ABSTRACT

Aims: To evaluate the influence of phytoestrogen Secoisolaciresinol diglicosídeo flaxseed over a long time period and its hormonal effects on weight loss in castrated rats. Materials and Methods: Wistar rats 21 that underwent bilateral oophorectomy for induction of menopause were randomly divided into three groups: Control group (n=7) received diet based on 10% casein, Flaxseed group (n=7) received flaxseed diet based on casein plus 25% flaxseed and the Modified control group (n=7) received modified diet based on 10% casein with the addition of lipid and fiber. All diets were prepared according to the recommendations of the AIN 93, and the animals received water *ad libitum*. The rats were anesthetized with Thiopentax® for blood collection by cardiac puncture to determine 17β-estradiol. The acquired data were statistically analyzed by the nonparametric Kruskal-Wallis test and post hoc Mann-Whitney U for comparison two by two through Software S-Plus version 8.0, with a 0.05 level for the significant differences. Results: At the end of 180-day experiment we observed that the results suggest that SDG is present in flaxseed at 13.5 ± 0.4 mg/g, feed intake in FG (1641.1 ± 0.9), CG (1478.4 ± 0.1) and MCG (1605.8 ± 1.8) showed no significant difference between groups. Have the mass on animals body it is observed that the FG (275.5 ± 33.9), CG (294.0 ± 33.1) and MCG (339.5 ± 24.6), suggesting that was no statistical difference between groups (p<0.002). Serum levels of 17β-estradiol showed higher values in FG (48.7 ± 12.31 pg/dl), GC (29.0 ± 5.4 pg/dl), MCG (36.0 ± 5.60 pg/dl), with statistical differences (p = 0.0012). Conclusion: The consumption of Secoisolaciresinol Diglicosídeo in food intake does not influence on body mass of animals and levels of estradiol in ovariectomized rats.

Key words: Phytoestrogen. Linseed. Hormonal. Body Weight. Ovariectomized.

RESUMO

Objetivo: avaliar a influência do fitoestrogênio Secoisolaciresinol Diglicosídeo por um longo período de tempo e os efeitos hormonais sobre a perda de peso em ratas castradas. Materiais e Métodos: 21 ratas Wistar submetidas à ooforectomia bilateral para indução da menopausa, divididas aleatoriamente em três grupos: grupo controle recebeu dieta à base de caseína a 10%, Grupo linhaça dieta acrescido de 25% de linhaça e grupo controle modificado dieta modificada à base de caseína 10% com a adição de lipídios e fibras. Para realização da ooforectomia as ratas foram anestesiadas com Thiopentax® e para determinar o 17β-estradiol no soro dos animais foi realizada coleta de sangue por punção cardíaca. Os dados obtidos foram analisados estatisticamente através do teste não paramétrico Kruskal-Wallis e post hoc de Mann-Whitney para comparar dois a dois através de um software versão S-Plus 8.0, com um nível de 0,05 para diferenças significativas. Resultados: Ao final de 180 dias de experimento os resultados apresentaram que o SDG está presente na semente de linhaça 13,55 ± 0,4 mg/g, o consumo do GL (1641,1 ± 0,9), GC (1478,4 ± 0,1) e GCM (1605,8 ± 1,8), não mostraram diferença significativa entre os grupos. A massa corporal relativa dos animais apresentou no GL (275,5 ± 33,9), GC (294,0 ± 33,1) e GCM (339,5 ± 24,6) diferenças estatísticas entre os grupos (p<0,002), os níveis séricos de 17β-estradiol apresentaram no GL (48,7 ± 12,31 pg/dl), GC (29,0 ± 5,4 pg/dl), GCM (36,0 ± 5,60 pg/dl), com diferença estatística entre os grupos (p=0,0012). Conclusão: Secoisolaciresinol Diglicosídeo não influenciou no consumo alimentar, influenciando no peso relativo corporal e níveis de estradiol das ratas ovariectomizadas.

INTRODUCTION

Flaxseed (Linum usitatissimum) is recognized as a major source of lignans (52.7 mg/100 g seed flour) on excellent way diglycosidic (Claupach and collaborators, 2002, Begum and collaborators, 2003).

The flaxseed is found in nature in the form diglicosidic, precursor of lignans breast enterodiol and enterolactone (Cardoso and collaborators, 2010), suffers action of bacterial enzymes, which by hydrolysis make them free from sugar molecules, pass firstly, the form of aglycones (which is dehydroxylated and demethylated to give the enterodiol, which oxidizes to enterolactone) compounds with similar chemical structure to estrogen (Ruggiero and collaborators, 2002), specifically secoisolaricirecinol diglycosidic (SDG) after absorbed through the gastrointestinal tract and distributed to the tissues have the Ability to bind to estrogen receptors (Wang and collaborators, 2005), acting as agonists or antagonists, depending on the amount present in the body (Carreau and collaborators, 2008).

Premenopausal lignans act the antagonists, reducing the action of this hormone (Stringheta and collaborators, 2007).

In the post-menopause and menopause period, when estrogen levels naturally cease, the lignans act the agonists, acting as estrogens, although less potent (Chilibeck and collaborators, 2008).

Among the estrogens, estradiol is the most important and abundant in the circulation, is released in an active form in various tissues with estrogen receptors (Dalais and collaborators, 1998).

Estrogen receptors are very sensitive and have a high affinity for a specific hormone estrogen, produced in the body (Bessa and collaborators, 2011).

Therefore, extremely low concentrations of hormone produce a particular effect, producing a natural response. However, these hormone receptors bind to other substances such as phytoestrogens, even at very low concentrations, and are capable of generating purposes, provoking thus agonistic or antagonistic responses (Brooks and collaborators, 2004).

The lignan is able to act beneficially in various conditions such as cardiovascular disease prevention (Cordeiro and collaborators, 2009), (Riedger and collaborators, 2008), reducing the risk of acute myocardial infarction (Kuijsten and collaborators, 2009), in combating the symptoms of menopause (Soares and collaborators, 2005), prevention of osteoporosis (Arjmandi and collaborators, 2001), certain types of cancer (Morris and collaborators, 2001), which develop under the influence of steroid hormones and prevention of chronic degenerative diseases, mainly due to its antioxidant action since most of these diseases is characterized by oxidative processes (Pelletier and collaborators, 2000).

Despite the benefits attributed to the consumption of flaxseed, excellent use during the menopause and post menopause period has sparked interest in the scientific community, the association is remains uncertain because of the prevalence of central obesity and overweight (Dodin and collaborators, 2008) among the female in this population stage, because of the complex physiological and psychological limiting factors involved, among them the reduction of lean mass (Carusi and collaborators, 2009; Figueiredo, 2011) and the reduction in metabolism that alters the hormonal balance and energy expenditure (Hallund and collaborators, 2006).

About the effect of flaxseed consumption exclusively at menopause, because study results have contradictory and greatly varying depending on the concentration used (Bessa and collaborators, 2011).

This study to observe the effects of consuming 25% of flaxseed over a long period on time and hormonal effects on weight loss of ovariectomized female rats.

MATERIALS AND METHODS

Materials

Ethical aspects

Project has approved by the Ethics Committee for Animal Research/University Federal Fluminense, under Protocol No. 00103-09 and followed the protocol standards set out in the Guide for Care and Use of Laboratory Animals published by U.S. National Institutes of Walt. This project conducted within the ethical principles of Good Practices of the Brazilian College of Animal Experimentation (COBEA).
Delineament Experiment

In the biological assay of 180 days, 21 newly weaned female Wistar rats (Rattus norvegicus, Albinus variety, order Rodentia mammalia, family Muridae) from the colony of the Experimental Nutrition Laboratory, Department of Nutrition and Diетetics, Nutrition Faculty, Universidade Federal Fluminense, Niterói, RJ, Brazil were used.

After weaning at approximately 21 days old, the animals received a commercial diet (Cr-1 Nuvilab Autoclavable - Nuvital ®) until adulthood. At 90 days, which is considered the time for a normal animal to reach adulthood after his birth, the rats underwent bilateral ovariectomy to induce early menopause (Biondo-Simôes and collaborators, 2005).

After ovariectomy they continued on the commercial diet for 30 days, which was long enough to allow the animals to reach a status of menopause. Then the animals were randomly selected and divided into groups, which will be detailed below.

At the end of the 180 day experiment, the animals were sacrificed. Anesthetized was performed with thiopental (0.15 ml/100g pc, ip) and blood collection by cardiac puncture to determine 17 β-estradiol was carried out.

The tubes containing blood were centrifuged (Sigma®) for 30 minutes at 3,500 RPM (Miller, 1977) to obtain serum and after it was put in the freezer-20°C for determination of serum 17 β-estradiol in animals.

The tissues were collected, weighed on a precision scale and stored in nitrogen at -80°C. Throughout the test, the animals were kept in plastic cages in an environment with constant temperature (22°C ± 2°C) and adequate lighting (12 hr light: dark cycle).

Water has offered ad libitum, diet, weight and feed intake were recorded every two days.

Experimental groups and diets

The animals were divided into three groups (n=7): Control group (CG) was fed a casein diet with 10% protein, 5% fiber, and 7% oil; Flaxseed Group (FG) was fed a casein diet with 10% protein, plus 25% of flaxseed meal, 7% fiber and 11% lipid and Modified Control Group (MCG) was fed a casein diet, with 10% protein, 7% fiber and 11% oil.

The insertion of the Modified Control Group is made by the chemical composition that flaxseed presents, and this is a good source of lipids and presents high fiber content. In order to obtain parameters for comparison between diets aimed to increase the concentrations of their constituents so that they present similar chemical compositions to their compositions.

All diets were added to the mixture of minerals and vitamins, according to rules of Laboratory Animal Diets On Committee, 1979, modified in accordance with the recommendations of the American Institute of Nutrition (AIN 93G), (Reeves and collaborators, 1993, AOAC, 1995).

MATERIALS AND METHODS

Isolation and quantification this Secoisolariciresinol diglucosido in flaxseed

Isolation of the lignan is consistent with the methodology described in flaxseed (Linum usitatissimum) and offered to animals in feed containing your meal.

For analysis by HPLC (High Performance Liquid Chromatography Efficiency) with Waters C18 column chromatography (Bondapak® C18, 15-20 mM 125 Å, 3.9 x 300 mm) and detection by spectrophotometer Ultra Violet (UV-Vis) at 280 nm and to evaluate the chromatographic separation was used a standard solution of SDG (secoisolariciresinol diglucoside) P ChromaDex.

For quantification of SDG in flaxseed was implemented the extraction method, where 0.5 g of dried and ground sample was added 10 mL of extraction solvent (70% methanol and 30% water) and kept in water bath at 60°C for 3 hours after centrifugation 2:00 mL of extract was removed and 0.5 mL of NaOH (0.5 mol/L) for hydrolysis for 3 hours, after 3 hours was added 0.5 mL of acetic acid (0.5 mol/L) for neutralization, after filtration of this extract was analyzed by HPLC-UV at 280 nm under the conditions.

Surgical: Bilateral Oophorectomy

This procedure followed the standards of vivisection of animals set by the Brazilian College of Animal Experimentation (COBEA).
The surgical procedures were performed in the morning. Before the procedure, the rats underwent eight hours of fasting. Intramuscular anesthesia with ketamine (Cristalia®-100mg/kg the animal IM) and xylazine (Anasedanâ Vetbrands ®-20 mg/kg of animal IM) was used. Moreover, the animals received analgesia with Tramadol (TramaLive Teuto®-5mg/Kg the animal IM), which was injected 1-2 minutes after surgical incision in order to minimize the suffering of rats in the postoperative period (Laboratory Animals published by U.S. National Institutes of Walt).

The abdominal cavity was opened, the ovaries identified and their heel ends were clipped and ligated using catgut 3.0 simple. The ovaries were then removed and held aponeurosis raffia e peritoneum. The skin was closed with separated mononylon 4.0.

**Biochemical Analysis: Hormonal**

The hormonal assays were determined by specific radioimmunoassay for each hormone, using the biochemical kit from Diagnostic Products Corporation-CPS, in apparatus VITALAB Selectra ®.

**Biological Analysis: Determination of the relative weight and food consumption**

To determine weight were weighed animals three times a week Filizola® and to determine the mass animals body, these were individually weighed, suspended by portion distal caudal and stored in cage polypropylene with the balance previously adjusted. To perform the calculation of relative body weight. The diets were offered on alternate days and weighed in the balance TOLEDO® with a maximum capacity of 3 kg and precision of 0.05g.

Post to the rest of the feed intake of each animal to determine the food consumption of rats. The measurements were taken and the data stored in electronic form for later analysis. Compilation of data was performed and for each parameter set, the most frequent finding was considered representative for each animal and for the group.

**Statistical Analysis**

The results are presented as mean and standard deviation (SD). The results obtained were applied to analysis of variance Kruskal-Wallis. When a significant difference was detected the post hoc Mann-Whitney U test was applied for a two by two comparison. Such statistical analyses were performed by the software S-Plus version 8.0, with a significance level of 0.05.

**RESULTS**

Identification, quantification of SDG in flaxseed and determination of SDG present in 25% flaxseed added to the experimental diet

The chromatogram observed the pattern of SDG and the extract of flaxseed is given by comparison of retention time. The analytical curve SDG provides a linear response range, with a correlation coefficient > 0.99. The SDG is present in 25% flaxseed in the diet added experimental 3.3mg/25g feed.

**Determination of relative body weight and food consumption**

At the end of the 180-day experiment we observed that feed intake in FG (1641.1 ± 0.9), CG (1478.4 ± 0.1) and MCG (1605.8 ± 1.8) showed no significant difference between groups.

Have the mass on animals' body it is observed that the FG (275.5 ± 33.9), CG (294.0 ± 33.1) and MCG (339.5 ± 24.6), suggesting that was no statistical difference between groups (p < 0.002).

**Determination of 17β-estradiol**

The concentration of 17β-estradiol in the GL (48.7 ± 12.31 pg/dl) in the CG (29.0 ± 5.4 pg/dl) in the GCM (36.0 ± 5.60 pg/l), suggesting that there was a statistical difference (P < 0.0012).

**DISCUSSION**

The demand for functional foods, such as flaxseed, has increased due to its beneficial
effects and role in disease prevention (Soares and collaborators, 2009).

This seed has been consumed by people of all ages and genders, pregnant women, nursing mothers and women during menopause (Cardoso and collaborators, 2010).

The increased consumption of phytoestrogens for women seeking treatment for symptoms typical of menopause has improved their well-being (Branca and Lorenzetti, 2005). This is due to close association of flaxseed with the hormone estrogen.

The Flaxseed is known as the largest source of lignans and phytoestrogen secoisolariciresinol diglicosidio (SDG) more abundantly found in nature (Claupach and collaborators, 2002), has a chemical structure similar to estrogen (Hu and collaborators, 2007).

Our results suggest that the SDG this in flaxseed, as observed resultsads the chromatogram retention time of SDG and in row and presence of SDG expressing a quantity of 13.5 mg/g and other lignanos. Where corroborates with study by M.R O’Neil (2009) in expressing SDG present in the seed showed value of approximately 14.53 mg/g.

The SDG present in flaxseed has activity similar to the hormone estrogen. Due to this characteristic, is widely used to minimize the symptoms of menopause (Prasad and collaborators, 2000), reduced body weight and body mass index (Hallund and collaborators, 2006).

In the present study we observe that the relative weight of the animal body presents differences between groups and consumption of experimental diets no difference between groups, since the composition of experimental diets with the percentage of lipids in the FG and MGC greater than the FG, which might be justifying a higher weight gain in the MCG and FG, since part of the ingested lipids can is being moved to storage of fat and the bioavailability of this fiber in the diet.

Soares and collaborators (2009), in a study of postmenopausal rats with 455 days of age, found an average daily consumption of 10g of food in their groups, similar to that observed in this study.

This fact suggests that the rats induced menopause have not had their food intake affected, since the study described the animals were not subjected to previous surgery.

Yet in a study performed reported that the ponderal gain of oophorectomized rats is approximately. 60g on 3 months, that resembles to data presented in this experiment in the GCM, although the experimental protocol has occurred in 180 days, being more long and with different experimental diets used in the study cited above.

The relative body mass of ovariectomized rats could be related to ovarian hormone deprivation, given that estrogen increases the energy consumption and reduced body weight (Schoppen and collaborators, 2005). Therefore, if there the estrogenic deprivation, energy consumption will be smaller and the animals will have weight gain.

Bhathena and collaborators (2002) when using similar method to present study verified weight gain greater in animals oophorectomized rats.

Have Szabo and collaborators (2000) conducted with hormone replacement ovariectomized rats with estradiol showed less weight gain.

It is suggested then, that estrogen action from linseed, specifically from SDG may be related with the decrease of mass relative body tailed by FG in relation to MCG.

According to MR O’Neil (2009) in a study with linseed 14.5 mg/g of SDG in ovariectomized sheep also exhibit changes in body mass and hormone levels of 17β-estradiol. Suggesting that SDG found that seed acted and decreased body mass on these animals.

In literature several studies have reported that the influence of phytoestrogens on hormones is still uncertain (Bessa and collaborators, 2011).

In study conducted over seven weeks, with rats menopausal, consumption 5-10 g/day linseed significantly reduced estrogen concentration (Hallund and collaborators, 2006) and randomized double blind performed by three months with 79 women in the period for post-menopausal consumed 100mg/day isoflavone capsule, observed that the levels of estradiol and BMI did not change.

Which presents a quantity of SDG approximately 15% above the consumed by rats of this study (3.37g/day). Thus, it was.
observed a greater influence of this seed in the experimental diets for a long period of time.

Several authors reported that foods rich in phytoestrogens, as linseed or soya, may promote adverse antiestrogenic as the inhibition of aromatase, causing decrease in endogenous estradiol and therefore reproductive alterations (Hutchins and collaborators, 2003).

The increase in estradiol in FG suggests that it is associated with high concentration of lipids present in the diet with added flaxseed, which stimulate the synthesis of this hormone by means other than the ovary.

Serum concentrations of 17β-estradiol in the FG have greater influence on estradiol levels when compared to the CG, MCG. Association suggests the similarity in chemical structure of this seed in the experimental diets FG, which together with the high concentration of lipids present in the experimental diets in the CG, and MCG suggests that it stimulates the synthesis of this hormone.

Under normal conditions the concentrations of estrogen in women of fertile age (Puig-Duran and collaborators, 1979), SDG binds to estrogen receptors and acts as antagonists, due to its ability to promote a negative feedback, reducing the action of this hormone.

Moreover, postmenopausal, when estrogen levels are generally lower, the SDG binds to estrogen receptors and acts as agonists, acting as estrogens, despite being less potent (Ruggiero and collaborators, 2002), but the mechanism receptor activation and the number of receptors present in tissues is still a matter of discussion (Serock and collaborators, 2008).

**CONCLUSION**

Our results suggest that secoisolaciresional diglicosido showed a pattern similar to estrogen, although it does not influence both food intake and relative body mass because it has excess weight gain which is common in menopause suggesting an influence on levels of estradiol.

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