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Original Article

Changes in the Hypothalamic-Pituitary-Gonadal Axis in Adult Male Rats Poisoned with Proteus and Biscaya Insecticides

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Abstract

Background: Insecticides may have negative effects on reproductive organs. Given the interaction between leptin and the hypothalamic-pituitary-gonadal (HPG) axis, we sought to investigate the changes in leptin and the HPG axis in adult male rats poisoned with Proteus and Biscaya insecticides.

Methods: Our experimental subjects were 110 adult male Wistar rats (80–90 days of age; average weight=200–210 g). They were randomly split into 11 groups of 10 rats: control, sham, and 9 experimental groups namely treatment with 2.75, 5.5, and 11 mg/kg/BW of Proteus, treatment with 1.5, 3, and 6 mg/kg/BW of Biscaya, treatment with 2.75 mg/kg/BW of Proteus+1.5 mg/kg/BW of Biscaya, treatment with 5.5 mg/kg/BW of Proteus+3 mg/kg/BW of Biscaya, and treatment with 11 mg/kg/BW of Proteus+6 mg/kg/BW of Biscaya. Intraperitoneal injections were performed over a 14-day period. For bloodletting at the end of the experiment, blood samples were withdrawn from the rats in order to investigate the serum concentration of luteinizing hormone (LH), follicle-stimulating hormone (FSH), gonadotropin- releasing hormone (GnRH), testosterone, and leptin. The data were analyzed using SPSS, version 16, via ANOVA and the Duncan test. A P value equal to or less than 0.05 was considered statistically significant.

Results: Our comparisons between the experimental groups (average and maximum compound concentrations of Proteus and Biscaya) and the control group showed a significant decrease in the mean serum levels of FSH (P=0.001), LH (P=0.001), GnRH (P=0.001), testosterone (P=0.005), and leptin (P=0.001) in all the experimental groups in a dose-dependent manner.

Conclusion: Proteus and Biscaya decreased GnRH, LH, FSH, and testosterone by reducing the serum level of leptin in the hypothalamus in a dose-dependent manner.

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Keywords ● Proteus ● Biscaya ● Testosterone ● Leptin ● Rats

What's Known

- Proteus and Biscaya, insecticides with a widespread use in agriculture, cause various negative effects on human health such as reducing reproduction or increasing the risk of testicular cancer.
- Effects of the simultaneous consumption of insecticides on sexual activity through impacts on the hypothalamic-pituitary-gonadal (HPG) axis have been discussed.

What's New

- Proteus and Biscaya decreased GnRH, LH, FSH, and testosterone by reducing the serum level of leptin in the hypothalamus in a dose-dependent manner.
- Use of pesticides affects the HPG axis and, thus, damages the growth and development of reproductive tissues.

Introduction

Insecticides are amongst the chemicals which are extensively used for agricultural pest control.[1] Acting as endocrine-disrupting chemicals, insecticides create negative effects on wildlife and humans[2] and may reduce reproduction or increase the risk of testicular, prostate, or thyroid cancer in men.[3]

Thiacloprid is a neonicotinoid class and Deltamethrin is a pyrethroid class of synthetic insecticides which are mainly used either alone or in combination.[4] Proteus (AKA Deltamethrin-Thiacloprid) and Biscaya (AKA Thiacloprid) are 2 insecticides which have been widely used in agriculture in recent years. The extensive use of these insecticides causes various negative effects on human health.[5]

Moreover, it has been found that these types of chemical compounds cause disturbances in the mechanism of testicular steroidogenesis.[6] It has also been reported that endocrine-disrupting chemicals play a crucial role in the reduction of the number of sperm and reproduction in a hormonal manner. In other words, they are significantly implicated in hypospadias, cryptorchidism, and reproductive cancers.[7]

Several studies have shown that pyridaben pesticides create histomorphological and hormonal changes in the reproductive actions of BALB/C rats; reduce the levels of luteinizing hormone (LH), follicle-stimulating hormone (FSH), and testosterone in a dose- and-time-dependent manner; and decrease the diameter of seminiferous tubules, thickness of epithelium, and distribution of the Leydig cells.[8]

There exist several reports on the effects of cyanotoxin on the hypothalamic-pituitary-gonadal (HPG) axis in adult male rats. In these experiments, the rats were treated with different concentrations of Microcystin-LR and it was indicated that the different concentrations of this toxin might directly or indirectly inhibit the synthesis of gonadotropin-releasing hormone (GnRH) in the hypothalamus, decrease the serum level of LH, and inhibit testosterone in testicles.[9] Furthermore, an investigation demonstrated that pyrethroid insecticide cypermethrin accelerated pubertal onset in male mice.[10] The results of another study revealed that neonatal exposure to estrogenic-disrupting compounds such as genistein advanced pubertal onset and induced premature anestrous in female rats.[11] In this regard, leptin is considered an important controller in the secretion of LH by the pituitary gland (hypophysis).[2, 12] On the other hand, genetic studies have shown that leptin is needed for the initiation of puberty[13] and it may act directly in the brain to initiate reproductive development.[14] Additionally, previous research has shown that the combination of several classes of pesticides has more toxic effects than the compound of their own class alone.[4, 15]

Given the increased consumption of toxins in contrasting reports on the effects of different toxins on body tissues and also the increase in puberty disorders, we conducted the present study with a view to controlling and reducing the negative effects of toxins on sexual activity and puberty. This is a comparative study on the toxic effects of Proteus and Biscaya, alone and in combination, on the HPG axis and GnRH, FSH, LH, testosterone, and leptin in adult male rats with regard to pertinent findings on humans. We also sought to investigate whether there is any significant relationship between leptin and changes in sex hormones.

Materials and Methods

The present study is a completely randomized and experimental research.

Animals and Their Classification

The current study complied with all the ethical issues vis-à-vis the care and use of laboratory animals during the research period. To that end, 110 adult male Wistar rats, aged between 80 and 90 days, with a mean weight of 200 g were selected for the current experimental study. The animals were kept in the Animal Breeding Room of Shiraz University of Medical Sciences for 1 week so that they could adapt themselves to the environment. The animals were kept on special shelves, made of transparent Poly Macrolone, under the conditions of 12 hours of light and 12 hours of dark at a room temperature of 23±2 ˚C and relative humidity of 50% to 55%. The rats had access to sufficient water and food. The effect and dose of each poison were determined via the LD50 test.[16] The animals were randomly divided into 11 groups of 10 (table 1).

Table 1: Different groups of animals

Group	Treatment
Control	Getting water and food naturally
Sham	Getting solvent of toxins (distilled water)
Experimental I	P 2.75 mg/kg/BW
Experimental П	P 5.5 mg/kg/BW
Experimental Ш	P 11 mg/kg/BW
Experimental IV	B 1.5 mg/kg/BW
Experimental V	B 3 mg/kg/BW
Experimental VI	B 6 mg/kg/BW
Experimental VП	B 1.5+P 2.75 mg/kg/BW
Experimental VШ	B 3+P 5.5 mg/kg/BW
Experimental IX	B 6+P 11 mg/kg/BW

BW: Body weight; P: Proteus; B: Biscaya

All injections were intraperitoneal with insulin syringes; the injection volume was 0.44 cc in all the groups. It took 2 weeks to fulfill the experiment. The intended poisons were produced by Bayer Crop Science Company. The protocol of the study was established in accordance with the guideline of animal ethics

Table 2: Parameters in the different groups (mean±SEM)

Variable / Group	Control	Sham	E-I	E-II	E-III	E-IV	E-V	E-VI	E-VII	E-VIII	E-IX	P value between Groups
GnRH (IU/L)	265.53±5.53	262.20±4.60	233.86±8.27	226.06±17.59	226.05±4.49	258.60±12.84	237.71±7.75	246.26±10.72	235.98±6.20	211.76±10.48 ac	200.70 ±7.17 ac	0.001
FSH (IU/L)	11.08±0.36 d	11.16 ±0.33 d	10.25±0.34	9.90±0.79	9.71±0.32	10.95±0.41 d	10.20±0.42 bcd	8.96±0.63 a	8.76±0.13 ac	8.18±0.17 ac	6.58±0.46 abcd	0.001
LH (IU/L)	8.83±0.22	8.71±0.14	8.03±0.44	7.48±0.17	7.51±0.45	8.11±0.25	7.96±0.46	7.43±0.20	6.96±0.32 a	6.85±0.44 a	6.41±0.58 a	0.001
Testosterone (IU/L)	24.85±1.06	24.45±1.53	21.06±0.86	22.23±0.71	20.40±1.81	22.25±0.85	20.41±1.16	21.21±1.54	19.46±0.71	19.26±0.58 a	19.00±1.10 a	0.005
Leptin (ng/mL)	4.88±0.13 bcd	4.61±0.11 bcd	3.50±0.13 a	3.56±0.41 a	3.13±0.37 a	3.43±0.36 a	3.30±0.36 a	3.18±0.14 a	2.95±0.26 a	3.05±0.34 a	2.71±0.23 a	0.001

E: Experimental; GnRH: Gonadotropin-releasing hormone; FSH: Follicle-stimulating hormone; LH: Luteinizing hormone
Anova test was used to compare each variable between the groups and a P value is generally given in the last column. The Duncan test was used for pairwisecomparisons.
a: Indicates a significant difference between the studied groups and the control group
b: Indicates a significant difference between the studied groups and the experimental E-I group
c: Indicates a significant difference between the studied groups and the experimental E–IV group
d: Indicates a significant difference between the studied groups and the experimental E-VII group
Data means are presented as mean±SEM
Level of P<0.05 is considered statistically significant

and welfare[17] and international regulations on the protection of laboratory animals and it was approved by the Ethics Committee of Islamic Azad University (IR.miau 13951206).

Bloodletting

Blood samples were withdrawn directly from the heart of the animals exactly 1 day after the last injection. Afterward, blood serums were collected via centrifuge (at 3000 rpm for 5 min) and stored at -20˚C until they were prepared for the experiment. The levels of FSH, LH, GnRH, testosterone, and leptin were measured using ELISA kits, specifically designed for rats. The ELISA kits were manufactured by Crystal Day Company in China (LOT=2015 10 14-2016 10 13).

Data Analysis

One-way ANOVA was used for data analysis. According to the Kolmogorov–Smirnov test, the data distribution was normal; hence, they were used in the next stages of the analysis of parametric tests. Additionally, the Duncan test was applied to determine the difference between the means if there was a statistically significant difference between the groups. The statistical analyses were done using SPSS, verison16, and the significance level was estimated at 5% (P<0.05). The data are presented as mean±standard error of the mean (SEM).

Results

Our comparisons between the experimental groups (different compound concentrations of Proteus and Biscaya) and the control group showed that there was a significant decrease in the mean serum levels of FSH (P=0.001) and LH (P=0.001) in a dose-dependent manner (table 2; figure 1). Furthermore, the comparisons between the experimental groups (average and maximum compound concentrations of Proteus and Biscaya) and the control group demonstrated a significant drop in the mean serum levels of GnRH (P=0.001) and testosterone (P=0.005) in a dose-dependent manner (table 2; figures 2 and 3). Moreover, there was a significant drop in the serum level of leptin in the experimental groups, treated with different concentrations of Proteus and Biscaya alone and in combination, in comparison to the control group in a dose-dependent manner (P=0.001) (table 2; figure 4).

Discussion

According to the results of the present study, different doses of Biscaya and Proteus insignificantly decreased the mean serum levels

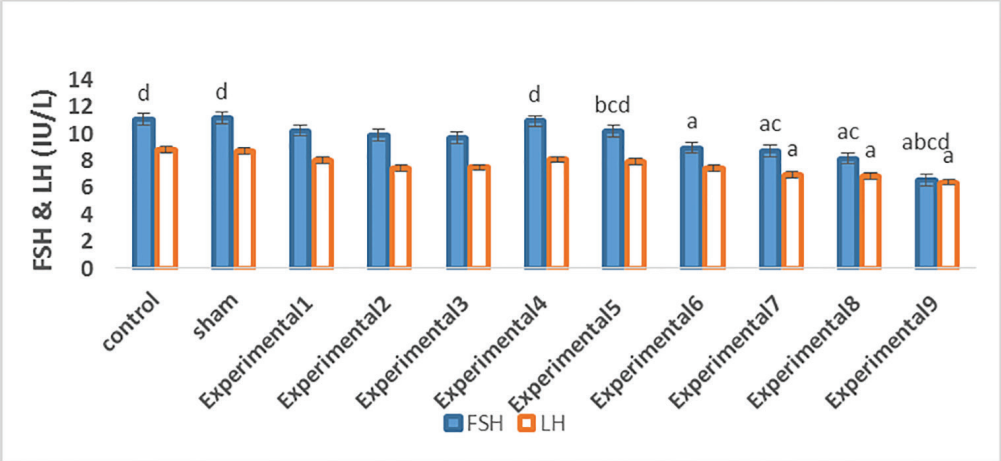

Figure 1: Reduction in the concentration of FSH and LH in the studied groups has been compared.
FSH: Follicle-stimulating hormone; LH: Luteinizing hormone
a: Indicates a significant difference between the studied groups and the control group
b: Indicates a significant difference between the studied groups and the experimental E-I group
c: Indicates a significant difference between the studied groups and the experimental E-IV group
d: Indicates a significant difference between the studied groups and the experimental E-VΠ group
Data means are presented as mean±SEM

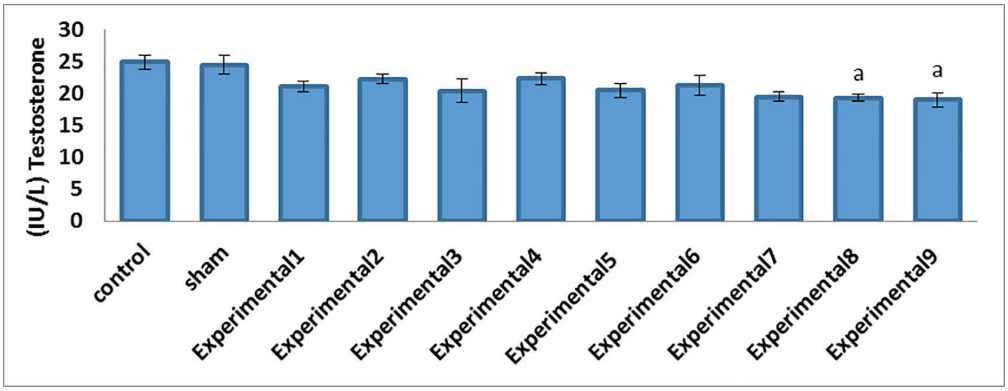

Figure 2: Reduction in the testosterone concentrations in the studied groups in comparison with the control group.
a: Indicates a significant difference between the studied groups and the control group
Data means are presented as mean±SEM

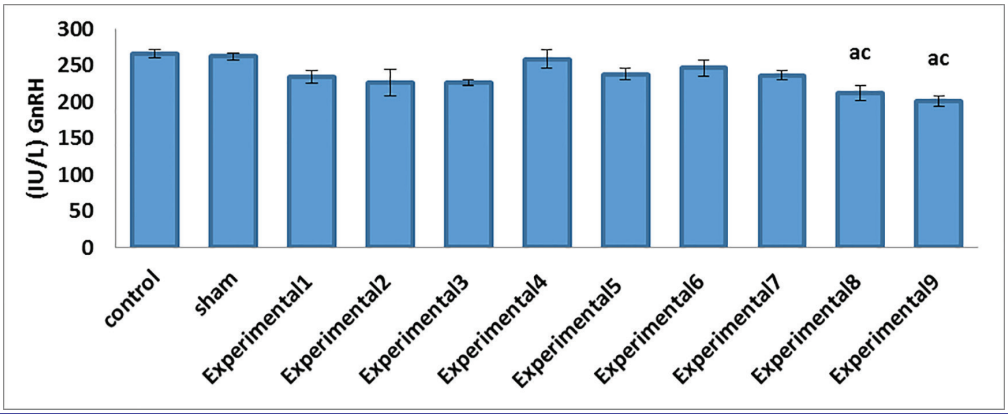

Figure 3: Decrease in the GnRH concentrations in the studied groups by comparison with the control group.
GnRH, Gonadotropin-releasing hormone
a: Indicates a significant difference between the studied groups and the control group
c: Indicates a significant difference between the studied groups and the experimental E–IV group
Data means are presented as mean±SEM

of FSH, LH, GnRH, and testosterone. In contrast, the average and maximum compound doses of Proteus and Biscaya significantly reduced the mean serum levels of the aforesaid hormones. The more severe effects of the abovementioned toxins on the combined doses may have been due to the effects of Thiacloprid, which is in both types of insecticides.

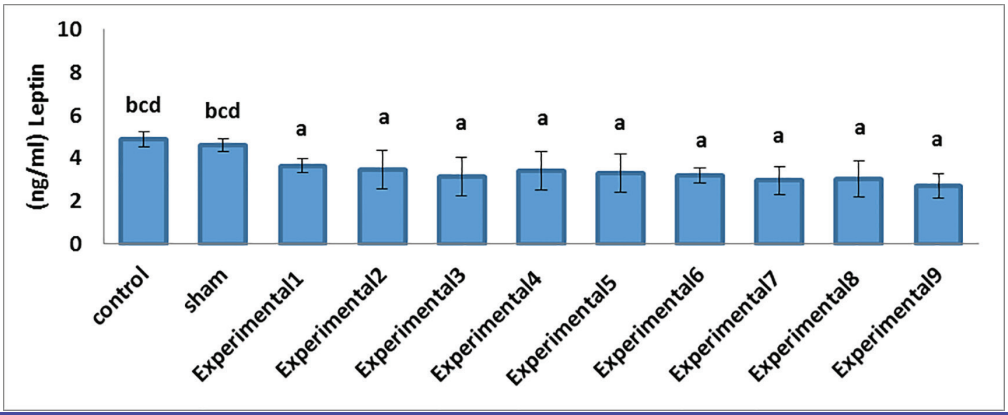

Figure 4: Decrease in the leptin concentrations in the studied groups in comparison to the control group.
a: Indicates a significant difference between the studied groups and the control group
b: Indicates a significant difference between the studied groups and the experimental E-I group
c: Indicates a significant difference between the studied groups and the experimental E-IV group
d: Indicates a significant difference between the studied groups and the experimental E-VΠ group
Data means are presented as mean±SEM

The reduction in the serum levels of FSH, LH, GnRH, and testosterone in the present study is consistent with the findings of other researchers, while it does not chime in with the findings of some other investigators. Loza et al.[11] studied the effects of genistein on pubertal development in female rats and found that genistein advanced pubertal onset and induced premature anestrous in the animals via the hypothalamic kisspeptin signaling pathways. Ye X et al.[10] reported that early postnatal exposure to cypermethrin at environmentally relevant doses significantly accelerated the age of pubertal onset in their male mice. Elsewhere, Xiolu et al.[9] and Ebadi et al.[8] studied the effects of cyanotoxins on the HPG axis as well as the effects of pyridaben pesticide on reproductive actions in BALB/C rats and reported that different concentrations of Microcystin-LR reduced the serum level of LH and inhibited testosterone. Furthermore, previous research has shown that pyridaben pesticide creates histomorphological and hormonal changes in the reproductive actions of BALB/C rats and decreases FSH, LH, and testosterone in a dose-dependent manner.[6, 8] The reduction in FSH, LH, and testosterone is most probably in consequence of a drop in leptin levels.

There are several studies on the relationship between leptin and the secretion of GnRH and its effects on puberty. Ahmed Sayed et al.[18] found that leptin facilitated the secretion of both GnRH through direct and indirect mechanisms and neuropeptide Y by neuropeptides. Previous research has also indicated that leptin, as an endogenous factor, expedites the onset of puberty and plays a crucial role in signaling the onset of puberty.[19, 20] The relationship between leptin and kisspeptin can directly activate the secretion of GnRH in GnRH neurons (i.e., GpR54 [G protein-coupled receptor-54] receptor in the preoptic area).[21, 22] Kisspeptin has a crucial role in signaling the onset of puberty.[21, 23, 24]

There are several reports regarding the relationship between kisspeptin and GnRH. In this regard, Liu et al.[25] concluded that the deficiency in the increase of GnRH might be associated with the downregulation of leptin receptors and the kisspeptin system (Kiss 1r) in the arcuate nucleus of adult male rates. Endocrine-disrupting chemicals such as estradiol benzoate and polychlorinated biphenyls can affect the kisspeptin system of GpR54 in rats and have significant effects on puberty.[26]

Joanne et al.[27] found that kisspeptin acted as a stimulator in the secretion of GnRH through upregulation and concluded that a rise in kisspeptin receptors resulted in early puberty. Crofton et al.[28] also highlighted the crucial role of GnRH in regulating the secretion of FSH, LH, and testosterone. Leptin acts as a pleiotropic regulator of several metabolic and neuroendocrine systems such as reproductive axis, mainly in the central area of the hypothalamus.[29, 30] There is also a study in the existing literature on the expression of leptin receptors and direct actions of leptin in male and female gonads.[31] It is, therefore, probable that Proteus and Biscaya, in combination, reduce the secretion of GnRH in hypothalamic neurons and subsequently lessen the levels of FSH, LH, and testosterone by reducing the serum level of leptin in a dose-dependent manner.

It has been previously posited that leptin has the role of a biochemical message between fat deposits and the reproductive axis so that the superficial injection of recombinant leptin can

restore fertility and reproduction in male and female OB/OB rats. A previous investigation demonstrated that an injection of leptin antiserum into the lateral ventricles of rats caused a decrease in LH pulse-shaped secretion and stopped estrus cycle.[32] There is considerable evidence indicating that, like estrogen, leptin is regulated by sex hormones.[33] A previous study showed that testosterone regulated the m-RNA expression and production of leptin in cultured human adipocytes.[34] The relative amount of androgens plays a crucial role in determining the sensitivity of the brain to the catabolic actions of leptin.[35] Leptin stimulates pituitary cells (hypophysis) and secretes FSH and LH, which accompany the onset of puberty.[12, 36] Not only does leptin enact its effects on the release of LH by increasing nitric oxide in the pituitary and hypothalamus,[37] but also it has an important role in puberty regulation by controlling the secretion of neuropeptide Y from neuropeptide Y neurons.[19]

Research has shown that both exogenous melatonin and androgen inhibit leptin levels.[38] Melatonin plays a significant role in controlling leptin production and regulating reproductive activities through testosterone concentration.[39] Finally, there are several reports indicating that insecticides may poison reproductive organs through either direct poisoning or interface with hormonal actions.[40] The other possible mechanism is that these poisons affect the secretion of GnRH through direct toxicity in reproductive organs or interface with hormonal action.

We recommended that further studies be undertaken on the effects of Proteus and Biscaya on the changes in kisspeptin in rats.

Conclusion

Our comparisons between the experimental groups (treated with different concentrations of Proteus and Biscaya) and the control group showed a significant decrease in mean serum levels of FSH, LH, and testosterone. Furthermore, there was a significant decrease in the mean serum level of GnRH in the experimental groups (treated with average and maximum compound concentrations of Proteus and Biscaya) by comparison with the control group in a dose-dependent manner. Additionally, the mean serum level of leptin was decreased in all the experimental groups compared to the control group. Consequently, it can be concluded that Proteus and Biscaya affect the HPG axis by reducing leptin and subsequently GnRH, as a result of which the mean serum levels of FSH, LH, and testosterone hormones are decreased in a dose-dependent manner.

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Conflict of Interest: None declared.

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