Reproductive impairment associated with the hydro ethanolic extract of *Calendula officinalis* (petals) on wistar strain of male albino rat (Rattus norvegicus)

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ABSTRACT

Ingestion of hydro ethanolic extract of Calendula officinalis flower (COH_{ETOX})to adult Sprague –Dawley male rats at the dose level of 300 mg /kg mg body weight for 60 days did not resulted in 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 was reduced highly ($P \leq 0.001$) significantly which resulted and 100 % negative fertility. Serum testosterone level showed highly significant ($P \leq 0.001$) reduction. Biochemical parameters like protein and sialic acid, in testis, epididymis, seminal vesicles and ventral prostate and glycogen and Lipid Peroxidation in testis and liver were decreased significantly where as testicular cholesterol concentration was elevated highly significantly TEC and TLC count, haemoglobin , haematocrit, Mean corpuscular volume , mean corpuscular haemoglobin , mean corpuscular haemoglobin concentration ., blood urea and Blood sugar, Serum Glutamate Pyruvate Transaminase, Serum Glutamate Oxalo Acetate, Acid Phosphataes, Alkaline phosphates, testosterone, Bilirubin,blood sugar and blood urea were remained in normal range reflecting no effect upon the normal mechanism of the body of male albino rat..

Keywords- Antispermatogenic ,bilirubin, Sialic acid, cholesterol Sperm motility, Calendula

INTRODUCTION

Investigations in to tradionational plant medicines conducted with modern theories and techniques can enrich western medicines by absorbing new ideas and concept from all over the world. Calendula officinalis known as (marigold), cultivated through out the world and also valued for its culinary and medicinal properties (Bisset 1994) It is also used in homeopathic medicines as a way to promote the healing of minor sun burns, scrapes, and skin irritations. (Alanso 2004) Calendula was associated with a fatal reduction in blood glucose, accompanied by decreased serum, lipid and protein .It is also act as a herpes simplex virus infection, urinary retention, uterus problem, menstrual period abnormalities (Krag et al 1976), fatigue ,diuretic. 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 is also used as antiseptic and anti-inflammatory disease (Cordova et al 2002 Potentially active chemical consistent identified were Sesquiterpene and flavonolglycosides (pieta et al 1992) some estrogenic activity has been reported in ovariectomized mice by Calendula officinalis extract. It has traditionally been thought to have harmful effect on sperm and to cause abortion. But there is no scientific evidence documented referring to the male antifertiity.it was there fore

of interest to evaluate the effective concentration of (COFEt) of calendula officinalis on reproductive system of male rat to explore new male contraceptive ,thus the present study is attempt to investigate the effect (COH_{ETEX}) on reproduction of albino rat without having any toxic effect to the body as no report of acute toxicity exposer related to Calendula officinalis have been made to poison control centre (poisedex 1991)

MATERIAL AND METHOD

The flowers of Calendula officinalis were purchased from market, Botanical identification was authenticated at the Botanical department of Rajasthan University in comparison with the pre existing vouchers specimen (RUBL 20102) the flowers were shade dried and powdered and 100 gm of powder were subjected to soxhelet extraction with 50% ethanol for 24 hours. The solvent was evaporated under reduced pressure to obtain the residue. 23.33%. Adult, healthy male of wistar strain 185-200 Gms were selected from the inbred animal colony for the experimental use and the animal were maintained according to the guide lines of care and use of animals for scientific research (INSA) The rats were divided in two groups having 10 rats in each group.

Group 1- Vehicle (distilled water) 0.5ml/rat/day for 60 days through pearl point needle.

Group 2- 300 mg/rat/day (COH_{ETEX})dissolved in 0.5ml of distilled water for 60 days through pearl point needle

FERTILITY AND EFFICACY 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 rats were cohabited with normal adult proestrous females in the ratio of 1:2. The matted females were separated separated for normal delivery. . On 16^{th} day of pregnancy Number of pregnant females, no Implantation sites, and number of (normal and absorbed foetus viable fetuses, and the implantation site 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 and vital organs (testis, epididymis, seminal vesicle, ventral prostate vas differens) and vital organs weight (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 caudal epididymis was dissected out, an incision was made caudal epididymis.Sperm fluid was then squeezed on to the microscope slide.Epididymal sperm motility was assessed by calculating motile spermatozoa per unit area and was expressed as percent motility Sperm density was assayed by the method of Briefly, total number of sperm were counted using haematocytometer after further diluted the sperm suspension from cauda epididymis and testis .the sperm density was calculated in million /ml as per the dilution (Yokoi

K et al 2003)

BLOOD AND SERUM ANALYSIS

The whole blood was estimated for total erythrocyte count (Schalm et al 1975), Total leukocyte count (Lynch et al 1969), haematocrit values (Benson et al 1992) Blood sugar (Astoor and king 1954) and blood urea (Varley 1969) M.C.V,M.C.H,,MC.H.C and .Serum biochemistry (serum glutamate pyruvate transaminase ,serum glutamate oxalo acetate, acid phosphates, alkaline acid phosphates, Bilirubin,) determinations were performed, by using the standard methods. And the testosterone was estimated by Radio Immuno assay (commercial kit).Serum luteinising harmone (LH) and follicular –stumulating harmone (F.S.H)and estradiol were measured using standard parameters.

BIOCHEMICAL ANALYSIS

Frozen testis, epididymis, seminal vesicle and ventral prostate were subjected for the analysis of protein (Bradford,1976), glycogen (Montogomery 1957), cholesterol (Oser 1965) ,Sialic acid (Warren 1959).and Lipid peroxidase by (Okhawa 1979)

RESULTS

Table 1

Designed to show that intragastric administration of (COH_{ETEX})flower extract had no significant effect on the body weight of treated male rats when compared with those of the control group. However the absolute and relative weight of weight of testis, epididymis, seminal vesicles and ventral prostate and vas deferens were reduced highly significantly ($p \leq 0.001$) .

Table 2 :

It shows that oral administration (COH_{ETEX})of brought about no significant changes in serum biochemistry serum glutamate pyruvate transaminase and serum glutamate oxaloacetate, acid phosphates, alkaline phosphates, , and Bilirubin, and circulating level of harmone like Estradiol ,F.SH and L.H level were in normal range. Table 3: It showed that sperm motility % and sperm density in cauda epididymis was severely impaired ($p<0.001$) and fertility test showed 100 % negative fertility along with number of pregnant females, number of implantation sites and number of viable fetuses ($p<0.001$) in comparison to control group.

Table 4 :

A marked significant reduction in protein, Sialic acid content of testis, epididymis, seminal vesicles and ventral prostate were observed along with testicular Glycogen and lipid peroxidase of treated animals when compare with that of control group and the cholesterol level was increased highly significantly in testis ,liver and that of adrenal gland.

Table 5 :

Designed to show that oral administration of Calendula officinalis resulted in no alteration in hematological parameters of male rats, it showed that total leukocyte count, Hemoglobin,

Hematocrit and Blood urea, Blood sugar , M.C.V,M.C.H,,MC.H.C, were in normal range.

DISCUSSION

Calendula officinalis flower extract is a potent antifertility agent with little or no apparent effect on organs other than those involved in the reproductive process (Table- 1). Significant decline in the testicular weight is due to decrease in the number of spermatogenic elements and spermatozoa (Sherins and Hawards,1978;Takihara et al .,1987). Reduction in the weight of accessory reproductive organs directly supports the reduced availability of androgens (Sharma and Jacob, 2001) and the process of spermatogenesis and function of accessory sex organs are androgen dependent .(Dym et al 1979)and (Desjardins et al 1978). The drug may act on the pituitary gland and decrease the main hormone of spermatogenesis like testosterone.

The plant material showed no toxicity as indicated by the normal values of several haematological parameters most importantly like haemoglobin, haematocrit, total number of R.B.C, W.B.C, and Serum biochemistry analysis also indicated that liver and kidney functions were not notably different from those of the control group.

calendula officinalis has been shown to induce reproductive abnormalities which was reflected in decreased sperm count (Mathew et al .,1992).Suppression of gonadotrophin might have caused decrease in sperm density in testis (Sinha et al .,1995)low caudal epididymal sperm density may be due to alteration in androgen metabolism. the 100% negative fertility test may be attributed to lack of forward progression and reduction in density of spermatozoa and altered biochemical milieu of cauda epididymis.

Mode of action of Calendula officinalis extract was not through pituitary gonadal axis, which was confirmed by unaltered level of F.S.H, L.H, and estradiol level

Protein contents of reproductive organs were decreased significantly due to low level of androgens (Chinoy and Bhattacharya 1997) Reduction in the protein contents of testis and reproductive accessories may be due to low androgen level as the androgens extract a profound infrequence on transcription and regulate the synthesis of protein by provision of more mRNA and functional ribosome (Farooq.,1991. Reduced level of glycogen might be due to some metabolic change induced by the drug as it may interfere in glucose metabolism (Bbadwal et al 1994) Nag et al 1977 have reported that an optimal level of sialic acid is essential for functional intrigity of spermatozoa. Therefore decrease in the contents of sialic acid in testis, epididymis, seminal vesicles and ventral prostate reported decrease in the testicular sialic acid concentration due to antifertility activity of Calendula officinalis (Gupta and Ahmed 1991). After the administration of COFEt increased testicular cholesterol might be due to arrest of steroidogenesis of testosterone (Gupta et al 2002).Lipid per oxidation of the testis and liver were decreased these result obtained suggest that the calendula officinalis posses a significant free radical scavenging and antioxidant activity (Cordova et al 2002)

From the present study it is concluded that the oral administration of crude (COFEt) may lead to fertility control in male rats due to interfere in the testicular androgens level which arrest the

process of spermatogenesis in testis.

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fig 1 chemical structure of calendic acid

Table 1. Body and Organ weight

Treatment	Body weight (gms)		Reproductive organs weight (mg/100 gm b. wt.)					Vital organ weight (mg/100gm b. wt.)			
	Initial	Final	Testes	Epididymis	Seminal Vesicle	Ventral Prostate	Vas deferens	Heart	Kidney	Liver	Adrenal
Group I control	–	270.20±5.1	1314.8±9.7	415.9±4.0	580.8±7.1	270.1±8.5	150±1.51	406.78±8.96	699.10±o1.20	3289±180.21	24.95±01.20
Group- II 100mg/kg b.wt./day for 60 days	189.10±5.6	192.00±10.5 ns	1002.00±41.6 ns	503.90±16.5 ns	498.0±15.8 ns	286.1±16.4 ns	140±2.51	392.68±7.89	697.12±02.21	3281±181.02	27.89±02.02
Group- III 300 mg/kg b.weight for 60 days	186.00±7.0	190.00±6.6 ns	888.60±29.00**	409.00±15.00**	468.00±10.5*	260.0±4.5*	100±1.02	365.76±5.29	687.12±o2.71	3120±170.12	24.02±03.02