HISTOMORPHOLOGICAL, HAEMATOLOGICAL AND BIOCHEMICAL EFFECTS OF METHAMPHETAMINE (CRYSTAL ICE) ON THE LIVER OF WISTAR RATS

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Received: 30th June, 2024 **Accepted:** 19th July, 2024 **Published**: 31st December, 2024

ABSTRACT

Background: Methamphetamine, commonly known as meth, is a powerful and highly addictive central nervous system stimulant. It is chemically related to amphetamine but has much stronger effects.

Aim: The research was aimed at evaluating the effect of haematological, biochemical, and histology of the liver of Wistar rats was studied.

Methodology: A total of 25 albino rats was used in this research. They were divided into five groups of five rats per group. Group A (Control) received rat pellet and water, B along with water and rat pellet received 0.5 mg/ kg body weight of Methamphetamine, C received 1.0 mg/kg body weight of meth, D received 3.0 mg/kg body weight of Meth, E received 5.0 mg/kg body weight of meth twice daily for a duration of six weeks. The rats were given a mild anaesthesia, with chloroform and sacrificed afterward, blood samples were taken for biochemical and haematological test, and the liver excised for histological examination.

Results: There were a significant reduction ($P<0.05$) in weight when compared to the control. There were also observable significant reduction ($P<0.05$) in hematocrit, white blood cell value when compared with the control. There was a significant increase ($P< 0.05$) in the value of monocytes and granulocytes when compared with the control. Also observed were significant increases ($p<0.05$) in the value of aspartate transaminase, alkaline phosphatase, ST, ALP, ALT, and TB when compared with the control. The histology of the various liver organs reveals no changes morphologically.

Conclusion: This study showed that the complications of METH were dose-dependent, and the highest dose caused the most damage. However, no observable damage histologically.

Keywords: Histomorphology, Haematology, Methamphetamine, Crystal Ice, Liver, Wistar Rats

INTRODUCTION

Methamphetamine (METH), a powerfully addictive psychostimulant, is also known as "speed," "crystal," "crank," "go," and "ice." It is estimated that approximately 35 million people worldwide abuse this substance (Office of Applied Studies, 2004). METH is primarily used as a recreational drug by young people, leading to excitement and hallucinations (Chomchai & Manaboriboon, 2012). Methamphetamine directly affects the brain, inducing feelings of happiness and excitement. According to Weng et al. (2020), METH is one of the primary amphetamine compounds with stimulating and addictive properties. Upon entering the central nervous system, METH triggers a rapid release of dopamine, a chemical mediator that stimulates brain cells.

Citation: Asibor, E., Iyare, G. I., Avioro, G. O., and Omorodion, N. T.[2] (2024): Histomorphological, Haematological and Biochemical Effects Of Methamphetamine (Crystal Ice) On the Liver of Wistar Rats *BJMLS 9(2): 46 - 55*

Scott et al. (2007) noted that METH use can lead to feelings of excitement, anxiety, decreased appetite and sleep, and increased physical activity. The harmful effects of METH are primarily due to alterations in physiological systems, particularly the autonomic nervous system. Acute activation of the sympathetic nervous system can lead to hypertension, tachycardia, elevated heart rate, peripheral hyperthermia, pupillary enlargement, diarrhea, and vasoconstriction (Nordahl et al., 2003). Other side effects include convulsions, serotonergic neurotoxicity, rhabdomyolysis, infectious diseases, and renal and hepatic failure, along with aggression, paranoia, hallucinations, headaches, tremors, and gastrointestinal irritation (Koriem et al., 2013; Luciano & Perazella, 2014). Clinical reports indicate that the liver is a primary target of METH toxicity, which can range from minor liver injury to fulminant hepatic impairment (Eskandari et al., 2014).

METH is increasingly abused worldwide as a central nervous system stimulant. Data from a tertiary care medical center in Hawaii indicate that methamphetamine addicts account for 40% of young patients with cardiomyopathy (Yeo et al., 2007). Liver issues caused by METH include hepatocellular carcinoma, hepatomegaly, centrilobular necrosis, and fibrosis. The toxicity of METH is attributed to its metabolism, enhanced neurotransmitter outflow, biogenic amine oxidation, and hyperthermia (Carvalho et al., 2004).

METH is available in various forms, including crystals, paste, and powder, and can be used through sniffing, oral ingestion, lung inhalation, and injection, with smoking being the most popular method (Karila et al., 2010; Hobkirk et al., 2015). Abuse of METH increases the risk of pulmonary hypertension, high blood pressure, elevated body temperature, adrenergic activation, cerebrovascular abnormalities, circulatory collapse, and renal failure (Moratalla et al., 2017). It also raises the risk of infections such as HIV and hepatitis.

The lungs absorb METH, and the liver metabolizes it into amphetamine and p-OHMA (Lin et al., 1997). Research indicates that METH and its metabolites can cause significant DNA damage, oxidative stress, and abnormal inflammatory responses (Jones et al., 1994; Carvalho et al., 1997; Wagner et al., 2017). These effects are linked to severe pathological changes in the liver, which may lead to cancer or hepatic failure (He & Karin, 2011; Wang et al., 2016). However, our understanding of METH's detrimental effects on the liver remains limited.

Most METH research has focused on the central nervous system, with its effects on other organs less clearly understood (Moratalla et al., 2017). Limitations of human postmortem evaluations include the small number of cases, difficulty in obtaining tissue samples, and challenges in sample preparation. Animal model studies can provide valuable data as they allow manipulation and control over the state at death, unlike human specimens that can only be collected post-mortem. Investigating the effects of methamphetamine on the liver is thus critical. Therefore, this study aimed to examine the histomorphological effects of METH on rat livers, along with conducting haematological assays and biochemical tests for alkaline phosphatase (ALP), aspartate transaminase (AST), and alanine transaminase (ALT).

MATERIALS AND METHODS

Study Area

This research was conducted in Ekpoma, the capital of Esan West Local Government Area in Edo State, Nigeria. Ekpoma is located in the transitional zone between savannah and rainforest in southwest Nigeria.

Study Design

The study involved twenty-four adult albino Wistar rats, weighing between 200 and 220 grams. These rats were sacrificed six weeks after treatment with methamphetamine.

Following the injection, a mild dose of chloroform was administered as an anesthetic, and their livers were removed for histological examinations. The livers were preserved in 10% neutral buffered formalin for 24 hours. In the grossing room, samples with a thickness of 3 mm were cut. The chosen tissues underwent histological processing after being meticulously labeled and arranged in tissue baskets.

Ethical Consideration

The study was approved by the Health Research Ethics Committee of Ambrose Alli University in Ekpoma, Edo State, Nigeria. The research adhered strictly to the guidelines for the care and use of research animals established by the World Health Organization (WHO; Petherick, 2003).

Experimental Animals/Housing Condition

The Wistar albino rats were obtained from the animal farm of the College of Medical Sciences at Ambrose Alli University, Ekpoma. They were then moved to the experimental laboratory located in the Faculty of Medical Laboratory Science's histology laboratory at the College of Medical Sciences, Ambrose Alli University, Ekpoma, Edo State. The animals were given a week to acclimate. They were kept in an environment with regulated temperatures and a 12-hour light-and-dark cycle and had access to water and food. To avoid cross-contamination, they were housed in wire mesh cages equipped with tripods that separated the animals from their excrement. Throughout this acclimation phase, the rats were fed growers' mash and had unlimited access to water. The maintenance and usage of the animals followed the standard guide for the care and use of laboratory animals.

Animal Grouping

The experimental animals were divided into five groups (A through E). Each group contained four rats (n = 4) and was housed in four large cages. Group A served as the control group, while groups B through E were the test groups. Graded doses of prepared methamphetamine (METH) were administered to groups B through E. Group A did not receive any METH; instead, they were given a standard diet (grower's mash) and water.

Substance Preparation

Methamphetamine (purity >98%) was purchased from a government-approved pharmacy in Ekpoma, Edo State, and verified by St. Kenny Research Consult in the same city. The lethal dose 50 (LD50) was computed to establish the dose administered. METH was given orally.

Substance Administration

A total of twenty-five adult Wistar rats were used for the investigation, divided into five groups, each consisting of five rats. Methamphetamine was administered orally. For six weeks, Group A (control) was fed growers' mash and distilled water or regular saline daily. Groups B through E received growers' mash and methamphetamine at varying doses twice a day for six weeks: 0.5 mg/kg body weight (Group B), 1.0 mg/kg body weight (Group C), 3.0 mg/kg body weight (Group D), and 5.0 mg/kg body weight (Group E). After administration, the rats were given mild anesthesia using chloroform, and blood was taken for biochemical and hematological analysis. Their livers were excised for histological examination.

Organ Collection and Analysis

The animals' weights were measured before METH administration and again after six weeks, and the average weight was computed.

Photomicrography

Slices of liver tissue were inspected using a light microscope, and photomicrographs were taken for each group. The results were interpreted using these photomicrographs (Fisher, 1998).

Blood Sample Collection

At the conclusion of the administration period, two milliliters of each rat's blood were extracted from the heart under light chloroform anesthesia.

The blood was placed into plain containers or serum-separating tubes (SST) with EDTA for hemoglobin estimation and labeled properly. The samples were centrifuged within two hours of collection at 3000 rpm for 5 minutes. The collected plasma was stored frozen at -20°C until analysis. Hemoglobin, alkaline phosphatase (ALP), aspartate transaminase (AST), and alanine transaminase (ALT) were analyzed in a lab.

Estimation of Biochemicals

The atomic absorption spectrophotometer (AAS) was used to determine the concentrations of the biological analytes using a direct technique as outlined by Kaneko (1999).

Hemoglobin Estimation

Hemoglobin levels were estimated using the Sysmex KX-21N Analyzer, a mechanical hematology analyzer. The blood sample was blended for approximately ten minutes using a blood mixer.

Data Analysis

Statistical analysis of the collected data was performed using SPSS (version 25). The values of the test groups were compared to those of the control group using ANOVA (Scheffe) at a 95% confidence level.

RESULTS

After six weeks of treatment, all rats given methamphetamine showed histological damage to their organs, including necrosis and degenerative alterations. The following sections provide independent explanations of the results.

Table 1: Showing the Effect of METH on the body weight of the Rats.

Weight (control group)	Weight (Exp. group)	t-value	p-value
210.0 ± 5.30	202.7 ± 3.11*	5.987	0.0001

KEYS: P<0.05 = Significant; P>0.05 = Not Significant

Note: The collated values were expressed in mean ± SD. P-value of less than 0.05 was considered significant. The average weight of animal after the 6 weeks administration of METH shows significant reduction (P<0.05) when compared with the control.

Table 2: The Effects of METH on Haematological Parameters

	Control	Experimental group	t-value	p-value
HCT	45.6 ± 1.50	41.0 ± 1.66*	20.917	0.0001
WBC	7.4 ± 1.92	2.3 ± 0.55*	9.039	0.004
LYM	52.2 ± 2.49	46.7 ± 12.98	0.925	0.369
MON	7.4 ± 1.14	17.2 ± 2.38*	-8.684	0.0001
GRAN	40.4 ± 1.82	32.8 ± 3.27*	4.876	0.0001

KEYS: P<0.05 = Significant; P>0.05 = Not Significant

Note: The value of the collated data was expressed in mean ± SD. P value less (P<0.05**)** was considered significant. The haematocrit and white blood cell count value were significantly reduced (P<0.05) in the test group when compared to the control. They were no significant changes in lymphocytes of the experimental group when compared with control. There was significant increase in monocytes count (P<0.05) in the test group in comparison compared with the control group.

The results presented in Table 4.3 indicated that METH caused a significant increase ($P < 0.05$) in serums AST, ALT, ALP, and bilirubin levels in rats. The increase in treatment dose of METH increases the AST, ALT, ALP, and bilirubin levels.

Table 3: Effect of METH on serums AST, ALT, total bilirubin, and ALP of METH-injected rats

Groups	Parameters			
	AST (U/L)	**ALT (U/L)**	**ALP (U/L)**	**Total bilirubin (mg/dL)**
Group A (Control)	123.1 ± 3.85	62.5 ± 2.48	210.5 ± 6.81	0.56 ± 0.08
Group B (0.5 mg/kg bw)	135.4 ± 4.17[a]	71.1 ± 2.9[b]	228.5 ± 6.59[b]	0.66 ± 0.04[b]
Group C (1.0 mg/kg bw)	138.3 ± 3.94[a]	73.5 ± 2.87[b]	232.1 ± 4.86[a]	0.68 ± 0.07[a]
Group D (3.0 mg/kg bw))	139.6± 2.94[a]	75.5 ± 2.58[b]	233.9 ± 6.18[a]	0.70 ± 0.05[a]
Group E (5.0 mg/kg bw)	140.2 ± 0.72*	82.0 ± 1.96**	241.2 ± 7.15**	0.75 ± 0.05**

Data presented as mean ± SE. Number of animals = 5 per group.
*Significant change ($P \leq 0.05$) compared to control rats.
**Highly significant change ($P \leq 0.01$) compared to control rats.
[a]Significant change ($P \leq 0.05$) compared to group B.
[b]Highly significant change ($P \leq 0.01$) compared to group B.
bw = body weight

Histopathological Findings of METH-treated Liver

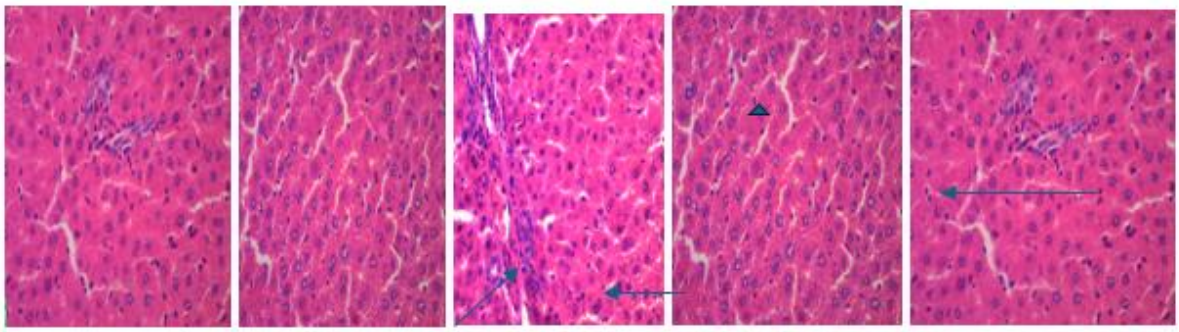

PLATE 1: Liver section of rats in group A (control section). Note the hepatocytes (arrow) with eosinophilic cytoplasm surrounding a centrally placed normochromic nuclei with distinct nucleoli. Features in keeping with Normal Hepatocytes, Liver Section of rats in group B administered 0.5mg/kg body weight of METH. The cytoplasm surrounds a centrally placed normochromic nuclei with distinct nucleoli. Features in keeping with normal liver morphology, Liver section of the rats in group C administered 1.0mg/kg body with of METH. Note hepatocytes (arrow) with eosinophilic cytoplasm containing normal morphology with central vein showing normal architecture, Liver section of the rats in group C administered 3.0mg/kg body with of METH. Note hepatocytes (arrow) with eosinophilic cytoplasm containing normal features. Liver section of the rats in group E administered 5mg/kg body weight of METH. Note hepatocytes (arrow) with eosinophilic cytoplasm containing normal histological features, the cytoplasm surrounds a centrally place nuclei with distinct nucleoli. H and E X400

DISCUSSION

Methamphetamine (METH) can be administered in various ways, with injection causing the most severe side effects. Injecting illegal substances also exposes users to additional hazards do not present with other administration methods. Domier *et al*. (2000) found that injectors experienced more psychotic states, hallucinations, loss of consciousness, sexual dysfunction, inability to function at work, and a higher risk of blood-transfusion infections. Injectors were also described as more dependent and severe addicts. Additionally, chronic METH abusers often use the drug in patterns that differ from single-day binge injections (Krasnova and Cadet, 2009).

METH poisoning is common due to the drug's euphoric effects, accessibility, and affordability worldwide. It disrupts dopamine, serotonin, glutamate, nitric oxide (Cadet, 2003), and noradrenaline (Fleckenstein *et al*., 2000). METH inhibits monoamine oxidase and dopamine reuptake, leading to a significant release of dopamine in the brain (Melo et al., 2005). Giros *et al*. (1996) reported that high doses deplete dopamine by damaging dopaminergic terminals, neurons, and transporters. Dopamine reacts with molecular oxygen to create reactive oxygen species (ROS), such as hydroxyl radicals, superoxide, and hydrogen peroxide, causing oxidative stress (Stumm *et al*., 1999). Oxidative stress is a major factor in METH-induced toxicity, yet previous research has not adequately compared its effects on various organs (Burrows and Yamamoto, 2003b; Tokunaga *et al*., 2006).

This study examined METH's histopathological effects on the liver at doses of 0.5, 1.0, 3.0, and 5.0 mg/kg/day. Organ weight is a crucial indicator of physiological and pathological states. Methamphetamine can cause significant weight loss due to its adverse effects on appetite (Werb *et al*., 2010).

Data values were reported as mean ± SD, with a p-value of less than 0.05 considered significant. Haematocrit and white blood cell counts were significantly lower ($P<0.05$) in the test group compared to the control. No significant differences were found in lymphocyte counts, but monocyte counts were significantly higher ($P<0.05$) in the test group. Platelets, which link inflammation and thrombosis, can release coagulation and inflammatory factors (Sarma *et al.*, 2002). METH-induced oxidative stress and inflammation may affect inflammatory markers such as WBC and platelet counts.

The liver, due to its role in drug metabolism, was chosen for toxicity research. METH-induced rats showed no nuclear fragmentation, contrary to findings by Suphakong *et al*. (2016) on liver tissue alterations due to dextromethorphan (DEX). Addictive drugs like DEX or METH may increase ROS production, inhibiting cell regeneration (Cao *et al*., 2013; Suphakong *et al*., 2016). This study found anatomical liver abnormalities in rats exposed to METH. The liver, which purges blood of toxins, undergoes composition changes with significant external substance toxicity (Li et al., 2015).

These findings contrast with Kamijo *et al*. (2002), who reported liver failure, psychosis, hyperthermia, and rhabdomyolysis after METH injection in a 41-year-old man. Liver biopsy showed ballooning degeneration and hepatic necrosis. Merchant et al. (2019) reported hepatic and pancreatic ischemia in a 35-year-old man who died from METH overdose, showing liver steatosis, necrotic bile duct hamartomas, and macrovesicular changes. METH-induced ischemia and vasoconstriction damage multiple organs. METH raises brain and plasma ammonia levels, contributing to hepatotoxicity. High METH doses cause hyperthermia and hepatocyte damage (de Silva *et al*., 2013; 2013b).

This study used repeated intraperitoneal METH doses, showing significant hepatotoxicity in rats. Liver function was evaluated using amino transferases, bilirubin, lactate dehydrogenase (LDH), and alkaline phosphatase (ALP). AST and ALT, released into the bloodstream upon liver damage, serve as early indicators of liver injury (Jagadeesan and Kavitha, 2006; Vermeulen et al., 1992). METH administration significantly increased AST, ALT, and ALP levels, indicating liver damage. Wang et al. found similar results with 15 mg/kg METH doses, showing increased ALT, AST, and ammonia levels, and hepatic injury confirmed by histopathology. Khaled et al. also reported oxidative stress and abrupt liver failure in METH-intoxicated mice, with increased serum liver injury markers and oxidative stress (Koriem and Soliman, 2014). Halpin et al. (2013) identified increased plasma ammonia and hyperthermia as key mechanisms of METH-induced hepatotoxicity.

Drug use is a global issue, with many suffering from addiction despite known health risks. A comprehensive approach is needed to educate communities about drug use's negative impacts, manage use, and treat addiction to substances like METH, alcohol, marijuana, and opiates. Variations in blood enzyme concentrations (AST, ALT, ALP, and bilirubin) have significant clinical implications (Plaa and Hewitt, 1989; Martin and Friedman, 1998). Elevated levels of these indicators reflect the hepatocellular impairment caused by METH injections in rats.

CONCLUSION

This study examined the effects of METH on the liver. We were unable to definitively attribute the observed hepatic alterations in our investigation solely to METH. It should be noted that delineating the direct mechanisms underlying the harmful effects of METH is challenging, as it is often not used alone and can be contaminated with other substances. Reductions in haematocrit and haemoglobin concentrations also increase the risk of anaemia. These findings contribute to our understanding of the pathobiological pathways associated with methamphetamine use and may help address issues arising from its use. This study demonstrated that METH side effects were dose-dependent, with the highest dose causing the most significant harm. To further elucidate the detrimental impacts of METH on various bodily systems across different doses and durations, molecular and immunohistochemical analyses are crucial. Understanding the etiology of METH addiction could aid in the development of innovative therapeutic strategies for affected individuals.

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