

APHRODISIAC ACTIVITY OF *VITIS VINIFERA* JUICE IN WISTAR RATS

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Abstract

Background: It has been known for over 6000 years that *Vitis vinifera*, grapes have therapeutic properties. The grape's leaves have been used to treat diarrhea. The grape leaves were also used to treat bleeding, inflammatory, and analgesic diseases. Sore throats have been treated with unripe grapes. Constipation was treated with raisins.

In this study, we assessed the aphrodisiac potential of *Vitis vinifera* juice in Wistar rats.

Material and method: In this study mounting behaviour test, mating performance test and orientation activities of male rats were assessed to observe the effect of the juice on the sexual behaviour of the rats. The juice was given orally by gavage at the two doses of 4 ml/kg and 8 ml/kg. Sildenafil citrate (5 mg/kg) was utilized as the control drug.

Result: The results of the analysis reveal that the number of mounts was significantly increased in treated groups when compared with the saline-treated group. the number of sperm positive females per cage was significantly increased in treated groups when compared with the saline-treated group. the number of anogenital sniffing, lickings, climbing's and genital grooming were significantly increased in treated groups when compared with the saline-treated group.

Conclusion: *Vitis vinifera* juice has shown a significant aphrodisiac activity in Wistar rats. This activity could be due to its elevating effect on noradrenaline levels in the brain of the animals.

Keywords: *Vitis vinifera*, Aphrodisiac activity, Mounting behaviour, Mating performance.

INTRODUCTION:

For at least 5,000 years, natural ingredients have been utilized as medicines. Plant medicines have been a part of human health care since prehistoric times. Ancient Egyptian, Chinese, Hebrew, Indian, Japanese, and Persian societies have all successfully employed herbal remedies in their medicinal practices. According to recent research, it continues to be the most popular form of healthcare for contemporary people. According to scientific research, about 85,000 plant species have been identified as medicinal out of 4,22,000 flowering plant species. A World Health Organization (WHO) survey found that between 75 and 80 percent of individuals worldwide take herbal medications (Byard, 2010).

It has been known for over 6000 years that *Vitis vinifera* grapes have therapeutic properties (Ziskind & Halioua, 2007). The grape's leaves have been used to treat diarrhea. The grape leaves were also used to treat bleeding, inflammatory, and analgesic diseases. Sore throats have been treated with unripe grapes. Constipation was treated with raisins (Ali, Maltese, Choi & Verpoorte, 2009). Some ancient Greek scholars praised the grapes' ability to recover more frequently when they were made into wine (Sun, Ribes, Leandro, Belchior & Spranger, 2006). According to Shi, Yu, Pohorly, and Kakuda (2003); rounded, ripe, and delicious grapes were used to treat a number of health issues, including smallpox, nausea, eye, liver, and skin diseases.

A growing body of research has linked the consumption of wine and grape juice to several health benefits, including a lower risk of heart disease, some types of cancer, type-2 diabetes, and other chronic illnesses (Preuss, Bagchi & Bagchi, 2002; Lasuncio, Castilla, Echarri, Ortega & Teruel, 2003; Brooker et al., 2006; Kar, Laight, Rooprai, Shaw & Cummings, 2009; Mellen, Daniel, Brosnihan, Hansen & Herrington, 2010). In this study, the aphrodisiac activity of the *Vitis vinifera* fruits is assessed in Wistar rats.

MATERIALS AND METHODS:

The collection of the herb and preparation of the juice:

The White Kishmish variety of *Vitis vinifera* fruits were purchased from local markets in Karachi, Pakistan. Following verification, the fruits were squeezed through a cotton cloth to extract fresh juice. The juice was extracted from fresh fruit each day. About 80–100 ml/100 g was the yield.

Animal Selection:

Wistar rats (160 to 220 g) were selected for this study. Polypropylene cages were used for the housing of the rats and six rats were housed in each cage. The animals were provided with standard rat pellets and water *ad libitum*. The animals were deprived of the food six hours before the drug administration and during the conduct of the experimental tests.

DIVISION OF RATS INTO DIFFERENT GROUPS:

Division of rats into different groups was carried out in the following manner.

Group I: Normal control, given normal saline 8 ml/kg, *p.o.*

Group II: Treatment group, given *Vitis vinifera* juice (VVJ) 4 ml/kg, *p.o.*;

Group III: Treatment group, given *Vitis vinifera* juice (VVJ) 8 ml/kg, *p.o.*;

Group IV: Positive control, given sildenafil citrate 5 mg/kg, *p.o.*

DOSE:

Vitis vinifera juice and normal saline and sildenafil citrate were administered through oral gavage. The dosing was carried out once daily at 10 a.m. for 60 days. Different parameters were observed to determine the aphrodisiac activity of the juice. These parameters include the mounting behaviour, mating performance, and orientation activities towards females, towards the environment, and towards self.

MOUNTING BEHAVIOUR TEST:

The mount is operationally characterized as the male expecting the copulatory position, yet neglecting to accomplish intromission. To evaluate mounting recurrence, the mounting behaviour test was conducted. Male rats, treated with the juice (4 ml/kg and 8 ml/kg; *p.o.*), were allowed to pair with non-oestrus female rats. The rats were kept under observation for 3 hours testing period and their practices were scored on the 7th, 15th, 30th, and 60th day of dosing. Male rats were put separately in glass cages. A non-oestrus female was brought into every cage after 15 min of acclimatization period. The quantity of mounts was noted amid a 15 min observation period at the start of the first hour. At that point the female was withdrawn from every enclosure for a time of 105 min. Again, the female was presented and the quantity of mounts was noted for 15 minutes as before at the third hour. Every one of the examinations were done between 9:00 a.m. to 12:00 p.m. amid daytime at room temperature (Tajuddin, Ahmad, Latif & Qasmi, 2003; Aslam & Sial, 2014).

ASSESSMENT OF MATING PERFORMANCE:

The female rats were brought into oestrus (heat) in an artificial manner by the successive administration of progesterone (0.5 mg/100 g body weight) and estradiol benzoate (10 g/100 g body weight) through subcutaneous injections, 4 hours and 48 hours, respectively. Male rats were individually introduced in glass cages. Following an acclimatization period of 15 min, five oestrus females were introduced in each cage and the rats were allowed to cohabit overnight. The next day vaginal smear of every female mouse was microscopically examined for the presence of any sperms. The quantity of sperm positive female rats was noted in every group (Tajuddin, Ahmad, Latif & Qasmi, 2003; Aslam & Sial, 2014).

ASSESSMENT OF THE ORIENTATION ACTIVITIES OF MALE RATS:

The evaluation of orientation behaviour of every male rat towards the environment, and towards self and towards females was carried out to confirm the aphrodisiac activity of the juice. Orientation behaviour test was conducted as per method defined elsewhere (Aslam & Sial, 2014). The number of climbing's, anogenital sniffing, genital grooming and lickings were noted for a period of one hour.

STATISTICAL ANALYSIS:

One-way ANOVA and Newman-Keuls *post hoc* test were used to calculate the statistical significance. The data is expressed as mean \pm SEM. Statistically significant difference was accepted at $P < 0.05$. GraphPad Prism version 5.00 was used as statistical software.

RESULTS:

MOUNTING BEHAVIOUR TEST:

The means of treated groups (VVJ 4 ml/kg and VVJ 8 ml/kg) were compared with the means of the normal control (saline-treated) group using Newman-Keuls *Post hoc* test. The results of the analysis reveal that the number of mounts was significantly increased in treated groups when compared with the saline-treated group. Moreover, administration of sildenafil citrate (5 mg/kg, *p.o.*) significantly increased the number of mounts (Figure 1 and 2).

The test was conducted on 7th, 15th, 30th and 60th day of the study, that is, the animals were examined 4 times during the 60 days of the study.

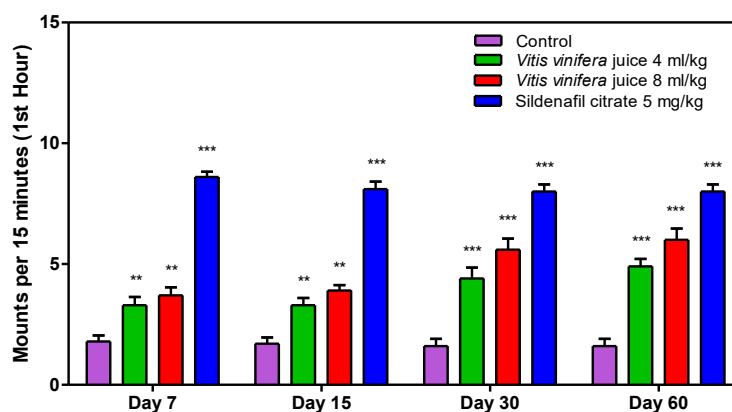


Figure 1. Effect of *Vitis vinifera* juice on number of mounts per 15 min during 1st hour.

Number of animals per group (n) = 10. The observations are given as mean \pm S.E.M.

*** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$; ANOVA followed by Newman-Keuls test.

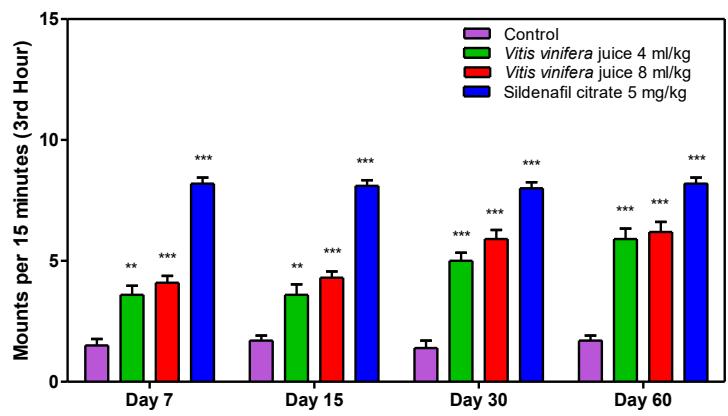


Figure 2. Effect of *Vitis vinifera* juice on number of mounts per 15 min during 3rd hour.

Number of animals per group (n) = 10. The observations are given as mean ± S.E.M.

***P<0.001, **P<0.01, *P<0.05; ANOVA followed by Newman-Keuls test.

MATING PERFORMANCE TEST:

The means of treated groups (VVJ 4 ml/kg and VVJ 8 ml/kg) were compared with the means of the normal control (saline-treated) group using Newman-Keuls *Post hoc* test. The results of the analysis reveal that the number of sperm positive females per cage was significantly increased in treated groups when compared with the saline-treated group. Moreover, administration of sildenafil citrate (5 mg/kg, *p.o.*) significantly increased the number of sperm positive females per cage (Figure 3).

The test was conducted on 7th, 15th, 30th and 60th day of the study, that is, the animals were examined 4 times during the 60 days of the study.

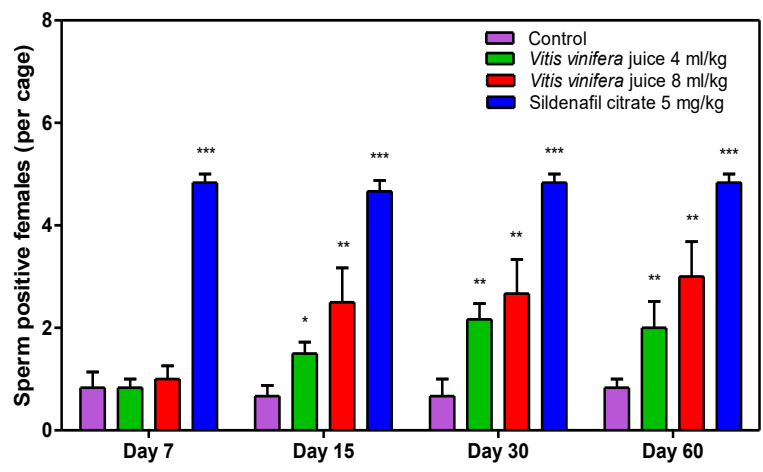


Figure 3. Effect of *Vitis vinifera* juice on mating performance of male rats.

Number of animals per group (n) = 10. The observations are given as mean ± S.E.M.

***P<0.001, **P<0.01, *P<0.05; ANOVA followed by Newman-Keuls test.

ORIENTATION BEHAVIOUR TEST:

The means of treated groups (VVJ 4 ml/kg and VVJ 8 ml/kg) were compared with the means of the normal control (saline-treated) group using Newman-Keuls *Post hoc* test. The results of the analysis reveal that the number of anogenital sniffing, lickings, climbing's and genital grooming's were significantly increased in treated groups when compared with the saline-treated group. Moreover, administration of sildenafil citrate (5 mg/kg, *p.o.*) significantly increased the number of anogenital sniffing, lickings, climbing's and genital grooming's (Figure 4, 5, 6 and 7). The test was conducted on 7th, 15th, 30th and 60th day of the study, that is, the animals were examined 4 times during the 60 days of the study.

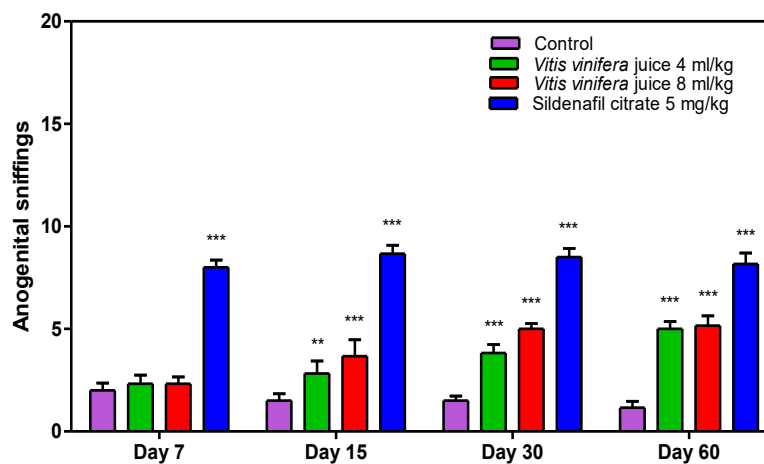


Figure 4. Effect of *Vitis vinifera* juice on anogenital sniffing.

Number of animals per group (n) = 10. The observations are given as mean \pm S.E.M.
 ***P<0.001, **P<0.01, *P<0.05; ANOVA followed by Newman-Keuls test.

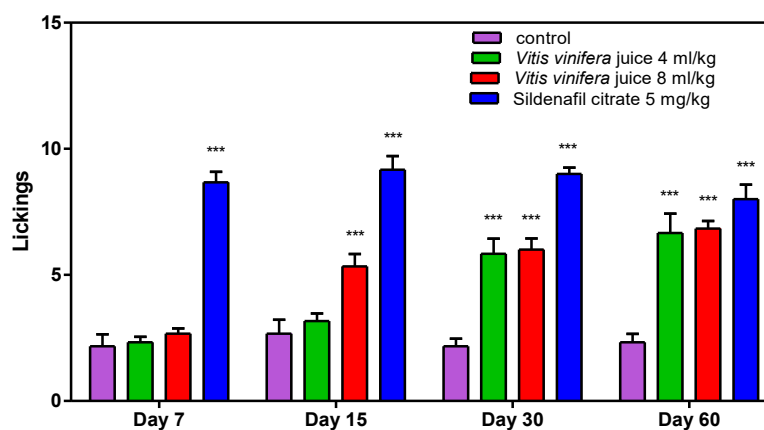


Figure 5. Effect of *Vitis vinifera* juice on number of lickings.

Number of animals per group (n) = 10. The observations are given as mean \pm S.E.M.
 ***P<0.001, **P<0.01, *P<0.05; ANOVA followed by Newman-Keuls test.

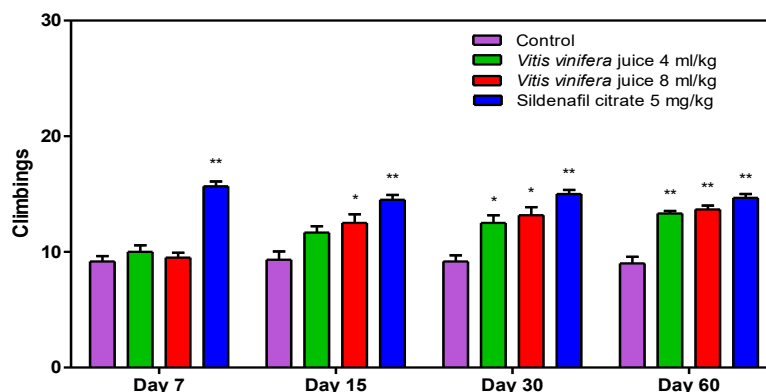


Figure 6. Effect of *Vitis vinifera* juice on climbing's.

Number of animals per group (n) = 10. The observations are given as mean \pm S.E.M.

***P<0.001, **P<0.01, *P<0.05; ANOVA followed by Newman-Keuls test.

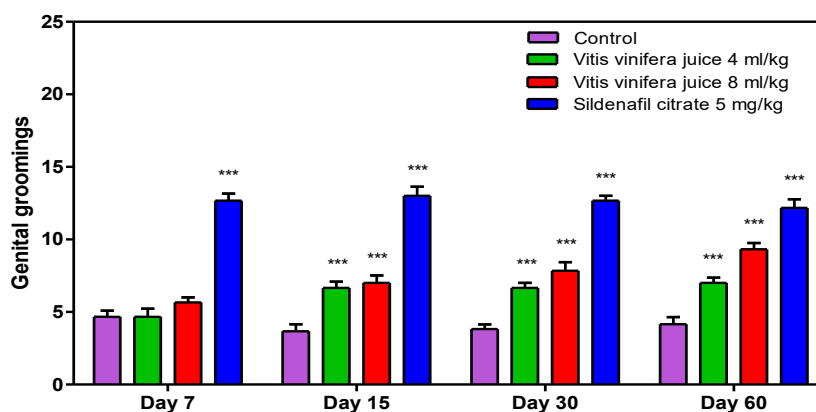


Figure 7. Effect of *Vitis vinifera* juice on genital grooming's.

Number of animals per group (n) = 10. The observations are given as mean \pm S.E.M.

***P<0.001, **P<0.01, *P<0.05; ANOVA followed by Newman-Keuls test.

DISCUSSION:

In this study, *Vitis vinifera* juice has enhanced the sexual activity of rats as evidenced by a significant increase in number of mounts in mounting behaviour test and significant increase in the number of sperm positive females in mating performance test. The aphrodisiac activity of the *Vitis vinifera* juice can be attributed to its neurochemical effect. Noradrenaline has a significant role in sexual behaviour of male rodents. Elevated levels have a positive effect on sexual behaviour (Hull & Dominguez, 2007). The results of our study show that *Vitis vinifera* juice caused significant elevations in the levels of noradrenaline in the rodent brain. This effect of the juice might be responsible for aphrodisiac activity in the rats. In addition, *Vitis vinifera* is a rich source of proanthocyanidins and procyanidins. Proanthocyanidins and procyanidins are potent antioxidant and free radical scavengers. They react with free radicals and form more stable radicals with lower toxicity. Proanthocyanidins have significant neuroprotective as well as cardiovascular protective effect (Natella, Belevi,

Gentili, Ursini & Scaccini, 2002; Sharma, Tyagi, Singh, Chan & Agarwal, 2004; Zhang et al., 2006). Therefore, it may be that the antioxidative potential of *Vitis vinifera* might have protected the adrenergic neurons against oxidative stress and their number has increased; correspondingly sexual behaviour has also been increased.

In another study, it was observed that with the consumption of *Vitis vinifera* juice the concentration of noradrenaline (NA) was significantly increased in the rodent brain, but the levels of dopamine were significantly decreased. As dopamine is converted into noradrenaline (NA) through the action of dopamine β -hydroxylase, so it might be that the levels of dopamine have been decreased due to the increased synthesis of noradrenaline (NA). (Aslam, 2015)

Noradrenergic drugs have been reported to facilitate sexual behaviour in rodents (Clark, Smith & Davidson, 1984; Clark, Smith & Davidson, 1985; Rodríguez-Manzo, 1999; Smith, Lee, Schnur & Davidson, 1987; Tallentire, McRae, Spedding, Clark & Vickery, 1996). The sexual behaviour enhancing effect of the *Vitis vinifera* juice may be due to its elevating effect on noradrenaline levels in the brain of the animals.

CONCLUSION:

Vitis vinifera juice has shown a significant aphrodisiac activity in Wistar rats. This activity could be due to its elevating effect on noradrenaline levels in the brain of the animals.

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