with possible antiophidic activity as the ancillary treatment (Gomes et al., 2010).

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Studies show that since 1980, a phytotherapic tincture prepared from Brazilian plants, have been used as supportive therapy in envenoming by snakebites, particularly in the north and northeast of Brazil (Nakagawa et al., 1982; Pierini et al., 1996). However, its use is currently being disseminated to other regions, such as southern Brazil. It is a hydroalcoholic extract of the root of a plant popularly known as "cabeça-de-negro" (*Cayaponia tuyuya* (Kell.) Cogn., *Cayaponia espelina* Cogn., *Annonna coriacea* (Mart.) and *Wibrandia sp*) (Militão et al., 2007) manufactured in Ceará (Brazil) and registered as Específico-Pessôa. Its antiophidic property is related to the presence of two pterocarpans, cabenegrin A-I and cabenegrin A-II, which were initially isolated by Nakagawa et al. (1982). The administration of these pterocarpans in mice previously envenomed with 2.5 times the lethal dose of venom of *Bothrops atrox*, restored to the physiological conditions of the animals within 24 h (Nakagawa et al., 1982). In beagle dogs, the cabenegrin A-I reversed the cardiovascular and cardiorespiratory effects induced by *B. atrox* venoms. In poisoned dogs and cats, the neuromuscular function was slowly restored within 24 h after the administration of these pterocarpans (Darko, 1984). Since then, studies has been developed and other biological properties were described for natural and synthetic pterocarpans as antibacterial, anti-inflamatory, antiproliferative and cytotoxic (Silva et al., 1997; Bodoh, 2007; Araújo et al., 2009; Zhou et al., 2009). The molecular mechanisms that lead to these properties are not yet clear; studies show that the action for some pterocarpans on inflammation and oxidative stress is related to the cholinergic system, via acetylcholinesterase and butyrylcholinesterase (Jung et al., 2010). However, acetyl- and butyrylcholinesterase are involved in the pathogenesis of neurodegenerative diseases and diabetes, respectively (Butterfield et al., 2007; Srinivas et al., 2012).

The use of this phytotherapic tincture has increased, not being more restricted in the regions north and northeast of Brazil. Despite these therapeutic advantages, little or no information is available in the literature about the safety/toxicity of this phytotherapic tincture in maximum dose. Therefore, the present study was designed to investigate the effect of the phytotherapic tincture Específico-Pessôa by maximum dose on acetyl- and butyrylcholinesterase and other biochemical parameters in male Wistar rats.

MATERIALS AND METHODS

Chemicals

Acetylthiocoline iodide (Ach), propionylthiocoline iodide (PTCh) and 5'5'-dithiobis(2-nitrobenzoic acid) (DTNB) were purchased from Sigma Chemical Co., USA. Ketamine hydrochloride and xylazine hydrochloride were obtained from Vetbrands (Brazil). All other chemicals were of the best available grade (98 to 99.8% purity).

Phytotherapic tincture and dosage formulation

The phytotherapic tincture Específico-Pessôa, a hydroalcoholic extract (manufactured in Ceará, Brazil; Register number 262 – Department of Public Health of Rio de Janeiro – ONSP) employed in the present work was purchased from drugstore local (Cascavel, Brazil). The usual adult dosage is 1.0 ml diluted in 14.0 ml of water, one to three times dialy. The dosage formulation was prepared by dilution of the test product in aqueous solution to produce the required dosing (ml/ml), according to recommendation of the guide that comes with the product.

Animals

Male albino rats (Wistar), weighing 210 to 360 g, provided by the Central Animal Facility of the University were fed *ad libitum* with a standard laboratory diet (Nuvilab®). They were housed at 22 ± 2°C in a room with a 12-h ligh/dark cycle in the propylene cages. Animal management was conducted according to the Brazilian regulations for the use of laboratory animals and the ethical principles for animal management.

Experimental design

Animals were divided into five groups each consisting of three to six animals. The phytotherapic tincture diluted was administered by gavage at a dosing volume of 0.75 ml kg^{-1} body weight. The dose level was selected by the guide that comes with the product, corresponding to three daily doses. Another group received high pure dose (without dilution) of phytotherapic tincture. The pure dose level was selected by reports of people using the pure product. Diluted phytotherapic tincture (0.75 ml kg^{-1} body weight) was given in a single dose (Group II and Group III) and the animals were sacrificed after 24 and 48 h, respectively. Group IV was treated with diluted phytotherapic tincture (0.75 ml kg^{-1} body weight) and on the 10th day, the animals were sacrificed. Animals of Group V received undiluted phytotherapic tincture (pure 0.75 ml kg^{-1} body weight) and on the 10th day the animals were sacrificed. Ten days was selected because the half-lives of butyrylcholinesterase and acethylcholinesterase were between 5 and 16 days (Solano et al., 2008) and 3 and 12 days (Krejci et al., 2006), respectively. Whereas, the objective of the study was to evaluate the anti-ophidian tincture and not the active compound alone, it was considered as control group (Group I), the rats treated with normal water by gavage. The animals were observed continuously for initial 30 min and intermittently for the next 6 h following gavage. Toxic manifestations, such as signs of toxicity and body weight changes, were monitored daily. At the end of the study, all rats were anesthetized for blood collection and subsequently sacrificed. The internal organs were weighed to determine relative organs weights and observed for gross lesions.

Collection of blood and organs

Under ketamine + xylazine anesthesia, the blood samples were obtained from intracardiac puncture and serum was separated by centrifugation at 3700 × g for 10 min. Subsequently, the animals were sacrificed by overdose of ketamine + xylazine anesthesia. The brain, heart, liver, lung and kidney were removed, weighed individually and calculated for organ weight ratio. Gross lesions were observed and organ weight ratio determined by the formula:

Ratio organs weights = (organ weight (g) / body weight (g)) × 100

Homogenate preparation for measurements of cholinesterase activities

Homogenates were prepared from rat brain and liver by adaptation of the methodology described by Cimasoni (1966). Animals were sacrificed by overdose of ketamine + xylazine anesthesia. Their brains and livers were removed immediately, weighted and cut into small pieces. These fragments were suspended in phosphate buffered Ringer's solution (pH 7.4) and 0.5% Triton X-100 Ringer's solution for liver and brain, respectively. Homogenization was carried out in the same medium by means of glass homogenizers on ice. Homogenization was followed by differential centrifugation at 536 × g for 10 min and 4000 × g for 10 min. Their supernatant fraction was used for biochemical assay. Acethylcolinesterase (AChE) and butyrylcholinesterase (BuChE) activities were measurement in brain and liver, respectively. Protein content of the homogenate was measured using Folin-phenol reagent and bovine-serum albumin as a standard (Lowry et al., 1951). The results were expressed in mg of protein ml^{-1}.

Measurement of cholinesterase activities in brain and liver

The acetyl- and butyrylcholinesterase activities were assayed by method of Ellman et al. (1961) using acethylthiocoline and propionylthiocoline as substrate, respectively. For determination of acetylcholinesterase, 0.25 mg protein of the brain supernatant fraction was added to the 100 mM phosphate buffer (pH 8.0) and 0.5 mM DTNB. After determination of the blank, the reaction was started by addition of 0.5 mM acetylthiocoline iodide. The change in extinction was recorded at 405 nm for 60 s. For butyrylcholinesterase activity was added 5 mM propionylthiocoline iodide and the change in extinction was recorded for 120 s. The specific enzyme activities were calculated as nmol of substrate hydrolysed min^{-1} mg $protein^{-1}$.

Estimation of biochemical parameters

Uric acid, albumin, alkaline phosphatase (ALKP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), cholesterol, creatinine, gamma glutamyltransferase (GGT), glucose, HDL-cholesterol, LDL-cholesterol, total protein, triglycerides and urea were assayed using diagnostic reagent kit by Ortho-Clinical Diagnostics VITROS 5,1 FS®. BuChE serum (BuChEs) was estimated by method of Ellman et al. (1961).

Treatment of data

Data are expressed as mean ± standard error of mean (SEM). The difference values between treated groups and control groups was evaluated using one-way analysis of variance (ANOVA) followed by Dunnet's t-test. Statistical significance was accepted at $P < 0.05$. GraphPad Prism® 3 software was used for statistical analysis.

RESULTS

Body weight gain and organ weight ratios

The absolute body weights and body weight gain of rats after 24, 48 h and 10 days of treatment are shown in Table 1 and Figure 1, respectively. After 24 and 48 h, the body weight gain of rats treated with phytotherapic tincture was higher than that of control group, with the ratios of phytotherapic tincture (g)/control (g) of 3.5 ($P < 0.0001$) and 1.4 ($P = 0.0308$), respectively. No change in body weight gain was observed in group V with the ratio of 1.0 ($P = 0.8421$), while a significant increase ($P < 0.0001$) was observed in the group IV as compared to control group, with the ratio of 2.3. As revelead by *t-test* analysis between groups IV and V ($P = 0.0033$), the influence of phytotherapic tincture after ten days on body weight was essentially dependent on its concentration, pure or diluted.

The phytoherapic tincture had no effect on liver and kidney weight ratio (Figure 2) in all treated groups, but the brain weight ratio in group V was reduced by 15.3% ($P < 0.01$) as compared to control group. The other groups showed unchanged brain weight ratio ($P > 0.05$), in addition statistic difference between IV and V groups was observed ($P = 0.0422$). The decrease in heart weight ratio was observed in groups IV (12.9%, $P < 0.01$) and V (20.6%, $P < 0.01$). The lung in both III and IV groups were increased by 13.2% ($P < 0.05$) as compared to the control group. However, no statistic difference between IV and V groups was observed ($P = 0.0738$). The groups treated with phytotherapic tincture showed agitation following drowsiness and grooming. The extract did not show any clinical adverse effect, like restlessness, diarrhea and muscle corrdinated movement on the animals. No mortality was observed. Macroscopic observation of organs did not show abnormalities.

Cholinesterasic activities in brain and liver

The acetylcholinesterase activity in brain homogenates of rats treated with phytotherapic tincture, except in the group V in which the increase was observed (16.5%, $P < 0.01$), remained practically unchanged in comparison to those from the control group ($P > 0.05$). AChE activity in control group was 60.1 ± 3.64 nmol min^{-1} mg $protein^{-1}$, while other groups had 61.8 ± 2.78 nmol min^{-1} mg $protein^{-1}$ (Group II), 65.3 ± 4.29 nmol min^{-1} mg $protein^{-1}$ (Group III), 67.6 ± 5.02 nmol min^{-1} mg $protein^{-1}$ (Group IV) and 70.0 ± 5.92 nmol min^{-1} mg $protein^{-1}$ (Group V) (Table 2). However, total proteins showed significant increase in all groups treated ($P < 0.01$) (Figure 3A). Quite similar results were obtained on total protein in liver with $P < 0.01$ in all groups treated (Figure 3B). On the other hand, the administration of undiluted phytotherapic tincture showed a decreased in BuChE activity around 2.50 nmol min^{-1} mg $protein^{-1}$ (32.5%, $P < 0.05$) as compared to the control group (6.93 ± 1.21 nmol min^{-1} mg $protein^{-1}$), while BuChE activities in the other groups were 7.70 ± 1.00 nmol min^{-1} mg $protein^{-1}$ (Group II), 7.13 ± 0.81 nmol min^{-1}

Table 1. Absolute body weights in control rats and rats treated with phytotherapic tincture.

Experimental group	Body weight (g)			
	Initial (n=6)	24 h/gain (n=6)	48 h/gain (n=6)	10 days/gain (n=6)
Control (Group I)	275.3 ± 22.31	279.2 ± 19.41 4.2 ± 0.543	285.0 ± 18.70 9.7 ± 0.882	295.0 ± 31.10 27.5 ± 5.57
Group II	277.3 ± 14.34	291.3 ± 14.00 14.0 ± 1.53***	—	—
Group III	250.7 ± 15.21	258.0 ± 18.33	265.0 ± 16.50 14.3 ± 3.67*	—
Group IV	237.3 ± 8.60	239.0 ± 8.62	262.7 ± 43.23	307.3 ± 24.18 70.0 ± 1.38***[a]
Group V	321.7 ± 23.28	328.3 ± 21.87	332.0 ± 21.90	350.8 ± 15.74 29.2 ± 9.28

Value represents mean ± SE obtained with 6 rats. The P values refer to *test t*. *P < 0.05, ***P < 0.0001 compared to the control group. [a]P < 0.001 compared to the group V. The number in parenthesis represents the number of the rats.

Table 2. Acetyl- and butyrylcholinesterase activities in brain and liver, respectively, in control rats and rats treated with phytoherapic tincture.

Parameter	(n=6)				
(nmol min^{-1} mg $protein^{-1}$)	Control (Group I)	Group II	Group III	Group IV	Group V
Acetylcholinesterase	60.1 ± 3.64	61.8 ± 2.78	65.3 ± 4.29	67.6 ± 5.02	70.0 ± 5.92**
Butyrylcholinesterase	6.93 ± 1.21	7.70 ± 1.00	7.13 ± 0.81	6.63 ± 1.50	4.41 ± 0.56*

Value represents mean ± SE obtained with 6 rats. The P values refer to Dunnett's test. *P<0.05 compared to the control group. **P<0.01 compared to the control group. The number in parenthesis represents the number of the rats.

mg $protein^{-1}$ (Group III) and 6.63 ± 1.50 nmol min^{-1} mg $protein^{-1}$ (Group IV) (Table 2).

Biochemical parameters

The results of serum biochemical parameters are shown in Table 3. Various parameters of lipid profile were measured, among them serum butyrylcholinesterase activity. The phytotherapic tincture treatment showed no significant effect on the levels of triglycerides (P > 0.05) and HDL-cholesterol (P > 0.05). However, an important increase was noted in total cholesterol (29%, P < 0.01) and LDL-cholesterol levels (148.7%, P < 0.01) in Group III when compared to control group. A decrease in plasma butyrylcholinesterase was observed in Goup V (38%, P < 0.01). The AST activity was clearly diminished in Group V (31%, P < 0.05), while no significant change was observed in other groups (P > 0.05). No signficant variability of AST activity was observed between groups IV and V (P > 0.05). Other parameters as ALT, ALKP, gamma glutamyltransferase (GGT), total protein serum, albumin, glucose, creatinine, urea and uric acid remained unchanged when compared with control group (Table 4).

DISCUSSION

The traditional or folkloric use of the plants extracts is a practice in most regions of the world, as complementary or alternative medicine (Gomes et al., 2010; Dey and De, 2012). However, the use of plants extracts without the availability of studies on their safety has elevated concerns on their efficacy and toxicity (Prasad and Shyma, 2012). Although there is knowledge of the therapeutic properties of cabenegrins, the literature shows no studies to phytotherapic tincture, particularly on the safety of its use. The present study was to evaluate the safety of this phytotherapic tincture by single dose in normal rats. The results observed in the present work show that phytotherapic tincture at the dose studied is active on physiologic system contributing to increases in

Table 3. Lipid profile and plasma butyrylcholinesterase activity in control rats and rats treated with phytoherapic tincture.

Parameter	Control (Group I)	Group II	Group III	Group IV	Group V
Tryglicerides (mg dl^{-1})	74.5 ± 9.87 (n=6)	92.7 ± 7.62 (n=5)	79.0 ± 3.51 (n=6)	72.3 ± 19.5 (n=4)	88.8 ± 10.2 (n=6)
Cholesterol (mg dl^{-1})	75.8 ± 5.32 (n=6)	68.3 ± 7.06 (n=6)	97.7 ± 2.33** (n=6)	77.0 ± 1.73 (n=6)	71.5 ± 1.71 (n=6)
HDL-cholesterol (mg dl^{-1})	43.8 ± 3.09 (n=6)	43.3 ± 3.84 (n=6)	44.7 ± 7.17 (n=6)	51.0 ± 1.00 (n=6)	47.7 ± 0.84 (n=6)
LDL-cholesterol (mg dl^{-1})	15.0 ± 3.42 (n=6)	6.7 ± 4.70 (n=6)	37.3 ± 9.68** (n=6)	9.3 ± 2.19 (n=6)	23.5 ± 8.23 (n=6)
BuChEs (ł)	2.9 ± 0.09 (n=6)	2.7 ± 0.12 (n=6)	2.5 ± 0.36 (n=3)	3.3 ± 0.43 (n=6)	1.8 ± 0.33** (n=6)

Value represents mean ± SE obtained with 3 to 6 rats. The P values refer to Dunnett's test. **P<0.01 compared to the control group. The number in parenthesis represents the number of the rats. łnmol min^{-1} mg $ptna^{-1}$.

Table 4. Hepatic indicators, renal markers and other biochemical parameters in control rats and rats treated with phytotherapic tincture.

Parameter	Control (Group I)	Group II	Group III	Group IV	Group V
AST (U L^{-1})	80.3 ± 8.93 (n=6)	68.3 ± 11.2 (n=6)	58.0 ± 2.00 (n=6)	55.7 ± 4.67 (n=6)	55.2 ± 5.29* (n=6)
ALT (U L^{-1})	43.7 ± 2.79 (n=6)	44.7 ± 2.85 (n=6)	57.0 ± 9.02 (n=6)	41.0 ± 7.37 (n=6)	39.7 ± 3.36 (n=6)
ALKP (U L^{-1})	239 ± 11.6 (n=6)	364 ± 33.0 (n=6)	359 ± 11.1 (n=6)	359 ± 17.0 (n=6)	276 ± 9.77 (n=6)
GGT (U L^{-1})	< 10 (n=5)	< 10 (n=5)	< 10 (n=3)	< 10 (n=4)	< 10 (n=5)
Total Protein (g dl^{-1})	3.6 ± 0.11 (n=6)	3.4 ± 0.10 (n=6)	3.7 ± 0.07 (n=6)	3.7 ± 0.03 (n=5)	3.6 ± 0.09 (n=6)
Albumin (g dl^{-1})	2.1 ± 0.18 (n=6)	1.9 ± 0.08 (n=5)	2.2 ± 0.21 (n=6)	2.1 ± 0.15 (n=6)	2.0 ± 0.07 (n=6)
Glucose (mg dl^{-1})	172 ± 13.2 (n=6)	213 ± 24.5 (n=6)	173 ± 2.60 (n=6)	240 ± 50.7 (n=6)	234 ± 30.9 (n=6)
Creatinin (mg dl^{-1})	0.36 ± 0.01 (n=6)	0.39 ± 0.02 (n=6)	0.43 ± 0.04 (n=6)	0.44 ± 0.06 (n=6)	0.46 ± 0.04 (n=6)
Urea (mg dl^{-1})	36.3 ± 0.99 (n=6)	33.3 ± 0.67 (n=6)	31.7 ± 2.19 (n=6)	33.7 ± 0.67 (n=6)	33.0 ± 0.63 (n=5)
Uric Acid (mg dl^{-1})	1.7 ± 0.32 (n=6)	0.9 ± 0.24 (n=6)	1.3 ± 0.29 (n=4)	0.8 ± 0.10 (n=6)	1.1 ± 0.39 (n=6)

Value represents mean ± SE obtained with 3-6 rats. The P values refer to Dunnett's test. *P<0.05 compared to the control group. The number in parenthesis represents the number of the rats.

body weight, protein synthesis in brain and liver, changes in lipid profile, decrease in serum butyrylcholinesterase and changes in AST activity and acetyl- and butyrylcholinesterase activities in brain and liver.

The data show that the tincture used in the dose recommended by package insert may contribute to increased body weight. Except for Group IV, the other groups treated with diluted tincture showed tendency to gain weight within 24 h. This did not occur with the group treated with undiluted tincture. Moreover, comparing the body weight gain between the undiluted group and the control group there was no statistically significant difference. Although studies show estrogenic effec of trifolirhizin pterocarpan on utero through the increased weight of this organ, the literature does not describe any study on the influence of pterocarpan on body weight gain (Abdel-Kader, 2010). On the other hand, there is the possibility of alcohol having influenced this parameter. An influence in weight gain has been reported by other authors. Studies show a correlation between the amount of alcohol intake and weight gain (Larue-Achagiotis et al., 1990). The authors show that the ingestion of a solution of alcohol 20% causes significant reduction in body weight while a solution to 10% leads to weight gain around the 8th day of treatment. The mechanism is complex. Somehow, alcohol affects the process of absorption, digestion,

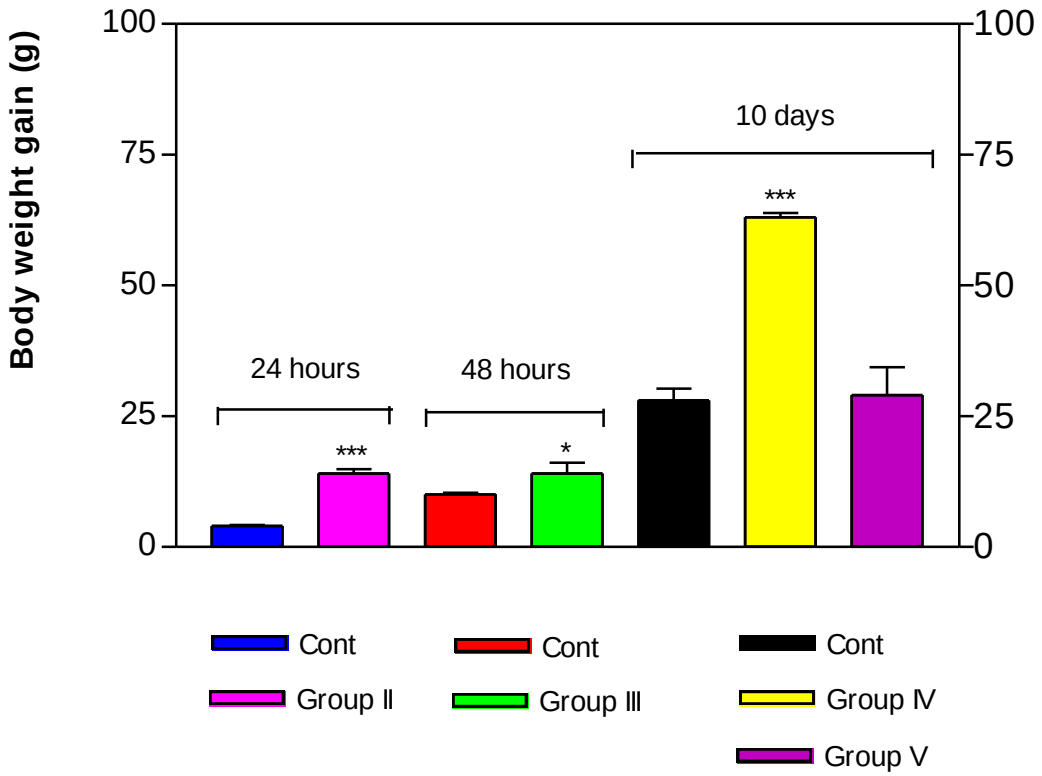

Figure 1. Body weight gain in control rats and rats treated with phytotherapic tincture. Value represents mean ± SE obtained with 6 rats. The P values refer to *test t*.
*P < 0.05, *** P < 0.0001 compared to the control group.

utiilization, storage and excretion of proteins, vitamins and minerals (MacDonald et al., 2010). The decreases in brain and heart weight ratios indicate a hypotrofic action on the development of these organs, while there is a hypertrophic action on the lung (Sellers et al., 2007). However, the causes for the variation of the actions cannot be inferred from the available data, whereas the structure of these organs was not microscopically examined.

A significant increase in total protein in brain and liver were also observed. Stimulation of the protein synthesis is an unexpected phenomenon because it is well established that pterocarpans, the main compound present in the phytotherapic tincture, has antimitotic activity by cell cycle arrest and apoptosis induction (Militão et al., 2007). Generally, these studies show the inhibition of protein synthesis being one of the processes involved in antimitotic activity (Militão et al., 2007; Thenmozhi and Mahadeva Rao, 2011).

The stimulus in protein synthesis, especially in brain and liver, may be a response in the change of absorption of dietary proteins and also the steroid action (Hayase et al., 1998; Wong et al., 1996). There are numerous examples in the literature showing that changes in quality and quantity of dietary protein intake affects brain protein synthesis, not only in young but also adult rats (Hayase and Yokogoshi et al., 1994; Tujioka et al., 2009). Moreover, it can directly reflect in the polyribosomal profile in the endoplasmatic reticulum in liver and consequently protein synthesis (Yokogoshi et al., 1980; Hayase et al., 1998). On the other hand, protein synthesis can also be caused by several factors, such as response to a period of stress, an adaptation of the organ, the presence of alcohol or a regenerative process (Baubet et al., 1996; Ogura et al., 2001; Zhu et al., 2013). It is recognized that alcohol, one the components of the tincture, is active on the biological system, especially on the liver metabolic system leading to several alterations in different organs (Epstein, 1997; Das et al., 2009; Toffolo et al., 2012). The interference of alcohol is a reasonable explanation considering that the control group had the same diet and experimental conditions, with no significant change in protein synthesis. In addition, the animals showed agitation and grooming after administration of phytotherapic tincture. It is believed that the alcohol present in the tincture has contributed to these effects including drowsiness observed after the period of agitation (Chabria, 2008).

The change in the acetylcholinesterase and butyrylcholinesterase activities only in Group V shows

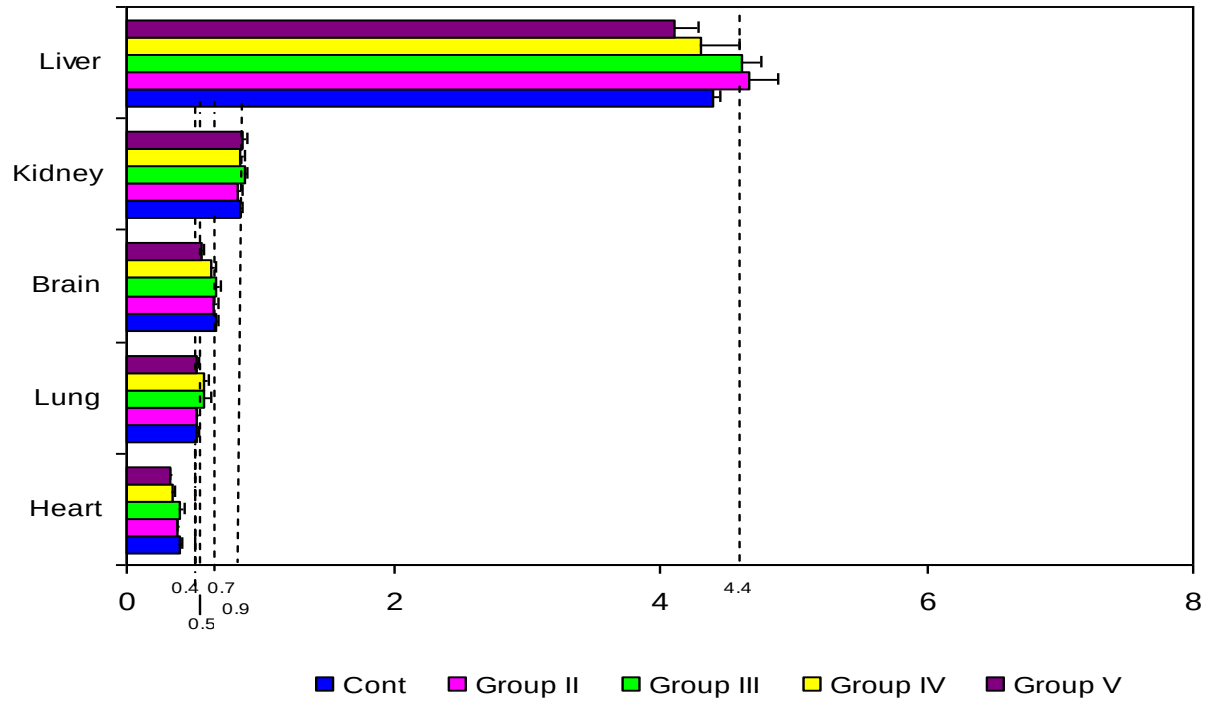

Figure 2. Organs weight ratio of the control rats and rats treated with phytotherapic tincture. Value represents mean ± SE obtainedwith 6 rats.

this effect is concentration-dependent. On the other hand, the difference in the effect of phytotherapic tincture on the activity of acetyl- and butyrylcholinesterase increase and decrease, respectively, is unclear. One possibility can be the difference in amino acid sequence between the two enzymes. Although acetyl- and butyrylcholinesterase have 65% amino acid sequence homologous, molecular structure and similar active site exhibit different specificity and catalityc rate (Perelman et al., 1990; Çokuğraş, 2003). Just comparing the primary structures of these two enzymes is not possible to deduce which can lead to different effects, this requires further study. The increase in the acetylcholinesterase activity is an important finding because studies show a link between reduced levels of acetylcholine in the brain and the presence of neurodegenerative diseases such as Alzheimer's disease (Lawrence and Sahakian, 1998). The ability of the phytotherapic tincture at pure 0.75 ml kg^{-1} body weight, increase the acetylcholinesterase activity, suggesting it may not be beneficial in the central nervous system and can result in neurological disorders.

The same dose significantly reduced serum and liver butyrylcholinesterase activities. Studies show butyrylcholinesterase as a marker of protein-energy malnutrition, obesity and liver and non liver diseases. The malnutrition, stress and inflammatory processes are factors that can lead to diminution in the activity of this enzyme (Santarpia et al., 2012). On the other hand, phenolic compounds, such as pterocarpans, are recognized for their ability to inhibit butyrylcholinesterase, among other enzymes. The inhibitory potential is attributed to the presence of groups, particularly –OH, which can form H-bonds with the amino acids residues at the active site of enzyme (Ahmad et al., 2006). Clinical studies showed positive association concerning butyrylcholinesterase activity with serum lipid levels, with possible involvement of this enzyme in lipid metabolism (Calderon-Margalit et al., 2006; Krnić et al., 2008). However, we found no evidence for butyrylcholinesterase in increased cholesterol and LDL levels, indicating that another mechanism may be involved, including an action of alcohol present in the phytotherapic tincture. Anyway, the ambiguous effect of the phytotherapic tincture on these two enzymes observed in the present work shows that despite similarity between the acetyl- and butyrylcholinesterase, the response after the same dose of xenobiotic is different. This certainly is related to each physiological function of each enzyme. This will be an important issue to be discussed in future work.

The increase in the cholesterol and LDL levels suggests that the phytotherapic tincture in 48 h can affect the carrier of cholesterol predisposes animals to cardiovascular risk (Oyedemi et al., 2010). Increased cholesterol level is a surprising phenomenon because

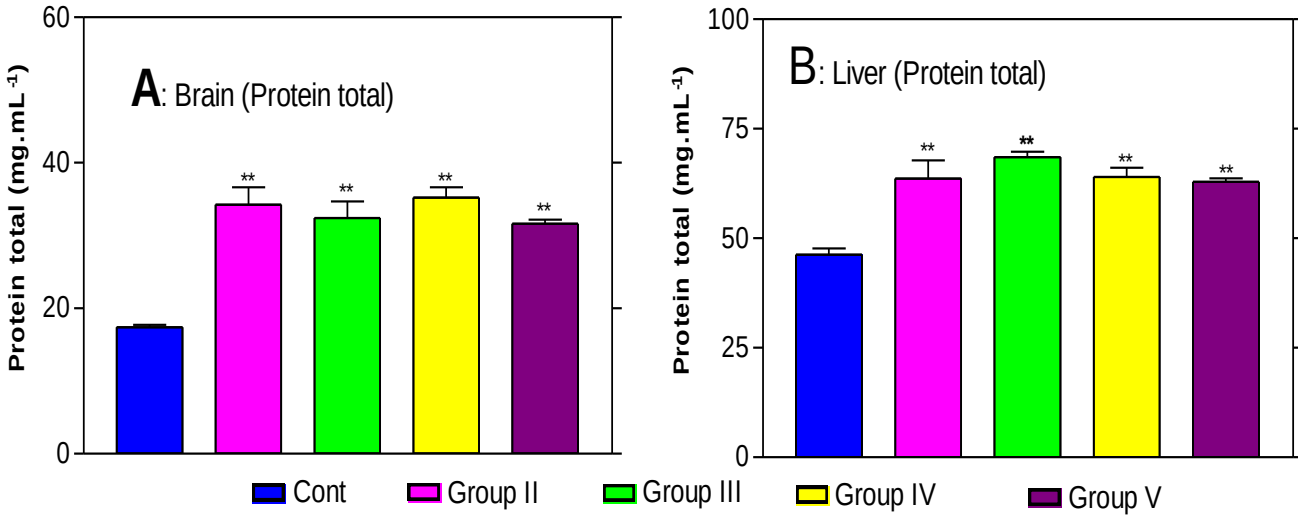

Figure 3. Protein total in brain (Panel A) and in liver (Panel B) of the control rats and rats treated with phytotherapic tincture. Value represents mean ± SE obtained with 6 rats.The P values refer to Dunnett's test. **P < 0.001 compared to the control group.

pterocarpan has been implicated in hypocholesterolemic effect. The molecular mechanisms for hypocholesterolemic effect of this compound remain not fully understood, maybe through their interference with steroid absorption process and LDL oxidation inhibitory activity (Lee et al., 2006). However, our experiment shows evidences of an interaction of phytotherapic tincture with lipidic metabolism, specifically on cholesterol levels. Some authors have reported the relationship between increase of cholesterol and protein metabolism with the channeling of peripheral amino acids for the hepatic protein synthesis (Goldberg et al., 1977) and others show that alcohol induces the absorption of cholesterol (Latour et al., 1999).

Regarding to the disease and toxicity to the liver, it is often revealed by increased transaminase levels. Our results showed that phytoherapic tincture specifically induced diminution in the AST level, demonstrating a direct effect on this enzyme and not on the ALT. Likewise the acetyl- and butyrylcholinesterase, AST and ALT have similarities, however they showed different response on the same substance. Decreases of AST may be associated with the low amounts of pyridoxal 5'phosphate, with the presence of inhibitors and toxic metabolites of nature proteins or some combination of these factors (Warnock et al., 1974; Hafkenscheid and Ven-Jongekrijg, 1979). Drugs with an aromatic ring are also potent inhibitors of the AST by mechanism of binding as by the solvation and steric effects (Bonsib et al., 1975). Perhaps this is a possible explanation for the decrease in AST level in this study, since ALT level remained unchanged.

Conclusion

Phytotherapic tincture Específico-Pessôa acts in a complex way on the physiologic organism. Probably some of the observed effects are due the action of alcohol of the tincture. These findings suggest that precaution should be taken in the utilization of this phytotherapic tincture and that some alterations on the biological system are concentration-dependent. No specific data are available for phytotherapic tincture in normal rats and more detailed investigations on this subject could help identify the mechanism of action of this tincture.

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Conflict of Interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this paper.

REFERENCES

Abdel-Kader MA (2010). Preliminary pharmacological study of the pterocarpans macckian and trifolirhizin isolated from the roots of

Ononis vaginalis. Pak. J. Pharm. Sci. 23(2):182-187.
Ahmad VU, Iqbal S, Nawaz AS, Choudhary MI, Farooq U, Ali ST, Ahmad A, Bader S, Kousar S, Arshad S, Tareen RB (2006). Isolation of four new pterocarpans from *Zygophyllum eurypterum* (Syn. Z. atriplicoides) with enzyme-inhibition properties. Chem. Biodivers. 3:996-1003.
Araújo RM, Lima MAS, Silveira ER (2009). Pterocarpans and a novel flavanone from *Harpalyce brasiliana* roots. J. Braz. Chem. Soc. 20(5):935-938.
Baubet V, Grance E, Sermet E, Giaume M, Fay N, Bobillier P (1996). Widespread increase in brain protein synthesis following acute immobilization stress in adult rat brain. Neurosci. Lett. 219(3):187-190.
Bodoh B (2007). Synthesis of Pterocarpan Cabenegrin A-II. Oshkosh Scholar, vol II. University of Wisconsin Board of Regents. pp. 71-79.
Bonsib SM, Harruf RC, Jenkins WT (1975). Factors contributing to the inhibition of aspartate aminotransferase by dicarboxylic acids. J. Biol. Chem. 250(22):8635-8641.
Butterfield DA, Reed T, Newman SF, Sultana R (2007). Roles of amyloid B-peptide-associated oxidative stress and brain protein modifications in the pathogenesis of Alzheimer's disease and cognitive impairment. Free Rad. Biol. Med. 43:658-677.
Calderon-Margalit R, Adler B, Abramson JH, Gofin J, Kark JD (2006). Butyrylcholinesterase activity, cardiovascular risk factors, and mortality in middle-aged and Elderly men and women in Jerusalem. Clin. Chem. 52(5):845-852.
Chabria SB (2008). Impatient management of alcohol withdrawal: a practical approach. Signa Vitae 3(1):24-29.
Cimasoni G (1966). Inhibition of cholinesterases by fluoride in vitro. Biochem. J. 99:133-137.
Çokuğraş AN (2003). Butyrylcholinesterase: structure and physiological importance. Turk. J. Biochem. 28(2):54-61.
Cruz LS, Vargas R, Lopes AA (2009). Snakebite envenomation and death in the developing world. Ethnic. Dis. 19:42-46.
Darko LL (1984). Method of treating mammals for effects of neuro- and cardiovascular toxins. United States Patent. Patent No. 4,443,472.
Das SK, Dhanya L, Varadhan S, Mukherjee S, Vasudevan DM (2009). Effects of chronic ethanol consumption in blood: a time dependent study on rat. Indian J. Clin. Biochem. 24(3):301-306.
Dey A, De JN (2012). Phytopharmacology and antiophidian botanicals: a review. Int. J. Pharmacol. 8(2):62-79.
Ellman GL, Courtney KD, Andres V, Featherstone RM (1961). A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem. Pharmacol. 7:88-95.
Epstein M (1997). Alcohol's impact on kidney function. Alcohol Health Res. World. 21(1):84-93.
Goldberg AC, Eliaschewitz FG, Quintão EC (1977). Origin of hypercholesterolemia in chronic experimental nephrotic syndrome. Kidney Int. 12(1):23-27.
Gomes A, Das R, Sarkhel S, Mishra R, Mukherjee S, Bhattacharya S, Gomes A (2010). Herbs and herbal constituents active against snake bite. Indian J. Exp. Biol. 48(9):865-878.
Hafkenscheid JCM, Ven-Jongekrijg J (1979). Influence of pyridoxal-5'-phosphate on the determination of the alanine aminotransferase and asparte aminotransferase of commercial test sera. J. Clin. Chem. Clin. Biochem. 17:219-223.
Hayase K, Yokogoshi H (1994). Age affects brain protein synthesis in rats. J. Nutr. 124:683-688.
Hayase K, Koie M, Yokogoshi H (1998). The quantity of dietary protein affects brain protein synthesis rate in aged rats. J. Nutr. 128:1533-1536.
Jung HA, Yokozawa T, Kim BW, Jung JH, Choi JS (2010). Selective inhibition of prenylated flavonoids from Sophora flavescens agains BACE1 and cholinsesterases. Am. J. Chin. Med. 38(2):415-429.
Krejci E, Valenzuela IM-P, Ameziane R, Akaaboune M (2006). Acetylcholinesterase dynamics at the neuromuscular junction of live animals. J. Biol. Chem. 281(15):10347-10354.
Krnić Z, Tiljak MK, Topić RZ, Bradamante V (2008). Correlation between serum butyrylcholinesterase activity and serum lipid concentrations in rats treated with diferente antagonists of the adrenergic system. Period. Biol. 110(1):57-62.
Lallo D, Theakston RDG (2003). Snake antivenoms. J. Toxicol. 41:277-290.
Larue-Achagiotis C, Poussard AM, Louis-Sylvestre J (1990). Alcohol drinking, food and fluid intakes and body weight gain in rats. Physiol. Behav. 47:545-548.
Latour MA, Patterson BW, Kitchens RT, Ostlund Jr RE, Hopkins D, Schonfeld G (1999). Effects of alcohol and cholesterol feeding on lipoprotein metabolism and cholesterol absorption in rabbits. Arterioscler. Thromb. Vasc. Biol. 19:598-604.
Lawrence AD, Sahakian BJ (1998). The cognitive psychopharmacology of Alzheimer's disease: focus on cholinergic systems. Neurochem. Res. 23:787-794.
Lee JH, Lee BW, Kim JH, Jeong TS, Kim MJ, Lee WS, Park KH (2006). LDL-antioxidant pterocarpans from roots of *Glycine max* (L.) Merr. J Agric Food Chem. 54(6):2057-2063.
Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951). Protein measurement with Folin phenol reagent. J. Biol. Chem. 193:265-272.
MacDonald IO, Olusola OJ, Osaigbovo UA (2010). Effects of chronic etanol administration of body weight, reduced glutathione (GSH), malondialdehyde (MDA) levels and glutathione-s-transferase activity (GST) in rats. NY Sci. J. 3(4):39-47.
Militão GCG, Pinheiro SM, Dantas INF, Pessoa C, Moraes MO, Costa-Lotufo L, Lima MAS, Silveira ER (2007). Bioassay-guided fractionation of pterocarpans from roots of Harpalyce brasiliana Benth. Bioorg. Med. Chem. 15:6687-6691.
Nakagawa M, Nakanishi K, Darko LL, Vick JA (1982). Structures of cabenegrins A-I and A-II, potent anti-snake venoms. Tetrahedron Lett. 38:3855-3858.
Ogura Y, Hamanoue M, Tanabe G, Mitsue S, Yoshidome S, Nuruki K, Aikou T (2001). Hepatocyte growth factor promotes liver regeneration and protein synthesis after hepatectomy in cirrhotic rats. Hepatogastroenterology 48(38):545-549.
Oyedemi SO, Yakubu MT, Afolayan AJ (2010). Effect of aqueous extract of Leonotis leonorus (L.) leaves in male Wistar rats. Hum. Exp. Toxicol. 29:377-384.
Perelman A, Abeijon C, Hirschberg CB, Inestrosa NC, Brandan E (1990). Differential association and distribution of acetyl- and butyrylcholinesterases within rat liver subcellular organelles. J. Biol. Chem. 265(1):214-220.
Pierini SV, Warrel Da, De Paulo A, Theakston RDG (1996). High incidence of bites and stings by snakes and other animals among rubber tappers and Amazonian indians of the Jurua Valley, Acre State, Brazil. Toxicon. 34(2):225-236.
Prasad AGD, Shyma TB (2012). Evaluation of anti-oxidante activity (in vitro) of Hecheria subpetalta (Willd.) Kunth. leaves. J. Med. Plants Res. 6(9):1562-1566.
Santarpia L, Grandone I, Contaldo F, Pasanisi F (2012). Butyrylcholinesterase as a prognostic marker: a review of the literature. J. Cachexia Sarcopenia Muscle.doi: 10.1007/s13539-012-0083-5.
Sellers RS, Morton D, Michael B, Roome N, Johnson JK, Yano BL, Perry R, Shafer K (2007). Society of Toxicologic Pathology Position Paper: Organ Weight Recommendations for Toxicology Studies. Toxicol. Pathol. 35:751-755.
Silva GL, Matos FJA, Silveira ER (1997). 4'-dehydroxycabenegrin A-I from roots of *Harpalyce brasiliana*. Phytochemistry. 46(6):1059-1062.
Silva JM, Coelho AL, Simas ABC, Moraes RAM, Pinheiro DA, Fernandes FFA, Arruda EZ, Costa PRR, Melo PA (2004). Synthesis and pharmacological evaluation of prenylated and benzylated pterocarpans against snake venom. Bioorg. Med. Chem. Lett. 14:431-435.
Solano MI, Thomas JD, Taylor JT, McGuire JM, Jakubowski EM, Thomson SA, Maggio VL, Holland KE, Smith JR, Capacio B, Woolfitt AR, Ashley DL, Barr JR (2008). Quantification of nerve agent VX-butyrylcholinesterase adduct biomarker from an accidental exposure. J. Anal. Toxicol. 32:68-72.
Srinivas K, Sridhar GR, Allam AP (2012). Secondary structure of butytylcholinesterase. J. Diabetes Metab. 3:199.
Thenmozhi A, Mahadeva Rao US (2011). Evaluation of antimitotic

activity of Solanum torvum using Allium cepa root meristamatic cells and anticancer activity using MCF-7-human mammary gland breast adenocarcinoma cells lines. Drug Invent. Today 3(12):290-296.
Toffolo MCF, Aguiar-Nemer AS, Silva-Fonseca VA (2012). Alcohol: Effects on nutritional status, lipidic profile and blood pressure. J. Endocrinol. Metab. 2(6):205-211.
Tujioka K, Ohsumi M, Horie K, Kim M, Hayase K, Yokogoshi H (2009). Dietary y-aminobutiric acid affects the brain protein synthesis rate in ovariectomized female rats. J. Nutr. Sci. Vitaminol. 55:75-80.
Warnock LG, Stone WJ, Wagner C (1974). Decreased aspartate aminotransferase (“SGOT”) activity in serum of uremic patients. Clin. Chem. 20(9):1213-1216.
Wong M, Thompson TL, Moss RL (1996). Nongenomic actions of strogens in the brain: physiological significance and cellular mechanisms. Crit. Rev. Neurobiol. 10(2):189-203.
Yokogoshi H, Sakuma Y, Yoshida A (1980). Relationships between nutritional quality of dietary proteins and hepatic polyribosome profiles in rats. J. Nutr. 110:383-387.
Zhou H, Lutterodt H, Cheng Z, Yu L (2009). Anti-inflammatory and antiproliferative activities of trifolirhizin, a flavonoid from *Sophora flavescens* roots. J. Agric. Food Chem. 57:4580-4585.
Zhu Y, Wang Y, Zhao B, Wei S, Xu M, Liu E, Lai J (2013). Differential phosphorylation of GluN1-MAPKs in rat brain reward circuits following long-term alcohol exposure. PLos ONE 8(1):e54930.

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Full Length Research Paper

Biocompatibility research of a novel biodegradable ion exchange resin

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The *in vivo* pharmacological and toxicological investigation of biodegradable ion exchange resin was carried out to provide evidence for further clinical utilizations. Acute toxicity study, general pharmacological studies, hemolytic experiments, systemic hypersensitivity experiments and vascular stimulation experiments were conducted. The general pharmacological effects of the biodegradable ion exchange resin on the nervous system of mice, the functional coordination of mice, the hypnosis of mice treated with nembutal at subliminal dose, the autonomic activities of tested mice, and the heart rate, blood pressure, electrocardiograph (ECG) and breathing of the anesthetic cats. The LD_{50} of biodegradable ion exchange resin to mice by tail intravenous injection was 129.37 mg kg^{-1}, and the 95% credible limit was 121.65 ~ 137.58 mg kg^{-1}. The biodegradable ion exchange resin did not have significant influence on the animals in the general pharmacological studies in the experimental conditions described in this study. Besides, the biodegradable ion exchange resin did not have hemolytic and erythrocyte aggregate effect and was qualified for allergy test under the dose condition in this experiment. Neither did it have obvious irritant effect on the blood vessels of rabbit ears. The desirable pharmacological and toxicological behaviors of the biodegradable ion exchange resin exhibited indicated that this novel formulation had great biocompatibility and had great potential for clinical utilizations.

Key words: Pharmacology, toxicology, biodegradable ion exchange resin, biocompatibility.

INTRODUCTION

For years, considerable attentions have been drawn to injectable biodegradable microspheres for their application on drug delivery systems (Okada et al., 1989; Lee et al., 2009) which stemmed from the merits including ease of application, localized delivery for a site-specific action, prolonged delivery periods, and improved patient compliance and comfort (Levy et al., 1996; Sultana et al., 2009). However, some properties of injectable biodegradable microspheres become obstacles for their future application, such as burst, incomplete or

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uncontrollable drug release (Kumar et al., 2001). Among all these disadvantages, initial burst release is the most major concern because it has the potential to increase side effects. Although efforts have been made to reduce the burst release (Soriano et al., 1996; Nahata and Saini, 2008), few microspheres without burst release have been reported.

Ion exchange resins are water-insoluble, cross-linked, high-molecular weight poly-electrolyte containing salt-forming groups in repeating positions on the polymer chain. Recently, they have been widely used as carriers in drug delivery systems, showing a number of improved properties, such as better stability, better taste, fewer side effects, and more uniform absorption and sustained release (Guo et al., 2009; Kouchak and Atyabi, 2010; Salve, 2011). One of the most impressive advantages of the ion exchange polymers is that theoretically the drug release from the ion exchange polymers is determined by the ion concentration in the surrounding medium. When the ion exchange polymers are introduced into the body, the body's natural counter ion concentration stabilizes drug release, hence eliminating burst release profile. So if we can combine the biodegradable polymer with the ion exchange technology to make the biodegradable and ion exchangeable microspheres, it may provide a new solution for the "burst release" problem.

In previous studies, we successfully prepared biodegradable and ion exchangeable resin and developed a drug delivery system, using ambroxol hydrochloride (AH) as a model drug (Liu et al., 2011). Briefly, we combined a biodegradable polymer, carboxymethyl chitosan and ion exchange technology to prepare the AH lung targeting microspheres. The microspheres were characterized and studied for the drug release *in vitro* in different ionic concentration dissolution mediums.

In this study, to further investigate the possibility of biodegradable ion exchange resin for clinical utilizations, we carried out pharmacological and toxicological studies. Acute toxicity study, general pharmacological studies, hemolytic experiments, systemic hypersensitivity experiment and vascular stimulation experiment were conducted. Animals including mice, cats, rabbits and guinea pigs were employed, respectively to finish these experiments. This study will provide valuable messages for the potential applications of this novel biodegradable ion exchange resin.

MATERIALS AND METHODS

Biodegradable ion exchange resin was prepared in our lab. Nembutal was purchased from Shanghai Chemical Reagent Company of China Pharmaceutical Group (Shanghai, China, batch number: F20110815). Animals were kindly provided by the Experimental Animal Center of Shenyang Pharmaceutical University (Liaoning, China), including male Kunming rats weighing 250 ± 20 g, male New Zealand white rabbits weighing 2.1 kg (license: SYXK (Liaoning) 2011-0013), male and female guinea pigs weighing 300 ~ 350 g (license: SYXK (Liaoning) 2011-0013), and male and female Kunming mice weighing 18 to 22 g (license: SCXK (Liaoning) 2011-009). Cats were purchased from the market by the Experimental Animal Center of Shenyang Pharmaceutical University. Autonomic activity tester for mice was provided by Beijing pharmaceutical institute (ZIR-2, Beijing, China). RM6240CD multi-channel bio-signal acquisition and processing system was purchased from Chengdu Instrument Factory (Sichuan, China).

Acute toxicity study of Intravenous injection

Pre-experiment: 16 mice were divided into four groups randomly with 4 in each group. According to the doses of 0.40, 0.28, 0.2, 0.14 ml/10 g, 4.5 mg L^{-1} (the largest concentration) of the ion exchange resin was administered through tail intravenous injection. The mice were observed of the toxic reaction and death. 100% estimated lethal dose (Dm) and 0% estimated lethal dose (Dn) were found out.

The effect on nervous system in mice by tail intravenous injection

The tested mice were divided into four groups at random with 10 in each group, half male and half female. 3, 6 and 12 mg kg^{-1} of the biodegradable ion exchange resin was administered by intravenous injection with 0.1 ml/10 g, and the control group was administrated with 5% glucose injection with 0.1 ml/10 g. Irwin behavior experimental method was employed to investigate the influence of the biodegradable ion exchange resin on the righting reflex, passive state, muscle twitching, salivation and nystagmus of mice before administration and at 5, 10, 30, 60 and 90 min after administration (Janice and Irwin, 1993). The righting reflex and passive state of tested mice were graded according to the standard documents, and muscle twitching, salivation and nystagmus were graded according to Table 1.

The effect on functional coordination of mice (rotating rods method)

The tested mice were screened, and the mice which could climb up for 3 min on rotating rods were qualified. The qualified mice were then divided into four groups at random, half male and half female in each group. 3, 6 and 12 mg kg^{-1} of the biodegradable ion exchange resin was administered to the three experimental groups with 0.1 ml/10 g by intravenous injection. 0.1 ml/10 g of 5% glucose injection was administered to the control group. At 30 min after administration, mice were put on rotating rods (16 r min^{-1}) to calculate the percentage of mice which fell within 1 min.

The effect on hypnosis of mice treated with nembutal at subliminal dose

80 mice were divided into four groups evenly with the same condition of sex and weight, half male and half female in each group. The control group was intravenously administered with 5% glucose injection, while the rest three groups were intravenously administered with the biodegradable ion exchange resin using the dose of 3, 6 and 12 mg kg^{-1}, respectively with 0.1 ml/10 g. After 30 min, 25 mg kg^{-1} nembutal was intraperitoneally injected to each tested mouse with 0.1 ml/10 g. If the righting reflex disappeared for 1 min, the mouse was regarded as being asleep. Each group was observed for the number of mice falling asleep at 30 min after the injection of nembutal.

Table 1. The score standard of the muscle twitching, salivation and nystagmus in mice.

Item	Degree	Score
Muscle twitching	None	0
	Lightly	4
	Strongly	8
Salivation	None	0
	Few	4
	Many	8
Nystagmus	None	0
	Lightly	4
	Strongly	8

The effect of the biodegradable ion exchange resin on the autonomic activities of tested mice

The tested mice were divided into four groups at random, half male and half female in each group. Before the determination of autonomic activity, the mice were put on the autonomic activity box, ZIR-2 (Beijing drug research institute) for 3 min to be adapted. The mice were recorded for their frequency of autonomic activity within 3 min as the index before administration. Then each experimental group was given the biodegradable ion exchange resin with doses of 3, 6 and 12 mg kg^{-1}, respectively by intravenous administration, and the control group was intravenously administered with 5% glucose injection. At 15, 30, 60, and 90 min after administration, the frequency of autonomic activities of each mouse in 3 min were determined. The frequency of autonomic activities in the experimental groups was compared with that in the control group with t-test.

The effect of the biodegradable ion exchange resin on heart rate, blood pressure, ECG and breathing of the anesthetic cats

24 cats were evenly divided into four groups with same sex and similar weight, the control group and the low, medium and high dose group, respectively. Cats were anesthetized by intraperitoneal injection of 20% urethane (1.0 g kg^{-1}) and fixed at the back. The anterior portion skin of mice was disinfected conventionally, and a central longitudinal incision with length of approximately 4 to 5 cm was made below the prominentia laryngea. The carotid artery on one side was separated, and an artery intubation was inserted and connected to RM6240 biological signal acquisition and processing system through the pressure transducer. The mean arterial pressure (MBP, mmHg) was recorded. A transverse incision was made at epigastric side with the length of approximately 4 to 5 cm and the muscle layer was incised to open the abdominal cavity. The position of duodenum was determined for administration. At the same time, ECG electrode was connected to record ECG and heart rate and processus xiphoideus was separated and connected with muscle tension transducer to synchronously record the breathing frequency and breathing depth. The administration was not conducted until each index became steady. 1, 2 and 4 mg·kg^{-1} of the biodegradable ion exchange resin were injected with 4 ml kg^{-1} through duodenum to jejunum. After administration we continuously observed for 90 min to respectively record changes of indexes above each cat before the administration, and 5, 15, 30, 60 and 90 min after the administration. The significance of each index between experimental group and control group was obtained by t-test of paired data.

Hemolytic experiments *in vitro*

10 ml of venous blood sample taken from rabbit ear margin was stirred for 10 min with a glass rod (top twined with absorbent cotton) to remove fiber protein, and then centrifuged (1500 rpm × 15 min). The upper clear liquid was removed and tenfold amount of 5% glucose was added to wash three times (upper clear liquid was abandoned after centrifuged) until the upper liquid showed no red color. Then 5% glucose was added to make 2% erythrocyte suspension. According to Table 2, various solutions were added into 7 test tubes. Among them, tube 6 was 5% glucose blank control and tube 7 was the positive control (distilled water). After gently shaken up, the 7 test tubes were incubated at 37°C water bath. The samples were observed for 3 h, every fifteen minutes in the first hour and every 1 h after the first hour. The result was evaluated according to the standard listed in Table 3.

The systemic hypersensitivity experiment in guinea pigs

Dose grouping

1. The low dose group of biodegradable ion exchange resin: Sensitization dose of 1 ml (0.2 mg)/time for five times (ip) and aggressive dose of 2 ml (0.4 mg)/time for one time (iv).
2. The high dose group of biodegradable ion exchange resin: Sensitization dose of 1 ml (0.4 mg)/time for five times (ip) and aggressive dose of 2 ml (0.8 mg)/time for one time (iv).
3. The positive control group of egg white injection: Sensitization dose of 1 ml/time for five times (ip) and aggressive dose of 2 ml/time for one time (iv).
4. The negative control group was 5% glucose injection with the same volume.

24 white healthy guinea pigs were randomly divided into four groups, named as the low, high dose group of biodegradable ion exchange resin, the positive control group of egg white injection and the 5% glucose negative control group, 6 in each group with half male and half female. On the next day, each guinea pig in each group was administered (ip) with the sensitization dose for continuous five times. The reaction of the guinea pigs was observed after each injection. At 10 days after the last administration, an aggressive dose was administered (iv) with 2 ml

Table 2. Application of sample in hemolytic test *in vitro.*

Application of sample (ml)	The number of the tube						
	1	2	3	4	5	6	7
2% RBC	2.5	2.5	2.5	2.5	2.5	2.5	2.5
The biodegradable ion exchange resin	0.1	0.2	0.3	0.4	0.5	0	0
5% Glu	2.4	2.3	2.2	2.1	2.0	2.5	0
Distilled water	0	0	0	0	0	0	2.5
Final concentration (mg/ml)	0.1	0.2	0.3	0.4	0.5	-	-

Table 3. The evaluation of hemolytic test result.

Degree	Sign	Phenomenon
No haemolysis	-	All of the RBC sink and the upper stratum solution is colourless and clear.
Haemolysis partly	±	The solution is red or marron, and there is a little of RBC depositing at the bottom of the tube.
Haemolysis	+	The solution is red and clear. None of the RBC deposited at the bottom of the tube.
Aggregation		The RBC is aggregating,and it didn't disperse after vibrating.

Table 4. The LD_{50} of the biodegradable ion exchange resin in mice by vein injection.

Group	Dose (mg/kg)	Number	Number of the death	Mortality (%)
1	200	10	10	100
2	180	10	9	90
3	162	10	8	80
4	146	10	8	80
5	131	10	8	80
6	118	10	5	50
7	106	10	1	10
8	95	10	0	0

of the corresponding drug or solvent. The guinea pigs were observed for 30 min to see if there were any allergic symptoms such as catching nose, standing hair, difficult breathing, spasm and shock until death. The results were comprehensively evaluated by the degree of allergic reactions, rate of appearance, dead situation and level of allergic reaction in guinea pigs.

Vascular stimulation experiment

1. The experimental group: The biodegradable ion exchange resin, 1 ml (5 mg)/kg/time × 1 time × 3 d.
2. The control group: 5% glucose injection, 1 ml/kg/time × 1 time × 3 d.

6 rabbits were divided into two groups randomly. The experimental group was administrated through left ear with 1 ml (5 mg)/kg/time. The control group was administrated through left ear with 5% glucose injection of the same volume as control. The two groups were administered continuously for 3 days with one time in one day. After 48 h from the last administration, the rabbits were sacrificed. The ears of the rabbits were cut at 1 and 5 cm under the needle spot. No swelling and maculopapular were observed by naked eyes. 10% formaldehyde was used for fixing, and paraffin slice was made dyed by HE. The vascular endothelium, subcutaneous tissue and thrombus were observed under an optical microscopy.

RESULTS AND DISCUSSION

Acute toxicity results of intravenous injection

The result showed that Dm was 180 mg kg^{-1} and Dn was 90 mg kg^{-1}. 8 groups were set up, 10 mice in each group with 5 male and 5 female. The dose distance was 1: 0.9. Rate of 200, 180, 162, 146, 131, 118, 106, and 95 mg kg^{-1} of the ion exchange resin was administered to the 8 groups, respectively with 0.2 ml/20 g to proceed acute toxicity experiment. 5 min after administration, dead mice began to appear in high dose group. The death time of mice mainly concentrated in 12 to 24 h. The toxic mice mainly behaved as reposing motionless, slow-moving, difficult breathing, restless moving before death, jumping and urinary incontinence. No obvious pathological changes were observed in the main organs by visual inspection. The experimental result could be seen in Table 4.

The LD_{50} of biodegradable ion exchange resin to mice by tail intravenous injection was 129.37 mg kg^{-1}, and the 95% credible limit was 121.65 ~ 137.58 mg kg^{-1}.

Table 5. Effect of the biodegradable ion exchange resin on the nervous system of mice.

Index	Before	After (min)				
	0	5	10	30	60	90
Righting reflex	0±0	0±0	0±0	0±0	0±0	0±0
Passive state	0±0	0±0	0±0	0±0	0±0	0±0
Muscle twitching	0±0	0±0	0±0	0±0	0±0	0±0
Salivation	0±0	0±0	0±0	0±0	0±0	0±0
Nystagmus	0±0	0±0	0±0	0±0	0±0	0±0

Table 6. Effect of 5% Glu on the nervous system of mice.

Index	Before	After (min)				
	0	5	10	30	60	90
Righting reflex	0±0	0±0	0±0	0±0	0±0	0±0
Passive state	0±0	0±0	0±0	0±0	0±0	0±0
Muscle twitching	0±0	0±0	0±0	0±0	0±0	0±0
Salivation	0±0	0±0	0±0	0±0	0±0	0±0
Nystagmus	0±0	0±0	0±0	0±0	0±0	0±0

Table 7. Effect of the biodegradable ion exchange resin on the functional coordination in mice (rotating rods method).

Group	Does (mg/kg)	Number	Rate of falling (%)
Control	-	10	0
The biodegradable ion exchange resin	3	10	0
	6	10	0
	12	10	0

P > 0.05, compared with control.

The effect on nervous system in mice by tail intravenous injection

Average scoring of each group was calculated, as shown in Tables 5 and 6. Though in the observation of righting reflex, passive state, muscle twitching, salivation and nystagmus of the biodegradable ion exchange resin and 5% glucose, the results indicated that by tail intravenous injection, biodegradable ion exchange resin did not have significant influence on nervous system and general behavior of sober mice with the dose used in this experiment.

The effect on functional coordination of mice (rotating rods method)

The result could be seen in Table 7. The result indicated that the biodegradable ion exchange resin with chosen range of dose by tail intravenous injection did not have significant influence on the functional coordination of mice by rotating rods method, showing good property of biodegradable ion exchange resin.

The effect on hypnosis of mice treated with nembutal at subliminal dose

The experimental groups and control group were compared by X^2 testing with the result shown in Table 8. Compared with the control group, the result of X^2 testing was $P > 0.05$, which indicated that the biodegradable ion exchange resin did not have obvious hypnotic effect on the mice treated with nembutal at subliminal dose.

The effect of the biodegradable ion exchange resin on the autonomic activities of tested mice

The result was shown in Table 9. The result showed that there was no significant difference in the frequency of autonomic activities before and after the administration of

Table 8. Effect of the biodegradable ion exchange resin on mice treated with nembutal at subliminal dose.

Group	Dose (mg/kg)	Number	Number of rats falling asleep	Rate of falling asleep (%)
Control	-	20	1	5
The biodegradable ion exchange resin	3	20	1	5
	6	20	0	0
	12	20	0	0

P > 0.05, compared with control.

Table 9. Effect of the biodegradable ion exchange resin on autonomic activities in mice.

Group	Dose (mg/kg)	Before	After (min)			
		0	15	30	60	90
Control	-	120.5±51.4	116.5±54.8	80.7±33.8	70.4±33.4	51.3±35.6
Biodegradable ion exchange resin	3	128.3±40.9	114.1±58.8	98.6±50.7	82.6±44.1	37.5±23.7
	6	115.7±47.1	83.0±43.8	64.5±30.8	48.6±26.5	50.0±23.0
	12	120.4±58.0	94.5±36.2	70.8±25.6	49.1±18.0	61.9±21.7

Mean ± SD, n = 10, P > 0.05, compared with control.

Table 10. Effect of the biodegradable ion exchange resin on heart rate in anesthetized cats.

Time	5% Glu	Biodegradable ion exchange resin		
	4 ml/kg	1 mg/kg	2 mg/kg	4 mg/kg
0′	175±30	194±35	206±29	208±11*
5′	171±27	196±36	204±28	211±15**
15′	176±29	206±28	219±28*	210±10*
30′	190±17	203±27	219±23*	211±13*
60′	184±15	209±25	218±18**	212±11**
90′	202±17	208±30	224±25	207±14

Mean ± SD, vices/min, n=6 ; *P<0.05, **P<0.01, compared with 5% Glu.

the biodegradable ion exchange resin, compared with the control group, indicating that the biodegradable ion exchange resin did not have the effect on the automomic activities of tested mice.

The effect of the biodegradable ion exchange resin on heart rate, blood pressure, ECG and breathing of the anesthetic cats

Results were shown in Tables 10 to 18. Mice were orally administered with 3, 6 and 12 mg kg^{-1} of the biodegradable ion exchange resin and there was no significant influence on nervous system, general behavior, function coordination and hypnotic effect of mice treated with nembutal at subliminal dose. Within the 90 min after 1, 2 and 4 mg kg^{-1} of the biodegradable ion exchange resin was injected to cat duodenum, the heart rate, blood pressure, breathing and ECG of the cats did not make significant changes in each experimental group compared with the control group, showing good biocompatibility of biodegradable ion exchange.

Hemolytic experiments *in vitro*

Hemolysis or part of hemolysis was not observed in the total three hours. All the erythocytes sank in the solution with upper colorless transparent liquid and was dispersed after mixed up. The 5% glucose control group did not show hemolytic and aggregate phenomena within three hours. The distilled water control group showed hemolysis at all time points. The result was shown in Table 19. This experiment indicated that biodegradable

Table 11. Effect of the biodegradable ion exchange resin on mean arterial blood pressure (MAP) in anesthetized cats.

Time	5% Glu	Biodegradable ion exchange resin		
	4 ml/kg	1 mg/kg	2 mg/kg	4 mg/kg
0′	31±5	29±2	27±7	27±4
5′	30±5	30±1	29±3	28±3
15′	30±5	29±2	27±3	28±3
30′	31±5	28±6	27±5	28±6
60′	29±6	28±3	27±4	28±3
90′	30±7	27±3	26±8	27±3

Mean ± SD; Kpa, n = 6. *P<0.05, **P<0.01, compared with control.

Table 12. Effect of the biodegradable ion exchange resin on breathing rate in anesthetized cats mean ± SD, vices/min, n = 6), *P<0.05,**P<0.01, compared with control.

Time	5% Glu	Biodegradable ion exchange resin		
	4 ml/kg	1 mg/kg	2 mg/kg	4 mg/kg
0′	36±14	42±15	46±21	43±18
5′	40±19	44±18	40±13	45±14
15′	40±12	44±16	44±18	44±17
30′	33±6	45±20	41±10	44±18
60′	32±8	45±19	44±14	48±19
90′	32±5	49±19	42±13	46±19

Table 13. Effect of the biodegradable ion exchange resin on Respiratory depth in anesthetized cats mean ± SD, g, n=6), P>0.05, compared with control.

Time	5% Glu	Biodegradable ion exchange resin		
	4 ml/kg	1 mg/kg	2 mg/kg	4 mg/kg
0′	7.84±3.22	7.06±2.89	8.13±3.91	7.55±1.54
5′	7.38±2.73	6.69±2.82	6.93±3.56	7.16±1.68
15′	7.34±2.67	7.09±2.70	7.51±3.75	7.21±2.05
30′	7.50±2.41	7.59±2.72	7.70±2.92	7.58±2.54
60′	8.80±2.27	7.86±2.53	7.74±3.21	7.60±2.81
90′	8.98±3.02	7.83±2.99	7.43±2.60	7.99±2.91

ion exchange resin did not have hemolytic and erythrocyte aggregate effect in the experimental conditions.

The systemic hypersensitivity experiment in guinea pigs

When the sensitization dose was administered (ip) for 5 times, the 4 groups all showed no abnormal reaction. After each administration, the guinea pigs in the low and high dose group of biodegradable ion exchange resin exhibited normal activity, ingestion and drinking as the negative control group (5% glucose injection). On the 10th day after the last administration, each group was attacked by administering (iv) an aggressive dose. In the experimental groups of low and high dose of biodegradable ion exchange resin, the guinea pigs did not have any obvious allergic reaction so the level of allergic reaction was 0. But in the positive control group (egg white injection) appeared serious and significant allergic symptoms, containing difficult breathing, twitching, urinary incontinence and then shock to death. The death time was within one minute and the death rate was 100%. The level of allergic reaction of the positive control group was the 4th grade. No abnormal reaction was observed in the negative control group of glucose injection. The experimental result indicated that the biodegradable ion exchange resin was qualified for allergy test under the

Table 14. Effect of the biodegradable ion exchange resin on P wave of electrocardiogram in anesthetized cats.

Time	5% Glu	Biodegradable ion exchange resin		
	4 ml/kg	1 mg/kg	2 mg/kg	4 mg/kg
0′	0.17±0.07	0.16±0.04	0.19±0.05	0.22±0.09
5′	0.14±0.07	0.17±0.06	0.17±0.04	0.19±0.06
15′	0.18±0.08	0.17±0.05	0.17±0.04	0.24±0.09
30′	0.21±0.10	0.18±0.06	0.20±0.05	0.23±0.09
60′	0.21±0.07	0.17±0.04	0.18±0.02	0.25±0.08
90′	0.23±0.07	0.14±0.05*	0.18±0.03	0.21±0.05

Mean ± SD, mV, n = 6. *P<0.05, compared with control.

Table 15. Effect of the biodegradable ion exchange resin on T wave of electrocardiogram in anesthetized cats.

Time	5% Glu	Biodegradable ion exchange resin		
	4 ml/kg	1 mg/kg	2 mg/kg	4 mg/kg
0′	0.45±0.15	0.28±0.13	0.29±0.11	0.38±0.15
5′	0.45±0.14	0.28±0.12	0.29±0.09	0.39±0.13
15′	0.46±0.16	0.29±0.13	0.28±0.12	0.36±0.14
30′	0.42±0.17	0.29±0.09	0.29±0.08	0.37±0.16
60′	0.40±0.15	0.25±0.10	0.29±0.07	0.42±0.18
90′	0.37±0.14	0.27±0.09	0.25±0.08	0.32±0.14

Mean ± SD, mV, n = 6. *P<0.05, compared with control.

Table 16. Effect of the biodegradable ion exchange resin on QRS wave of electrokardiogram in anesthetized cats.

Time	5% Glu	Biodegradable ion exchange resin		
	4 ml/kg	1 mg/kg	2 ml/kg	4 mg/kg
0′	29±11	24±10	23±5	27±6
5′	29±9	27±9	25±5	29±8
15′	28±8	26±9	27±7	27±6
30′	26±8	26±7	25±9	27±5
60′	29±10	27±7	25±5	26±6
90′	25±8	23±8	28±8	26±5

Mean ± SD, ms, n=6. P>0.05, compared with control.

Table 17. Effect of the biodegradable ion exchange resin on P-Rinterval of electrokardiogram in anesthetized cats.

Time	5% Glu	Biodegradable ion exchange resin		
	4 ml/kg	1 mg/kg	2 mg/kg	4 mg/kg
0′	48±6	46±3	44±3	47±8
5′	43±5	45±7	42±3	46±7
15′	47±4	45±3	43±6	46±7
30′	45±5	47±3	46±4	45±5
60′	45±6	45±6	43±7	47±8
90′	48±4	45±8	43±4	47±5

Mean ± SD, ms, n = 6. P>0.05, compared with control.

Table 18. Effect of the biodegradable ion exchange resin on Q-T interval of electrocardiogram in anesthetized cats.

Time	5% Glu	Biodegradable ion exchange resin		
	4 ml/kg	1 mg/kg	2 mg/kg	4 mg/kg
0′	237±25	231±40	216±17	213±12
5′	241±29	232±32	216±13	212±13
15′	239±25	221±28	203±14*	213±14
30′	233±17	221±29	201±12**	211±10*
60′	229±14	221±25	204±8**	211±15
90′	219±15	214±21	205±11	217±20

Mean ± SD, ms, n = 6. *P<0.05, **P<0.01, compared with control.

Table 19. The hemolytic test result of the biodegradable ion exchange resin *in vitro.*

Time (h)	Number of cuvette						
	1	2	3	4	5	6	7
0.5	-	-	-	-	-	-	+
1	-	-	-	-	-	-	+
3	-	-	-	-	-	-	+
4	-	-	-	-	-	-	+

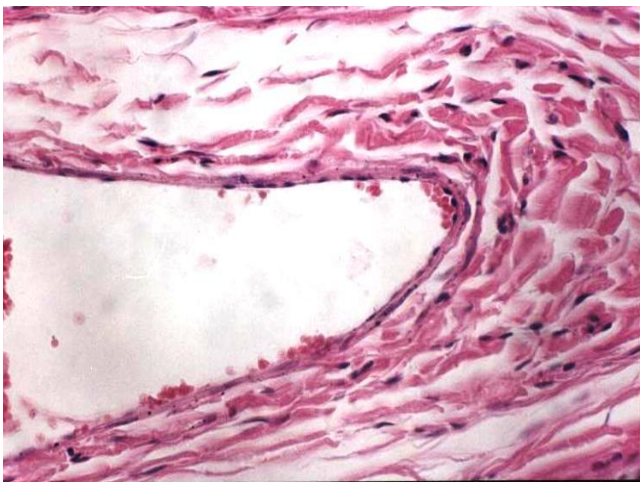


Figure 1. The pathological histology picture of the rabbit ear vein, 1 cm under the injection site of biodegradable ion exchange resin.

Vascular stimulation experiment

1. Naked eye observations: The veins in ears for injection in experimental group did not have obvious changes like congestion, exudation, edema, necrosis etc.
2. Microscopic observations: (1) The experimental group: In the 3 cases of blood vessels at 1 cm under the injection site, different amount of erythrocytes were observed. The vascular endothelium did not have swelling hyperplasia and no thrombus appeared in the vein. No infiltration and necrosis of inflammatory cells were observed in the surrounding tissues of the blood vessels. In the 3 cases at 5 cm under the injection site, the vascular endothelium did not have swelling hyperplasia and no thrombus appeared in the vein. No infiltration and necrosis of inflammatory cells were observed in the surrounding tissues of the blood vessels. (2) 5% glucose injection group: In the 3 cases at 1 cm under the injection site and the 3 cases at 5 cm under the injection site, there were no apparent pathological changes in and out of the blood vessels. Results could be seen in Figures 1 to 4. Biodegradable ion exchange resin did not have

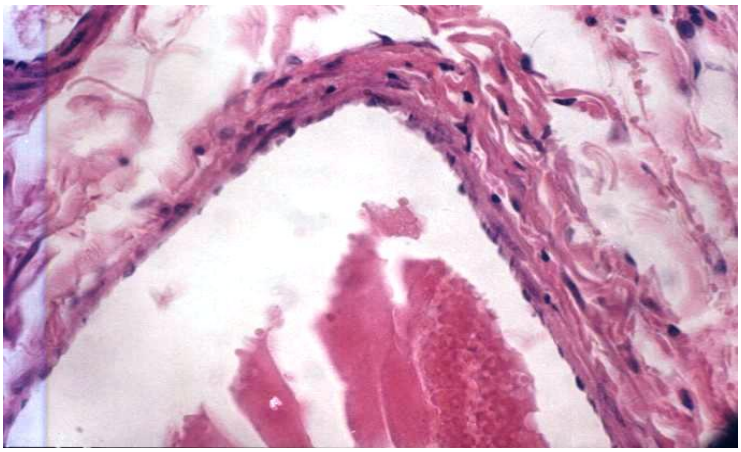


Figure 2. The pathological histology picture of the rabbit ear vein, 1 cm under the injection site of 5% glucose.

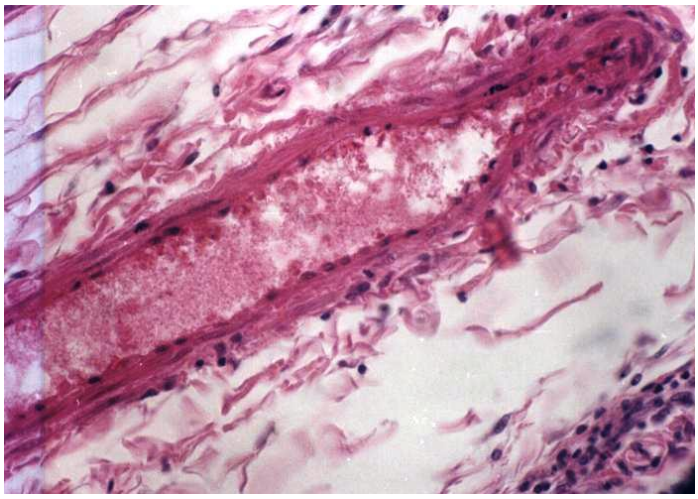


Figure 3. The pathological histology picture of the rabbit ear vein, 5 cm under the injection site of biodegradable ion exchange resin.

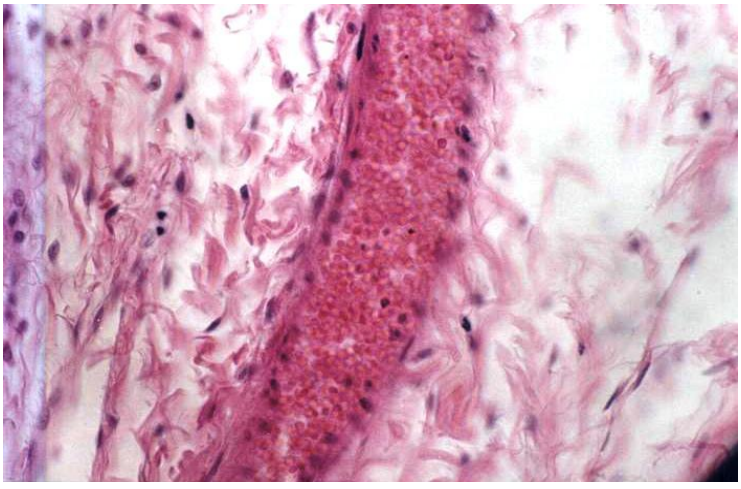


Figure 4. The pathological histology picture of the rabbit ear vein, 5 cm under the injection site of 5% glucose.

obvious irritant effect on the blood vessels of rabbit ears.

Conclusion

In the present study, *in vivo* pharmacological and toxicological research was investigated for the novel biodegradable ion exchange resin. Acute toxicity study, general pharmacological studies, hemolytic experiments, systemic hypersensitivity experiment and vascular stimulation experiment were conducted. The acute toxicity study results showed that LD_{50} of biodegradable ion exchange resin to mice by tail intravenous injection was 129.37 mg kg^{-1}. The biodegradable ion exchange resin did not have significant influence on the animals in the general pharmacological studies. Besides, the biodegradable ion exchange resin did not have hemolytic and erythrocyte aggregate effect and was qualified for allergy test under the dose condition in this experiment. Neither did it have obvious irritant effect on the blood vessels of rabbit ears. The desirable pharmacological and toxicological behaviors of the biodegradable ion exchange resin exhibited indicate that this novel formulation has great potential for clinical utilizations.

REFERENCES

Guo X, Chang RK,Hussain MA (2009). Ion-exchange resins as drug delivery carriers. J. Pharm. Sci. 98:3886-3902.

Janice ZR, Irwin LG (1993). The relationship between organizational transfer climate and positive transfer of training. Human Resourc. Dev. Qua. 4377–4390

Kouchak M, Atyabi F (2010). Ion-exchange, an approach to prepare an oral floating drug delivery system for diclofenac. Iran. J. Pharm. Res. 3:93-97.

Kumar N, Majeti NV, Ravikumar, Domb AJ (2001). Biodegradable block copolymers.Adv. Drug Deliv. Rev. 53:23-44.

Lee J, nTan CY, Lee SK, Kim YH, Lee KY (2009). Controlled delivery of heat shock protein using an injectable microsphere/hydrogel combination system for the treatment of myocardial infarction. J. Control Release 137:196-202.

Levy RJ, Labhasetwar V, Strickberger SA, Thomas U, James D (1996). Controlled release implant dosage forms for cardiac arrhythmias: Review and perspectives. Drug Deliv. 3:137-142.

Liu HF, He Y, Zhao Y,Ke P(2011). Preparation of ambroxol hydrochloride carboxymethyl chitosan micropheres without burst release. Afr. J. Pharm. Pharmacol. 5:1063-1069.

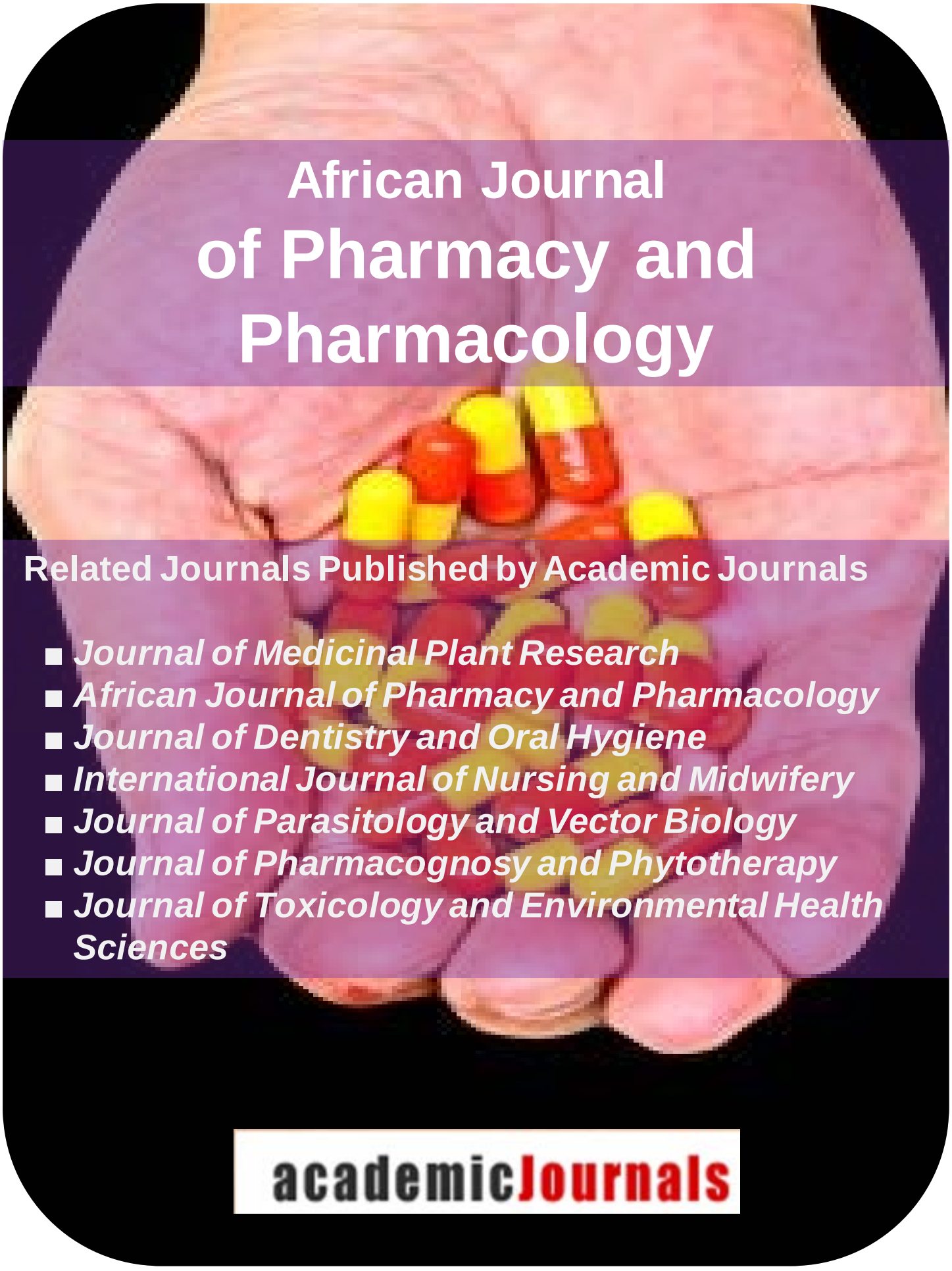
Nahata T, Saini TR (2008). D-optimal designing and optimization of long acting microsphere-based injectable formulation of aripiprazole. Drug Dev. Ind. Pharm. 34:668-675.

Okada H, Heya T, Igari Y, Ogawa Y, Toguchi H, Shimamoto T (1989). One-month release injectable microspheres of leuprolide acetate inhibit steroidogenesis and genital organ growth in rats. Int. J. Pharm. 54:231-239.

Salve P (2011). Development of sustained release beads for salbutamol sulphate using ion exchange resin. Asian J. Pharm. Tech. 1(4):104-118.

Soriano I, Delgado A, Kellaway I, Evora C (1996). Effect of surfactant agents on the in vitro release of insulin from DL-PLA microspheres. Drug Dev. Ind. Pharm. 22:1009-1012.

Sultana Y, Mall S,Maurya DP,Kumar D,Das M(2009). Preparation and in vitro characterization of diltiazem hydrochloride loaded alginate microspheres. Pharm. Dev. Technol. 14(3):321-331.



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