THE EFFECTS OF ESTRADIOL BENZOATE INJECTION TO INTACT AND CASTRATED MALE RABBITS ON GLUCOSE, TOTAL PROTEIN, ALBUMIN, CALCIUM, UREA AND CREATININE CONCENTRATIONS

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ABSTRACT

This study was conducted to estimate the effects of estradiol benzoate injection to intact and castrated male rabbits on glucose, total protein, albumin, calcium, urea and creatinine concentrations. A total of Seventy two adult male rabbits were used in this study. The rabbits were randomly divided in to two groups (Thirty sex per group). One group was used as intact group, the other group of rabbits was subjected to bilateral orchiectomy, and used as castrated group. Each group was further divided randomly to four sub-group(six for each sub-group).Three sub-groups of intact and castrated rabbits were treated once each alternative day with the intramuscular injections of estradiol benzoate (Estradol® Animal health care Australia) at a doses of (40, 80 and 120 μgm/ rabbit), respectively for thirty days, whereas the fourth sub-group of each receive placebo and act as control group. After the end of experiment period 30 days, a blood samples were collected, and the serum samples were being harvested for analysis of glucose, total protein, albumin, calcium, urea and creatinine concentrations. The study found that glucose concentration was significantly (P<0.05) increase and decrease in intact male rabbits, significantly (P<0.05) increase in castrated male rabbits, total protein significantly (P<0.05) increase or not changed in intact male rabbits, and significantly (P<0.05) decrease in castrated male rabbits. Albumin and calcium were not change (P>0.05) in intact and castrated male rabbits. Urea was not changed in intact male rabbits and in castrated male rabbits not changed or significantly (P>0.05) increased. Creatinine was not changed or significantly (P>0.05) decrease in intact and castrated male rabbits. It conclude that estradiol benzoate may induced increase or decrease in glucose and total protein, increase in urea or no change, decrease in creatinine or no change, while albumin and calcium were not affected.

INTRODUCTION

Estrogens are group of steroid hormones include estrone, estradiol, and estriol which animal body is produce them naturally (Deborah Moskowitz, 2006). Although estrogen was a female hormone, but it had biological role in the male reproduction (Todioda, 2010). Exogenous estrogens have been used in food animalsto increase the secretory characteristics of growth hormone (Misztal et al., 2007 and Colak et al., 2011) for weight gain purposes. Xenosterogens have been defined by The United States Environmental Protection Agency as “an exogenous agent” that interferes with synthesis, secretion, transport, metabolism, binding action or elimination of natural blood borne hormones that are present in the body and which are responsible for homeostasis, reproduction and developmental process (Evanthia et al., 2009). However, there was differences between the natural and synthetic steroids in their effects on body due to difference in affinity for binding to globulin, biological activity in plasma, metabolism, and half-life (Anna-Maria and Niels, 1999). The present objective of this study was to investigate the changes on glucose, total protein, albumin, calcium, urea and creatinine concentrations of intact and castrated males rabbits due to exposure to different doses of estradiol benzoate via intramuscular injection route each alternative day for 30 days period.

MATERIALS AND METHODS

Animals: Seventy two adult male rabbits weighing 2.1±0.1 kg housed under individual cage 50 x 50 cm were used in this study. They had free access to standard rabbit food and tap water.
**Table 1.** The effect of estradiol benzoate on glucose, T-protein, albumin, calcium, urea and creatinine concentrations of intact male rabbits

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>40 μg Estradiol</th>
<th>80 μg Estradiol</th>
<th>120 μg Estradiol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose mg/dL</td>
<td>88.5±3.11</td>
<td>143.5±21.83*</td>
<td>155.5±03.87*</td>
<td>143.5±25.32*</td>
</tr>
<tr>
<td>T-protein g/L</td>
<td>06.3±1.05</td>
<td>05.89±0.13*</td>
<td>05.58±0.17*</td>
<td>05.73±0.34*</td>
</tr>
<tr>
<td>Albumin g/L</td>
<td>04.5±0.29</td>
<td>04.28±0.13</td>
<td>04.08±0.17</td>
<td>04.35±0.21</td>
</tr>
<tr>
<td>Calcium mg/dL</td>
<td>14.3±0.68</td>
<td>14.40±0.24</td>
<td>13.85±0.19</td>
<td>13.90±0.64</td>
</tr>
<tr>
<td>Urea mg/dL</td>
<td>60.00±1.17</td>
<td>70.75±1.58</td>
<td>56.75±7.93</td>
<td>59.50±1.24</td>
</tr>
<tr>
<td>Creatinine mg/dL</td>
<td>01.43±0.51</td>
<td>01.13±0.43</td>
<td>01.05±0.30</td>
<td>01.18±0.15*</td>
</tr>
</tbody>
</table>

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

**Table 2.** The effect of estradiol benzoate on glucose, total protein, albumin, calcium, urea and creatinine concentrations of castrated male rabbits

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>40 μg Estrogen</th>
<th>80 μg Estrogen</th>
<th>120 μg Estrogen</th>
</tr>
</thead>
<tbody>
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<td>Glucose mg/dL</td>
<td>88.5±03.11</td>
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<td>05.73±0.34*</td>
</tr>
<tr>
<td>Albumin g/L</td>
<td>04.5±0.29</td>
<td>04.28±0.13</td>
<td>04.08±0.17</td>
<td>04.35±0.21</td>
</tr>
<tr>
<td>Calcium mg/dL</td>
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</tr>
</tbody>
</table>

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

**Experiment Design:** The rabbits were randomly divided in to two groups (Thirty sex per group). One group was used as intact group, the other group of rabbits was subjected to bilateral orchiectomy, and used as castrated group. Each group was further divided randomly to four sub-group (six for each sub-group). Three sub-groups of intact and castrated rabbits were treated once each alternative day with the intramuscular injections of estradiol benzoate (Estradal ® Animal health care Australia) at a doses of (40, 80 and 120 μg/ rabbit), respectively for thirty days, whereas the fourth sub-group of each receive placebo and act as control subgroup.

**Sample collection:** After the end of experiment period 30 days, a five ml of blood samples were collected into plan plastic containers using disposable syringe from the heart, and the serum samples were being harvested into Eppendorf tubes, deep-frozen for later analysis of glucose, total protein, albumin, calcium, urea and creatinine concentrations.

**Analysis of serum samples**

Automate chemistry analyzer (MINDARY) and reagent Biosystem® Spain made were used for analysis of glucose, total protein, albumin, calcium, urea and creatinine concentrations in to serum samples.

**Statistical analysis**

The obtained data were analyzed by independent T-test using SPSS statistical program, version 20 for Windows (IBM SPSS Statistics 20 IL, USA), the results were expressed in form of mean ± standard deviation. The difference between the means in this study were considered statistically significant when the P value was less than 0.05.

**RESULTS**

The effects of estradiol Benzoate on glucose, total protein, albumin, calcium urea and creatinine concentrations of intact male rabbits are show in table (1). There is a significant (P<0.05) increase in total protein in the 40 μg estradiol treated sub-group and significant (P<0.05) increase in glucose in the 80 μg estradiol treated sub-group. On the other hand there was significant (P<0.05) decrease in glucose concentration in the 40 μg estradiol treated sub-group, glucose and Creatinine in the 120 μg estradiol treated sub-group. However there was no significant difference (P>0.05) between 40 μg,80 μg and 120 μg estradiol treated and control sub-groups in albumin, calcium urea. Urea, total protein between 80 μg, 120 μg estradiol treated and controlsub-groups, Creatinine between 40 μg, 80 μg estradiol treated and controlsub-groups. The effects of different estradiol benzoate doses on serum Concentration of Glucose, total protein, albumin, calcium, urea, and creatinine of castrated male rabbit were presented in table (2). There was significant (P<0.05) increase of glucose in the 40 μg, 80μg and 120 μg estradiol treated sub-group and significant (P<0.05) increase of urea concentration in the 80 μg and 120 μg estradiol treated sub-group. On the other hand there was significant (P<0.05) decrease of total-protein and globulin concentrations in the 40 μg, 80 μg, and 120 μg estradiol treated sub-groups, and creatinine in the 40 μg and120 μg estradiol treated sub-groups. However there is no significant difference (P>0.05) between 40 μg,80 μg and 120 μg estradiol treated and control sub-groups in albumin, calcium between 40 μg, 80 μg, 120 μg estradiol treated and control sub-groups, urea between 40 μg estradiol treated and control sub-groups , creatinine between 80 μg estradiol treated control sub-groups.

**DISCUSSION**

A number of studies have suggested that estrogens have a profound modulating effect on systemic glucose homeostasis (Barros et al., 2009, and Foryst-Ludwig and Kintscher, 2010). In the present study the result of glucose response to injection of estradiol benzoate to intact male rabbits were ranged from significant (P<0.05) increase in glucose concentration in the 80 μg estradiol treated sub- groups to significant (P<0.05) decrease in the 40 and 120 μg estradiol treated sub-groups, and significant (P<0.05) increase of glucose in the 40 μg, 80μg and
120 μg estradiol treated sub groups of castrated rabbits. This variation in results were documented by several studies; (Nagira et al., 2006) found that in adipocytes increase in glucose level due to inhibition of insulin level by the 17β-estradiol, while in rat (Verma et al., 2005) showed that 17β-estradiol lowered glucose because of raising insulin level. However, in male rabbits plasma glucose level was not change due to injection of estradiol valerate (Nematbakhsh et al., 2001). Total-protein in the current study was found to be significantly (P<0.05) increase in 40 μg estradiol treated sub-group of intact rabbits but slightly as similar result of (Indu, 2009). While the significantly (P<0.05) decrease in the 40 μg, 80 μg, and 120 μg estradiol treated sub-groups of castrated rabbits was agree with the similar that obtained by (Woo et al., 1993;Stevenson et al., 2005 and Elnagar and Abd-Elhady, 2009) which they found decrease in total protein. On the other hand no significantly (P>0.05) changed of total protein was found in 80 μg, 120 μg estradiol treated sub-groups of intact rabbits were disagree with above studies.

The albumin synthesis is occurs in the liver (Reece, 2005). And the impact of exogenous estrogens on the liver is dependent on the route of administration and the type and dose of estrogen (Schoultz et al., 1989). The result of albumin in this study was not significantly changed (P>0.05) in 40 μg, 80 μg and 120 μg estradiol treated sub-groups of intact and castrated male rabbits, as (Schoultz et al., 1989) noted that parenteral administration of native estradiol has very little influence on these aspects of liver function, the result of albumin in this study was disagree with that obtained by (Elnagar and Abd-Elhady, 2009). The response of calcium in 40 μg, 80 μg and 120 μg estradiol treated sub-groups of intact and castrated rabbits in present study were not significantly (P>0.05) different in compared to control sub-group . this result was disagree with the similar study done in fish by (Ching-Lin and Li-Hsueh, 2000) which they found estradiol at 50 or 100 mg/kg diet significantly increased serum calcium levels dose-dependently in both gonadectomized males and females. So according to above study the effect of estradiol on serum calcium may be depend of dose of hormone. The Insignificant (P>0.05) decreased result in urea of intact male rabbits in the 40 μg and 80 μg sub-groups were agree with that similar of (Islam, 2013), but the result of 80 μg and 120 μg estradiol treated sub-groups of castrated male rabbits were disagree. The results of significant (P<0.05) decrease in Creatinine of the 120 μg Estradiol treated sub-group of intact rabbits, and in the 40 μg, 120 μg estradiol treated sub-groups of castrated rabbitswere disagree with the study in male mice by (Islam, 2013), which found no significant changed observed in Creatinine.

Conclusion
This study was concluded that the injection of estradiol benzoate at dose of 40 μg/rabbits IM to intact male rabbits induce significant increase in totalprotein, significant decrease in glucose. While in castrated male rabbits induce significant increase in glucose, significant decrease in total-protein, and creatinine. At the dose of 80 μg/rabbits IM induce significant increase of glucose in intact and castrated male rabbits , significant increase in urea and significant decrease in total-protein in castrated male rabbits. At the dose of 120 μg/rabbits IM induce significant decrease in glucose, and creatinine in intact male rabbits. While in castrated male rabbits induce significant increase in glucose and urea, significant decrease in totalprotein and creatinine .while albumin and calcium were not changed in both groups intact and castrated.

Conflict of interest
The authors declare that they have no conflict of interest.

Acknowledgments
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REFERENCES


