Original Article

# Effects of Spironolactone on the Testes

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**ABSTRACT**

**Purpose:** To evaluate the anti-androgenic effects of spironolactone on the testes in a rodent model.

**Materials and Methods:** 42 male mice of age ranging from 30 to 60 days were used in the study. These were equally divided at random into control groups A1, A2 and A3 and experimental groups B1, B2 and B3 each comprising of 7 animals. Each control group received aqueous suspension of gum acacia (vehicle) 3ml/kg/day orally, A1 for 10, A2 for 20 and A3 for 30 days. Each experimental group received spironolactone 200 mg/kg/day orally, B1 for 10, B2 for 20 and B3 for 30 days. The animals were weighed and sacrificed 24 hours after last respective dose and testes removed. The weight and volume of testes were measured. Student-t test was applied to compare the mean data of control and experimental groups.

**Results:** Animal body weight decreased significantly in B2 and B3. There was significant reduction in weight and volume in all three experimental groups. No significant change was found in the relative tissue weights.

**Conclusion:** From the results of present study it is concluded that the treatment with spironolactone causes significant reduction in the weight and volume of the testes and hence the testicular function. Moreover the testes of animals at younger age (of sexual maturation) are more sensitive to spironolactone.

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**INTRODUCTION:**

Fertility of human being, the least fertile among the mammals, is certainly on the decline. Many drugs used in various conditions like acid pepticdisease and hypertension impair male fertility by interfering with spermatogenesis, sperm motility, or by interfering with the fertilizing capacity of spermatozoa. (P. Nicolopoulou-Stamati, L. Hens and C.V. Howard, 2007). Widely used spironolactone, a mineralocorticoid receptor antagonist and a potassium-sparing diuretic also has anti-androgen effects (Steelman et al., 1969; Wilson, 1996; Snyder PJ, 2006). It is used in variety of conditions. It is very effective drug for the management of refractory edema associated with secondary hyperaldosteronism in cardiac failure, hepatic cirrhosis, nephritic syndrome and severe ascites. It is also used in the treatment of essential hypertension, hypokalaemia, diabetes insipidus, and migraine and is particularly useful for the treatment of primary hyperaldosteronism due to adrenal adenomas or bilateral adrenal hyperplasia (Wade A, 1977; Jackson, 1996 and 2006).

As an anti-androgen it is used in variety of conditions e.g., benign hyperplasia and carcinoma of prostate, acne (Klus et al., 1996; Sciarra et al.,1990), hirsutism (Cumming et al., 1982), androgenic alopecia and ovulatory dysfunctions, polycystic ovarian syndrome and increased bone mineral density (Moghetti et al., 2000; Diamanti-Kandarakis E et al., 1999; Prezelj and Kocijancic, 1994). Acting on the kidney, spironolactone competes with mineralocorticoid receptors in the epithelial cells of distal tubules and conducting ducts. This results in reduced reuptake of NaCl which causes diuresis (Jackson, 1996 and 2006).

As an antiandrogen it acts in two ways: (1) Affecting the Leydig cells of testes and adrenocortical cells of adrenal glands it inhibits the steroidogenesis in these glands by inhibiting their cytochrome p-450 steroid hydroxylases. As a result androgen synthesis is blocked and testosterone production is reduced and hence the spermatogenesis is badly affected, leading to reduction in testicular mass and volume (Corvol et al., 1975 and 1976, Jackson, 2006). (2) It has some affinity towards androgen and progesterone receptors in the target tissues. Spironolactone interacts with androgen receptors of these tissues and inhibits the action of androgen on the testes, epididymis, ductus deferens, seminal vesicles, prostate, cartilage, sebaceous glands and hair (Mahoudeau et al.,1976; Basinger and Gittes, 1974; Pita et al., 1975; Klus et al., 1996; Neumann and Kalmus, 1991).

About 70-80% of testicular mass consists of seminiferous tubules and the spermatogenesis is reflected mainly by the testicular volume (Setchell BP and Brooks DE, 1988). Studies in infertile men show that the testicular volume has a direct correlation with semen profiles. In men with normal testicular function total volume of (the two) testes is about 30 ml (Arai T et al., 1998).

Aim of this study was to assess the testicular function in term of changes in the testicular weight and volume due to spironolactone.

## MATERIALS AND METHODS

**Animals:** The study was conducted in the Zoology Department of Punjab University and Anatomy Department of King Edward Medical University, Lahore. Colonies of Mus-musculus Swiss Webster albino mice were raised at animal house and 42 male mice of age ranging from 30 to 60 days were separated for study. The housing and treatments of animals were in accordance with International and institutional guidelines and all procedures were approved by Punjab University, Lahore. Optimum light and temperature was provided in the animal room. The room was maintained at 25 $\pm$ 2˚C under a 12/12 hour light/dark cycle. Animals were fed on commercial diet No.14 of National Feeds Company of Pakistan. Water was provided ad labitum in glass bottles. General condition and body weight of the animals were noted daily.

**Chemicals Used**

1. Spironolactone, available as Aldactone 25 mg and 100 mg tablets, a product of Searle Pakistan Limited was used in this study. It was given orally to the experimental groups as an aqueous suspension in vehicle.
2. Gum acacia (aqueous suspension) was used as vehicle.

The suspensions were made as under:

1. The vehicle was made by dissolving 50 mg of gum acacia in 100ml of water using heat to get fresh thin aqueous suspension and kept in bottle no.1.
2. 2 tablets of 100 mg spironolactone (200 mg) were ground to fine powder. This powder was mixed in 3 ml of vehicle in bottle no. 2.

**Grouping of Animals:** 42 male mice of body weight ranging from 25 to 35 gm used in the study were divided at random into a control group A and experimental groups B, each comprising of 21 animals. These were treated according to the treatment plan (Table. 1).

Control group A was divided at random into subgroups A1, A2 and A3 comprising of 7 animals each, kept in separate cages. The animals of these subgroups were given vehicle (bottle no. 1) orally in a dose of 3 ml / kg / day for 10, 20 and 30 days respectively.

Experimental group B was divided into subgroups B1, B2 and B3 comprising of 7 animals each, kept in separate cages. The animals of these subgroups were given spironolactone (bottle no.2) in a dose of 200mg (as 3ml suspension) / kg / day orally for 10, 20 and 30 days respectively.

**Table 1: Animal grouping and treatment plan.**

| **Group** | **Control-A** | **Experimental-B** | **Treatment duration**(days) |
|---|---|---|---|
| **Sub-groups** | A1 | B1 | 10 |
| | A2 | B2 | 20 |
| | A3 | B3 | 30 |

**Collection of Organs:** 24 hours after the last respective dose, the general condition and weight of each animal was noted. Using the

glass desiccator these animals were anaesthetized with ether. Then inguinal canal and scrotal sac of each side were opened**.** The fat around internal spermatic vessels, testicular artery, caput epididymidis, corpus epididymidis, cauda epididymidis, ductus deferens and testis

Suspensions of Spironolactone were prepared daily. The suspensions were given by 1 ml insulin syringe having 10 large divisions, each equal to 0.1 ml and each large division having 10 small sub-divisions, each equal to 0.01ml. 1.5 cm long flexible plastic tubing, taken from scalp infusion set was used in place of steel needle.

## MORPHOLOGY

**Body weight:** The body weight of each animal was recorded daily by digital balance during the experimentation and 24 hours after the last dose before they were sacrificed.

**Photography:** Testes were photographed using 7.2 mega pixels digital camera (Panasonic Lumix TZ 15) and dissecting microscope (Labomed CZ 6).

**Organ Weight:** The weight of paired testes and the prostate was measured using electronic balance (Shimadzu Corporation of type A x 120 having readability = 0.1 mg and capacity = 120 gm).

**Relative Tissue Weight Index:** The Relative Tissue Weight Index (RTWI) was calculated using following formula:

$$RTWI = \frac{\text{Mean weight of the organ}}{\text{Mean body weight}} \times 100$$

**Volume of the testes**: 1 ml insulin syringe cylinder (readability = 0.01 ml) containing 0.5 ml normal saline was used to measure the volume of each testis.

was removed on each side. The testicular vessels were excised close to the testis. Shape, colour and vascularity of the testes were noted. The testis was separated from epididymis and removed on each side, washed in the saline and bloated before morphological study.

## STATISTICAL ANALYSIS

SPSS 16.0 software was used for statistical analysis of the study. Student-t test was applied to compare the mean data of control and experimental groups.

## RESULTS

**Effect on animal body weight:** After 10 days treatment there was a decrease in the mean body weight in B1 group but not significant (P = 0.298). The decrease in the mean weight of B2 group was significant (P<0.05). Similarly after 30 days treatment the mean body weight of B3 group decreased significantly (P<0.05) (Table 2).

**Table 2: Effect of spironolactone on animal body weigh**

| Parameter | n | Group | Mean | t-value | P-value |
|---|---|---|---|---|---|
| **Body weight (g) after 10 days** | 7 | **A1** | 27.143 ± 6.362 | 1.0870 | 0.298 |
| | 7 | **B1** | 24.000 ± 4.243 | | |
| **Body weight (g) after 20 days** | 7 | **A2** | 28.714 ± 3.302 | 4.073 | 0.002 |
| | 7 | **B2** | 20.857 ± 3.891 | | |
| **Body weight (g) after 30 days** | 7 | **A3** | 31.429 ± 3.309 | 3.389 | 0.005 |
| | 7 | **B3** | 25.571 ± 3.155 | | |

(P<0.05 is significant)

**Table 3: Effect of spironolactone on weight of testes**

| Parameter | n | Group | Mean | t-value | P-value |
|---|---|---|---|---|---|
| **Organ weight (mg) after 10 days** | 7 | **A1** | 207.57 ± 18.174 | 6.219 | 0.000 |
| | 7 | **B1** | 142.930 ± 20.642 | | |
| **Organ weight (mg) after 20 days** | 7 | **A2** | 221.140 ± 15.269 | 3.917 | 0.002 |
| | 7 | **B2** | 132.140 ± 58.145 | | |
| **Organ weight (mg) after 30 days** | 7 | **A3** | 236.57 ± 18.183 | 3.932 | 0.002 |
| | 7 | **B3** | 173.140 ± 38.607 | | |

(P<0.05 is significant)

**Effect on weight of testes:** After 10 days treatment the mean paired testes weight of group B1 was a decreased significantly (P < 0.05). Mean testes weight of group B2 after 20 days treatment decreased significantly (P<0.05). Similarly after 30 days treatment the mean testes weight of group B3 decreased significantly (P<0.05) (Table 3).

**Effect on relative tissue weight:** After 10 days treatment there was a significant in the mean relative tissue weight in the experimental group B1 (P < 0.05). Mean relative tissue weight of group B2 decreased insignificantly (P=0.145). Similarly after 30 days treatment the decrease in the mean relative tissue weight of group B3 was also not significant (P=0.134) (Table 4).

**Table 4: Effect of spironolactone on relative organ tissue weight**

| Parameter | n | Group | Mean | t-value | P-value |
|---|---|---|---|---|---|
| **RTWI after 10 days** | 7 | **A1** | 0.797 ± 0.173 | 2.462 | 0.030 |
| | 7 | **B1** | 0.606 ± 0.111 | | |
| **RTWI after 20 days** | 7 | **A2** | 0.779 ± 0.102 | 1.561 | 0.145 |
| | 7 | **B2** | 0.622 ± 0.245 | | |
| **RTWI after 30 days** | 7 | **A3** | 0.758 ± 0.078 | 1.606 | 0.134 |
| | 7 | **B3** | 0.676 ± 0.111 | | |

RTWI=Relative tissue weight index. (P<0.05 is significant)

**Table 5: Effect of spironolactone on volume of testes**

| Parameter | n | Group | Mean(ml) | t-value | P-value |
|---|---|---|---|---|---|
| **Organ volume after 10 days** | 7 | **A1** | 0.166 ± 0.024 | 4.919 | 0.000 |
| | 7 | **B1** | 0.084 ± 0.032 | | |
| **Organ volume after 20 days** | 7 | **A2** | 0.188 ± 0.040 | 3.718 | 0.003 |
| | 7 | **B2** | 0.100 ± 0.048 | | |
| **Organ volume after 30 days** | 7 | **A3** | 0.170 ± 0.018 | 2.375 | 0.035 |
| | 7 | **B3** | 0.136 ± 0.032 | | |

(P<0.05 is significant)

**Effect on volume of paired testes:** After 10 days treatment the decrease in the mean paired testes volume was very significant in the experimental group B1 ($P < 0.05$). Mean testes volume of group B2 decreased significantly ($P < 0.05$). Similarly after 30 days treatment the mean testes volume of group B3 animals decreased significantly ($P<0.05$) (Table 5).

**DISCUSSION:**

The results of present study suggest that the prolonged administration (20 and 30 days) of spironolactone causes significant reduction in the body weight in mice ($P \leq 0.005$) and insignificant in those which were given the drug for 10 days ($P = 0.298$). In a study 12.5 mg of spironolactone given to 10 rats subcutaneously twice daily for 10 days, produced significant reduction in the body weight ($P < 0.05$) (Basinger GT and Gittes RF, 1974). In addition to anti androgenic effect, spironolactone probably reduces the body weight due to its diuretic effect (Jackson, 2006) and its effect on lipid metabolism (Wada T, Kenmochi H, Miyasshita Y, et al., 2010).

Significant reduction in testes weight of all three groups ($P \leq 0.002$) suggests anti androgenic activity of spironolactone. Our study also reveals that testes of growing mice (10 days treatment group) are more sensitive ($P = 000$) as compared to older mice of 20 and 30 days treatment groups ($P = 0.002$) to spironolactone. One previous study with an antiandrogen anandrone has revealed similar differential effect (Dhar JD and Setty BS, 1990).

In our study, there is no significant change in the relative organ tissue weight in 20 and 30 days treatment groups (P value not less than 0.05), whereas there is significant reduction in relative organ tissue weight in 10 days treatment group. This might again be due to the differential sensitivities of the testes to the spironolactone.

The results of our study reveal differential effect of spironolactone also on the volume of the testes. There is significant reduction in the testicular volume in all three groups ($P< 0.035$). The effect is maximum in 10 days treatment group ($P=0.000$) and minimum in 30 days treatment group. This again shows that the testes of younger group have maximum sensitivity to spironolactone and this sensitivity decreases as they grow. This is in agreement with the results of JD Dhar (1990).

**CONCLUSION:**

Spironolactone due to its anti-androgenic activity, affects the spermatogenesis badly leading to arrest in maturation and decrease in number of germ cells. As a result total mass of the testes is decreased which is reflected in reduction in their weight and volume and hence the testicular function. It is predicted that the treatment with spironolactone affects the seminal profile which leads to sub-fertility and infertility

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