Research Article

Histopathological effects of galanin on reproductive hormones and reproductive organs of rabbit (*Oryctolagus cuniculus*)

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Citation

Suleman Hussain Shah, Razia Iqbal, Mubashar Hussain, Muhammad Faheem Malik, Bilal Mukhtar and Muhammad Saqib Sajid. Histopathological effects of galanin on reproductive hormones and reproductive organs of rabbit (*Oryctolagus cuniculus*). Pure and Applied Biology. Vol. 8, Issue 2, pp1523-1534.
<http://dx.doi.org/10.19045/bspab.2019.80093>

Received: 31/01/2019

Revised: 03/05/2019

Accepted: 16/05/2019

Online First: 18/05/2019

Abstract

Effects of galanin, on reproductive hormones and histopathology of reproductive organs of rabbit was assessed by exposing adult rabbits to low, medium and high doses of 0.2, 0.4 and 0.8 µg/ml per kg body weight, respectively. Adult rabbits were reared and managed separately in cages (2 x 2 x 2 m³) under controlled conditions of photoperiod (12 light: 12 dark) whereas temperature was maintained at 23 ± 2 °C. The measured quantities of Alfalfa fodder plant as a sole food was provided to all groups simultaneously with free access to water. The increase in the weight of testes and ovaries was recorded in males and females, respectively. The histopathological examination of gonads revealed no adverse effects, however, the significant differences in the levels of testosterone (4.21ng/dl, 4.33ng/dl, 4.57 ng/dl), estrogen (123.33 pg/ml, 135.33 pg/ml, 151.33 pg/ml) and progesterone (196.33 ng/ml, 217.00 ng/ml, 229.33 ng/ml) were recorded in the low, medium and high dose treated groups, respectively. The increase in the aggressive sexual behavior was noticed which correlated with the increase in the concentration of reproductive hormones. Galanin had no negative effects on the testes and the ovaries of rabbits but induced the production of reproductive hormones in addition to the increase in the weight of gonads.

Keywords: ELISA; Estrogen; Gonads; Testosterone

Introduction

Galanin (GAL) is a neuropeptide comprising of 29-30 amino acids found enormously throughout the endocrine system as well as in nervous system especially in the hypothalamus [1-6]. In order to perform various functions, galanin interacts with three kinds of G-protein coupled receptors viz. galanin receptor 1 (GalR1), galanin receptor 2 (GalR2) and galanin receptor 3 (GalR3) [7-

9]. GalR1 and GalR3 play its role through inhibition of Cyclic adenosine monophosphate/ protein kinase A (cAMP/PKA pathway, whereas GalR2 activates phospholipase-C [10].

Galanin primarily serves as a neurotransmitter for the transmission of impulses in the central as well as peripheral nervous system [11-13]. Recent studies has demonstrated that galanin (GAL) is involved

in certain other processes like reproduction, gonadotropin-releasing hormone (GnRH), luteinizing hormone (LH), LH-releasing hormone (LHRH) [14], uterine contraction regulation [15], regulation of estrogen [16], growth hormones [17], ingestion [18], enhanced prolactin secretion, growth of pituitary gland [11], inhibition of insulin secretion [9], vasopressin regulation, osmoregulation [19], muscle contraction, bowel movement [20], development, differentiation [21], maintenance of fat ingestion [22], skin, sweat glands [23], sensory reception [24], nociception, homeostasis of energy [25], appetite, seizure regulation [26], mood balance [8], inflammation, metabolism, acquired immunity, formation of bone and galial cell [10].

Galanin is involved in numerous pathological conditions like acute pancreatic necrosis [27], treatment of cancer [28], obesity [25], smoking [29], atrial pressure regulation [30], increased alcohol utilization [31], treatment of Alzheimer's disease, loss of memory, learning disabilities [32], epilepsy [33], emotions [34], anxiety [35], stress and depression [36]. Although galanin performs various functions, however, the objective of this work was to evaluate its effect on reproductive hormones and histopathology of reproductive organs of rabbit.

Materials and methods

Experimental animal

The male and female rabbits (*Oryctolagus cuniculus*) weighing approximately 1.0 kg to 1.5 kg were obtained from the Veterinary Research Institute, Lahore, Punjab, Pakistan. The rabbits were kept under photoperiod (12 hrs:12 hrs) and temperature 23 ± 2 °C with free access to corresponding water and diet [37].

Chemical and dose preparation

Galanin (powder form) was purchased from Merck Sigma Aldrich, U.K. A measured quantity of galanin 1000 µg was dissolved in

1000 ml water to prepare 1µg/1ml dose. Low, medium and high doses were prepared through serial dilution method. All groups were treated with different doses of galanin T₁ (0.2), T₂ (0.4) and T₃ (0.8) µg/ml per kg body weight. The dose was administered daily for a month at an interval of 24 h. Galanin doses were administered through jugular vein [38].

ELISA and histopathology

Blood samples were drawn from each group to perform ELISA after two hours of dose administration [39]. Blood sample collection was carried out by standard instructions [40]. In addition, six rabbits from each group were slaughtered on 10th, 20th and 30th day of the 1st dose administration followed by microtomy for histopathological examination of gonads. The blood samples were collected and serum was taken from red blood cells within 6 hours of sampling in gelatin serum tubes and preserved [41]. Blood samples were collected in gel tubes, centrifuged at 1600Xg for about 15 min at 4 °C and the plasma collected for further use and stored at 70 °C.

Weight of testes and ovaries

The testes and ovaries were properly removed from the slaughtered rabbits, their weight was recorded with the help of electronic balance and histological examination of the sex organs was carried out [42].

Tissue processing

The tissue processing and staining was performed [42, 43] and to avoid the decomposition of tissues, they were put in Bouin's fixative for about 7-8 hours.

Sectioning block

The standard method was used for the sectioning of tissue and slide preparation [44]. Block cutting was done by using the microtome [45, 46].

Experimental design and data analysis

The study was laid down in Completely Randomized Design (CRD) with three

replications. Adult breeding rabbits were divided into four groups, each group containing 18 rabbits with equal gender ratio. Data were subjected to statistical analysis and ANOVA was performed by using SPSS 21.

Results

Effect of galanin on testes weight

The weight of testes of male rabbits increased after the exposure to galanin. The data analysis indicated that the mean values for the treated group i.e. T₁ (1.01 g, 1.06 g, 1.12 g), T₂ (1.13 g, 1.17 g, 1.22 g) and T₃ (1.25 g, 1.32 g, 1.39 g) were significantly higher as compared to untreated group T₀ (0.74 g, 0.84 g, 0.95 g). Moreover, testes weight of male rabbits was high in treated group but regularly increased with respect to days and on 30th day it was significantly higher as compared to 10th day (Table 1).

Effect of galanin on ovaries weight

The weight of ovaries of female rabbits increased after the exposure to galanin. The statistical analysis revealed that mean values T₁ (0.31 g, 0.34 g, 0.39 g), T₂ (0.42 g, 0.48 g, 0.55 g) and T₃ (0.53 g, 0.61 g, 0.66 g) were significantly higher as compared to untreated group (0.20 g, 0.25 g, 0.28 g) at 10th, 20th and 30th days of experiment for low, medium and high doses, respectively. Moreover, maximum weight of ovaries of female rabbits increased in the high dose group at 30th day (Table 2).

Effect of galanin on testosterone

The level of testosterone was elevated significantly. In addition, statistical analysis displayed that the mean values for each of the treated group i.e. T₁ (3.43 ng/dl, 3.55 ng/dl, 3.64 ng/dl), T₂ (3.79 ng/dl, 3.93 ng/dl, 4.01 ng/dl) and T₃ (4.21 ng/dl, 4.33 ng/dl, 4.57 ng/dl) were comparatively higher than the control group (1.70 ng/dl, 1.92 ng/dl, 2.15 ng/dl). Moreover, testosterone hormone level was significantly higher in the treated group and regularly increased with respect to days (Table 3).

Furthermore, the results of current study were in confirmation with other researchers who suggested that galanin modulated sex hormones like testosterone and LH by stimulation of pituitary gland of rat [47]. Galanin significantly enhanced testosterone abundantly in rats [48]. Galanin increased the plasma level of testosterone in sannan goats [38].

Effect of galanin on estrogen

The levels of estrogen in female rabbit have risen significantly in dose response. The statistical data analysis depicted that mean values for each of the treated groups (low, medium and high dose) i.e. T₁ (88.66 pg/ml, 97.00 pg/ml, 114.33 pg/ml), T₂ (103.67 pg/ml, 117.66 pg/ml, 136.33 pg/ml) and T₃ (123.33 pg/ml, 135.33 pg/ml, 151.33 pg/ml) were comparatively higher than those of control group (36.00 pg/ml, 40.67 pg/ml, 52.67 pg/ml). Estrogen hormone levels were highest in the high dose group at 30th day (Table 4).

Our results were compatible with earliest studies involving intravenous administration of galanin in human beings in order to determine its effects on pituitary hormone (estrogen and growth hormone) secretion [49]. In 1987, it was revealed that galanin is a strong neuromodulator of LH and estrogen secretion [50]. Several studies revealed that galanin was a remarkable neuromodulator of reproductive hormones like LH and FSH secretion through GnRH-stimulation as well as the release of prolactin and oxytocin in mammals including human being, sheep, rodent like rat and mice. Thus, it might be a potent modulator of reproduction [51]. In 1998, an experiment on rats revealed that galanin regulated the plasma level of LH and estrogen. These findings suggest that galanin is a strong neuromodulator of LH and estrogen secretion in female rats [52].

Effect of galanin on progesterone

In addition, statistical data analysis reveals that the mean values for each of the treated

group i.e. T₁ (125.67 ng/ml, 148.33 ng/ml, 167.00 ng/ml), T₂ (161.00 ng/ml, 179.67 ng/ml, 195.67 ng/ml) and T₃ (196.33 ng/ml, 217.00 ng/ml, 229.33 ng/ml) are comparatively higher than those of control group (55.67 ng/ml, 71.00 ng/ml, 96.67 ng/ml). Moreover, progesterone hormone level is enormously highest in high dose group at 30th day (Table 5).

Our results were compatible with earliest studies including intravenous administration of galanin in rat in order to increase the level of LH by increasing the concentration of progesterone [50]. These findings suggest

that galanin plays a vital role in the modulation of LH and progesterone secretion in female rats [52].

Histopathology results

At 10th, 20th and 30th day, histological examination showed no difference in testes and ovaries both in galanin treated (low, medium and high) and untreated groups (control). The histopathological studies shown the normal tissue like sertoli cells were appeared in testes as well as oocytes and follicles were also present in ovaries (Figure 1-6).

Table 1. Effect of Galanin on Testes Weight (g) of Male Rabbits at 10th, 20th, and 30th Days (Mean ± S.E.M)

Duration (in days)	Treatment			
	T ₀ (Control)	T ₁ (0.2µl)	T ₂ (0.4µl)	T ₃ (0.8µl)
10	0.74 ± 0.018 ^a	1.01 ± 0.020 ^b	1.13 ± 0.029 ^c	1.25 ± 0.027 ^d
20	0.84 ± 0.018 ^a	1.06 ± 0.017 ^b	1.17 ± 0.029 ^c	1.32 ± 0.015 ^d
30	0.95 ± 0.015 ^a	1.12 ± 0.011 ^b	1.22 ± 0.026 ^c	1.39 ± 0.018 ^d

Means with different superscript in row are significantly different from one another (p≤0.05) Tukey's Test

Table 2. Effect of Galanin on Ovaries Weight (g) of Female Rabbits at 10th, 20th, and 30th Days (Mean ± S.E.M)

Duration (in days)	Treatment			
	T ₀ (Control)	T ₁ (0.2µl)	T ₂ (0.4µl)	T ₃ (0.8µl)
10	0.20 ± 0.01 ^a	0.31 ± 0.026 ^b	0.42 ± 0.023 ^c	0.53 ± 0.020 ^d
20	0.25 ± 0.023 ^a	0.34 ± 0.026 ^b	0.48 ± 0.021 ^c	0.61 ± 0.015 ^d
30	0.28 ± 0.026 ^a	0.39 ± 0.033 ^b	0.55 ± 0.026 ^c	0.66 ± 0.026 ^d

Means with different superscript in row are significantly different from one another (p≤0.05) Tukey's Test

Table 3. Effect of Galanin on Testosterone Level (ng/dl) Of Male Rabbits at 10th, 20th, and 30th Days (Mean ± S.E.M)

Duration (in days)	Treatment			
	T ₀ (Control)	T ₁ (0.2µl)	T ₂ (0.4µl)	T ₃ (0.8µl)
10	1.70 ± 0.052 ^a	3.43 ± 0.046 ^b	3.79 ± 0.083 ^c	4.21 ± 0.067 ^d
20	1.92 ± 0.032 ^a	3.55 ± 0.057 ^b	3.93 ± 0.087 ^c	4.33 ± 0.046 ^d
30	2.15 ± 0.035 ^a	3.64 ± 0.071 ^b	4.01 ± 0.072 ^c	4.57 ± 0.090 ^d

Means with different superscript in row are significantly different from one another (p≤0.05) Tukey's Test

Table 4. Effect of Galanin on Estrogen Level (pg/ml) of Female Rabbits at 10th, 20th, and 30th Days (Mean $\pm$ S.E.M)

Duration (in days)	Treatment			
	T ₀ (Control)	T ₁ (0.2 μ l)	T ₂ (0.4 μ l)	T ₃ (0.8 μ l)
10	36.00 $\pm$ 2.309 ^a	88.66 $\pm$ 2.028 ^b	103.67 $\pm$ 2.027 ^c	123.33 $\pm$ 2.027 ^d
20	40.67 $\pm$ 2.028 ^a	97.00 $\pm$ 2.309 ^b	117.66 $\pm$ 2.028 ^c	135.33 $\pm$ 2.028 ^d
30	52.67 $\pm$ 2.603 ^a	114.33 $\pm$ 2.0278 ^b	136.33 $\pm$ 1.763 ^c	151.33 $\pm$ 2.028 ^d

Means with different superscript in row are significantly different from one another ($p \leq 0.05$) Tukey's Test

Table 5. Effect of Galanin on Progesterone Level (ng/ml) of Female Rabbits at 10th, 20th, and 30th Days (Mean $\pm$ S.E.M)

Duration (in days)	Treatment			
	T ₀ (Control)	T ₁ (0.2 μ l)	T ₂ (0.4 μ l)	T ₃ (0.8 μ l)
10	55.67 $\pm$ 3.528 ^a	125.67 $\pm$ 4.630 ^b	161.00 $\pm$ 4.618 ^c	196.33 $\pm$ 4.055 ^d
20	71.00 $\pm$ 6.429 ^a	148.33 $\pm$ 4.055 ^b	179.67 $\pm$ 2.9623 ^c	217.00 $\pm$ 3.055 ^d
30	96.67 $\pm$ 4.631 ^a	167.00 $\pm$ 4.619 ^b	195.67 $\pm$ 2.906 ^c	229.33 $\pm$ 2.028 ^d

Means with different superscript in row are significantly different from one another ($p \leq 0.05$) Tukey's Test

Figure 1. Shows Comparative Effects of Galanin on Histological Parameter of Testes between Group T₁ (Low), Group T₂ (Medium), Group T₃ (High) and Group T₀ (Control) at 100X Magnification Power under Light Microscopes at 10th day

Figure 2. Shows Comparative Effects of Galanin on Histological Parameter of Testes between Group T₁ (Low), Group T₂ (Medium), Group T₃ (High) and Group T₀ (Control) at 100X Magnification Power under Light Microscopes at 20th day

Figure 3. Shows Comparative Effects of Galanin on Histological Parameter of Testes between Group T₁ (Low), Group T₂ (Medium), Group T₃ (High) and Group T₀ (Control) at 100X Magnification Power under Light Microscopes at 30th day

Figure 4. Shows Comparative Effects of Galanin on Histological Parameter of Ovaries between Group T₁ (Low), Group T₂ (Medium), Group T₃ (High) and Group T₀ (Control) at 100X Magnification Power under Light Microscopes at 10th day

Figure 5. Shows Comparative Effects of Galanin on Histological Parameter of Ovaries between Group T₁ (Low), Group T₂ (Medium), Group T₃ (High) and Group T₀ (Control) at 100X Magnification Power under Light Microscopes at 20th day

Figure 6. Shows Comparative Effects of Galanin on Histological Parameter of Ovaries between Group T1 (Low), Group T2 (Medium), Group T3 (High) and Group T₀ (Control) at 100X Magnification Power under Light Microscopes at 30th day

Discussion

The findings of the current study suggested that galanin significantly modulated the reproductive hormone secretion i.e. testosterone in adult males and estrogen and progesterone in adult females. In addition, it was examined that testes and ovaries' weight was significantly increased in both male and female rabbits. However, the increase in testes' weight was relatively greater as compared to that of ovaries. Contrary to untreated group, an aggressive sexual behavior was observed in treated groups (male and female). Nevertheless, after dissection on day 10th, 20th, and 30th it was observed that there was no significant alteration or damage present in testes and ovaries of male and female rabbits respectively. On 30th day, sertoli cell was prominent in testes as well as oocyte and follicles in ovaries of rabbits. Subsequently, the histological study of testes and ovaries showed that galanin had no pronounced negative effects on gonadal tissues of rabbits. Several studies revealed that galanin was a remarkable neuromodulator of reproductive hormones like testosterone, estrogen and progesterone secretion through GnRH-stimulation in mammals including human being [14], sheep [12], rodent like rat [47, 53-55] and mice [56], pig [15] and sannan goat

[38]. These findings were compatible with those of prevailing study suggesting that galanin may be a potent modulator of reproduction. However, various studies revealed that the concentration of testosterone [51], estrogen [49] and progesterone [52] was elevated by galanin at high dose as compared to other doses.

Conclusion

Galanin has no adverse effects on reproductive organs but it stimulates the reproductive hormone secretion and induces aggressive sexual behavior in dose dependent manner. These findings suggest that galanin administration in animals of commercial importance may increase their reproductive performance. However, further studies are required by altering the model organism and by changing the galanin concentration since its effects may vary by trying different doses as well as from species to species.

Authors' contributions

Conceived and designed the experiments: SH Shah, R Iqbal, Performed the experiments: SH Shah, MS Sajid, Analyzed the data: MS Sajid, M Hussain & MF Malik, contributed reagents/ materials/ analysis tools: SH Shah, B Mukhtar & R Iqbal, Wrote the paper: B Mukhtar & M Hussain & SH Shah.

Acknowledgments

The authors profess the diligence and meticulous support provided by the Laboratory staff of Department of Zoology, University of Gujrat.

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