UNIVERSIDADE FEDERAL DO PAMPA

GRACIELE DOS SANTOS TAUHEIMANN

EFEITO DO GAMA ORIZANOL NOS PARÂMETROS OXIDATIVOS, ENDÓCRINOS E BIOQUÍMICOS DE CAMUNDONGAS OVARIECTOMIZADAS

Itaqui
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Trabalho de Conclusão de Curso apresentado ao Curso de Nutrição da Universidade Federal do Pampa, como requisito parcial para obtenção do Título de Bacharel em Nutrição.

Orientador: Prof. Dr. Cristiano Ricardo Jesse

Co-orientadora: Prof. Dra. Silvana Peterini Boeira

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Dedico este trabalho à Deus que me deu sabedoria e me guiou em toda a trajetória acadêmica. Nos momentos de dificuldades, renovou minhas forças e sempre me ajudou a propor um novo mundo de possibilidades.

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“A sabedoria não se transmite, é preciso que nós a descubramos fazendo uma caminhada que ninguém pode fazer em nosso lugar e que ninguém nos pode evitar, porque a sabedoria é uma maneira de ver as coisas.”

“Uma verdadeira viagem de descobrimento não significa, necessariamente, encontrar novas terras, mas, isto sim, adquirir um novo olhar.”

Marcel Proust

RESUMO

Introdução: O aumento da expectativa de vida, sobretudo no sexo feminino, tornou-se um fenômeno global. Isso significa que as mulheres passaram a viver mais tempo com os efeitos da menopausa, caracterizada pela cessação da menstruação e declínio do hormônio estrogênio. A deficiência de estrogênio está relacionada a diversos sintomas e modificações no perfil lipídico e glicêmico, o qual podem ser minimizados com terapias hormonais e alguns fármacos. Porém, apresentam efeitos colaterais e como alternativa para o tratamento, destaca-se a administração do gama orizanol, uma mistura de ésteres de ácido ferúlico de fitoesteróis e de álcoois triterpênicos. Considerado um potente fitoesterol com propriedades hipolipidêmica, anti-inflamatória e antioxidante. Neste sentido, o objetivo deste estudo é avaliar o efeito do gama orizanol nas alterações fisiológicas verificadas em um modelo experimental de pós-menopausa no plasma em camundongos. **Métodos:** Foram utilizados 32 camundongos fêmeas, o qual foram divididas em 4 grupos. O gama orizanol na dose de 50 mg/Kg e o veículo (óleo de canola - 10 ml/Kg) foram administrados por via oral, via gavagem durante 28 dias consecutivos. Sendo que um dia antes do início do tratamento, os animais foram submetidos à cirurgia. E após os 28 dias, o sangue dos animais foi coletado por punção cardíaca para a análise dos parâmetros oxidativos, endócrinos e bioquímicos. **Resultados:** Durante o período experimental, os grupos submetidos a ovariectomia ou cirurgia sham, apresentaram aumento do peso corporal, diminuição dos níveis de estrogênio e aumento nos níveis de glicose, insulina, colesterol e suas frações lipídicas. A administração do gama orizanol foi eficaz na reversão do aumento desses níveis e responsável pelo aumento do potencial antioxidante total e redução das espécies reativas de oxigênio, evidenciando seu importante efeito protetor. **Conclusão:** Portanto, o gama orizanol promoveu melhorias metabólicas essenciais na manutenção do perfil lipídico, glicêmico e demais benefícios relacionados a sua capacidade antioxidante. Sendo uma excelente alternativa terapêutica para o tratamento das complicações presentes na pós-menopausa, bem como, evitar um quadro de síndrome metabólica, bem prevalente nas mulheres neste período.

PALAVRAS-CHAVE: menopausa, óleo do farelo de arroz, estrogênio, fitoesteróis.

ABSTRACT

Introduction: The increase of the life expectancy, especially in female sex, it has become a global phenomenon. This means that women passed to live longer with the effects of menopause, characterized by the cessation of menstruation and decline of the hormone estrogen. The estrogen deficiency it is related to various symptoms and changes in lipid and glucose profile, which can be minimized with hormonal therapies and some drugs. But, present side effects and as alternative for the treatment, stands out the administration of the gamma-oryzanol, a mixture of ferulic acid esters of phytosterols and triterpenic alcohols. Considered the phytosterol potent hypolipidemic with hypolipidemic properties, anti-inflammatory and antioxidant. In this sense, the objective of this project is to evaluate the effect of gamma-oryzanol on physiological changes occurring in a postmenopausal experimental model in mice plasma. **Methods:** 32 female Swiss albino mice were used, were divided into 4 groups. The gamma-oryzanol at a dose of 50 mg/kg and vehicle (canola oil - 10 ml/kg) are administered orally via gavage for 28 consecutive days. Being that one day before the initiation of treatment, the animals were submitted to surgery. And after 28 days, the o blood of the animals was collected by cardiac puncture for the analysis of oxidative, endocrine and biochemical parameters. **Results:** During the experimental period, the groups submitted to ovariectomy or sham surgery, presented increase of the body weight, decrease in levels of estrogen and increase in levels of glucose, insulin, cholesterol and lipid fractions. The administration of gamma-oryzanol it was effective in reversal these levels and responsible for increasing of the total reactive antioxidant potential and decrease of reactive species of oxygen, evidencing its important protective effect. **Conclusion:** Therefore, the gamma-oryzanol promoted essential metabolic improvements in maintaining the lipid profile, glycemic and other benefits related to their antioxidant capacity. Thus, an excellent therapeutic alternative for the treatment of complications present in postmenopausal, as well as, avoid a picture of metabolic syndrome, well prevalent in women during this period.

KEYWORDS: Menopause, rice bran oil, estrogen, phytosterols.

LISTA DE FIGURAS

SUMÁRIO

MANUSCRITO

EFFECT OF GAMMA-ORYZANOL IN OXIDATIVE, ENDOCRINE AND BIOCHEMICAL PARAMETERS IN OVARIECTOMIZED MICE

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1. INTRODUCTION

The life expectancy of the Brazilian population has increased in recent decades, especially in female sex. Thus, women were living longer with the effects of menopause, which although it is a physiological phenomenon promotes a negative impact on quality of life (PANAZZOLO et al., 2014).

According to the World Health Organization (WHO), by 2030, more than 1 billion women will be in menopause, that being in Brazil alone, there are over 13,5 million who pass through menopause, indicating along with advancing age, a risk factor for cardiovascular disease (COLPANI, 2015).

Menopause is the stage of a woman's life, in that occurs permanent cessation of menstruation, characterized by the decline in female hormone levels, mainly estrogen. The significant decrease of estrogen is related to several changes such as heat waves, progressive atrophy of the urogenital system and central nervous system disorders. And after menopause, these decreased concentrations of estradiol they are related to depression, sleep disturbances, irritability, anxiety and cognitive dysfunctions (MOREIRA, 2014).

It is known that the incidence of obesity in this age range is greater, hypoestrogenism due to be associated with changes in the metabolic profile, favoring the increase of fat, especially, in the abdominal region, it is a crucial factor for the development of comorbidities (CORREA et al., 2015).

The symptoms of menopause can be minimized by Hormone Replacement Therapy (THM) which is currently the most widely used treatment. However, accumulate studies showing its side effects, as the risk for developing breast cancer, thromboembolism and cardiovascular diseases (PARDINI, 2014).

Furthermore, there are selective modulators o estrogen receptors drugs, as tamoxifen and raloxifen, used as substitutes of THM, however, they are associated with side effects, including, uterine cancer, venous thrombosis, teratogenicity and cataracts (MUHAMMAD et al., 2013; BUNRATSAMIA et al., 2015).

Considering the health risks that current therapies provide, the need arises to seek compounds that can contribute to the improvement of symptoms without causing further complications. In this sense, some phytosterols has been the subject of several studies, as the gamma-oryzanol found in rice is bran oil that has aroused interest because of its beneficial properties to health (PAN et al., 2014).

The gamma-oryzanol is a mixture of ferulic acid esters of phytosterols and triterpenic alcohols. Several line of evidence involving animal models, show that the gamma-oryzanol has antioxidant activity, anti-inflammatory and property of reducing serum cholesterol, improve the lipid profile and acting on platelet aggregation. This phytosterol also acts on the hormonal system, increasing the levels of growth hormone, testosterone and other anabolic hormones (SAENJUN et al., 2012; ESLAMI et al., 2014).

A recent study involving mice, proved the positive effects of gamma-oryzanol in markers of metabolic syndrome, indicating to be effective substance in the treatment of dyslipidemia, diabetes mellitus, hypertension and in the inhibition of liver and kidney damage (WANG et al., 2015).

From the concern about the side effects that hormonal and no hormonal therapies cause, which although they are effective in relieving the symptoms of menopause, there is a growing body of evidence about their health complications. Therefore evidencing the importance of studies in animal models, in order to contribute to the knowledge of the physiopathogeny of menopause, as well as, investigate new alternative therapies for the treatment of their symptoms and prevention of pathologies this period.

Therefore, the present study aimed to evaluate the effects of gamma-oryzanol on physiological changes occurring in a postmenopausal experimental model in female mice.

2. MATERIALS AND METHODS

2.1. Animals and reagents

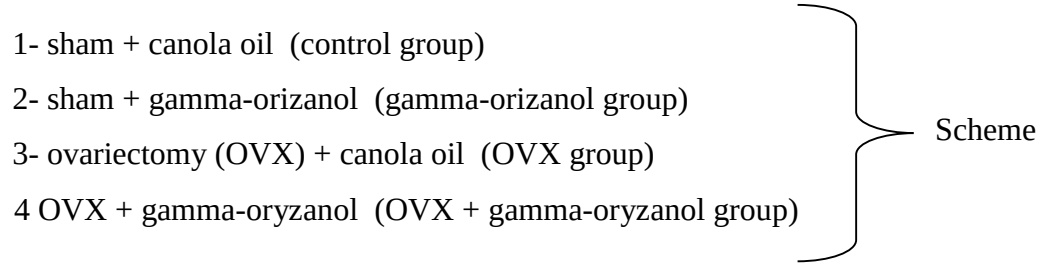
Female Swiss albino mice (25–30 g in weight and 90 days old) were used. Animals were housed in groups of 5 in Plexiglas cages (41 cm × 34 cm × 16 cm) with the floor covered with sawdust. They were kept in a room with light–dark cycle of 12 h with the lights on between 7:00 and 19:00 h and temperature controlled (20–25◦C) and received water and food ad libitum.

The animals were maintained and used in accordance with the guidelines of the Committee on Care and Use of Experimental Animal Resources (process #**021/2014**) of the Federal University of Pampa, Brazil.

2.2. Experimental design and sampling

32 animals were weighed and divided into 4 groups. The gamma-oryzanol at a dose of 50 mg/kg and vehicle (canola oil - 10 ml/kg) are administered orally via gavage for 28

consecutive days. Thus, during the 28 days the animals of groups 2 and 4 receive the gamma-oryzanol and the animals of groups 1 and 3 receive the vehicle (canola oil, 10 ml/kg). One day before the initiation of treatment, animals were submitted to surgery (sham: sham surgery groups 1 and 2; ovariectomy in groups 3 and 4). After 28 days of administration of gamma-oryzanol, the animals receive a dose of pentobarbital (180 mg/kg intraperitoneally) and blood is collected by cardiac puncture from where they are transferred into tubes containing heparin (anticoagulant).



2.3. Biochemical parameters

Plasm is used for biochemical determinations of glucose, total cholesterol, HDL (High Density Lipoprotein) cholesterol, LDL (Low Density Lipoprotein) cholesterol and triglycerides according to the instructions of each kit. Reagents for the determination of biochemical parameters are obtained from Labtest LTDA (BRAZIL).

2.4. Endocrine parameters

The concentrations of estrogen and insulin in the plasm of animals were performed using ELISA kits (Phoenix Pharmaceuticals®, Burlingame, USA).

2.5. Oxidative Profile

2.5.1. GSH (Glutathione) levels

GSH content was determined fluorometrically using ortho-phthalaldehyde (OPA) as the fluorophore (HISSIN & HILF, 1976). S1 (100 mL) was incubated with 100 mL of OPA (0.1% in methanol) and 1.8 mL of 0.1 M phosphate buffer (pH 8.0) for 15 min at room temperature in the dark. Fluorescence was measured with a fluorescence spectrophotometer at the excitation wavelength of 350 nm and at the emission wavelength of 420 nm. GSH levels were expressed as nmol/g of tissue.

2.5.2. ROS (Reactive Oxygen Species) levels

To determine ROS levels, S1 (fresh preparation) was diluted (1:10) in 50 mM Tris-Hcl (pH 7.4) and incubated with 10 mL of 20 ,70 - dichlorofluorescein diacetate (DCHF-DA; 1 mM) at 37C for 30 min. ROS levels were determined by a spectrofluorimetric method using the DCHF-DA assay, as previously described (2005). The DCHF-DA is enzymatically hydrolyzed by intracellular esterases to form nonfluorescent DCFH, which is then rapidly oxidized to form highly fluorescent 20 ,70 -dichlorofluorescein (DCF) in the presence of ROS. DCF fluorescence intensity is proportional to the amount of ROS that is formed. The DCF fluorescence intensity emission was recorded at 520 nm (with 480 nm excitation) 30 min after the addition of DCHF-DA to the medium. ROS levels were expressed in arbitrary units.

2.5.3. TRAP (total reactive antioxidant potential)

The non-enzymatic antioxidant potential of the plasm was estimated by the TRAP (LISSI et al., 1995). The reaction is initiated by adding luminol (5-amino-2,3-dihydro- 1,4-phthalazinedione, 4 mM), an external probe for monitoring radical production, as well as AAPH (2,20 -azobis–2-methylpropionamidine–dihydrochloride, 10 mM), a free radical source that produces peroxyl radical at a constant rate, in glycine buffer (0.1 M) pH 8.6 at room temperature, resulting in a steady luminescence emission (system counts). Chemiluminescence was read in a liquid scintillation counter (Agilent Care Elipse) as counts/min. The sample addition decreases the luminescence proportionately to its antioxidant potential. The luminescence emission was followed for 40 min after the addition of the sample (100 g of protein) in a TRAP protocol, and the area under the curve (AUC) was quantified. In the TAR protocol, results were expressed as the percentage of radical production (e.g., system counts considered to be 100% of radical production).

2.6. Protein determination

Protein content was measured colorimetrically by the method of Bradford (1976), using bovine serum albumin (1 mg/ml) as standard.

2.7. Statistical analysis

Data were analyzed by two-way ANOVA and post hoc analyses were carried out by the Newman–Keuls test. When appropriate, data were transformed (Y = Log(Y)) to meet

ANOVA requirements. A probability of $P < 0.05$ was considered significant, and all data are reported as mean and S.E.M.

3. RESULTS

3.1. Effect of gamma-oryzanol in weight, food intake and plasma levels of estrogen

The results demonstrate that there was an significant increase in body weight of ovariectomized mice compared to the control group ($p < 0.001$, Fig. 1A). The treatment with the gamma-orizanol caused a protection of weight gain induced by ovariectomy. The animals treated with gamma-oryzanol obtained a reduction in feed consumption (Fig. 1B). A significant reduction in estrogen levels was observed in OVX groups, additionally, in the OVX/vehicle group there was a greater decline in these levels when compared to the control group ($p < 0.001$). However, the group treated with gamma-oryzanol showed a partial effect compared to the control group (Fig. 1C).

3.2. Effect of gamma-oryzanol in glucose and insulin levels

A significant increase in glucose levels was observed in OVX/vehicle group compared to the control group ($p < 0.001$), and treatment with gamma-oryzanol prevented against this change (Fig. 2A). Besides, it was also observed a significant increase of insulin in the OVX/vehicle group compared with the control group ($p < 0.001$), but the treatment with gamma-oryzanol promoted protective effect (Fig. 2B).

3.3. Effect of gamma-oryzanol in lipid profile

In relation the concentrations of cholesterol, the results revealed a significant increase in total cholesterol in OVX/vehicle group compared with the control group ($p < 0.001$). Furthermore, the gamma-oryzanol was able to protect against increased total cholesterol (Fig. 3A).

There was a significant increase in plasma triglyceride levels in mice of OVX/vehicle group compared to the other experimental groups. Treatment with gamma-oryzanol reversed the increase, but did not get effect on the group Sham/Gamma-oryzanol (Fig. 3B). The administration of gamma-oryzanol was able to reduce LDL cholesterol levels and ovariectomy caused a significant increase of LDL cholesterol in OVX/vehicle group

compared with the control group ($p < 0.01$) (Fig. 3C). In addition, it proved a great protection factor, able to increase HDL levels (Fig. 3D).

3.4. Antioxidant effect of gamma-oryzanol

In relation to total antioxidant potential, it was observed that the gamma-oryzanol promoted an increase in groups that received this antioxidant. While in OVX/vehicle group, the removal of the ovaries significantly decreases the total antioxidant potential. Interestingly, treatment with gamma-oryzanol protected against the reduction of total antioxidant potential in serum of mice (Fig. 4A).

There was a significant decrease in GSH levels in the OVX group/vehicle when compared to the other experimental groups. The administration of gamma-oryzanol not increase GSH, in other words, it is not through an increase of GSH, than the gamma-oryzanol increase the TRAP (Fig. 4B).

Ovariectomy induced a significant increase of reactive oxygen species in serum of mice when compared to control group. The administration of the gamma-oryzanol promoted a reduction of the reactive oxygen species compared to their respective control.

4. DISCUSSION

Menopause is characterized by the irreversible suspension of ovarian function with declining estrogen secretion. In the absence of estrogen, is observed the increased in incidence of obesity, dyslipidemias, diabetes mellitus type 2, and consequently, a metabolic syndrome (PANAZZOLO et al., 2014).

Based on our results is possible realize that ovariectomy resulted in several changes of great impact, such as changes in body weight, endocrine, biochemical changes and oxidative status.

Whereas the association between the reduction of estrogen and increased adiposity is well established, it was observed that animals submitted to ovariectomy procedure showed higher body weight gain. These results are similar to those found by Dantas (2011), differing only in relation to the feed intake, on which the higher body weight gain was associated with increased energy intake of these groups. Differently of the animals involved in our study, where the increase of the body weight is not related to excessive intake of energy, once that the mice consumed the same amount of energy and continued increasing the weight.

Estrogens can control food intake through their central actions in the hypothalamus mediated by estrogen receptor alpha (ERα) and beta estrogen receptors (SOUZA, 2012).

Accordingly, the weight gain observed on our results may be associated with the decline in estrogen levels due to ovariectomy. Moreover, it is postulated that the energy balance is the relation between the amount of energy consumed and the total energy expenditure. Thus, the energy balance is a possible explanation for this weight gain, independent of food intake, it would be some metabolic change, wherein the metabolic tax decreases and the lipogenesis increases.

Some authors suggest than the gamma-oryzanol as a mixture of substances with anorexigenic effect, is capable of reduce the food intake (DANTAS, 2011). Therefore, our results demonstrate the beneficial action of gamma-oryzanol in reducing food intake and protect against the increase in ovariectomized mice weight.

The ovariectomy reduced estrogen levels and the gamma-oryzanol in turn, increased. This protection is related to the structure of the gamma-oryzanol, composed by a mixture of esters ferulic acid with phytosterols, which the cycloartenyl ferulate, 24-methylenecycloartanyl ferulate, sitosteryl ferulate and campesteryl ferulate are the main components (KLONGPITYAPONG; SUPABPHO; SUPABPHOL, 2013). In this sense, the structure of these 4 main compounds is esters and due our body has large amounts of esterase enzymes, responsible for the breakdown and release of ferulic acid. It is believed that the gamma-oryzanol have estrogen-like effect, being capable of reducing the effects of the absence of estrogen and thus act as a mimic the hormone.

There are evidences demonstrating the relation of gamma-oryzanol in glucose control, as shown in the study of Wang et al., (2015) where treatment with gamma-oryzanol and ferulic acid resulted in a significant improvement of hyperglycemic rats. In our study, the gamma-oryzanol protects significantly increased blood glucose due to the ovariectomy. The gamma-oryzanol can be acting on improving adiponectin concentrations, which is a hormone with action in the regulation of blood glucose. Second Iman et al., (2012), the gamma-oryzanol present in the rice bran oil, has anti-diabetes effect and one of the mechanisms by which it acts improving glucose levels, beyond afoid the hipoadiponectinemia which is involved in the reduction of insulin sensitivity.

Another explication for the effects of gamma-oryzanol on glycemic control and insulinic, it was suggested by Kaup et al., (2012), which, related to their participation in liberation of insulin, thus promoting, a reduction in the hyperinsulinemic response.

Among the negative effects of the estrogen deficiency, stand out the changes related to lipid profile, in particular, increase LDL, reduction HDL and increased deposition of fat. After

the menopause, women tend to present several changes in lipid profile and the increase of these parameters can exert cytotoxic effects on the arterial wall modulating various events for atherosclerosis disease (GONÇALVES et al., 2012).

The gamma-oryzanol treatment range proved to be an excellent protective factor against the development of one atherogenic profile, after all, it was beneficial in significant reduction of the plasma total cholesterol levels, LDL and triglycerides with cominate increased of the HDL, which corroborates the results found in several other studies involving animal models (SCHMIDT et al., 2012; SAWADIKIAT & HONGSPRABHA, 2014; WANG et al., 2014).

According Mäkynen et al., (2012), hypocholesterolemic property orizanol range is due to their structural similarity to cholesterol. The gamma-oryzanol is capable of causing interference with the cholesterol incorporation into micelles digestive preventing intestinal absorption of cholesterol, with consequent reduction in the amount of circulating cholesterol molecules. Furthermore, the phytosterol may be associated with inhibition of HMGCoA reductase activity, which is the limiting enzyme of cholesterol biosynthesis speed.

In addition to hypolipidemic and hypocholesterolemic properties, gamma-oryzanol has recognized antioxidant activity. This antioxidant activity is attributed to its complex composition of ferulic acid esters. According to Andrade (2010), ferulic acid esterified plant sterols, such as gamma-oryzanol, increases the antioxidant activity by favoring access to the molecular hydrophobic components that are more susceptible to oxidative cellular destruction.

The glutathione (GSH) system, which is responsible for removing free radicals and maintaining protein thiols in their appropriate redox state, is an important protective mechanism for minimizing oxidative stress (AITKEN & ROMAN, 2008). It should be noted that the levels of GSH and TRAP were significantly reduced in the OVX animals compared with those in the sham-operated group and that this reduction was protected by gamma-oryzanol.

Menopause has been reported to be associated with increased oxidative stress and metabolic disorders among women worldwide. It is suggested that the hormonal changes of menopause cause a redox imbalance leading to increased reactive oxygen species (BEHR; SCHNORR & MOREIRA, 2011). Increased production of ROS is considered to be one of the major causes of several age-related diseases. These species are continuously generated in physiological conditions and effectively controlled/eliminated by intracellular and extracellular antioxidant systems. Oxidative stress has been defined as an unbalance between

increased ROS production and inadequate antioxidant activity (HALLIWELL & GUTTERIDGE, 2007). Our data corroborate this information since in ovariectomized mice there was an increase of ROS and the oryzanol gamma acted as an antioxidant effective.

5. CONCLUSION

The results of this study indicate that gamma-oryzanol promotes metabolic improvements that may be beneficial in maintaining adequate levels of total cholesterol and lipid fractions, glycemic control and other benefits related to their antioxidant capacity. Thus, it can be considered an excellent therapeutic alternative for the treatment of common complications in the postmenopausal period and reduce the chances of other comorbidities.

6. REFERENCES

AITKEN, R. J.; ROMAN, D. R. Antioxidant systems and oxidative stress in the testes- a review. **Oxidative Medicine and Cellular Longevity**, n. 14, p. 15-24, 2008.

ANDRADE, M. L. **Óleo de arroz rico em gama orizanol e alterações morfofisiológicas em ratos treinados**. Maringá: UEN, 2010. Disponível em: <http://www.def.uem.br/geraMonografia.php?id=233> Acesso em: 23 mar. 2015.

BEHR, G.; SCHNORR, C. E.; MOREIRA, J. C. Increased blood oxidative stress in experimental menopause rat model: the effects of vitamin A low-dose supplementation upon antioxidant status in bilateral ovariectomized rats**. Fundamental & Clinical Pharmacology**, n. 2, p. 235-249, 2011.

BRADFORD, M. M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principles of protein-dye binding. **Analytical Biochemistry**, n. 3, p. 248-54, 1976.

BUNRATSAMIA, S.; UDOMUKSORNB, W.; KUMARSITC, E.; VONGVATCHARANOND, S.; VONGVATCHARANONA, U. Estrogen replacement improves skeletal muscle performance by increasing parvalbumin levels in ovariectomized rats. **Acta Histochemica**, n. 117, p.163–175, 2015.

COLPANI, V. **Fatores de risco cardiovascular em uma coorte de mulheres na menopausa no sul do Brasil**. Porto Alegre: UFRGS, 2015. Disponível em: <http://www.lume.ufrgs.br/handle/10183/115036> Acesso em: 18 abr. 2015.

CORREA, C. S.; TEIXEIRA, B. C.; BITTERNCOURT, A.; OLIVEIRA, A. R. Effects of strength training on blood lipoprotein concentrations in postmenopausal women. **Jornal Vascular Brasileiro**, n. 13, p. 312-317, 2014.

DANTAS, A. C. S. **Impacto da ovariectomia sobre alterações metabólicas e inflamatórias em modelos de obesidade induzida por dieta em ratas**. Vitória da Conquista: Universidade Federal da Bahia, 2011. Disponível em: <http://repositorio.ufba.br/ri/handle/ri/10442> Acesso em: 12 mai. 2015.

ESLAMI, S.; ESA, N. M.; MARANDI, S. M.; GHASEMI, G.; ESLAMI, S. Effects of gamma-oryzanol supplementation on anthropometric measurements & muscular strength in healthy males follwing chronic resistance training. **Indian Journal of Medical Research**, n. 139, p. 857-862, 2014.

GONÇALVES, R. C.; FARIA, K. R. M.; SILVA, P. I.; FILHO, R.; MALAFAIA, G. Lipid profile and risk factors for disease atherosclerosis in metallurgical of quirinópolis-go. **Enciclopédia biosfera**, n.14; p. 1615- 2012, 2012.

HALLIWELL, B.; GUTTERIDGE, J. M. C. **Free Radicals in Biology and Medicine**. 4. ed. New York: Oxford Universiy Press, 2007. 888 p.

HISSIN, P. J.; HILF, R. Afluorometric method for determination of oxidized and reduced glutathione in tissues. **Analytical Biochemistry**, n. 1, p.214–226, 1976.

IMAN, M. N.; AZMI, N. H.; BHANGER, M. I.; ISMAIL, N.; ISMAIL, M. Antidiabetic Properties of germinated brown rice: a systemic review. **Hindawi Publishing Corporation**, n. 4, p. 125-137, 2012.

KAUP, R. M.; KHAYYAL, M. T.; VERSPOHL, E. J. Antidiabetic Effects of a Standardized Egyptian Rice Bran Extract. **Phytotherapy Research**, n. 2, p. 264-71, 2012.

KLONGPITYAPONG, P.; SUPABPHO, R.; SUPABPHOL, A. Antioxidant Effects of Gamma-oryzanol on Human Prostate Cancer Cells**. Asian Pacific Journal of Cancer Prevention**, n. 9, p. 5421-5425, 2013.

LISSI, E.; SALIM, H. M.; PASCUAL, C.; CASTILLO, M. D. Evaluation of total antioxidant potential (TRAP) and total antioxidant reactivity from luminolenhanced chemiluminescence measurements. **Free Radical Biology & Medicine,** n. 18, p.153-161, 1995.

MÄKYNEN, K.; CHITCHUMROONCHOKCHAI, C.; ADISAKWATTANA, S.; FAILA, M.; ARIYAPITIPUN, T. Effect of gamma-oryzanol on the bioaccessibility and synthesis of cholesterol. **European Review for Medical and Pharmacological Sciences**, n. 16, p. 49-56, 2012.

MOREIRA, S. F. da S. **Avaliação de modelo de menopausa em ratas: parâmetros fisiológicos, comportamentais, bioquímicos e novas estratégias terapêuticas**. Porto Alegre: UFRGS, 2014. Disponível em: < http://www.lume.ufrgs.br/handle/10183/104123>. Acesso em: 14 abr. 2015.

MUHAMMAD, S. I.; MAZNAH, I.; MAHMUD, R. B.; SAEED, M. I.; IMAM, M. U.; ISHAKA, A. Estrogen receptor modulatory effects of ferminated brown rice bioactives in the uterus of rats through the regulation of estrogen-induced genes. **Drug Design, Development and Therapy,** n. 7, p. 1409-1420, 2013.

PAN, Y.; CAI, L.; HENZI, S.; ZHANG, Z. Pharmacokinetics study of ferulic acid in rats after oral administration of γ-oryzanol under combined use of Tween 80 by LC/MS/MS. **European Review for Medical and Pharmacological Sciences**, n. 18, p. 143-150, 2014.

PANAZZOLO, D. G.; SILVA, L. H. A.; LEÃO, L. M. C. M.; AGUIAR, L. G. K. Effects of menopausal hormone therapy on body fat. **Review HUPE**, v.13, n. 1, 2014.

PARDINI, D. Hormone replacement therapy in menopause. **Archives of Endocrinology and Metabolism**, n. 58, p. 172-181, 2014.

SAENJUM, C.; CHAIYASUT, C.; CHANSAKAOW, S.; SUTTAJIT, M.; SIRITHUNYALUG, B. Antioxidant and anti-inflammatory activities of gamma-oryzanol rich extracts from Thai purple rice bran. **Journal of Medicinal Plants Research**, n. 6, p. 1070-1077, 2012.

SAWADIKIAT, P.; HONGSPRABHA, P. Phytosterols and y-oryzanol in rice bran oils and distillates from physical reting process. **International Journal of food Science and Techonology**, n. 49, p. 2030-2036, 2014.

SCHMIDT, L; BERLEZE, K. J.; BRUSQUE, A. M. B. Hypolipidemic effect of rice bran diet. **Revista Ciência & Saúde**, n. 2, p. 92-98, 2012.

SOUZA, S. I. **Alterações renais e metabólicas induzidas pela dieta hiperlipídica e hipersódica em ratas ovariectomizadas**. Vitória da Conquista: Universidade Federal da Bahia, 2012. Disponível em: <http://repositorio.ufba.br/ri/handle/ri/10441> Acesso em: 20 mai. 2015.

WANG, Y. X.; LI, Y.; SUN, A. M.; WANG, F.; YU, G. P. Hypolipidemic and Antioxidative Effects of Aqueous Enzymatic Extract from Rice Bran in Rats Fed a High-Fat and -Cholesterol Diet. **Nutrients**, n. 6, p. 3696-3710, 2014.

WANG, O.; LIU, J.; CHENG, Q.; GUO, X.; WANG, L.; ZHOU, B. Effects of Ferulic Acid and γ-Oryzanol on High-Fat and High-Fructose Diet-Induced Metabolic Syndrome in Rats. **PLOS ONE**, n. 10, p. 1360-1371, 2015.

FIGURE LEGENDS

Figure 1 - Effect of gamma-orzyzanol (50 mg/kg, p.o. for 28 days) administration on body weight (A), food intake (B) and estrogen levels (C) in blood in ovariectomy (OVX)- or sham-operated mice. Data are mean + S.E.M. for n= 8 animals in each group. **Indicates a significant difference ($p<0.01$) compared with sham/vehicle. ***Indicates a significant difference ($p<0.001$) compared with sham/vehicle. $^{\#}$Indicates a significant difference ($p<0.01$) compared with OVX/vehicle. $^{@}$Indicates a significant difference ($p<0.05$) compared with Sham/gamma-oryzanol.

Figure 2 - Effect of gamma-orzyzanol (50 mg/kg, p.o. for 28 days) administration on glicose (A) and insulin (B) levels in blood in ovariectomy (OVX) or sham-operated mice. Data are mean + S.E.M. for n= 8 animals in each group. ***Indicates a significant difference ($p<0.001$) compared with sham/vehicle. $^{\#}$Indicates a significant difference ($p<0.01$) compared with OVX/vehicle. $^{@}$Indicates a significant difference ($p<0.05$) compared with Sham/gamma-oryzanol.

Figure 3 - Effect of gamma-orzyzanol (50 mg/kg, p.o. for 28 days) administration on cholesterol (A), triglyceride (B), LDL (C) and HDL (D) levels in blood in ovariectomy (OVX) or sham-operated mice. Data are mean + S.E.M. for n= 8 animals in each group. **Indicates a significant difference ($p<0.01$) compared with sham/vehicle. ***Indicates a significant difference ($p<0.001$) compared with sham/vehicle. $^{\#}$Indicates a significant difference ($p<0.01$) compared with OVX/vehicle.

Figure 4 - Effect of gamma-orzyzanol (50 mg/kg, p.o. for 28 days) administration on total reactive *antioxidant* potential (*TRAP*) (A), glutathione (GSH) (B) and reactive species (ROS) (C) levels in blood in ovariectomy (OVX)- or sham-operated mice. Data are mean + S.E.M. for n= 8 animals in each group. *Indicates a significant difference ($p<0.05$) compared with sham/vehicle. **Indicates a significant difference ($p<0.01$) compared with sham/vehicle. ***Indicates a significant difference ($p<0.001$) compared with sham/vehicle. $^{\#}$Indicates a significant difference ($p<0.01$) compared with OVX/vehicle. $^{@}$Indicates a significant difference ($p<0.05$) compared with Sham/gamma-oryzanol.

Figura 1

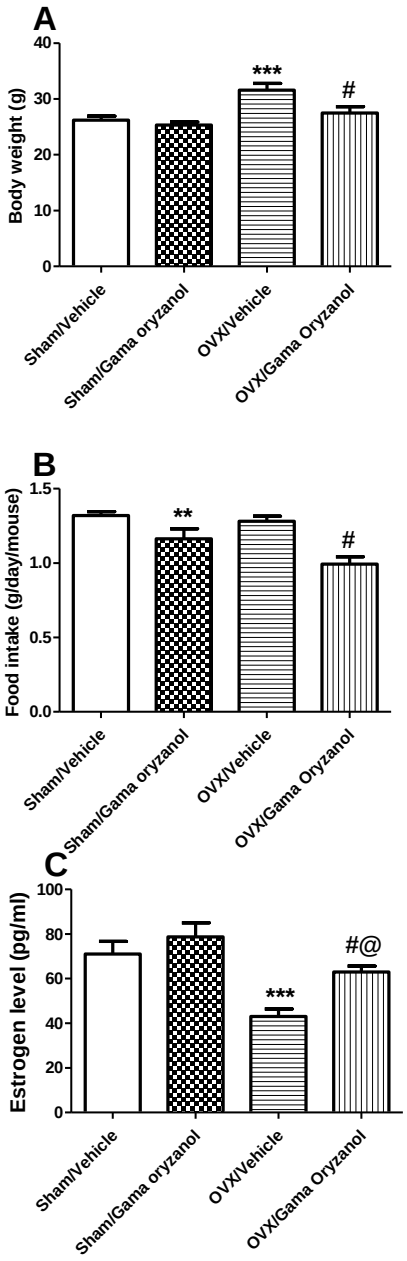

A
Body weight (g)
40
30
20
10
0
***
#
Sham/Vehicle
Sham/Gama oryzanol
OVX/Vehicle
OVX/Gama Oryzanol
B
Food intake (g/day/mouse)
1.5
1.0
0.5
0.0
**
#
Sham/Vehicle
Sham/Gama oryzanol
OVX/Vehicle
OVX/Gama Oryzanol
C
Estrogen level (pg/ml)
100
80
60
40
20
0
***
#@
Sham/Vehicle
Sham/Gama oryzanol
OVX/Vehicle
OVX/Gama Oryzanol

Figura 2

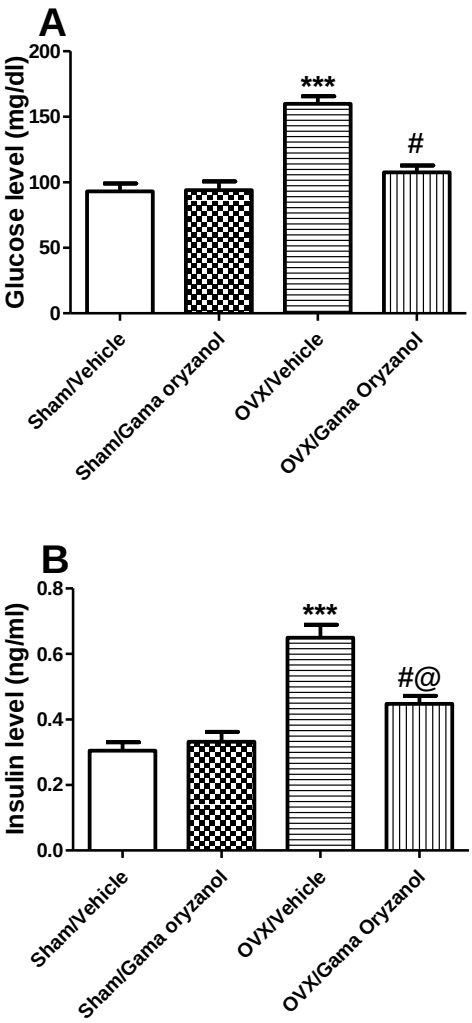

A
Glucose level (mg/dl)
200
150
100
50
0
***
#
Sham/Vehicle
Sham/Gama oryzanol
OVX/Vehicle
OVX/Gama Oryzanol
B
Insulin level (ng/ml)
0.8
0.6
0.4
0.2
0.0
***
#@
Sham/Vehicle
Sham/Gama oryzanol
OVX/Vehicle
OVX/Gama Oryzanol

Figura 3

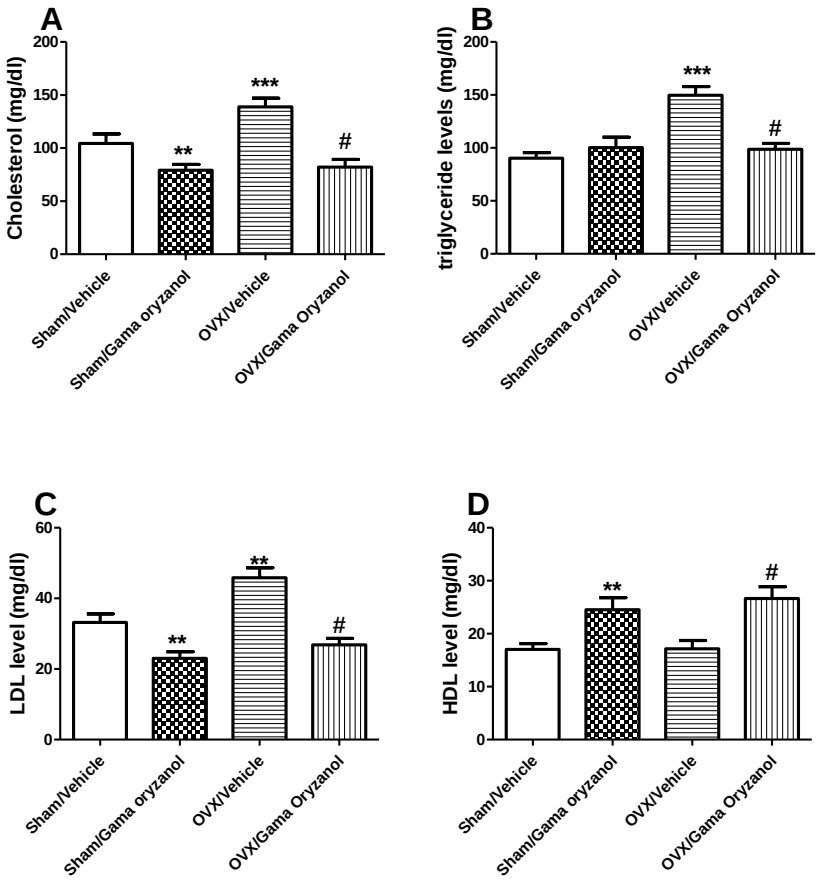
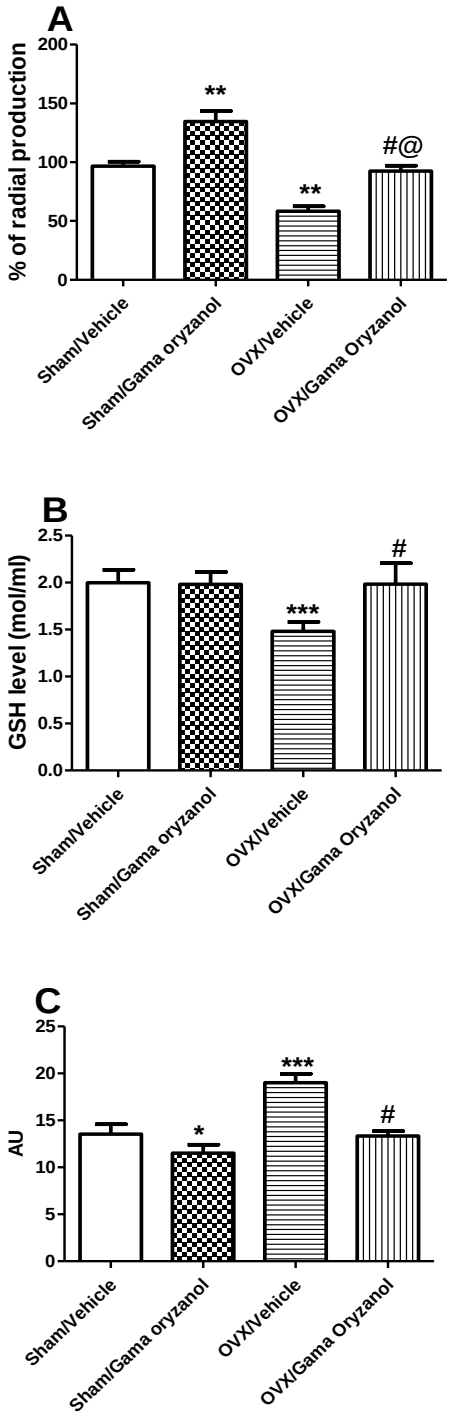

A
Cholesterol (mg/dl)
200
150
100
50
0
**
***
#
Sham/Vehicle
Sham/Gama oryzanol
OVX/Vehicle
OVX/Gama Oryzanol
B
triglyceride levels (mg/dl)
200
150
100
50
0
***
#
Sham/Vehicle
Sham/Gama oryzanol
OVX/Vehicle
OVX/Gama Oryzanol
C
LDL level (mg/dl)
60
40
20
0
**
**
#
Sham/Vehicle
Sham/Gama oryzanol
OVX/Vehicle
OVX/Gama Oryzanol
D
HDL level (mg/dl)
40
30
20
10
0
**
#
Sham/Vehicle
Sham/Gama oryzanol
OVX/Vehicle
OVX/Gama Oryzanol

Figura 4


A
% of radial production
200
150
100
50
0
**
**
#@
Sham/Vehicle
Sham/Gama oryzanol
OVX/Vehicle
OVX/Gama Oryzanol
B
GSH level (mol/ml)
2.5
2.0
1.5
1.0
0.5
0.0
***
#
Sham/Vehicle
Sham/Gama oryzanol
OVX/Vehicle
OVX/Gama Oryzanol
C
AU
25
20
15
10
5
0
*
***
#
Sham/Vehicle
Sham/Gama oryzanol
OVX/Vehicle
OVX/Gama Oryzanol

ANEXO A

CHEMICO-BIOLOGICAL INTERACTIONS
A journal of molecular, cellular and biochemical toxicology

GUIDE FOR AUTHORS

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We now differentiate between the requirements for new and revised submissions. You may choose to submit your manuscript as a single Word or PDF file to be used in the refereeing process. Only when your paper is at the revision stage, will you be requested to put your paper in to a 'correct format' for acceptance and provide the items required for the publication of your article. **To find out more, please visit the Preparation section below.**

INTRODUCTION

CHEMICO-BIOLOGICAL INTERACTIONS publishes research reports and review articles that examine the molecular, cellular, and/or biochemical basis of toxicologically relevant outcomes. Special emphasis is placed on toxicological mechanisms associated with interactions between chemicals and biological systems. Outcomes may include all traditional endpoints caused by synthetic or naturally occurring chemicals, both in vivo and in vitro. Endpoints of interest include, but are not limited to carcinogenesis, mutagenesis, respiratory toxicology, neurotoxicology, reproductive and developmental toxicology, and immunotoxicology. CBI discourages papers that are descriptive in nature and that do not address toxicological mechanisms (e.g., reports of toxicological effects following chemical exposure in absence of mechanistic experiments). CBI also discourages papers reporting on toxicological effects from materials, such as plant extracts or herbal medicines, that have not been chemically characterized.

Types of paper

Chemico-Biological Interactions will publish, (1) Papers reporting results of original mechanistic research; (2) Review articles; (3) Letters to the Editor; (4) Announcements and advertisements. Review Articles - Outlines of papers for these sections should be submitted to the Editor in Chief. Announcements and Advertisements - Organisers of relevant meetings

may submit announcements through the Editor-in-Chief for publication free of charge as space permits. Advertising rates can be obtained upon request.

BEFORE YOU BEGIN

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Divide your article into clearly defined and numbered sections. Subsections should be numbered 1.1 (then 1.1.1, 1.1.2,...), 1.2, etc. (the abstract is not included in section numbering). Use this numbering also for internal cross-referencing: do not just refer to 'the

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Abstract

A concise and factual abstract of approx. 200-300 words is mandatory. The abstract should state briefly the purpose of the research, the principal results and major conclusions. An abstract is often presented separately from the article, so it must be able to stand alone. For this reason, References should be avoided, but if essential, then cite the author(s) and year(s). Also, non-standard or uncommon abbreviations should be avoided, but if essential they must be defined at their first mention in the abstract itself.

Introduction

State the objectives of the work and provide an adequate background, avoiding a detailed literature survey or a summary of the results.

Material and methods

Provide sufficient detail to allow the work to be reproduced. Methods already published should be indicated by a reference: only relevant modifications should be described.

Theory/calculation

A Theory section should extend, not repeat, the background to the article already dealt with in the Introduction and lay the foundation for further work. In contrast, a Calculation section represents a practical development from a theoretical basis.

Results

Results should be clear and concise.

Discussion

This should explore the significance of the results of the work, not repeat them. A combined Results and Discussion section is often appropriate. Avoid extensive citations and discussion of published literature.

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The main conclusions of the study may be presented in a short Conclusions section, which may stand alone or form a subsection of a Discussion or Results and Discussion section.

Appendices

If there is more than one appendix, they should be identified as A, B, etc. Formulae and equations in appendices should be given separate numbering: Eq. (A.1), Eq. (A.2), etc.; in a subsequent appendix, Eq. (B.1) and so on. Similarly for tables and figures: Table A.1; Fig. A.1, etc.

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Example: '..... as demonstrated [3,6]. Barnaby and Jones [8] obtained a different result ....'

List: Number the references (numbers in square brackets) in the list in the order in which they appear in the text.

Examples:

Reference to a journal publication:

[1] J. van der Geer, J.A.J. Hanraads, R.A. Lupton, The art of writing a scientific article, J. Sci. Commun.163 (2010) 51–59.

Reference to a book:

[2] W. Strunk Jr., E.B. White, The Elements of Style, fourth ed., Longman, New York, 2000.

Reference to a chapter in an edited book:

[3] G.R. Mettam, L.B. Adams, How to prepare an electronic version of your article, in: B.S. Jones, R.Z.Smith (Eds.), Introduction to the Electronic Age, E-Publishing Inc., New York, 2009, pp. 281–304.

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Divide the article into clearly defined sections. Please ensure the text of your paper is double-spaced– this is an essential peer review requirement.

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Please ensure the figures and the tables included in the single file are placed next to the relevant text in the manuscript, rather than at the bottom or the top of the file.

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