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Atropine Sulphate Induced Changes in Uterine, Adrenal, Liver and Thyroid Gland in Female Albino Rats

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Abstract: In the present study, effect of atropine sulphate on uterine cytotoxicity, gravimetric changes, histopathology and biochemical analysis has been evaluated. Three groups of healthy adult female albino rats having six rats in each group were taken. The rats of groups II and III were administered atropine sulphate at the dose level 0.1 mg and 0.2 mg/100 g b.wt., respectively intraperitoneally everyday between 10:00 and 11:00 am for 30 days. However, the rats of group I (control) were given saline alone. After the experimental periods, the rats were sacrificed and the histopathological study of uteri was performed. The uterine tissue of the rats of group II and III showed marked vascular congestion, epithelial necrosis and fibrous tissue proliferation. The fibrosis was extensive resulting into compression of endometrial glands. Desquamation of glandular epithelium was also observed. Histometric changes observed in uterine parameters like diameter, thickness of myometrium and endometrium and surface epithelial cell height were reduced significantly. Biochemical changes are parallel to the gravimetric changes, the protein and glycogen contents are reduced significantly with respective administration of graded dose of atropine sulphate. Although, the gravimetric analysis of adrenal, liver and thyroid gland were increased significantly due to administration of atropine sulphate.

Key words: Uterus, rat, atropine sulphate, cytotoxicity

INTRODUCTION

Atropine is used in premedication as anesthesia, it decreases bronchial and salivary secretions block the bradycardia associated with some drugs used in anesthesia such as halothane, saxamethonium and neostimine. They are known to inhibit pituitary hormonal secretion and reduce the adrenocortical secretion during the period of sedation (Harwood and Mason, 1957; Everett, 1961). Atropine, an anticholinergic drug is known to inhibit the preovulatory surge of LH and thereby block the ovulatory in rats and rabbits

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(Everett, 1949; Sawyer *et al.*, 1951; Meyer *et al.*, 1974). Besides, these sedatives may interfere in the conversion of pregnenolone to progesterone, thus altering the steroidal environment essential for ovulation (Meyer *et al.*, 1971). Prolonged treatment of sedatives to immature or pregnant rats decreases the uterotrophic potency to synthetic estrogens or cause fetal resorption or abortion, respectively (Champakamalini and Rao, 1967; Levin *et al.*, 1968; Sindagi and Rao, 1973). Atropine, the principle alkaloid in *Atropa belladonna* plant influence the CNS mechanism, there by having much adverse effect on nervous and endocrine system (Schmidt *et al.*, 1957). Secretion of pituitary gonadotrophins are regulated by brain and neurons situated in the anterior part of the hypothalamus which synthesize the gonadotrophin releasing hormones (GnRH) (Krieger *et al.*, 1982; Terasawa and Davies, 1983; Batten and Ingleton, 1987). Therefore, it is of interest to study the chronic administration of atropine sulphate on uterine activity and other endocrine organs of albino rats.

MATERIALS AND METHODS

Animals

Colony bred virgin female albino rats Wistar strain (160-180 g) were maintained under controlled standard animals house conditions with access to food and water *ad libitum*. Vaginal smears from each rats were monitored daily, only rats with normal estrous cycles (Hariham, 1980) were selected for the experiment, to study the effect of atropine sulphate on the estrous cycle, the above selected animals were divided into three groups containing 6 animals in each groups.

Experimental Design

The treatment was started when the animals were in estrous phase (Murthy *et al.*, 1997). The group I received vehicle only (0.2 mL saline) and served as control. Group II and III received atropine sulphate at doses of 0.1 and 0.2 mg for 100 g body weight in 0.2 mL saline, respectively. The treatment was given for 30 days intraperitoneally between 10:00 to 11:00 am to cover 6 regular estrous cycles and vaginal smear from the experimental animals was observed every morning.

Autopsy Schedule

On day 31st, 24 h after last treatment, all the animals from each groups sacrificed, the uterus, adrenal, liver and thyroid gland were dissected out, freed from extra depositions and weighed to the nearest mg on an electronic balance. One side of uterus from each animals was fixed in Bouin's fluid for histological, cytological and histometrical studies. The histometric measurement like diameter of uterus, thickness of endometrium and myometrium and height of endometrial epithelial cells were made from randomly chosen 20 sections from each group using ocular and stage micrometer (Deb *et al.*, 1964). Uterus from other side were used for biochemical estimations like proteins (Lowry *et al.*, 1951) and glycogen (Carroll *et al.*, 1956).

Statistical Analysis

All the values were statistically analyzed by Student's t-test using SPSS (11.0.1.). Data are expressed as the Mean±SE Statistical significance was set at $p < 0.05$, $p < 0.01$ and $p < 0.001$.

RESULTS

Changes in the Body Weight

There is non-significant change in the body weight after administration of atropine sulphate (Fig. 1).

Change in the Uterus

Gravimetric Changes

Administration of 0.1 mg atropine sulphate has reduced the weight of uterus significantly ($p < 0.01$) with 28.96% of inhibition. But, the 0.2 mg atropine sulphate administration showed highly significant ($p < 0.001$) reduction in uterine weight 53.90% of inhibition when compared with control (Fig. 2).

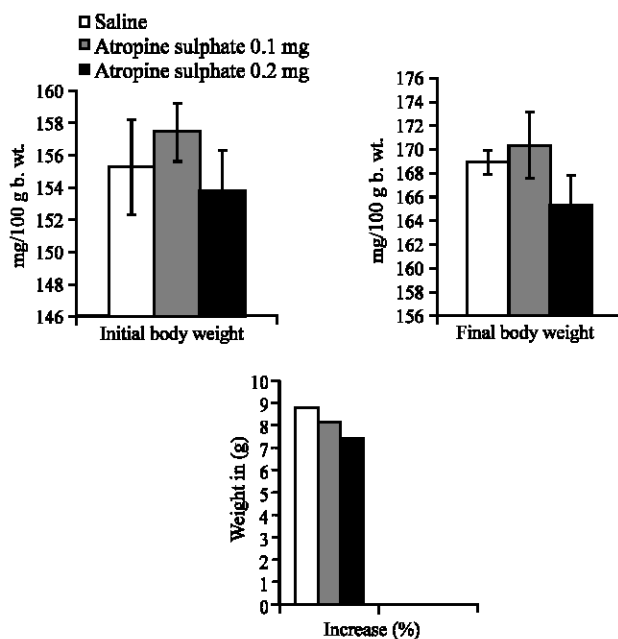


Fig. 1: Effect of graded doses of atropine sulphate on the body weight of albino rats

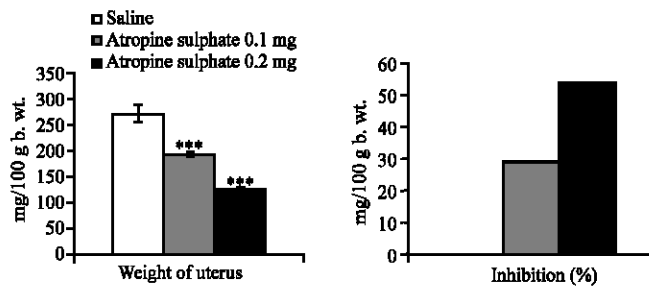


Fig. 2: Effect of graded doses of atropine sulphate on the gravimetric changes of uterus.
 $p < 0.01$, $**p < 0.01$

Changes in the Estrous Cycle

The duration of proestrus is reduced significantly ($p < 0.01$) with 0.1 mg and highly significantly ($p < 0.001$) with 0.2 mg doses, whereas the reduction of estrus and metaestrus phases were highly significant ($p < 0.001$) with both the doses of experimental animals. The diestrus phases is increased highly significantly ($p < 0.001$) with both the doses of atropine sulphate administration (Fig. 3).

Histopathological Changes of the Uterus

On microscopic examination, the uterine tissue (Fig. 5, 6) of the rats of group II and III treated with atropine sulphate showed marked vascular congestion, epithelial necrosis and fibrous tissue proliferation as against (Fig. 4) group I (Control). The fibrosis was extensive resulting into compression of endometrial glands. Desquamation of glandular epithelium was also noticed.

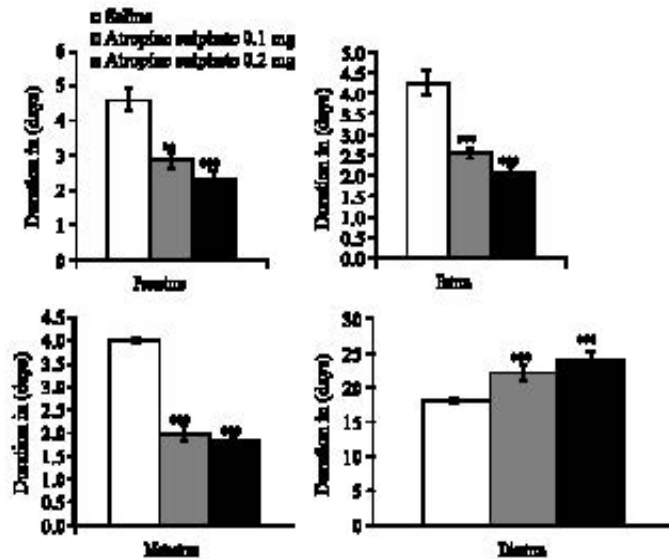


Fig. 3: Effect of graded doses of Atropine sulphate on the duration of various stages of Estrous cycle in albino rats, * $p < 0.01$, ** $p < 0.001$

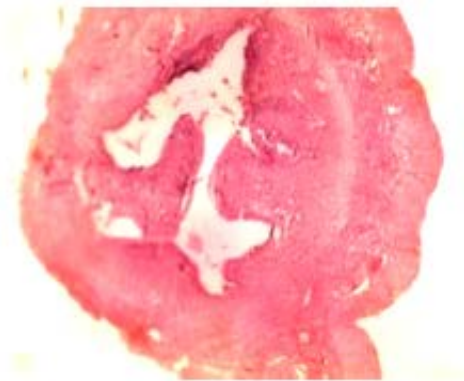


Fig. 4: Photomicrograph of uterus treated with vehicle showing normal endometrium with endometrial glands and luminal epithelial cells (Magx100)

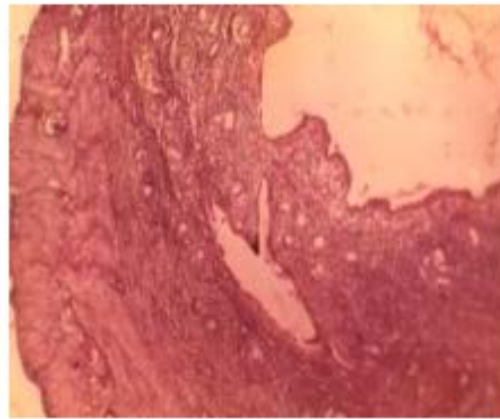


Fig. 5: Photomicrograph of uterus treated with 0.1 mg atropine sulphate showing hypertrophied endometrium, endometrial glands and luminal epithelium (Magnification x20)

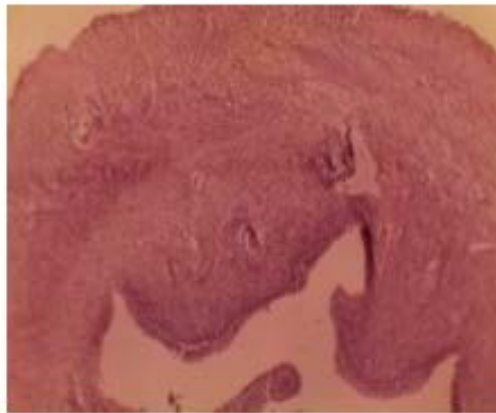


Fig. 6: Photomicrograph of uterus treated with 0.2 mg atropine sulphate showing slightly hypertrophied endometrium, endometrial glands and luminal epithelium (Magnification x20)

Histometric Changes of the Uterus

The histometric measurements such as diameter of the uterus, thickness of endometrial and myometrial gland and thickness of surface epithelial cells height were proportional to that of the uterus weight. The diameter of uterus is reduced significantly ($p < 0.01$) with 0.1 mg and highly significantly ($p < 0.001$) with 0.2 mg of atropine sulphate administration. Whereas, the thickness of myometrium is reduced almost significantly ($p < 0.05$) with 0.1 mg and significant ($p < 0.01$) with 0.2 mg and thickness of endometrium also reduced almost significantly ($p < 0.01$) and highly significant ($p < 0.001$) with administration of low and high doses of atropine sulphate. The surface epithelial cell height was also decreased almost significant ($p < 0.01$) with 0.1 mg and significant ($p < 0.01$) with 0.2 mg of atropine sulphate administration (Fig. 7).

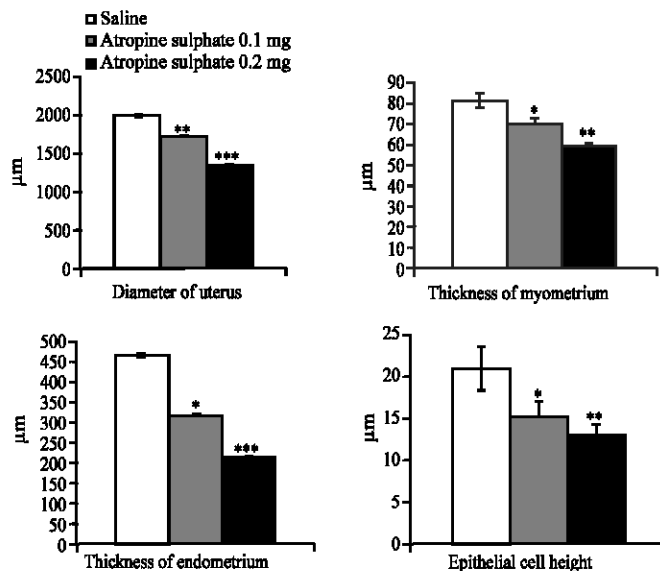


Fig. 7: Effect of graded doses of atropine sulphate on the histometric changes of uterus.
* $p < 0.01$, ** $p < 0.001$

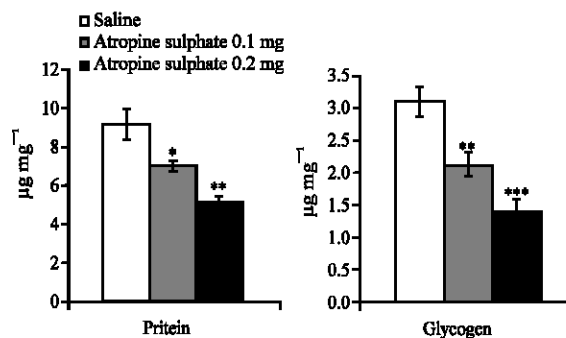


Fig. 8: Effect of graded doses of atropine sulphate on the biochemical changes of uterus.
* $p < 0.01$, ** $p < 0.001$

Biochemical Changes

The biochemical changes are parallel to the gravimetric changes. The protein content of the uterus has reduced almost significantly ($p < 0.05$) with 0.1 mg and significantly ($p < 0.01$) with 0.2 mg atropine sulphate administration. Similarly, the glycogen content of the uterus is reduced significantly ($p < 0.01$) and highly significant ($p < 0.001$) with respective administration of low and high dose treatment of atropine sulphate (Fig. 8).

Gravimetric Changes of Adrenal, Liver and Thyroid Gland

As the treatment of atropine sulphate decreases the gravimetric, morphometric, histometric and biochemical parameters of uterus, it is of interest to note that the weight of these glands. The weight of adrenal gland, liver and thyroid gland were increased almost significantly ($p < 0.05$) with 0.1 mg and significantly ($p < 0.01$) with 0.2 mg of atropine sulphate administration for 30 days (Fig. 9).

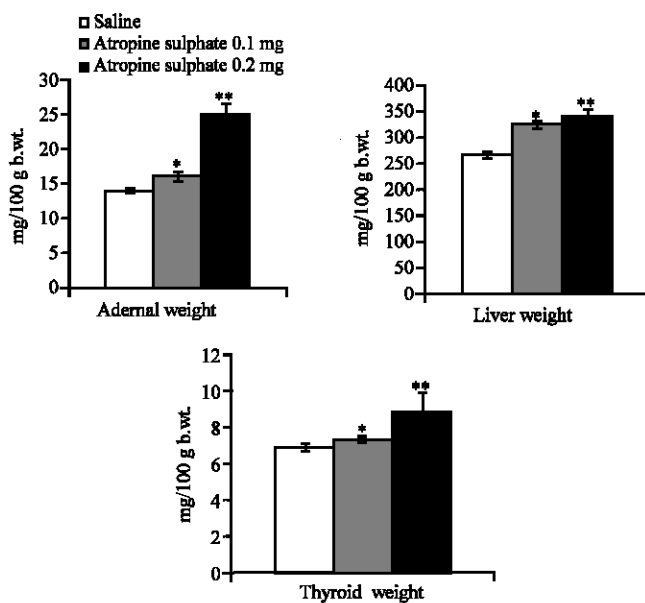


Fig. 9: Effect of graded doses of atropine sulphate on the gravimetric changes of adrenal, liver and thyroid gland

DISCUSSION

Atropine sulphate is a drug which has varied activities depending on the condition being treated and used. However, it depresses the food and water intake in the rat (Schmidt *et al.*, 1957). In the present experiment, atropine sulphate is injected between 10 to 11 am for 30 days. The percent growth of experimental animals is reduced but not significantly which may be because of depressed intake of food and water as noticed by Schmidt *et al.* (1957). In the present investigation, the weight of uterus is reduced significantly due to the administration of atropine sulphate. As the drug is administered between 10 to 11:00 am every day, there is possibility of lowering the so called Critical period for cyclic LH surge, necessary for ovulation; thus postponing the ovulation for one day by interfering with 24 h periodicity for gonadotrophin release (Lawton and Sawyer, 1968). Low levels of plasma FSH and LH and high concentration of pituitary of gonadotrophin and prolactin are observed after atropine sulphate treatment by some investigator (Anderson *et al.*, 1982). It is well known that hypothalamus regulates the rhythmic releases of pituitary gonadotrophins i.e., FSH, LH and prolactin through the neural stimulus to gonadotrophin releasing hormone-GnRH (Carmel *et al.*, 1976).

Various uterine abnormalities including cytological and histopathological changes after administration of atropine sulphate have been observed. Meissener *et al.* (1957) reported that stilbesterol (synthetic estrogen) administration induced uterine damage and cytotoxicity in rabbits. Estrogen induced damage has also been reported by Pandey *et al.* (2006) in liver and Madhuri *et al.* (2006) in uterus of rats. The estrogen induced uterine cytotoxicity leading to cancer has been further cited by many authors (Hertz, 1976; Mitchell and Stancel, 2001). All these reports corroborate the result of atropine sulphate administration of rats in the present study.

Uterine growth depends upon the ovarian estrogen secretion. Estrogen primarily acts upon the surface epithelium and the glands within endometrium (Jalikhani, 1980). Progesterone acts on estrogen epithelium from proliferative to secretory state (Jalikhani, 1980). In the present investigation, reduction in the uterine diameter, reduced thickness of its myometrium and endometrium and reduced secretions from endometrial glands indicates the inhibition of ovarian steroid biosynthesis necessary for growth of the uterus and reproductive cyclicity.

As the uterine growth and reproductive cyclicity requires rhythmic production of ovarian steroid hormones (Jalikhani, 1980; Vrontakis *et al.*, 1993; Findlay, 1994), uterine growth and reproduction cyclicity is hampered in atropine sulphate treated rats. Estrogens are known to increase the glycogen content in the uterus of rats (Gregoire *et al.*, 1967). The decreased glycogen and protein content of the uterus indicate retarded uterine growth and lowered availability of estrogen to the major reproductive tract.

Estrogen levels are highest during estrus phase and decrease gradually during diestrus phase to reach a peak at the proestrus phase (Michel *et al.*, 1969; Smith *et al.*, 1975). The FSH and prolactin are low during estrous phase and lowest during diestrus and highest during proestrus phase, whereas, the LH is low during the estrous and diestrus phases but highest during proestrus phase. The progesterone hormone is low during estrus phase and higher during diestrus phase and highest during proestrus phase (Smith *et al.*, 1975). The decrease in the duration of diestrus phase in the treated rats indicates further decrease of estrogen and FSH levels upon administration of the atropine sulphate (Patil and Rao, 1992).

CONCLUSION

The significant prolongation of diestrus phase in treated rats indicates the decrease in the levels of either any one of above mentioned hormones or all of them at the same time due to administration of atropine sulphate.

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