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Research Article

Hepatoprotective effect of high dose of vitamin A on the liver in toxic dose of methamphetamine induced adult male Wistar rats

Ezejindu Damian Nnabuihe[1], Alozie Osinachi Prince[1*], Ekelemchukwu Chinelo Juliet[1], Udodi Sopuluchukwu Princewill[1], Enemuo Ijeoma[1], Okeke Somadina Nnamdi[1], Ogbuokiri Doris K[1], Okeke Henry Kachikwuru[1], Ekoh Augustine Alobu[1], Benedict Nzube Obinwa[1], Chinyere Elizabeth Eze[1], Nwaefulu Kester Eluemunor[2], Sobanke A. Omolara[2], Chidinma Ifeyinwa Mmaju[2], Okafor Anulika Jacinta[2], Chuka-Onwuokwu Ngozi Cynthia[2], Ejiogu Ikedichukwu Chibueze[3], Nwoko Sebastine Okechukwu[3], Wuraola Serah Nnaemeka[3], Ebi Victory Chinecherem[4], Agu Augustine Uchenna[5], Ugwu Augustus Uchenna[5], Nwodo Ndubuisi Francis[6], Elemuo Chukwuebuka Stanley[7], Agbai Johnson Ukwa[8], Muorah Chinecherem Onyekachi[9], Ozoemena Chiadiobi Lawrence[10]

[1]Department of Anatomy, [2]Department of Physiology, [3]Department of Biochemistry, Faculty of Basic Medical Sciences, Nnamdi Azikiwe University, Nnewi Campus, Anambra State, Nigeria.
[4]Department of Medicine, College of medicine, University of Calabar, Cross River State, Nigeria.
[5]Department of Anatomy, Faculty of Basic Medical Sciences, University of Nigeria, Enugu Campus, Enugu State, Nigeria.
[6]Department of Human Anatomy, Faculty of Basic Medical Sciences, Madonna University, Rivers state, Nigeria.
[7]Department of Anatomy, Chukwuemeka Odumegwu Ojukwu University, Uli Campus, Anambra State, Nigeria.
[8]Department of Human Anatomy, Faculty of Basic Medical Sciences, Abia State University, Uturu, Abia State, Nigeria.
[9]Department of Nursing science, Christopher University, Ogun State, Nigeria.
[10]Department of Anatomy, Fculty of Basic Medical Sciences, Igbo-Eno, Enugu State, Nigeria.

[*]**Correspondence**
Alozie Osinachi Prince
ossyalozie@gmail.com

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Methamphetamine (METH) is a potent psychostimulant that induces oxidative stress and hepatotoxicity following prolonged or high-dose exposure. Vitamin A, a fat-soluble antioxidant, has been reported to exert protective effects against oxidative tissue injury. This study evaluated the effect of high-dose vitamin A on methamphetamine-induced liver toxicity in adult male Wistar rats. Twenty adult male Wistar rats were randomly assigned to four experimental groups (n = 5 per group). Group A served as the control and received standard feed and water only; Group B received methamphetamine (10 mg/kg body weight) administered at 3-hour intervals daily; Group C received high-dose vitamin A (2.5 mg/kg body weight); and Group D received methamphetamine in combination with high-dose vitamin A. All experimental animals had free access to standard feed and water throughout the study. The administration was done orally by gavage for 28 consecutive days. Twenty-four hours after the final administration, the animals were anesthetized and sacrificed. The livers were excised, weighed, and fixed in 10% neutral buffered formalin for histopathological examination using haematoxylin and eosin (H&E) staining. Blood samples were collected for the determination of serum liver enzyme activities, including alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP). Body weight results revealed reduction in body weight of the rats in METH treated group while co-administration with high-dose vitamin A mitigated the weight loss. The organ weight assessment revealed an increase in relative liver weight

across the experimental groups compared with the control group. However, the methamphetamine-treated group (Group B) exhibited a significantly higher relative liver weight than the control group ($P < 0.005$). Histological observation revealed that methamphetamine caused liver damage such as hepatocellular degeneration and inflammatory infiltration. Co-treatment with high-dose vitamin A showed improved liver cytoarchitecture suggesting protective effect. In conclusion, methamphetamine caused hepatotoxicity in Wistar rats of the animal models studied while high-dose vitamin A offered hepatoprotection against methamphetamine-induced liver damage.

Keywords: Vitamin A, Wistar rat, Hepatoprotective, Toxic dose, Methamphetamine

Introduction

Methamphetamine (METH) is a potent central nervous system (CNS) stimulant that produces marked euphoria, heightened alertness, and appetite suppression (Partridge *et al.,*2018*).* Methamphetamine primarily exerts its effects by promoting the release of the neurotransmitters dopamine and serotonin within the central nervous system (Yang *et al.,*2021). METH abuse is associated with significant damage to various peripheral organs, including the liver. Clinical and preclinical studies consistently report that METH can induce hepatotoxicity, characterized by elevated liver enzymes (e.g. AST. ALT), hepatocellular damage, steatosis (fatty liver), fibrosis, and even acute liver failure (Liu *et al.,*2019). Experimental studies using animal models have demonstrated that methamphetamine exposure induces inflammatory responses and oxidative stress, both of which are implicated in the development of respiratory, cardiovascular, and neurological disorders (Jayanthi et al., 2020). In addition, methamphetamine has been shown to promote hepatic inflammation and elicit several neurotoxic effects, including neuroinflammation, oxidative stress, autophagy, and neuronal degeneration (Seki et al., 2021). The extensive availability and abuse of methamphetamine, together with its severe adverse health consequences, have contributed to increasing morbidity and mortality worldwide (Castelli et al., 2019). Methamphetamine (METH) induces hepatotoxicity through multiple interconnected mechanisms, including disruption of hepatic metabolic activities, increased oxidative stress, and hyperthermia (Gottardi et al., 2020). These pathological processes have been linked to liver injury and may promote hepatocyte apoptosis by interfering with fundamental cellular functions such as cell cycle regulation and cell proliferation (Zhang et al., 2020). Furthermore, the accumulation of bile salts, particularly within the bile canaliculi, represents a characteristic feature of the underlying pathology. Impaired hepatocyte function or reduced hepatocyte viability has also been suggested to compromise plasma membrane integrity, thereby contributing to hepatic dysfunction (Geladariss et al., 2024). Methamphetamine (METH) exposure produces marked hyperthermia, which contributes to neuronal injury by disrupting normal thermoregulatory mechanisms. The elevation in core body temperature (39–42 °C) observed after METH administration is thought to result from excessive monoamine release triggered by the drug. This hyperthermic response is mediated through a combination of increased locomotor activity, altered metabolic processes, dysregulation of hypothalamic neurotransmission, and peripheral vasoconstriction. Moreover, METH-induced hyperthermia has been reported to produce hepatocellular morphological alterations comparable to those observed during heat stroke, with similar hepatic changes evident within 24 hours following acute METH exposure (Kumar et al., 2020). Vitamin A comprises a group of fat-soluble compounds, including retinol, retinal, and retinyl esters, that are essential for maintaining normal physiological functions. Dietary vitamin A exists in two principal forms: preformed vitamin A, consisting of retinol and retinyl esters, which is obtained exclusively from animal-derived foods such as liver, fish, and dairy products, and provitamin A carotenoids, which are abundant in fruits, vegetables, and plant oils (Gilbert et al., 2013). Vitamin A is indispensable for vision, immune competence, cellular differentiation, growth, and reproduction. The liver serves as the primary site for vitamin A metabolism, storage, and transport, with most reserves localized within hepatic stellate cells. Despite its physiological importance, excessive vitamin A intake, particularly through prolonged high-dose supplementation, can result in hypervitaminosis A, a condition characterized by vitamin A toxicity. Because vitamin A is fat-soluble, it is not readily eliminated from the body and therefore accumulates in hepatic tissues with sustained overconsumption. Chronic exposure to excessive amounts of vitamin A is a well-recognized cause of hepatotoxicity, producing hepatic alterations that range from reversible liver injury to advanced fibrosis and cirrhosis. The hepatotoxic effects of vitamin A have been attributed to several interconnected mechanisms, including activation of hepatic stellate cells, increased oxidative stress, and direct injury to hepatocytes.

Methamphetamine and high-dose vitamin A have each been reported to exert hepatotoxic effects through several overlapping mechanisms, including oxidative stress, mitochondrial impairment, and inflammatory responses. Although the individual hepatic effects of these agents have been extensively investigated, limited information is available regarding their combined influence on liver function. The simultaneous exposure to toxic-dose methamphetamine and high-dose vitamin A may modify hepatic metabolism, potentiate liver injury through synergistic mechanisms, or alter inflammatory pathways involved in hepatocellular damage. Therefore, this study aimed to evaluate the effects of high-dose vitamin A on methamphetamine-induced liver injury in adult male Wistar rats by assessing biochemical indices of hepatic function together with histopathological alterations, in order to determine its potential protective or therapeutic role.

Methods

Materials of the Study

The materials used in this study included twenty-four adult male Wistar rats, iron cages fitted with wire mesh covers, sawdust as bedding material, grower and finisher mash animal feed, laboratory coats and gloves, dissecting instruments, a stopwatch, a digital video recorder, measuring cylinders, a weighing balance, a water bath, sample bottles, specimen labels,10% neutral buffered formalin (prepared from sodium phosphate monobasic, sodium phosphate dibasic, 37% formaldehyde, and distilled water), graded ethanol solutions (50%, 70%, 95%, and absolute ethanol), xylene, paraffin wax, embedding plates and pots, paraffin dispenser, tissue cassettes, slide storage boxes, glass slides, coverslips, DPX mounting medium, hematoxylin and eosin stains, a rotary microtome, a knife sharpener, an orbital shaker, a hot plate, a light microscope, a diamond pencil, micropipettes with appropriate pipette tips, trays, cotton wool, chloroform for anesthesia, notebooks, and writing materials.

Procurement and Housing of Experimental Animals

A total of twenty-four healthy adult male Wistar rats, weighing 180–250 g, were procured from the Research Enterprise Farm, University of Ibadan, Oyo State, Nigeria. Following procurement, the animals were randomly distributed into four treatment groups, with six rats allocated to each group. They were maintained in well-ventilated Perspex cages fitted with wire-mesh lids under standard laboratory housing conditions. Prior to the experimental procedures, the rats were allowed a two-week acclimatization period in the Animal House of the College of Health Sciences, Nnamdi Azikiwe University, Nnewi Campus. During this period, the environmental temperature was maintained between 27 and 30°C. Throughout the acclimatization and experimental phases, the animals were provided with standard grower mash feed and clean drinking water ad libitum.

Experimental Design

Twenty-four (24) adult male Wistar rats were randomly assigned into four experimental groups, with six (6) animals in each group.
Group A (Control): Received standard feed and clean drinking water only.
Group B (Methamphetamine): Received methamphetamine at a dose of 20 mg/kg body weight.
Group C (Vitamin A): Received high-dose vitamin A at 2.5 mg/kg body weight.
Group D (Methamphetamine + Vitamin A): Received methamphetamine (20 mg/kg body weight) concurrently with high-dose vitamin A (2.5 mg/kg body weight).
All treatments were administered orally by gavage once daily for a period of 28 consecutive days. Throughout the experimental period, all procedures were conducted under strict supervision in accordance with the approved experimental protocol.

Termination of Treatment

Twenty-four (24) hours after the final administration of the experimental treatments, the animals were weighed using a digital weighing balance to obtain their final body weights before sacrifice. The rats were subsequently anesthetized with chloroform, after which a midline abdomino-pelvic incision was made using blunt scissors to minimize damage to the visceral organs. Each animal was positioned in the supine position with the limbs secured laterally to a dissecting board to facilitate tissue dissection. The liver was carefully identified, excised, and examined macroscopically for any visible abnormalities or lesions. The harvested liver was weighed and subsequently fixed in 10% neutral buffered formalin for preservation and histopathological processing. Histological analyses were carried out in the Histology Laboratory, Department of Anatomy, Nnamdi Azikiwe University, Nnewi Campus.

Tissue Processing

To facilitate microscopic examination, the liver tissues were processed through a series of standard histological procedures, including fixation, dehydration, clearing, paraffin infiltration, embedding, sectioning, and staining. These steps were performed to preserve tissue architecture and produce high-quality sections suitable for histopathological evaluation.

Fixation

Tissue fixation was performed to preserve cellular and tissue architecture, prevent autolysis and microbial decomposition, and maintain the structural integrity of the specimens for histological examination. The harvested liver tissues were immersed in 10% neutral buffered formalin, prepared from sodium phosphate monobasic (4.0 g), sodium phosphate dibasic (6.5 g), 37% formaldehyde (100.0 mL), and distilled water (900.0 mL). The tissues were fixed for 48 hours, after which they were rinsed under running tap water to remove residual fixative before further histological processing.

Dehydration

Following fixation, the liver tissues were dehydrated to remove water and prepare them for subsequent clearing and paraffin infiltration. Dehydration was carried out using ascending concentrations of ethanol (50%, 70%, 95%, and absolute ethanol). The tissues were immersed twice in each concentration, with each immersion lasting 2 hours, except for absolute ethanol, where each change was maintained for 1 hour to ensure complete dehydration.

Clearing

Following dehydration, the liver tissues were cleared in two successive changes of xylene, each for 2 hours, to remove the absolute ethanol and render the tissues receptive to paraffin wax infiltration. Xylene was selected as the clearing agent because it is miscible with both absolute ethanol and molten paraffin wax, thereby facilitating the replacement of ethanol within the tissues before embedding. Upon completion of the clearing process, the tissues were subjected to paraffin wax infiltration.

Infiltration

The tissues were transferred to two changes of molten paraffin wax for 2 hours each. I ensured that the amount of wax was 25-50 times the volume of the tissue. Infiltration was carried out to replace xylene in the tissue spaces with molten paraffin wax. The temperature of the paraffin was kept at 60^{o}C.

Embedding

The tissues were embedded by submerging them in two changes of molten paraffin wax contained in cassette metal moulds at a constant temperature of 52-60C in an oven of paraffin wax for 2 hours each time. The tissues were well-oriented in the moulds to ensure the presentation of the desired parts of the tissues to be examined in the tissue sections.

Sectioning

This is the process by which a thin slice of the tissue of about 4µm thick is made using a rotatory microtome (Leica RM 2125 RST).

Staining

Paraffinized tissue sections were initially deparaffinized by immersing them in xylene for 1 minute. The sections were then rehydrated by passing them through a series of graded ethanol solutions in decreasing concentrations, followed by washing with distilled water. Hematoxylin staining was carried out for 20 minutes, after which differentiation was performed using 2% acid alcohol for approximately 2 seconds. The slides were immediately rinsed with tap water to remove residual acid alcohol and subsequently exposed to running tap water for 10 minutes to achieve optimal bluing of the hematoxylin stain. Counterstaining was performed with 1% aqueous eosin for 30 seconds. Following staining, the tissue sections were dehydrated through ascending concentrations of ethanol, cleared with xylene, and permanently mounted using DPX mounting medium. Finally, the stained slides were observed under a light microscope to evaluate histopathological alterations.

Statistical Analysis

All statistical analyses were conducted with IBM SPSS Statistics Version 27.0.1. Experimental data are reported as mean ± SD. Group differences were assessed by one-way ANOVA, and a P-value below 0.05 was considered indicative of statistical significance.

Results

Behavioural and Physical Observations

At the commencement of the study, all experimental animals appeared healthy, active, and physically agile. During the two-week acclimatization period, the rats remained clinically stable and produced normal stools. Following methamphetamine administration, varying degrees of toxicity were observed in the treated groups, with the severity of clinical signs increasing in a dose-dependent manner. The affected animals exhibited tremors, increased agitation accompanied by aggressive behaviour, impaired balance, and a staggering gait. These manifestations became progressively more pronounced with continued methamphetamine exposure.

Changes in Body Weight

Table 1. Comparison of Mean Initial Body Weight, Final Body Weight, and Body Weight Change Among Experimental Groups (A–D)

GROUPS	INITIAL BODY WEIGHT(g)	FINAL BODY WEIGHT(g)	P-VALUE
A	130.40±2.03	145.96±0.02	0.007
B	136.20±4.83	121.04±6.01	0.001
C	146.40±2.64	163.10±9.23	0.003
D	143.04±1.36	154.30±0.00	0.000

Data were analysed using one-way analysis of variance (ANOVA). Results were expressed as mean ± standard deviation (SD), and differences were considered statistically significant at $P < 0.05$.

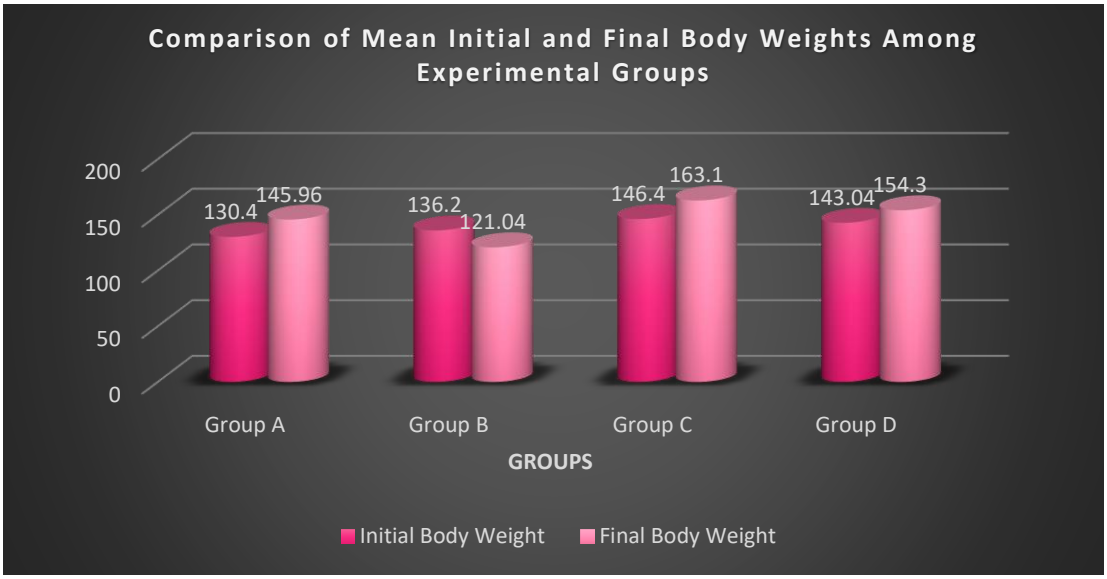

Figure 1. Bar chart showing Comparison of Mean Initial and Final Body Weights Among Experimental Groups

The body weight results showed that methamphetamine administration significantly impaired normal weight gain in adult male Wistar rats, while high-dose vitamin A treatment mitigated this effect. The control group (Group A) exhibited a significant increase in body weight from 130.40 ± 2.03 g to 145.96 ± 0.02 g ($p = 0.007$), reflecting normal physiological growth throughout the experimental period. In contrast, the methamphetamine-treated group (Group B) experienced a significant reduction in body weight from 136.20 ± 4.83 g to 121.04 ± 6.01 g ($p = 0.001$), indicating that toxic methamphetamine exposure induced weight loss, likely due to its appetite-suppressing effects, increased metabolic rate, and systemic toxicity. Rats treated with high-dose vitamin A alone (Group C) showed a significant increase in body weight from 146.40 ± 2.64 g to 163.10 ± 9.23 g ($p = 0.003$), demonstrating that vitamin A did not adversely affect growth and may have supported normal metabolic function. The group that received both methamphetamine and high-dose vitamin A (Group D) exhibited a significant increase in body weight from 143.04 ± 1.36 g to 154.30 ± 0.00 g ($p < 0.001$). This improvement, despite concurrent methamphetamine exposure, suggests that high-dose vitamin A attenuated methamphetamine-induced weight loss, possibly through its antioxidant and hepatoprotective effects, which may have preserved hepatic function, improved nutrient metabolism, and reduced oxidative damage. The bar chart illustrates the mean initial and final body weights of adult male Wistar rats across the experimental groups following the treatment period. The control group (Group A) exhibited a normal increase in body weight over the course of the study. In contrast, rats treated with methamphetamine alone (Group B) showed a significant reduction in body weight. Animals administered high-dose

vitamin A alone (Group C) recorded the greatest increase in body weight, while those receiving methamphetamine in combination with high-dose vitamin A (Group D) also demonstrated a significant improvement in body weight compared with the methamphetamine-treated group. These findings indicate that high-dose vitamin A attenuated methamphetamine-induced weight loss and promoted normal body weight gain.

Table 2. Comparison of Mean Relative Kidney Weight Across Experimental Groups

Test groups	Mean ±SEM	P-value	F-value
Group A	3.10±0.00		
Group B	3.50±0.07	0.005	23.934
Group C	3.15±0.96		
Group D	3.20±0.01		

Data were analyzed using one-way analysis of variance (ANOVA), followed by Fisher's least significant difference (LSD) post hoc multiple comparison test. Results were expressed as mean ± standard deviation (SD), and differences were considered statistically significant at $P < 0.05$.

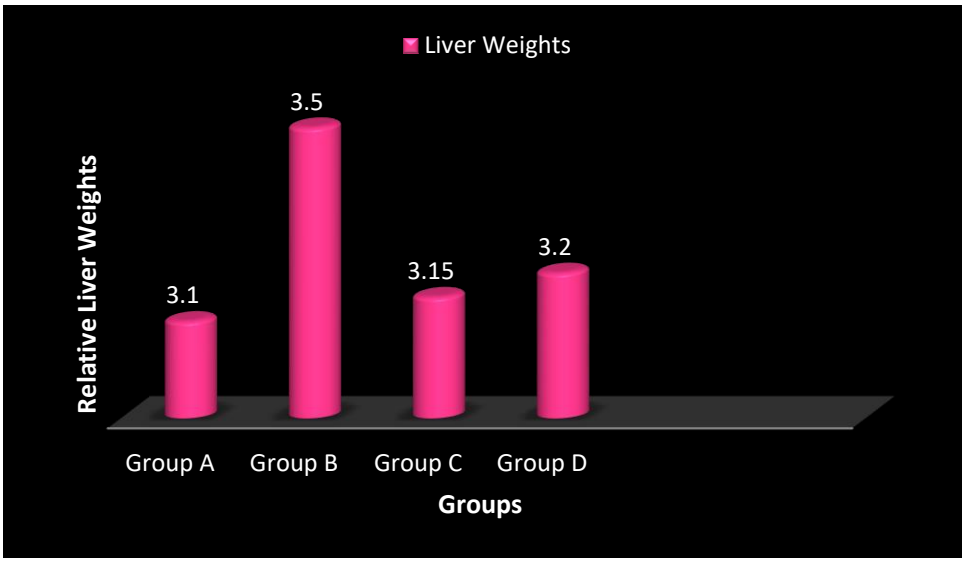

Figure 2. Bar chat showing Comparison of mean relative liver weight experimental groups

Table 3. Showing Activities of Serum Levels of Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT) And Alkaline Phosphatase (ALP) across experimental groups.

GROUPS	ALP	ALT	AST
A	106.04 ± 0.27	29.20 ± 0.29	25.10 ± 0.76
B	79.00 ± 0.42	42.13 ± 1.32	37.49 ± 0.10
C	105.00 ± 0.10	29.40 ± 1.00	25.23 ± 0.23
D	103.78 ± 0.23	30.34 ± 0.23	26.14 ± 1.02
F-RATIO	17.021	12.643	5.043
PROB. OF SIG.	<0.005	<0.005	<0.005

Statistical analysis demonstrated a significant difference in mean relative liver weight among the experimental groups ($F = 23.934$, $P = 0.005$). The control group (Group A) exhibited a mean relative liver weight of 3.10 ± 0.00**,** which served as the baseline value for comparison with the treated groups. Rats treated with methamphetamine alone (Group B) exhibited the highest relative liver weight **(**3.50 ± 0.07**),** suggesting hepatomegaly, which is likely due to methamphetamine-induced hepatic inflammation, oxidative stress, and cellular injury. Rats treated with high-dose vitamin A alone (Group C) had a relative liver weight **(**3.15 ± 0.96**)** comparable to the control group, indicating that vitamin A did not adversely affect liver size. The methamphetamine plus high-dose vitamin A group (Group D) showed a reduced relative liver weight **(**3.20 ± 0.01**)** compared with the methamphetamine-only group, suggesting that high-dose vitamin A attenuated methamphetamine-induced liver enlargement. The bar chart presents the mean relative liver weights of the experimental groups following the treatment period. Rats administered methamphetamine alone (Group B) exhibited the highest relative liver weight, suggesting hepatomegaly associated with methamphetamine-induced hepatic injury. In comparison, the control group (Group A), the high-dose vitamin A group (Group C), and the methamphetamine plus high-dose vitamin A group (Group D) displayed relatively similar liver weights. The lower relative liver weight observed in Group D compared with Group B indicates that high-dose vitamin A attenuated methamphetamine-induced liver enlargement, suggesting a protective effect against hepatic damage.

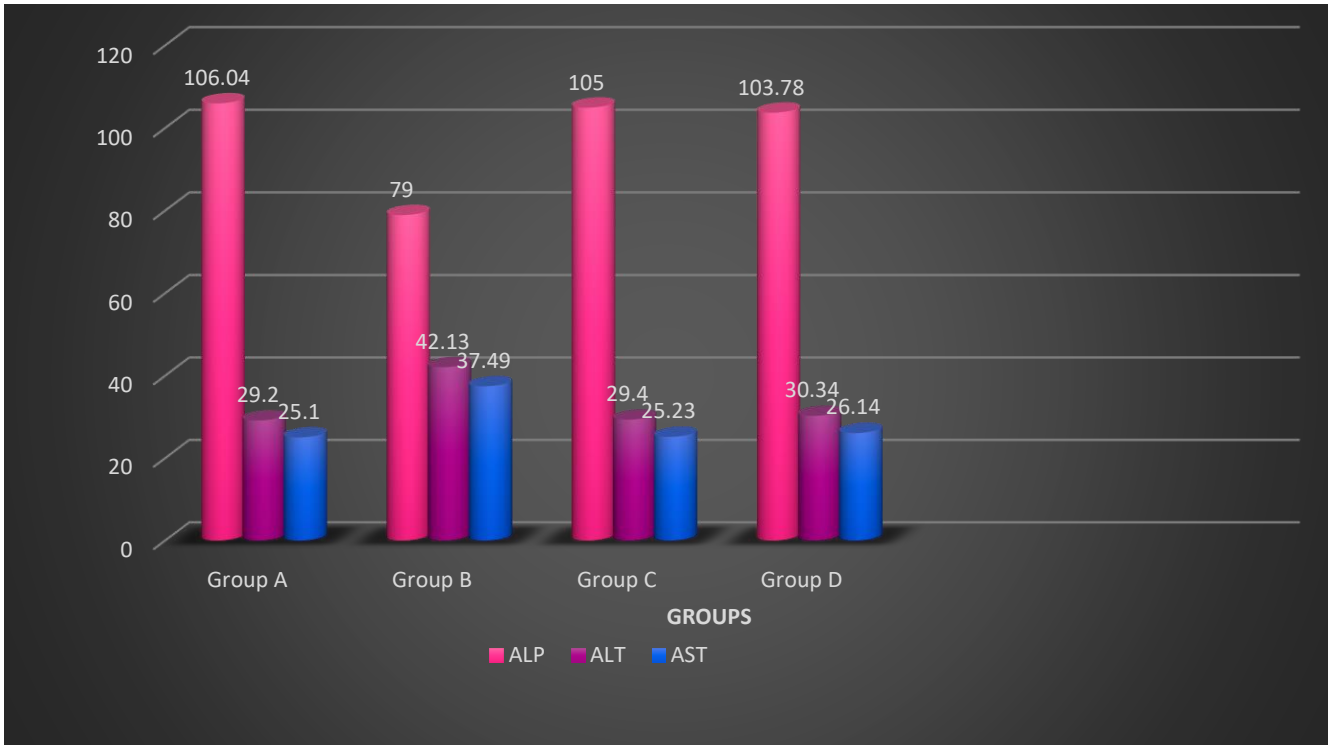

Figure 3. showing Bar Chart Representation of Activities of Serum Levels of Aspartate aminotransferase (AST), Alanine aminotransferase (ALT) & Alkaline phosphatase (ALP) across experimental groups

The table show the activities of serum liver enzymes ALP, ALT, and AST across the experimental groups. The control group (Group A) recorded normal enzyme levels (ALP: 106.04 ± 0.27 U/L, ALT: 29.20 ± 0.29 U/L, AST: 25.10 ± 0.76 U/L). Methamphetamine-treated rats (Group B) exhibited a marked decrease in ALP (79.00 ± 0.42 U/L) and significant increases in ALT (42.13 ± 1.32 U/L) and AST (37.49 ± 0.10 U/L), indicating liver injury. Rats treated with high-dose vitamin A alone (Group C) showed enzyme levels comparable to the control (ALP: 105.00 ± 0.10 U/L, ALT: 29.40 ± 1.00 U/L, AST: 25.23 ± 0.23 U/L), while the methamphetamine plus high-dose vitamin A group (Group D) also demonstrated near-normal values (ALP: 103.78 ± 0.23 U/L, ALT: 30.34 ± 0.23 U/L, AST: 26.14 ± 1.02 U/L). These findings indicate that high-dose vitamin A attenuated methamphetamine-induced liver damage by restoring serum liver enzyme activities toward normal levels. Statistical analysis revealed significant differences in the serum activities of all three liver enzymes among the experimental groups (ALP: $F = 17.021$; ALT: $F = 12.643$; AST: $F = 5.043$; $P < 0.005$). The bar chart illustrates the serum activities of ALP, ALT, and AST across the experimental groups. The methamphetamine-treated group (Group B) showed the lowest ALP level and the highest ALT and AST levels, indicating significant liver injury. The control (Group A), high-dose vitamin A alone (Group C), and methamphetamine plus high-dose vitamin A (Group D) groups exhibited enzyme levels close to normal. The restoration of ALP, ALT, and AST levels in Group D suggests that high-dose vitamin A protected against methamphetamine-induced hepatic damage by preserving normal liver function.

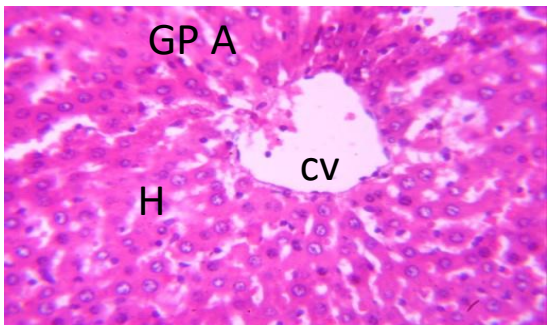

Figure 4. Photomicrograph of the liver section from Group A (Control) (H&E, ×400) showing normal hepatic architecture with a well-defined central vein (CV) surrounded by well-preserved, active hepatocytes (H).

Figure 4 shows a photomicrograph of the liver tissue from group A (control) stained with H&E at ×400 magnification. The liver exhibits normal histological architecture, characterized by a well-defined central vein (CV), well-organized

hepatic cords, and healthy, active hepatocytes (H). No evidence of cellular degeneration, inflammation, or tissue damage is observed, indicating normal hepatic morphology.

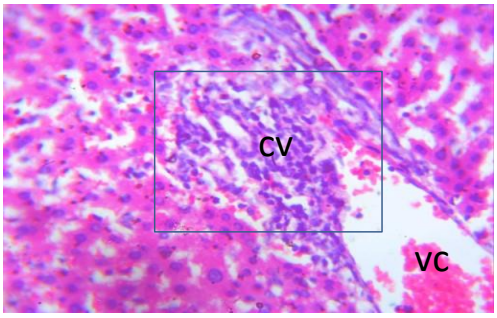

Figure 5. Photomicrograph of the liver section from Group B (methamphetamine-treated) stained with H&E (×400) showing severe hepatocellular degeneration, marked aggregation of intrahepatic inflammatory cells (IHI), and pronounced vascular congestion (VC). The overall histopathological features are consistent with chronic hepatitis.

Figure 5 shows a photomicrograph of the liver tissue from the methamphetamine-treated group (Group B) stained with H&E at ×400 magnification. The section reveals severe hepatic degeneration characterized by marked intrahepatic inflammatory cell infiltration (IHI) and vascular congestion (VC). These histopathological alterations are consistent with chronic hepatitis, indicating that methamphetamine induced significant liver injury and inflammation.

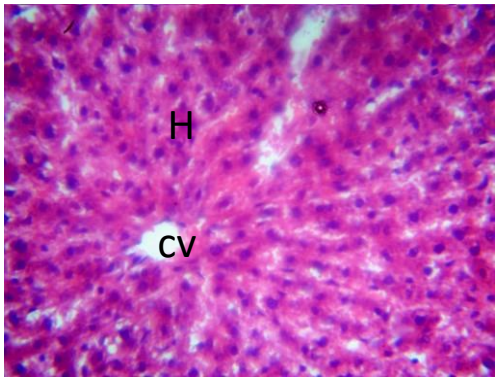

Figure 6. Photomicrograph of the liver section from Group C (high-dose vitamin A-treated) stained with H&E (×400) showing preserved hepatic architecture with a well-defined portal triad (PT) and normal, active hepatocytes (H).

Figure 6 shows a photomicrograph of the liver tissue from the high-dose vitamin A-treated group (Group C) stained with H&E at ×400 magnification. The liver exhibits normal histological architecture with well-preserved hepatic tissue, a clearly defined portal triad (PT), and healthy, active hepatocytes (H). No evidence of tissue degeneration or inflammatory changes is observed, indicating that high-dose vitamin A did not adversely affect liver morphology.

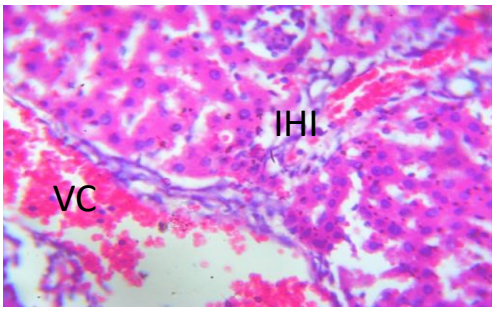

Figure 7. Photomicrograph of the liver section from Group D (methamphetamine-induced and treated with high-dose vitamin A) stained with H&E (×400) showing moderate hepatic regeneration with mild intrahepatic inflammatory cell infiltration (IHI) and mild vascular congestion (VC).

Figure 7 shows a photomicrograph of the liver tissue from the methamphetamine plus high-dose vitamin A-treated group (Group D) stained with H&E at ×400 magnification. The section demonstrates moderate hepatic regeneration with only

mild intrahepatic inflammatory cell infiltration (IHI) and mild vascular congestion (VC). These findings indicate that high-dose vitamin A ameliorated methamphetamine-induced liver damage and promoted recovery of the hepatic tissue.

Discussion

Methamphetamine (METH) is a potent psychostimulant belonging to the amphetamine class that exerts profound effects on the central nervous system. Although its clinical use is highly restricted, methamphetamine is prescribed in certain countries for the management of attention-deficit/hyperactivity disorder (ADHD) and, in selected cases, obesity because of its stimulant and appetite-suppressing properties (Cruickshank & Dyer, 2009). Beyond its medical use, methamphetamine is widely abused as a recreational drug. It induces euphoria, increased energy, and alertness, but prolonged use leads to addiction, neurotoxicity, and systemic toxicity. According to (Volkow et al.,2001), chronic METH use can damage dopaminergic and serotonergic neurons, leading to cognitive decline and emotional disturbances. Several studies have reported that Methamphetamine induces oxidative stress, apoptosis, and inflammation in vital organs including the brain, liver and kidneys (Yamamoto et al.,2010). In the liver particularly, METH use has been associated with incre4ase lipid peroxidation and elevated serum enzymes, indicating he4patocellular injury (Cadet et al.,2003). Other studies have also shown that methamphetamine negatively affects body and organ weight, disrupts normal physiological functions, and alters biochemical markers, highlighting its systemic toxicity (Thanos et al.,2007). The findings of the present study demonstrated that methamphetamine exposure induced significant hepatocellular injury, as evidenced by elevated serum activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP). These observations are consistent with previous reports indicating that psychostimulants promote oxidative stress and lipid peroxidation in hepatocytes, leading to hepatic injury (Ali et al., 2015; Udem et al., 2019).

The changes in body weight observed among the experimental groups indicated a normal increase in body weight in the control group (Group A), which is likely attributable to normal physiological growth, as the animals received only standard feed and water throughout the experimental period. A significant increase in body weight was also observed in Groups C and D, which received high-dose vitamin A alone and methamphetamine in combination with high-dose vitamin A, respectively. In contrast, rats administered methamphetamine alone (Group B) exhibited a significant reduction in body weight compared with the control group. This reduction may be attributed to the appetite-suppressing properties of methamphetamine, coupled with its ability to increase metabolic activity, resulting in decreased food intake and subsequent body weight loss. This result agrees with previous studies according to (Wang et al.,2017), chronic METH exposure led to significant weight loss in rodents, primarily due to decreased food intake and increased energy expenditure. The groups that were treated with vitamin A alone, vitamin A + Methamphetamine showed significant increase ($P<0.05$) in body weight in relation to the control group. In this context, vitamin A functions as a protective antioxidant. It helps counteract the oxidative stress and metabolic disturbances caused by methamphetamine, potentially Improving appetite and reducing tissue damage, which may explain the observed increase in body weight in the vitamin A + METH- treated group. This result is in line with the finding o Saleh et al.,2020), who reported that vitamin A supplementation helped mitigate weight loss and oxidative damage in drug-induced toxicity models by enhancing antioxidant defense and preserving metabolic balance. The relative organ weight result also showed significant differences in groups. There was significant increase ($P>0.05$) in group B animals administered Methamphetamine when compared with the control group A and groups C and D. This could be due to methamphetamine-induced hepatotoxicity and inflammation, leading to cellular damage and enlargement of liver tissues, which contributes to increased organ weight. This finding agrees with the report of (Ajibade et al.,2020), who observed that exposure to methamphetamine resulted in increased liver weight due to oxidative stress and inflammatory responses. Similarly, the study by (Singh et al.,2017) supported that chronic methamphetamine administration causes pathological changes in the liver, including swelling, congestion and infiltration, which contribute to the observed increase in relative liver weight. The result of groups C and D relative organ weight were statistically similar with the control group A suggesting that vitamin A might have offered protective effects. This result agrees offered protective effects. This result agrees with (El-Nekeety et al.,2011), who observed that vitamin A reduced organ damage and oxidative stress caused by toxins, leading to liver weights comparable to untreated controls.

The assessment of tissue and serum marker enzyme activities is widely employed to evaluate the extent of organ injury and the toxic effects of chemical agents (Malomo et al., 2000; Yakubu et al., 2003). Alterations in enzyme activities are considered sensitive biochemical indicators of cellular damage and may be detected before the onset of observable histopathological changes (Akanji et al., 1986). Consequently, serum and tissue enzyme activities are routinely used to assess the toxicological effects of xenobiotics in experimental animals (Coodley et al., 1970). Alkaline phosphatase (ALP) is a membrane-bound enzyme (Wright & Plummer, 1974), whereas alanine aminotransferase (ALT) and aspartate aminotransferase (AST) are predominantly cytosolic enzymes (Schmidt et al., 1965). Because these enzymes are abundant within hepatocytes, their release into the circulation occurs when hepatocellular membrane integrity is compromised or

when cellular necrosis develops, resulting in elevated serum enzyme activities (Cotran et al., 1989; Ngaha et al., 1981).A rise in serum level or decrease in tissue level of these intracellular enzymes is an index of damage to the liver and kidney cells (Moss, Rosalki et al.,1996). In the present study, there was a significant difference in the serum levels of AST, ALT and ALP among the groups, particularly in group B, which received METH alone. It showed a significant increase in the ALT and AST levels compared to control group A and other groups which indicates liver cell damage, hepatocellular injury or liver stress. This result aligns with previous studies that have reported hepatocellular damage following METH exposure. According to (Cadet et al.,2003) methamphetamine induces liver toxicity, including elevation of ALT and AST levels in rodents due to oxidative stress and mitochondrial dysfunction. Group B had decreased significantly in ALP compared to the control group A. Similarly, (Yamamoto et al.,2010) confirmed that chronic METH use results in increased liver enzymes, indicating liver injury in rodents. Overall, enzyme activities in groups C and D showed no significant difference from control. This suggests biliary dysfunction or enzyme suppression linked to METH toxicity, while the similarity in groups C and D suggests vitamin A may offer protection, helped maintain normal enzyme levels, showing protective or antioxidant effects. The normalization of enzyme levels in group C and D agrees with the findings of (El-Nekeety et al.,2011), who demonstrated that vitamin A possess antioxidant properties that help protect the liver by reducing oxidative damage and stabilizing enzyme levels in toxin-exposed rats.

Histological findings of the present study indicated normal liver tissues in the control group A and group C. The group B animals administered Methamphetamine caused intense damage to the liver tissue architecture as seen in photomicrograph 2, severe degeneration and aggregate of intra hepatic inflammatory (IHC), moderate regeneration with intra hepatic inflammatory were recorded in group D. This could be as a result of the oxidative and inflammatory damage triggered by methamphetamine, leading to hepatocellular degeneration and intrahepatic inflammatory response, as supported by previous studies (Moszczynska et al.,2004; Northrop, Yamamoto et al.,2015). These lesions align with those described by (Lin et al.,2017), who reported centrilobular necrosis and sinusoidal dilation following chronic methamphetamine administration in rodents. Conversely, Vitamin A treatment preserved hepatic architecture and normalized serum enzymes, suggesting its hepatoprotective action. Vitamin A's known antioxidant and membrane-stabilizing properties likely counteracted oxidative damage (Shang et al.,2019; Oyinloye et al.,2020). The co-administration of Vitamin A with methamphetamine resulted in partial restoration of normal hepatic cords and reduced inflammatory infiltration, showing a dose-dependent amelioration similar to that observed by (Saleh et al.,2021; Azeez et al. 2020). Thus, these findings support the hypothesis that Vitamin A mitigates methamphetamine-induced liver damage by enhancing endogenous antioxidant defenses and stabilizing hepatocyte membranes (Ajibola et al.,2021; Nwaogu et al.,2022).

Conclusion

From the findings of this study, Methamphetamine administration induced hepatic toxicity, confirmed by histological degeneration and elevated liver enzymes. Vitamin A alone maintained normal hepatic structure, indicating it is non-toxic and supports hepatocellular integrity. Combined Vitamin A and methamphetamine treatment significantly reduced hepatic damage, confirming its hepatoprotective potential. The mechanism of Vitamin A's protective effect is linked to its antioxidant and anti-inflammatory properties. Vitamin A supplementation could be a potential supportive intervention against oxidative liver injury.

Recommendation

This study recommends increased public awareness of the harmful effects of methamphetamine, particularly its toxic impact on the liver. Health authorities should strengthen preventive measures and provide rehabilitation support for users. Routine liver function monitoring is advised for individuals with a history of methamphetamine use to enable early detection of damage. The observed protective role of antioxidants suggests their potential as supportive therapy, warranting further investigation. Finally, stricter regulations on methamphetamine production and distribution are essential to reduce its abuse and related health risks.

Author contributions

Concept and study design: Ezejindu Damian Nnabuihe, Alozie Osinachi Prince.
Data collection: Alozie Osinachi Prince, Ekelemchukwu Chinelo Juliet, Udodi Sopuluchukwu Princewill, Enemuo Ijeoma, Okeke Somadina Nnamdi, Ogbuokiri Doris K., Okeke Henry Kachikwuru, Ekoh Augustine Alobu, Benedict Nzube Obinwa, Chinyere Elizabeth Eze, Muorah Chinecherem Onyekachi.
Laboratory investigations and histological analysis: Alozie Osinachi Prince, Nwaefulu Kester Eluemunor, Sobanke A. Omolara, Chidinma Ifeyinwa Mmaju, Okafor Anulika Jacinta, Chuka-Onwuokwu Ngozi Cynthia.

Data analysis and interpretation: Ezejindu Damian Nnabuihe, Alozie Osinachi Prince, Ekelemchukwu Chinelo Juliet, Ejiogu Ikedichukwu Chibueze, Nwoko Sebastine Okechukwu, Wuraola Serah Nnaemeka.
Manuscript drafting: Alozie Osinachi Prince.
Critical revision of the manuscript: Ezejindu Damian Nnabuihe, Agu Augustine Uchenna, Ugwu Augustus Uchenna, Nwodo Ndubuisi Francis, Elemuo Chukwuebuka Stanley, Agbai Johnson Ukwa.
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Ethics approval

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AI tool usage declaration

No AI tool was used in manuscript preparation.

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