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Can Uncaria rhynchophylla alleviate damages produced by ketamine?

Final report

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Background

The genus *Uncaria* belongs to the family of Rubiaceae and the genus alone contains many species including the most common ones, namely *Uncaria tomentosa*, *Uncaria rhynchophylla* and *Uncaria sinensis*. *Uncaria rhynchophylla* and *Uncaria sinensis* are the species present in China. In Hong Kong, most of the imported species for medical usage were the above two species while the local indigenous species is the *Uncaria sinensis*. One of the sites where it can be located is on the cliff of Victoria peak facing towards Wanchai. *Uncaria* as a genus that grows in the tropical regions in China and even in some parts of Japan and South America from Peru all the way through the Amazons. The plant itself is a climbing vine with the dichotomy of two rows of leaves facing each other. The stem of the vine contains curve hooks (figure 1.1) and thus the Spanish name of uña de gato which was used as the name for *Uncaria tomentosa* - the cats hook. Today, cats hook commonly refers to the *Uncaria* species. *Uncaria* species has been employed for centuries as folk medicine in many countries. In South America, it has been used as an anti-inflammatory agent while in China and Japan, *Uncaria* was primarily used in central nervous system ailments.

Figure 1.1 Dry stems of *Uncaria* sp. with hooks

The chemical constituents in *Uncaria* were analyzed only recently by Aquino et al. (1991), Laus (2004) and Zhang et al. (2017) (Aquino, De Feo, De Simone, Pizza, & Cirino, 1991; Laus, 2004; Zhang et al., 2017). It contains 26 alkaloids, 6 flavonoids, 2 triterpenoids, 2 chlorogenic acid and 2 unknown chemicals. The detailed pharmacological effects of each component have to be worked out. However, the following are known: 1) the anti-epileptic effect of *Uncaria* probably acted through blocking of NMDA receptors, 2) the bark of the stem and the hooks affected the nervous system. One of the effects was its ability to bind to serotonin receptors *Uncaria* also has strong neuroprotective effects (Liu et al., 2015), 3) the leave extract would bind to melatonin receptors and 4) alkaloids do not contribute to all therapeutic effects of the plant (Zhang et al., 2017).

Uncaria rhynchophylla (Gouteng in Chinese) has been suggested for the treatment of symptoms relevant to drug addiction (Shi, Yu, Chen, & Xu, 2003). Besides possessing many other beneficial effects such as anti-hypertensive, anti-anxiety, anti-arrhythmic, anticonvulsant, and neuroprotective effects (Chou et al., 2009; Yuan & Chang, 1962; Zhou & Zhou, 2010), its bioactive ingredient rhynchophylline has attracted considerable interest due to its potent effects on the central nervous system such as acting as an "antagonist" of the glutamatergic N-methyl-D-aspartate (NMDA) receptor (Kang et al., 2002; Lee et al., 2003), the receptor which involves ketamine addiction (Mion & Villevieille, 2013). As such, *Uncaria rhynchophylla* could play a role in modulating ketamine damages, likely by competing with ketamine for the receptors and either inhibit or enhance its effects.

Among the common psychotropic substances of abuse, ketamine has been stably topping the list as the most popular substance for more than ten years (2005-2015) (CRDA, 2016). Despite the recently drifted trend for methylamphetamine (ice) in the first quarter of 2016, ketamine is usually associated with polysubstance use. It was among the top three drugs to be used simultaneously with other drugs (CSEW, 2012). In Hong Kong, 35% of a sample of patients presenting to the emergency department with acute ketamine toxicity reported coingestion of drugs (Ng, Tse, Ng, & Lau, 2010). The well-received polysubstance use of ketamine may potentially regain its already high popularity (27% took ketamine among all psychotropic substances in 2016 (CRDA, 2016). Besides, ketamine is substantially popular in clinical settings, recorded as one of the core medicines in the Essential Drugs List of the World Health Organization (WHO, 2015). Although it is regarded as a safe anesthetic within normal clinical dosages, up to 40% of patients may experience side effects such as dizziness, blurred vision, altered hearing, hypertension, nausea and vomiting, vivid dreams, hallucinations, and other dissociative reactions (Quibell, Prommer, Mihalyo, Twycross, & Wilcock, 2011; Youssef-Ahmed, Silver, Nimkoff, & Sagy, 1996). Since the society is inevitably in touch with ketamine, the search for substances to inhibit its psychotropic effects and limit its damage is necessary, thus the postulated beneficial effect of *Uncaria rhynchophylla* is worth testing. On the other hand, Gouteng is cheap and readily available over the counter as a Chinese herbal medicine. If it acts pharmacologically akin to ketamine and produce comparable desirable short-term dissociative sensations, it may invite illegal exploitation from both dealer and abusers. In fact, a novel low-cost extraction process of rhynchophylline from the herb with high efficiency was patented recently (SIPO 2012). Therefore, understanding *Uncaria rhynchophylla*'s pharmacological nature and its reaction with ketamine ahead of time is also of immense

importance.

The initial research in China on the herbal complex rhynchophylline however pointed out in cell culture that this herb with its chemical constituents can block the receptors of ketamine, the NMDA receptor (Yang et al., 2018). The subsequent mechanisms and limits on damage are however unknown. For example, would this interaction between the herb and ketamine be able to alleviate the negative effect of ketamine in the long term and short term addiction? In the nervous system, for instance, would such herbal treatment in the addicts prevent or lessen the amount of cell death and perhaps prevent excessive memory loss? These potentials would have to be tested in an animal model of ketamine. On the other hand, by binding to the same receptor as ketamine, the herb may have the potential to mimic the effects of ketamine and bring about similar deleterious effects and toxicity. Therefore, its effects and toxicity on different organs shall be investigated. Subsequently, our study will include an evaluation of the drug *Uncaria rhynchophylla* and its dose related toxicity on organs of intact animals.

Brief methods

Establishment of animal models (mice)

In this study, one hundred and sixty mice were divided into 8 groups (n=20 for each group) and received the following treatments: The first two groups were treated with *Uncaria rhynchophylla* (human equivalent dosage of 10g or 20g, respectively) daily for three months; the second two groups were treated with ketamine (30mg/kg body weight) plus with *Uncaria rhynchophylla* (human equivalent dose of 10g or 20g, respectively) daily for three months; the fifth group was treated with ketamine (30mg/kg body weight) daily for 1 month, followed by *Uncaria rhynchophylla* (human equivalent dose of 10g) daily for 2 months; the sixth group was treated with ketamine (30mg/kg body weight) daily for 2 months, followed by *Uncaria rhynchophylla* (human equivalent dose of 10g) daily for 1 month; The seventh group was treated with ketamine (30mg/kg body weight) daily for 3 months while the last group was treated with normal saline (0.045g NaCl/ kg body weight) daily for 3 months (table 2.1).

Due to death of surgery and other experimental procedures, the final number of animals of each group was 15. As objectives of the project were accomplished, no additional animals were added in order to prevent waste of animals.

	#	Group name	treatments
Gouteng groups	1	Gouteng, low dose	*Uncaria rhynchophylla* (human equivalent dose of 10g) daily for 3 months
	2	Gouteng, high dose	*Uncaria rhynchophylla* (human equivalent dose of 20g) daily for 3 months
Ketamine plus Gouteng groups	3	Ketamine plus Gouteng, low dose	Ketamine (30mg/kg) + *Uncaria rhynchophylla* (human equivalent dose of 10g) daily for 3 months
	4	Ketamine plus Gouteng, high dose	Ketamine (30mg/kg) + *Uncaria rhynchophylla* (human equivalent dose of 20g) daily for 3 months

Ketamine plus Gouteng groups (one after another)	5	1 month ketamine and 2 month Gouteng	Ketamine (30mg/kg) daily for 1 month, followed by *Uncaria rhynchophylla* (human equivalent dose of 10g) daily for 2 months
	6	2 month ketamine and 1 month Gouteng	Ketamine (30mg/kg) daily for 2 months, followed by *Uncaria rhynchophylla* (human equivalent dose of 10g) daily for 1 month
Ketamine group	7	ketamine	Ketamine (30mg/kg) daily for 3 months
Control group	8	control	Normal saline (0.045g NaCl/kg) daily for 3 months

Table 2.1 a table showing treatments of different groups

1, Behavioral testing

Among studies on cognitive impairment of ketamine, ketamine abusers exhibited profound impairments in both short- and long-term memory (Morgan & Curran, 2006). Spatial working memory was also hampered (Stewart, 2001). Apart from memory loss, cerebella activity in both humans and mice was as well down-regulated in ketamine users, and the number of cerebella apoptotic cells significantly increased (Chan et al., 2012). In this part we tested whether *Uncaria rhynchophylla* has the same deleterious effect on memory and motor coordination as ketamine in behaving mice, and whether it interacts with ketamine or attenuate the damages by ketamine.

A, Morris water maze

Morris water maze was used to assess the spatial learning and memory of mice (Vorhees & Williams, 2006). A circular water tank of 100cm diameter was filled with 25±1°C water of

20cm depth. Milk was added to make the water opaque. A transparent platform 10 cm below the water level was placed in the center of a quadrant (platform quadrant). Four fluorescent distal cues of different shapes were placed around the water tank. Mice were trained for 5 consecutive days and there were 2 trials in each training day and 10-15 minutes intervals between each trial. In each trial, a mouse was placed at a starting location changed every day and was allowed to swim and navigate for the platform. When the mouse located and climbed onto the platform, it is allowed to stay on the platform for 10 seconds. If the mouse did not locate the platform in 120 seconds, it would be guided to the platform and allowed to stay on the platform for 10 seconds. On the sixth day, the platform was removed from the water tank. There was only one trial for each mouse. In each trial, a mouse was placed at a starting location and allowed to swim and navigate in the tank for 120 seconds. The time of the mice staying in the platform quadrant were recorded. The results were compared among all groups.

B, Consolidative memory study

This test improved the measurement of memory versus the traditional Morris Water Maze test in the sense that it not only eliminates factors of water aversiveness and swimming capability, it also indicates how well the mice's memory was by measuring the waiting time at the site of food. A longer waiting time indicates better memory. It also rules out the randomness of the water maze test by providing multiple figures as the objects to be remembered: A box of 50cm x 50cm was used as apparatus. Four different figures were placed at the four corners and the mouse was trained to recognize one of these figures no matter where it was placed. Once the correct figure was recognized, the animal was rewarded with a piece of food. During test day, no food was given. The arrival time at the correct figure and the duration of their stay near the figure was recorded and used as the measurement of memory. The results were compared among all groups.

C, Gait analysis

The footprint test has long been used to unravel neurological impairments in laboratory rodents (de Medinaceli, Freed, & Wyatt, 1982). The animal's paws will be coated with ink and the animal is allowed to walk over a sheet of paper to generate a footprint pattern. Static step

parameters including hindlimb stride length, hindlimb stride width, hindlimb stride ratio (hindlimb stride length / limbstride width), feet support and footprint spread will be analyzed. The results were compared among all groups.

2, Effect on Central Nervous System (CNS)

A, Neurotransmitters

Ketamine may potentiate the effects of gamma-aminobutyric acid (GABA) synaptic inhibition and induce activation of dopamine release (Lorrain, Baccei, Bristow, Anderson, & Varney, 2003). It also inhibits neuronal uptake and increases serotonergic activity (Yamamoto et al., 2013), which is thought to be the underlying basis of nausea and vomiting. The elevated glutamate and dopamine levels can provoke dose-dependent positive and negative schizophrenia-like symptoms. In addition, glutamate is an excitatory neurotransmitter abundant in brain. Both glutamate, ketamine and rhynchophylline of Gouteng bind on NMDA receptor. To investigate whether *Uncaria rhynchophylla* brings about the same effects on the CNS or alleviate the effect of ketamine, animals in each group were sacrificed at the end of the experiments; their brains were processed. Enzyme-linked immunosorbent assay (ELISA) was used to detect the level of gamma-aminobutyric acid (GABA), dopamine (DA), serotonin (5-HT) and glutamate in the whole brain of all groups. The results were compared among all groups.

B, Apoptosis

Ketamine is known to initiate apoptosis even within simple cell cultures. For example, this drug has been shown to have a proapoptotic effect on SH5Y5Y neuroblastoma cells. In the primate, short-term ketamine exposure caused widespread neuronal apoptosis (Creeley et al., 2013). In this study, terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) was used to map apoptotic cells (withering cell death) in the whole brain of all animals. The DNA fragmentation indicative of apoptosis and the topographic distribution of apoptotic cells were examined. The results were compared among all groups.

C, Neurophysiology

Electroencephalography (EEG) is the staple of measuring brain electrical activity. In this study EEG pattern was accessed in each group of mice after treatment period. The results were compared among all groups.

3, Toxicity on different organs

To investigate the toxicity of *Uncaria rhynchophylla* on different organs, morphological changes of the organs of the mice after different treatments were studied. Histological studies were performed on sections of the heart, kidney, urinary bladder and liver. The results were compared among all groups.

Biochemically, ketamine inhibits the reuptake of catecholamines, stimulating the sympathetic nervous system and resulting in cardiovascular symptoms. In this study, the serum cardiac troponin I (cTnI) was quantified with ELISA and electrocardiogram (ECG) was performed to record patterns of cardiovascular activities. Results were compared among all groups.

Ketamine is known to cause fibrosis in the liver (M. Wai, W. Chan, A. Zhang, Y. Wu, & D. Yew, 2012). Aspartate transaminase (AST) and Alanine transaminase (ALT) test were used as an indicator for liver functions of the mice. In order to identify collagen deposits and the stage of fibrosis in the liver, sirius red stain was employed and was compared with the results of collagen I immunostaining. Biochemical quantitation of liver collagen was performed and the results were compared among all groups.

Damage of kidney by ketamine was also reported (Braden, O'Shea, & Mulhern, 2005; S. K. Chu, Ma, To, Yiu, & Man, 2011; M. Wai et al., 2012; L. Yeung, Rudd, Lam, Mak, & Yew, 2009). Serum creatinine level was measured and used as a marker for assessing renal function. The results of the mice of all groups were compared.

Through the above experiments, the toxicity of *Uncaria rhynchophylla* on the mice on different organs at different dosages will be analyzed and compared with the ketamine controls. Its interactive effect with ketamine will as well be explored. Any *Uncaria rhynchophylla*-induced amelioration or aggravation of ketamine effects will be investigated.

Results and discussion

Part 1: Brain

Results

Histology and Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) in situ identification of apoptotic cells in brain regions

For the prefrontal cortex, the histological images from ketamine plus Gouteng, Ketamine and control groups were depicted in figures 3.1, 3.2 and 3.3 respectively while ketamine plus Gouteng and Gouteng groups were compared at a higher magnification of 200X (figures 3.4 and 3.5). Overall, ketamine group had much fewer cells in the prefrontal cortex compared with Gouteng groups while the Gouteng and ketamine plus Gouteng groups did not show much difference in the prefrontal cortices (figures 3.4 and 3.5). TUNEL studies confirmed more apoptotic cells were present in all layers of the cortex of ketamine group (figure 3.6) than cortices of other groups e.g. ketamine plus Gouteng group (figure 3.7) and control group (figure 3.8). For the hippocampus, in all groups, most apoptotic cells were in the stratum oriens and stratum reticularis, while the pyramidal cell layer had less apoptotic cells (figure 3.9). When all groups were compared, again the ketamine group (figure 3.10) had more apoptotic cells than other groups e.g. Gouteng plus ketamine group and Gouteng group (figure 3.11). For cerebellum, most of the apoptotic cells were seen in the deep nuclei of the cerebellum and in the ketamine group, again with the most cell death amongst all groups. In H&E staining cerebellum sections, more cells were observed in deep nuclei of brain stem in ketamine plus Gouteng group (figure 3.12) than that of ketamine group (figure 3.13). TUNEL staining confirmed the apoptotic cell death in the ketamine group (figure 3.14). The quantitative counting of TUNEL-positive (apoptotic) cells per 700μm^2 field (100X) from each region and each group (3 mice in each group and 4 fields from each mouse were acquired) and results were depicted in figure 3.15, 3.16, 3.17, 3.18 and 3.19. In general, ketamine group always had more dying cells than the Gouteng and Gouteng plus ketamine group. The latter two were more than control.

Morris water maze

Spatial memory of mice was evaluated by Morris Water Maze. Ketamine group (group 7) showed poorer memory than Gouteng groups low dose (group 1) and high dose (group 2) while Gouteng alleviated the effect of ketamine slightly but not significantly (group 3, 4 and 5). In group 6, a longer ketamine treatment (2 month) followed by a shorter treatment (1

month) of Gouteng produced no beneficial effect at all on spatial memory. On the whole, effect of Gouteng on spatial memory was not substantial (figure 3.20).

Consolidated memory study (Object recognition test)

In consolidated memory study, where the mouse identified a certain figure with the award of food afterwards in training and in experiment, no food was given and the longer the animal stayed in front of the figure, the more consolidated memory it had acquired. In other words, the mice remembered the figure and stayed at the vicinity of the figure for a much longer time though food was finally not given. The ketamine group (group 7) had the poorest memory while all Gouteng-treated mice (groups 1 and 2) and those with Gouteng and ketamine co-treatments (groups 3-6) had better memory. In most cases, the differences are statistically significant ($p < 0.05$), indicating Gouteng had a beneficial effect on consolidated memory (figure 3.21).

Gait analysis

Hindlimb stride ratio (figure 3.22) and hindlimb stride length (figure 3.23) were slightly better in ketamine group (group 7) than Gouteng group (groups 1, 2). Adding ketamine together with Gouteng (groups 3, 4, 5 and 6) did not show much benefit. Likewise, hindlimb stride width (figure 3.24) indicating stability was slightly better in the Gouteng groups than ketamine groups. This may be related to greater anesthetic of ketamine over Gouteng. On the whole, difference was not statistically significant.

Neurochemistry

Brain glutamate level

Glutamine and its metabolite glutamate are excitotoxic for the brain and was the highest in the ketamine group (group 7). It was higher than the Gouteng groups (groups 1 and 2) and all the Gouteng plus ketamine groups of different dosages and sequences (groups 3, 4, 5 and 6), while Gouteng and ketamine plus Gouteng group had a decrease of roughly 15% on average (figure 3.25).

Brain serotonin level

Serotonin level was higher in the ketamine group (group 7) than Gouteng group (groups 1 and 2). Combining Gouteng with ketamine treatment simultaneously or in sequence (group 3, 4, 5 and 6) did not manage to upregulate the serotonin level of ketamine treated mice. Thus, Gouteng did not have any anti-depressive trend (increase of serotonin) on ketamine treated mice (figure 3.26). Those mice treated with Gouteng alone had about on average less than 25% of serotonin when compared with that of ketamine.

Brain GABA level

GABA is an inhibitory transmitter, being useful to calm the mood down. GABA production in the ketamine group (group 7) was slightly lower than the Gouteng and Gouteng plus ketamine groups whether used simultaneously or in sequence (groups 2-6). Noteworthy was the increase of GABA in the Gouteng-ketamine groups (3-6), particularly in group 3 (figure 3.27).

Interestingly, GABA level in the brains of Gouteng treated alone was actually higher than the level of GABA in the brains of ketamine treated mice by about 20% on average. However, in the brains of mice with combined Gouteng and ketamine treatment, the increase of GABA was very little except for group 3 (Gouteng plus ketamine, low dose) and was not a simple addition effect (figure 3.27). In group 4 (Gouteng plus ketamine, high dose), thus the effect went down showing might be low dose of Gouteng was optimal.

Brain dopamine level

Dopamine ELISA indicated in the brains of Gouteng treated animals, the dopamine contents were below that of ketamine and control group (groups 7 and 8). For all groups of Gouteng combined with ketamine (groups 3, 4, 5 and 6), generally speaking, the dopamine levels were all lower than those treated by ketamine alone (group 7). This appeared to indicate that Gouteng can downregulate the level of dopamine increase induced by ketamine. If dopamine is related to the excitatory phase of ketamine addiction, then the Gouteng can possibly ameliorate or calm the excitation (figure 3.28).

From the above neurotransmitter experiments, the more remarkable points were that level of serotonin was high in the ketamine group and ketamine plus Gouteng group did not increase the serotonin when compared with the ketamine group. That in fact led to the suggestion that

ketamine might be used as an antidepressant (because of serotonin) but Gouteng could not increase the serotonin production which is same as suggested by Xian et al. (2017) (Xian, Fan, Ip, Mao, & Lin, 2017). The other remarkable point was that Gouteng and ketamine cotreatment could decrease the dopamine content in the brain. This is an important regulation further to maniac or hypomaniac (excitatory) condition produced by dopamine. Further, Gouteng addition appeared to be related to the increase of primary alpha wave or mu waves in Gouteng and ketamine EEG versus the slow wave of only treatment by ketamine (figures 3.29 - 3.32). This could be done with modulation of the decrease of serotonin normally related to happiness in mood and sleep.

Discussion

Our results indicated that when ketamine was administered with Gouteng, in the hippocampus and prefrontal cortex, cell death was insignificant, while those with ketamine alone induced a lot of cell death. Cellular protection was one of the features of the extracts from this plant, either through protection from oxidation (C. Li et al., 2018) or inhibition of excessive Ca^{2+} influx (Y. Shimada et al., 1999). Other alternative mechanisms of cellular protection include upregulation of myocyte transcript factor, regulation of bal/bak pathway and potentiation of PI3 kinase/AKT pathway via downregulation of GSK3-beta (Hu et al., 2018).

This study showed that apoptosis was highest in quantity in the CNS of the ketamine treated mice in comparison with Gouteng-treated, Gouteng-ketamine treated and control saline treated. The presence of cell death in the CNS after ketamine treatment had been documented (*Ketamine: Use and Abuse*, 2015). What was new here was that the prefrontal cortex had much more apoptotic cells than hippocampus and cerebellum after ketamine insult in the mice. Further, different layers of the same region might not be equally vulnerable. For example, cells in the strata oriens and reticularis were most vulnerable in the hippocampus. In addition and most importantly was that when Gouteng treatment was performed together with ketamine, the damage on the CNS as revealed by apoptosis and memory tests appeared to be less apparent, with the exception of the water maze test. This seemed to indicate a protective role of Gouteng on ketamine damage of the CNS. Gouteng, when used alone on the CNS of the mice with an equivalent dosage proportion as that of the human (10g/20g per 60 kg) in this study, had no detrimental effect on the brain when compared with saline control. Presently there is a lack of treatment for the CNS in ketamine addicts, neuroprotective agents like Gouteng might indeed be useful as a supplement for this purpose.

Our results also pointed out that consolidated memory of recognition with an award (feeding) in ketamine-Gouteng cotreated mice was improved over ketamine treatment alone, though spatial memory was not. Memory improvement can be further triggered by:

1. Inhibition of NMDA receptors by Gouteng (Chen, Li, Zhang, Xia, & Zhang, 2016; Yang et al., 2018)
2. Antiacetylcholinesterase activity initiated by Geissoschizine methyl ether N-oxide or catechin, components of Gouteng (Jiang et al., 2015)

These reactions increased muscle activity and memory (Chen et al., 2016; Jiang et al., 2015). The ability of this herb to suppress beta-amyloid further supported its other role in counter degeneration (Yang et al., 2018). Our chemical analyses on serotonin production showed ketamine mice actually produced more serotonin than Gouteng treated or Gouteng-ketamine treated mice, thus ketamine alone did have an antidepressant effect with increase of serotonin to produce a happy mood (Ener, Meglathery, Van Decker, & Gallagher, 2003). In addition, the antidepressive effect of Gouteng may have come from the norepinephrine and serotonin elevation by the constituent isorhynchophylline in Gouteng too (Xian et al., 2017). Another suggestion was that catechin of Gouteng acted also on melatonin receptor, could have antidepressive effect as well (Geng et al, 2018). As for modulation of epilepsy and anxiety, the mechanism appears to be related to the RAGE protein of Gouteng as well (Tang et al., 2017).

Our studies pointed out that *Uncaria rhynchophylla* has an effect on protecting against cell death, and improving consolidative memory but without being able to help spatial memory, which is a cardinal sign of Alzheimer's disease. In fact, both amyloid and tau had been located in the brains of mice and monkey treated with ketamine (Sun et al., 2014; L. Y. Yeung et al., 2010).

Our neurochemical part of this study further demonstrated that

1. Both Gouteng and ketamine treated mice had brains with serotonin, GABA, dopamine and glutamate.
2. Serotonin and glutamate level were on average higher in the ketamine treated alone mice than the other groups whilst GABA was higher on average in the brains of Gouteng treated alone mice.
3. Decrease of dopamine after combined Gouteng ketamine treatment than the ketamine alone, but close to Gouteng alone and saline.

Serotonin was long known to have an excitatory effect on mood, but too much serotonin could lead to social phobia, agitation, anxiety, tremor and rise in temperature (Ener et al., 2003). The action of serotonin was usually associated with 1A and 2A receptors for the mental domains. In this study, it was evident that ketamine alone induced more serotonin production than the other groups studied while on the other hand, Gouteng induced slightly higher average GABA level in the brain. Since ketamine was recorded to tie to GABA receptors (Tan, Lam, Wai, Yu, & Yew, 2012; Tan, Rudd, & Yew, 2011), the hypnotic effects of GABA would be likely also present in Gouteng. Furthermore, ketamine also led to more production of glutamate in the brain while interaction between ketamine and Gouteng only decrease the level slightly. Glutamate was known to be an excitotoxic transmitter and once bound to NMDA receptors would increase the cellular calcium leading to neuronal cell death (Platt, 2007). As our TUNEL studies did show that Gouteng could exert neuroprotective abilities quite well on the ketamine treated brain, but the Gouteng-ketamine interaction here showed only minimal downregulation of glutamate, the neuroprotective effect on cell death probably were not through just downregulating the glutamates but rather via the cellular pathways mentioned previously in this paper.

One very interesting thing is the decrease of dopamine associated with excitation after Gouteng and ketamine cotreatment to a level, less than ketamine alone, but close to Gouteng alone and control. Since dopamine is excitatory in most circumstances, therefore the co-administration of Gouteng and ketamine would block excitatory mode of drug abuser of ketamine.

Electroencephalography (EEG)

In recordings during the day, alpha waves were only detected in the control saline group (Figures 3.29 and 3.30), the Gouteng fed alone group (Figure 3.31) but not in the ketamine alone group and the ketamine plus Gouteng group (Figure 3.32). In the control group (Figure 3.29), alpha wave constituted 31 plus/minus 2% of the total waves while the delta, theta waves commonly and collectively termed as slow waves constituted 63 plus/minus 15%. The rest were beta waves and k complexes. The alpha waves in the control could be divided into three groups according to amplitudes under a magnification of three folds, the highest amplitude was about 120 μV while the lowest was about 12 μV. Besides that, in the control group during sleeping (Figure 3.30), there was no alpha wave and slow waves constituted 85 plus/minus 2.7% of the EEG. In the Gouteng treated animals (Figure 3.31), alpha waves constituted 18 plus/minus 0.5% of the total waves and with amplitudes between 8-26 μV, while the slow waves

constituted 66 plus/minus 2.55% of the EEG. In the Gouteng plus ketamine treated mice (Figure 3.32), like that of ketamine treated mice, there were no alpha wave and the slow wave constituted 66 plus/minus 1.5%, similar to the Gouteng group but significantly lower than the proportion of slow wave in the ketamine treated alone group, indicating that the downside of drowsiness introduced by ketamine alone was ameliorated. The drowsiness (slow wave) might be associated high serotonin in the ketamine only group. In some Gouteng and ketamine treated animals, a return of the excitatory alpha wave was again observed and constituted to 5% of the total waves, perhaps related to the decrease of serotonin.

Neurobehavioral tests

Neurobehavioral tests comprised of three tests, the water maze tests for spatial memory, the consolidated memory study and the test for walking stability (gait analysis). In short, the water maze did not confirm any increase of spatial memory after Gouteng ketamine cotreatment versus those treated by ketamine alone but the consolidated memory was elevated in the Gouteng and ketamine group than the ketamine alone group. This may be related to less cell death in the brains of the Gouteng ketamine cotreatment group presented in the histology part of this section. Finally, Gouteng ketamine interaction did not seem to increase stability in walking than the ketamine group.

In summary, as Gouteng interacted with ketamine could produce neuroprotective effects and ameliorated the damages on neurons and the addition of Gouteng produce little toxicity on the brain, this herb does have the potential to serve as adjunct treatment for the brain of addicts on ketamine. It is important to note, however, Gouteng would not be able to reverse or even ameliorate all defects produced by ketamine.

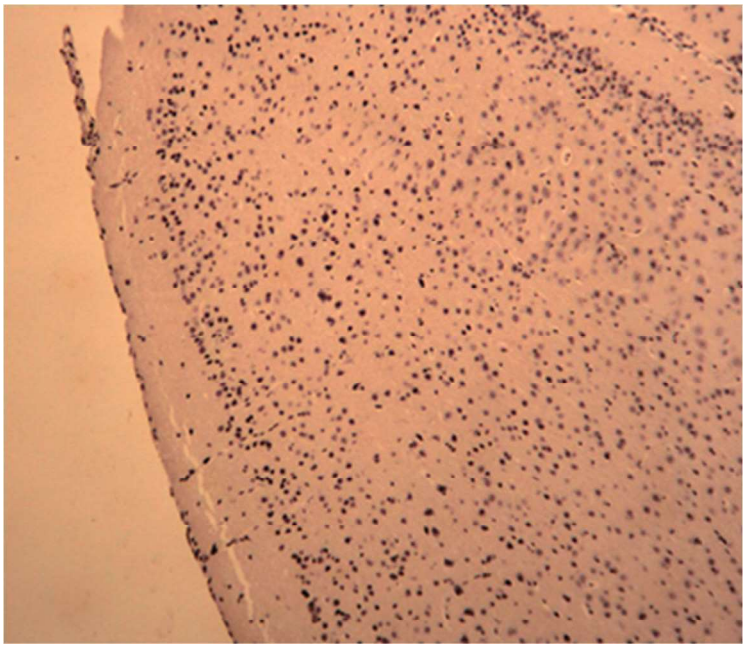

Figure 3.1 H&E stain of a prefrontal cortex section of ketamine plus Gouteng group (100X)

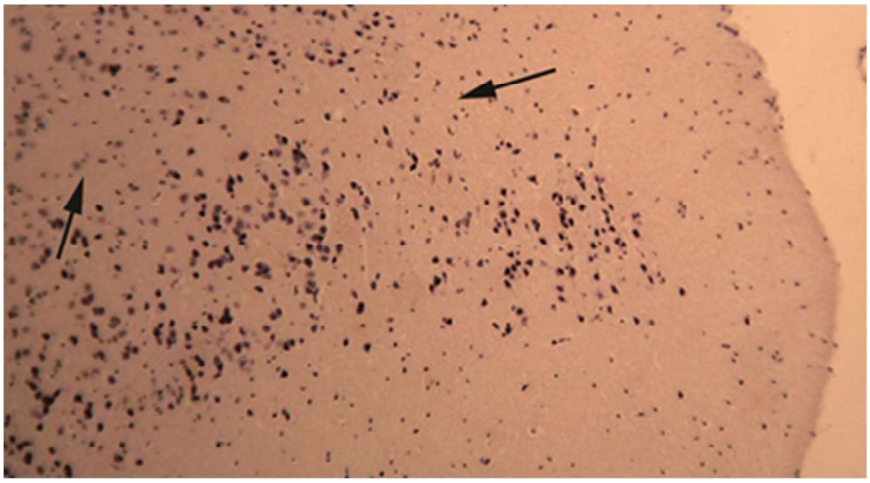

Figure 3.2 H&E stain of a prefrontal cortex section of ketamine group. Note a large area of less neurons (arrows) (100X)

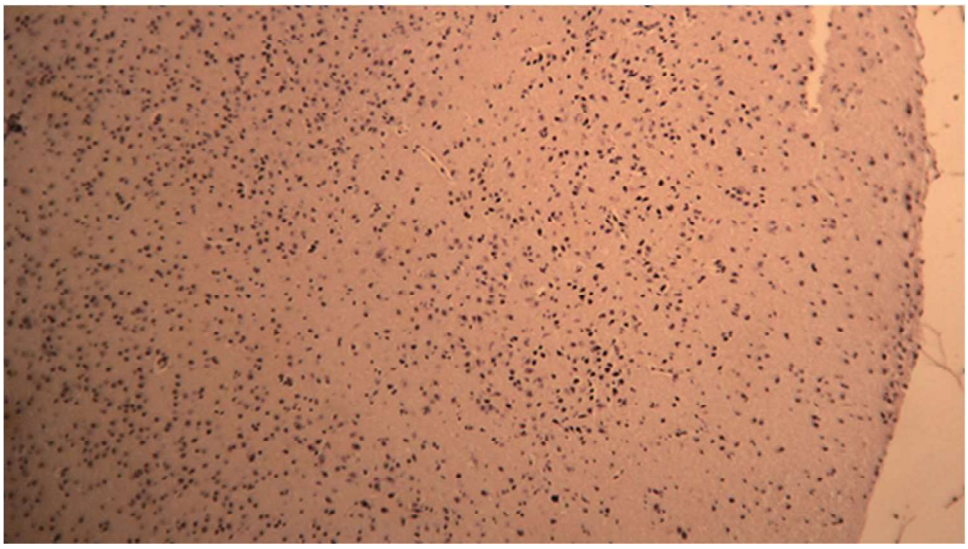

Figure 3.3 H&E stain of a prefrontal cortex section of control group (40X)

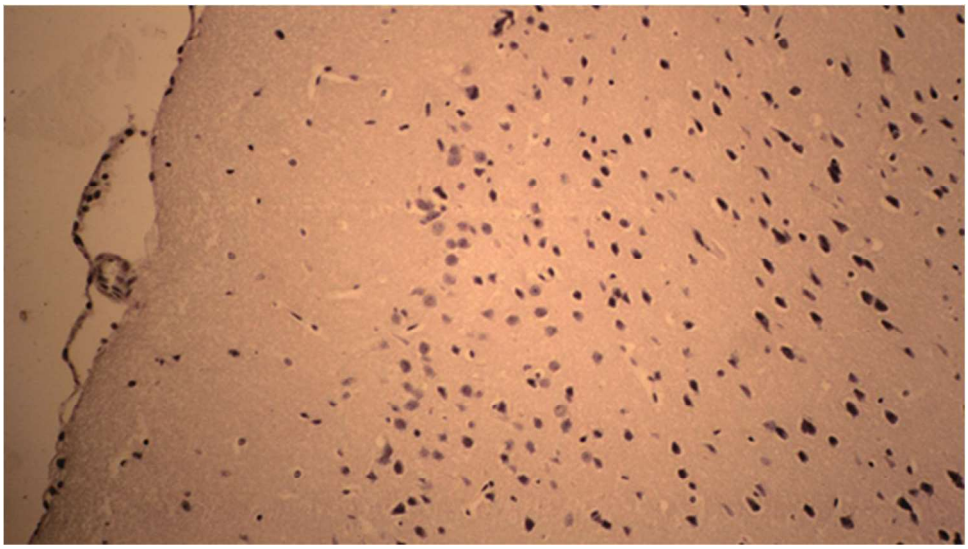

Figure 3.4 H&E stain of a prefrontal cortex section of Gouteng group (200X)

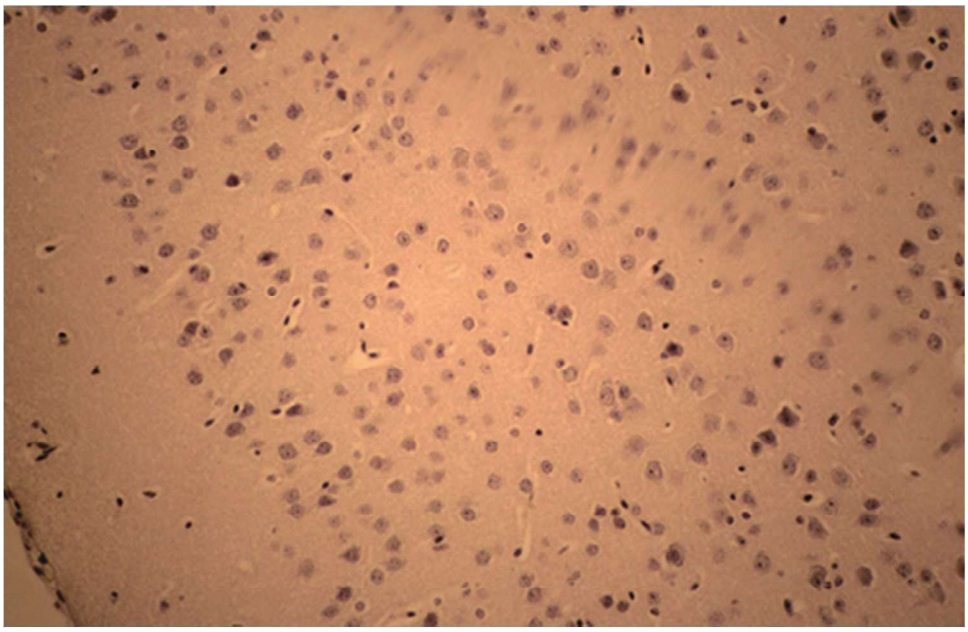

Figure 3.5 H&E stain of a prefrontal cortex section of ketamine plus Gouteng group (200X)

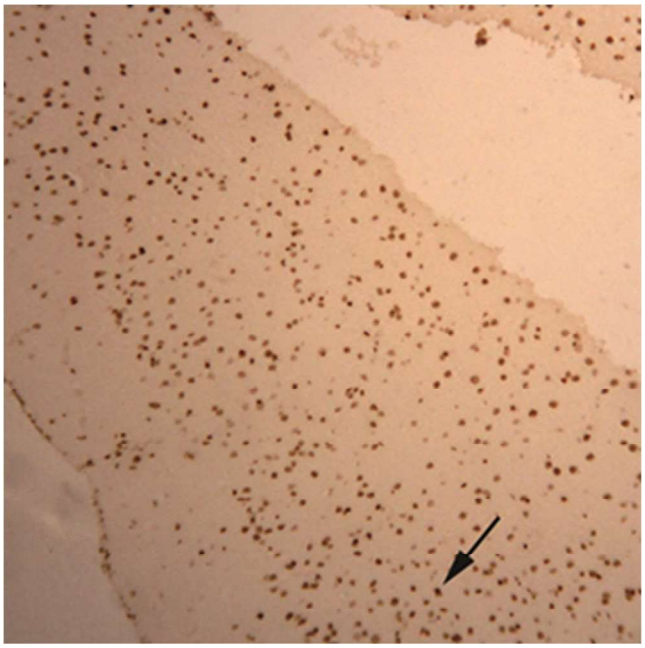

Figure 3.6 TUNEL staining of a prefrontal cortex section of ketamine group. Arrow denotes a TUNEL positive (apoptotic) cell. (100X)

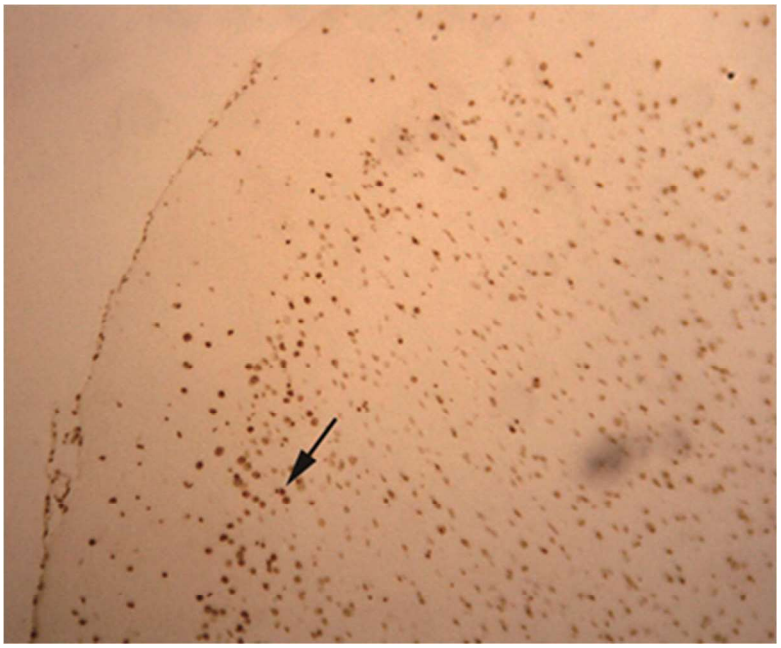

Figure 3.7 TUNEL staining of a prefrontal cortex section of ketamine plus Gouteng group with less TUNEL positive (apoptotic) cells (arrow) than ketamine group in figure 3.6 (100X)

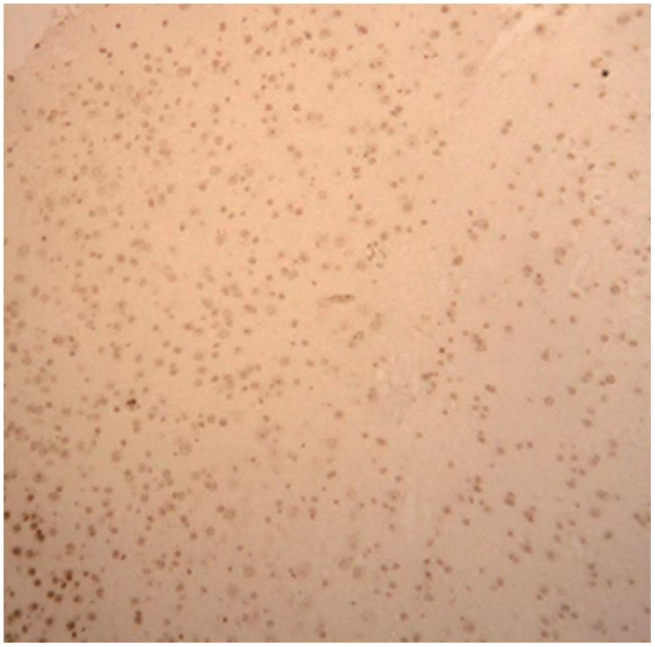

Figure 3.8 TUNEL staining of a prefrontal cortex section of control group with the least TUNEL positive (apoptotic) cells (100X)

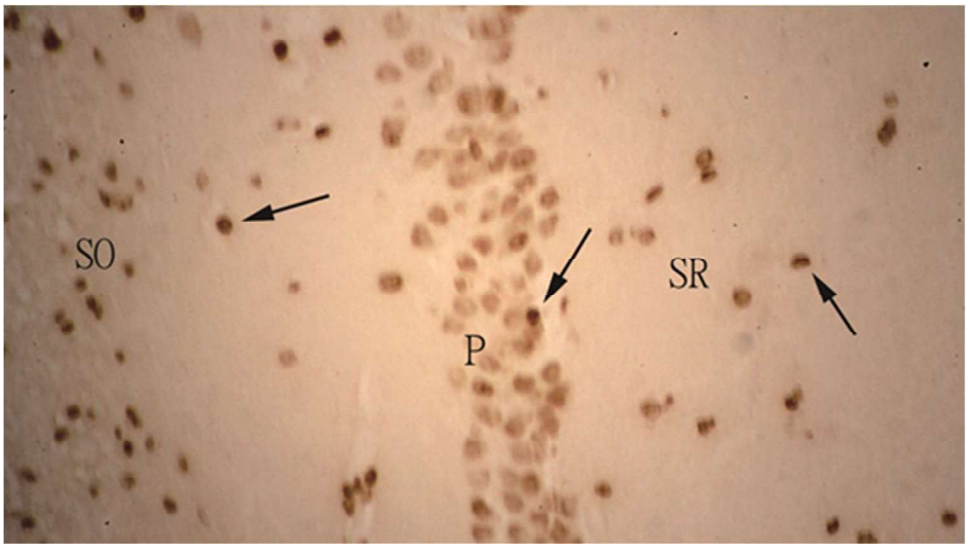

Figure 3.9 TUNEL staining of a hippocampus section of ketamine group. TUNEL positive cells in ketamine treated mice hippocampus (arrows). SO denotes stratum oriens; P denotes pyramidal cells and SR denotes stratum reticularis. Note that pyramidal layer had least dying cells. (400X)

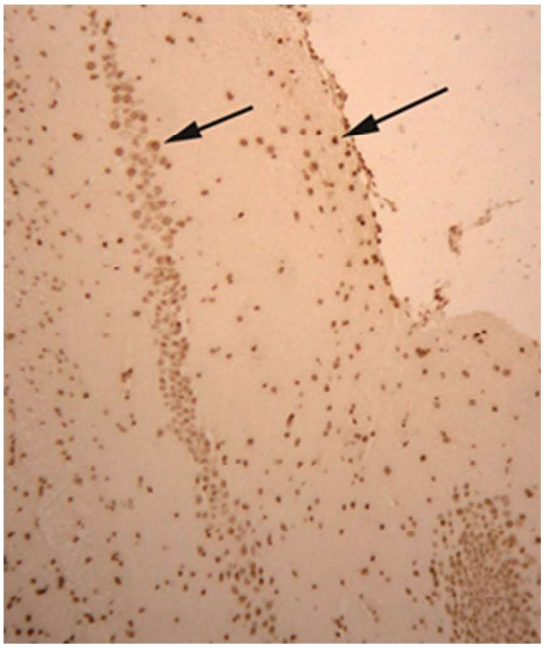

Figure 3.10 TUNEL staining of a hippocampus section of ketamine group showing apoptotic cells with darker nuclei (arrows) (100X)

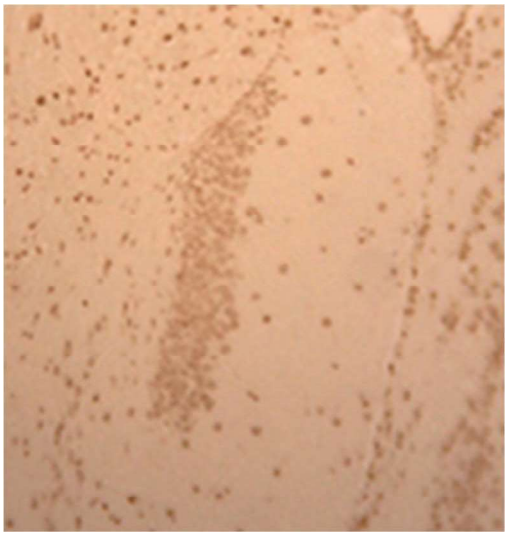

Figure 3.11 TUNEL staining of a hippocampus section of Gouteng group showing fewer apoptotic cells (100X)

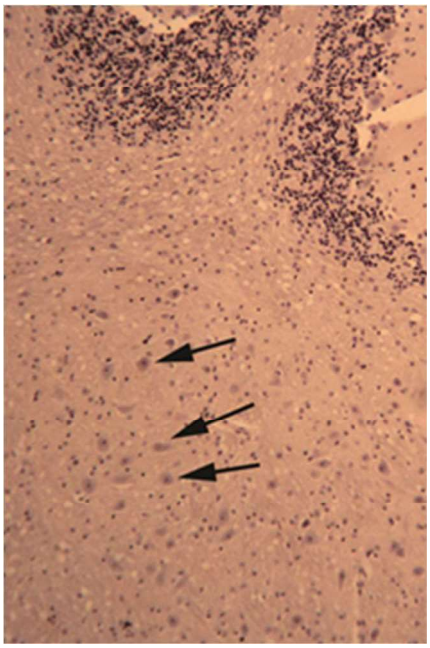

Figure 3.12 H&E staining of a cerebellum section of ketamine plus Gouteng group showing many cells (arrows) in the deep nuclei (40X)

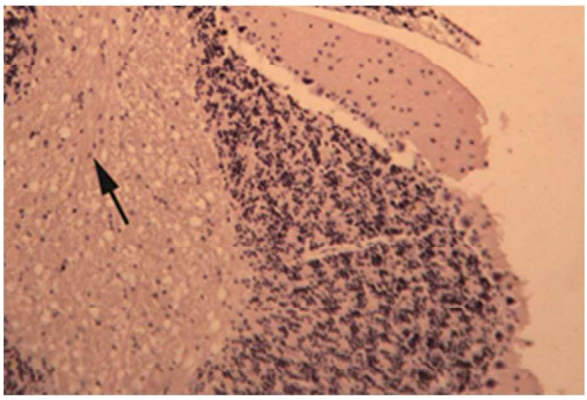

Figure 3.13 H&E staining of a cerebellum section of ketamine group. Fewer neurons were seen in the deep nuclei of the cerebellum (arrow) than ketamine plus Gouteng group (Figure 3.12) (40X)

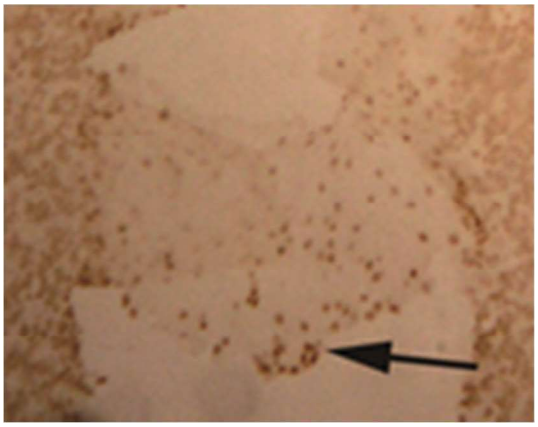

Figure 3.14 TUNEL staining of cerebellum section of ketamine group. Note TUNEL-positive (apoptotic) cells in the deep nuclei of the cerebellum (in brainstem) (100X)

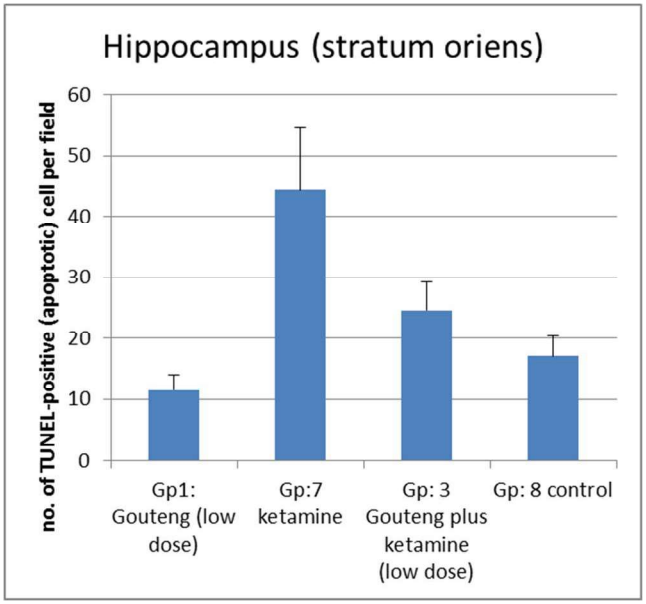

Figure 3.15 a graph showing the number of TUNEL positive (apoptotic) cell per field in stratum oriens of hippocampus in different groups of mice.

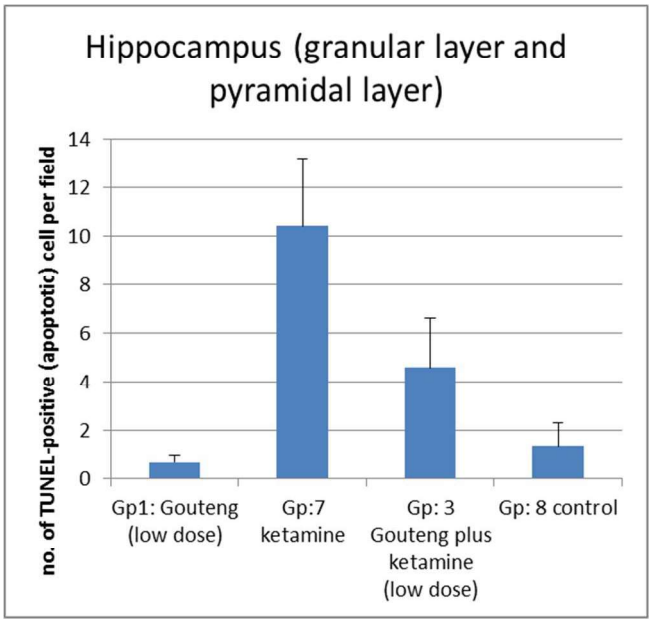

Figure 3.16 a graph showing the number of TUNEL positive (apoptotic) cell per field in granular layer and pyramidal layer of hippocampus in different groups of mice.

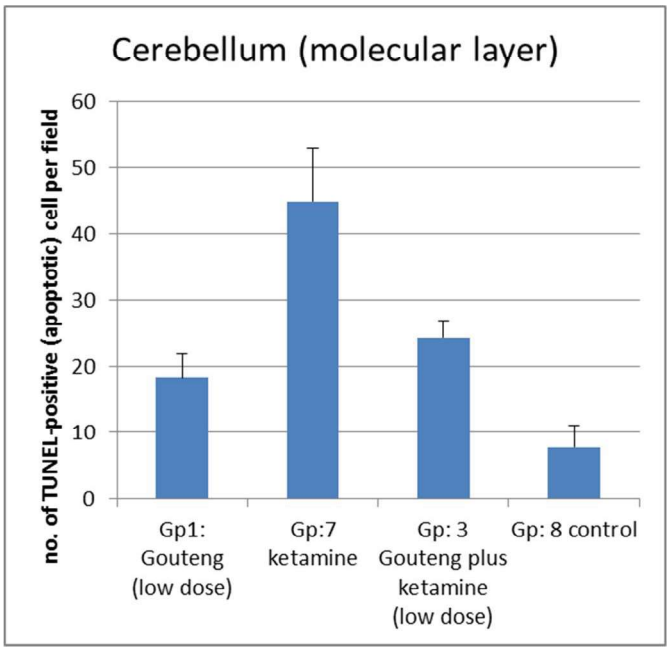

Figure 3.17 a graph showing the number of TUNEL positive (apoptotic) cell per field in molecular layer of cerebellum in different groups of mice.

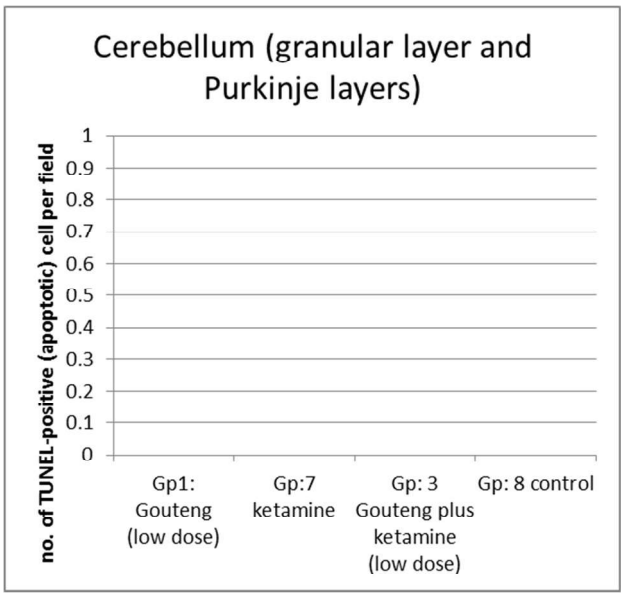

Figure 3.18 a graph showing the number of TUNEL positive (apoptotic) cell per field in granular layer and Purkinje layer of cerebellum in different groups of mice overall. No apparent apoptotic cell was found in this area of those groups.

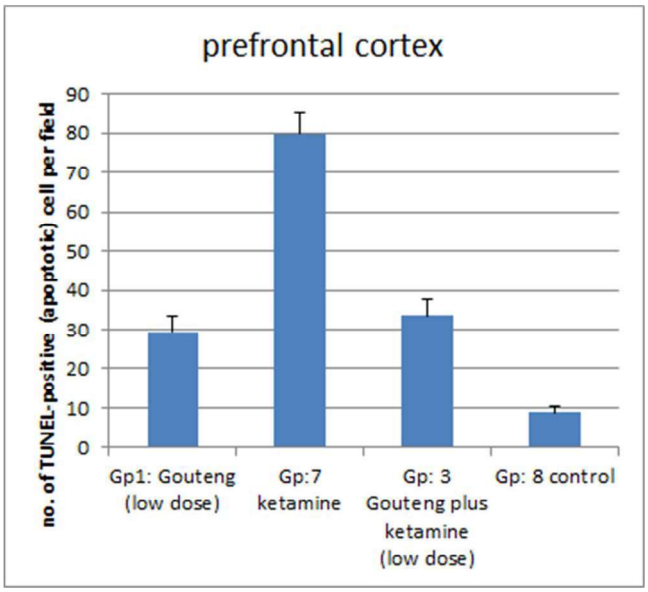

Figure 3.19 a graph showing the number of TUNEL positive (apoptotic) cell per field in prefrontal cortex in different groups of mice.

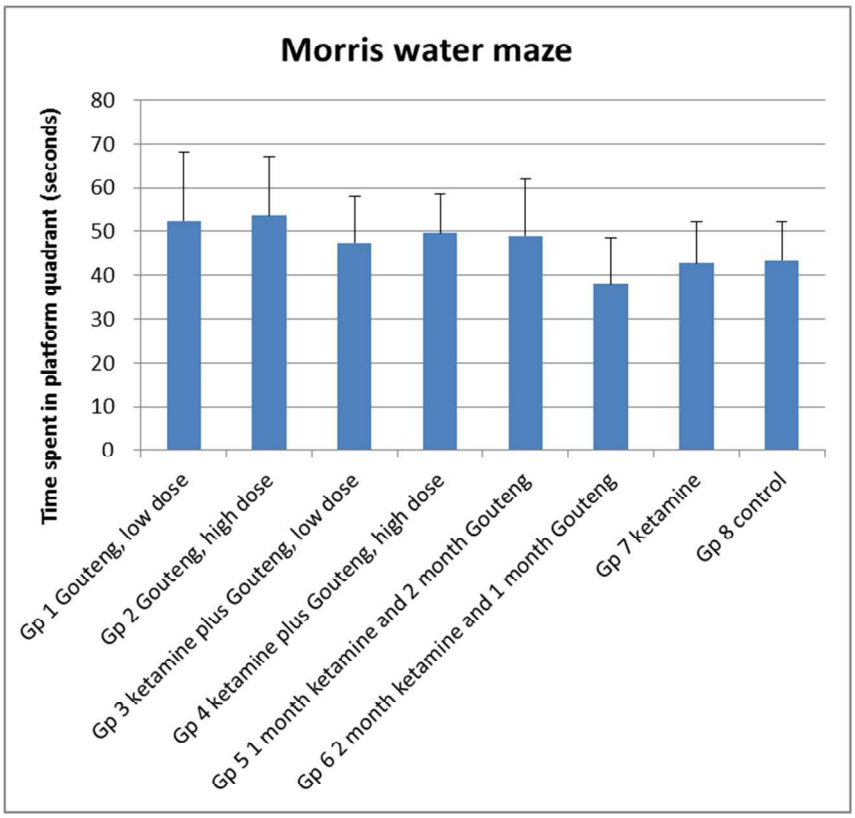

Figure 3.20 a graph showing the time spent in platform quadrant of different groups of mice in Morris water maze

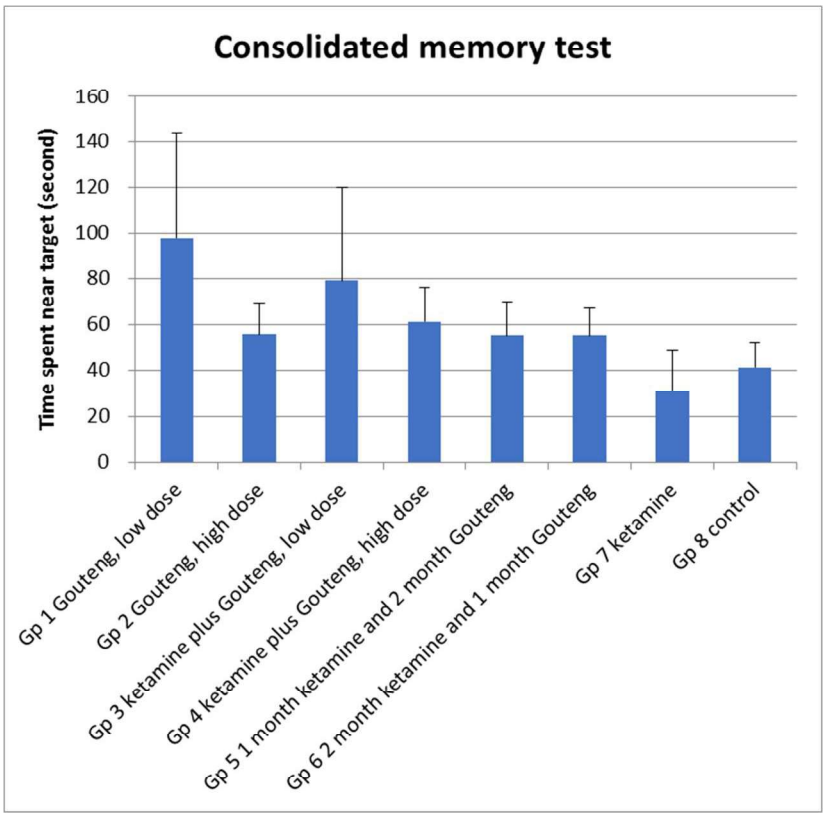

Figure 3.21 a graph showing the time spent near target of different groups of mice in consolidated memory test

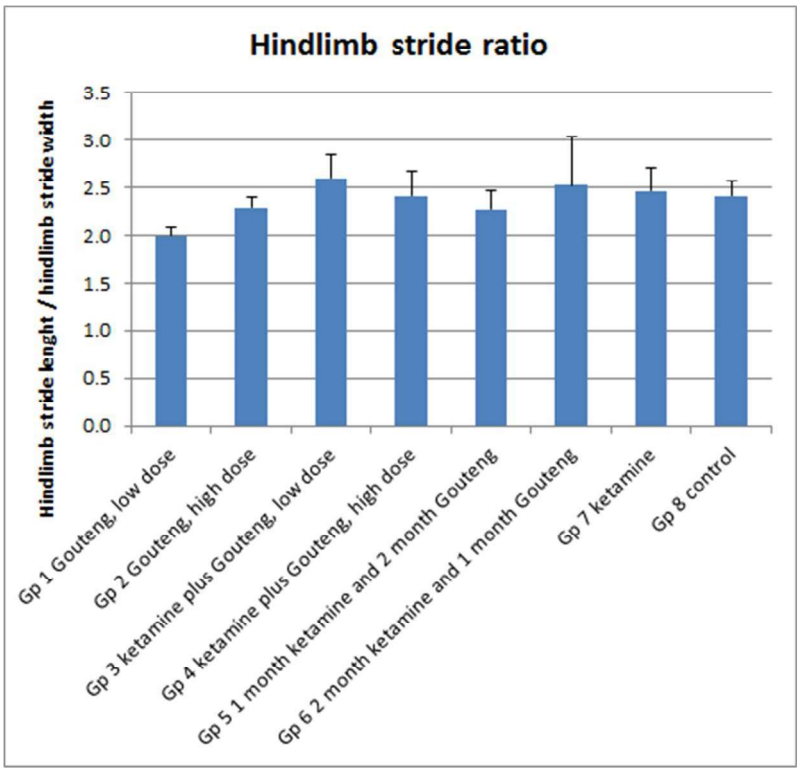

Figure 3.22 a graph showing the hindlimb stride ratio of different groups of mice in gait analysis

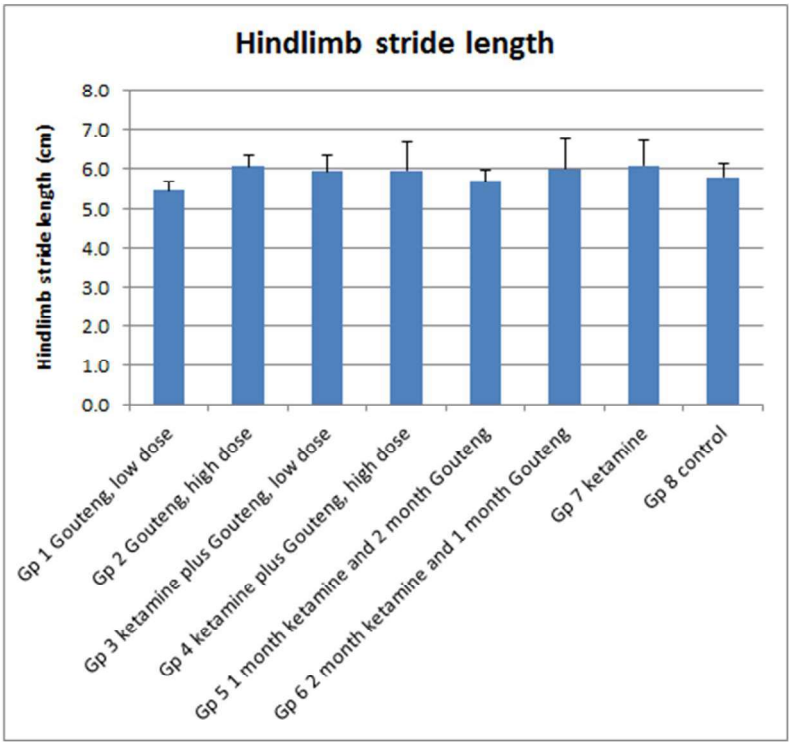

Figure 3.23 a graph showing the hindlimb stride length of different groups of mice in gait

analysis

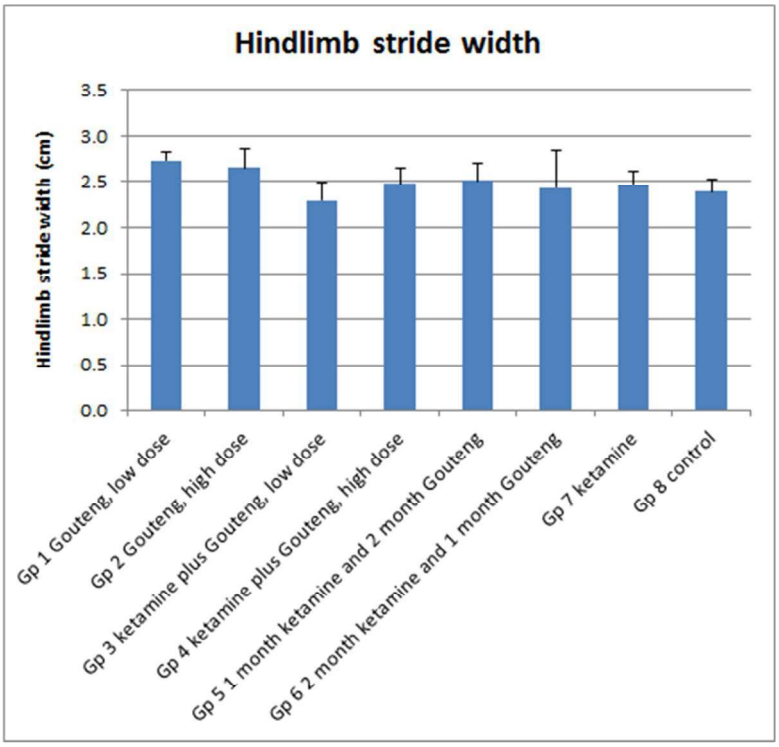

Figure 3.24 a graph showing the hindlimb stride width of different groups of mice in gait analysis

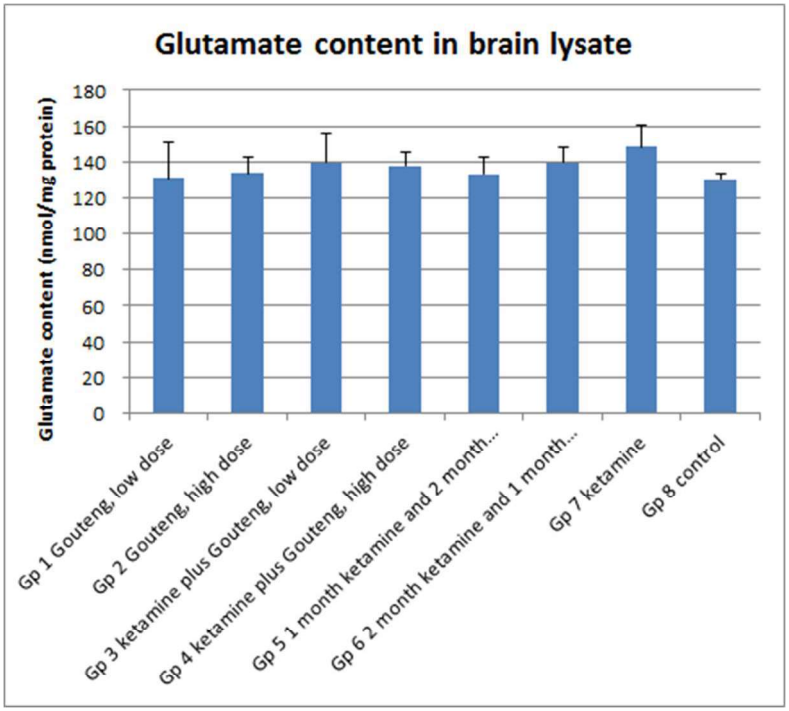

Figure 3.25 a graph showing glutamate content in brain lysate of different groups of mice

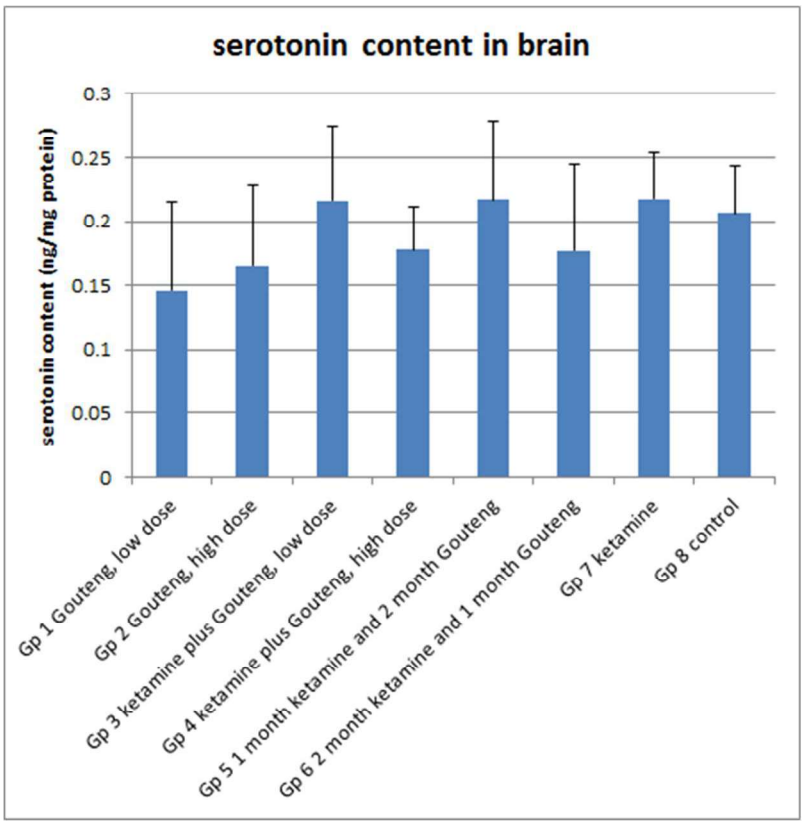

Figure 3.26 a graph showing serotonin content in brain lysate of different groups of mice

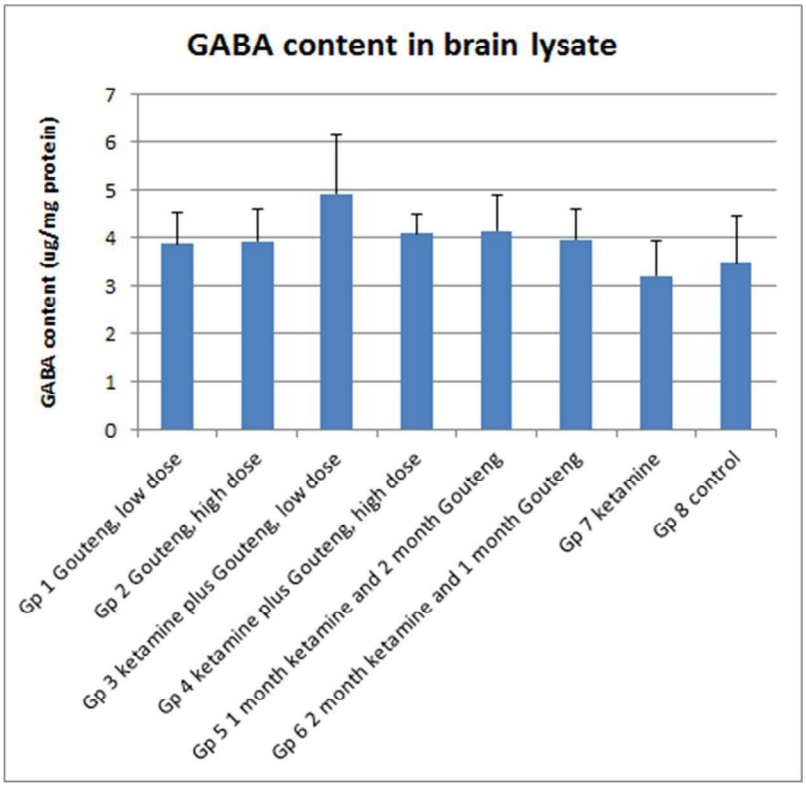

Figure 3.27 a graph showing GABA content in brain lysate of different groups of mice

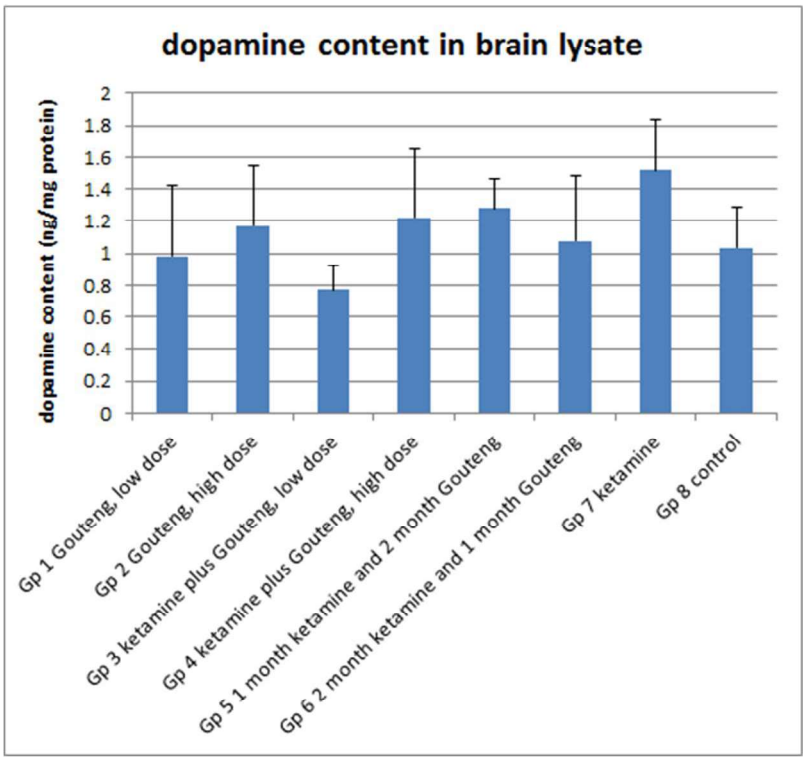

Figure 3.28 a graph showing dopamine content in brain lysate of different groups of mice

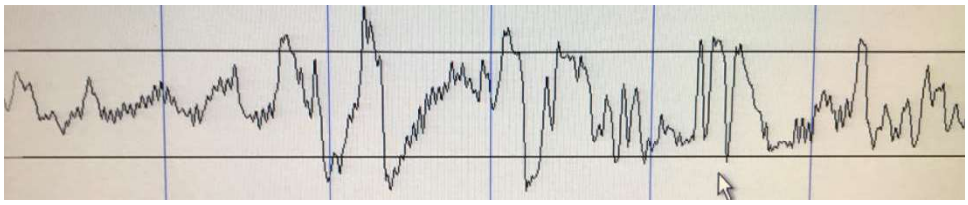

Figure 3.29 shows a normal large alpha wave.

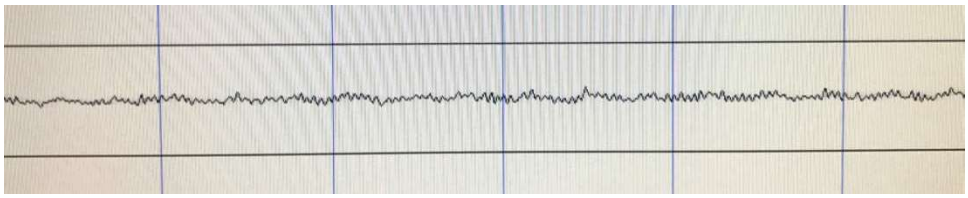

Figure 3.30 shows a routine smaller alpha wave in mice.

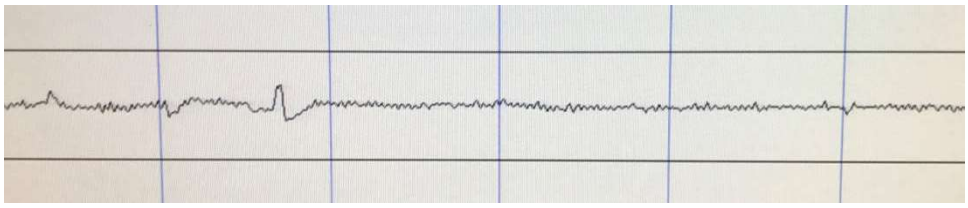

Figure 3.31 shows the presence of immature alpha wave mu wave after Gouteng treatment.

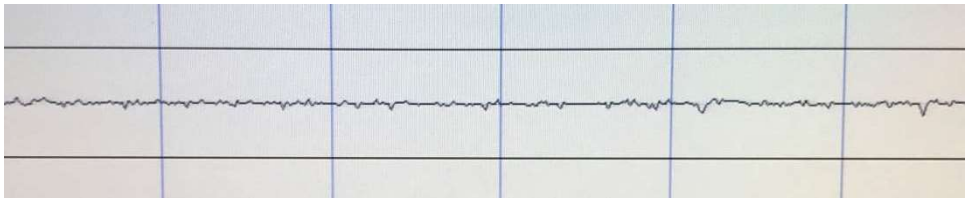

Figure 3.32 shows a sleeping or semiconscious wave of Gouteng and ketamine cotreated alone mice.

Part 2: Heart

Results

The histological studies of the heart reveal degenerative changes of myofibril in the cells of the heart of the ketamine treated group (figure 4.1), as presented in former studies (Chan, Liang, Wai, Hung, & Yew, 2011). In groups Gouteng, Gouteng and ketamine combined, and control, histological studies do not reveal any significant abnormality (figures 4.2, 4.3, 4.4).

Cardiac troponin I (cTnI) is a marker for acute damage of heart muscles. Enzyme-linked immunosorbent assay (ELISA) of cTnI of serum samples confirmed the increase of serum cTnI level in ketamine group, signifying a possible damage whereas in all other groups, the serum cTnI level was decreased comparing with ketamine group (figure 4.5), aligning well with the histopathology.

In electrocardiogram (ECG) evaluations, in ketamine group, there were recordings with ST inversions indicating ischemia (figure 4.6) as reported by us previously (Chan et al., 2011). On top of this, the present investigation recorded brachycardia (slowing of heart beat, figure 4.6) and with the decrease of ventricular contraction amplitude (figure 4.6). In groups of ketamine plus Gouteng, none of these recordings were obtained and all tracings were by at large normal as in control (figure 4.7), with an occasional mild atropic in the Gouteng groups (figure 4.8). Mild arrhythmias were also observed by Varkevisser et al (2015) in rabbits upon ketamine (Varkevisser, Vos, Beekman, Tieland, & Van Der Heyden, 2015).

Discussion

Since our discovery of ketamine toxicity on the heart (Chan et al., 2011), there were subsequently many papers on the forms of ketamine toxicity on the hearts of animals and humans (Ahiskalioglu et al., 2015; Y. Li et al., 2012; Varkevisser et al., 2015; Wai et al., 2013). Our findings here that ketamine caused low amplitude of ventricular tracing coincided with the suggestion of AV conducting system or muscular defect suggested by Varkevisser et al. (Varkevisser et al., 2015). The defect would also cause slowing of heart rates. Recently, it was further documented the ectopic irregularities could be augmented via the use of beta blockers like metoprolol (Ahiskalioglu et al., 2015).

Overall, studies on the heart revealed that Gouteng and ketamine treatment could alleviate some of the damages caused by ketamine treatment alone.

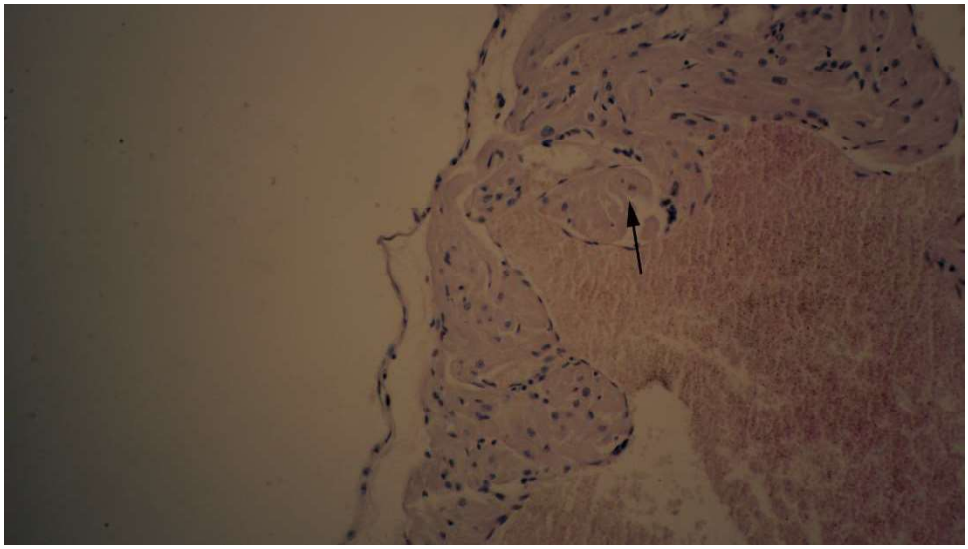

Figure 4.1 H&E stain of a heart tissue section of the ketamine group (100X). Arrow indicated degeneration of myofibril.

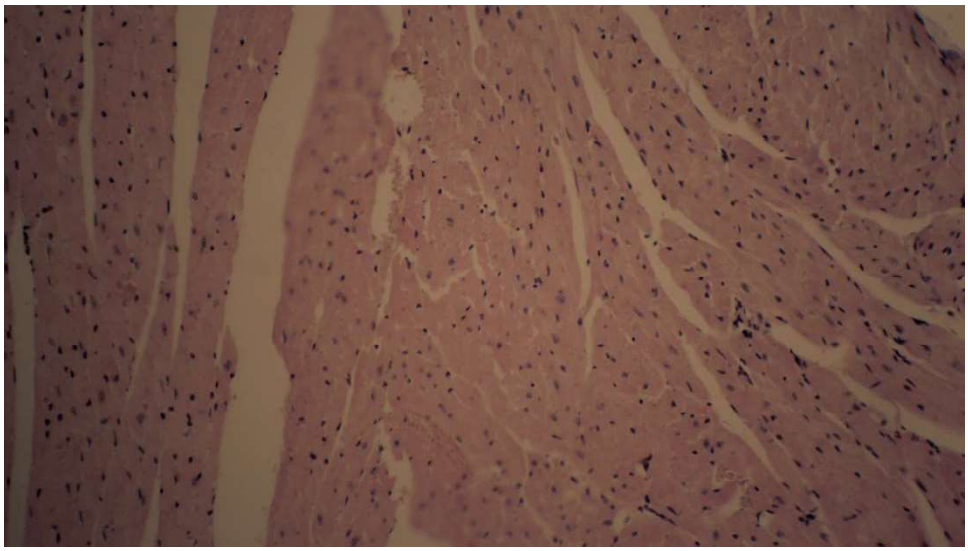

Figure 4.2 H&E stain of a heart tissue section of Gouteng group (100X). No significant degeneration.

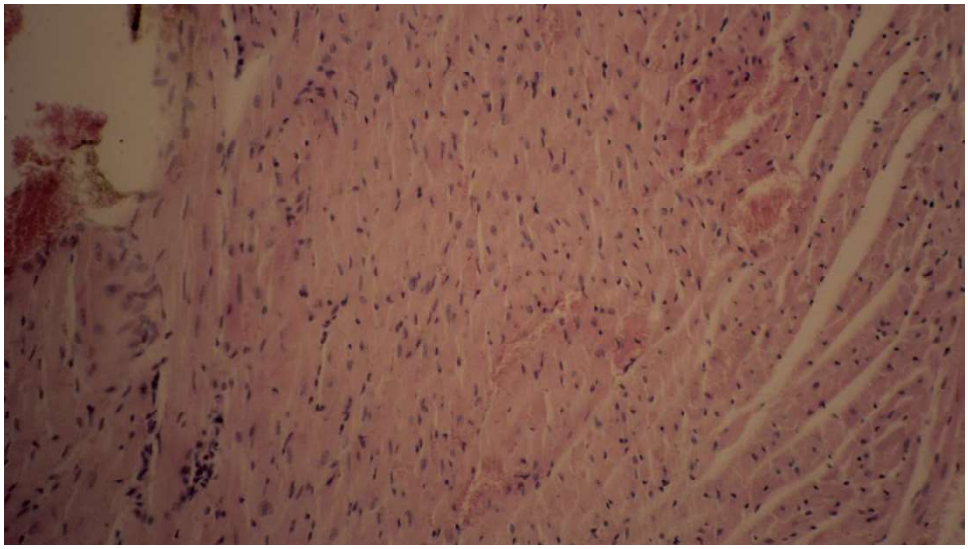

Figure 4.3 H&E stain of a heart tissue section of group of ketamine plus Gouteng (100X). No significant degeneration was observed.

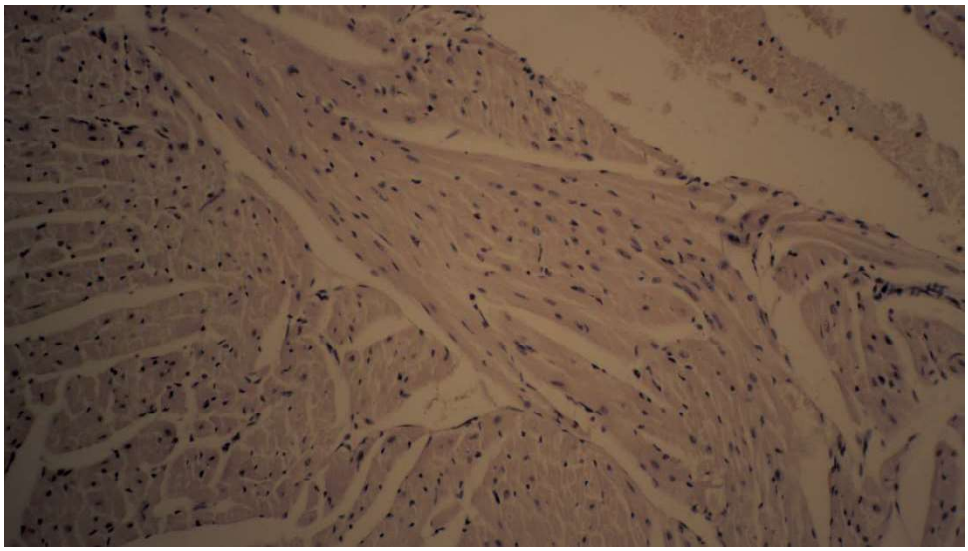

Figure 4.4 H&E stain of a heart tissue section of control (100X)

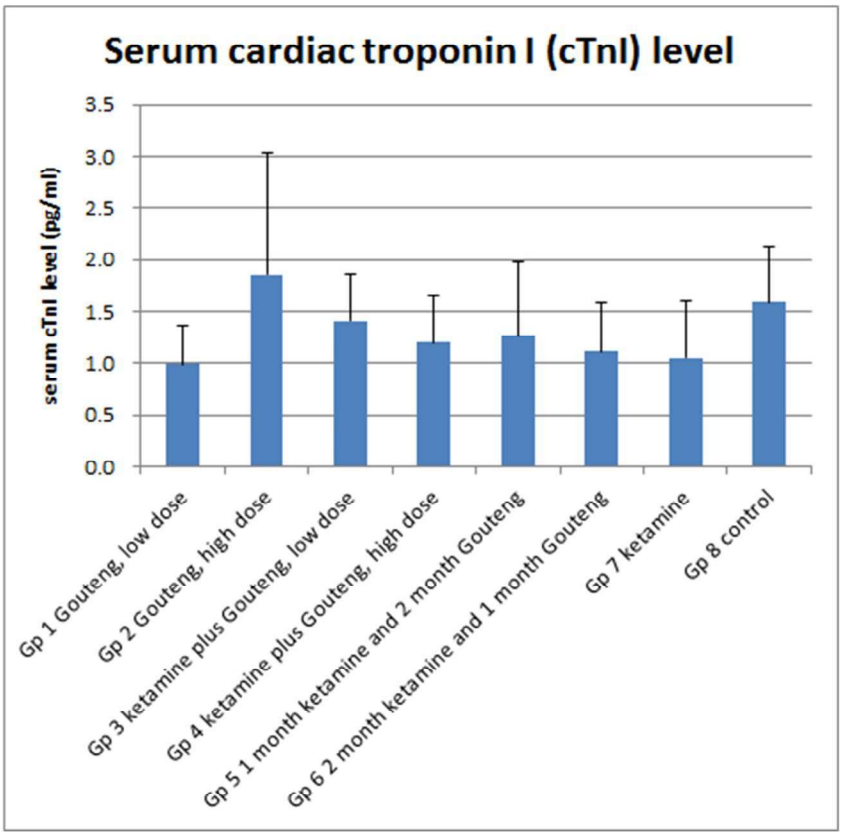

Figure 4.5 a graph showing serum cardiac troponin I concentration of different groups

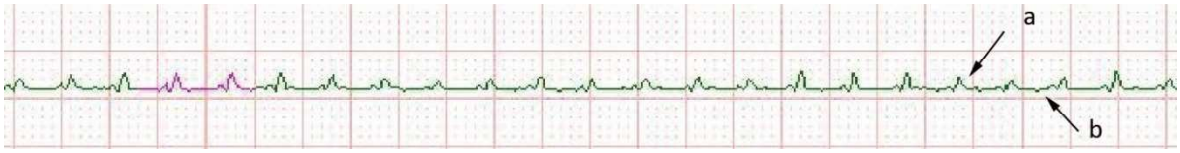

Figure 4.6 ECG of ketamine group. a indicates possible ST inversion and b indicates brachycardia.

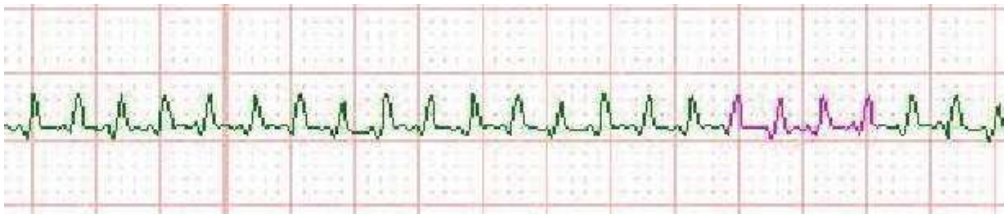

Figure 4.7 ECG of control group.

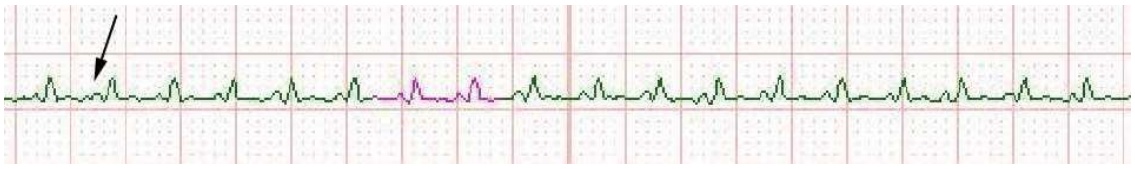

Figure 4.8 ECG of ketamine plus Gouteng groups. Arrow points to an ectopic beat.

Part 3: Liver

Results

The liver of ketamine treated mice displayed many cells had fatty degenerations (figure 5.1) as reported earlier in other works (M. S. Wai, W. M. Chan, A. Q. Zhang, Y. Wu, & D. T. Yew, 2012), while those treated by Gouteng had less liver cells with degeneration (rough estimation of about 50% less, figure 5.2). In the normal and the ketamine and Gouteng cotreated mice, the histology of the liver looked normal (figure 5.3). Enzyme determination revealed however both serum aspartate transaminase (AST) and alanine transaminase (ALT) were high for both ketamine treated alone and Gouteng treated alone mice, except that in the Gouteng and ketamine cotreated mice, serum AST decreased in these animals (figure 5.4). This shows a possible inhibitory effect of Gouteng on ketamine toxicity when acting together and it coincides with the normal histology observed in these groups. It seems that although Gouteng and ketamine are both toxic, they inhibited each other when acting together, a sort of competitive inhibition on target (liver), although the inhibition is not perfect e.g. no decrease in ALT level (figure 5.5). It is important to note that ALT was also involved in glucose regulation in stressful conditions, thus it was arguable whether it was a good biomarker for liver recently (McGill, 2016). It was also in this experiment that collagen did not show substantial differences in between groups (figure 5.6) indicating as in previous works, fibrosis of liver started after several months (M. S. Wai et al., 2012). Sirius red staining and immunohistochemistry (IHC) targeting on collagen I revealed that Gouteng had no or few positive fibers in the liver (figures 5.7 and 5.8) while ketamine plus Gouteng liver showed insignificant positive fibers (figures 5.9 and 5.10). Ketamine treated liver had more positive fibers (figures 5.11, 5.12 and 5.13). Control animals had no positive fibers in liver (figures 5.14 and 5.15). In fact in this cohort of ketamine treated animals, beginning of fibrosis was already evident.

Discussion

It is now well known that liver and the bile ducts can be affected by ketamine toxicity. In the human, ketamine affected the common bile duct and the liver of 9.8 percent of 297 chronic users of ketamine. Amongst these, seven out of nine liver patients had bile duct lesions and two actually developed fibrosis or cirrhosis at young ages (Wong et al., 2014). In the blood of these patients, increase of alkaline phosphatase, bilirubin and alanine aminotransferase were features (Wong et al., 2014). Liver damage was seen not only in Chinese patients but in Caucasian as well (Bevan & Burke, 2014; Sassano-Higgins, Baron, Juarez, Esmaili, & Gold,

2016). Ketamine can also interact with alcohol to aggravate the damage on the liver and liver cirrhosis was common in models of more than three-month treatment of ketamine (M. S. Wai et al., 2012) or even before.

In this study, we have explored the effect of *Uncaria rhynchophylla* in the treatment of organic damage in the ketamine addicts in our model of mice. Firstly, it was evident that liver damage was very clear in the histopathology sections of the liver, predominantly as a form of fatty degeneration. On the other hand, in the groups treated by *Uncaria* (Gouteng in common term), in the control and in the Gouteng plus ketamine combined treatment groups, no clear pathology was observed histologically. However, it was noted that Gouteng would also increase the amount of transaminase in the blood of these animals, though as compared with ketamine treated alone group, the level of transaminase was less except when at 20mg/kg dose. This was in fact not surprising as even western medicines went through their metabolism via the liver or the kidney. The more significant finding was that in the AST evaluation, the Gouteng plus ketamine groups (whether treated at the same time groups 3,4 or those treated by Gouteng first and then ketamine groups 5,6) had lower AST levels than the ketamine treatment alone. This appeared to indicate that Gouteng and ketamine would interact and lower the AST level in the liver. This did align well with the lack of pathology in the Gouteng plus ketamine group. It is tempting to point to a beneficial effect of Gouteng on the ketamine damaged liver. However, it is likely this protection is not total as the other transaminase level of ALT did not align with this preposition as ALT could be involved in glucose regulation in physiology (McGill, 2016).

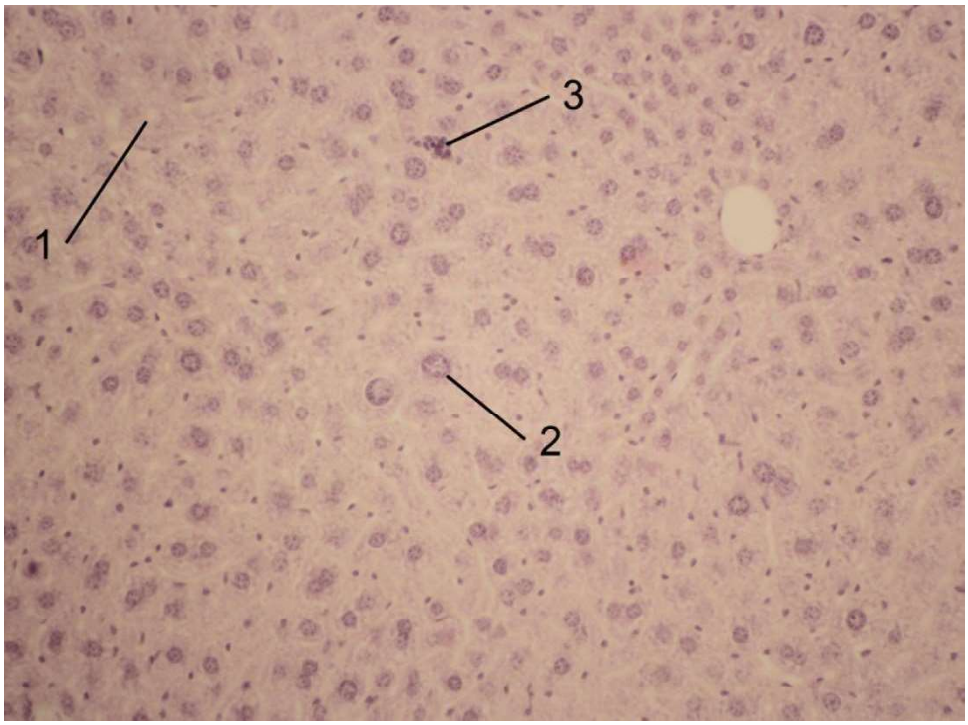

Figure 5.1 H&E stain of a liver section of ketamine treated mouse (group 7). Note cells of fatty degeneration (1), nuclear degeneration (2) and infiltration of lymphocytes (3) (100X).

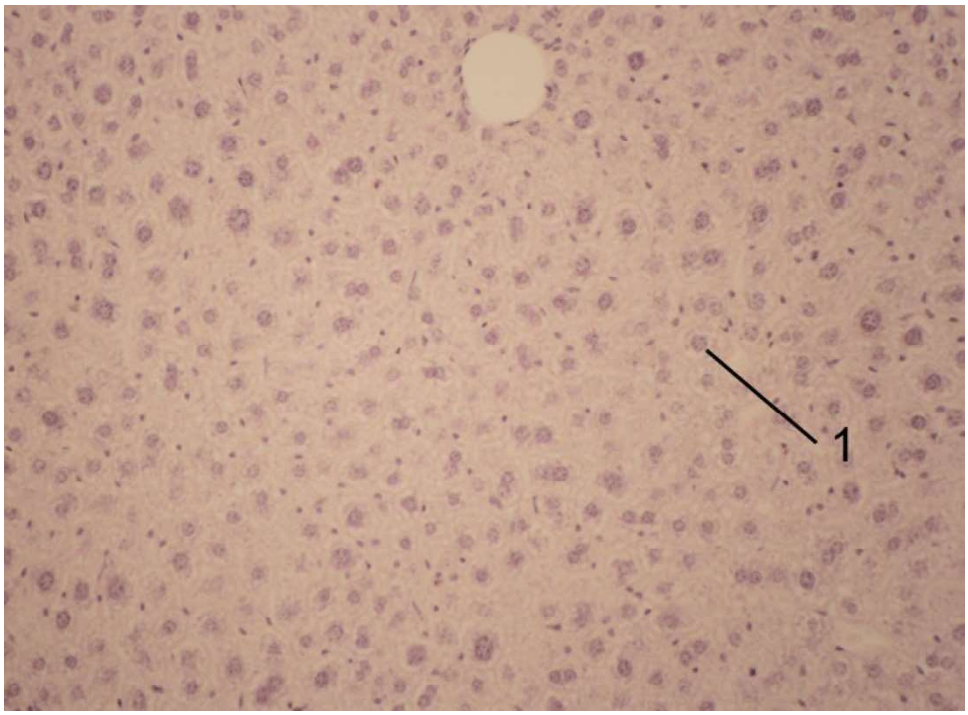

Figure 5.2 H&E stain of a liver section Gouteng treated mouse (group 1). Liver had lesser cells with degeneration except 1 (100X).

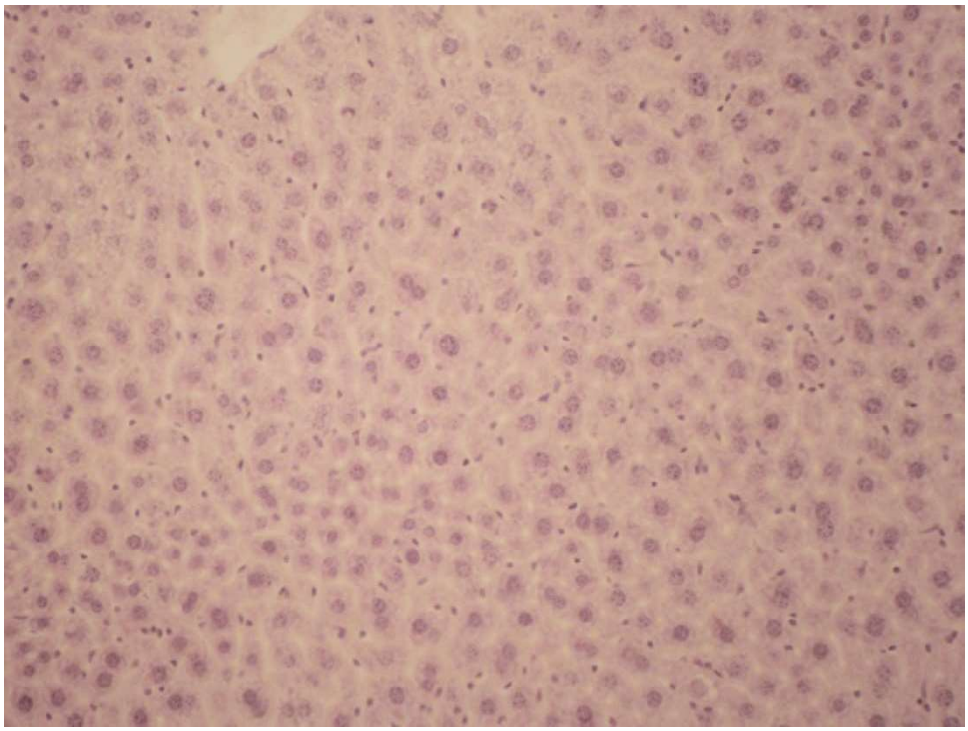

Figure 5.3 H&E stain of a liver section ketamine and Gouteng co-treated mouse (group 4). Liver was normal in architecture and few degenerative cells. (100X)

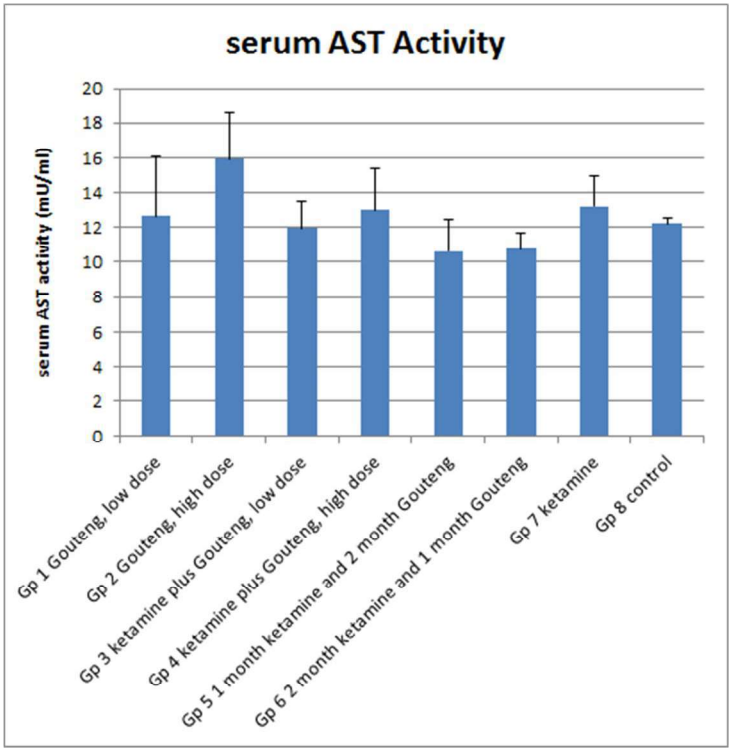

Figure 5.4 serum AST activities of different groups of mice

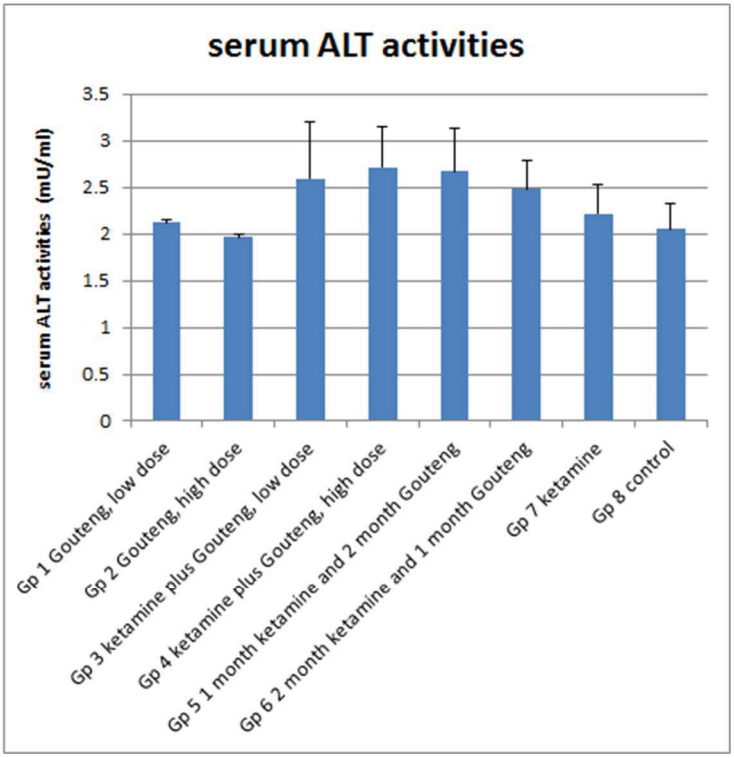

Figure 5.5 serum ALT activities of different groups of mice

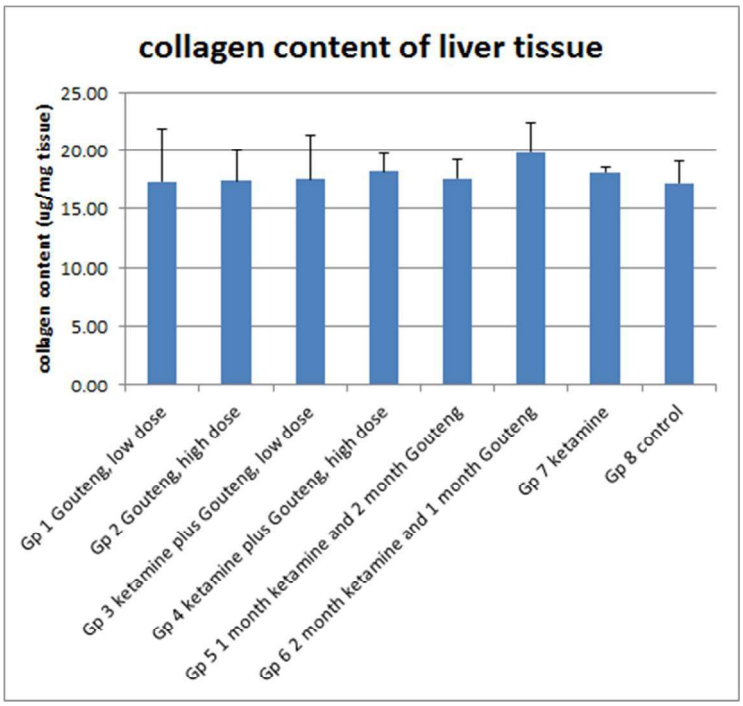

Figure 5.6 collagen content of liver tissue of different groups of mice

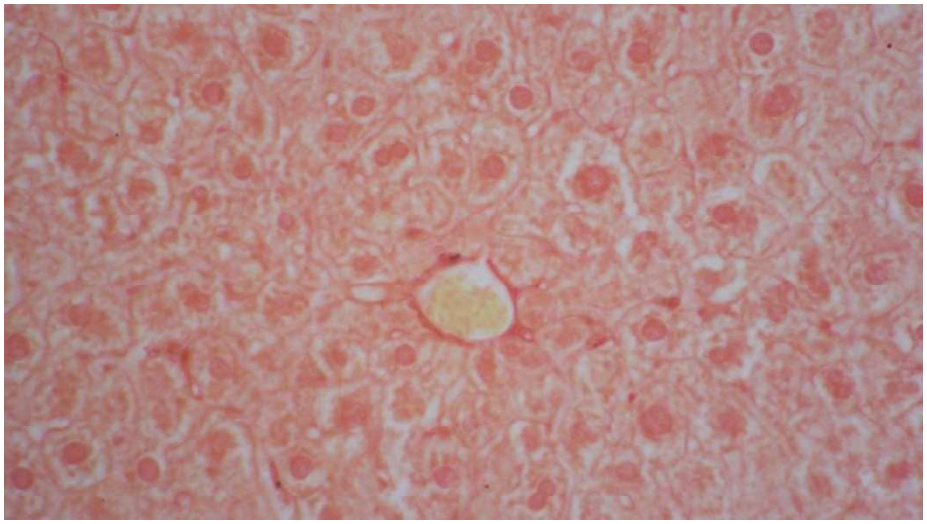

Figure 5.7 sirius red stain of a liver section of Gouteng treated mice (group 1) showing few red collagen fibers (400X)

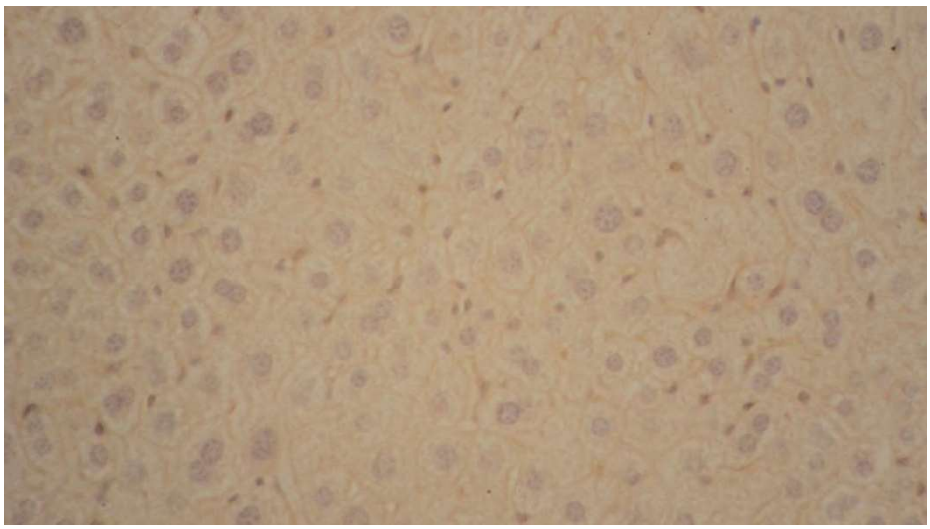

Figure 5.8 IHC (collagen I) of a liver section of Gouteng treated mice (group 1) showing few collagen fibers (400X)

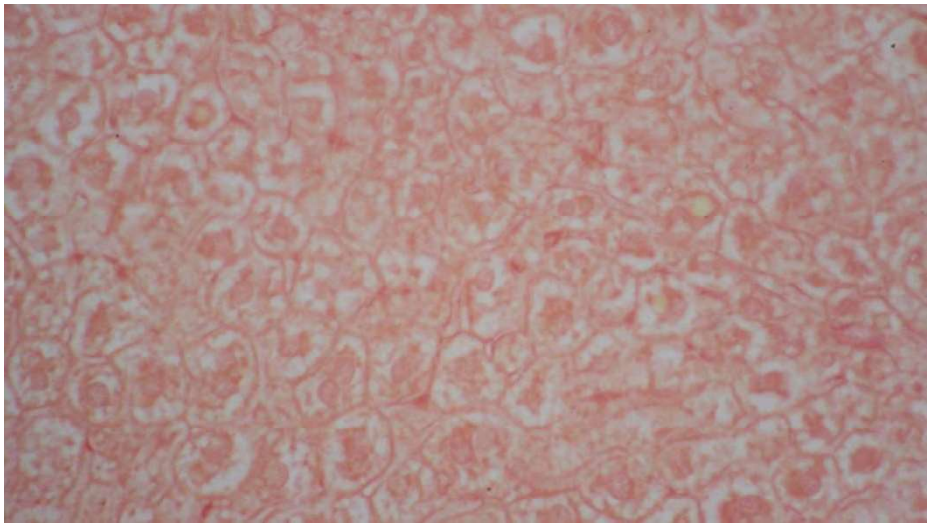

Figure 5.9 sirius red stain of a liver section of Gouteng plus ketamine treated mice (group 3) also showing few red collagen fibers (400X)

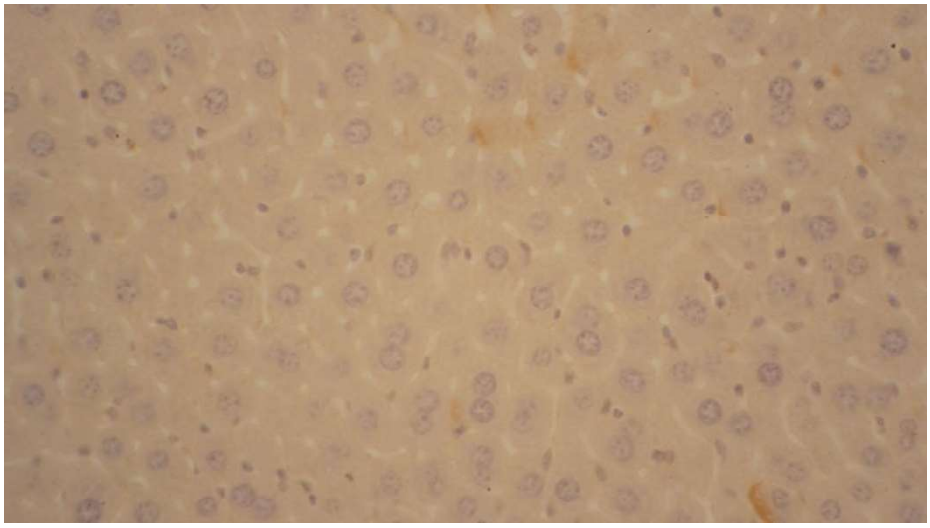

Figure 5.10 IHC (collagen I) of a liver section of Gouteng treated mice (group 1) showing few collagen fibers (400X)

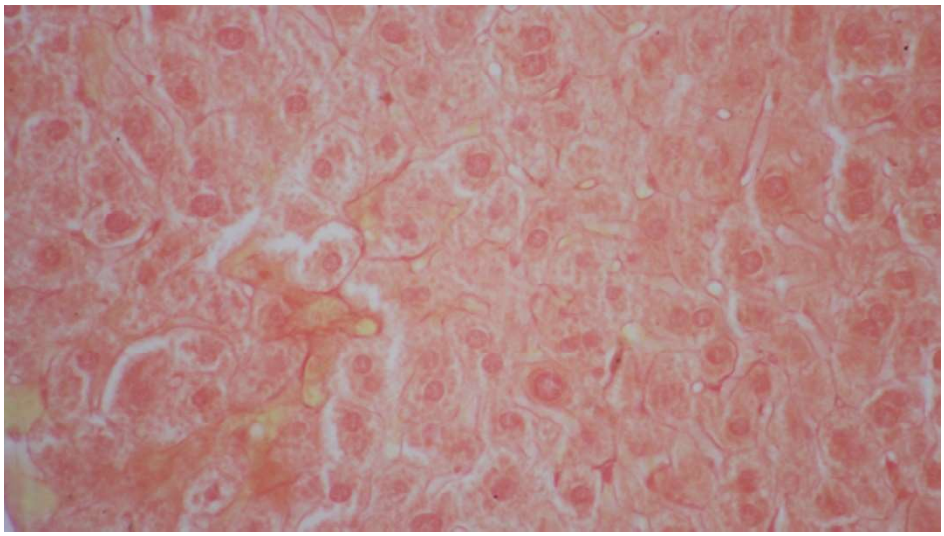

Figure 5.11 sirius red stain of a liver section of ketamine treated mice (group 7) showing a few red collagen fibers (400X)

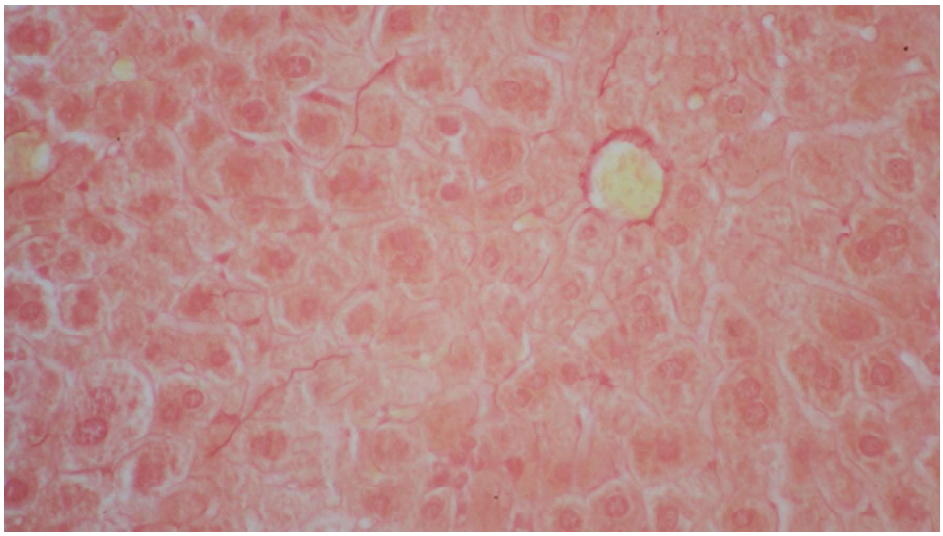

Figure 5.12 sirius red stain of a liver section of ketamine treated mice (group 7) showing a few red collagen fibers (400X)

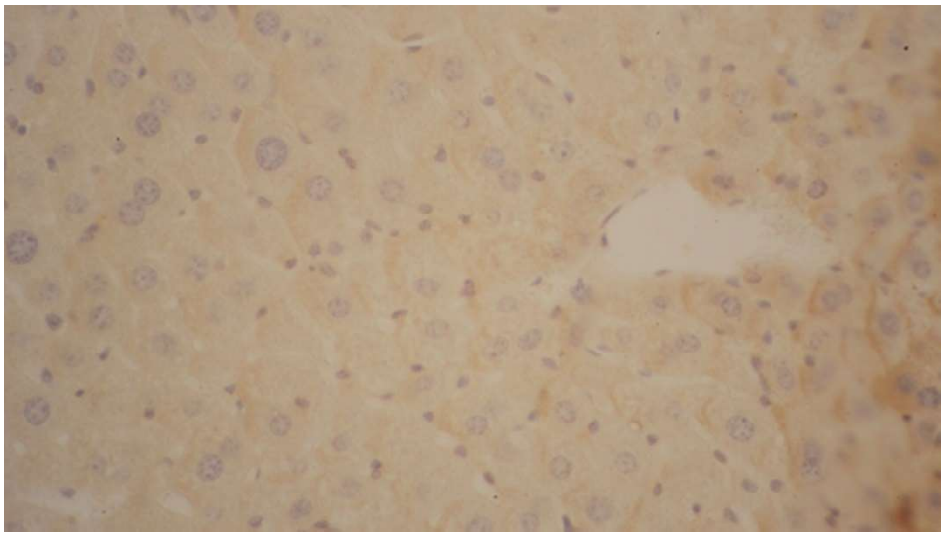

Figure 5.13 IHC (collagen I) of a liver section of ketamine treated mice (group 7). A few deposits of collagen I were observed. (400X)

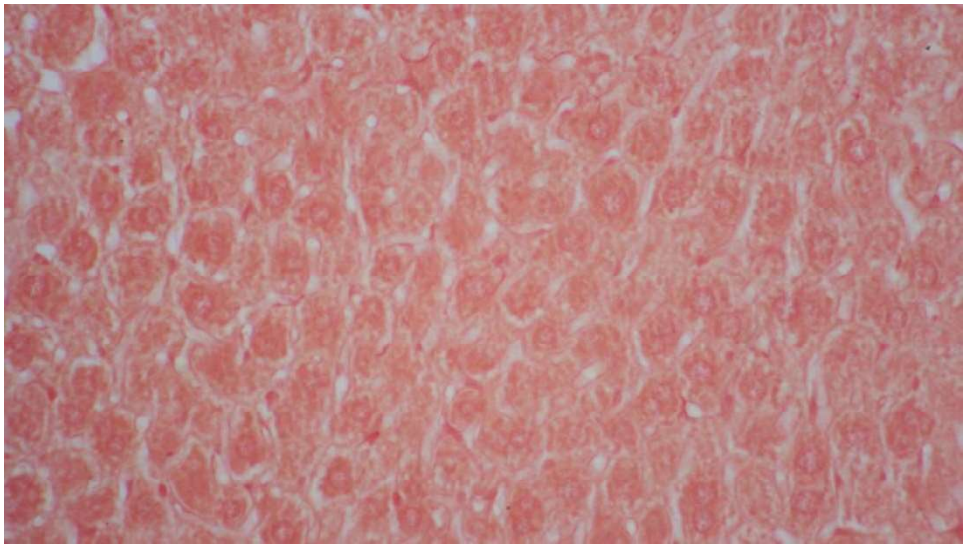

Figure 5.14 sirius red stain of a liver section of control mice (group 8) without red collagen fibers (400X)

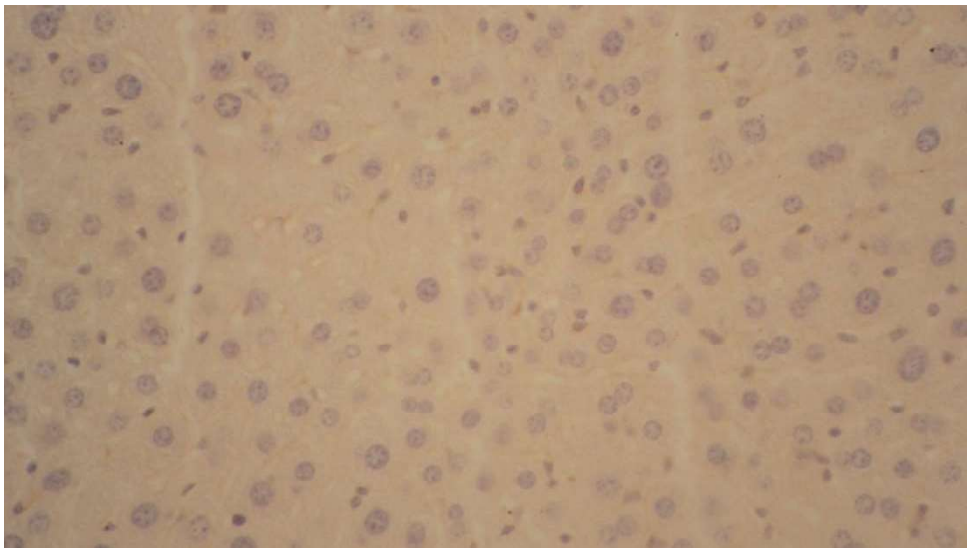

Figure 5.15 IHC (collagen I) of a liver section of control mice (group 8) showing no positive collagen I in the liver. (400X)

Part 4: Urinary system

Results

In bladders of the ketamine treated animals, loci of dystrophic and disoriented groups of cells which we termed as “metaplasia” was revealed in the epithelium (Figure 6.1). Lymphocytes were observed in the lumen of the bladder (Figure 6.1), as well as in the lamina propria and submucosa of these urinary bladders (Figures 6.2 & 6.3). There were also swollen muscle fibers (Figure 6.4) in these bladder walls of ketamine treated mice. Furthermore, the ketamine treated bladder lumen contained macrophages and epitheloid cells as well (Figures 6.5 & 6.6). In the mice that had combined treatment of ketamine and Gouteng and in the Gouteng treated mice, the bladders had relatively normal epithelial and normal muscle cells and no significant infiltration of lymphocytes in the bladder wall or lumen (Figures 6.7, 6.8, 6.9 & 6.10).

In ketamine treated mice, kidneys demonstrated degenerative glomeruli, lymphocytes migration into kidney and pyknotic tubular cell death, along with shedding and dislodged tubular cells which were seen (Figures 6.11, 6.12 & 6.13) together with dilated tubular space (Figure 6.14), while Gouteng-ketamine co-treated animals and Gouteng treated animals alone had relative normal morphology in kidneys (Figures 6.15 & 6.16), with the exception of two cases in the cohort of sixteen animals in the Gouteng groups, one had protein cast (Figure 6.17) and another had degenerated glomerular space with sclerosis (Figure 6.18).

Degeneration of glomeruli included lysis and shrinkage of glomeruli. For the latter, shrinkage was defined if the area of each glomerulus occupied less than 30% of the total area inside the Bowman’s capsule, to avoid sectioning artifact. An estimation of degeneration of glomeruli in 500 counted glomeruli in this way were 4.5% ± 1% in saline control, 15.6% ± 2% in Gouteng group and 19.9% ± 2.2% in ketamine group whilst in the combination of Gouteng and ketamine group. It was 12.5% ± 3.3% (n = 3 animals in each group). A decrease in a number of degenerative glomeruli was noted after ketamine and Gouteng combined treatment than ketamine treatment alone. But also note that in Gouteng, like ketamine, high dose of Gouteng could increase serum creatinine level in long term (Figure 6.19).

Discussion

Ketamine addicts usually presented contracted small bladders of one-third the sizes as that of the normal (P. S. Chu et al., 2008). Cell disorientation and cell death in the epithelium, lymphocyte infiltration, and degeneration of muscle were observed in the mice treated with

ketamine model within 1-3 months of treatment while fibrosis was seen later (S. Tan et al., 2011; L. Yeung et al., 2009). This was similar to the present ketamine model in this study. By 6 months of ketamine treatment, fibrosis then occupied the bladder and the bladder constricted (S. Tan et al., 2011; L. Yeung et al., 2009). What was interesting here was that Gouteng, a species of *Uncaria*, which had no toxic effect on the bladder and adding Gouteng to ketamine treatment, could arrest the growth of disorientated and dystrophic (metaplasia) epithelium and arrested the inflammatory response of invasion of lymphocytes into the bladder and its lumen caused by ketamine. Recent studies had indicated that in certain cancers, *Uncaria* species could arrest apoptosis in adenocarcinoma while in colon and lung cancers and breast, cancer cell cycle could be affected by the oxindole alkaloid of other *Uncaria* species. The blockage of cell death in the bladder and other areas by *Uncaria* via downregulation of lactic dehydrogenate, DR4 death receptor, glutamate induced Bid cleavage and upregulation of Bcl2 was also illustrated (Ji Yeon Jang et al., 2014; J. Y. Jang et al., 2012) and anti-inflammatory response had been documented by various *Uncaria* species (Aguilar et al., 2002; Goncalves, Dinis, & Batista, 2005; Rojas-Duran et al., 2012). One of the components of Gouteng, Isorhynchophylline had a wide effect of suppressing superoxide formation as well as inhibited inflammatory molecules like nuclear factor kappa B, prostaglandin E 2, nitric oxide and cyclooxygenase 2 (Hsieh et al., 2009). Anti-inflammatory response could further act on cytokines (mitraphylline) or worked against free radicals but not on cyclooxygenase. After ketamine treatment, the presence of inflammatory cells (lymphocytes in the majority) in the lumen of the bladder implied these cells were in the urine. Evaluation of the quantity of these cells in the urine could also give some hints to the prognosis. Our studies also indicated that Gouteng-ketamine combined treatment erased the presence of inflammatory and epitheloid cells in urine.

In the kidney, ketamine could cause tubular degeneration, detachment and dislodging of proximal tubular cells and in addition, infiltration of immune cells (lymphocytes) as well as degeneration of glomeruli. These were similar to that reported earlier (Dargan, Tang, Liang, Wood, & Yew, 2014; M. S. Wai et al., 2012; Wai et al., 2013). In Gouteng treated, and Gouteng-ketamine combinely treated groups, tubular necrosis and degeneration were no longer obvious, and detachment of tubular cells in the proximal tubules were not demonstrated. *Uncaria* with its oxindole alkaloids had been studied for its effect against cell death in the nervous system, its actions were mainly on Bax/Bcl2 ratio, calcium influx, PI3 Kinase/AKT signaling pathway (Lee et al., 2003; Yutaka Shimada et al., 1999). It is likely that the tubular cells of the kidney got protected in the same way from cell death. Other tubular changes like dislodgement, decoupling or change of permeability related to the loss of E-cadherin. It is likely that Gouteng also controlled these pathways in the kidney (Tian et al., 2011). Furthermore, *Uncaria* species, being anti-inflammatory (Goncalves et al., 2005), reduced lymphocytes entry and this helped to curb the invasion of lymphocytes in the

Gouteng-ketamine group. One thing arising from this study was that the number of degeneration glomeruli in Gouteng treated, and Gouteng-ketamine treated were still higher than normal, though not as high as ketamine treated. Creatinine measurement was high for both Gouteng and ketamine after long term treatment. It is therefore suggested that Gouteng treatment when performed should be in cycles of less than a month and with free a month in between.

In conclusion, it appeared that in the combined ketamine-Gouteng treated animals, the kidney had less pathology than those of ketamine treated, in the aspects of epithelial cells shedding in the tubules and lymphocyte infiltration in the kidney. On the other hand, Gouteng treatment did not protect all glomeruli damaged by ketamine. There were still an observable number of degenerative glomeruli even after Gouteng-ketamine interaction, howbeit less than ketamine treatment alone. This reflected that the kidney was functionally not at the normal level as control even after Gouteng treatment.

As importantly, the fact that long term treatment of Gouteng led to increase of serum creatinine level. This probed us to consider long term treatment of Gouteng must be performed carefully. Contrary to the kidney, in the ketamine induced urinary bladder, Gouteng treatment curbed the lymphocyte infiltration, metaplasia of epithelium and muscular edema. It is therefore useful for treating ketamine induced urinary bladder pathology. In addition, lymphocytes disappeared in the lumen of the bladder, along with epithelioid cells revealed a good prognosis after treatment in the bladder.

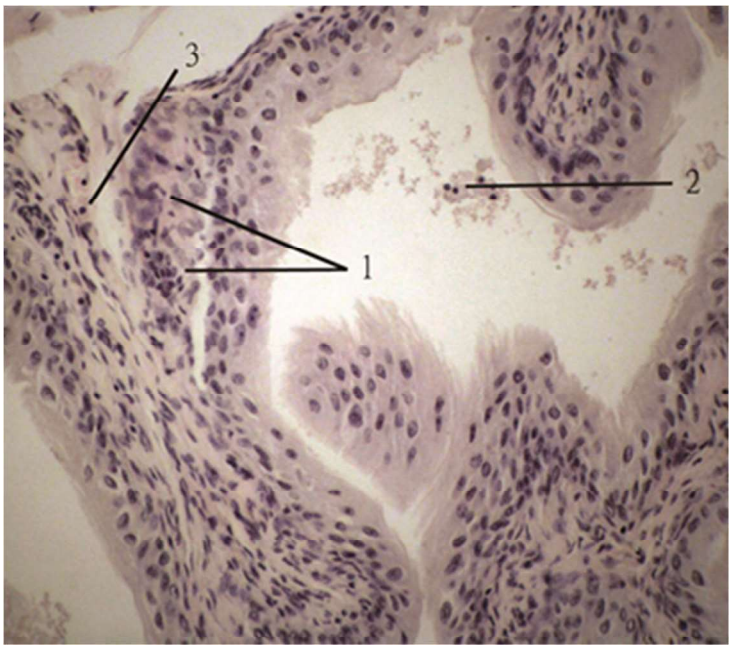

Figure 6.1 H&E stain of a bladder section of a ketamine treated mouse. 1 denotes areas of dystrophic and disoriented epithelium. 2 denotes lymphocytes in bladder lumen. 3 denotes lymphocyte in lamina propria. (200X)

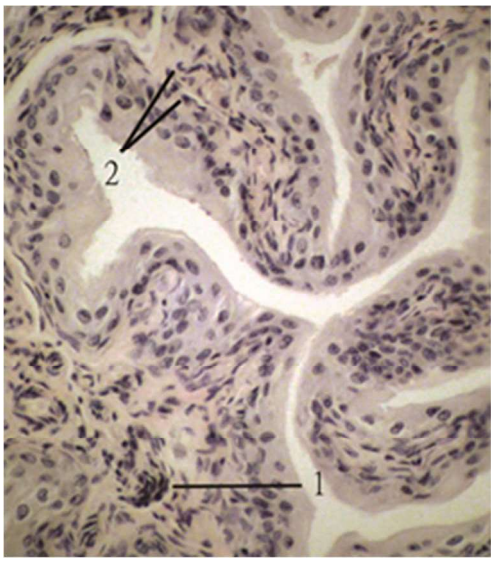

Figure 6.2 H&E stain of a bladder section of a ketamine treated mouse. 1 denotes gathering of fibroblasts and inflammatory cells in submucosa of bladder. 2 denotes constricted and twisted nuclei of dying cells. (200X)

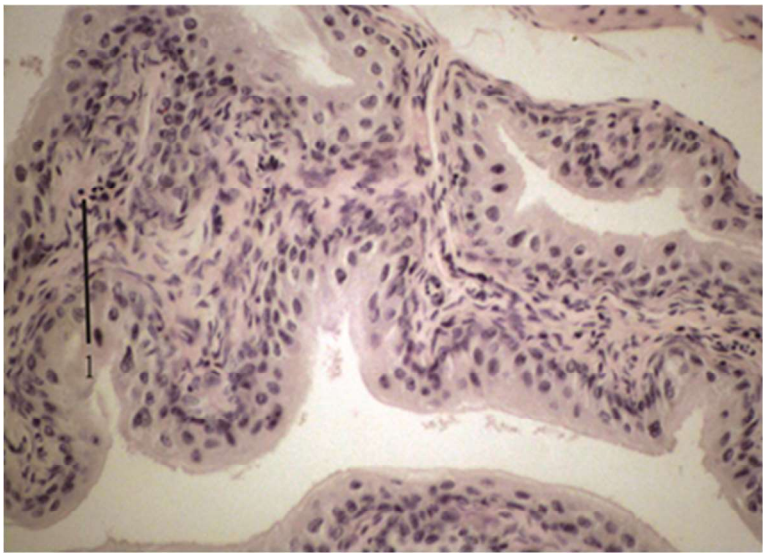

Figure 6.3 H&E stain of a bladder section of a ketamine treated mouse. 1 denotes collection of lymphocytes and fibroblasts in lamina propria of bladder. (100X)

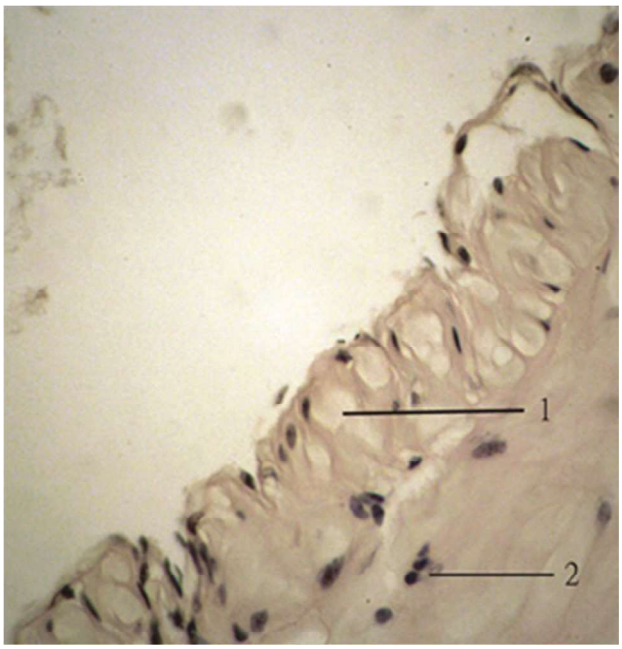

Figure 6.4 H&E stain of muscle fibers in the bladder wall of a ketamine treated mouse. Note loss of myofibril in cross sectional swollen fiber (1) and the presence of lymphocyte (2). (400X)

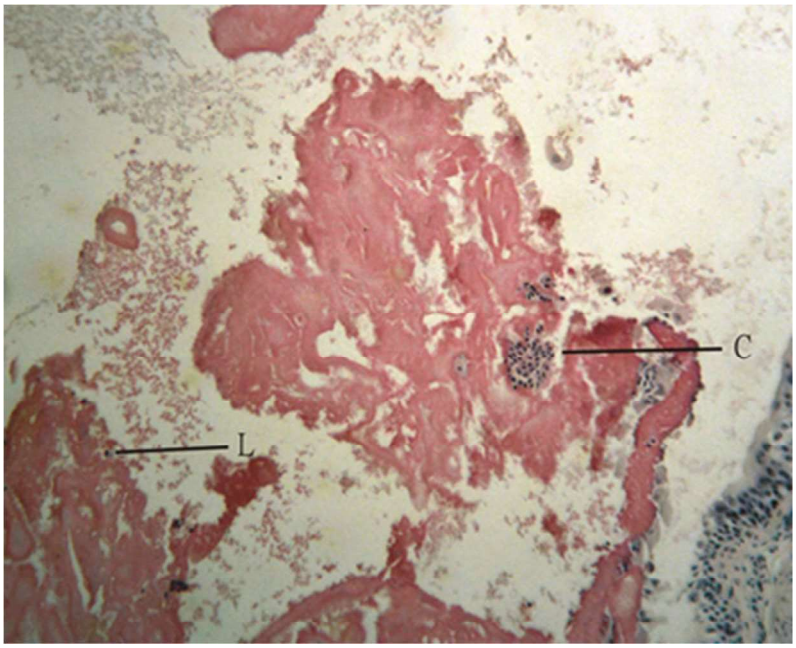

Figure 6.5 H&E stain of the lumen of a bladder of a ketamine treated mouse. Note inflammatory cells in the lumen (C) with lymphocyte (L). (400X)

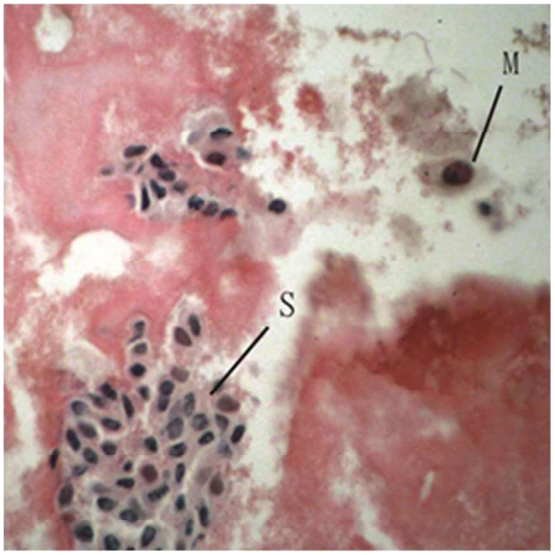

Figure 6.6 H&E stain of the lumen of a bladder of a ketamine treated mouse. Other cells include macrophage (M) and shedded epitheloid cells (S). (400X)

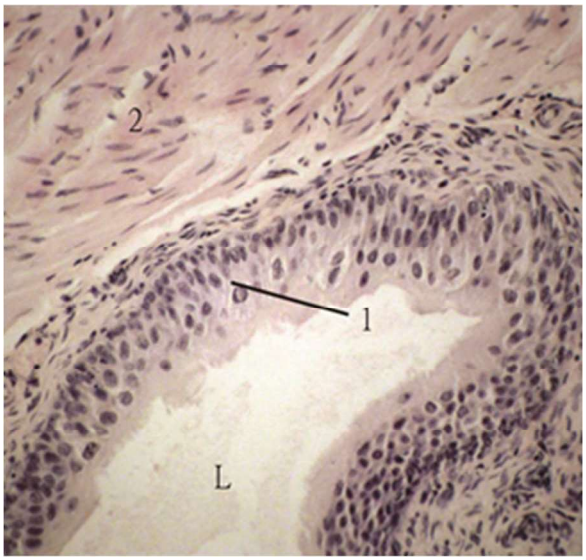

Figure 6.7 H&E stain of a bladder section of a ketamine and Gouteng treated mouse showing normal epithelium (1) and non-swollen muscles (2). No significant accumulation of cells in lumen (L). (200X)

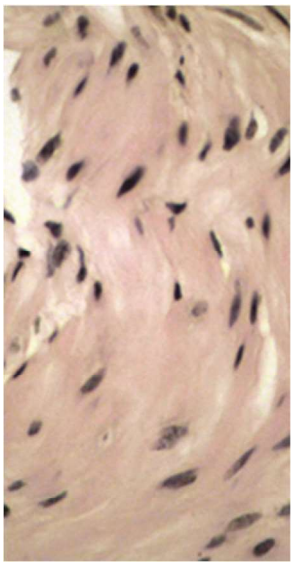

Figure 6.8 H&E stain of a bladder section of a Gouteng treated mouse. Muscles in the bladder was normal. (400X)

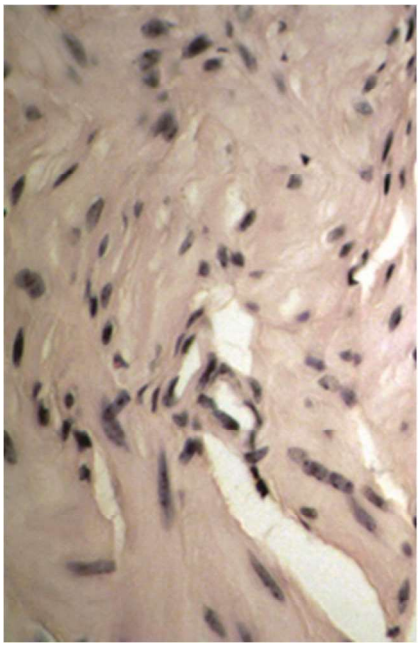

Figure 6.9 H&E stain of a bladder section of a ketamine and Gouteng treated mouse. Muscles in the bladder showed no pathology though a bit disoriented. (400X)

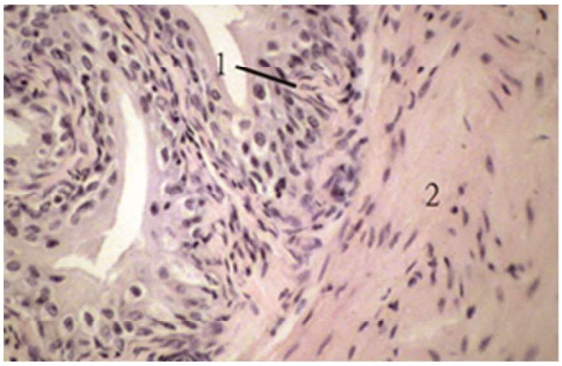

Figure 6.10 H&E stain of a bladder section of a Gouteng treated mouse. Epithelium (1) and muscle (2) were normal. (200X)

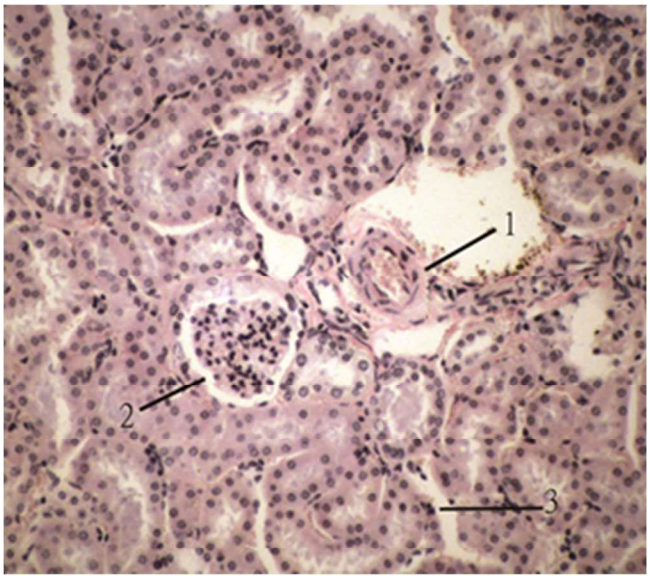

Figure 6.11 H&E stain of a kidney section of a ketamine treated mouse showing a degenerating glomerulus (1) while 2 denotes a normal glomerulus. 3 denotes pyknotic nucleus in tubular cells. (200X)

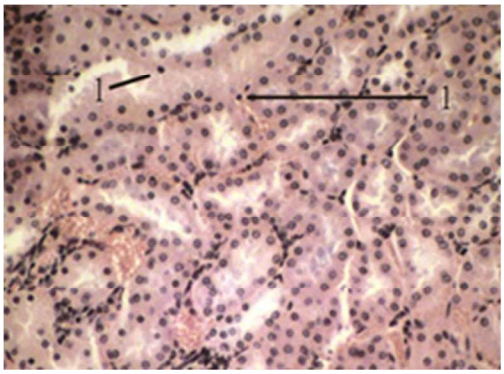

Figure 6.12 H&E stain of a kidney section of a ketamine treated mouse showing lymphocytes (1). (200X)

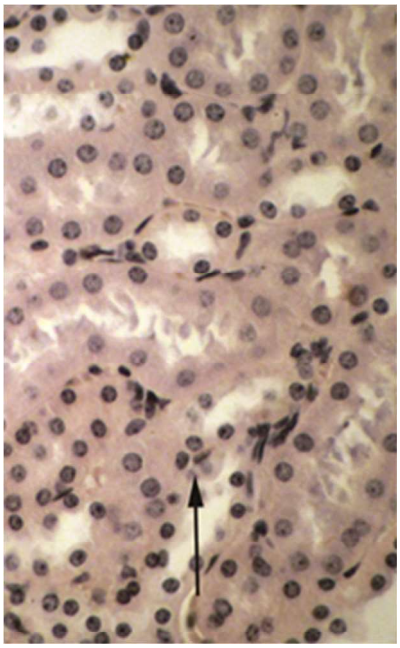

Figure 6.13 H&E stain of a kidney section of a ketamine treated mouse. Shedding of tubular cells from proximal tubule (arrow). (400X)

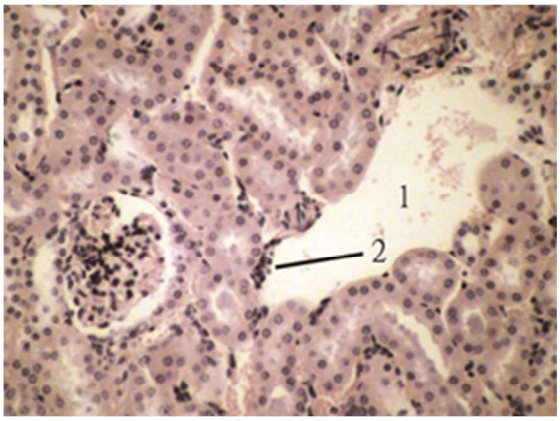

Figure 6.14 H&E stain of a kidney section of a ketamine treated mouse showing dilated tubule (1) and group of lymphocytes (2). (200X)

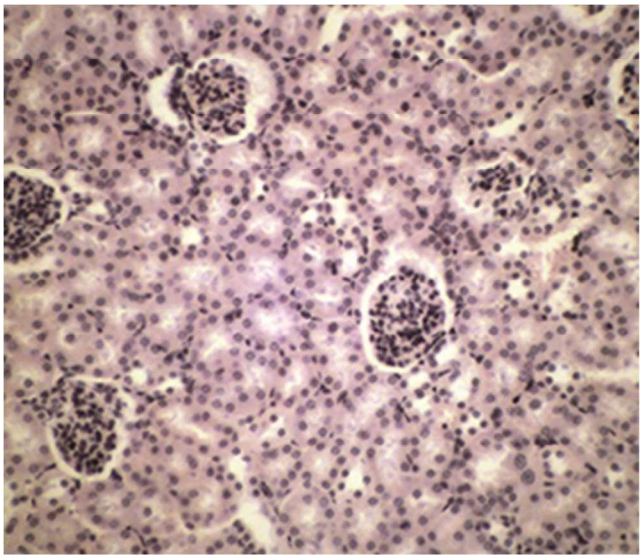

Figure 6.15 H&E stain of a kidney section of a ketamine and Gouteng treated mouse showing rather normal kidney. (200X)

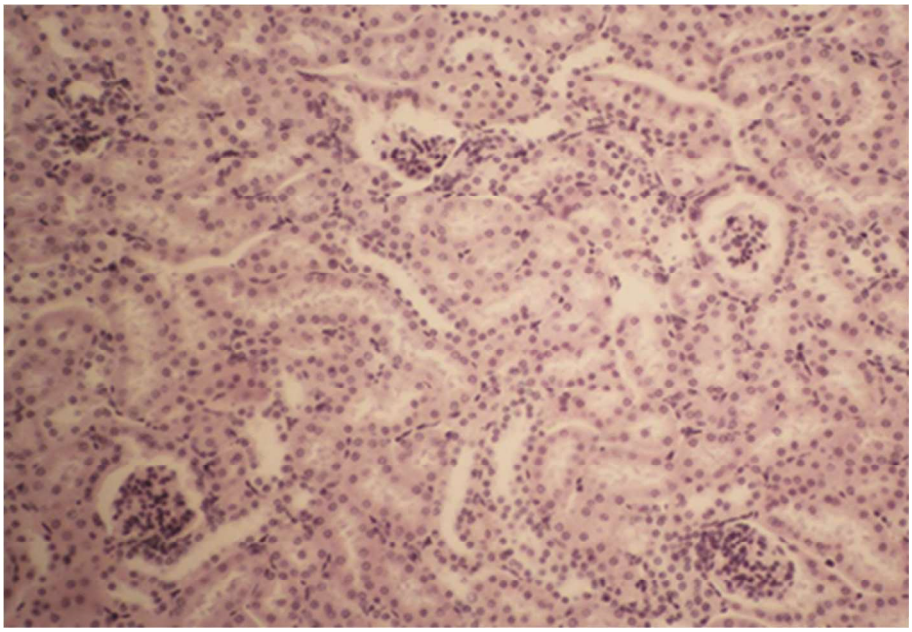

Figure 6.16 H&E stain of a kidney section of a Gouteng treated mouse. Kidney is relatively normal in Gouteng treated animal. (200X)

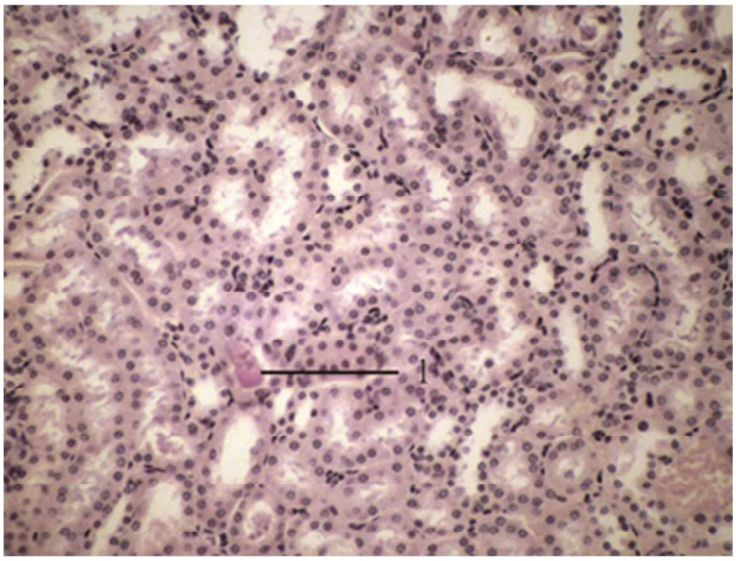

Figure 6.17 H&E stain of a kidney section of a Gouteng treated mouse (Group 1) with a protein cast (1). (200X)

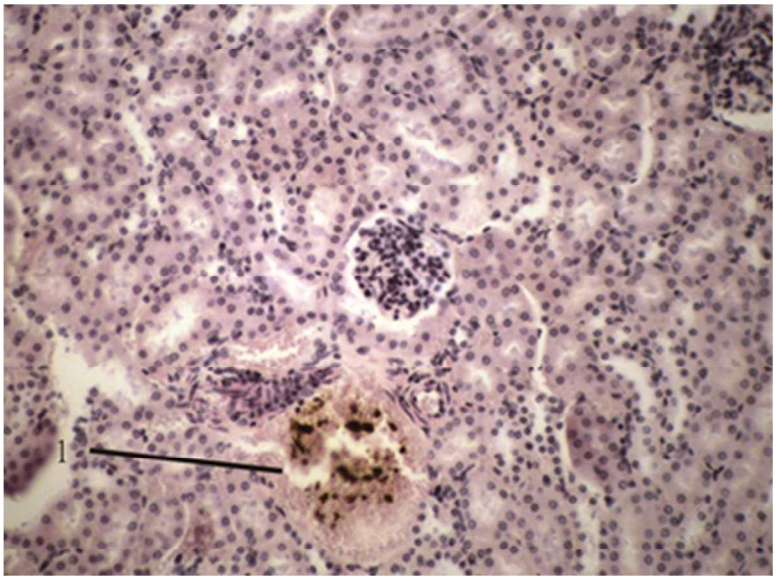

Figure 6.18 H&E stain of a kidney section of a Gouteng treated mouse (group 2) with a degenerated glomerulus (1) with sclerosis. (200X)

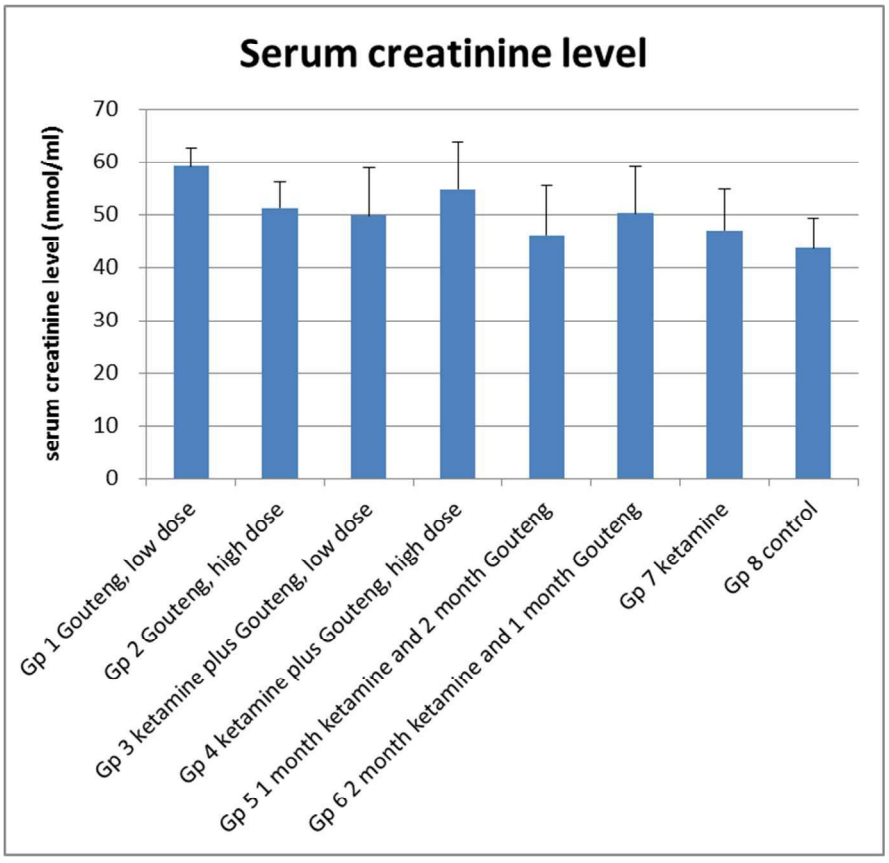

Figure 6.19 serum creatinine level of different groups of mice

Part 5: Final conclusion

Our studies point out that Gouteng has the potential to be included as one of the therapeutics for the treatment of ketamine induced damages. From the histopathology of various organs and the biochemical analyses, it is evident that Gouteng in several settings can alleviate the damages induced by ketamine.

In the liver, for example, less degenerative changes were revealed in Gouteng and ketamine cotreated animals. However, Gouteng only decreased serum AST levels when combined with ketamine but not serum ALT levels. But ALT levels had recently be questioned as a liver injury marker (McGill, 2016).

In the kidney, Gouteng and ketamine cotreatment downregulated the number of degenerating glomeruli and suppressed lymphocyte infiltration into the kidney. In the urinary bladder, the protective effect of Gouteng on ketamine-induced damage was outstanding. There was reduction of inflammatory cells in urine, protection of the transitional epithelium against metaplasia and protection against degeneration of muscle fibers in the muscular layer. However, Gouteng fed at the present dosage for a lengthy time can induce high levels of creatinine (increase related to dosage) which had not been reported before except in a paper on oral toxicity by Valerio and Gonzales (2005) (Valerio & Gonzales, 2005) and this work.

In the heart, the number of degenerative cells also decreased with Gouteng treatment and RCG illustrated that conditions adhered with ketamine toxicity, i.e. brachycardia and occasional ST inversions, were all downregulated upon Gouteng ketamine cotreatment.

In the central nervous system, Gouteng cotreatment with ketamine resulted in less cell death in the prefrontal, hippocampal and cerebellar regions, evident via morphometry and histopathology.

Behavioral studies illustrated an improvement of consolidation memory upon cotreatment with Gouteng and ketamine although water maze test on spatial memory did not record significant improvement. Neurochemical studies pointed to a significant decrease of dopamine upon Gouteng ketamine cotreatment versus ketamine alone. This may indicate Gouteng could control excitability in the ketamine treated mice. EEG, on the other hand, showed slow waves in the brain after ketamine treatment and these waves were equated with dizziness and sleep due to high serotonin in ketamine treated mice. Gouteng treatment also induced the formation of mu waves (a sort of primary alpha waves) in animals treated with ketamine which had no alpha waves. Thus, Gouteng could play a role in reviving some alertness in addicted animals, probably due to the lower of serotonin in Gouteng ketamine cotreated brains.

An often-asked question may be: what is the better mode of treatment, cotreatment of Gouteng and ketamine concomitantly or one treatment after another. In our experiments, the results indicated the former and others indicated the latter, thus it depends highly on the parameters being measured. Some might indicate improvement either way, for instance, both mode of combined treatment would decrease level of creatinine in the serum but the rotation of one drug then another had better results. In the case of AST, Gouteng and ketamine cotreatment or one followed by another both illustrated downregulation of AST levels.

The dosages of 10g and 20g in the mice was higher than that of the human at an average of 15g per human, given the very high metabolism of the rodents, such dosages were warranted. In most of the results, significant benefits were not seen with the higher dosage, indicating 10g perhaps was already the optimal for improvement.

As for the mechanism of Gouteng benefits, we can summarize into three points: 1) protection against cell death, 2) halting inflammatory responses probably via TNF alpha, NF kappaB, cytokines and T cells, and 3) anti-neoplastic responses. These possibilities were outlined by Aquino et al. (1991), Laus (2004), Sandoval et al. (2002), Valerio & Gonzales (2005). (Aquino et al., 1991; Laus, 2004; Na et al., 2004; Sandoval et al., 2002; Valerio & Gonzales, 2005).

Part of the results will be published in academic journal. There were, to this date, two manuscripts ready for submission.

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