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ORIGINAL ARTICLE

# Development of spermatic granuloma in albino rats following administration of water extract of *Heliotropium bacciferum* Forssk

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**KEYWORDS**

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**Abstract** A spermatic granuloma is a chronic inflammatory reaction produced in response to extravasated sperm within the intertubular connective tissue. The present study investigates the possible toxic effects of water extract of *Heliotropium bacciferum* on the reproductive system of male albino rats and the associated potential for the development of spermatic granulomas. *H. bacciferum* is a herbal plant used in traditional medicine and reported to have cytotoxic effects due to pyrrolizidine alkaloids. Histological examinations revealed no changes in the tissues of the testes, although, some changes were detected in the cauda epididymis, the most important of which was the development of small lesions of spermatic granulomas. Clear gaps were observed between the epithelial linings of the epididymal tubules.


## 1. Introduction

It has been reported earlier that the herbal plant *Heliotropium bacciferum* has cytotoxic effects due to the presence of pyrrolizidine alkaloids including Heliotrine, Heleurine, Supinine, Europine (Schoental, 1957; Farrag et al., 1996). Bick and Jackson (1968) reported that Heliotrine is categorized as an alkylating agent and may cause mutations and liver lesions through its reaction with the phosphate group of DNA to form a trialkyl phosphate resulting in the breakdown of DNA into small fragments (Culvenor et al., 1969; Mattocks, 1969). It has also been shown that pyrrolizidine alkaloids cause

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chromosomal damage in plants, bacteria, fungi and cell cultures (Bull et al., 1968; McLean, 1970; Bick and Culvenor, 1971; Mattocks, 1986).

Spermatic granulomas are a chronic inflammatory reaction produced in response to extravasated sperm within intertubular connective tissue. Spontaneous spermatic granulomas of the epididymis have been described in both domestic and laboratory animals (Ashdown and Ford, 1967; King, 1978; Jones and Hunt, 1983). In rats, however, the microscopic changes associated with spermatic granulomas were not well characterized until the work of Yamasaki (1990) in Sprague-Dawley rats. The aim of the present study is to investigate the possible toxic effects of *H. bacciferum* on the reproductive system of albino rats and the potential that these may be associated with the development of spermatic granulomas.

## 2. Materials and methods

Specimens of *H. bacciferum* were collected from the Thumama zone (northeast of the city of Riyadh in the Kingdom of Saudi Arabia). The plants were identified by specialists from the department of Botany, Faculty of Science, King Saud University. The plants were allowed to dry at room temperature and were then ground and dissolved in boiled distilled water for 5–10 min. The resulting solution was filtered and incubated in Petri dishes at 70 °C until the fluid had evaporated. The extract was then collected from the Petri dishes and stored in dark bottles in a refrigerator at 4 °C. At the time of administration, the extract was once again dissolved in distilled water.

The animals used in the present study were the Wistar strains of the albino rats *Rattus norvegicus*, obtained from the animal house of the Faculty of Pharmacy at King Saud University. The experimental animals were sexually mature males, ranging in age from eight to ten weeks and weighing between 230 and 290 g. The animals were randomly divided into four groups with each group comprising ten rats. The *H. bacciferum* plant extract was administered in doses of 0.5 g/kg body weight, 1 g/kg body weight and 1.5 g/kg body weight for groups 1, 2 and 3 respectively. Group 4 was treated as a control group, with the animals being administered distilled water without any extracts. The treatments were administered two weeks after captivity to give enough time for acclimatization. Daily administration was then carried out for nine weeks: this period being sufficient for spermatogenesis to take place and for sperm to be transported to the epididymis. During the experimental period the animals were closely monitored and examined for any abnormal signs or death. At the end of the experimental period, the animals were sacrificed and dissected. The weight of the testes, epididymis, seminal vesicles and prostate were measured by using a digital balance (Mettler Toledo, Switzerland). The relative organ weight was calculated as described by Andrade et al. (2002) and Yu et al. (2008) using the following formula:

$$\text{The relative organ weight} = \frac{\text{The absolute organ weight}}{\text{Total body weight}}$$

After weighing, the testes and epididymis from each animal were fixed in Bouin's solution followed by dehydration, paraffin embedding, sectioning (3–5 micron), and staining with haematoxylin and eosin (H&E) for microscopic analysis (Hess and Moore, 1993).

### 2.1. Statistical analysis

Results were expressed as mean ± SE and were statistically analyzed using a SAS software and a student's *t*-test applying a significance level of $p < 0.05$ (Sokal and Rohlf, 1981).

## 3. Results

### 3.1. Mortality

No cases of mortality were reported in the control group. Two cases were recorded in group 1, one on the fifth week and the other on the seventh. On the other hand, one case of mortality was reported in group 2 following administration of the fifth dose and two cases in group 3 in the fifth and sixth weeks, respectively.

### 3.2. Body/organ weight

Statistical evaluation of the body weights did not show any significant changes compared with the control group. The relative organ weight did, however, show a significant decrease in the epididymis of group 1 ($p < 0.01$) and group 3 ($p < 0.05$) and in the seminal vesicles of group 3 ($p < 0.05$) (Fig. 1).

### 3.3. Microscopic analysis

Microscopic analysis of the histological sections revealed that the examined organs in the control group had a normal appearance. On the other hand, in group 1, atrophy was observed in some epididymal tubules together with a decrease in the number, or complete absence of, sperm in addition to duplication of the epithelial cell lining of the lumen (Fig. 2). In group 2 some spermatic granulomas were observed in the intertubular connective tissue. A spermatic granuloma is a

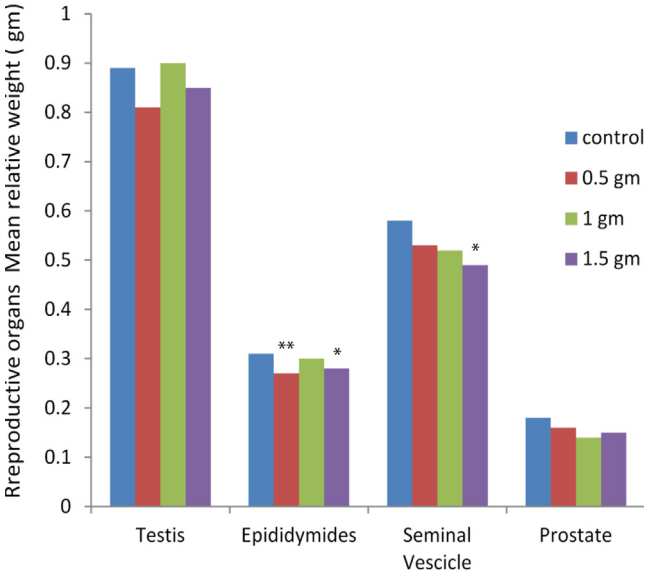


**Figure 1** Histogram showing the effect of different doses of water extract of *Heliotropium bacciferum* on the relative weight of reproductive organs of male albino rat.

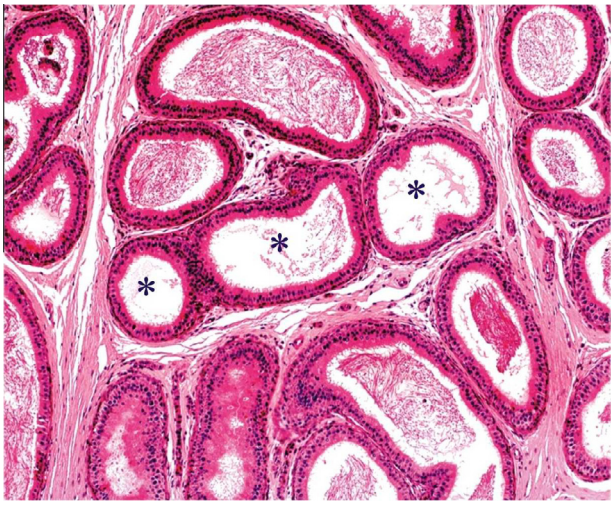

**Figure 2** Photomicrograph of a section of cauda epididymis of a rat from group 1, showing atrophied epididymal tubules. Some tubules (black stars) are empty and lacking sperm (H&E, 100×).

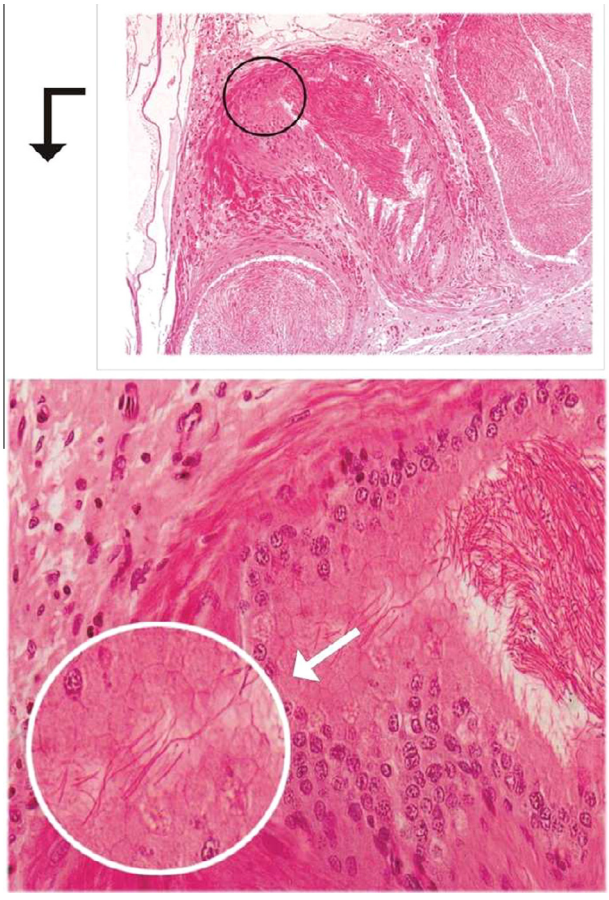

**Figure 3** Photomicrograph of a section of cauda epididymis of a rat of group 2, showing a ruptured epithelial lining. Spermatic granulomas start to develop around extravasated sperm (arrow) within the intertubular connective tissue (H&E, 100×).

small lesion consisting of chronic inflammatory cell infiltration surrounding a group of sperm extravasated from the tubules of the cauda epididymis (Fig. 3). The chronic inflammatory cells are lymphocytes and plasma cells, which reflect an early onset of the inflammatory reaction. In addition, clear gaps were observed between the epithelial cell linings the epididymal tubules associated with extravasated sperm (Fig. 4) and some white blood cells within the lumen (Fig. 5).

## 4. Discussion

Body weight after the end of the experimental period is considered to be a general indicator for the systemic toxicity of any administrated agent. As a general rule, a decrease of an average body weight is significant only if they reduced to 10% of the control group (Keller and Banks, 2006). In the present study no significant decrease in body weight was detected in any of the three treated groups. Regarding the relative organ weight, however, a significant decrease was detected in the epididymis of groups 1 and 3. It needs to be mentioned here that the decrease in the relative organ weight can be considered to be a parameter for toxicity while the absolute organ weight may reflect a general decrease in the body weight without a significant toxic effect from an agent (Sellers et al., 2007). The decrease in the relative weight of the epididymis in group 1 can be explained by the histological finding that some of the tubules were atrophied. In the present study, small lesions of spermatic granulomas were observed in the intertubular connective tissue of the cauda epididymis. The lesions consisted of chronic inflammatory cells surrounding a group of extravasated sperm. Since the plant under investigation contains chemicals that are toxic for human and experimental animals (Schoental, 1957; Braginskii and Bobokhodzhaev, 1965; Stillman et al., 1977; Huxtable, 1980), it is possible that the histological changes in the tubular wall of the cauda epididymis may result from a toxic effect of the plant under investigation. This is supported by the presence of extravasated sperm in the

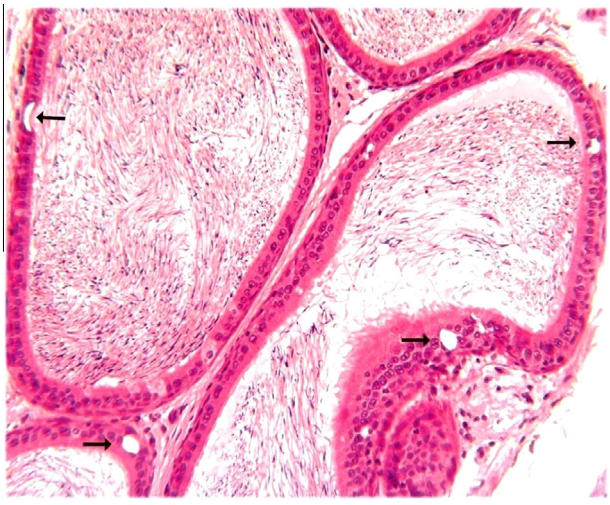

**Figure 4** Photomicrograph of a section of cauda epididymis of a rat from group 3, showing gaps within the epithelial lining of the epididymal tubules (arrows) (H&E, 100×).

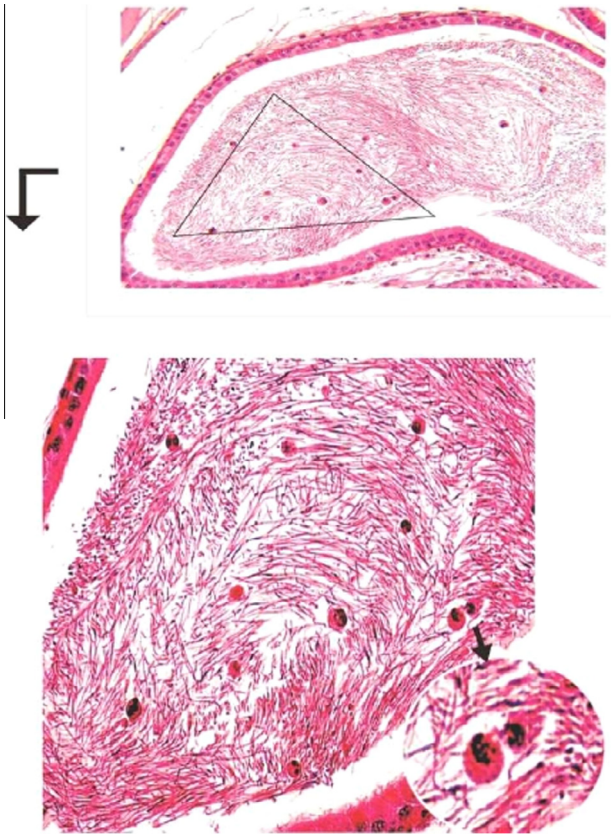

**Figure 5** Photomicrograph of a section of the cauda epididymis of a rat from group 3, showing extravasated leucocytes within the lumen of some epididymal tubules (arrows) (H&E, 100×).

intertubular connective tissue and the appearance of leucocytes in the tubular lumen (Figs. 3 and 5). The extravasated sperm in the interstitium of the epididymis acts as an irritant that stimulates the connective tissue to develop a chronic inflammatory reaction which begins with the accumulation and infiltration of lymphocytes and plasma cells (Yamasaki, 1990). The finding that the spermatic granuloma developed in the animals of group 3 are small lesions is explained by the fact that the experimental period in the present study was not enough to develop a typical pathological lesion, as the chronic inflammatory reaction requires several weeks or even months to develop. Spontaneous spermatic granuloma of the epididymis is known to occur in domestic and laboratory animals (Ashdown and Ford, 1967; Jones and Hunt, 1983; King, 1978). Yamasaki (1990), in a study on Sprague-Dawley rats, reported the development of spontaneous spermatic granuloma in 10 rats out of 152 rats used in this experiment. The lesion was grossly described as the presence of white nodules in the epididymis and numerous spermatozoa in the center of chronic inflammatory cells. The presence of ruptured basement membrane of the tubules with spillage of sperm into interstitium was also reported. King (1978) suggested that the histological picture is a function of the age of lesion. In the earliest lesions, the inflammatory response is associated with the invasive sperm while later on it was characterized by increasing accumulations of lymphocytes, plasma cells and macrophages. Rats may develop spermatic granuloma in response to administration of some chemical toxins for prolonged periods (Tani et al., 2005). Several other studies showed that the development of spermatic granuloma in rats treated with cadmium, methyl chloride and other toxic agents is due to the direct lesions in the tubular epithelial lining (Benson and Clare, 1966; Mason and Young, 1967; Chapin et al., 1984). The development of spermatic granuloma in the epididymis rather than in the testis is due to the resistance of the testicular tubular wall for sperm extravasation in the absence of mechanical trauma (Itoh et al., 1999). Later, some studies suggested that the rapid lymphatic drainage of the testis prevents the accumulation of any germ cells or spermatozoa and does not give enough time for the reaction to develop.

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