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Histopathology and Biochemical Analysis of Pentazocine Effect on Ovary, Uterus, Adrenal Gland and Thyroid Gland of Female Albino Rats

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Abstract

Many drugs are known to interfere with the functions of CNS including hypothalamus there by modify the activity of hypophysis and gonads. Due to that many functions alters by them, such as the secretion and release of pituitary follicle stimulating hormone (FSH), luteinizing hormone (LH) and prolactin (PRL) are directly dependent on gonadotropic releasing hormones (GnRH). The effect of pentazocine on endocrine organs like ovary, uterus, thyroid and adrenal glands, there estrous cycle, gravimetrical changes, histological changes and biochemical changes has been evaluated. Three groups of healthy adult female albino rats having six rats in each group were taken. The rats of groups II and III were administered pentazocine at the dose level 0.5mg and 1.0mg/100g body weight respectively intraperitoneally/daily for 30 days. However, the rats of group I (Control) were given saline alone.After the experimental periods, the rats were sacrificed and the histological study of ovary and uteri, thyroid and adrenal gland were performed. Body weight, estrous cycle, ovarian histometric elements of the follicles were non significant and histometrical changes of uterine parameters like diameter, thickness of myometrium and endometrium and surface epithelial cell height increased significantly. Biochemical changes of endocrine glands are parallel to the gravimetrical changes, the protein and glycogen contentsincreased significantly and reduced cholesterol content significantly with respective administration of both the dose level of pentazocine. Also, the gravimetric analysis of thyroid and adrenal gland increased significantly due to pentazocine administration. The study indicates that pentazocine has slightly stimulatory action on endocrine activities related to female reproduction.

Keywords: Pentazocine, Endocrine glands, Estrous cycle, Ovary, Endometrium

Introduction

The principle of central nervous system (CNS) influencing drugs are the anticonvulsants, antiparkinsonism, opioid and non-opioid analgesics, appetite suppressants, antiemetics, analgesics, antipyretics , certain stimulants, neutolectics, transquilizers sedatives and hypnotics.

These drugs are known to interfere with the functions of CNS including hypothalamus there by modify the activity of hypophysis and gonads. The secretion and release of FSH, LH and PRL are directly dependent on (GnRH). Therefore, the drugs which modify the functions of CNS may also effect the pituitary functions. As a result, the function of gonads may also be modified. Therefore, the structure and biological activities of some CNS influencing drugs, the actions of which modify the reproductive and endocrine activities is briefly summarized below. The effect of these drugs on female endocrine activities were undertaken.

Pentazocine was synthesized as part of deliberate effort to develop an effective analgesic with little or no abuse potential. The pharmacology of pentazocine has been reviewed by Brogden and associates (1). Pentazocine a benzomorphine derivative has the analgesic activity of the recemate mainly due to the 1-isomer (2).

Pentazocine is an opioid with mixed agonist and antagonist properties (3). Gilbert and Martin (4) proposed that pentazocine is antagonist at μ-receptor and an agonist at both the K and δ-receptor. Bouchard and Quirion (5) have described the binding sites for pentazocine in the rat brain at various sites including thalamic and hypothalamic nuclei. Though pentazocine produces significantly greater analgesia among females than in males, no significant difference was observed in analgesic among females during different phases of the menstrual cycle (6, 7). Tifluadom, selective K-agonist elevates the 5-hydoxytryptophine (5-HT) and norepinephrine content, which have both facilitator and permissive role on LH secretion and ovulation (8, 9).

Material and Methods

Animals

Healthy, sexually matured, regularly cycling, colony bred virgin female rats of Wistar strain (*Rattus norvegicus*) aged three months and weighing 150 -200 gm were purchased from National Institute of Nutrition, Hyderabad. The rats were housed in polypropylene cages measuring 12”x10”x8”, under well ventilated animal

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house conditions (temperature: 28-31°C). The rats were fed with balanced diet as per CFTRI formula and tap water *ad libitum.* They were maintained as per the principles of laboratory animals care (NIH Publication No. 85-88, 1985 (10). The animals were divided into five groups, each consisting of six rats in each group and treated as follows.

Experimental design

The treatment was started when the animals were in estrous phase. The group I received vehicle only (0.2ml saline) and served as control. Group II and III received pentazocine at dose level of 0.5 and 1.0mg for 100g body weight in 0.2ml saline respectively. The treatment was given for 30 days intraperitoneally between 10:00 to 11:00AM to cover 6 regular estrous cycles and vaginal smear from the experimental animals was observed every morning.

Ethical issue

The experimental protocol was approved by the Animal Ethical Committee in accordance with the guidelines for care and use of laboratory animals prepared by the Institutional Animal Ethics Committee registered number 34800.

Autopsy schedule

On day 31st, 24h after last treatment, all the animals from each group sacrificed, the ovary, uterus, thyroid and adrenal gland were dissected out, freed from extra depositions of adherent tissue and weighed to the nearest mg on an electronic balance. One side of ovaries, uterus, thyroid and adrenal gland from each animals were fixed in Bouin's fluid for histological, cytological and histometrical studies. The histometric measurement like diameter of uterus, thickness of endometrium and myometrium and height of endometrial epithelial cells were made from randomly chosen 20 sections from each group using ocular and stage micrometer(11) as well as diameter of ovarian follicles were made.Ovaries and uterus from other side were used for biochemical evaluationof protein(12), glycogen(13) and cholesterollevel (14).

Statistical analysis

All the values were statistically analysed by Student's-'*t*' test using SPSS(11.0.1.)(15). Data were expressed as the Mean + S.E. Statistical significance was set at $p<0.05$, $p<0.01$ and$p<0.001$.

Results

Behavior

The rats which received the chronic treatment of pentazocine were as active as the control rats and the total intake of feed/day was not much altered due to drug administration.

Mortality

No mortality was observed in either control group or in the experimental group that received pentazocine.

Changes in the body weight

The body weight of the rats which received low or high doses of pentazocine have showed slight increase in the body weight which does not deserve significant consideration for treatment of 30 days (Table 1).

Changes in the estrous cycle

Control rats showed regular estrous cycle and there length of cycles were observed similar to that of control rats within 30 days. Pentazocine treatment has showed no noticeable change either in the number of cycle or its length. The different phases of estrous cycle like pro-estrus, estrus, metaestrus, diestrus also were not different when compared to that control rats (Table 2).

Gravimetric changes

The increase in the ovarian weight was seen in pentazocine treated rats. This increase was significant ($P<0.01$) with low dose and highly significant $P<0.001$) with high dose of pentazocine administration (Table 3).

Biochemical changes

Availability of pituitary gonadotropins is very essential for the conversion of cholesterol into steroid hormones in the ovary. Administration of pentazocine caused reduction in the cholesterol level. Also significant reduction in the cholesterol content was observed with low dose ($P<0.05$) and high dose ($P<0.001$) of pentazocine treatment. Ovarian growth and activities reflected through its protein and glycogen content. Significant ($P<0.05$) increase in the protein content was observed with low dose and high dose ($P<0.001$) of pentazocine treatment. Glycogen content of the ovary is increased significantly ($P<0.01$) with the treatment of both doses of pentazocine (Table 3).

Ovarian follicular kinetics

Administration of pentazocine caused non-significant change in the ovarian follicular number and size (Table 4, Figure 1 & 2).

Number and histometric changes in follicles

The number of developing follicles increased non-significantly with low dose of pentazocine treatment, whereas, it was almost significant ($P<0.05$) with high dose. The number of antral follicles increased significantly ($P<0.01$) with both the doses of pentazocine. Also number of Graafian follicles increased significantly with low and high doses of pentazocine ($P<0.01$ and $P<0.001$ respectively) (Table 4).

The histometric changes of follicles were parallel with that of number of follicles. Non-significant changes were observed in the diameter of developing follicles, antral and Graafian follicles with the treatment of low and high doses of pentazocine treatment.

Atretic follicles

The number of atretic follicles decreased significantly ($P<0.05$) with both doses of pentazocine treatment and the average diameter of atretic follicles of ovaries which received low and high doses of pentazocine shows non-significant change (Table 4).

Corpus luteum

The number of corpus lutea increased almost significantly ($P<0.05$) with both doses of pentazocine and they were as healthy as that of control without any significant change in their diameter (Table 4).

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Changes in the uterus

Uterine growth, biochemical contents, histopathology histometry observation were depends on dose of the pentozocine treatment.

Gravimetric changes

Both the doses of pentazocine stimulated the ovarian growth, as a result slight but non-significant increase in its weight was observed when compared with control (Table 6).

Biochemical changes

Significant (P<0.05) reduction was seen in the cholesterol level of the uterus of pentazocine treated rats. The protein content increased significantly (P<0.05) and (P<0.001) with respect to treatment of low and high dose of pentazocine. The glycogen content of the uterus treated with both the doses of pentazocine increased significantly (P<0.05) (Table 6).

Histometric changes of the uterus

The histometric measurements of the uterus are parallel to that of protein content of uterus. The pentazocine treatment has increased the diameter of uterus, thickness of its myometrium, endometrium and height of the epithelial cell of endometrium. This increase was significant (P<0.05) only with its diameter. The changes were observed in all these parameters were dose dependent (Table 7; Figure 3-4).

Changes in the thyroid and adrenal glands

Thyroid gland showed no significant change in its weight in pentazocine treated rats. The histological observations of thyroid in pentazocine treated rats, showed healthy follicles with normal secretion as same as that of normal rats. Pentazocine treatment, did not have any effect on adrenal gland as any significant change either in its weight or histological observation was not observation (Table 8; Figure 5-8).

Table 1: Effect of pentazocine on the body weight of female albino rats

Groups	Initial Body Weight	Final Body Weight	Percent Change
Control (I)	150.00 ± 3.66	160.00 ± 3.32	6.66
Group II	168.30 ± 4.80	178.33 ± 4.44	6.68
Group II	165.00 ± 4.47	175.00 ± 4.27	10.06

M ± SE = Mean ± Standard error. *P<0.05, **P<0.01, ***P<0.001, when compared to saline treated rats

Table 2: Effect of pentazocine on estrous cycle in female albino rats

Groups	No. of cycles/rat	Length of cycles (days)	Duration of phases (days) / cycle			
			Estrus	Metaestrus	Diestrus	Proestrus
Control (I)	5.40 ± 0.21	5.41 ± 0.30	6.81 ± 0.19	6.34 ± 0.10	10.21 ± 0.68	4.41 ± 0.31
Group II	5.33 ± 0.10	5.49 ± 0.40	6.91 ± 0.31	6.30 ± 0.24	10.00 ±0.45	5.32 ± 0.21
Group II	5.33 ± 0.26	5.32 ± 0.96	7.21 ± 0.43	5.92 ±0.32	10.00 ± 0.56	5.64 ±0.30

M ± SE = Mean ± Standard error. *P<0.05, **P<0.01, ***P<0.001, when compared to saline treated rats

Table 3: Effect of pentazocine on ovarian gravimetric and biochemical parameters in female albino rats

Groups	Ovary mg/100gm body weight	Cholesterol µg/mg	Protein µg/mg	Glycogen µg/mg
Control (I)	42.53± 2.2	4.16 ± 0.23	3.61± 0.14	1.89± 0.09
Group II	47.40**± 2.09	3.26*± 0.32	4.02*± 0.21	2.13**± 0.10
Group II	50.61**± 2.3	2.50***± 0.17	4.68***± 0.17	2.22**± 0.12

M ± SE = Mean ± Standard error. *P<0.05, **P<0.01, ***P<0.001, when compared to saline treated rats

Table 4: Effect of pentazocine on ovarian kinetics in female albino rats

Groups	Developing follicles	Antral follicles	Graafian follicles	Atretic follicles	Corpus luteum
Control (I)	5.50 ± 0.22	1.83 ±0.06	1.83 ± 0.22	1.89 ± 0.09	1.83 ± 0.06
Group II	5.92 ±0.20	2.05* ± 0.03	2.01** ± 0.18	0.60** ± 0.05	2.05* ± 0.11
Group II	6.08* ± 0.26	2.09** ± 0.02	2.25*** ± 0.20	0.58** ±0.04	2.09* ±0.08

M ± SE = Mean ± Standard error. *P<0.05, **P<0.01, ***P<0.001, when compared to saline treated rats

Table 5: Effect of pentazocine on ovarian histometric elements of the follicles

Groups	Developing follicles (µm)	Antral follicles (µm)	Graafian follicles (µm)	Atretic follicle (µm)	Corpus luteum (µm)
Control (I)	9.06 ± 0.48	25.42 ±0.55	33.48 ± 0.24	34.21 ± 0.32	48.21 ± 0.57
Group II	9.02 ±0.42	26.21 ±0.48	32.21 ±0.39	35.21 ±0.42	47.48 ± 0.62
Group II	9.21 ± 0.45	28.42 ± 0.63	34.29 ± 0.38	33.93 ± 0.71	50.48 ± 0.60

M ± SE = Mean ± Standard error. *P<0.05, **P<0.01, ***P<0.001, when compared to saline treated rats

J Infertil Reprod Biol, 2018, Volume 6, Issue 3, Pages: 9-15. https://doi.org/10.47277/JIRB/6(3)/9

Table 6: Effect of pentazocine on uterine gravimetric and biochemical parameters in female albino rats

Groups	Uterus Wt. mg/100g body wt.	Cholesterol µg/mg	Protein µg/mg	Glycogen µg/mg
Control (I)	205.91 ± 8.59	4.18 ±0.09	8.18 ±0.2	1.98 ± 0.05
Group II	204.22 ±1.82	3.70* ± 0.08	9.50* ± 0.2	2.29* ±0.03
Group II	208.25 ± 2.51	3.90* ± 0.06	10.17** ± 0.31	2.50* ± 0.06

M ± SE = Mean ± Standard error. *P<0.05, **P<0.01, ***P<0.001, when compared to saline treated rats

Table 7: Effect of pentazocine on uterine histometric parameters in female albino rats

Groups	Uterus diameter (µm)	Thickness of myometrium (µm)	Thickness of endometrium (µm)	Height of epithelial cell (µm)
Control (I)	631.96 ± 22.16	169.61 ± 8.04	390.78 ± 7.56	32.33 ± 1.40
Group II	789.83* ± 23.82	170.17 ± 4.55	429.33 ± 5.15	34.83 ± 1.73
Group II	795.67** ± 25.12	181.83 ± 3.03	435.67 ±4.01	35.01 ± 1.41

M ± SE = Mean ± Standard error. *P<0.05, **P<0.01, ***P<0. 001, when compared to saline treated rats

Table 8: Effect of pentazocine on gravimetric changes of thyroid and adrenal gland in female albino rats

Groups	Thyroid		Adrenal	
	mg/100g body Wt.	Percent change	mg/100gbody Wt.	Percent change
Control (I)	10.68 ± 0.52	3.08	26.88 ±0.57	4.50
Group II	10.80 ± 0.63	4.09	25.1 ± 1.16	5.89
Group II	11.04 ± 0.33	4.12	25.18 ± 0.87	5.95

M ± SE = Mean ± Standard error. *P<0.05, **P<0.01, ***P<0.001, when compared to saline treated rats

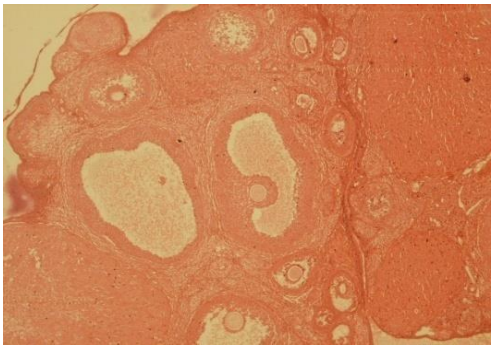

Figure 1. Cross section of the ovary of control rat showing normal ovarian components

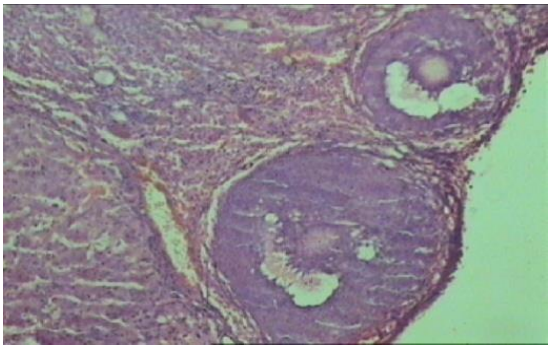

Figure 2. Cross section of the ovary of pentazocine treated rat showing hyperactivity of ovarian components

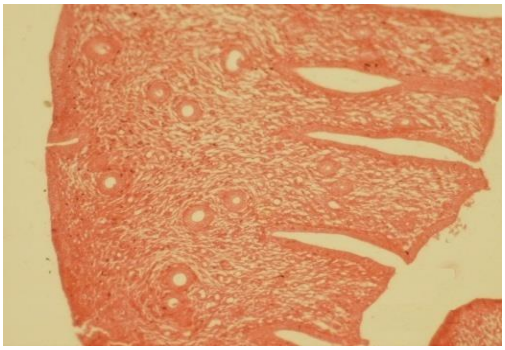

Figure 3. Uterus cross section of control rat showing normal growth of uterine components

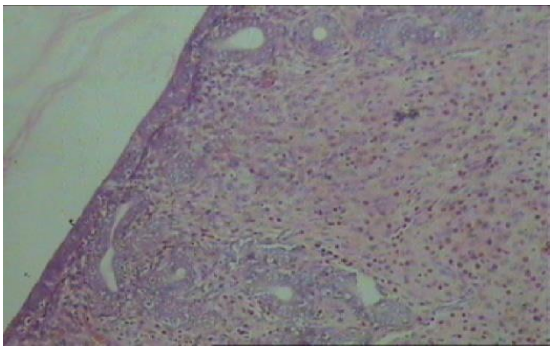

Figure 4. Cross section of the uterus of pentazocine treated rat showing increased activity of the uterine components

J Infertil Reprod Biol, 2018, Volume 6, Issue 3, Pages: 9-15. https://doi.org/10.47277/JIRB/6(3)/9

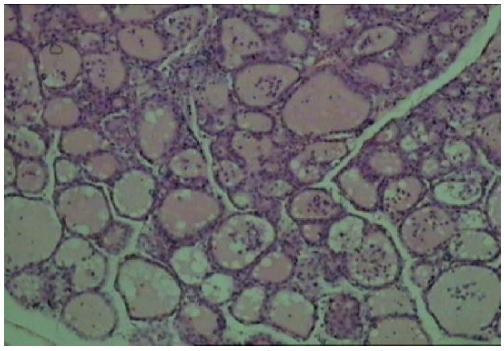

Figure 5. Cross section of thyroid of control rat showing normal follicles

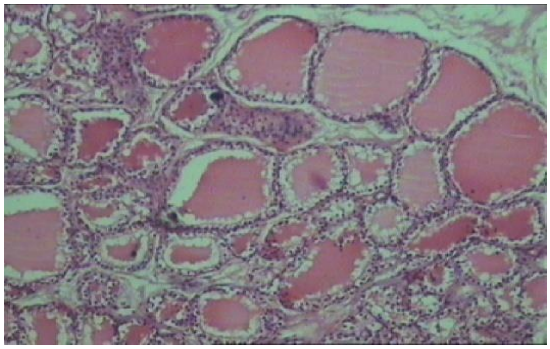

Figure 6. Cross section of the thyroid of pentazocine treated rat showing increased the size of follicular cells and hormone secretion

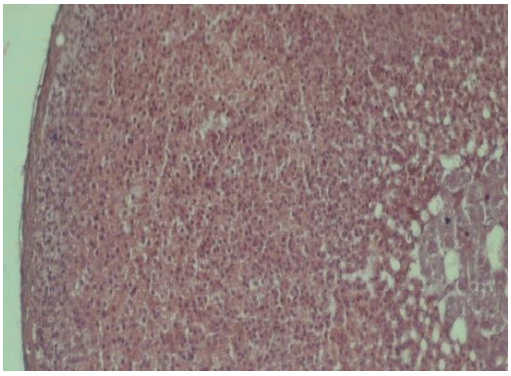

Figure 7. Cross section of adrenal of control rat showing normal cortex and medulla

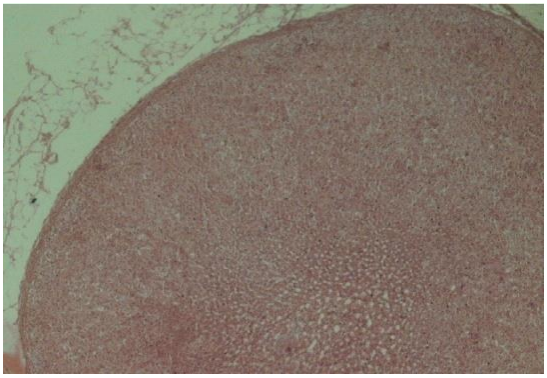

Figure 8. Cross section of the adrenal of pentazocine treated rat showing slightly decreased the size of the cortex and medulla parts also hormone secretion

Discussion

Pentazocine using it as opioid for pain medication, also called a narcotic drug. It is used to treat moderate to severe pain in adults and children who are at least 12 years old. It is also considered as anesthetic drug to use during surgery. According to researchers opiate receptor work it as antagonists to increase in GnRH release and acting through μ-receptors of hypothalamus, which stimulates the gonadotropins secretion (16-19).

Pentazocine is a competitive antagonist at the μ-receptor and against at the K- and δ-receptors (2). It produces a type of analgesia that differs from morphinedrug and act as CNS depressant (1). The analgesic effect of pentazocine was mainly due to agonistic actions at K-opioid receptors (20).

There is a paucity of information regarding the direct effect of mixed opioid like pentazocine on GnRH or pituitary gonadotropins release and secretion. Therefore, it may be attributed that the stimulatory effects of this mixed opioid might have been mediated through its antagonistic activity at μ-receptor, which was caused to increase the pituitary gonadotropins secretion, it was evidenced in the case of another μ-receptor antagonist naloxone (19). Similar studies on the action of other opioids such as morphine and pethidine is also mediated through the hypothalamo–hypothphyseal axis (21-26).

The folliculogenesis depends upon the pituitary FSH and LH surge is essential for the ovulation (27-30). Increased availability of pituitary FSH and LH supports the growth of follicles and prevents atretia as observed by increase in the follicular dynamics and decrease in atretic follicle in pentazocine treated rats. As, LH stimulates steroidogenesis through utilization of cholesterol store, level of cholesterol is decreased in the ovary. The increase in the uterine weight and its protein content may be attributed to this increase in the steroid hormone production in pentazocine treated rats. FSH is also known to stimulate the protein synthesis in the gonads (31, 32). The increased histometric parameters of the uterus like its diameter thickness of myometrium, endometrium and surface epithelial cell height in pentazocine treated rats may also be due to the more availability of ovarian steroid hormones.

The study of estrous cycle in pentazocine treated rats indicates the non- significant results. Regular estrous cycle is observed in these rats. Though the chronic treatment of pentazocine for 30 days has resulted in hypertrophied activity of the ovary of pituitary and ovary (33-36). The rhythmicity of these endocrine glands along with the activities of uterine growth and vaginal cornification has not lost.

In the present study administration of pentazocine has not altered the thyroid and adrenal weight and there histometric measurements may be because of low/ no influence of these drugs on neuroendocrine system responsible for the synthesis and release of TSH and ACTH. Similar findings were observed with many medicinal plants and plant products in the form of their molecular medicine as fraction, compounds or metabolites (37, 38).

Conclusion

The study conclude that effect of pentazocine on ovary, uterus, thyroid and adrenal gland histopathology and biochemical analysis by understanding the influence of drug to be considered asstimulatory action on endocrine activities and it is clearly evidenced by all the parameters elevated in their functions.

Conflict of interests

The author declares that there is no conflict of interest.

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