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Research Article

EFFECT OF ATROPINE SULPHATE ON OVARIAN ACTIVITIES IN ALBINO RATS

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ABSTRACT

Atropine sulphate at the dose level of 0.1mg & 0.2mg/100gm body weight administration for 30 days to the cycling albino rats, caused decrease in the ovarian weight, showing a decreasing number of developing follicles, *Graafian* follicles and corpora lutea, and an increased number of atretic follicles in histological sections. The estrous cycles of these rats were irregular with prolonged diestrus and reduced proestrus, estrus and metaestrus phases also support the decreased estrogen synthesis. Responsible for cornification of vaginal smear in Atropine sulphate treated rats. The hisometric changes of diameter of the ovarian follicles are reduced significantly. The total cholesterol content of the ovary was increased; protein and glycogen content were decreased.

Key words: Atropine sulphate, Rats, Ovary, *Graafian* follicle, Atretic follicle, Corpora lutea, Estrous cycle.

INTRODUCTION

Atropine, is a naturally occurring alkaloid of plant "*Atropia belladonna*". The other sources are *Dattura inoxia, Dattura stramonium*. It is a competitive antagonist of muscarimic cholinergic drug. Generally, Atropine sulphate is used as atropine sulphate injection and chemically designed as 1αH, 5αH-Tropan-3-αOL (±) - tropate (ester) sulphate (2:1) (salt) monohydrate, $(C_{17}H_{23}NO_3)_2$ H_2SO_4 H_2O[1]. A single subcutaneous injection of atropine on proestrus day delays ovulation for several hours in mice[2]. The studies of Redmond[3] indicate that, the atropine effectively blocks the progesterone induced ovulation in rats. In male rats the administration of this drug into autonomic nerve inhibits the testicular development[4]. All the facets of activity exhibited by nervous system are susceptible to pharmacological manipulation. The anaesthetic gases, the aliphatic alcohols, the barbiturates, the nicotine and atropine, interfere in the activities of the CNS therapy modify the action of the gonads and associated organs. CNS depressants acts on the hypothalamus and inhibit the release of gonadotrophin releasing hormone (GnRH) and corticotrophin releasing factor (CRF) thus decreasing the circulating concentrations of luteinizing hormone (LH), follicle stimulating hormone (FSH), adrenocorticotropic hormone (ACTH) and β-endorphin[5]. Secretions of pituitary gonadotrophins are regulated by brain and neurons situated in the anterior parts of the hypothalamus that synthesize the GnRH[6]. According to several investigators CNS influencing drugs inhibit the release of FSH and LH from the pituitary action through hypothalamus, blocking the neural stimulus to the gonadotrophin releasing hormone[7-9]. Though there are many indirect evidences of atropine sulphate on reproduction, so far, no direct action has been reported. Therefore, in the present study is aimed to understand the effect of Atropine sulphate on ovarian activities which are dependent on hypophysical gonadotrophins in albino rats[10, 11].

MATERIALS AND METHODS

Animals

Sexually matured, healthy, colony bred virgin female rats of Wistar strain; aged 3 months and weighing 160-180g were used for the experimentation. The rats were housed in polypropylene cages measuring 12"×10"×8", under well ventilated animal house conditions (Temperature: 28-31°C; Photoperiod: 12h natural light and 12h darkness: humidity: 50-55%). The rats were fed with balanced diet as per CFTRI, Mysore, INDIA formula and water *ad libitum*. The rats were divided into three groups of six animals each.

Group 1: Received 0.2ml saline/100g body weight for 30 days.

Group 2: Received 0.1mg Atropine sulphate in 0.2ml saline/100gm body weight for 30 days.

Group 3: Received 0.2mg Atropine sulphate in 0.2ml saline/100gm body weight for 30 days.

The treatment was started from estrus phase only, as the ovarian activities changes markedly from one phase to another phase of oestrous cycle. The saline or Atropine sulphate was administrated intraperitoneally everyday between 10:00 to 11:00AM

All the rats were sacrificed on 31st day, 24 hour after the last treatment. The ovaries were dissected out immediately and separated out from the adherent tissue and weighed to the nearest mg on an electronic balance. Organ from one side of each rat were fixed in Bouin's fluid, embedded in paraffin wax, sectioned at 5µm, stained with haemotoxylin-eosin for histological studies. Ovarian follicular diameter and morphologies were used to classify follicles by using established method[12, 13]. Morphometric studies of the ovary were made by using stage and ocular micrometer and organ from the other side was used for biochemical estimations like protein[14], glycogen[15] and cholesterol[16].

RESULTS

Changes in the body weight [Table-1]

There is non-significant change in the body weight after administration of Atropine sulphate.

Changes in the Ovary

Gravimetric changes [Table-2]

Administration of 0.1mg Atropine sulphate showed almost significant reduction ($p<0.05$) in the ovarian weight with 15.56% inhibition. But, the administration of 0.2mg atropine sulphate showed significant ($p<0.01$) reduction in the ovarian weight with 45.81% inhibition when compared that of saline treated control.

Table 1: Effect of of Atropine Sulphate on the body weight of albino rats

Treatment	Initial body weight	Final body weight	% Increase	Weight of the Ovary	% Inhibition
Saline	155.25 ± 2.95	168.96 ± 0.97	8.83	39.57 ± 1.91	—
Atropine sulphate (0.1mg/100g body wt.)	157.42 ± 1.80	170.37 ± 2.85	8.22	33.41± 1.01*	15.56
Atropine sulphate (0.2mg/100g body wt.)	153.76 ± 2.54	165.23 ± 2.70	7.46	21.44 ± 1.10**	45.81

Duration: 30days, 6 animals are maintained in each group. M ± S.E. = Mean ± Std. Error, * = P<0.05, ** = P<0.01, *** = P<0.001

Biochemical changes [Table -2]

The Atropine sulphate administration has shown inhibitory effect on ovarian activities. Cholesterol the precursor for steroid biosynthesis is increased significantly (p<0.01) with 0.1mg and highly significant (p<0.001) with 0.2mg of Atropine sulphate administration. The protein content is decreased significantly (p<0.001) with 0.1mg and highly significantly (p<0.001) with 0.2mg treatment of Atropine sulphate, whereas, glycogen content of the ovary, the energy reservoir of female reproductive activities were decreased highly significantly (p<0.001) with both the doses.

Table 2: Effect of Atropine Sulphate on the Biochemical changes of Ovary

Treatment	Weight of ovary	Cholesterol (µg/mg ovary)	Protein (µg/mg ovary)	Glycogen (µg/mg ovary)
Saline	39.57 ± 1.91	27.50 ± 1.37	23.53 ± 0.43	6.13 ± 0.29
Atropine sulphate (0.1mg/100g body wt.)	33.41 ± 1.01*	32.28 ± 0.63**	18.09 ± 0.38**	3.05 ± 0.13***
Atropine sulphate (0.2mg/100g body wt.)	21.44 ± 1.10**	36.21 ± 0.30***	15.75 ± 0.35***	2.08± 0.16***

Duration: 30days, 6 animals are maintained in each group. M ± S.E. = Mean ± Std. Erro, * = P<0.05, ** = P<0.01, *** = P<0.001

Histological changes [Table -3; Figure-1-3]

The histological sections of the ovaries of Atropine sulphate administration has decreased in number of healthy follicles and increased in the number of regressing follicles.

The number of healthy follicles like primary follicles has reduced significantly (p<0.01) with 0.1mg and highly significantly (p<0.001) with 0.2mg doses. The decrease in the number of secondary follicles non-significantly with 0.1mg and significantly (p<0.05) and with 0.2mg doses, the highly significant reduction (p<0.001) with both the doses and number of corpora lutea which are formed after the ovulation were decreased significantly (p<0.01) with 0.1mg and highly significantly (p<0.001) with 0.2mg of Atropine sulphate administration. The regressing follicles like atretic follicles were increased almost significantly (p<0.05) with 0.1mg and significantly with 0.2mg of Atropine sulphate administration.

Table 3: Effect of Atropine Sulphate on the Histological changes of Ovary

Treatment	Primary Follicles	Secondary Follicle	*Graafian* Follicles	Atretic Follicles	Corpora Lutea
Saline	3.80 ± 0.24	3.50 ± 0.16	3.70 ± 0.15	1.30 ± 0.15	4.70 ± 0.82
Atropine sulphate (0.1mg/100g body wt.)	2.90 ± 0.23*	3.40 ± 0.21	2.20 ± 0.24***	1.67 ± 0.16*	3.50 ± 0.16**
Atropine sulphate (0.2mg/100g body wt.)	2.30 ± 0.29***	3.02 ± 0.21*	1.50 ± 0.16***	1.90 ± 0.17**	3.10 ± 0.23***

Duration: 30 days, 6 animals are maintained in each group. M ± S.E. = Mean ± Std. Error ,* = P<0.05, ** = P<0.01, *** = P<0.001

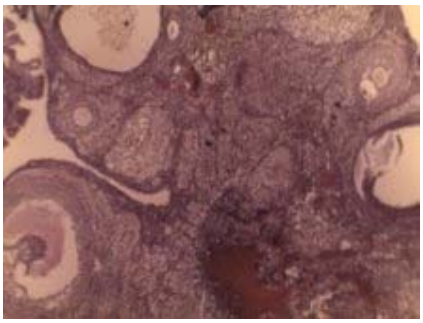

Fig. 1: Photomicrograph of ovary treated with vehicle showing normal fully developed primary, secondary follicles and *Graafian* follicle with healthy oocyte (x 100).

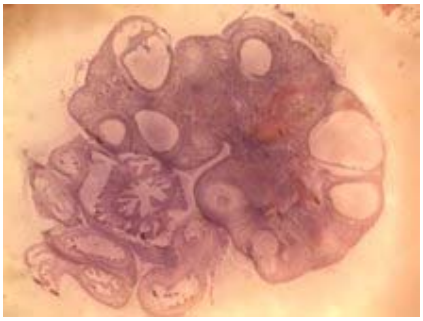

Fig. 2: Photomicrograph of ovary treated with 0.1mg of Atropine Sulphate showing under developed and degenerating follicles (x 100).

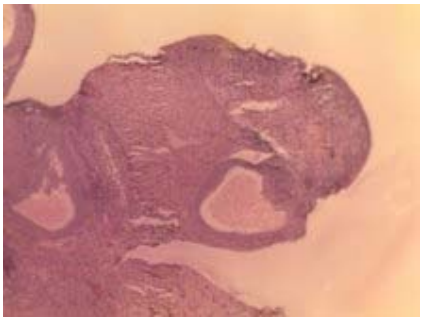

Fig. 3: Photomicrograph of ovary treated with 0.2mg of Atropine Sulphate showing degenerative follicles (x 120).

Histometric changes of ovarian components [Table-4; Figure-1-3]

The histomertic measurement of ovarian diameter of the ovarian components like primary follicles, secondary follicles, *Graafian* follicles, atretic follicles and corpora lutea were decreased their diameter almost significant ($p<0.05$) with 0.1mg and significantly ($p<0.01$) with 0.2mg of Atropine sulphate administration, these results are parallel to that of ovarian weight and number of follicles of the experimental studies.

Changes in the oestrous cycle [Table -5]

The duration of proestrus is reduced significantly ($p<0.01$) with 0.1mg and highly significant ($p<0.001$) with 0.2mg doses, whereas, the reduction of estrus and metaestrus phases were highly significant ($p<0.001$) with both the doses of experimental animals. The diestrus phase was increased highly significantly ($p<0.001$) with both doses of Atropine sulphate administration.

Table 4: Effect of Atropine Sulphate on the Histometric changes of Ovary

Treatment	Primary Follicles	Secondary Follicle	*Graafian* Follicles	Atretic Follicles	Corpora Lutea
Saline	9.09 ± 0.09	26.47 ± 0.28	36.16 ± 0.88	34.12 ± 0.25	39.20 ± 0.21
Atropine sulphate (0.1mg/100g body wt.)	7.19 ± 0.14*	21.94 ±0.37*	29.02 ± 0.18*	31.02 ± 0.24*	34.02 ± 0.24*
Atropine sulphate (0.2mg/100g body wt.)	5.97 ± 0.27**	15.03 ± 0.30**	21.08 ± 0.21**	28.14 ± 0.29**	29.20 ± 0.21**

Duration: 30 days, 6 animals are maintained in each group. M ± S.E. = Mean ± Std. Error, * = P<0.05, ** = P<0.01, *** = P<0.001

Table 5: Effect of Atropine Sulphate on the duration of various stages of Estrous Cycle in albino rats

Treatment	Proestrus	Estrus	Metaestrus	Diestrus
Saline	4.62 ± 0.30	4.21 ± 0.31	3.98 ± 0.06	18.08 ± 0.32
Atropine sulphate (0.1mg/100g body wt.)	2.89 ± 0.18**	2.56 ± 1.42***	1.98 ± 0.14***	21.97 ± 1.28***
Atropine sulphate (0.2mg/100g body wt.)	2.34 ± 0.24***	2.09 ± 0.12***	1.87 ± 0.02***	24.01 ± 1.68***

Duration: 30 days, 6 animals are maintained in each group. M ± S.E. = Mean ± Std. Error, * = P<0.05, ** = P<0.01, *** = P<0.001

DISCUSSION

In the present investigation the weight of ovary is reduced significantly due to the administration of Atropine sulphate. As the drug is administrated between 10 to 11:00 AM every day, there is possibility of covering the so called "Critical period" for cyclic LH surge, necessary for ovulation; thus postponing the ovulation for one day by interfering with 24 hours periodicity for gonadotrophin release[17,18]. Low levels of plasma FSH and LH with high concentration of pituitary gonadotrophin and prolactin are observed after Atropine sulphate administration by some investigators[7, 9]. It is well known that hypothalamus regulates the rhythmic release of pituitary gonadotrophin i.e., FSH, LH and prolactin through the neural stimulus to gonadotrophin releasing hormone (GnRH) [19]. The orderly event of follicular growth and ovulation depends upon the pituitary FSH, LH & prolactin. FSH stimulates the differentiation of granulosa cells and promotes the follicular development[20-22].

In the present study the ovaries of treated rats have reduced significantly with retarded follicular growth, differentiation of granulosa cells in the follicles, underdeveloped follicles and reduction in the ovulatory follicles may be attributed to the non availability of gonadotrophin and these are very essential for maintenance of ovarian activities.

The continuous presence of FSH within the follicles prevents the follicle undergoing atresia[23]. The large number of atretic follicles along with degeneration of granulosa cells and disappearance of antrum in Atropine sulphate treated rats may be due to inadequate supply of pituitary FSH. As ovulation needs increased concentration of plasma LH and FSH[24-26]. Atropine sulphate might have resulted in the inhibition gonadotrophin release resulting in the blockade of ovulation as evidenced by decreased in the number of freshly formed corpora lutea.

The low protein content of ovary indicates the retarded ovarian growth as FSH is essential for protein synthesis in gonads[27]. High accumulation of cholesterol content in the ovary of drug treated rats may be attributed to the lowered steroidogenesis which is dependent on availability of pituitary gonadotrophins[28, 29].

The energy source for female reproductive activities is ovarian glycogen that is oestrogen dependent[30]. The supply of glycogen to different reproductive organs in female has been reported to be controlled by the ovarian estrogen and progesterone[31]. The decreased level of glycogen in the Atropine sulphate treated ovary may be due to low ovarian steroidogenesis, which is attributed to low availability of pituitary gonadotrophins.

Oestrous cycle is regulated by the secretion and release of ovarian estrogen and progesterone production which in turn controlled by pituitary gonadotrophins. The administration of atropine sulphate prolongs the length of diestrus phase significantly resulting in decrease in number of cycles. This may be the effect of reduced steroidogenesis of the ovary as the estrogen which is essential for cornification of vaginal epithelial cell during estrus phase.

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