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Effect of Curcumin-Loaded Chitosan Nanoparticles and phenylalanine on Behavioral Performance and Brain Histology in Haloperidol-Treated Male Rats

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Abstract Neurotoxicity from agents like heavy metals, pesticides, chemicals, and drugs disrupts neurotransmission and causes oxidative stress, leading to neuronal degeneration. This study tested the neuroprotective effects of curcumin-loaded chitosan nanoparticles and phenylalanine against haloperidol-induced neurotoxicity in male rats. Fifty healthy rats (3–4 months, 250–300 g) from Al-Qadisiyah University were divided into five groups (n=10). Group 1 was controls, receiving distilled water for 30 days. Groups 2–5 received haloperidol (2 mg/kg i.p.) for 14 days. Group 2 was positive control; Groups 3 and 4 received curcumin-chitosan nanoparticles (100 mg/kg) and phenylalanine (2 mg/kg), respectively; Group 5 received both for 30 days after haloperidol. Behavioral tests (Open Field, Y-Maze, Forced Swimming) were conducted. Brain tissues were examined after fixation. Haloperidol reduced locomotion, exploration, and memory, increasing immobility. Curcumin nanoparticles and phenylalanine improved behaviors; their combination nearly normalized activity. Histology showed neuronal contraction, pyknosis, chromatin fragmentation, vascular congestion, and gliosis, indicating severe neurotoxicity. Both treatments reduced these effects; the combination significantly restored neuronal structure and decreased necrosis and gliosis. In conclusion, combined curcumin-loaded chitosan nanoparticles and phenylalanine provided strong neuroprotection against haloperidol toxicity, enhancing activity and neuronal integrity.

Key words: Curcumin, Phenylalanine, Brain, Chitosan.

Introduction Exposure to a spectrum of neurotoxic substances, including heavy metals (such as lead and mercury), pesticides, and other environmental pollutants, may trigger neuronal damage or necrosis, thereby prompting the development of neurological disorders characterized by motor impairment and cognitive disability (1). The etiological mechanisms that have been recognised as the most important in forming the basis of neurotoxicity and the development of neurodegenerative diseases, such as Alzheimer's disease and Parkinson's disease, include oxidative stress, neuroinflammation, and disproportionate levels of neurotransmitters (2, 3). Existing studies have attempted to outline these processes to develop preventive and therapeutic interventions that can reduce neuronal damage. Plants are considered one of the primary sources of neuroprotective agents, which provide bioactive molecules that help maintain and repair neural activity. Of them,

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flavonoids, which are secondary plant metabolites with strong antioxidant and anti-inflammatory effects, have been shown to protect neurons against oxidative damage and extend cell life. Extracts of the Ginkgo biloba, green tea [Camellia sinensis], and Panax ginseng presented significant neuroprotective activity, which has shown the pharmacological significance of these extracts (4). Curcumin stands out as one of the few plant-based compounds with strong neuroprotective properties in the arena of plant-based compounds. It reduces neurotoxicity by preventing oxidative stress and free-radical damage, which are among the main contributors to neuronal degeneration (3), as well as inhibiting chronic neuroinflammation, a key factor in the pathogenesis of Alzheimer's disease and Parkinson's (5). Curcumin balances cellular processes related to survival, apoptosis, and inflammation, primarily by suppressing the action of nuclear factor-kappa B (NF-κB) and enhancing the transcription of endogenous antioxidant enzymes superoxide dismutase and catalase (6). Besides its antioxidant and anti-inflammatory properties, curcumin also exhibits anti-amyloid properties, binding to amyloid-β plaques and suppressing the aggregation of the protein, which in turn reduces Alzheimer-related neurotoxicity and cognitive impairment (7, 8). Despite its therapeutic promise, curcumin's clinical application is limited by poor bioavailability; however, advanced formulations—such as nanoparticles, liposomal carriers, and phospholipid complexes—are being developed to enhance its absorption and efficacy (9).

In parallel, phenylalanine plays a vital role in maintaining normal central nervous system function as a precursor for the neurotransmitters tyrosine, dopamine, epinephrine, and norepinephrine. Through sequential enzymatic conversion, phenylalanine is converted into dopamine, which is essential for regulating mood, cognition, and motor control. Since dopamine cannot cross the blood–brain barrier, its synthesis from phenylalanine and tyrosine within the brain is critical for sustaining neurotransmitter balance and cognitive performance (10).

The selection of phenylalanine as the subject of this study is predicated upon its essential biochemical function as the direct precursor to the neurotransmitter dopamine. Neurotoxicity induced by haloperidol is primarily attributed to the inhibition of dopaminergic D2 receptors, leading to a functional deficiency of dopamine, oxidative stress, and subsequent motor and cognitive impairments. Curcumin impedes the oxidative and inflammatory pathways that contribute to neurotoxicity, while phenylalanine facilitates dopamine synthesis at the cellular level, thereby directly addressing the neurotransmitter imbalance. Consequently, the combination of curcumin-loaded chitosan nanoparticles and phenylalanine represents a scientifically justified, multi-targeted therapeutic strategy: curcumin mitigates downstream cellular dysfunctions such as oxidative stress, inflammation, and apoptosis, whereas phenylalanine counteracts upstream neurochemical imbalances. It is hypothesized that this synergistic approach will offer superior neuroprotection compared to either agent alone, by simultaneously targeting the molecular insults and neurochemical deficits associated with haloperidol's adverse effects.

Materials and Methods

Ethical approval

The researchers obtained ethical approval from the research Ethical Approval Committee of the College of Life Sciences, University of Al-Qadisiyah.

Preparation of Curcumin Nanoparticles

According to (11), the dose of Curcumin nanoparticle that was used in the present study was 100 mg/kg.B.W per day . So, an amount of 2.5 mg of curcumin nanoparticle, the main active compound extracted from turmeric (Curcuma longa), was dissolved in 10 ml of methanol, and the volume was completed to 90 ml with distilled water.

Preparation of nanoparticles for loading of the materials (ion gelation methods) was carried out weekly in the Postgraduate Laboratory of Physiology, Chemistry and Pharmacy

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Preparation of Chitosan Nanoparticles (CNP)

The concentrations were prepared from a solution of chitosan provided by (Beijing) company according to the modulating method of (12), 2.5 g of chitosan was prepared by dissolving it in 250 ml of distilled water with 2.5 ml of glacial acetic acid and left for 24 hours at room temperature. Then, by continuous movement during stirring by a magnetic-bar in a hotplate stirrer for 30 minutes at 900 rpm, which leads to the formation of semi-colloidal solution. The pH was adjusting at 4.6 by a pH meter by adding NaOH (0.1N), and exposure to sonication with a probe sonicator for 3 minutes, after which the solution was filtered with filter paper (400-800).

Preparation of Tripolyphosphate (TPP) Solution

The TPP solution (Supplied by Daejung Chemicals and Metals Company) was prepared according to the method of (13) by adding 250 mg of sodium tripolyphosphate powder to 100 ml of deionized distilled water to obtain a ratio of 0.25% W/V.

Loading of Curcumin on Chitosan Nanoparticles (SCNPs)

Chitosan nanoparticles were placed on a magnetic stirrer and heated at a moderate temperature. Then, curcumin nanoparticle and chitosan solutions were mixed together using a dropwise addition technique, ensuring uniform distribution and proper encapsulation. A dose volume of 1 ml/100 g B.W. of the prepared solution was introduced orally (by the Gavage metallic tube) via stomach once daily for 30 days (12).

Characterization of Nanoparticles

1. Particle Size Analysis

The particle sizes of unloaded and curcumin-loaded chitosan nanoparticles were measured using laser diffraction (0.5–50,000 nm) over 90 seconds. Data, including average particle size and distribution curves, were recorded electronically (14). Measurements were conducted at the Nanotechnology and Advanced Materials Research Center, University of Technology.

2. Zeta Potential Analysis

Zeta potential analysis was performed to determine the surface charge of CNPs and G-CNPs, which is essential for assessing their stability and suitability for biological applications. Measurements were obtained using a zeta potential analyzer with a detection range of +150 to −150 mV (15) at the Center for Nanotechnology and Advanced Materials Research, University of Technology.

3. FTIR (Fourier Transform Infrared Spectroscopy) analysis

FTIR spectroscopy was used to identify functional groups and confirm the formation of new chemical bonds in the prepared materials and the nanoparticle-loaded compounds. After centrifugation at 10,000 rpm for 15 minutes, residues were washed three times with distilled water, dried at 40 °C, and analyzed using a TENSOR 27 FTIR spectrophotometer. Samples were mixed with KBr and compressed into pellets. Spectra were recorded in the 400–4000 cm^{-1} range to detect characteristic peaks corresponding to specific functional groups (16, 15). The test was conducted at the Center for Nanotechnology and Advanced Materials Research / University of Technology.

Experimental animals

Fifty healthy male rats, aged 3–4 months and weighing 250–300 grams, were used. They were obtained from Al-Qadisiyah University's animal facility. After arrival, the rats were housed in specialized cages under optimal conditions for a month of acclimatization, fed a standard pellet diet and tap water ad libitum. Housing conditions included a temperature of 20–25 °C and a 12-hour light/dark cycle in an air-conditioned room. The bedding, mulch, was replaced twice weekly for hygiene and comfort. The rats were divided into five groups (n=10),

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three treatment groups and one negative control group and one positive control group that were allocated to following dosing regimen according to the following:

- G1 group was given 1ml of distilled water daily for 30 days consecutive considers as negative control group.
- G2, G3, G4, and G5 groups were given Haloperidol at dose of (2 mg/ kg B.W i.p) dissolved in 1 ml of distilled water for 14 days neurotoxicity induction (17)
- G2 group was considered as positive control group.
- G3 group was given 1ml of curcumin- loaded chitosan nanoparticles at dose of 100 mg/kg B.W for 30 days (11).
- G4 was given Phenylalanine at a dose of 2 mg/kg B.W dissolved in 1 ml of distilled water for 30 days (18).
- G5 was given curcumin-loaded chitosan nanoparticles and Phenylalanine for 30 days..

Histopathological studies

The animal was anesthetized by intraperitoneal injection of ketamine and xylazine (9 and 10 mg/kg/B.W, respectively), then scarified, and its brain was subsequently extracted. The brain was kept in plastic containers with formaldehyde solution 10% for routine histological examination. The Histo-Line ATP1000 automatically dehydrates and clears the tissue. Dehydrated tissues were embedded in paraffin using the HESTION TEC2900 system, controlled by a TEC2900 Thermal Console. Tissue blocks were sectioned at 4-5 µm with a Histo-Line MRS3500 microtome, floated in a water bath at 37°C, and mounted on slides. Sections were stained with Hematoxylin and Eosin (H&E) and examined under a light microscope at 40× and 10× magnifications (20, 21).

Data Analysis

Experiment data were analyzed using one-way analysis of variance (ANOVA), and significant differences were tested using the L.S.D. test at a 0.05 probability level using the SPSS program Ver. 26. (22)

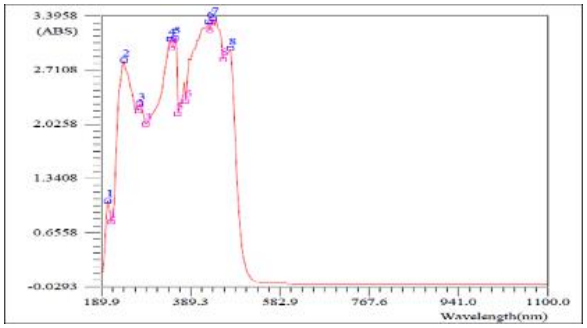
Result

Characterization of Nano-particles

UV-visible spectroscopy

As shown in Figure 1, the UV-Visible Spectroscopic analysis of the curcumin-loaded chitosan nanomaterial sample, scanned from 190 nm to 1100.0 nm, demonstrates strong characteristic absorption in the ultraviolet and visible regions. The spectrum is dominated by several distinct peaks, with the highest absorbance (best absorption) recorded for the peak at 438.4 nm. Other significant peaks indicative of strong light absorption are observed around 204.2 nm, 275.9, 354.13 nm, and 354.1nm.

Figure 1 .UV- Visible Spectrophotometer of Curcumin -Chitosan Nanoparticles



X-Ray Diffraction (XRD) analysis

As shown in Figure 2, the curcumin-loaded chitosan nanoparticles' X-ray diffraction (XRD) pattern shows a broad, low-intensity peak (amorphous halo) that is centered roughly between 18° and 22° (in 2). This is typical of the chitosan polymer matrix and indicates that the prepared material is primarily amorphous or nanocrystalline with very small crystallite size.

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Curcumin has been successfully encapsulated within the chitosan matrix, as evidenced by the absence of the sharp, intense peaks that are usually observed for highly crystalline pure curcumin. This likely leads to curcumin's conversion to an amorphous or disordered-

crystalline phase, which greatly increases its solubility and is consistent with the nanoparticles' morphology.

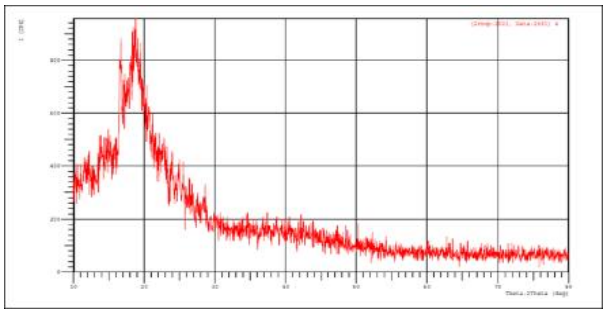

Figure 2. Dynamic Light Scattering (DLS)

As shown in the figure, curcumin-chitosan nanoparticles were subjected to a Dynamic Light Scattering (DLS) examination at 25° C and a scattering angle of 15°. The results showed a combined effective diameter of 256.2 nm and a high polydispersity Index (PDI) of 0.502, indicating a wide range of particle sizes. The distribution graph also sheds light on the presence of more than one population of particles, with the primary distribution centered on the larger size ranges and the subsidiary one on the nanometer scale. The analysis using the lognormal distribution model yielded a mean diameter of 313.9nm, while the multimodal analysis indicated a mean diameter of 477.5nm. A high polydispersity index indicates strong heterogeneity or aggregation in the sample.

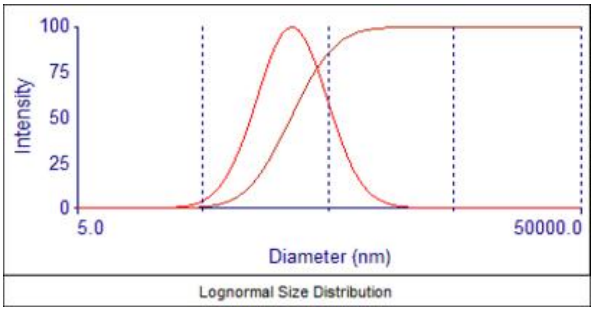

Figure 3 .Dynamic Light Scattering – DLSZeta potential

As shown in Figure 4, the zeta-potential analysis of curcumin-loaded chitosan nanoparticles was performed under aqueous medium and under pH values of 6.30 and temperature of 24.0 °C, in which the zeta potential of the nanoparticles was 160.99 + mV. This significantly high value indicates good colloidal stability of the nanoparticles. It is in agreement with the positive charge contributed by the protonated amine group of the chitosan polymer (NH3+) at the studied pH, indicating strong electrostatic repulsion between particles, which is the primary factor preventing their aggregation. The average electrophoretic mobility was 12.35 (m s-1)/(V cm-1).

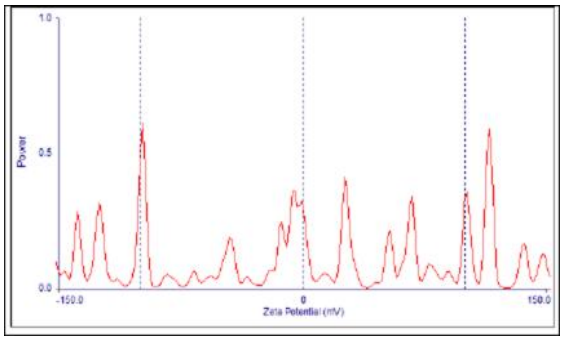

Figure 4. Zeta Potential

Open Field Test

The difference in behaviour between the experimental groups became apparent after the open-field test. In G2, which was treated with haloperidol, locomotor and exploratory activity were significantly reduced as demonstrated by the number of crossings (15.2-0.66) and rearings (3.1-0.19). On the other hand, Group G3 was treated with a significant degree of recovery in the activity (24.5 ± 0.89 crossings; 6.3 ± 0.28 rearings). Figure 4 shows that the performance of Group G4 was also better (20.1 0.76 crossings; 5.2 0.22 rearings). Surprisingly, in Group 5, administered concomitantly with curcumin-chitosan nanoparticles and phenylalanine, behavioural metrics were almost normal (28.7⁻ - 0.95 crossings; 7.1- -0.32 rearings), similar in distribution to control values, and significantly better than those with individual treatments.

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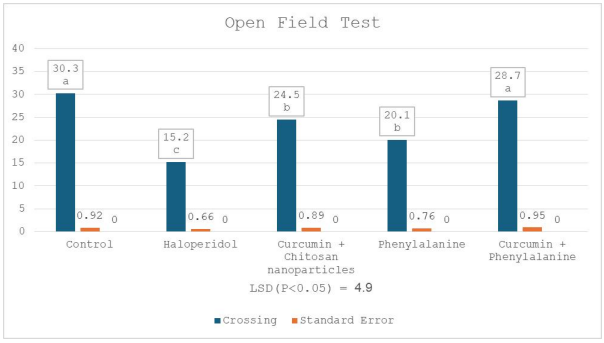


Figure 5. Open Field Test Crossing

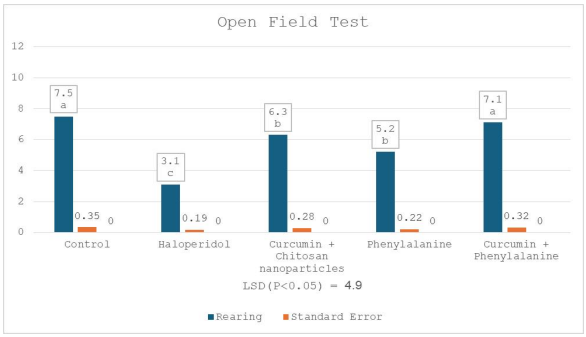


Figure 6. Open Field Test Rearing

Forced Swimming Test (FST)

The Forced Swimming Test (FST) yielded results showing that the experimental groups differed significantly in terms of immobility time (IM), indicating that the administered treatments clearly modulated the morphine- or saline-induced behavioral despair-like reaction.

The total immobility time (180 ± 3.8s) in rats receiving haloperidol (G2) increased significantly compared with the control group (G1; 100 ± 2.5s). On the other hand, rats subjected to curcumin-loaded chitosan nanoparticles (G3) exhibited a significant decrease in immobility time (130 ± 3.2s) compared to the haloperidol group, as shown in Figure 7.

Likewise, the effect of phenylalanine (G4) resulted in a strong reduction in the immobility period (145 ± 3.5 s), whereas the impact on the haloperidol-treated rats was moderate. Surprisingly, the co-encouragement of curcumin-loaded chitosan nanoparticles with phenylalanine (G5) resulted in a significant reduction in immobility time (110.2s), which was similar to that of the control group

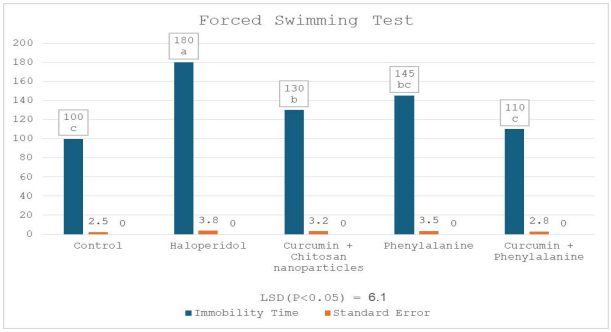
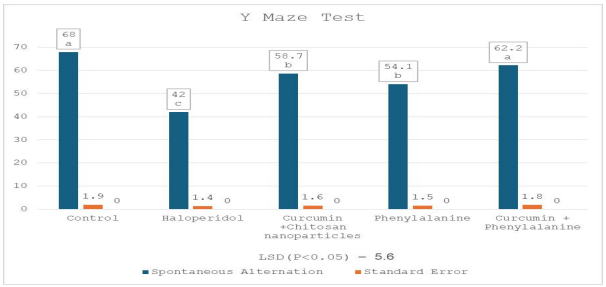


Figure 7. Forced Swimming Test

Y-Maze Test

The outcome of the Y-maze spontaneous alternation test in Figure 8 exhibited a high degree of variation across experimental groups, reflecting differences in changes in spatial working memory and cognitive flexibility. A clear example was observed in rats treated with haloperidol (G2), which exhibited a severe decrease in spontaneous alternation, with a mean of 42.1% compared to the control group (G1: 68.1%, 1.9%), indicating a severe impairment of spatial memory and executive functioning. Rats fed on curcumin-loaded chitosan nanoparticles (G3), on the other hand, showed an intermediate



outcome (58.7 ± 1.6 percent), which is indicative

Figure 8. Y-Maze Test

of partial restoration of cognition. Similarly, treatment with phenylalanine (G4) showed a

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significant increase in free alternation (54.1±1.5) compared to haloperidol. Interestingly, the simultaneous use of curcumin-loaded chitosan nanoparticles and phenylalanine (G5) resulted in a significant enhancement (65.2⁻ ± 1.8), comparable to the control values and even higher than those of each agent.

Histopathological Findings

The findings revealed a clear gradient among the five experimental groups. In Figure 9, the control group (G1) showed almost no abnormalities, with well-organized neurons, no cellular necrosis, no vascular congestion, or gliosis. In contrast, the haloperidol-only group (G2) exhibited significant neuropathology, including many pyknotic or shrunken neurons with fragmented chromatin. Severe vascular congestion, widespread reactive gliohistone degeneration, and spongiform neurodegeneration of the neuropil were also observed in Figure 10. These findings indicate extensive neural toxicity likely caused by haloperidol. The damage shown in Figure 11 in G3 was less severe: neurons remained organized, with less shrinkage and gliosis; the changes were moderate compared to G4. Figure 12 shows that the G4 showed partial recovery, with most neurons maintaining normal structure, though minor lesions (necrosis, congestion) persisted alongside moderate gliosis and spongiosis. The combination group (G5) demonstrated the best recovery, with brain histology resembling normal tissue, featuring aligned, intact neurons, minimal congestion or glial scarring, and few signs of degeneration (Figure 13).

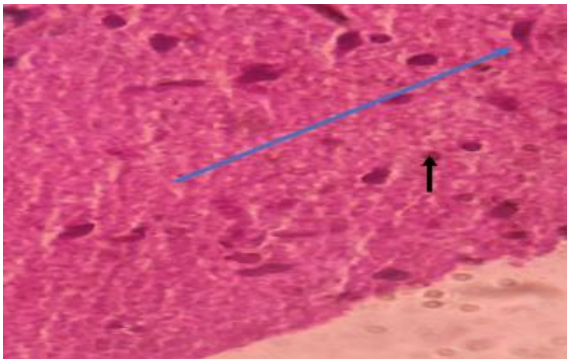

Figure 9. Histopathological image of the rat brain (negative – without Haloperidol) shows no pathological changes, normal neurons (blue arrows), and normal glial cells (black arrows), X400; H&E stain.

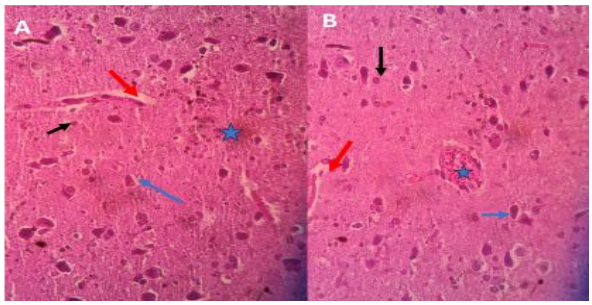

Figure 10. A&B: histopathological images of the brain in rats (Group G2: positive control) show severe pathological changes, including neuron shrinkage (blue arrows), severe microvasculature congestion (blue stars), gliosis (black arrows), and spongiosis (red arrows).

Figure 11. The histopathological image of the brain in rats (G3 treated with curcumin-chitosan nanoparticles) shows fewer pathological changes, less neuronal shrinkage (blue arrows), less gliosis (black arrows), and less spongiosis (red arrows), X400; H&E stains.

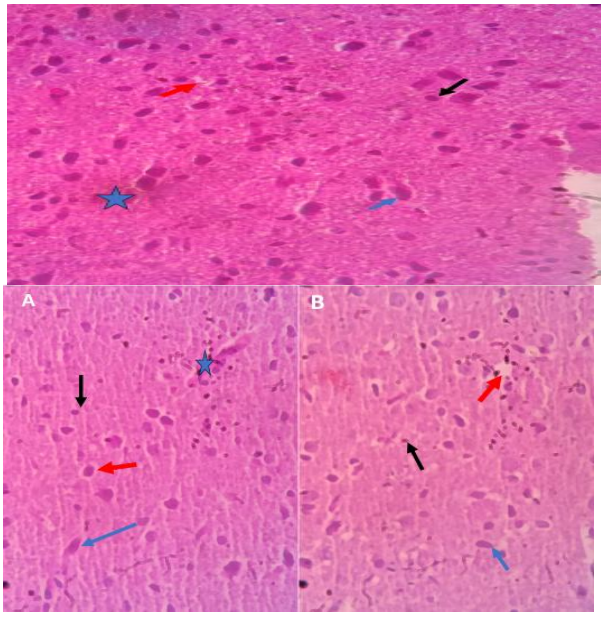

Figure 12. A&B: histopathological image of the brain in rats (G4 treated with Phenylalanine) showed moderate pathological change, less severe shrinkage of neurons (blue arrows), moderate congestion of microvasculature (blue

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stars), gliosis (black arrows), and spongiosis (red arrows). X400; H&E stains.

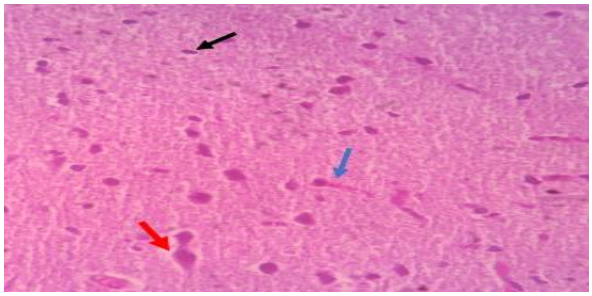

Figure 13. The histopathological image of the brain in rats (G5 treated with Phenylalanine and curcumin-chitosan nanoparticles) showed less pathological change, less shrinkage of neurons (blue arrows), less congestion of microvasculature (blue stars), less gliosis (black arrows), and less spongiosis (red arrows), X400; H&E stains.

Discussion

Neurobehavioral Study

Open field test

Animals treated with haloperidol demonstrated a significant decline in locomotor and exploratory activities. This result aligns with the well-documented extrapyramidal side effects of this antipsychotic agent, such as rigidity and akinesia (23). As a dopamine D_2 receptor antagonist, haloperidol reduces dopaminergic signaling, thereby diminishing the motivation to explore novel environments, which manifests behaviorally as sustained immobility or "freezing" (24).

Conversely, rats subjected to the combination of curcumin and phenylalanine (G5) exhibited significantly better performance in open-field tests compared to the haloperidol-only group and the single-agent group. This increased behavioral recovery, particularly the enhanced spontaneous locomotion and exploration, is indicative of a synergistic interaction between curcumin and phenylalanine that outperforms the additive effects of each substance when used separately. Curcumin is a potent neuroprotective phytochemical that was widely reported to reduce motor dysfunction and anxiety-like behaviours in neurodegenerative models. Its ability to maintain dopaminergic homeostasis and prevent neuroinflammation helps sustain exploratory behavior (25).

Phenylalanine serves as a biochemical precursor to tyrosine, which is then converted to L-DOPA, which is an essential precursor in dopamine production. Under neurotoxic conditions, such as when exposed to haloperidol or rotenone, phenylalanine can restore striatal dopamine depletion and counteract motor deficits (23). Therefore, the simultaneous challenge of curcumin and phenylalanine is assumed to have complementary activity in that curcumin provides antioxidative and dopamine-sparing effects, and phenylalanine enhances dopamine production. This synergy of mechanisms can explain the improved locomotor recovery observed in the co-treatment group (G5) compared to single treatments.

Altogether, the above-presented results support the hypothesis that dual therapeutic approaches, i.e., combining neuroprotective antioxidants with agents that enhance dopamine levels, might produce better results in alleviating the effects of haloperidol on motor disturbances. The significantly better behavioural outcomes of the G5 group support the possible use of such combined interventions to recover the motor functions and dopaminergic homeostasis.

Forced swimming test

The current behavioral results indicated that the administration of haloperidol contributed to a significant increase in the duration of immobility during the Forced Swim Test (FST), which showed a strong phenotype of depression in rats. This finding is consistent with the earlier findings published those traditional antipsychotics like haloperidol may enhance depressive and avolitional behaviours in preclinical models (26). Haloperidol has a consistent and significant effect in rodents, causing strong increases in FST immobility, which is contrary to the expected antidepressant agent effects or the development of negative affect and motivational depletion (26). These preclinical findings are similar to clinical data

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indicating the possibility of haloperidol worsening mood disorders and inducing dysphoria in patients, which implies that the use of haloperidol monotherapy facilitates the behavioural despair and not the alleviation of it.

Conversely, the co-treated rats with curcumin and phenylalanine (Group 5) had a significantly shorter immobility time than the curcumin-alone and single curcumin groups, and exhibited a higher antidepressant-like effect. The increased potency of the synergistic regimen suggests a synergistic interaction between curcumin and phenylalanine. The multifaceted neuroprotective effects are known to confer antidepressant-like effects of curcumin on FST. It has been found to reduce immobility and increase active coping in animals that are depressed, which has been supported by biological findings indicating that it increases neurotrophic factors and alters monoaminergic systems (27). In particular, curcumin increases brain-derived neurotrophic factor (BDNF) and serotonin levels in stressed rats, resulting in a significant decrease in immobility times, similar to the effect of typical antidepressants (28).

Phenylalanine, a biosynthetic product of tyrosine and consequently catecholamine, could elevate the levels of neurotransmitters during a state of deficiency. Phenylalanine supplementation has been reported to restore normal levels of dopamine and enhance behavioral performance in neurotoxic and stress-induced paradigms. It is important to note that antidepressant-like effects are possible through an increase in the supply of precursors (29). D-Phenylalanine, a raised trace amine (e.g., phenylethylamine), has also been proposed to decrease FST immobility and increase active coping behaviours in rodent subjects. Its ability to reduce immobility was equal to tricyclic antidepressants (imipramine) and selective serotonin reuptake inhibitors (fluoxetine) (29). What is found here is the therapeutic potential of precursor supplementation in reversing depressive-like behaviours, by restoring catecholamine synthesis, and reversing monoamine depletion linked to chronic stress or dopamine receptor blockage. Among the effects of haloperidol on behavioural despair is the observation of phenylalanine to reinstate dopaminergic and noradrenergic transmission to relieve the immobility and helplessness associated with D2 receptor antagonism.

Y maze test

The performance of the haloperidol administration created a significant effect of deficiency in the spatial working memory illustrated by a considerably lower spontaneous alternation score in the Y-maze test. This observation aligns with the well-documented cognitive side-effect profile of haloperidol (30). The deficits support the previous studies suggesting that chronic blocking of D2 receptors by haloperidol inhibits exploratory alternation behaviour and impairs spatial memory in rodent models (30). These cognitive disturbances are attributed to the dysregulation of dopaminergic systems in the hippocampal and frontal circuits, which can be explained by the dominant role of dopamine in executive functioning and memory processing (31). On the other hand, the group of cohorts that received a combination of curcumin and phenylalanine (G5) showed significantly improved performance compared to the haloperidol-only group (G2) and the single-compound treatment groups (G3 and G4). The interactional enhancement of spontaneous alternation is an indication of enhanced maintenance and recovery of cognitive ability whenever curcumin and phenylalanine are used concurrently. Curcumin is known for its neuroprotective and pro-cognitive properties through multiple mechanisms, including stimulation of neurogenesis and synaptic plasticity (e.g., increased BDNF levels), as well as attenuation of neuroinflammation and oxidative stress in brain regions critical for memory (32). In models of neurodegeneration and chemically induced memory deficits, curcumin has been shown to improve memory performance and reduce neuronal damage via upregulation of the Nrf2 antioxidant pathway and suppression of hippocampal pro-inflammatory mediators (32).

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ISSN P: 1818-5746
E: 2313-4429
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Phenylalanine, on the other hand, provides biosynthetic support for neurotransmitter recovery. As a precursor to L-tyrosine and L-DOPA, it contributes to the replenishment of brain dopamine stores depleted by haloperidol-induced stress. Although tyrosine and phenylalanine have limited permeability across the blood-brain barrier, their role as precursors to DOPA facilitates increased dopamine synthesis. The dopaminergic signal is crucial for optimal performance in Y-maze alternation tasks and other cognitive trials (31).

Histopathological results

Histopathology of cerebral tissue revealed significant differences in neuronal damage and recovery among the five experimental groups. These degenerative alterations indicate the oxidative and inflammatory neurotoxicity typically earlier linked to chronic oxidative and inflammatory exposure to haloperidol, which is due mainly to dopamine D2 receptor blockage and resultant redox imbalance (33). These adverse changes were significantly reduced by curcumin treatment (G3). There was increased neuronal organization in brain sections from G3, with decreased cell shrinkage and gliosis relative to G2, suggesting some restoration of tissue integrity. Mechanistically, these effects can be attributed to curcumin's well-documented antioxidant and anti-inflammatory properties, which are mediated through activation of the Nrf2 pathway, free-radical scavenging, and suppression of pro-inflammatory cytokines (31). Thus, the relatively preserved neuronal morphology and limited gliosis in G3 substantiate the hypothesis that curcumin mitigates haloperidol-induced oxidative injury by re-establishing redox homeostasis and suppressing microglial activation, as corroborated by previous neurodegeneration models. Administration of curcumin chitosan nanoparticles (CNPs) produced visible restoration of neuronal architecture, reduced apoptosis, and lowered pro-inflammatory TNF-α levels in brain tissue (34).

These improvements are attributed to enhanced brain delivery of curcumin via chitosan-based carriers, which reduce oxidative stress, inflammation, and mitochondrial dysfunction, collectively enabling better preservation of histological integrity.

In the phenylalanine-treated group (G4), histological findings revealed moderate amelioration, with most neurons retaining near-normal morphology and only mild residual gliosis and congestion. Overall tissue integrity was superior to that of G2. The noted partial neuroprotective effect appears to be at least partially attributable to the impact of the biochemical precursor of the catecholaminergic neurotransmitters, particularly dopamine, namely phenylalanine. The haloperidol exposure results in a severe impairment of dopaminergic synthesis and turnover (31); however, restoring dopamine levels with phenylalanine could reduce excitotoxic stress and protect neuronal function, thereby preventing morphological damage despite its poor antioxidant abilities. The results support the hypothesis that restoring neurotransmitter levels, in the context of ineffective redox correction, may induce a partial counter-response to a dopaminergic blockage-induced neurodegenerative cascade. The most significant level of neuroprotection was in the combination therapy cohort (G5). Histologic examination revealed nearly standard tissue architecture, with well-aligned, intact, and minimally congested neurons, and showed no signs of gliosis or degeneration. The higher result in G5 suggests the presence of a synergistic effect between curcumin and phenylalanine, in which the antioxidative and anti-inflammatory effects of curcumin enhance the ability of phenylalanine to replenish the number of neurotransmitters. Such a combined effect likely stabilized redox balance and dopaminergic signaling, facilitating structural and functional recovery. The effectiveness of such combination therapies, which modulate oxidative stress through Nrf2 activation and metabolic support, has been further supported by recent evidence demonstrating their efficacy in treating haloperidol-induced neurodegeneration (32). The general upward trends in G2 to G5 in the dose-response indicate the existence of a biochemical normalization and histological recovery dose-response relationship, confirming

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ISSN P: 1818-5746
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qjvms@qu.edu.iq

the critical role of oxidative stress and neurotransmitter dysregulation in haloperidol-induced neurotoxicity. The comodulation of curcumin-chitosan nanoparticles with phenylalanine effectively re-establishes neuronal structure and reduces gliosis, thereby achieving the therapeutic benefits of multimodal interventions that simultaneously target oxidative and inflammatory mechanisms and neurotransmitter-related pathways.

Conclusion

Haloperidol induced neurotoxicity in male rats, manifested by behavioral impairments, oxidative stress, and histopathological brain alterations. Curcumin significantly attenuated these effects due to its antioxidant and anti-inflammatory properties. Phenylalanine showed limited neuroprotection; however, its combination with curcumin produced a synergistic effect. Notably, curcumin nanoparticles, particularly when combined with phenylalanine, demonstrated superior neuroprotective efficacy and helped restore normal brain function.

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CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest in the current study.

AUTHOR CONTRIBUTION

The authors made equal contributions.

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