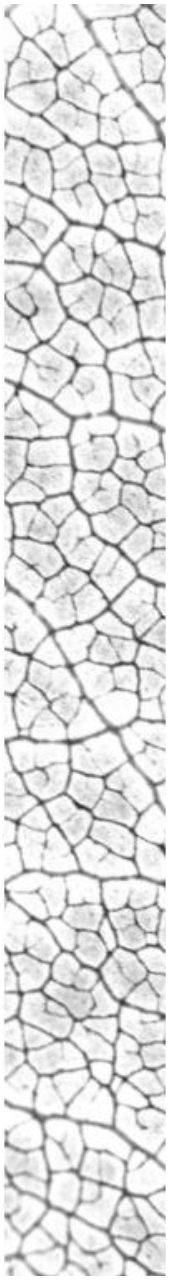

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Effect of the rutin on azathioprine-induced toxicity in reproductive function male rats

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Abstract

The current examination directed to research the defensive capability of flavonoid Rutin (Rt) against Azathioprine (AZA)- incited testicular and epididymal injury in experimental male rats. Sixty grown-up male rodents were partitioned into six gatherings. Control gathering (C): was given distiller water orally, The first animals gathering (T1): was treated as a dose of (2.5) mg/kg bw of Azathioprine, Second treatment gathering (T2): received oral gavage (50) mg/kg of Rutin only, Third treatment gathering (T3): was given with (2.5) mg/kg Azathioprine with Rutin at portion 50 mg/kg bw in combination, Fourth treatment gathering (T4): was given Azathioprine orally at 2.5 mg/kg body weight for month and afterward Rutin oral given at a dose of 50 mg/kg toward the finish of the analysis, Fifth treatment gathering (T5):was given Rutin orally at 50 mg/kg of body weight for month and afterward Azathioprine oral given at a dose of 2.5 mg/kg toward the finish of the test. Results indicated that AZA caused a significantly decline ($P<0.05$) in plasma luteinizing hormone (LH), follicle stimulating hormone (FSH), and testosterone levels which significantly increased as a result of co-treatment with rutin. Likewise, the co-treatment of these animals with rutin, produced a potential increase of the spermatogenesis in testes, epididymal sperm count, ability of motility, viability and percentage of morphology, are lowered in AZA administered, as compared to normal control. Rutin inhibited oxidative damage in each of testis and epididymis of AZA-treated experimental rats. The rutin treatment may decrease AZA-stimulated male reproductive injury as a powerful antioxidant component. The results proved treating with rutin lessen damaging effects of AZA therapy by reduction of the generating ROS and ameliorate reproductive toxicity in male rats, by restoring normal spermatogenesis, and hormones levels. Consuming of foods that contain rutin might be deem as an alternative and advantageous way to safeguard male fertility from chemotherapy-induced reproductive injury.

Keywords: biological tests, chemotherapy, damage, reduction agents

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INTRODUCTION

Since the beginning of civilization, mankind has used various plant materials to meet the needs of life, as natural plants have been in use since several centuries BC by the Egyptians, Chinese, Indians, Syrians, Babylonians and Hebrews (Said and Aydagnehum, 2013; El Sheika, 2015). Most medical studies confirm the need to return to medicinal plants and natural pharmaceuticals as a safe source, in addition to the fact that the method of obtaining them is easier and the cost is low compared to manufactured treatments (ABC, 2005), as it has attracted the attention of researchers recently to medicinal plants that contain different materials Such as alkaloids, glycosides, flavonoids, phenols and cannabinoids due to their pharmacological properties (Mohammad et al, 2017). Rutin is a glycoside flavonol consisting of flavonol quercetin and glucose-rhamnose and also called vitamin P (Ganeshpurkar and Saluja, 2017). Rutin is a citrus flavonoid found in a wide range of plants, including citrus fruits such as oranges and lemons, and is found in tomatoes and tobacco, as well as in other vegetables and fruits such as buckwheat, berries, apricots and cherries (Ashraf et al, 2012). Rutin has been shown to have important biological, pharmacological and medical properties. Rutin has an anti-cancer effect against a wide range of cancers such as anti-neuroblastoma, colorectal cancer and leukemia (Chen et al, 2013; Araujo et al, 2011; Lin et. al, 2007), as it mitigates the negative effects generated by chemotherapy treatments (Bai et al, 2020; Kamel et al, 2014). It is also considered a strong antioxidant (Yeung et al, 2019), an anti-toxin (Alhoshani et al, 2017), an anti-diabetic (Tuhin et al, 2017), an anti-parasite (Chauhan et al, 2018), an anti-inflammatory (Gul et al, 2018), heart

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protection (Wang et al, 2017) and liver protection (Liang et al, 2018). Azathioprine (AZA) is an immunosuppressive drug and is used to prevent the body from rejecting transplanted organs and in cancer and autoimmune diseases. Which interferes with adenine ribonucleotides required for the synthesis of DNA and RNA and thus azathioprine inhibits DNA and RNA, causing DNA abnormalities such as broken DNA strands, damage to the chromatids, and thus AZA suppress the proliferation of B and T lymphocytes and inhibited bone marrow activity, and it is used to suppress leukemia (Pritish Medical Association and Royal Pharmaceutical Society, 2013; Tripathi et al, 2003).

MATERIALS AND METHODS

Chemicals

Rutin and Azathioprine were purchased from Sigma-Aldrich chemical company (St Louis, MO, USA).

Animals and treatment

Sixty grown-up male Wistar rodents (Rattus norvegicus, weighing 180 ± 10 gm) while the ages extended from 12-14 weeks. Six gatherings each are containing ten creatures. The creatures were kept up on a 12-hr light/12-hr dim cycle, took care of standard rodent chow and water not indispensable. Multi week was permitted preceding the beginning of trial for the creatures to adjust to their general condition and human contact.

Trial structure

The creatures were partitioned into sixty gatherings as appeared:

1. Control gathering (C): Typical saline is given distinctly for two months.
2. First treatment gathering (T1): Azathioprine is given at a dosage of 2.5 mg/kg for two months.
3. Second treatment gathering (T2): Rutin is given at a dosage of 50 mg/kg orally for two months.
4. Third treatment gathering (T3): Azathioprine is given orally at 2.5 mg/kg body weight and afterward rutin oral given with a dosage of 50 mg/kg in combination for two months.
5. Fourth treatment gathering (T4): Oral azathioprine with a grouping of 2.5 mg/kg body weight for one month, and oral rutin given 50 mg/kg to the furthest limit of the test.
6. Fifth treatment gathering (T5): Oral rutin is given a dose of 50 mg/kg body weight for one month, and afterward azathioprine is orally given at a dosage of 2.5 mg/kg weight until finishing of the examination.

RESULTS

Hormonal profile

The consequences of the current investigation, **Table 1** recorded a significant decline in the levels hormones of testosterone, FSH, and LH in the gathering of animals treated with azathioprine (TI) contrasted for the control and the rest of the treatments. On the other hand, the group treated with rutin (T2) experienced a considerable increase in the levels of hormones (Testosterone, FSH, LH) contrasted to control and other treatments. While it was found that (T3, T5) registered a significantly decrease contrasted with the control and each of (T4, T2), while it was found that (T4) recorded a significant increase contrasted to the control, also (T3) registered a significantly augmentation comparing to (T5).

Table 1. The effect of rutin treatment on the average concentrations of testosterone, follicle-stimulating hormone (FSH) and luteinizing hormone (LH) in male rats treated with azathioprine for two months

Groups	LH (IU/L)	FSH (IU/L)	Testosterone (ng/ml)
C	11.38±0.51 C	13.47±1.02 C	0.61±0.004 C
A (T1)	7.14±0.45 F	8.49±0.85 F	0.22±0.002 F
R (T2)	13.61±1.06 A	16.24±0.95 A	0.75±0.005 A
AR (T3)	9.74±0.58 D	11.39±1.05 D	0.46±0.003 D
A+R(T4)	12.47±0.95 B	14.63±1.07 B	0.64±0.002 B
R+A (T5)	8.51±0.65 E	10.25±0.75 E	0.34±0.005 E
LSD	0.721	1.105	0.0201

Table 2. The effect of rutin treatment on sperm parameters represented by (sperm concentration - percentage of motile sperms - percentage of normal sperms - percentage of live sperms) in male rats treated with azathioprine for two months

Groups	Morphology	Viability %	Motility %	Count*10/ml
C	76.47±1.62 C	82.10±2.95 C	85.11±2.56 C	55.92±2.05 C
A (T1)	44.39±1.08 F	45.03±1.25 F	45.19±1.54 F	36.83±1.52 F
R (T2)	85.01±2.08 A	90.35±2.05 A	93.07±2.85 A	75.04±2.06 A
AR (T3)	72.42±1.87 D	77.06±1.63 D	80.54±2.64 D	51.29±1.65 D
A+R (T4)	77.21±1.05 BC	84.52±2.08 B	87.54±2.35 BC	59.13±1.75 BC
R+A (T5)	66.16±0.97 E	66.36±1.02 E	65.86±1.74 E	43.87±1.02 E
LSD	3.025	2.001	3.251	4.210

Sperm parameters

As the consequences of the current examination indicated **Table 2** a significantly decline in sperm count, motility, viability and morphology in the tail of the epididymis of animals treated with azathioprine (T1), then again, the current investigation demonstrated a significantly augmentation in studied sperm parameter, in the gathering of rats treated with Rutin (T2) contrasted to the control gathering and the remainder of the treatments, while it was discovered that (T3, T5) recorded a significantly lessening contrasted to the control and (T4, T2), while they registered a significantly increment contrasted to (T1) in All sperm parameters recorded in **Table 2**, while it was found that the treatment (T4) recorded a significantly increment contrasted to (T1), also there are no significantly different recorded between the treatment (T4) and the control group in each of (count, Motility, Morphology) while the increase was significant in viability.

DISCUSSION

Hormones profile

The results of the current study showed a significant decrease in the level of testosterone hormones and FSH, LH in the group of animals treated with azathioprine (TI), and the results of the present study agreed with (Ramadan et al, 2018; Morgan et al, 2015; Akinlolu et al, 2014).

Akinlolu et al, (2014) found that azathioprine caused a dysfunction in the pituitary gland, which led to disturbances in FSH and LH levels in male rats treated with the drug, which resulted in a significant decrease in the level of testosterone hormone. The results of the current study agree with Ramadan et al (2018), where the researcher found that azathioprine affects the axis of the pituitary gland-gonads and causes a decrease in the levels of the hormone testosterone, LH, and FSH, and this is due to the oxidative stress caused by this drug, where he noticed a decrease in weight testes of rats treated with azathioprine, this decrease is due to the anabolic role of testosterone in the testicles. This is confirmed by the previous study conducted by (Qasim & Al-Mayali) (2019) on male rats treated with the chemical anti-cancer drug (Gemcitabine), where he observed a significant decrease in the levels of the sex hormones FSH, LH, and testosterone, as a result of the negative effects of the drug used that lead to high lipid peroxidation, and thus he occurrence of oxidative stress as a result of the accumulation of free radicals generated by these treatments, which negatively affected the gene expression of the genes responsible for the production of these hormones.

While the group treated with rutin (T2) witnessed a significantly augmentation in the levels of hormones (Testosterone, FSH, LH), due to the importance of flavonoids in protecting tissues and reproductive organs from the effect of free radicals and contributing to maintaining the efficiency of cells, as well as may contribute to the increase of secretions of gonadotropic hormones. Mortin and Touaibia (2020) found that the use of flavonoids, including rutin, improves the production of steroid hormones in aging men, have male hypogonadism, as the level of testosterone production decreases in old age by Leydig cells in the testis, and the use of flavonoids and isoflavonoids to ameliorate the production of testosterone hormone which participate to the natural spermatogenesis and the prevention of age-correlating degenerative diseases related with a lack of testosterone, and it was found that flavonoids stimulate the expression of the steroidogenic regulating protein (StAR), which is responsible of the introduction the cholesterol into the mitochondria, which leads to increased production process of testosterone from the Leydig (interstitial) cells in the testis. Al-Roujeaie et al, (2016) also found in their study of streptozotocin-induced diabetes mice that rutin has an important role in increasing the testosterone hormone.

The marked improvement in testosterone, LH, and FSH levels for groups (T3, T4, T5) compared with the control group, could reflect the positive role of rutin as an antioxidant in reducing the negative effect of azathioprine at the level of each of these parameters as well as reducing the damage to the membranes of testicular and pituitary cells, as well as the ability of flavonoids in general to protect the testis and the reproductive organs from oxidative stress damage, that the cells are exposed to as a result of the accumulation of free radicals generated by chemotherapy, by increasing the effectiveness of endogenous antioxidant enzymes such as (SOD, CAT, GSH-Px) as well as increasing the activity of non-enzymatic endogenous antioxidants (GSH) (Hamza et al, 2015). The results of this study also agreed with Salem et al (2016), who found that treating male rats with rutin and the anti-cancer drug Adriamycin (ADP) led to an improvement in the levels of the sex hormones testosterone, LH, FSD and reaching the normal levels of these hormones, which was accompanied by a severe decrease in the level of lipid peroxidation, thus rutin plays an effective role in preserving the pituitary-gonadal axis by inhibiting lipid peroxidation and enhancing body's antioxidant defenses. Rutin also works to inhibit apoptosis, which preserves the cells of these glands from oxidative damage, as the oxidative stress caused by the accumulation of free radicals causes' damage to macromolecules, including lipids, which leads to a decrease in the production of sex hormones (Ceccatelli et al, 2007). Thus, the properties of rutin as an active antioxidant, which are attributed to several pathways that include scavenging of free radicals, chelating mineral ions such as iron and copper, and inhibiting enzymes responsible for generating of free radicals (Benavente - Garcia et al, 1997), that enable it to play a significant role in activating and protecting a process of building steroids, also maintaining optimal levels of testosterone and sex hormones. The results of our current study also agreed with Elsawy et al, (2019), whose study showed that rutin showed a moderating effect on the toxicity induced by carbon tetrachloride (CCl_4) in the liver, kidneys, and gonads of male rats, where it was found that rutin works to enhance hormone levels. FSH, LH and testosterone, which decreased as a result of exposure to Carbon-tetrachloride, and the reason for this is due to the ability of rutin to scavenge free radicals, suppress the resulting oxidative stress and support the antioxidant system in the tissues and thus protect the cells in the tissues responsible for the production of sex hormones.

Sperm parameters

The devastating effects of anticancer treatments on spermatogenesis are well documented, as infertility is

one of the serious side effects of cytotoxicity in young patients in the reproductive stage (Das et al, 2002; Silva et al, 2002) as high concentrations of ROS, generated as a result of exposure to anti-cancer treatments, chemical compounds, and harmful radiation, have an important role in physiological disorders of spermatozoa in humans, and therefore oxidative stress is a main cause of male infertility, as studies have shown that a large percentage of men who suffer from infertility have a considerable augmentation in activity of reactive oxygen species levels in their semen (Beigi Boroujeni et al, 2017; Moozamian et al, 2015).

Therefore, there are several laboratory studies that have recorded changes in sperm parameters, especially in sperm concentration, vitality, activity and movement, as well as the percentage of normal sperm in cases of treatment with anti-cancer treatments such as (Cyclophosphamide, 6-MP, Cisplatin, Methotrexate and Azathioprine)

(Hamzeh et al, 2019; Morgan et al, 2015; Aldemir et al, 2013; Elelaimy et al, 2012).

In the group of animals treated with azathioprine (T1), which witnessed a significant decrease in the sperm parameters, the results were in agreement with study (Elfiky et al, 2012),which recorded deformed spermatozoa compared to the control group, as well as a significant depletion of the content of glutathione in the testis and epididymis and the occurrence of fragmentation in DNA, as a result of oxidative stress caused by oxidation of lipids in the sperm cell membranes as a result of the accumulation of free radicals generated by the metabolism of azathioprine in male rats**.**

The ratio of sperm concentration in the tail of the epididymis is an important parameter that can reflect the normal testicular function and give a clear evaluation of the semen (Ezer and Robaire, 2002). Several studies have found that treatment with anti-cancer chemical treatments causes an increase in apoptosis of germ cells and atrophy of the seminiferous tubules. This causes oligospermia or azoospermia as well as decreased motility (Jahnukanen et al, 2011; Stumpp et al, 2006). The decrease in sperm concentration may explain the negative effects of azathioprine, represented by the increase in oxidative stress caused by the accumulation of free radicals and the formation of lipid peroxidation, which negatively affects the process of spermatogenesis, where the high concentricity of reactive oxygen species ROS play a significant role in physiological disorders of sperm in humans, As considered Oxidative stress is the main cause of infertility (Agarwal et al, 2014). The significant decline in a count of sperm in the epididymis may be correlating to a decline in the testosterone hormone level in laboratory animals, as it was found that there is a decrease in the quality of sperm parameters when the level of testosterone is also low (Abarikwu et al, 2015; Pires et al, 2013), as well as causing AZA increases the oxidative stress caused by the overproduction of ROS, and it is also known that ROS promotes apoptosis (Park et al, 2017), and this could be the mechanism that leads to a decrease in the number of sperms and deterioration of sperm parameters. It was found that the use of azathioprine causes an acceleration of the apoptosis of testicular cells through an increase in TNF-α and caspase-9, and a decrease in the anti-necrosis protein Bcl-2, in addition to a decrease in the levels of the sex hormones Testosterone, FSH, and LH, which causes necrosis and atrophy in the testicular cells, this negatively affected the process of spermatogenesis, and resulted in a decrease in sperm count, motility and viability (Mohammad et al, 2018).

Or perhaps due to the effect of azathioprine on mitochondrial function by generating free radicals, free radicals may cause oxidation of cell membrane lipids and mitochondria as lipid peroxidation causes changes in membrane permeability and disturbances in cell membrane integrity, resulting in cell damage (Zendedel et al, 2017; Khaksary et al, 2016; Gholami et al, 2014), thus their lack of mobility and activity. The damage can be attributed to the disruption of the hormonal regulation of the pituitary-testis axis, which leads to a decrease in the levels of the hormone (testosterone, FSH, LH), which was recorded by the current study, as a previous study indicated that the decrease in the testosterone hormone in laboratory animals is closely related with the decrease of the sperm parameters (Pires et al, 2013).

The current study showed a significant increase in sperm parameters in the group of animals treated with Rutin (T2) compared with the control group and the rest of the treatments, and the reason for this is due to the antioxidant role of flavonoids and their ability to protect cells, as a study conducted by (khaki et al, 2011) in rats given citrus flavonoids, there was an improvement in sperm parameters represented by sperm viability, motility and concentration, with a significant decrease in MAD, as it was observed that citrus flavonoids possessed antioxidant activity and had beneficial effects on the spermatogenesis process and fertility parameters. The researcher (Xu et al, 2020) also found that rutin has the ability to protect sperm in vitro, increase their activity, movement, acrosome capacity, sperm mitochondria, and cell membrane integrity.

Also, in the current study, the treatment with rutin showed moderating effects on sperm parameters and stimulating the spermatogenesis in animals treated with azathioprine (T3, T4, T5), possibly due to the antioxidant capabilities of the rutin (Ismail et al, 2016). Rutin, with its free radical scavenging nature such as superoxide dismutase and hydroxyl radical, makes it a powerful antioxidant compound (Mirani et al, 2012). In addition, rutin has the ability to chelating with the iron ion Fe^{+2} and thus prevents it from binding with hydrogen peroxide, which in turn is able to create a highly reactive

free radical that may lead to cell damage (Chow et al, 2005), as one possible explanation for the mechanism of action is rutin as an antioxidant is that rutin can donate electrons that are direct targets for free radicals such as hydroxyl radical and superoxide radical (Ghiasi et al, 2012). Moreover, its ability to inhibit oxidative stress has been demonstrated by increasing the activities of antioxidants defenses such as (GSH, GSH-Px, CAT, SOD) in the somatic cell (Annapurna et al, 2013), which lead to inhibition of lipid peroxidation and to enhance antioxidant defenses. The increase in sperm concentration in the current study can be attributed to the increase in testosterone hormone and FSH hormone, as the level of these two hormones have a direct effect on the concentration of sperm for their direct effect on the initiation and completion of the spermatogenesis (Duffield et al, 2003). It is known that the testosterone hormone is essential in the spermatogenesis process, because it stimulates the division of spermatocytes located in the germinal epithelium of the seminal tubules, and it also participates with the follicle stimulating hormone (FSH) in completing the process of sperm maturation (Yent et al, 1999). This is consistent with what was shown by Salem et al. (2016), who found that rutin has a therapeutic effect against the reproductive toxicity induced by Adriamycin (ADP), where rutin protects the testicles from the negative effects of the anti-cancer drug by inhibiting lipid peroxidation, which results in a rise in levels of sex hormones, and an increase in testicular enzymes (ALP, ACP, h-Glu, g-GT), which are indications of the activity of the spermatogenesis, leading to the clear increment in sperm concentration and a considerable improvement in the rest of the sperm parameters. It was also found that administering rutin to male rats contributes to the protective effect against testicular and fertility disorders induced by exposure (CCl4), by inhibiting oxidative stress caused by accumulation of ROS and promote the endogenous antioxidants (GSH-Px, CAT, SOD), which led to the enhancement of Levels of the proliferative hormones FSH,LH and testosterone, which resulted in stimulating the process of sperm formation, greatly improving sperm concentration, reducing sperm abnormalities and increasing sperm motility (Elsawy et al, 2019).

CONCLUSION

In the current work, apparently the management of rutin participates to its defensive impact against AZA-stimulated male infertility through restraint of oxidative stress damages as well as through increase sex hormonal levels and improving spermatogenesis. Thus, in the futurity, oral rutin intake as an assistant natural therapy may be approved to protect against restrained effects of anticancer chemotherapy drugs on male reproductive function.

REFERENCES

Abarikwu S O, Akiri O F, Durojaiye M A, Adenike A (2015). Combined effects of repeated administration of Bretmont Wipeout (glyphosate) and Ultrazin (atrazine) on testosterone, oxidative stress and sperm quality of Wistar rats. Toxicology Mechanisms and Methods, 25, 70–80.

Agarwal A, Virk G, Ong C, du Plessis S S, (2014). Effect of oxidative stress on male reproduction. The World Journal of Men's Health., 32, 1–17.

Akinlolu A A, Akinlola O B, Khobe P, Obasi K and Dada O (2014). Azathioprine and Methotrexate impaired the morphology and functions of the testes in adult wistar rats. Journal of Morphological Sciences, 31 (2), 75- 81.

Aldemir M, Okulu E, Kösemehmetoğlu K, Ener K, Topal F, Evirgen O, Avcı A. (2013). Evaluation of the protective effect of quercetin against cisplatin-induced renal and testis tissue damage and sperm parameters in rats. Andrologia, 46(10), 1089–1097.

Alhoshani A R, Hafez M M, Husain S, Al-sheikh Abdel Malek, Alotaibi M R, Al Rejaie S S et al. (2017). Protective effect of rutin supplementation against cisplatin-induced Nephrotoxicity in rats. BMC Nephrology, 18(1),194.

Al-Roujeaie A S, Abuohashish H M, Ahmed M M, Alkhamees O A (2016). Effect of rutin on diabetic-induced erectile dysfunction: Possible involvement of testicular biomarkers in male rats. Andrologia, 49(8), 1-9.

American Botancial Council (2005). Integrative Medical Communication Monographs Austin (Internet). http://www.abc@herbalgram.com.

Annapurna A, Ansari M A, Manjunath PM (2013). Partial role of multiple pathways in infarct size limiting effect of quercetin and Rutin against cerebral ischemia-reperfusion injury in rats. European Review for Medical and Pharmacological Sciences, 17(4), 491–500.

Araújo J R, Gonçalves P, Martel F. (2011). Chemopreventive effect of dietary polyphenols in colorectal cancer cell lines. Nutrition Research, 31(2), 77–87.

Ashraf J, Nagma J S, Nagma M, Mirani N and Rub A. (2012). Protective effect of rutin against carbon tetrachloride-induced hepatotoxicity in mice. International Journal of Drug Development & Research, 4(2), 352-357.

Bai L, Li A, Gong C, Ning X, Wang Z. (2020). Protective effect of rutin against bleomycin induced lung fibrosis: Involvement of TGFβ1/α-SMA/Col I and III pathway. BioFactors,1-8. doi: 10.1002/biof.1629.

Beigi Boroujeni M B, Shahrokhi S S, Birjandi M, Abbaszadeh A, Beyranvand F, Hamoleh S, Gholami M (2017). Effects of pomegranate peel extract on histopathology, testosterone levels and sperm of testicular torsion–detorsion induced in adult Wistar rats. Journal of Complementary and Integrative Medicine, 14(4),1-4

Benavente-García O, Castillo J, Marin F R, Ortuño A, Del Río J A (1997). Uses and properties of citrus flavonoids. Journal of agricultural and food chemistry, 45(12), 4505-4515.

British Medical Association and Royal Pharmaceutical Society. (2013). British National Formulary. United Kingdom: Pharmaceutical Press. P. 583-585.

Ceccatelli S, Tamm C, Zhang Q, Chen M, (2007). Mechanisms and modulation of neural cell damage induced by oxidative stress. Physiology & Behavior, 92(1-2), 87-92.

Chauhan K, Kaur G, Kaur S (2018). Activity of rutin, a potent flavonoid against SSG-sensitive and -resistant Leishmania donovani parasites in experimental leishmaniasis. International Immunopharmacology, 64,372–385.

Chen H, Miao Q, Geng M, Liu J, Hu Y, Tian L, Pan J, Yang Y. (2013). Anti-tumor effect of rutin on human neuroblastoma cell lines through inducing G2/M cell cycle arrest and promoting apoptosis. Scientific World Journal, 2013(20), 1-8

Chow J M, Shen S C, Huan S K, Lin H Y, Chen Y C (2005). Quercetin, but not rutin and quercitrin, prevention of H2O2-induced apoptosis via anti-oxidant activity and heme oxygenase 1 gene expression in macrophages. Biochemical pharmacology, 69(12), 1839-1851.

Das U B, Mallick M, Debnath J M, Ghosh D. (2002). Protective effect of ascorbic acid on cyclophosphamide-induced testicular gametogenic and androgenic disorders in male rats. Asian journal of andrology, 4(3), 201-208.

Duffield T. (2000). Subclinical ketosis in lactating dairy cattle. Veterinary Clinics of North America: Food animal practice, 16(2), 231-253.

El Sheikha A F (2015). Rumex nervosus: an overview. International Journal of Innovative Horticulture, 4(2),87–95.

Elelaimy I A, Elfiky S A, Hassan A M, Ibrahim H M, Elsayad R I (2012). Genotoxicity of anticancer drug Azathioprine (Imuran): role of Omega-3 (ω-3) oil as protective agent. Journal of Applied Pharmaceutical Science, 2 (4), 14-23.

Elfiky S A, Elelaimy I A, Hassan A M, Ibrahim H M, Elsayad R I (2012). Protective effect of pumpkin seed oil against genotoxicity induced by azathioprine. The Journal of Basic & Applied Zoology, 65, 289–298.

Elsawy H, Badr G M, Sedky A, Abdallah B M, Alzahrani A M, Abdel-Moneim A M, (2019). Rutin ameliorates carbon tetrachloride (CCl4)-induced hepatorenal toxicity and hypogonadism in male rats. Peer J, 7,e7011.

Ezer N, and Robaire B (2002). Androgenic regulation of the structure and functions of the epididymis. In: The Epididymis From Molecules to Clinical Practice. Robaire, B. and Hinton, B.T. (eds). P. 297-316.

Ganeshpurkar A, Saluja A K, (2017). The Pharmacological Potential of Rutin. Saudi Pharmaceutical Journal. 25(2), 149–16

Ghiasi M, Azadnia A, Arabieh M, Zahedi M (2012). Protective effect of Rutin (vitamin p) against heme oxidation: A quantum mechanical approach. Computational and Theoretical Chemistry, 996, 28–36.

Gholami M, Saki G, Hemadi M, Khodadadi A, Mohammadi-asl J (2014). Melatonin improves spermatogonial stem cells transplantation efficiency in azoospermic mice. Iranian journal of basic medical sciences, 17(2), 93-99.

Gul A, Kunwar B, Mazhar M, Faizi S, Ahmed D, Raza Shah M, Simjee S U (2018). Rutin and rutin-conjugated gold nanoparticles ameliorate collagen-induced arthritis in rats through inhibition of NF-κB and iNOS activation. International Immunopharmacology, 59, 310–317.

Hamza R Z, El-Shenawy N S, and Ismail H A (2015). Protective effects of blackberry and quercetin on sodium fluoride-induced oxidative stress and histological changes in the hepatic, renal, testis and brain tissue of male rat. Journal of Basic and Clinical Physiology and Pharmacology, 26(3),237- 251.

Hamzeh, M., Hosseinimehr, S.J., Karimpour, A., Mohammadi, H.R., Khalatbary, A.R., & Talebpour Amiri, F. (2019).Cerium oxide nanoparticles protect cyclophosphamide-induced testicular toxicity in mice. International Journal of Preventive Medicine, 10(1),5-14.

Ismail H, Maksimović J D, Maksimović V, Shabala L, Živanović B D, Tian Y, Shabala S. (2016). Rutin, a flavonoid with antioxidant activity, improves plant salinity tolerance by regulating K+ retention and Na+ exclusion from leaf mesophyll in quinoa and broad beans. Functional Plant Biology, 43(1), 75-86.

Jahnukainen K, Ehmcke J, Hou M, Schlatt S (2011). Testicular function and fertility preservation in male cancer patients. Best practice & research Clinical endocrinology & metabolism, 25(2), 287-302.

Kamel K M, Abd El-Raouf O M, Metwally S A, Abd El-Latif H A, El-sayed M E, (2014). Hesperidin and Rutin, Antioxidant Citrus Flavonoids, Attenuate Cisplatin-Induced Nephrotoxicity in Rats. Journal of Biochemical and Molecular Toxicology, 28(7), 312–319.

Khaki A, (2011). Anti-oxidative effects of citro flavonoids on spermatogenesis in rat. African Journal of Pharmacy and Pharmacology, 5(6), 721–725.

Khaksary M M, Gholami M R, Najafzadeh V H, Zendedel A, Doostizadeh M. (2016). Protective effect of quercetin on skeletal and neural tube teratogenicity induced by cyclophosphamide in rat fetuses. In Veterinary Research Forum, 7 (2), 133-138.

Liang W, Zhang D, Kang J, Meng X, Yang J, Yang L, Xue N, et al. (2018). Protective effects of rutin on liver injury in type 2 diabetic db/db mice. Biomedicine & Pharmacotherapy, 107, 721–728.

Lin J-P, Yang J-S, Lu C-C, Wu S-Y, Lan S-H, Liao S-S, Chung J-G (2007). Rutin inhibits WEHI-3 leukemia cells in Balb/C mice in vivo. The FASEB Journal, 21 (6), A1190-A119.

Martin L J, Touaibia M. (2020). Improvement of Testicular Steroidogenesis Using Flavonoids and Isoflavonoids for Prevention of Late-Onset Male Hypogonadism. Antioxidants., 9(3), 237.

Mirani N, Ashraf N J, Siddique J, Rub A. (2012). Protective effect of rutin against cadmium induced hepatotoxicity in Swiss albino mice. Journal of Pharmacology and Toxicology, 7, 150-157.

Moazamian R, Polhemus A, Connaughton H, Fraser B, Whiting S, Gharagozloo P, Aitken R J (2015). Oxidative stress and human spermatozoa: diagnostic and functional significance of aldehydes generated as a result of lipid peroxidation. Molecular Human Reproduction, 21(6), 502–515.

Mohammad F E, Hasan W A, Mohamed E G (2017). Natural antioxidant flavonoids in formalin-induced mice paw inflammation; inhibition of mitochondrial sorbitol dehydrogenase activity. Journal of Biochemical and Molecular Toxicology, 31(7), 1-8.

Mohammad S A, Ramadan B K, Schaalan M F (2018). Mitigation of Azathioprine-induced Testicular Atrophy by Taurine; An Impact on Inflammation, Oxidative Perturbations and Apoptosis. Canadian Journal of Physiology and Pharmacology, 96(3), 1–43.

Morgan J A, Lynch J, Panetta J C, Wang Y, Frase S, Bao J, Schuetz J D (2015). Apoptosome activation, an important molecular instigator in 6-mercaptopurine induced Leydig cell death. Scientific Reports, 5(1),1-11.

Park H J, Choi Y J, Lee J H, Nam M J (2017). Naringenin causes ASK1-induced apoptosis via reactive oxygen species in human pancreatic cancer cells. Food and Chemical Toxicology, 99, 1–8.

Pires V C, Gollücke A P, Ribeiro D A, Lungato L, D'Almeida V, Aguiar O (2013). Grape juice concentrate protects reproductive parameters of male rats against cadmium-induced damage: A chronic assay. British Journal of Nutrition, 110, 2020–2029.

Qasim M and Al-Mayali H K (2019). Investigate the relation between Baicalin effect and gene expression of LH, FSH, Testosterone in male rats treated with Gemcitabine drug. Research Journal of Pharmacy and Technology,12 (9),4135-4141.

Ramadan B K, Schaalan M F, Mahmoud E S (2018). Protective Effect of Taurine on Thiopurine-Induced Testicular Atrophy in Male Albino Rats. Journal of Steroids & Hormonal Science, 9(1),192.

Salem E A, Salem N A, Hellstrom W J (2016). Therapeutic effect of ozone and rutin on adriamycin-induced testicular toxicity in an experimental rat model. Andrologia, 49(1),e12603.

Salem E A, Salem N A, Hellstrom W J (2016). Therapeutic effect of ozone and rutin on adriamycin-induced testicular toxicity in an experimental rat model. Andrologia, 49(1),1-7.

Seid M A, Aydagnehum S G (2013). Medicinal plants biodiversity and local healthcare management system in Chencha district; Gamo Gofa, Ethiopia. Journal of Pharmacognosy and Phytochemistry, 2(1),284–293.

Silva C A, Hallak J, Pasqualotto F F, Barba M F, Saito M I, Kiss M H (2002). Gonadal function in male adolescents and young males with juvenile onset systemic lupus erythematosus. The Journal of Rheumatology, 29(9), 2000-2005.

Stumpp T, Freymüller E, Miraglia S M, (2006). Sertoli cell function in albino rats treated with etoposide during prepubertal phase. Histochemistry and cell biology, 126(3), 353-361

Tripathi K D, (2003).Essentials of Medical Pharmacology. 5th ed. India : Jaypee Brothers Medical Publishers, p. 769-774.

Tuhin RH, Begum MM, Rahman MS, Karim R, Begum T, Ahmed SU, Mostofa R, Hossain A, Abdel-Daim M, Begum R. (2017).Wound healing effect of Euphorbia hirta linn. (Euphorbiaceae) in alloxan induced diabetic rats. BMC Complementary and Alternative Medicine,17(1),423.

Wang Y D, Zhang Y, Sun B, Leng X W, Li Y J, Ren L Q (2017). Cardioprotective effects of rutin in rats exposed to pirarubicin toxicity. Journal of Asian Natural Products Research, 20(4), 361–373.

Xu D, Wu L, Yang L, Liu D, Chen H, Geng G, Li Q (2020). Rutin protects boar sperm from cryodamage via enhancing the antioxidative defense. Animal Science Journal, 91(1),1-10.

Yent S S C, Jaffe R B, and Barbieri R L (1999). Reproductive Endocrinology, Physiology, Pathophysiology and Clinical management. 4th ed.

Yeung A W K, Tzvetkov N T, El-Tawil O S, Bungău S G, Abdel-Daim M M, Atanasov A G (2019). Antioxidants: Scientific Literature Landscape Analysis. Oxidative Medicine and Cellular Longevity, 2019,1–11.

Zendedel A, Gharibi Z, Anbari K, Abbaszadeh A, Khorramabadi R M, Soleymaninejad M, Gholami M (2017). Selenium ameliorate peripheral nerve ischemic-reperfusion injury via decreased TNF-α. Biological trace element research, 176(2), 328-337.