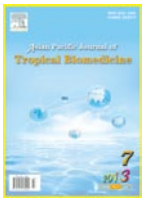
Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Biomedicine

journal homepage: www.elsevier.com/locate/apjtb



Document heading doi: 10.1016/S2221-1691(13)60114-8

Sensibility of male rats fertility against olive oil, *Nigella sativa* oil and pomegranate extract

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PEER REVIEW

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Comments

This research demonstrated the protective activity of olive oil, *Nigella sativa* oil and pomegranate extract against testicular and spermatozoal lipid membrane damage due to excess free radicals in rats. This activity was assessed based on examination of the reproductive organs, body weight, sperm criteria (number, motility and abnormal forms) beside hormonal assays for testosterone, FSH, LH, inhibin-B and lipid peroxidation determined by the level of MDA and determination of antioxidant activity determined by GSH-Px, GSH, and catalase.
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ABSTRACT

Objective: To clarify the modulatory effects of daily consumption of pomegranate extract (PE), olive oil (OO) and *Nagilla sativa* oil (NSO) on antioxidant activity, sperm quality and pituitary-testicular axis of adult male wistar rats. **Methods:** Thirty-two adult male Wistar rats were divided into four equal groups, eight rats each. Using rat gastric tubes, 1.0 mL distilled water, 1.0 mL PE, 0.4 mL NSO and 0.4 mL OO were orally administered daily for 6 weeks in the first, second, third and fourth groups, respectively. Reproductive organs, body weight, sperm criteria, testosterone, FSH, LH, inhibin-B, lipid peroxidation, and antioxidant enzyme activities were investigated. At the end of the study protocol, analyses occurred at the same time. Data were analysed by ANOVA test and $P<0.05$ was considered to be a significant value. **Results:** In all studied groups, malondialdehyde level was significantly decreased accompanied with an increases in glutathione peroxidase and glutathione. Rats treated with PE showed an increase in catalase activities accompanied with an increase in sperm concentration which was also observed in NSO group. In PE treated group, sperm motility was also increased accompanied with decreased abnormal sperm rate. NSO, OO and PE treated groups shows an insignificant effect on testosterone, inhibin-B, FSH and LH in comparison with control group. **Conclusions:** These results show that administration of PE, NSO and OO could modify sperm characteristics and antioxidant activity of adult male wistar rats.

KEYWORDS

Pomegranate extract, *Nagilla sativa* oil, Olive oil, Testosterone, FSH, LH, Inhibin-B, Lipid peroxidation, Sperm characteristics, Antioxidant enzymes

1. Introduction

Pomegranate scientifically known as *Punica granatum*, olive oil (OO) and *Nagilla sativa* oil (NSO) have been used in the herbal medicine of different populations specifically in the Middle East[1]. Pomegranate extract (PE) is found to be rich in vitamin C and polyphenolic compounds such as ellagic, punicalagin, anthocyanins and gallic acid[2,3].

Pomegranate has become more famous due to its important pharmacological properties, including anti-cancer[4], anti-proliferative, apoptotic[3], topical antimicrobial, HIV-I entry inhibitory, cardioprotective and hypolipidemic effects[5-7].

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Foundation Project: Supported by Deanship of scientific research, King Faisal University, KSA (Grant no. 170323-2012).

Article history:
Received 9 Mar 2013
Received in revised form 1 May, 2nd revised form 6 May, 3rd revised form 22 May 2013
Accepted 5 Jun 2013
Available online 28 Jul 2013

Additionally, many investigators have documented that pomegranate and its extracts have a marked antioxidant activity and free radical scavenger effect[8].

Reactive oxygen species (ROS) that belong to the class of free radicals are highly reactive oxidizing agents. Production of ROS in various tissues like testis is a common event; however, the abnormal increase in its synthesis could stimulate the DNA damage and oxidation of many cells[9]. The sperm plasma membrane contains a high level of unsaturated fatty acids. So, it is liable to peroxidative damage. Lipid peroxidation could lead to the damage of lipid matrix structure in spermatozoal membranes, and could be associated with impaired motility[10].

Antioxidants are compounds which scavenge and decrease the synthesis of ROS and lipid peroxidation. Biological antioxidants include glutathione (GSH), catalase (CAT), glutathione peroxidase (GSH-Px) and superoxide-dismutase (SOD) that have a very crucial role in scavenging of free radicals. Therefore, ROS scavenger's application is likely to improve sperm quality[9].

Furthermore, there are lots of contradictory reports concerning the effects of PE, OO and NSO on male fertility[8-10]. In the present study, the modulatory effects of PE, OO and NSO on male fertility were investigated. We investigated epididymal sperm characteristics, levels of testosterone, inhibin-B, FSH, LH (Pituitary-testicular axis), antioxidant enzyme activity and lipid peroxidation in adult male wistar rats, to which these herbal extract were given orally for 6 weeks.

2. Materials and methods

2.1. Chemicals

OO, NSO and pomegranate were got from herbal public market in Al-Hufof, Al-Ahsa, eastern region, KSA.

2.2. Plant extraction

Pomegranate was obtained from the market (3 500 g) was transformed to juice and mixed with 1 000 mL 80% methanol and distilled water for 3 d with repeated stirring. Hydro-alcoholic extract was obtained by repeating the extraction procedure for 3 consecutive times. Vacuum evaporator was used for making extract filtered and concentrated to obtain the final solid residue. Final pomegranate extract was weighed and kept refrigerated until further use[11]. The extract was dissolved in water and doses of rat treatment were prepared (100 mg/kg body weight in 1 mL distilled water)[12].

2.3. Animals

Thirty two adult male Wister rats with average body weight of 1 505 g were obtained from the animal house of college of Veterinary Medicine, KFU, Al-Ahsa, KSA. Rats were housed in well ventilated polypropylene cages (8 animals/cage) and had 12/12 h dark and light cycles with constant temperature 24 °C through the study period. Rats were acclimatized to laboratory conditions for 1 week before start of the study protocol. Rats received a standard food of commercially available rat feed pellet (ARASCO, Riyadh, KSA) and distilled water. Standard rat food pellets include minerals, cereals, soya, molasses, alfalfa, wheat-bran and vitamins. The food pellets contain crude proteins amount 13%, fats 2% and fibers 10% in the food particles. Rats were handled according to WHO guideline for animals care and the study protocol was approved by the committee of research ethics at King Faisal University.

2.4. Experimental design and treatment schedule

Rats were equally divided into four groups with eight animals in each. The first control group rats received 0.4 mL distilled water orally by oro-gastric tube every day. The second group rats received orally 0.4 mL OO/day. The third group rats received orally 0.4 mL NSO/day. The fourth group rats received orally 0.4 mL PE extract (100 mg/kg body weight) in distilled water. All were given for 6 weeks[12].

2.5. Blood sampling

At the end of study period, all rats were anesthetized with Di-ethyl ether and decapitated. Blood was collected in dry propylene test tubes without anticoagulant. Blood samples were left to clot at room temperature for 30 min and centrifuged for 10 min at 5 000 r/min. Sera were separated and stored at -70 °C until assayed. Testes, Cowper glands, seminal vesicles, prostate and epididymides were removed, cleared of adhering tissues and weighed.

2.6. Evaluation of sperm parameters

The epididymal sperm concentration was investigated according to Koksal *et al*[13] with the use of a hemocytometer equipped with an Improved Neubauer slide (LABART, Darmstadt, Germany). Sperms were counted using a light microscope at 200× magnification. The percentage of forward progressive sperm motility was evaluated as described by Wittayarat *et al*[14], using a light microscope with heated stage. Motility estimates were provided from three different fields in each sample. The final motility score was performed by getting the mean values of the three successive estimations. Percentage of morphologically abnormal spermatozoa was determined by staining the slides with eosin-nigrosin (10.0% nigrosin, 1.6% eosin and 0.1 mol/L sodium citrate). The slides were viewed using a light microscope at 400× magnification.

2.7. Lipid peroxidation level

The serum lipid peroxidation levels were determined according to the concentration of thiobarbituric acid reactive species according to the method of Sadek *et al*[15], and the

amount of produced malondialdehyde (MDA) was used as an index of lipid peroxidation.

2.8. GSH level and GSH-Px activity

Serum reduced GSH contents were measured according to the method of Sedlak and Lindsay(in nmol/mL)[16]. GSH-Px activity was measured according to the method of Padma *et al*[17].

2.9. CAT activity

The serum CAT activity was determined according to Sharma & Sharma[18](in kU/L).

2.10. Testosterone, inhibin-B, FSH and LH

The serum testosterone level was determined by the ELISA method using a testosterone kit (DRG Instruments, GmbH, Germany) according to the kit manufacturer's instructions. ELISA kits for serum FSH, LH and inhibin-B were purchased from Genzyme (Cambridge, USA).

2.11. Statistical analysis

The data are presented as mean±SE. Statistical analysis was done using one-way ANOVA test for the significant interrelation between the various groups[19]. $P<0.05$ will be considered statistically significant, <0.01 will be considered statistically highly significant and <0.001 will be considered statistically very highly significant.

3. Results

3.1. Reproductive organs and body weight

Herbal extracts of PE, OO and NSO were found to produce an statistically insignificant effect on body weight of the rats in comparison with control group. Changes in weight of testes, epididymes, seminal vesicles, prostate and Cowper glands of rats treated with OO, NSO and PE in comparison with that of the control group proved to be statistically insignificant (Table 1).

3.2. Epididymal sperm criteria

The effects of OO, NSO and PE on epididymal sperm concentration, sperm motility and abnormal sperm rate are shown in Table 1. While both NSO and PE could significantly increase the sperm concentration in comparison with the control group, an insignificant change was observed in the OO group. Furthermore, sperm concentration of rats in PE group was also significantly higher than that in the OO groups.

Both OO and NSO groups were found to produce an insignificant elevation in sperm motility associated with an insignificant decrease in abnormal sperm concentration in comparison with the control group. However, there were a significant increase in sperm motility and a decrease in total abnormal sperm concentration of PE group in comparison with the control group.

Table 1

Reproductive organ weights, epididymal sperm concentration, sperm motility and abnormal sperm rate in all study groups.

Weight	Control	OO	NSO	PE
Testis (mg)	1 301.9±33.8	1 402.3±55.8	1 485.7±60.4	1 441.0±66.5
Epididymis (mg)	504.7±17.0	529.6±8.4	512.7±15.2	513.0±17.1
Seminal vesicles (mg)	1 062.0±41.3	1 257.1±98.8	1 081.7±65.2	1 108.1±42.7
Prostate (mg)	603.3±44.2	663.3±52.0	610.7±47.1	625.0±31.0
Cowper gland (mg)	34.7±4.7	35.7±4.3	41.0±4.7	39.0±3.5
Epididymal sperm concentration (million/g)	222.3 ± 12.1^{A}	250.2 ± 13.8^{AB}	298.5 ± 15.7^{BC}	335.2 ± 21.0^{C}
Sperm motility (%)	73.3 ± 4.3^{a}	78.1 ± 4.9^{ab}	83.0 ± 4.1^{ab}	95.2 ± 2.4^{b}
Abnormal sperm rate (%)	12.9 ± 1.2^{a}	11.3 ± 1.2^{a}	7.6 ± 0.7^{ab}	6.6 ± 0.8^{b}

Data are presented as mean±SE. The mean differences between the values having different upper cases (A, B, C; $P<0.01$), lower cases (a, b; $P<0.05$) Within the same row are statistically significant.

Table 2

Serum MDA, GSH levels and GSH-Px, CAT activities in all study groups.

Serum	Control	OO	NSO	PE
MDA (nmol/mL)	1.33 ± 0.13^{A}	0.61 ± 0.07^{B}	0.84 ± 0.02^{B}	0.68 ± 0.02^{B}
GSH (nmol/mL)	$0.093\ 5\pm0.002\ 7^{a}$	$0.093\ 3\pm0.000\ 8^{a}$	$0.108\ 0\pm0.004\ 6^{b}$	$0.107\ 0\pm0.002\ 5^{b}$
GSH-Px (IU/L)	7.57 ± 0.24^{A}	8.75 ± 0.13^{B}	8.72 ± 0.08^{B}	9.77 ± 0.14^{C}
CAT (kU/L)	27.53 ± 1.72^{A}	33.52 ± 2.72^{A}	38.61 ± 2.64^{A}	56.16 ± 3.94^{B}

Data are presented as mean±SE. The mean differences between the values having different upper cases (A, B, C; $P<0.001$) and lower cases (a, b; $P<0.01$) within the same row are statistically significant.

3.3. Serum lipid peroxidation level and antioxidant enzyme activities

The MDA, GSH-Px, GSH levels, and CAT activities of all the groups are demonstrated in Table 2. OO, NSO and PE were found to produce significant decreases in serum MDA levels in comparison with control group.

There were significant differences in serum GSH levels between OO, NSO and PE groups in comparison with control group. OO, NSO and PE caused a significant increases in serum GSH-Px. OO and NSO increased serum CAT activities when compared to the control group, but the difference was insignificant. However, the increase observed in serum CAT activities of PE group was statistically significant in comparison with the control and the other groups.

3.4. Serum testosterone, inhibin-B, FSH and LH levels

The levels of serum testosterone, inhibin-B, FSH and LH in all the groups are shown in Table 3. Testosterone, inhibin-B, FSH and LH levels in the rats treated with OO, NSO or PE were found to produce an insignificant change in comparison with the control group.

Table 3
Levels of serum testosterone, inhibin-B, FSH and LH in all study groups.

Serum	Control	OO	NSO	PE
Testosterone (ng/mL)	0.83±0.06	0.87±0.06	0.91±0.17	1.01±0.11
Inhibin B (pg/mL)	31.34±5.70	31.24±3.40	29.77±4.20	32.85±3.80
FSH (ng/mL)	7.07±2.40	6.78±1.80	7.35±2.66	6.82±2.40
LH (ng/mL)	4.51±0.78	4.97±0.65	5.01±0.44	4.22±0.33

Data are presented as mean±SE.

4. Discussion

Daily oral administration of NSO and PE for 6 weeks could produce a significant increase in epididymal sperm concentration and sperm motility accompanied with decreased abnormal sperm concentration that could be related with decreased lipid peroxidation in wistar male rats.

PE is considered as one of the most important sources of hydrolyzable tannins, punicalagin, anthocyanins, and punicalin[4], ellagic and gallic acids, and it also contains vitamin C[2]. Both free radical scavenging and antioxidant activity of phenolic compounds that is derived from pomegranates and vitamin C have been documented by many investigators[10,14]. In the present study, it was observed that PE had an insignificant effect on body and reproductive organ weights, levels of serum testosterone, inhibin-B, FSH and LH levels in comparison with the control group.

Many cellular metabolites can cause an increase of the concentration of electrophilic radicals, which can react with oxygen giving rise to ROS. The main sources of free radicals include singlet-oxygen ($^1O_2$), hydroxyl radical (OH) and H_2O_2. ROS are normally generated in many vital metabolic processes for living cells including the spermatozoa; however, marked generation of ROS produced by spermatozoa[10,20] or by the combinations of xenobiotics and immunosuppressive agents can induce the production of toxic lipid peroxides[2,21]. Cells exhibit defensive pathways using various antioxidants.

The main detoxifying systems for peroxides are GSH and CAT. CAT is an antioxidant enzyme that destroys H_2O_2 which can synthesize a highly reactive OH. On participation of the glutathione redox cycle, GSH together with GSH-Px converts H_2O_2 and lipid peroxides to non-harmful products[9,23]. Phenolic compounds derived from pomegranate[6]. Vitamin C, lycopene and melatonin have been reported to be used as antioxidant agents to buffer various lipid-peroxidation-induced damages in different tissues[13,14,21,22,29]. In the present study, a significant decrease in MDA level, which is a by-product of lipid peroxidation, and marked increases in GSH, CAT and GSH-Px activities of serum samples of rats receiving OO, NSO and PE were observed. These findings demonstrate that they have potent anti-oxidative effects.

In addition, ROS are highly reactive molecules that can react with many intra-cellular structures, mainly unsaturated fatty acids and trans-membrane proteins. The oxidation of these molecules can produce disturbance in cellular membrane permeability. Spermatozoa are highly susceptible to peroxidative damage due to existence of high concentration poly-unsaturated fatty acids, which are responsible for regulation of sperm maturation, capacitation, acrosome reaction, spermatogenesis and membrane fusion, with low antioxidant capacity. Moreover, sperm lipid peroxidation could destroy the structure of the lipid matrix in spermatozoal membranes, accompanied with a rapid decrease of intracellular ATP that leads to decreased sperm viability, axonemal damage and increased mid-piece morphological defects, and it could dramatically finally inhibit spermatogenesis[9]. The level of serum inhibin-B could be considered as a marker that reflects the capability of testicular spermatogenesis[24].

Results of the present study showed that epididymal sperm concentration and sperm motility of rats receiving PE were significantly higher than those of the control group. In addition, it was found that PE could significantly decrease abnormal sperm rate in comparison with the control. In this study, improvements observed in sperm quality may be attributed to prevention of excessive generation of free radicals, produced by antioxidant property of OO, NSO and PE.

In addition, consumption of OO is thought to have many beneficial influences on human health[25]. Recently, it has been reported that OO phenols are strong antioxidants that have biological activities which may account for the

observed beneficial influences of the Mediterranean diet[26].

Virgin OO contains many phenolic compounds including oleuropein and hydroxytyrosol which are thought to be the principal components conferring the characteristic taste and stability of OO[26]. The results of the present study could be explained by what was observed by Kano *et al*[27], who reported that dietary supplementation with oleuropein could alter the hormonal level of those associated with protein anabolism by increasing urinary noradrenaline and testicular testosterone hormone levels and decreasing plasma corticosterone level in rats.

Moreover, data obtained in the present study are in contrast to those reported by Kano *et al*[27], who documented that oleuropein aglycone in extra virgin OO is responsible for the increase of LH secretion via the enhancement of the pituitary gland by the increase in noradrenaline plasma level.

Furthermore, it was reported that consumption of thymoquinone, which is the major active constituent of *Nigella sativa*, could lead to decreased total antioxidant capacity and could prevent the increase in the myelo-peroxidase activity, explaining data obtained in the present study[28].

Improved sperm quality could be produced by increased antioxidant capacity that could protect spermatozoa against per-oxidative damage in healthy rats. Hence, it can be said that there is a positive correlation between OO, NSO and PE consumptions and enhanced sperm parameters.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

This study was funded by Deanship of scientific research, King Faisal University, KSA (Grant No.: 170323-2012).

Comments

Background

Male fertility power has been frequently correlated with herbal extracts. The aim of the present study was to clarify the modulatory effects of daily consumption of PE, OO and NSO on antioxidant activity, sperm quality and pituitary-testicular axis of adult male wistar rats.

Research frontiers

The present research work investigated the effects of OO, *Nigella sativa* oil and pomegranate extract on antioxidant activity, sperm quality and number, and pituitary testicular axis in adult male wister rat and assessed different antioxidant parameters, sperm count and quality, serum testosterone, FSH, LH and inhibin-B hormones .

Related reports

Pomegranate extract, OO and *nigella sativa* oil were used frequently in herbal medicine for treatment and prevention of a variety of diseases as they are considered as strong antioxidants. Their role in improving the seminal quality and sperm concentration was to investigate in the present study. Furthermore, there are a lot of contradictory reports concerning the effects of pomegranate, OO and NSO on male fertility.

Innovations and breakthroughs

Improved sperm quality could be produced by increased antioxidant capacity that could protect spermatozoa against per-oxidative damage in healthy rats, so these herbal non-toxic agents can be used safely for treartment of male infertility.

Applications

OO, NSO and PE could be used safely for treatment of male infertility resulted from excess oxidative free radicals accumulation in testicular tissues affecting the sperm quality and motility.

Peer review

This is a valuable research work in which authors have demonstrated the protective activity of OO, NSO and PE against testicular and spermatozoal lipid membrane damage due to excess free radicals in rats. This activity was assessed based on examination of the reproductive organs, body weight, and sperm criteria (number, motility and abnormal forms). Besides, hormonal assays for testosterone, FSH, LH, inhibin-B and lipid peroxidation determined by the level of MDA and determination of antioxidant activity determined by GSH-Px and GSH.

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