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Neuroprotective Effects of Cloves on Albino Rats Induced with Mercury

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Abstract

This study evaluated the neuroprotective potential of clove (*Syzygium aromaticum*, CV) in albino rats exposed to mercury chloride (MC), utilizing a diabetic rat model to investigate both neurological and immunological responses. Clove is known for its potent antioxidant properties, with applications spanning the pharmaceutical, food, cosmetic, and agricultural sectors. Mercury chloride, a recognized neurotoxin, is commonly ingested through carbohydrate-rich foods subjected to high-temperature processing. The experimental design included five groups: Group I (Control: corn flour diet), Group II (MC-treated: 20 mg/kg body weight), Group III (CV-treated: 200 mg/kg BW), Group IV (CV 100 mg/kg BW + MC 20 mg/kg BW), and Group V (CV 200 mg/kg BW + ascorbic acid 20 mg/kg BW), with treatments administered over 21 days. MC exposure induced oxidative stress and DNA damage in brain tissue, evidenced by elevated malondialdehyde (MDA) levels and histopathological degeneration in the hippocampal MA3 region and granular layer (HL). Clove administration significantly mitigated these effects

by increasing glutathione (GSH) and superoxide dismutase (SOD) levels, reducing MDA concentrations, lowering gamma-aminobutyric acid (GABA), and elevating acetylcholinesterase (AChE) activity, thereby restoring biochemical parameters toward normal levels. Histological findings further confirmed the protective effect of clove against MC-induced neuronal damage. These results suggest that clove confers neuroprotection through its antioxidative and neurochemical modulation properties, highlighting its potential therapeutic value in preventing heavy metal-induced neurotoxicity.

Keywords: Clove; Mercury Chloride; Neurotoxicity; Oxidative Stress; Hippocampus; Antioxidant Therapy

INTRODUCTION

Mercury chloride induces oxidative stress, leading to cellular damage in the brain. It disrupts antioxidant defenses by increasing lipid peroxidation and reducing total antioxidant capacity, which contributes to neuronal death through cytotoxicity and apoptosis.

Effects in Animals: Studies in rats show that chronic exposure to low doses of $HgCl_2$ impairs motor coordination and balance. It also causes degeneration of neurons and astrocytes in the motor cortex, highlighting its neurodegenerative potential. [1]

Human Impact: In humans, mercury exposure—especially in its methylated form—can result in cognitive, motor, and neurological dysfunction. Symptoms include tremors, irritability, headaches, hallucinations, and in severe cases, death. Mercury can cross the blood-brain barrier and accumulate in neural tissue, exacerbating its toxic effects (Chamoli & Karn, 2024)

Sources of Exposure: Mercury chloride exposure typically occurs through industrial emissions, contaminated food (especially fish), and high-temperature processing of carbohydrate-rich foods. Environmental contamination has increased due to industrialization, raising concerns about bioaccumulation and biomagnification (Naik & Gamare, 2023)

The neurotoxicity of mercury chloride is well-documented across both animal and human studies. Its ability to induce oxidative stress, disrupt neurotransmitter balance, and cause structural brain damage underscores the need for continued research and public

health interventions. Understanding its mechanisms and sources of exposure is critical for developing protective strategies and therapeutic approaches (Teixera et al., 2018)

Syzygium aromaticum, commonly known as clove, is a medicinal spice widely recognized for its potent antioxidant properties. Its bioactive compounds, particularly eugenol, gallic acid, and flavonoids—play a crucial role in neutralizing free radicals and reducing oxidative stress. These compounds enhance the activity of endogenous antioxidant enzymes such as superoxide dismutase (SOD) and glutathione (GSH), which are vital for cellular defense. In vitro studies have demonstrated clove's high radical scavenging capacity, making it a promising candidate for managing oxidative stress-related disorders (Aziz et al., 2023).

Beyond its antioxidant effects, clove exhibits significant anti-inflammatory activity. Eugenol, the primary active constituent, inhibits the synthesis of pro-inflammatory mediators including tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), and cyclooxygenase-2 (COX-2). This suppression of inflammatory pathways helps reduce tissue swelling, pain, and cellular damage. Animal models have shown that clove extracts can effectively reduce inflammation induced by agents like carrageenan and also stabilize cell membranes through anti-proteinase and anti-hemolytic actions (Bouddine et al., 2022).

Given its dual therapeutic potential, clove has found applications across pharmaceutical, food, and cosmetic industries. Its antioxidant and anti-inflammatory properties support its use in treating chronic conditions such as arthritis, neurodegenerative diseases, and skin disorders. Moreover, clove's natural origin and safety profile make it an attractive alternative to synthetic drugs. Continued research into its mechanisms and bioavailability could further enhance its role in integrative medicine and functional food development (Ozuah, 2023).

Therefore, the present study was conducted to investigate the neurotoxicity and DNA damage induced by MC in rats and the ameliorative effect of CV.

Figure 1: Showing Cloves seeds

MATERIALS AND METHODS

Chemicals: MC was obtained from Sigma-Aldrich (USA) as a white powder (99% purity), while CV was obtained from yola Local Market.

Experimental animals:

Fifty adults male Wistar albino rats (185-210 g) were obtained from the HAUEM Animal farm in Federal housing Bajabure, Gerie Local Government Adamawa State. Nigeria. Rats were kept in stainless steel cages and standard pellet diet and tap water ad libitum were provided. Ethical Committee approved the study protocol.

Experimental design:

Following 2 weeks of experimental acclimation, the rats were grouped into 5 groups (each of 10 rats). Group (I) were administered corn flour as vehicle. Group (II) MC were administered at a dose of 20 mg/kg BW (Rahangadale et al., 2012). Group (III) CV (200 mg/kg BW). Group (IV) CV (100 mg/kg BW) and MC (20 mg/kg BW). Group (V) CV (200 mg/kg BW) and MC (20 mg/kg BW). The doses of CV; 100 and 200 mg/kg BW (El-Hadary and Ramadan Hassanien, 2016).

All treatments were given orally, once daily for 28 days. At the end of experiment, blood samples were collected through direct heart puncture for separation of serum which was kept at -20°C until biochemical analysis of AChE.

Measurement of serum AChE, brain GABA and brain lipid peroxidation and antioxidant status: Serum AChE activity was determined according to Here are the latest references

you can cite for updated methodologies and validation of biochemical assays measuring serum AChE, brain GABA, and oxidative stress markers like GSH, SOD, and MDA:

AChE and Brain Neurotransmitters Rahman, H., & Eswaraiah, M. C. (2012), Oxidative Stress Biomarkers (GSH, SOD, MDA) Martins, M. C., et al., 2022, and Redox Biomarker Overview by XL Biotec. (2025, May 8).

Brain samples for comet assay:

Brains from each rat in each group were immediately dissected out after scarification and put in ice cooled physiological saline 9% then prepared for Comet assay to estimate DNA damage parameters (Tail length, DNA in tail and tail moment) (Olive and Banath, 2006).

Histopathology: The brain of each rat was removed completely, the hippocampus was isolated and fixed in 10% formalin for 72h, washed, dehydrated, embedded in paraffin wax, serially sectioned with a microtome at 3µm thickness and stained with hematoxylin and eosin (H&E) for histopathological investigation.

Statistical analysis:

Analysis was done by SPSS (24) software (SPSS Inc., Chicago, USA). The results were expressed as mean±SE using the analysis of variance test (one way ANOVA) followed by Duncan's multiple range test to determine the differences between the averages.

RESULTS

Effect of MC and/or CV on serum AChE and brain GABA: Table 1; showed, significant increase in brain GABA concentration after MC administration (103.21 ± 1.54^{a}) as compared to control group (70.01 ± 1.77^{d}). While co-administration of CV in both low (94.23 ± 1.58^{b}) and high doses with MC (83.02 ± 1.55^{c}), produced significant decrease in brain GABA concentration when compared to MC treated group (Table 1). Serum AChE activity was significantly decreased in MC treated group (33.88 ± 1.97^{d}) as compared to control group (93.72 ± 1.64^{d}). While coadministration of CV in both low (56.13 ± 2.14^{c}) and high doses with MC (77.11 ± 2.46^{b}), significantly increased serum AChE activity when compared to MC treated group .

Brain Lipid Peroxidation and Antioxidant Status:

MC administration at a dose of 20 mg/kg in rats significantly increased the oxidative stress marker MDA (8.9±0.32[a]) when compared to the control group (4.87±0.45[d]). Furthermore, MC significantly reduced brain antioxidant capacity as indicated by declines in GSH (0.91±0.03[d]) and SOD levels (34.21±1.36[d]) when compared to the control group (4.43±0.25[a]and 71.68±5.23[a]), respectively as reported in Table 1. CV administration restored the antioxidant capacity in the 4th and 5th groups of rat's brains towards the normal capacity.

Comet Assay of Brain Samples:

As shown in Table (2) in MC treated group, tail length, DNA in tail%, and tail moment were significantly increased (14.51±1.04[a]; 8.98±0.92[a] and 0.91±0.06[a]) compared with control (6.47±0.27[c]; 7,16±0.69[b] and 0.58±0.03[b]) and CV treated groups (7.33±0.12[c]; 7.07±0.23[b] and 0.52±0.07[b]), respectively. These elevations were decreased in other treated groups with MC in combination with CV.

Table 1: Effect of MC and/or CV on serum AchE (U/L), brain GABA (µg/g) and brain antioxidant biomarkers [MDA (nmol/g), SOD (U/g) and GSH (mg/g)] in rats (n=10)

Groups	AchE (U/L)	GABA (µg/g)	MDA (nmol/g)	SOD (U/g)	GSH (mg/g)
I	**93.72±1.64[d]**	**70.01±1.77[d]**	4.87±0.45[d]	71.68±5.23[a]	4.43±0.25[a]
II	**33.88±1.97[d]**	**103.21±1.54[a]**	8.9±0.32[a]	34.21±1.36[d]	0.91±0.03[d]
III	95.03±1.05[a]	72.33±1.12[d]	4.07±0.23[d]	75±212.74[a]	4.13±1.16[a]
IV	56.13±2.14c	**94.23±1.58[b]**	7.15±0.22[b]	45.37±1.35[c]	2.34±0.08[c]
V	77.11±2.46[b]	**83.02±1.55[c]**	5.88±0.28[c]	58.22±2.78[b]	3.41±0.15[b]

Values are mean ± SE; Means with different alphabets as superscripts differ significantly (P<0.05); Group (I); control. Group (II); MC treated group. Group (III); CF treated group. Group (IV); MC and CV (100 mg) treated group. Group (V); MC and CV (200 mg) treated group.

Table 2: Comet assay (DNA damage) in control, MC and/or CV treated groups

Groups	Comet (%)	Tail Length (PX)	DNA in Tail (%)	Tail Moment
I	15.84±1.514[b]	6.47±0.27[c]	7,16±0.69[b]	0.58±0.03[b]
II	23.68±1.57[a]	14.51±1.04[a]	8.98±0.92[a]	0.91±0.06[a]
III	14.03±1.05[b]	7.33±0.12[c]	7.07±0.23[b]	0.52±0.07[b]

Groups	Comet (%)	Tail Length (PX)	DNA in Tail (%)	Tail Moment
IV	23.13±2.14^{ab}	9.63±0.58^{b}	8.15±0.22^{ab}	0.83±0.05^{a}
V	17.77±1.46^{ab}	9.52±0.55^{b}	8.11±0.28^{ab}	0.72±0.03^{ab}

Values are mean ± SE; Means with different alphabets as superscripts differ significantly (P<0.05); Group (I); control. Group (II); AA treated group. Group (III); Co treated group. Group (IV); AA and CO (100 mg) treated group. Group (V); AA and CO (200 mg) treated group

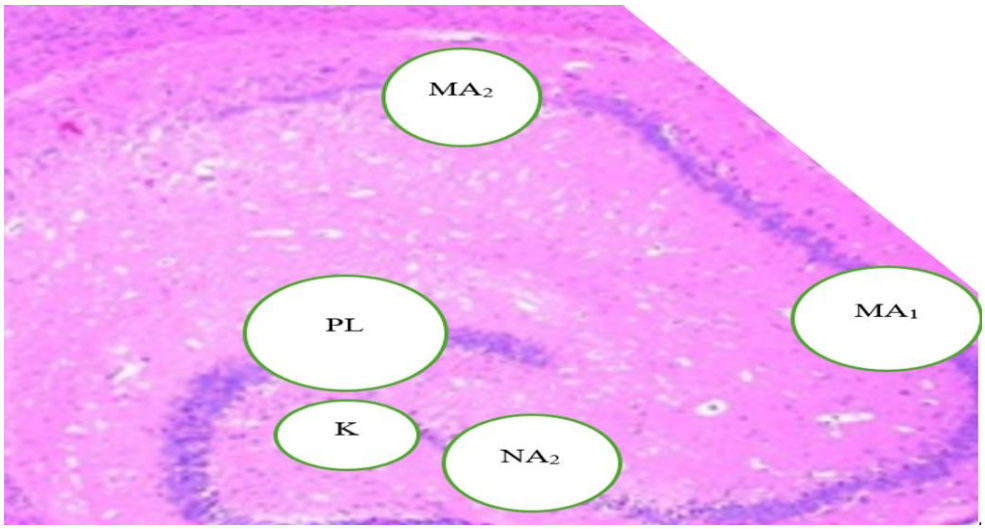

Figure 2: Hippocampus; Rat; Control group, panoramic view: MA1, MA1 pyramidal layer; MA2, MA2 pyramidal layer; MA3, MA3 pyramidal layer; K, hilus; PL, granular layer. HE stains, Lieca EZ4 D stereomicroscope.

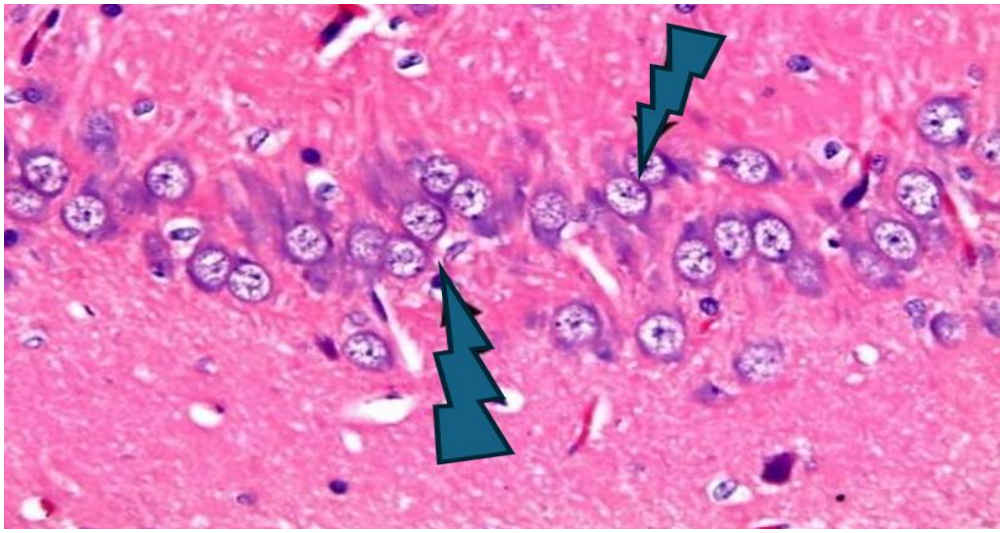

Figure 3: MA_3 region, Hippocampus, Rat. A) CV treated group: Showing normal histological architecture; Pyramidal cells (arrow).

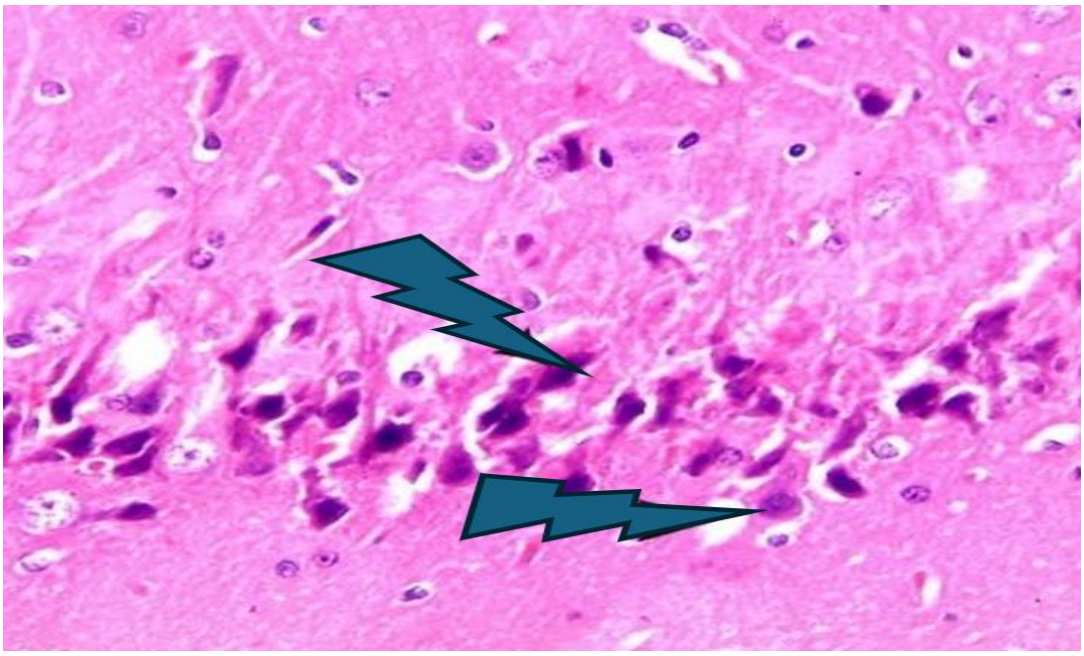

Figure 4: MC and CV (100mg) treated group: showing degeneration/ necrosis of sporadic pyramidal neuron (arrow).

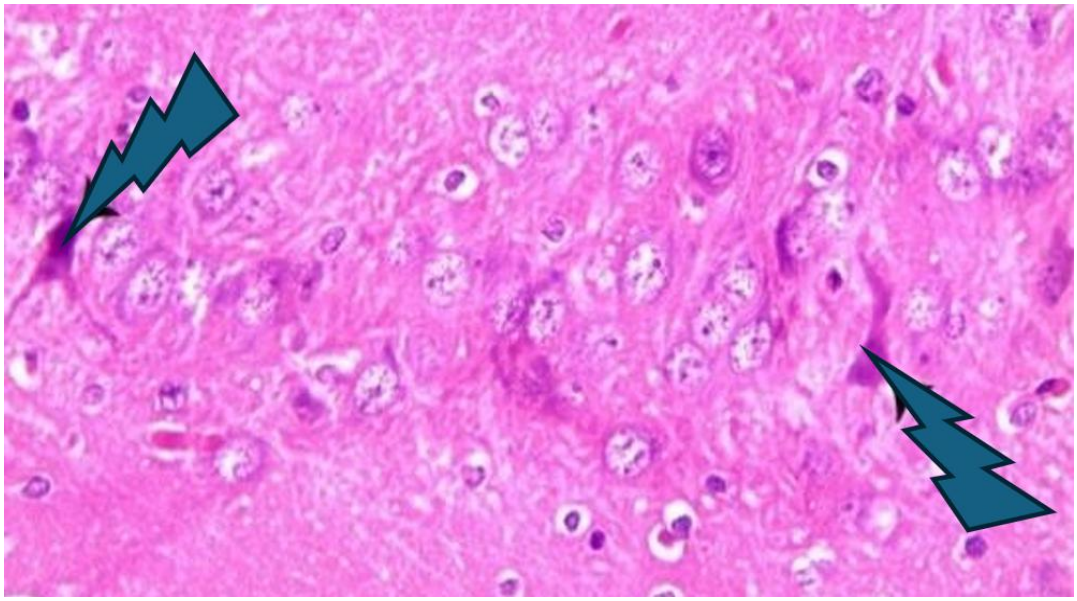

Figure 5: showing MC and CV (200mg) treated group: showing degeneration/ necrosis of sporadic pyramidal neuron (arrows). HE stains, X 20.

DISCUSSION

The brain, due to its high metabolic demand and relatively low antioxidant defenses, is particularly vulnerable to oxidative stress. Its elevated oxygen consumption rate

facilitates the generation of reactive oxygen species (ROS), which can overwhelm endogenous antioxidant systems and lead to cellular damage (Samarghandian et al., 2017). Mercury Chloride (MC), a compound formed during high-temperature cooking of carbohydrate-rich foods, has been identified as a potent neurotoxic agent in both humans and experimental animal models (Uthra et al., 2017; Zhao et al., 2022).

ROS are chemically reactive molecules that can inflict oxidative damage on cellular components, including lipids, proteins, and nucleic acids. This oxidative burden compromises DNA integrity and contributes to neurodegenerative processes. The body's primary antioxidant defense mechanisms—superoxide dismutase (SOD), glutathione (GSH), and malondialdehyde (MDA)—play crucial roles in neutralizing ROS and maintaining cellular homeostasis. Dietary antioxidants further bolster these defenses, enhancing resistance to oxidative insults and preserving genomic stability (Jayakumar & Kanthimathi, 2012).

Studies have consistently shown that Mercury Chloride (MC) exposure leads to significant oxidative damage in the brain, evidenced by increased lipid peroxidation and elevated MDA levels, a biomarker of oxidative injury (Badgujar et al., 2015). SOD and GSH serve as the first line of defense against such oxidative breakdown, mitigating the harmful effects of free radicals (Gill & Dumka, 2016).

Clove (Syzygium aromaticum), commonly referred to as CV, is rich in bioactive compounds such as eugenol, which exhibit strong antioxidant and anti-genotoxic properties. These constituents have demonstrated the ability to counteract oxidative stress and protect neural tissues from Mercury Chloride (MC)-induced damage (Ogata et al., 2000; Sharma et al., 2011). Clove's phenolic compounds not only scavenge free radicals but also modulate key neurotransmitter systems, including cholinergic and glutamatergic pathways, thereby reducing NMDA receptor-mediated neurotoxicity.

Mercury Chloride (MC) is a food-processing contaminant, exacerbating oxidative stress by depleting GSH and altering antioxidant enzyme activity. MC reacts with GSH to form glycidamide, a genotoxic metabolite known to induce DNA adduct formation, leading to mutations and potential carcinogenesis (Klaunig, 2008; Alturfan et al., 2012). This compound contributes to the accumulation of harmful radicals such as superoxide, nitric oxide, and hydroxyl radicals, further amplifying oxidative damage.

The genotoxic effects of Mercury Chloride (MC) and its metabolites are primarily mediated through oxidative stress mechanisms. Glycidamide has been implicated in mutagenesis and cancer development due to its high affinity for DNA binding and adduct formation (Uthra et al., 2017). Clove's therapeutic potential in this context is noteworthy; its antioxidant-rich profile has shown efficacy in reducing lipid peroxidation and protecting DNA from oxidative insults (Jayakumar & Kanthimathi, 2012).

Experimental evidence from rodent models supports the neuroprotective role of clove, demonstrating significant improvements in oxidative stress markers following treatment. These findings underscore the potential of clove-derived compounds as natural interventions against neurotoxicity induced by food-borne contaminants like MC.

CONCLUSION

Mercury Chloride (MC), a neurotoxic compound formed during high-temperature cooking of carbohydrate-rich foods, poses a significant threat to brain health by inducing oxidative stress and genotoxicity. Its interaction with antioxidant systems, particularly the depletion of glutathione (GSH) and elevation of malondialdehyde (MDA)—leads to cellular damage, DNA mutations, and potential neurodegeneration. The brain's vulnerability to oxidative stress, due to its high oxygen consumption and limited antioxidant defenses, exacerbates this risk.

Clove (Syzygium aromaticum), rich in antioxidant compounds like eugenol, demonstrates strong protective effects against MC-induced oxidative and genotoxic damage. It enhances endogenous antioxidant defenses, scavenges reactive oxygen species (ROS), and modulates neurotransmitter systems to reduce neurotoxicity. Experimental evidence supports clove's efficacy in mitigating oxidative markers and preserving neural integrity, highlighting its potential as a natural therapeutic agent against food-borne neurotoxins like Mercury Chloride. Clove offers promising neuroprotective benefits and may serve as a dietary intervention to counteract the harmful effects of Mercury Chloride exposure.

Conflict of Interest

The authors affirm that there are no conflicts of interest associated with this publication.

Authors' Declaration

The authors confirm that the research presented in this article is entirely original. They accept full responsibility for any claims or issues arising from the content herein.

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