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Research Article

EFFECT OF KETAMINE ADMINISTRATION ON TESTICULAR FUNCTIONS IN ALBINO RATS

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ABSTRACT

Ketamine, a composed mixture of (S)- and (R)-ketamine is used for anesthesia in clinical purpose since 1970. In the present study, effect of Ketamine administration on testicular functions like biochemical, gravimetrical and histological analysis has been studied. Three groups of healthy adult colony breed male albino rats having six rats maintained in each group were used for the experimental studies. The rats of groups II and III were administered Ketamine, intraperitoneally, at the dose level 1 and 3 mg/100 gm body weight respectively daily between 10:00 and 11:00 am for 21 days and group I was maintained as control. After the experimental duration, the rats were sacrificed and studied their gravimetric, biochemical and histological analyses of testis. In the results, testicular weights of the rats of group II and III showed significant reduction, histological, biochemical changes were also observed which marked cytotoxicity and inhibition of testicular functions. Histometric changes of testis diameter and surface epithelial cell height were reduced significantly. Biochemical changes are analogous to the gravimetric results. The protein and cholesterol contents are elevated significantly with the graded dose of Ketamine administration. While, in the gravimetric analysis of accessory organs like epididymis, vas deferens, seminal vesicle and prostate gland weights were decreased significantly due to the administration of Ketamine and exhibited as endocrine disruptive drug.

Keywords: Accessory organs, Biochemical, Histormetric, Gravimetric, Ketamine, Testis.

1. INTRODUCTION

Ketamine drug is characterized for its dissociative anesthetic activities; it exhibits analgesic, anti-inflammatory and antidepressant properties. The drug has been proved to have therapeutic effect according to dose, mode of administration and the time schedule during the useful application [1]. Ketamine induces general anesthesia and exhibited dissociative effects in animals [2] and humans [3]. Furthermore, ketamine is also used as a potential adjunct drug for local anesthesia in veterinary and in humans from the last 6 decades [4]. Various experimental evidences were observed for ketamine's role as antidepressant drug since from 1970s. In many preclinical findings, ketamine exhibited similar effects to those of standard administration of classic antidepressant drugs like tricyclic antidepressants and monoamine oxidase inhibitors in rodent's models [5]. Oral administration of ketamine to mice reversed reserpine-induced hyperthermia at the dose of 40 mg/kg and prevented tetrabenazine-induced apoptosis with an ED_{50} of 27.6 mg/kg [5], which mimics classical antidepressants [6]. Early evidence of ketamine's possible antidepressant properties in humans was described in 1973 by Khorramzadeh and Lotfy [7], who reported that intravenous (i.v.) administration of ketamine at the subanesthetic doses of 0.2-1.0 mg/kg (i.v. bolus) resulted in emotional discharge and facilitation of psychotherapy in a cohort of 100 psychiatric inpatients. However, the specific depression symptoms were improved with ketamine administration; it is not well defined in the context of modern diagnostic approach in anesthetic drug as safer in therapeutic applications.

The most common psychoactive effects reported after a single subanesthetic i.v. administration of ketamine include dissociation (distortions in visual, auditory, or somatosensory stimuli, or alterations in the perception of self or time), positive psychotomimetic effects (conceptual disorganization, hallucinations, suspiciousness, unusual thought content), and negative psychotomimetic effects (blunted affect, emotional withdrawal, motor

retardation) [8-10]. Prolonged recreational use of ketamine is associated with urological complications that include dysuria, increased frequency and urgency of urination, incontinence, pain, hematuria, and ulcerative cystitis [11, 12]. So far now only higher dose of intraperitoneal injection of ketamine (20, 40 or 60 mg/kg) every 3 days for 7 times exhibited reproductive toxicity via breaking the hypothalamic-pituitary-testicular equilibrium [13]. Furthermore, Tan et al., [14] demonstrated that chronic administration of ketamine affected the genital system. Hence, current study was planned to explore low dose concentration of Ketamine and its effect on male reproductive system in albino rats.

The objectives of the present work was to evaluate the testicular functions like biochemical, gravimetrical, histological and histometrical changes and also their impact on accessory organs by chronic intraperitoneal administration at the graded dose of Ketamine in male albino rats.

2. MATERIAL AND METHODS

2.1. Experimental animals

Healthy colony breed male albino rats of Wistar strain, weighing 150-180gm, of 60-90 days old were maintained at room temperature of 20-28°C with lighting schedule of 12 h light and 12 h darkness. They were maintained in individual cages and divided in groups each containing six animals and fed with balanced diet as described by CFTRI (Central Food and Technological Research Institute) Mysore, Karnataka, India and water *ad libitum*. The acclimatization of the animals lasted for 7 days before Ketamine administration for the experiments. It was carried out in accordance with ethical regulations for the animal care and use of laboratory animals. The study parameters like gravimetrical, histological, histometrical, biochemical and statistical analysis of testis weights by inducing graded dose of Ketamine through intraperitoneal route.

2.2. Experimental drugs

Ketamine Hydrochloride (Ketam) generic; an opioid analgesic, was purchased as commercial product of Sun Pharmaceutical Industries Ltd, India from local drug houses.

2.3. Experimental groups

The animals were divided into following groups

Group-I: Received 0.2 ml saline/100 g body weight i.p. for 21 days and served as control.

Group-II: Received 1 mg Ketamine/100 g body weight i.p. for 21 days in 0.2 ml saline.

Group-III: Received 3 mg Ketamine/100g body weight i.p. for 21 days in 0.2 ml saline.

All the animals were sacrificed by cervical dislocation after 24 h of the last injection. The testis were dissected out immediately and separated from adherent tissue, weighed up to the nearest mg on electronic balance to determine gravimetry. Organs from one side of each animal were fixed in Bouin's fluid for histological studies. They were embedded in paraffin, sectioned at 5 μ, stained with Ehrlich hematoxylin and Eosin. The micrometric measurements like diameter of testis, its epithelial cell height were made from randomly chosen 20 sections appearing round at cross sections from each group using ocular and stage micrometers [15]. Spermatogenic elements count was made from randomly chosen 20 round cross section, taken from the middle part of the testis [16]. Organs from other side were used for biochemical estimations of the protein content [17], total cholesterol [18] and glycogen [19] of testicular tissues were estimated.

2.4. Experimental statistical analysis

All the values were statistically analysed by Student's-'*t*' test using SPSS (19.0.1.). Data are expressed as the Mean + S.E. Statistical significance was set at $p<0.05$ and $p<0.0$ 1[20].

3. RESULTS

3.1. Changes in the testis

3.1.1. Gravimetric changes in testis

Administration of the 1mg/100g body weight of Ketamine significantly decreased ($P<0.05$) the weight of testis, whereas 3mg/100g body weight was reduced the testis weight significantly ($P<0.01$), when compared to control rats (Table 1 & 2).

3.1.2. Gravimetric changes in accessory organs

Administration of the 1mg/100g body weight of Ketamine significantly decreased ($P<0.05$) the weight of epididymis (caput & cauda), vas deferens, seminal vesicle, prostate gland, whereas 3mg/100g body weight reduced all the studied organ weight significantly ($P<0.01$), when compared to control rats.

3.2. Changes in the sperm count

Administration of the 1mg/100g body weight of Ketamine significantly decreased ($P<0.05$) the sperm count, whereas 3mg/100g body weight reduced significantly ($P<0.01$), when compared to control rats.

Table 1: Effect of Ketamine on the weight of testis and accessory organs in mature rats

Groups	Testis weight and accessory organ weight mg/100g body weight						
	Testis (g/100g body wt)	Epididymis		Vas deferens	Seminal vesicle	Prostate gland	Sperm count (million/ml)
		Caput	Cauda				
Control	1.023 ± 0.25	368.16 ± 1.36	287.36 ± 1.23	80.15 ± 1.45	269.49 ± 3.43	105.66 ± 2.45	50.85 ± 2.65
1mg	1.104 ± 0.17*	357.57 ± 1.65*	276.36 ± 1.69*	69.93 ± 1.34*	248.82 ± 2.69*	90.16 ± 3.95*	44.56 ± 3.66*
3mg	1.183 ± 0.22**	356.09** ± 4.23	262.64 ± 2.09**	54.88 ± 1.58**	212.26 ± 3.07**	78.65 ± 2.55**	38.35 ± 2.56**

*Six animals were maintained in each group. M±SE = Mean ± Standard error. *P<0.05, **P<0.01.*

Table 2: Effect of Ketamine on histometric changes in the testis of male rats

Groups	Diameter of testis		
	Testis (µm)	Seminiferous tubules (µm)	Leydig cells (µm)
Control	6025.00 ± 9.45	303.25 ± 3.25	6.55 ± 1.45
1mg	6001.15± 4.85*	293.45 ± 3.45*	5.45 ± 1.55*
3mg	5890.45 ± 7.35**	289.55 ± 2.55**	4.95 ± 1.05**

*Six animals were maintained in each group. M±SE = Mean ± Standard error. *P<0.05, **P<0.01.*

3.3. Histological changes

The histological changes in the testis were exhibited as a significant reduction in the number of spermatogonia, spermatocytes and spermatids. Necrosis in tubular epithelial cells and shrinkage of sertoli cells were recorded. None of the spermatozoa were observed in the lumen of seminiferous tubules in the Ketamine administered rats. The Leydig cells in both the dose administered groups showed significant degeneration (Fig. 1).

3.4. Histometric changes

A significant (P<0.05) reduction in the diameter of testis was observed due to the administration of 1mg/100g body weight, whereas, 3mg/100g body weight reduced the diameter of testis highly significant (P<0.01) when compared to control rat groups (Fig. 1).

The diameter of seminiferous tubules were reduced significantly (P<0.05) at the dose 1mg/100g body weight and highly significant (P<0.01) at 3mg/100g body weight of Ketamine administration, when compared to control rat groups.

The diameter of Leydig cell nucleus is reduced significantly (P<0.05) at 1mg/100g body weight and highly significant (P<0.01) with 3mg/100g body weight of Ketamine administration, when compared to control rat groups.

3.5. Biochemical changes

There is decrease in the protein and glycogen content of testis significantly (P<0.05) due to the 1mg/100gm body weight of Ketamine administration, whereas cholesterol content is increased significantly (P<0.05) at 1mg/100gm body weight. The administration of Ketamine at 3mg/100g body weight decreased significantly (P<0.01) protein and glycogen content of testis whereas in total cholesterol content increased significantly (P<0.01) when compared to control rat groups (Table 3).

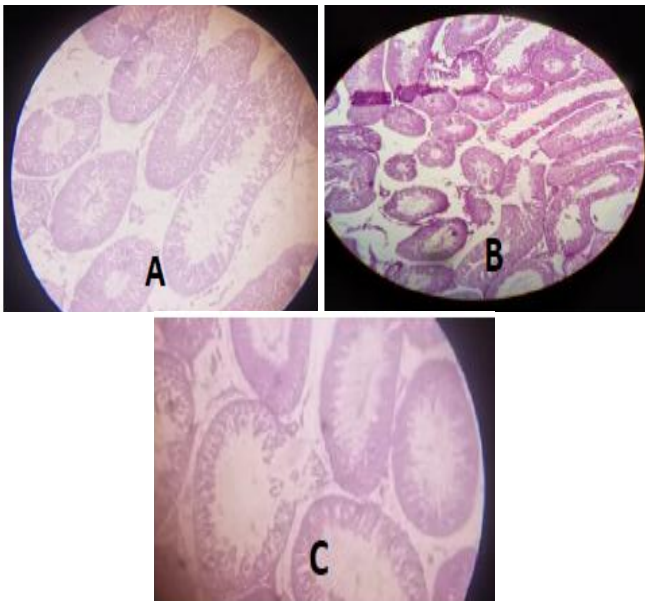

Fig. 1: Cross section of rat testis

A. Cross section of the testis of control rat showing normal spermatogenic elements with the presence of all types of spermatogenic and Leydig cells.

B. Cross section of the testis rat administered with 1mg/100gm body weight Ketamine drug showing reduction in the tubular diameter, number of spermatogenic elements and degenerating Leydig cells.

C. Cross section of the testis of rat treated with 3mg/100gm body weight Ketamine drugshowing reduction in the tubular diameter, number of spermatogenic elements and enhanced degeneracy of Leydig cells.

Table 3: Effect of Ketamine on biochemical changes in the testis of male rats

Groups	Biochemical changes		
	Protein (µg/mg)	Cholesterol (µg/mg)	Glycogen (µg/mg)
Control	71.24 ± 2.14	7.08 ± 2.15	1.11 ± 1.01
1mg	55.05 ± 1.15*	8.95 ± 1.95*	0.90 ± 0.98*
3mg	45.25 ± 2.45**	11.55 ± 2.23**	0.68 ± 0.55**

*Six animals were maintained in each group. M±SE = Mean ± Standard error. *P<0.05, **P<0.01.*

3.6. Sperm morphology and number

The caudal epididymis sperms of normal rat exhibited sickle shaped head and straight tail piece morphologically. But in Ketamine administered rats, the sperm morphology were abnormal as their head part reduced and the tail is wrinkled or coiled. A significant reduction in sperm population were observed in both the doses of Ketamine administration, whereas in the sperm count of cauda epididymis with 1mg Ketamine was significantly reduced ($P<0.05$) and exhibited highly significant ($P<0.01$) reduction at the 3mg Ketamine administration when compared to control rat groups (Table 1).

4. DISCUSSION

Sperm maturation is a function of physiological process whereas spermatozoa exhibit fertilizing potential during their transitional approach by the epididymis. Recent studies observed that androgen-dependent activity is mediated by factors of epididymis during the endocrine function regulation [21]. In the current study, Ketamine drug administration inhibited the testicular functions to exhibit antispermatogenic potential due to the indication of the significant decrease in the weight of testis and their diameter, number of spermatogonic elements like spermatogonia, spermatocytes and spermatids activities, expressing inhibition ultimately due to lack of pituitary gonadotrophins, especially follicle stimulating hormone, which is critically helpful in spermatogenesis process [22, 23].

The increased level of cholesterol indicates the non-utilization of hormones and these are the precursors for steroidogenesis which may alter the availability of gonadotrophins inhibition such as LH or FSH which are essential to stimulate the germinal epithelium for necessary action [24]. The glycogen content in the testicular cells like Leydig, sertoli, and spermatogonial cells indicates the storage of energy, they also secrete substrates from the blood stream and they provide reserved carbohydrates energy source for seminiferous tubules and the glycogen content has remarkably held responsible for synthesis of steroidal hormones for all the normal functions [25]. The decreased content of glycogen in the testis due to the Ketamine administration might be correlated with the reduction in spermatogenic number due to lower level of glycogen source for spermatogenic process. Furthermore, the testosterone hormone plays a significant role in sperm maturation, behavioral index and maintenance of all the accessory reproductive organs [26, 27]. Administration of Ketamine has significantly affected the spermatogenesis, steroidogenesis and androgen production inhibition and also it may alter the reproductive parameters to exhibit as antifertility agent. The two graded concentration of Ketamine, found effective, highly significant at the 3mg/100g body weight is potent in causing antispermatogenic and antisteroidogenic property. The findings of Patil et al., [28] with various pharmaceutical drugs like morphine, nicotine, pentazocin eand pethidine reported for their antispermatogenic and androgenic activities, such remarks were contributed by Londonkar et al., [29-31] in the administration of various opiod and non-opoid CNS drugs in male albino mice and rats. Opioids are observed acting through the hypothalamus function inhibitionby the release of GnRH and CRF and it has decreased the concentrations of LH, FSH and ACTH circulation [32].

5. CONCLUSION

These research findings state that long term administration of Ketamine decreased the testicular functions and produced deleterious effects to the male reproductive system of experimental rats. Hence, it has been advised that such drug should be used with very low level of concentration with all the major precaution and dose duration should be minimized to avoid its significant adverse inhibitory effects on male fertility, which also interrupts the mechanism of action in reproduction.

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Conflict of interest

The authors declare no conflict of interest.

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