TRAMADOL INDUCED OVARIAN AND UTERINE CHANGES IN ALBINO RAT

Abstract : Tramadol at the dose levels of 1mg and 3mg/100 g body weight was administered to normal cycling rat for 20 days through intraperitoneal routes. At autopsy on 21st day significant reduction in the ovarian and uterine weight was observed. Histological observations showed decreased in the number and size of Graafian follicles, corpora lutea and increase in the atretic follicles in the ovary. The uterus showed absences of endometrial glands, decrease in the height of myometrium, endometrium and its epithelial cells. The total protein content of the ovary and uterus is decreased whereas the cholesterol content is increased. This action of tramadol to gonadotrophins is discussed.

Key words : Tramadol, atretic follicles, ovarian steroids

INTRODUCTION

Tramadol is an analgesic with opioid-like effects(Volk R et al.,2017) and (Schnabel A *et al*., 2015). It has pharmacodynamic and pharmacokinetic profile relative to other opioids. The treatment of mild to severe pain at dose of 200 or 300 milligrams per day is the formulations in both immediate and extended-release in many parts of the world.(Vijayan R et al., 2018) and (Santos Garcia JB *et al*., 2017)

The toxicity of tramadol includes central nervous system depression, nausea, vomiting, dizziness, anorexia, seizures and hypotension which may occur in therapeutic or toxic doses (Beakley.B.D. *et al.,*2015). Fatal toxicity of tramadol have been reported as a result of overdose. In these cases, death has been result due to cardiopulmonary arrest and liver failure, in addition hypoglycaemia (Daubin *et al.,*2007) and (Mugunthan 2012).

In the year 2013 more than 44 million people were prescribed tramadol in United States, making it one of the most used opioids. (Patterson, 2017). Many countries of the world use moderate to severe medications of opioid-based analgesics. During the last 20 years, there is significant increase and also higher demand of opioids globally(Berterame S *et al*., 2016). The acceptability and availability of opioids for the treatment of pain differs throughout the world(Kunnumpurath S et al., 2018) and (Pastrana T *et al*., 2017)

As over development or reproductive toxicity, endogenous opioid peptides are said to be located in different tissues on the reproductive system which indicate that they might be involved in the reproductive functions. (Subiran N, 2011, Vuong C, 2010).

As peptides induces their effect on opioid receptors. So, in men it can cause loss of libido and erectile and ejaculatory dysfunctions and can inhibit the proliferation of uterine cells which mediate mainly by mu opiate receptor (Dziekonski, 2015). Chronic administration of tramadol can cause reproductive dysfunction and increased average of sterility (El-Ghawet, H 2015). The

abuse of opiate leads to hypogonadism, primarily by decreasing the release of gonadotropin releasing hormone(GnRH), deficiency of testosterone and infertility. Many researchers have demonstrated that long-term administration of tramadol had dose dependent adverse effects on testicular tissue(Azari. O *et al.*(2014). Some other studies have showed that rats received subcutaneous injections of tramadol(40 mg/kg body weight) three times per week for 8 weeks showed reduced plasma levels of luteinizing hormone, follicle stimulating hormone(Ahmed. M.A, 2014). In addition, tramadol caused a concentration-dependent inhibition of potassium chloride-induced myometrium contractility(Vazzana. M, 2015).

MATERIALS AND METHODS

Normal cycling healthy female albino rats of wistar strain were maintained at room temperature of 28 ± 2^{o}c with lighting schedule of 12 hours light and 12 hours darkness. They were grouped in individual cages, each containing six animals. They were fed with a standard pellet diet (VRK Nutrition, Pune) and water *ad libitium*. Approval at the Institutional Animal Ethics Committee (IAEC) of Luqman College of Pharmacy, Gulbarga was taken for conducting experimental activities.

The animals were divided into the following groups:

Group 1- Received 0.2ml saline interaperitonally for 20 days and served as control group.
Group 2- These rats received tramadol 1mg/100g body weight interaperitonally for 20 days.
Group 3- These rats received tramadol 3mg/100g body weight interaperitonally for 20 days.

The treatment was started from estrous phase of the cycle only as the ovarian and uterine activities change markedly from one phase to another phase. One group was treated with 3mg tramadol/100g body weight. The doses were given interaperitoneally(i.p). Group-1 served as suitable saline treated controls were maintained. The treatment was given once a day between 10:00 AM to 11:00 AM for 20 days. All the experimental rats were sacrificed by decapitation on 21st day 24 hours after the final dose.

The body weight was recorded. Ovary and Uterus were dissected out, freed from adherent tissue and weighed on Anamed electronic balance. Organs from left side of each animal were processed for histological studies. The number of Graafian follicle, atretic follicle and corpora lutea was made from randomly choosen 20 sections from each group. Micrometric measurements such as diameter of uterus, thickness of myometrium, endometrium and epithelial cell height were also made from randomly selected 20 sections which appeared round incross section from each group. Micrometric measurements were made by using stage and ocular micrometer.

Protein content of ovary and uterus was estimated by Lowry's method(Lowry OH, 1951). Cholesterol content from the right side ovary and uterus was estimated by Libermann and Burchard's reaction as described by Peter and Vanslyke. The glycogen content of ovary and uterus was estimated by Carrol *et al.,* Stastical analysis was carried out by using student "t" test.

RESULTS AND DISCUSSION

Body weight – There is no significant change in the body weight of the rat due to treatment of tramadol for 20 days, intraperitoneally compared to their respective control groups.

TABLE 1 : Effect of Tramadol on gravimetric and biochemical changes of ovary and in albino rat

	Weight (mg/100g body wt.) Ovary	Cholesterol (µg/mg) Ovary	Protein (µg/mg) Ovary	Glycogen (µg/mg) Ovary
Saline	46.51±0.94	3.23±0.03	6.01±0.05	2.43±0.03
1mg Tramadol	43.40±0.69**	4.41±0.05**	5.16±0.18**	1.71±0.05**
3mg Tramadol	40.66±1.17**	5.63±0.07**	4.31±0.06**	1.54±0.06**

M±S = Mean ± Standard Error

*P<0.05; **P<0.01, compared to respective control.

Biochemical changes (Table - I)

Highly significant (P<0.01) increase in the cholesterol content of ovary was significantly through intraperitonally. Whereas the protein and glycogen content of the ovary and was decreased significantly (P<0.01). Tramadol administered group compared to the control group of rats.

TABLE 2: Effect of Tramadol on gravimetric and biochemical changes of ovary and in albino rats

Treatment	Class I SPAF	Class II LPAF	Class III SAF	Class IV MSAF	Class V LSAF	Class VI GF
Saline	58.61±3.1	49.17±2.17	11.02±0.76	5.18±0.81	3.38±0.03	2.46±0.37
1mg Tramadol	34.26±1.33**	31.89±0.40**	7.82±0.31**	4.61±0.08**	2.23±0.33**	1.01±0.02**
3mg Tramadol	29.38±0.51**	28.51±1.12**	7.40±0.03**	3.44±0.04**	1.17±0.19**	0.68±0.01**

M±S = Mean ± Standard Error

*P<0.05; **P<0.01, compared to respective control.

TABLE 3 : Effect of Tramadol on gravimetric and biochemical changes of uterus in albino rats

	Weight (mg/100g body wt.) Uterus	Cholesterol (µg/mg) Uterus	Protein (µg/mg) Uterus	Glycogen (µg/mg) Uterus
Saline	228.21±4.21	4.18±0.02	8.28±0.09	1.64±0.05
1mg Tramadol	201.81±3.61*	5.51±0.14**	5.16±0.02**	1.49±0.07*

3mg Tramadol	169.67±2.28**	6.75±0.05**	4.37±0.03**	1.31±0.04**

M±S = Mean ± Standard Error
*P<0.05; **P<0.01, compared to respective control.

Biochemical changes (Table - 2)

Highly significant (P<0.01) increase in the cholesterol content of uterus was significantly through intraperitonally. Whereas the protein and glycogen content of the ovary and was decreased significantly (P<0.01). Tramadol administered group compared to the control group of rats.

TABLE – 4 : Effect of Tramadol on histometric changes of uterus in albino rat.

	Diameter of uterus (µm)	Thickness of myometrium (µm)	Thickness of endometrium (µm)	Height of epithelium (µm)
Saline	2139.49±7.38	241.01±2.29	433.00±2.08	39.89±1.02
1mg Tramadol	1914±8.23**	183.01±2.04**	349.18±3.49**	29.28±2.12**
3mg Tramadol	1737.36±9.27**	170.17±3.21**	317.59±5.89**	19.24±0.8**

M±S = Mean ± Standard Error
*P<0.01; **P<0.01, compared to respective control

Histological and histometric changes in uterus (Table- 3). There was significant reduction in the diameter of uterus, thickness of endometrium and myometrium and epithelial cell height (P<0.01) in tramadol treated groups compared to their respective control group. A reduction in the secretion of endometrial gland was observed.

It is also known that hypothalamus regulates the rhythmic release of pituitary gonadotrophins, i.e., FSH, LH and prolactin through neural stimules to GnRH(Carmel PW, *et al*,.1976). The orderly event of follicular growth and ovulation depends upon the pituitary FSH, LH and prolactin. Investigations on tramadol indicate that tramadol being a central nervous system influencing drugs inhibits the release of gonadotrophins from pituitary (Salah *et al.*,2020). The studies also indicate that tramadol blocks ovulation by inhibiting the LH surge from pituitary in rats.(Ahmed MA and Kurkar A 2014). In the present study, as the drug was administered between 10.00 and 11.00 AM every day, it covers the “critical period” of LH surge, thus postponing the ovulation for one day by interfering with 24 hours periodicity for gonadotrophin release(Lawton I *et al*., 1968)(Sindagi SB, 1975). Decreased level of protein content in tramadol treated rats indicates hampered growth and source of energy. Increased in cholesterol and glycogen content indicated hampered steroidogenesis.

FSH stimulates the differentiation of granulose cells and promotes the follicular development (Channing CP, 1970, Goldenberg RL *et al*., 1972). In the present investigation, their is reduction in the number of Graffian follicle in the ovary of tramadol treated rat, thus indicating the inhibition of follicular growth which is gonadotrophin dependent. There is decrease in the number of corpora lutea in tramadol treated rat indicating reduction in the rate of ovulation leading to follicular atresia.

The growth of uterine cavity depends upon the ovarian estrogen secretion. Primarily estrogen acts upon the surface epithelium and the glands within endometrium (Richards JS *et al.,*). Progesterone acts on estrogen primed uterus and thus prepares the uterine epithelium from proliferative to secretory state.

CONCLUSION In the present study reduction in the diameter of uterus, thickness of endometrium, myometrium and epithelial cell height may be attributed to the non-availability of steroids necessary for uterine growth due to decresed levels of gonadotrophins in tramadol treated rats.

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