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Long Term Ketamine and Ketamine Plus Alcohol Toxicity - What can we Learn from Animal Models?

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Abstract: This review addressed the adverse effects of the frequently-used recreational drug, ketamine through using mice and monkey models. Our laboratory has documented initially that ketamine can induce the formation of hyperphosphorlated tau (hypertau), which is a hallmark of Alzheimer's disease (AD), in the cerebral cortex of both mice and monkeys as well as apoptosis in neurons in these species. Besides the cerebral cortex, other centers in the central nervous system (CNS) and peripheral nervous system (PNS) are also influenced by ketamine. Cerebellum was found to be down-regulated in both mice and humans after long-term of ketamine administration and it was caused by the apoptosis of Purkinje cells. Deleterious effects in other organs reported in long-term ketamine users include of kidney dysfunction leading to proteinuria, fibrosis of the urinary bladder and reduction in size of the urinary bladder leading to frequent urination, increase of liver fibrosis and cardiac problems such as premature ventricular beats. Moreover, ketamine is usually co-administrated with other chemicals such as caffeine or alcohol. It has been reported increased harmful effects when ketamine was used in combination with the above substances. Mechanisms of damages of ketamine might be due to 1) up-regulation of NMDA receptors leading to overestimation of glutamatergic system or 2) the metabolite of ketamine which was a hydroquinone exerted toxicity.

Keywords: Ketamine, animal, liver, urinary, cerebellum, toxicity, interaction, alcohol.

INTRODUCTION

Ketamine is a cyclohexane (Fig. **1**) which has been employed as an anesthetic. It is a dissociative anesthetic which was widely used in pediatric surgery and veterinary medicine [1,2]. Although its mechanism is far from clear, it has been documented to have an antagonistic effect on excitatory amino acids and interact particularly with NMDA receptors to block these receptors [3]. There are two isomers of ketamine, S and R. The S isomer has a higher affinity to bind to NMDA receptors [4]. In the past ten years, ketamine abuse became very popular in Asia and extensive use of ketamine in rave party has been documented. In Hong Kong alone, as much as 30% of the drug abusers used ketamine as the main recreational agent [5]. As a reasonably inexpensive and abusive drug, ketamine has been used by adults as well as children. This has caused extensive concerns in the community, both locally and in other parts of Asia. Recently, ketamine injection amongst young drug abusers has also been found in the USA [6] and England [7].

Ketamine was introduced to the market in the 60's under the trades names of Vatalar (for animals) and Katalar (for humans). It could be used as an anesthetic, sedative, amnesiac and analgesic [2]. It has a rapid induction when injected intravenously (i.v.) and can be used with propofol, midazolam and xylazine [2]. In the children, ketamine can be administered orally, nasally, rectally and intramuscularly (i.m.) [2], but was frequently used *via* the nose and through the nasal mucosa [8]. Among the drug abusers, most of the ketamine uptake is *via* the nasal mucosa. In the normal adult, ketamine is used as anesthetic in the surgery of high risk patients.

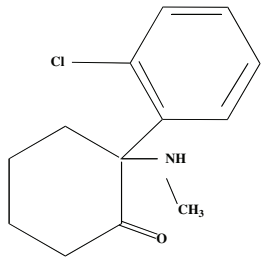

Fig. (1). The molecular structure of ketamine.

Using adult mice and monkeys, many novel studies in the past few years had been conducted by our team and we had studied various regions of the cerebral cortex of both species under the influence of ketamine. Our fMRI studies in the monkey indicated the regions targeted were widespread and

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included the prefrontal cortex, the hippocampus, the fusiform gyrus, the corpus striatum and the basal forebrain (Fig. **2**). As well, novel report by our group on the monkey indicated chronic exposure resulted in hypoactivities of the ventral tegmentum, substantic nigra and the posterior cingulate and cerebellum while hyperactivity resulting from chronic treatment of ketamine was observed in the striatum and entorhinal cortex [9]. Dysfunction of brain areas led to dysfunction of circuitry, followed by genesis of psychiatric disorders, putting burden on the community. In the mouse, regions in the prefrontal cortex, sensorimotor cortex and hippocampus were the frequently affected areas for degeneration (Fig. **3**). Fig. (**4**) showed the sensorimotor cortex with loss of pyramidal neurons in the ketamine treated mice when compared with the control mice (Fig. **5**). Most alarmingly, however, was the detection of hyperphosphorylated tau (hypertau) positive sites in the prefrontal cortices of both of these species after long term ketamine treatment by our team. In the addicted mice, however, hypertau was found mostly in the outer cortical layers while hypertau in the addicted monkey was mainly located in the inner cortical layers [10]. Since hypertau formation in the brain was a feature of Alzheimer disease, an abnormal aging procedure might have been triggered after long term ketamine treatment which was similar to Alzheimer degeneration. In normal circumstances, the tau protein is a constitutive protein of the microtubules. If the tau protein is bonded to an extra phosphate group in one of its amino acid sites (e.g. Ser-99), then the tau protein is said to be hyperphoshorylated and this process affects the rigidity of the microtubules. The event will not only influence transmission, but in fact will lead to cell death (apoptosis). In a study of the brains of Alzheimer patients, it was documented that hypertau positive cells colocalized with activated caspases including caspases 3 and 6, which were death execution enzymes in the cell death pathway [11,12]. Fortunately, however, very few amyloid plaques were observed in the brains of our addicted animals [10]. Effect of ketamine may be related to NMDA receptors [1] but it can also affect other systems of transmitters directly or indirectly. For example, we have documented on upregulation of GABA5 receptors after long term abuse of ketamine [13]. Neuronal degeneration was even observed in culture cells of neuroblastoma cells (SH-SY5Y cell line (ATCC number CRL-2266)) and PC12 cells (ATCC number CRL-1721), after treatment with various dosages of ketamine [14]. For the neuroblastoma cells, it could be differentiated through the addition of retinoic acid [14]. Comparing the undifferentiated neuroblastoma cells with the more differentiated ones, it was interesting to note that the more differentiated cells would go into degeneration only after higher dosages of ketamine treatment [14], thus implying that immature neuronal tissue was more vulnerable to ketamine.

On the other hand, the different CNS centers in the long term ketamine abuse model reacted differently. While most centers of the CNS appeared to increase in activities, the cerebellum was downregulated [15]. This downregulation of the cerebellum in the long term ketamine abused humans was actually translated to the apoptosis of Purkinje cells in the cerebellum of long term ketamine mouse model. The apoptosis of the Purkinje cells might imply that the larger neurons in this organ were more susceptible [15]. Dying by apoptotic cells would lead to dysfunction of the cerebellum i.e. ataxia and disturbance of balance which were two characteristics of ketamine abuse in the human. Nephrotic toxicity was also indicated in addition to neuronal damage [16]. Our study [17] repeated interstitial nephritis and apoptosis of the cells in kidney tubules (Fig. **6**) as well as the glomerulus (Fig. **7**). As a consequence, large amount of protein leaked out from the kidney. In a summary of several hundred mice used for the ketamine experiments in

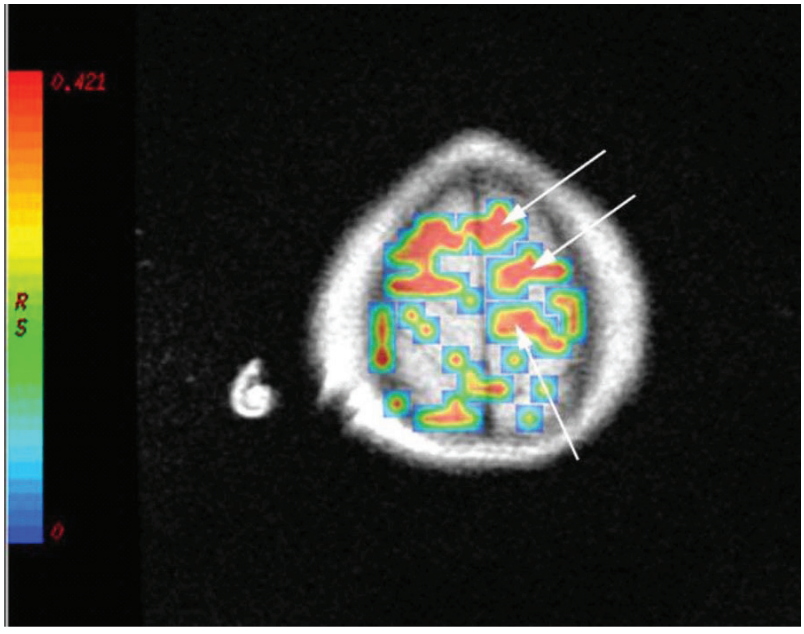

Fig. (2). The fMRI of the brain of the monkey which had received 6 months of ketamine treatment (dosage: 1 mg/kg intraperitoneal (i.p.) injection daily) Simple movement like flexion of the limbs evoked activations in many parts of the cortex (arrows), including the prefrontal cortex (P).

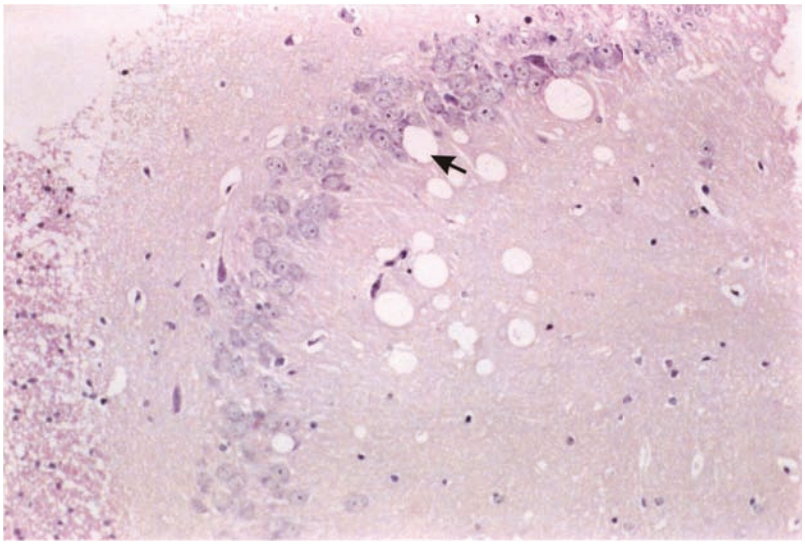

Fig. (3). Degeneration of the mice hippocampus after 6 months of ketamine intoxication (dosage: 30 mg/kg intraperitoneally (i.p.) injection daily). Arrow points to a degenerating spot X400.

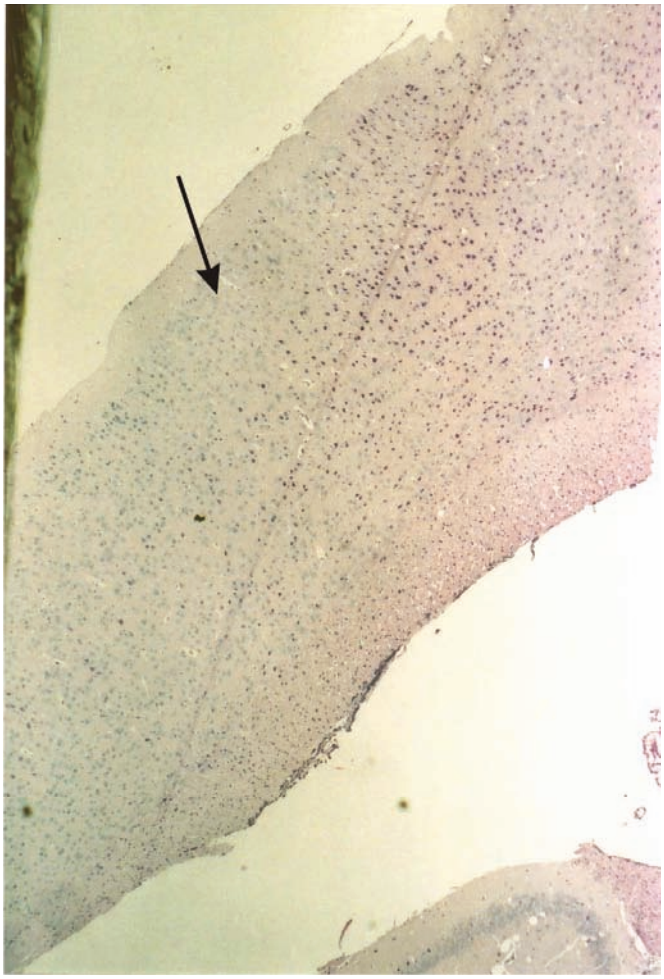

Fig. (4). Median segittal section of the cerebral cortex of the mouse which had received 6 months of ketamine. Note the decrease in the amount of cells in the sensorimotor part of cortex (arrow) X100.

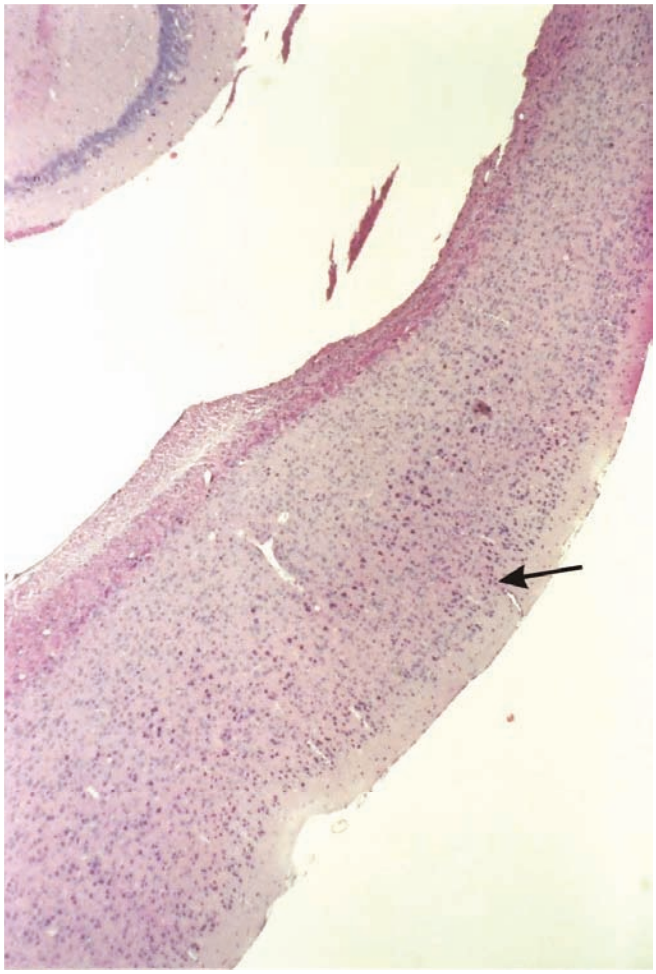

Fig. (5). Control mouse had a lot of cells in the sensorimotor part of the cortex (arrows) X100.

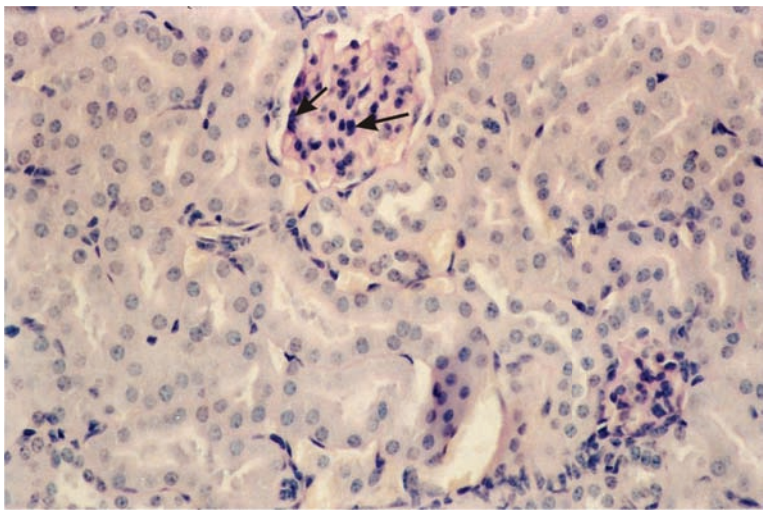

Fig. (6). The pyknotic nuclei (arrows) in the glomerulus of the kidney of the mouse which had received 6 months of ketamine X400.

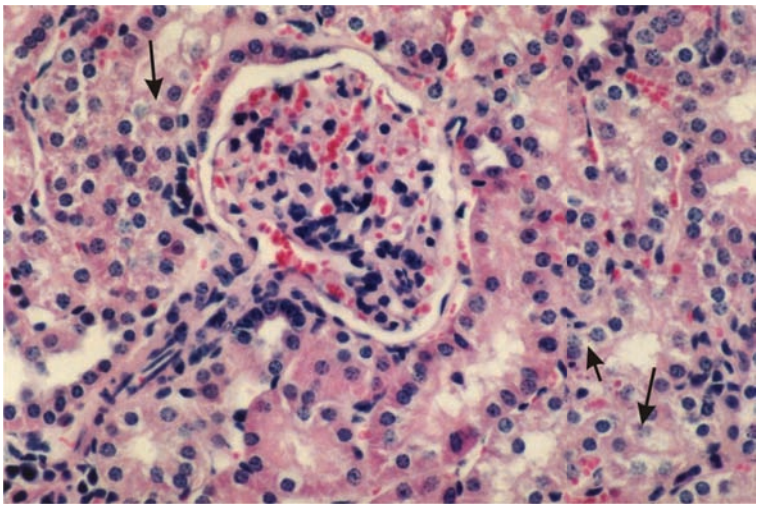

Fig. (7). Degeneration in the kidney tubules of the mouse addicted for 6 months of ketamine. Arrows point to almost empty or swollen and empty cytoplasm, typical of hydropic degeneration X400.

our laboratory in the past two years, protein analysis of the urine detected at least 50% of these mice developed proteinuria which had one month to three months of ketamine treatment. Most of the protein leak was seriously high, as much as 100 mg/ml in the majority of the ketamine treated group. About 5 % of these animals had protein concentration in the urine of up to 500 mg/ml.

The first deleterious effect observed in long term ketamine human users was initially reported in the bladder [18] where cystitis like symptoms appeared along with a small bladder. The novel data reported by our group on histopathological changes in the bladder appeared to come in sequence after long term ketamine exposure in the animal model [18]. To begin with, mild gatherings of lymphocytes were present in the lamina propria in the wall of the bladders. The mucosal surface would demonstrate degenerative changes. After one month of ketamine abuse in our mouse model, some epithelial cells went into apoptosis. By three months of ketamine abuse, the muscular layer demonstrated loss of muscle fibers, again *via* apoptosis. The neuromuscular junctions in the bladder wall decreased in quantity [17]. It was, however, not certain whether this decrease of the neuromuscular junctions was a consequence of the loss of muscle fibers or in fact occurred at the same time as independent events. Replacement of the muscles was by fibrosis, an increase of collagen fibers proliferation. In the six-month ketamine treated model, the connective fibers at the neck of the bladder appeared profusely and with heavy branching. The bladder therefore lost its elasticity and became smaller in size. In the patient, this would cause frequent urination as a small bladder could hold less urine. Ketamine usage also affected the genital system and we reported recently the decrease in the number of motile sperms in the long term addicted mice [17].

In our research with ketamine, we have had the opportunity to deal with different models including rodents (mice and rat), monkey and even some human abusers. Although seemingly, we had been using higher dose on the

rodents (e.g. 30 mg/kg on the mice) in these experiments than monkey (1-5 mg/kg in monkey), this was because the rodents e.g. mice had a very high metabolism, which was ten times that of the human. The anesthetic dosage recommended for the mice was therefore at least 150 mg/kg [19] to 200mg/kg (University of California, San Francisco, Department of Comparative Medicine). In our laboratory, we initially analyzed the motility of the limbs of mice by putting them into water and noted that it was after 40-60 mg/kg of ketamine treatment that one pair of limbs (hindlimbs) started to show decrease of activity [16]. For subanesthetic dosage to induce long term abuse, we therefore resolved to use 30mg/kg as a standard in the rodents. In the human addicts of ketamine, it has been reported that they could use ketamine in very high doses, often at several hundred milligrams a night (information obtained from addicts).

Since ketamine metabolism rested in the liver, long term treatment of abuse of ketamine would affect the liver. Studies on the mouse liver in our laboratory indicated increase of collagen fibers in the liver parenchyma after 30 mg/kg of ketamine intraperitoneally (i.p.) each day for six months (Fig. **8**) [20]. There was a further increase after ketamine and ethanol were used in combinations (Fig. **9**). Ketamine had been reported to induce liver cell cultures into apoptosis [21]. Spordic reports on repeated clinical usages of ketamine in the human patients resulted in increase of liver enzymes [22]. In the heart, there were necrotic like lesions in cardiac muscles of the ventricles and increase of premature ventricular beats as well as ST elevation in our rodent models of long term ketamine treatment [23]. In the human, ST wave elevation of the ECG had been reported in one patient [24] and pulmonary vasoconstriction has been noted during anesthesia with ketamine [25].

It is difficult at this stage to conclude whether ketamine itself or any of its metabolite causes the damage. Ketamine, being an organic structure had a toxic chemical residue of benzene. There have been some reports on benzene and its metabolite hydroquinone is prime suspect leading to induce cell death, DNA damages and even induce inflammation [26]. The intermediate metabolite of ketamine- hydroquinone, by

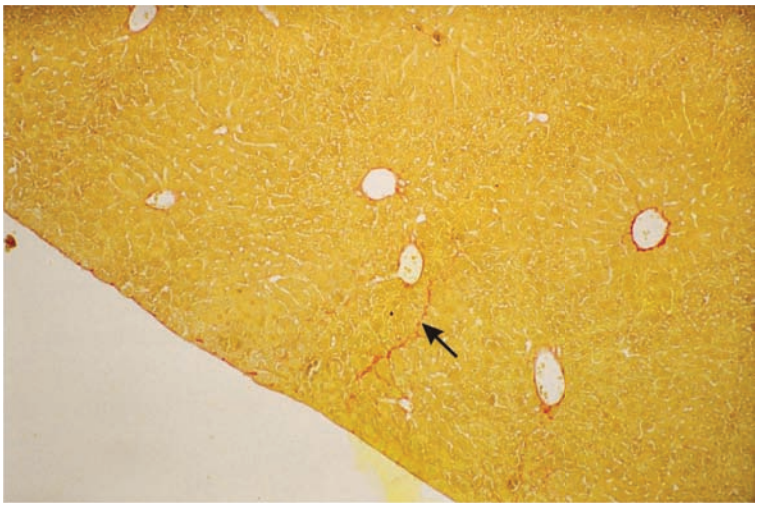

Fig. (8). Liver of mouse showing fibrosis (arrow on collagen fiber red in staining) after 6 months of ketamine treatment X50.

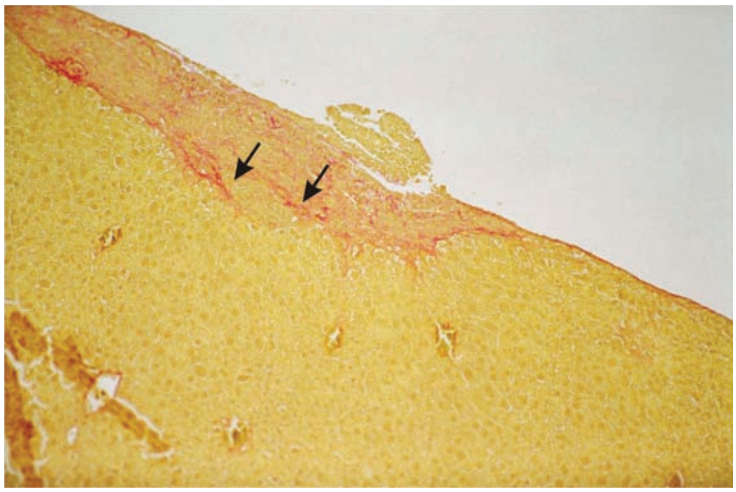

Fig. (9). Liver of mouse showing increased fibrosis (arrows) after 6 months of ketamine treatment plus with alcohol consumption in the last 4 weeks X50.

itself is even more toxic. It affected not only the central nervous system (resulting in hyperactivity, stupor, tinnitis, nausea, dizziness, delirium, headache and sweating), but would also increase respiration and cyanosis and caused gastrointestinal dysregulation [27-29]. In fact, ketamine metabolized *via* hydroxylation and demethylation [30]. Once a 'hydroxyl' group was added on the benzene ring of ketamine. That part of the structure became a hydroquinone. In the animal studies, central nervous system (CNS) reactions also common, including tremor, convulsion and reduced activity as a consequence of hydroquinone toxicity [16]. Neuropathological examination revealed the damages in the CNS and the peripheral nervous system (PNS), including changes of the myelinated axons. The other possibility is of course related to the binding of NMDA receptors by ketamine which leads to an overstimulation of the glutamatergic system causing excitotoxicity [31].

Of much interest was whether ketamine interacts with other chemical agents. As an example, it had been documented that the packages that addicts bought on the street probably contained caffeine and caffeine interacted with ketamine, leading to even worse scenarios [32]. Besides this, ketamine usage was mainly found in parties where drinking of alcohol was a norm. Since both of ketamine and alcohol acted on NMDA, interaction became unavoidable. In fact, studies in our laboratory indicated the two drugs potentiated each other to result in more cell death (apoptosis) in the brain [19]. In addition, Fig. (**10**) showed the increase in the level of the liver enzyme, glutamic-oxaloacetic transaminase (GOT) and alanine transaminase (ALT) after 16 weeks of ketamine treatment with addition of ethanol administration (0.5 ml, 10% ethanol per day during the last 4 weeks) when compared with the livers of those animals which received ketamine alone. This result pointed to that a more serious inflammation in the liver. The results agreed well with the increase of fibrosis of the liver in Fig. (**9**) after ketamine alcohol combination. Chronic treatment of ketamine for only 16 weeks will lead to increase of GOT by 20% and when treated with alcohol together, the increase would be 40%.

For the urinary system, it had only been documented by our group that ketamine and alcohol together caused more muscular death and fibrosis [17]. The mechanism was unclear yet but might be an indirect effect of action *via* NMDA or a direct effect of ketamine derived hydroquinone interacting with alcohol in, for example, the urinary bladder.

Ketamine addicts using ketamine for long term is a potential health problem and will lead to grave consequences. This, however, does not rule out that ketamine's usage as an anesthetic which is probably acceptable as most of its deleterious effects are from long

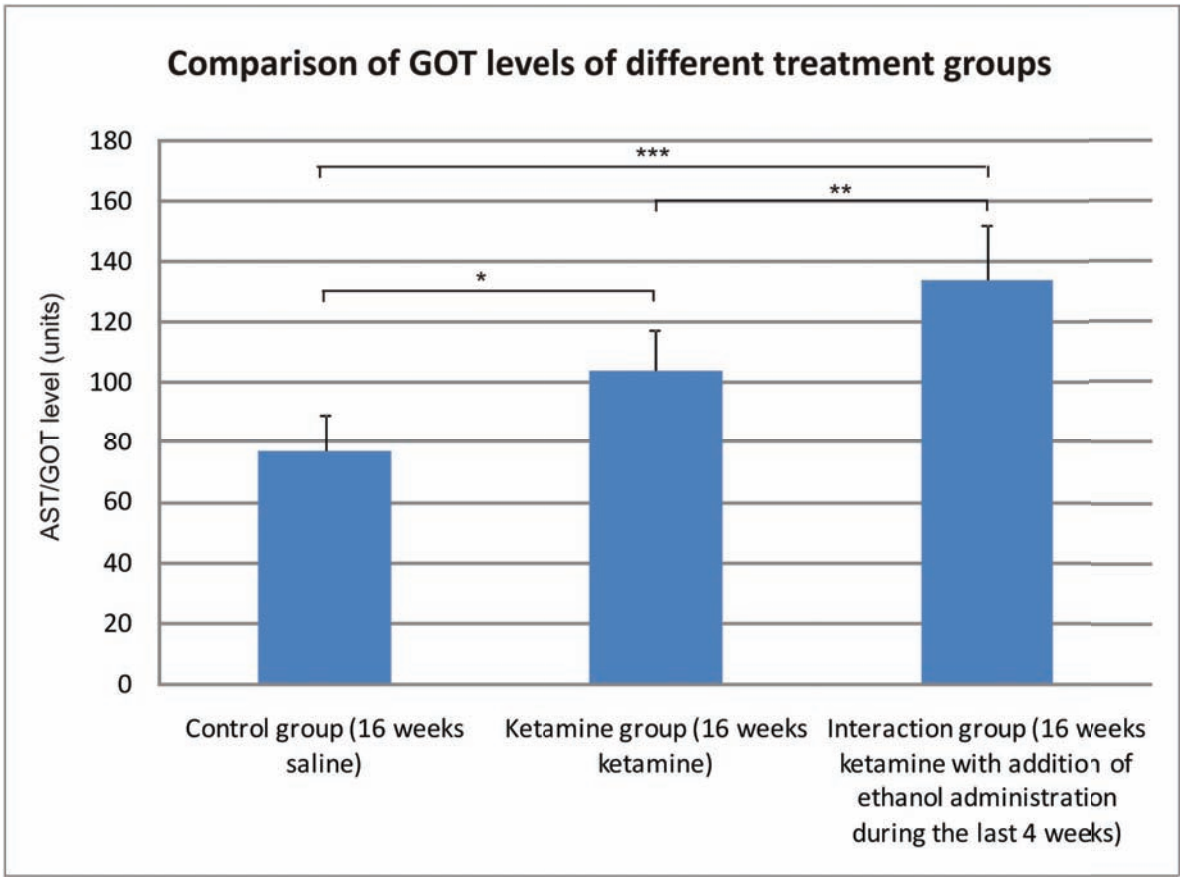

Fig. (10). The level of liver enzyme (GOT) in the mice increased significantly after 16 weeks of ketamine treatment (* $p = 0.02$). A further increase was detected with coadministration of alcohol (0.5 ml of 10% ethanol given daily to each mouse during the last 4 weeks of ketamine treatment) (** $p = 0.01$; *** $p < 0.001$).

term usage. Ketamine itself, as an anesthetic is fast acting and of short duration and its use was reliable and with stable outcome in most cases if carefully monitored. Its use in neonatals as anesthetic or as an analgesic for the long term, however, needs to be carefully explored.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflicts of interest.

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