



ISSN: 0976-3376

Available Online at <http://www.journalajst.com>

ASIAN JOURNAL OF  
SCIENCE AND TECHNOLOGY

Asian Journal of Science and Technology  
Vol. 08, Issue, 01, pp.4161-4164, January, 2017

## RESEARCH ARTICLE

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

1,\***TamourElkhier**, 2**Shams-Eldein Hassaballa** and 2**Shadia Abdo-Elatti Omer**

<sup>1</sup>Department of Physiology and Biochemistry, Faculty of Veterinary Science, University of Nyala, Nyala- Sudan

<sup>2</sup>Department of Basic Science, Collage of Veterinary Medicine and Animal Production, Sudan University of Science and Technology, Khartoum North, Kuku- Sudan

#### ARTICLE INFO

##### Article History:

Received 07<sup>th</sup> October, 2016

Received in revised form

20<sup>th</sup> November, 2016

Accepted 24<sup>th</sup> December, 2016

Published online 31<sup>st</sup> January, 2017

##### Key words:

Estradiol Benzoate,  
Intact,  
Male rabbits,  
Calcium,  
Urea

#### 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 six per group) .One group was used as intact group, the other group of rabbits was subjected to bilateral orchietomy, 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.

Copyright©2017, TamourElkhier et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

#### 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 (Todiada, 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. Xenoestrogens 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  $2.1\pm 0.1$  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.

\*Corresponding author: TamourElkhier,

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

**Table 1. the effect of estradiol benzoate on glucose, T-protein, albumin, calcium, urea and creatinine concentrations of intact male rabbits**

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
Urea mg/dL	60.00± 1.17	70.75± 1.58	56.75± 7.93	79.50± 1.24
Creatinine mg/dL	01.43± 0.51	01.13± 0.43	01.05± 0.30	01.18± 0.15*

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**

	Estradiol doses			
	Control	40 µ Estrogen	80µg Estrogen	120 µg Estrogen
Glucose mg/dL	88.50±03.11	143.50± 21.83*	155.50± 03.87*	143.50± 25.32*
T- protein g/L	06.30± 1.05	05.89± 0.13*	05.58± 0.17*	05.73±0.34 *
Albumin g/L	04.53± 0.29	04.28± 0.13	04.08±0.17	04.35± 0.21
Calcium mg/dL	14.33± 0.68	14.40± 0.24	13.85± 0.19	13.90± 0.64
Urea mg/dL	60.00± 1.17	60.00± 5.29	75.75± 4.35*	72.00± 5.24*
Creatinine mg/dL	01.43± 0.51	01.23± 0.10*	00.90± 0.37	00.98± 0.17*

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 six per group) .One group was used as intact group, the other group of rabbits was subjected to bilateral orchietomy, 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 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 , 120µg estradiol treated and control sub-groups in albumin, calcium urea. Urea, total protein between 80 µg, 120 µg estradiol treated and controls sub-groups, Creatinine between 40 µg, 80 µg estradiol treated and controls sub-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 and 120 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 found that 17β-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 rabbits were 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.

## Conflic of interest

The authors declare that they have no conflict of interest.

## Acknowledgments

We thankfully acknowledge the authority of the University of Nyala and DAAD for their supporting this study.

## REFERENCES

- Anna-Maria, A.M. and Niels, N.E .1999. Exposure to exogenous estrogens in food: possible impact on human development and health. *Eur J Endocrinol.* 140:477–485.
- Barros, R.P. Gabbi, C.,Morani, A. Warner, M.Gustafsson, J.A .2009. Participation of ER alpha and ER beta in glucose homeostasis in skeletal muscle and white adipose tissue. *Am J PhysiolEndocrinolMetab.* 297: 124-133.
- Ching-Lin, T. and Li-Hsueh, W. 2000. Sex differences in the responses of serum calcium concentrations to temperature and estrogen in Tilapia, *Oreochromismossambicus*. *Zool. Stud.* 39: 55-60 .
- Colak, M. Shimizu ,T. Matsunaga, N. Murayama ,C. Nagashima, S. Kataoka, M. Kawashima, C. Matsui ,M. van Dorland, H.A. Bruckmaier, R.M. Miyamoto, A.2011. Oestradiol enhances plasma growth hormone and insulin-like growth factor-I concentrations and increased the expression of their receptors mRNAs in the liver of ovariectomized cows. *ReprodDomest Anim.* 46: 854-861.
- Deborah Moskowitz, ND .2006. A Comprehensive Review of the Safety and Efficacy of Bioidentical Hormones for the Management of Menopause and Related Health Risks. *Altern Med Rev.* 11:208-223.
- Elnagar, S.A . and Abd-Elhady, A.M .2009. Exogenous Estradiol: Productive and Reproductive Performance and Physiological Profile of Japanese Quail Hens. *Intern. J. Poultry Scie.* 8 : 634-641.
- Evanthia, D.K. Jean-Pierre, B. Linda, C.G. Russ, H. Gail, S. Prins, A.M. Soto ,R. Thomas, Z. and Andrea, C.G .2009. Endocrine disrupting chemicals: An endocrine society scientific statement. *Endocr Rev.* 30:293-342.
- Foryst-Ludwig, A. and Kintscher. U .2010. Metabolic impact of estrogen signalling through ER alpha and beta. *J Steroid BiochemMol Biol.* 122: 74-81.
- Indu, K .2009. Text book of medical physiology., Reprint 1st Ed, Elsevier publications , p859-860.
- Islam, M.D.S. 2013. Effects of butter and estrogen on blood biochemistry and some selected organs in reference to the development of obesity in mice. Bangladesh Agricultural University, The Digital Repository of the Library materials (Thesis).
- Misztal, T. Wańkowska, M. Górski ,K. Romanowicz, K.2007. Central estrogen-like effect of genistein on growth hormone secretion in the Ewe. *ActaNeurobiolExp (Wars)* 67:411-419.
- Nagira,k.Sasoaka,T. Wada , T. Fukui, K.Ikubo, M. Hori, S.Tsuneki,H.Satio, S. kobavashi, M. 2006. Altered sub

- cellular distribution of estrogen receptor alpha is amplified in estradiol –induced regulation of insulin signaling in 3T3- 11 adipocytes. *Endocrinol.* 147:1020-1028.
- Nematbakhsh, M. Samarian , H.Sarafzadegan, N.Sharifi, M.R.Ghorvi, M.Amini, M.Mohammadifard, N. Alikhalesi, H. and Soltani, N. 2001. The effect of estrogen on plasma insulin and glucose level, and cholesterol- HDL and LDL- HDL ratio in normal and cholesterol- red male rabbits. *Pajouhesh Dar Pezeshki.* 25:17-24.
- Reece, W.O .2005. Dukes, physiology of domestic animals. 12th ed. Panima publishing Co. New Delhi/Bangalore. P47-48.
- Schoultz, B.V.Carlstorm, K. Collste, L. Eriksson, A.Henriksson, P. Pousette, A. and Stege, R .1989. Estrogen therapy and liver function—metabolic effects of oral and parenteral administration. *The Prostate* 14: 389-395.
- Todioli, M. 2010. The effects of exogenous estrogens on estrogen receptors in male reproductive organs. *Interdiscip J. Health. Sci* 1:73-82.
- Verma, S .Srivastava, R.K.Sood, S . Sharma, S. 2005.Effect of estrogen on hypoglycemia- induce neurological impairment in ovariectomized rats. *Methods Find Exp Clin Pharmacol.* 27:405 -409.

\*\*\*\*\*