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Research Article

Effectiveness of Pomegranate (*Punica granatum L.*) Fruit Extract on the Sexual Function in Rats

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ABSTRACT

Background: Pomegranate (*Punica granatum* L.) has been mentioned to be of value in the management of male sexual disorders. This study investigated the effects of pomegranate fruit extract on healthy male rats as the animal model.

Materials and methods: 50 adult male and female Albino rats of Wistar strain weighing 250-350 g and 200-250 g respectively were used in this study. The pomegranate extract was administered (500, 1000 and 1500 mg/kg) to different groups of male rats on a once-daily regime throughout the experiment period. The general mating behaviour, libido, potency and testosterone concentration were studied and compared with sildenafil citrate.

Results: Administration of the pomegranate extract orally at the dose of 1500 mg/kg produced significant augment of sexual activity in male rats. The mounting frequency, intromission frequency, mounting latency, intromission latency, ejaculation latency and post ejaculation interval did not vary significantly. However, the mounting frequency varied significantly between control, pomegranate crude extracts and sildenafil. In addition, the potency of the extracts did not vary significantly between control, pomegranate crude extracts and sildenafil. The testosterone levels varied significantly between pomegranate, sildenafil and control.

Conclusion: Pomegranate extracts have the potential of increasing sexual behaviour in rats. There is need for mass production of pomegranate extracts for use in enhancing sexual behaviour in human beings.

Keywords: Pomegranate; Mating; Libido; Potency; Testosterone

INTRODUCTION

Sexual behaviour reflects the normal functioning of the hypothalamo-pituitary-gonadal axis. It encompasses sexual motivation as well as sexual performance [1]. In males, sexual performance depends on the integration and coordination of various anatomical and physiological factors that eventually brings about a rise in the corporal system and provides the requisite penile tumescence and rigidity for successful sexual activity [2]. Apart from reproduction, sexuality is practiced in humans for purposes of providing pleasure, bolstering self-esteem, fostering intimacy, and reducing anxiety or tension. Both males and females experience normal sexual activity throughout adult life which declines with aging. Apart from old people, healthy people who are unable to carry out sexual act normally suffer from psychogenic, organic or mixed etiologies leading to erectile dysfunction (ED). Erectile dysfunction (also called impotence) is the inability to attain and maintain a penile erection that is sufficient to sustain satisfactory sexual activity for both partners [3-5].

Global prevalence of ED in 1995 was estimated to be over 152 million men and the projections for 2025 show a prevalence of approximately 322 million with ED, an increase of nearly 170 million men. The largest projected increases were in the developing world such as Africa, Asia and South America. Inability to achieve or sustain an erection and an inability to ejaculate is common among men over 40, where about 5% will experience ED and rates increase with age [6,7]. There are no current statistics available for Kenya, on what age group is more susceptible to erectile dysfunction but in USA about 10% of men are believed to be affected. By the age of 45 years, most men have experienced erectile dysfunction at least once. The incidence increases with age: about 5% of men at the age of 40 and between 15% and 25% of men at the age of 65 suffer from erectile dysfunction and the percentage grows

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to 70% as men reach 80 years of age. At old age, men are reported to lose sexual desires as well as ED, which are unavoidable features of ageing. Men with chronic illnesses, low testosterone level, those taking certain medications or alcohol users, are more vulnerable to experience. Other causes may include emotional instability, exhaustion, injury or physical damage. Young men who experience ED can often relate the condition to underlying health conditions, such as heart disease, diabetes, and side effects of certain drugs and medications. Psychological trauma can also be the cause of ED in men under 40 and treatment may involve counseling [8-11].

The pomegranate, *Punica granatum* L. is a fruit-bearing deciduous shrub or small tree in the family Lythraceae that grows between 5 and 8 metres (16 and 26 ft) tall. The fruit is typically in season from September to February and from March to May. The pomegranate originated in the region of modern-day Iran, and has been cultivated since ancient times throughout the Mediterranean region and Northern India. Pomegranates were known in Ancient Israel as the fruits which the scouts brought to Moses to demonstrate the fertility of the "promised land". It was introduced into Spanish America in the late 16th century and into California by Spanish settlers in 1769. Today, it is widely cultivated throughout the Middle East and Caucasus region, north and tropical Africa, the Indian subcontinent, Central Asia, and the drier parts of Southeast Asia [12-15].

Pomegranate fruit can be consumed pure or used to make pomegranate juice, and it's a healthy addition to any diet. Both the pomegranate seeds and the surrounding pulp are edible and nutritious [16-17]. Research shows that the fruit increases the level of oxygen in the body particularly in the heart, fights arthritis (ageing of bones) and helps fights erectile dysfunction among men in Kenya. Not only is pomegranate fruit packed with beneficial vitamins, minerals, phytonutrients and antioxidants, but clinical evidence supports its ability to protect against heart disease and certain types of cancer. They are also associated with health, fertility and rebirth. Therefore, there is need to look for a sustainable and nutritious alternative in management of male sexual disorders and this study investigated the effectiveness of pomegranate fruit extract on the sexual function [18-20].

MATERIALS AND METHODS

Study site

The study was carried out at Egerton University, Njoro, Kenya. The university is located approximately 182 kilometers, by road, northwest of Nairobi, the capital city of Kenya. The university: lies at 0°22'11.0" S, 35°55'58.0" E (Latitude: 0.369734; Longitude: 35.932779) [21].

Animals

Three months old male and female albino rats of Wistar strain weighing 250-350 g and 200-250 g respectively were used in this study. The rats were obtained from the University of Eldoret in Kenya and transported to Egerton University. All rats were housed singly in separate standard propylene cages and maintained under standard laboratory conditions (temperature 24-28°C, relative humidity 60-70% and 12/12- h light-dark cycle). They were fed with rat pellets (Unga Farm Care (E.A.) Limited, Kenya) and water was provided *ad libitum*. Rats were divided into three groups of five male rats each. Group 1: Control–treated with distilled water; Group 2: test group treated with pomegranate; and Group 3: standard group–treated with sildenafil [22].

Ethical approval

Ethical approval was obtained from the Biosafety, Animal use and Ethics Committee of the Faculty of veterinary medicine, University of Nairobi with the reference number FVMBAUEC/2019/213.

Pomegranate extraction preparation

Fresh pomegranate fruits were obtained from a farm in Njoro, Kenya. They were transported in cartons to the laboratory in the Department of Biological Sciences, Egerton University. Pomegranate fruits were cut into two halves and the seeds scooped into a mixing bowl. Using a blender, the seeds were blended until they were crushed and pulpy. Two pieces of cheese cloth were placed on a flat surface, one on top of the other. The blended seeds were poured into the centre of the cheese cloth, then the ends of the cheese cloth were brought together to make a bag. The cheese cloth bag was held over a beaker and squeezed until juice stopped coming out. The seed casings remained in the cheese cloth, and the pomegranate extract was collected in the container. The pomegranate extract was covered with a container lid and stored in refrigerator at 4°C [23].

Chemicals and drug preparation

Sildenafil citrate was obtained from Teva Pharmaceuticals (Nairobi, Kenya). Estradiol and Progesterene were procured from Sun Pharmaceutical Industries Limited (Mumbai, India), 5% xylocaine ointment was obtained from Lexicare Pharma PVT. LTD. (Nairobi, Kenya). Pomegranate was suspended in distilled water using Tween-80 (1%) for oral use. Similarly, sildenafil citrate and estradiol were suspended in distilled water separately, using Tween-80 (1%) for oral administration. Progesterone was dissolved in olive oil for subcutaneous injection. All the drug solutions were prepared just before administration [24].

Mating behaviour test

Healthy male rats were divided into three experimental groups of 5 each. Group 1, the control group, received 10 ml/kg of distilled water orally, once a day for 7 days at 18:00 h. Group 2, the test group, was treated with pomegranate extract at a dosage of 500, 1000 and 1500 mg/kg orally in a once-daily regime throughout the study period. Group 3, the standard group, was treated with sildenafil drug which was administered orally at the dose of 5 mg/kg 1 h prior the commencement of the experiment. Since rats should not be tested in an unfamiliar circumstance, they were brought to the laboratory and exposed to dim light (in 1 W fluorescent tube in a laboratory of 14' × 14') at the stipulated time of testing daily for 6 days before the experiment. Each rat was weighed using an electronic weighing balance and its weight recorded once a week [25].

Female rats allow mating only during the estrus phase. Thus they were artificially brought into oestrus. They were given a suspension of ethinyl oestradiol orally at the dose of 100 µg/animal 48 h prior to the pairing plus progesterone injected subcutaneously at the dose of 1 mg/animal 6 h before the experiment. The receptivity of the female rats was confirmed before the test by exposing them to male animals other than the control, test and standard animals. The most receptive females were selected for the study. The experiment was carried out on the 7th day after commencement of the treatment of the male rats. The experiment was conducted at 20:00 h in the same laboratory and under the light of same intensity. The receptive female rats were introduced into the cages of male rats on a ratio of 1 female to 1 male [26].

The observation for mating behaviour was immediately commenced and the following parameters were recorded: Mount frequency (MF) and Intromission frequency (IF); Mount latency (ML) and Intromission latency (IL); finally, Ejaculation latency (EL) and Post ejaculatory interval (PEI). The experiment was terminated when each male rat began to mount each female again after a brief period of inactivity or following 30 minutes of sexual inactivity by the male rat from the time of introduction of the female into the testing chamber [27].

Test for libido

The test was carried out using the method of Shravan et al. The Wistar male rats were divided into three experimental groups of five rats each and kept singly in separate propylene cages during the experiment. Group 1 represented the control group, which received 10 ml/kg of distilled water orally, once a day for 7 days at 18:00 h. Group 2 received suspension of pomegranate extract orally at the dose of 500, 1000 and 1500 mg/kg, daily for 7 days at 18:00 h. Group 3 served as standard group and was given suspension of sildenafil citrate orally at the dose of 5 mg/kg 1 h prior to the commencement of the experiment. The female rats were made receptive by hormonal treatment and all the animals were accustomed to the testing condition as previously mentioned in mating behavior test. The animals were observed for Mount frequency (MF) on the evening of 7th day at 20:00 h. The penis was exposed by retracting the sheath and 5% xylocaine ointment was applied 30, 15 and 5 min before starting observations. Each animal was placed in a cage and receptive female rat were placed in the same cage. The number of mountings were noted. The rats were also observed for intromission and ejaculation [28,29].

Test for potency

The test was carried out using the method described by Qu et al. [30]. The male rats were divided into three groups each consisting of five rats and placed individually in separate propylene cages during the experiment. Group I represented the control group, which received 10 ml/kg of distilled water orally daily for 7 days. Group II received pomegranate extract orally at the dose 500, 1000 and 1500 mg/kg daily for 7 days. Group III served as the standard group and received suspension of the sildenafil drug orally at the dose of 5 mg/kg, 1 h prior to the commencement of the experiment. On the 8th day the test for penile reflexes was carried out by placing the rat on its back, in a glass cylinder for partial restraint. The preputial sheath was pushed behind for a period of 15 minutes. Such stimulation elicited a cluster of genital reflexes. The following parameters were recorded: Erection (E), Quick flips (QF) and Long flips (LF).

Determination of the concentration of testosterone

The study was carried out on male rats and concentration of testosterone was determined at the end of the experiment. The male rats were divided into five groups each consisting of five rats and placed individually in separate propylene cages during the experiment. Group 1 represented the control group, which received 10 ml/kg of distilled water orally daily for 8 weeks. Group 2 received pomegranate extract orally at the dose of 500, 1000 and 1500 mg/kg respectively daily for 8 weeks. Group 3 served as the standard group and did receive suspension of the sildenafil drug orally at the dose of 5 mg/kg, 1 hour prior to the commencement of the experiment.

After eight weeks of treatment, the rats were sacrificed using ether anaesthesia. Enough volume of blood was collected from each rats using a cardio-puncture method. All the samples were collected in the morning in order to minimize the diurnal variation of hormone levels. Bicinchoninic acid assay chemical (BCA) tablets were powdered to increase the dissolution surface area. Briefly, 25 mg of powder was transferred to a 100 ml volumetric flask. A volume of 60 ml methanol was added and solution centrifuged for total dissolution for 15 minutes at 1600 g. Methanol was added to the volume of 100 cm^3 and solution filtered through filter paper. Further dissolutions were made using methanol to get concentrations of 10 mg/ml. Absorbance was determined and the standard curve generated at 272 nm. The absorbance values were obtained from male rats treated with pomegranate extracts, sildenafil positive control and distilled water negative control administered orally to compare sexual parameters based on those treatments. Testosterone concentrations were calculated based on the absorbance values.

Data analysis

Data collected was computed to find the mean values and summarized in tables. The frequency of parameters observed in control, test and standard groups was statistically analyzed by using one-way analysis of variance (ANOVA) method. The significance of difference between the mean was determined with post-hoc 't' test. All the results were expressed as mean ± standard of the mean error and the level of significance for comparisons set at $p<0.05$.'

RESULTS

Effects of pomegranate extract on mating behavior in male rats

Mounting frequency in control group varied from 11.4 ± 0.2 to 13 ± 0.1, intromission frequency (4.4 ± 0.2-12.2 ± 0.2), mounting latency (11.8 ± 0.1-35.8 ± 0.2), intromission latency (15.0 ± 0.2-40.0 ± 0.1), ejaculatory latency (253.2 ± 0.2-344.0 ± 0.3) and post ejaculation interval (98.6 ± 0.3-364.0 ± 0.2) per second. The mounting frequency, intromission frequency, mounting latency, intromission latency, ejaculation latency and post ejaculation interval did not vary significantly ($p=0.997388$) (Table 1).

Effects of pomegranate extract on libido in male rats

The results obtained with the test for libido showed that the mounting frequency varied significantly between control, pomegranate and sildenafil ($p=2.67$). Intromission and Ejaculation were absent in control, pomegranate and sildenafil groups (Table 2).

Effect of pomegranate fruit extract on potency in male rats

The number of erections in control group varied from 7.6 ± 0.1 to 12.4 ± 0.2, quick flips (5.2 ± 0.1-17.2 ± 0.2), long flips (2.4 ± 0.2-12.0 ± 0.3) and total penile reflexes (15.2 ± 0.1-48. ± 20.1) per second. The potency did not vary significantly between control, pomegranate and sildenafil ($p=0.137802$) (Table 3).

Testosterone concentrations

The testosterone levels varied from 2.47 mg/ml in test animal 3 to 5.04 mg/ml in test animal 1. When sildenafil was used, the testosterone ranged between 3.88 mg/ml in test animal 3 to 5.64 mg/ml in test animal 5. However, the testosterone concentration varied from 0.086 mg/ml in test animal 1 to 3.22 mg/ml in test animal 5. The testosterone levels varied significantly between pomegranate, sildenafil and control group ($p=0.000229$) (Table 4).

Table 1: Mating behavior in male rats in seconds.

Parameters	Control	Pomegranate			Sildenafil
	10 ml/kg	500 mg/kg	1000 mg/kg	1500 mg/kg	5 mg/kg
Mounting frequency	11.4 ± 0.2	14.4 ± 0.2	24 ± 0.3	43.8 ± 0.1	48.2 ± 0.3
Intromission frequency	4.4 ± 0.2	4.4 ± 0.3	8.0 ± 0.2	12.2 ± 0.2	24.2 ± 0.1
Mounting latency	35.2 ± 0.2	35.8 ± 0.2	28.6 ± 0.3	22.8 ± 0.3	11.8 ± 0.1
Intromission latency	40.0 ± 0.1	37.8 ± 0.2	34.2 ± 0.2	27.6 ± 0.1	15.0 ± 0.2
Ejaculatory latency	246.8 ± 0.3	253.2 ± 0.2	268.0 ± 0.3	294.0 ± 0.2	344.0 ± 0.3
Post Ejaculation interval	364.0 ± 0.2	336.2 ± 0.2	301.2 ± 0.3	224 ± 0.2	98.6 ± 0.3

Tabular values are mean ± SEM, n=5.

Table 2: Effect of pomegranate fruit extract on mounting frequency (libido test) in male rats.

Parameters	Control	Pomegranate			Sildenafil
	10 ml/kg	500 mg/kg	1000 mg/kg	1500 mg/kg	5 mg/kg
Mounting frequency	6.40 ± 0.24	7.40 ± 0.51	8.00 ± 0.71	14.60 ± 0.51	22.60 ± 1.03
Intromission frequency	Nil	Nil	Nil	Nil	Nil
Ejaculation	Absent	Absent	Absent	Absent	Absent

Tabular values are mean ± SEM, n=5.

Table 3: Effect of pomegranate fruit extract on penile reflexes (test for potency) in male rats.

Parameters	Control	Pomegranate			Sildenafil
	10 ml/kg	500 mg/kg	1000 mg/kg	1500 mg/kg	5 mg/kg
Erections	7.6 ± 0.1	7.4 ± 0.2	8.2 ± 0.3	12.4 ± 0.2	19.0 ± 0.2
Quick flips	5.2 ± 0.1	5.4 ± 0.1	5.6 ± 0.2	8.4 ± 0.1	17.2 ± 0.2
Long flips	2.4 ± 0.2	3.4 ± 0.2	4.6 ± 0.1	8.4 ± 0.2	12.0 ± 0.3
Total penile reflexes	15.2±0.1	16.4±0.2	18.4±0.2	29.2±0.3	48.2±0.1

Table 4: Testosterone concentrations in mg/ml × 10^{-3}.

Test animal	Pomegranate	Sildenafil	Control
1	5.04 ± 0.1	5.46 ± 0.2	0.086 ± 0.2
2	3.90 ± 0.2	4.86 ± 0.2	0.199 ± 0.1
3	2.47 ± 0.3	3.88 ± 0.1	0.236 ± 0.2
4	2.50 ± 0.2	4.02 ± 0.3	2.88 ± 0.2
5	4.80 ± 0.2	5.64 ± 0.3	3.22 ± 0.1
Mean	**3.74 ± 0.1**	**4.77 ± 0.2**	**1.32 ± 0.1**

Tabular values are mean ± SEM, n=5.

DISCUSSION

The results on mounting frequency obtained in this study indicated that the mounting frequency increased with increase in the concentration of pomegranate extract. The mounting frequencies from the highest concentration of pomegranate extract was comparable with those of sildenafil. These results agreed with a study by Fedder et al.. The possible results could be attributed to extraction of the same compounds [31].

In the current study, intromission frequency increased with concentration in pomegranate. However, the intromission frequencies in the highest concentration of pomegranate were lower than in sildenafil. This may be attributed to the crude nature of pomegranate as compared to the pure form of sildenafil [32].

The mean mounting latency increased from sildenafil to control group. In addition, the mean mounting latency reduced with concentration in pomegranate. This agreed to a study carried out by Fayed et al. in Egypt on the effects of pomegranate peel on semen quality in rabbits. This may be attributed to the plants from which the crude extracts were obtained accumulating the same active compounds [33].

However, the mean intromission latency reduced from control to sildenafil. The intromission latency reduced with concentration of pomegranate crude extracts. This can be associated with increased concentration of the active compounds within the crude extracts. This disagreed with a study by Hussen on the protective role of pomegranate peel extract on testis in adult male rabbits treated with pomegranate crude extract. The differences in the observed results in the two studies may be associated to differences in the ecological environment in which the pomegranate were growing [34].

The ejaculatory latency increased from control, pomegranate and sildenafil in the first series which was also the trend in the second series. The highest concentration of pomegranate gave almost similar results with sildenafil. This suggested that an increase in the concentration of pomegranate can give the same results as sildenafil. However, the post ejaculation interval reduced from control group to sildenafil. These results concurred with those of a

study by Misaka et al., on effect of pomegranate juice consumed by Japanese volunteers for two weeks [35].

The effect of the pomegranate fruit extract, on libido was studied by assessing the Mounting frequency (MF) after genital anaesthetization which does away with the reinforcing effect of intrinsic sexual desire. During the experiment the pomegranate fruit extract produced a significant increase in mounting frequency of sexually normal male rats. However, the highest concentration in pomegranate crude extracts didn't yield the same result as sildenafil. This could be attributed to low concentration of the active compounds in crude pomegranate [36]. The test for libido revealed that Intromission and Ejaculation were absent in all groups of animals, as the genital sensations which are absent due to penile anaesthetization are necessary for the development of these two events. Thus, it may be inferred that the pomegranate fruit extract, produced a striking increase in the intrinsic sex drive or 'pure' libido.

The results on mean potency of crude extract obtained in the current study agreed with a study carried out on protective role of pomegranate peel extract on testis in adult male rabbits treated with pomegranate crude extracts by Riad et. Possible reason could be use of the same solvents with the same polarity in extracting active compounds from pomegranate. The quick flips increased with increase in concentration of the pomegranate extract. The same trend was observed for the long flips and the total penile reflexes indicating high presence of the active metabolites associated with high concentration of the crude extracts. The possible reason for the difference could be the soil physico-chemical characteristics in which the plants were growing. Lansky et al. asserted that the composition of the soil that pomegranate grows in greatly influences the metabolites it will synthesize [37-42].

The testosterone concentration increased from the animals treated with distilled water to those treated with sildenafil. However, the testosterone concentration in animals treated with pomegranate was comparable to those treated with sildenafil. The results agreed with a studies carried out on effect of pomegranate pre-treatment on the oral bioavailability of buspirone in male albino rats. Similarity in the metabolic activities of the rats may be a contributing factor [43,44].

CONCLUSION AND RECOMMENDATION

Pomegranate fruit extracts has the potential of enhancing sexual behaviour in rats. There is need for mass production of pomegranate extracts for use in enhancing sexual behaviour in human beings. Further research is also needed for the identification of its active constituent (s) responsible for sexual function improving activities.

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