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Administration *Senna occidentalis* Seeds to Adult and Juvenile Rats: Effects on Thymus, Spleen and in Hematological Parameters

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Abstract: The effects of daily administration of *Senna occidentalis* (So) seeds in various concentrations [1% (So1), 2% (So2) and 4% (So4)] to animal feed were investigated in adult and juvenile rats. Additionally, this study evaluated the effects of the same amount of feed without So, seeds on rats (PF-rats). Food consumption and body weight gain were evaluated during 14 day. Moreover, hematological parameters, lymphoid organ weight and histopathology were also performed. We found major alterations in the follow parameters: diminished in food consumption in all treated So adult rats, reduction in the total body weight gain in both adult and juvenile rats from So2 and So4 groups. Lymphoid organs evaluation revealed that *So* seeds can induce immunotoxic effect on thymus and splenomegaly in adult and juvenile rats. These results provide the first evidence that *S. occidentalis* has a direct toxic effect in thymus how a target organ in mammals and suggested that alterations in lymphoid organs are probably associated with the direct toxic effects of this plant and are not due to malnutrition.

Key words: *Senna occidentalis*, toxicology in rats, hematology, lymphoid organs

INTRODUCTION

Herbal drugs and teas have been used since ancient times as a medicine for the treatment of a range of diseases (Cragg and Newman, 2001). Medicinal plants have played a key role in health science, worldwide. According to the World Health Organization (WHO), because of the poverty and the lack of access to modern medicines, about 65-80% of the world's population living in developing countries depends essentially on plants for primary health care. However, it should be noticed that the idea that herbal drugs are safe and present no side effects is misleading. Since, plants can contain not only the therapeutic principle, but also many other compounds that can cause toxicity. In fact, the potential applications and possible side effects of botanical medications are being intensively investigated by a number of studies (Calixto, 2000).

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In Brazil, due to the cultural diversity and the multiple traditional communities and ethnic groups, the use of folklore plants is widespread (Giorgetti *et al*., 2007). In this context, one of the main natural drugs used for the treatment of constipation, not only in Brazil, but also in many other countries (Soyuncu *et al*., 2008) is the leguminous *Senna occidentalis-So* (formerly *Cassia occidentalis*) (link). This plant is native from tropical South America, but it can be also found throughout many tropical and subtropical regions of the world and contains anthranoid compound. Anthranoids are a group of naturally-occurring substances commonly used in clinical practice and as a self-medication for chronic constipation (Seybold *et al*., 2004). *Senna occidentalis* has also been used for other therapeutic purposes, such as expectorant, antibacterial, anti-inflammatory and antiplatelet agent, vermifuge and as a remedy for the treatment of liver diseases (Chopra *et al*., 1980; Adam *et al*., 2001; Nadal *et al*., 2003).

Although, some argue for the safety of short-term use of *S. occidentalis* as a laxative (Bin-Hafeez *et al*., 2001), loss of fluids, hypokalemia and diarrhea in humans are well documented with the use of this plant (Spiller *et al*., 2003). Other side effects include abdominal pain, excessive bowel activity, diarrhea and diaper rash (Spiller *et al*., 2003). In addition, results from *in vitro* (Silva *et al*., 2008) *in vivo* and animal studies suggested that this plant has miotoxic (Haraguchi *et al*., 1998), hepatotoxic (Soyuncu *et al*., 2008) and neurotoxic (Barbosa-Ferreira *et al*., 2005) effects. Moreover, a study using low concentrations of *S. occidentalis* seeds in animal feed during 3 weeks, performed by our laboratory (Silva *et al*., 2003), described alterations in lymphoid organs of broiler chickens, suggesting an immunotoxic effect for this plant.

MATERIALS AND METHODS

Plant

Ripe seeds of *S. occidentalis* were collected from a culture grown at the Biological Institute of São Paulo, State of São Paulo, Brazil, in October 2003. This plant was identified at the species level at the Maria Eneida Fidalgo Herbarium of the Botanical Institute of São Paulo, State of São Paulo, Brazil. The voucher herbarium specimen was deposited in the Botanical Institute of São Paulo (SP) under number SP-363817.

After harvesting, the seeds were dried, frozen in liquid nitrogen and then immediately triturated and incorporated into the animal feed at different concentrations.

Animals

The protocol employed by us met the guidelines of the Bioethics Committee of the School of Veterinary Medicine and Animal Science, University of São Paulo, following the Guide for the Care and Use of Laboratory Animals (NIH publication No. 85-23 text available at http://www.nap.edu/readingroom/books/labrats/. All efforts were made to minimize animal suffering.

Adult and juvenile male Wistar rats with aged approximately 60 days (150-200 g) and 21 days old (100-125 g), respectively inbred in the Department of Pathology, School of Veterinary Medicine and Animal Science, at the University of São Paulo, Brazil were employed. The animals were kept in plastic cages measuring 40×50×20 cm in artificially lighted rooms on a 12 h light/12 h dark cycle (lights on at 07:00 h), with controlled temperature (24-26°C) and free access to food and water.

Experimental Design

Fifty adult rats were randomly divided into four group that received diets with 0 (control group), 1 (So1), 2 (So2) or 4 (So4) of So seeds incorporated into ration, during 14 days; the

rats from control group (0) were fed with pure feed no *S. occidentalis*. In addition, another group of animal (n = 10) was designed as Peer-Feeding (PF) group which animals received the same amount of food without So consumed by So4 group one day before. Thus, rats from So4 and PF groups received the same amount of food. The experimental designs were performed from December 2008 to March 2009.

At the end of experimental period, all animal were killed. Blood sample were harvested for hematological analysis and white blood cell differential counts. After macroscopic evaluation thymus and spleen were collected to perform their relative weight (organ weight/100 g of body weight) and cellularity of thymus and spleen was determined after brief single cell suspensions in RPMI-1640 (10^6 cell mL^{-1}) and following viable cells counting by trypan-blue dye exclusion method. In addition, the above-mentioned organs fragment were sampled and fixed in 10% neutral-buffered formalin for histopathological examination and stained with Haematoxylin and Eosin (HE).

Statistical Analysis

Student's t-test was used to compare two groups (So4 and PF) and ANOVA followed by Dunnet's post hoc test was employed with more than two groups, with the level of significance set at $p<0.05$. Non parametric data were analyzed by Kruskal-Wallis test followed by Dunn's multiple comparison tests to compare the treated rats with the control group, in the analysis of food consumption and hematological parameters. All data are expressed as Mean±SD.

RESULTS AND DISCUSSION

No animal from any group died during the study. All experimental adult animals showed significant decrease ($p<0.05$) in food consumption (Fig. 1), when compared with control rats, as well as with the juvenile group that received the same treatment. In the total body weight gain parameter (Fig. 2), both adult and juvenile rats from So2 and So4 showed decrease

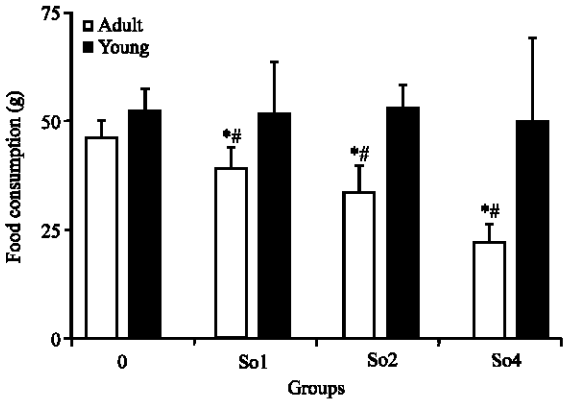

Fig. 1: Food consumption: means (g) for rats fed on a commercial rat feed containing with 0 (control), 1% (So1), 2% (So2) or 4% (So4) of ground *S. occidentalis* seeds and PF group during 14 consecutive days (n = 10/group). Data are reported as Means±SD with Kruskal-Wallis non-parametric test followed by Dunn's multiple comparisons test. *$p<0.05$ different from the adult control group. #$p<0.05$ different from the juvenile group given the same treatment

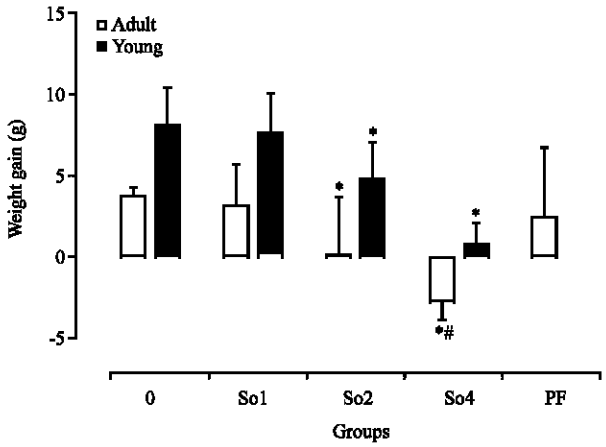

Fig. 2: Total weight body gain: means (g) for rats fed on a commercial rat feed containing with 0 (control), 1% (So1), 2% (So2) or 4% (So4) of ground *S. occidentalis* seeds and PF group during 14 consecutive days (n = 10/group). Data are reported as Means±SD by ANOVA followed by Dunnet's test. *p<0.05 different from control group. #p<0.05 different from the PF group (Student t-test)

Table 1: Hematological parameters

Groups	RBC ------(x10^6 mm^{-3})-------	WBC	HGB (g dL^{-1})	HCT (%)	MCV (μ^3)	MCH (µg)	MCHC (%)
0	64.0±0.9	6.10±0.6	1.53±0.2	42.2±1.5	75.3±0.5	27.5±0.9	3.66±1.0
So1	6.60±0.1	15.6±0.4	14.4±0.9	42.5±2.6	74.1±0.7	25.2±0.5	33.6±1.3
So2	6.10±0.1	5.80±0.4	16.0±0.6	40.0±3.0[a]	65.9±1.0[a]	25.0±0.5	33.5±0.7
So4	6.50±0.1	5.70±0.3	15.2±0.7	39.5±2.5[ab]	67.5±1.5[ab]	24.6±0.9	23.8±1.5[a]
PF	6.80±0.5	5.80±0.7	16.5±0.7	44.5±2.8	77.4±1.0	25.7±1.3	35.7±1.6

Means for adult rats fed on a commercial rat feed containing with 0 (control), 1% (So1), 2% (So2) or 4% (So4) of ground *S. occidentalis* seeds and PF group during 14 consecutive days (n = 10/group). Data are reported as means and respective SD.[a]Significantly different from the adult control group at p<0.05 (Kruskal-Wallis non-parametric test followed by Dunn´s multiple comparisons test). [b]Significantly different from the PF group at p<0.05 (Student t-test)

(p<0.05) in this parameter when compared with control group. The evaluation of the hematological parameters revealed a significant decrease (p<0.05) of HCT, MCV in adult rats from So2 and So4 groups and reduction in MCHC percentage in those animals treated with the highest dose of So seeds (Table 1). In the white blood cell differential counts related to *S. occidentalis* seeds in the diet, no alteration was observed in animals from the experimental groups. Adult and juvenile rats treated with the highest concentration of seeds (So2 and So4) displayed an increase (p<0.05) in the relative spleen organ weight (Fig. 3) when compared with control group. On the other hand, adult rats from So1, So4 and PF showed decrease (p<0.05) in relative thymus organ weight (Fig. 4); we observed the same in juvenile animals from So4 groups when compared with control groups. In the cellularity of spleen no alteration was observed in both adult and juvenile So animals. However, all experimental juvenile rats displayed decrease (p<0.05) in the cellularity of thymus, when compared with the control group (Fig. 5). Histopathology of thymus and spleen of the juvenile and adult rats treated with *S. occidentalis* seeds were performed; however no alteration was observed.

In this study, we observed a decrease in the food consumption in all So rats group. Since, the animals showed reduction in food consumption only during the second week of *S. occidentalis* administration, this arguably suggests that such reduction was not

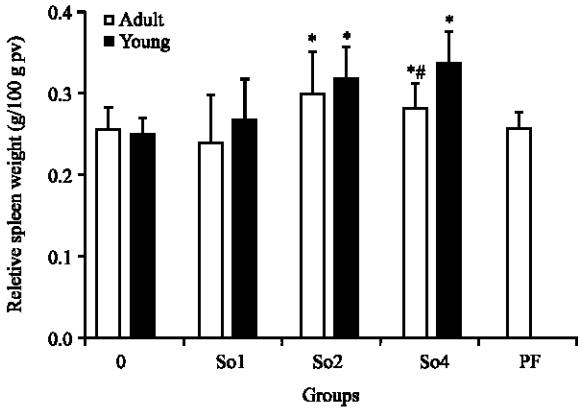

Fig. 3: Relative spleen weight (g/100 g pv): means for rats fed on a commercial rat feed containing with 0 (control), 1% (So1), 2% (So2) or 4% (So4) of ground *S. occidentalis* seeds and PF group during 14 consecutive days (n = 10/group). Data are reported as Means±SD by ANOVA followed by Dunnet's test. *p<0.05 different from control group. #p<0.05 different from the PF group (Student's t-est)

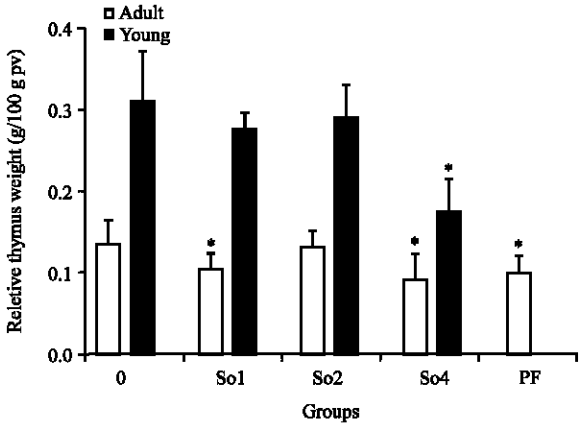

Fig. 4: Relative thymus weight (g/100 g pv): means for rats fed on a commercial rat feed containing with 0 (control), 1% (So1), 2% (So2) or 4% (So4) of ground *S. occidentalis* seeds and PF group during 14 consecutive days (n = 10/group). Data are reported as Means±SD by ANOVA followed by Dunnet's test. *p<0.05 different from control group

associated with the low palatability of this plant, but mainly with its anorexic effects. In fact, some data confirm that anorexia is associated to the *Senna* abuse consumption in humans that take this plant as laxative agent for weight loss (Stickel and Schuppan, 2007; Soyuncu *et al*., 2008). Moreover, spontaneous intoxication with *S. occidentalis* in domestic animals (Barros *et al*., 1999) and several experimental studies performed in laboratory animals (Tasaka *et al*., 2000; Nadal *et al*., 2003; Barbosa-Ferreira *et al*., 2005) showed that anorexia is a common feature in *S. occidentalis* toxicosis.

In the present study, we observed a decrease in body weight gain in both juvenile and adult rats from So2 and So4-groups. Hypothetically, this effect might relate only to the

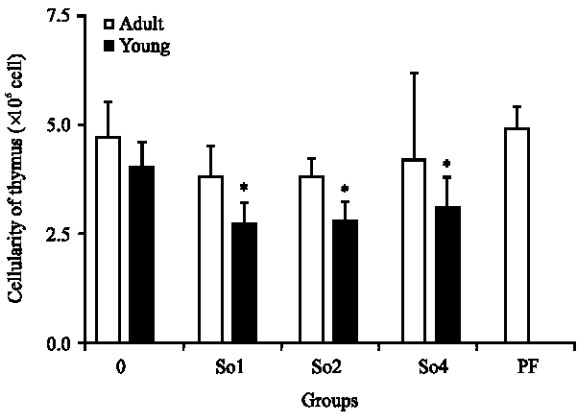

Fig. 5: Cellularity of thymus (x10^6 cel): means for rats fed on a commercial rat feed containing with 0 (control), 1% (So1), 2% (So2) or 4% (So4) of ground *S. occidentalis* seeds and PF group during 14 consecutive days (n = 10/group). Data are reported as means and respective SD. *p<0.05 different from control group

anorexia produced by the plant. However, it should be considered that PF-rats did not show any alteration in weight gain; hence, other factors could have contributed for this effect. *S. occidentalis* contains anthranoids that are widely used as laxative agents (Adrian, 2000) and, accordingly, in the present study we observed that So-treated rats showed increased volume and softening of faeces.

Besides, it is fairly known that *S. occidentalis* produces hepatotoxicity, according to reports on its use for phytotherapic purposes in humans (Vashishtha *et al*., 2007; Soyuncu *et al*., 2008). Hepatic lesions were also described in spontaneous intoxication confirmed in cattle (Barros *et al*., 1999). Moreover, experimental intoxication in different animal species as rabbits (Tasaka *et al*., 2000), goats (Suliman and Shommein, 1986), broiler chickens (Haraguchi *et al*., 1998) and rats (Barbosa-Ferreira *et al*., 2005) has suggested that the hepatotoxicity is one of the main toxic effects of *S. occidentalis*. According to Beuers *et al*. (1991), the induction of the hepatotoxicity produced by *S. occidentalis* is probably due to the effect of antraquinone, which is a compound of the plant.

In fact, data from several studies with humans (Jacobs and Hirsch, 2000; Stickel *et al*., 2000) and experimental animals (Cui *et al*., 2009) showed a direct relation between hepatotoxicity and loss weight after *S. occidentalis* consumption. Therefore, it can be suggested that another factor that possibly contributed to the loss weight in So2 and So4-groups showed in this study was the plant hepatotoxicity as demonstrated by Barbosa-Ferreira *et al*. (2005).

The complexity of the immune system results in multiple potential target sites for the pathological effects of immunotoxic effect of xenobiotics (De Jong and Van Loveren, 2007). Our experiments showed that *S. occidentalis* seeds produced alterations in rat lymphoid organs, as well as in hematologic parameters. Thus, the analyses of the thymus of juvenile and adult animals of both So groups revealed a decrease in size as well as in the cellularity, suggesting an immunotoxic effect like the one that occurred in chickens (Silva *et al*., 2003); these results strongly augment the above a *S. occidentalis* toxic effect in lymphoid organs tends in this case a thymus as target organ in mammals.

However, while we observed a clear immunotoxic effect of *S. occidentalis* in rats, Bin-Hafeez *et al*. (2001) studying mice treated with aqueous extract of *S. occidentalis* for two

weeks, demonstrated the potent immunoprotective effect of this plant. A hypothesis to justify this discrepancy between the two studies is that rat's immune system could be less resistant to the toxic effects of the active principle of the plant than in mice. On the other hand, it should be emphasize that in the aqueous extract of *S. occidentalis*, as used in the Bin-Hafeez's experiment, the liposoluble components, such as anthraquinone, a well characterized toxic principle of this plant, are not present (De Witte, 1993); nevertheless, when the plant whole seeds are administered, as we used in the present study, the animals are exposed to all *S. occidentalis* constituents including these liposoluble and toxic substances.

Thus, an additional and more feasible hypothesis to give an explanation between the opposite results found in ours and in Bin-Fafeez's experiments is the presence or the absence of toxic constituents in the whole *S. occidentalis* seeds and in the aqueous extract of this plant, respectively.

We observed an increase in the relative spleen weight in juvenile and adult rats from So2 and So4 groups. In this context, general parameters like organ weight, which may indicate target organ specific toxicity, play an important role as a first indicator for the presence of direct immunotoxicity (De Jong and Van Loveren, 2007). However, at this moment we do not have a way to clarify the toxic mechanism of *S. occidentalis* involved in the enhanced spleen weight. Thus, in order to clarify this question, we aim to perform additional studies in our laboratory.

It is well known that malnutrition has a proportionately greater impact on the size of lymphoid tissues, particularly the thymus (Prentice, 1999; Savino, 2002). Since, both ours' and Silva *et al.* (2003) studies showed that *S. occidentalis* produces a significant decrease in food intake, we can argue that alterations seen in bursa of Fabricius and thymus, respectively, could be due to the nutritional deficiency and not to a toxic effect of *S. occidentalis* itself. In fact, reinforcing this theory, we have data from the PF group, where animals also showed the same thymus changes.

The concern over the immunotoxicity has been heightened by the realization that the developing immune system may be more sensitive than the adult immune system, at least in response to some well characterized immunotoxic chemicals (Luebke *et al*., 2006). In fact, the present study observed a decrease in size and cellularity of thymus in juvenile animals from So2 and So4 groups. Considering that juvenile rats did not show any change in food consumption, it is reasonable to suggest that this effect on thymus in young animals was due to a direct toxicity produced by *S. occidentalis*.

Overall, the present study showed that the *S. occidentalis* seeds lead to injury to both the lymphoid organs and the hematopoietic system and these alterations are probably associated with the direct effect of the toxic principle of the plant and not due to malnutrition. Our findings suggest that the evaluation of both systems should be an integral part of investigations on the *S. occidentalis* chronic effects in different animal species.

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