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Aphrodisiac activity of *Cordia dichotoma forst .f.* fruits

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ABSTRACT

The aphrodisiac activity of extracts of *Cordia dichotoma Forst.f.* fruits (300mg/kg body wt) was studied in albino rats of Wistar strain. The extractions of *C.dichotoma Forst.f.* fruits were carried out using ethanol. This extract was fractionated using petroleum ether, solvent ether, ethyl acetate, butanol and butanone in succession. These extracts were studied for aphrodisiac activity using different components of copulatory behaviour viz. sniffing, genital grooming, mounting frequencies and number of mating in albino rats. A significant increase in sexual activity was observed in petroleum ether, ethyl acetate and butanol extracts of fruits of *C.dichotoma Forst.f.* The animals showed improved responses, thus showing the efficacy of drug as an aphrodisiac and its use in the traditional Indian system of medicine.

INTRODUCTION

C.dichotoma Forst.f. a plant belonging to family Boraginaceae is medium sized tree with a short, usually crooked trunk 3-4 ft. in girth[1]. The fruits are globose, yellowish-brown, pink or black and pulpy. The plant grows in India, Sri Lanka and other warmer regions. The medicinal attributes of *C.dichotoma* have been known since a long time. The fruits of the plant are used as cooling, astringent, emollient, expectorant, anthelmintic, purgative and diuretic[2]. A number of pharmacological properties such as analgesic, antiinflammatory and hepatoprotective have been reported[3-5]. A survey of literature revealed that no systemic work has been carried out to establish the potential of the fruits in spite of its frequent and reputed use against sexuality debility. The present work is an attempt to verify the claims made in the traditional system of medicine.

MATERIALS AND METHODS

Preparation of plant extracts

The fruits of *C.dichotoma* were purchased from local market of Hubli and were authenticated by Prof V.S.Huddar, Department of Botany, H. S. K Science and S. K Arts College, Hubli. The collected fruits were shade dried, reduced to coarse powder (2 kg) and subjected to repeated exhaustive extraction in batches with ethanol in a soxhlet extractor. After complete extraction the alcoholic extract (68 gm) was then suspended in water and further fractionated using petroleum ether (8 gm), solvent ether (2 gm), ethyl acetate (4 gm) butanol (5.5 gm) and butanon (2.5) in succession. These extracts were vacuum dried and used for aphrodisiac activity. Tween 80 (1%) was used as vehicle to suspend the extracts.

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TABLE 1: Qualitative chemical examination of various extracts obtained by successive solvent fractionation of the *Cordia dichotma forst.f.* fruits

Sl. no.	Phytoconstituents	Pet. ether fraction	Solvent ether fraction	Ethyl acetate fraction	Butanone fraction	Butanol fraction
1.	Alkaloids	+	+	+	+	+
2.	Carbohydrates	+	+	+	+	+
3.	Phytosterols	-	-	-	-	-
4.	Flavanoid	-	-	+	-	+
5.	Tannins	-	-	-	-	-
6.	Proteins and amino acids	+	+	+	+	+
7.	Saponins	+	+	-	-	-

Phytochemical screening

All the extracts were subjected to phytochemical analysis as per standard procedures[6] to know the nature of phytoconstituents present (TABLE 1). The major active constituents were found in extracts includes flavanoids, alkaloids and saponins. Among all active constituents flavanoids were found to be in higher concentrations.

Animals

The experiments were initiated only after approval Institutional Animal Ethical Committee. Albino mice weighing 25-30 g and albino rats weighing 150-200 gm were obtained from central animal house, K.L.E.S's College of Pharmacy, Hubli, Karnataka. They were maintained at standard housing conditions and fed with commercial diet (Hindustan Lever Ltd., Bangalore) and water ad libitum during the experiment.

Acute toxicity studies

The acute toxicity studies were carried out as per stair case or "Up and down method[7]. Accordingly the LD_{50} of all extracts were found to be 3000 mg/kg bodyweight. One tenth of this dose was selected for the study of aphrodisiac activity.

Experimental design for aphrodisiac activity

The Albino rats of Wistar strain are divided into six groups of six animals each. Group I served as control where as group II. III. IV, V and VI were administered with petroleum ether, solvent ether, ethyl acetate, butanol and butanone respectively.

Selection of the male rats

Males were trained individually with normal adult females in estrous in a transparent arena. Only such males, which attempted to mount any female introduced into the cages, were used for subsequent experiments. To provide sexual experience, each male rat was allowed 30 minutes exposure to a stimulus female in behavioral estrous, several days before testing for copulatory performance. The animals were tested three times over 21 days periods for copulatory behaviour.

Selection of female rats

Adult female rats were ovariectomies. They were allowed to recover from the effect of operation for 10 days. Ovariectomisation and confirmation of estrous were carried out according to standard procedure[7]. After Ovariectomisation, the animals were in diestrous stage. To bring them to estrous, they were given 2µg/kg of estrogens 48 hours before and 500µg/kg of progestesterone 6 hours before copulatory test.

Evaluation of the efficiency of *C.dichotoma*

From 2 weeks prior to the screening tests until the end of the study, the rats were housed individually at 25 ± 2^0C under reversed light and dark cycle (with light from 11P.M.to11A.M.). The rats were fed with commercial pellet rats chow and water *ad libitum*.

The LD_{50} of the used extracts were found to be 3000-mg/kg body weight[6]. The extracts of *C.dichotoma* were given to male rats orally in the dose of 300mg/kg of body weight daily. Female ovariectomies rats were given 2µg/kg of estrogens 48 hours before the experiments; 500g/kg of progestesterone was also given 6 hours before the starting of the experiment. Six hours after administration of progesterone, the ovariectomies female rats were observed for estrous stage by observing the vaginal smear of the rat. The female rats, which were in estrous stage, were employed for the study. The highly receptive female was introduced into male's cage and each male rat was observed for 30 min for copulatory behaviour under dim red light. Simi-

lar procedure was followed for all groups and the following parameters were recorded.

1. Sniffing at female genitals

This behaviour of male rats towards the female genitals was observed and recorded for a fixed period of time.

2. Male genital grooming

Male rats chase the female and in between start grooming its own genitals, which was counted for a fixed period of time.

3. Number of mounts (Mounting frequency)

Number of mounting act carried out in a particular period of time was recorded.

4. Assessment of mating

For the assessment of mating, six males were placed individually and five estrous females were admitted in to each cage and they were cohabited over night. The vaginal smear of each female was examined under a microscope for the presence of sperm. The number of sperm positive female was recorded in each group. The mating assessment study was carried out on 1st, 7th, 14th and 21st days of treatment.

5. Effect on body weight

During the aphrodisiac study the body weight of all the male rats was recorded before the experiment and finally after completing the observations but before sacrificing them.

6. Determination of sperm motility

On the 21st days after the behavioural observations male rats of all the groups were sacrificed by cervical dislocation. The cauda epididymis was identified and cut longitudinally into three segments. The compacted spermatozoa were released from the cut tubules by gentle pressure and were deposited on a clean prewarmed (37^0C) microscopic slide. It was covered with a cover slip and ringed with petrolatum. Motility was evaluated by scanning several fields with high dry objective until a total of at least 100 spermatozoa were observed. It is essential to focus through the entire depth of a given field so as to include non-motile sperms, which may have settled to the bottom of the medium. The percentage of sperm showing actual progressive motion was recorded.

7. Determination of sperm count

The sperm suspension remaining after the motility determination was counted using a haemocytometer chamber following initial dilution in a white blood cell pipette. The sperm sample was mixed thoroughly and drawn an aliquot to the 0.5 mark on the pipette and diluted till 11 marks with the diluting solution, which was prepared by taking Sodium bicarbonate-5g. Formalin (neutral) 1 ml. and Distilled water 100ml. After charging the haemocytometer chamber, 2 min was allowed for the immobilized sperm to settle. The spermatozoa in 4 sq. mm (four large squares) were counted. This number multiplied by 50,000 gives the number of spermatozoa per ml.

8. Effect on fertility

After the mating experiments, the sperm positive females were identified by vaginal smear observations and were watched for pregnancy and birth of offspring. Number of male and female pups was recorded in each case.

Statistical analysis

All the results were reported as mean ± S.E. Significance of the difference between "control" and" drug treated" were determined by the student "t" test

RESULTS

The LD_{50} of the extracts were found to be 300mg/kg body weight by Up and Down method and 1/10th of the LD_{50} i.e. 300mg/kg body weight was considered as therapeutic dose. All the results were analysed by student "t" test and level of significance was $P<0.001$. 1) Sniffing at female genitals- There was significance increase in sniffing- On the 14th days in the petroleum ether, ethyl acetate, butanol and butanone groups of animals compared to the control group (TABLE 2). This trend was observed on 21st days also. On the 21st days sniffing was more than double of that of control group. This result showed significant increase ($P<0.001$) in sniffing. *2*) Genital grooming- When observed on the 21st day of administration of the drug revealed that there was increase in this parameter when compared to control group (TABLE 3), indicating the efficacy of drug.

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TABLE 2: Effect of *C.dichotoma* fruits extracts on male rat sniffing at female genitals (number of times)

Group	1st Day	7th Day	14th Day	21st Day
Control	1.83 ±0.307	3.5 ±0.428	3.83 ±0.60	3.5 ±0.422
Pet. Ether	4.5 ±0.619**	6.66 ±0.333***	8.5 ± 0.420***	8.66 ±0.494***
Sol. Ether	2.66 ±0.499	3.66 ±0.333	4.16 ±0.470	4.66 ±0.210
Eth.acetate	4.33 ±0.557**	6.8 ±0.307***	8.66 ± 0.333***	8.33±0.494***
Butanol	5.66 ± 0.496***	7.66 ±0.421** *	9.5 ± 0.422***	10.33 ±0.55***
Butanone	5.33 ± 0.660***	7.5 ±0.420***	9.5 ±0.423***	10.16 ±0.791***

The values are expressed as Mean ± SE, The significance on comparison with control group is indicated by *mark, *** p<0.001 ** p<0.01

TABLE 3: Effect of *C.dichotoma* fruits extracts on male genital grooming (number of times)

Group	1st Day	7th Day	14th Day	21st Day
Control	1.5±0.223	2.5 ±0.223	1.5 ±0.223	2.3 ±0.210
Pet. Ether	2.66±0.494***	3.16± 0.307***	4.66 ± 0.494***	5.16 ±0.477***
Sol. Ether	2.0 ±0.258	3.33 ±0.210	3.63 ±0.307***	4.5 ±0.428***
Eth.acetate	3.66 ± 0.333	4.66 ± 0.494	6.33 ± 0.494***	8.83± 0.307***
Butanol	3.0 ± 0.258	4.33 ±0.333	6.33± 0.555	8.99± 0.307***
Butanone	3.33 ± 0.660***	3.95 ±0.420***	5.96 ± 1.16***	7.66 ± 0.333***

The values are expressed as Mean ± SE, The significance on comparison with control group is indicated by *mark, *** p<0.001 ** p<0.01

TABLE 4: Effect of C.*dichotoma* fruits extracts on mounting frequency (number of times)

Group	1st Day	7th Day	14th Day	21st Day
Control1	1.0 ±0.258	1.5 ±0.223	2.33 ±0.210	2.83 ±0.307
Pet. Ether	2.68±0.107**	3.84± 0.307***	7.33 ± 0.421***	8.0 ±0.577***
Sol. Ether	1.84 ±0.307**	2.18 ±0.210***	2.58± 0.730**	3.83± 0.494***
Eth.Acetate	2.14 ±0.307**	3.5 ±0.428**	5.0± 0.730**	6.83± 0.793***
Butanol	2.35± 0.223**	3.67 ±0.333**	5.33± 0.730**	7.28± 0.494***
Butanone	2.5 ± 0.428**	3.84 ±0.477**	5.0 ± 0.577**	7.66 ± 0.667***

The values are expressed as Mean ± SE, The significance on comparison with control group is indicated by *mark, *** p<0.001 ** p<0.01

TABLE 5: Effect of *C.dichotoma* fruits extracts on assessment of mating (number of sperm positive females) in rats

Group	1st Day	7th Day	14th Day	21st Day
Control	0.5 ±0.223	1.5 ±0.223	1.33 ±0.210	1.5 ±0.223
Pet. Ether	1.83 ± 0.166***	3.84± 0.307***	3.66 ± 0.421***	4.5 ±0.427***
Sol. Ether	1.33 ±0.210	1.68 ±0.210***	2.33 ±0.421	3.33 ±1.03***
Eth.acetate	1.33 ± 0.333	3.55 ± 0.428**	4.5 ± 0.494***	5.83± 0.307***
Butanol	2.0 ± 0.365**	3.67 ±0.333*	4.2± 0.524**	4.95± 0.342***
Butanone	1.5 ± 0.223**	3.84 ±0.477**	3.16 ± 0.3071***	4.66 ± 0.413***

The values are expressed as Mean ± SE, The significance on comparison with control group is indicated by *mark, *** p<0.001 ** p<0.01

As far a mounting frequency is concerned, significant increase was found on 14th and 21st day in petroleum ether, ethyl acetate, butanol and butanone treated groups when compared to control group (TABLE 4). Similarly as far an assessment of mating is concerned, significant increase was found on 14th and 21st day in petroleum ether, ethyl acetate, butanol and butanone treated groups when compared to control group (TABLE 5). Also there was marked increase in body weight, sperm motility, sperm count and fertility compared to control are indications of the effectiveness of drug as aphrodisiac. To attain maximum benefit, administration of the drug for a month is preferred.

DISCUSSION

The drug reported to be aphrodisiac in traditional system of medicine and used for that purpose has been shown in rat's significantly increased sexual activity. The phytochemical study of fruits shows presence of flavonoids. In the present study, the findings reveal that the tested extracts showed significant aphrodisiac ac-

tivity. Thus from the results of the current investigation it may be inferred that the petroleum ether, ethyl acetate, butanol and butanone extracts of Cordia dichotoma Forst.f. fruits possess potent aphrodisiac activity. C.dichotoma fruit is an essential constituent in traditional medicine for the treatment of sexual impotence. It is likely that this effect reflects the tonic, restorative and adptogenic, properties reported. However, experimental studies have indicated a specific action for such an effect. R.Hesham et al.[8] have shown that flavonoids relax rabbit corpus cavernosum and it is effect mediated by nitric oxide, released from endothelial or neural cells. These endothelial and/or nitrogenic effects of flavonoids in inducing relaxation of the carpus cavernasum may account for the aphrodisiac effect of C.dichotoma fruits. Consistently with this nitric oxide linked mechanism, several recent studies have suggested that the antioxidant and organ-protective actions enhanced nitric acid. Further study regarding isolation and characterization of active principal responsible for aphrodisiac activity is currently under progress.

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