EFFECT OF POLYHERBAL FORMULATION ANDROCARE, FOR ITS APHRODISIAC ACTIVITY

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Summary

To study the aphrodisiac activity of polyherbal formulation, Androcare in different models of experimental animals, mice and rats. The polyherbal formulation, Androcare was gifted from SHRUSHTI, an Herbal Pharma Industry from Bangalore and LD_{50} value for the Androcare was conducted as per as OECD guidelines No. 425 upto the dose level of 2000 mg/kg. The aphrodisiac activity was evaluated in various experimental animal models like Effect on fertility in mice, Effect on sperm properties in mice and Sexual behavior on prolonged immobilization stress in rats. The polyherbal formulation, Androcare even upto the dose level of 2000mg/kg. It has not produced any lethal effect. In Effect on fertility model, Androcare only 200 & 400 mg/kg dose treated groups but not 100 mg/kg dose had shown a significant increase in litter size but no effect on M/F ratio. Androcare was tested for its effect on sperm properties with different dose levels and all doses (100,200 & 400 mg/kg) have shown a significant increase in spermatogenic activity with scant intertubular spaces between the tubules. Sexual behavior in prolonged immobilization stress induced model, a significant increase in number of mounts and thrusting and decrease in mounting latency were recorded with Androcare 200 & 400 mg/kg treated doses only but not with 100 mg/kg treated group. The present investigation revealed that the polyherbal formulation, Androcare was found to possess aphrodisiac activity.

Key words: Polyherbal formulation, Androcare, Aphrodisiac activity, Mice, Rats.

Introduction

Erectile dysfunction is defined as the inability to achieve and maintain an erection sufficient to permit satisfactory sexual intercourse. It has been estimated to affect 20 million to 30 million men in the United States. Result from psychological, neurologic, hormonal, arterial or cavernosal impairment or from a combination of these factors[1]. The underlying causes for sexual disorders may be psychological, psychiatric, organic, interpersonal or related to pharmacological factors and treatment should be provided accordingly, further 49% of 40 years age group and 67% of 70 year age group men suffer from sexual dysfunction disorders[2].

In recent years, there has been phenomenal rise in the interest of scientific community to explore the pharmacological actions or to confirm the veracity of claims made about herbs in the official books of Ayurveda and Siddha. reported to possess aphrodisiac activity.

Review of various published works it has been revealed that a good number of plant based drugs i.e., Aalooka (Tuber), Badara (Root, Bark, Fruit), Dhanya (Seed), Dugdhika (Whole plant), Kapikachchu (Root, Seed, Hairs), Kokilaksha (Root, Seed), Musali (Root), Rushabha (Tuber), Talamuli (Root), Kottai karanthai (Root, Leaf, Flower, Seed), Mullangi (Root, Leaf, Seed), Neermuli (Flower, Seed)[3]reported with aphrodisiac activity.

Apart from the above mentioned individual herbal drugs, there are certain polyherbal formulations launched by the various companies in market like "Tentex Forte, Tentex Royal (Himalaya Drug Company Bangalore), Vita-Ex-Gold (Baidyanath) are also recommended for aphrodisiac activity.

In the present study a polyherbal formulation "Androcare" was chosen which is traditionally used for aphrodisiac activity.

Recently scientific interest in the pharmacology of sexual behavior has been given impetus by the discovery of drugs (indigenous) that can stimulate or inhibit such behavior[4, 5, 6].

AYURVEDA, an ancient Indian system of healing, described various plants for the treatment of sexual disorders particularly like erectile dysfunction and SHRUSHTI, a Herbal Pharma Industry in Bangalore, has come out with a aphrodisiac formulation consisting of plant ingredients of *Bacopa monnieri, Asparagus adscendens, Astercantha longifolia, Asparagus recemosus, Mucuna pruriens and Withania somnifera*. So the present study was aimed to assess the aphrodisiac activity of this herbal formulation in different models of experimental animals, mice and rats.

Methods

Drugs:

Tentex Forte (Himalaya Drug Company, Bangalore), Androcare (SHRUSHTI Herbal Pharma Industry, Bangalore)

Animals:

Albino mice of either sex weighing between 18-22 gm procured from Shri Venkateswara Enterprises, Bangalore were used in this study. All the animals were acclimatized for 7 days and housed in groups of six under standard husbandry condition like room temperature 26 ± 2^0C, relative humidity 45-55% and light/ dark cycle of 12 hours. All the animals under strict hygienic conditions[7,8].were fed with synthetic standard diet (Amrut Laboratories Pranava Agro Industries Ltd. Sangli) and water was supplied *ad libitum*

Pharmacological activities

a) Determination of acute toxicity (LD_{50})

b) Aphrodisiac activity in different animal models.

a) Determination of acute toxicity (LD_{50}):

The acute toxicity of Androcare was determined by using female albino mice of 20-30g those maintained under standard husbandry conditions. The animal were fasted 3 hrs prior to the experiment and up and down procedure (OECD guideline no.

425) of CPCSEA was adopted for toxicity studies[9]. Animals were administered with single dose of polyherbal formulation and observed for its mortality during 48 hours study period (short term toxicity). Based on short-term profile of drug, the dose for the next animals was determined. The LD_{50} of the polyherbal formulation was calculated using AOT 425 software provided by Environmental protection agency, USA[10,11]. From the LD_{50} dose 1/20, 1/10 & 1/5 th doses were selected and considered as low, medium and high dose respectively.

b) Models for aphrodisiac activity:

1) Effect on fertility in mice [12,13]:

Experimental Procedure:

Adult swiss albino male mice of (25-35g) each consisting of 6 animals was divided in to following groups

Group I	:	Normal control (Gum acacia 10ml/kg, p.o)
Group II	:	Standard drug (Tentex Forte 171 mg/kg, p.o)
Group III	:	Low drug of Androcare (100 mg/kg, p.o)
Group IV	:	Medium drug of Androcare (200 mg/kg, p.o)
Group V	:	High drug of Androcare (400 mg/kg, p.o)

In the evening (17.00 to 18.00) different groups of mice were treated as mentioned above and then each male mouse was placed in separate cage. After one hr, one oestrous female with proven fertility was admitted into each cage and cohabitated overnight. Later these females were watched for pregnancy and birth of offsprings. With the litter size and number of male and female pups were recorded in each group. Similarly aphrodisiac activity of standard drug was also evaluated.

2) Effect on Sperm Properties in mice [14]:

Experimental Procedure:

Adult swiss albino male mice of (25-35g) each consisting of 6 animals were divided in to following groups

Group I	:	Normal control (Gum acacia 10ml/kg, p.o)
Group II	:	Standard drug (Tentex Forte 171 mg/kg, p.o)
Group III	:	Low dose of Androcare (100 mg/kg, p.o)
Group IV	:	Medium dose of Androcare (200 mg/kg, p.o)
Group V	:	High dose of Androcare (400 mg/kg, p.o)

Animals were administered with vehicle/standard drug/formulation for a period of daily once for 30 days and at the end of treatment period animals were sacrificed by overdose of ether anesthesia. Histopathological studies of testis were done by fixing the testes in Bouin's fluid and passed through ascending series of ethanol and then through xylene, and embedded in paraffin wax. Tissues were sectioned at 5 mm and stained with haematoxylene and eosin.

3) Sexual behavior on prolonged immobilization-induced stress in rats[15]:

Experimental Procedure:

Adult albino rats of (150-200g) each consisting of 6 animals were divided in to following groups.

Group I	:	Normal control (Gum acacia 10ml/kg, p.o)
Group II	:	Stress Control
Group III	:	Standard drug (Tentex Forte 1 gm/kg, p.o)
Group IV	:	Low dose of Androcare (100 mg/kg, p.o)

Group V : Medium dose of Androcare (200 mg/kg, p.o)
Group V : High dose of Androcare (400 mg/kg, p.o)

Different groups of prepubertal (40 days of age) male albino rats were housed under controlled environmental conditions and had free access to laboratory chow foodand tap water. In the morning time animals were treated as mentioned above. Immobilization Stress was induced by wrapping the animals in wire mesh daily 3 h for a day during the light period, starting at 8:00 A.M., for 15 days. Control animals for adaptation were left undisturbed in their cages. The males were placed in the observation 2 h after the beginning of the dark phase and 10 min before the females dropped into the cage. The latency, number of mounts and thrusting were recorded in red light of 40-watt capacity simultaneously by two investigators with light provided. In the mount behavior the male places his forepaws on the female without pelvic movements, while in the thrusting behavior it executes repeated deep pelvic thrusts.

Statistical Analysis

All values are expressed as mean ± SEM from 6 animals and results are subjected for statistical analysis using one-way ANOVA (analysis of variance) followed by Post hoc test (Dennett's 't' test). $P<0.05$ will be considered as statistically significant.

Results

1. Determination of acute toxicity (LD_{50}):

The polyherbal formulation, Androcare when administered orally to different groups of mice with different dose levels even upto the dose level of 2000mg/kg dose did not produced any mortality.

2 Aphrodisiac activity:

2.A Effect of Androcare on fertility in mice

When compared to control group Tentex Forte and Androcare with 200 and 400mg/kg doses but not 100mg/kg treated groups have shown an increase in the litter size. Similarly when compared to control group Tentex Forte (171mg/kg) and Androcare (100, 200 and 400mg/kg) treated groups does not exhibit a significant increase in M/F ratio.

Table 1. Effect of Androcare on litter size in female mice (Fertility model):

S.No	Group	Dose (mg/kg)	mean ± SEM
1	Control	10ml	4.500 ± 1.455
2	Standard	171mg	11.333** ± 0.3333
3	Low Dose Androcare	100 mg	6.667ns ± 1.358
4	Medium Dose Androcare	200 mg	9.167** ± 0.3073
5	High Dose Androcare	400 mg	10.167** ± 0.3073
One way ANOVA		F df	8.954 29

n=6 in each group; Significance at *$P<0.05$, ** $P<0.01$ & ns-not significance Vs control

Figure: 1. Effect of Androcare on litter size in female mice (Fertility model)

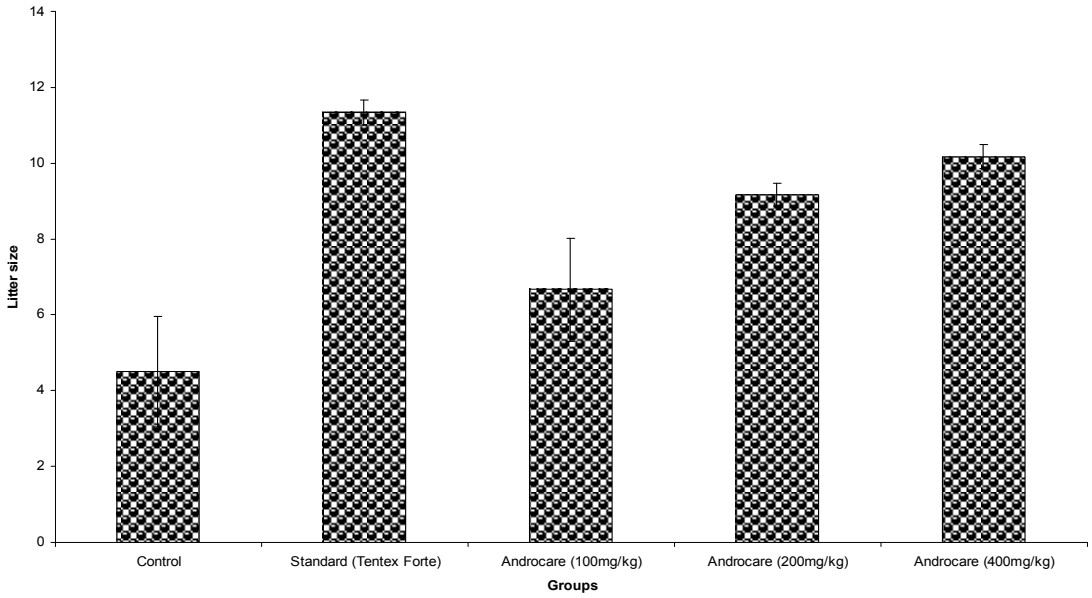
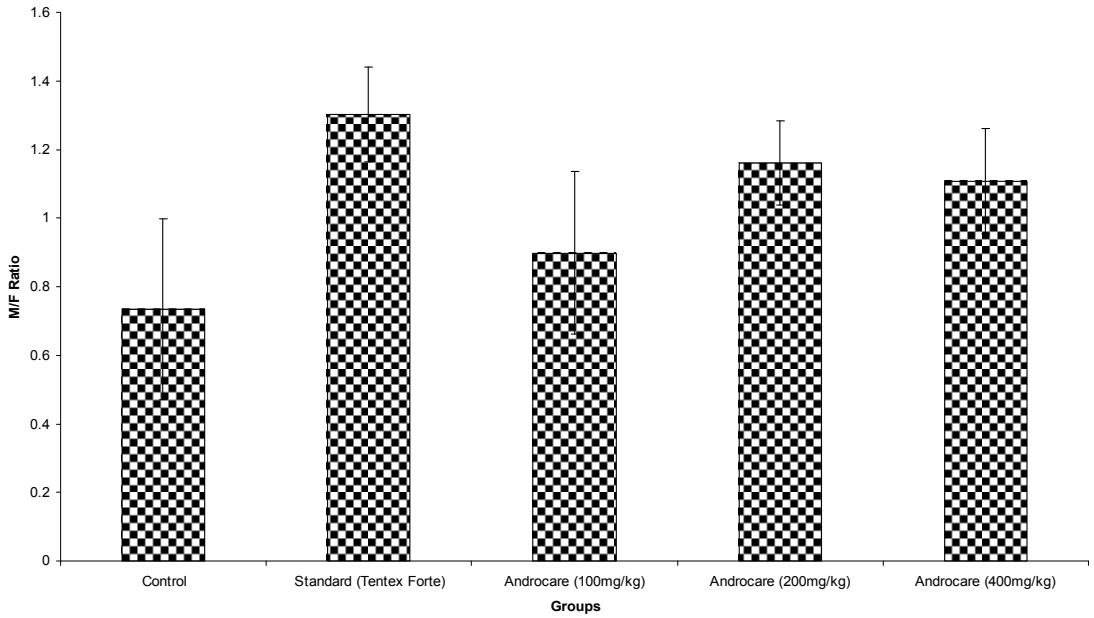


Table: 2 Effect of Androcare on M/F ratio in female mice (Fertility model):

S.No	Group	Dose (mg/kg)	mean ± SEM
1	Control	10ml	0.7350 ± 0.2631
2	Standard	7gm	1.302 ± 0.1371
3	Low Dose Androcare	100	0.8983± 0.2368
4	Medium Dose Androcare	200	1.160 ± 0.1221
5	High Dose Androcare	400	1.108 ± 0.1534
One way ANOVA		F	1.374
		df	29

n=6 in each group
Significance at **P*<0.05, ** *P*<0.01 & ns-not significance Vs control

Figure: 2 Effect of Androcare on M/F ratio in female mice (Fertility model):



2.B Effect of Androcare on sperm properties in mice:

When compared to the control animals histopathology of the testis in Standard (Tentex Forte 171mg/kg) and Androcare 400mg/kg treated groups has showed an outstanding increase in spermatogenic activity, with scant intertubular spaces where as low and medium dose of androcare has shown mild and moderate increase in the spermatogenic activity, with scant intertubular spaces between the tubules.

2.C Effect of Androcare on Sexual behavior in prolonged immobilization-induced stress in rats:

When compared to stress control animals, Tentex Forte (1gm/kg) and Androcare (200, 400 mg/kg) treated groups have shown a significant increase in the number of mounts, thrusts and decrease in the mounting latency. But low dose of Androcare 100 mg/kg did not exhibited a significant effect on number of mounts, thrusting and mounting latency.

Table 3 Effect of Androcare on mounting latency, number of mounts and thrusting in Stress induced altered sexual behavior in rats:

S.No	Group	Dose mg/kg	Mounting Latency in sec/30 min mean ± SEM	Number of Mounts mean± SEM	Thrusting mean ± SEM
1	Control	10 ml	925.83 ± 34.169	9.833 ± 0.6009	6.667 ± 0.4216
2	Standard Tentex Forte	1gm	59.167** ± 3.301	33.167 ** ± 2.272	31.333** ± 2.186
3	Low dose Androcare	100	965.83ns ± 5.764	14.167ns ± 1.302	11.167ns ± 0.6009
4	Medium dose Androcare	200	204.50** ± 20.013	22.000** ± 1.506	20.000** ± 1.342
5	High dose Androcare	400	73.667** ± 5.136	31.500** ± 0.7638	29.000** ± 1.265
One way ANOVA				F df	704.35 35

n=6 in each group

Significance at *P<0.05, ** P<0.01 & ns-not significance Vs control

Figure: 3 Effect of Androcare on sexual behavior in immobilization stress induced mounting latency in rats:

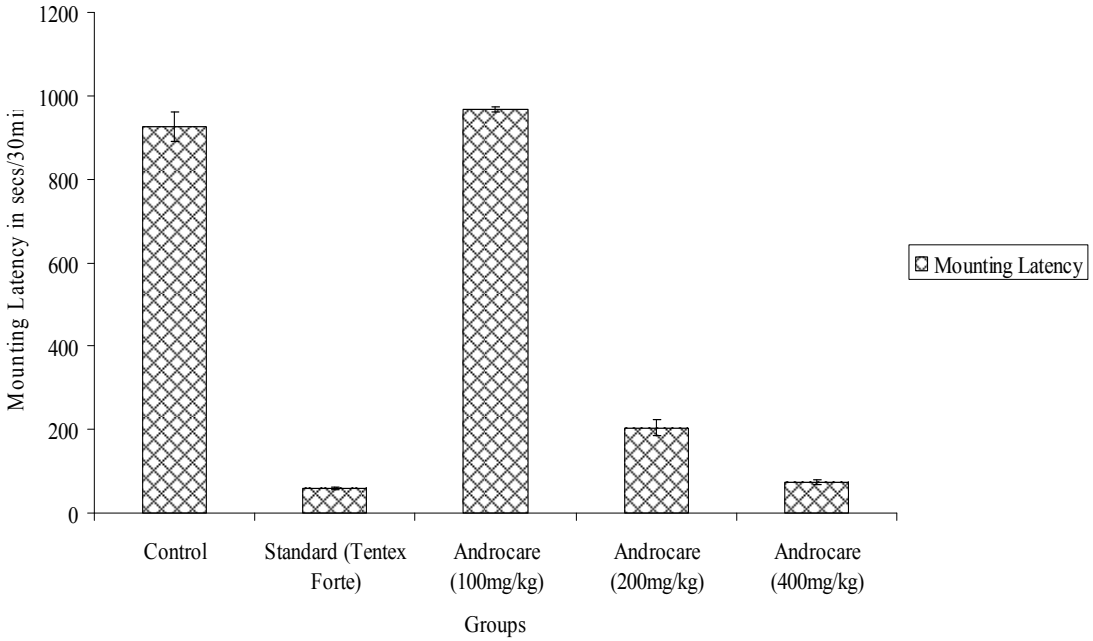


Figure 4 Effect of Androcare on sexual behavior in immobilization stress induced mounting latency in rats:

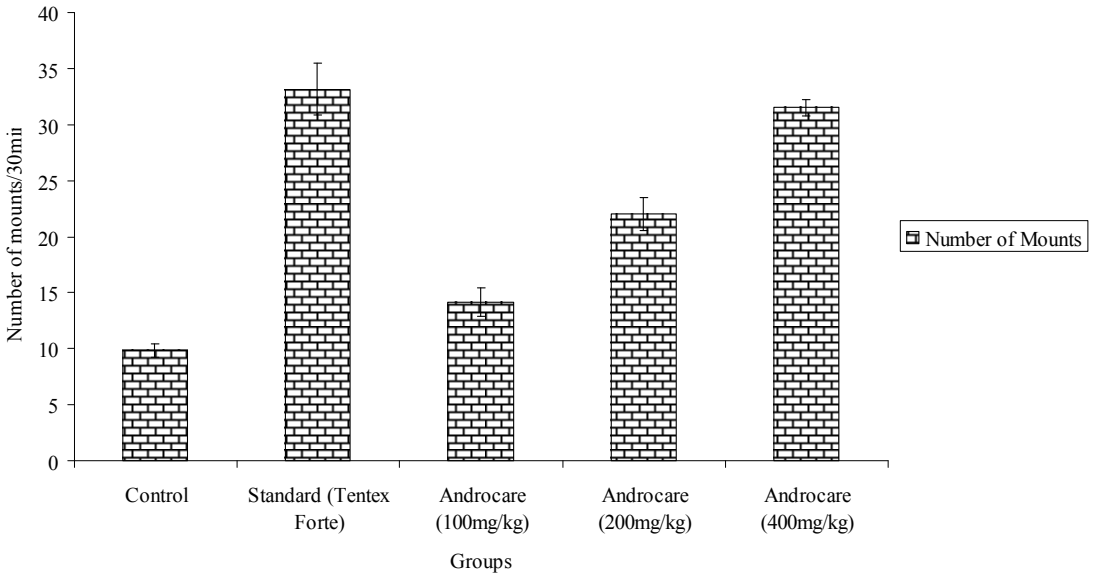
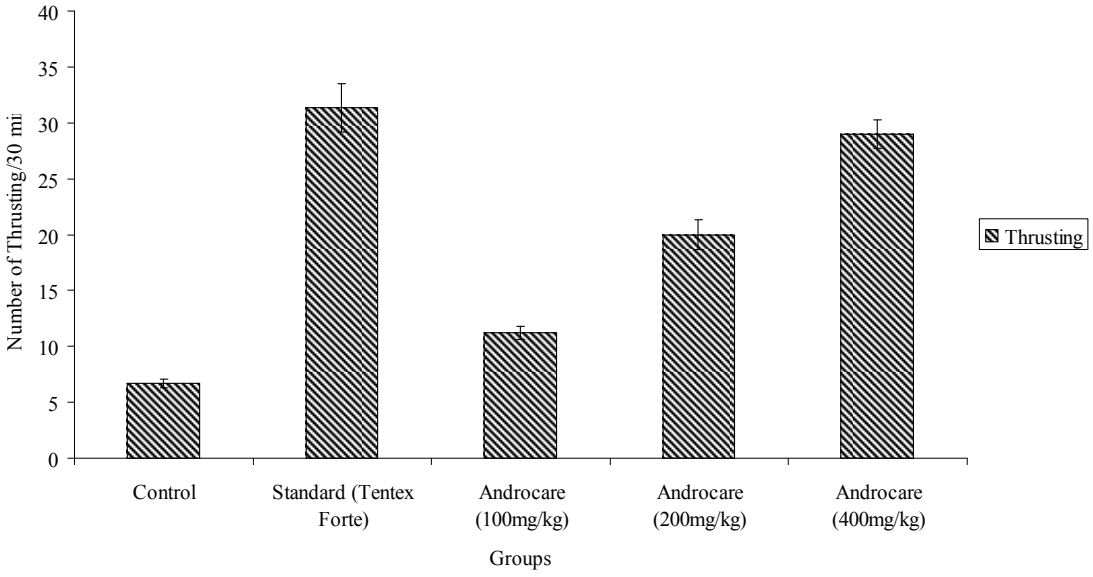


Figure: 5: Effect of Androcare on number of thrusting in Stress induced altered sexual behavior in Rats:



Discussion

Erectile dysfunction is more prevalent in males and so, it is more conventional to focus on male sexual difficulties. It has been discovered that men between 17 and 96 years old could suffer sexual dysfunction as a result of psychological or physical health problems.

Further it may result from psychological, neurologic, hormonal, arterial or cavernosal impairment or from a combination of these factors. The underlying causes for sexual disorders may be psychological, psychiatric, organic, interpersonal or related to pharmacological factors and treatment should be provided accordingly. Generally, a prevalence of about 10% cases occurs across all ages and sexual dysfunction is an inevitable process of aging, the prevalence is over 50% in men between 50 and 70 years of age. As men advances with age, the absolute number of Leydig cells decreases by about 40%, and the vigour of pulsatile leutenizing hormone release is dampened. In association with these events, free testosterone level also declines by approximately 1.2% per year. All these contributed in no small measure to prevalence of sexual dysfunction in the aged males[16].

Male sexual behavior is regulated by a range of redundant mechanism involving several neuropeptides like oxytocin and galanin, with inhibitory activity and neurotransmitters (mainly dopamine, serotonin, noradrenaline and NO). The stimulatory effect of oxytocin on male sexual behavior is proportionately greater in sexually sluggish than in sexually potent animals. Low non-stereotypy-inducing doses of direct or indirect dopaminiergic drugs improve the copulatory performance of sluggish/impotent males, while a further improvement of the sexual behavior of vigorous copulators is not always clearly apparent. Finally, facilitation of central noradrenergic transmission, either by alpha 2-adenoreceptors blockade or by stimulation of beta 2-adrenorecpetors, while having either no effect or a worsening effect in sexually potent animals, improves copulatory behavior in sexually sluggish animals[17].

Many central neurotransmitters and neuropeptides are involved in the control of male sexual behavior. Increased brain noradrenergic and dopaminergic activities may improve parameters of copulatory activity, indicating their facilitatory role in the process. The effects of serotonin and dopamine on male copulatory behavior seem to occur by interaction with testosterone. A proper androgenic status is also necessary for a normal sexual performance, the deleterious effects of castration being reversed by hormonal replacement; Moreover, chronic treatment of prepubertal rats with testosterone can precipitate the onset of first mount, thrusting and ejaculation, probably by stimulation of sexual arousal. It is possible that increased plasma testosterone concentration, in addition to the higher catecholamine and serotonin levels expected to occur after prolonged stress, might account for the enhanced sexual performance described at the onset of puberty.

As a result of hypothalamic-pituitary-adrenal axis activation, prolonged stress may inhibit the male reproductive functions through a depression of the hypothalamicpituitary- testicular axis. Chronic intermittent immobilization-induced stress caused a significant decrease in plasma LH of both pubertal and adult rats, whereas plasma testosterone was lower than control levels in adult stressed rats but was more than twofold higher in pubertal animals, suggesting that prolonged stress probably acts in a different way on the gonadal axis during distinct phases of sexual development. Since adrenergic innervation seems to play a pivotal role in testicular steroidogenesis around the onset of puberty, it is we proposed that sympathetic over stimulation might explain the increased testosterone levels observed in pubertal stressed rats. Prolonged immobilization caused no significant change in plasma FSH but induced a significant delay in testicular maturation, in addition to a decrease in spermatid production and sperm density in both pubertal and adult animals[18].

Testosterone supplementation improves sexual function and libido, in addition to the intensity of orgasm and ejaculation which is likely to improve. Testosterone in the blood exists in three different forms namely: free, albumin-bound and sex-hormone binding globulin (SHBG). While it is generally considered that SHBG bound testosterone is not available for uptake by tissues, opinion is mixed as to whether the biologically active testosterone is restricted to the small quantity of the hormone that is free (2%) or includes the larger amount of albumin-bound hormone (20–80%). However, investigations suggest that both free and albumin-bound testosterone is biologically available. Generally, elevated testosterone level also enhances the sexual behaviour in humans. Therefore, an increase in testicular and serum free testosterone concentration can confirm aphrodisiac potential inherent in the plant extract. Generally sexual behaviors is enhanced by elevated testosterone levels, and Drug induced changes in neurotransmitter levels or their action in the cells could also changes sexual behavior.

The purpose of the present study is to determine whether prolonged immobilization- induced stress from prepuberty interferes with the onset of sexual behavior at puberty and with fertility during adulthood. The exposure to stress decrease male sexual activity and cause longer latencies with decrease in number of mounts and thrusting.

In order to evaluate whether the polyherbal formulation, Androcare has effect on litter size and M/F ratio, the experiment fertility in mice model was selected and Androcare has shown an increase in the litter size but no effect on M/F ratio.

Further whether the formulation, Androcare has modifying the effect of mounting latency, mounts and thrusting in stress induced animals in prolonged immobilization stress model ,was sexual behavior in rats to assess I sever stress.

The above all functional and histological parameters noted and photomicrography of testicular tissue clearly depicts that the polyherbal formulation, Androcare possessed aphrodisiac activity.

Conclusions

It is interesting to note that during acute toxicity study, the polyherbal formulation, Androcare was non-toxic and has not produced any lethal effect even upto the dose level of 2000mg/kg in mice. When compared to control even after prolonged immobilization induced stress, Androcare has shown an significant effect which were indicated by increase in litter size in Fertility model, increase in spermatogenic activity and Sperm properties in mice model and increase in thrusting, number of mounts and decrease in mounting latency in Sexual behavior model in rats.

The formulation, Androcare has not shown any influence on m/f ratio in Fertility model in mice.

Thus the results concluded that the formulation Androcare, has a definitely aphrodisiac activity.

In order to understand exact mechanism of aphrodisiac effect of Androcare it is necessary to perform assays for neuronal nitric oxide synthase and Androgen receptor protein in different models of experimental animals.

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