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Original Research Article

Momordica charantia Leaf Extract Attenuates Cyanide Toxicity by Enhancing Antioxidant, Anti-inflammatory and Cholinergic response in Rats' Brain

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ABSTRACT

Medicinal plants have been exploited over centuries for the management and treatment of diseases and as antidote against acute poisoning. Here, the antioxidant, anticholinergic and anti-inflammatory impacts of methanol extract of *Mormodica charantia* (MMC) leaf were investigated in the cortex and hippocampus of cyanide-intoxicated rats. Except for animals in control and potassium cyanide (KCN) groups that were orally administered distilled water and KCN, other groups were co-administered 0.4 mg/kg of freshly prepared KCN and varying dose (100, 200 and 400 mg/kg) of MMC and 200 mg/kg thiosulphate. Animals were treated for 21 days and thereafter sacrificed for sample collections for biochemical and histopathological studies. Findings from this study revealed enhanced reversal of oxidative stress markers near normalcy in rats' brain cortex and hippocampus. Acetylcholine esterase (AChE) and butyrylcholine esterase (BChE) activities were significantly reduced ($p<0.05$) in animals that were administered varying doses of MMC and thiosulphate relative to KCN untreated group. The level of hippocampal TNF–α and IL–6 significantly decreased ($p<0.05$) in MMC treated animals compared to KCN groups. Micromorphology section of both brain and hippocampus revealed improvement in MMC treatment as no degenerative changes was observed in these sections. This study has demonstrated that *Mormodica charantia* methanol extract is medicinally potent against cyanide toxicity and presumably contain lead compound that could be harnessed for the treatment of oxidative damage, inflammatory and neuronal responses in cyanide poisoning.

Keywords: Cyanide, Hippocampus, Inflammation, Oxidative Stress, Thiosulphate, Acetylcholine Esterase.

Introduction

Cyanide is a known industrial contaminant and a possible suicide, homicidal and chemical warfare agent. Environmental sources of cyanide include effluent from electroplating processes, metallurgy, gold mining, and petroleum industries. Iatrogenic source of cyanide such as sodium nitroprusside has also been reported.[1] The harmful action of cyanide is due largely to the development of anoxia after inhibition of mitochondrial respiratory enzyme (cytochrome c oxidase).[2] The central nervous system (CNS) is the principal target site of cyanide poisoning and the associated neurological disorders of its acute poisoning include convulsions, non-coordinated movements, seizures, and tremors.[3,4] The sensitivity of CNS to the lethal activities of cyanide is presumably related to its hypoxic state, low energy stores, and high energy demands.[2] Aside anthropogenic exposures, chronic exposure from dietary sources (cyanogenic glycosides in food materials) have also been reported to cause blindness and neuropathy, endemic goitre and sometimes death.[5]

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In addition to the modifications in structure and function of hemoglobin and cytochrome oxidase, multiple processes have been reported to surface during cyanide poisoning. Termination of the electron transport chain owing to cyanide is reported to be associated with energy deprivation, elevated reactive oxygen species, altered calcium homeostasis, increased lipid peroxidation and apoptosis of neuronal cells.[6] Previous neuropathological evidences also revealed significant decline of neurotransmitters in rats' brain, neuronal infiltration, reactive gliosis and upregulations of inflammatory indices in brain distinct parts (cortex and hippocampus) of cyanide induced intoxicated animal models.[2,4] Several supportive care such as administration of oxygen, intravenous fluids, and vasopressor support in patients with refractory hypotension have been developed to alleviate the toxic effects of cyanide. Antidote kits such as sodium thiosulfate, sodium nitrite, amyl nitrite, isosorbide dinitrate, nitrites and dicobalt edetate are also available as synthetic kits to counteract the toxic effects of cyanide if detected early.[7] However, these synthetic antidotes are reported to be associated with toxicities and other limitations such as slow acting, minimal attenuating effects and poor permeation of the blood brain barrier.[4,8] Previous report has established that the use of alternative therapy, essentially from plants origin are effective against cyanide poisoning and its complications. For instance, the administration of aqueous garlic extract[9], *Tetrapleura Tetraptera* Fruit[10], *Telfairia occidentalis*[11] were reported to reduce the toxic effects and the degree of inflammation in cyanide induced animal models.

Momordica charantia (*M. charantia*), commonly called bitter gourd or melon belongs to the family 'Cucurbitaceae'. Different parts of the herbaceous vine have been reported to be therapeutically effective against diseases such as hypertension, diabetes mellitus, microbial infections, obesity, cancer and possesses antioxidant activity.[12,13] Previous review has revealed that different extracts of *M. charantia* confers neuroprotection by attenuating oxidative stress, neuroinflammation and cell death. The anti-depressant and memory improvement of mice following the administration of methanol extract of *M. charantia* have also been reported.[14] In bid to bridge the toxic effects and other limitations that are associated with the use of

chemically synthesized cyanides antidotes, the use of ecofriendly treatments with little or no side effects against neuronal damage in cyanide poisoning remain significant. This study is design to investigate the effects of *M. charantia* leaf extract on oxidative stress, neurochemical changes and inflammatory responses in brain of cyanide-intoxicated rats.

Materials and methods

Materials

Potassium cyanide, sodium thiosulphate, thiobarbituric acid, acetylthiocholine iodide, butyrylthiocholine iodide, 5,5[1]-dithiobis-2-nitrobenzoic acid (DTNB), Sodium citrate and hydrogen peroxide were obtained from Sigma-Aldrich (St Louis, Missouri). Monobasic sodium phosphate (NaH_2PO_4), dibasic sodium phosphate (Na_2HPO_4), potassium permanganate, ethylenediamine tetra acetic acid (EDTA), methanol, sodium dodecyl sulfate (SDS), sodium bicarbonate, sodium hydroxide pellets, hydrochloric acid, Tris–HCl buffer, trichloroacetic acid are product of Sigma-Aldrich and were purchased from Pascal Scientific, Nigeria. Adrenaline, pyrogallol, acetylthiocholine iodide, butyrylthiocholine iodide (Merck, Darmstadt, Germany). Rat ELISA kits used for determination of IL–6 and TNF–α were purchased from ElabScience, Texas USA. All reagents and solvents were analytical grades and aqueous solutions were glass distilled.

Collection and Identification of leaf sample

Fresh leaves of *Momodica charantia* were harvested in March, 2022 in Okitipupa local farmlands, Ondo state, Nigeria and authenticated at the herbarium unit, biological sciences department, OAUSTECH with the herbarium reference code OAUSTECH/H/0657.

Extraction of Plant Sample

The leaves were thoroughly cleaned under running water to remove sand particles, air-dried under shade for 10 days and pulverized into powder using electric blender. A portion of 300 g powdered sample was soaked in 900 mL methanol for 48 hours with recurrent agitation. The methanol filtrate that was sieved from the mixture was further concentrated under reduced pressure using rotary evaporator (40 ^{0}C) to get a slurry of methanol extract of *Momordica charantia* (MMC).

Experimental Animals

Male albino rats (Wistar strains) were purchased from the department of chemical sciences animal house, Ladoke Akintola University of Science and Technology, Osun state, Nigeria. The rats were kept in clean and aerated rat's enclosure, accustomed for two (2) weeks in a ventilated room, and allowed access to clean water and pelletized rat chow. Treatment of the animals strictly followed the ethical manual guide of the National research council[15], of laboratory animal care and the Institution ethical committee with reference code (**OAUSTECH/ETHC-BCH/2022/006**).

Experimental Protocol

A total of thirty (30) healthy rats between 130–150 g body weights (bw) were systematically grouped into six (6) divisions of five animals per division with the following treatment order: Group I; control (NC) received 0.5 mL/kg distilled water; group II; KCN 0.4 mg/kg ; group III: 100 mg MMC/kg + KCN (MMC1); group IV: 200 mg MMC/kg b.w + KCN (MMC2); group V: 400 mg MMC/kg b.w + KCN (MMC3) and group VI; 400 mg thiosulphate /kg b.w + KCN (Thiosulphate). Cyanide was administered orally at intervals of 3 days using gavage while *M. charantia* leave extract was administered daily for 21 days following the same route of administration. The dose of *M. charantia* extract was chosen to contain lower, middle and upper dose following previous therapeutic dose range.[16]

Termination of Experiment

After 21 days of extract administration, animals were deprived of feed overnight and sacrificed by cervical dislocation. Craniotomy was done to remove the whole brain and hippocampal separation from the cortex was performed on ice. The tissues (brain cortex and hippocampus) were separately homogenized in ice-cold phosphate buffer (0.1 M, pH 7.4) and centrifuged at 3000 x g for 15 mins to obtain respective homogenates for biochemical analysis. Portions of the cortex and hippocampus were fixed in buffered formalin for histology.

Oxidative Stress Indices

Oxidative stress indices that were performed on the tissues (brain cortex and hippocampus) include total protein (TP) concentration[17], catalase (CAT) activity[18], glutathione Peroxidase (GPx) activity[19], reduced glutathione (GSH) concentration[20], superoxide dismutase activity (SOD)[21] and lipid peroxidation (LPO) level[22]. All reaction mixtures were quantified at different wavelength using T70 PG UV/Vis spectrophotometer, United Kingdom.

Neurochemical Studies

Neurochemical changes in the brain tissues (hippocampus and brain cortex) was performed by analysing the level of acetylcholine esterase (AChE) and butyrylcholine esterase (BChE) following the method of Ellman[23]. The principle of analysis follows the appearance of yellow anion 5, 5'-dithio-bis-nitrobenzoic acid that is quantified at 412 nm for every 120 seconds using T70 PG UV/Vis spectrophotometer.

Estimation of TNF–alpha (TNF–α) and Interleukin–6 (IL–6) Levels

The concentration of TNF–α and IL–6 levels in the hippocampus were determined using commercial ELISA kit following the manufacturer's manual guide. The optical density value of reaction mixtures in each well were quantified with microplate reader at 450 nm.

Histology Study

Tissues (hippocampus and brain cortex) for histology were initially preserved in formalin and thereafter processed for histopathological examination by slicing tissues (to 5 µm thickness) using microtome cutter and embedded tissue samples in paraffin. Samples were stained with hematoxylin and eosin, and histological changes in the organs were examined at 400 x magnification using Olympus binocular research microscope (Olympus, New Jersey, USA) and a 5.0MP Amscope Camera (Amscope inc, USA).

Statistical analysis

Data are presented as mean ± standard error of mean. Analyses were carried out using one-way analysis of variance and differences in mean values were considered to be statistically significant at $p<0.05$ using the SPSS statistical package. Graphical representation of results was performed using the GraphPad Prism 5.01 package.

Results and Discussion

Result revealing the total protein concentration in brain cortex and hippocampus is presented in Figure 1. The result revealed a non-significant decrease in protein concentration in brain cortex and hippocampus of groups that received KCN only compared to the control.

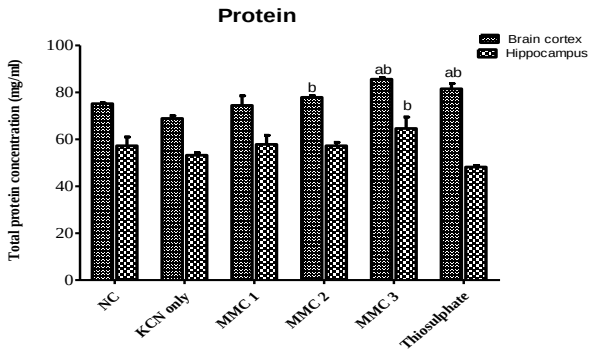

Figure 1: Total protein concentrations in brain cortex and hippocampus. Values mean ± SEM ($n = 4$-6), [a]$p<0.05$, significant when treated groups were compared to control. [b]$p<0.05$ is significant when treated groups were compared with KCN only group. NC (normal control), MMC 1 (100 mg MMC/kg b.w + KCN), MMC 2 (200 mg MMC/kg b.w + KCN), MMC3 (400 mg MMC/kg b.w + KCN) and thiosulphate (400 mg thiosulphate /kg bw + KCN). Where MMC = Methanol extract of *Momordica charantia*

Treatments with 200 and 400 mg MMC/kg body weight caused significant increase in brain cortex total protein compared to KCN only group while on 400 mg MMC/kg caused significant rise in hippocampal total protein concentration compared to KCN untreated group. Proteins such as hemoglobin, cytochrome oxidase and other important proteins that are rich in cysteine are one of the primary targets of cyanide since they form interaction with the sulphide groups of these proteins. From this study, the unperturbed concentration of the brain cortex and hippocampal protein could be due to stability in the protein synthetic machinery in the brain region. In addition, studies have demonstrated that cyanide intoxication might not potentially alter protein concentration since approximately 60 % of the cyanide form complexes or adducts with proteins, thereby altering their biological functions.[24] Contrast to animals that received KCN only, rats that were administered MMC3 demonstrated significant ($p<0.05$) increase in hippocampal protein relative to KCN group. Similarly, a significant increase was also obtained in brain cortex of animals that received MMC2, MMC3 and thiosulphate relative to KCN group. These improvements in protein concentration could be linked to the protein constituents in the extract, stability of endoplasmic reticulum modification process of nascent polypeptides and increased protein synthesis possibly by MMC bioactive constituents. Active compounds in the extracts at high dose might have also caused redox changes in cyanide ion (CN-) from intact cyanylated proteins thereby stabilizing the protein disulfide-bridge and concentrations.[1] Cyanide intoxicated patient with protein deficiency have been reported to be sensitive to cyanide-induced thyroid abnormalities.[24]

The results of oxidative stress indicators in both hippocampus and brain cortex of cyanide-intoxicated rat is presented in Table 1 and 2 respectively. In the brain cortex, animals that were administered KCN only demonstrated significant decrease in GSH level, catalase and SOD activities.

Table 1: Oxidative stress indicators in the brain cortex of cyanide intoxicated rats

	Catalase (U/ g Protein) $x10^{-1}$	GSH (µmol/g Protein)	SOD (Unit/ g protein) $x10^{-2}$	GPx (µmol/ mg Protein)	LPO (µmol/ mg Protein) $x10^{-2}$
NC	4.37 ± 0.14^{b}	0.33 ± 0.02^{b}	1.35 ± 0.11^{b}	0.27 ± 0.02	26.75 ± 0.25^{b}
KCN only	3.81 ± 0.09^{a}	0.27 ± 0.01^{a}	1.04 ± 0.05^{a}	0.14 ± 0.01	30.75 ± 0.25^{a}
MMC 1	3.09 ± 0.14^{ab}	0.29 ± 0.02	1.47 ± 0.07^{b}	0.37 ± 0.07^{b}	28.75 ± 1.97
MMC 2	6.21 ± 0.15^{ab}	0.29 ± 0.02	1.78 ± 0.08^{ba}	0.31 ± 0.05^{b}	26.00 ± 0.00^{b}
MMC 3	8.57 ± 0.05^{ab}	0.32 ± 0.01	1.64 ± 0.06^{ab}	0.34 ± 0.06^{b}	23.40 ± 0.40^{ba}
Thiosulphate	14.85 ± 0.26^{ab}	0.40 ± 0.02^{ba}	1.47 ± 0.14^{b}	0.24 ± 0.01	24.75 ± 0.63^{b}

Values mean ± SEM ($n = 4$-6), $^{a}p<0.05$, significant when treated groups were compared to control. $^{b}p<0.05$ is significant when treated groups were compared with KCN only group. NC (normal control), MMC 1 (100 mg MMC/kg b.w + KCN), MMC 2 (200 mg MMC/kg b.w + KCN), MMC3 (400 mg MMC/kg b.w + KCN) and thiosulphate (400 mg thiosulphate /kg bw + KCN). Where MMC = Methanol extract of *Momordica charantia*

Table 2: Oxidative stress indicators in the hippocampus of cyanide intoxicated rats

	Catalase (U/ g Protein) $x10^{-1}$	GSH (µmol/g Protein)	SOD (Unit/ g protein) $x10^{-2}$	GPx (µmol/ mg Protein)	LPO (µmol/ mg Protein) $x10^{-2}$
NC	0.35 ± 0.03^{b}	0.43 ± 0.04^{b}	3.24 ± 0.20	1.38 ± 0.08^{b}	10.33 ± 0.67^{b}
KCN only	0.17 ± 0.03^{ab}	0.30 ± 0.0^{a}	2.99 ± 0.02	0.48 ± 0.08^{a}	15.67 ± 0.88^{a}
MMC 1	0.97 ± 0.02^{ab}	0.39 ± 0.02^{b}	3.20 ± 0.22	0.74 ± 0.10^{a}	6.33 ± 0.33^{ab}
MMC 2	1.01 ± 0.04^{ab}	0.45 ± 0.04^{b}	3.20 ± 0.09	1.60 ± 0.19^{b}	7.67 ± 0.88^{ab}
MMC 3	1.18 ± 0.03^{ab}	0.31 ± 0.01^{a}	2.87 ± 0.21	0.99 ± 0.18^{ab}	7.67 ± 0.33^{ab}
Thiosulphate	0.37 ± 0.03^{b}	0.55 ± 0.03^{ab}	3.84 ± 0.05^{ab}	1.60 ± 0.01^{b}	7.33 ± 0.33^{ab}

Values mean ± SEM ($n = 4$-6), $^{a}p<0.05$, significant when treated groups were compared to control. $^{b}p<0.05$ is significant when treated groups were compared with KCN only group. NC (normal control), MMC 1 (100 mg MMC/kg b.w + KCN), MMC 2 (200 mg MMC/kg b.w + KCN), MMC3 (400 mg MMC/kg b.w + KCN) and thiosulphate (400 mg thiosulphate /kg bw + KCN). Where MMC = Methanol extract of *Momordica charantia*

Similarly, hippocampal catalase, GPx and GSH in KCN only administered animals compared to control. From this study, treatment with varying doses of MMC caused significant rise in antioxidant system in the brain cortex and hippocampus while lipid peroxidation in both tissues declined significantly when compared to KCN only treated group. Intoxication with KCN caused significant rise of lipid peroxidation in hippocampus and brain cortex of KCN treated animals while treatment with varying doses of MMC caused noticeable and steady decrease in LPO compared to KCN untreated group.

In consonance with previous studies, findings from this research revealed positive relationship between cyanide intoxication and oxidative stress generation in animal model.[4,25] The significant decrease in antioxidant (SOD, GSH, GPx and GSH) and significant rise in LPO agrees with the concept that inhibition of mitochondria cytochrome C oxidase triggers surge in reactive oxygen species (ROS). Elevated reactive oxygen species in mitochondria have been reported to systematically activates the Fenton reaction thereby initiating lipid peroxidation and culminating oxidative stress in the tissues.[26] Several research groups have reported the essential antioxidant effects of *M.*

charantia leaf extracts in different animal studies.[27,28] From this finding, multiple mechanisms including activation of several cyanide detoxification and elimination routes,[1] recycling and resynthesis of other antioxidant (ascorbate and tocopherol),[29] and enhancement of electron transport enzymes and ATP production,[4] are thought to underlie the ameliorative effects of MMC. Further, the presence of amino acids containing sulfur might have also contributed significantly to the overall detoxification process, aiding potential recovery from the toxic effects of KCN.[30]

Data from this study showed that cyanide administration caused disturbance in cholinergic systems as demonstrated by significant increase in AChE and BChE of KCN only treated group (Figure 2). Treatment with varying doses of *M. charatia* leaf extract significantly ($p<0.05$) decrease the activities of both enzymes in the brain regions (hippocampus and brain cortex) relative to the KCN untreated group. The central cholinergic system plays crucial role in brain function and regulation of memory. Alterations in acetylcholine metabolism through activation of acetylcholinesterase increases the breakdown of acetylcholine in the hippocampus precipitating cognitive deficiencies in the brain.[31,32] The significant difference of AChE and BChE activity in KCN only treated group could be due to imbalances in intracellular calcium (Ca^{2+}) level and oxidative effects of reactive oxygen species (superoxide and hydroxyl radical), possibly mediated by cyanide interaction with key proteins of the mitochondrion electron transport chain.[33,34] The peroxidation of lipids in the brain by hydroxyl radicals cannot be overemphasized.[6] The observed significant reduction in MMC treated groups could possibly be due to AChE and BChE inhibition by bioactive constituents in the extract. Inhibition of these enzymes by the extract could suggest improvement in cholinergic neurotransmitters with concomitant enhancement of cognitive functions. Important polyphenols with antioxidant potentials can also move via the blood-brain barrier to protect the brain and nervous system by suppressing the activities of free radicals.[35] Different plants metabolites such as flavonoids, alkaloids, glycosides, protein inhibitors, phenolics (gallic acid) and polysaccharides have been reported to be present in *M. charantia* leaves and they play important neuroprotective functions.[36]

In the current study, cyanide intoxication caused significant rise of the inflammatory indices (TNF-α and IL-6) in the hippocampus of KCN only group when compared to the control (Figure 3). Treatments with doses of *M. charantia* leaf extract for 21 days significantly ($p<0.05$) suppressed the hippocampal inflammatory mediators (TNF-α and IL-6) when compared to the KCN only treated group. However, while only MMC doses caused significant reduction in TNF-α compared to the control, IL-6 was significantly elevated across the treated groups compared to the control (Figure 3).

One crucial pathogenic mechanism that are associated with memory dysfunction due to neurodegeneration includes exacerbation of neuroinflammation. Evidences from previous studies have indicated that chronic exposure to cyanide induces inflammatory responses in the brain through expression of inflammatory mediators.[37,38] Possible mechanisms by which KCN intoxication elevated TNF-α and IL-6 could be linked to the nuclear binding and activation of redox sensitive transcription factors such as NF-κB. Reactive oxygen species (such as hydroxyl radical and hydrogen peroxide) that are generated from KCN-altered respiratory chain have been reported to be one of the mainstays for the activation of the NF-κB pathway.[39,40] The anti-inflammatory effect in the hippocampus of animals that were administered *M. charantia* extract could suggest the presence of important bioactive compounds with anti-neuroinflammatory properties. Important polysaccharides in *M. charantia* leaf have been reported to exert neuroprotective effects in the hippocampal neuronal cells of experimental rat.[41] In addition, other probable means by which MMC exerted these effects could be linked to the suppression of NF-κB induced neuroinflammation.[42,43] Previous study has demonstrated the neuroprotective effects *M. charantia* through downregulation of pro-inflammatory cytokines (IL-6, TNF-α) in mice hippocampus.[44]

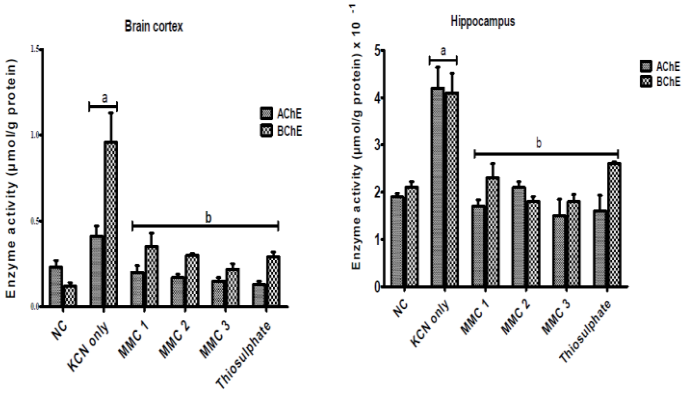

Figure 2: Effects of *M. charantia* extract on acetylcholinesterase and butyrylcholinesterase in brain cortex and hippocampus

Values mean ± SEM (n = 4-6), $^{a}p<0.05$, significant when treated groups were compared to control. $^{b}p<0.05$ is significant when treated groups were compared with KCN only group. NC (normal control), MMC 1 (100 mg MMC/kg b.w + KCN), MMC 2 (200 mg MMC/kg b.w + KCN), MMC3 (400 mg MMC/kg b.w + KCN) and thiosulphate (400 mg thiosulphate /kg bw + KCN). Where MMC = Methanol extract of *Momordica charantia*

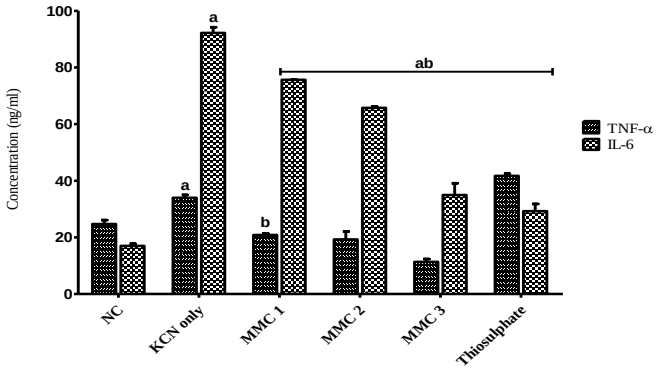

Figure 3: Effects of *M. charantia* extract on TNF-α and IL-6 in hippocampus of cyanide intoxicated rats.

Values mean ± SEM (n = 4-6), $^{a}p<0.05$, significant when treated groups were compared to control. $^{b}p<0.05$ is significant when treated groups were compared with KCN only group. NC (normal control), MMC 1 (100 mg MMC/kg b.w + KCN), MMC 2 (200 mg MMC/kg b.w + KCN), MMC3 (400 mg MMC/kg b.w + KCN) and thiosulphate (400 mg thiosulphate /kg bw + KCN). Where MMC = Methanol extract of *Momordica charantia*

The photomicrographs showing magnified views of hippocampus and brain cortex of KCN–intoxicated experimental rats are presented in Figure 4 & 5 respectively. The results revealed normal cellular architecture that is devoid of lesion in the brain cortex and hippocampus of animals in the control group. Group that was administered KCN only showed degree of damage in the brain cortex and hippocampus while noticeable reversal was observed in MMC treated groups. The layer II and III as well as the granule and pyramidale neuron of their brain cortex are visible and conspicuous at high magnification. Similarly, there was fine array of cells within the hippocampus, distinctively lined from the Cornu amonus (CA) to the dentate gyrus (DG). However, the brain and hippocampus of KCN-only treated group revealed significant observable degenerative changes (red arrow) that was characterised by degenerative layers, fragmented pyramidal and granule neurons with necrotic stellate neurons and loss of cytoplasmic content.

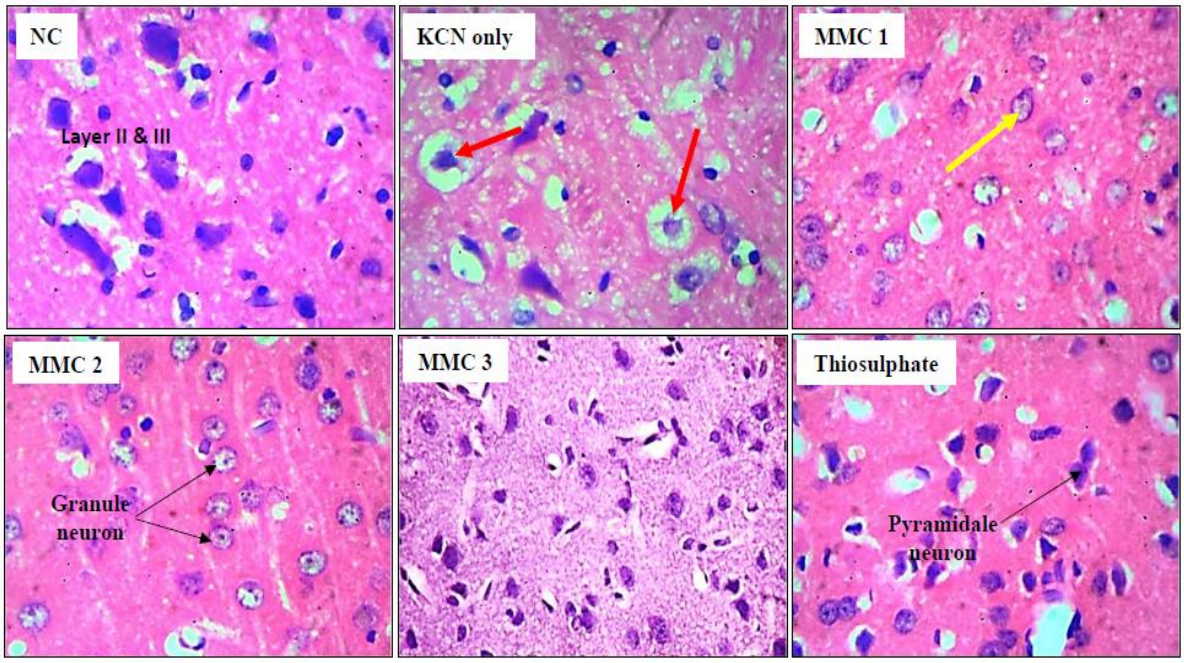

Figure 4: Photomicrographs showing magnified views of brain cortex of rats across the various study groups 1-6 using hematoxylin and eosin stain (X400).

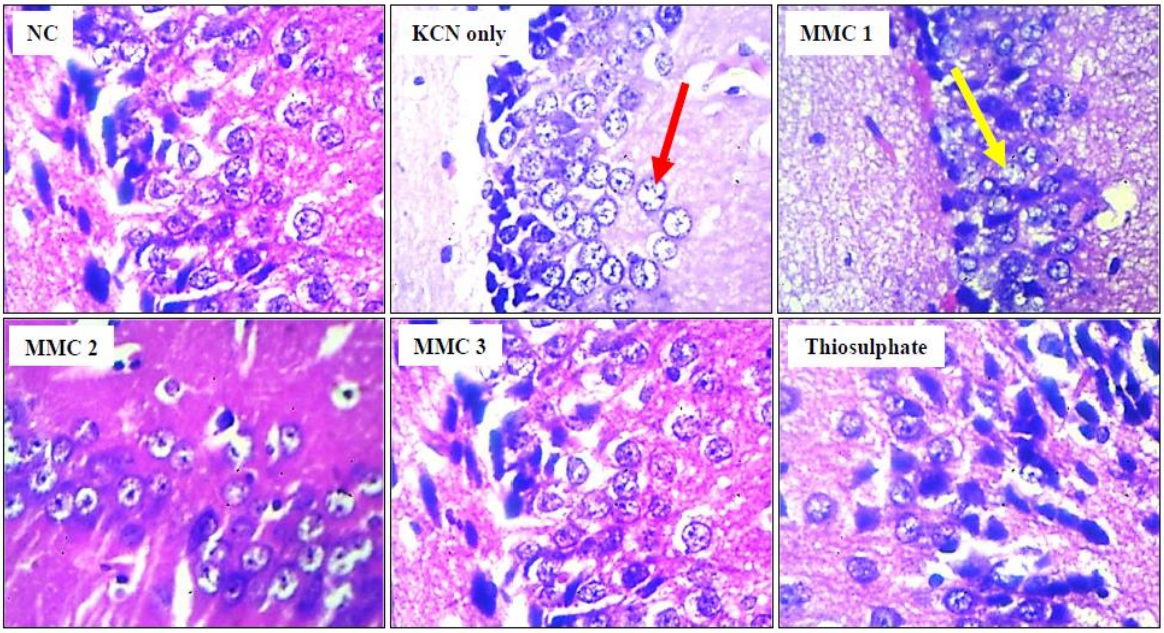

Figure 5: Photomicrographs showing magnified views of hippocampus dentate gyrus morphological presentations in rats across the various study groups 1-6. Hematoxylin and Eosin stain (X400).

This group also showed exacerbation of neuro-inflammation with significant increase in cell bulkiness of hippocampal granular layer. There were mild neurodegenerative lesions in cortex of MMC1 treated animals with mild neuronal cell congestion (yellow), fragmented pyramidal and granule cell layer with mild loss of cellular processes, nuclear and cytoplasmic content. Similar to the KCN-only treated group, there was also mild degenerative alterations in the hippocampus which was characterised by comparative increased cell bulkiness in the granular layer. Administration of MMC2, MMC3 and thiosulphate conferred neuroprotection of rat brain cortex and hippocampus against KCN induce neuronal damage. These groups did not show any significant observable alteration in the architecture of the brain cortex and the hippocampus relative to the control. The cerebral cortex in these groups revealed normal layer II and III, the granule and pyramidale neuron are visible without lesion and conspicuous at high magnification. Similarly, the cellular density within these groups appear normal across all hippocampal layers. The central nervous system has been reported to be the main target of cyanide intoxication with observed neurodegeneration changes in the brain.[4] From this study, the ameliorative effects of methanol extract of *M. charantia* leaf against KCN-induced brain toxicity could suggest potential neuroprotection of the plant extract.

Conclusion

This study has demonstrated that methanol extract of *M. charantia* leaves possesses the ability to reduce the toxic effects of cyanide in the

brain. The extract might possess a plethora of active compounds (such as polyphenols, flavonoid, terpenes and glycosides) with multifaceted mechanisms for extenuating the inflammatory, oxidative and neurodegenerative effects of cyanide poisoning in the brain. Owing to the significant effects of the extract in brain function, it is possible that the pharmacologically active compounds in *M. charantia* leaf extract have the potential to penetrate the blood–brain barrier to exert their neuroprotective effects. Sequel to the establishment of the potent neuroprotective effects of *M. charantia* leaf in cyanide-intoxicated rats, this study hereby suggests the exploration of its bioactive constituents for the formulation of cyanide antidotes. Further studies on the bioactivity-guided isolation of potent active compounds and possible mechanisms by which these compounds traverse the blood-brain barrier can be explored.

Conflict of interest

The authors declare no conflict of interests.

Authors' Declaration

The authors hereby declare that the work presented in this article are original and that any liability for claims relating to the content of this article will be borne by them.

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References

1. Zuhra K, Szabo C. The two faces of cyanide: an environmental toxin and a potential novel mammalian gasotransmitter. Fed Euro Biochem Soc J 2022; 289: 2481–2515. https://doi.org/10.1111/febs.16135
2. Hariharakrishnan J, Satpute RM, Bhattacharya R. Cyanide-induced changes in the levels of neurotransmitters in discrete brain regions of rats and their response to oral treatment with a-ketoglutarate. Indian J exp bio 2010; 48: 731-736.
3. Husain K, Ansari RA, Ferder L. Pharmacological agents in the prophylaxis/treatment of organo-phosphorous pesticide intoxication. Indian J Exp Biol 2010; 48(7): 642-650.
4. Gitonga F, Biwott K, Gitau GW, Wafula OP, Amwayi P, Isaac AO, Nyariki JN. Coenzyme Q10 Ameliorates potassium cyanide-induced toxicosis in a mouse model. Sci African, 2021; *12*, e00815. https://doi.org/10.1016/j.sciaf.2021.e00815
5. Adamolekun B. Neurological disorders associated with cassava diet: a review of putative etiological mechanisms. Metab Brain Dis. 2011; 26: 79–85. https://doi.org/10.1007/s11011-011-9237-y
6. Zhang D, Lee B, Nutter A, Song P, Dolatabadi N, Parker J, Sanz-Blasco S, Newmeyer T, Ambasudhan R, McKercher SR., Masliah E, Lipton SA. Protection from cyanide-induced brain injury by the Nrf2 transcriptional activator carnosic acid. J Neurochem 2015; 133(6): 898-908. https://doi.org/10.1111/jnc.13074
7. Alqahtani RM, Alyousef MY, Alwatban ZH, Ghandour MK. Long-Term Neuropsychiatric Sequelae in a Survivor of Cyanide Toxicity Patient with Arterialization. Cureus 2020; 12(6): e8430. https://doi.org/10.7759%2Fcureus.8430
8. Borron WS, Baud JF. Antidotes for acute cyanide poisoning. Curr Pharm Biotechnol 2012; 13(10): 1940–1948 https://doi.org/10.2174/138920112802273182
9. Muhammad, A., Muhammad, S.K., Muhammad, A.K., Kamran, A., Zahoorul, H., Jawaria AK, Nisar A. Assessing the efficacy of aqueous garlic extract, sodium nitrite and sodium thiosulfate against prolonged oral cyanide exposure in Rabbits, Pak J Pharm Sci 2018; 31(2): 411–419
10. Kadiri HE, Okoro IO, Ichipi-Ifukor PC. *Tetrapleura Tetraptera* Fruit Protects Against Cyanide Induced Toxicity in Rats. Iraqi J Sci 2020; 61(10): 2504–2514. https://doi.org/10.24996/ijs.2020.61.10.7
11. Bolaji OM, Olabode OO. Modulating effect of aqueous extract of *Telfairia occidentalis* on induced cyanide toxicity in rats. Niger J Physiol Sci. 2011; 26: 185–191
12. De Campos OC, Layole MP, Iheagwam FN, Rotimi SO, Chinedu SN. Effects of aqueous extract of *Momordica charantia* on survival, locomotive behavior, and antioxidant status of *Drosophila melanogaster*. Trop J Nat Prod Res. 2021; 5(1): 178-181. doi.org/10.26538/tjnpr/v5i1.23
13. Fan M, Kim E-K, Choi Y-J, Tang Y, Moon S-H. The Role of *Momordica charantia* in resisting obesity. Inter J Environ Res Public Health 2019; 16(18): 3251–3251. https://doi.org/10.3390/ijerph16183251
14. Ishola I, Akinyede A, Sholarin A. Antidepressant and Anxiolytic Properties of the Methanolic Extract of *Momordica charantia* Linn (Cucurbitaceae) and its Mechanism of Action. Drug Res 2013; 64(07): 368–376. https://doi.org/10.1055/s-0033-1358712
15. National Research Council. Occupational Health and Safety in the Care and Use of Research Animals. Washington, DC: The National Academies Press 1997. https://doi.org/10.17226/4988.
16. Ofuegbe SO, Oyagbemi AA, Omobowale TO, Adedapo AD, Ayodele AE, Yakubu MA, Oguntibeju OO, Adedapo AA. Methanol leaf extract of *Momordica charantia* protects alloxan-induced hepatopathy through modulation of caspase-9 and interleukin-1β signalling pathways in rats. 2020; www.veterinaryworld.org/Vol.13/August-2020/6.pdf
17. Bradford KM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analyt Biochem 1976; 72(1-2): 248-254. https://doi.org/10.1016/0003-2697(76)90527-3
18. Cohen G, Dembiec D, Marcus J. Measurement of catalase activity in tissue extracts. Ann Biochem 1970; 34(1): 30–38
19. Nyman M. Serum hatoglobin; methodological and clinical studies. Scandinavian J Clini Lab Investi. 1959; 11(Supp 39): 1–169.
20. Ellman GL. Tissue sulfhydryl groups. Arch Biochem Biophys 1959; 82: 70–77.
21. Misra HP, Fridovich I. The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. J Biol Chem 1972; 247(10): 3170–3175
22. Buege JA, Aust SD. Microsomal lipid peroxidation. Meth Enzymol 1978; 52: 302–310. https://doi.org/10.1016/S0076-6879(78)52032-6
23. Ellman GL, Courtney KD, Andres V Jr, Feather-Stone RM. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem Pharmaco 1961; 7(2): 88–95.
24. Fasco MJ, Hauer CR, Stack RF, O'Hehir C, Barr JR, Eadon GA. Cyanide Adducts with Human Plasma Proteins: Albumin as a Potential Exposure Surrogate. Chem. Res. Toxicol. 2007; 20: 677–684. https://doi.org/10.1021/tx6003425
25. Sabourin PJ, Kobs CL, Gibbs ST, Hong P, Matthews CM, Patton KM, Sabourin CL, Wakayama EJ, Characterization of a mouse model of oral potassium cyanide intoxication. Int. J. Toxicol. 2016; 35(5): 584–603.
26. Zhang F, Lin L, Xie J. A mini-review of chemical and biological properties of polysaccharides from *Momordica charantia*. Inter J Bio Macromol 2016; 92: 246–253. https://doi.org/10.1016/j.ijbiomac.2016.06.101
27. Maneenin C, Burawat J, Maneenin N, Nualkaew S, Arun S, Sampannang A, Iamsaard S. Antioxidant capacity of

Momordica charantia extract and its protective effect on testicular damage in Valproic acid-induced rats. Int J Morphol 2018; 36(2): 447–453. http://dx.doi.org/10.4067/S0717-95022018000200447
28. Wang L, Clardy A, Hui DF, Gao AW, Wu Y. Antioxidant and antidiabetic properties of Chinese and Indian bitter melons (*Momordica charantia* L.). Food Biosci 2019; 29: 73–80. https://doi.org/10.1016/j.fbio.2019.03.010
29. Akintimehin ES, Karigidi KO, Omogunwa TS, Adetuyi FO. Safety assessment of oral administration of ethanol extract of *Justicia carnea* leaf in healthy wistar rats: hematology, antioxidative and histology studies. Clin. Phytosci. 2021; 7: 2. https://doi.org/10.1186/s40816- 020- 00234-4
30. Tshala-Katumbay DD, Ngombe NN, Okitundu D, David L, Westaway SK, Boivin MJ, Mumba ND, Banea JP. Cyanide and the human brain: perspectives from a model of food (cassava) poisoning. Ann N Y Acad Sci. 2016; 1378(1): 50–57. https://doi.org/10.1111/nyas.13159
31. Blake MG, Krawczyk MC, Baratti CM, Boccia MM. "Neuropharmacology of memory consolidation and reconsolidation: insights on central cholinergic mechanisms." J Physiol Paris. 2014; 108(4–6): 286–291. https://doi.org/10.1016/j.jphysparis.2014.04.005
32. Park HR, Lee H, Park H, Cho WK, Ma JY. "Fermented Sipjeondaebo-tang alleviates memory deficits and loss of hippocampal neurogenesis in Scopolamine-induced amnesia in mice. Sci Rep. 2016; 6: 22405. https://doi.org/10.1038/srep22405
33. Chen F, Jiang L, Yang B. Visual loss caused by acute cyanide poisoning: a case report. Clin Toxicol 2011; 49(2): 121–123. https://doi.org/10.3109/15563650.2011.556643
34. Martins NO, de Brito IM, Araujo SSO, Negri G, Carlini EA, Mendes FR. Antioxidant, anticholinesterase and antifatigue effects of *Trichilia catigua* (catuaba). BMC Complement Alternat Med 2018; 18: 172. https://doi.org/10.1186/s12906-018-2222-9
35. Kayode AAA, Kayode OT, Rotimi DE. Pharmacological actions of phytoconsitutnts on neurodegenration disorders. Trop J Nat Prod Res. 2022; 6(7): 1019-1046. https://10.26538/tjnpr/v6i7.2
36. Valarmathi N, Sree RS, Rajan TS. Neuroprotective effects of *Momordica charantia*: A review from preclinical Studies. Int J Res Pharm Sci 2020; 11(2): 1902–1907
37. Zhu L, Xu YJ, Du F, Qian ZM. Ginkgolides protect primary cortical neurons from potassium cyanide-induced hypoxic injury. Exp Brain Res 2007; 179(4): 665–671. https://doi.org/10.1007/s00221-006-0823-x
38. Ozel AB, Cilingir-Kaya OT, Sener G, Ozbeyli D, Sen A, Sacan O, Yanardag R, Yarat A. Investigation of possible neuroprotective effects of some plant extracts on brain in bile duct ligated rats. J Food Biochem 2021; 45: e13835. https://doi.org/10.1111/jfbc.13835
39. Collins T, Cybulsky MI. Firdausi SR, Nur'aini RAR, Izzah FN, Nabilah SN, Christiana YI, Dwijayanti DR, Rahayu S, Rifa IM, Djati MS. *Elephantopus scaber* ethanol extract suppresses inflammation via regulation of the NF-*K*b pathway expression in pulmonary fibrosis. Trop J Nat Prod Res. 2024; 8(9): 8554-8560 https://doi.org/10.26538/tjnpr/v8i9.44
40. Zhu L, Chen T, Chang X, Zhou R, Luo F, Liu J, Zhang K, Wang Y, Yang Y, Long H, Liu Y, Yan T, Ma C. Salidroside ameliorates arthritis-induced brain cognition deficits by regulating rho/ROCK/ NF-kappaB pathway. Neuropharmaco 2016; 103: 134–142. https://doi.org/10.1016/j.neuropharm.2015.12.007
41. Gong J, Sun F, Li Y, Zhou X, Duan Z, Duan F, Shen J. *Momordica charantia* polysaccharides could protect against cerebral ischemia/reperfusion injury through inhibiting oxidative stress mediated c-Jun N-terminal kinase 3 signaling pathway. Neuropharmacol 2015; 91: 123–134. https://doi.org/10.1016/j.neuropharm.2014.11.020
42. Qaid HR,Aljunaid MA, Kaid N, Ridwan RD, Budi HS, Diyatri I, Kamila NT, Alkadasi BA, Alaghbari SG, AAlqhtani AS. Epigallocatechin-3-gallate modulates NF-*k*B in *Porphyromonas gingivalis*-induced periondotitis. Trop J Nat Prod Res. 2024; 8(8): 8032-8036. https://doi.org/10.26538/tjnpr/v8i8.15
43. Nerurkar PV, Johns LM, Buesa LM, Kipyakwai G, Volper E, Sato R, Shah P, Feher D, Williams PG, Nerurkar VR. *Momordica charantia* (bitter melon) attenuates high-fat diet-associated oxidative stress and neuro-inflammation. J Neuroinflamm 2011; 8(1): 64. https://doi.org/10.1186/1742-2094-8-64
44. Deng Z, Yuan C, Yang J, Peng Y, Wang W, Wang Y, Gao W. Behavioural defects induced by chronic social defeat stress are protected by *Momordica charantia* polysaccharides via attenuation of JNK3/PI3K/AKT neuroinflammatory pathway. Ann Transl Med 2019; 7(1): 6. https://doi.org/10.21037%2Fatm.2018.12.08