APHRODISIAC ACTIVITY OF GEYENAPHRO: A POLY HERBAL FORMULATION IN RATS

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Summary

Geyenaphro' a polyherbal formulation, used in the treatment of sexual inertia, premature ejaculation and erectile dysfunction. The present study was aimed at investigating the aphrodisiac effect of 'Geyenaphro' in male and female albino rats in comparison with L-dihydroxyphenylalanine (L-dopa). 2-3 months old sexually inactive male and female rats were used for the study. In male rats, latency for mount, intromission, ejaculation and number of mounts, intromission and ejaculation and in female rats, number of lordosis and rejection were observed as parameters of assessment of aphrodisiac activity. 'Geyenaphro' significantly enhanced sexual activity in both male and female rats, both in single dose (100 mg/kg, orally) and chronic dose study (100 mg/kg /day orally for 28 days). 'Geyenaphro' was found to be superior to (L-Dopa) in male rats and equipotent in female rats.

Key words: Aphrodisiac activity, Mount latency, Ejaculation latency, Intromission latency, Lordosis.

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Introduction

'Geyenaphro' is a poly herbal formulation available in the form of capsules. This drug is an alleged cure for sexual inertia, erectile dysfunction and premature ejaculation. 'Geyenaphro' is formulated in accordance with Ayurvedic literatures^[1] and each capsule contains following powdered plant parts: *Mucuna pruriens* (seed), *Withania somnifera* (root), *Pueraria tuberosa* (root), *Tribules terrestris* (fruit), *Myristica fragrans* (seed), *Curculigo orchioides* (rhizome). The individual plants of this formulation were mentioned in Indian Materia Medica^[1] as aphrodisiacs. Aphrodisiac property of *Mucuna pruriens*, was studied by Ananth Kumar et al^[2]. *Curculigo orchioides* known to possess estrogenic activity^[3]

In the present study the effect of 'Geyenaphro' on sexual behaviour of male and female albino rats was carried out to know whether the alleged therapeutic effect can be confirmed in animal studies.

Materials and methods

Materials

'Geyenaphro' (Manufactured by the Geyenayurvedics, Shivalli Industrial Area, Manipal) was procured from Geyenayurvedics, SINEMET[®] tablets containing L-Dopa 100 mg and carbidopa 10 mg were procured from Pharmacy section of Kasturba Hospital, Manipal.

'Geyenaphro' was suspended in water using 4% w/v gum acacia and was orally administered to groups of rats at different doses to determine its therapeutic dose. On the basis of this study a dose of 100 mg/kg body weight was used in the present study.

Animals and experimental set-up

Male and female albino rats of age 2-3 months were used in the study. Animals were housed in a temperature and humidity controlled room, a 12 hour light and 12 hour dark cycle was maintained throughout the experiment period. All animals were given food and water *ad libitum*.

Study on Male rats

This was done according to the method of Burses^[4]. Normal albino male rats of 2-3 months age were trained for sexual experience by keeping 3 males with one receptive female rat in their home cage for about 30 minutes.

Selection of male rats

Sexual behaviour of each male was assessed individually twice for two consecutive weeks. The animals were divided into sexually active and sexually inactive group, based on their sexual behaviours. Sexually inactive rats were used in the study.

Selection of the female rats

Female rats of 2-3 months age were ovariectomised. A 10 days interval was given for the animals to recover after ovariectomy. Ovariectomisation and the confirmation of estrous were carried out according to the standard procedure^[5]. After ovariectomisation the animals were in diestrous stage. To bring them to the estrous stage they were given 50 μ g of estradiol benzoate 48 hours before and 500 μ g of hydroxyprogesterone 6 hours before the test.

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Sexually inactive male rats were divided into 3 groups, each consisting of 6 rats. Group 1: was administered 'Geyenaphro' at a dose of 100 mg/kg, daily for 28 days. Group 2: was treated with a single dose of (100 mg/kg) of Geyenaphro 2 hour prior to the test. Group 3: was treated with L-dopa at a dose of 100 mg/kg, daily for 28 days. All drugs were administered orally.

A highly receptive female (in estrous) was introduced into the male's cage and each male rat was observed for sexual behaviour for 30 minutes under dim red light. Rats of group 1 and 3 were tested on 0th, 7th, 14th, 21st and 28th day. Rats of group 2 were tested only one day, 2 hours after the drug administration.

The following parameters were recorded; mount latency, number of mounts, intromission latency, number of intromission, ejaculation latency and number of ejaculations.

Study on female rats

This was done according to the method described by Venkatraman and Joseph^[6]. Two groups of ovariectomised rats each consisting of 6 rats were selected and they were prepared for sexual receptivity by injecting subcutaneously $2 \mu g/kg$ of estradiol benzoate 48 hours before and 500 μg of hydroxyprogesterone 6 hours before the test.

Group 1: was treated with 'Geyenaphro' at a dose of 100 mg/kg.

Group 2: was treated with L-dopa at a dose of 100 mg/kg.

All the drugs were administered orally 2 hours prior to the test.

Female sexual behaviour

Behavioural observations were made by introducing sexually active male rats into each ovariectomised female rat's cage. The following parameters were recorded for a period of 5 minutes.

Number of mounts by the males.

Number of lordosis responses shown by the female.

Number of rejections of the male by the female as shown by the female turning towards the male and resisting the mount.

The lordosis quotient (LQ) and rejection quotient (RQ) were then calculated using the following formulae,

LQ = total number of lordosis responses / total number of mounts by the male.

RQ = total number of rejections of male / total number of mounts or mounts attempts.

This study was conducted in accordance with the latest CPCSEA guidelines and the experimental protocol was approved by Institutional Animals Ethics Committee.

Statistical Analysis

One way ANOVA followed by Scheffe's test was used to analyse the difference in the sexual activity between before treatment and subsequent days of observations.

The difference in the sexual activities between before treatment and after treatment following single dose of 'Geyenaphro' and L-dopa in male and the female rats were analysed by paired t-test.

Results

Selection of sexually inactive male rats

Animals of disturbed sexual behaviours with a significant increase in latency for mount intromission, ejaculation and number of intromissions. With a decrease in the number of ejaculation were chosen as sexually inactive animals.

Effect of chronic treatment of Geyenaphro and L-dopa on male sexual behaviour

'Geyenaphro' produced a significant (p<0.01) decrease in the latency for mount from 14th day to 28th day as compared to before treatment, while L-dopa showed a significant (p<0.01) decrease only on the 21st and 28th day (Table-1). 'Geyenaphro' produced a significant (p<0.01) decrease in latency for intromission from 7th day to 28th day as compared to before treatment, while L-dopa showed significant (p<0.01) decrease on 0, 14th, 21st and 28th day (Table-2).

Table 1. Effect of chronic treatment of Geyenaphro and L-dopa on mount latency of sexually inactive male rats

	Mount Latency (seconds) Mean±S.E.M					
	Before treatment	0 day	7 th day	14 th day	21 st day	28 th day
Geyenaphro						
(n = 6)	97.25±39.69	25±12.05	20.66 ± 9.86	6.33 ± 1.83^{a}	3.16 ± 1.08^{a}	1.33 ± 0.16^{a}
L-dopa (n = 6)	10.58±3.68	2.66±1.08	4.66±1.4	3.16±0.65	1.83±0.31 ^a	1.16±0.16 ^a

a= p<0.01 Vs before treatment

Table 2. Effect of chronic treatment of Geyenaphro and L-dopa on intromission latency of sexually inactive male rats

	Intromission Latency (seconds) Mean±S.E.M					
	Before treatment	0 day	7 th day	14 th day	21 st day	28 th day
Geyenaphro						
(n=6)	224.66±97.99	24±5.41	17.5 ± 4.47^{a}	10.66 ± 2.46^{a}	5.16 ± 1.08^{a}	2.33±0.21 ^a
L-dopa (n=6)	18.17±5.36	4.33±1.38 ^a	8.66±2.11	5.0 ± 0.82^{a}	3.16±0.4 ^a	2.16±0.16 ^a

a= p<0.01 Vs before treatment

'Geyenaphro' produced a significant (p<0.01) decrease in latency for ejaculation on 28^{th} day as compared to before treatment. While L-dopa produced a significant (p<0.01) decrease on 0, 14^{th} and 28^{th} day as compared to before treatment (Table-3).

	Ejaculation Latency (seconds) Mean±S.E.M					
	Before treatment	0 day	7 th day	14 th day	21 st day	28 th day
Geyenaphro	722.58	663.58	624.25	341.66	237.5	117.83
(n=6)	± 147.35	±112.26	±111.33	±84.2	± 38.9	$\pm 24.8^{\mathrm{a,b,c}}$
L-dopa (n=6)	503.75	235.83	343.66	213.00	205.66	173.5
	± 36.65	$\pm 44.86^{a}$	± 48.94	$\pm 42.56^{a}$	$\pm .26.17^{a}$	$\pm 25.46^{a}$

Table.3 Effect of chronic treatment of Geyenaphro and L-dopa on ejaculation latency of sexually inactive male rats

a = p < 0.01 Vs before treatment; b = p < 0.01 Vs 0 day; c = p < 0.01 Vs 7th day

'Geyenaphro' and L-dopa produced a significant (p<0.01) increase in the number of ejaculations from 14th day to 28th day as compared to the before treatment (Table-4).

Table 4. Effect of chronic treatment of Geyenaphro and L-dopa on number of ejaculations of sexually inactive male rats

	Number of ejaculations [Mean ± S.E.M.]					
	Before treatment	0 day	7 th day	14 th day	21 st day	28 th day
Geyenaphro(n=6)	1.33 ± 0.16	1.16±0.17	1.16±0.17	1.66±0.33 ^a	2.16±0.16 ^a	2.83±0.16 ^a
L-dopa (n=6)	1.5±0.18	2.33±0.21	2.0±0.36	2.66±0.21 ^a	2.83±0.16 ^a	3.0±0.0 ^a

a = p < 0.01 Vs before treatment

A single dose treatment with 'Geyenaphro' produced a significant decrease in latency for mount (p<0.05), intromission (p<0.05), ejaculation (p<0.01), and number of intromissions (p<0.01), with an increase in the number of ejaculations (p<0.01) as compared to before treatment (Table-5).

Table 5. Effect of single dose (100 mg/kg) of Geyenaphro on sexual activities of sexually inactive male rats

Parameters	Before treatment (n=6) Mean±S.E.M	After treatment (n=6) Mean±S.E.M
Mount latency (seconds)	15.16±4.16	3.66±1.05 ^a
Number of mounts	11.16±1.49	8.5 ± 1.43^{NS}
Intromission latency (seconds)	59.75±18.32	6 ± 1.65^{a}
Number of intromissions	26.75 ± 1.86	16 ± 1.81^{b}
Ejaculation latency (seconds)	670.25±76.47	271.33±43.78 ^b
Number of ejaculations	1.42±0.15	2.66±0.21 ^b

Values represent the mean (\pm S.E.M) of two weekly tests. a=p<0.05, b=p<0.01 Vs before treatment (paired t-test). NS= statistically not significant.

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Effect of Geyenaphro and L-dopa on female sexual behaviour

Both 'Geyenaphro' (p<0.01) and L-dopa (p<0.02) produced significant increase in the lordosis quotient as compared to before treatment (Table-6).

L-dopa produced significant (p<0.02) decrease in the rejection quotient as compared to before treatment. However, there is no significant difference between before treatment and in 'Geyenaphro' treatment group (Table-6).

Table 6. Effect of Geyenaphro and L-dopa on female sexual behaviour

	Lordosis Qu Mean±		Rejection Quotient (RQ) Mean±S.E.M		
	Before treatment		Before treatment	After treatment	
Geyenaphro (n=6)	0.486±0.07	$0.658{\pm}0.07^{a}$	0.23±0.11	0.088±0.03 ^{NS}	
L-dopa (n=6)	0.385±0.03	0.643 ± 0.07^{b}	0.23±0.11	0.123 ± 0.02^{b}	

a = p < 0.01, b = p < 0.02 Vs before treatment (paired t-test).

NS = statistically not significant.

Discussion

In male rats latency for mount and intromission are considered to be indicators of the sexual motivation, while number or of intromission and ejaculation are considered as behavioural indication of sexual performance and facilitations^[7]. After treatment with the 'Geyenaphro' there was a significant decrease in the latency for mount and intromission indicating an enhancement of sexual motivation, which was predominant from 7th day of observation. Also an increase in the number of ejaculations with a decrease in the ejaculation latency indicated an increase in the sexual performance. L-dopa produced a similar response as that of the 'Geyenaphro'.

In single dose study it was observed that there was a significant improvement in the sexual motivation and performance. Thus the study indicates that single dose treatment is as effective as chronic treatment in male rats.

There was a significant increase in the lordosis quotient in 'Geyenaphro' treated female rats, which was comparable to L-dopa. Lordosis quotient is a standard measure of sexual receptivity of the female rat to a male^[4].

Thus from the present study the 'Geyenaphro' was found to enhance sexual activity in sexually inactive male rats and female rats. In Ayurveda, the formulation and ingredients of the formulation are reported to be aphrodisiac in sexual debilities, which were further confirmed by this study.

Dopamine plays an important role in sexual behaviour ^[6]. In consistence with this report L-dopa showed a significant enhancement in sexual activity in male rats. The aphrodisiac activity of *Mucuna pruriens*^[2], one of the ingredients of the 'Geyenaphro', has already been reported in our laboratory. One of the phytochemical constituent of this plant is L-dopa^[8], which might have contributed, in enhancing the sexual activity. Constituents of the other plants of the 'Geyenaphro' also could be involved in the effect on sexual behaviour.

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