

Aphrodisiac Activity of Pomegranate (*Punica granatum* L.) fruit extract on the sexual function in rats.



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ABSTRACT

Punica granatum L. has proven to possess aphrodisiac effects in the management of male sexual disorder. There being no scientific validation of these claims, *Punica granatum* fruit extract was investigated for its aphrodisiac activity in male rats.

Fifty (50) adult male and female albino rats of *Wistar* strain weighing 250-350 g and 200-250g respectively were used in this study. The pomegranate fruit extract was orally administered (500, 1000 and 1500 mg/kg) to different groups of male rats on a once-daily regime for seven (7) days.

Oral administration of *Punica granatum* L. fruit extract at the dose of 1500 mg/kg produced a significant increase of sexual activity in male rats. It caused a significant increase in Mounting Frequency,

Intromission Frequency, Intromission Latency and caused a significant reduction in Mounting Latency and Post Ejaculatory Intervals. It also significantly increased Mounting Frequency with Penile anaesthetization as well as frequencies of Erection, Quick and Flips, Long Flips and the aggregate of penile reflexes with penile stimulation. In addition, the extract was also observed to be devoid of any adverse effects and acute toxicity.

The resultant significant and sustained increase in the sexual activity of normal male rats without any conspicuous adverse effects indicate that *Punica granatum* certainly has aphrodisiac activity particularly at the dose of 1500 mg/kg and lends support to the traditional claim.

Keywords: Pomegranate (*Punica granatum* L.), Aphrodisiac, Sexual behavior, Erectile dysfunction.

INTRODUCTION

Erectile dysfunction (ED) also called impotence, is the inability to attain and maintain a penile erection that is sufficient to sustain satisfactory sexual activity for both partners (Njila et al., 2018). Erectile dysfunction is one of the most prevalent kinds of sexual debilitation which is set to increase over time. The global prevalence of ED in 1995 was estimated to be over 152 million men and the projections for 2025 shows a prevalence of approximately 322 million with ED, an increase of nearly 170 million men (Rakuambo et al., 2012). Just over half of all men experience erectile dysfunction (ED) and at age 40 and above, about 40% experience light to moderate ED and by age 70, men will experience 70% chance of erectile dysfunction. Impotence can trigger a feeling of low esteem and depression which further leads to anticipatory anxiety. It also affects one's masculinity and confidence, and emotional fall out as well for their partners. Men suffering from sexual dysfunction can be successful at reversing their problem by a focus on lifestyle factors and not just relying on conventional aphrodisiac drugs. Many drugs like sildenafil (Viagra) have side

effects ranging from sudden vision loss, chest pain, nausea, general ill-health to irregular heart-beat. Recent focus has shifted to the effectiveness of natural products on sexual function such as pomegranate which has been shown to have significant biological effects.

Pomegranate a predominant member of the *Punicaceae* family has proven to exhibit important physiological properties, such as anticancer (Afaq et al., 2005; Lansky, 2005) anti-proliferative, apoptotic (Seeram et al., 2005), HIV-I entry inhibitory (Neurath et al., 2004), cardioprotective, antihyperlipidemic, anti-inflammatory, anti-mutagenic, anti-bacterial activities and as a powerful antioxidant and antifungal substance (Tehranifar et al., 2011). Animal studies have shown that pomegranate juice intake increases testosterone levels in men, one of the main hormones behind sex drive (Emad and Nacer, 2014) also increases the number of motile sperm (Fedder et al., 2014) and on semen quality (Fayed et al., 2012; Turk et al., 2008). Therapeutically beneficial constituents of pomegranate include ellagic acid, ellagitannins, punicalagins, punic acid, flavonoids, anthocyanidins,

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Cite This Article: Katana, L.K., Maina, C.I., Orenge, C.O. 2020. Aphrodisiac Activity of Pomegranate (*Punica granatum* L.) fruit extract on the sexual function in rats. *Discovery Phytomedicine* 7(2): 65-71. DOI: 10.15562/phytomedicine.2019.121

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antocyanins and estragenic flavones (Seeram et al., 2005; Bachoual et al., 2011).

Throughout history, pomegranate symbolizes fertility, rebirth, health and wellbeing (Mansour et al., 2013). It has been postulated to enhance sexual drive and believed to help fight erectile dysfunction among men but there was no scientific validation of these claims. This prompted an investigation on the aphrodisiac activity of pomegranate (*Punica granatum* L.) fruit extract on sexual function in male rats hence the present study.

MATERIALS AND METHODS

Experimental Animals

Health adult male and female albino rats of *Wistar* strain of three months old 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*. 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.

Extract preparation

Fresh *Punica granatum* fruits were obtained from a market in Njoro, Kenya. They were transported in cartons to the laboratory in the Department of Biological Sciences, Egerton University, Kenya. *Punica granatum* fruits were washed and rinsed with water. They were dried under sunlight until water droplets completely evaporated. Using a surgical blade the 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 cheesecloth were placed on a flat surface, one on top of the other. The blended seeds were poured into the centre of the cheesecloth, then the ends of the cheesecloth were brought together to make a bag. The cheesecloth bag was held over a beaker and squeezed until juice stopped coming out. The seed casings remained in the cheesecloth, and the *Punica granatum* extract was collected in the container. The pomegranate extract was covered with a container lid.

Chemicals and drug preparation

Sildenafil citrate was obtained from Teva Pharmaceuticals industries limited (Nairobi,

Kenya). Estradiol and Progesterone were procured from Sun Pharmaceutical Industries Limited (Mumbai, India), 5% xylocaine ointment was obtained from Lexicare Pharma PVT. LTD. (Nairobi, Kenya). And all other chemical and reagent used were of analytical reagent quality. Pomegranate extract was administered orally as a fine suspension in tween-80 (1%). Similarly, sildenafil citrate and ethinyl oestradiol were suspended in distilled water separately, using Tween-80 (1%) for oral use. Progesterone was dissolved in olive oil for subcutaneous injection. All the drug solutions were prepared just before administration.

Adverse effects

All treated rats were observed at least once daily for any overt sign of toxicity (salivation, rhinorrhea, lachrymation, ptosis, writhing, convulsions and tremors), stress (far erection and exophthalmia) and changes in behaviour such as spontaneous movement in the cage, climbing, cleaning of face. In addition, food and water intake was noted.

Acute toxicity testing

Determination of acute toxicity of the pomegranate fruit extract was done using the method described by Lorke (1983). Adult *Wistar* rats were randomly divided into five groups each consisting of five rats. The rats in group I, II, III and IV were administered with the aid of an orogastric tube, single doses (1000, 2000, 4000 and 5000 mg/kg body weight respectively) of the *Punica granatum* fruit extract orally. The last group served as control and was administered 1 ml distilled water orally. All the animals used for the acute toxicity study were observed continuously for behavioural changes and mortality and for the initial 4 hours and then intermittently for the next 6, 24, and 48 hours after dosing. The behaviour parameters observed were convulsion, hyperactivity, sedation, grooming, loss of right reflex and increased respiration. The rats were kept under the same conditions throughout the study.

Mating behaviour test

The effect of the *Punica granatum* extract on mating behaviour was studied according to the methods described by Dewsbury and Davis (1970). Healthy male rats were divided into five 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: 00h. Group 2-4, the test group, was treated with pomegranate extract at a dosage of 500 mg/kg, 1000 mg/kg and 1500 mg/kg respectively, orally in a once-daily regime for 7 days at 18: 00 h. Group 5, the standard group, was treated with sildenafil

drug which was administered orally at the dose of 5 mg/kg 1 h prior to the commencement of the experiment. The rats were brought to the laboratory for acclimatization and were exposed to dim light (Dewsbury, 1972) (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.

Female rats were artificially brought into oestrus by the method of Szechtman (1981). They were administered 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 (Srilatha, 1998). 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 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 animals were introduced into the cages of male animals with 1 female to 1 male (Yehia et al., 2011).

The occurrence of events and phases of mating were recorded on a video camera. Later, the frequencies and phases were determined from the video transcriptions: 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 (this period of refractoriness of the male rat to the female is usually observed after ejaculation) or following 30 minutes of sexual inactivity by the male rat from the time of introduction of the female into the test chamber (Zeweil et al., 2013).

Test for libido

The test was carried out using the method of Shravan et al. (2011). 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 2-4 received a suspension of pomegranate extract orally at the dose of 500 mg/kg, 1000 mg/kg and 1500 mg/kg respectively, daily for 7 days at 18: 00 h. Group 5 served as standard group and given a suspension of the standard drug (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 behaviour 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 was placed in the same cage. The number of mounting was noted and the animals were also observed for intromission and ejaculation (Teixeira da Silva et al., 2013).

Test for sexual potency

The test was carried out using a method described by Zeweil et al. (2013). 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 10ml/kg of distilled water orally daily for 7 days. Group 2-4 received pomegranate extract orally at the dose 500 mg/kg, 1000 mg/kg and 1500 mg/kg respectively daily for 7 days. Group 5 served as the standard group and did receive 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 15 min. Such stimulation elicits a cluster of genital reflexes. The following parameters were recorded: Erection (E), Quick Flips (QF) and Long Flips (LF) (Tehranifa et al., 2011).

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 (SEM) and the level of significance for comparisons set at $p < 0.05$, $p < 0.01$, and $p < 0.001$.

RESULTS

Adverse effects

There were no treatment-related overt signs of toxicity, stress and changes in behaviour observed. The food and water intake of all the treated rats remained similar in those of control group.

Acute toxicity studies

There were no mortality and changes in the behaviour observed in all the treated and control groups of rats up to a dose of 5000 mg/kg.

Effect of *Punica granatum* extract on mating behaviour

The results of mating behaviour test show that the *Punica granatum* fruit extract at the dose of

1500 mg/kg, significantly increased the Mounting Frequency (MF) ($P < 0.001$), Intromission Frequency (IF) ($P < 0.001$), Ejaculation Latency in first series (EL_1) ($P < 0.001$), Ejaculatory Latency in second series (EL_2) ($P < 0.001$). The extract caused a significant decrease in the Mounting Latency (ML) ($P < 0.001$), Intromission Latency ($P < 0.01$) and Post Ejaculatory Interval (PEI) ($P < 0.001$), as compared to control group. The dose of 1000 mg/kg of the extract significantly augmented the MF ($P < 0.001$), IF ($P < 0.01$) and significantly decreased the ML ($P < 0.01$), PEI ($P < 0.001$), IL ($P < 0.05$), EL_1 ($P < 0.05$) but did not significantly alter the EL_2 in comparison with the control group. Whereas, the extract at the dose of 500 mg/kg, significantly increased the MF ($P < 0.01$) and PEI ($P < 0.05$), but did not affect the

Table 1 Effect of *Punica granatum* extract on mating behaviour in male rats

Parameters	Mean \pm SEM				
	Control	Pomegranate			Sildenafil citrate
	10 ml/kg	500 mg/kg	1000 mg/kg	1500 mg/kg	5 mg/kg
Mounting frequency (MF)	11.40 \pm 0.51	14.4 \pm 0.24**	24.00 \pm 0.45***	43.80 \pm 0.37***	48.20 \pm 0.37***
Intromission Frequency (IF)	4.40 \pm 0.40	4.40 \pm 0.40 ^{NS}	8.00 \pm 0.32**	12.20 \pm 0.37***	24.20 \pm 0.45***
Mounting Latency (ML, in sec)	35.20 \pm 0.45	35.80 \pm 0.37 ^{NS}	28.60 \pm 0.40**	22.80 \pm 0.37**	11.80 \pm 0.37***
Intromission Latency (IL, in sec)	40.00 \pm 0.32	37.80 \pm 0.37 ^{NS}	34.20 \pm 0.63*	27.6 \pm 0.28***	15.00 \pm 0.89***
Ejaculatory Latency in first series (EL_1 , in sec)	198.60 \pm 0.40	206.80 \pm 3.22 ^{NS}	218.00 \pm 0.32*	235.00 \pm 0.77***	344.00 \pm 6.17***
Ejaculatory Latency in second series (EL_2 , in sec)	297.33 \pm 0.71	299.60 \pm 0.68 ^{NS}	318.00 \pm 0.32 ^{NS}	335.00 \pm 0.77***	344.00 \pm 6.17***
Post Ejaculatory Interval	364.00 \pm 1.05	336.20 \pm 1.07	301.20 \pm 0.86**	224.50 \pm 0.55	98.60 \pm 0.20***

Tabular values are mean \pm SEM, n = 5 (number of animals in each group); significant difference from control, NS: Not significant, *p < 0.05; **p < 0.01; ***p < 0.001.

Table 2 Effect of *Punica granatum* extract on mounting frequency (test for libido) in male rats

Parameters	Mean Frequency \pm SEM				
	Control	Pomegranate			Sildenafil citrate
	10 ml/kg	500 mg/kg	1000 mg/kg	1500 mg/kg	5 mg/kg
Mounting frequency (MF)	6.40 \pm 0.24	7.40 \pm 0.51*	8.00 \pm 0.71**	14.60 \pm 0.51***	22.60 \pm 1.03***
Intromission Frequency (IF)	0	0	0	0	0
Ejaculation (E)	0	0	0	0	0

Tabular values are mean \pm SEM, n = 5 (number of animals in each group); significant difference from control, *p < 0.05; **p < 0.01; ***p < 0.001

Table 3 Effect of *Punica granatum* extract on penile reflexes (test for potency) in male rats

Parameters	Mean \pm SEM				
	Control	Pomegranate			Sildenafil citrate
	10ml/kg	500 mg/kg	1000 mg/kg	1500 mg/kg	5 mg/kg
Erection (E)	7.60 \pm 0.24	7.40 \pm 0.24 ^{NS}	8.20 \pm 0.20*	12.40 \pm 0.24***	19.00 \pm 0.32***
Quick Flips (QF)	5.20 \pm 0.20	5.40 \pm 0.24 ^{NS}	5.60 \pm 0.24 ^{NS}	8.40 \pm 0.24***	17.20 \pm 0.20***
Long Flips (LF)	2.40 \pm 0.24	3.40 \pm 0.24 ^{NS}	4.60 \pm 0.24**	8.40 \pm 0.24***	12.00 \pm 0.24***
Total Penile Reflexes (TPR)	15.20 \pm 0.37	16.40 \pm 0.24 ^{NS}	18.40 \pm 0.24*	29.20 \pm 0.37***	48.20 \pm 0.20***

Tabular values are mean \pm SEM, n = 5 (number of animals in each group); significant difference from control, NS: Not significant. *p < 0.05; **p < 0.01; ***p < 0.001.

IF, ML, IL, EL₁ and EL₂ in a significant manner as compared to control group. However, the standard drug, sildenafil drug, increased the MF ($P < 0.001$), IF ($P < 0.001$), EL₁ ($P < 0.001$), EL₂ ($P < 0.001$) and PEI ($P < 0.001$) as well as decreased the ML ($P < 0.001$) and IL ($P < 0.001$) in a highly significant manner as compared to control animals (Table 1).

Effect of *Punica granatum* extract on libido

The results obtained with the test for libido show that the *Punica granatum* extract at the dose of 1500 mg/kg, 1000 mg/kg and 500 mg/kg, significantly augmented the Mounting Frequency (MF) ($p < 0.001$, $p < 0.01$ and $p < 0.05$ respectively) as compared to control group. The standard drug also significantly increased the MF ($p < 0.001$) as compared to control animals. Intromission and Ejaculation were absent in control, test and standard groups (Table 2).

Effect of *Punica granatum* extract on sexual potency

The test for potency revealed that the *Punica granatum* extract at a dose 1500 mg/kg, significantly increased the frequency of Erections (E) ($p < 0.001$), Quick Flips (QF) ($p < 0.001$) and Long Flips (LF) ($p < 0.001$) as well as the aggregate of these penile reflexes (TPR) ($p < 0.001$) in comparison with the control group. The test drug at the dose 1000 mg/kg significantly increased the E ($p < 0.05$), LF ($p < 0.01$) and TPR ($p < 0.05$) but did not significantly affect the QF. Whereas the *Punica granatum* extract at the dose of 500 mg/kg, did not alter the E, QF, LF and TPR. The standard drug also significantly increased the E ($p < 0.001$), QF ($p < 0.001$), LF ($p < 0.001$) and TPR ($p < 0.001$) with respect to the control animals (Table 3).

DISCUSSION

The study exhibits a marked change in sexual behaviour of male rats. The results of this investigation show that the test drug significantly increased the Mounting Frequency (MF) and Intromission Frequency (IF) as compared to control group. However, the standard drug produced a greater increase in these parameters. The results agreed with a study by Fedder et al., (2014). The possible results could be attributed to extraction of the same compounds in the two studies.

The significant increase in the Ejaculatory Latency (EL) suggests that the extract and standard drug prolonged the duration of coitus. The significant increase in the EL in both first and second series as well as the decrease in Post Ejaculatory

Interval (PEI), i.e. the refractory period between first and second series of mating, suggest that the test drug intensified sexual activity in a sustained manner. These results concurred with those of a previous study carried out in Japan (Misaka et al., 2011).

The *Punica granatum* fruit extract also caused a significant reduction in the Mounting Latency (ML) and Intromission Latency (IL) as compared to control animals, while a highly significant decrease was observed in the ML, of animals treated with the referent drug. This agreed with a study carried out by Zeweil et al., (2013) in Egypt. This may be attributed to the plants from which the extracts were obtained accumulating the same active compounds. This also provides evidence for aphrodisiac effect of the test drug (Sonu and Ashish, 2017). These findings show that the test drug, *Punica granatum*, produces a striking enhancement of over-all sexual performance of normal animals.

Mounting Frequency (MF) after penile anaesthetization of rats is a reliable index of libido and penile reflexes of the rats are a good model of potency (Davidson, 1982). Therefore, in this study, the extract was also studied for effect on these components of sexual behaviour. The effect of the test drug, *Punica granatum*, 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 test drug produced a significant increase in MF of sexually normal male rats. Whereas, the MF was much reduced in control, test and standard animals in comparison with the MF of corresponding groups in mating behaviour test where the penis had not been anaesthetized. However, 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 test drug produced a striking increase in the intrinsic sex drive.

The test for sexual potency exhibited that the *Punica granatum* fruit extract significantly increased the frequency of all the components of penile reflexes: Erections (E), Quick Flips (QF) and Long Flips (LF) as compared to control group, but comparatively less than the standard drug, sildenafil citrate. The aggregate of these penile reflexes (TPR) was also significantly increased in both test and standard animals. This indicates that the pomegranate (*Punica granatum*) fruit extract increases potency also. This agreed with a previous study carried out on the protective role of pomegranate peel extract on testis in adult male rabbits treated

with pomegranate crude extracts by (Hussen and Arrack, 2014). Possible reason could be the use of the same solvents with the same polarity in extracting the active compound from *Punica granatum* in the two studies (Chauhan and Dixit, 2010).

When an extract or active fraction of a drug is used it is better to evaluate its possible toxicity. Although it is the normal practice to determine the LD₅₀ value, now it is acceptable to limit the study to an acute toxicity test using multiple doses including reasonably high doses of the drug (Babu et al., 2003). In this connection, the *Punica granatum* fruit extract was subjected to an acute toxicity testing and it was tested up to a high concentration of 5000 mg/kg, orally. The acute toxicity study carried out on the fruit extract revealed no mortality at any of the doses used within the first 24 hours and during the 48 hours of observation. The absence of mortality at doses of 5000 mg/kg suggests that these plants are very safe explaining why they are consumed without restrictions.

CONCLUSION

Punica granatum fruit extract appears to possess sexual enhancement properties as reflected in the sexual behaviour studies. The extract produced a striking increase in the intrinsic sex drive. Along with increasing libido, the extract also increases potency. The resultant significant and sustained increase in the sexual activity of male rats, without any conspicuous adverse effects and toxicity, suggests that *Punica granatum* (pomegranate) possesses clinical applicable aphrodisiac properties, and lends support to the claims for its traditional usage as sexual function enhancing medicine. Further, the study also indicates that the aphrodisiac effects of the pomegranate fruit extract may be due to an effective and safe alternative remedy in sexual disorders.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

ACKNOWLEDGEMENT

The authors are grateful to Muriuki Githaiga and Dickson Ayeka of zoology section, Department of Biological Sciences, University of Egerton, Njoro, Kenya, for their assistance in handling and treating the animals under study. The authors are also grateful to Peter Amwoga Department of Biological Sciences, Edith Bett and Collins Kipkorir Kirui for their support in data collection during research.

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