Iranian Journal of Pharmaceutical Research (2011), 10 (3): 585-596
Received: November 2009
Accepted: June 2010

Protective Effect of Rutin and Naringin on Sperm Quality in Streptozotocin (STZ) Induced Type 1 Diabetic Rats

Abstract

sperm quality. Bioflavonoids, Rutin 10 mg/Kg and Naringin 10 mg/Kg were evaluated for their

rats. Mass drug administration (MDA) levels were increased and superoxide dismutase (SOD) catalase levels were decreased. Histopathological changes were evident and in accordance with the above results. In the treatment groups, both Rutin and Naringin in combination with

parameters, decreased the MDA levels and increased the SOD and catalase levels. Protection was evident in histological examination. Our data suggests that the possible protection of

Keywords: Type 1 diabetes; Oxidative stress; Male infertility; Rutin; Naringin.

Introduction

known as juvenile onset diabetes which occurs

streptozotocin (STZ) induced diabetic rats supported the relation between male infertility and diabetes mellitus (1, 2). It is indicated that

Hyperglycemia associated biochemical

species (ROS) is mainly due to glucose autoxidation, activation of polyol pathway,

kinase C) and hexosamine pathway (1, 2). Increased reactive oxygen species (ROS) cause

macro molecules like DNA (14-16).

oxygen species (ROS) and decreased efficiency of anti-oxidant enzyme defenses (3). Several

motility (17), DNA damage by gene mutations, denaturation and DNA base pair oxidation

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(14). DNA fragmentation (15, 16) is associated with declining motility, count, viability and

Cellular antioxidant defenses are classified into primary antioxidant enzymes which include superoxide dismutase (SOD), Catalase (CAT)

carotene. SOD converts

peroxide to water (H
) where as glutathione peroxidase catalyzes

hydroperoxide into water and corresponding alcohol at the expense of Glutathione (GSH).

defense against ROS during chronic diabetes

term complications caused by ROS (18).

male infertility (11). In 2008, Hulya Aybek reported beneficial effects of vitamin E on sperm parameters in type I diabetic rats. Bioflavonoids like rutin and Naringin were proved as antioxidants. Bioflavonoids have also shown simulative effects on sperm parameters. 19).

In the present study, we selected (STZ) induced diabetic rats as a model for type I diabetes mellitus and evaluated the effects of Rutin and Naringin on sperm parameters and oxidative stress in type I diabetic male rats. In this study, we estimated the sperm count, sperm

concentrations in streptozotocin-induced type I diabetic rats. To the best of our knowledge, this is the first study to evaluate the effects of Rutin and Naringin on testicular oxidative stress in type 1

Experimental

Rutin, Naringin and streptozotocin were purchased from Sigma chemicals Ltd (Sigma, USA). Thiopental sodium injection was purchased from Neon Laboratories Ltd (Neon, MUMBAI). All other chemicals and reagents used were of analytical grade.

The 4-month-old young male albino Wistar rats purchased from National Institute of Nutrition (Hyderabad, India), weighing 175-200 g were used in the study. Animals were maintained under the standard laboratory conditions at 25 ± 2°C, relative humidity of 50 ± 15% and normal photoperiod (12 h dark/ 12 h light). Commercial

India) and water were provided

No. 516/01/A/CPCSEA).

The rats were randomly divided to eight groups each containing of six. The groups were treated

of 45 days. These groups were as follows: Group 1: normal control. Group 2: diabetic control. Group 3: vehicle control; diabetic rats treated with 0.1% sodium carboxymethylcellulose (sodium cmc); Rutin and Naringin were dissolved in 0.1% sodium cmc and administered intra-peritoneally (IP). Group 4: diabetic animals treated with insulin SC 3 U/100 g body weight per day. Group 5: diabetic animals treated with both insulin and rutin, 3 U/100 g body weight per day and 10 mg/kg per day, respectively. Group 6: diabetic animals treated with rutin 10 mg/kg per day alone. Group 7: diabetic animals treated with both insulin and Naringin, 3 U/100 g body weight per day and 10 mg/kg per day, respectively. Group 8: diabetic animals treated with Naringin10 mg/kg per day alone.

Diabetes was induced by a single Intravascular/Intravenous (IV) injection of STZ, 45 mg/Kg of body weight, dissolved in citrate buffer (pH 4.5), into the tail vein of animals lightly anaesthetized with ether. Diabetes was confirmed after the third day of STZ injection

analyzer (Screen master 3000)

On the 46 day, animals were sacrificed

Table 1. Effect of insulin, Rutin and Naringin on fasting blood glucose levels (mg/dL) of diabetic rats.

Groups	Sperm motility (%)	Sperm count (10^6/mL)	Sperm viability (%)
	61.83 ± 50.83	28.91 ± 2.48	74.66 ± 0.95
	18.16 ± 0.48 *	5.83 ± 1.17*	25.16 ± 0.6*
Diabetic + 0.1% Sodium CMC	18.83 ± 0.98*	6.46 ± 1.08*	23.83 ± 1.25*
Diabetic + Insulin	41.66 ± 0.56 *	15.67 ± 1.5*	53.33 ± 0.71*
Diabetic + Insulin + Rutin	58.16 ± 0.6 *	26.16 ± 0.64*	67.33 ± 0.88*
	25.17 ± 3.48*	10.33 ± 2.8	29.5 ± 2.85
Diabetic + Insulin + Naringin	50.83 ± 1.68*	21.170.83 *	59.17 ± 0.75 *
Diabetic + Naringin	20.67 ± 3.45	9.17 ± 3.75	28.17 ± 2.06

with lethal ether anesthesia and laporatomy was conducted. Testes and epididymis were collected. The epididymis was used for the evaluation of sperm parameters. The right testis was processed for histopathological studies and the left one was

tissue were measured by the method developed by Ohkawa . (20). This is based on the

were expressed as nmol/g tissue. Super oxide dismutase (SOD) activity was determined by

method was based on the inhibition of superoxide radicals' reaction with phenyl tetrazolium chloride. The specific activity was expressed in

activity was measured based on the Aebi method (22). The activity of catalase was based on the disappearance of hydrogen peroxide. It was

expressed as µM of H metabolized/mg protein/min. One unit was defined as 1 pmol of H consumed per min and the specific activity was reported as units per milligram of protein. Protein was estimated by the method developed by Lowry (23).

Epididymal spermatozoa were collected

warmed to 37°C. Sperm was forced out of the cauda epididymis with fine forceps by putting pressure on lower region of cauda epididymis,

cells. In this study, sperm motility, count, and viability were evaluated by using conventional methods (24-26). Progressive sperm motility was done immediately after the collection of

was calculated per unit area and expressed as sperm motility percentage. Sperm counts were

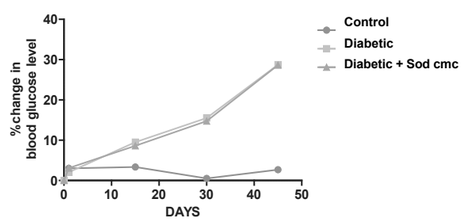

Figure1. Graph showing effect of sodium CMC (vehicle) on

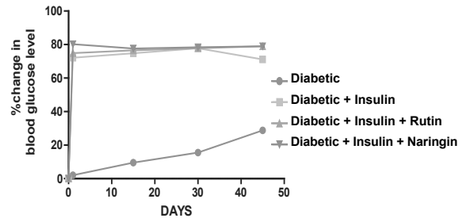

Figure 2. Graph showing effect of insulin, Rutin and Naringin

Table 2. Effect of insulin, Rutin and Naringin on sperm parameters of diabetic rats.

Groups	0th day	45th day	Change%
	99 ± 1.0	96.33 ± 0.99	2.69
	317 ± 3.56	408.17 ± 6.48	28.76
Diabetic + 0.1% Sodium CMC	319.33 ± 3.33	410.83 ± 4.99	28.64
Diabetic + Insulin	451.16 ± 2.77	103.17 ± 2.02	
Diabetic + Insulin + Rutin	473 ± 2.41	99.17 ± 1.54	78.92
	317 ± 3.56	393.33 ± 3.18	24.07
Diabetic + Insulin + Naringin	474 ± 5.52	99.833 ± 2.06	78.93
Diabetic + Naringin	321.1 ± 2.60	399.17 ± 3.74	24.31

represents the means ± SEM of six animals per group* $p < 0.01$, compared as below

Control vs. diabetic + Sodium CMC (diabetic control)

Diabetic + Insulin vs. diabetic + Insulin + Rutin

Diabetic + Insulin vs. diabetic + Insulin + Naringin

Diabetic control vs. Diabetic + Naringin g (NS)

Diabetic + Insulin + Rutin vs. Diabetic + Insulin + Naringin

done using hemocytometer and the results were expressed as millions/mL of suspension. Sperm viability was done using Eosin and Nigrosin stain. The dead sperm took up the stain. Hundred sperm cells were counted in order to obtain the percentage of live/death ratio.

The testis were fixed in 10% formalin and embedded in paraffin. Five-micron thick sections were prepared and stained with hematoxylin and eosin (H and E). The tissue sections were

The results are expressed as mean ± SD. Differences in tissue lipid peroxide levels, SOD and CAT were determined by factorial one-way analysis of variance. Individual groups were compared using Tukey's test. Differences with $p < 0.001$ were considered statistically significant. Statistical analysis was performed using Graph Pad Prism software (Version 5).

Results and Discussion

Streptozotocin-induced diabetic rats had shown significant increase in blood glucose

in Table 1. Diabetic animals treated with insulin

Diabetic animals treated with Rutin alone and Naringin alone and in combination along with

Sperm count, sperm motility percentage and sperm vitality percentage were significantly ($p < 0.001$) decreased in diabetic control group in comparison with sham control group. Values

vitality percentage were given in Table 2. Insulin treatment has significantly ($p < 0.001$) increased

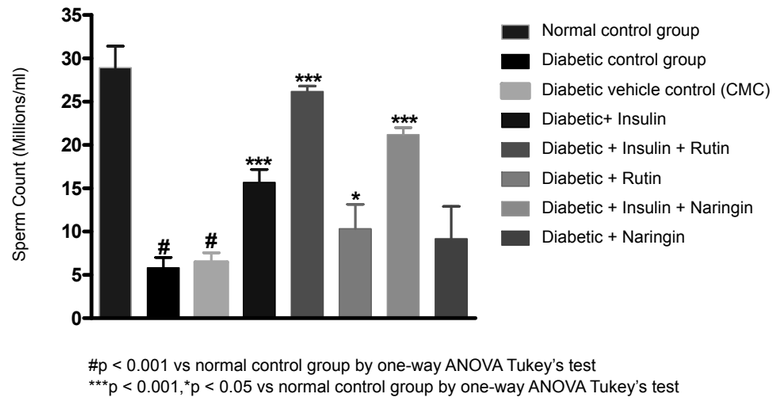

Figure 3. Effect of insulin, Rutin and Naringin on sperm count of diabetic rats.

rats. Insulin treatment with Rutin has further significantly ($p < 0.001$) increased the above sperm parameters. Rutin alone also shown significant ($p < 0.001$) increase in all the sperm

alone has produced better efficacy. Interestingly, insulin in combination with rutin has shown much better efficacy than individual treatments of insulin and rutin. Similarly, insulin treatment with Naringin has also shown significant ($p < 0.001$) improvement in the sperm parameters. Naringin alone has not shown any significant improvement (Figures 3-4 and 5).

with TBA has been widely adopted as a sensitive

levels have significantly ($p < 0.001$) increased

control group. Values of MDA were given in

Rutin and Naringin, significantly decreased when given alone to diabetic rats. But insulin has shown better efficacy in comparison with

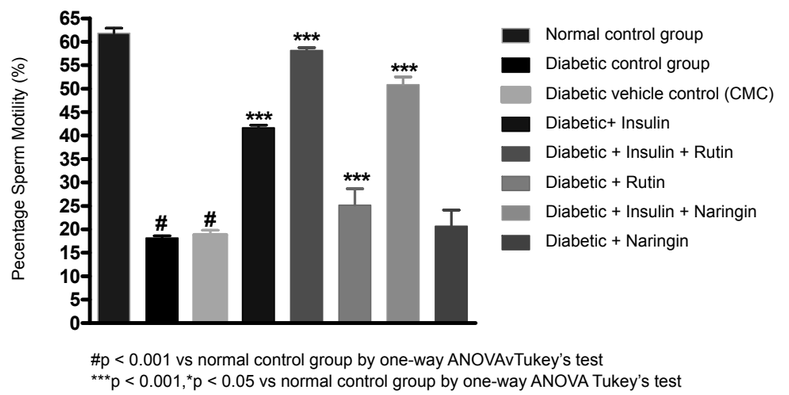

Figure 4. Effect of insulin, Rutin and Naringin on sperm motility of diabetic rats.

Table 3. Effect of insulin, Rutin and Naringin on biochemical parameters of diabetic rats.

Groups	MDA (nmol/g tissue)	SOD (u/mg protein)	Catalase (u/mg protein)
	190.75 ± 3.78	1755.52 ± 7.91	25.17 ± 1.25
	380.32 ± 5.04*	606.27 ± 5.70*	7.83 ± 0.44*
Diabetic + 0.1% Sodium CMC	382.75 ± 7.31*	577.68 ± 25..21*	8.50 ± 0.43*
Diabetic + Insulin	274.57 ± 5.59*	1020.53 ± 19.55*	15.67 ± 0.77*
Diabetic + Insulin + Rutin	225.28 ± 3.29*	1540.12 ± 25.57*	21.17 ± 0.64*
	304.88 ± 3.26 *	927.45 ± 18.41*	11.5 ± 0.70*
Diabetic + Insulin + Naringin	238.15 ± 3.60*	1481.95 ± 11.14*	19.83 ± 0.65*
Diabetic + Naringin	329.3 ± 4.75 *	811.76 ± 4.54*	10.66 ± 0.88

represents the means ± SEM of six animals per group* $p < 0.01$, compared as below

Control vs. diabetic + Sodium CMC (diabetic control)

Diabetic + Insulin vs. diabetic + Insulin + Rutin
Diabetic + Insulin vs. diabetic + Insulin + Naringin

Diabetic control vs. Diabetic + Naringin
Diabetic + Insulin + Rutin vs. Diabetic + Insulin + Naringin (NS)

Rutin and Naringin. Insulin treatment with rutin and insulin treatment with Naringin has further significantly ($p < 0.001$) decreased the MDA levels in diabetic rats. Interestingly, Insulin in combination with rutin has shown much better efficacy than all other groups.

In diabetic control group animals, the such as SOD and CAT in testicular tissue were significantly ($p < 0.001$) reduced compared SOD and catalase were given in Table 3. Insulin treatment has significantly ($p < 0.001$) increased the SOD and catalase levels in diabetic rats. Insulin treatment with rutin and insulin treatment with Naringin has further significantly ($p < 0.001$) increased the SOD and catalase levels in diabetic rats. Insulin alone, rutin alone and Naringin alone also significantly ($p < 0.001$) increased the SOD and catalase levels in diabetic rats. But Insulin treatment alone has produced better efficacy than Rutin and Naringin. Interestingly, Insulin in combination with rutin has shown much better efficacy than individual treatments of insulin and rutin (Figures 6-7 and 8).

Histopathological examination of sections of diabetic rats' testes showed lesions on cell with complete destruction of spermatogenic germ cell detachment (Figure 10). These were however, absent in the normal control rats which had intact seminiferous tubules (Figure 9).

Insulin treated rats, Rutin alone treated rats and Naringin alone treated rats have shown which have been treated only on insulin have shown maximum degree of protection against architecture. Diabetic animals treated with insulin in combination with rutin and insulin in combination with Naringin, have shown almost with normal control rats (Figures 11 and 12).

Discussion and Conclusion

metabolic disorder. Type 1 DM is associated with

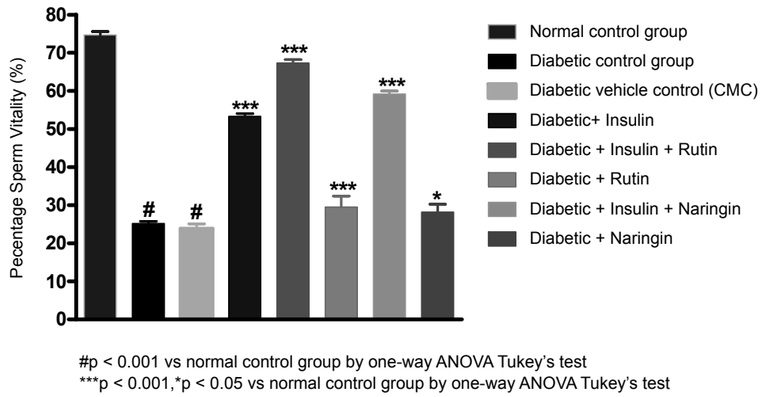

Figure 5. Effect of insulin, Rutin and Naringin on sperm vitality of diabetic rats.

many complications including male infertility. It

DM with sustained high blood glucose levels causes oxidative stress (27). Increased lipid damage in patients with DM. Lipoperoxidation reflects oxidative stress. Cellular and tissue (28). Studies have detected increased semen ROS levels in 25% to 40% of infertile men (29, 30).

Experimentally, streptozotocin (STZ) induces DM, probably through the generation of ROS, between oxidant and antioxidant species has been balance. Bioflavonoids considered as efficacious antioxidants. Rutin and Naringin belong to the class of bioflavonoids widely distributed in fruits

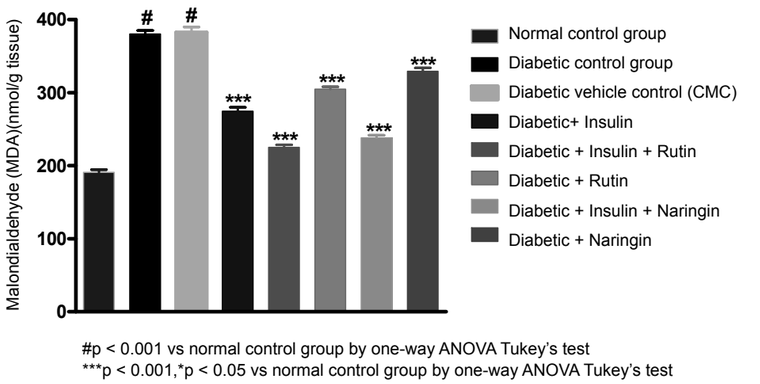

Figure 6. Effect of insulin, Rutin and Naringin on Malondialdehyde

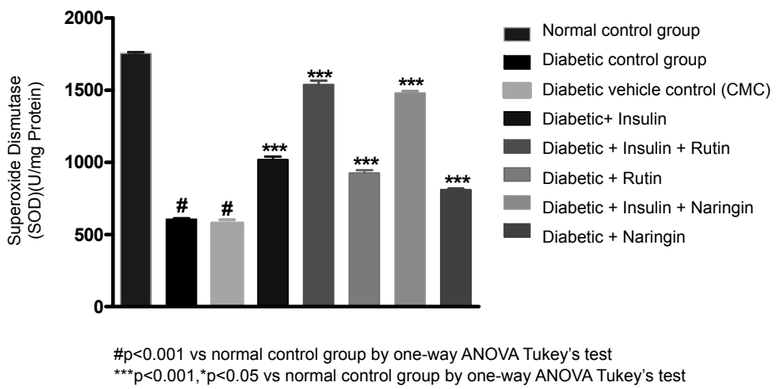

Figure 7. Effect of insulin, Rutin and Naringin on sod levels of diabetic rats.

Naringin were tested for their antioxidant activity in our laboratory. They were evaluated for their antioxidant and sperm

In this study, streptozotocin treated rats showed high levels of blood glucose and insulin and Naringin at 10 mg/kg/day had no effect on diabetic rats. But Rutin and Naringin combined with insulin showed effective control of blood glucose compared to rats treated with only

flavonoids and their complexes with vanadium (33). In the current study, Rutin and Naringin induced diabetic rats (34). In another study, administration of rutin (100 mg/kg) to diabetic rats for a period of 45 days resulted in significant

study, Naringin 10 mg/kg has not shown any

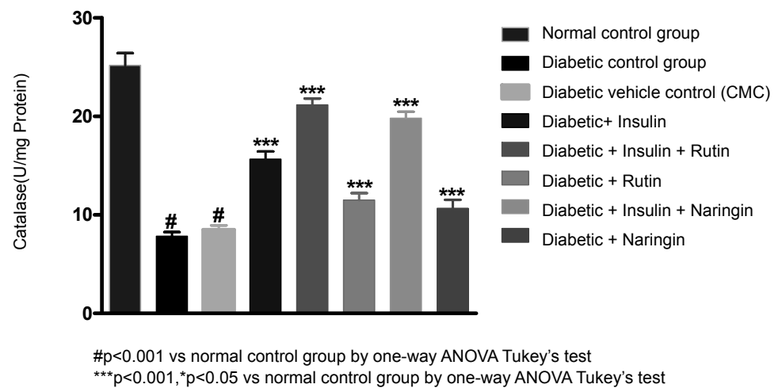

Figure 8. Effect of insulin, Rutin and Naringin on catalase levels of diabetic rats.

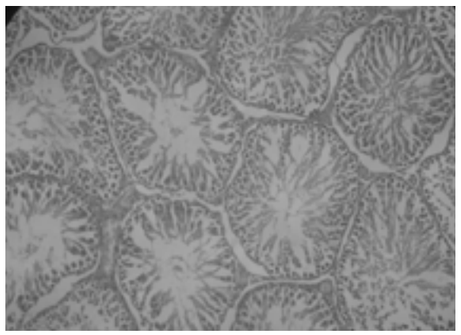

Figure 9. Histological picture of normal testis.

Figure 10. Histological picture of diabetic control rats testis.

of Rutin and Naringin shows anti-hyperglycemic

that bioflavonoids had shown anti-diabetic In our study, we 10 mg/kg

These results are in accordance with the previous control group had shown significant reduction

count and sperm viability, in comparison with normal rats. Earlier works had reported that

on male reproductive system. Type I diabetes

spermatogenesis (36, 37). In diabetes, mellitus hyperglycemia increases oxidative stress (ROS) and it causes DNA damage in all tissues like

acids (PUFA) in the sperm cell membrane and

peroxidation causes DNA damage in sperm cell (3). Therefore, it was suggested that oxidative

associated with diabetes mellitus.

In the present study, bioflavonoids, like Rutin and Naringin, had shown significant stimulating along with insulin, compared to insulin 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 (38). Previous study of N.R. Desroches 2005, demonstrated that Blueberry leaf extract

study (29). They suggested that the flavonoids

after 3-4 h incubation. Antioxidants, like vitamin E, had shown improved steroidogenesis and ROS in diabetic rat testis (13). Some anti diabetic

Rutin and Naringin along with insulin treated groups shows stimulating effects on all sperm

sperm viability, in type I diabetic rats. Rutin with the dose of 10 mg/kg along with insulin offers

in comparison with all other groups.

In our study, testicular MDA levels were

and the antioxidant parameters were reduced (SOD and Catalase). Oxidative stress-mediated

of DNA and on germ cell leads to deterioration of sperm quality (1, 3). With the administration of insulin, Rutin and Naringin alone or in combination, MDA levels were effectively reduced and the SOD increased; Catalase levels

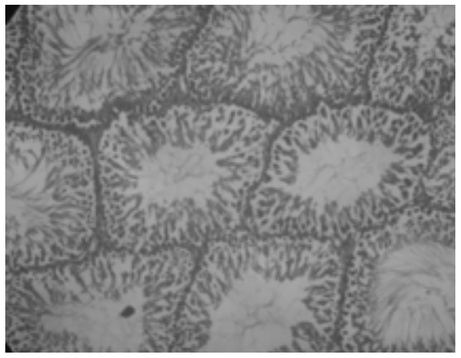

Figure 11. Histological picture of rats' testis after treating with

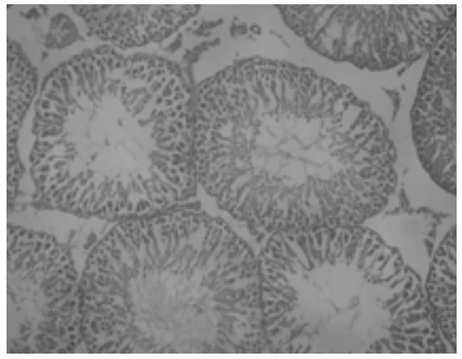

Figure 12. Histological picture of rats' testis after treating with insulin and Naringin.

comparable with the corresponding normal control value were also achieved. These results

leading to enhanced oxidative stress. Several

and Naringin as antioxidants. Rutin reduces
method (40).

hydroxyl and superoxide radicals (41). Jeon s.m
. (2002), suggested that Naringin reduces the

scavenging free radicals that are generated. (42)
., (2004) reported that

hyperglycemia (43, 44). In 2006, Jungsook Cho
.,proved that, flavonoid hesperidin protects brain by scavenging free radicals (45, 46).

elevations in specific oxidant stress markers in both experimental STZ and human diabetes mellitus, together with reduced total antioxidant

(47). Increased prooxidant levels increase lipid

activity of antioxidative enzymes CAT, SOD, and GPX as well as total antioxidant status (48).

In our study, rutin has shown better effect in comparison with naringin. Rutin belongs to the class of flavonols and naringin belongs to flavonones. It is well noted and proved in several studies that flavonols are much active in delivering therapeutic benefit, compared to flavonones. (49)

In conclusion, both rutin and naringin in combination with insulin restored normal

SOD and catalase levels reduction in MDA

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