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Acute and subchronic toxicological study of senna in rodents

[Toxicología aguda y subcrónica de una pasta de sen en roedores]

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Abstract

Senna crude herbal paste, CIRUELAX®, laxative used in America and Spain, was administered by oral gavage to mice and rats once daily in doses from 2 to 10 g/kg for 7 days. LD_{50} was rated as higher than 10 g/kg. To determine subchronic peroral toxicity (SPT), rats were treated with 1, 2.7 and 6 g/kg of CIRUELAX® for 90 days. Body weight (BW) data and weights of liver, spleen, kidney, heart, gonads and lung were determined each week until 90th day. No decrease in weight gain was observed; males BW increased about 10% after 60 days. Liver and gonad relative weight in males and females, respectively, showed a slight increase at lower doses. Organs were free from discernible lesions. Microscopical examination showed no morphological changes. Rat males treated with CIRUELAX® showed blood hypokalemia. Potassium decreased about 25% after 90 days. Low doses of CIRUELAX® decreased WBC count in 19.61%. Monocytes count increased about five times after 90 days with 2.7 g/kg of CIRUELAX®. Absence of hepatocellular injury was inferred by AST and ALT activities. Soluble alkaline phosphatase (SAP) activity was not modified. CIRUELAX® higher dose equivalent to 200 times the dose used to treat human constipation induced no acute or subchronic toxic effects in rodents.

Keywords: Cassia angustifolia, Senna alexandrina; *senna; laxative; anthranoids; Phytotherapy.*

Resumen

CIRUELAX®, es una pasta herbal cruda preparada a base de sen, *Senna alexandrina* Mill., ampliamente utilizada en América y España. Fue administrada oralmente a ratas y ratones una vez al día en dosis de 2 a 10 g/kg durante 7 días. La Dosis Letal 50, LD_{50}, fue estimada como superior a 10 g/kg. Para determinar la toxicidad subcrónica por vía oral, un grupo de ratas fueron tratadas con 1; 2,7 y 6 g/kg de CIRUELAX® durante 90 días. Se determinaron los datos de peso corporal, peso del hígado, bazo, riñón, corazón, gónadas y pulmones cada semana hasta el día 90. No se apreció disminución en peso corporal, los machos experimentaron 10% de aumento de peso tras 60 días. Los pesos relativos de hígado y gónadas en machos y hembras, respectivamente, mostraron un ligero aumento en dosis bajas de CIRUELAX®. Los órganos no presentaron lesiones visibles y el examen microscópico no mostró variación de las características morfológicas. Las ratas machos tratadas con CIRUELAX® mostraron hipokalemia sanguínea. El potasio disminuyó alrededor de 25% tras 90 días de tratamiento. Bajas dosis del laxante disminuyeron alrededor de 19 % el recuento de glóbulos blancos. Los monocitos aumentaron alrededor de 5 veces tras 90 días de tratamiento con 2,7 g/kg de CIRUELAX®. La ausencia de daño hepatocelular fue inferida de la determinación de actividades de enzimas hepáticas (AST y ALT). La actividad de fosfatasa alcalina soluble (SAP) no fue modificada. La dosis superior de CIRUELAX® equivalente a 200 veces la dosis que se emplea para tratar estreñimiento en humanos, no indujo efectos tóxicos en roedores.

Palabras Clave: Cassia angustifolia; Senna alexandrina; *sen; laxantes naturales; antranoides; Fitoterapia.*

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INTRODUCTION

Chronic constipation occurs in all age groups, but apparently increases with age. Modern style of living has increased the prevalence of chronic constipation due to bad alimentary habits, low-fiber diet, a higher degree of sedentarism, and medication. In Germany 1995, it was determined that 25% of women and 10% of men acknowledged to be suffering some kind of difficulty to defecate and constipation (Knopf et al., 1995).

Since its introduction by Arabians to occidental medicine in the 19th century, anthranoids laxatives have been widely used. Among the anthranoids containing plants (*Rhamnus frangula, Rhamnus purshiana Rheum palmatum*), Senna (*Senna alexandrina* ex *Cassia angustifolia*) is used worlwide to treat constipation and is the most studied anthranoid laxative (Leng-Peschlow, 1992).

Due to their natural origin, specificity, low oral toxicity, efficacy and because they do not require medical prescription, anthranoids are commonly used to treat constipation. Unfortunately, this popularity has led to abuse of natural anthranoids. In England, 1993, 2% of the population between 40 and 59 years and 20 to 30% of the people older than 60 years used laxants more than once a week (Cooke, 1981). In Germany, 80% of the people under chronic use of laxants preferred anthranoids (May, 1982). Although long-term daily use can produce severe diarrhoea that causes hyponatremia, hypokalemia and dehydration, use for two to three times per week has been considered safe and effective (Ralevic et al., 1990; Wald, 2000).

Numerous studies have characterized toxic status of anthranoids. Hietala et al. (1987) distinguished between pure sennosides and other fractions of senna, since it had been found that it was possible to differentiate between the laxative and toxic effects of various senna fractions; the typical mixed preparations contain impurities that are three- to five –folds more toxic than pure sennosides which are hardly toxic, but less laxative. Those studies however, considered only acute toxicity (24 h) and were performed using very high doses.

Sennosides are the main active metabolites of senna and exhibit a very low toxicity in rats (Hietala et al., 1987). Sennosides genotoxicity both in bacterial strains and mammal cells has been observed, to be very low (Sandnes et al., 1992; Mukhopadhyay et al., 1998; Heidemann et al., 1993; Mereto et al., 1996).

Toxicological and genotoxic status of crude extract of senna is considered to be different. While mean LD_{50} of sennosides at 98% purity is 4100 mg/kg, LD_{50} for senna extract standardized at 5.5% sennosides is 384 mg/kg (Hietala et al., 1987). Others anthranoids as chrysantine, hidroxyanthraquinones (emodin, aloe-emodin, rhein), which are present as minor components in senna, have a toxicologic and mutagenic status, different from that of the sennosides, and presently is a very controversial issue. Emodin and aloe-emodin resulted positive in genotoxic assays in *Salmonella typhimurium*, V79-HGPRT cells, rat hepatocytes and mice fibroblasts (Westendorf et al., 1990), while resulted negative in the study of Heidemann et al. (1993). Mori et al. (1990) observed formation of neoplasms in stomach, intestine and liver of rats before 480 days of dietary exposure to 1% hydroxianthraquinones. In addition, out from Siegers et al. (1993) clinical work, it has been speculated that chronic use of anthranoids laxative is a risk factor for colorectal cancer development. Others investigators disagreed with these observations (Sandnes et al., 1992; Heidemann et al., 1993; Mascolo et al., 1999; Mengs et al., 1999). The aim of this work was to determine acute and subchronic 13-weeks toxicity of CIRUELAX®, an herbal paste containing linseed, prune and senna leaves and pods, as laxatives compounds.

MATERIALS AND METHODS

Animal preparation

All animals were supplied by the animal house of the Universidad Católica of Chile, Santiago of Chile. All were individually identified by ear tatoo. Upon arrival, experimental and control animals were caged separately. A period of 7 days of acclimatization was allowed before experimentation. Animals received a high quality food made by Champion S.A., Santiago, Chile, with the following composition: Protein 20.5%; fiber 5.0% and humidity 4.0%. Water was given ad libitum. Environmental conditions were as followed: temperature 20-24 ºC, relative humidity 60% and light-dark cycle of twelve hours each. The cleansing procedures were daily and the bed (wood shavings) was changed daily too. All procedures were authorized by the ethical comitee of the Universidad Austral de Chile.

Substance preparation

CIRUELAX® herbal paste contained (per 100 g): Powdered senna, *Senna alexandrina* Mill. –Fabaceae, standardized to 0.12-0.22% of anthraquinones calculated as sennosides B, 6.6 g; fruit pulp 48.8 g; white sugar 18.0 g; honey 9.5 g; linseed (seeds of *Linum usitatissimum*), 2.5 g; caramel 1.9 g; citric acid 1.0 g; sodium benzoate 0.07 g; potassium sorbate 0.07 g; water q.s.f. 100 g.

Quantification of sennosides was performed by HPLC. Column LUNA 250 x 4.6 mm, temperature: 40 °C, flow rate 1.0 mL/min; pressure 160 bar. Mobile phase acetonitrile: buffer pH 2.77 TEA 15:85. Sennosides A and B were detected at 265 nm by UV detector. The respective peak areas were integrated by comparison with external standard calibration curves.

The sample of CIRUELAX® herbal paste was homogenized with destilled water in an ultraturrax and given by oral gavage using buco-oesophagial cannulae at different doses as shown below. Maximum dosage volumes were 1 mL in mice and 3 mL in rat.

Acute peroral toxicity in mice and rats

100 Rockefeller mice of both sexes; body weight 25-30 g and 100 Sprague-Dawley rats of both sexes body weights 150 ± 8 g were randomly assigned to different treatment groups. The animals were fasted for 24 h before the administration of a single dose of CIRUELAX®. Single increased doses were: 2, 4, 6, 8 and 10 g/kg.

Afterwards, the animals were observed daily for 7 days. Mortality LD_{50} was estimated by method of Miller and Tainter (1944), designed to assess the ED_{50} and its error by means of logaritmic-probit graph paper. This method allows estimating toxicity from all-nothing responses considering fixed doses given to a group, instead of individual doses. Clinical signs, time of onset, duration and reversibility of toxicity were recorded. Gross necropsies were performed in all animals after the observation period.

Subchronic peroral toxicity in rats

Four groups of 11-13 rats per dose and per sex, with an initial body weight of 140 ± 10 g, were randomly assigned to treatment and control groups. CIRUELAX® doses were 1.0 g/kg (Group 1), 2.7 g/kg (Group 2) and 6.0 g/kg (Group 3). Control group received distilled water. The duration of the study was 90 days. At the end of this period, animals previously anaesthetized with ether were sacrificed by decapitation and different organs dissected. Every 3-4 days the product was mixed in a know quantity of water and administered during the whole experiment. The doses were daily adjusted to body weight (weights of groups) and weekly by individual weight.

Body and organ weight

Body weight data was obtained at the beginning and every 7 days until the end of the 90 day period, by using an electronic scale (Soehnle).

At the end of the 90 days, liver, spleen, kidneys, heart, gonads, and lung obtained from control and treated groups were weighted, after removal of connective and fat tissue, in a Sartorius analytical Scale.

Hystopathological examination

Tissues from heart, lung, kidney, spleen, stomach, duodenum, colon, liver, ovary, uterus and testicles of control and CIRUELAX® treated groups were prepared for optical and electronic microscopical examination. To light microscopy, tissues were undergone to 10% formaldehyde and routine hematoxylin eosine stainining. To electron microscopy examination the tissues were fixed in 1% buffered osmium tetroxide and processed by standard procedures.

Haematological and blood chemistry tests

Sampling of blood from each animal was performed at the first, 7^{th}, 30^{th}, 60^{th} and 90^{th} day. The blood was drawn by retro-orbital puncture under light ether anesthesia. The following analyses were done: red blood cells (RBC), white blood cells (WBC), volume packed RBC, haemoglobin, differential count of lymphocytes, monocytes, neutrophil segmenters, neutrophil bands, eosinophils and basophils.

Blood samples were added (1:9) to a 1% ethylenediaminetetraacetic acid (EDTA) solution previous to the standard laboratory procedures.

Blood samples were kept during 2 h at 4ºC and then were centrifuged to obtain serum for analysis of the following parameters: urea-N, protein, alkaline phosphatase (SAP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), sodium, potassium, calcium.

Data analysis

All hematological and biochemical data were evaluated according to the following statistical

methods: basic statistics including mean (M), standard error of the mean (SEM), standard deviation (SD), number (N), minimum, maximum, range, variance, Skewness (G1), Kurtosis(G2), sum and coefficient of variation (C.V.), Bartlett test for homogeneity or variance, ANOVA ONE WAY and multiple Tukey's comparison test were used. After that a Kruskal-Wallis non-parametric test was utilized for all variables. Finally, an ANOVA TWO WAY (factors: treatment and sex) was used and, when significant a Scheffe's test was also used. Sequentially, a box–design graphic statistics was employed.

Same descriptive statistics as described above was used for the evaluation of body weight. An ANCOVA TWO WAY (covariance analysis) was utilized. All data were analyzed with the software SYSTAT 5.04 and the level of significance defined was $p< 0.05$.

RESULTS

Oral Acute Toxicity

The study for acute toxicity was performed with a total of 100 Sprague Dawley rats and 100 Rockefeller mice. Doses used were 2, 4, 6, 8 and 10 g/kg. Only at maximal doses animals showed a reduction in the stool consistence, i.e. this dose represents a laxative dose in rats. During the first week following a single administration of CIRUELAX® paste no animal died.

Subchronical Toxicity

Mean body weight of female rats remained without statistically significant changes with respect to control group throughout the study. After 60 days, mean body weight of males treated with 1.0 and 2.7 g/kg of CIRUELAX® increased about 10% compared to control group ($p< 0.05$), see Fig. 1. Two female and five male rats died during ether anaesthesia.

At the end of the treatment period, both liver and ovary relative weight of female rats exposed to 1 g/kg of CIRUELAX® increased 12 and 150% in comparison to control group, however, this effect was not observed with higher doses. In male rats treated with 1 and 2.7 g/kg, liver relative weight increased about 19 and 13% respectively, compared with control group (Table 1). This effect was not observed with 6 g/kg dosis of CIRUELAX®.

Transaminase enzymes level was determined as an indicator of hepatocellular injury. Aspartate aminotransferase (AST) activity of males exposed to CIRUELAX® was similar to control group (Fig. 2A). After 30 days, AST activity of treated female rats, with all doses, increased respect to control while at day 60th the AST activity increased only in rats treated with 1 and 2.7 g/kg. At day 90^{th}, no difference in AST activity between experimental and control group (Fig. 2B), was observed.

Alanine aminotransferase (ALT) activity increased about 9% in male rats treated with 1g/kg at day 30, with respect to control. No differences were observed either in ALT of males treated with higher doses or in ALT of females exposed at different doses (Fig. 3A and B).

Alkaline phosphatase (SAP) activity showed a great variation throughout the study, however no significant differences were found among experimental and control groups (Fig. 4).

Urea nitrogen and serum protein levels in both, males and females treated with CIRUELAX® remained unaltered with respect to control groups (data not shown).

Potassium levels decreased 31, 22 and 20% at day 90 in males but not in females treated with 1, 2.7 and 6 g/kg of CIRUELAX® (Fig. 5A and B). Sodium and calcium electrolytes in males and females were not statistically different from controls throughout the 90 days of treatment. In comparison to Control group, no changes were observed in red blood cells (RBC) count, packed volume of RBC (PVC), hemoglobin and leukocytes, in males treated with CIRUELAX® (data no shown). WBC count in females rats treated with 1 g/kg of CIRUELAX® decreased 19.6% at 90^{th} day. Also in females, monocytes count at day 90^{th} was 1.2% in the control group and 5.8% in the group treated with CIRUELAX® 2.7 g/kg (Figs. 6 and 7).

Examination of heart, lung, kidney, spleen, stomach, duodenum, colon, liver, ovary, uterus and testicles of control and treated groups showed them to be free from specific macroscopically discernible lesions under the described experimental conditions.

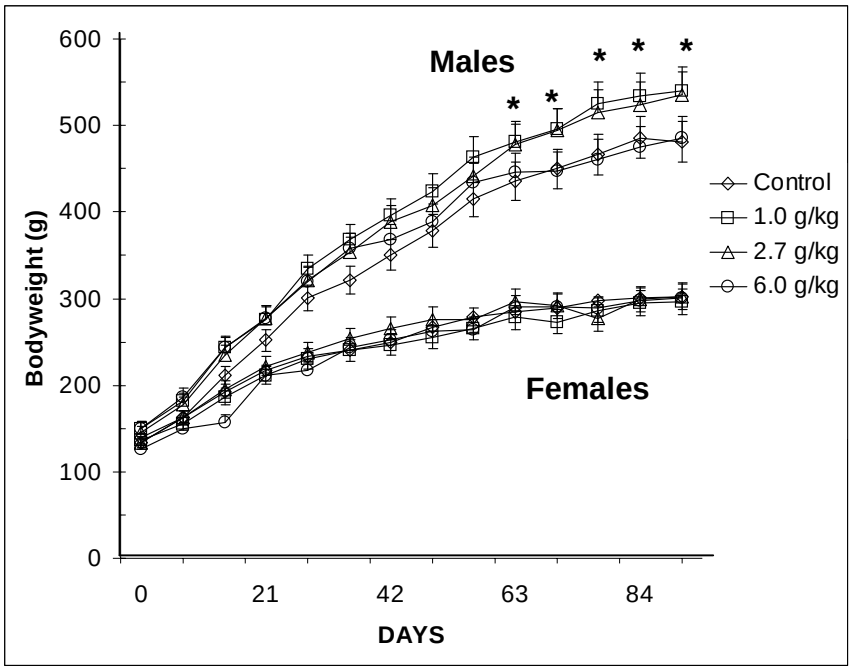
Optical and electronic microscopical examination of tissues from different organs, showed no microscopic morphological variations or differences among control and CIRUELAX® treated groups.

Table 1. Liver, kidney, heart and gonads relative weight, standardized to 100 g of body weight (somatic index) after 90 days of administration of CIRUELAX® paste.

		CIRUELAX® paste Doses (g/kg)		
	CONTROL	1	2.7	6
Males				
Liver	1.99± 0.037	2.37 ± 0.060 *	2.25 ± 0.058 *	2.08 ± 0.050
Kidney Right	0.30 ± 0.010	0.30 ± 0.001	0.29 ± 0.007	0.29 ± 0.008
Heart	0.29 ± 0.007	0.29 ± 0.008	0.28 ± 0.012	0.29 ± 0.008
Gonad Right	0.34 ± 0.011	0.32 ± 0.010	0.33 ± 0.008	0.35 ± 0.011
Gonad Left	0.34 ± 0.011	0.32 ± 0.008	0.32 ± 0.008	0.35 ± 0.016
Females				
Liver	2.84 ± 0.062	3.18 ± 0.090 *	3.06 ± 0.0120	2.82 ± 0.075
Kidney Right	0.33 ± 0.012	0.34 ± 0.009	0.33 ± 0.009	0.32 ± 0.010
Heart	0.33 ± 0.014	0.30 ± 0.015	0.33 ± 0.009	0.31 ± 0.013
Gonad Right	0.02 ± 0.002	0.05 ± 0.005 *	0.02 ± 0.001	0.02 ± 0.001
Gonad Left	0.02 ± 0.003	0.06 ± 0.009 *	0.02 ± 0.001	0.02 ± 0.002

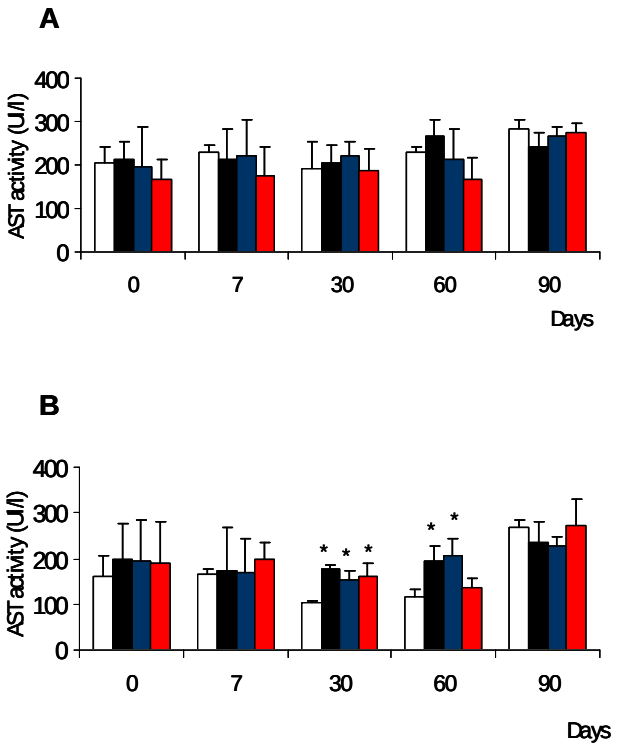
* p< 0.001, statistically significative with respect to control. Values are the mean ± standard deviation of 11-13 rats.

Figure 1. Effect of different doses of CIRUELAX® on male (A) and female (B) body weights throughout the study.



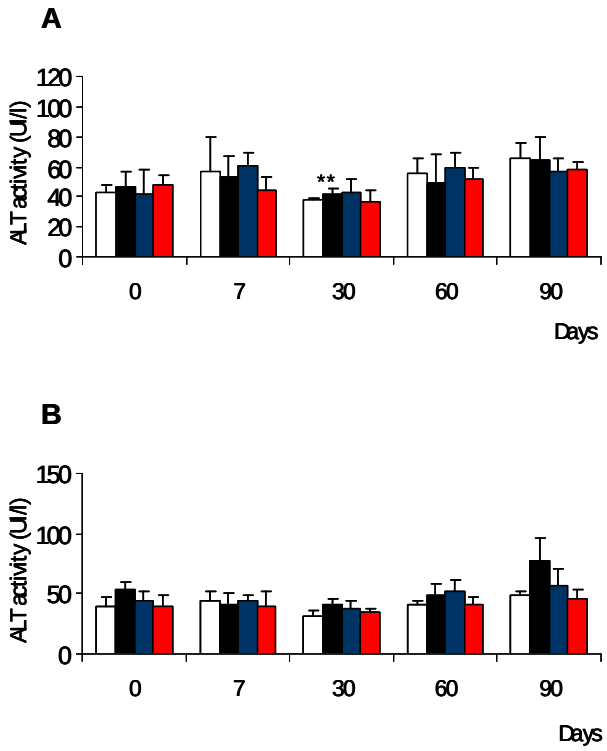
Each point represents the mean ± SEM of 11-13 rats. * p< 0.05 with respect to the control.

Figure 2. Effect of CIRUELAX® on male (A) and female (B) seric AST activity of rats at 0, 7, 30, 60 and 90 days of treatment.



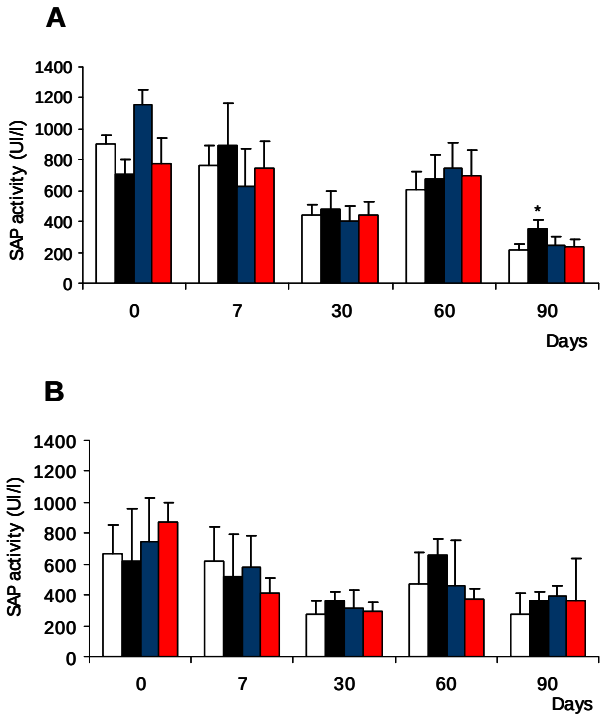
0 g/kg (□); 1 g/kg (■); 2.7 g/kg (■); 6.0 g/kg (■) of CIRUELAX®. Each bar represents the mean ± SD of 11 – 13 rats. * p< 0.05 with respect to the control.

Figure 3. Effect of CIRUELAX® on male (A) and female (B) seric ALT activity of rats at 0, 7, 30, 60 and 90 days of treatment.



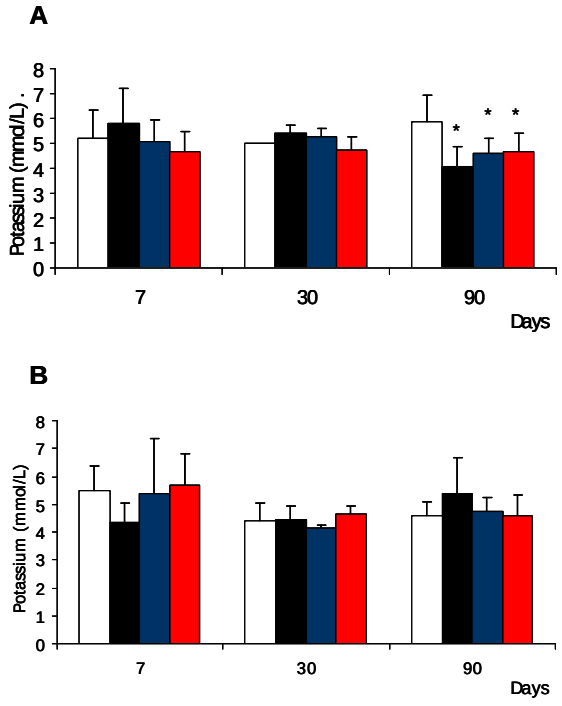
0 g/kg (□); 1 g/kg (■); 2.7 g/kg (■); 6.0 g/kg (■) of CIRUELAX®. Each bar represents the mean ± SD of 11 – 13 rats. ** p<0.01 with respect to the control.

Figure 4. Effect of CIRUELAX® on male (A) and female (B) seric SAP activity at 0.7, 30, 60 and 90 days of treatment.



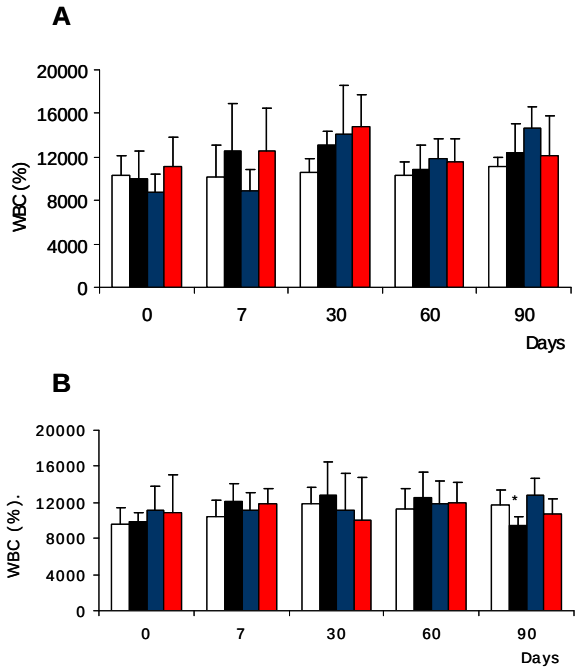
0 g/kg (□); 1 g/kg (■); 2.7 g/kg (■); 6.0 g/kg (■) of CIRUELAX®. Each bar represents the mean ± SD of 11 – 13 rats.* $p < 0.05$ with respect to the control.

Figure 5. Effect of CIRUELAX® on male (A) and female (B) plasmatic potassium levels at 7, 30 and 90 days of treatment.



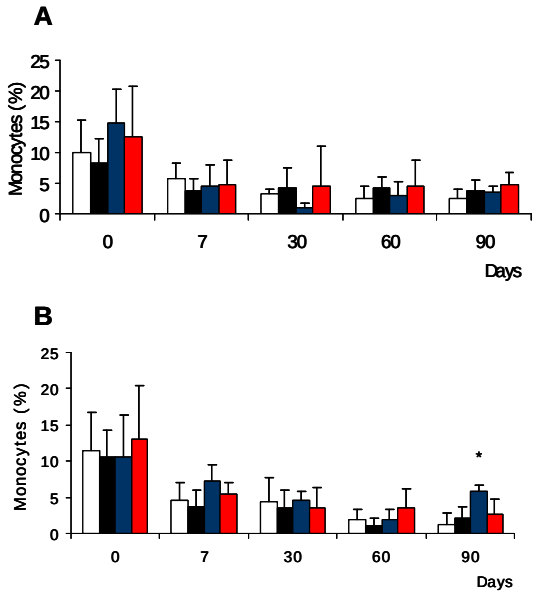
0 g/kg (□); 1 g/kg (■); 2.7 g/kg (■); 6.0 g/kg (■) of CIRUELAX®. Each bar represents the mean ± SD of 11 – 13 rats. * $p < 0.05$ with respect to the control.

Figure 6. Effect of CIRUELAX® on male (A) and female (B) white blood cells (WBC) count at 0, 7, 30, 60 and 90 days of treatment.



0 g/kg (□); 1 g/kg (■); 2.7 g/kg (■); 6.0 g/kg (■) of CIRUELAX®. Each bar represents the mean ± SD of 11 – 13 rats. * $p < 0.05$ with respect to the control.

Figure 7. Effect of CIRUELAX® on male (A) and female (B) monocytes count at 0, 7, 30, 60 and 90 days of treatment.



0 g/kg (□); 1 g/kg (■); 2.7 g/kg (■); 6.0 g/kg (■) of CIRUELAX®. Each bar represents the mean ± SD of 11 – 13 rats. *$p < 0.05$ with respect to the control.

DISCUSSION

No deaths occurred in the rat oral acute toxicity study with CIRUELAX® doses 2, 4, 6, 8 and 10 g/kg, so that LD_{50} for both sexes has to be rated as higher than 10 g/kg. It is important to mention that at 10 g/kg, CIRUELAX® induced a definite reduction in the stool consistence only in some animals. It is known that acute toxicity of pure sennosides is very low: LD_{50} of sennosides 98% pure equals 4100 mg/kg in *i.v.* acute toxicity using mice (Hietala et al., 1987) while LD_{50} for a 93% sennosides extract as 5200 and 3530 mg/kg for males and females, respectively in oral toxicity study using Wistar rats (Mengs, 1988). LD_{50} for extracts of senna is different: LD_{50} of calcium sennosides 5.5% (senna extract) is 384 mg/kg in acute *i.v.* toxicity study in mice (Hietala et al., 1987). CIRUELAX® is a preparation that contains 6.6% of senna leaf, so that, maximal dosis of 10 g/kg of CIRUELAX® used in this work, could be equivalent to 660 mg/kg of senna powder, and to about 79 mg/kg of anthraquinones calculated as sennosides B. The low acute oral toxicity of CIRUELAX® observed in this study is consistent with observations from other studies (Mengs, 1988; Mengs et al., 2004). In spite of previously observations reported elsewhere (Mengs et al., 2004), in subchronic toxicity study, no decrease in weight gain was observed. In contrast, male body weight increased about 10% after 60 days of treatment. This body weight increase was not correlated with laxative dose. Liver relative weight increased lightly in groups treated with lower but no maximal doses of CIRUELAX®. In addition, gonads relative weight was increased in females at lower but no maximal doses of CIRUELAX®. Therefore, increase of liver and gonads relative weight were not dose correlated. No changes with respect to control group were observed in kidney

relative weight, contrary to previous reported results after treatment with pure sennosides and senna powder (Mengs, 1988; Mengs et al., 2004). This different result is probably due to lower senna doses used in the present work (maximal doses: 660 mg/kg) when compared to that used by Mengs et al. (2004) (maximal doses: 1500 mg/kg). Potassium level decreased in comparison to control group in males but not in females that received doses of 1, 2.7 and 6 mg/kg of CIRUELAX® at end of treatment. This result is consistent with hypokalemia due to abuse of stimulant laxatives (Knopf et al., 1995; Cummings, 1974). Sodium and chloride serum levels in treated animals were similar to control group. In other subchronic senna pod study (Mengs et al., 2004) it was described that sodium and chloride serum levels increased in rats treated with 750 and 1500 mg/kg of senna powder, while potassium levels exhibited no differences between treated and control groups. These discrepancies are probably due to lower doses of senna used in the protocol of subchronic toxicity in the present study (maximal dosis: 396 mg/kg). It has been described that chronic use or abuse of stimulant laxatives produces disturbance of electrolyte balance, especially potassium deficiency (Mengs et al., 2004; Mitchell, 2006).

Haematological evaluation resulted in a slight decrease of WBC count and an increase in monocytes count in rats treated with 1 g/kg and 2.7 g/kg with respect to control at day 90th of treatment. No other changes were observed. It has been described elsewhere that the invasion of colonic mucosa by monocytes is a result of stimulant laxatives use (Van Gorkom et al., 1998), but further studies are necessary to determine if the invasion of colonic mucosa by monocytes produces an increase of plasmatic monocytes count.

Many morphologic changes associated to anthranoids use have been reported in literature, although some of them are still controversial: pseudomelanosis coli, a reversible pigmentation of intestine considered harmless, apoptotic cells increment on colonic mucosa (Van Gorkom et al., 2001), higher proliferative activity in mucosa cells (Kleibeuker et al., 1995), swelling and basophilia of cortical tubular epithelial cells in kidney and epithelial hyperplasia in forestomach and large intestine (Mengs et al., 2004) and damage of the myenteric plexus (Smith., 1973; Defour and Gendre, 1984). Further hystopathologic research with chronic CIRUELAX® is necessary to determine probable effects of this laxative in long term abuse. A recently study of oral toxicity of senna in the rat reported no histo-pathological abnormalities after 13-week of treatment with 750 and/or 1500 mg/kg senna extract per day (Mengs et al., 2004).

CONCLUSION

From the results obtained in the present work and under the experimental procedures described above, no acute or subchronic toxic effects of CIRUELAX® have been observed. It is useful to emphasise that at present, there is no hard proof that short term or occasional use of this kind of laxative possesses a threat of toxic effects. If any, it could be attributed to chronic use of these substances.

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