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RESEARCH ARTICLE

INFLUENCE OF AQUEOUS EXTRACT OF *CALENDULA OFFICINALIS* (FLOWER) ON THE REPRODUCTIVE FUNCTION OF ADULT MALE RATS

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ABSTRACT

Oral administration of aqueous extract of *Calendula officinalis* flower to male rats at the dose level of 100mg/kg body weight for 60 days did not cause body weight loss but decreased the weight of testis, epididymis, seminal vesicle and ventral prostate in a significant manner. Sperm motility as well as sperm density were reduced highly significantly which resulted in 45% negative fertility. Serum testosterone level showed highly significant reduction. Biochemical parameters like total protein and Sialic acid in testis, epididymis, seminal vesicles and ventral prostate were decreased significantly where as testicular cholesterol concentration was elevated. All the haematological parameters were in normal range when compared with the control group animals. It is concluded that oral administration of aqueous extract of *C. officinalis* (Plant) showed a significant effect on fertility in male rats without interfering general physiology of rats.

Key words: Calendula officinalis, Antispermatogenic, Sialic acid, Sperm motility, Sperm density.

INTRODUCTION

The population is a leading cause of poverty, malnutrition and pollution in developing countries. Several potential approaches for infertility have been investigated over a long period, including chemical, hormonal and immunological approaches.(Joshi *et al*., 2010). Many plant products inhibit fertility in male and female and may be developed into contraceptive. Even though, many plants have been shown to prevent the birth, only few plants have so far been investigated for antifertility activity (Ramya *et al*., 2009). In many developing countries, traditional medicines are widely utilized in the treatment of various ailment on an empirical basis. A variety of plants have been used for the treatment of diabetes and (Upadhyay *et al*., 2004) and male reproduction (Das *et al*., 2004). Medicinal plant is an important element of indigenous medicinal system. The Ethno botany and ubiquitous plant provide a rich resource for natural drug research and development.

In recent years the use of traditional medicines information on plants research has again received considerable interest and the need for basic scientific investigation on medicinal plants using indigenous medicinal system become imminent. *Calendula officinalis* belongs to the family compositae is commonly known as 'marigold'. Extract of dried flower from *Calendula officinalis* were examined for their ability to inhibit the human immunodeficiency virus type - 1 (HIV-1) replication (Kalvatchev *et al*., 1997). It has traditionally being used for gastric ulcers and menstrual discomforts (Szakiel *et al*., 2005) skin disorders, antiseptic and anti-inflammatory disease (Cordova *et al*., 2002). Contraceptive like properties have been reported in females by local tribes of Rajasthan they use it for birth control. The contraceptive efficacy of this plant has not been investigated scientifically hence the present investigation has been under taken to investigate the effect of aqueous extract of *Calendula officinalis*. In this communication a pronged approach involving haematological and biochemical parameters has been used and antispermatogenic activity of *Calendula officinalis* is discussed.

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MATERIAL AND METHOD

The flowers of *Calendula officinalis* were collected from University Campus identified in Department of Botany (RUBL 20102), shade dried and powdered and 100 gm dry powder was macerated in 200ml of distilled water and stayed for 36 hours at room temperature and filtered to obtain a final crude extract in the form of powder. 24.75% yield was obtained from 100 gms of flower. This powder was dissolved in distilled water which was administered to the male rats while control group rat received equal amount of distilled water. Adult, healthy male albino rats of wistar strain 16-18 week old were selected from the inbred colony and the animal were maintained according to the guide lines for care and use of animals for scientific research (Indian National Science Academy, 2000) through out the course of investigation. The rats were divided in two groups having 10 rats in each group.

Group I- Vehicle treated i.e. 0.5ml/rat/day distilled water for 60 days.

Group II- 100mg/rat/day *C. officinalis* (COFAq) dissolved in 0.5ml of distilled water for 60 days.

Fertility test

The mating test of control and treated groups were performed on day 55-60 using the method of W.H.O (W. H. O Protocol 1990) the females were separated for normal delivery. On 16th day of pregnancy the implantation site (normal and absorbed foetus) were recorded.

Autopsy

After 24 hours of last dose rats were weight and autopsied under light ether anesthesia. The blood was collected from heart in pre-heparinized tubes for hematological studies and serum was also separated from non-heparinized tubes for RIA studies. The animal were autopsied, the reproductive (testis, epididymis, seminal vesicle, ventral prostate) and vital organs (liver, adrenal and kidney) were taken out and trimmed free of fat and weight separately on electronic balance.

Sperm motility and density

At autopsy, the testes epididymis were exposed and spermatozoa were taken out by cutting cauda epididymis for sperm motility (Srikanth *et al.,* 1999) and testes cauda epididymis for sperm density (Zaneveld and Polakoski 1997).

Haematology

Total erythrocyte Count (Schalm *et al.,* 1975), Total leukocyte count (Lynch *et al.,* 1969), haematocrit, (Schalm *et al.,* 1975), haemoglobin (Makarem *et al.,* 1974), blood sugar (Astoor and King 1954) and blood urea (Varley 1969) were estimated while serum was assessed for the estimation of testosterone by Radio Immuno assay (commercial kit).

Tissue Biochemistry

Frozen testis, epididymis, seminal vesicle and ventral prostate were used for the estimation of protein (Lowry *et al.,* 1951), glycogen (Montogomery 1957), cholesterol (Oser 1965) and Sialic acid (Warren 1959).

Hormones

Estimation of testosterone was performed with the help of ELISA technique.

Statistical analysis

The mean and standard error of mean (SEM) were calculated from the data obtained by the experiment and the treated groups were compared to the control using the student's 't' test (Ipstein and poly 1970).

RESULTS

Body and organ weight

Oral administrations of *Calendula officinalis* flower extract (COFAq) did not cause any change in the body weight when compared to their initial body weight. However it showed significant reduction in weight of testes, epididymis, seminal vesicles and ventral prostate ($p \leq 0.001$) in comparison to the control group (Table -1)

Sperm dynamics

Percentage of sperm motility, sperm density were decreased significantly ($p<0.01$) where as fertility rate was 45% negative after the administration of *Calendula officinalis* (flower) aqueous extract. Number of pregnant females; number of implantation sites and number of viable fetuses were also declined in *C. officinalis* treated rats (Table-2).

Serum testosterone

Serum testosterone levels were decline in all of the treatment groups in comparison to control group (Table-2).

Tissue Biochemistry

Total protein and Sialic acid content of testis, epididymis, seminal vesicles and ventral prostate were decreased significantly following the administration of *Calendula officinalis (*flower) aqueous extract (COFAq) Glycogen level in testis and liver reduced slightly where as cholesterol level was increased slightly (Table-3).

Blood Profiles

After treatment with *C. officinalis* showed that total erythrocytes count, total leukocyte count, haemoglobin, haematocrit, blood sugar and blood urea were in normal range (Table-4).

DISCUSSION

Oral administration of *C, officinalis* flower extract (COFAq) showed body weight of rats were not altered, but there was a loss of testicular weight which could be attributed to the loss of germ cell. (D'souza & Narayana 2001) and reduction in the weight of epididymis, seminal vesicles and ventral prostate and other accessory sex organs might be due to low level of androgens (Sharma and Jacob, 2001), which was reflected in decreased serum testosterone level in treated rats. Sperm motility and density in cauda epididymis and testis were decreased which shows alteration in maturation and production of sperm (Sarkar *et al.,* 2000). Protein content of reproductive organs were significantly decreased due to low level of androgens (Chinoy and Bhattacharya 1997) which was confirmed in low concentration of serum testosterone.Decreased level of Sialic acid in testis, epididymis, seminal vesicles and ventral prostate reflected loss of androgens (Gupta *et al.,* 2001). After the administration *Calendula officinalis* extract, increased testicular cholesterol might be due to arrest of steroidogenesis of testosterone (Gupta *et al.,* 2002) so to accumulate in the testis. A significant depletion in Glycogen reserve in testes attributed to the inhibition of glycogenolysis (Murthy and Devi ; 1982). From the present study it is concluded that the oral administration of crude ethanolic extract of *Calendula officinalis* may lead to fertility control in male rats due to interfere in the testicular androgens level which arrest the process of spermatogenesis in testis without disturbing general metabolism.

Table 1 : Effect of aqueous extract of *Calendula officinalis* (Coftaq) on body and reproductive organ weights of adult male rat

Treatment	Body Weight (gm)		Reproductive organs weight (mg/100gm)				vital organs weight (mg/100gm)			
	Initial	Final	Testis	Epididymis	Seminal Vesicle	Vas defence	Ventral prostate	Heart	Kidney	Liver
Group- I Control	185.30 ±08.01	204.20 ±7.20	1021.40 ±60.20	426.60 ±21.56	519.60 ±26.56	151.960 ±1.51	294.29 ±01.20	450.98 ±8.96	705.92 ±1.20	4.9561 ±1.21
Group II 100 mg/kg.b.wt/ day for 60 days	180.28 ±2.03	200.00 ±5.26	939.67* ±62.21	384.5* ±26.69	477.56 ±26.69*	114.50 ±2.61 *	224.66 ±02.10*	440.96 ns ±7.98	696.52 ns ±1.69	4.894 ns ±1.01

(Mean+ SEM of 10 animals) Group II compared with Group I
ns = Non-significant
* = P< 0.01 - Significant
** = P< 0.001 – Highly Significant

Table 2: Effect of *Calendula officinalis* (Coftaq) on the sperm dynamics, fertility test and serum testosterone level male rat

Treatment	No. of males	No. of females	No. of pregnant females	No. of implantation sites	No. of viable fetuses	Sperm motility	Sperm density (million/ml)		Fertility Test %	Serum Testosterone ng/dl
						(Cauda epididymis) %	Testes	Cauda epididymis		
Group- I Control	10	20	20/20	10.6 ±2.6	9.6 ±1.6	85.328 ±1.25	4.2 ±0.16	48.20 ±3.4	100(+)	4.71 ±0.12
Group II 100 mg/kg.b.wt/ day for 60 days	10	20	11 /20	6.2 * ± 2.3	8.63 ns ±1.27	58.52** ±1.26	2.02** ±0.43	23.10** ±0.50	55(-)	1.87** ±0.09

(Mean+ SEM of 10 animals) Group II compared with Group I
ns = Non-significant
* = P≤ 0.01 - Significant
** = P≤ 0.001 – Highly Significant

Table 3 : Effect of *Calendula Officinalis* (Coftaq) on the tissue biochemistry of adult male rat

Treatment	Protein mg/100gm				Sialic Acid mg/100gm				Glycogen mg/100gm		Cholesterol mg/100gm	
	Testes	Epididymis	Seminal Vesicle	Ventral Prostate	Testes	Epididymis	Seminal Vesicle	Ventral Prostate	Testes	Liver	Testes	Liver
Group- I Control	245.16 ±6.27	210.18 ±5.17	195.18 ±5.27	181.69 ±0.26	5.89 ±0.19	4.60 ±0.67	5.86 ±0.27	5.96 ±0.69	3.78 ±0.39	7.69 ±1.43	7.99 ±0.49	11.90 ±2.31
Group II 100 mg/kg.b.wt/ day for 60 days	215.27 ±6.19*	189.17* ±7.98	158.17* ±6.01	134.68* ±1.28	4..38 * ±0.26	4.02. * ±0.89	4.11* ±0.67	4.12* ±1.67	3.51 ns ±0.52	6.05 * ±2.78	10.66* ±1.92	8.46 * ±0.76

(Mean+ SEM of 10 animals) Group II compared with Group I
ns = Non-significant
* = P≤ 0.01 - Significant
** = P≤ 0.001 – Highly Significant

Table 4: Effect of aqueous extract of *Calendula Officinalis* (Coftaq) on haematological parameters of adult male rat

Treatment	Total Erythrocyte Count (TEC) million/mm^3	Total Leucocyte Count (TLC) (–/mm^3	Haemoglobin gm%	Haematocrit % (pcv)	Blood sugar mg/100ml mg/dl	Blood Urea mg/dl mg/100ml
Group- I Control	5.02 ±0.62	8.602.3±406.9	14.0 ±0.39	45.23±2.6	96.86±0.23	46.86±0.23
Group II 100 mg/kg.b.wt/ day for 60 days	5.06 ns ±0.69	8.216.00 ns ±460.01	14.4 ns ±0.37	45.26 ns ±6.8	95.87 ns ±0.69	39.02 ns ±3.8

(Mean+ SEM of 10 animals); Group II compared with Group I ; ns = Non-significant

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