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A protocol for evaluating the safety of herbal preparations in a rat model: the case of a supercritical fluid extract of Saw Palmetto

[Un protocolo para evaluar la seguridad de preparaciones herbales en un modelo de ratas: el caso de un extracto fluido supercrítico de Saw Palmetto]

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Abstract

Herbal extracts must be evaluated for their efficacy and safety. *In vivo* acute toxicity studies must consider the different mechanisms by which active compounds may elicit toxicological outcomes. Thus, a methodology to test general parameters related to acute toxicity responses in a murine model was developed, using a Saw Palmetto extract (HiPower®): adult male Sprague-Dawley rats were treated orally with two doses of HiPower® (the recommended dose for humans and a dose 10-fold higher) for 10 days, to examine general homeostatic parameters (hemogram and clinical chemistry) as well as morphological features of tissues involved in the response to xenobiotics (liver, kidney, spleen, and lymphatic ganglia). None of the parameters analyzed underwent significant changes during treatment, suggesting that HiPower® displays a good safety profile for the period tested. This method may be adopted for testing the *in vivo* acute toxicity of herbal extracts.

Keywords: Saw Palmetto, safety profile, Sprague-Dawley rats, acute toxicity.

Resumen

Los extractos herbales deben ser evaluados en cuanto a eficacia y seguridad. Estudios de toxicidad aguda *in vivo* deben considerar los diferentes mecanismos por los cuales los principios activos pueden producir toxicidad. Por consiguiente, se desarrolló una metodología para examinar parámetros generales relacionados con las respuestas de toxicidad aguda en un modelo murino, utilizando un extracto de Saw Palmetto (HiPower®): ratas Sprague-Dawley macho fueron tratadas con dos dosis de HiPower® (la dosis recomendada para humanos y una dosis 10 veces mayor) durante 10 días, para ensayar parámetros generales homeostáticos (hemograma y perfil bioquímico), así como características morfológicas de tejidos involucrados en la respuesta a xenobióticos (hígado, riñón, bazo y ganglios linfáticos). Ninguno de los parámetros analizados sufrió cambios significativos durante el tratamiento, sugiriendo que HiPower® presenta un buen perfil de seguridad durante el periodo evaluado. Este método puede ser adoptado para ensayar la toxicidad aguda *in vivo* de extractos herbales.

Palabras Clave: Saw Palmetto, perfil de seguridad, ratas Sprague-Dawley, toxicidad aguda.

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INTRODUCTION

Compounds occurring in plants have been used for millennia to treat diseases; this use has set the basis for the isolation of such compounds for therapeutic use. Only recently, however, scientific research on herbal extracts has begun to fully validate their therapeutic use and safety. Most of this research is focused on the therapeutic application of compounds found in herbal extracts. For instance, extracts from leaves of plants are rich in antioxidant compounds, such as polyphenols, which can be useful for the treatment of pathologies associated to oxidative stress (e.g. neurodegenerative diseases). Nevertheless, assessing the safety of herbal extracts is a challenging endeavour, due to their complex nature. In contrast to purified compounds, herbal extracts display a number of different molecules with potentially very different biological targets; interaction of herbal compounds with their targets may lead to beneficial and/or toxic consequences. Therefore, it becomes necessary to develop a methodology that evaluates the safety profile of herbal extracts containing more than one active compound.

The present work is aimed to establish a methodology for assessing the acute toxicity of a particular herbal extract of Saw Palmetto (*Serenoa repens* W. Bartram). Saw Palmetto belongs to the *Arecaceae* (*Palmae* or *Palmaceae)* family. It is also known as *Serenoa serrulatum* Schultes, *Serenoa serrulata* (Michaux) Nichols, or *Sabal serrulata* (Michaux) Nutall ex Schultes (Wilt et al., 2000)).

The therapeutic benefits of Saw Palmetto appear to be related to its fruits, rich in natural oils. Currently, Saw Palmetto extract is widely used for the treatment of benign prostate hyperplasia (BPH, prostate enlargement) (Bent et al., 2006; Gerber and Fitzpatrick, 2004; Hizli and Uygur, 2007; Wilt et al., 2000; Wilt et al., 1998). The type and relative abundance of characteristic compounds (phenolic compounds, phytosterols, flavonoids, polyprenoids, sugars, fatty acids, etc.) of the oily extract from Saw Palmetto depends on the extraction procedure. Currently, the most used procedures to obtain Saw Palmetto extracts are: 1) *n*-hexane (100%) extraction that produces a liposterolic extract (LESP) (Carraro et al., 1996); 2) ethanol (70-95% w/w) extraction (Derakhshani et al., 1997); or 3) supercritical fluid extraction with liquid CO_2 (Cristoni et al., 1997).

Although there are several reports regarding the therapeutical applications of Saw Palmetto extracts (Bent et al., 2006; Carraro et al., 1996; Lowe and Fagelman, 2004; Ulbricht et al., 2006; Vallancien and Pariente, 2001; Wilt et al., 2000), there are few classical toxicological studies in animals. Only a small amount of studies have shown significant toxic effects of a particular Saw Palmetto extract (PC-SPES), which has been removed from the market (de la Taille et al., 2000; de la Taille et al., 1999; Small et al., 2000; Sovak et al., 2002). More recent clinical studies in humans have found no serious adverse effects of Saw Palmetto extracts (Avins et al., 2008; Boyle et al., 2004; Ernst, 2002; Hizli and Uygur, 2007; Willetts et al., 2003). Some hepatotoxic effects have been associated to a *n*-hexane-based Saw Palmetto extract (Hamid et al., 1997); indeed, a classical toxicity study in rats has been reported, showing an increase in oxidative stress associated with the intake of 2X and 5X the maximum dose recommended for humans (480µl/day) for this type of preparation (Singh et al., 2007). Nonetheless, no toxicological studies have been reported using the supercritical fluid extract of Saw Palmetto. Therefore, to perform the first acute toxicological study in rats of a supercritical fluid Saw Palmetto extract (commercial name "HiPower®"), Sprague-Dawley rats were fed with 1X and 10X the recommended dose for humans of this product (doses adjusted for rat metabolism) for 10 days. Rat blood samples obtained at different time intervals were analyzed through hemogram and clinical chemistry parameters; biopsies from liver, spleen, thymus and lymphatic ganglia were also collected and analyzed for possible morphological changes indicative of tissue damage. This study showed no statistically significant changes compared to normal ranges of any of the parameters analyzed; in addition, no significant changes were found in the morphological profile of the studied tissues. This demonstrates the safety of this particular Saw Palmetto extract at the doses and periods tested.

The protocol followed for this study evaluates general homeostasis and the function of liver and kidney, organs responsible for xenobiotic biotransformation and excretion, respectively. Therefore, the type of study presented here may be adopted as a method for assessing the acute toxicity of herbal extracts.

MATERIALS AND METHODS

Saw Palmetto extract HiPower®

HiPower® is a supercritical fluid extract from Saw Palmetto fruits and was provided by Madreselva

Desarrollo y Producción Ltda. (Santiago, Chile). This extract contains: 3.8% palmitic acid, 1.7% stearic acid, 14.8% oleic acid, 44.2% linoleic acid, and 34.3% linolenic acid.

Animals

Male Sprague-Dawley rats (200-230g) were maintained with normal pellet diet (Kimber), access to water ad libitum, in a 12:12 light/dark cycle at 21°C. Animals were maintained in the vivarium of the School of Chemical and Pharmaceutical Sciences (Universidad de Chile, Santiago, Chile). All procedures were performed according to the protocols approved by the Ethical Committee of the institution and to the "Guide for the Care and Use of Laboratory Animals" (NRC, USA).

Treatment of rats

Dosage of HiPower® was calculated from the 1X recommended dose for humans (480µl/day), and considering 70kg for normal human weight and that rats display a 4-fold higher metabolic rate than humans. Doses were diluted in sunflower oil for oral delivery. Rats were distributed in 3 experimental groups of 40 rats each: two groups that were given 28 (HiPower® 1X group) and 280µl (HiPower® 10X group) extract/Kg/day, in two oral (gavage) doses, respectively, and a control group that received sunflower oil alone in equivalent volumes (Sunflower oil group). Following 2, 4, 6, 8, and 10 days of daily treatment, 8 rats from each group were anaesthetized with ether and sacrificed by exsanguination through cardiac puncture. Blood samples were used for hemogram and biochemical profile, and biopsies of liver, thymus, spleen and lymphatic ganglia were collected for histopathology analysis.

Hemogram study

It was performed by the Central Laboratory of the Clinical Hospital of the Universidad de Chile (Santiago, Chile). Parameters analyzed were red blood cell count (RBC), hematocrit, haemoglobin, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), and white blood cell, lymphocyte and platelet counts.

Clinical chemistry study

It was performed by the Central Laboratory of the Clinical Hospital of the Universidad de Chile (Santiago, Chile). Parameters analyzed were: calcium, phosphorus, glucose, blood ureic nitrogen, cholesterol, total protein, albumin, total bilirubin, acid phosphatase (AP), lactate dehydrogenase (LDH), and glutamyl oxaloacetic transaminase (GOT).

Histopathology studies

These studies were performed by the Cyto-Histopathology Laboratory BiopsCyt (Santiago, Chile). Histomorphology studies were performed with haematoxylin-eosin on the biopsies obtained at the different intervals during treatment.

Statistical Analyses

Data presented in this study correspond to mean ± 95% confidence intervals (95% CI). Statistical significances between means of hemogram and clinical chemistry data were obtained through ANOVA. Statistical significances between medians of data and reference values were obtained through Wilcoxon Signed Rank tests. Reference values for adult Sprague-Dawley rats were obtained from observations from the Institute of Public Health of Chile (Uribe et al., 1995) or according to Lillie et al., 1996. All statistical analyses were performed using GraphPad Prism 5.0. Significances were set at 95% confidence

RESULTS

1. Effect of HiPower® on hemogram parameters of Sprague-Dawley rats.

Adult male Sprague-Dawley rats were treated orally for up to 10 days with vehicle (Sunflower oil group), 1X (HiPower® 1X group), or 10X (HiPower® 10X group) the recommended dose of HiPower® for humans, as detailed in Material and Methods. Following 2, 4, 6, 8, and 10 days of each treatment, rats were sacrificed and blood samples were collected for hemogram analysis.

As shown in Figure 1, HiPower® treatment did not significantly alter the following parameters, compared to normal ranges: red blood cells count (RBC) or hematocrit (Figure 1); haemoglobin or mean corpuscular haemoglobin (MCHC, Figure 2); white blood cells count (WBC, Figure 3); and platelet count (Figure 4). We also found complete absence of bacilliform white cells, basophiles, eosinophiles, metamyelocytes, or myelocytes (not shown).

Figure 1. Effect of HiPower® acute treatment on red blood cell count (RBC) and hematocrit of Sprague-Dawley rats. Animals were treated with vehicle (sunflower oil) or with two different doses of HiPower® (HiPower® 1X and HiPower® 10X cohorts), as detailed in Material and Methods. RBC and hematocrit were analyzed at different time points of a 10-day treatment. Data points represent the mean of each determination and error bars depict the 95% CI. Horizontal dotted lines represent the upper and lower limits of reference values for each parameter.

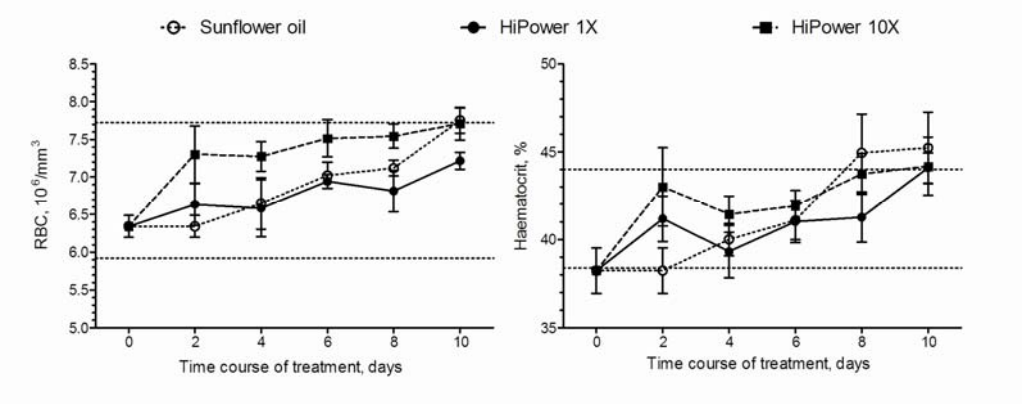


Figure 2. Effect of HiPower® acute treatment on red blood cell mean corpuscular volume (MCV) and hemoglobin-related parameters of Sprague-Dawley rats. Animals were treated with vehicle (sunflower oil) or with two different doses of HiPower® (HiPower® 1X and HiPower® 10X cohorts), as detailed in Material and Methods. Hemoglobin, MCV, mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were analyzed at different time points of a 10-day treatment. Data points represent the mean of each determination and error bars depict the 95% CI. Horizontal dotted lines represent the upper and lower limits of reference values for each parameter. Stars indicate parameters with a median significantly different ($p<0.05$) than their corresponding reference range, according to Wilcoxon Signed Rank test.

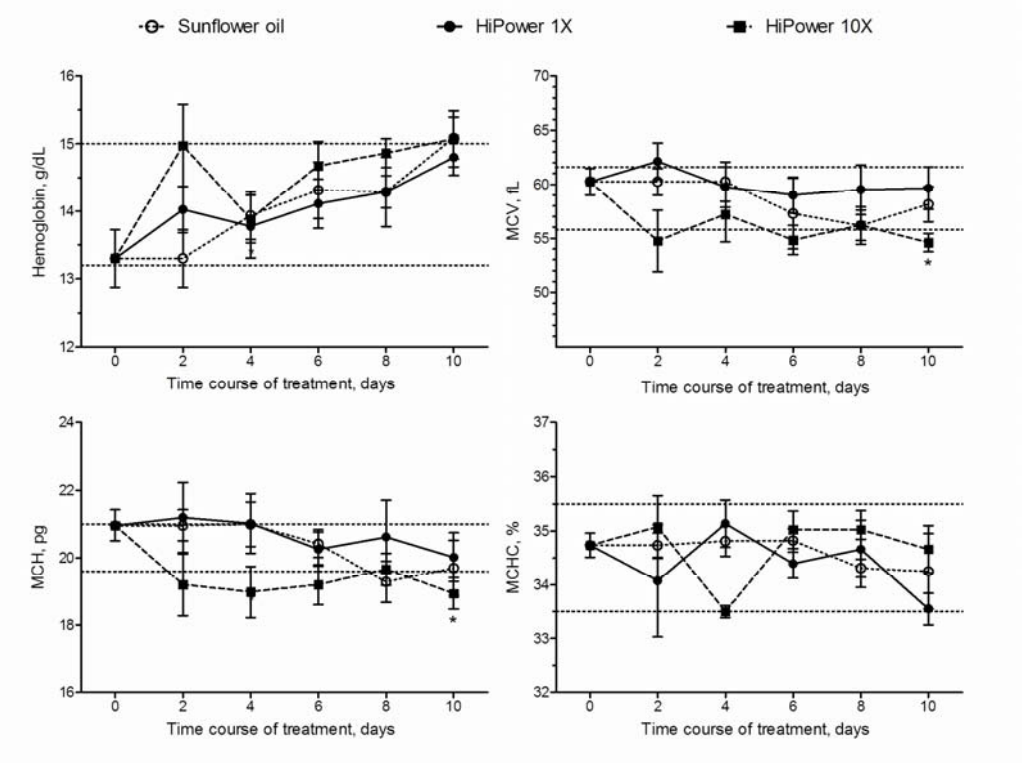


Figure 3. Effect of HiPower® acute treatment on white blood cell count (WBC) and lymphocytes of Sprague-Dawley rats. Animals were treated with vehicle (sunflower oil) or with two different doses of HiPower® (HiPower® 1X and HiPower® 10X cohorts), as detailed in Material and Methods. WBC and lymphocytes were analyzed at different time points of a 10-day treatment. Data points represent the mean of each determination and error bars depict the 95% CI. Horizontal dotted lines represent the upper and lower limits of reference values for each parameter. Stars indicate parameters with a median significantly different ($p<0.05$) than their corresponding reference range, according to Wilcoxon Signed Rank test.

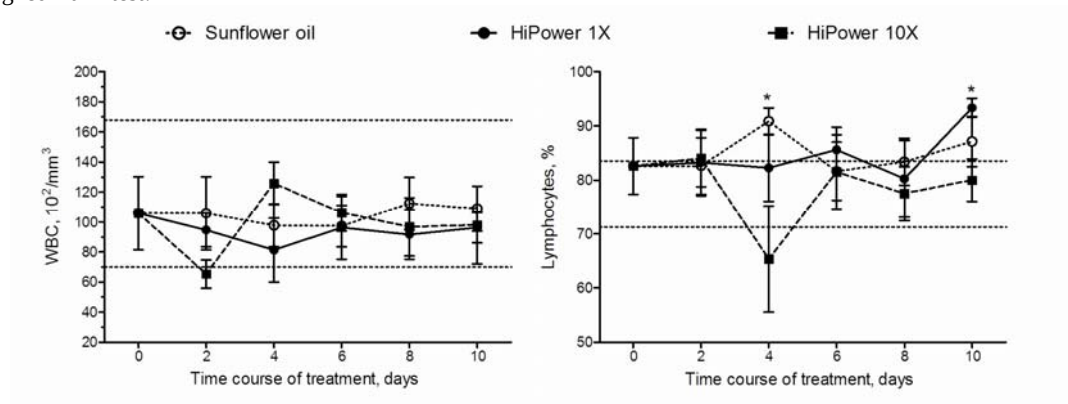


Figure 4. Effect of HiPower® acute treatment on platelets of Sprague-Dawley rats. Animals were treated with vehicle (sunflower oil) or with two different doses of HiPower® (HiPower® 1X and HiPower® 10X cohorts), as detailed in Material and Methods. Platelets were analyzed at different time points of a 10-day treatment. Data points represent the mean of each determination and error bars depict the 95% CI. Horizontal dotted lines represent the upper and lower limits of reference values for each parameter.

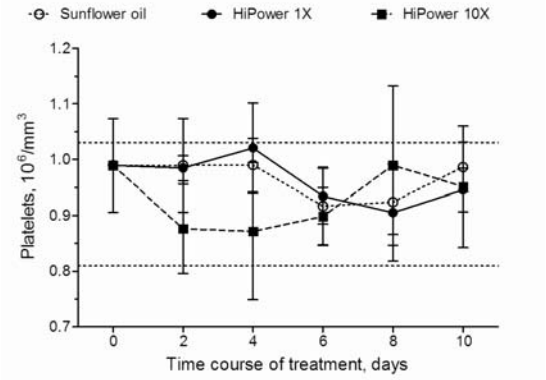


Figure 5. Effect of HiPower® acute treatment on blood levels calcium and phosphorus of Sprague-Dawley rats. Animals were treated with vehicle (sunflower oil) or with two different doses of HiPower® (HiPower® 1X and HiPower® 10X cohorts), as detailed in Material and Methods. Blood levels of calcium and phosphorus were analyzed at different time points of a 10-day treatment. Data points represent the mean of each determination and error bars depict the 95% CI. Horizontal dotted lines represent the upper and lower limits of reference values for each parameter.

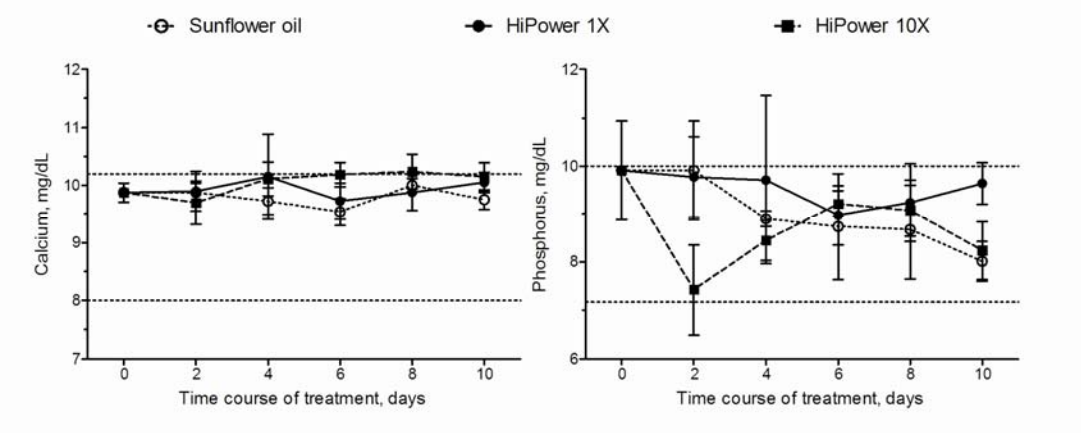


Figure 6. Effect of HiPower® acute treatment on blood levels of albumin and total protein of Sprague-Dawley rats. Animals were treated with vehicle (sunflower oil) or with two different doses of HiPower® (HiPower® 1X and HiPower® 10X cohorts), as detailed in Material and Methods. Blood levels of albumin and total protein were analyzed at different time points of a 10-day treatment. Data points represent the mean of each determination and error bars depict the 95% CI. Horizontal dotted lines represent the upper and lower limits of reference values for each parameter.

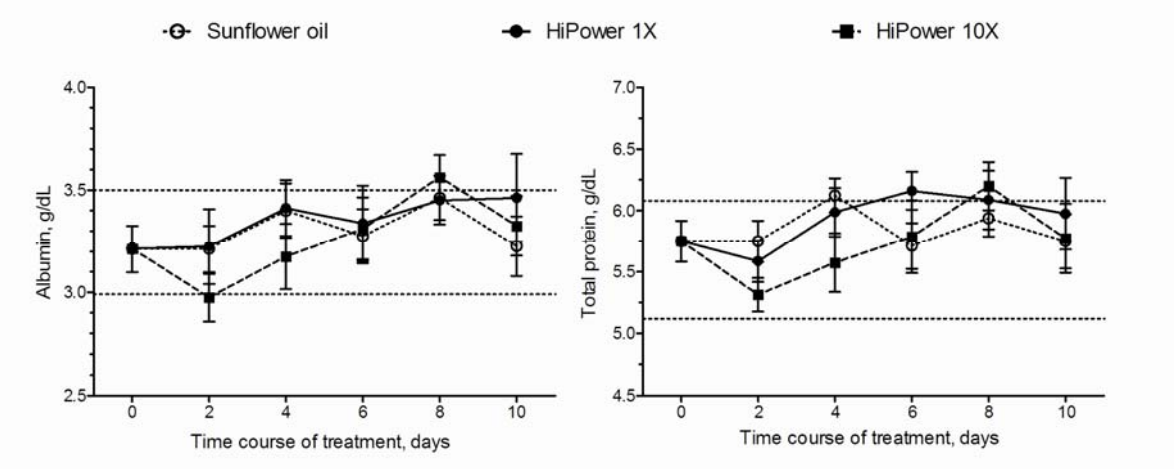


Figure 7. Effect of HiPower® acute treatment on blood levels of glucose, cholesterol, bilirubin, and urea nitrogen of Sprague-Dawley rats. Animals were treated with vehicle (sunflower oil) or with two different doses of HiPower® (HiPower® 1X and HiPower® 10X cohorts), as detailed in Material and Methods. Blood levels of glucose, cholesterol, bilirubin, and urea nitrogen were analyzed at different time points of a 10-day treatment. Data points represent the mean of each determination and error bars depict the 95% CI. Horizontal dotted lines represent the upper and lower limits of reference values for each parameter.

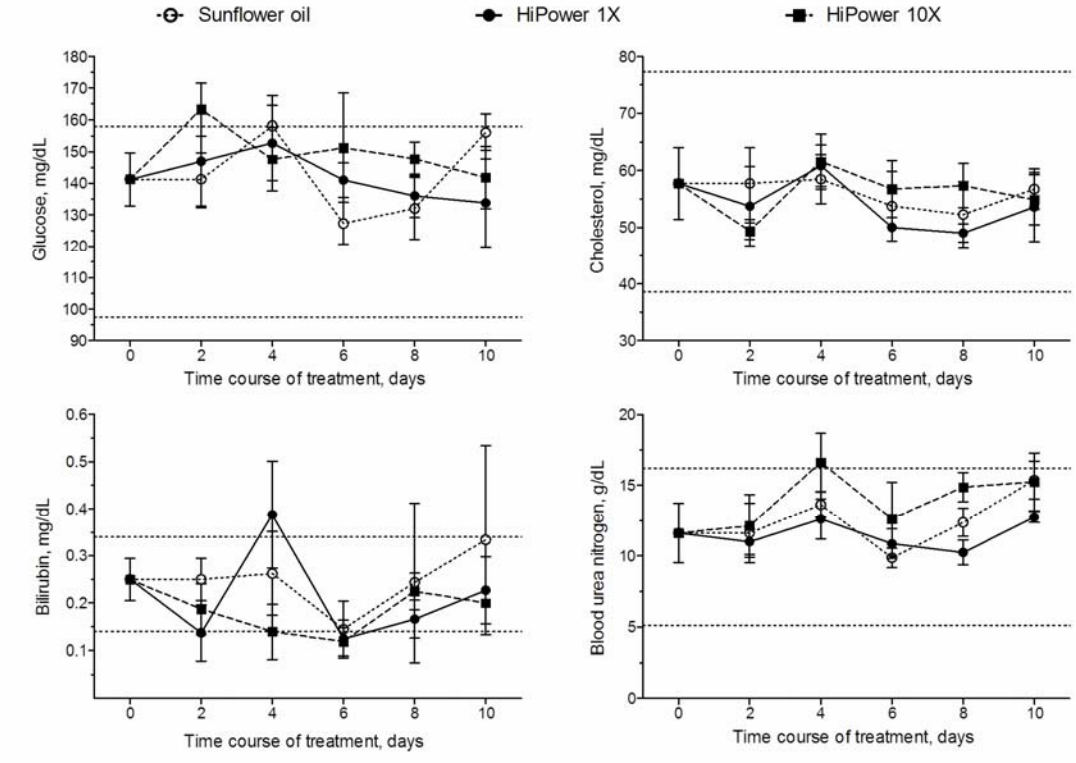


Figure 8. Effect of HiPower® acute treatment on blood levels of alkaline phosphatase, aspartate aminotransferase, and lactate dehydrogenase of Sprague-Dawley rats. Animals were treated with vehicle (sunflower oil) or with two different doses of HiPower® (HiPower® 1X and HiPower® 10X cohorts), as detailed in Material and Methods. Blood levels of alkaline phosphatase, aspartate aminotransferase, and lactate dehydrogenase were analyzed at different time points of a 10-day treatment. Data points represent the mean of each determination and error bars depict the 95% CI. Horizontal dotted lines represent the upper and lower limits of reference values for each parameter. Stars indicate parameters with a median significantly different ($p<0.05$) than their corresponding reference range, according to Wilcoxon Signed Rank test.

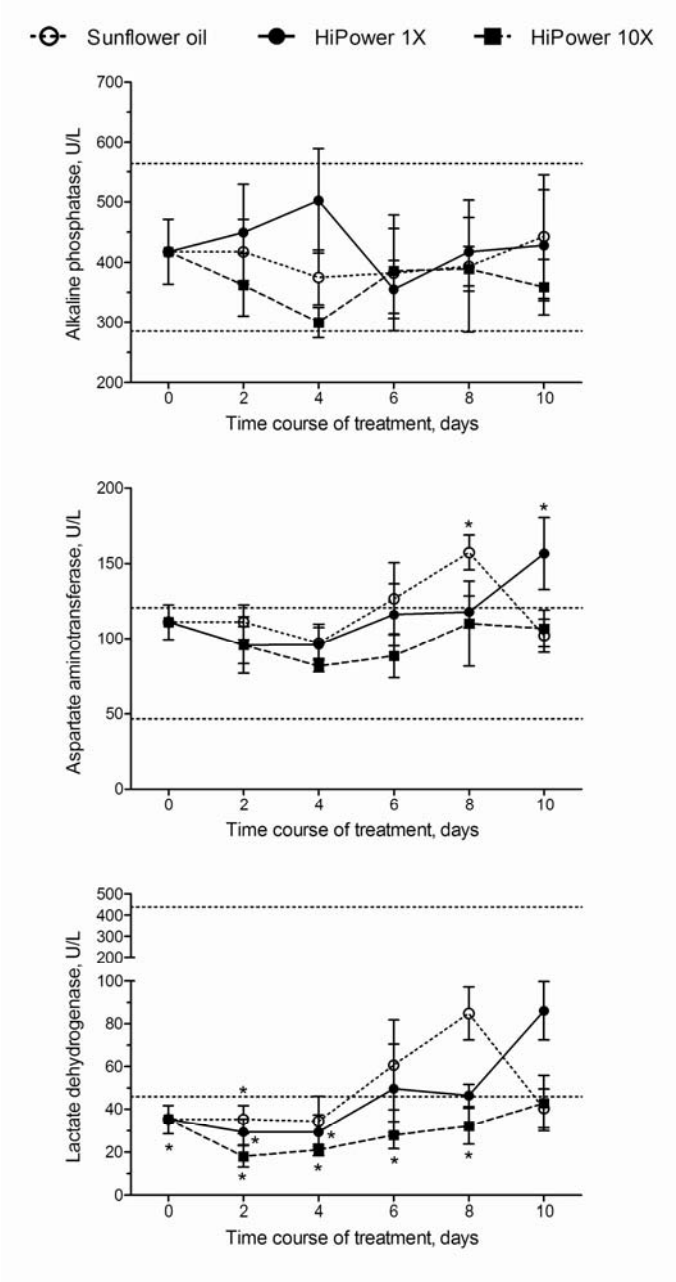
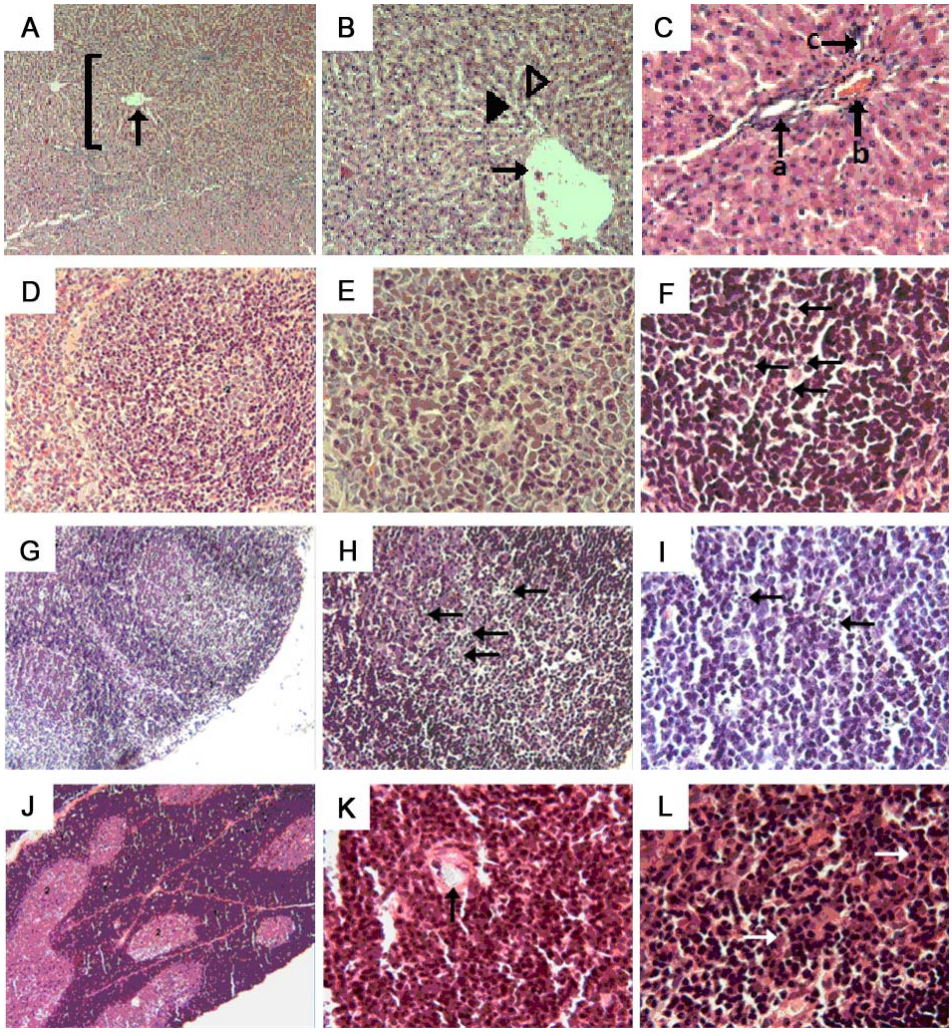


Figure 9. Effect of HiPower® acute treatment on morphological features of liver, spleen, lymphatic ganglia, and thymus biopsies from Sprague-Dawley rats. Animals were treated with vehicle (sunflower oil) or with two different doses of HiPower® (HiPower® 1X and HiPower® 10X cohorts), as detailed in Material and Methods. Biopsies from liver, spleen, lymphatic ganglia, and thymus were collected at different time points of a 10-day treatment. Representative images from haematoxylin-eosin staining of samples are shown. **A.** 4X magnification showing a typical hepatic lobule (bracket) with a central vein (arrow). **B.** 10X magnification of a hepatic biopsy showing a better view of the central vein (arrow); hepatic trabecules (solid arrowhead) and sinusoids (open arrowheads) are also distinguishable. **C.** 20X magnification showing a portal triad, with the portal venule (a), the bile duct surrounded by a cuboid epithelium (b), and the hepatic arteriole (c). **D.** 20X magnification of a spleen biopsy showing white and red pulps, with a germinative center in the latter. **E.** Germinative center of a splenic red pulp at 40X magnification. **F.** Germinative center of a splenic red pulp depicting apoptotic bodies (arrows). All structures shown are representative of all groups and treatment intervals. **G.** 10X magnification of a lymphatic ganglion biopsy, in which cortex and part of the medulla can be distinguished. Two lymphoid follicles can be discriminated, with their respective germinative centers. **H.** 20X magnification of a germinative center of a ganglion lymphoid follicle, with numerous apoptotic bodies (arrows). **I.** 40X magnification of a germinative center of a ganglion lymphoid follicle, with a better view of apoptotic bodies (arrows). **J.** 4X magnification of thymus lobule structure, with clear discrimination of cortex (1) and medulla (2) **K.** 40X magnification of a Hassall body (black arrow), with normal architecture. **L.** 40X magnification of a medullar zone of a thymus lobule, displaying scarce apoptotic bodies (white arrows).



Wilcoxon Signed Rank tests showed that particular cohorts displayed medians significantly different from reference ranges in mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and the % lymphocytes (stars in Figures 2 and 3). Two-way ANOVA tests, however, showed that these differences are not a reflection of significant deviations compared to the vehicle ($p>0.05$).

2. Effect of HiPower® on clinical chemistry parameters of Sprague-Dawley rats.

In addition to hemogram analysis, blood samples were also analyzed for several clinical chemistry parameters. As shown in Figures 5-8, HiPower® treatment did not change the following parameters: calcium or phosphorus (Figure 5); blood levels of albumin or total proteins (Figure 6); blood glucose, cholesterol, bilirubin, or urea nitrogen (Figure 7); and the activity of alkaline phosphatase (AP, Figure 8).

Wilcoxon Signed Rank tests showed that some cohorts displayed medians significantly higher than the upper limit of the normal range for aspartate aminotransferase activity (stars in Figure 8, middle panel). Two-way ANOVA tests, however, showed that these differences are not a reflection of significant deviations compared to the vehicle ($p>0.05$). Also, most cohorts showed medians significantly lower than the lower limit of the normal range for lactate dehydrogenase (LDH) activity (stars in Figure 8, bottom panel). Two-way ANOVA analysis demonstrated that most of these deviations were not significantly different from the vehicle cohort, except for the cohorts treated with 1X HiPower® (8 and 10 days of treatment, $p<0.001$) and 10X HiPower® (6 and 8 days of treatment, $p<0.001$).

3. Effect of HiPower® on morphological features of rat tissues.

We also obtained biopsies (hepatic, splenic, thymic, and lymphatic) from each group at different time intervals of the treatments. These biopsies were used to perform histopathology analysis of tissue sections with haematoxylin-eosin, as detailed in Material and Methods.

All liver biopsies, regardless of the group or the time of collection, showed a conserved histological architecture (Figure 9A-C). We observed classical structures, such as hepatic lobules with a central vein (Figure 9A and 9B) and portal triads constituted by blood vessels and bile ducts (Figure 9C). Hepatocytes displayed a conserved structure, without noticeable degeneration or necrosis. Most liver biopsies displayed small hematopoietic loci, with the same occurrence regardless of the cohort analyzed or the collection time. Liver parenchyma displayed no mitosis count per mm^2 in most samples, with a mitosis count of 1-4 per mm^2 in a few biopsies, regardless of the cohort or collection time.

Spleen biopsies had also normal architecture of red or white pulp, regardless of the cohort or treatment interval analyzed (Figure 9D-F). All samples displayed hematopoietic activity and a mitotic count in germinative centers of 0-4 per mm^2, regardless of the treatment or collection interval (Figures 9D and 9E). Vehicle cohort displayed virtually no apoptotic bodies, while 1X HiPower® and 10X HiPower® cohorts showed some apoptotic bodies in seldom cases (Figure 9F).

Lymphatic ganglia biopsies from all three cohorts displayed conserved histological structures (Figure 9G-I). We found lymphocytic elements and reticulo-histocytic cells in the lymphoid tissue. Most biopsies, regardless of the cohort or collection time, displayed secondary follicles with germinative centers (Figure 9G). With low frequency, some sinusal edema was found regardless of the cohort or the collection time. At the level of lymphoid follicles, some apoptotic activity was evidenced by the occurrence of apoptotic bodies in the cytoplasm of reticulo-histocytic cells (Figures 9H and 9I). The abundance of apoptotic bodies, however, was not associated to a cohort or collection time. On the other hand, at the level of germinative center, we observed a mitotic count of 2-25 per mm^2. Variability of mitotic count was unchanged among cohorts or collection times.

Histological structures of the thymus were conserved in all biopsies tested (Figure 9J-L), regardless of the cohort or the collection time, with normal architectures of cortex and medulla (Figure 9J). Normal lymphoid and epithelial (Hassall bodies, Figure 9K) components were also visualized. Apoptotic scores did not reveal significant differences between cohorts or collection times (Figure 9L). Mitotic count in germinative centers was 1-2 per mm^2, displaying no significant differences between cohorts or collection times.

DISCUSSION

Our study was aimed to develop a methodology that allows the evaluation of the safety profile of herbal extracts, using a supercritical fluid Saw Palmetto extract (HiPower®) as a start point. To this end, we evaluated possible changes in classical hemogram and clinical chemistry parameters (as a measure of general homeostasis) from Sprague-Dawley rats fed for 10 days with two different doses of HiPower®, compared with rats fed with vehicle (sunflower oil). We also evaluated potential gross morphological alterations in key organs involved in xenobiotic metabolism (to address possible toxicity associated with xenobiotic biotransformation) and immune system (to address potential toxic immunological response). With this purpose, blood samples and tissue biopsies were collected from rats at different intervals during the 10-day treatment.

Essentially none of the parameters tested underwent any statistically significant changes following this treatment, as assessed by Wilcoxon Signed Rank test. Although these analyses showed some deviations of medians from the normal ranges, most of such deviations were not significantly different from those of the vehicle cohort. This may be the reflection of parameters with high inter-individual dispersion. Noteworthy, lactate dehydrogenase activity values were lower than the lower limit of normal ranges for this parameter in all cohorts, especially at the beginning of the treatment. Although this parameter reached the normal interval in the case of the vehicle cohort, it remained under the lower limit for the 1X HiPower® and 10X HiPower® cohorts. Since lactate dehydrogenase activity is a classical marker for liver damage, these data suggest that HiPower® may display hepatoprotective activity.

In summary, hemogram and clinical chemistry data show that the treatment of Sprague-Dawley rats with a supercritical fluid Saw Palmetto extract led to undetectable alterations of red blood cells integrity, haematopoiesis, immune or inflammatory responses, coagulation, metabolism of glucose, nucleic acids, proteins, fatty acids or steroids, and function of liver, spleen, kidney or parathyroid gland. The latter was also corroborated by the absence of gross morphological changes in liver, spleen, lymphatic ganglia or thymus. Therefore, we concluded that this acute treatment did not lead to any classic toxicological outcomes, even with doses that differ in one order of magnitude.

It has been shown that some Saw Palmetto extracts can be toxic in acute toxicological studies (Hamid et al., 1997; Singh et al., 2007). On the other hand, several studies have demonstrated the safety of these extracts in humans (Avins et al., 2008; Boyle et al., 2004; Ernst, 2002; Hizli and Uygur, 2007; Willetts et al., 2003). It is very difficult to address the safety of a Saw Palmetto extract due to the diversity of extraction methods (Habib and Wyllie, 2004). Although this extract appears to be more concentrated than other commercial Saw Palmetto extracts, we postulated that the absence of organic solvents in the supercritical fluid extraction of Saw Palmetto fruits, leading to the preparation of an extract with a good safety profile. Due to their oily nature, it is possible that Saw Palmetto extracts used for the treatment of benign prostate hyperplasia contain lipoperoxides; these substances must be controlled due to their potential for promoting lipid peroxidation in tissues. Noteworthy, the particular Saw Palmetto extract used in this study did not lead to an increase in basal lipoperoxidation of rat liver microsomes (data not shown), a system routinely used by our lab to test oxidative damage (Letelier et al., 2007). The absence of pro-oxidant activity corroborates the good safety profile of this Saw Palmetto extract. In light of our data, we can conclude that HiPower® displays a safety profile of at least one order of magnitude in dosage.

The general strategy used for the present study indeed allowed the assessment of potential alterations in general homeostasis and morphological features of key organs in xenobiotic biotransformation (liver) and excretion (kidney). Therefore, we conclude that this type of studies may be adopted for future evaluations of safety profiles of herbal extracts.

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