DOI: https://doi.org/10.36811/ojpsr.2021.110014 **OJPSR: November-2021: Page No: 47-55**

Open Journal of Pharmaceutical Science and Research

Review Article **Open Access**

Tramadol injection as dosage form -Induced deterioration in reproductive performance of male rabbits

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Received Date: *Oct 07, 2021 /* ***Accepted Date:*** *Nov 15, 2021 /* ***Published Date:*** *Nov 24, 2021*

Abstract

Injections are usually more expensive than tablets, but they may be required less often since they are more concentrated, whereas a person may need to take a tablet more regularly, thus consuming a large volume. when we administer a drug via oral rout ,its meant to be absorbed by stomach or intestinal mucosa ,but its so happens that, gastric acid, food ,other drugs and many other factor come into the play and retarded the drug absorption ,metabolized in intestinal mucosal cells even before it can reach to liver or target tissue(first-pass metabolism)thus decrease the effective amount reaching to circulation, Now, a drug given through injection reaches circulation at cent percent level and show prompt action which is earnestly required at the moment ,it also decrease the dose and systemic side effects . also the tablets formulation contain the excipient like filler ,diluents for the purpose of long -term stabilization ,bulking up tablet formulations making concentration of drug less in tablets and need more dose to give the therapeutic action. Tramadol is a potent analgesic effective in the treatment of mild to severe pains. However, the use of the drug can pose a threat to other organs and systems. Therefore, this study was designed to investigate the effect of tramadol injection as dosage form on reproductive parameters in male rabbits. Tenth rabbits were randomly divided into two equal groups (each group five rabbits). The first group was used as a control. The second group was used to study the effect of tramadol (50 mg/kg body weight) for six weeks. Results obtained showed that tramadol significantly ($P<0.05$) decreased libido (by increasing the reaction time), ejaculate volume, sperm concentration ,total sperm output, sperm motility (%), total motile sperm per ejaculate (TMS), packed sperm volume (PSV), total functional sperm fraction (TFSF), normal and live sperm and semen initial fructose. While initial hydrogen ion concentration (pH) and dead and abnormal sperm were increased ($P<0.05$). Live body weight (LBW) and relative weights of testes (RTW) were significantly ($P<0.05$) decreased. Concentrations of thiobarbituric acid-reactive substances (TBARS) were significantly ($P<0.05$) increased in plasma of rabbits treated with tramadol compared with control. The study showed the harmful effects of tramadol on the reproductive performance on male rabbits.

Keywords: Tramadol Injection; Rabbits; Semen; Testosterone

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Cite this article as: Abtisam SA, Osama HA, Nosiba EB, et al. 2021. Tramadol injection as dosage form -Induced deterioration in reproductive performance of male rabbits. Open J Pharm Sci Res. 3: 46-55.

Introduction

Injections are commonly used when a quick result is required some medications may provide further benefits in addition to speed when injected. For example, a pain relief medication that's injected will better target the affected area, whereas a tablet will only provide general pain relief and may cause certain side effects, such as fatigue. A tablet pain reliever may also need to be taken regularly for a person to experience results, whereas the pain relief from an injection may last several weeks or months. Analgesics are a group of drugs that act in different ways on the central nervous system to analgesia and relief from pain [1]. In moderate and severe pain cases, opioid analgesic drugs are mainly used [2]. Opioid analgesic drugs might be obtained from natural sources (e.g. morphine, codeine) or from synthetic sources as Tramadol, heroin [3]. Tramadol hydrochloride is narcotic-like pain reliever drug centrally effective analgesic used for acute pain conditions include neuropathic, cancer and postoperative surgical pain [4].

Figure 1: Tramadol injection.

Nowadays, tramadol is widely used as an analgesic drug in human medicine [5]. Its mechanism of action is based on the inhibition of ascending pain to the central nervous system by its binding to μ- opiate receptors and inhibit the reuptake of norepinephrine and serotonin [6]. Continuous tramadol administration leads to the appearance of its toxic effects on various organs of the body [7]. Tramadol has toxic effects on the structure and function of hepatic, renal and testicular tissues of male albino rats [8]. In addition, the neurotoxicity of tramadol has been reported by [9]. High doses of tramadol cause neuronal degeneration in the rat brain [10] and alter brain neurotransmitter levels [11]. Tramadol can also bring on feelings of intense euphoria, which may be what prompts some individuals to start abusing it. Tramadol abuse among adolescents becomes a widely spread all over the world [12]. Generally, opioids are used as analgesic drugs without considering the several side effects already known. One of the side effects that is rarely considered is hypogonadism [13]. In recent times, it has been observed that intrathecal and oral opioids are capable of suppressing testosterone secretion throughout their period of administration [14]**.** Opioids, both endogenous and exogenous, modulate gonadal function primarily by acting on opioid receptors in the hypothalamus [15], inducing the decreased release or disruption of the normal pulsatility of gonadotropin releasing hormone secretion. This results in a reduction in the release of the luteinizing hormone (LH) and follicle-stimulating hormone (FSH) from the pituitary gland and of testosterone or estradiol (E2) from the gonads. Opioids can also have direct effects on the pituitary gland and the testes [16]. Tramadol abuse among

DOI: https://doi.org/10.36811/ojpsr.2021.110014 OJPSR: November-2021: Page No: 47-55

adolescence has many health and social consequences Adolescence is a critical period for neurodevelopment. Exposure to addictive substances during this period leads to various alterations in brain histological structure that can be translated into functional consequences throughout life [17]. Although tramadol is a weak opioid, its long-term use may result in wide psychiatric and physical symptoms. The chronic administration of tramadol is associated with oxidative stress, inhibition of neurogenesis, apoptosis and mitochondrial dysfunction [18]. The frontal lobe control emotions, behavior, attention, judgment and plays a key role in future planning including self-management and decision-making Tramadol abuse lead to behavior and emotional disturbances, loss of confidence, neglect healthy social interactions and education regression [19]. The present study was designed to study the effects of tramadol in male rabbits on the reproductive performance.

Materials and Methods

In this study tramadol was purchased from pharmacy alsalam hospital in El -Beida-Libya. The dosage of tramadol was 50 mg/kg BW each other day [20]**.** The animals were weighted and received the calculated dose of the drug according to their weight. Male New Zealand White rabbits (age of 6 months and initial weight of 1.892±50.79kg) were used. Animals were individually housed in cages and weighed weekly throughout 6-weeks experimental period. Rabbits nourished pellets which comprised of 30% berseem (Trifolium alexandrinum) roughage, 25% yellow corn, 26.2% wheat bran, 14% soybean supper, 3% molasses, 1% $CaCl_2$, 0.4% NaCl, 0.3% blend of minerals and vitamins, and 0.1% methionine. The vitamin and mineral premix per kg contained the taking after IU/gm for vitamins or minerals: vit A-4000,000, vit D3-5000, 000, vit E-16,7 g, K-0.67 g, vit B1-0.67 g, vit B2-2 g, B6-0.67 g, B12-0.004 g, B5-16.7 g, Pantothinc acid-6.67 g, Biotein-0.07 g, Folic acid-1.67 g, Choline chloride-400 g, Zn-23.3 g, Mn-10 g, Fe-25 g, Cu-1.67 g, I-0.25 g, Se-0.033g, and Mg-133.4 g (Rabbit premix created by Holland Nourish Connect. Co.).The chemical analysis of the pellets [19] showed that they contained 15.8 % crude protein, 11.3 % crude fiber, 3.7 % ether extract, 7.2 % ash, 92.9 % organic matter and 62.4 % nitrogen free extract % as DM basis.

A total of 10 mature male rabbits were randomly divided into two equal group. Group 1 served as control, while groups 2 was given tramadol (50 mg/kg body weight). The doses of tramadol was calculated according to the animal's body weight on the week before dosing. The proper doses of tramadol for each animal were placed into a syringe that was inserted orally with the help of plastic tube directly into the oesopharyngeal region. Semen collection was done weekly and continued throughout the 6-week experimental period. Ejaculates were collected using an artificial vagina and a teaser doe. The volume of each ejaculate was recorded (utilizing a graduated collection tube) after takeoff of the gel mass. A weak eosin solution [20] was used for evaluation of sperm concentration by the improved Neubauer haemocytometer slide (GmbH+Co., Brandstwiete 4, 2000 Hamburg 11, and Germany). Add up to sperm yield calculated by increasing semen ejaculate volume and semen concentration. Determination of initial fructose concentration in seminal plasma was determined immediately after semen collection according to [21]. Assessments of dead and normal spermatozoa were performed using an eosin-nigrosine blue staining mixture [22]. The percentages of motile sperm were estimated by visual examination under low-power magnification (10×) using light microscope. Add up to number of motile sperm was calculated by duplicating the rate of motile sperm and add up to sperm yield. Reaction time was chosen as the diminutive of subjecting a doe to the buck until the completion of erection; it was measured in seconds.

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Starting hydrogen particle concentration (pH) was determined immediately after collection using pH cooperative paper (Universalindikator pH 0-14 Merck, Merck KgaA, 64271 Darmstadt, Germany). Packed sperm volume (PSV) was recorded. Add up to useful sperm division (TFSF) was calculated as the item of add up to sperm yield, motility (%), and ordinary morphology (%) [23. Plasma thiobarbituric acid-reactive substances (TBARS) were measured by the strategy of [24. The relative weight of organs (%) was calculated as g/100 g body weight. Serum was obtained by centrifugation of blood samples at 860×g for 20 min, and was stored at (–20^{oC}) until used for analysis Testosterone hormone concentration were assayed by using commercial kit that was supplied by Coat – A – Count testosterone RIA, from Diagnostic Systems Laboratories (DSL), from Texas, USA. Statistical analysis: Where applicable, statistical analysis was carried out in Minitab software (version17) statistical significance was assessed using ANOVA analysis with Tukey multiple comparison test after detection normal distribution to the information and suitable $P < 0.05$ consider critical.

Results

Observation of animal's tramadol-fed rabbits showed varying degrees of clinical signs few days after dose. The testicular weight in group tramadol was significantly lower than control group (Figure 1).

Figure 2: Morphology effect of tramadol after 6weeks on testes of male rabbits.

Table 1: The overall means (±SE) of body weight, relative testes weight , blood plasma testosterone concentration and Thiobarbituric acid-reactive substances (TBARS) in plasma and testes during treatment of male rabbits with tramadol.

Parameters	Animal Groups	
	Control	Tramadol
BW (g)	1892± 50.79^{a}	1529± 64.85^{c}
RTW (g/100 g BW)	4.432± 0.486^{ab}	2.774± 0.424^{b}
Testosterone (ng/mL)	1.570± 0063^{b}	0987± 0112^{c}
TBARS (nmol/ml)	2.673± 0.025^{a}	3.113 ± 0.087^{a}
Testes TBARS (nmol/g tissue)	14.9±1.39^{b}	28.5±0.25^{a}
^{abc} Within row, means with different superscript letters differ significantly ($p<0.05$).		

DOI: https://doi.org/10.36811/ojpsr.2021.110014 OJPSR: November-2021: Page No: 47-55

Body weight (BW) relative weight of testes and testosterone were significantly (P<0.05) decreased in rabbits treated with tramadol compared to control animals (Table 1). Results obtained showed that tramadol significantly (P<0.05) decreased libido (by increasing the reaction time), ejaculate volume, sperm concentration, total sperm output, sperm motility (%), total motile sperm per ejaculate (TMS), packed sperm volume (PSV), total functional sperm fraction (TFSF), normal and live sperm and semen initial fructose. While initial hydrogen ion concentration (pH) and dead and abnormal sperm were increased (P<0.05). Live body weight (LBW) and relative weights of testes (RTW) were significantly (P<0.05) decreased. Concentrations of thiobarbituric acid-reactive substances (TBARS) were significantly (P<0.05) increased in plasma of rabbits treated with tramadol compared with control (Table 2).

Table 2: The overall means (±SE) of semen characteristics during treatment of male rabbits with tramadol.

Parameters	Animal Groups	
	Control	Tramadol
Ejaculate volume (ml)	0.75 ± 0.024^{ab}	0.70 ± 0.024^{b}
PH	7.84 ± 0.032^{b}	8.08 ± 0.074^{a}
Reaction time (s)	4.00 ± 0.79^{b}	5.13 ± 0.33^{a}
Packed sperm volume (%)	14.6 ± 0.17^{ab}	13.34 ± 0.22^{c}
Sperm concentration ($\times10^{6}$ ml^{-1})	248 ± 5.7^{b}	237 ± 5.3^{b}
Total sperm output ($\times10^{6}$)	185 ± 6.5^{b}	166 ± 6.9^{b}
Sperm motility (%)	$66.2.\ \pm0.95^{b}$	62.5 ± 1.1^{b}
Total motile sperm ($\times10^{6}$)	123 ± 5.0^{b}	104 ± 4.6^{b}
Live sperm (%)	73.7 ± 1.2^{b}	65.8 ± 1.24^{a}
Dead sperm (%)	26.3 ± 1.19^{b}	34.2 ± 1.24^{a}
Normal sperm (%)	82 ± 0.5^{a}	78 ± 0.9^{b}
Abnormal (%)	18 ± 0.2^{b}	22 ± 06^{a}
Total functional sperm fraction ($\times10^{6}$)	100 ± 4.2^{b}	82 ± 42^{c}
Initial fructose (mg/dl)	264 ± 5.2^{b}	229 ± 5.8^{a}
^{abc}Within row, means with different superscript letters differ significantly (p<0.05).		

Discussion

Tramadol addiction and abuse is now a major public health menace in libya. The rampancy of tramadol abuse and the consequential leap in crime in libya led to Government banning the manufacture, sale and use of tramadol. The design of the current study investigated the short- and long-term implications of tramadol abuse on semen quality indices using an animal model. Tramadol abuse has increased in the Middle East region, tramadol use was common among adolescents and over one third of tramadol users had drug-related problems [17]. This study has shown that the treatment of rabbits with tramadol caused significant reduction in body weight of rabbits at a later stage during the treatment period. This suggests that tramadol increases the catabolism of lipids in the adipose tissue, resulting in significant reduction in body weight of rabbits at a later stage during the treatment period. Similar results were reported by [25]in Persea americana leaf extractstreated rats. Results obtained revealed that tramadol treatments had significant effect on the body weight, sperm count and testicular integrity which agrees with the findings of [26-28] who reported that tramadol caused disorganization of the seminiferous tubules with almost missing of sperm and comparatively decreased spermatogenic cells. Results obtained are also in line with previous report on the gonadotoxic

DOI: https://doi.org/10.36811/ojpsr.2021.110014 OJPSR: November-2021: Page No: 47-55

effects of tramadol in male animal models by [29,30] who reported that tramadol significantly decreased sex hormones and degeneration of spermatogonia, distortion of Sertoli cell tight junctions, and accumulation of electron-dense bodies in Sertoli cells. Histological findings clearly revealed that the normal architecture and integrity of the testes of tramadol treated animals were altered causing atrophy and necrosis of the spermatogenic cells (Sertoli and Leydig cells). Sertoli cells are considered as nursery units for the developing sperms [31]. Also, [32] reported that Sertoli cells are major supporters of spermatogenesis and germ cells because of the secretion of proteins such as core protein histone, androgen binding protein, and androgen binding protein–heat shock protein, Ncadherin, and desmoglein. The Leydig cells play an important role in the function and structure of seminiferous tubules and in the synthesis of testosterone, which is vital for the regulation of spermatogenesis. Reduced intra-testicular testosterone results in apoptosis of germ cells [33,34] added that steroid biosynthesis is a multistep process controlled by pituitary hormones, and this process is accelerated by the hormone dependent organelle communication network mediated by protein-to-protein interactions and inter-organelle trafficking, resulting in the efficient and timed delivery of cholesterol into the mitochondria for steroid synthesis. Therefore, reduced steroidogenesis results in altered spermatogenesis and spermatic failure. This could be the underlying cause of the significant reduction in the sperm count and weight of testes observed in tramadol treated animals. This assertion is corroborated by [35,36]. More so, [37] reported that the distortion in fertility in male mammals is directly correlated with the disruption of spermatogenesis and the hormone regulatory mechanisms and pathways.Also, Tramadol caused significant reduction in sperm motility. This suggest that the drug has an inhibitory effect on fertilizing capacity, since it has been reported that sperm motility is of importance with regard to sperm fertilizing capacity [38]. Similar report was given by 18 in rats treated with Pueraria tuberosa root extract. Youths are known to continue using these drugs despite the major risk behaviours with its accompanied physical and mental health complications [39].

Studies have also shown that increased reactive oxygen species (ROS) levels and oxidative stress correlates positively with decreased sperm parameters [40-42], where it reported that oxidative stress causes significant damage to biological molecules such as lipid peroxidation, DNA damage and testicular histopathology as well as decline in sperm quality. It has also been reported that tramadol suppresses testosterone by inducing nitric oxide (NO) [43]. A wellcharacterized consequence of NO compounds is the reduction in steroidogenic enzyme activities [44]. Inhibition of LH stimulated steroidogenesis may be reinforced by NO in Leydig cells [45]. Excessive NO production might inhibit the production of testicular adenosine 3, 5-cyclic monophosphate, which helps to transport cholesterol to the inner mitochondrial membrane, culminating in lower testosterone release [46]. All these reports suggest that the degenerative changes in germ cells observed in this study might be attributed to hormonal deficiency. A significant decrease in testosterone levels were seen in this study which correlates with [47] suggesting that long term opioid therapy results in clinically relevant suppression of both hypothalamopituitary-adrenal and –gonadal axes with suppression in testosterone, estrogen and cortisol. This effect as seen in this sub-acute study may be attributed to suppression of the hypothalamic-pituitary-gonadal axis (HPG axis) by opioids [48,49]. Testosterone and estrogen (the male and female principal sex hormones) are produced by the testes and ovaries respectively by the action of FSH and LH (referred to as gonadotropins) which are produced by the pituitary gland following the stimulation of gonadotropinreleasing hormone (GnRH) which in turn is produced by the hypothalamus. It is expected that as the level of sex hormones rises

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a negative feedback loop should trigger the hypothalamus to reduce production of GnRH.

Conclusion

Parenteral products are liquid preparations administered directly into body tissues rather than via the oral route. “Parenteral” is itself derived from the Greek words para (beside) and enteron (the intestine) and most often refers to either subcutaneous (SC), intramuscular (IM), or intravenous (IV) administration of drugs. Liquid preparations administered by injection are characterised by three qualities possessed by no other type of pharmaceutical dosage form: sterility, freedom from pyrogens, and particulate matt. The findings of the study provide substantial evidence that tramadol injection as dosage form has an adverse effect on sperm profile and reproductive organs of male albino rabbits in terms of body weight, weight of testes, TBARS, sperm count, sperm viability, sperm motility as well as sperm head abnormalities, it is recommended that moderation should be exercised in the consumption of this drug by those taking it for therapeutic purpose.

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