NATURAL REMEDIES
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FINAL REPORT

STUDY TITLE

EVALUATION OF APHRODISIAC ACTIVITY OF VigRx IN MALE ALBINO WISTAR RATS

Report No.: R401.07

STUDY DIRECTOR

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SPONSOR

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PREFACE

General

Study title	:	Evaluation of aphrodisiac activity of VigRx in male albino Wistar rats
Sponsor	:	AIBMR Life Sciences, Inc., 4117, South Meridian, Puyallup, WA 98373
Report No.	:	R401.07

OBJECTIVES

The purpose of this study is to evaluate the aphrodisiac activity of VigRx in male albino Wistar rats. The results of this study will provide preliminary information about the efficacy of VigRx to be used as an aphrodisiac agent.

MATERIALS AND METHODS

Test substance

Sponsor	:	AIBMR Life Sciences, Inc., 4117, South Meridian, Puyallup, WA 98373
Label on substance	:	**VigRx Tablet Blend** Lot No. : 120657
Characteristics of sample	:	Consistency - Solid (powder) Colour - Brown

Preparation of dose		
Dose	:	225, 335 and 450 mg/kg body weight.
Dose volume	:	10 ml/kg.
Vehicle	:	Demineralised water.
Procedure	:	The test substance was taken in demineralised water to obtain 22.5, 33.5 and 45.0 mg/ml strength. The test substance was administered in the dose volume of 10 ml/kg body weight for 14 days. The formulation was prepared fresh on each day of dosing.

Justification for selection of doses

The rat doses were selected based on the information provided by the sponsor and as agreed in the protocol.

Tablet weight - 1200 mg as per COA SF 2226 (12/06)

No. of tablets per day	Total human dose mg/human/day	Rat dose mg/kg rat b.w	Approximated Rat dose mg/kg rat b.w
2	2400	216	225 (Low dose)
3	3600	324	335 (Mid dose)
4	4800	432	450 (High dose)

Test system		
Species	:	Albino rats.
Strain	:	Wistar.
Source	:	Central Animal Facility, R&D centre, Natural Remedies Pvt. Ltd.
Sex	:	Male and Female.
Body weight range	:	200 to 250 g.
No. of animals per dose	:	Eight male rats.
Acclimation	:	One month in reversed light dark cycle (light from 21.00 h to 9.00 h).
Veterinary examination	:	Before allocation of animals to individual groups.
Identification of animals	:	By cage card number.
Diet	:	Pelleted feed *ad libitum* supplied by Gold Mohur Foods & Feeds Ltd., Bangalore.
Water	:	U.V. purified water *ad libitum.*
Housing and environment	:	One animal per polypropylene cage provided with bedding of husk. The temperature was maintained between 25 ± 2°C and relative humidity between 30% and 70% with optimal air changes per hour and reversed light dark cycle (light from 21.00 h to 9.00 h) was maintained.

Randomization of animals

Healthy adult male rats, acclimatized to reversed light dark cycle for one month prior to dosing, were used in this study. Animals were randomly assigned to the cages.

Drugs/chemicals used

Testosterone enanthate (*Source*: German Remedies; *Batch No.*: GG1054; *Mfd*: 01/07;
Exp: 12/09; *Trade Name*: Testoviron; *Qty*: 250 mg) was used at the dose of 15 mg/kg rat body weight.

Estradiol valerate (*Source*: German Remedies; *Batch No.*: HFF287; *Mfd*: 01/07; *Exp*: 12/09; *Trade Name*: Progynova; *Qty*: 2 mg) was used at the dose of 10 µg/kg rat body weight.

Progesterone (*Source*: Ferring Pharmaceuticals; *Batch No.*: FO2511K; *Mfd*: 12/05;
Exp: 07/08; *Trade Name*: Gestone; *Qty*: 100 mg) was used at the dose of 500 µg/kg rat body weight.

INDUCTION OF ESTRUS IN FEMALE RATS

The female rats were brought to estrus by sequential administration of estradiol valerate
(10 µg/kg rat body weight) and progesterone (500 µg/kg rat body weight), through subcutaneous injections, 48 h and 4 h before the copulatory studies respectively.

SELECTION OF MALE RATS

Male rats were trained for sexual behavior with primed female rats in estrus individually for 15 min in a mating arena (60 x 30 x 18 cm) for a period of 10 days prior to the start of experiment. Then, the trained animals were screened for selecting the active males. Only those males that attempted to mount the female more than 5 times in 5 min were considered sexually active and were selected for the study.

EXPERIMENTAL DESIGN

Eight sexually active males were randomly allotted to each group. Group I served as vehicle control (Demineralized water; 10 ml/kg; *p.o*). Group II was administered with testosterone enanthate subcutaneously at the dose of 15 mg/kg. Groups III, IV and V were administered orally with VigRx at the doses of 225, 335 and 450 mg/kg rat body weight respectively.

Study design:

Groups	Treatment	Dose (rat b.w.)
I	Vehicle control	10 ml/kg
II	Testosterone	15 mg/kg
III	VigRx	225 mg/kg
IV	VigRx	335 mg/kg
V	VigRx	450 mg/kg

OBSERVATIONS

Behavioral signs

The sexual behavior was observed after 1 h of treatment in a four quadrant mating arena under dim red illumination. The experimental animal (male rat) was placed in the mating arena 10 min before the observation period for acclimation. The receptivity of the female rat was confirmed before the behavior test by exposing them to male rats other than the experimental animals. The receptive female rat was introduced to the male rat in the mating arena in 1:1 ratio. The rats were then observed for 30 min for copulatory behaviors such as mounting, intromission and ejaculation on 0^{th}, 1^{st}, 7^{th} and 14^{th} day of treatment and the parameters such as mount latency, mount frequency, intromission latency, intromission frequency, ejaculation latency, ejaculation frequency and post ejaculatory interval were recorded. The observers were blinded about the treatment to avoid any bias.

Testosterone

After 14 days of treatment, animals were kept for overnight fasting and blood was collected (on 15^{th} day) through retro-orbital sinus and serum was separated for testosterone estimation.

Histopathology

Testes, epididymides and seminal vesicles were collected for histopathological evaluation.

STATISTICS

The data were analyzed using one way ANOVA followed by Bonferroni method as post-hoc test. In case of heterogeneous data after transformation dunnetts's T3 method was used. All values were reported as mean ± SEM. Statistical significance was set at $p \leq 0.05$.

RESULTS

Mount latency (Table 1)

The mean mount latency on different days is presented in Table 1. No significant difference in mount latency between different groups was observed in basal value when compared to vehicle control. No significant difference in mount latency was observed on day 1 when compared to vehicle control. Significant decrease in mount latency was observed in testosterone treated group on day 7 and 14 as compared to vehicle control. VigRx treated groups showed a non significant decrease in mount latency on day 1, 7 and 14 with a significant decrease observed on day 14 at the dose of 450 mg/kg as compared to vehicle control.

Mount frequency (Table 2)

The mean values of mount frequency on different days are presented in Table 2. No significant difference in mount frequency between different groups was observed in basal value as compared to vehicle control. The testosterone and VigRx treated groups did not show any significant difference in mount frequency on day 1, 7 and 14 when compared to vehicle control.

Intromission latency (Table 3)

The mean intromission latency on different days is presented in Table 3. No significant difference in intromission latency between different groups was observed in basal value when compared to vehicle control. Significant decrease in intromission latency was observed in testosterone treated group on day 1, 7 and 14 when compared to vehicle control. A significant reduction in intromission latency was observed on day 7 and 14 in VigRx high dose group.

Intromission frequency (Table 4)

The mean values of intromission frequency on different days are presented in Table 4.

No significant difference in intromission frequency between different groups was observed in basal value when compared to vehicle control. The intromission frequency observed in treated group on day 1, 7 and 14 did not show any significant difference when compared to vehicle control.

Ejaculation latency (Table 5)

The mean ejaculation latency on different days is presented in Table 5. No significant difference in ejaculation latency between different groups was observed in basal value and on day 1 when compared to vehicle control. A significant decrease in ejaculation latency was observed in testosterone treated group on day 7 and 14 when compared to vehicle control. The VigRx treatment at the doses of 225, 335 and 450 mg/kg on day 14 showed significant decrease in ejaculation latency when compared to vehicle control.

Ejaculation frequency (Table 6)

The mean values of ejaculation frequency on different days are presented in Table 6.

No significant difference in ejaculation frequency was observed in basal value between different groups when compared to vehicle control. The treatment groups did not show any significant difference in ejaculation frequency on day 1 when compared to vehicle control. A significant increase in ejaculation frequency was observed in testosterone treated group on day 14 when compared to vehicle control. VigRx treated group at the dose of 450 mg/kg on day 7 showed a

significant increase in ejaculation frequency with a non significant increase on day 14 as compared to vehicle control.

Post ejaculatory interval (Table 7)

The mean values of post ejaculatory interval on different days are presented in Table 7.

No significant difference in post ejaculatory interval between different groups was observed in basal value when compared to vehicle control. No significant difference in post ejaculatory interval in treated groups was observed on day 1 and 7 when compared to vehicle control. A significant decrease in post ejaculatory interval was observed in testosterone treated group and VigRx treatment at all the doses on day 14 when compared to vehicle control.

Testosterone (Table 8)

The mean values of testosterone concentration are presented in Table 8. A significant increase in serum testosterone concentration was observed in testosterone treated group when compared to vehicle control. VigRx at the doses of 335 and 450 mg/kg showed a marginal increase in testosterone concentration as compared to vehicle control.

Histopathology

The mean number of spermatogonia cells on histopathological examination in five seminiferous tubules of the testes is presented in Table 9. An increase in the number of spermatogonia cells was observed in treated groups when compared to vehicle control, but was not found to be statistically significant.

The histopathological examination of epididymides and seminal vesicles did not reveal any significant changes between the normal and treated groups.

CONCLUSION

Based on the observations from the present study, VigRx exhibited aphrodisiac activity by significantly reducing the ejaculation latency and post ejaculatory interval at all the tested doses on day 14 and by significantly reducing the mount latency (on day 14) and intromission latency (on day 7 and 14) at 450 mg/kg when compared to vehicle control. Treatment with VigRx at the dose of 450 mg/kg showed a significant increase in ejaculation frequency on day 7 and a non significant increase on day 14 with marginal increase in testosterone concentration in serum and number of spermatogonia cells in seminiferous tubules of testes.

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DISCLAIMER

1. The results listed above pertain only to the tested samples and applicable parameters.

2. Samples will be disposed after one month from the date of issue of test report unless otherwise specified.

3. This report is not to be reproduced either wholly or in part and cannot be used as an evidence in the court of law and should not be used in any advertising media without prior written permission.

TABLE 1

Effect of VigRx on mount latency in male albino Wistar rats

Treatment group	Basal value	Day 1	Day 7	Day 14
I Vehicle control (10 ml/kg)	20.75 ± 2.59	22.38 ± 2.20	28.50 ± 4.85	26.75 ± 5.53
II Testosterone (15 mg/kg)	21.50 ± 3.57	17.38 ± 2.25	4.88 ± 0.61*	4.38 ± 0.84*
III VigRx (225 mg/kg)	24.13 ± 4.15	18.50 ± 2.69	20.88 ± 3.63	16.50 ± 3.67
IV VigRx (335 mg/kg)	24.13 ± 2.52	20.13 ± 1.85	23.38 ± 3.41	18.38 ± 2.76
V VigRx (450 mg/kg)	23.88 ± 3.46	19.00 ± 2.09	15.38 ± 2.92	9.75 ± 1.21*

Values are expressed as mean ± SEM; n=8; Unit – Seconds.
*$p \leq 0.05$ Testosterone / VigRx Vs Vehicle control.

TABLE 2

Effect of VigRx on mount frequency in male albino Wistar rats

Treatment group	Basal value	Day 1	Day 7	Day 14
I Vehicle control (10 ml/kg)	34.00 ± 2.99	33.25 ± 4.62	35.00 ± 4.12	31.50 ± 3.55
II Testosterone (15 mg/kg)	36.88 ± 2.69	36.38 ± 3.17	20.38 ± 1.81	30.00 ± 3.54
III VigRx (225 mg/kg)	38.75 ± 4.87	31.13 ± 3.94	37.38 ± 4.40	33.25 ± 3.76
IV VigRx (335 mg/kg)	34.88 ± 4.27	32.13 ± 4.54	42.25 ± 5.17	32.63 ± 3.40
V VigRx (450 mg/kg)	28.25 ± 3.21	26.88 ± 3.50	31.88 ± 3.34	28.88 ± 3.28

Values are expressed as mean ± SEM; n=8; Unit – Numbers.

TABLE 3

Effect of VigRx on intromission latency in male albino Wistar rats

Treatment group	Basal value	Day 1	Day 7	Day 14
I Vehicle control (10 ml/kg)	51.88 ± 6.90	39.88 ± 3.11	58.00 ± 5.32	54.13 ± 7.07
II Testosterone (15 mg/kg)	47.13 ± 5.86	22.13 ± 3.12*	14.75 ± 2.19*	11.50 ± 1.95*
III VigRx (225 mg/kg)	47.63 ± 6.61	31.50 ± 3.72	58.50 ± 6.41	54.50 ± 7.08
IV VigRx (335 mg/kg)	51.13 ± 7.37	39.25 ± 5.41	47.38 ± 6.29	35.25 ± 4.78
V VigRx (450 mg/kg)	49.38 ± 6.08	32.75 ± 3.73	35.25 ± 4.13*	23.50 ± 3.83*

Values are expressed as mean ± SEM; n=8; Unit – Seconds.
*$p \leq 0.05$ Testosterone / VigRx Vs Vehicle control.

TABLE 4

Effect of VigRx on intromission frequency in male albino Wistar rats

Treatment group	Basal value	Day 1	Day 7	Day 14
I Vehicle control (10 ml/kg)	28.00 ± 2.75	30.50 ± 2.71	25.88 ± 3.71	27.75 ± 3.07
II Testosterone (15 mg/kg)	35.88 ± 3.14	27.50 ± 3.63	32.75 ± 2.04	27.25 ± 3.60
III VigRx (225 mg/kg)	30.25 ± 3.46	32.13 ± 3.44	32.25 ± 3.56	27.25 ± 3.10
IV VigRx (335 mg/kg)	26.75 ± 1.69	29.75 ± 2.20	25.75 ± 3.27	25.63 ± 1.75
V VigRx (450 mg/kg)	33.63 ± 1.63	32.13 ± 3.26	31.88 ± 2.32	29.25 ± 1.74

Values are expressed as mean ± SEM; n=8; Unit – Numbers.

TABLE 5

Effect of VigRx on ejaculation latency in male albino Wistar rats

Treatment group	Basal value	Day 1	Day 7	Day 14
I Vehicle control (10 ml/kg)	537.50 ± 26.31	664.25 ± 61.60	650.63 ± 77.28	674.00 ± 30.49
II Testosterone (15 mg/kg)	477.50 ± 35.59	446.38 ± 52.29	205.13 ± 41.67*	178.50 ± 22.68*
III VigRx (225 mg/kg)	464.25 ± 48.53	433.13 ± 43.61	426.63 ± 66.69	378.75 ± 44.01*
IV VigRx (335 mg/kg)	502.75 ± 53.43	507.88 ± 60.25	516.75 ± 52.31	424.13 ± 34.47*
V VigRx (450 mg/kg)	548.63 ± 59.00	675.75 ± 69.66	435.13 ± 48.01	375.50 ± 35.85*

Values are expressed as mean ± SEM; n=8; Unit – Seconds.
*$p \leq 0.05$ Testosterone / VigRx Vs Vehicle control.

TABLE 6

Effect of VigRx on ejaculation frequency in male albino Wistar rats

Treatment group	Basal value	Day 1	Day 7	Day 14
I Vehicle control (10 ml/kg)	2.50 ± 0.19	1.75 ± 0.31	2.50 ± 0.19	2.25 ± 0.37
II Testosterone (15 mg/kg)	2.50 ± 0.19	2.75 ± 0.25	3.13 ± 0.23	3.88 ± 0.23*
III VigRx (225 mg/kg)	2.63 ± 0.26	2.38 ± 0.38	2.75 ± 0.45	3.00 ± 0.38
IV VigRx (335 mg/kg)	2.50 ± 0.33	2.38 ± 0.32	2.75 ± 0.25	3.13 ± 0.30
V VigRx (450 mg/kg)	2.50 ± 0.33	2.38 ± 0.32	3.38 ± 0.18*	3.25 ± 0.25

Values are expressed as mean ± SEM; n=8; Unit – Numbers.
*$p \leq 0.05$ Testosterone / VigRx Vs Vehicle control.

TABLE 7

Effect of VigRx on post ejaculatory interval in male albino Wistar rats

Treatment group	Basal value	Day 1	Day 7	Day 14
I Vehicle control (10 ml/kg)	348.75 ± 21.87	431.50 ± 27.91	373.50 ± 17.60	423.75 ± 17.28
II Testosterone (15 mg/kg)	383.25 ± 24.55	382.25 ± 29.40	356.25 ± 10.68	316.25 ± 9.34*
III VigRx (225 mg/kg)	310.88 ± 21.17	385.75 ± 28.42	391.50 ± 20.08	355.25 ± 12.35*
IV VigRx (335 mg/kg)	355.50 ± 21.10	354.13 ± 16.35	339.88 ± 21.44	347.75 ± 16.68*
V VigRx (450 mg/kg)	349.13 ± 30.31	346.50 ± 18.31	341.88 ± 19.20	353.25 ± 19.21*

Values are expressed as mean ± SEM; n=8; Unit – Seconds.
*$p \leq 0.05$ Testosterone / VigRx Vs Vehicle control.

TABLE 8

Effect of VigRx on testosterone level in male albino Wistar rats

Treatment group	Testosterone (ng/ml)
I Vehicle control (10 ml/kg)	3.54 ± 0.96
II Testosterone (15 mg/kg)	23.00 ± 1.83*
III VigRx (225 mg/kg)	2.45 ± 0.31
IV VigRx (335 mg/kg)	4.66 ± 0.89
V VigRx (450 mg/kg)	5.93 ± 1.61

Values are expressed as mean ± SEM; n=8.
*p ≤ 0.05 Testosterone / VigRx Vs Vehicle control.

TABLE 9

Effect of VigRx on number of spermatogonia in male albino Wistar rats

Treatment group	Number of spermatogonia
I Vehicle control (10 ml/kg)	356.00 ± 9.42
II Testosterone (15 mg/kg)	415.13 ± 28.21
III VigRx (225 mg/kg)	368.50 ± 15.70
IV VigRx (335 mg/kg)	376.13 ± 25.29
V VigRx (450 mg/kg)	406.00 ± 30.38

Values are expressed as mean ± SEM; n=8.