Vitamin D Co-Supplementation Against Methamphetamine-Induced Nephrotoxicity in Wistar Rats

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ABSTRACT

Methamphetamine (METH) is a white, odorless, bitter-tasting crystalline psycho-stimulant drug related to amphetamine. It is highly addictive and often employed for the potent euphoric effects it produces, similar to cocaine. Vitamin B complex and vitamin D are noted for their potential protective effects against kidney damage. This study is aimed at investigating the effect of co-administration of vitamin B complex and vitamin D on the kidney in methamphetamine-induced nephrotoxicity in adult male Wistar rats. A total of twenty-four (24) adult male Wistar rats weighing 120–140g were grouped into six groups of n=4 each, labeled A to F. Group A served as the control (feed and water only), group B received 10 mg/kg of methamphetamine only, group C received 100 mg/kg of vitamin B complex, group D received 20 mg/kg of vitamin D, group E received 10 mg/kg of methamphetamine and 100 mg/kg of vitamin B complex, while group F received 10 mg/kg of methamphetamine and 20 mg/kg of vitamin D. All substances were administered orally for the duration of twenty-one (21) days. The results showed a statistically significant decrease in total body weight in group B ($P<0.05$), while groups C, D, E, and F showed significant increases in body weight ($P<0.05$). The result on organ weight showed a significant increase in group B, but no significant impact in groups C, D, E, and F ($P>0.05$) when compared to A. The result on urea and creatinine levels are indicated respectively, a 37.99% and 40% significant increase in group B ($P<0.05$), a slight 3.76% and 10% non-significant decrease in groups C and D ($P>0.05$), and a slight 5.8% and 7.67% non-significant increase in groups E and F ($P>0.05$) when compared to A.

Keywords: Methamphetamine, Urea, Creatinine, Vitamin B complex, Vitamin D.

BACKGROUND

Methamphetamine, often known as crystal Meth or *Mkpurummiri* in Igbo language is a shiny blue-white, ice-block like addictive drug used by a great percentage of addicts in Nigeria, especially youths (Seye, 2021). The crystal form of the drug looks like glass fragments and is chemically similar to amphetamine, a drug used to treat attention-deficit hyperactivity disorder (ADHD) and narcolepsy, a sleep disorder (Majeed, 2021). The abuse of this drug has increased rapidly in developing countries including Nigeria and individuals are affected

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on different multiple levels from its addiction. Abuse of this drug has been linked with severe addiction and accompanying neuronal damage, Neuro-inflammation, oxidative stress and impaired cognition, memory, and attention (Golsorkhdan *et al*., 2020). Methamphetamine is available in different forms including powder, paste, and crystals. Sniffing, oral intake, lung inhalation, and injection are the routes of utilization while Smoking is the most common method of administration.

Vitamin B deficiency, amongst other factors, may contribute towards the development of chronic kidney disease (CKD), considering the progressing inflammatory and oxidative stress processes that are associated with impairment of kidney function and development of kidney histopathology. An emerging topic of discussion is the association between each vitamin B sub-form (B1 (Thiamine), B2 (Riboflavin), B3 (Niacin/Nicotinamide), B5 (Pantothenic Acid), B6 (Pyridoxine), B8 (Biotin), B9 (Folate), and B12 (Cobalamin)) and CKD. A recent study showed that Vitamin D administration can ameliorate Cd-induced nephrotoxicity (Abdelghany et al., 2023). Vitamin D deficiency is a potential risk factor for IC- or Gd-induced nephrotoxicity most likely due to imbalance in intrarenal vasoactive substances and oxidative stress. (Weverton et al., 2015).

METHODOLOGY

Ethical Approval

Ethical approval was obtained from the animal ethical committee, Faculty of Basic Medical Sciences, College of Health Sciences, Nnamdi Azikiwe University.

Materials

1. Twenty-four (24) adults wistar rats
2. Iron cages with iron nettings
3. Saw dust (litter)
4. Animal feed (grower and finisher mash) and water
5. Laboratory coat and gloves
6. One stopwatch
7. Clean caps of 15 ml conical tubes
8. 55cm wide 2-mm thick metallic wire
9. Measuring cylinder
10. Weighing balance
11. Water bath
12. Sample bottle
13. 10% Neutral Buffered Formalin (Sodium Phosphate Monobasic 4.0gm, Sodium Phosphate Dibasic 6.5gm, Formaldehyde 37% 100.0ml, Distilled Water 900.0ml)
14. Cotton and anesthesia (chloroform)
15. Graded alcohol (50%, 70%, 95% and absolute alcohol)
16. Glass slide and slide rack
17. Hot plate
18. Xylene
19. Paraffin wax
20. Embedding plate and pot
21. Deepex (DPX) mountant
22. Haematoxylin and eosin
23. Cover slip
24. Light microscope
25. Dissecting kit
26. Diamond pencil
27. Rotatory microtome
28. Ethanol for tissue processing
29. Micro pipette

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30. Specimen labels and bottles
31. Ethylenediamine tetra acetic acid (EDTA) bottles
32. Trays
33. Paraffin dispenser
34. Pipette tips (various sizes)
35. Knife sharpener
36. Notebook and biro

Procurement of Methamphetamine

Methamphetamine was procured from Madobi pharmaceutical stores, Nnewi, Anambra State and 10mg/kg of methamphetamine was administered to the experimental animals by oral route.

Acute Toxicity Test (LD_{50}) of Methamphetamine

The acute toxicity test of the mixture of Methamphetamine was carried out in the Department of Biochemistry, Nnamdi Azikiwe University, Nnewi campus, according to the method employed by Dietrich Lorke (1983).

Acquisition of drug dosage for administration

The liquid form of the drugs was generated by dissolution of the substances in water. The animals in each group were administered in a well-ventilated room with the specific dose of drug required as obtained in the LD50. The administration of methamphetamine was maintained for a specified length of time depending on the group. This was necessary to ensure the accurate concentration of the drugs within the experimental time.

Experimental design

A total of twenty-four (24) adults male wistar rats weighing 120-140g were grouped into six groups of n=4 each (Groups A to F).

Group A: Negative control (just feed and water only).

Group B: High dose (10mg/kg) of methamphetamine.

Group C: High dose (100mg/kg) of vitamin B complex.

Group D: High dose (20mg/kg) of vitamin D.

Group E: High dose (10mg/kg) of methamphetamine and (100mg/kg) of vitamin B complex.

Group F: High dose (10mg/kg) of methamphetamine and (20mg/kg) of vitamins D.

All experimental protocols were observed under strict supervision and administration was done using an oral gavage needle.

Treatment Termination

Twenty-four hours after the last exposure, following a behavioral test, the animals were weighed, then anaesthetized under chloroform vapor. The animal abdomens were then dissected by vertical midline incision to harvest and weigh the kidneys.

Data Analysis

Data was analyzed using One-way ANOVA followed by multiple comparison using LSD and data were considered significant at $P<0.05$. *$P<0.05$ means significant and $P>0.05$ means not significant.

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RESULTS

Table 3.1: Effect of the Co- administration of vitamin B complex and vitamin D on the kidney of Methamphetamine induced nephrotoxicity on total body weight.

GROUPS	WEIGHT (g)	MEAN± SEM	p-value
GROUP A	Initial Final	140.47±1.32 160.33±0.05	0.005
GROUP B	Initial Final	125.06±0.33 100.56±0.41	0.001*
GROUP C	Initial Final	128.07±0.02 150.76±2.31	0.000*
GROUP D	Initial Final	132.14±0.02 156.26±4.71	0.000*
GROUP E	Initial Final	120.65±3.02 131.75±0.02	0.001*
GROUP F	Initial Final	126.45±3.02 138.55±0.02	0.001*

Table 3.2: Effect of the Co- administration of vitamin B complex and vitamin D on the kidney of Methamphetamine induced nephrotoxicity on organ weight.

Test groups	Mean ±SEM (g)	P-value	F-value
Group A	1.00±0.00		
Group B	1.35±0.00	0.005	25.351*
Group C	1.00±0.56		
Group D	1.00±0.00		
Group E	1.01±0.00		
Group F	1.02± 0.01		

Table 3.3: Effect of the Co- administration of vitamin B complex and vitamin D on the kidney of Methamphetamine induced nephrotoxicity on biochemical results.

GROUP	UREA	CREATININE
A	22.06±7.58	1.50±0.00
B	30.44±2.21	2.10±0.14
C	21.40±0.39	1.30±0.34
D	21.06±0.39	1.40±4.34
E	23.42±0.00	1.42±2.87
F	23.26±0.00	1.35±6.32
F-RATIO	6.88	1.421
PROB. OF. SIG	<0.005	<0.005

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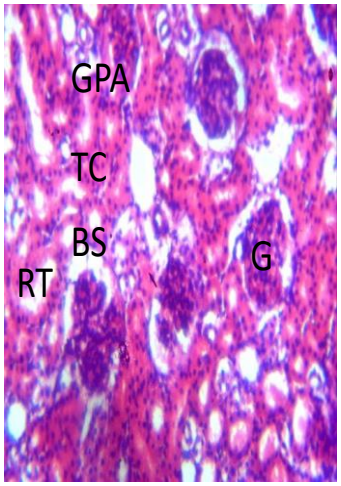
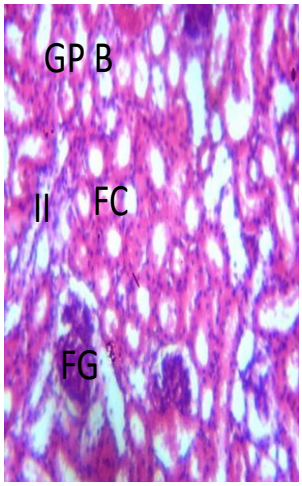
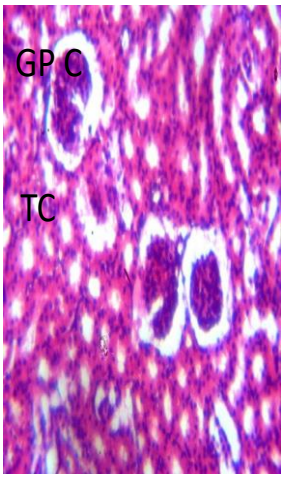
Histological Results



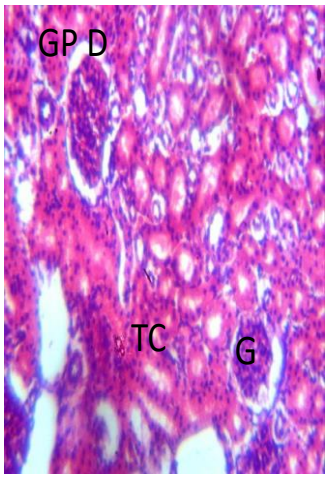
Photomicrograph of group A control section of kidney shows normal renal architecture with glomeruli (G), bowman space (BS), renal tubules (RT) and active tubular cell (TC)



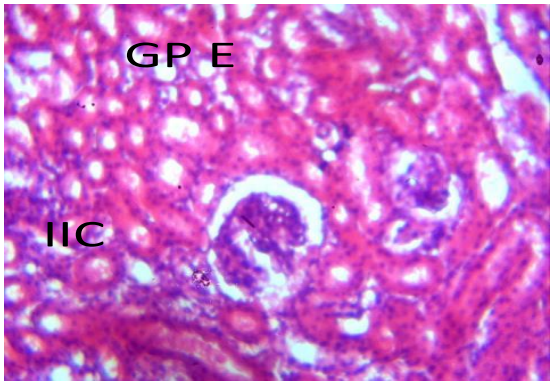
Photomicrograph of group B section of kidney induced with 10mg/kg of methamphetamine (high dose) shows sever renal degeneration with sever fatty change (FC), severe pyknotic glomeruli (PG) and interstitial inflammation (II).



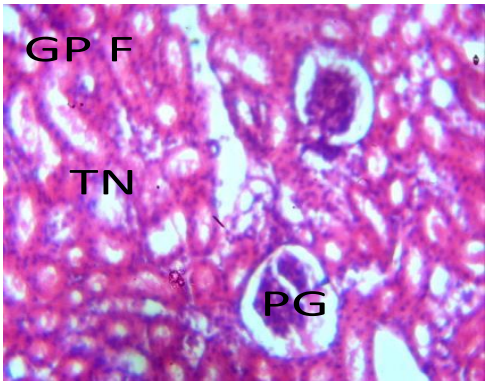
Photomicrograph of group C section of kidney induced with vitamin B complex 100mg/kg (High Dose) show renal tissue with active tubular cells (TC)



Photomicrograph of group D section of administered with vitamin D 20mg/kg (high dose) shows renal tissue with well outlined active tubular cells (TC) and glomeruli (G)



Photomicrograph of group E section of Kidney induced with 10mg/kg (high dose) and vitamin B complex 100mg/kg (high dose) shows moderate regeneration with fatty changes (FC) and mild infiltration of inflammatory cells (IIC)



Photomicrograph of group F section of kidney induced with 10mg/kg of methamphetamine (high dose) and Vitamin D 20mg/kg (high dose) shows mild regeneration with moderate pyknotic glomeruli (PG) and tubular necrosis (TN)

DISCUSSION, CONCLUSION AND RECOMMENDATION

Discussion

Body weight

Group A (Control) and Group D (20mg/kg) show significant body weight gain, suggesting a beneficial effect of proper feeding pattern. A significant reduction in body weight is observed in Group B indicating severe weight loss due to methamphetamine-induced nephrotoxicity. Group C, Group E, and Group F show weight gain, indicating that Vitamin B complex and Vitamin D have a positive impact on weight despite nephrotoxic stress. This is consistent with studies suggesting that combined supplementation can improve overall health outcomes (Biesalski & Dorey, 2017).

Organ Weight

The organ weight result shows notable differences, particularly in Group B, which has a significantly higher organ weight compared to other groups ($p<0.05$). This could indicate methamphetamine-induced damage, exacerbated by nephrotoxicity (Yang et al., 2019). The absence of significant changes in other groups indicates that the co-administration of Vitamin B complex and Vitamin D may not have uniformly affected kidney weight in this study.

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Biochemical Result

Biochemical result for Group A shows lower levels of urea and creatinine, suggesting relatively normal kidney function or less severe nephrotoxicity. Group B shows significantly elevated urea and creatinine levels, indicating severe nephrotoxicity. Methamphetamine exposure is associated with impaired kidney function, as reflected by these markers (Yang et al., 2019). The lack of significant differences in creatinine levels across Group C, D, E, and F might indicate variability in how Vitamin B complex and Vitamin D impact kidney function.

Renal Histology

Histological examination reveals the presence of active tubular cells, suggesting some level of regenerative response. However, the persistence of fatty changes and mild inflammatory cell infiltration indicates that vitamin B complex does not fully counteract the nephrotoxic effects of methamphetamine. Previous studies have demonstrated that B vitamins, particularly B6 and B12, possess antioxidant and anti-inflammatory properties which could contribute to cellular protection and repair (Hsu et al., 2013; Li et al., 2017). Despite these benefits, the extent of recovery observed here suggests that vitamin B complex alone may be insufficient for complete nephroprotection in severe cases of drug-induced damage.

Vitamin D demonstrates a more pronounced protective effect against methamphetamine-induced nephrotoxicity. The observed preservation of kidney architecture, including well-defined glomeruli and active tubular cells, indicates a substantial restorative effect. Vitamin D's nephroprotective role has been well-documented, with evidence suggesting that it mitigates oxidative stress and inflammation in renal tissues (Zhao et al., 2015; Piwowar et al., 2016). Vitamin D's ability to modulate calcium homeostasis and exert anti-inflammatory effects could explain its superior efficacy in ameliorating kidney damage compared to vitamin B complex.

Conclusion: Vitamin D appears to be more effective than vitamin B complex in mitigating methamphetamine-induced nephrotoxicity.). Though this study was limited to a small sample size, the observed histological improvements with vitamin D administration suggest its potential as a therapeutic agent in protecting against renal damage caused by nephrotoxins. Although both vitamins contribute to kidney health, their combined use may not fully resolve nephrotoxicity, highlighting the need for further research into optimal dosing and combination strategies for renal protection.

RECOMMENDATIONS

1. Additional studies should be conducted to investigate the mechanisms behind Vitamin D's nephroprotective effects and to determine optimal dosages for renal health.
2. Implement regular monitoring of kidney function in patients using nephrotoxic substances, alongside supplementation, to assess the effectiveness of interventions.
3. Patient should be educated on the importance of nutrition and supplementation in mitigating the effects of nephrotoxicity, emphasizing the role of vitamins in kidney health.

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