

EFFECTS OF CURCUMA ZEDOARIA RHIZOME GUMMY CANDY ON SPATIAL MEMORY AND HIPPOCAMPAL CA1 HISTOPATHOLOGY IN ALCOHOL-INDUCED MALE *Rattus norvegicus*

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ABSTRACT

Background: Memory impairment is a critical issue affecting cognitive function and quality of life, often associated with oxidative stress and neuronal damage in the hippocampal CA1 region. *Curcuma zedoaria* (white turmeric) contains curcuminoids and flavonoids with antioxidant and neuroprotective properties, yet its potential in an innovative gummy candy dosage form remains underexplored. This study aimed to evaluate the neuroprotective effect of gummy candy containing *Curcuma zedoaria* rhizome fraction on spatial memory and hippocampal CA1 histopathology in an alcohol-induced rat model.

Methods: This experimental study used a randomized post-test-only control group design involving 25 male *Rattus norvegicus* divided into five groups. Animals received gummy candy containing *Curcuma zedoaria* rhizome fraction at doses of 150, 200, and 250 mg, alongside negative (0.5% CMC-Na) and positive (Ginkgo biloba) controls, for 7 days, followed by induction with 70% ethanol (0.5 mL/rat/day) for 7 days. On day 15, histopathological analysis of the hippocampal CA1 region was performed using hematoxylin–eosin staining, with neuronal necrosis quantified across three microscopic fields per sample and analyzed using one-way ANOVA followed by Tukey's post hoc test.

Results: Treatment groups showed reduced neuronal necrosis and improved cellular architecture compared with the negative control. The 250 mg dose demonstrated the strongest neuroprotective effect, with the lowest mean neuronal damage (4.33) compared to the negative control (12.26), a result comparable to the positive control (8.59) ($p < 0.05$).

Conclusion: *Curcuma zedoaria* gummy candy exhibits dose-dependent neuroprotective effects against alcohol-induced hippocampal CA1 damage, supporting its potential as an innovative functional neuroprotective agent.

Keywords : *Curcuma zedoaria*, gummy candy, hippocampus, neuroprotection, histopathology

INTRODUCTION

Memory is a fundamental cognitive function that enables individuals to process, store, and retrieve information necessary for daily functioning, identity formation, and adaptive behavior. Impairment of memory is a hallmark of various neurodegenerative and neuropsychiatric conditions and can significantly reduce quality of life. Although memory decline is commonly associated with aging, increasing evidence indicates that cognitive impairment may also occur in younger populations due to environmental, metabolic, and lifestyle-related factors^{1,2}.

One of the principal mechanisms underlying memory impairment is oxidative stress, which disrupts neuronal homeostasis and contributes to structural and functional damage in the brain, particularly in the hippocampus^{3,4}. The hippocampal

CA1 region is especially vulnerable to oxidative damage due to its high metabolic activity and sensitivity to reactive oxygen species (ROS), which are associated with neuronal degeneration and impaired cognitive performance^{4,5}. Furthermore, oxidative stress has been recognized as a central factor linking neuroinflammation, mitochondrial dysfunction, and progressive neuronal loss in neurodegenerative disorders^{3,6}.

Natural products with antioxidant and anti-inflammatory properties have attracted increasing attention as potential neuroprotective agents^{7,8}. Among these, *Curcuma zedoaria* (white turmeric) contains bioactive compounds such as curcuminoids and flavonoids that have been reported to modulate oxidative stress pathways and improve cognitive function⁹. Studies on curcumin, a major bioactive compound of turmeric, have demonstrated its ability to enhance antioxidant enzyme activity,

attenuate neuronal degeneration, and protect hippocampal regions, including CA1, from oxidative damage^{7,10}. Moreover, recent findings suggest that curcumin may improve spatial memory and reduce neuroinflammation through modulation of glial cell activity and maintenance of blood-brain barrier integrity^{10,11}.

Despite these promising findings, several critical gaps remain. Most studies have focused on curcumin as an isolated compound, while limited research has explored the neuroprotective potential of *Curcuma zedoaria* in innovative pharmaceutical dosage forms that may enhance bioavailability and patient compliance^{11,12}. In addition, the application of functional food-based delivery systems, such as gummy candy formulations, in improving cognitive function and protecting hippocampal structure remains underexplored, particularly with respect to histopathological changes in the CA1 region under induced conditions¹³.

Therefore, this study aims to evaluate the neuroprotective effects of gummy candy containing the rhizome fraction of *Curcuma zedoaria* on spatial memory performance and histopathological changes in the hippocampal CA1 region in an induced animal model. This study offers several novelties, including the use of *Curcuma zedoaria* rhizome fraction in a gummy candy dosage form as an innovative functional neuroprotective approach, the specific evaluation of histopathological alterations in the hippocampal CA1 region, and the integration of behavioral (spatial memory) and structural (histopathology) assessments in an induced cognitive impairment model.

MATERIALS AND METHODS

This study employed an experimental design using a randomized post-test-only control group approach to evaluate the preventive neuroprotective effects of gummy candy containing the rhizome fraction of *Curcuma zedoaria* against alcohol-induced cognitive impairment. A total of 25 male *Rattus norvegicus* (aged

Table 1. Gummy Candy Formulation

Ingredient	F1	F2	F3	Function
White turmeric (<i>Curcuma zedoaria</i>) fraction	7,5	10	12,5	active ingredient
Gelatin	15	15	15	gelling Agent
Sucrose	50	50	50	sweetener
Citric acid	0,3	0,3	0,3	acidulant
Propylen glycol	10	10	10	solvent
Essence	q.s	q.s	q.s	flavoring agent
Colorant	q.s	q.s	q.s	coloring agent
Aquadest	ad 100	ad 100	ad 100	solvent

The quality of the gummy candy was evaluated through organoleptic assessment (color, shape, aroma, taste, and texture), pH measurement using a calibrated pH meter (1 g sample dispersed in 10 mL distilled water), and moisture content determination using the gravimetric method (oven drying at 100–102°C). The acceptable moisture content was $\leq 20\%$ in accordance with standard guidelines (25,13).

For the treatment protocol, all groups received their respective treatments orally three times daily for 7 consecutive days (Day 1–7). Subsequently, from Day 8 to Day 14, cognitive impairment

2–4 months; body weight 150–200 g) were used and randomly assigned into five groups (n = 5 per group): a negative control group (0.5% sodium carboxymethyl cellulose, CMC-Na), a positive control group (*Ginkgo biloba*), and three treatment groups receiving gummy candy formulations at doses of 150 mg, 200 mg, and 250 mg, respectively.

All animals were housed under standard laboratory conditions (22–25°C, 12 h light/dark cycle) with ad libitum access to food and water and were acclimatized for 3 days prior to the experiment. Body weights were recorded before treatment initiation. All experimental procedures were conducted in accordance with internationally accepted guidelines for the care and use of laboratory animals (26,27).

The rhizomes of *Curcuma zedoaria* were extracted using 70% ethanol by maceration, followed by concentration to obtain a viscous extract. Fractionation was performed by liquid-liquid extraction using n-hexane and ethyl acetate. Briefly, 10 g of extract was dissolved in distilled water and partitioned sequentially with n-hexane and ethyl acetate using a separatory funnel. The collected fractions were concentrated using a rotary evaporator to obtain viscous fractions, and the process was repeated until approximately 40 g of the ethyl acetate fraction was obtained (30).

The gummy candy formulation was prepared based on the method described by Muadifah et al. (31) with slight modifications. Gelatin was dispersed in distilled water and heated at 60–70°C to form a homogeneous gel, followed by the addition of a sucrose solution to produce the gummy base. The *Curcuma zedoaria* ethyl acetate fraction was dissolved in propylene glycol and incorporated into the base at approximately 50°C. Citric acid and colorant were added, followed by flavoring at approximately 30°C. The mixture was poured into molds and allowed to solidify at room temperature, with optional refrigeration for 24 h to enhance stability. The composition of the gummy candy formulations was presented in **Table 1**.

was induced by oral administration of 70% ethanol at a dose of 0.5 mL per rat per day^{14,15}. This design was intended to assess the preventive effect of the formulation against alcohol-induced neurotoxicity. Animals were monitored daily for general health and behavioral changes.

On Day 15, animals were euthanized, and brain tissues were collected for histopathological analysis. The hippocampus was isolated and fixed in 10% neutral buffered formalin for 24 h. Tissue processing was carried out using standard histological techniques, including dehydration in graded ethanol, clearing in

xylene, embedding in paraffin, sectioning at 3–4 μm using a rotary microtome, and staining with hematoxylin and eosin (H&E) (16,29). Histological evaluation focused on the CA1 region of the hippocampus, with assessment of neuronal morphology such as pyknosis, cell shrinkage, and neuronal loss¹⁶. Quantification was performed by counting damaged neurons in multiple microscopic fields under 400 $\times$ magnification, and observations were conducted in a blinded manner to reduce bias.

Statistical analysis was performed using IBM SPSS Statistics 22 with a 95% confidence level. Data were expressed as mean $\pm$ standard deviation (SD). Normality was assessed using the Shapiro–Wilk test, and homogeneity of variance was evaluated using Levene’s test. For normally distributed and homogeneous data, one-way analysis of variance (ANOVA) was applied, followed by Tukey’s post hoc test. For non-normally distributed data, the Kruskal–Wallis test was used, followed by the Mann–

Whitney U test for pairwise comparisons. A p-value < 0.05 was considered statistically significant (28).

RESULTS

Histopathological evaluation of neuronal necrosis in the hippocampal CA1 region of male *Rattus norvegicus* was performed using the hematoxylin–eosin (H&E) staining method. Neuronal necrosis was identified based on characteristic morphological features, including cell shrinkage due to cytoplasmic condensation and nuclear pyknosis, which appeared as intensely dark (hyperchromatic) nuclei under light microscopy¹⁶. The number of necrotic neurons was quantified manually across three independent microscopic fields of view for each sample. The quantitative results of neuronal necrosis in the hippocampal CA1 region were presented in Table 2.

Table 2. Mean of Necrotic Neurons in the Hippocampal CA1 Region

Group	Jumlah Nekrosis Sel Neuron				Mean
	Field 1	Field 2	Field 3	Mean per animal	
Negative Control	12	13	15	13.33	12.26
	13	12	14	13	
	10	13	14	12.33	
	15	12	10	12.33	
	10	12	9	10.33	
Positive Control	9	9	9	9	8.59
	5	5	6	5.33	
	7	9	6	7.33	
	9	15	13	12.33	
	9	7	11	9	
Group 3 (150 mg)	4	9	10	7.66	7.59
	3	9	10	7.33	
	11	9	7	9	
	5	5	11	7	
	11	9	12	12	
Group 4 (200 mg)	4	4	5	4.33	6.91
	7	5	6	6	
	8	9	5	7.33	
	7	5	9	7	
	8	5	9	7.33	
Group 5 (250 mg)	5	4	6	5	4.33
	6	3	4	4.33	
	3	3	5	3.66	
	5	3	3	3.66	
	6	5	4	5.00	

Field: Microscopic field of view

Representative histopathological images of neuronal necrosis in the hippocampal CA1 region

of male *Rattus norvegicus*, stained with hematoxylin eosin (H&E), were shown in Figure 1.

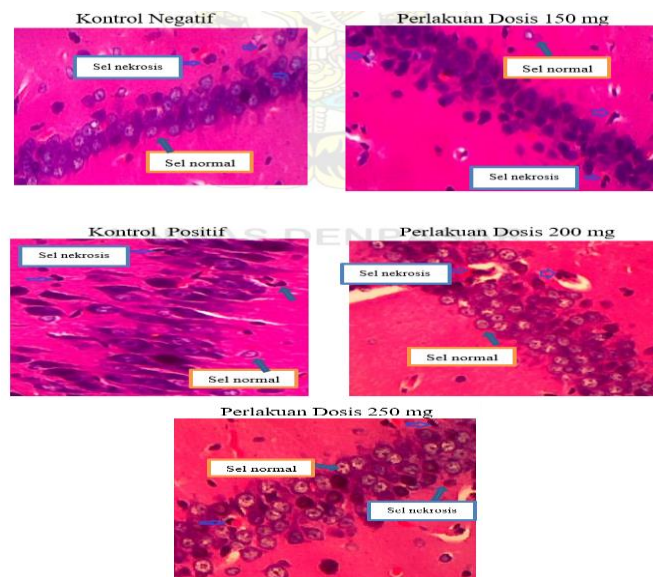


Figure 1. Histopathological Features of Neuronal Necrosis in Male *Rattus norvegicus*

DISCUSSION

The present study provides compelling evidence that gummy candy containing the rhizome fraction of *Curcuma zedoaria* exerts a significant neuroprotective effect against alcohol-induced neuronal damage in the hippocampal CA1 region. The findings demonstrate a clear dose-dependent attenuation of neuronal necrosis, with the highest dose (250 mg) yielding the most pronounced protective effect. This trend is not only statistically meaningful but also biologically relevant, as it reflects a progressive preservation of neuronal integrity under oxidative stress conditions.

Ethanol-induced neurotoxicity is primarily mediated through excessive generation of reactive oxygen species (ROS), which disrupt cellular redox homeostasis and initiate lipid peroxidation, mitochondrial dysfunction, and ultimately neuronal death^{1,3,14}. The hippocampal CA1 region is particularly vulnerable to such insults due to its high synaptic activity and metabolic demand, making it a critical locus for early neurodegenerative changes^{4,15}. The elevated necrotic cell count observed in the negative control group confirms the robustness of the alcohol-induced injury model employed in this study.

Importantly, the histopathological improvements observed across treatment groups—characterized by reduced nuclear pyknosis, decreased cellular shrinkage, and improved neuronal architecture—indicate that *Curcuma zedoaria* effectively mitigates irreversible neuronal injury. These morphological findings strongly align with established criteria for necrotic cell death and provide direct structural evidence of neuroprotection¹⁶.

From a mechanistic perspective, the observed effects are likely driven by the synergistic activity of bioactive constituents in *Curcuma zedoaria*, particularly curcuminoids and flavonoids. These compounds have been extensively reported to exert multimodal neuroprotective actions, including ROS scavenging, upregulation of endogenous antioxidant defenses (e.g., superoxide dismutase [SOD] and glutathione peroxidase [GPx]), and suppression of neuroinflammatory signaling pathways such as NF- κ B^{7,17,18}. Beyond antioxidant activity, emerging evidence

suggests that curcuminoids also modulate mitochondrial biogenesis and synaptic plasticity, thereby enhancing neuronal resilience under stress conditions^{6,19}.

A particularly noteworthy finding in this study is that the highest dose group demonstrates a neuroprotective effect that approaches—and in some observations surpasses—that of the positive control (*Ginkgo biloba*). Given that *Ginkgo biloba* is a well-established neuroprotective agent with clinically recognized efficacy, this comparison highlights the therapeutic potential and competitive relevance of *Curcuma zedoaria* as a novel neuroprotective candidate²⁰.

The novelty of this study lies not only in the use of *Curcuma zedoaria* but also in its formulation as a gummy candy dosage form, representing an innovative convergence between pharmaceutical science and functional food technology. Unlike conventional solid or liquid dosage forms, gummy-based delivery systems may enhance patient compliance, particularly for long-term preventive interventions. Moreover, the semi-solid matrix may facilitate improved stability and controlled release of bioactive compounds, potentially enhancing bioavailability—an aspect that warrants further pharmacokinetic investigation^{13,21}.

Despite these strengths, several limitations should be acknowledged. First, the study relies primarily on histopathological endpoints without direct quantification of oxidative stress biomarkers (e.g., MDA, SOD, GPx), which are essential to confirm the proposed mechanistic pathways^{3,22}. Second, although structural improvements are clearly demonstrated, functional correlation through behavioral cognitive tests (e.g., spatial memory performance) remains limited. Third, molecular-level analyses, such as expression of apoptotic or inflammatory markers, are not included in this study^{23,24}. Future studies integrating histological, biochemical, molecular, and behavioral parameters will be critical to establish a comprehensive mechanistic framework.

Taken together, this study provides strong experimental evidence that *Curcuma zedoaria* gummy candy exerts a dose-dependent neuroprotective effect against alcohol-induced

hippocampal damage. The integration of a novel delivery system with a bioactive-rich plant extract represents a promising strategy for the development of functional neuroprotective agents. These findings not only contribute to the growing body of evidence supporting phytochemical-based neuroprotection but also open new avenues for translational research in preventive neurology and functional food therapeutics.

CONCLUSION AND RECOMMENDATION

This study demonstrates that gummy candy containing the rhizome fraction of *Curcuma zedoaria* exerts a significant neuroprotective effect against alcohol-induced neuronal damage in the hippocampal CA1 region of male *Rattus norvegicus*. The formulation produced a clear dose-dependent reduction in neuronal necrosis, with the 250 mg dose showing the strongest protective effect. Histopathological findings confirmed improved neuronal integrity, characterized by reduced nuclear pyknosis and preservation of cellular architecture. These results suggest that *Curcuma zedoaria* has promising potential as a functional neuroprotective agent, particularly when delivered in an innovative gummy-based dosage form.

Further studies are recommended to strengthen and expand these findings. First, future research should include biochemical analyses of oxidative stress markers, such as malondialdehyde (MDA), superoxide dismutase (SOD), and glutathione peroxidase (GPx), to confirm the underlying mechanisms. Second, behavioral assessments of cognitive function, particularly spatial memory tests, should be integrated to correlate structural improvements with functional outcomes. Third, molecular investigations involving inflammatory and apoptotic pathways (e.g., NF- κ B, caspase signaling) are needed to elucidate the neuroprotective mechanisms more comprehensively. Finally, pharmacokinetic and bioavailability studies are essential to evaluate the effectiveness of the gummy candy delivery system and support its potential translation into clinical applications.

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