ARTIGO ORIGINAL

TOXICOLOGICAL EVALUATION OF THE FLAVONOID RUTIN ON THE REPRODUCTIVE SYSTEM OF WISTAR RATS

AVALIAÇÃO TOXICOLÓGICA DO FLAVANOIDE RUTINA NO SISTEMA REPRODUTOR DE RATOS WISTAR

Juliana Marques Raso Becho[*], Vera Maria Peters[**], Renato Marques Macedo[***], Leda Marília Fonseca Lucinda[****], Martha de Oliveira Guerra[*****]

Resumo

Introdução: A rutina é um flavonoide comumente encontrado na natureza que tem atividades antimitótica, vasoprotetora e anti-hiperlipidêmica Quando hidrolizada em quercetina, promove a inibição da motilidade do espermatozoide, alterações na próstata, e nos níveis da testosterona e dihidrotestosterona. Objetivo: Este estudo teve como objetivo avaliar a toxicidade da rutina em ratos Wistar. Métodos: Os animais foram divididos em: Controle (1 ml de água destilada), Tratado I, II e III, respectivamente recebendo 5, 10 e 20 mg/kg/dia de rutina por sete dias consecutivos. A eutanásia foi realizada depois de 10, 42 e 60 dias do experimento, seguiu-se o procedimento de laparotomia, em que removeu-se os testículos, próstata, vesícula seminal, epidídimo, fígado, baço e rins. A contagem dos espermatozoides removidos da cauda do epidídimo foi realizada, assim como testes hematológicos e bioquímicos. Resultados: Foi observada hepatomegalia, e no sistema reprodutor, o peso do epidídimo estava reduzido, não foi observado efeito em nenhum outro órgão estudado. Conclusão: Exceto pela redução do peso do epidídimo, que foi reversível após 42 dias de cessado o tratamento, não houve dados sugestivos de que a rutina tenha causado toxicidade no sistema reprodutor de ratos Wistar adultos.

Palavras-chave

Flavonoides. Rutina. Ratos. Epidídimo. Toxicidade.

Abstract

Introduction: Rutin, a flavonoid commonly found in nature, has anti-mitotic, vasoprotective, and antihyperlipidemic activity. When hydrolyzed as quercetin, it promotes inhibition of spermatozoa motility, alterations in the prostate, and in the levels of testosterone and dihydrotestosterone. Objective: This study aimed to evaluate the toxicity of rutin in Wistar rats. Methods: Animals were divided into Control (1 ml of distilled water), Treated I, II and III, respectively receiving 5, 10 and 20 mg/kg/day of rutin for seven consecutive days. When euthanasia was performed after 10, 42 and 60 days into the experiment, a laparotomy was performed and the testicles, prostate, seminal vesicles, epididymis, epididymal spermatozoa to be counted, as well as the liver, spleen and kidneys were removed. Hematological and biochemical tests were performed. Results: Hepatomegaly was observed and in the reproductive system, the weight of the epididymis was reduced, not affecting any other organ examined. Conclusion: Except by the reduction of the weight of the epididymis, which is reversible at 42 days of completion of treatment, no suggestive data of the toxicity of rutin on the reproductive system of adult rats were found.

Keywords

Flavonoids. Rutin. Rats. Epididymis. Toxicity.

Correspondence author: Leda Marília Fonseca Lucinda. ledamarilia@yahoo.com.br. Tel: 55(32)2102-2351. Fax: 55(32)2102-3255

[*] MSc. Centro de Biologia da Reprodução – Universidade Federal de Juiz de Fora, Campus Universitário, Rua Jose Lourenço Kelmer s/n, Bairro Martelos, CEP: 36036330. julianabecho@gmail.com.

[**] PhD. Centro de Biologia da Reprodução – Universidade Federal de Juiz de Fora, Campus Universitário, Rua Jose Lourenço Kelmer s/n, Bairro Martelos, CEP: 36036330. peters.vera@ufjf.edu.br.

[***] MSc. Centro de Biologia da Reprodução – Universidade Federal de Juiz de Fora, Campus Universitário, Rua Jose Lourenço Kelmer s/n, Bairro Martelos, CEP: 36036330. renatimdalapa@hotmail.com.

[****] PhD. Centro de Biologia da Reprodução; Departamento de Morfologia-ICB – Universidade Federal de Juiz de Fora, Campus Universitário, Rua Jose Lourenço Kelmer s/n, Bairro Martelos, CEP: 36036330. ledamari-lia@yahoo.com.br.

[*****] PhD. Centro de Biologia da Reprodução – Universidade Federal de Juiz de Fora, Campus Universitário, Rua Jose Lourenço Kelmer s/n, Bairro Martelos, CEP: 36036330. martha.guerra@ufjf.edu.br.

Received: 01/2015
Accepted: 11/2015

1 INTRODUCTION

Rutin is a flavonoid belonging to the subclass of flavonols that have a disaccharide (glucose + rhamnose) linked to position 3 of the pyran ring. It is found in numerous dietary sources such as onions, grapes, buckwheat, red beans, apples, tomatoes, and beverages such as red wine and black tea (THOMSON; BLOCH; HASLER, 1999; HOLLMAN; HERTOG; KATAK, 1996).

It has been attributed to rutin an improvement in the symptoms of the lymphatic and venous vessels insufficiency, associated with some bleeding disorders or hypertension by promoting the standardization of resistance and

permeability of the wall of these vessels (PATHAK; PATHAK; SIGLA, 1991). Other symptoms of capillary fragility are also improved, including the reduction in the loss of visual acuity and visual field changes (PATHAK; PATHAK; SIGLA, 1991). It also acts as a noncompetitive inhibitor of angiotensin II and prostaglandin E2 and as a smooth muscle relaxant (YILDZOGKE-AR et al., 1991). We also observed its efficiency in treating arthritis by *Candida albicans* and anti-Candida activity (HAN, 2009), antihyperlipidemic activity (SANTOS et al., 1999), anticonvulsant effect in rats (NASSIRI-ALS; SHARIAT-RAD; ZAMANSOLTAN, 2008), cellular immunity suppression (MIDDLETON; KANDASWAM; THEOHARIDES, 2000), anticarcinogenic activity (MACHADO, 2006), and anti-inflammatory effect (GUARDIA et al., 2001). It significantly reduced levels of cholesterol and triacylglycerols in hyperlipidemic Wistar rats and it also appears to be a cholesterol modulator (PARK et al., 2002).

Rutin needs to be hydrolyzed and converted to quercetin by the enterobacteriaceae of the intestine, before it can be absorbed, both in humans and mice (WALLE, 2004), since the absorption in the small intestine is hampered due to the sugars attached to its molecule (MUROTA; TERAO, 2003).

Manach et al. (1997) as well as Walle et al. (2004) showed that rutin is completely hydrolyzed by glycosidases produced by enterobacteriaceae, resulting in quercetin-3-glucoside and aglycone quercetin (BOKKENHEUSER; SHACKLETON; WINTER, 1987).

After the hydrolysis of sugars, these molecules begin to present greater affinity for membranes of epithelial cells and, therefore, are better absorbed (MACHADO, H., 2006). However, Hollman et al. (1995) described that quercetin glycosylated (Q3G) is better absorbed than quercetin in the aglycone form since Q3G binds to glucose transporters (SGLT-1) in epithelial cells.

Hasumura et al. (2004) demonstrated that a prolonged use of rutin in the diet of rats did not cause hematological alterations or clinical signs of toxicity. In addition in the researched literature were not found evaluations of the reproductive toxicity of rutin. However, the effects of quercetin, one of its metabolites, on the male reproductive system are well known. Such studies show controversy when analyzed “in vitro” and “in vivo”. In the first case, inhibitory effects on spermatozoa motility and viability were demonstrated (NASS-ARDEN; BREITBART, 1990; KHANDUJA; VERMA; BHARDWAJ, 2001), but in vivo studies indicate a protective effect of quercetin against exposure to pesticides (IZAWA et al., 2008), and a stimulating effect on spermatozoa quality (TAEPONGSORAT et al., 2008). At higher doses (300mg/body weight of quercetin) there was an initial reduction of the fertility rates of rats during the first two matings with females, which was recovered in subsequent matings (ARAVINDAKSHAN; CHAUHAN; SUNDARAM, 1985), as well as changes in the prostate (MA et al., 2004).

Given these observations, we decided to conduct this study in order to determine the reproductive toxicity of rutin administered to adult rats for seven consecutive days.

2 MATERIALS AND METHODS

The methodology used in this study was approved by the Ethics Committee for Animal Experimentation (Protocol nº 005/2007 – CEEA, Federal University of Juiz de Fora), which follows the international ethical principles for animal experimentation.

2.1 BIOLOGICAL MODEL

180 adult male Wistar rats, weighing between 250 and 300 grams from the vivarium of the Center for Reproductive Biology (CBR) of the Federal University of Juiz de Fora (UFJF), were used. The animals were kept in polypropylene cages, lined with industrialized wood shavings, and fed with pelletized (Nuvilab/Nuvital®) feed and filtered water. The cages were kept in climatized cabinets (Alesco), located in an environment with temperature and 12-hour light/dark cycle automatically controlled.

2.2 FLAVONOID

The flavonoid used was rutin hydrate, minimum 95% HPLC from the company Sigma®, lot 085K0196, reference R5143-50G.

2.3 EXPERIMENTAL DESIGN

The methodology used was described by D’Souza (2003). The animals were randomly divided into four experimental groups: Control (C) – received 1 ml of distilled water (n = 45); Treated T1 (n = 45), T2 (n = 45) and T3 (n = 45) which received 5, 10 and 20mg rutin/kg of body weight respectively, intragastrically for seven days, in a single dose,

always between 2 and 3 pm. The LD50 of quercetin for rats was calculated in 161mg/kg of body weight (SULLIVAN; FOLLIS; HILGARTNER, 1951); however, it was not used to calculate the doses to be administered, preferring to use the corresponding dose which was effective in reducing the number of neoplastic cells in the experimental model of Ehrlich tumor (MACHADO, 2006), followed by half and twice this dose.

After 10, 42 and 60 days of the beginning of treatment, 15 animals from each group were euthanized. The choice of days is due to the fact that lesions in spermatozoa, spermatids, spermatocytes or spermatogonial can be identified in spermatozoa counts (D'SOUZA, 2006). Moreover, one could assess the reversibility of effects. All animals were observed for 60 minutes after treatment and thereafter once a day to check signs of clinical toxicity (ZENIIK et al., 1994).

Body weight and feed intake were measured in the first and last day of treatment, followed by weighing every seven days until the day of euthanasia, when they were weighed again. Feed intake was estimated by the difference in weight between what was provided in one day and what remained 24 hours later. Water was provided *ad libitum*.

2.4 EUTHANASIA

The animals were euthanized by asphyxiation after having their diaphragms ruptured, which was performed under deep anesthesia with ketamine (intraperitoneal) and xylazine (intramuscular) at doses of 90mg/kg and 10mg/kg of body weight, respectively. Once euthanasia was performed, the right epididymal was exposed and the tail punctured for removal of secretions and analysis of spermatozoa concentration (MORAES, 1994; DOSTAL; FABER; ZANDEE, 1996; SEED et al., 1996).

2.5 EVALUATION OF SPERMATOZOA

To count the spermatozoa, small incisions in the right epididymis tail were made with the aid of scissors. The material was immediately placed in a drop of saline (50µl) previously prepared in a Petri dish placed on a heated plate (36°C). Then, with the aid of an automatic pipette (Eppendorf®), 20µl of this solution found in this drop were withdrawn and dissolved in 6ml of distilled water. After dilution, a sample was collected and placed in a Neubauer® chamber to count the spermatozoa. The total number of spermatozoa is obtained by the average of two countings which correspond to the upper and lower field of the Neubauer® chamber.

Afterwards, a laparotomy was performed in search of macroscopic damage to internal organs followed by removal and weighing of left testicles, left epididymis, prostate, empty seminal vesicles, right and left kidney, liver, and spleen.

2.6 HISTOPATHOLOGICAL ANALYSIS

The organs were fixed in Baker's calcium formaldehyde, embedded in paraffin, cut in microtome (5µm) and stained with hematoxylin and eosin; except the epididymis, which was stained with Gomori trichrome following the procedures of the Center for Biology of Reproduction of the Federal University of Juiz de Fora, for future analysis under light microscopy, in case alterations in weight or macroscopic lesions were found.

2.7 HISTOMORPHOMETRIC ANALYSIS OF THE EPIDIDYMIS

The height of the tubular epithelium of the epididymis was measured through digital measurements in images captured by the AxioVision Release 4.7 (Zeiss®) program. Five epididymis per experimental group were used for histomorphometry. In each section, 10 circular tubules along the epididymis were randomly selected in which eight measurements along its circumference were obtained (Figure 1). The mean value was considered representative of the height of the tubule.

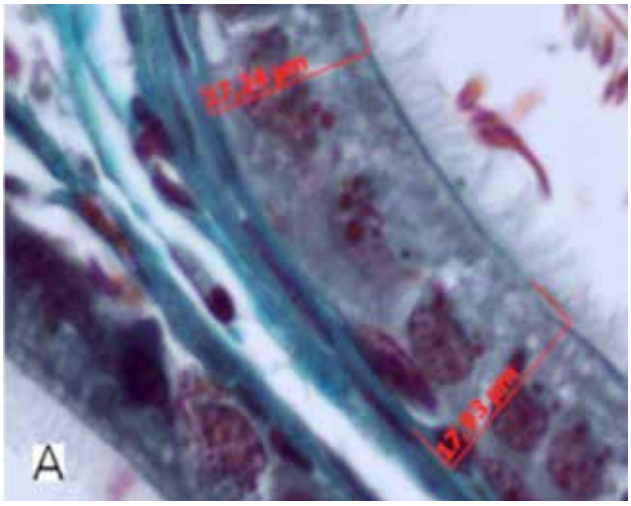

Figure 1: Original Magnification 400X. Epithelium Measurements of epididymis stained with Gomori trichrome.
Fonte: Acervo dos autores.

2.8 STATISTICAL PROCESSING

The collected data were processed by variance analysis – one way and a post-hoc Dunnet T3 test (α = 0.05), and Kruskal-Wallis test with significance level α = 0.05. To analyse the epididymal epithelium, we used one way – ANOVA followed by Dunnet post hoc test (α = 0.05).

3 RESULTS

No signs or symptoms of clinical toxicity were found in any animal of any of the experimental groups.

The animals' body weight in the beginning and end of treatment as well as in the day of their euthanasia is shown in Table 1.

Table 1: Comparison of initial, final and euthanasia weights (g) among animals in the groups treated with rutin TI (5 mg/kg/day), TII (10 mg/kg/day), and TIII (20 mg/kg/day) and control group (1ml distilled water) that were euthanized at 10, 42 and 60 days after the beginning of treatment.

Groups		Control	TI	TII	TIII
10 days	Initial W.	281.33±23.38	286.78±26.64	264.73±30.98	260.81±26.85
	Final W.	280.03±26.36	282.81±25.50	262.09±27.22	257.63±21.96
	Euthanasia W.	267.07±20.16	274.22±18.72	261.59±15.82	260.53±13.20
42 days	Initial W.	274.62±26.33	272.26±34.76	261.90±31.24	255.56±27.88
	Final W.	262.98±25.39	258.03±27.01	259.47±31.54	252.13±27.88
	Euthanasia W.	274.99±13.40	263.05±24.00	256.03±30.82	244.85±29.07*
60 days	Initial W.	263.81±24.36	260.05±31.85	267.15±27.82	255.05±21.07
	Final W.	255.96±25.90	256.19±32.07	261.10±21.22	249.99±18.12
	Euthanasia W.	268.87±28.71	258.13±28.27	261.45±29.20	268.73±35.54

Initial W., Final W., Euthanasia W. = Initial, Final and Euthanasia Wheigth. The results are expressed in mean standard deviation ±.
* p<0.05. Dunnett T3 test. A reduction in body weight of Group TIII rats in the day of euthanasia (42 days) was observed.
Fonte: Acervo dos autores.

Table 2: Absolute weights of liver, left and right kidney, and spleen of animals in groups treated with rutin TI (5 mg/kg/day), TII (10mg/kg/day), TIII (20mg/kg/day) and control group (1ml distilled water). Euthanasia at 10, 42 and 60 days after the beginning of treatment.

Variables		Liver Weight	R. Kidney Weight	L. Kidney Weight	Spleen Weight
10 Days	Control	8.87±0.57	1.00±0.10	1.00±0.09	0.50±0.07
	TI	9.51±0.54*	1.04±0.05	1.01±0.06	0.51±0.06
	TII	8.79±0.53	0.99±0.06	0.94±0.03	0.46±0.04
	TIII	8.89±0.58	0.96±0.06	0.95±0.06	0.49±0.08
42 days	Control	8.94±1.03	0.98±0.11	0.96±0.08	0.46±0.06
	TI	8.90±1.38	0.91±0.12	0.88±0.12	0.45±0.07
	TII	8.95±1.15	0.91±0.11	0.90±0.12	0.47±0.08
	TIII	8.32±1.47	0.91±0.09	0.89±0.10	0.45±0.05
60 days	Control	8.61±1.51	0.94±0.13	0.93±0.13	0.47±0.08
	TI	8.12±1.69	0.99±0.14	0.94±0.14	0.45±0.08
	TII	7.55±1.43	0.90±0.13	0.88±0.10	0.44±0.05
	TIII	8.90±0.96	0.94±0.10	0.92±0.11	0.43±0.05

The results are expressed in mean standard deviation. * p<0.05. Dunnett T3 test.
Note the increase in liver weight between the animals in group T1 – 10 days, compared to the control.
Fonte: Acervo dos autores.

Table 3. Absolute weights of epididymis, seminal vesicles, and prostate of animals in groups treated with rutin TI (5 mg/kg/day), TII (10mg/kg/day), TIII (20mg/kg/day) and control group (1ml distilled water). Euthanasia at 10, 42 and 60 days after the beginning of treatment.

Variables	Groups	S V Weight	Prostate Weight	Epididymis weight
10 days	Control	0.54±0.07	0.42±0.12	0.54±0.04
	TI	0.51±0.11	0.43±0.11	0.49±0.03*
	TII	0.47±0.07	0.36±0.06	0.50±0.02*
	TIII	0.43±0.03	0.35±0.07	0.48±0.03*
42 days	Control	0.41±0.05	0.45±0.09	0.50±0.03
	TI	0.37±0.08	0.38±0.12	0.47±0.05
	TII	0.39±0.05	0.44±0.09	0.51±0.04
	TIII	0.37±0.11	0.39±0.10	0.48±0.03
60 days	Control	0.41±0.07	0.45±0.11	0.52±0.04
	TI	0.39±0.08	0.45±0.11	0.53±0.05
	TII	0.40±0.11	0.42±0.14	0.51±0.05
	TIII	0.42±0.08	0.47±0.12	0.51±0.04

RT = right testicle; LT = left testicle, SV = seminal vesicle. The results are expressed in mean standard deviation. * p<0.05.
Dunnett T3 test. It is observed that the weight of the epididymis of animals euthanized at 10 days was lower than in the control group.
Fonte: Acervo dos autores.

The height of the seminiferous epithelium (μm) was 14.42 ± 1.83 (Control); 15.01 ± 1.84 (TI); 14.84 ± 2.07 (TII); and 14.43 ± 1.65 (TIII). Each average represents 50 tubules.

Regarding the number of spermatozoa observed in the tail of the epididymis, there was no significant statistical alteration among the groups compared to control.

Table 4: Biochemical and hematological parameters in animals treated with rutin TI (5 mg/kg/day), TII (10 mg/kg rutin) and TIII (20 mg/kg rutin) and control group (1ml of distilled water). Euthanasia at 10 after the beginning of treatment.

Variables	Control	TI	TII	TIII
ALT	58±8.28	66.8±8.11	66±5.57	58±8.28
Creatinine	0.57±0.04	0.51±0.04	0.60±0.04	0.57±0.04
MGV	51.9±0.7	50.92±0.8	51.5±1.13	51.9±0.7
MGH	19.38±0.34	18.5±0.46*	18.88±0.39	19.38±0.34
Hemoglobin	16±0.52	15.18±0.82	15.42±0.90	16±0.52
Hematocrit	43.3 ± 1:40	41.78 ± 2.15	42.04 ± 2.56	43.3 ± 1:40
MGHC	36.96±0.46	36.34±0.40	36.68±0.22	36.96±0.46
Leuk.counts	8120±2152.21	6040±1507.65	7640±2306.08	8120±2152.21
Hematimetry	8348000±264140.1	8208000±434073.7	8158000±327826.2	8348000±264140.1

ALT = alanine aminotransferase, MGV = mean globular volume, MGH = mean globular hemoglobin, MGHC= mean globular hemoglobin concentration, Leuk.counts = Leukocyte counts. The results are expressed in mean standard deviation ±. * p<0.05. Dunnett T3 test. The values of MGH were significantly lower among the animals in group T1, compared to control.
Fonte: Acervo dos autores.

4 DISCUSSION

In the experimental model used, the animal was exposed to rutin for seven days, covering the entire period of epididymal transit. Upon the proceeding of the euthanasia on subsequent days, it can be assessed, through spermatozoa count in the secretion of the tail of the epididymis, toxic effects on epididymal spermatozoa (euthanasia at 10 days), primary spermatocytes (euthanasia at 42 days), secondary spermatocytes and spermatogonia (euthanasia at 60 days) – considering a cycle of 12.9 days and the total duration of 58 days of spermatogenesis in mice (FRANÇA et al., 1998). The same model was used by D'Souza (2003). Furthermore, it is possible to observe the possible reversal of acute toxic effects.

Systemic toxicity can be diagnosed by clinical signs of toxicity such as piloerection, ambulation changes, diarrhea or increased diuresis; data that indicate the general health condition of animals. Another indication of toxicity is obtained by the increase or decrease of body mass of the organs of the animals as well as the emergence of behavioral alterations (MELLO, 2001). The changes observed for body weight and feed intake were specific and not accompanied by other indicative signs of toxicity, which suggest that treatment with rutin may not have changed the general state of the animal's body, data that concur with the findings of Hasumura et al. (2004).

Although it is known that rutin metabolizes into quercetin and that the latter causes prostate changes, increase of testosterone and decrease of dihydrotestosterone levels in rats (MA et al., 2004), in this study no changes in prostate weight were observed and the morphological data do not suggest hormonal changes.

Hepatomegaly was noted in the group of animals treated with the lowest dose of rutin, but this was a finding that appears to lack biological significance since there were no changes in concentrations of ALT, besides it was not manifested in higher doses, as well as it did not remain at 42 and 60 days.

Assessment of reproductive organs showed that there was a reduction in the weight of the epididymis of animals in all groups treated with rutin and euthanized at 10 days (three days after the last day of treatment). Epididymis is an androgen-dependent organ essential for spermatozoa maturation thus, the reduction of its weight could be attributed to the decrease of testosterone concentration (CORNIWALL, 2009). However, the seminal vesicles and prostate, which are also androgen-dependent organs, were not affected. The height of the epithelium of the epididymis did not change with treatment, which also suggests the absence of reduced levels of testosterone and, furthermore, spermatozoa concentration did not differ among the groups. The disruption of spermatogenesis due to the blocking the exit of the testicle or to a decrease in its production would also be a cause of weight reduction of the epididymis (LANNING et al., 2002); however, as aforementioned, the concentration of spermatozoa in the tail of the epididymis showed no significant difference between the experimental groups together with the fact that testicle weight was not changed.

In conclusion, except by the reduction of the weight of the epididymis, which is reversible at 42 days of completion of treatment, no suggestive data of the toxicity of rutin on the reproductive system of adult rats were found. The change in epididymis weight does not seem to be caused by lack of androgens or changes in spermatozoa production; therefore, more studies need to be conducted to explain this change.

5 ACKNOWLEDGEMENTS

We would like to thank Redes Mineira de Bioterismo (Rede 172/08) and Mineira de Toxicologia and Farmacologia de Produtos Terapêuticos (Rede 173/08) for the financial support.

6 REFERENCES

ARAVINDAKSHAN, M.; CHAUHAN, P.S.; SUNDARAM, K. Studies on germinal effects of quercetin, a naturally occurring flavonoid. **Mutation Research**, v. 144, p. 99-106, 1985.

BOKKENHEUSER, V. D.; SHACKLETON, C. H.; WINTER, J. Hydrolisis of dietary flavonoid glycosides by strais of intestinal bacteroides from humans. **Biochemical Journal**, v. 348, p. 953-956, 1987.

CORNIWALL, G.A. New insights into epididymal biology and function. **Human Reproduction Update**, v. 15, p. 213-227, 2009.

D'SOUZA, U. J. A. Toxic effects of 5-Fluorouracil on sperm count in Wistar rats. **Malaysian Journal of Medical Sciences**, v. 10, p. 43-45, 2003.

DOSTAL, L. A.; FABER, C. K.; ZANDEE, J. Sperm motion parameters in vas deferens and cauda epididymal rat sperm. **Reproductive Toxicology**, v. 10, p. 231-235, 1996.

FRANÇA, L.R.; OGAWA, T.; AVARBOCK, M. R.; BRISNTER, R. L.; RUSSEL, L. D. Germ genotype controls cell cycle during spermatogenesis in the rat. **Biology of Reproduction**, v. 59, p. 1371-1377, 1998.

GUARDIA, T., ROTELLI, A. E.; JUAREZ, A. Q.; PELZER, L. E. Anti-inflamatory properties os plant flavonoids. Effect of rutin, quercetin and hiperidin on adjuvant arthritis in rat. **II Farmaco**, v. 56, p. 683-687, 2001.

HAN, Y. Rutin has therapeutic effect on septic arthritis caused by *Candida albicans*. **International Immunopharmacology**, v. 9, p. 207-221, 2009.

HASSAMURA, M.; YASUHARA, H.; TAMURA, T.; IMAI, T.; MITSUMORI, K.; HIROSE, M. Evaluation of the toxicity of enzymatically decomposed rutin with 13-weeks dietary administration to Wistar rats. **Food and Chemical Toxicology**, v. 42, p. 439-444, 2004.

HOLLMAN, P. C.; VAN TRIPP, J.; BUYSMAN, M. N.; VAN DER GAAG, M. S.; MENGELERS, M. B. Relative bioavailability of the flavonóide quercetin from various foods in man. **Federation of European Biochemical Societies Leters**, v. 418, p. 152-156, 1995.

HOLLMAN, P. C. H.; HERTOG, M. G. L.; KATAK, M. B. Analysis and health effects of flavonoids. **Food Chemistry**, v. 57, p. 43-46, 1996.

IZAWA, H.; KOHARA, M.; AIZAWA, K.; SUGANUMA, H.; INAKUMA, T.; WATANABE, G. et al. Alleviative effects of quercetin and onion on male reproductive toxicity induced by diesel exhaust particles. **Bioscience, Biotechnology, Biochemistry,** v. 72, p. 1.235-1.241, 2008.

KHANDUJA, K. L.; VERMA, A.; BHARDWAJ, A. Impairment of human sperm motility and viability by quercetin is independent of lipid peroxidation. **Andrologia**, v. 33, p. 277-281, 2001.

LANNING, L. L.; CREASY, D. M.; CHAPIN, R. E.; MANN, P. C.; BARLOW, N. J.; REGAN, K. S. et al. Recommended approaches for de evaluation of testicular and epididymal toxicity. **Toxicologic Pathology**, v. 30, p. 507-520, 2002.

MA, Z.; NGUYEN, T. H.; HUYNH, T. H.; DO, P. T.; HUYNH, H. Reduction of rat prostate weight by combined quercetin-finasteride treatment is associated with cell cycle deregulation. **Journal of Endocrinology**, v. 181, p. 493-507, 2004.

MACHADO, H. **Atividade dos flavonoides rutina e naringina sobre o tumor ascítico de Erlich "in vivo"**. 125 f. 2006. Dissertacão (Mestrado)–Programa de Pós-Graduação em Bioquímica Agrícola, Universidade Federal de Viçosa, Viçosa, MG, 2006.

MANACH, C.; MORAND, C.; DEMIGNE, C.; TESIER, O.; REGERAT, F.; REMESY, C. Bioavailability of rutin and quercetin in rats. **Federation of European Biochemical Societies**, v. 409, p.12-16, 1997.

MELLO, F. B. **Estudo dos efeitos de Lantana camara (Verbenaceae) sobre a fertilidade e reprodução de ratos**. 120 f. 2001. Dissertação (Mestrado em Ciências Veterinárias)– Programa de Pós-Graduação em Ciências Veterinárias, Universidade Federal do Rio Grande do Sul, Porto Alegre, 2001.

MIDDLETON, E. J. R.; KANDASWAM, C.; THEOHARIDES, T. C. The effects of plant flavonoids on mammalian cells: implications for inflammation, heart disease and cancer. **Pharmacological Reviews**, v. 53, p. 673-751, 2000.

MORAES, G. E. S. **Espermocitograma**. Porto Alegre: Médica Missau, 1994. p. 61-93.

MUROTA, K.; TERAO, J. Antioxidative flavonoid quercetin: implication of it's intestinal absortion and metabolism. **Archives of Biochemistry and Biophysics**, v. 417, p. 12-17, 2003.

NASS-ARDEN, L.; BREITBART, H. Modulation of mammalian sperm motility by quercetin. **Molecular Reproduction and Development**, v. 25, p. 3.369-3.373, 1990.

NASSIRI-ALS, M.; SHARIATI-RAD, S.; ZAMANSOLTAN, F. Anticonvulsive effects of intracerebroventicular administration of rutin in rats. **Progress in Neuro-Psychopharmacology & Biological Psychiatry**, v. 32, p. 989-993, 2008.

PARK, S. Y.; BOK, S. H.; JEON, S. M.; PARK, Y. B.; LEE, S. J.; JEONG, T. S. et al. Effect of rutin and tannic acid supplements on cholesterol metabolism in rats. **Nutrition Research**, v. 22, p. 283-295, 2002.

PATHAK, D.; PATHAK, K.; SINGLA, A. K. Flavonoids as medicinal agents: recent advances. **Fitoterapia**, v. 57, p. 371-389, 1991.

SANTOS, K. F. R.; OLIVEIRA, T. T.; NAGEM, T. J.; PINTO, A. S.; OLIVEIRA, M. G. A. Hypolipidaemic effects of neringenin, rutin, nicotinic acid and their associations. **Pharmacology Research**, v. 40, p. 493-496, 1999.

SEED, J.; CHAPIN, R. E.; CLEGG, E. D.; DOSTAL, L. A.; FOOTE, R. H.; HURTT, M. E. et al. Methods for assessing sperm motility, morphology, and counts in the rat, rabbit, and dog: a consensus report. **Reproductive Toxicology**, v. 10, p. 237-244, 1996.

SULLIVAN, M.; FOLLIS, R. H.; HILGARTNER JUNIOR, M. Toxicology of podophyllin. **Proceedings of the Society for Experimental Biology and Medicine**, v. 77, p. 269-272, 1951.

TAEPONGSORAT, L.; TANGPRAPRUTGUL, P.; KITANA, N.; MALAIVIJITNOND, S. Stimulating effects of quercetin on sperm quality and reproductive organs in adult male rats. **Asian Journal of Andrology**, v. 10, p. 249-258, 2008.

THOMSON, C,; BLOCH, A.; HASLER, C. M. Position of the American Dietetic Association: functional foods. **Journal of American Dietetic Association**, v. 99, p. 1.280-1.281, 1999.

WALLE, T. Absortion and metabolism of flavonoids. **Free Radical Biology & Medicine,** v. 36, p. 829-837, 2004.

YILDZOGKE-AR, N.; ALTAN, V. M.; ALTINKUT, O.; OZTURK, Y. Pharmacological effects of rutin. **Phytotherapy Research**, v. 5, p. 19-23, 1991.

ZENIK, H.; CLEGG, E. D.; PERREAULT, S. D.; KLINEFELTER, G. R.; GRAY, L. E. Assessment of male reproductive toxicity. In: HAYES, A. W. **Principle and methods of toxicology.** New York: Raven Press, p. 937-980, 1994.