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Histopathological studies of cardiac lesions after an acute high dose administration of methamphetamine

Dissertation submitted as partial fulfillment for the Degree of Bachelor of Science (Health) in Forensic Science

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CERTIFICATE

This is to certify that the dissertation entitled

"Histopathological studies of cardiac lesions after an acute high dose administration of methamphetamine"

is the bonafide record of research work done by

Mr. Arthur Kong Sn Molh

during the period of December 2006 to April 2007

under my supervision.

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ABSTRACT

Male Wistar rats aged 6 weeks were injected intraperitoneally with methamphetamine hydrochloride in saline at a dosage of 50 mg/kg. The dosage of the drug used was determined to allow the rats to show signs of intoxication and to survive for 24 hours (Maruta et al., 1997). The rats were sacrificed and their hearts were collected to be studied for cardiac lesions under light microscopy. Hematoxylin and eosin staining, Masson's Trichrome stain and immunohistochemistry methods involving anti-myosin reagents were used to enhance visibility of changes in the myocardium. Five rats injected with methamphetamine hydrochloride died within four hours and their hearts were collected on the same day. Another rat survived for two days after injection of methamphetamine hydrochloride before being sacrificed.

Microscopic examination of the myocardium of the rats that died on the same day injection did not show any of the expected sign of necrosis, with most test samples being non-discernible from the control samples, as the rats had died too soon after administration of the drug before the myocardium could develop any necrotic changes. However, there was loss of nuclei in some myocytes in the sub-endocardium region, indicating cell deaths. Other areas in the sub-endocardium region also showed internalisation and enlargement of myocyte nuclei, indicating regeneration of cells. There were very few foci of necrosis observed in these samples.

The heart sample from the single rat that survived the injection for two days showed foci of infiltration of macrophage-like cells with large nuclei and little cytoplasm within the sub-endocardium layers, but they were subsequently identified as regenerating

myocytes. Within these foci of cell infiltrations were also found macrophages and a few leucocytes. There was also presence of spindle-like fibroblasts, but the overall appearance of the myocardium does not indicate any inflammatory response of the cells. The expected signs of necrosis including eosinophilic changes and contraction band necrosis were not observed in this sample.

These results suggest that there may be a need to re-evaluate the toxic and lethal dosages of methamphetamine for use in animals testing. The cause of death in these rats were suspected to be due to failure of other major organs from the acute dosage administration of methamphetamine, which may then indirectly caused heart failure, but death occurred within a time period where significant changes due to necrosis may not be evident in the myocardium. Thus, further testing procedures such as examination of changes in other major organs and detection of serum levels of methamphetamine may be necessary to help detect deaths due to acute intake of methamphetamine. The use of electron microscopy in conjunction with light microscopy is also recommended, as they will allow clearer differentiation of the necrotic changes in myocardium or other major organs.

Keywords: methamphetamine, acute dose administration, myocardium, cardiac lesions

INTRODUCTION

The discovery and raiding of several large clandestine methamphetamine laboratories in Malaysia in the year 2006 and 2007 reflects the modern trend where such "designer" drugs are becoming increasingly the drug of choice among abusers. Methamphetamine (MA), amphetamine-type stimulants (ATS) and other modern hallucinogenic drugs are known collectively as "designer" drugs, due to their synthetic nature and the ease with which each type of drug can be altered into different forms with similar or increasing potency according to the users' liking. The use of MA along with other "designer" drugs have seen a dramatic increase beginning from the 1990s, as more drug abusers seek cheaper, more potent alternatives to the "traditional" stimulants such as cocaine. (Derlet and Heischober, 1990; Jacobs, 2006; Wijetunga et al., 2003)

In the United States of America, inhalers containing amphetamine were introduced in the 1930s as a treatment for rhinitis and asthma. The stimulant, euphoriant and anorectic effects of amphetamines, which were discovered soon after, led to its eventual abuse. The widespread abuse of amphetamine began after the mid-1940s, particularly noted in Japan and Sweden. The United States enacted legislatures in the 1950s to ban the abuse of amphetamines but it continued to be abused by some students, athletes, shift workers and truck drives for its reputed effect of enhancing intellectual and physical performance through increased wakefulness. Increasing legislature caused the gradual decline in the availability of pharmaceutically manufactured amphetamine, but in turn clandestine laboratories began increasing the synthesis of methamphetamine. (Derlet and Heischober, 1990)

The euphoric effect of MA is similar to cocaine, bringing about similar behaviour in animal tests of MA and cocaine. MA in the form of hydrochloride crystals are volatile and smokeable, bringing an immediate euphoria that lasts longer than cocaine. The hydrochloride salt of MA is the most common form of MA found to be abused, with street names such as "ice", "crystal" and syabu. MA became the current choice for many abusers due to its wide availability, low cost, and a longer duration of action compared to cocaine. Apart from being smoked, MA is also taken orally and intravenously by abusers. (Derlet and Heischober, 1990; Ellinwood et al., 2000)

MA is much easier to synthesize in clandestine laboratories compared to D-amphetamine (the more potent isomer of amphetamine) although both sympathomimetic drugs are derivatives of phenylethylamine. The main difference in the chemical structure of d-amphetamine and MA is the presence of a methyl group that attaches to the terminal nitrogen in MA. One common method of synthesis utilises L-ephedrine, which is then reduced to MA using hydroiodic acid and red phosphorus. The process may be varied in different laboratories by using a different acid or catalyst or by using chloroephedrine or methylephedrine in place of ephedrine. These processes results in D-methamphetamine, an isomer that is several times more active than the L-methamphetamine form. These processes produce a volatile, lipid-soluble, pure base form of MA that needs to be converted using hydrochloride into the water-soluble MA-hydrochloride form. (Derlet and Heischober, 1990; Yu et al., 2003)

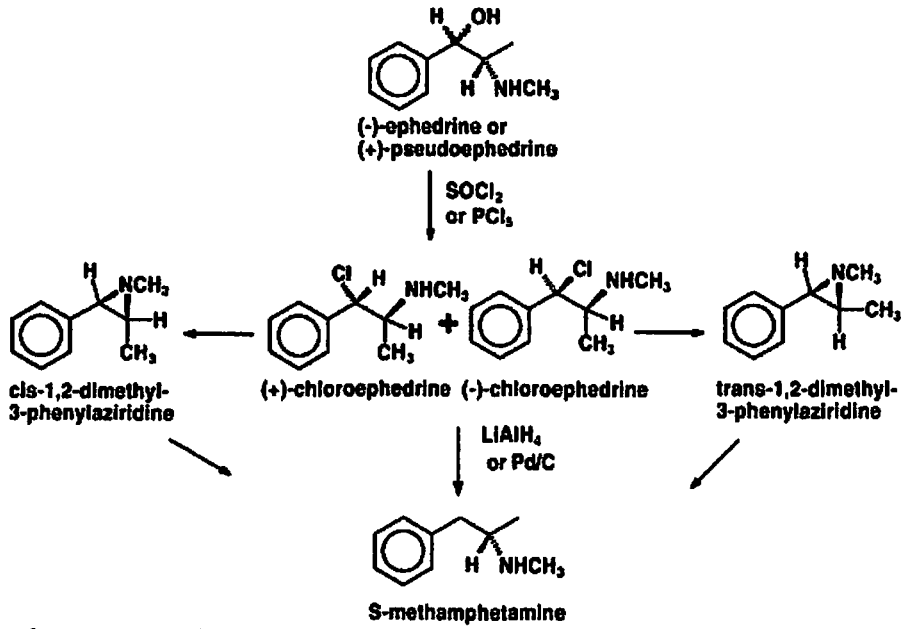

***Figure 1:** Common synthesis pathways of MA in clandestine laboratories (Yu et al., 2003)*

Physiological effects of MA intake include central nervous system (CNS) stimulation leading to euphoria, increased alertness, intensified emotions, altered self-esteem, and allegedly increased sexuality. This effect is due to the displacement of dopamine from specific nerve terminals leading to hyperstimulation of dopaminergic receptor neurons in the synaptic cleft. These in turn stimulate various CNS pathways and sympathetic nervous system. Direct peripheral and organ stimulation may also be possible. Toxic doses of MA result in agitation, anxiety, hallucinations, delirium and seizures, and possibly, death. Cardiovascular symptoms related to MA toxicity include chest pain, palpitations, dyspnoea, hypertension, tachycardia, atrial and ventricular arrhythmias, and myocardial ischaemia. (Derlet and Heischober, 1990; Ellinwood et al., 2000; He et al.[1], 1996; Maneo et al., 2000; Matoba, 2001; Rostagno et al., 1996; Yu et al., 2002)

MA abusers often go through a repeated pattern of frequent drug administrations (binge) followed by a period of abstinence. This pattern of chronic MA abuse can significantly alter cardiovascular function and cardiovascular reflex function and produce serious cardiac pathology. (Varner et al., 2002) However, tachyphylaxis occurs with MA

abuse, with long-term abusers being able to tolerate higher doses with fewer symptoms. MA has been known to cause death at an ingested dose as low as 1.5 mg per kg body weight, while long-time abusers developing drug tolerance may use as much as 5,000 to 15,000 mg per day. (Derlet and Heischober, 1990)

MA sold in the streets is usually mixed with other stimulants such as cocaine, phenypropanolamine hydrochloride, D-amphetamine, ephedrine, or pseudoephedrine, and also with other adulterants such as caffeine and baking soda. This reduces the purity of the MA sold and increases the profit of the MA manufacturer and trafficker. Street MA in the United States were reported to be highly impure and only found in 40% of street-purchased drugs in the late 1980s. However, law enforcement laboratories have discovered that recent available MA was nearly pure. (Derlet and Heischober, 1990) This discrepancy in the purity of MA available leads to the question whether the abuser may be taking high dosages far too toxic to the body, which may result in sudden death of the abusers.

Given the pattern of MA abuse, previous studies have focused largely upon the chronic effect of MA intake to major organs, such as the brains and the heart, by using animal testing. (Islam et al., 1995; Maeno et al., 2000; Riviere et al. 2000; Shaw et al., 1995; Yu et al., 2002) However, there is a lack of research into the effects of acute dose intake of MA, especially pertaining to the heart. Sudden death due to acute MA intoxication has been suggested to be similar to acute myocardial infarction, where pathological changes to the myocardium generally are hard to detect, even under microscopy. (Smith et al. 1976) Thus, there is a need to review the effects of acute dosages of MA intake to the heart, which can help medical examiners differentiate myocardium changes due to acute MA intake from those of other cardiovascular diseases.

REVIEW OF LITERATURE

Previous studies aiming to elucidate the relationship between MA intake and its effects relied on animal studies using rats, and also through autopsy reports of known MA abusers or patients receiving drugs with similar physiologic action as MA, while one study focused on direct effects of MA on cell cultures of adult rat ventricular cardiomyocytes. Derlet and Heischober (1990) wrote an introductory paper and a general review on methamphetamine; its history, illicit production and current trends in the United States of America; clinical pharmacology; toxic effect; and treatment of central nervous system and cardiovascular toxicities of MA. They suggested MA as a dangerous drug matching or superseding cocaine in inducing euphoric symptoms, and being competitive to cocaine for long-term or recreational abuse.

Post-mortem Studies

Effects of chronic administration of MA were investigated by a few researchers as most abusers exhibit a repeated pattern of frequent MA administrations (binge) followed by a period of abstinence. (Varner et al., 2001) An early study into the effects of amphetamine intake towards cardiomyopathy was conducted by Smith et al. (1976) through a post mortem study on a patient with congestive heart failure in the absence of coronary heart disease, valve lesions, or other demonstrable cause, and was receiving long-term dextroamphetamine treatment at 100 mg per day prior to her death. The results of this study suggested a causal relationship between the observed cardiomyopathy and amphetamine ingestion. The researchers noted that there was absence of convincing reports suggesting that clinical heart disease may be caused by chronic ingestion of sympathomimetic amines, even though there has been many experimental evidence

suggesting that acute large dose administration of catecholamines, especially isoproterenol, may induce myocardial necrosis. A similar phenomenon to myocardial necrosis was also demonstrated in experiments involving amphetamine. The findings of the study suggested that chronic exposure to high doses of amphetamine caused the hypertrophy of heart, with widespread interstitial oedema with scattered predominantly lymphocytic and histiocytic cellular infiltrate present in between muscle fibres, particularly marked in the left ventricular myocardium. Individual muscle fibre degeneration and small areas of muscle necrosis were present, with these areas being more prominently surrounded by cellular infiltrate containing scattered cells appearing like Anitschkow myocytes.

Huang et al. (1993) also presented a case report of acute myocardial infarction following transnasal inhalation of amphetamine in a drug addict taking amphetamine five times a day. The electrocardiogram and cardiac enzyme changes detected in the study were compatible with acute myocardial infarction rather than acute cardiomyopathy induced by catecholamine release, and there have been suggestions that the mechanism for acute myocardial infarction induced by amphetamine may be similar to those related to cocaine abuse. Coronary artery vasoconstriction, or spasm of normal coronary artery or at sites of pre-existing atherosclerotic stenosis was suggested to be the mechanism of acute myocardial infarction in both types of drug abused. The common combination of amphetamine with cocaine in street drugs may have a synergistic effect in inducing myocardial injury.

The result of the above study was echoed by Rostagno et al. (1996) who reported that the use of amphetamines and amphetamines-like drugs, like the use of other

sympathomimetic drugs, has been associated with severe cardiovascular syndromes such as myocardial infarction, arrythmias, rupture of ascending aorta, endocarditis, and dilated cardiomyopathy. They studied the case of a patient affected by dilated cardiomyopathy with severe impairment of left ventricular function apparently induced by long-term administration of an amphetamine-like drug, phendimetrazine. The endomyocardial biopsy shows some degree of myocardial hypertrophy and interstitial fibrosis without evidence of active myocarditis. However, the precise mechanism of cardiomyopathy induced by sympathomimetic drugs is still unclear. Myocarditis and myocardial impairment have been reported after exposure to high concentrations of both endogenous, as in phaechromocytoma, and exogenous catecholamines. Catecholamines may exert a direct toxic effect on myocardial cells by inducing endothelial damage and can cause myocardial damage through ischaemia induced by increasing myocardial oxygen demand with concurrent decrease of oxygen supply resulting from coronary vasoconstriction.

Animal Studies on Chronic Administration of Methamphetamine (MA)

Rats and mice were used in the majority of animal studies on the effects of chronic administration of MA as the myocardial lesions in rats treated with MA resemble the cardiomyopathy associated with MA abuse in humans. (He et al.[1], 1996) The effects of chronic dose intake of MA and the reversibility of the changes on the myocardium after withdrawal of MA intake were reported by Islam et al. (1995). Histological, immunohistochemical and electron microscopic changes in the myocardium of rats were examined following daily intraperitoneal administration of MA at a dose of 1 mg per kg body weight for 4, 8, and 12 weeks before sacrifice. The cardiotoxic action of MA has been of particular interest since standardized dosage consistently produces myocardial lesions. Light microscopic changes found in the myocardium of the MA-treated group

included atrophy, hypertrophy, patchy cellular infiltration, eosinophilic degeneration and disarray, oedema myolysis, fibrosis, and the appearance of vacuoles. Ultrastructurally, nuclei and normal mitochondria had various shapes and there were dilated T tubules and sarcoplasmic reticulum, the accumulation of glycogen granules and fat droplets. Intra- and extra-cellular oedema and intramyocytic vacuoles were often found. Withdrawal of MA at the twelfth week in another group of rats evidenced gradual recovery of the myocardial changes, commencing at 3 weeks after withdrawal.

Cardiovascular complications associated with methamphetamine abuse have increasingly been reported but chronic cardiotoxicity of methamphetamine is not experimentally well documented. (He et al.[1], 1996) In this study, MA (1 mg/kg/day) was subcutaneously injected into 5-week-old male Wistar Kyoto rats. After 14 and 56 days, hearts were examined by light and electron microscopy, which revealed foci of myocytic degeneration and necrosis appeared in the subendocardial areas on day 14 of methamphetamine exposure. Myocytic degeneration and necrosis became more extensive on day 56 with patchily distributed myocytolysis, contraction bands, atrophied myocytes, and spotty fibrosis throughout the myocardium in most of rats treated with MA. The accompanying ultrastructural features included marked degeneration of cardiac mitochondria with fractured and disrupted cristae, hypercontraction of myofibrils, and loss of myofilament.

Using similar dosage of MA as the above study, He et al.[2] (1996) studied the morphological and morphometrical effects of chronic administration of MA on myocardium in experimental models. MA (1 mg/kg/day) was subcutaneously injected to five-week-old male Wistar Kyoto rats (WKY) for 8 weeks. Light microscopy revealed

scattered cytomyolysis, vacuolisation, contraction bands and prominent disarray of myofibres in MA-treated group. Examination using electron microscopy showed degenerated mitochondria with disrupted cristae, myofibrillar hypercontraction and dissolution. Morphometric analysis was carried out using electron photographs and an image analyser. The sizes of mitochondria and the number of mitochondria per unit area (100 microns^2) in methamphetamine-treated group were significantly smaller than in the controls. No statistically significant difference was found regarding the percentage of myofibrillar area in cytoplasm between methamphetamine-treated and control groups. These findings show that chronic administration of methamphetamine can cause serious cardiac lesions and decrease in mitochondrial function.

There have been several cardiac lesions reportedly found in the myocardium of MA abusers including hypertrophy, atrophy, disarrangement of myofibrils and fibrosis. These changes were suggested to be mediated indirectly by excess release of peripheral catecholamines from nerve terminals. (Maneo et al. 2000) Chronic exposure to MA on isolated cell cultures of adult rat ventricular cardiomyocytes (ARCs) induced cardiomyocyte hypertrophy and high dose administration may lead to cardiac function disorder with disruption of microtubules and actin. (Maneo et al., 2000)

Riviere et al. (2000) conducted a study on the disposition of MA and its metabolite amphetamine in brain and other tissues in rat by comparing the concentration in serum. The study showed that MA accumulated highest in kidneys, followed by spleen, brain, liver, heart and serum. They also observed that high MA concentrations in the brain occurred immediately after intravenous bolus dosing, suggesting that there is essentially

no hindrance to passage of MA at the blood brain barrier. The study also concluded that MA distributes very rapidly to all tissues studied except the spleen.

The study by Varner et al. (2001) attempted to characterise the effect of "binge" pattern of MA use on cardiovascular function, by using radiotelemetry to record the cardiovascular responses elicited during three successive MA binges (3 mg/kg, b.i.d. for 4 days) in conscious rats, with each binge followed by a 10-day MA-free period. The hearts from treated rats showed focal inflammatory infiltrates with abundant monocytes and occasional necrotic foci. These results indicate that this binge pattern of MA administration can significantly alter cardiovascular function and cardiovascular reflex function and produce serious cardiac pathology.

Matoba (2001) reported that various cardiac lesions such as hypertrophy, disarray and fibrosis similar to HCM, were often found in the heart of methamphetamine (MA) abusers. Myolysis, eosinophilic changes, contraction band necrosis and small round cell infiltration were also observed. Male ddy mice were administered MA at a dose of 1 mg/kg subcutaneously every day for 4 weeks. Their hearts revealed many cardiac changes such as hypertrophy, myolysis, contraction band necrosis, disarrangement of myofibres, saw-like cytoplasm, side-to-side connection of cardiac cells and vascuolative degeneration microscopically, and crysterosis of mitochondria, enlargement of sarcoplasmic reticulum and hypercontraction electronmicroscopically. These changes are thought to be similar to that of MA abusers, so it is certified that MA has toxic effect on the heart.

Yu et al. (2002) reported that MA increases catecholamine levels, which have detrimental effects on heart function through vasoconstriction, myocardial hypertrophy,

and fibrosis. The study investigated the effects of chronic MA treatment on heart functions of uninfected and retrovirus-infected mice. MA is a derivative of amphetamine with strong effects on the central nervous system; it is highly addictive both physically and psychologically. Autopsies on sudden deaths of MA abusers show cardiac lesions or dilated cardiomyopathy, interstitial oedema, cardiac hypertrophy, disarrangement of myofibres, rupture of myocardium, and fibrosis. Acute and overdose of MA use can cause sudden congestive heart failure leading to sudden death while chronic administration of MA may cause cardiomyopathy in humans. MA stimulates the CNS indirectly by promoting the release while inhibiting the breakdown of catecholamines including norepinephrine, detrimentally affecting the heart. Chronic MA treatment lowered the heart function in uninfected, saline injected mice while no significant cardiac effects in retrovirus-infected mice.

Yu et al. (2003) also reported that MA not only affects the nervous system but also has cardiac toxicity and immunosuppressive properties. Chronic use of MA causes cardiomyopathy including cellular infiltration, myocardial hypertrophy, myocardium rupture and fibrosis. The increased catecholamine levels are responsible for the cardiac lesions induced by MA.

Effects of Acute High Dose Methamphetamine (MA)

One landmark study on the effects of acute dose administration of MA and similar stimulant drugs was done by Maruta et al. (1997). Their study examined the acute poisoning caused by MA, morphine and cocaine using six weeks old male Wistar rats. Their study was focused on detecting the early histopathological changes in the heart, lungs, liver and kidneys using light microscopy, as well as measuring the concentration of

the drugs and metabolites in blood using gas chromatography/mass spectrometry (GC/MS) method. The MA test group was injected intraperitoneally with 50 mg/kg body weight of MA and the rats sacrificed at intervals of 5 minutes, 1 hour, 2.5 hours, 6 hours and 18 hours after injection. The blood MA concentration was found to be at a maximum 5 minutes after injection. The hearts of the MA group rats was reported to show diffuse eosinophilic changes after 2.5 hours of injection, while partial inflammatory cell infiltration and contraction band necrosis were only visible in the rats sacrificed after 18 hours of injection.

The significance of contraction band necrosis was explored by Armiger and Smeeton (1986) who conducted a study based on autopsies on sudden deaths considered to be caused by cardiac related reasons and were not complicated by other findings. They reported a characteristic pattern of myocardial cell alteration known as "contraction band necrosis" was present in experimentally induced acute regional myocardial infarcts of 2 or more hours' duration, which could be useful in assessing the myocardial status in sudden cardiac death. Contraction band necrosis was frequently encountered, characterised in 3 main patterns that correlate to coronary artery pathology and case history, the most frequent pattern being a regional distribution consistent with early subendocardial or transmural infarction, yet not characterised by coagulative necrosis, associated in most cases with a recent thrombotic event in the relevant supply artery. Thus, presence of a specific pattern of contraction band necrosis may frequently facilitate the definitive diagnosis of sudden cardiac death. However, an unequivocal historical diagnosis of myocardial infarction cannot be usually made if death occurs within 12 hours of the onset of myocardial infarction. Previous studies show that contraction band necrosis is clearly detectable about 2 hours after occlusion of a major coronary artery and develops in a

characteristic pattern correlated with transmural gradients in blood flow and consequently in extent and severity of the ischaemic injury.

Apart from these, the study conducted by Shaw et al. (1995) showed that self-administration models for rats fed with acute dose (50 μg/ml) of MA revealed a various degree of pulmonary lesion with mild exudates formation. Ellinwood et al. (2000) stated that catecholamine release and activation of receptors are important in acute toxicity of amphetamines. Ellinwood et al. (2000) suggested that deaths directly attributable to the pharmacological response to amphetamines relate to several phenomena, including: 1) hypertensive cerebrovascular hemorrhage (confirmed pathologically); 2) cardiovascular collapse secondary to ventricular fibrillation, with the majority of these cases in individuals less than 30 years of age with no evidence of pre-existing heart disease; 3) hyperpyrexia in the range of 40°C and 4) miscellaneous causes, such as septicemia with bacterial endocarditis or necrotizing angiitis. In general, acute fatal drug reactions to amphetamine are more common in the occasional user than in the tolerant, chronic, high-dose abuser. (Ellinwood et al., 2000)

Wijetunga et al. (2003) reported previous studies describing myocardial infarction, pulmonary oedema, and aortic dissection related to methamphetamine use. However, cardiomyopathy due to methamphetamine exposure has been rarely described. Jacobs (2006) reported that cardiotoxicity (manifested as cardiomyopathy, acute myocardial infarction/necrosis, heart failure, or arrhythmia) after the recreational (mis)use of amphetamine and its synthetic derivatives has been documented but is rather rare but is probably a genuine entity that should be considered both in forensic and clinical/emergency medicine because of its potential medicolegal implications.

From the review of previous studies, there are strong indications that intake of MA whether in chronic or acute dose can induce cardiac myopathy that may be varying in severity and the mechanism of which is still not well understood. There is also a lack of research describing the effects of acute high dose administration of MA. Thus, this study aims to reveal more of the important myocardial changes pertaining to the acute high dose administration of MA to facilitate the proper diagnosis in cases of deaths related to MA abuse.

OBJECTIVES OF STUDY

Previous studies focused more on the chronic effects of methamphetamine intake through various methods on the major organs of the body. However, there is a lack of research done to show the effects of acute dosage methamphetamine intake to the body, particularly the impact it has on the heart. Thus, this study has been designed with the following objectives:

- To determine the major pathological changes after methamphetamine administration in acute high dosage.
- To analyse the pathological condition of the myocardium after acute high dosage administration of methamphetamine.
- To examine early pathological changes after methamphetamine administration in acute high dosage through immunohistochemistry.
- To provide a guide to forensic pathologists when handling cases involving methamphetamine abusers.

MATERIALS AND METHODS

Materials

Eighteen male Wistar rats aged of six weeks were reared in the animal house of Universiti Sains Malaysia, Kubang Kerian, Kelantan under standard atmospheric conditions in three 12 (w) X 24 (l) X 8 (h) inch cage. Each cage was labelled according to the three groups the rats were divided into, namely the Control, Placebo, and Methamphetamine (MA) injected groups:

1. **Control Group** – The six rats in this group were kept under normal rearing condition
2. **Placebo Group** – The six rats in this group were given intraperitoneal injection of 0.3ml of 0.9% (w/v) saline each
3. **Methamphetamine (MA) Injected Group** – The six rats in this group were given intraperitoneal injection of methamphetamine hydrochloride dissolved in 0.9% (w/v) saline for pathological observation

The methamphetamine hydrochloride used in this experiment was requested through the Department of Chemistry Malaysia, Petaling Jaya, with a total amount of 50 milligrams. The saline was prepared fresh on the day of injection and another fresh batch was prepared for use as sample preservative solution on the day of sacrifice.

For the harvest of heart samples, the equipment used consisted of a rat immobilising mount, gauze, surgical kits (scalpel, blades, forceps, scissors, etc), electronic balance, and sample bottles. Reagents used for the sacrifice and harvesting of the hearts include chloroform, 0.9% (w/v) saline, and 10% (w/v) formalin.