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# Adverse Effects of Propranolol on Reproductive Function in Adult Male Mice

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**Abstract:** Ingestion of propranolol at 10 and 15 mg $kg^{-1}$ body weight for 35 days by adult male mice was investigated for its effects on fertility. Body weight and absolute and relative testes weights were reduced and the average weights of epididymis, ventral prostate and seminal vesicle decreased significantly. A significant decline of spermatogenesis in testes due to a decrease in the number of primary, secondary spermatocytes and spermatids in the treatment group 2 (15 mg $kg^{-1}$) is attributed to a significant decrease in testosterone, LH and FSH. Sperm motility and density were also significantly decreased in the cauda epididymis and in the testes of group 2 treated male mice. In addition, the treatment markedly increased the number of fetal resorptions in female mice impregnated by the group 2 males, thereby reducing their fertility.

**Key words:** Propranolol, antifertility, fetal resorption, spermatogenesis, male mice

## INTRODUCTION

β-adrenoceptor antagonist are widely used in cardiovascular medicine (Bhatt *et al*., 2007). They protect the heart by blocking the effect of adrenaline to maintain a normal heart beat and normal blood pressure (Ojeda *et al*., 2002; Zelaszczyk and Kiec-Kononowicz, 2007). Propranolol has been associated with male and female sexual side effects (Adamowicz and Kala, 2005; Ko *et al*., 2002). It has been previously reported that systemic administration of β-adrenoceptor antagonists differentially alters sexual function in male rats (Smith *et al*., 1990). Specifically, the nonselective β-adrenoceptor antagonists inhibited male rat sexual behavior (Smith *et al*., 1990), while selective β1-drenoceptor antagonists did not (Smith *et al*., 1990; Weiner, 1985). The sexual inhibition was evidenced by the decrease in the number of males copulating to ejaculation and by increases in the intervals between copulatory events and a decreased copulatory efficacy. Propranolol effects were not only limited to copulation, but also to deficits in erectile and ejaculatory reflexes (Smith *et al*., 1995).

Therefore, the present research was designed to investigate the detailed effects of propranolol on sexual maturation, fertility and testicular functions in male Albino mice.

## MATERIALS AND METHODS

Thirty adult male and 60 female Albino mice weighing approximately 40 g were bred in the Animal House Unit at JUST, School of Medicine, between January and February 2007. Mice were maintained at a controlled temperature of 21±1°C and under a 12-h-light: 12-h-dark schedule. Food and water were supplied *ad libitum*.

Generic propranolol was purchased from a local pharmacy. This compound was then dissolved in distilled water and administered orally to mice using animal feeding intubation needles (Popper and Sons, New York) in a concentration of 10 and 15 mg $kg^{-1}$ body weight (1 mL volume) as single daily doses. Similarly, the controls received gastric infusions of 1 ml distilled water, the same way the experimental mice did.

Male mice were randomly assigned to control or experimental groups. Experimental male mice were divided into 2 groups: group 1 and 2 were fed 10 and 15 mg $kg^{-1}$ body weight propranolol for 35 days, respectively. All male mice were healthy and continued to receive their respective drinking water and food throughout the experimental period.

To estimate the fertility in both experimental and control male mice; each male was placed in an individual cage with two virgin untreated females of the same strain for 10 days, brought into estrus by sequential

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subcutaneous treatment with 0.9 mg of estradiol benzoate (Sigma Chemical Co. St Louis, MO, USA) 54 h before testing and 0.1 mg of progesterone (Gift from Roussel Uclaf, Paris, France) 6 h before testing. The hormones were dissolved in corn oil (ALFCO: Arab International Food and Oil Processing Co.) in a total volume of 0.1 mL. They were left together for 10 days during which two estrus cycles should have elapsed (Rugh, 1968). One week after the removal of the males, the females were killed by cervical dislocation under light ether anesthesia and the number of pregnant females, implantation sites, viable fetuses and fetal resorptions were recorded (Prasad *et al*., 1972).

All males were sacrificed after 35 days of exposure. Blood was collected by heart puncture using a sterile syringe and serum was separated and stored at -20°C for biochemical analysis. Body weight and weights of paired testes and seminal vesicles (stripped of fluid) were recorded. The reproductive organs of male mice including the testes, epididymides, ventral prostrate, seminal vesicle and vas deferens were fixed in Bouin's fixative for histological studies.

The sperm motility and sperm counts of cauda epididymis were determined by the method described earlier Quantitative motility (%) was determined by counting both motile and immotile spermatozoa per unit area. Cauda epididymis and testicular sperm counts were performed by routine procedure and expressed as millions $mL^{-1}$ of suspension (Abercrombie, 1946). Histological evaluation and sperm analyses were performed by a person who is unfamiliar with mice groups in this experiment. The Bouin's fixed reproductive organs were processed for paraffin embedding, sectioning (5 μm) and staining (Harris haematoxylin and eosin).

Using Camera Lucida, 100 circular appearing seminiferous tubules were traced at ×80 and the diameter of each tubule was measured separately. The measurement was expressed in terms of mean of all the traced tubules. Similarly, Leydig cell nuclei were traced at ×800. The epithelial cell height of caput and cauda epididymis and seminal vesicle were also traced at × 360. The spermatogonia, spermatocytes and spermatids were counted in 5 μm thick cross sections of 10 seminiferous tubules in 10 animals of each group. All raw counts were transformed into true counts by an adaptation of Abercrombie formula {T = C (s/s+dx) 100 [T = True counts; C = Crude counts; S = Thickness of section; d = Diameter of nuclei]} (Dixon, 1975) from germ cell diameter measurement. Interstitial cell types (such as fibroblast, immature and mature Leydig cells and degenerating cells) were counted, applying a differential count over a 200-cell population and statistically verified by the binomial distribution (Ipstein and Poly, 1970).

Serum testosterone concentration was measured by Enzyme Immunoassay Kits (Cayman Chemical, USA). Serum LH (Luteinizing Hormone) and FSH (Follicle Stimulating Hormone) concentrations were measured by a mice LH enzyme immunoassay and mice FSH enzyme immunoassay systems (Amersham, England), respectively.

Data were expressed as mean±SD and Median (statistical package for social sciences [SPSS, version 11.5]. Differences between control and propranolol exposed male groups were analyzed using either the Chi-square test or Student t-test. A p-value of $<0.05$ was considered statistically significant (Ipstein and Poly, 1970).

## RESULTS

Table 1 shows that intragastric administration of propranolol significantly decreased body weight of treated males when compared to those of control group. Furthermore, their relative weights of testes, epididymides, seminal vesicles, ventral prostate and vas deferens were significantly reduced in group 2.

Table 2 shows that sperm motility in cauda epididymis, sperm density and serum levels of testosterone, FSH and LH were significantly decreased in group 2 treated animals in comparison with controls.

Table 3 shows that administration of propranolol caused a significant decrease in the germinal cell population: spermatocytes (primary and secondary) and spermatids were decreased to a significantly lower level in group 2. Furthermore, in group 1 there was a significant decrease in secondary spermatocytes, spermatids, fibroblasts, immature Leydig cells and mature Leydig cells. Similarly, the numbers of fibroblasts, immature and mature

Table 1: Body and organ weights of male mice ingested propranolol

| | Body weight (g) | | Testes | Epididymides | Seminal vesicle | Ventral prostate |
|---|---|---|---|---|---|---|
| Treatments | Initial | Final | (mg/100 g body weight) | | | |
| Control (n = 10) | 30.8±1.80 | 31.9±1.65 | 710.1±25.21 | 247±2.61 | 687.8±14.38 | 86.0±4.10 |
| Group 1 propranolol (n = 10) | 31.1±1.95 | 30.4±1.87 | 707.0±11.80 | 236±2.33 | 669.3±11.03 | 81.2±4.11 |
| Group 2 propranolol (n = 10) | 31.0±1.31 | 29.3±1.32* | 620.0±10.36** | 202±1.36** | 401.6±12.69** | 74.4±3.18** |

Results are expressed as mean±SD. *p<0.05, **p<0.01 significantly different from control group (Student's t-test). Group 1 received propranolol 10 mg $kg^{-1}$ body weight. Group 2 received propranolol 15 mg $kg^{-1}$ body weight

Table 2: Fertility parameters and sperm dynamics of male mice ingested propranolol

| Treatments | Sperm motility (%) | Sperm count million $mL^{-1}$ | | Testosterone ($\mu mol\ L^{-1}$) | FSH ($ng\ mL^{-1}$) | LH ($ng\ ml^{-1}$) |
|---|---|---|---|---|---|---|
| | | Testes | Cauda epididymides | | | |
| Control (n = 10) | 84.10±1.94 | 6.55±0.14 | 12.18±0.88 | 13.92±2.53 | 76.3±15.5 | 14.2±4.2 |
| Group 1 propranolol (n = 10) | 78.45±2.21* | 5.43±0.14 | 9.33±0.57 | 11.56±2.09 | 73.2±12.8 | 11.5±6.32 |
| Group 2 propranolol (n = 10) | 53.26±1.08** | 3.75±0.47** | 6.00±0.94** | 8.38±1.89** | 56.0±13.7* | 7.37±6.21** |

Results are expressed as mean±SD.*p<0.05, **p<0.001 significantly different from control group (Student's t-test). Group 1 received propranolol 10 mg $kg^{-1}$ body weight. Group 2 received propranolol 15 mg $kg^{-1}$ body weight

Table 3: Testicular cell population dynamics of male mice ingested propranolol

| Treatments | Germinal cell types | | | | Interstitial cell type | | | |
|---|---|---|---|---|---|---|---|---|
| | Spermatogonia | Spermatocyte (primary) | Spermatocyte (secondary) | Spermatids | Fibroblast | Immature leydig cell | Mature leydig cell | Degenerating cell |
| Control (n = 10) | 27.05±4.44 | 27.96±2.41 | 77.970±3.73 | 89.23±5.82 | 78.66±1.33 | 81.660±1.65 | 86.66±0.78 | 13.00±0.76 |
| Group 1 propranolol (n = 10) | 25.57±5.33 | 24.54±2.66 | 73.180±4.86* | 65.77±6.33* | 71.23±1.87** | 74.900±2.13* | 77.37±1.16* | 15.78±1.44 |
| Group 2 propranolol (n = 10) | 23.99±0.93* | 18.85±1.80** | 46.126±5.51*** | 47.17±6.87*** | 63.83±1.64* | 65.195±3.47** | 70.64±1.03** | 19.34±1.67*** |

Results are expressed as mean±SD. *p<0.05, **p<0.001, ***p<0.001 significantly different from control group (Student's t-test). Group 1 received propranolol 10 mg $kg^{-1}$ body weight. Group 2 received propranolol 15 mg $kg^{-1}$ body weight

Table 4: Effect of propranolol ingestion on fertility in adult male mice

| Treatments | No. of male | No. of female | No. of pregnant females | No. of implantation sites | No. of viable fetuses | No. of resorption/total No. of implantation |
|---|---|---|---|---|---|---|
| Control group | 10 | 20 | 18/20 (90%) | 11.37±1.31 | 10.38±1.54 | 11/187 (5.88%) |
| Group 1 propranolol (n = 10) | 10 | 20 | 17/20 (85%) | 10.55±1.31* | 9.76±1.54* | 19/166 (11.44%) |
| Group 2 propranolol (n = 10) | 10 | 20 | 9/20 (45%) | 9.62±1.66** | 8.82±1.16** | 33/79ξ (41.77%) |

Results are expressed as mean±SD. *p<0.05, **p<0.001, significantly different from control group (Student's t-test and Chi-square test ξ). Group 1 received propranolol 10 mg $kg^{-1}$ body weight. Group 2 received propranolol 15 mg $kg^{-1}$ body weight

Leydig cells were also significantly decreased in group 2. The number of degenerating cells, however, was significantly increased in group 2.

Table 4 shows that propranolol exposure reduced fertility as indicated by the smaller number of pregnant females impregnated by the propranolol-exposed males. However, the number of implantations and number of viable fetuses were significantly decreased in those females impregnated by groups 1 and 2 males. The total number of resorptions was also significantly increased in females impregnated by group 2 males.

## DISCUSSION

The animal model used in this work has previously been used to assess the effect of various extracts obtained from medicinal plants on reproductive functions in male (Nusier and Bataineh, 2005; Bataineh and Nusier, 2005; Bataineh *et al*., 1998).

Spermatogenic process in mice requires 35 days, out of which spermatozoa spend the last 6 to 7 days in the final transit through epididymides (Ke and Tso, 1982). Propranolol was administrated for one complete spermatogenic cycle.

The present investigation shows that oral administration of propranolol reduced fertility in male albino mice. The weights of reproductive organs were markedly decreased. The weight, size and secretory function of testes, epididymis, seminal vesicles, ventral prostate and vasa deferentia are closely regulated by androgens (Choudhary and Steinberger, 1975). The process of spermatogenesis and accessory reproductive organs function are androgen dependent (Agrawal *et al*., 1986; Dym *et al*., 1979). Histological studies of testes, epididymis, seminal vesicles, ventral prostate and vasa deferentia also confirmed the results (Agrawal *et al*., 1986). The drug may act on pituitary gland and decrease the main hormone of spermatogenesis (Agrawal *et al*., 1986). Decrease androgen production is reflected by decreased number of mature Leydig cells and their functional status. In the present study, the number of degenerating Leydig cells was significantly increased; this may reflect the decrease of androgen levels. It was further confirmed by the decreased number of spermatocytes (primary and secondary) and spermatids as these stages are completely androgen dependent (Agrawal *et al*., 1986). The decreased weight and histometry of reproductive organs further confirmed the decrease in androgen levels. A significant increase in the sperm motility of cauda epididymis was observed in the treated group.

Results presented in this manuscript also showed that the ingestion of propranolol by adult male mice caused a slight decrease in the number of females impregnated by the treated males. However, the number of implantations sites and the number of viable fetuses

were reduced. These observations may be due to the decrease in sperm motility and function. Reduction in implantation and phytotoxic effects might be due to cytotoxic effects which can result in decreased fertility, failure of preimplantation or postimplantation death. Cytotoxic agents can disrupt pregnancy possibly by interfering with mitotic division of the fetus (Desjardins, 1978; Working *et al*., 1985).

The increased resorption is further evidence, along with the reduced implantation rate, that propranolol at 15 g $kg^{-1}$ $day^{-1}$ is having an adverse effect on male sperm.

These results may suggest that propranolol imposes toxic effects on fertility in male mice.

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