

Research Article

Investigations on *Citrullus colocynthis* seeds for aphrodisiac activity in male albino rats

Shain Kumar Singh, Alok Pal Jain*, Manisha Tandon

RKDF College of Pharmacy, SRK University, Hoshangabad Road, Misrod, Bhopal - 462026 (MP) India

Received: 2 May 2020

Revised: 4 June 2020

Accepted: 13 June 2020

Abstract

Objective: The aim of the study was to evaluate the effect of the aqueous and ethanolic extract of *Citrullus colocynthis* on reproductive abilities of male albino rats. **Materials and methods:** Plant extracts at doses of 200 and 400 mg/kg body weight were administered for 21 days. Activity was observed by assessment of counting number of mounts, mating performance, hormonal analysis, testes-body ratio and sperm count. **Results:** The ethanolic extract of *Citrullus colocynthis* L. seeds at higher concentration (400 mg/kg body weight) showed significant aphrodisiac activity on male wistar albino rats as evidenced by an increase in number of mounts, mating performance, hormonal analysis, testes-body ratio and sperm count. On the other hand, ethanolic extract at lower dose (200 mg/kg. body weight) and aqueous extract (400 mg/kg body weight) showed moderate aphrodisiac property. The extract was also observed to be devoid of any adverse effects and showed negative results for acute toxicity. **Conclusion:** Thus, the results of the present study demonstrate that ethanolic extract of *C. colocynthis* seeds enhance sexual behavior in male rats.

Keywords: *Citrullus colocynthis*; Seeds; Aphrodisiac; Mating; Herbal medicine, Male Rat, sexual behavior

Introduction

An aphrodisiac is defined as an agent that arouses sexual desire. Many natural substances have historically been known as aphrodisiac (Ang and Sim, 2001). Sexual dysfunction is a repeated inability to achieve normal sexual intercourse, which includes various forms like premature ejaculation, retrograded, or retarded ejaculation, erectile dysfunction, arousal difficulties, etc. Several management options employed are associated with some serious side effects and are not readily available and expensive. The search for natural supplement from medicinal plants is being intensified, probably because of reduced side effect, its ready availability and reduced cost.

Therefore, the increasing used for search and screening of medicinal plants with aphrodisiac potential in male has been necessitated (Yakubu et al., 2007).

Citrullus colocynthis has the traditional use in remedy for

cancer, carcinoma, endothelioma, leukemia, tumors of the liver and spleen, even the eye. Roots may also be used as a purgative against as cures for jaundice, urinary diseases, rheumatism and for snake poison. This plant is available in the southern coastal areas of the Bay of Bengal (Chaturvedi et al., 2003).

Citrullus colocynthis is commonly known as the colocynth, bitter apple, bitter cucumber. It resembles a common watermelon vine, but bears, small, hard fruit with a bitter pulp. The main chemical contain of fruit pulp is colocynthin (the bitter principle up to 14%), colocynthein (resin), colocynthetin, pectin gum. Seed contain a fixed oil (17%) and albuminoids (6%). *Citrullus colocynthis* is widely used in folk medicine for centuries and as an energy source also e.g. Oilseed and biofuel. The leaves are diuretic and used in the treatment of jaundice and asthma. The root is useful in inflammation of the breasts, amenorrhea, rheumatism, joint pains and is used externally in ophthalmia and uterine pains. The fruit is pungent, cooling purgative, anthelmintic, antipyretic carminative. It cures, tumors, leucoderma, ulcers, asthma, bronchitis, urinary discharge, enlargement of spleen, tuberculosis glands of

*Address for Corresponding Author:

Dr. Alok Pal Jain

RKDF College of Pharmacy, SRK University, Hoshangabad Road, Misrod, Bhopal - 462026 (MP) India

Email: dralokpaljain@gmail.com

DOI: <https://doi.org/10.31024/apj.2020.5.3.6>

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the neck, dyspepsia, constipation, anemia's and throat diseases. The fruit pulp is purgative, diuretic, antiepileptic, and

is used against gonorrhoea (Dhanotia et al., 2011; Marzouk et al., 2010; Qazan et al., 2007).

But to the best of our knowledge, there is no information in the open scientific literature that has substantiated or refuted the aphrodisiac claims of *Citrullus colocynthis* seeds in the folklore medicine. Hence then, the present work was undertaken to validate scientifically the aphrodisiac role of *Citrullus colocynthis* seeds as acclaimed by the traditional tribal user of region of Bhopal district, Madhya Pradesh.

Materials and methods

Collection of Plant Material

Seeds of *Citrullus colocynthis* were collected from Vidisha district, Bhopal, India during the flowering period of September to February, identified and authenticated by Department of Botany, Barkatullah University, Bhopal.

Procurement and rearing of experimental animal

Healthy wistar strain male albino rats, two months old and weighing 200- 300 g were procured from RKDF College of Pharmacy, Bhopal (Madhya Pradesh). The rats were housed in polypropylene cages and maintained under environmentally controlled room provided with a

12:12 hours light and dark cycle approximately at 25°C. They were fed on pellets and tap water *ad libitum*. The rats were allowed to acclimatize to laboratory environment for 15 days before experimentation.

Preparation of extract

The seeds of *Citrullus colocynthis* were collected, shade dried, powdered and subjected to soxhlet extraction with distilled water, ethanol. The extract was evaporated to near dryness on a water bath, weighed and kept at 4°C in refrigerator until further use.

Phytochemical screening

The presences of various constituents in the seed extract of *Citrullus colocynthis* were determined by preliminary

phytochemical screening as per Obasi (Obasi et al., 2010).

Acute toxicity study

Healthy male albino rats were starved for 3-4 hr and subjected to acute toxicity studies as per (OECD) Organization of Economic Co-operation and Development guidelines No: 423 (OECD, 2004).

Aphrodisiac activity study

Experimental details: The sexually active male rats were chosen separately and divided into 6 groups; each group consisting of 6 animals. The animals in the divided group received the treatment orally. Different groups of animals which received the plant extract and the control are as follows in table 1.

Mating behavior study

Mating behavior studies were carried out in a separate room under dim red illumination according to the standard procedure. Healthy male albino rats showing brisk sexual activity and female animals showing regular oestrus cycle were selected for the study. The male rats were placed in a rectangular plexiglass chamber, 10 minutes before the introduction of a primed female and get acclimatized to the chamber conditions. The primed female was then introduced into the chamber with one female to one male ratio and the mating behaviors observed for first week and third week after commencement of the PHF treatment. The following mating behavior parameters were recorded: (a) Mount frequency (MF) (b) Intromission frequency (IF) (c) Mount latency (ML) (d) Intromission latency (IL) (e) Ejaculation latency (EL) (f) Post-ejaculatory interval (PEI). The experiment was terminated when the male rat begins to mount the female followed by intromission after a brief period of inactivity (which normally results following ejaculation). The values of the observed parameters were measured at first week and third week of drug administration and compared with control as well as standard (Gauthaman et al., 2002; Subramoniam et al., 1997).

Table 1. Different groups of animals which received the plant extract and the control

Groups	Treatment	Dose
I	Control (0.5% CMC) (Group I)	2 ml/kg b.wt.
II	Standard (Sildenafil citrate) (Group II)	5 mg/kg b.wt.
III	Aqueous extract of Seeds of <i>Citrullus colocynthis</i> (Group III)	200 mg/kg b.wt.
IV	Aqueous extract of Seeds of <i>Citrullus colocynthis</i> (Group IV)	400 mg/kg b.wt.
V	Ethanollic extract of Seeds of <i>Citrullus colocynthis</i> (Group V)	200 mg/kg b.wt.
VI	Ethanollic extract of Seeds of <i>Citrullus colocynthis</i> (Group VI)	400 mg/kg b.wt.

Statistical analysis

All the data are expressed as Mean ±S.E. Statistical analysis was done by Student's t-test and one way ANOVA (Mahajan, 1997).

Results and discussion

The phytochemical study of *Citrullus colocynthis* gives valuable information about the chemicals present in the plant. The various qualitative chemical tests showed the presence of diterpenoids, saponin, sterols, flavonoids, carbohydrate and alkaloids. Aromatic acid, gums and mucilage and tannin were totally absent in the seed of this plant.

From (Table 2 and Figure 1), the data reveals the effect of prepared extracts at the doses of Group V and Group VI on various parameters of mating behaviour. Daily administration of prepared extracts for 3 weeks to male rats resulted in increase in the mating

behaviour as compared to the control group. It is observed that extremely significant results were obtained by Group V and Group VI when compared to control.

In (Table 3 and Figure 2), daily administration of prepared extracts for 3 weeks to male rats resulted in a dose-dependent increase in the mating performance as compared to the control group. The prepared extracts at the doses of Group- III, Group- IV, Group- V and Group- VI, showed 53.75%, 63.95%, 68.98% and 75.05% mating performance, respectively, against 38.65% of the control group, whereas the standard showed 82.07% mating performance. The prepared extract of EECC-400 showed closely resemblance with standard treatment and plays a significant role in mating performance of rats as compared to control.

Table 2. Effect of aqueous and ethanolic extracts of *Citrullus colocynthis* on mating behavior after 3 weeks treatment in male rats

Treatment Groups	Dosage (mg/kg body wt)	ML	IL	EL	PEI	NM	MF	IF
Group- I (Control)	Vehicle	11.65±0.81	11.16±1.35	255±193	520±2.18	5.47±0.38	68.30±7.48	79.49±5.18
Group- II Standard	5	1.56±0.35	1.27±145	1215±2.65	4.26±2.46	7.46±0.56	224±6.46	214±5.56
Group- III (Aq. Extract)	200	8.36±1.35	8.37±1.56	258±2.37**	426±2.69	6.45±0.96	75.34±6.84	124.75±5.7**
Group- IV (Aq. Extract)	400	7.47±0.96**	8.74±1.35**	523±2.75	684±1.45	6.85±0.64	39.94±5.45	184.74±5.76***
Group- V (Ethanolic Extract)	200	5.95±0.65**	5.75±0.35**	5.76±0.56**	5.03±0.98**	5.86±0.98**	5.86±0.54**	5.97±0.45**
Group- VI (Ethanolic Extract)	400	3.76±0.56***	3.74±0.74***	3.86±0.45***	3.74±0.36***	3.95±0.67***	3.86±0.34***	3.98±0.35***

Paired t-test: All values were expressed as Mean±SD (n=6); ***P<0.0001 considered extremely significant as compared to control, **P<0.01 considered significant as compared to control

Table 3. Effect of aqueous and ethanolic extracts of seed of *Citrullus colocynthis* on mating performance in male rats

Treatment groups	% of Sperm positive females
Control	38.65
Standard	82.07
AECC-200	52.75
AECC-400	63.95***
EECC-200	68.98***
EECC-400	75.05***

Paired t-test: All values were expressed as Mean ± SD (n=6); ***P<0.0001 considered extremely significant as compared to control

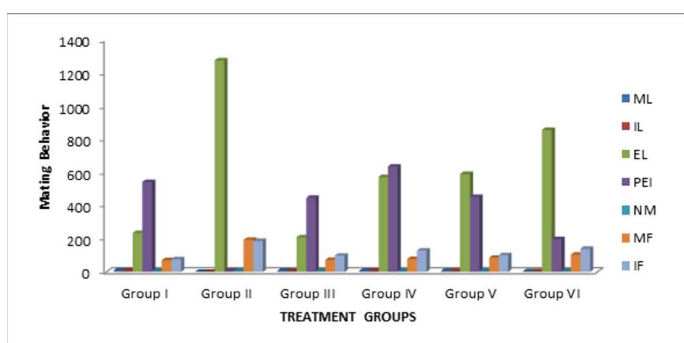


Figure 1. Effect of aqueous and ethanolic extracts of *Citrullus colocynthis* on mating behavior after 3 week treatment in male rats

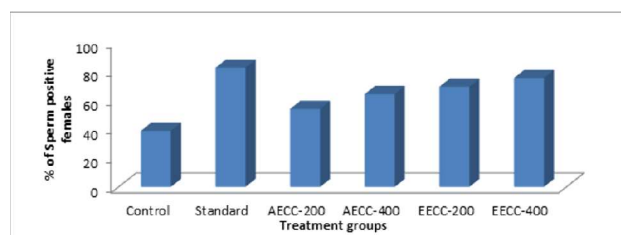


Figure 2. Effect of aqueous and ethanolic extracts of seed of *Citrullus colocynthis* on mating performance in male rats

Conclusion

This aphrodisiac activity study lends support to the claim for traditional usage of *Citrullus colocynthis* as a sexual function enhancing medicine. Thus, this study may prove to be an effective and safe alternative remedy in sexual disorders. Work is in progress on the isolation and characterization of the aphrodisiac principle in the plant extract, the actual mechanism of action of the crude extract and bioactive agents.

Acknowledgment

Authors are thankful to Management, SRK University, Bhopal MP (India) for providing lab facility and financial support.

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