CODEN [USA]: IAJPBB ISSN : 2349-7750

INDO AMERICAN JOURNAL OF
PHARMACEUTICAL SCIENCES

SJIF Impact Factor: 7.187
https://doi.org/10.5281/zenodo.6820851

Available online at: http://www.iajps.com *Research Article*

APHRODISIAC ACTIVITY OF AQUEOUS EXTRACT OF AMARANTHUS TRICOLOR L. IN WISTAR RATS

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Article Received: May 2022 **Accepted:** June 2022 **Published:** July 2022

Abstract:
The Leaves of Amaranthus tricolor are popular for their aphrodisiac and immunostimulatory properties. The herbs have been traditionally used as Vajikaran Rasayana herbs because of their putative positive influence on sexual performance in humans. Hydroalcoholic extract of Amaranthus tricolor leaves were studied for sexual behavior effects in male albino rats and compared with untreated control group animals. The rats were evaluated for effect of treatments on anabolic effect. Seven measures of sexual behavior were evaluated. Administration of 300 mg/kg body weight of the Hydroalcoholic extract of Amaranthus tricolor leaves had pronounced anabolic effect in treated animals as evidenced by weight gains in the body and reproductive organs. There was a significant variation in the sexual behavior of animals as reflected by reduction of mount latency, ejaculation latency, post ejaculatory latency, intromission latency, and an increase of mount frequency. Penile erection (indicated by Penile Erection Index) was also considerably enhanced. Reduced hesitation time (an indicator of attraction towards female in treated rats) also indicated an improvement in sexual behavior of extract treated animals. The observed effects appear to be attributable to the testosterone-like effects of the extracts.

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*Please cite this article in press Balweer SIngh Kirar et al,**Aphrodisiac activity of aqueous extract of amaranthus tricolor l. In wistar rats.,**Indo Am. J. P. Sci, 2022; 09(7).*

INTRODUCTION:

Male sexual dysfunction, which includes erectile dysfunction (ED) and premature ejaculation, is the most common problem that contributes to infertility, distress, relationship problems, deterioration of self-image, and quality of life.[1,2] Erectile dysfunction has been identified as the persistent inability to attain and maintain penile erection sufficient for satisfactory sexual performance. [3] The predisposing factors for ED include chronic heart disease, high cholesterol, diabetes mellitus, smoking, alcohol, drug abuse, stress, food habits, and increasing age. Epidemiological studies have demonstrated a high prevalence of ED in developed countries, and therefore it is considered to be an important health problem. [1] Currently, modalities including psychotherapy, surgery, mechanical devices, drugs, and penile implants are used for the management of ED.[1] Drug therapy today mainly focuses on phosphodiesterase type 5 inhibitors, which increase the levels of cyclic Guanosine Mono-Phosphate (cGMP) in the cavernosal vasculature, leading to facilitation and prolongation of penile erection. [4]

However, as sexual dysfunction has been shown to be more prevalent in the aging population, [1] the evaluation of investigational drugs in aged and sexually sluggish males for their aphrodisiac and sexual tonic activity is a better predictor for their efficacy in the clinical setting.

The present study was thus carried out to evaluate the aphrodisiac activity of single and repeated dose administration of Hydroalcoholic extract of *Amaranthus tricolor* (HEAT) ,in aged and sexually sluggish male albino rats. Additionally, in order to evaluate the effect of the Hydroalcoholic extract of *Amaranthus tricolor*(HEAT)treatment on testosterone levels and its feedback inhibition of gonadotropin release, we estimated the testosterone levels and sperm count after repeated dose (HEAT) administration.

MATERIAL AND METHOD:

Aphrodisiac Activity:

Animals:

Wistar strain albino rats of either sex weighing 220–225 g were fed on standard pellet diet and water ad libitum. A total of 60 male rats naive to sexual behavior prior to experimentation were used for the present study. The animals were housed at room temperature (24 ± 2C) on a reversed daynight cycle (06:00 h to 18:00 h). All the animals were allowed to acclimatize in the test cage 7 days prior to experimentation. The experiment was conducted under dim red light and the behavioral aspects were video recorded using a digital camera. Observational and behavioral analyses were performed in a wooden chamber with a glass wall (70 x 40 x 60 cm) under diffused red light in the dark phase of the light-dark cycle. The chamber had a special small opening at the side for introducing the female as stimulus.

Preparation of Test Samples:

Hydroalcoholic extract of Leaves of *Amaranthus tricolor* administered orally as suspension in 0.2% PVP solution (vehicle) using metal canula. Marketed preparation of testosterone (Aquaviron, Nicholas Piramal India Ltd.) was purchased from the market, suspended in arachis oil, and administered intramuscularly.

Treatment:

Sexually naive Wistar strain male albino rats weighing between 220 ± 2 gm were divided in groups of six each and marked appropriately. The rats were randomly divided into 8 groups comprising 6 animals each.

The first frou groups were randomized as Set 1 animals. The animals of this set were treated for 28 days continuously and were subjected to sexual behavior analysis on Day 28 of treatment. Similarly, the other four groups were considered as Set 2 animals. This set of animals was specifically subjected to analysis of hesitation time, attraction towards female rats, and anabolic activity.

Two different sets were used in order to ensure that the animals for evaluation of sexual behavior (Set 1) remained naive to sexual interaction until the experimentation was carried out on Day 28 and, therefore, the possibility of a carryover effect was minimized.

The treatment for various groups of rats comprising Set 1 and Set 2 was as follows:

Group I was administered vehicle only and served as control. Group II was administered a daily dose of 200 mg/kg b.w. of Hydroalcoholic extract of *Amaranthus tricolor*,III was given 300 mg/kg b.w. of Hydroalcoholic extract of *Amaranthus tricolor* while Group IV was administered 0.5 mg/kg b. w. suspension of testosterone propionate in arachis oil twice weekly intramuscularly. Female animals were of the same strain and were prepared for experimentation by using the method reported by Agmo (2003) [5].

Group I—Control (treated with vehicle only)
Group II—Hydroalcoholic extract of *Amaranthus tricolor* (200 mg/kg b.w.) p.o. daily

Group III— Hydroalcoholic extract of *Amaranthus tricolor* (300 mg/kg b.w.) p.o. daily
Group IV—Testosterone in arachis oil (0.5 mg/kg b.w.) twice weekly by intramuscular route

In brief, the ovariectomized females were injected with estrogen followed by progesterone after 48 h and then they were used as a stimulus for evaluation of sexual behavior.

Attraction Towards Female and Determination of Hesitation Time:
Determination of attraction towards sexually receptive female was done using the methods reported [6] and modified by Thakur and Dixit (2007) [7]. A female rat was placed in a cage which had a wooden barrier of 15 cm separating male and female compartments which could be passed by a motivated male rat.

The hesitation time was recorded as the time (in sec) required by the male rat before making an attempt to cross the barrier. In the same way, a scoring for attraction towards female was recorded by a score between 0–5 during an observation period of 15 min. A complete cross of the partition by the male rat each time was given a score of 5 while an attempt to climb was given a score of 2 and disinterest to climb was rated as 0. The readings were recorded on Days 0, 7, 14, 21, and 28 of treatment. This test is useful in determining the willingness of a male rat to cross an aversive or obstructive position, thus indicating the intent of sexual attraction [8] Male rats of all the groups were subjected to experimentation and their scores for attraction as well as hesitation time were recorded.

Sexual Behavior Analysis:
Each male rat was placed in the observation chamber for 5 min to acclimatize with the cage environment. A sexually receptive female rat was then introduced silently from one side of the chamber as stimulus.

The whole pattern was digitally recorded and observations for various parameters were made as follows:
Mount latency (ML) was calculated as the time from the introduction of the female to the occurrence of the first mount.

Intromission latency (IL) was considered as the time for first intromission after introduction of the female in the cage. Intromission frequency was total number of intromissions observed during the observation period. Intromission ratio was determined by dividing the number of intromissions by the sum of number of mounts and number of intromissions [9] .

Post ejaculatory interval (PEI) was calculated as time from ejaculation until next intromission.

Penile Erection (PE) was determined using the method reported [10]. The rats in all the groups were given the treatment 30 min prior to experimentation. The rats in each group were placed in observation cages (6 at a time) and continuously observed for a period of 30 min. The PE was recorded when the rats bent down to lick their erect penis. These observations were recorded on Days 0, 7, 14, 21, and 28 of treatment.

Penile Erection Index (PI) was determined by multiplying the percentage of rats exhibiting at least one episode of PE during 30-min observation period with the mean number of PEs [11].

Copulatory rate was calculated by determining the number of mounts plus number of intromissions divided by the time from the first mount until ejaculation.

$$\text{Copulatory rate} = \frac{\text{Number of mounts} + \text{Number of intromissions}}{\text{Time from first mount till ejaculation}}$$

A mount bout, which has been defined as a sequence of mount with or without vaginal penetration, was also observed and recorded [12,13].

Anabolic Effect:
For Set 1 animals, two male rats from each group were sacrificed on Day 0, and the rest were sacrificed 28 days after different treatments and the body weight of animals was recorded. They were euthanized and their testes, prostate, and seminal vesicles were removed and weighed [14].

Statistical Analysis:
Results are reported as M ± SE. The data were subjected to one-way analysis of variance (ANOVA) using Dunnet's test for determining statistical significance.

Aphrodisiac Activity:
Anabolic Effects:
Table 1 shows the data on body and organ weights as a function of treatment condition. The results of studies undertaken suggest that plant extracts have an anabolic effect. The results for Day 30 among different groups were compared with that of control by using a one-way ANOVA using Bonferroni's test. The animals gained body weight with the treatments and weight of secondary sexual organs, i.e., testis,

prostate, and seminal vesicles were also increased. In the case of the untreated group, no significant change was observed. The effect of various treatments on body and organ weights was found to be significant ($p<0.01$) in case of all the treated groups.

Table 1: Effect of drug treatment on body and organ weights

Group	Weight of animal (g) (M ± SE)		Weight of testes (g) (M ± SE)		Weight of prostate (mg) (M ± SE)	
	0 days	30 days	0 days	30 days	0 days	30 days
Group I	221.5± 0.84	222.3± 0.84	0.86 ± 0.01	0.88 ± 0.01	97.2 ± 1.5	99.6 ± 1.4
Group II	223.3 ± 0.8	240.2± 1.65**	0.87 ± 0.01	0.94± 0.02*	96.5 ± 1.6	117.2± 2.18*
Group III	222.8± 0.94	244.9± 0.93**	0.83 ± 0.02	0.98± 0.01**	94.5 ± 2.9	124.3± 1.3**
Group IV	220.9 ± 0.81	242.7 ± 0.91**	0.85 ± 0.02	0.99 ± 0.03**	97.1 ± 1.1	125.1 ± 1.1**

* p<.05, ** p<.01

The results from Table 1 demonstrate the effect of various treatments on general sexual behavior. Administration of lyophilized extracts of all three herbs influenced the behavior of the treated animals, which were more attracted towards the female. In the 300mg/kg extract treated group, a 2.5 fold increase in attraction towards female was found (p\.01) compared to a two fold increase in the 200mg/kg extract treated group (p\.05). Testosterone treated groups also exhibited a significant increase in attraction (p\.05). The ANOVA by post-hoc test demonstrated that the most significant difference among the observations within the group was between Day 7 and Day 28 observations.

Sexual Behavior:

In case of the data for hesitation time, a significant difference was observed in control group and different treated groups on Day 28 of treatment. The analysis of individual group data by post-hoc test showed that the most significant difference was in the observations for various groups between Day 7 and Day 28 observations. Hence, the data for day 28 were subjected to a one-way ANOVA. It was determined that the maximum reduction in hesitation time was in 300mg/kg group (p<.01)

Table 2 Effect of treatment on hesitation time and attraction towards female rats using barrier method

Treatment	Hesitation time (in seconds) M and SE after treatment for (days)				Cumulative score for attraction towards female M and SE after (days)			
	7	14	21	28	7	14	21	28
Group I	350 ± 11	340± 12	337 ± 10	330 ± 8	20 ± 2	21 ± 3	23 ± 1	22 ± 2
Group II	254 ± 10	228 ± 8	198 ± 7	166 ± 5**	36 ± 1	45 ± 2	67 ± 2	84 ± 3**
Group III	227 ± 9	200 ± 6	176 ± 7	100 ± 8**	35 ± 2	47 ± 7	59 ± 6	92 ± 2***
Group IV	222 ± 12	198 ± 12	165 ± 8	138 ± 7*	42 ± 2	58 ± 4	61 ± 3	76 ± 1**

* p<.05, ** p<.01, *** p<.005

Treatment with lyophilized extracts of the test drugs influenced the sexual behavior of the animals. The display of copulation by treated animals increased and the mount bouts increased significantly as well. Standard testosterone treated group also exhibited greater performance than the control group and a three-fold increase in bout frequency was observed (Table 2).

Penile Erection Index, which is an indicator of increased nitric oxide-based activity, was also increased in the treated groups; it was significantly higher in all the treated groups. 300 and 200mg/kg were at par in this aspect (p<.01) Also, an increase in percentage of ejaculating animals was observed in all the treated groups.

The sexual behavior of the animals was influenced after treatment with aqueous extract and testosterone. Overall sexual performance was improved as evidenced by the different parameters studied. The ML, IL, and PEI was significantly reduced in extract treated groups.

ML time was reduced by 37% in 300mg/kg, 34% in 200mg/kg treated, and 34% in testosterone treated group as compared to the control group.

Intromission (IL) and post ejaculatory latency (PEL) time was reduced by 36% in 300mg/kg, 32% in 200mg/kg, ($p<.05$) whereas only a 17% reduction was observed in the testosterone treated group. Significant increases in mount and intromission frequency were also observable after treatment. In terms of overall analysis, the most significant improvement was observed in case of 300mg/kg group activity as compared to testosterone in most of the parameters for sexual behavior evaluated in the present investigation.

Table 3: Behavior analysis and Penile Erection Index after 28 days of treatment

Animal groups	Mount bout	Penile Erection Index	%Ejaculating animals	Copulatory rate
GroupI (control)	0.6 ± 0.3	21.2 ± 1.1	68.6 ± 0.3	1.2
Group II	1.33 ± 0.3	51.7 ± 2.8**	79.3 ± 0.8	2.4
Group III	2.16 ± 0.16	56.3 ± 1.8**	84.1 ± 1.1	2.7
Group IV	1.83 ± 0.3	51.2 ± 1.1**	81 ± 1.05	1.9

Table 4: Effect of drug treatment on sexual behavior parameters after 28 days of treatment

Parameters	Group I	Group II	Group III	Group IV
Mount latency	169.9 ± 12.6	127.6 ± 7.6*	115.4 ± 7.9*	128.1 ± 3.2
Intromission latency	313.2 ± 10.8	285.9 ± 12.9	259.8 ± 9.8**	262.7 ± 5.4*
Post ejaculatory interval	496.7 ± 5.8	423.2 ± 6.8*	410.8 ± 8.9**	426.6 ± 7.4
Mount frequency	15.6 ± 3.9	28.7 ± 2.8	31.1 ± 0.92**	27.1 ± 2.4*
Intromission frequency	6.2 ± 0.1	11.1 ± 0.31*	16.7 ± 16**	11.2 ± 1.0
Ejaculation frequency	3.1 ± 0.9	3.8 ± 2.9	5.6 ± 1.6*	4.1 ± 1.1

* $p<.05$, ** $p<.01$

CONCLUSION:

Prognosis of sexual activity has been directly correlated to the enhancement of sexual pleasure. The effects observed in all the groups was much more pronounced when compared to the administration of testosterone, which only improved PE and ejaculation frequency. Administration of Hydroalcoholic extract improved orientation as well as sexual intent, suggesting a better sexual performance after administration of the extract. A very high PI observed in the treated group clearly suggests involvement of a nitric oxide based mechanism which may be responsible for an increased blood flow to penis therefore bringing out improvement in erectile function have shown that plant phytochemicals were effective in protecting against testicular damage and promote rejuvenation of testicular architecture. Present results provide evidence that the Hydroalcoholic extracts are not only effective in overall sexual performance but may also be effective in erectile dysfunction. The results therefore substantiate the folkloric claims that these plants have aphrodisiac activity and may be helpful in improving the sexual behavior and performance. The studies provide scientific evidence to recommendation of usage of these plants in traditional Indian medicine.

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