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 density lipoproteins (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).
Studies show that since 1980, a phytotherapeutic 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 espeilina Cogn., Annona coriacea (Mart.) and Wibrandia sp) (Militão et al., 2007) manufactured in Ceará (Brazil) and registered as Especifico-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 after the administration of Bothrops atrox venoms. In poisoned 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 have been developed and other biological properties were described for natural and synthetic pterocarpans as antibacterial, anti-inflammatory, 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 phytotherapeutic tincture has increased, not being more restricted in the regions north and northeast Brazil. Despite these therapeutic advantages, little or no information is available in the literature about the safety/toxicity of this phytotherapeutic tincture in maximum dose. Therefore, the present study was designed to investigate the effect of the phytotherapeutic tincture Especifico-Pessôa by maximum dose on acetyl- and butyrylcholinesterase and other biochemical parameters in male Wistar rats.

MATERIALS AND METHODS

Chemicals

Acetylthiocholine iodide (Ach), propionythiocholine iodide (PTCh) and 5'-S-thiosulfato(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).

Phytotherapeutic tincture and dosage formulation

The phytotherapeutic 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 daily. 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 light/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 phytotherapeutic tincture diluted was administrated 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 phytotherapeutic tincture. The pure dose level was selected by report of people using the pure product. Diluted phytotherapeutic 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 undiluted phytotherapeutic tincture (0.75 ml kg⁻¹ body weight) and on the 10th day, the animals were sacrificed. Animals of Group V received undiluted phytotherapeutic 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 acetylcholinesterase 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 antiophidic 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 the 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:

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organ weight ratio = (organ weight / body weight) × 100
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GraphPad Prism® 3 software was used for statistical analysis. Dunnet's t-test. Statistical significance was accepted at P < 0.05.

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. Acetylcholinesterase (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 acetylthiocholine and propionylthiocholine 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 acetyliothiocholine iodide. The change in extinction was recorded at 405 nm for 60 s. For butyrylcholinesterase activity was added 5 mM propionylthiocholine 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 phytotherapeutic tincture was higher than that of control group, with the ratios of phytotherapeutic 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 phytotherapeutic tincture after ten days on body weight was essentially dependent on its concentration, pure or diluted.

The phytotherapeutic 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 phytotherapeutic tincture showed agitation following drowsiness and grooming. The extract did not show any clinical adverse effect, like restlessness, diarrhea and muscle coordinated 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 phytotherapeutic 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 phytotherapeutic 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.

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>Body weight (g)</th>
<th>Initial (n=6)</th>
<th>24 h/gain (n=6)</th>
<th>48 h/gain (n=6)</th>
<th>10 days/gain (n=6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (Group I)</td>
<td></td>
<td>275.3 ± 22.31</td>
<td>279.2 ± 19.41</td>
<td>285.0 ± 18.70</td>
<td>295.0 ± 31.10</td>
</tr>
<tr>
<td>Group II</td>
<td></td>
<td>277.3 ± 14.34</td>
<td>291.3 ± 14.00</td>
<td>14.0 ± 1.53</td>
<td>27.5 ± 5.57</td>
</tr>
<tr>
<td>Group III</td>
<td></td>
<td>250.7 ± 15.21</td>
<td>258.0 ± 18.33</td>
<td>265.0 ± 16.50</td>
<td>14.3 ± 3.67*</td>
</tr>
<tr>
<td>Group IV</td>
<td></td>
<td>237.3 ± 8.60</td>
<td>239.0 ± 8.62</td>
<td>262.7 ± 43.23</td>
<td>307.3 ± 24.18</td>
</tr>
<tr>
<td>Group V</td>
<td></td>
<td>321.7 ± 23.28</td>
<td>328.3 ± 21.87</td>
<td>332.0 ± 21.90</td>
<td>350.8 ± 15.74</td>
</tr>
</tbody>
</table>

Value represents mean ± SE obtained with 6 rats. The P values refer to *t* test. *P < 0.05, ***P < 0.0001 compared to the control group. **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.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control (Group I)</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
<th>Group V</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetlycholinesterase (nmol min⁻¹ mg protein⁻¹)</td>
<td>60.1 ± 3.64</td>
<td>61.8 ± 2.78</td>
<td>65.3 ± 4.29</td>
<td>67.6 ± 5.02</td>
<td>70.0 ± 5.92**</td>
</tr>
<tr>
<td>Butyrylcholinesterase</td>
<td>6.93 ± 1.21</td>
<td>7.70 ± 1.00</td>
<td>7.13 ± 0.81</td>
<td>6.63 ± 1.50</td>
<td>4.41 ± 0.56*</td>
</tr>
</tbody>
</table>

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 phytherapeutic 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 Group 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 significant 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 cabenegins, the literature shows no studies to phytherapeutic tincture, particularly on the safety of its use. The present study was to evaluate the safety of this phytherapeutic tincture by single dose in normal rats. The results observed in the present work show that phytherapeutic 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 phytoherapeutic tincture.

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

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.

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

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

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 effect 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,
utilization, storage and excretion of proteins, vitamins and minerals (MacDonald et al., 2010). The decreases in brain and heart weight ratios indicate a hypotrophic 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 phytotherapeutic 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; Tajioka 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 phytotherapeutic 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 1.** Body weight gain in control rats and rats treated with phytotherapeutic 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.
Figure 2. Organs weight ratio of the control rats and rats treated with phytotherapic tincture. Value represents mean ± SE obtained with 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 catalytic rate (Perelman et al., 1990; Čokuğraş, 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; Kmić 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
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 phytotherapeutic 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 phytotherapeutic 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**

Phytotherapeutic tincture Especifico-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 phytotherapeutic tincture and that some alterations on the biological system are concentration-dependent. No specific data are available for phytotherapeutic 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.

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