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Aphrodisiac Activity of *Alocassia macrorhiza (L.)* on Ethanol Induced Testicular Toxicity in Male Rats

**[**]A. Saravana Kumar, [1]S. Mohana Lakshmi

[*,1]Sree Vidyanikethan College of Pharmacy, Tirupathi , Andhra Pradesh , India-517102.

Abstract

According to Indian Systems of Medicine, *Alocassia macrorhiza (L.)* belonging to the family **(**Araceae**)**, were used for treating male sexual disorders since ancient times. Aim of this study to evaluate the phytochemical constituents and the aphrodisiac potential of the petroleum ether extract of leaves of *Alocassia macrorhiza (L.)* on ethanol induced testicular toxicity in albino wistar rats. Phytochemical screening revealed the presence of alkaloids and saponins. All the doses resulted in significant increase in mount frequency, intromission frequency and significantly prolonged the ejaculatory latency ($P < 0.05$) and reduced mount and intromission latency ($P < 0.05$). There was also a significant increase in serum testosterone concentrations in all the groups in a manner suggestive of dose-dependence ($P < 0.05$). Results of this study concluded that the petroleum ether extract of *Alocassia macrorhiza (L.)* increased the blood testosterone concentrations and this may be the mechanism responsible for its aphrodisiac effects and various masculine behaviors. It may be used to modify impaired sexual functions in animals, induced testicular toxicity in albino wistar rats.

Introduction

Erectile dysfunction is defined by the National Institutes of Health as the inability to attain or maintain an erection sufficient for satisfactory sexual performance [1]. It is a highly prevalent and often under treated condition. Although a wide range of risk factors contributes to the development of Erectile dysfunction, vasculogenic Erectile dysfunction is recognized as the most common organic etiology (70%), about (30%) of it related to diabetes mellitus [2]. Psychogenic causes (30%) include depression, performance anxiety, relationship problems and psychosocial stressors. Combination of organic and psychogenic factors is common [3].

Alocassia macrorhiza (Linn.) (Family: Araceae) is probably native to Indo-malesia but widely distributed by aboriginal peoples throughout South-East Asia into the tropical Pacific. According to literature review its constituents, oxalic acid, calcium oxalate, flavonoids, cyanogenic glycosides, alocasin, cholesterol, beta-sitosterol, stigmatosterol, camposterol, fucosterol, amino acids, citric acid, malic acid, ascorbic acid, succinic acid, glucose, fructose and sucrose. Arabino-galactan proteins and betalectins [4,5]. This plant traditionally using, In Fiji, the sap of the stem is used to treat ear ache or boils in the ear. The wood is used to treat stomachache and diarrhoea. In New Guinea, headaches are treated with the sap and the leaves. Sexual disorders are treated by eating the leaves cooked in coconut milk [4,6]. Therefore, the present study was performed to verify the folklore claim of aphrodisiac activity of *Alocassia macrorhiza (L.)* on ethanol induced testicular toxicity in albino wistar rats.

Corresponding Author

A. Saravana Kumar
E-mail: sarganjune1@gmail.com

Materials and Methods

Plant collection

The Plant material of *Alocassia macrorhiza (L.)* was collected from Tirupati, Andhra Pradesh, in the month of August 2009. The plant was authenticated by Prof. P. Jayaraman, Director of National Institute of Herbal Science, West Tambaram, Chennai. The voucher sPEAMmen (PARC/2009/350) of the plant was deposited at the college, for further reference.

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Preparation of extracts

The leaves of the plants were dried in shade, separated and made to dry powder. It was then passed through the 40 mesh sieve. A weighed quantity (80gm) of the powder was subjected to continuous hot extraction in Soxhlet Apparatus. The extract was evaporated under reduced pressure using rotary evaporator until all the solvent has been removed to give an extract sample. Percentage yield of petroleum ether extract of *Alocassia macrorhiza (L.)* was found to be 18.5 % w/w.

Preliminary phytochemical screening

The phytochemical examination of petroleum ether extract of leaves of *Alocassia macrorhiza (L.)* was performed by the standard methods [7].

Animals used

Twelve-week-old female (body weights around 150–200 gm) and male (body weights around 200–250 gm) albino rats of Wistar strain were used for the present study. They were obtained from the animal house in Sree Vidyanikethan College of Pharmacy, Tirupati. The animals were maintained in a well-ventilated room with 12:12 hour light/dark cycle in polypropylene cages. The animals were fed with standard pellet feed (Hindustan Lever Limited., Bangalore) and water was given *ad libitum*. Ethical committee clearance was obtained from IAEC (Institutional Animal Ethics Committee) of CPCSEA (Ref No. IAEC / XIII / 04 / SVCP / 2009 - 2010).

Experimental Design

Healthy male albino rats showing brisk sexual activity were selected for the study. The male rats were divided in 5 groups each consist of 6 rats. The entire group was fed 6% ethanol for 30days in drinking water except group I. Group I received normal saline 10 ml/kg b.w per orally for a period of 30 days and served as control. Group II received ethanol 6% v/v in water feeding bottle as aqueous solution for period of 30days and served as negative control. Group III and IV received PEAM 200 and 400 mg/kg/p.o, daily for a period of 30 days respectively. Group V received Sildenafil citrate (5 mg/kg b.wt) daily for period of 30 days.

Mounting behavior

Mounting behavior was carried out by method of Agmo (1997) [8]. Healthy male albino rats and Female rats showing non-oestrus cycle were used for mating behaviour analysis. Female rats with maximum receptivity with male rats were selected for the experiment. The tests for sexual desire were carried out on 20th and 30th day after treatment of PEAM. All sexual behavior studies were carried out between 13:00 and 16:00 at room temperature 26 ºC–28 ºC. Sexual behavior studies were monitored in a separate room for 2 h following the administration and were given 20 min adaptation period, after which a female rat was placed in the same cage with the male in 1:1 ratio. The male and receptive female rats were introduced into the mating cages, with 1:1 ratio. The mating behaviours were monitored, including: number of mounts without Intromission until ejaculation or mounting frequency (MF), number of intromission from the time of introduction of the female until ejaculation or intromission frequency (IF), the time interval between the introduction of the female time to the first mount by the male or mounting latency (ML), the interval from the time of introduction of the female to the first intromission by the male or intromission latency (IL), time from the first intromission of a series up to the ejaculation or ejaculatory latency (EL). The values of the observed parameters for control, standard and PEAM treated groups were recorded.

Hormonal analyses

The blood was collected from retro orbital venous plexus of all animals at the 20th and 30th day of the experiment. Testosterone was estimated after separation of serum by using Radio Immunoassay (RIA) [9]. The RIA was carried out in diagnostic Endocrinology and clinical Biochemistry service, No. 56/64, First Avenue, Indra Nagar Adyar, Chennai-20.

Histological Study:

For histological work, tissues were fixed in Bouin's fluid and processed for routine microtomy. 6µm thick paraffin sections were made stained with Cason's trichrome and Haematoxylin-Eosin procedures. From the well-stained sections of the testes and the epididymis of various groups, observations were made and photomicrographs taken. Well stained sections were mainly used for the cytometric measurements. The quantitative data were recorded properly for analysis.

Statistical analysis

The data were expressed as mean ± standard error mean (S.E.M).The Significance of differences among the groups was assessed using one way and multiple way analysis of variance (ANOVA). The test followed by Dunnet's test *P* values less than 0.05 were considered as significance.

Results

Phytochemical screening

The results of preliminary phytochemical screening of petroleum ether extract of leaves of *Alocassia macrorhiza (L.)*, revealed that presence of steroids, flavanoids and carbohydrates.

Male sexual behavior

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Increase in the sexual vigor of MF and IF (table 1) were observed in all dosed groups (namely 200 mg/kg and 400 mg/kg body weight) in a dose dependent manner that was statistically significant ($P < 0.05$) when compared with the control. By the 30th day of the experimental period in the highest dosed group (400mg/kg), both MF and IF had increased to 2 times of their respective control values. In contrast, the mount latency (Figure 3) and intromission latency (Figure 4) decreased significantly with the doses and as the experimental period increased ($P < 0.05$). There was also statistically significant prolongation of ejaculatory latency ($P < 0.05$) following the administration of various doses of the plant stem extract.

Hormonal analysis

Hormonal analysis revealed that the levels of testosterone increased gradually in all the experimental groups. Particularly on the 30th day, the levels of both hormones increased in a significant manner. However, the increase in testosterone (Groups III–IV) was lower when compared to the standard drug (Sildenafil citrate) group (Group V).

Histopathological studies of testis:

Control – Seminiferous tubule of the control rat testis showing different germ cell types with abundant spermatids and mature sperms.

Negative control – Negative control rat's testis showing different germ cell types with abnormal spermatids and immature sperms.

PEMA 200mg/kg – Testicular section of the male albino rats after oral administration of PEMA 200mg/kg. The regressed seminiferous tubules show the presence of secondary spermatocyte, spermatid and spermatozoa. Note the presence of spermatids within the tubule.

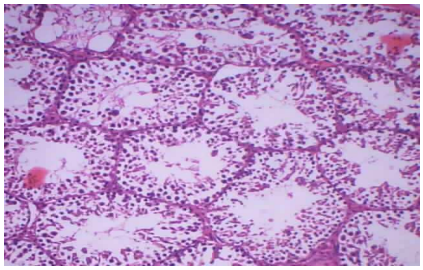
PEMA 400mg/kg - A necrotic structure of testicular section after oral administration of PEMA 400mg/kg. Sertoli cells, spermatogonial cells and primary spermatocytes are present within the tubules. Note the lumen of the seminiferous tubule, with the presence of more spermatozoa compare to that of control animal.

Table 1: Effect of ***Alocassia macrorhiza*** (L.) on mating behavior in ethanol treated male rats

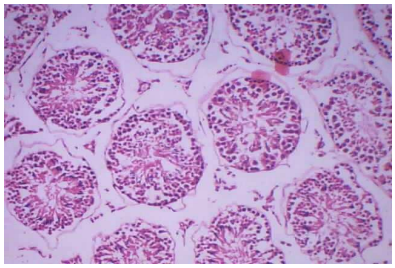
Group I (Control-normal saline 10 ml/kg, p.o)			Group II (6% ethanol in drinking water)		Group III (PEAM 200mg/kg)		Group IV (PEAM 400mg/kg)		Group V (Sildenafil citrate 5mg/kg,s.c)	
Mating behavior	20 th day	30 th day	20 th day	30 th day	20th day	30 th day	20 th day	30 th day	20 th day	30 th day
ML	12.12 ±0.56	12.15±0.48	13.15±0.43	12.45±0.51	11.24±0.25	9.26±0.56**	4.24±0.36*	5.26±2.31*	2.52±0.24**	2.18±0.16**
IL	11.01±0.34	11.42±1.41	12.17±0.23	13.15±1.10	6.56±0.54	6.24±0.31*	2.64±0.57*	2.12±2.13*	0.98±0.34**	1.94±0.15**
EL	2.61±1.50	2.54±1.14	235±1.13	215±2.18	282±1.35	293±2.10	395±3.51*	1214±4.45**	384±2.65**	1522±2.56**
MF	68.01±1.40	67.45±2.16	70.10±1.20	64±2.24	88.50±1.24	147.14±1.45**	90.50±1.43*	162±1.56**	133±2.26**	186.65±1.23**
IF	72.05±2.15	71.64±2.53	78.41±2.24	68±2.45	97.54±3.21	141±1.74**	95.54±1.85*	152±2.17**	130.24±1.17**	180±2.54**

ML: mounting latency, **IL**: intromission latency, **EL**: ejaculation latency, **MF**: mounting frequency and **IF:** intromission frequency.
Statistical significant test for comparison was done by ANOVA, followed by Dunnet's 't' test *p<0.05; ** p<0.01.
Mating behaviour scores of various groups in 20th, 30th days of experiment. Values are expressed as mean ± SEM of six observations. Comparison between: Group I &II Vs group III, IV&V.

Fig 2:Histopathological studies of testis in male mice (10X)



Group I
(**Control-normal saline 10 ml/kg, p.o)**



Group II
(**6% ethanol in drinking water**)

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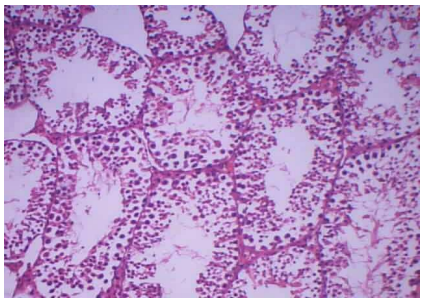
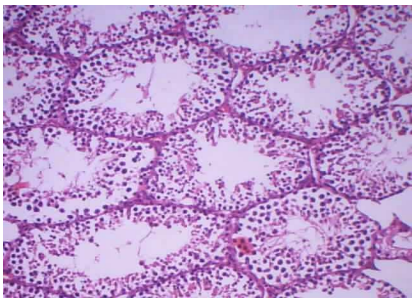
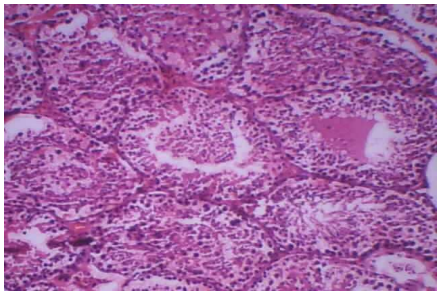


Group III
(PEAM 200mg/kg/kg, p.o)



Group IV
(PEAM 400mg/kg, p.o)



Group V
(Sildenafil citrate 5mg/kg, p.o)

DISCUSSIONS AND CONCLUSIONS

Erectile dysfunction (ED) is a common condition that may result from psychological, neurologic, hormonal, arterial or cavernosal impairment, or from a combination of these factors [10]. Ethanol in small amounts was shown to improve erection and to increase libido because of its vasodilator effect and suppression of anxiety, but in large amounts it can cause central sedation, decreased libido, and transient ED [11].

The results of preliminary phytochemical screening of the petroleum ether extract of leaves of *Alocassia macrorhiza L.* revealed that presence of alkaloids and saponins. The Present studies have implicated the saponin component of plants in enhancing aphrodisiac properties due to its androgen increasing property [12].

Saponins present in the petroleum ether extract of this plant might have assisted in stimulating an increase in the body natural endogenous testosterone levels by raising the level of leutinizing hormones (LH). This LH released normally by the pituitary gland helps to maintain testosterone levels; as LH increases, so does the testosterone [12]. The increase in testosterone seemed to have translated into the male sexual competence observed in this study. Furthermore, this study suggests that the aphrodisiac action may be mediated through a change in the blood testosterone level.

PEAM treated group's rats, such increase in the frequency of mount and intromission suggests that libido, sexual vigor and sexual performance were unimpaired [13]. The prolonged ejaculatory latency indicates enhancement of sexual function and suggests an aphrodisiac action. It has been documented previously that sexual behavior and erection are dependent on an androgen that may be acting both centrally and peripherally [14]. Testosterone supplementation has previously been shown to improve sexual function and libido, in addition to the intensity of orgasm and ejaculations which might also be expected to improve [15,16]. The continued administration of the PEAM for 30days at various doses which led to the significant increase in serum testosterone may be responsible for the marked effect on sexual behavior indices of the male rats. Increase in testosterone levels in the present study may thus account for the observed masculine behavior [17].

It was clear that the administration of PEAM not only increases aphrodisiac activity but also enhances the spermatogenic potential, as the action may be in the hormonal level. With Group V (400mg/kg) showing best results, it was concluded that changes were dose dependent. The result was close to the effects produced by Sildenafil Citrate (Group VI), which was used as the standard reference drug in the experiment.

Moreover, research should aim at isolating the active principle(s) responsible for aphrodisiac activity and the mechanism by which the drug enhances sexual function. From the present investigation, we conclude that the petroleum ether extract of *Alocassia macrorhizaL.*400mg/kg body weight possesses potent aphrodisiac activity in ethanol treated male albino rats. This result is the scientific evidence in favour of the claims made in Indian Systems of Medicine that the PEAM is clinically useful as sexual invigorator in males.

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