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Full Length Research Paper

Preliminary phytochemical analysis and the effect of *Agave sisalana* on body weight and defensive behaviours in ovariectomized rats

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Agave sisalana **is a plant belonging to the Agavaceae family. Sisal juice is constituted of steroidic saponins which are precursor molecules of many pharmacologically active steroids. These precursor molecules can act in brain structures that are related to the modulation of emotional disorders. They can act on energy metabolism or directly on the absorptive process of fats. The objective of this research was to identify the biocompounds present in *A. sisalana* juice, and to evaluate the effect of different extracts on the expression of defensive behaviours of rats in elevated plus maze and open field tests, and body weight, in a condition that corresponds to an induced menopause. Wistar rats were subjected to bilateral ovariectomy or to a sham surgical procedure under anaesthesia. Following surgery, they were treated gavage with sisal juice - concentrated crude juice (CCJ): 500 or 1000 mg/kg; dried extract (DE): 50, 100 or 200 mg/kg; dry mucilage extract (DME): 25, 50 or 100 mg/kg; and intermediate product (IP): 25, 50 or 100 mg/kg; or distilled water. The results showed the presence of coumarins, flavonoids, condensed tannins, free anthraquinones, alkaloids and saponins in the sisal juice. Both CCJ and DE (100 mg/kg) caused weight loss without alteration of defensive behaviours related to the manifestation of anxiety. As saponins were identified in DE in significant amounts, the observed effects were attributed to this component. Such findings point to *A. sisalana* as a plant that could potentially be used to treat weight gain during menopause.**

Key words: *Agave sisalana,* steroidic saponins, body weight, anxiety, plus-maze, females, ovariectomy.

INTRODUCTION

Agave sisalana is a plant belonging to the family Agavaceae, and it is popularly known in Brazil as sisal (Martin et al., 2009). The fibre is the main product of *A. sisalana* culture; it is used in the automotive industry and in the manufacture of ropes, twines, sea cables, carpets, bags, brooms, upholstery and crafts (Silva and Beltrão,

1999; Martin et al., 2003). The fibre-loosening process generates about 95% waste, which corresponds to the watery part of the plant and the bagasse (Pizarro et al., 1999). When not disposed of, the fibre is used as animal feed, biofertilisers and in the production of drugs, particularly hormones (Martin et al., 2009; Ribeiro et al., 2013).

In an ethnopharmacological approach, the juice of *A. sisalana* (that is, the liquid residue of the mucilage) is applied topically to treat skin diseases (El-Hilaly et al., 2003). It has also been administered orally for the treatment of indigestion, bloating, jaundice, constipation and diarrhoea (Bown, 1995); as an analgesic (Duke and Ayensu, 1985); and as a uterine stimulant (Sharaf and Zahran, 1967).

Several organic compounds have been isolated that make up sisal juice, such as oxalic acid, cortisone and saponins (Azevedo et al., 2003). The structure of saponins consists of a glycidic portion linked to an aglycone portion called a sapogenin; in a steroidal skeleton structure it is called steroidal sapogenin (Hostettmann and Marston, 1995; Simons et al., 2006). Sapogenins that are found in *A. sisalana* are tigogenin, diosgenin and hecogenin (Pizarro et al., 1999). The steroidal saponins are of considerable economic importance as precursors to many pharmacologically active steroids (Oashi, 1999). The use of different saponins, for example, *Panax ginseng* (Attele et al., 2002), *Panax japonicas* (Yun, 2010) and *Platycodi radix* (Han et al., 2000; Zhao et al., 2005) has been validated in different models to prevent or decrease obesity. Studies report that saponins might be able to interfere with the metabolism of cholesterol and fat absorption by inhibiting pancreatic lipase activity (Dickel et al., 2007). In addition, it has been reported that treatment with saponins that were isolated from the plant *Panax quinquefolium* caused an anxiolytic effect in the elevated plus maze (EPM), among other tests (Wei et al., 2007). These findings indicate a possible relationship between the presence of saponins that were identified in *A. sisalana* and effects such as weight loss and reduced anxiety.

Periods during which sex steroids in women are found in low concentrations in the serum have been found to be correlated with signs of anxiety, depression and other physiological changes. During the climacteric, the transition phase from the reproductive to non-transition phase from the reproductive to non-reproductive phases of life, which is part of the ageing process in women, often results in hot flashes or hot flushes (Sclowitz et al., 2005), high levels of cholesterol and an increase in body weight (Toth et al., 2000). The accumulation of weight during menopause, especially in the abdominal region, is related to an increase in cardiovascular diseases, diabetes mellitus type 2, cancer and knee osteoarthritis (Milewicz and Jedrzejuk, 2006; Popov et al., 2007). Metabolic and hormonal changes could be responsiblefor considerably damage in the psychosocial aspects, leading to a reduction in quality of life (Silva et al., 2003).

Considering that the hormone replacement therapy (HRT) have controversies, and their use is contraindicated in patients at risk for some types of cancer and other pathologies (Lima and Baracat, 1995; Bonduki et al., 2006), several studies have been conducted to analyse the therapeutic potential of plants that have phytoestrogens, in the treatment of symptoms of menopause (Rachev et al., 2000; Clapauch et al., 2002; Chandeying and Lamlertkittikul, 2007).

The hypothesis investigated in this study was that the steroidal saponins present in sisal juice could reverse some of the effects caused by the loss of ovarian function. Thus, the objectives of this investigation were: (a) to determine the phytochemical profile of *A. sisalana* juice and the concentration of saponins in different extracts of *A. sisalana* that have pharmacological activity; and (b) to characterise the effects of *A. sisalana* extracts on the body weight of rats in a condition that corresponds to an induced climacteric and to characterise its effects on defensive behaviours related to the manifestation of anxiety.

MATERIALS AND METHODS

Obtaining the plant materials of origin

The mucilage that was obtained by refining *A. sisalana* leaves was sourced from farmers in the city of Valente-Bahia, north-eastern Brazil (latitude: 11° 44' 24" S, longitude: 39° 27' 43" W, altitude: 358 m), through a partnership with the Syndicate of Plant Fiber Industries of the State of Bahia. The mucilage was frozen and transported to the Laboratory of Pharmaceutical Technology in Herbal Medicines of the Faculty of Sciences and Letters of São Paulo State University (UNESP), Assis. *A. sisalana* juice was acquired by pressing and filtering the mucilage.

Animals

The study was approved by the local Ethics Committee on the Use of Animals (CEUA 013/2012). All procedures were conducted in accordance with international ethical standards concerning animal experimentation.

Wistar rats, obtained from the Central Vivarium of UNESP, Botucatu, were used. The mean weight of the rats was 200 g at the beginning of the experimental sessions. The animals were housed in polypropylene cages (five animals per box). Food (Nuvilab CR-1 kibble) and water were available *ad libitum*. The vivarium was maintained with controlled temperatures (23±2°C) and with an artificial lighting programme that corresponded to 50 lux (lights on at 7:00 am and off at 7:00 pm). The animals were handled for the exchange of boxes three times per week.

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Analysis of the chemical composition of *A. sisalana* juice

All phytochemical screening was performed according to the recommendations from previous study (Collins and Braga, 1988; Simões et al., 2010; Bessa et al., 2013). The tests were carried out with *A. sisalana* juice. The different phytochemical compounds present in this juice were characterised by performing chemical reactions that resulted in the development of foam, colouring or precipitation. The following compounds analysed and reactions used were: saponins, agitation-froth test, coumarins, reaction with aqueous potassium hydroxide solution; alkaloids, reaction with Dragendorff's reagent; flavonoids, Shinoda, Taubock, aluminium chloride and Pew tests; anthraquinones, Bornträger's reaction (direct and with prior acid hydrolysis); tannins, identification tests of hydrolysed and condensed tannins with solutions of ferric chloride, acetic acid and lead acetate, and by Stiasny's reaction. A positive or negative result was determined for each phytochemical compound based on whether the expected characteristic reaction was or was not observed.

Preparation of *A. sisalana* extracts

In this study, concentrated crude juice (CCJ) was obtained by heating *A. sisalana* juice to 100°C and reducing the volume six-fold. In addition, three extracts from the mucilage of *A. sisalana* were developed: (1) dried extract (DE) that resulted from centrifugation (Fanem FR 22) of the natural juice at 4000 rpm for 20 min; (2) dry mucilage extract (DME) was obtained after mucilage was dried in the sun for a week then crushed, rehydrated to a smaller volume, pressed, filtered and centrifuged at 4000 rpm for 20 min; (3) intermediate product (IP) was obtained from *A. sisalana* juice that was initially subjected to sulphuric acid to obtain a pH of 0.4 to 0.8, heated to a high temperature (120°C) for 150 min and centrifuge (Fanem FR 22) at 4000 rpm for 20 min. All extracts were dried in a greenhouse (Fanem 002CB model) at 50°C until a constant weight was achieved.

Because the glycosidic portion of saponins makes these large molecules water soluble (Simões et al., 2010), all extracts were dissolved in distilled water. However, considering that chemical hydrolysis of the IP extract is able to remove the sugar chain (the hydrophilic portion of saponins), the lipophilic portion or the steroidal sapogenins (Bodeiko and Kintya, 1975), it was necessary to dissolve in 2% Tween 80 mixed with distilled water.

Application of *A. sisalana* extracts

CCJ (500 or 1000 mg/kg), DE (50, 100 or 200 mg/kg), DME (25, 50 or 100 mg/kg) and IP (25, 50 or 100 mg/kg) were administered through the orogastric pathway using a syringe attached to a gavage probe. Doses were administered in the morning in a volume of 2 mL per animal. The same procedure was followed for animals of the control group (sham and ovariectomized rats); they received only the vehicle (distilled water) in the same way as the treated animals and with the same volume. Another control group was designed in which the rats were treated with distilled water plus 2% Tween 80 because the IP was prepared with this substance. The animals were weighed three times per week, immediately before the morning dose administration, to ensure appropriate dosage. The doses were defined based on previous results obtained by a research group in other studies (unpublished).

Study description

After arriving from the central vivarium, the rats were grouped (five animals per box) and left for a week to acclimatise. The treatment, as described previously, was started after the one-week acclimatisation period, and it lasted for a period of 15 days. On day 15, the rats were subjected to an ovariectomy with the goal of interrupting their hormonal cycle. The sham animals underwent a surgical procedure, which was identical to those of the treatment animals except for removal of the ovaries. Twenty-one days after the procedures, behavioural assessments were performed (on day 36 after the first application of the extract or control solution).

Two experiments were developed. The first experiment was designed to evaluate the effect of *A. sisalana* CCJ (CCJ 500 or 1000 mg/kg) in comparison to a control group of females that underwent sham surgery and ovariectomized rats treated only with distilled water (2 mL/animal) (sham, n = 15; ovariectomized, n = 15; CCJ 500 mg/kg, n = 15; and CCJ 1000 mg/kg, n = 14).

The second experiment was designed to evaluate the effects of different preparations and their controls. The experimental groups were: Sham females that received daily doses of distilled water (2 mL/animal), n = 10; ovariectomized rats that received daily doses of distilled water (2 mL/animal), n = 18; ovariectomized + Tween 80, rats that received daily doses of distilled water plus 2% Tween 80 (2 mL/animal), as a parameter to the IP that was diluted with the aid of this emulsifier and surfactant, n = 9; DE 50 mg/kg, n = 9; DE 100 mg/kg, n = 10; DE 200 mg/kg, n = 8; DME 25 mg/kg, n = 9; DME 50 mg/kg, n = 10; DME 100 mg/kg, n = 10; IP 25 mg/kg, n = 10; IP 50 mg/kg, n = 7; and IP 100 mg/kg, n = 9. All groups received doses or volumes in a single application (during the morning) for 36 days, as previously described.

Behavioural assessment

The tests were performed under low illumination, similar to the vivarium lighting (50 lux). Other environmental conditions were exactly the same as the vivarium. The tests were recorded through a video system and analysed using the EthoLog 2.25 program (Ottoni, 2000).

The animals were evaluated in an EPM test, which allows for an assessment anxiety. It was elevated 50 cm from the floor and it consisted of two open arms (50 × 10 cm) and two closed arms (50 × 10 × 40 cm) that are arranged in such a way that the closed arms were perpendicular to the open arms (Pellow et al., 1985). To avoid animals falling off, the edges of the open arms were protected by wood that measured 3 mm high and 2 mm thick. Animals were assessed for a period of 5 min. The results were expressed as the percentage of entries and time in open arms in relation to total entries and time in open and closed arms, respectively. The number of entries in the closed arms was measured as an index of motor activity. Entrance into an arm or across the centre was considered to have occurred with the complete passage of all four legs.

Immediately after being tested in the EPM, the animals were evaluated in a 60 x 60 cm wooden arena (open field) that was made up of 20 × 20 cm demarcated squares (20 × 20 cm) for a period of 5 min. The animals were initially placed in the central quadrant of the box. In order to check if the motor activity of the tested animals influenced the behavioural test, the number of quadrants traversed by the animals was counted. An animal was counted as having crossed a square if it crossed the intersection of the lines with all four legs.

After the behavioural evaluations, each animal was carefully anesthetised and subsequently sacrificed. Some organic structures were removed and weighed, such as, spleen, adrenals, thymus, uterine tubes and uterus.

Body weight

Animals were weighed through the duration of the experiment in

order to check possible differences in weight variation between groups. For this, the differential weight of animals were used, which was the difference between the final weight (observed on the day of behavioural evaluation) and starting weight (noted at the beginning of treatment) of each animal.

Detection of total saponin concentrations

The concentration of saponins in *A. sisalana* extract that presented the best result was evaluated using a spectrophotometric analysis, according to the recommendations from previous study (Clark et al., 1993; Vigo et al., 2003). The analysis was performed in a UV-Vis spectrophotometer. The *A. sisalana* dried extract was dissolved in distilled water at three concentrations: 0.25, 0.35 and 0.50 mg/mL. An aliquot of 1 mL of each extract solution was added to 1 mL of 0.2% cobalt chloride chromogenic reagent and 1 mL concentrated sulphuric acid. To verify the absorbance, the reading of the solution occurred at 284 nm 20 min after the start of the reaction. In this analysis, the saponin (Merck, 0.2 mg/mL) was used as a positive control.

The concentration of total saponins was expressed in milligram of saponins per milliliter of extract solution and in milligram of saponins per gram of the dry extract. For the calculation of these concentrations, a linear regression curve was used to establish a straight line equation, which was then applied at concentrations of 0.08 to 0.28 mg/mL.

Data analysis

The behavioural data in each experiment, as well as the body weight, the weight of organic structures (such as spleen, adrenals, thymus, uterine tubes and uterus) and the consumption of water and food were analysed by an analysis of variance (ANOVA), followed by Duncan's *post hoc* test. In all cases, results with a p value less than or equal to 0.05 were considered significant.

RESULTS

Phytochemical analysis

The tests for detecting and prospecting the chemical constituents of *A. sisalana* juice indicated the presence of saponins, coumarins, flavonoids, tannins and anthraquinones (Table 1). A high saponin content in *A. sisalana* juice was detected by observing foam permanence, even after the addition of hydrochloric acid. The presence of coumarin was detected by the reaction of this compound with potassium hydroxide. In the search for flavonoid, using metallic magnesium powder and concentrated hydrochloric acid, the presence of chalcones, aurones, dihydrochalcones and isoflavones in *A. sisalana* juice were identified by the cyanidin or Shinoda reaction. The presence of flavones, flavonols and flavonones occurred by a reaction of *A. sisalana* juice with aluminium chloride, and flavanones and isoflavones with the boric acid and oxalic acid solutions, also known as reaction Taubock. Using Pew's reaction, no anthocyanins were detected in the *A. sisalana* juice. Ferric chloride and Stiasny's reaction were used for detection of condensed tannins. The presence of hydrolysable tannins was not detected. Using Bornträger's direct reaction, the presence of free anthraquinones was determined by observing a red colouration in the region that made contact with the juice and by observing a brown colour after homogenisation. Bornträger's reaction with prior acid hydrolysis was used to identify anthraquinones, glycosides and dimers. The presence of these types of anthraquinone in the juice was not identified because the reaction did not result in a rosy-coloured reaction, which is indicative of a positive result. The formation of precipitates through direct survey in the samples was not observed, suggesting the absence of alkaloids.

Behavioural and body weight analysis

Experiment 1: Effect of treatment with A. sisalana CCJ (500 or 1000 mg/kg) on the manifestation of defensive behaviours, body weight and some structures of ovariectomized females

The two doses of *A. sisalana* CCJ used in this study did not cause behavioural changes in the EPM. Specifically, a statistical change was not observed in the percentage of entries in open arms/total [$F(3.54) = 1.07$; $p = 0.368$]; the percentage of time in open arms/total [$F(3.54) = 1.34$; $p = 0.272$] (Figure 1); or entries in closed arms [$F(3.54) = 0.88$; $p = 0.458$] or in the arena [$F(3.54) = 1.58$; $p = 0.204$] (Figure 2). Also, there were no alterations observed in the weight of organic structures (such as spleen, adrenals, thymus, uterine tubes and uterus), change in the amount of water or change in the amount of food ingested ($p > 0.05$). However, there was a significant observation of a loss of body weight [$F(3.55) = 28.26$; $p < 0.001$] (Figure 3).

Experiment 2: Effect of treatment with different preparations of A. sisalana (DE, DME or IP) on the manifestation of defensive behaviours, body weight and some structures of ovariectomized females

In the EPM, an analysis of the percentage of entries in open arms/total, ANOVA showed that in relation to different doses of DE, DME and IP, the result was not significant [$F(11.11) = 1.87$; $p = 0.051$]. However, considering that the result was borderline, Duncan's test showed the ovariectomized females treated with DE at 50 and 200 mg/kg, DME at 100 mg/kg and IP at 50 mg/kg IP explored the open spaces of the EPM less than the control group females (ovariectomized + Tween 80; $p < 0.01$). For the percentage of time in open arms/total, ANOVA showed that there were no differences in a comparison of the effects of treatments at different doses of DE, DME and IP [$F(11.11) = 1.20$; $p = 0.292$]. Figure 1 illustrates these results.

Table 1. Phytochemical prospecting tests of the sisal juice.

Chemical constituent	Positive reaction	Result observed
Saponins	Stable foam layer for more than 30 m	Positive
Coumarins	Blue fluorescence under ultraviolet light	Positive
Flavonoids	Reaction with aluminium chloride; yellow fluorescence under ultraviolet light for flavones and flavonols; blue-green for flavanones	Positive for flavones, flavonols and flavonones
	Shinoda's reaction: Yellow to red for flavones; red to bloody red for flavanols and dihydroflavonols; red to purple for flavanones; and red to pink for anthocyanic derivatives	The reaction was colourless, indicating the presence of chalcones, aurones, dihydrochalcones and isoflavones
	Negative reaction without staining for chalcones, aurones, dihydrochalcones and isoflavones	
	Pew's reaction: Reddish for anthocyanins	Negative
	Taubock's reaction: Yellow fluorescence under ultraviolet light for flavonols. Flavones and isoflavones do not present fluorescence. Anthocyanic compounds stain but do not produce fluorescence	The reaction did not present fluorescence, indicating the presence of flavones and isoflavones
Tannins	Stiasny's reaction: Red precipitate for condensed tannins and blue precipitate for hydrolysable tannins	Red precipitate for condensed tannins
	Reaction with ferric chloride: Blue for hydrolysable tannins and green for condensed tannins	Green colouration, indicating the presence of condensed tannins
	Reaction with acetic acid and acetate of lead: Whitish precipitate for hydrolysable tannins	Negative
Anthraquinones	Bornträger's direct reaction: Red tinge in the area in contact with the juice for free anthraquinones (brown when homogenised)	Positive
	Bornträger's reaction with prior acid hydrolysis: Rosy colouring to identify anthraquinones, glycosides and dimers	Negative
Alkaloids	Reddish-orange precipitate	Negative

In relation to entry in the closed arms (Figure 2), there were significant effects between the treatments and their respective doses of *A. sisalana* [F (11.11) = 3.24; $p < 0.001$], as can be seen in Figure 2. Based on results from Duncan's test, rats that received doses of 100 mg/kg of DME ($p < 0.01$) showed a significant increase in activity in relation to the control group (ovariectomy + Tween 80).

For the number of squares covered in the arena (Figure 2), there was an effect between the treatments and their respective doses of *A. sisalana* [F (11.11) = 3.94; $p < 0.001$]. Results from Duncan's test demonstrated that ovariectomized rats treated with distilled water + Tween 80 showed a significant increase in motor activity compared to ovariectomized rats and those treated only with distilled water ($p < 0.05$). Rats treated with DE 50, 100 and 200 mg/kg showed a decrease in motor activity only when compared to the control group (ovariectomized + Tween 80; $p < 0.05$). Results were also different for rats treated with IP (25 to 100 mg/kg), which showed an increase in motor activity compared to the specific control group, especially those treated with DE 50 mg/kg.

When the mean differential variation of weight was analysed by ANOVA (Figure 3), the results showed that there was an effect between the treatments and their respective doses of *A. sisalana* [F (11.11) = 8.45; $p < 0.001$]. Duncan's test indicated that ovariectomy caused an increase in body weight. Rats treated with DE at 100 and 200 mg/kg, loss weight when compared with control rats (ovariectomized with or without Tween) ($p < 0.001$). Rats treated with DE at 50 mg/kg were only different from ovariectomized + Tween 80 rats ($p < 0.05$), compared to the control group (ovariectomized + Tween 80).

Because the rats treated with DE at 100 mg/kg did now show any changes in behaviours in the EPM or open field tests compared to the rats that were only ovariectomized, other variables were analysed. It was found that this extract, DE, caused an increase in the weight of the reproductive structures and of the adrenals ($p < 0.01$), but it did not cause changes in the weight of the other organs, such as the spleen and liver, or a change in the

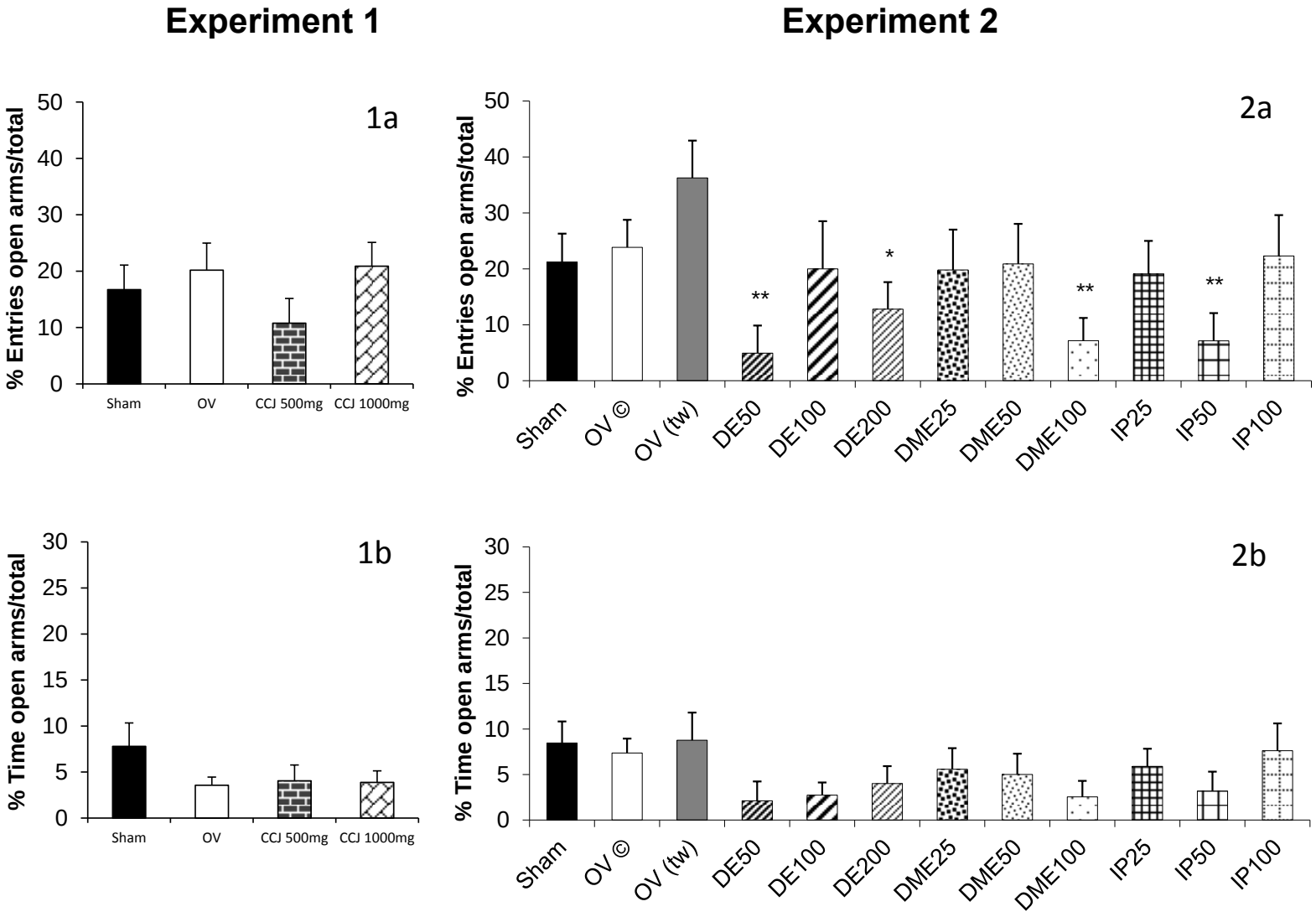

Figure 1. Manifestation of defensive behaviours related to anxiety in the elevated plus maze. Mean + standard error of the mean (SEM) of the percentage of entries and time in open arms in relation to total entries and time in open and closed arms, respectively. On the left are data from Experiment 1 (1a: % entries/total; 1b: % time/total): treatment with *Agave sisalana* concentrated crude juice (CCJ, 500 or 1000 mg/kg) and control group. On the right are data from Experiment 2 (Panel 2a: % entries/total; Panel 2b: % time/total): Different doses of the dried extract (DE), dry mucilage extract (DME) and intermediate product (IP) of *A. sisalana* and control groups. A p-value of less than 0.05 (*) and less than 0.01 (**) represents the significant differences in relation to the control group of ovariectomized + Tween 80 (analysis of variance followed by Duncan's post hoc test).

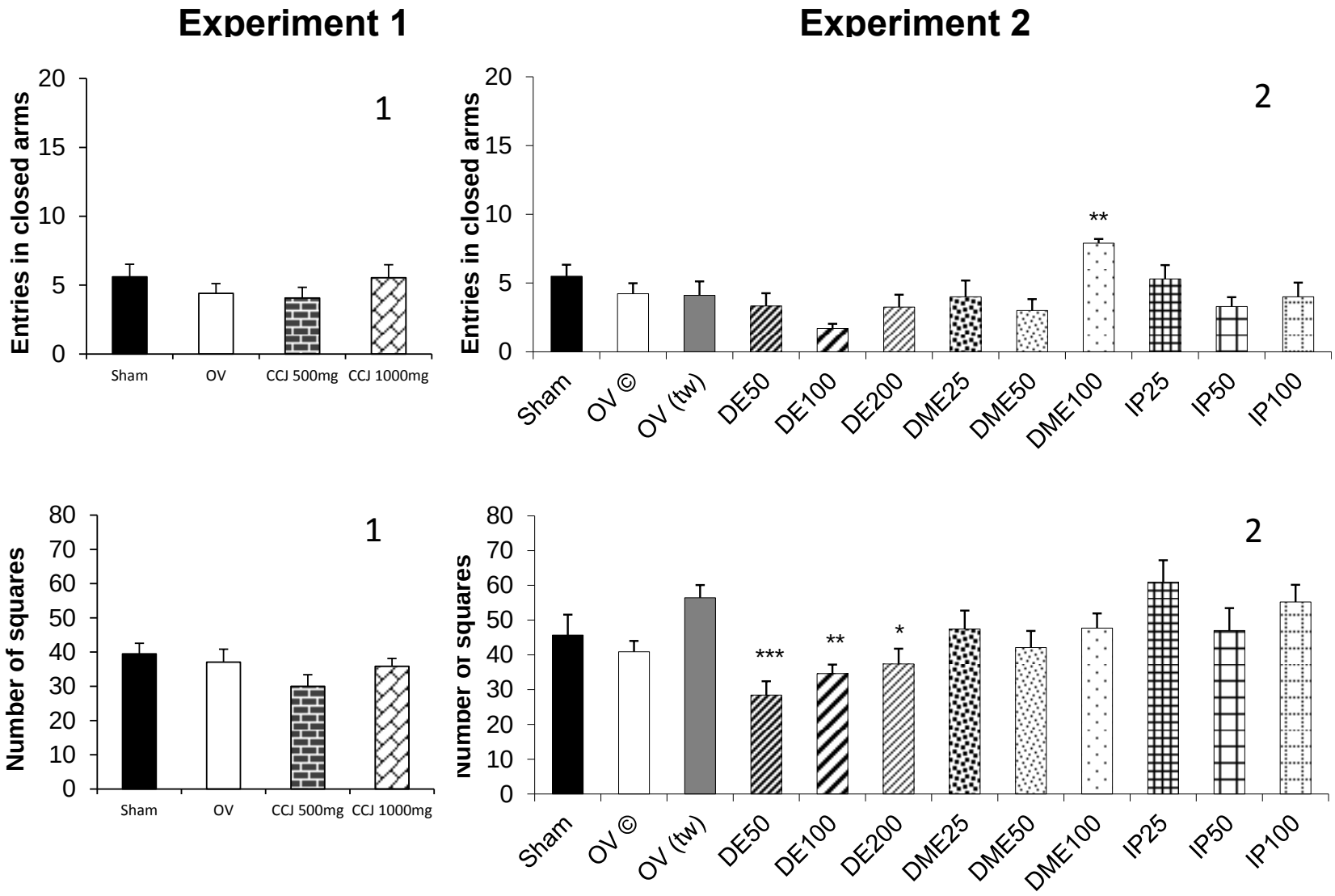

Figure 2. Motor activity of females in the elevated plus maze and the arena. Mean + standard error of the mean (SEM) of the percentage of entries and entries in open arms in relation to total entries and time in open and closed arms, respectively. On the left are data from Experiment 1 (1a: % entries/total; 1b: % time/total): *A. sisalana* concentrated crude juice - CCJ, 500 or 1000 mg/kg groups and control group. On the right are data from Experiment 2 (2a: % entries/total; 2b: % time/total): different doses of dried extract (DE), dry mucilage extract (DME) and intermediate product (IP) of *A. sisalana* and control groups. A p-value of less than 0.05 (*) and less than 0.01 (**) represents the significant differences in relation to the control group of ovariectomized + Tween 80 (analysis of variance followed by Duncan's post hoc test); $p > 0.05$, result not significant (ANOVA followed by Duncan's post hoc test).

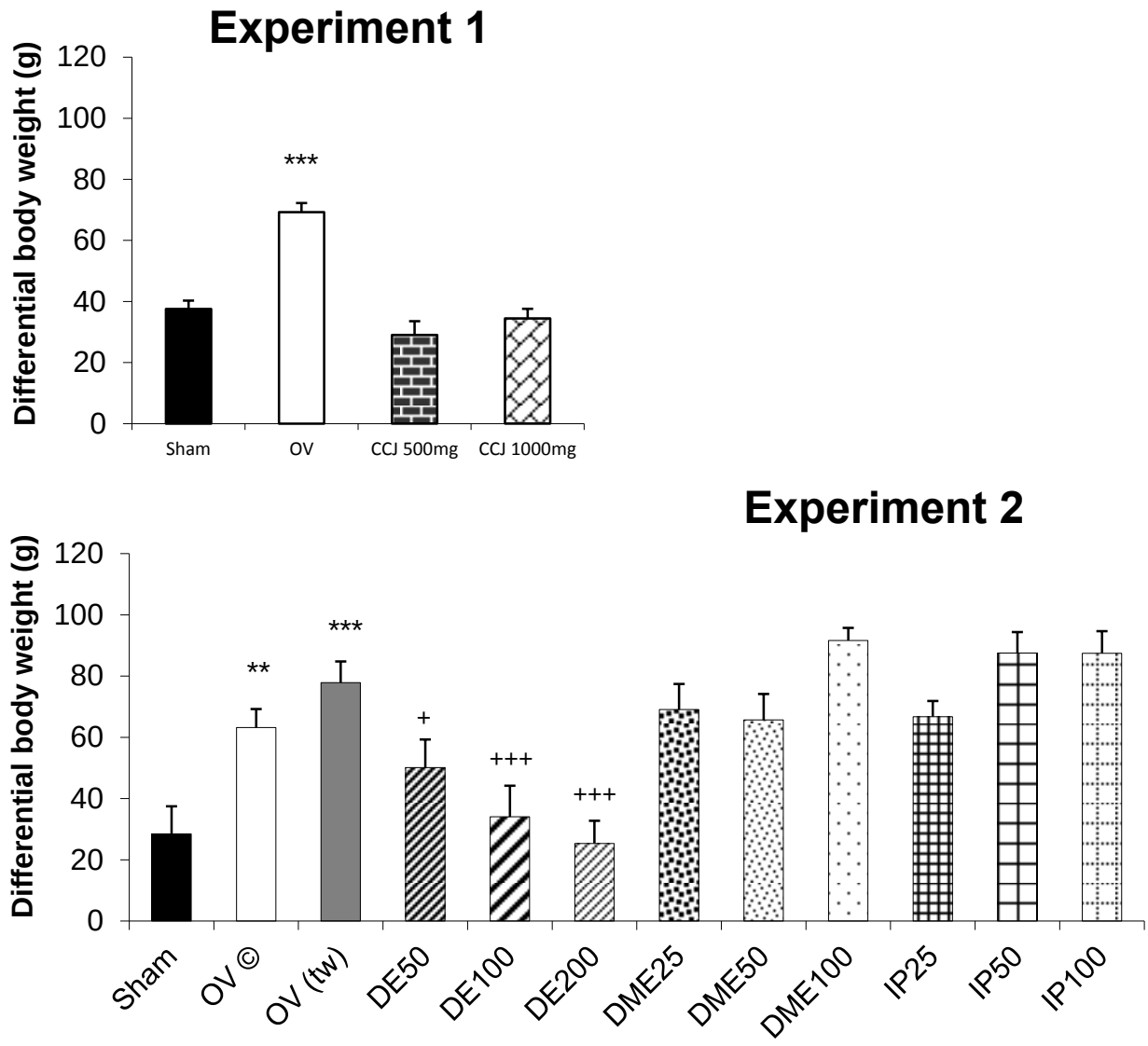

Figure 3. Differential body weight of females (change from initial weight measurement to final weight measurement). Mean + standard error of the mean (SEM) of the differential body weight of ovariectomized females. On the left are data from Experiment 1: *A. sisalana* concentrated crude juice - CCJ, 500 or 1000 mg/kg groups and control group. On the right are data from Experiment 2: Different doses of dried extract (DE), dry mucilage extract (DME) and intermediate product (IP) of *A. sisalana* and control groups (analysis of variance followed by Duncan's post hoc test). A p-value of less than 0.05 (*) and less than 0.001 (**) represents the significant differences in relation to the control group of ovariectomized + Tween 80.

amount of water and food ingested ($p > 0.05$).

Detection of total saponin concentrations

Because three different concentrations of DE were associated with a decrease in body weight in ovariectomized rats without affecting behaviour in the EPM or in the arena, this extract was analysed to determine the concentration of total saponins. To calculate the concentration of total saponins present in the three concentrations (Table 2), a straight equation ($y=1.9841x + 0.0995$) was established using the curve of a linear regression with the saponin (Merck). In the straight equation, x corresponds to the analysed concentration of saponins (mg/ml) and y corresponds to the absorbance measured. A line obtained with $R2=0.9963$, confirms the linearity of the reading of the concentrations that were used.

In addition, starting with the dilution factor of the DE solution analysed, the concentration of total saponins in "milligram" per "gram" of the dry extract was also expressed. The average of the values obtained in this analysis showed a high concentration of total saponins

Table 2. Values of total saponins presented in dried extract.

Concentration of extract (mg/mL)	Absorbance	Concentration of saponins (mg/mL)*	Concentration saponins (mg/g)**
0.25	0.537	0.22	882.68
0.35	0.737	0.32	918.01
0.50	1.017	0.46	924.52

*Results were expressed in milligram of total saponins per milliter of dried extract solutions analysed. **Results were expressed in milligram of total saponins per gram of the dried extract.

(908.40 mg/g) in this extract; this was a proportion of 90.84% of the weight of the dry extract.

For more assurance about the accuracy of the obtained data, 0.2 mg/mL of the saponin (Merck) was used as positive control for the analysis of the values of absorbance of the DE solutions; an absorbance of 0.471 was reported. For comparison purposes, using the straight equation (y=1.9841x + 0.0995), a value of 0.496 was obtained for the absorbance of 0.2 mg/mL of saponin, which confirmed the precision of the method used.

DISCUSSION

The phytochemical study of *A. sisalana* juice revealed the presence of a diverse group of secondary metabolites, including coumarins, flavonoids, condensable tannins, alkaloids, free anthraquinones and mainly saponins. These secondary metabolites are described as having many biological properties and therapeutic actions. Emphasis should be given to the antioxidant, anti-inflammatory and antitumour activities of flavonoids (Machado et al., 2008; Simões et al., 2010) and especially to the biological properties of saponins, whose presence was largely detected in this preliminary phytochemical study. Saponins have numerous activities, such as antifungal, anti-allergic, anti-inflammatory, antibiotic and antitumour activities (Francis et al., 2002; Sparg et al., 2004). Furthermore, saponins exhibit an anxiolytic effect in the EPM (Wei et al., 2007), and they are able to interfere in the metabolism of cholesterol and fat absorption by inhibiting pancreatic lipase activity (Dickel et al., 2007).

Based on the biological properties associated with secondary metabolites, especially the saponins, it has been suggested that *A. sisalana* juice could contribute to the pharmacological effects related to the objectives of this investigation (that is, the behavioural and physiological assessments).

In this regard, ovariectomy, as expected, caused an increase in the body weight of females in both experiments (Chen and Heiman, 2001), and treatment with two different concentrations of CCJ caused weight loss in ovariectomized rats. Similarly, rats treated with DE (50, 100 or 200 mg/kg) also demonstrated a reduction in body weight.

Contrary to observations reported previously (Lagunas et al., 2010), the ovariectomy did not cause any behavioural modification in the EPM or open field tests. However, considering that many pharmacological treatments of obesity cause several adverse effects to the body, including an increase in anxiety and in general activity (Ioannides-Demos et al., 2011; Carter et al., 2012; Haslam, 2016; Nuffer et al., 2016), it has become of extreme importance to verify if the preparations of *A. sisalana* that cause weight loss modify the anxiety profile or cause motor disorders.

A. sisalana CCJ did not modify the behavioural responses of ovariectomized rats in the EPM and open field tests, which indicate that it is a potent candidate for the treatment of weight gain that is associated with menopause. Therefore, it is important to highlight the results obtained with DE at doses of 100 and 200 mg/kg because those doses promoted a reduction in body weight that was acquired after an ovariectomy, and it did not affect the manifestation of behaviour related to anxiety and motor activity when compared with ovariectomized rats treated only with distilled water (without Tween). In addition, the treatment with DE at 100 mg/kg caused an increase in the weight of the structures of the reproductive system.

The presence of saponins in *A. sisalana*, CCJ, and especially in DE extract, may explain the observed reduction in body weight. In fact, the spectrophotometric analysis of DE showed a high proportion of total of saponins (90.84%) in relation to the weight of the dry extract. Thus, the reduction in body weight observed in ovariectomized rats and the increase in the weight of the reproductive system structures seem to be related to the presence of saponins. According to Francis et al. (2002), the effects of saponins appear to be related to interactions with steroid receptors, once the basic structures of saponins are similar to steroidal hormones.

As already mentioned, studies with plants used for weight loss reported that the saponins in these plants might be able to interfere in the metabolism of cholesterol and fat absorption by inhibiting pancreatic lipase activity (Dickel et al., 2007). Natural and synthetic saponins inhibit the absorption of cholesterol in the intestine,

reduce the concentration of plasma cholesterol in laboratory animals and are used for their pharmacological potential in the treatment of hypercholesterolaemia (Harwood Jr. et al., 1993). In recent studies, Liu et al. (2015) reported that the dioscin, a natural steroid saponin that exists widely in various agave plants (Sidana et al., 2016), could cause gradual weight loss without inhibiting appetite or increasing the physical activity of obese mice. In addition, Leal-Díaz et al. (2016) demonstrated that steroidal saponins extracted from *Agave salmiana* were able to reduce obesity-related metabolic abnormalities by promoting an abundance of *Akkermansia muciniphila* in the intestinal lumen. The presence of these bacteria is inversely associated with insulin resistance, altered adipose tissue metabolism, the onset of inflammation and obesity development during diet-induced obesity in mice (Schneeberger et al., 2015).

Besides that, in toxicity studies, any change in the weight of reproductive or non-reproductive organs may be a good indication of toxicity that is promoted by one or more phytochemical compounds from medicinal plants. On the basis of this information, a toxicological study was carried out by a research group in adult female Wistar rats using a hydrolysed extract obtained from *A. sisalana* (unpublished). Results show that oral treatment (gavage) for 30 consecutive days promoted a slight increase in the absolute weight of ovarian tissues; however, the relative weight was unaltered in the group administered sisal juice. The uterus, heart, liver and kidney weights were not affected by exposure to *A. sisalana* extract. Furthermore, the plant did not cause mortality in experimental groups, and it did not promote signs of toxicity such as changes in behaviour, ataxia, salivation, vomiting, diarrhoea, polyphagia, fever, weakness, tremors or convulsions (Viel et al., 2017). In addition, a lack of toxicity was observed when the hexane fraction obtained from the hydrolysed extract of *A. sisalana* was administered in an acute dose to rats and mice; the chemical high-performance liquid chromatography analyses of the hexane fraction of *A. sisalana* confirmed the presence of two steroidal sapogenins, hecogenin and tigogenin (Dunder et al., 2013).

The results obtained in this study are of high clinical relevance. This is because one of the major problems associated with the loss of ovarian function, whether by ovary removal surgery or functional loss resulting from natural ageing in women (menopause), is body weight gain. In addition to involving health problems such as an increase in rates of cholesterol (low-density lipoprotein) that can lead to cardiovascular diseases, weight gain can severely affect a woman's self-esteem, increase anxiety and manifest as depression in this stage of life.

Conclusions

Together, the results obtained indicate that chronic treatment with *A. sisalana* CCJ and with DE (100 mg/kg) caused a decrease in body weight without affecting behavioural manifestations related to anxiety or motor alteration, but with relevant stimulation of the structures of the reproductive system. As an expressive concentration of saponins was identified in DE, the effects observed were attached to this component. Such findings point to *A. sisalana* as a potential product in the treatment of weight gain in menopause, as it did not interfere with the manifestation of defensive behaviours, and it also brought on stimulation of internal structures of the reproductive system as a potent phytoestrogen. To find a compound that causes weight loss without an increase in anxiety would be a major breakthrough, which strengthens the relevance of this investigation.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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