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Research Article

ANTIFERTILITY EFFICACY OF 50% ETHANOLIC EXTRACT OF *CALENDULA OFFICINALIS* IN MALE RATS

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ABSTRACT

The reproductive effect of the ethanolic extract of *C.officinalis* was evaluated by oral administrated at the dose level of 150, 250 and 500 mg/rat/day for 60 days. After 55 days of treatment, male rats were kept for mating with female rats. At termination on 61st day the rats were sacrificed & the blood was collected by cardiac puncture for hematological & serological studies. The reproductive organs (testes, epididymis, vas deferens, seminal vesicle and prostate gland) were dissected out and processed for biochemical estimation. The body weights were not affected whereas the weights of reproductive organs viz. testes, epididymis, seminal vesicles, vas deferens and prostate gland were decreased significantly (P<0.01).The value of glycogen, protein, sialic acid and fructose of reproductive organs were also decline significantly (P<0.01). The level of cholesterol and lipid per oxidation were significantly increased however glutathione in testes showed low values. Serum testosterone was decline after the treatment. Hematological profiles were remained in normal range. The sperm motility and density of cauda epididymis were declined highly significantly (P≤0.001).It is concluded that oral administration of 50% ethanolic extract of *C.officinalis*(Plant) showed a significant effect on fertility in male rats without interfering general physiology of rats.

Keywords: Reproductive organs, Lipid per oxidation, Glutathione, Testosterone

INTRODUCTION

The population of India is multiplying at alarming rate. The control of human fertility in the sense of its limitation is the most important and urgent of all biosocial and medical problem confronting mankind today [1]. During the recent past decades a large number of plant species mentioned in old Material, Medica and Ayurvedic literature have been screened and searched thoroughly for their antifertility effect on males[2-3]. Natural products are in great demand owing to their extensive biological properties and bioactive components which have proved to be useful against large number of diseases.[4]

Calendula officinalis Linn. (Asteraceae) commonly known as Pot Marigold, is an important medicinal plant. *C. officinalis* contains flavonol glycosides, triterpene Oligoglycosides, oleanane type triterpene glycosides, saponine and sesquiterpenes glucosides [5-6]. *C. officinalis* is phytoterapic plant rich in biologicallyactive metabolites like sesquiterpens, alcohol, triterpene ,flavonoids,hydroxycumarin ,carotenoids,tannins and volatile oils (0.1-0.2%)[7-8]. Pharmaceutical studies have suggested that Calendula extract have anti-inflammatory properties[9]. *Calendula* has long been a topical staple remedy of homeopathic medicine widely used for open wound ,ulcer and for promoting tissue formation [10].*Calendula* has been reported to possess many pharmacological activities which include antioxidant [11], antiviral [12], antifungal [13]. It also possess cytotoxic as well as tumor reducing potential [14]. Little is known about its effect on fertility. Hence, present study had been an attempt on possible contraceptive efficacy of *C. officinalis* in male rats.

MATERIAL AND METHOD

Plant Extract

Calendula officinalis (plant) was collected from Nursery, University of Rajasthan Jaipur .Botanical identification was authenticated at the Department of Botany, University of Rajasthan in comparison with the pre existing voucher specimen (RUBL 20541). The plant was shade dried and powered. 100 gm. powder was subjected to soxhelet extraction with 50% ethanol. The solvent was evaporated under reduced pressure. Thus the solid obtained was used for experiment and termed as COEtEx.

Animal Model

Healthy, adult male albino rats (*Rattus norvegicus*)180-220 gm.were selected for the experimental use. Male rat were housed in plastic cages under standard condition of light and dark (12h:12h) with an ambient temperature of 24ºc±2ºc. They were fed with standard laboratory chow and water *ad libitum*. The guidelines for care and use of animals for scientific research [15] were strictly followed throughout the course of investigation.

Before starting the experiment the male rat were kept with normal oestrous female for normal mating.

Experimental Set up

The animals were divided in four groups having 10 animals in each group. All animals were treated orally with different doses of COEtEx daily for 60 days.

Group I - Vehicle (Distilled water) 0.5 ml/day/rat.
Group II – Animal received (COEtEx) 150 mg/ kg b.wt/day/rat.
Group III - Animal received (COEtEx) 250mg/ kg b.wt/day/rat.
Group IV - Animal received (COEtEx) 500mg/ kg b.wt/day/rat.

Fertility Test

The mating expoure test of control and treated group were performed on day 50-60 using the method of W.H.O [16]. The mated females were separated and the implantation sites and number of foetuses were recorded on 21stday of pregnancy through laprotomy.

Autopsy

The rats were weighed and autopsied under light ether anesthesia, 24 hour after last dose of treatment. The blood from heart was collected in pre heparinzed tubes for hematological studies and serum was separated for serological studies.

Body and organ weight measurements

Initial and final body weights of the animals were recorded. At autopsy, the reproductive organs (Testes, Epididymis, Seminal Vesicle, Prostate, Vas deferens) and vital organs (Adrenal, Heart, Liver, Kidney,) were taken out trimmed free of fat and weighed separately on electronic balance to the nearest milligram.

Sperm motility and density

After anesthetizing the rats, the epididymis was exposed by scrotal incision and spermatozoa were expressed out by cutting the distal end of the cauda epididymal tubules.

Spermatozoa with epididymal fluid was diluted with physiological saline and placed on a Neubauer's chamber slide and motility (rate

and percentage) of 100 spermatozoa per rat was observed under microscope using pre calibrated ocular micrometer [17].

Spermatozoa were counted by placing the sperm suspension on both slides of Neubauer's hemocytometer and allowed to settle in a humid chamber for one hour. The number of spermatozoa in the appropriate squares of Neubaures camber slide was calculated under the microscope at 100 X magnification [18].

Blood and Serum analysis

Blood was collected in the heparinized tubes by cardiac puncture and the value of R.B.C. and W.B.C. counts [19], Hematocrit and Hemoglobin were estimated. The value of M.C.V., M.C.H., and M.C.H.C. were also calculated from the value of R.B.C. counts, Hematocrit, and Hemoglobin %. The serum was separated to estimate the SGOT, SGPT, Alkaline phosphatases and Acid phosphatase according to the standard methods.

Tissue Biochemistry

Biochemical estimation of fresh frozen tissues were done by Colorimeter. The tissues were analyzed for the various parameters. Total protein in testes, epididymis, seminal vesicle and prostate [20], Glycogen in testes and liver [21] Fructose in seminal vesicle [22] Total cholesterol in testes, and liver [23] Lipid peroxidation in testes [24] Glutathione (GSH) in testes [25].

Hormones

Estimation of testosterone, FSH (Follicle stimulating hormone) and LH (Leutinizing hormone) were performed with the help of ELISA technique.

Statical Analysis

All of the recorded values of body/organ weight, sperm dynamics, hematological parameters and testicular cell dynamics were expressed in terms of mean ± SEM. The treated groups will be compared to control using the student's' test.

RESULTS

Oral administration of *Calendula officinalis* at all the dose level did not cause any significant change in the body weight of rats (Table 1). However COEtEx caused a highly significant decreased in the weight of testes, epididymis, seminal vesicles, ventral prostate and vas deferens in dose dependent manner (Table 1). Feeding of COEtEx to male rats caused significant to highly significant drop in the number of implantation sites, viable fetuses, sperm motility and concentration of cauda epididymal sperms (P<.001) at the all dose level (Table 2). The fertility rate of rats reduced upto 90% after the oral administration of COEtEx (Table 2). Sperm testosterone levels were decline in all of the treatment groups in comparison to control group(table 3).In contrast, serum FSH and LH level were similar to control in all treatment groups (Table 3).

Protein and sialic acid level in testes, epididymis, seminal vesicles and prostate were significantly lower (P<0.01) in dose dependent manner after administration of COEtEx (Table 4A). A significant decrease (P<0.001) was noticed in testicular glycogen and seminal vesicle's fructose level (Table 4B). Cholesterol and lipid per oxidation in testes was significantly higher than control values (Table 4B). No alteration were observed in liver glycogen and cholesterol. However, Glutathion in testes showed lower values in comparison to control group (Table 4B). Hematological parameters (RBC and WBC counts, Haemoglobin, Haematocrit) (Table 5) and serological parameters (SGOT, SGPT, Acid phosphates and Alkaline phosphates) showed non significant change in all the treatment groups (Table 6).

DISCUSSION

Body weight of rats treated with COEtEx were not altered, but there was a loss of testicular weight which could be attributed to the loss of germ cell 26. Decrease weight of accessory sex glands indicate the atrophy of glandular tissue and diminished secretary ability reflects the decrease level of testosterone as these organs are androgen dependent 27 A dose dependent lowering of cauda epididymal sperm motility and density suggested an undersupply of testosterone to epididymis there by possibly causing impaired epididymal function. The impaired epididymal function may also be due to the reduced activity of testes, which affect the normal passage of testicular fluid into the epididymis 28 The principal cells of the epididymis synthesized proteins that play an important role in maturation of spermatozoa [29] In the present study protein were also decreased due to COEtEx supplementation. Reduced glycogen reflects a decreased in the number of post-meiotic germ cells which are thought to be the sites of glucose metabolism. A decrease in glycogen content in testes indicative of decreased number of post meiotic germ cells (spermatids), a site for glucose metabolism [30] . Increased level of Cholesterol may be due to decreased androgen production, which resulted in accumulation of Cholesterol in testes and thus impaired spermatogenesis [31]. Decreased in Sialic acid content of testes and sex accessory organs may be correlated with loss of androgen [32]. Fructose is main source of energy required by spermatozoa. The results from this study indicate COEtEx decreased the fructose level, because the inhibition of fructose and the decrease in sperm motility were always correlated [33].

Antioxidants exert their mode of action by suppressing the formation of reactive oxygen species either by inhibition of enzyme or by chelating trace element.[34] Administration of COEtEx extracts caused a significant elevation in TBARS in testes indicating increased oxidative stress. High level of TBARS might elicit germ cell apitosis and necrotic change in germ cell [35].Decline in testosterone may lead to increase in Lipid peroxidation and decline of GSH levels. A similar increase in Lipid peroxidation with concornitant decline in GSH leading to impairment of epididymal function have been reported by Chitra [36].

Table 1: Body and organ weight of control and *calendula officinalis* extract (50% etoh) treated rats

Treatment	Body weight (gms)		Reproductive and Vital organ weight (mg / 100 gm body weight)								
	Initial	Final	Testes	Epididymis	Seminal Vesicle	Prostate	Vas defence	Heart	Kidney	Liver	Adrenal
Group I Control (vehicle treated)	190.00 ±9.01	200.00 ±8.14	1016 ±12.01	458.33 ±10.69s	485.22 ±14.25	300.00 ±23.42	145.26 ±7.72	506.78 ±18.96	699.10 ±1.20	3770.71 ±101.00	25.18 ±1.44
Group II 150mg/kg.bwt/ day for 60 days	189.55 ns ±13.24	211.98** ±5.06	989.56 ns ±7.12	388.78** ±6.72	462.43** ±11.27	224.42** ±6.87	125.88 ns ±3.55	497.32 ns ±3.17	688.31 ns ±12.11	3699.31 ns ±113.34	21.30 ns ±2.35
Group III 250mg/kg.bwt/day for 60 days	192.60 ns ±3.20	208.24* ±4.90	850.60** ±6.8	332.32** ±11.51	450.10 ** ±11.24	189.37** ±10.70	122.47 ns ±2.68	499.32 ns ±3.33	683.40 ns ±22.66	3554.74 * ±32.43	22.96 ns ±2.04
Group IV 500mg/kg.bwt/day for 60 days	187.51 ns ±2.14	202.41 ns ±5.26	716.28** ±4.74	278.91** ±14.29	391.64** ±13.27	154.22** ±7.29	114.49* ±1.88	482.59 ns ±1.29	657.81 ns ±2.92	3515* ±98.42	22.22 ns ±2.24

(Mean ± SEM of 10 Animals)

ns = non-significant (P<0.05), * = significant (P<0.01), ** = highly significant (P<0.001) Group II, III, IV Compared with group I

Table 2: Sperm dynamics and fertility tests of control and *calendula officinalis* extract (50% etoh) treated rats

Treatment	No. of males	No of females	No of pregnant females	No of implantation sites/rat	No. of viable fetuses	Sperm density (million/ml)		Sperm motility Cauda epididymis %	Fertility %
						Testis	Cauda epididymis		
Group I Control (vehicle treated)	10	20	20/20	11.25 ±1.92	8.25 ±2.16	4.55 ±0.43	56.5 ±1.6	79.01 ±1.7	100(+ve)
Group II 150mg/kg.b.wt/ day for 60 days	10	20	10/20	7.21* ±2.31	7.01* ±0.78	3.80 ns ± 0.7	40.21** ±1.1	39.57** ±1.88	50(-ve)
Group III 250 mg/kg.b.wt/day for 60 days	10	20	3/20	4.43** ±1.48	2.34** ±.061	1.91** ±0.2	18.66** ±0.27	16.05** ±8.24	85(-ve)
Group IV 500 mg/kg.b.wt/ day for 60 days	10	20	2/20	2.27** ±1.19	1.09** ±0.32	1.21** ±0.41	15.45** ±1.4	10.92** ±6.23	90(-ve)

(Mean ± SEM of 10 Animals)

ns = non-significant (P<0.05), * = significant (P<0.01), ** = highly significant (P<0.001) Group II, III, IV Compared with group II

Table 3: Changes in testosteron, follicle stimulating hormon (fsh) and leutinizing hormon(lh) after the treatment of *c.officinalis*

Treatment	Testosteron (□g/g□ng/ml	Follicle Stimulating Hormon (FSH) mIu/ml	Leutinizing Hormon (LH) mIu/ml
Group I Control (vehicle treated)	4.30 ±0.49	0.66 ±0.08	5.17 ±0.45
Group II 150mg/kg.b.wt/ day for 60 days	3.68 ns ±0.57	0.45 * ±0.04	4.47 ns ±0.42
Group III 250 mg/kg.b.wt/day for 60 days	2.84* ±0.20	0.31** ±0.01	3.12** ±0.21
Group IV 500 mg/kg.b.wt/day For 60 days	1.67** ±0.15	0.18** ±0.08	2.64 ** ±0.65

(Mean ± SEM of 10 Animals)

ns = non-significant (P<0.05), * = significant (P<0.01), ** = highly significant (P<0.001) Group II, III, IV Compared with group II

Table 4 a: Tissue biochemistryof control and *calendula officinalis* extract (50% etoh) treated rats

Treatment	Protein mg/gm				Sialic acid (mg/gm)			
	Testis	Epididymis	Seminal vesicles	Prostate	Testis	Epididymis	Seminal vesicles	Prostate
Group I Control (vehicle treated)	212.43 ±4.15	218.90 ±6.02	204.81 ±4.32	246.31 ±11.90	6.90 ±0.09	6.20 ±0.09	5.10 ±0.19	5.86 ±0.34
Group II 150mg/kg.b.wt/day for 60 days	183.83 ns ±6.94	195.52 ns ±5.72	199.66 ns ±9.84	119.73** ±9.84	4.4** ±0.20	4.12** ±0.20	4.70 ns ±0.17	3.92** ±0.24
Group III 250 mg/kg.b.wt/day for 60 days	167.81 ns ±5.98	180.63 ns ±4.56	179.54 * ±5.22	97.87** ±5.40	3.7** ±0.58	3.40** ±0.58	4.14** ±0.18	3.40** ±0.09
Group IV 500 mg/kg.b.wt/day for 60 days	132.24** ±5.19	139.29** ±2.94	149.93** ±2.05	82.95** ±4.18	3.18** ±0.32	2.9 ** ±0.63	3.93** ±0.24	3.27** ±0.17

(Mean ± SEM of 10 Animals)

ns = non-significant (P<0.05), * = significant (P<0.01), ** = highly significant (P<0.001) Group II, III, IV Compared with group I

Table 4b: Tissue biochemistry of control and *calendula officinalis* extract (50% etoh) treated rats

Treatment	Cholesterol (mg/gm)		Glycogen (mg/gm)		Fructose mg/ml Seminal Vesicle	Lipid per oxidation (n mol/mg) Testes	Glutathion Testes
	Testes	Liver	Testes	Liver			
Group I Control (vehicle treated)	7.96 ±0.36	16.38 ±0.29	4.89 ±0.14	7.52 ±0.22	5.07 ±0.32	1.98 ±0.28	3.80 ±0.29
Group II 150mg/kg.b.wt/day for 60 days	8.61 ns ±0.79	16.24 ns ±0.31	3.54** ±0.32	8.72 ns ±0.26	4.74 ns ±0.56	3.29** ±0.10	2.68* ±0.24
Group III 250 mg/kg.b.wt/day for 60 days	9.32** ±0.15	16.78 ns ±0.44	2.84** ±0.24	8.52 ns ±0.24	3.83** ±1.41	5.60** ±0.21	2.13** ±1.22
Group IV 500 mg/kg.b.wt/day for 60 days	7.19** ±0.28	15.81 ns ±0.26	2.57** ±0.11	7.21 ns ±0.19	3.16** ±1.08	4.13** ±1.07	1.93** ±0.72

(Mean ± SEM of 10 Animals)

ns = non-significant (P<0.05), * = significant (P<0.01), ** = highly significant (P<0.001) Group II, III, IV Compared with group I

Table 5: Hematological changes in control and *calendula officinalis* extract (50% etoh) treated rats

Treatment	Total Erythrocyte Count (TEC) (million/mn^3)	Total Leucocyte Count (TLC) (–/mm^3)	Haemoglobin (gm %)	Haematocrit (PCV) (%)	Mean Corpuscular Volume (M.C.V.) (□m^3)	Mean Corpuscular Haemoglobin (M.C.H.) (Pg.)	Mean Corpuscular Haemoglobin Concentration (M.C.H.C.) (%)
Group I Control (vehicle treated)	5.40 ±0.16	8476.66 ±21.8	14.23 ±0.72	46.00 ±0.94	85.18 ±6.20	26.35 ±0.75	30.93 ±0.85
Group II 150mg/kg.b.wt/day for 60 days	4.83 ns ±0.39	8450.88** ±22.44	14.70 ns ±0.11	45.95 ns ±1.22	84.5 ns ±3.5	25.2 ns ±2.1	31.14 ns ±0.76
Group III 250 mg/kg.b.wt/day for 60 days	4.80 ns ±0.4	8216 ** ±14.60	14.50 ns ±0.3	45.6 ns ±3.1	81.0 ns ±4.2	27.3 ns ±0.3	32.58 ns ±2.85
Group IV 500 mg/kg.b.wt/day for 60 days	4.21 ns ±0.67	8185** ±21.57	13.92 ns ±0.83	43.72 ns ±2.75	78.43 ns ±5.3	25.71 ns ±3.5	30.24 ns ± 1.5

(Mean ± SEM of 10 Animals)

ns = non-significant (P<0.05), * = significant (P<0.01), ** = highly significant (P<0.001) Group II, III, IV Compared with group I

Table 6: Serological changes of control and *calendula officinalis* extract (50% etoh) treated rats

Treatment	SGPT (u/ml)	SGOT (u/ml)	Acid phosphates (u/mg)	Alkaline phosphates (u/mg)
Group I Control (vehicle treated)	25.2 ±2.29	36.6 ±2.55	5.37 ±0.65	4.16 ±0.79
Group II 150mg/kg.b.wt/day for 60 days	26.1 ns ±1.65	35.4 ns ±4.57	5.32 ns ±0.26	4.12 ns ±0.24
Group III 250 mg/kg.b.wt/day for 60 days	25.5 ns ±1.68	36.11 ns ±0.80	5.71 ns ±0.28	4.66 ns ±0.24
Group IV 500 mg/kg.b.wt/day for 60 days	23.8 ns ±1.74	34.27 ns ±3.63	5.08 ns ±0.47	4.18 ns ±0.84

(Mean ± SEM of 10 Animals)

ns = non-significant (P<0.05), * = significant (P<0.01), ** = highly significant (P<0.001) Group II, III, IV Compared with group I

CONCLUSION

From the above results it can be concluded that of 50% ethanolic extract of *C.officinalis*(Plant) alters the fertility of rats. Further it reduces the testosteron, androgen dependent parameters and glutathion but increase LPO in testes.

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