Preclinical study of an antiobesity herbal medicine, PholiaNegra (X´Tract Vetorized)™, in male and female rats fed with high-fat diet: comparison with sibutramine

Estudo pré-clínico de um medicamento antiobesidade de ervas, PholiaNegra (X'Tract Vetorized)™, em ratos machos e fêmeas alimentados com dieta hipercalórica: comparação com sibutramina

Maria Martha Bernardi[1,2], Helenice de Souza Spinosa[1], Esther Lopes Ricci[1], Thiago Marinho Reis-Silva[1]

[1]School of Veterinary Medicine, University of São Paulo, São Paulo-SP, Brazil; [2]School of Veterinary Medicine, University Paulista, São Paulo-SP, Brazil.

Abstract

Objective – To assess, in this preclinical study, the effectiveness of a herbal medicine developed from a group of plants of the genus *Ilex* in the adjuvant treatment of obesity in rats fed with a high-fat diet. Phytotherapy is becoming increasingly popular both for the results it yields in several pathologies and because they are growing herbal medicine studies showing its effectiveness. **Methods** – Male and female rats were fed with a high-fat diet for one month. The diet was then replaced by a chow diet. All male and female rats received the PholiaNegra (X´Tract Vetorized)™ or water. The treatment was orally administered twice a day over 30 days. Body weight gain was assessed weekly and, at the end of treatment, the total body weight gain was calculated. A positive control with sibutramine (7.5 mg/kg, twice a day, orally, over 30 day was also included. **Results** – A significant reduction in weekly body weight gain, as well as in total weight gain, in both male and female rats after the herbal medicine administration. The index of body weight loss showed that PholiaNegra (X´Tract Vetorized)™ was more effective in reducing body weight in female than in male rats. The sibutramine treatment showed the same profile as PholiaNegra (X´Tract Vetorized)™ treatment. **Conclusion** – The present data indicate that PholiaNegra (X´Tract Vetorized)™ herbal medicine was effective in decreasing body weight in male and female rats submitted to a high-fat diet, and showed a similar profile to that of sibutramine.

Descriptors: Phytotherapy; Obesity; Plants, medicinal; Weight loss

Resumo

Objetivo – Avaliar, neste estudo pré-clínico, a eficácia de um medicamento desenvolvido a partir de um grupo de plantas do gênero *Ilex* no tratamento adjuvante da obesidade em ratos alimentados com uma dieta hipercalórica. A fitoterapia está se tornando cada vez mais popular, tanto pelos resultados positivos em diversas doenças e porque estão crescendo estudos de medicina de ervas que mostram a sua eficácia. **Métodos** – Ratos machos e fêmeas foram alimentados com uma dieta rica em gordura durante um mês. A dieta foi então substituída por uma ração normal do biotério. Todos ratos e ratas foram tratado com PholiaNegra (X´Tract Vetorized)™ ou água. O tratamento foi administrado por via oral, duas vezes por dia durante 30 dias. O ganho de peso corporal foi avaliado semanalmente e, no final do tratamento, o ganho de peso total foi calculado. Como controle positivo empregou-se a sibutramina (7,5 mg/kg, duas vezes por dia, por via oral, durante 30 dias. **Resultados** – Observou-se redução significativa no ganho de peso corporal semanal, bem como do ganho de peso total, tanto nos ratos machos e fêmeas, após a administração do medicamento à base de plantas. O índice de perda de peso corporal mostrou que PholiaNegra (X´Tract Vetorized)™ foi mais eficaz na redução do peso corporal nas fêmeas do que em ratos machos. O tratamento com sibutramina mostrou o mesmo perfil obtido com o tratamento com PholiaNegra (X´Tract Vetorized)™. **Conclusão** – Os presentes dados indicam que PholiaNegra (X´Tract Vetorized)™ foi eficaz em diminuir o peso corporal em ratos machos e fêmeas submetidos a uma dieta rica em gordura, e mostrou um perfil semelhante ao da sibutramina.

Descritores: Fitoterapia; Obesidade; Plantas medicinais; Perda de peso

Introduction

Obesity and overweight are associated with several disorders, including cancer, diabetes, and heart disease, and have become two of the most important risk factors for morbidity and mortality in both men and women[1]. The evidence that obesity is a health problem that is difficult to control is apparent not only in statistical data but also in observations of the general public. The development of new medicines to control weight is the object of much recent attention by the food and drug industries.

The leaves of *Ilex paraguariensis* St. Hill (Aquifoliaceae), popularly known as *maté* (mate), *yerba-mate*, or *erva-mate*, have been used in the preparation of several types of tea, beverages, and soft drinks in South America. Mate trees grow naturally and have been cultivated in southern Brazil, Argentina, and Paraguay, and the popularity of its preparations is increasing worldwide, particularly in North America and Europe. Tea made from the leaves of *I. paraguariensis* has central nervous system stimulant properties due to the presence of methylxanthine alkaloids[2-3], antioxidant properties attributed to acids and caffeoylquinic acid derivatives[4-6], and other beneficial properties such as hepatoprotective[7], choleretic[8], diuretic[8], hypocholesterolemic[7,9], antirheumatic, antithrombotic[3], anti-inflammatory[10-11] and antiobesity[12] effects.

Chronic administration of *I. paraguariensis* tea has been shown to reduce hyperglycemia and serum insulin levels[13] and to lead to significant improvement in insulin sensiti-

vity in a metabolic syndrome mouse model[14]. Studies have also indicated that *I. paraguariensis* tea slows gastric emptying, lowers body weight, and reduces food intake. Evidence of weight loss induction with mate intake suggests possible roles of satiation increase and energy intake reduction[12]. Related species growing in the same habitat (*I. brevicuspis*, *I. theezans*, *I. microdonta*, *I. dumosa var. dumosa*, *I. taubertiana*, *I. pseudobuxus*, *I. integerrima* and *I. argentina*) are locally used as substitutes or adulterants of *I. paraguariensis*[15].

Phytotherapy is becoming increasingly popular both for the results it yields in several pathologies and because they are growing herbal medicine studies showing its effectiveness. Nowadays, it is possible to find herbal formulations that maintain the plant-specific characteristics and have undergone microbiological and analytical tests. In this preclinical study, we assessed the effectiveness of an herbal medicine, PholiaNegra (X´Tract Vetorized)™ developed from a group of plants of the genus *Ilex* in the adjuvant treatment of obesity in rats fed with a high-fat diet.

Methods

Animals

Adult male (200-250 g and 60 days old) and female (150-200 g and 60 days old) Wistar rats (Department of Pathology, School of Veterinary Medicine, University of São Paulo, Brazil) were used. The animals were housed in polypropylene cages (40 × 50 × 20 cm) at a regulated temperature (20 ± 2°C) and humidity (70 ± 5%) on a controlled light schedule (12h light: 12 h dark), with lights on at 6:00 AM. The animals used in this study were kept in accordance with the guidelines of the Committee on the Care and Use of Laboratory Animal Resources of the School of Veterinary Medicine, University of São Paulo (protocol nº 2041/2010 in 27/10/2010, FMVZ-USP). These guidelines are based on those of the U.S. National Institutes of Health. The experiments were performed in accordance with good laboratory practice protocols and with quality-assurance methods.

Herbal medicine

The following manipulated commercial product was employed: capsules with 150 mg of PholiaNegra (X´Tract Vetorized)™ + excipients: 30 mg mannitol, 0.75 mg aerosil, 1.5 mg magnesium estearate, cellulose/ talc (1:1) qs 100%. The capsules were manipulated in the Pharmacopeia® CIL Laboratories (Brazil).The gelatin capsules were made with *pullulan* to protect the extract and increase its shelf-life. This herbal preparation was registered in ANVISA nº 25352.716809/2010-78. It contains among its phytochemicals methylxantines, saponins, and pholianegrosides.

Drug

Sibutramine was presented as 15 mg capsules plus excipients, qs 100%. The capsules were acquired in an existing pharmacy (São Paulo, SP, Brazil – CNPJ 61.744.595/0001-84).

Treatments and experiment design

Thirty male and 30 female rats were fed with a high-fat diet (60% kcal, Rhoster Indústria e Comércio Ltda., Rua José Egon Knittel, 120 – Jardim Tonelli, Araçoiaba da Serra/SP – CEP 18190-000, Brazil) for 30 days. Then, the rats were divided into 6 groups (3 male and 3 female groups) with 10 rats in each group. The high-fat diet was replaced with a chow diet (3.3 kcal) and the 3 groups of male rats received twice-a-day oral administrations of water (control group), 150 mg/kg of PholiaNegra (X´Tract Vetorized)™, or 7.5 mg/kg of sibutramine. The same procedure was performed with the remaining 3 groups of female rats. These treatments were administered for 30 days. The rats in all groups were weighed daily during treatments and observed for gross signs of toxicity. Weekly weight gain was calculated at the end of the experiment and the delta of weight loss (DWL) *in vivo* was computed as

$$DWL = \frac{(WIT - WFT) \times 100 - (WIP - WFP) \times 100}{WIT \times WIP}$$

where

WIT = Initial body mass on the first day of the phytotherapic treatment.

WFT = Final body mass on the last day of the phytotherapic treatment.

WIP = Initial body mass on the first day of the placebo treatment.

WFP = Final body mass on the last day of the placebo treatment.

At the end of the treatments, the rats were euthanized in CO_2 and examined for lesions or other alterations.

Statistical analysis

Repeated measures two-way ANOVA was used to compare data of weight gain. One-way ANOVA was employed to compare the total weight gain between the control and experimental groups of male or female rats. In all cases, values of $P < 0.05$ were considered statistically significant. The statistical analyses were performed using GraphPad Prism software, version 5 (GraphPad, San Diego, CA, USA).

Results

Figure 1 (A and B) shows the weekly weight gain of male and female rats fed with hypercaloric or normal diets. In male rats (Figure 1A), the two-way ANOVA showed that treatments [$F_{2/108} = 5111.59$, $P < 0.0001$] and number of weeks [$F_{3/108} = 184.09$, $P < 0.0001$] influenced the results, with significant interactions between the factors [$F_{6/108} = 45.48$, $P < 0.0001$]. In relation to the control group, the Bonferroni post hoc test revealed that weight gain of male rats treated with PholiaNegra (X´Tract Vetorized)™ and sibutramine was significantly reduced ($P < 0.0001$); PholiaNegra (X´Tract Vetorized)™ and sibutramine data did not differ ($P > 0.05$). Also, the weight gain in both experimental groups was lower than that in the

control group ($P < 0.0001$) throughout all four weeks of the treatments.

In female rats (Figure 1B), the two-way ANOVA showed that treatments [$F_{2/108} = 4764077.50$, $P < 0.0001$] and number of weeks [$F_{3/108} = 115317.44$, $P < 0.0001$] influenced the results, with significant interactions between the factors [$F_{6/108} = 972844.13$, $P < 0.0001$]. In relation to the control group, the Bonferroni *post hoc* test revealed that weight gain of female rats treated with PholiaNegra (X´Tract Vetorized)™ and sibutramine was significant reduced ($P < 0.0001$); also weight gain with PholiaNegra (X´Tract Vetorized)™ was decreased in relation to that with sibutramine in all weeks of treatments ($P < 0.0001$).

Figure 2 shows the data for total weight gain (A) and the DWL index (B). In relation to the control group, male rats treated with PholiaNegra (X´Tract Vetorized)™ and sibutramine had significantly reduced total weight gain [$F_{2/29} = 348.9$, $P < 0.0001$]; no differences were detected between treatments ($P = 0.163$).

Treatments significantly reduced the total weight gain in female rats in relation to the control group [$F_{2/29} = 16110.0$, $P < 0.0001$]. Data from the treated female rats differed, with total weight gain with PholiaNegra (X´Tract Vetorized)™ being lower than that with sibutramine ($P < 0.05$).

The DWL index obtained was 5.35 ± 0.10 in male rats and 10.35 ± 0.1 in female rats. Finally, no gross signs of toxicity were observed during the experiment and no hepatic and kidney lesions were detected at the end of the treatments.

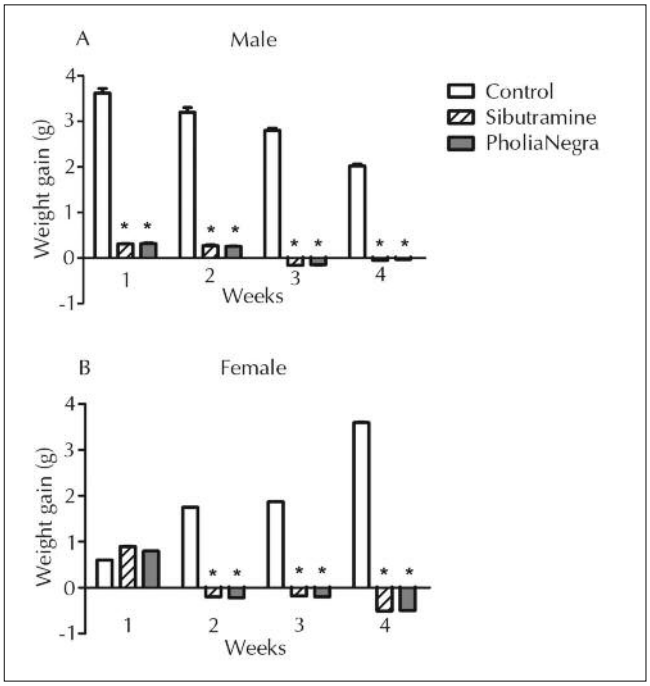

Figure 1. Weekly body weight gain (g) in male (A) and female (B) rats fed previously with a high-fat diet (1 month) and treated with PholiaNegra (X'Tract Vetorized)™ (150 mg/kg) or sibutramine (positive control group – 7.5 mg/Kg) or water (control group) twice a day. During treatments, rats received the normal chow. Data are presented as means ± SEM. *p< 0.0001, two way ANOVA followed by the Bonferroni test, in relation to control group

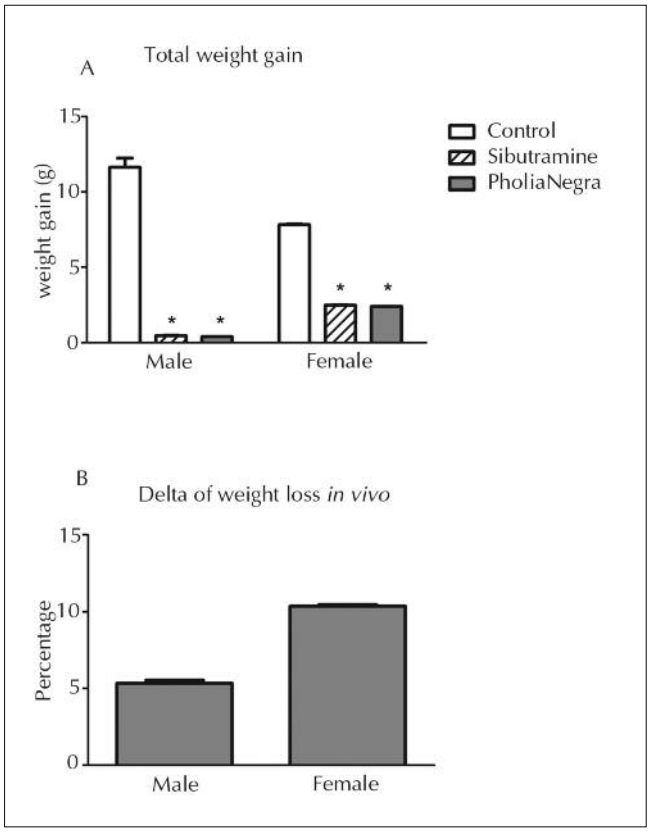

Figure 2. Total weight gain (A) and the delta of weight loss *in vivo* (B) of male and female rats fed previously with a high-fat diet (1 month) and treated with PholiaNegra (X'Tract Vetorized)™ (150 mg/kg) or sibutramine (positive control – 7.5 mg/Kg) or water (control group) twice a day. During treatments, rats received the normal chow. Data of total weight gain are presented as means ± SEM. *p< 0.0001, one way ANOVA followed by the Bonferroni test, in relation to control group. Data of delta of weight loss *in vivo* are presented in percentage

Discussion

The herbal medicine evaluated in the present study significantly reduced the weight gain of male and female rats. The loss in weight gain of the phytotherapic group was examined weekly and at the end of treatment, and showed a high level of efficacy. These data, when compared with that of the anti-obesity drug sibutramine, were very similar in male rats. Female rats treated with the herbal medicine presented greater reductions in body weight gain than those treated with sibutramine. No gross signs of toxicity, organs lesions, or hemorrhage were detectable by visual examination at the end of the experiment.

Male and female rats received a hypercaloric diet for one month to induce weight gain and then it was tested whether PholiaNegra (X´Tract Vetorized)™ had an anti-obesity effect. The weekly weight gain data revealed that in male rats there was an abrupt decrease in body weight in the experimental group relative to the control group, which lasted until the last week of treatment. A different profile was observed in female rats; in the first week of treatment, body weight loss in the experimental group was similar to that of the control group. However, in the following weeks, while the control group showed a gradual increase in body weight gain, the herbal medicine

led to significant body weight decrease in the experimental group.

These different profiles of the anti-obesity properties of PholiaNegra (X´Tract Vetorized)™ in male and female rats could be attributed to sexual dimorphism on pharmacokinetics. In fact, sex-based differences in pharmacokinetics and pharmacodynamics are widely recognized[16-18] and can be important sources of individual differences in drug responses. Sex-based differences in pharmacokinetics reflect differences in bioavailability, distribution, metabolism, and/or excretion. Sex hormones influence bioavailability through effects on gastrointestinal motility; for example, estrogen inhibits gastric emptying. Sex differences in pharmacokinetics can result from sex differences in distribution, which can be caused by differences in body weight (lower in women), body fat (higher in women), plasma volume (lower in women, but varies throughout the menstrual cycle and during pregnancy), and organ blood flow (higher in women)[16].

The total weight gain data showed that, in relation to the control group, male experimental rats treated with the herbal medicine had a decrease in total weight gain that was apparently higher than the decrease in female rats. Nevertheless, these differences could be compared to the control data. The male control rats had lower weight loss than the female control rats, probably due to sex differences in the distribution and quantity of proteins and fat, which are characteristics of male and female body weight[19-20]. To avoid bias caused by sexual dimorphism on body weight, we employed the DWL index, which allowed the minimization of these interferences. Thus, when we accounted for the initial body weight of the control and experimental groups, the DWL showed that the herbal medicine was more effective in female than in male rats in inducing body weight loss. Interestingly, sibutramine, a drug used to control overweight and obesity and employed here as a positive control, showed the same profile of reduction in body weight as the herbal medicine .

Although the present results do not provide data on the mechanism by which this herbal medicine reduced the weight of the animals, some indications of the *Ilex* plant, particularly *Ilex paraguariensis,* suggest that the slowing of gastric emptying, increased satiation, and energy intake reduction are the most important mechanism of its anti-obesity effect[21]. Also, the bioactives, cafeiol, cafeic acid, and chlorogenics present in this herbal medicine have antioxidant and antiglicant properties[8,22]. Reductions in cholesterol levels and abdominal fat have been attributed to flavonoids[23].

Conclusion

The present data indicate that PholiaNegra (X´Tract Vetorized)™ herbal medicine was effective in decreasing body weight in male and female rats submitted to a high-fat diet and showed a similar profile to that of sibutramine. Although this compound has been commercially available in Brazil, its efficacy has never been assessed in a randomized preclinical trial until now.

References

1. Ogden CL, Carroll MD, Curtin LR, McDowell MA, Tabak CJ, Flegal KM. Prevalence of overweight and obesity in the United States, 1999-2004. JAMA. 2006;295:1549-55.

2. Vieira MA, Maraschin M, Pagliosa CM, Podesta R, de Simas KN, Rockenbach II *et al.* Phenolic acids and methylxanthines composition and antioxidant properties of mate (*Ilex paraguariensis*) residue. J Food Sci. 2011;75:C280-5.

3. Heck CI, de Mejia EG: Yerba mate tea (*Ilex paraguariensis*). A comprehensive review on chemistry, health implications, and technological considerations. J Food Sci. 2007;72:R138-51.

4. Cunha FL, Silva CM, Almeida MG, Lameiro TM, Marques LH, Margarido NF *et al.* Reduction in oxidative stress levels in the colonic mucosa without fecal stream after the application of enemas containing aqueous *Ilex paraguariensis* extract. Acta Cir Bras. 2011;26:289-96.

5. Hussein G, Matsuda H, Nakamura S, Hamao M, Akiyama T, Tamura K *et al*. Mate tea (*Ilex paraguariensis*) promotes satiety and body weight lowering in mice: Involvement of glucagon-like peptide-1. Biol Pharm Bull. 2011;34:1849-55.

6. Coentrão PA, Teixeira VL, Netto AD. Antioxidant activity of polyphenols from green and toasted mate tea. Nat Prod Commun. 2011;6:651-6.

7. Lima IFP, De Dea Lindner J, Soccol VT, Parada JL, Soccol CR. Development of an innovative nutraceutical fermented beverage from herbal mate (*Ilex paraguariensis* A.St.-Hil.) extract. Int J Mol Sci. 2012;13:788-800.

8. Filip R, Lopez P, Giberti G, Coussio J, Ferraro G. Phenolic compounds in seven South American Ilex species. Fitoterapia. 2001; 72:774-8.

9. Mosimann AL, Wilhelm-Filho D, da Silva EL. Aqueous extract of *Ilex paraguariensis* attenuates the progression of atherosclerosis in cholesterol-fed rabbits. Biofactors. 2006;26:59-70.

10. Puangpraphant S, Berhow MA, Vermillion K, Potts G, Gonzalez de Mejia E. Dicaffeoylquinic acids in yerba mate (*Ilex paraguariensis* St. Hilaire) inhibit NF-kappaB nucleus translocation in macrophages and induce apoptosis by activating caspases-8 and -3 in human colon cancer cells. Mol Nutr Food Res. 2011;55: 1509-22.

11. Wnuk M, Lewinska A, Oklejewicz B, Bugno M, Slota E, Bartosz G. Evaluation of the cyto- and genotoxic activity of yerba mate (*Ilex paraguariensis*) in human lymphocytes *in vitro*. Mutat Res. 2009; 679:18-23.

12. Kang YR, Lee HY, Kim JH, Moon DI, Seo MY, Park SH *et al*. Anti-obesity and anti-diabetic effects of yerba mate (*Ilex paraguariensis*) in C57BL/6J mice fed a high-fat diet. Lab Anim Res. 2012;28:23-9.

13. Klein GA, Stefanuto A, Boaventura BC, de Morais EC, Cavalcante LS, de Andrade F *et al.* Mate tea (*Ilex paraguariensis*) improves glycemic and lipid profiles of type 2 diabetes and pre-diabetes individuals: a pilot study. J Am Coll Nutr. 2011;30:320-32.

14. Hussein GM, Matsuda H, Nakamura S, Akiyama T, Tamura K, Yoshikawa M. Protective and ameliorative effects of mate (*Ilex paraguariensis* on metabolic syndrome in TSOD mice. Phytomedicine. 2011;19:88-97.

15. Giberti GC. Los parientes silvestres de la yerba mate y el problema de su adulteración. Dominguezia. 1989;7:1-22.

16. Waxman DJ, Holloway MG. Sex differences in the expression of hepatic drug metabolizing enzymes. Mol Pharmacol. 2009;76: 215-28.

17. Fletcher CV, Acosta EP, Strykowski JM. Gender differences in human pharmacokinetics and pharmacodynamics. J Adolesc Health. 1994;15:619-29.

18. Franconi F, Brunelleschi S, Steardo L, Cuomo V. Gender differences in drug responses. Pharmacol Res. 2007;55:81-95.

19. Kotani K, Tokunaga K, Fujioka S, Kobatake T, Keno Y, Yoshida S *et al*. Sexual dimorphism of age-related changes in whole-body fat distribution in the obese. Int J Obes Relat Metab Disord. 1994; 18:207-12.

20. Lovejoy JC, Sainsbury A. Sex differences in obesity and the regulation of energy homeostasis. Obes Rev. 2009;10:154-67.

21. Andersen T, Fogh J. Weight loss and delayed gastric emptying following a South American herbal preparation in overweight patients. J Hum Nutr Diet. 2005;14:243-50.

22. Gugliucci A. Antioxidant effects of *Ilex paraguariensis*: induction of decreased oxidability of human LDL *in vivo*. Biochem Biophys Res Commun. 1996;224:338-44.

23. Pedroso GL, Mendes RH, Persch K, Jahn M, Kucharski LC. Efeito do extrato aquoso de *Ilex paraguariensis* sobre o metabolismo de ratos machos. Rev HCPA. 2010;30:241-6.

Corresponding author:

Maria Martha Bernardi
Departamento de Patologia
Faculdade de Medicina Veterinária
Universidade de São Paulo
Av. Prof. Dr. Orlando Marques de Paiva, 87
São Paulo-SP, CEP 05508–270
Brazil

E-mail: marthabernardi@gmail.com

Received June 30, 2012
Accepted August 2, 2012