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RESEARCH ARTICLE

Antispermatogenic Effect of Caesalpinia bonduc (L.) Roxb. Seeds

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ABSTRACT

Caesalpinia bonduc (L.) Roxb. are variously used in traditional health system. Present study was carried out to test antispermatogenic effect of *Caesalpinia bonduc* (L.) Roxb. seeds on male albino rats. Aqueous extract of seeds of *C. bonduc* administered orally for 21 days resulted in decreased sperm count in male albino rats. It significantly reduced sperm density and exhibited 9.06%, 25.29% and 39.79% average increase in antispermatogenic activity with treatment at concentrations of 50, 100 and 150 mg/kg respectively. Results suggest that the *C. bonduc* seeds can be used to develop a safe and effective male contraceptive.

Keywords: *Caesalpinia bonduc* seeds, Antispermatogenic, Sperm count.

INTRODUCTION

In recent times, focus on plant research has increased all over the world and a large body of evidence has collected to show immense potential of medicinal plants used in various traditional systems. Today, we are witnessing a great deal of public interest in the use of herbal remedies. Furthermore many western drugs have their origin in plant extracts. There are many herbs, which are predominantly used to treat cardiovascular problems, liver disorders, central nervous system, digestive and metabolic disorders. Given their potential to produce significant therapeutic effect, they can be useful as drug or supplement in the treatment / management of various diseases.

Caesalpinia bonduc (L.) Roxb. is a large, scandent shrub; armed with hooks and straight hard yellow or hooked prickles. Common in hedges around the fields and wild throughout the plains of India.

Found up to an altitude of 1,000 m in Himalaya; it also occurs in deltaic region of western, eastern and southern India; particularly along the seacoast throughout the hotter parts of India.

Seeds are bitter, astringent, acrid, thermogenic, anodyne, antiperiodic (checks symptoms of periodic, febrile disease, sometimes specific antimalarial), febrifuge, anti-inflammatory, anthelmintic, digestive, stomachic, liver tonic, depurative, expectorant, laxative, blood purifier, antifungal, antidiarrhoeal, antipyretic, antirheumatic, contraceptive, aphrodisiac and tonic; used in menstrual disorders and skin diseases. Seed oil is emmolient, used to stop discharges from ear (Jain 1991, Chopra *et al*., 1996, Warrier *et al*., 1996, Dhiman 2005, and Khare 2007). Sugalis of Andhra Pradesh give seed powder mixed with palmyra palm sugar orally for promoting sterility in men (Jeevan Ram *et al*., 2002).

Ethnobotany has been advancing towards becoming a more experimental science (Reyes-Garcia *et al*., 2007). Several ethnic uses of *C. bonduc* also has been experimentally proved. Seeds are shown to be antidiabetic (Parmeshwar *et al*., 2002, Chakrabarti *et al*., 2005; Kannur *et al*., 2006), antitumor and antioxidant (Archana *et al*., 2005), antifilarial (Gaur *et al*., 2008) and anti-inflammatory (Kale *et al*., 2010). Seeds also possess antimicrobial property (Saeed and Sabir, 2001). Moon *et al*. (2010) and Singh and Raghav (2012) has reviewed various activities of *C. bonduc.* However, the property of seeds to promote sterility in male has been tested here for the first time.

MATERIALS AND METHODS

Preparation of Extract: Since traditionally seed powder is used as drug here only aqueous extract was tested to validate the ethnic claim. Seeds of *C. bonduc* were collected from village Salona in Melghat (Dist. Amravati) and shade dried. Dried material was powdered; 50 gm powder was extracted in 100-150 ml distilled water with the help of Soxhlet's extraction assembly for 24 hrs at 50^0C. After cooling, the extract was collected and evaporated on water bath to dryness. The extract was stored at room temperature. For experimentation the extract was completely dissolved in double distilled water and solution obtained was used for treatment. Male albino rats (Wistar strain) of age between 11-14 weeks were obtained from National Centre for Laboratory Animal Sciences, National Institute of Nutrition, Hyderabad and allowed to acclimatize in the animal house. The animals were maintained and housed in wire mesh cages under standard environmental conditions. They were feed with pellet diet and water *ad libitum.* The animal room was well ventilated with a temperature range of 25-27^{o}C under day/night 12-12 hour photoperiod. All experiments were carried out in quiet laboratory settings with ambient illumination and temperature close to those of the animal house. During the experimentation, maximum care was taken to minimize animal suffering, and in addition, the number of rats used was kept at a minimum. The protocol was approved by the Institutional Animal Ethical Committee Registration No. 817/04/AC/CPCSEA (IAEC/2/2005-06).

Male albino rats of proven fertility were divided into following 4 groups of 6 each:

Group I - Control : Distilled water (Vehicle)

Group II - administered with aqueous extract (50mg/kg) body weight for 1-21 days.

Group III - administered with aqueous extract (100mg/kg) body weight for 1-21 days.

Group IV - administered with aqueous extract (150mg/kg) body weight for 1-21 days.

Aqueous extract of seeds was fed orally to fertile male rats for 21 days before taking the sperm count. Though the seed coat is very hard seeds were pounded along with seed coat. The extracts were fed orally with catheter; control animals were given only the vehicle. The duration of the experiment was 21 days. Sperm count was done

using the method prescribed by Mukherjee (1988). Rats were weighed before and after treatment. At the end of the experimental period the rats were fasted overnight. All animals in the above four groups were sacrificed on the 22nd day. The left and right epididymis were isolated and freed from adjoining fat. The cauda portion was cut off so as to separate it from the caput epididymis portion and 5 ml of saline solution was aspirated into each cauda epididymis and the aspirate from both the cauda was collected together to give a sperm suspension.

This suspension was diluted in ratio of 1:20 with 9% saline solution. This diluted suspension was then used for the sperm count on a Neubauer haemocytometer.

Sperm count / ml = N x 50,000

(N = Number of sperms counted in a square on haemocytometer scale)

$$\text{No. of sperms /ml of dilute suspension} = \frac{\text{Sperm count X 20}}{\text{4 X 0}} \text{ X 1000}$$

The results obtained for antispermatogenic effect of *Caesalpinia bonduc* were statistically analyzed using Microsoft Excel 2007 for one way ANOVA for comparison among the four groups (Mungikar 1997).

RESULTS AND DISCUSSION

The treatment results in gradual decrease of average sperm density from 27.63 (Million/Ml) in control to 25.11, 20.63 and 16.63 at the dose of 50, 100 and 150 mg/kg respectively. (Table 1).

Table 1: Antispermatogenic Effect of Caesalpinia bonduc (L.) Roxb.

Sr. No.	Group & Treatment	Number of Males Used	Body Wt. (gm)	Sperm Density in Cauda Epididymis (Million /Ml)	Average Sperm Density in Cauda Epididymis (Million/Ml)
1	Control (Vehicle)	6	150-200	26.5, 26.7, 27.8, 27.8, 28.6, 28.4	27.63 ± 1.1
2	Aqueous Extract (50mg/Kg)	6	150-200	24.7, 24.8, 24.9 25.1, 25.5, 25.7	25.11 ± 0.6
3	Aqueous Extract (100mg/Kg)	6	150-200	20.2, 20.4, 20.7 20.8, 20.8, 20.9	20.63 ± 0.3
4	Aqueous Extract (150mg/Kg)	6	150-200	15.9, 16.5, 16.7, 16.8, 16.9, 17.0	16.63 ± 1.1

Table 2: Percent antispermatogenic effect of Caesalpinia bonduc on individual male rats

Treatment Group Animals	Percent Sperm Count After Treatment			
	Control	50 mg/kg body wt.	100 mg/kg body wt.	150 mg/kg body wt.
1	0.000	6.790	23.770	40.000
2	0.000	7.120	23.600	38.200
3	0.000	10.430	25.540	39.930
4	0.000	9.710	25.180	39.570
5	0.000	10.840	27.270	40.910
6	0.000	9.510	26.410	40.140

Table 3: Statistical Analysis

Groups	Count	Sum	Average	Variance
Control	6.000	0.000	0.000	0.000
50 mg/kg body wt.	6.000	54.398	9.066	2.918
100 mg/kg body wt.	6.000	151.769	25.294	2.085
150 mg/kg body wt.	6.000	238.748	39.791	0.801

Source of Variation	**Df**	**ss**	**mss**	**F**
Replicate	5	17.69113	3.538227	4.685055
Treatment	3	5584.434	1861.478	2464.83
Error	15	11.32823	0.755216	
Total	23	5613.453		
S.E.	0.501736			
C.D. 5 %	1.068697			
C.D. 1 %	1.48012			

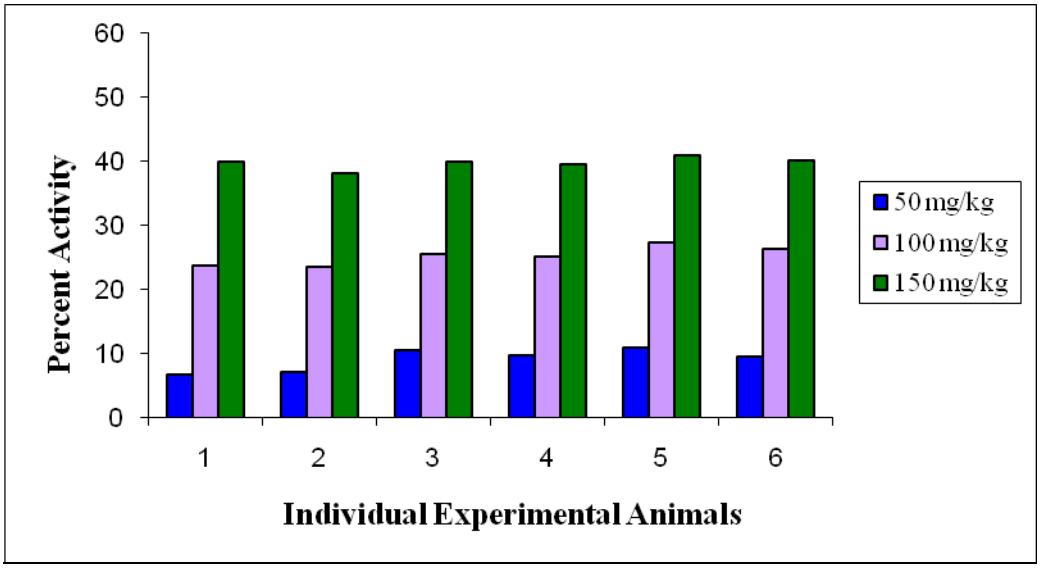

Fig.1: Caesalpinia bonduc (L.) Roxb.

The results showed that the seed extract significantly reduced sperm density and rats show (9.06%), (25.29%) and (39.79%) average increase in antispermatogenic activity with increasing dose of concentrations (F = 2464.83). The variation amongst the individuals within the groups was also significant (Table 2). The value of critical difference C.D. (1.06) at 5% indicated that all reduction was significant at p = 0.01 (Table 3).

The maximum spermicidal activity noted for *C. bonduc* seed extracts is 40.91% (Graph) i.e. 41%. In present study aqueous extracts were used, as tribals either use crude powder or decoction. The results obtained are quite significant and indicate the efficacy of traditional health system. Basically in any culture contraception/birth control is considered as sole responsibility of females. Even today there is no change in the attitude of society.

Fig. 2: *Caesalpinia bonduc* (L) Roxb.

Invention of simple oral male contraceptives can make the change. Oral administration of aqueous extract of seeds of *Caesalpinia bonduc* (L.) Roxb. (Caesalpiniaceae) resulted in 40% reduction in sperm count. Here now is hope to develop oral male contraceptive from the species.

CONCLUSION

Considering that the animals were given small drug doses which resulted in very encouraging results; systematic scientific exploitation will result in development of safe drug for birth

control programmes. Taking into account the fact that isolation of compounds from plants has not yet provided a single birth control drug, rejuvenation of traditional practices need to be advocated. However, clinical trials under supervision of expert physicians will be the first essential step in this direction.

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