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CALOTROPIN – A NOVEL COMPOUND FOR FERTILITY CONTROL

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ABSTRACT: Calotropin isolated and characterized from the roots of Calotropis procera when administered to gerbils (25mg/kg b.wt) and rabbits (25mg; kg b.wt) each day for a period of 30 days inhibited the process of spermatogenesis. The population of spermatids was depleted by 65% and 94% in gerbils and rabbits. The seminiferous tubules and the Leydig cell nuclei diameters were reduced in both the species.

The production of mature Leydig cells were decreased by 51.2% and 33.9% in gerbils and rabbits. The number of fibroblast like cells remain unchanged. Reduced protein, sialic acid, and glycogen contents of tests indicate dimished androgenesis. Abortifacient activity was also notived in female rats on the 12^{th} day of pregnancy. In conclusion, Calotropin was found to inhibit spermatogenesis in male and induced abortion in pregnant females.

Introduction

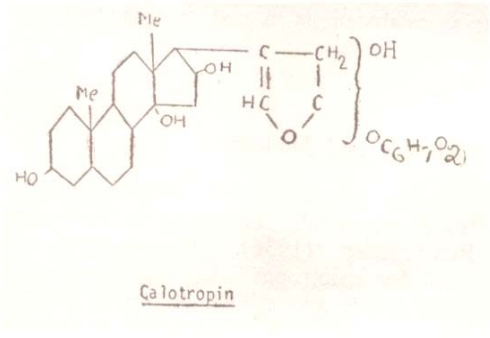
Calotropin isolated and characterized from Calotropis procera (Ait) R.Br. commonly known as "AAK" is a xerophytic perennial shrub belonging to the family Asclepiadaceae. In indigenous system of medicine all the parts of Calotropis procera affected respiration, blood pressure and involuntary muscle action in rat and rabbit (Derasari and Shah, 1965). The petroleum ether extract of flowers and leaves of Calotropis gigantean was found to be abortifacient in female albino rats. (Khana et al. 1969). The present study reports the results of a quantitative analysis of germ cells and the Leydig cells in the testis of gerbils and rabbits made infertile with calotropin, an active principle isolated from the flowers of Calotropis procera. Abortifacient or interceptive activity was also evaluated in pregnant females.

Materials and Methods

The extraction and isolation of calotropin was made according to Hesse and Reicheneder (1936). The chemical formula for calotropin is C29,H40,O9 m.p.221 Male gerbils and rabbits of proven fertility were numbered and maintained in metallic cages 2" x2"x2". The rabbits and gerbils were given standard food and water ad libitum. A total of 30 male gerbils and 10 rabbits were used. Calotropin was administered through gavage 25 mg/kg body wt. each day for a period of 30 days to male gerbils and rabbits. On day 31, testes, epididymides, seminal vesicles, ventral prostate and adrenals were removed. Tissues were fixed in Bouin's fluid. Paraffin sections were made and examined for pathological

changes. The pieces of testes were frozen and total protein, sialic acid, glycogen and cholesterol were determined (Lowry et al. 1951; Warren, 1959; Montogomery 1957; c.f. Oser 1965). Abortifacient activity was determined by administering calotropin (25 mg/kg b.wt.) from day 1 to day 12 of pregnancy. On day 16 the rats were laparotomized under light ether anaesthesia to examine the resorbed embryos.

The evaluation of cell population dynamics is based on the calculations made for each cell types per cross tubular section. All raw counts were transformed to nuclear points by an adaptation of Abercrombie's formula (Abercrombie, 1946). Interstitial cell types such as fibroblast, mature and degenerating Leydig cells were estimated applying a differential count over 200 cells of this cell population and statistically verified by the binomial distribution (Dixon and Massey, 1957). Mean tubular diameters were determined by measuring and tracing an average of 100 selected seminiferous tubules. Diameters of Leydig cell nuclei were measure at x800. The results were analysed using student "t" test.



Results

Calotropin did not bring about any significant change in the body weights of treated gerbils and rabbits. There was no significant change in the weights of testes, epididymides and seminal vesicle in gerbils whereas calotropin feeding did reduce the weights of testes, epididymides and seminal vesicle in rabbits (Table I).

Cell population dynamics

The production of spermatids was inhibited by 65.2 and 94.0% respectively in calotropin treated gerbils and rabbits as compared to controls. The total number of primary an secondary spermatocytes were significantly depressed by 38.2; 47.3% in gerbils and 51.7; 84% in rabbits while the population of spermatogonia did not change. The mature Leydig cells were reduced by 51.2% in gerbils and 33.9% in rabbits whereas the population of fibroblast like cells remain unaffected in both the animal species (Table-II).

In addition calotropin inflicted tubular atrophy. Few tubules contained only vacuolated sertoli cells. The walls of these tubules were thickened. Over all tubules were markedly decreased in both animal subjects. Leydig cells were atrophic.

Biochemical changes: Calotropin brings about a marked reduction in the testicular contents of protein, sialic acid and glycogen in both subjects. Whereas testicular cholesterol were significantly raised ($P<0.001$ Table III).

Abortifacient activity: Calotropin at the dose of 25 mg/kg b.wt. induced absolute

resorption of developing embryos in pregnant rats.

(Implantation before treatment: 10.5 ± 0.86; after treatment% developing fetuses = NIL, only black scars could be noticed.)

Discussion

The reduced testicular weights in rabbits and shrunken seminiferous tubular dimensions indicate the widespread testicular damages (keel and Abney, 1980).

Depopulation of germinal epithelium (Spermatocytes and spermatids) is of similar magnitude as observed by Weinbauer et al. (1987) following GnRH against administration.

Reduced volume and number of mature Leydig cells in gerbils and rabbits resulted in diminished androgen production. Thus affecting fertility (Monet Kuntz, 1984).

TABLE I

Changes in body weight and the weights of testes, epididymides, seminal vesicle and adrenal together with seminiferous tubule and Leydig cell nuclear diameter after calotropin treatment.

GERBILS

	Body weight (gms)	Testes	Epididymides Mg/100gm b.wt	Seminal vesicle	Adrenal	Seminiferous tubular diameter	Leydig cell nuclear diameter µm
Control	77±5	666±48	239±15	378±50	51.2±3.5	209±11	10.8±0.2
Calotropin 25 mg/kg body weight each day for a period of 30 days	61.6±8.6^{ns}	685±71^{ns}	228±20^{ns}	365±76^{ns}	150.3±18.8^{b}	169±9^{a}	7.3±0.2^{c}
				RABBIT			
Control	1400±100	150±5.7	43.4±1.7	85.0±6.5	32.0±3.0	192.0±16.0	7.1±0.3
Calotropin 25 mg/kg body weight each day for a period of 30 days	1150±150^{ns}	68.8±7.1^{c}	34.6±1.9^{b}	26.2±3.5^{c}	36.1±2.0^{ns}	100.4±8.0^{c}	5.95±0.25^{c}

Levels of significance ^{a} P<0.05; ^{b} P<0.01; ^{c} P<0.001; ^{ns} – Non significant

TABLE II

Testicular cell population dynamics following calotropin treatment

GERBIL

	GERMINAL CELL TYPES					INTERSTITIAL CELL TYPES		
	Spermatogonia	Spermatocytes (Pri)	Spermatocytes (Sec)	Spermatid	Spermatozoa	Fibroblast like cells	Mature Leydig cells	Degenerating cells
Control	7.87±0.25	44.41±6.1	54.37±5.6	55.7±4.5	(+++)	109.6±7.2	64.76±7.2	33.9±5.7
Treatment	6.59±0.62^{ns}	27.44±2.9	28.61±5.6^{b}	19.4±2.7^{c}	(-)	91.7±10.5^{ns}	31.58±3.9^{c}	76.66±11.2^{b}
%Deviation	-28.97	-38.2	-47.3	-65.0		-16.33	-51.2	2.2 Fold
			RABBIT					
Control	5.7±0.6	44.32±2.0	51.27±3.7	49.2±4.0	(+++)	102.7±12.0	75.2±6.2	22.3±7.2
Treatment	5.3±1.4^{ns}	21.42±3.8^{c}	8.18±1.3^{c}	2.7±0.2^{c}	(-)	84.9±12.9^{ns}	49.7±5.1	65.3±7.5^{b}
%Deviation	-8.1	-51.7	-84.0	-94.0		-17.31	-33.9	3fold

Levels of significance ^{a} P<0.05; ^{b} P<0.01; ^{c} P<0.001; ^{ns} – Non significant

(+++) Number. (-) NIL

TABLE III

Biochemical changes in testes following Calotropin treatment

GERBIL

	Protein	Sialic acid	Glycogen	Cholesterol
Control	195±15	6.3±0.8	1.9±0.19	2.89±0.5
Treatment	132±13[a]	3.4±0.4[a]	0.99±0.1[b]	7.94±0.6[c]
		RABBIT		
Control	213.4±22.8	5.65±0.3	1.76±0.31	3.6±0.58
Treatment	82.4±7.4[c]	2.89±0.35[c]	1.0±0.03[a]	7.6±0.5[c`]

Levels of significance a P<0.05; b P<0.01; c P<0.001; ns – Non significant

The androgenic parameters such as sialic acid and proteins declined in the testes (Peyre and Laporte, 1966). Androgens exert a profound influence on transcriptional events and regulate the synthesis of proteins by the provision of more-m-RNA and functional ribosomes (Williams Ashman and Reddi, 1971; Villee et al. 1975). The increase in the cholesterol content could be due to non-utilization of the substrate for androgen biosynthesis by fewer Leydig cells present. Reduced testicular glycogen was correlated wit diminished postmeiotic germ cells (secondary spermatocyte and spermatids) which are the site of glucose metabolism (Gunaga et al., 1972). Calotropin seems to be a very promising interceptive or abortifacient agent in females. The tem interceptive implies interruption of pregnancy after establishment of implantation (Brotherton, 1976) it may interfere with hormonal surge require for maintenance of pregnancy.

In summary the present study demonstrates that calotropin can be used for sustained suppression of testicular function an as a potent abortifacient of interceptive agent for unwanted pregnancies.

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