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Original Article

Effect of estradiol benzoate injection to male rabbits on glucose, total protein, albumin, calcium concentrations and prostate tissue

Tamour Elkhier, Shams-Eldein Hassaballa, Shadia Abdo-Elatti Omer and Abdelnasir Adam

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AFFILIATIONS

- Tamour Elkhier

Department of Physiology and Biochemistry, Faculty of Veterinary Science, University of Nyala. Nyala-Sudan.

- Shams-Eldein Hassaballa
- Shadia Abdo-Elatti Omer

Department of Basic Science, Collage of Veterinary Medicine and Animal Production- Sudan University of Science and Technology, Khartoum North, Kuku-Sudan.

- Abdelnasir Adam

Department of Pathology, Faculty of Veterinary Science, University of Nyala. Nyala-Sudan.

CORRESPONDENCE

Tamour Elkhier
Department of Physiology and Biochemistry, Faculty of Veterinary Science, University of Nyala. Nyala-Sudan.
E-mail: elkhiertmur@gmail.com

ABSTRACT

Objective: This study was carried out to investigate the effects of estradiol benzoate (EB) injection to male rabbits on glucose, total-protein, albumin, calcium concentrations and prostate tissue, and the role of prolactin as an important mediator of estrogen action in prostate.
Materials and methods: Fifty four adult male rabbits were used in this study. The rabbits were randomly divided into two groups. Group A contained 36 male rabbits, which were further randomly divided into four sub-groups, three of them contained 10 rabbits and one sub-group contained 6 rabbits as control. Group B contained 18 male rabbits, which were divided randomly into three equal sub-groups. Three sub-groups of Groups A and B were treated once each on alternative day with the intramuscular injections of EB dosed at 40, 80 and 120 µg/rabbit, respectively for 20 days, whereas the fourth sub-group of Group A received no estradiol, and Group B received 1 mg Bromocriptine Mesilate in addition to EB through oral route on each alternative day. Blood samples were collected for measuring glucose, t-protein, albumin and calcium levels. Prostate tissue samples were collected from all the rabbits for histological studies.
Results: Glucose was significantly ($P<0.05$) increased as a result of 80 µg EB injection, while significantly ($P<0.05$) decreased due to 40 and 120 µg EB injection. Total protein significantly ($P<0.05$) increased due to injection of 40 µg EB, however t-protein was not changed due to 40 and 120 µg injection. On the other hand, the results of albumin and calcium were not affected ($P>0.05$) by EB. In prostate tissues, EB induced hyperplasia with dysplasia or dysplasia only, but this effect was mild due to inhibition of prolactin.
Conclusion: The injection of EB to male rabbits increased or decreased glucose level, increased t-protein level mildly or not changed, while albumin and calcium levels were not affected. EB induced hyperplasia on prostate tissue, and this effect was reduced by prolactin inhibition indicating that prolactin might have a role on the action of estrogen.

KEYWORDS

Calcium; Estradiol; Glucose; Rabbit; Total protein

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INTRODUCTION

Estrogen is one of the main reproductive hormones that affects growth, development, maturation and functioning of reproductive tract as well as the sexual differentiation and the behavior (Balthazart et al., 2009). Although the estrogen is the female hormone, there is evidence that it has also biological role in the male reproduction (Todiodi, 2010). The effects of estrogen on glucose varied, as reported by Nematbakhsh et al. (2001). However, overectomized rats showed that 17β-estradiol lowered glucose levels (Verma et al., 2005), On the other hand, Nagira et al. (2006) found that 17β-estradiol increased the glucose level. Several reports suggested that estrogens had profound modulating effects on systemic glucose homeostasis (Barros et al., 2009; Foryst-Ludwig and Kintscher., 2010).

Estrogen causes positive nitrogen balance due to growth promoting effect which causes slight increase in the total body proteins (Indu, 2009). 17β-estradiol was found to cause decrease in total protein (Stevenson et al., 2005; Elnagar and Abd-Elhady, 2009).

Albumin is the most abundant protein in the blood and it binds steroids and other small lipophilic molecules non-specifically. The albumin synthesis was occurred in the liver (Reece, 2005). A study done in Japanese Quail Hens by Elnagar and Abd-Elhady (2009) demonstrated that egg albumin increased significantly as a result of estradiol injection.

Calcium plays important roles in cellular structural and biochemical functioning through signaling within and external to the cell. Besides, calcium is essential for normal neuromuscular function and proper functioning of the coagulation factors. Calcium concentration in plasma is regulated by parathyroid hormone, vitamin D and calcitonin (Bazydlo et al., 2014). A study on postmenopausal osteoporosis revealed that estrogen treatment increased calcium absorption by increasing serum 1,25-(OH)2D; this effect appeared to be mediated indirectly through stimulation of renal alpha-hydroxylase by increased serum PTH (Gallagher et al, 2013). A study in Tilapia on *Oreochromis mossambicus* demonstrated that estrogen (E2) had a hypercalcemic effect on both male and female, with this effect being greater in males (Tsai and Wang, 2000).

The effects of estrogen on prostate tissue were documented by many authors (Risbridger et al., 2001; Prins et al., 2001; Prins and Korach, 2008). Prolactin is one of the non-steroidal factors involved both in prostate cell proliferation (Van Coppenolle et al., 2004) and in the development of benign prostatic hyperplasia (Van Coppenolle et al., 2000, 2001). Recent study documented significant interactions between estrogen and prolactin systems. Estrogen stimulates prolactin secretion and can up-regulate prolactin receptor gene expression, which stimulates growth of tumor genesis in human (Dong et al., 2006). Estradiol is a major stimulator of prolactin secretion mediated by increasing the number of lactotrophs (Kansra et al., 2005, 2010; Nolan and Levy, 2009). Estrogen induced hyperprolactinemia as an important mediator of estrogen action in prostate carcinogenesis (Tam et al., 2010). The objective of this study was to investigate the changes in glucose, total protein, albumin, calcium concentrations as a result of injection of estradiol benzoate (EB) in male rabbits. The effect of EB on prostate tissue and the role of prolactin on estrogen action in prostate was also investigated.

MATERIALS AND METHODS

Animals: Fifty four adult male rabbits weighing 2.0±0.1 kg were used for this study. The rabbits were housed under individual cage 50x50 cm having free access to standard rabbit chow and tap water. The necessary ethical approval for the animal experimentation was taken from the Nyala University's Animal Welfare Committee.

Treatments: The rabbits were randomly divided into two groups. Group A (n=36) were further randomly division into four sub-groups; three of them contained 10 rabbits in each, and one sub-group contained 6 rabbits as control. The Group B (n=18) divided randomly into three sub-groups (six in each sub-group). The animals of the three sub-groups of Groups A and B were treated once each on alternative day with the intramuscular injections of estradiol benzoate (Estradol® Animal health care Australia dosed at 40, 80 and 120 µg/rabbit, respectively for 30 days, whereas the fourth group of Group A received no estradiol, and acted as the control sub-group.

Inhibition of prolactin secretion: Prolactin secretion was inhibited by Bromocriptine Mesilate 2.5 mg tablet Brameston®, Cyprus (EU). A tablet of 2.5 mg was dissolved in 2.5 mL normal saline (NS) *i.e.*, 1 mL contained 1 mg Bromocriptine. In Group B, each rabbit was drenched 1 mg of Bromocriptine orally on every alternative day over a period of 30 days.

Blood collection: After 30 days of treatment, 5 mL of blood samples were collected using disposable syringe from the heart of the rabbits, and the serum samples were prepared. The serum samples were kept at frozen

condtion for analyzing glucose, t-protein, albumin and calcium levels.

Prostate tissue collection: After the end of the experiment, the animals were scarified, and about 1 cm^3 of prostate tissue specimens were collected from all sub-groups of Group A and B immediately by using a sharp knife or a razor blade, and were fixed immediately in 10% formal saline, the volume ration of tissue to fixative was 1:10. The tissues were left at room temperature for fixation before they were processed.

Analysis of serum samples: Automate chemistry analyzer (MINDARY) and reagent Biosystem® Spain made was used for analysis of glucose, t-protein, albumin and calcium levels.

Prostate tissue study: The prostate tissues were left at room temperature for fixation before they were processed. Tissue processing method was made by automatic tissue processor (LEICATP1020), and then embedded in paraffin wax. Sections of 5 µ thick were cut by a rotary microtome, and were stained in Mayer's hematoxylin and Eosin (H&E) (Bancrof et al., 1996). All sections were examined under the 40X of light microscope (Olympus) to describe the microscopic changes and imaged by using digital camera (Dewinter-DigiEye).

Statistical analysis: The independent t-test was done using SPSS statistical program, version 20 for Windows (IBM SPSS Statistics 20 IL, USA). The results were expressed in the form of mean±standard deviation. The difference between the mean in glucose, t-protein, albumin and calcium levels in this study were considered statistically significant when the *P* value was less than 0.05.

RESULTS AND DISCUSSION

The effects of EB on glucose, t-protein, albumin, calcium are show in **Table 1**. There is a significant ($P<0.05$) increase in t-protein level in the 40 µg estradiol treated sub-group, and increase in glucose concentration in the 80 µg estradiol treated sub-group. On the other hand, there was a significant ($P<0.05$) decrease in glucose concentration in the 40 µg and 120 µg estradiol treated sub-groups as compared to control sub-group. However, there was no significant difference ($P>0.05$) among 40 µg, 80 µg and 120 µg estradiol treated and control sub-groups.

The effects of EB in prostate histology of Group A showed hyperplasia with dysplasia or dysplasia only (**Figure 1**). In Group B, when prolactin was inhibited by Bromocriptine Mesilate, the effect of EB on prostate histology was demonstrated the presence of mild hyperplasia in epithelia as compared to Group A.

The effects of estradiol on glucose concentration in previous studies were varied. In male rabbits, plasma glucose level was found to be unchanged as a result of injection of estradiol valerate (Nematbakhsh et al., 2001). Another study on overectomized rats showed that 17β-estradiol lowered glucose by raising insulin level (Verma et al., 2005). However, Nagira et al. (2006) found that 17β-estradiol inhibited the insulin level, and increased glucose level. On the other hand, a number of studies suggested that estrogens have a profound modulating effect on systemic glucose homeostasis (Barros et al., 2009; Foryst-Ludwig and Kintscher., 2010).

In the current study, glucose levels as a result of EB injection in Group A were ranged from significant ($P<0.05$) increase in the 80 µg estradiol treated sub-group to significant ($P<0.05$) decrease in the 40 and 120 µg EB treated sub-group. This finding was supported by several researchers; Nagira et al. (2006) found that adipocytes increase the glucose level due to inhibition of insulin by the 17β-estradiol, while in rat 17β-estradiol lowered glucose level by by raising insulin level (Verma et al., 2005).

The t-protein levels were found to be significantly ($P<0.05$) increased in 40 µg estradiol treated sub-group of Group A, but slightly as similar result was reported by Indu (2009). And, the increasing of t-protein was disagreed with the findings of Stevenson et al. (2005) and Elnagar and Abd-Elhady (2009) who found decreased level of t-protein. On the other hand, insignificantly ($P>0.05$) change in t-protein was shown in 80 µg, 120 µg estradiol treated sub-groups in Group A; these results were disagreed with the studies of Stevenson et al. (2005), Elnagar and Abd-Elhady (2009) and Indu (2009).

The albumin concentration in this study was not significantly changed ($P>0.05$) in 40 µg, 80 µg and 120 µg estradiol treated sub-groups of Group A; this result differed from the report of Elnagar and Abd-Elhady (2009). This variation could be attributed to the doses of EB which were used in this study might had not any effect on albumin because the impact of exogenous estrogens on the liver (which synthesis the albumin) is dependent on the route of administration and the type and dose of estrogen.

The influence of EB on calcium in the present study was shown as insignificant (P>0.05), as shown in Group A. A similar study done with fish by Tsai and Wang (2000) showed that the increase in serum calcium levels were dose-dependent in both gonadectomized male and female fishes. Based on the above studies, the effect of EB on serum calcium minght be depended of dose of EB, and this doses were used in this study might not quite enough to change in calcium concentration. The hyperplasia of prostate tissue as a result of EB injection in this study was agreed with other similar studies (Risbridger et al., 2001; Prins et al., 2001; Prins and Korach, 2008). In the presence of Prolactin inhibitor Bromocriptine Mesilate, the injection of EB induces mild hyperplasia (**Figure 2**).

Table 1: The effect of estradiol benzoate in concentration of glucose, t-protein, albumin, and calcium of male rabbits in Group A:

Parameters	**Estradiol doses**			
	Control	**40 µg Estradiol**	**80µg Estradiol**	**120 µg Estradiol**
Glucose mg/dL	88.50± 3.11	75.50± 9.11*	96.75± 1.59*	37.25±1.03*
Total protein g/L	06.30± 1.05	06.53± 0.15*	05.76± 0.56	06.13±0.34
Albumin g/L	04.53± 0.29	4.40± 0.08	04.28±0.36	04.40± 0.04
Calcium mg/dL	14.33± 0.68	16.48± 0.96	14.40± 1.07	14.38± 0.62

Means with superscript stars within the row were significantly different (P<0.05).

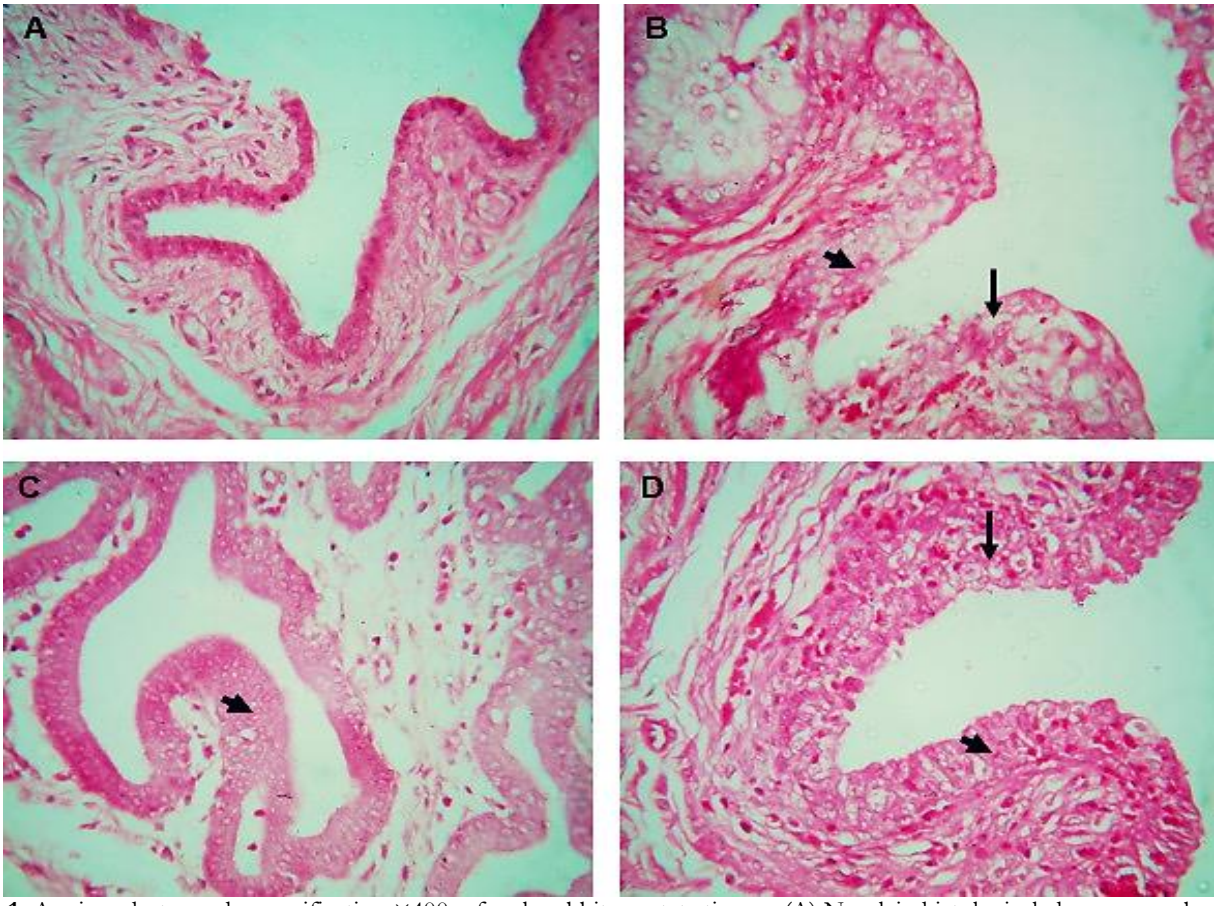

Figure 1. A microphotograph magnification ×400 of male rabbit prostate tissues, (A) No plain histological change was observed in the prostate tissues of the control . (B-D) 40, 80 and 120 µg estradiol benzoate treated shown the presence of hyperplastic (short arrows) and dysplastic (long arrows) lesions.

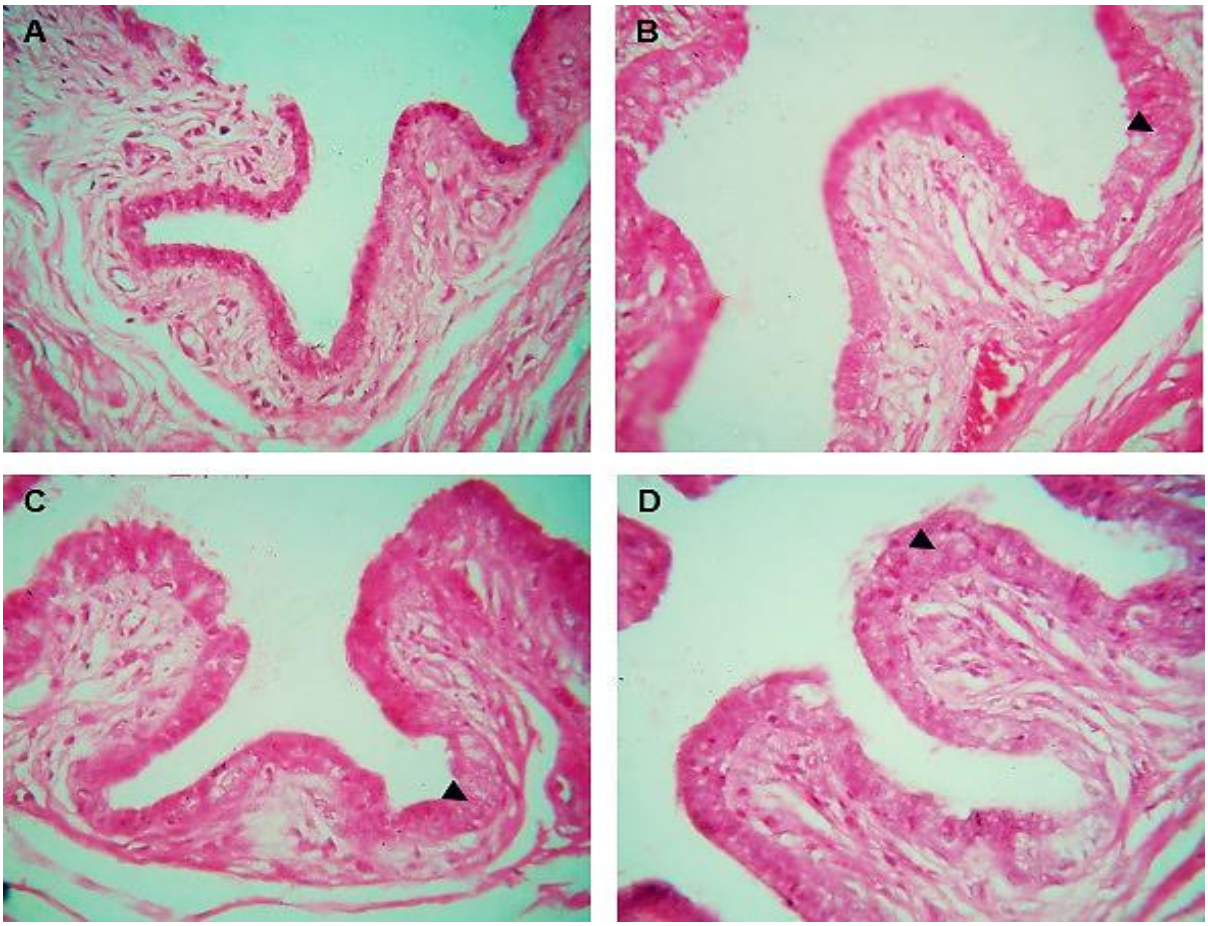

Figure 2. A microphotograph magnification ×400 showed the effect of Estradiol benzoate in present of prolactin inhibitor (Bromocreptein mysaylate) on the prostate histology of male rabbit's (A) Representative prostate section of control group showed no histological changes. (B-D) 40, 80 and 120µg estradiol benzoate treatment sub-groups showing the presence of mild hyperplasia (arrow heads) in epithelia.

This result may indicate to the direct effect of estrogen in prostate as a result of elicitation of external hormone (Harkonen and Makela, 2004), and this elicit hormone could be Prolactin influencing pituitary lactotrophs (Van Coppenolle et al., 2000, 2001, 2004; Khurana et al, 2000). Prolactin has been shown to be involved in the differentiation and proliferation of numerous tissues, including the prostate gland (Crepin et al., 2007). And estrogen appears to be induced hyperprolactinemia as an important mediator of estrogen action in prostate carcinogenesis (Tam et al., 2010).

CONCLUSION

Injection of EB dosed at 40 µg/heat though intramuscular route to male rabbits induces significant increase in t-protein while induce significant decrease in glucose, and dosed at 80 µg/head through intramuscular route induces significant increase in glucose level, while dosed at 120 µg/head induces significant decrease in glucose level. Injection of EB is induced hyperplastic and dysplastic lesion into prostate tissue but in concomitant with Prolactin inhibitor (Bromocreptein mysaylate) induces mild hyperplasia, and this indicate that the prolactin may play a role as mediator in Estrogen action.

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CONFLICT OF INTERESTS

The authors declare that they have no conflict of interest.

AUTHORS' CONTRIBUTION

TE, SHE and SAEO conceived the study and the design. TE and AA carried out the field works. TE analysed the data, and drafted the manuscript. All the authors were

involved in revising the manuscript and approved the final version.

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