SUBCHRONIC TOXICITY AND MOTOR COORDINATION OF ANIMALS TREATED WITH ASSOCIATION OF *p*-SYNEPHRINE, EPHEDRINE, SALICIN AND CAFFEINE

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Introduction: The association of *p*-synephrine, ephedrine, salicin and caffeine in dietary supplements and weight loss products is very frequently in worldwide and largely used, although the ephedrine was prohibit in many countries. The security and effectiveness of these products are controversy and none study about their toxicity can be found on the scientific literature. Previous toxicological studies found acute toxicity of the mixture of these compounds in male and female mice indicating the necessity of more studies regarding this subject.
Objective: The aim of this study was evaluate the subchronic toxicity and motor coordination of the association of *p*-synephrine, ephedrine, salicin and caffeine.
Materials and Methods: To subchronic toxicity test male and female Wistar rats (n=8 per group) were treated for 28 consecutive days by oral gavage with water (control) or 50, 75, 100 and 150 mg/kg of the association of *p*-synephrine, ephedrine, salicin and caffeine (proportion of 10:4:6:80 respectively). Daily, body weight was measured and animals were observed for signs of toxicity, morbidity and mortality. At the end of the period animals were sacrificed and necropsied. Blood were collected for hematological, biochemical and oxidative stress evaluation, including hematocrit, hemoglobin, alanine and aspartate aminotransferase (ALT and AST respectively), creatinine, creatine kinase MB fraction (CK-MB), malondialdehyde (MDA), reduced glutathione (GSH), delta-aminolevulinate dehydratase (ALA-D) and glutathione peroxidase (GPx). The results were analyzed by ANOVA/Bonferroni. To locomotor coordination test, male mice CF1 (n=7 per group) were treated by oral gavage with water (control) or 300 and 400 mg/kg of the mixture, submitted to rota-rod apparatus, and the latency to fall from the equipment (one 60s trial) was determined 30, 60, 90 and 120 min after administration. These results were analyzed by ANOVA of repeated measures/Bonferroni. All the experimental protocols were approved by CEP/UFRGS-2007982.
Results and Discussion: Repeated-dose oral subchronic toxicity study of the mixture of *p*-synephrine, ephedrine, salicin and caffeine showed no significative alterations in hematological and biochemical parameters both in male and female rats as well as no clinical signs of toxicity, weight alterations and deaths occurred. However some oxidative alterations could be observed. In males, MDA increased significantly ($p<0,05$) in group treated with 150mg/kg in comparison with controls, 50 and 75mg/kg treated groups, indicating oxidative damages. In females were observed alterations in antioxidant system expressed by GPx increase in groups treated with 75 and 150mg/kg. Amphetamines and analogues as *p*-synephrine and ephedrine could increase the production of free radicals and lead to imbalance in anti/oxidant system. The rota-rod test in mice showed that the latency to fall was significantly decreased ($p<0,05$) in 400mg/kg group compared with controls and 300 mg/kg group. These results highlight the motor incoordination provoked by the association, corroborating results found in previously acute toxicity test when the toxic signs were more intense at 400 than at 300mg/kg. All the substances used in this test have a direct or indirect adrenergic stimulation. So, the association of them could be potentiate the stimulus and promote toxicity.
Conclusions: The results demonstrated that the association of *p*-synephrine with ephedrine, salicin and caffeine showed low subchronic toxicity in the tested doses in rats, but they can alter the oxidative metabolism. More long-term studies such 90-days toxicity should be made to better clarification of the association's effects. In the motor coordination test was possible to observe toxicity as had already been found in previous experiments. So, the use of products containing the questioned association should be used with caution until the toxicological profile to be total elucidated.

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