ORIGINAL COMMUNICATION

Anatomy Journal of Africa. 2022. Vol 11 (2): 2218-2223

AMELIORATIVE POTENTIAL OF QUERCETIN AND RUTIN ON DEXTROMETHORPHAN-INDUCED TOXICITY IN SPRAGUE-DAWLEY RATS

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ABSTRACT

Dextromethorphan as an antitussive has been reported to have deleterious effect on the testicular function. Quercetin is an extensive class of polyphenolic flavonoid compounds found in plant sources like green vegetables and tea. It is considered to be a strong antioxidant due to its ability scavenge free radicals and bind transition metal ions. Rutin is a flavonoid of the flavonol-type that is found in plant kingdom and a nutritional component of foodstuffs in apples, onions and black tea. In this study, we determined the effect of Quercetin and Rutin on Dextromethorphan-induced toxicity in males using Sprague-Dawley rats as models. Eighty male rats (150 ± 30 g) divided into four (N=20; A-D) were used for a duration of 16 weeks. Group A, control received distilled water (DW); group B-C received 20, 40 and 80 mg/kg of DM respectively. At the end of treatment period, 5 animals were selected and euthanized from each group. Seminal parameters and Hormonal milieu were analysed. The remaining 15 rats were divided into 3 groups (N=5; E-G). They received Quercetin (50 mg/kg) Rutin (25 mg/kg) and DW respectively for 16 weeks to ascertain recovery rate. The rats were sacrificed and the above parameters were analysed. Significant dose-dependent reduction in seminal parameters and hormones was observed in DM-treated groups. An increase in seminal parameters and hormonal milieu was observed when DM-treated and recovery-alone groups were compared to Rutin and Quercetin groups. The supplementation of Rutin and Quercetin showed significant increases in the parameters which could mitigate the toxic effect of Dextromethorphan and in turn translates into improved fertility in males.

Key words: Dextromethorphan, Quercetin, Rutin Semen parameters

DOI: https://dx.doi.org/10.4314/aja.v11i2.10

INTRODUCTION

Dextromethorphan as an antitussive has been reported to a major constituent of about 125 over-the-counter-medications (1). Also, it has been used to relief pain as well as other psychological applications. According to the International Union of Pure and Applied Chemistry (IUPAC), Dextromethorphan is known as (+)-3-methoxy-17-methyl-9α, 13α, 14α-morphinan. It is a dextrorotatory isomer of Levomethorphan; the codeine analogue of Levophanol. When abused, it has been reported that individuals and hallucinate and have dissociative tendencies (2). The mechanism for its action is as an N-methylD-aspartate (NMDA) receptor antagonist, having similar effects to those of ketamine and phencyclidine. It produces a range of toxicities depending upon the dose or components of the specific formulation that was ingested. At recommended doses, some of the reported adverse effects include drowsiness, dizziness, coma, respiratory depression nausea, gastrointestinal upset,

constipation, abdominal discomfort tachycardia, warm sensations, inability to concentrate, dry mouth and throat. Our previous study showed that it has deleterious effect on the testicular function by affecting the cyto-architecture of the seminiferous tubules, significantly reduced hormonal milieu (follicle stimulating hormone, luteinizing hormone, testosterone), testicular and epididymal micronutrients (Zinc, Calcium, Selenium, Ascorbic acid and Vitamin E) that participates vital roles during spermatogenesis and seminal parameters (sperm count, sperm motility and morphology) when used at therapeutic doses by suppressing the secretion of gonadotropin releasing hormone (GnRH) from the hypothalamus with a consequence of a decrease or inhibition of spermatogenesis, dysfunction of secondary sex organs and accessory sex glands and impairment in sexual behaviour (2). The aim of this study is to investigate the possible ameliorative potentials of Quercetin and Rutin on Dextromethorphan-induced toxicity in testes using Sprague-Dawley rats as experimental models.

Quercetin is a uniquely bioflavonoid that is commonly found apples, berries, Brassica vegetables, capers, grapes, onions, shallots, tea, and tomatoes, as well as many seeds, nuts, flowers, barks, and leaves, in the form of a glycoside, are widely distributed in the plant kingdom (3). The best described property of Quercetin is its ability to act as antioxidant. Quercetin seems to be the most powerful flavonoids for protecting the body against reactive oxygen species, produced during the normal oxygen metabolism or are induced by exogenous damage (4).

Rutin is a flavonol found in a variety of food commonly consumed. It is found in teas, fruits, buckwheat seeds leaves and petioles of Rheum species and Aspargus in the fruits and flowers and fruits of Pagoda tree. Also abundant in citrus, berries like cranberries and nulberry (5). It has been reported to be safe (6). Studies have shown that it can prevent neuroinflammation and promote neural crest survival (7). It has been reported to afford protective effect on damage to human sperm induced by lipid peroxidation (8), hence the choice as one of the antioxidants chosen for this study.

MATERIALS AND METHODS

During Animals

A total of eighty male rats weighing between 150 ± 30 g were used and divided into four groups as A-D of 20 animals were used for duration of 16 weeks. Group A served as control and received 1 ml of distilled water (DW); treatment groups; B received 20 mg/kg, group, C received 40 mg/kg and group D received 80 mg/kg of DM for duration of 16 weeks. At the end of the treatment period with Dextromethorphan, 5 animals were randomly selected from each group and were euthanized. Histology of testes, seminal parameters (Count, Motility and Morphology) and Hormonal milieu (FSH, LH and Testosterone) were analysed. The remaining 15 rats from each group were further subdivided into three groups as E-G for duration of 16 weeks to determine the recovery-rate. Group E received Quercetin (50 mg/kg), Group F received Rutin (25 mg/kg) and group G received 1 ml of distilled water to ascertain recovery rate without the administration of Dextromethorphan. The rats were sacrificed and the above parameters were analysed. All experimental procedures and techniques were approved by the Health Ethics committee of the college of medicine, University of Lagos, Nigeria (CM/HREC/09/16/054) with strict compliance with the university's guiding principles for research involving animals

Seminal Fluid Analysis

Incisions were made on the caudal epididymis; it was placed in 0.5 ml of 0.9% normal saline and fluid collected by pipette. 5 ml of the epididymal fluid was delivered onto a glass slide and covered with a cover slip. The sperm progressive motility was determined according to the method described by Bearden and Fluquary. The spermatozoa were counted by haemocytometer using the improved Neubauer (Deep 1/10 mm, LABART, Germany) chamber as described by Pant and Srivastava. Microscopic examinations of the seminal smears were stained with Eosin and the stains were carried out to determine the percentages of sperm morphology. The slides were then examined under light microscope (Mag × 400).

Blood Sampling and Hormonal Assay

Blood was collected from ocular sinuses of the eye using capillary tubes and left to clot for separating the serum after centrifugation at 3000 rpm for 10 minutes. The sera were kept in a freezer at -80◦C for hormonal assay was performed. Testosterone, Luteinising (LH), and Follicle-Stimulating Hormones (FSH) were measured as described by our previous study (2)

Statistics

The data obtained from all the groups were compiled and statistically analysed using ONE WAY-ANOVA using Graph pad software version 6. The results of the data were expressed as mean ± SEM (standard error of mean) where $p<0.05$ was taken as significant.

RESULTS

Ameliorative potential of Quercetin and Rutin on Dextromethorphan-Induced toxicity on hormonal milieu in males.

A dose dependent significant decrease was recorded in the values of FSH, LH and Testosterone when DM-treatment groups were compared to control. Similar significant decrease was noticed when medium and high doses were compared to low dose and when high dose was compared to medium dose. Significant increase was recorded when Recovery-alone, Rutin and Quercetin groups were compared DM-treated groups. Also, significant increase was recorded when Rutin and Quercetin groups were compared Recovery-alone. Significant increase was seen when Quercetin group was compared to Rutin group.

Ameliorative potential of Quercetin and Rutin on Dextromethorphan-Induced toxicity on seminal parameters in males.

A dose dependent significant decrease was recorded in the values of seminal motility, count and morphology when DM-treatment groups were compared to control. Similar significant decrease was noticed when medium and high doses were compared to low dose and when high dose was compared to medium dose. Significant increase was recorded when Recovery-alone, Rutin and Quercetin groups were compared DM-treated groups. Also, significant increase was recorded when Rutin and Quercetin groups were compared Recovery-alone. Significant increase was seen when Quercetin group was compared to Rutin group.

Table 1: Ameliorative potential of Quercetin and Rutin on Dextromethorphan-Induced toxicity on hormonal milieu in males.

HORMONAL MILIEU												
16 weeks	FSH (miu/ L)				LH (miu/ L)				TESTOSTERONE (nmol/L)			
Group	CL	LD	MD	HD	CL	LD	MD	HD	CL	LD	MD	HD
DM-treated	62.08 ± 2.42	37.04 ± 1.81a	24.77 ± 0.09ab	15.98 ± 1.65abc	10.51 ± 0.14	5.02 ± 0.04a	3.14 ± 0.02ab	1.51 ± 0.05abc	13.97 ± 0.27	7.23 ± 0.32a	4.18 ± 0.06ab	1.88 ± 0.30abc
Recovery-alone	61.09 ± 3.38	36.36 ± 0.44a	25.19 ± 0.19ab	18.69 ± 0.35abc	10.58 ± 0.15	5.09 ± 0.06a*	3.18 ± 0.02ab*	1.61 ± 0.02abc*	14.11 ± 0.33	7.92 ±0.23a*	4.67 ± 0.18ab	2.24 ± 0.19abc
Rutin	65.44 ± 1.77	48.21 ± 0.17a*#	33.70 ± 0.66ab*#	23.99 ± 0.25abc*#	10.56 ± 0.09	7.69 ± 0.17a*#	5.80 ±0.04ab*#	3.80 ± 0.04abc*#	14.21 ± 0.37	10.19 ± 0.12a*#	7.37 ± 0.44ab*#	4.54 ± 0.15abc*#
Quercetin	65.94 ± 1.47	48.31 ± 0.45a*#	34.19 ± 0.56ab*#	24.82 ± 0.31abc*#	10.42 ± 0.06	7.91 ± 0.14a*#	5.90 ± 0.04ab*#	3.90 ± 0.03abc*#	14.44 ± 0.23	10.77 ± 0.20a*#	7.88 ± 0.09ab*#	4.98 ± 0.06abc*#

Values are expressed as Mean= Standard Error Mean (SEM) *p<0.05 significant compared with control; ap<0.05 significant compared with low dose; bp<0.05 significant compared with medium dose: cp<0.05 significant compared with high dose; *p<0.05 significant compared with Recovery-alone group: #p<0.05 significant compared with Rutin group.

Table 2: Ameliorative potential of Quercetin and Rutin on Dextromethorphan-Induced toxicity on seminal parameters in males.

SEMINAL PARAMETERS												
16 weeks	MOTILITY (%)				COUNT (**10^6 /mL)**				MORPHOLOGY (%)			
Group	CL	LD	MD	HD	CL	LD	MD	HD	CL	LD	MD	HD
DM-treated	93.20 ± 2.24	47.22 ± 0.69a	33.00 ± 0.82ab	23.60 ± 1.52abc	87.16 ± 2.04	52.96 ± 1.08a	39.46 ± 0.71ab	21.74 ± 0.26abc	93.34 ± 0.28	51.30 ± 0.71a	43.90 ± 0.30ab	30.52 ± 0.61abc
Recovery-alone	92.00 ± 2.35	50.60 ± 0.68a*	39.46 ± 0.26ab*	28.56 ± 0.31abc*	90.60 ± 3.13	57.52 ± 0.50a*	43.52 ± 0.46ab*	25.76 ± 1.16abc*	89.20 ± 1.44	55.22 ± 0.86a*	49.72 ± 0.41ab*	33.12 ± 0.40abc*
Rutin	91.40 ± 1.47	54.06 ± 0.05a*#	43.10 ± 0.30ab*#	36.14 ± 1.89abc*#	91.24 ± 2.07	57.10 ± 0.73a*	42.54 ± 0.56ab	28.48 ± 0.21abc*	93.66 ± 0.42	55.78 ± 0.57a*	53.18 ± 0.28ab*#	34.32 ± 0.27abc*
Quercetin	90.22 ± 3.34	56.56 ± 0.52a*#	49.36 ± 0.26ab*#$	39.38 ± 0.47abc*#	94.00 ± 1.16	62.78 ± 0.40a*#$	50.22 ± 0.53ab*#$	35.02 ± 0.17abc*#$	93.60 ± 1.11	59.24 ± 0.18a*#$	55.98 ± 0.45ab*#$	38.18 ± 0.15abc*#$

Values are expressed as Mean= Standard Error Mean (SEM) *p<0.05 significant compared with control; ap<0.05 significant compared with low dose; bp<0.05 significant compared with medium dose: cp<0.05 significant compared with high dose; *p<0.05 significant compared with Recovery-alone group: #p<0.05 significant compared with Rutin group.

DISCUSSION

In the present study, bioflavonoids, like Rutin and Quercetin showed significant increase in seminal parameters stimulating effects on sperm parameters like sperm motility, sperm count and sperm normal morphology when compared to Dextromethorphan treated group. These results are in agreement with the previous studies of bioflavonoids effect on male reproductive system. Bioflavonoid Quercetin shows effect on the function of prostate by interacting with prostatic type II sites (13). A study demonstrated that Quercetin reduces thelipid peroxidation (14) and improves capacitation of spermatozoa in in-vitro study (15). They suggested that the flavonoids Quercetin and Rutin can improve sperm motility of rat sperm.

Apart from the production of spermatozoa, testis is involved in the production of hormones that are required for various functions in the body, including maintenance of secondary sexual functions, and feedback on the hypothalamus and the pituitary to control the secretion of the gonadotrophins (16). In the absence of any obvious alteration in plasma concentrations of triiodothyronine and tetraiodothyronine, the remarkable decrease in the plasma concentrations of LH, FSH and testosterone was clearly demonstrated in Dextromethorphan-treated rats in this study.

The Leydig cells in the testis are almost exclusively responsible for the biosynthesis and secretion of testosterone which is the male primary steroid hormone. The secretion of testosterone is dependent upon the secretion of LH by the pituitary gland (17). Upon administration of Quercetin and Rutin, there was significant increase in a dose-dependent manner in the values of these reproductive hormones and seminal parameters. This may be due to the protective effect and the proliferative capacity of Quercetin on testicular steroidogenesis and spermatogenesis due to its free radical scavenging properties (18).

CONCLUSION

Quercetin and Rutin were considered over other antioxidants as they are known to decrease the risk of degenerative and reproductive diseases and functions (19). The supplementation of Rutin and Quercetin showed significant increases in the parameters checked which could ameliorate the toxic effect of Dextromethorphan and in turn translates into improved fertility in males.

CONFLICTS OF INTEREST

The authors affirmed that there are not any conflicts of interest.

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