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RESEARCH ARTICLE

Antifertility Effect of Aqueous Leaf Extract of *Prosopis cineraria* in Male Albino Rats

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ABSTRACT

Objective: The objective of the study was to evaluate the fertility effects of aqueous leaf extract of *Prosopis cineraria* treatment effects on fertility indices in male albino rats. **Materials and Methods:** The aqueous leaf extract was prepared according to conventional methods. The rats were randomly divided into three groups with six individuals in each group. Groups-I animals were used as control and given 0.5 ml distilled water by orally intubated. Group-II animals were administered 150 mg body weight of the aqueous extract for 35 days. Group-III animals were administered 250 mg/kg body weight of the aqueous extract for 35 days. **Results:** The aqueous leaf extract administered animals showed decrease in the weight of testis, epididymis, and seminal vesicles. In addition, sperm motility, sperm viability, and changes in testosterone levels were seen changes reproductive system, especially reduction of sperm count, motility and abnormal head and tail condition were also observed in the 250 mg/kg body weight extract administered of the animals, when compared to the control group. **Conclusion:** The aqueous leaf extract of *P. cineraria* showed antifertility effect in male albino rats. After the experimental period of 65 days without any oral administration of the plant extract, remarkable changes such as increase in the weight of the testis, epididymis, and seminal vesicle and also in the increment of the sperm count were found.

Keywords: *Prosopis cineraria*, Antifertility, Male Albino Rats, Testosterone, Epididymis, Seminal vesicles

INTRODUCTION

Increase in human population throughout the world, more particularly in developing and an underdeveloped countries, has its detrimental effects on the life supporting system on earth. The possibility of an effective check on human fertility may soon be realized through biological means. Fertility regulation comprising contraception and management of infertility forms an important component of reproductive health (Allag and Rangari, 2002; Singh and Kala, 2011).[1-5] Although considerable progress has been made in the development of highly effective, acceptable, and reversible methods of contraception among females, progress and possibilities on males are still sluggish and limited. With recent progress toward a better understanding of male reproductive physiology, there is a need to develop new

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contraceptives. Man has been using plant materials for medicinal purposes since time immemorial. It may be difficult to ascertain how and where this aspect of man-plant relationship had taken its roots, but on the basis of empirical experience through generations, human societies have developed a certain system of herbal medicines.[6-12]

Herbal medicines are comparatively safer and more hygienic than synthetic drugs. Plant-based traditional knowledge has the distinction of being recognized as a principal tool in the search for new sources of drugs and pharmaceuticals (Sharma and Mujundar, 2003; Jagatheeswari, 2012). An ethnobotanical survey can bring out many different clues for the development of drugs to treat human diseases.

Ayurveda, the science of life, is a comprehensive traditional system of health care in India for more than 5000 years (Mishra *et al.,* 2001). This system is responsible for making preparations from Indian medicinal plants. Ayurveda remains a vital system of medicine and drug therapy not only in India but also elsewhere (Borchardt, 2003). In addition to the problem of efficacy, traditional medicine has a problem of safety. People think that herbs are "safe" and "harmless" since they are natural and are not synthesized in the laboratory. However, utilization of herbs may possibly expose the patient to unknown dangers (Magee and Loiacono, 2004). Although traditional health systems are locally accessible and culturally relevant, they must first be rendered safe. Most importantly, poor documentation, lack of standardization, and the absence of regulatory mechanisms for traditional health-care practice in many countries were seen as problems to be solved (Bodeker *et al*., 2000).

Prosopis cineraria (Fabaceae) (Vanni Maram – Tamil), flower is pounded, mixed with sugar, and used during pregnancy as safeguard against miscarriage. The bark of the tree is dry, acrid, and bitter with a sharp taste; cooling anthelmintic; tonic, cures leprosy, dysentery, bronchitis, asthma, leucoderma, piles, and tremors of the muscles. The bark is used as a remedy for rheumatism, in cough colds, asthma. The bark is prescribed for scorpion sting. The smoke of the leaves is good for eye problems. The fruit is dry and hot, with a flavor, indigestible, causes biliousness, and destroys the nails and the hair. The pod is considered astringent in Punjab. The plant is recommended for the treatment of snakebite (Khasgiwal *et al*., 1969; Madan *et al*., 1972).[13-20]

MATERIALS AND METHODS

Plant material

The leaves of *P. cineraria* (Fabaceae) were collected in and around Vellore district, Tamil Nadu. The plant materials were cleaned with distilled water, shade dried at room temperature, and authenticated at the Department of Zoology, Thiruvalluvar University, Serkadu, Vellore district, Tamil Nadu.

Plants extract preparation

The shade dried plant materials were powdered separately in an electrical blender and stored at 5°C until further use. One hundred grams of the dried plant powder were taken separately mixed with 500 ml of distilled water and then magnetically stirred in separate containers overnight at room temperature. The residue was removed by filtration and the aqueous extracts were lyophilized and concentrated under vacuum to get solid yield 10% (leaves).

Animals

Adult male Wistar albino rats weighing around 180–220 g were purchased from Tamil Nadu Veterinary and Animal Sciences University, Chennai, India. The animals were kept in polypropylene cages (four in each cage) at an ambient temperature of 25±2°C and 55–65% relative humidity. 12±1 h light and dark schedule was maintained in the animal house till the animals were acclimatized to the laboratory conditions. They were fed with commercially available rat chow (Hindustan Lever Ltd., Bengaluru, India) and had free access to water. The experiments were designed and conducted in accordance with the institutional guidelines.

Experimental Design

Dose and duration of treatment

The daily dose of the plant extract was freshly dissolved in 0.5 mL of distilled water and orally

administered to each experimental animal every morning for 35 days.

Group I: Control rats received 0.5 mL/day of the vehicle, that is, distilled water.

Group II: Rats treated with *P. cineraria* at 150 mg/kg body wt. orally using intragastric tubes for 35 days.[21-27]

Group III: Rats treated with *P. cineraria* at 250 mg/kg body wt. orally using intragastric tubes for 35 days.

The plant extract was administered orally to each treated animal every morning for 35 days. After 35 days of aqueous leaf extract treatment, half of the animals of Groups II and III were autopsied under light ether anesthesia. The remaining animals were kept for recovery for a period of 65 days and designated as recovery groups, that is, II and III.

Estimation of sperm motility and count

The spermatozoa were obtained by making small cuts in cauda epididymis and vas deferens and placed in 1 ml of modified Krebs-Ringer bicarbonate buffer (pH 7.4). The sperm suspension was evaluated for sperm count and percentage of motility. The percentage of motility was determined by the number progressive and non-progressive movements of sperms observed under a compound microscope. The sperm count was determined under a Neubauer hemocytometer (Zaneveld, 1977; Srikanth *et al.,* 1999). To evaluate the abnormalities of spermatozoa abnormalities, the sperm suspension was stained with eosin; smears were made on slides, air dried, and made permanent.

Serum testosterone

Serum levels of testosterone were assayed in duplicate using specific RIA method (WHO, Method manual 1987). Serum samples were separated by standard procedure and stored at 20°C for subsequent analysis.

Histological changes

The testis was removed and stored immediately in 10% formalin initially for 48 h; thereafter, the materials were transferred to 70% alcohol and stored. After fixation, the tissue was subsequently put in paraffin. Thin (5 μm) sections were drawn using a microtome and then stained with hematoxylin and eosin and mounted in neutral di-styrene-dibutyl propylene medium and examined using photomicroscopy (Strate *et al*., 2005).

RESULTS

Herbal medicines are popular as remedies for diseases among a vast majority of world's population as remedies for diseases. From the dawn of civilization, humans have relied on plants and their products as a source of drugs for their primary health care. In recent years, their use as a popular alternative to modern medicine has increased considerably even in developed countries (Qureshi *et al*., 2006; Souad *et al*., 2007). A number of plants have been identified and evaluated by various researchers for fertility regulation in males (Melis, 1999). In the present study, no changes were observed in the body weight of the treated animals. However, testicular and epididymal weight decreased significantly after 35 days of treatment in both 150 and 250 mg/kg body wt. treated animals. Abnormal spermatozoa, predominantly deformed head and tailless spermatozoa, were also observed after 35 days of treatment with higher dose regimen. The sperm counts decreased. The sperm motility was inhibited [Table 1]. In *P. cineraria* aqueous leaf extract treated mice, the cauda epididymidal sperm parameters showed evidence of dose-dependent toxicity. The plant extract intoxication exerted a significant ($P < 0.001$) decrease in epididymal sperm concentration, sperm progress motility, live sperm count, and increased abnormal sperm rate. The reduction of sperm count and sperm motility was significantly higher in plant extract 250 mg/kg body wt. treated animals when compared to 150 mg/kg body wt. treated and control animals. The levels of serum testosterone in two dosage groups were found to have significantly reduced by when compared to the levels in control groups (Group-I) [Table 1]. In the recovery Groups II and III, the alteration in the concentrations of serum

Table 1: Antifertility activity of *Prosopis cineraria* aqueous leaf extract on male albino rats: Body weight, testis, epididymis, and seminal vesicle weight of the animal

Experiment	Body weight (g)		Reproductive organs weight (g)			Total sperm count m/ml	Motility %	Abnormal %		Testosterone ng/ml
	Initial	Final	Testis	Epididymis	Seminal vesicle			Head	Tail	
Group-I control	210.16±1.47	212.66±4.71	1.45±0.55	1.14±0.41	0.83±0.11	8.20±1.69	95.83±1.56	2.01±0.62	2.02±0.62	4.92±0.55
Group-II (150 mg/kg body wt.)	216.16±2.31	214.66±3.71	1.41±0.62*	1.01±0.63*	0.69±.28*	6.20±1.67*	58.50±2.50*	8.03±1.69*	8.03±1.51*	3.04±0.54*
Group-III (250 mg/kg body wt.)	216.17±2.36	214.66±7.08	0.56±0.82*	0.68±0.34*	0.61±.34*	4.70±0.63*	58.50±1.69*	16.01±1.98*	14.01±1.69*	2.22±059*
Recovery groups	212.51±3.26	218.18±1.44	1.43±0.27	0.75±0.13	0.72±0.18	10.08±0.466	95.81±0.67	3.35±0.28	3.30±0.30	4.71±0.66

testosterone was not significant alteration when compared to that of control animals. The light microscopy examination of the testis of the control rats showed normal structure completely enveloped by a thick capsule, tunica albuginea, which is composed mainly of dense collagenous fibrous connective tissue. The structural components of the testis are the seminiferous tubules and interstitial tissues. The seminiferous tubules are two types of cells, the Sertoli cells, resting on the thin basal lamina (basement membrane) and the spermatogenic cells. The testes of the plant extract treated group (II and III) animals showed normal features with successive stages of transformation of the seminiferous epithelium into spermatozoa. Leydig cells were situated in between the tubules.

DISCUSSION

In the present study, it was found that treatment with aqueous leaf extract of *P. cineraria* [Figure 1] at a dose of 250 mg/kg body wt. was more effective in producing antifertility effect when compared to 150 mg/kg body wt. dose for 35 days. After 65 days, the antifertility effect was reversible. The present study indicated that extract treatment did not cause alterations in body weight of the treated animals, suggesting that the treatment had no toxic effect. Rajalakshmi, 1992, and Angela *et al*., 2010, reported a reduction in the number of spermatozoa in cauda epididymides in *P. cineraria* treated rat which appeared to be due to the suppressive effect of *P. cineraria* treatment on spermatogenesis in the sperm number recovered to control levels after restoration of spermatogenic activity following cessation of treatment, while the alterations in sperm motility, viability, and morphology might have resulted from disturbances in epididymal function. A similar result was observed in the present study on albino rats treated with *Andrographis paniculata* (Sathiyaraj *et al*., 2011). The treatment also caused significant reduction in the weight of the seminal vesicle in treated rats when compared to control animals. Furthermore, the treatment also caused a marked reduction in the level of the seminal vesicle in treated rat, suggesting that *P. cineraria* treatment had an adverse effect on the secretory function of the gland.

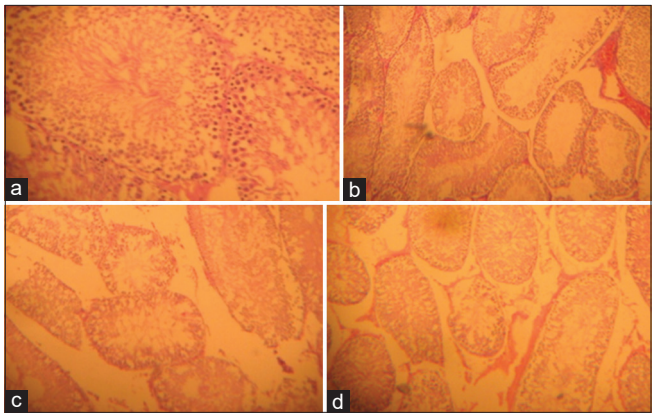

Figure 1: Plant 1 (*Prosopis cineraria*) (a) Normal testis of rat (b) Testis of 150 mg/kg body wt. plant extract fed rat showing severity of lesions of the seminiferous epithelium (c) Testis of 250 mg/kg body wt. plant extract fed rat showing reduced size of seminiferous tubules and alteration Leydig cells. (d) Testis of the recovery rat

In *P. cineraria* plant extract treated group, there was a significant depletion in serum levels of testosterone when compared to that of control group. It is well known that testosterone plays a major role in the maintenance of spermatogenesis (O'Donell *et al.*, 2006). Thus, it is probable that *P. cineraria*-induced suppression of spermatogenesis in rat testes in the present study was due to deficiency of testosterone. At the testicular level of testosterone secretion which is responsible for diminished spermatogenesis and hence, reduction in sperm counts (Mali *et al*., 2002, Newman and Cragg, 2007; Sathiyaraj *et al*., 2010; Thirumalai *et al*., 2012). The present study also shows the reversible nature of *P. cineraria* plant extract, since after the recovery period of 65 days, the animals became fertile and all the altered parameters were restored to normal level when compared to control. This study confirms that the aqueous leaf extract of *P. cineraria* leaves possesses anti-androgenic and anti-spermatogenic activity and is likely to arrest spermatogenesis. It also showed the reversible nature of the effect of the extract after withdrawal of aqueous extract treatment. An anti-androgenic effect is suggested based on the regression and disintegration of Leydig cells. Alterations of histological features were more pronounced at high dose of 350 mg/kg; disruption in seminiferous tubular arrangement was observed with fewer Leydig cells in the present study in male rat (Purohit and Dixit, 1991; Mishra and Singh, 2009), had earlier reported alteration of Leydig cell function in rats treated with aqueous extracts of *Prosopis cineraria.* The nuclei became pyknotic. Leydig cells revealed signs of atrophy. Contrary to this, no morphological changes were seen in the Sertoli cells.

CONCLUSION

This study reveals that the aqueous leaf extract of *P. cineraria* has anti-fertility property when administered alone. The toxic effects of the plant on the testis may be due to direct deleterious effects on the seminiferous tubules which are the important testicular structures involved.

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