Original Article

Effects of Rutin on Testicular Antioxidant Enzymes and Lipid Peroxidation in Rats

Rahmat Ali Khan[1*], Muhammad Rashid Khan[2], Mushtaq Ahmed[1], Mir Sadiq Shah[1], Shoiab Ur Rehman[1], Jahangir Khan[3], Masroor Husain[1], Farman Ali Khan[4], Muhammad Mohibullah Khan[5], Muhammad Shahzad Shifa[6]

[1]Department of Biotechnology, Faculty of Biological Sciences, University of Science and Technology Bannu, Khyber Pakutunkhwa, PAKISTAN.
[2]Department of Biochemistry, Faculty of Biological Sciences, Quaid-i-Azam University Islamabad, PAKISTAN.
[3]Department of Pharmacy, University of Malakand, Chakdara Dir, Khyber Pakutunkhwa, PAKISTAN.
[4]Department of Biochemistery, Abdul Wali Khan University Mardan, 23200, Khyber Pakutunkhwa, PAKISTAN.
[5]Department of Plant Breeding and Genetics
[6]Department of Physics Government College University Faisalabad

ABSTRACT

Background: Rutin, a polyphenolic flavonoid, possesses antioxidant and hypoglycemic effects. **Objective:** The present study was conducted to check the effect of rutin on serum reproductive hormonal level and sperm concentration in male rat. **Methods:** 30 rats were equally divided into 3 groups (10 rats per group). Group I was control (untreated), group II was given rutin 50 mg/kg bw while group III was given 100 mg/kg bw intragastric twice a week for four weeks respectively. The effect of rutin on antioxidant enzymes (POD, SOD, GSHpx, GST, GSR), glutathione contents and TBARS contents were checked. **Results:** The results revealed that rutin caused significant gain in body weight, antioxidant enzymes (POD, SOD, GSHpx, GST, GSR), glutathione contents, with significant ($P < 0.01$) decrease in thiobarbituric acid reactive substance (TBARS) concentrations. **Conclusion:** It may be concluded that rutin may be promising as a natural therapeutic agent in male reproductive dysfunction.

Key words: Rutin, TBARS contents, Antioxidant enzymes, Flavonoids, Catalase.

INTRODUCTION

Traditional medicine is the sum of knowledge, skills and practices based on theories, beliefs and experiences, indigenous to different cultures that are used to maintain health, as well as to prevent, diagnose, improve or treat physical and mental illness.[1] Virtually every illness has a remedy made from concoctions of various parts of the useful flora used for gastro- intestine disorders, cardiovascular, respiratory or nervous diseases as well as fertility problems such as sexual dysfunctions, which is common in 31% of men.[2] Traditional medicine or folk medicine practice is based on the use of medicinal plants and their extracts. Flavonoids are main constituents of plants and more than 4000 polyphenilc flavonoids are used as a component of human diet.[3] Flavonoids acquire an astonishing range of activities accredited, at slightest moderately, to their scavenging behavior of reactive oxygen species.[4,5] Flavonoids are implicated to exercise valuable effect in a range of disorders including infertility and oxidative stress.[6] The most commonly occurring dietary flavonoids such as rutin, hypersoid, quercetin, catchine and myricetin exhibit an extensive range of activities, including antimicrobial, anti-inflammatory and anticancer.[7,8] Rutin is an important source of dietary flavonoid.[4] It has been distributed in many foods such as onion, apples and in buck wheat, many vegetables, fruits and plant-derived beverages such as tea and wine. Rutin works as scav-

Submission Date: 13-12-2016;
Revision Date: 24-01-2017;
Accepted Date: 15-02-2017

DOI: 10.5530/ijper.51.3.69

Correspondence:
Dr. Rahmat Ali Khan,
Department of Biotechnology, Biotech, UST Bannu, KP,PAKISTAN.
Phone: 92 928633425
Fax: 92 51 9205753
E-mail: rahmatgenetics@gmail.com

www.ijper.org

enger of ROS by donating hydrogen atoms to peroxy radicals, superoxide anions, singlet oxygen and hydroxyl radicals and as terminator and chelators of metal ions that are capable of oxidizing lipid peroxidation.[7] Rutin has been shown to function as anticancer, antiviral, antibacterial, anti-inflammatory and used as a therapeutic in cardiovascular and neurodegenerative disorders which is the result of its high radical scavenging activity and antioxidant capacity.[6] Additionally results presented in different studies suggested that rutin alters signal transduction, causes activation of transcription factors, gene expression and may also protect DNA by interacting with carcinogens that have escaped detoxification processes.[8] These studies suggest that potential role for dietary intake of rutin containing foods in lowering the risk of certain pathophysiologies that have been associated with free radical-mediated diseases. The present study is therefore aimed at investigating the effect of rutin on hormonal secretion in Sprague-Dawley male rat.

EXPERIMENTAL

Chemicals

Rutin, Reduced glutathione (GSH), oxidized glutathione (GSSG), glutathione reductase, gamma-glutamyl p-nitroanilide, glycylglycine, bovine serum albumin (BSA), 1,2-dithio-bis nitro benzoic acid (DTNB), 1-chloro-2,4-dinitrobenzene (CDNB), reduced nicotinamide adenine dinucleotide phosphate (NADPH), Flavine adenine dinucleotide (FAD), glucose-6-phosphate, Tween-20, 2,6-dichlorophenolindophenol, thiobarbituric acid (TBA), picric acid, sodium tungstate, sodium hydroxide, trichloroacetic acid (TCA) and perchloric acid (PCA) were purchased from Sigma Chemicals Co. St. Louis, USA.

Animals and treatment

Six-week-old male Sprague-Dawley rats weighing 180±5 g were provided with food and water ad libitum and kept at 20-22°C on a 12-h light-dark cycle. All experimental procedures involving animals were conducted in accordance with the guidelines of National Institutes of Health (NIH guidelines Islamabad, Pakistan). The study protocol was approved by Ethical Committee of Quaid-i-Azam University, Islamabad. The rats were acclimatized to laboratory condition for 7 days before commencement of experiment.

The following experimental groups (n = 10 rats per group) were studied.

Group I: (control); the animals were remained untreated

Group II: animals were treated with 50 mg/kg bw rutin, intragastric twice a week for four weeks

Group III: was treated with 100 mg/kg bw rutin, intragastric twice a week for four weeks

After the completion of dosages rats were dissected. Half of tissues were treated with liquid nitrogen and stored at -80°C for further analysis.

Assessment of antioxidant profile

Testicular tissue was homogenized in 10 volume of 100 mmol KH_2PO_4 buffer containing 1 mmol EDTA (pH 7.4) and centrifuged at 12,000 × g for 30 min at 4°C. The supernatant was collected and used for enzymatic studies. Protein concentration of testis supernatant was determined by the method of Lowry *et al*.[9] using crystalline BSA as standard.

Catalase assay (CAT)

CAT activities were determined by the method of Chance and Maehly[10] with some modifications. The reaction solution of CAT activities contained: 2.5 ml of 50 mmol phosphate buffer (pH 5.0), 0.4 ml of 5.9 mmol H_2O_2 and 0.1 ml enzyme extract. Changes in absorbance of the reaction solution at 240 nm were determined after one min. One unit of CAT activity was defined as an absorbance change of 0.01 as units/min.

Superoxide dismutase assay (SOD)

SOD activity was estimated by the method of Kakkar *et al*.[11] Reaction mixture of this method contained: 0.1 ml of phenazine methosulphate (186 µmol), 1.2 ml of sodium pyrophosphate buffer (0.052 mmol; pH 7.0), 0.3 ml of supernatant after centrifugation (1500 × g for 10 min followed by 10000 × g for 15 min) of testis homogenate was added to the reaction mixture. Enzyme reaction was initiated by adding 0.2 ml of NADH (780 µmol) and stopped after 1 min by adding 1 ml of glacial acetic acid. Amount of chromogen formed was measured by recording color intensity at 560 nm. Results are expressed in units/mg protein.

Estimation of lipid peroxidation assay (TBARS)

The assay for lipid peroxidation was carried out with modified method of Iqbal *et al*.[12] The reaction mixture in a total volume of 1.0 ml contained: 0.58 ml phosphate buffer (0.1 mol; pH 7.4), 0.2 ml homogenate sample, 0.2 ml ascorbic acid (100 mmol), and 0.02 ml ferric chloride (100 mmol). The reaction mixture was incubated at 37°C in a shaking water bath for 1 h. The reaction was stopped by addition of 1.0 ml 10% trichloroacetic acid. Following addition of 1.0 ml 0.67% thiobarbituric acid, all the tubes were placed in boiling water bath for 20 min and then shifted to crushed ice-bath before cen-

trifuging at 2500 × g for 10 min. The amount of malonaldehyde formed in each of the samples was assessed by measuring optical density of the supernatant at 535 nm using spectrophotometer against a reagent blank. Tetramethoxypropane was used as an external standard. The results were expressed as nmol of TBARS/min/mg tissue protein.

Glutathione-S-transferase assay (GST)

The reaction mixture of glutathione-S-transferase activity consisted of 1.475 ml phosphate buffer (0.1 mol, pH 6.5), 0.2 ml reduced glutathione (1 mmol), 0.025 ml (CDNB; 1 mmol) and 0.3 ml of tissue homogenate in a total volume of 2.0 ml. The changes in the absorbance were recorded at 340 nm and enzymes activity was calculated as nmol CDNB conjugate formed/min/mg protein using a molar extinction coefficient of 9.6 × $10^3 M^{-1}cm^{-1}$.[13]

Glutathione reductase assay (GSR)

Glutathione reductase activity was determined with the protocol of Carlberg and Mannervik.[14] The reaction mixture consisted of 1.65 ml phosphate buffer: (0.1 mol; pH 7.6), 0.1 ml EDTA (0.5 mmol), 0.05 ml oxidized glutathione (1 mmol), 0.1 ml NADPH (0.1 mmol) and 0.1 ml of homogenate in a total volume of 2 ml. Enzyme activity was quantitated at 25ºC by measuring disappearance of NADPH at 340 nm and was calculated as nmol NADPH oxidized/min/mg protein using molar extinction coefficient of 6.22 × $10^3 M^{-1}cm^{-1}$.

Glutathione peroxidase assay (GSH-px)

Glutathione peroxidase activity was assayed by the method of Mohandas *et al.*[15] The reaction mixture consisted of 1.49 ml phosphate buffer (0.1 mol; pH 7.4), 0.1 ml EDTA (1 mmol), 0.1 ml sodium azide (1 mmol), 0.05 ml glutathione reductase (1 IU/ml), 0.05 ml GSH (1 mmol), 0.1 ml NADPH (0.2 mmol), 0.01 ml H_2O_2 (0.25 mmol) and 0.1 ml of homogenate in a total volume of 2 ml. The disappearance of NADPH at 340 nm was recorded at 25ºC. Enzyme activity was calculated as nmol NADPH oxidized/min/mg protein using molar extinction coefficient of 6.22 × $10^3 M^{-1}cm^{-1}$.

Reduced glutathione assay (GSH)

1.0 ml sample of homogenate was precipitated with 1.0 ml of (4%) sulfosalicylic acid. The samples were kept at 4ºC for 1 h and then centrifuged at 1200 × g for 20 min at 4ºC. The total volume of 3.0 ml assay mixture contained: 0.1 ml filtered aliquot, 2.7 ml phosphate buffer (0.1 mol; pH 7.4) and 0.2 ml DTNB (100 mmol). The yellow color developed was read immediately at 412 nm on a SmartSpecTM plus Spectrophotometer. It was expressed as µmol GSH/g tissue.[16]

Statistical analysis

The values were expressed as the mean ± SEM for the 10 rats in each group. Differences between groups were assessed by one-way analysis of variance (ANOVA) using the Statistical Package for Social Sciences (SPSS) software package for Windows (version 13.0). Post hoc testing was performed for intergroup comparisons using the least significant difference (LSD) test. A value corresponding to $P<0.05$ was deemed to be statistically significant.

RESULTS

Effect of rutin on body weight, testis weight, relative testis weight in rat

Body weight play important role in assessment of oxidative dysfunction. The effects of rutin on body weight, testis weight and relative testis weight are shown in Table 1. Administration of rutin non-significantly ($p<0.05$) improved body weight, testis weight and relative testis weight compare to control group.

Table 1: Effect of rutin on body weight, testis weight, relative testis weight in rat

Treatment	Tissue weight (g)	Relative testis weight (g)	% Body weight (g)
Control	7.1±0.16 a	0.034±0.016 a	6.8±0.9 a
50 mg/kg bw rutin	7.5±0.16 a	0.035±0.001 a	7.0±1.6 a
100 mg/kg bw rutin	7.8±0.08 a	0.038±0.004 a	7.1±1.0 a

Each value in the table is represented as mean ± SD (n = 10)
Means not sharing the same letter are significantly different (LSD) at P < 0.01 probability level in each column

Table 2: Effect of rutin on tissue protein, activities of CAT and SOD in male rat

Treatment	Protein (µg/mg tissue)	CAT (U/min)	SOD (U/min)
Control	82.5 ±2.0 a	60.8 ±1. 0 a	50.6 ±1.6 a
50 mg/kg bw rutin	96.0 ±1.9 b	83.1 ±2.1 b	71.6 ±3.09 a
100 mg/kg bw rutin	102.5±2.3 b	100.4±2.0 b	85.2±3.1 b

Each value in the table is represented as mean ± SD (n = 10)
Means not sharing the same letter are significantly different (LSD) at P < 0.01 probability level in each column

Table 3: Effect rutin on testis GST, GSH-Px, GSR, GSH and TBARS in male rat

Treatment	GSH-Px (nM/mg protein)	GSR (nM/min/mg protein)	TBARS (nM/ min/ mgprotein)	GSH (µM/g tissue)	GST(nM/min/mg protein)
Control	120 ± 2.3 [a]	702.3 ± 1.6 [a]	8.3 ± 0.3 [a]	3.7 ± 0.010 [a]	160 ± 2.3 [a]
50 mg/kg bw rutin	131 ± 1.8 [a]	98.2 ± 1.4 [b]	5.0 ± 0.03 [b]	5.8 ± 0.014 [b]	176 ± 3.8 [b]
100 mg/kg bw rutin	140 ± 2.9 [b]	99.8 ± 1.5 [b]	4.3 ± 0.01 [b]	5.9 ± 0.075 [b]	179 ± 2.5 [b]

Each value in the table is represented as mean ± SD (n = 10)
Means not sharing the same letter are significantly different (LSD) at P < 0.01 probability level in each column

Effect of rutin on tissue protein, activities of CAT and SOD in male rat

Antioxidant enzymes are very important for detoxification of reactive oxygen species (ROS) and maintaining cellular balance. The effects of various concentrations of rutin on tissue soluble protein, CAT and SOD are shown in Table 2. Oral administration of 50 and 100 mg/kg body weight rutin showed marked improvement on the antioxidant enzyme level as compare to control group behave as an excellent antioxidant.

Effect rutin on testis GST, GSH-Px, GSR, GSH and TBARS in male rat

Rutin play a crucial role on the activity of secondary defense level of antioxidant enzymes for improvement of sound health. The effects of various concentrations of rutin on GSH-Px, GSR, GST, GSH and TBARS are shown in Table 3. Oral administration of 50 and 100 mg/kg body weight showed marked increment on the activity of enzyme and decreased testicular tissue lipid peroxidation i.e. TBARS comparatively to control group.

DISCUSSION

In this investigation, results revealed that rutin a polyphenolic compound is caused changes in body weight, testis weight and relative testis weight (Table 1), in agreement with previous studies.[17] It has been shown that increased testis weight reflects the increment of lipid peroxidation that in turn related to fatty accumulation and alteration of weight.[18] Antioxidant enzymes play important role in detoxification of oxidative damages constitute a mutually supportive team of defense against reactive oxygen species (ROS). Activity of antioxidant and phase II metabolizing enzymes were reduced due to decreased content of GSH. Depletion of antioxidant responses has been implicated with fertility. Present study revealed that the activities of antioxidant enzymes including glutathione-S-transferase, glutathione reductase, superoxide dismutase, catalase, glutathione peroxidase and glutathione contents was significantly reduced in rats (Table 2 & 3) which possesses infertility and reproductive dysfunction.[19] Similar effects were observed in reports of Yousef *et al*.[20] and Khattab *et al*.[21]

CONCLUSION

This study provided the scientific proof that diet containing rutin is useful for reproductive dysfunction in male via increasing their antioxidant defense level and decreasing lipid peroxidation.

CONFLICT OF INTEREST

We are thankful for Vice Chancellor for provision of Research facilities.

ABBREVIATION USED

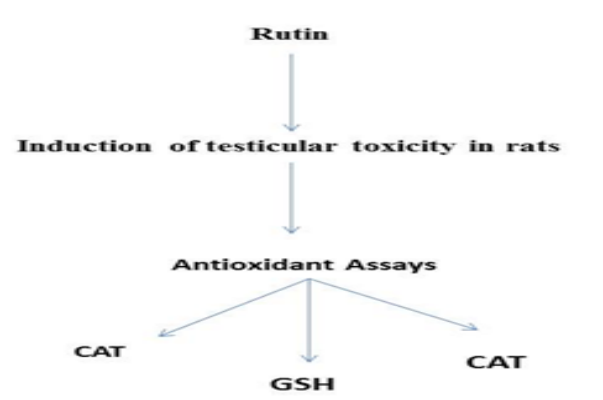
None

REFERENCES

1. Acarya D, Shrivastava A. Indigeneous Herbal Medicine. Tribal Formulations and Traditional Herbal Practices, Aavishka Publisher Distributor, Jaipur, India. ISBN: 9788179 102527. 2008;40
2. Brock GB, McMahon CG, Chen KK. Efficacy and safety of tadalafil for the treatment of erectile dysfunction: Results of integrated analyses. J Urol. 2002;168(4):1332-6.
3. Noroozi M, Angerson WJ, Lean ME. Effects of flavonoids and vitamin C on oxidative DNA damage to human lymphocytes. American J Clin Nutr. 1998;67(6):1210-8.
4. Middleton EJ, Kandaswami C, Theoharides TC. The effects of plant flavonoids on mammalian cells: implications for inflammation, heart disease, and cancer. Pharmacol Rev. 2000;52(4):673-751.
5. Williams RJ, Spencer JP, Rice-Evans C. Flavonoids: Antioxidants or signaling molecules?. Free Rad Biol Med. 2004;36:838-49. doi: 10.1016/j.freeradbiomed.2004.01.001.
6. Kampkötter A, Nkwonkam CG, Zurawski RF, Timpel C, Chovolou Y, Wätjen W, *et al*. Investigations of protective effects of the flavonoids quercetin and rutin on stress resistance in the model organism Caenorhabditis elegans. Toxicology. 2007;234(1):113-23.
7. Hanasaki Y, Ogawa S, Fukui S. The correlation between active oxygen scavenging. Free Radic Biol Med. 1994;16:845-50.
8. Srinivasan M, Rukkumani R, Ram SA, Menon VP. Ferulic acid, a natural protector against carbon tetrachloride induced toxicity. Fundam Clin Pharmacol. 2005;19(4):491-6.
9. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem. 1951;193(1):265-75
10. Chance B, Maehly A. Assay of catalases and peroxidases. Methods in Enzymol. 1955;2:764-75.

11. Kakkar P, Das B, Viswanathan P. A modified spectrophotometric assay of superoxide dismutase. Indian J Biochem Biophys. 1984;21:130-2.
12. Iqbal M, Sharma SD, Zadeh HR, Hasan N, Abdulla M, Athar M. Glutathione metabolizing enzymes and oxidative stress in ferric nitrilotriacetate (Fe-NTA) mediated hepatic injury. Redox Report. 1996;2(6):385-91.
13. Habig WH, Pabst MJ, Jakoby WB. Glutathione-S-transferases: The first enzymatic step in mercapturic acid formation. J Biol Chem. 1974;249:7130-9.
14. Carlberg I, Mannervik EB. Glutathione level in rat brain. J Biol Chem. 1975;250:4475-80.
15. Mohandas J, Marshal JJ, Duggin GG, Horvath JS, Tiller DJ. Differential distribution of glutathione and glutathione-related enzymes in rabbit kidney. Possible implications in analgesic nephropathy. Biochem Pharmacol Biochem Pharmacol. 1984;33(11):1801-7.
16. Jollow DJ, Mitchell JR, Zampaglione N, Gillete JR. Bromobenzene induced liver necrosis. Protective role of glutathione and evidence for 3,4-bromobenzene oxide as a hepatotoxic metabolite. Pharmacol. 1974;11(3):151-69.
17. Cadenas S, Barja G. Resveratrol, melatonin, vitamin E and PBN protect against renal oxidative DNA damage induced by kidney carcinogen KBrO3. Free Rad Biol Med. 1999;26(11):1531-7.
18. Kurokawa Y, Maekawa A, Takahashi M, Hayashi Y. Toxicity and carcinogenicity of potassium bromate–a new renal carcinogen. Envirn Health Persp. 1990;87:309-35.
19. Al-Saaidi JAA, Al-Khuzai ALD, Al-Zobaydi NFH. Effect of alcoholic extract ofNigella sativaon fertility in male rats. Iraqi J Vet Sci. 2009;23(Suppl II):123-1220.
20. Yousef MI, Salama AF. Propolis protection fromreproductive toxicitycaused by aluminium chloride inmalerats. Food and Chem Toxicol. 2009;47(6):1168-75
21. Khattab IKF. Histological and ultrastructural studies on the testis ofrat after treatment with aluminium chloride. Austr J Basic Appl Sci. 2007;1(1):63-72.

PICTORIAL ABSTRACT



SUMMARY

- Rutin, a polyphenolic flavonoid, possesses antioxidant and hypoglycemic effects.
- In the present study was conducted to check the effect of rutin on serum reproductive hormonal level and sperm concentration in male rat. Rats were equally divided into 3 groups. The effect of rutin on antioxidant enzymes (POD, SOD, GSHpx, GST, GSR), glutathione contents and TBARS contents were checked which showed potent results due to the presence of bioactive constituents.

ABOUT AUTHORS

Dr. Rahmat Ali Khan (Biochemist): Main area of research Pharmacology and Toxicology. Dr. Khan is also focusing on the Pharmacological potentials of medicinal plants.

Dr. Muhammad Rashid Khan: Working on the Pharmacological potentials of medicinal plants

Dr. Mushtaq Ahmed: Working on the Pharmacological potentials of medicinal plants

Dr. Shoaib ur Rehman: Working as an assistant professor (Molecular Biology) in the Department of biotechnology university of Science and Technology Bannu, KPP, Pakistan.

Dr. Mir Sadiq Shah (Microbiologist): Main area of research is Clinical & Food Microbiology. Dr. Shah is also focusing on the antimicrobial potentials of medicinal plants.

Dr. Masroor Hussain: Assistant Professor (Microbiology) at Department of Biotechnology, University of Science and Technology, Bannu.

Dr. Farman Ali Khan: He is biochemist working on biochemical properties of plants.

Dr. Jahangir Khan (Pharmacy): Main area of research Pharmacology and Toxicology.

Dr. Muhammad Muhib Ullah Khan: He is working on plant breeding and genetics.

Dr. Muhammad Shazad Shifa: He is working on biophysics.

Cite this article: Khan RA, Khan MR, Ahmed M, Shah MS, Rehman SU, Khan J, Husain M, Khan FA, Khan MM, Shifa MS. Effects of rutin on testicular antioxidant enzymes and lipid peroxidation in rats. Indian J of Pharmaceutical Education and Research. 2017;51(3):412-7.