

ORIGINAL ARTICLE

Oral ketamine induced pathological changes of the urinary tract in a rat model

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Abstract

Introduction: In recent years, prolonged ketamine abuse has been reported to cause urinary tract damage. However, there is little information on the pathological effects of ketamine from oral administration. We aimed to study the effects of oral ketamine on the urinary tract and the reversibility of these changes after cessation of ketamine intake. **Methods:** Rats were fed with illicit (a concoction of street ketamine) ketamine in doses of 100 (N=12), or 300 mg/kg (N=12) for four weeks. Half of the rats were sacrificed after the 4-week feeding for necropsy. The remaining rats were taken off ketamine for 8 weeks to allow for any potential recovery of pathological changes before being sacrificed for necropsy. Histopathological examination was performed on the kidney and urinary bladder. **Results:** Submucosal bladder inflammation was seen in 67% of the rats fed with 300 mg/kg illicit ketamine. No bladder inflammation was observed in the control and 100 mg/kg illicit ketamine groups. Renal changes, such as interstitial nephritis and papillary necrosis, were observed in rats given illicit ketamine. After ketamine cessation, no inflammation was observed in the bladder of all rats. However, renal inflammation remained in 60% of the rats given illicit ketamine. No dose-effect relationship was established between oral ketamine and changes in the kidneys. **Conclusion:** Oral ketamine caused pathological changes in the urinary tract, similar to that described in exposure to parenteral ketamine. The changes in the urinary bladder were reversible after short-term exposure.

Keywords: ketamine, inflammation, kidney, bladder, rat

INTRODUCTION

Ketamine is a well-known anesthetic drug used predominantly in veterinary settings. Ketamine has the ability to cause hallucinations and sensory distortion. Hence in the past several years, it has been more popularly known as a choice for abuse among young partygoers. The long-term use of ketamine especially abuse of ketamine has been confirmed to cause urinary tract damage. This was first described by Shahani *et al* in 2007 and approximately 20-30% of ketamine abusers are noted to have this problem.¹⁻⁴

The clinical manifestations of ketamine-abuse are lower urinary tract symptoms with a decreased bladder capacity caused by chronic bladder inflammation.^{1,4,5} Moreover in the progressive stages of the disease, hydronephrosis, papillary necrosis and renal impairment are the common

clinical features.² Although the clinical symptoms are well established, the exact underlying mechanism is still unknown.

The most postulated mechanism has been the disruption of the urothelial barrier leading to the leakage of urine constituents that may lead to chronic inflammation and urinary dysfunction. However, this is most likely to be a consequence of ketamine cystitis rather than the cause based on findings from Rajandram *et al* (2016).⁶ Therefore, the need to investigate other possible mechanisms that may be involved is crucial as the development of proper treatment for the disease will be hindered if no clear mechanism is established.

Ketamine may have direct microvasculature toxicity, which causes ischaemia and fibrosis or neural toxicity which induces nerve fibre

degeneration.^{6,7} Ketamine may also cause an autoimmune reaction, which results in bladder inflammation.⁶ It was originally suspected that these inflammatory changes were contributed by impurities present in street ketamine. However, studies with prolonged intraperitoneal exposure of pure ketamine injection in mice yielded the typical urinary tract pathological changes as described in human subjects who abused ketamine for a prolonged period.^{7,8} Although less commonly reported, patients on ketamine for chronic pain could also be similarly affected.⁹ Currently, ketamine cessation is the only known effective management strategy.^{10,11} Patients who discontinue ketamine use may experience symptomatic relief. However, the potential for reversing the pathological changes in affected organ systems upon stopping ketamine is uncertain.

Currently, there is no report on pathological changes in the urinary tract after prolonged oral ketamine exposure as all animal studies to date involved parenteral administration of pure ketamine. There is also a lack of studies on illicit ketamine tablets which often contain adulterants such as caffeine.¹² Our study aimed to examine the histopathological changes in the urinary tract of rats administered with oral ketamine (illicit combination). The study was extended to determine the potential recovery from the pathological changes after cessation of ketamine intake.

MATERIALS AND METHODS

Animals

Animal experimentation was approved by the Animal Use and Care Committee, University of Malaya. Eight-week old rats (male, Sprague-Dawley) were obtained from the University of Malaya Laboratory Animal Centre. The rats were housed in aluminium cages with shredded recycled paper as bedding. They were maintained on rat feed supplied by Specialty Feeds Pty. Ltd. (Australia) and water *ad libitum*. The rats were allowed to adjust to their new surrounding environment for two days before the start of the study.

Chemicals and drug administration

An illicit ketamine combination (38% ketamine hydrochloride, 55% caffeine, 7% magnesium stearate and coloring) were supplied by the Malaysian National Chemistry Department Laboratory. The composition of the illicit

ketamine was determined from a sample seized by the police. The ketamine was dissolved in distilled water and administered to the rats orally with a gavage feeding needle (Animal feeding needle, 18Ga, Cadence Science, USA). Feeding was carried out daily for four weeks and rats were weighed twice a week for dose adjustment.

Treatment and dosage

In the treatment groups, rats were randomly assigned into groups fed with 100 mg/kg or 300mg/kg of illicit ketamine combination. With this assignment, there were two treatment groups, i.e., two groups on the stated doses of illicit ketamine, with 12 rats per dosage group. For each treatment group, half of the rats were euthanized after four weeks while the other half were kept alive to observe any effect after eight weeks of ketamine cessation. Control rats (N=15) were given distilled water instead of ketamine.

Tissue sampling

Rats were euthanized with carbon dioxide inhalation. The kidneys, ureters and bladder were then removed and fixed overnight with 10% formalin in 0.1 M phosphate buffer. Subsequently, specimens were processed with increasing concentration of ethanol, three changes of xylene and paraffin embedding. Tissues embedded in paraffin blocks were then sectioned and slides stained with haematoxylin and eosin. The slides were then examined for general morphology and histopathological changes using conventional light microscopy.

Immunohistochemistry (CD3 and PAX5)

Heat antigen retrieval was carried out in citrate buffer (0.1 M, pH 6.0). After antigen retrieval, immunohistochemistry was performed using an automated machine with optimized protocols on Ventana's Benchmarck ULTRA (USA). Antibodies used were CD3 (Dako, USA) at 1:200 dilution and PAX5 at 1:20 dilution (Neomarkers, USA). The positive control (human tonsil) and negative control (without primary antibody) were used for each batch of staining.

Statistical analysis

Data analysis was carried out using Fisher's exact test for incidence of inflammation. A p value < 0.05 was considered as statistically significant.

TABLE 1: Incidence of kidney and bladder inflammation

Treatment groups (Dosage)	Incidence of inflammation after 4-week drug exposure		Incidence of inflammation after 8-week drug cessation	
	Kidney	Bladder	Kidney	Bladder
Control	0/9	0/9	0/6	0/6
Illicit ketamine 100 mg/kg	6/6*	0/6	3/5	0/5
Illicit ketamine 300 mg/kg combination	2/6	4/6*	3/5	0/5

*p<0.05 significantly different from the control using Fisher’s exact test

RESULTS

Bladder

Mild to moderate chronic bladder inflammation was observed in 66.7% (4/6) rats given 300mg/kg illicit ketamine (Table 1) (Fig. 1a). Inflammatory cells consisting of mainly lymphocytes were seen in the bladder submucosal layer. In comparison, there was no inflammation in the bladders of the 100 mg/kg illicit ketamine dosage and control groups of rats (Fig. 1b). Taken as a whole group, regardless of the dosage, bladder inflammation

was present in 33.3% (4/12) of the rats in the illicit ketamine groups. Inflammatory aggregates or clusters in the bladder of rats treated with 300 mg/kg illicit ketamine showed positive immunostaining for CD3, which is a T cell marker, as well as for PAX5, a B cell marker (Fig. 2).

Kidney

No histological change was detected in the kidneys of rats in control groups (Fig. 1c). However, in rats fed with illicit ketamine,

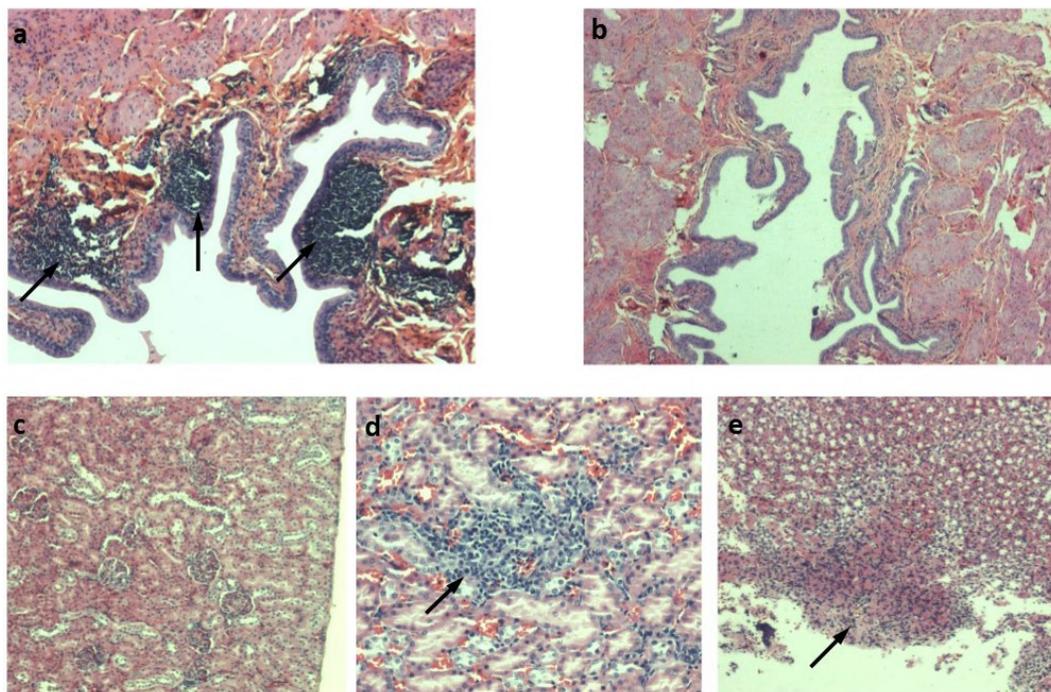


FIG. 1: Haematoxylin and eosin staining of the bladder and kidney. The arrows indicate infiltration of inflammatory cells. (a) Bladder section from a rat in the 300 mg/kg illicit ketamine combination group with focal aggregates or clusters of predominantly lymphocytes in the submucosa. 40x. (b) Bladder section from a control rat revealing normal histology. 20x. (c) Kidney section of a control rat showing normal tubules and glomeruli. 40x. (d) Presence of mononuclear inflammatory cells in between tubules in a rat treated with 100 mg/kg illicit ketamine combination. 100x. (e) Renal papillae with evidence of fibrinoid necrosis between the tubules in a rat treated with 300 mg/kg illicit ketamine. 40x

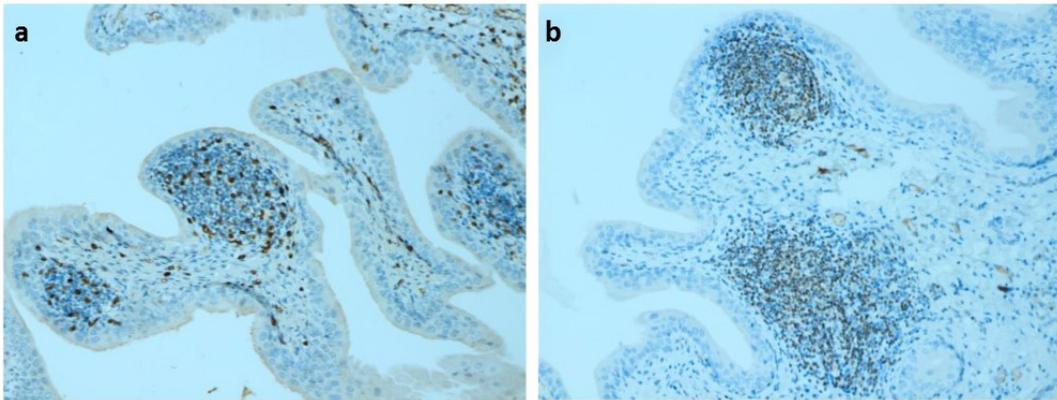


FIG. 2: Positive immunohistochemistry staining of the lymphocyte aggregates in a rat bladder from the 300 mg/kg illicit ketamine combination group. 200x. (a) CD3 staining (b) Pax5 staining

interstitial nephritis developed in six (6/6) of the rats on 100mg/kg dosage ($p = 0.0002$) (Table 1) (Fig. 1d). Glomeruli and tubules appeared normal. Papillary necrosis was observed in two of the rats (2/6) on the 300 mg/kg dosage (Fig. 1e). In total, inflammatory changes in the kidney was present in 66.7% (8/12) of the rats fed with illicit ketamine.

Rodent body weights and other findings

Figure 3 shows the comparison of average weight gains in rats from the control and

treatment groups (100 mg/kg and 300 mg/kg illicit ketamine) during the 4 weeks treatment period. In weeks 1 and 2, the average weight gains of rats in treatment groups were less compared to the control group. However, there was statistically significant difference only in the 100 mg/kg illicit ketamine group compared to control group in the first week ($p = 0.03$). In weeks 3 and 4, the average weight gains of the illicit ketamine treatment groups have caught up with the control group.

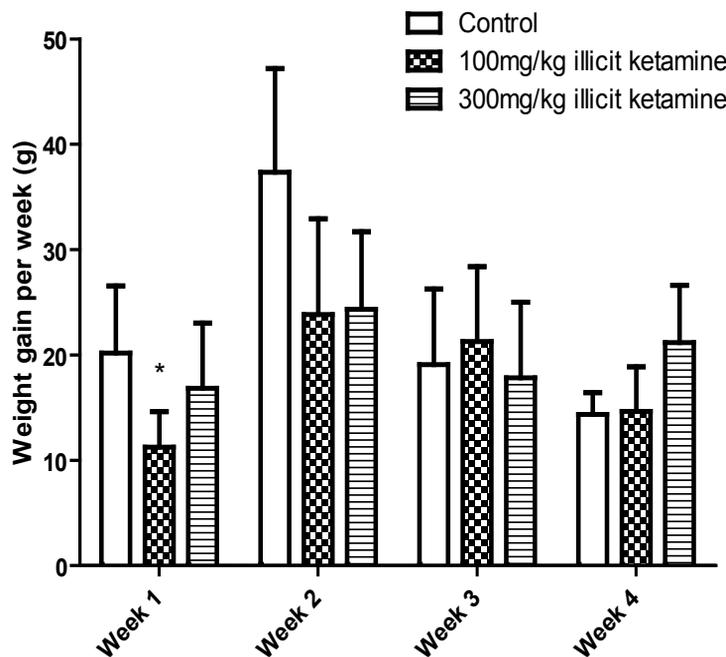


FIG. 3: Comparison of mean weight gains in rats from control and illicit ketamine treatment groups during the treatment period. *Denotes statistically significant difference ($p < 0.05$) compared to the control group

There were two rat mortalities in the groups given 100 and 300 mg/kg illicit ketamine after 3 weeks of treatment (one mortality per dosage group). On the whole, there was no clear dose dependency between ketamine and the development of inflammation in the kidney. No histological change was observed in the ureters of all rats.

After eight weeks without ketamine (in cessation groups), no bladder inflammation was observed but interstitial nephritis was still present in 60% of the rats in the 100 and 300 mg/kg groups (Table 1).

DISCUSSION

The current study is the first to show that oral feeding with illicit ketamine combination causes inflammatory changes in the kidney and bladder in an animal model. Inflammation was observed in the bladder of rats fed with 300 mg/kg illicit ketamine. The pathological changes in the urinary tract appeared to occur in the bladder where urine, along with the ketamine metabolites, is stored for a longer duration compared to the upper urinary tract (ureter). This finding is consistent with the clinical manifestation in ketamine abusers where early symptoms and damage are reported to occur in the bladder.

Rats given illicit ketamine combination developed interstitial nephritis even at lower doses. Although this study was the first to experiment using a concoction of ketamine used in the illicit setting, it was not designed to identify the exact causative agent in the pathogenesis of ketamine associated uropathy. As such, we can only postulate that adulterants in the illicit ketamine might contribute to the pathological changes. Caffeine, present in the illicit combination, has been implicated to increase the risk of analgesic nephropathy although the mechanism is unknown and the evidence inconclusive.¹³ It is postulated that adenosine receptors in the kidney could have a role in this process.¹⁴ Ketamine and caffeine, both inhibitors of adenosine receptors, may interfere with the regulation of glomerular filtration and blood flow in the kidney.^{15,16} However, chronic ingestion of caffeine alone in the rat has been shown to not cause significant pathological changes in the kidney.¹⁴ Whether caffeine potentiates the inflammatory effect of ketamine on the kidney will require further investigation.

Rats with inflammatory changes in the urinary tract in this study showed infiltration

predominantly of lymphocytes. The nature of submucosal infiltrates in the bladder of rats in this study was comparable to the histopathology of patients with interstitial cystitis.¹⁷ However, contrary to the typical histopathological appearance in interstitial cystitis, denudation of the urothelium was not observed in this study. This suggests that, in the early phase of pathological damage, the inflammation may not be caused by a compromised urothelial barrier at the macroscopic level. This was supported in a study by Rajandram *et al* where voiding dysfunction in mice induced by ketamine was not accompanied by disruption of urothelial barrier function.⁶ They postulated that the disruption of the urothelial barrier, and possibly inflammatory response, may be a consequence of stress caused by ketamine induced voiding dysfunction. The presence of CD3 (T cell marker) and Pax 5 (B cell marker) immunostaining suggests that there is a cellular autoimmune response which could be attributed to ketamine and its metabolites.¹⁸

With regards to the microscopic examination of other component cells in the bladder, recent animal studies (all using parenteral ketamine administration) have indicated the importance of neurogenic damage in the pathogenesis of ketamine cystitis. Yeung *et al* demonstrated a loss of cholinergic neurons in rats with prolonged ketamine administration.⁷ Furthermore, dysregulation of purinergic neuron transmission was suggested as a possible cause of ketamine induced bladder or voiding dysfunction in a study by Meng *et al*.¹⁹ All these local neural damages could be a result of ketamine and its metabolites. Future studies in this direction will reveal more information regarding the exact mechanisms of this disorder.

It is worthy of note from this study that bladder inflammation occurred even with short-term exposure to oral ketamine. This has clinical implications for patients who are taking ketamine for chronic pain. In this group of patients, microscopic bladder inflammatory changes could occur with short term use. Thus, it would be best to avoid the use of ketamine in this setting and consider other alternatives. The clinical symptoms of patients with ketamine induced cystitis are potentially reversible with timely discontinuation of ketamine but in patients who have abused ketamine for years or have a severely contracted bladder, the pathological damage is likely to be irreversible.^{2,8} In a report by Mak *et al*, adults taking ketamine more than three times per week over a period

exceeding two years experienced significant changes in their bladder function.²⁰ However, the potential for recovery after abstaining from ketamine for one year has been reported as well.²⁰ This clinical observation was reflected in the current study which demonstrated, for the first time in an animal model, the reversibility of histopathological changes induced by short-term ketamine exposure. Our results showed that bladder morphology normalized after cessation of ketamine for eight weeks. With regards to kidney changes, however, the inflammation still remained in a significant number of the rats fed with illicit ketamine combination. It is likely that kidney inflammation takes longer to resolve. In clinical practice, most patients with drug induced interstitial nephritis have been shown to recover with early intervention or discontinuation of the offending drug although recovery may take a longer period of time.²¹

Regarding the dosage of ketamine administration, 100 mg/kg was a subanaesthetic dose in rats as they caused ataxia while 300 mg/kg caused some rats to be completely immobilized. In rats, central nervous system (CNS) effects could be observed within 10 minutes of oral administration due to the rapid absorption of ketamine through the gastrointestinal tract.²² The CNS effect from oral ketamine is comparable to intravenous ketamine. Overall, there was no clear dose effect relationship between different doses of ketamine and the development of inflammation in the kidney in this study. As ketamine is a basic drug, the ionization status of the drug is a matter of concern as it may affect the rate of drug absorption. This highlighted the wide variability in drug absorption through the gastrointestinal tract. Parenteral administration is likely to give more consistent results in an experimental setting. In humans, the bioavailability of oral ketamine is 16-24% as the drug undergoes massive first pass metabolism, where ketamine is rapidly converted to norketamine.^{23,24} Our study demonstrated that, despite the low bioavailability of oral ketamine, it could still cause pathological damage to the urinary system. This shows that oral ketamine abuse is not any safer than parenteral use.

The limitations of the study include the variable enteral absorption of oral ketamine as pointed out above. In future studies, the blood and urine of rats can be analysed to determine the absorption efficiency along with the amount of ketamine and its metabolites in the urine. The pathogenesis of ketamine related urinary tract damage remains unclear at the present time as

there is a paucity of data in this aspect. Our study had focused on short term ketamine exposure and the observation of pathological changes in the urinary tract after such exposure. This will provide important basic histopathological information on the early changes in oral ketamine related organ damage which will serve as a basis for comparison with long term pathological changes in future studies.

In conclusion, oral ketamine is able to induce inflammatory changes in the urinary tract of rats after four weeks of exposure. Results from illicit ketamine administration suggest that adulterants present in street ketamine could affect the inflammatory response in these organs. Recovery was seen in the bladder after eight weeks of drug cessation while inflammation in the kidney was more persistent. This indicates that ketamine cessation at an early stage is important to prevent irreversible damage to the urinary tract.

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Ethics review

The study complied with the institution's ethical standard for animal experimentation. (Animal ethics approval number: 5/05/05/2010/OT(R))

Conflict of interest

The authors report no conflict of interest.

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