Egyptian Journal of Biology, 2007, Vol. 9, pp
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Impact of 50% ethanolic extract of *Calendula officinalis* (flower) on the reproductive function of male albino rats (*Rattus norvegicus*)

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Abstract

Oral administration to male rats of 200mg kg^{-1} body weight of an extract of *Calendula officinalis* flowers every day for 60 days did not cause loss of body weight, but decreased significantly the weight of the testis, epididymis, seminal vesicle and ventral prostate. Sperm motility as well as sperm density were reduced significantly, resulting in 80% loss of fertility. Serum testosterone levels showed highly significant reduction. Total protein and sialic acid in the testis, epididymis, seminal vesicles and ventral prostate decreased significantly, and testicular cholesterol was elevated. All measured haematological parameters were unchanged.

Keywords: antispermatogenic, sialic acid, sperm motility

Introduction

Herbal medicines are popular as remedies for diseases for a vast majority of the world's population. Various plants have been used for the treatment of many diseases such as ulcers, diabetes (Upadhyay *et al* 2004) and male reproduction (Das *et al* 2004). *Calendula officinalis* (Compositae) is commonly known as 'marigold'. It has traditionally been used for gastric ulcers and menstrual discomfort (Szakiel *et al* 2005), skin disorders, as an antiseptic, and for anti-inflammatory disease (Cordova *et al* 2002). Contraceptive-like properties have been reported in women by local tribes of Rajasthan, who use it for birth control.

The antifertility potential of this plant has not been investigated scientifically, and hence the present investigation. A two-pronged approach involving haematological and biochemical parameters was used, and the antispermatogenic activity of *Calendula officinalis* discussed.

Materials & Methods

The flowers of *Calendula officinalis* were collected from the University of Rajasthan campus, identified by the Department of Botany (RUBL 20102), shade-dried, powdered and subjected to soxhelet extraction with 50% ethanol (WHO 1983a). The ethanol was evaporated under reduced pressure to obtain the crude extract in the form of a powder. 23% yield was obtained from 100 g of flower. The powder was dissolved in distilled water and administered to male rats of the treated group.

20 adult, healthy male albino rats 16-18 weeks old of the wistar strain were selected from an inbred colony and maintained according to guidelines for the care and use of animals for scientific research (Indian National Science Academy, 2000) throughout the course of the experiment. The rats were divided into two groups of 10 rats. The first (control) group was vehicle-treated, i.e. each received 0.5 ml d^{-1} distilled water for 60 d. Each rat of the second (treated) group received 200 mg d^{-1} for 60 d of the *Calendula officinalis* extract dissolved in 0.5 ml of distilled water.

Mating was performed on day 55-60 using the standard method (WHO 1990) and females were separated for normal delivery: on the 16th day of pregnancy the implantation site (normal and absorbed foetuses) were recorded.

24 h after the last dose, rats were weighed; under light ether anesthesia, blood was collected from the heart in pre-heparinized tubes for hematological studies, and serum was also

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separated from non-heparinized tubes for RIA studies. Animals were autopsied, and the reproductive and vital organs (testis, epididymis, seminal vesicle, ventral prostate, liver, adrenal and kidney) were taken out, trimmed free of fat and weighed separately on an electronic balance. At autopsy the epididymis was exposed and spermatozoa taken out (by cutting the cauda epididymis) for sperm motility (Srikanth *et al* 1999) and density (Zaneveld & Polakoski 1997) measurement.

Total erythrocyte (Schalm *et al* 1975) and total leukocyte (Lynch et al 1969) counts were made, and haematocrit by the microhaematocrit method (Schalm *et al* 1975). Haemoglobin levels were estimated by the cynomethanoglobin method (Makarem *et al* 1974), and blood sugar (Astoor & King 1954) and blood urea (Varley 1969) also estimated. Serum was used for estimating testosterone by radio-immunoassay (commercial kit).

The 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).

The mean and standard error are displayed in the tables. The treated group was compared to the control using Student's t-test (Ipstein & Poly 1970), where ns = not significant, and *** = $p<0.001$.

Results

Oral administrations of *Calendula officinalis* flower extract did not cause any significant change in body weight relative to initial body weight. However there were significant reductions in the weight of the testes, epididymis, seminal vesicles and ventral prostate ($p<0.001$) of the treated group in comparison to the control (Table 1).

The percentage of sperm motility and sperm density decreased significantly ($p<0.01$), and rat fertility was 80% negative, after the administration of *Calendula* extract. The serum testosterone level was reduced significantly. The number of pregnant females, number of implantation sites and number of viable fetuses also declined in the treated group (Table 2).

Total protein and the sialic acid content of the testis, epididymis, seminal vesicles and ventral prostate decreased significantly in the treated group. The glycogen level in the testis and liver reduced slightly, whereas the cholesterol level increased slightly (Table 3).

Total erythrocyte count, total leukocyte count, haemoglobin, haematocrit, blood sugar and blood urea were all within the normal range (Table 4).

Table 1: Effect of *Calendula* extract on body and organ weights. Significance tests represent the Treated group compared to the Control using t-tests

	Body Weight (gm)		**Organ weight (mg/100 gm body weight)**			
	Initial	**Final**	**Testis**	**Epididymis**	**Seminal vesicle**	**Ventral prostate**
Control	170 ± 4.5	210 ± 5.1	1314.8 ± 9.7	415.9 ± 4.0	580.8 ± 7.1	270.1 ± 8.5
Treated	185 ± 4.5 ^{ns}	200 ± 2.0 ^{ns}	847.7 ± 8.9***	354.0 ± 36.2***	447.0 ± 19.3***	144.2 ± 5.5***

Table 2: Effect of *Calendula officinalis* on sperm dynamics, fertility and serum testosterone level. Significance tests represent the Treated group compared to the Control using t-tests

	No. of males	No. of females	No. of pregnant females (of 20)	No. of implantation sites	No. of viable fetuses	Sperm motility (%)	Sperm density (million/ml)		Fertility Test (%)	Serum Testosterone (ng/dl)
							Testes	Cauda epi-didymis		
Control	10	20	19	10.6 ± 2.7	9.4 ± 1.2	73.4 ± 1.2	4.3 ± 0.2	48.2 ± 3.4	95	4.7 ± 0.1
Treated	10	20	4	5.9 ± 2.9 ***	2.8 ± 1.7 ***	29.7 ± 1.7 ***	1.3 ± 0.1 ***	9.1 ± 0.5 ***	20	1.9 ± 0.1 ***

Table 3: Effect of *Calendula officinalis* on tissue biochemistry. Significance tests represent the Treated group compared to the Control using t-tests

Measure	Organ	Control	Treated
protein (mg/100gm)	testes	225.1 ± 6.1	187.2 ± 7.1 ***
	epididymis	205.2 ± 7.6	156.8 ± 6.6 ***
	seminal vesicle	193.2 ± 4.3	154.8 ± 5.0 ***
	ventral prostate	181.6 ± 4.1	156.2 ± 1.8 ***
sialic acid (mg/100gm)	testes	4.2 ± 0.1	3.1 ± 0.02 ***
	epididymis	4.9 ± 0.2	3.4 ± 0.1 ***
	seminal vesicle	5.4 ± 0.9	3.5 ± 0.4 ***
	ventral prostate	5.8 ± 0.4	3.8 ± 0.1 ***
glycogen (mg/100gm)	testes	3.6 ± 0.1	3.0 ± 0.5 ns
	liver	7.1 ± 0.4	6.3 ± 0.7 ns
cholesterol (mg/100gm)	testes	7.9 ± 0.5	9.2 ± 0.6 ns
	liver	11.9 ± 0.6	12.9 ± 0.6 ns

Table 4: Effect of *Calendula officinalis* on haematological parameters. Significance tests represent the Treated group compared to the Control using t-tests

	Total erythrocyte count (mm^{-3}) x10^6	Total leucocyte count (mm^{-3})	haemoglobin (gm %)	haematocrit (%)	blood sugar (mg/100 ml)	blood urea (mg/dl)
Control	4.4 ± 0.4	8853 ± 405	14.5 ± 0.3	45.6 ± 2.9	86.0 ± 0.9	38.9 ± 3.6
Treated	4.1 ± 0.8 ns	8216 ± 460 ns	14.2 ± 0.3 ns	43.6 ± 3.1 ns	90.0 ± 3.1 ns	43.1 ± 3.8 ns

Discussion

Oral administration of *Calendula officinalis* flower extract caused reduction in the weight of the testes, epididymis, seminal vesicles and ventral prostate. This weight reduction of the testis and other accessory sex organs might be due to a low level of androgens (Sharma & Jacob 2001), reflected in the decreased serum testosterone level in the treated rats. Sperm motility and density in the cauda epididymis and testis were decreased, showing alteration in

the maturation and production of sperm (Sarkar *et al* 2000). The protein content of the reproductive organs was significantly decreased, again perhaps due to a low level of androgens (Chinoy & Bhattacharya 1997). The decreased level of sialic acid in the testis, epididymis, seminal vesicles and ventral prostate also reflects loss of androgens (Gupta *et al* 2001).

The mode of action of *Calendula officinalis* extract was therefore through the pituitary-gonadal axis, confirmed by the decreased serum testosterone level. After the administration *Calendula officinalis* extract, the increased testicular cholesterol might be due to arrest of steroidogenesis of testosterone (Gupta *et al* 2002) and hence accumulation in the testis.

From the present study, we conclude that the oral administration of crude ethanolic extract of *Calendula officinalis* can lead to fertility control in male rats due to interference in the level of testicular androgens, which arrests the process of spermatogenesis in the testis, apparently without disturbing general metabolism.

Acknowledgements: The authors thank the coordinator of the Centre for Advanced Studies and the Head of the Department of Zoology, University of Rajasthan, Jaipur for providing necessary facilities.

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الملخص العربى

تأثير تركيز 50 % من المستخرجات الايثانولية من نبات *كالانديولا اوفيسنالس* على الوظيفة التناسلية لذكر فأر الألبينو *راتس نورفيجيكس*

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تم دراسة تأثير الجرعة 200 ملليجرام من مستخلص نبات *كالانديولا اوفيسنالس* لكل واحد كيلو جرام من ذكر فئران الألبينو راتس نورفيجيكس وذلك كل يوم لمدة 60 يوماً. وأوضحت الدراسة انه لاينتج عن ذلك فقد الوزن ولكنه يقلل من وزن الخصية، البربخ، الحويصلة المنوية، البروستاتا. كما تقل حركة الحيوان المنوى وتقل كثافته، والتى نتج عنها فقد 80% من الخصوبة. وتشير نسبة التستيستيرون الى انخفاض النسبة فى الدم. وانخفاض نسب البروتين و حمض السيليك فى الخصية، البربخ، الحويصلة المنوية و البروستاتا مع ارتفاع نسبة الكوليسترول الخصوى ولكن لم تتغير القياسات المتعلقة بالدم.