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IN VIVO SAFETY ASSESSMENT OF CURCUMIN-ENCAPSULATED ZIF-8 AND ZIF-67 NANOCOMPOSITES: A TOXICITY STUDY

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Abstract

Background: Curcumin, derived from the dried root rhizome of Curcuma longa, exhibits a spectrum of therapeutic effects, including antifungal, antibacterial, anti-inflammatory, antispasmodic, antioxidant, and antiarthritic properties. Despite these benefits, its low absorption limits its efficacy. Enhancing bioavailability through loading onto zeolitic metallic organic frameworks such as zeolitic imidazolate framework-8 (ZIF-8) and zeolitic imidazolate framework-67 (ZIF-67) presents a promising solution.

Objective: This study aimed to evaluate the acute toxicity of curcumin and its composites in male albino wistar rats.

Methods: Curcumin composites were synthesized using a solvothermal method. Male albino wistar rats were divided into seven groups (n=6/group) and administered curcumin, curcumin zeolitic imidazolate 8 (CZ8), and curcumin zeolitic imidazolate 67 (CZ67) at varying oral doses (30mg/kg, 60mg/kg) to experimental groups, and normal control group was treated with distilled water for 14 days. Throughout the study, body weight, behavior, and mortality were monitored. Blood and tissue samples were collected for analysis on the final day, with histopathological examination of vital organs (liver, kidney, spleen, brain, and heart) performed. Liver and kidney function tests, along with hematological parameters, were evaluated.

Results: No adverse effects or mortality were observed at the maximum dose of 60 mg/kg for either curcumin composite. Histopathological analysis revealed normal morphology in most organs at both doses. Slight alterations in liver tissue morphology were observed only at the 60mg/kg dose for CZ8 and CZ67. Liver and kidney function tests, and hematological parameters remained within normal ranges in all treatment groups.

Conclusion: Curcumin composites CZ8 and CZ67 demonstrated good biocompatibility and safety at doses up to 60mg/kg in this acute toxicity study.

Key words: Curcumin, ZIF-8, ZIF-67, toxicolgy, *In vivo*

Graphical abstract

Introduction:

Curcumin, a vivid yellow chemical compound extracted from the rhizomes of the *Curcuma longa* plant, known as turmeric, has been a staple ingredient in Indian and Southeast Asian cuisine for centuries. Its vibrant color and earthy flavor have made it an essential ingredient in curries, rice dishes, and other culinary creations. Beyond its culinary applications, curcumin has garnered significant attention for its potential health benefits, with extensive research exploring its therapeutic properties (Sharifi-Rad et al., 2020). Curcumin's remarkable biological activities stem from its unique molecular structure, characterized by a diaryl heptanoid backbone with β-diketone groups (Küpeli Akkol et al., 2022). This structure confers upon curcumin a range of pharmacological properties, including antioxidant (Dehzad, Ghalandari, Nouri, & Askarpour, 2023), anti-inflammatory (Peng et al., 2021), anti-microbial, (Michael Oghenejobo, Opajobi, Bethel, & Uzuegbu, 2022) and anti-cancer activities. Curcumin's antioxidant capacity arises from its ability to scavenge free radicals, preventing oxidative damage to cells and DNA (Dehzad et al., 2023). Its anti-inflammatory effects are attributed to its modulation of various signaling pathways involved in inflammation (Peng et al., 2021). Curcumin's anti-microbial properties have demonstrated efficacy against bacterial, fungal, and viral pathogens (M Oghenejobo & Bethel, 2017). Its anti-cancer potential has been investigated in various studies, suggesting its ability to inhibit tumor growth, induce apoptosis, and modulate gene expression (Ravindran, Mhatre, Koroth, Narayan, & Choudhary, 2023).

Curcumin's potential health benefits span a wide spectrum, encompassing various chronic diseases and conditions (Benameur et al., 2021; Darvesh et al., 2012). Its antioxidant and anti-inflammatory properties make it a promising contender for the management of alzheimer's disorder, parkinson's disorder, and cardiovascular diseases. Curcumin's anti-inflammatory effects may also prove beneficial in treating arthritis, inflammatory bowel disease, and other inflammatory conditions. Its antimicrobial properties indicate potential applications in fighting infections and facilitating wound healing. Furthermore, its anti-cancer properties have prompted research into its potential role in both cancer prevention and treatment.

Despite the promising evidence supporting curcumin's therapeutic potential, its low bioavailability, a common challenge with many natural compounds, limits its effectiveness. Curcumin undergoes fast metabolism and conjugation in the body, leading to inadequate absorption and short circulation time (Khezri, Saeedi, Mohammadamini, & Zakaryaei, 2021; Ma, Wang, He, & Tang, 2019). To address this issue, researchers are exploring various strategies to enhance curcumin's bioavailability, such as encapsulating curcumin within nanocarriers, such as liposomes, polymeric nanoparticles, and metal-organic frameworks (MOFs), to improve its pharmacokinetic profile and therapeutic efficacy, formulation with adjuvants, and structural modifications.

As research into curcumin's therapeutic properties continues to expand, the potential for this natural compound to revolutionize healthcare becomes increasingly evident. Its diverse range of biological activities, coupled with its relatively low toxicity profile, make it a promising prospect for the management and alleviation of several long term conditions. With ongoing efforts to improve its bioavailability, curcumin may soon emerge as a powerful tool in the fight against disease and the promotion of overall well-being.

Structural Formula:

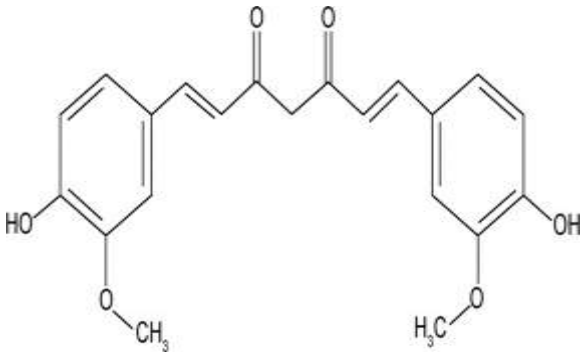

Figure 1: Structure of curcumin

Metal organic frameworks (MOFs):

Metal organic frameworks (MOFs) are a new sort of porous hybrid crystalline material made up of metal ion connectors and organic bridging linkers that are characterized by significant porosity, huge surface area, programmable forms and pore sizes, and controlled surface functions. Due to their small diameters and high porosity, nanoscale MOFs (NMOFs) promises to be a robust platform for the delivery and controlled release of pharmaceutical substances when scaled down into the nanoregime (Sun et al., 2020). MOFs have sparked interest not just in the chemical engineering community, but also in physics, energy, biology, materials engineering, nanotechnology, medicine, and environmental engineering, because of their unique properties (Bieniek et al., 2021). The zeolitic imidazolate frameworks are a type of metal-organic framework (MOF) that shares structural similarities with zeolites while also incorporating features of MOFs. ZIFs exhibit three-dimensional networks consisting of transition metal cations, such as Zn^{2+} (found in ZIF 8) and Co^{2+} (found in ZIF 67), which are linked to four imidazolate rings via nitrogen atoms of 2-methylimidazole (Hmim). This coordination structure bears resemblance to the linkage observed in zeolites, where silicon and aluminum atoms are covalently connected by bridging oxygen atoms. ZIFs exhibit a MImM (where M represents the metal and Im represents the imidazolate linker) angle of approximately 145 degrees, which is comparable to the SiOSi angle observed in zeolites. With adjustable pore structures, strong crystallinity, elevated surface area and outstanding chemical and thermal stability, these materials exhibit remarkable properties . ZIFs exhibit an exceptional ability to encapsulate hydrophobic molecules such as curcumin within their porous structure, rendering them highly promising for diverse applications in drug delivery systems (DDS) and harnessing the therapeutic potential of curcumin in biomedical fields. (Pillai, Archana, Rhee, Park, & Asif, 2019).

In this study, we aimed to assess the *in vivo* safety profile of curcumin-encapsulated ZIF-8 and curcumin encapsulated ZIF-67 formulations through a comprehensive toxicity evaluation. Recognizing the potential adverse effects of these formulations is imperative for facilitating their transition into clinical settings and maximizing their therapeutic applications.

Materials and Methods:

Preparation of curcumin composite of ZIF-8 and ZIF-67:

Solvothermal method was used for the fabrication of ZIF-8 and ZIF-67 curcumin composites followed established protocols with slight modifications (Pillai et al., 2019). Drug (curcumin) and 2 methyl imidazole were dissolved in methanol whereas metal salts (zinc nitrate hexahydrate and cobalt nitrate hexahydrate) were dissolved in distilled water using magnetic stirrer. The 2-methyl imidazole solution was added in curcumin solution on stirring and then the aqueous zinc nitrate or cobalt nitrate solution were added in it for the synthesis of Curcumin ZIF-8 composite and curcumin ZIF-67 composite respectively. The reaction mixture was allowed to agitate for 24 hours. Following agitation, the solutions were centrifuged for fifteen minutes at 10,000 rpm. The collected nanocomposites were then washed thrice with a solution of 20 ml methanol and water (2:1) to

ensure complete removal of unreacted reagents. Subsequently, the washed nanocomposites were dried overnight in an oven.

Experimental design for toxicity evaluation:

Albino wistar male rats weighing 165±15 g were employed for this model. Rats were housed in various cages with husk as bedding at a constant temperature of 27±2 °C, with pellet diet and water being given to each animal on a regular basis (*ad libitum*) and were acclimatized for 7 days before experiment. Before conducting the experiment, the Institutional Animal Ethics Committee (IAEC) and protocol permission was taken.

For oral toxicity assessments, the study was carried out for 14 days by following the Organization for Economic Co-operation and Development (OECD) guidelines 407 and 423 (Abbas et al., 2021). The animals were divided into seven groups of six animal per group as shown in Table 1.

Group 1 contains the normal control group received distilled water(D/W).
Group 2 - 3: Receive varying oral dose of curcumin.
Group 4 - 5: Receive varying oral dosages of CZ8.
Group 6 - 7: Receive varying oral dosages of CZ67

Table 1. Summary of Treatment Groups, Number of Rats, and Doses Administered

Groups	Treatment	Number of Rats	Dose/kg
G1	Normal	6	D/W
G2	CCM	6	30mg
G3	CCM	6	60mg
G4	CZ8	6	30mg
G5	CZ8	6	60mg
G6	CZ67	6	30mg
G7	CZ67	6	60mg

Parameters to be analyzed:

Body weight:

The body weight of all animals was monitored on day 1, 4, 7, 10 and 14. The changes in weight were calculated by formula:

$$\%\, weight\ change = \frac{W_t - W_o}{W_t} \times 100$$

W_o = Mean weight at 0 day
W_t = Mean weight at particular day.

The results were statistically compared with those of the normal control group.

Each animal's body weight was determined prior to administration of dose, considered as day 0 and dose was calculated in accordance to body weight. Following the oral dosage of curcumin (30mg/kg, 60mg/kg), CZ8 (30mg/kg, 60mg/kg), CZ67 (30mg/kg, 60mg/kg),

Behavioral changes:

The animals were observed for behavioral changes, clinical symptoms and mortality of toxicity during the first 30 minutes then at interval of 1, 2, 4, 8, 12, and 24 hours and then daily thereafter for 14 days. Changes in behavior as well as other factors like locomotory activity, food intake, respiration, water intake, temperature, convulsions, tremors, variation in eye and skin color were examined.

Hematological examination:
At 14th day, blood sample was taken via cardiac puncture under anesthesia. Hematological parameters i.e HGB count, total RBC's, WBC's, PCV were evaluated by an automated hematology analyzer and biochemical parameter such as, LFT's and RFT's were also analyzed.

Histopathological evaluation:
Animals were slaughtered under anesthesia and their liver, heart, spleen, brain and kidney organs were separated for histopathological study. Tissue samples were fixed, sectioned, and stained with hematoxylin and eosin (H&E) for microscopic evaluation of structural changes and pathological alterations (Park et al., 2020).

Statistical Analysis:
Statistical analysis and graphical representation were performed using SPSS software. Data were expressed as mean ± standard error. Unless specified otherwise, the statistical significance of the results was determined and analyzed using one-way analysis of variance (ANOVA).

Results and Discussion:
The acute toxic effect of curcumin ZIF 8, curcumin ZIF 67, and curcumin alone was evaluated using a limit test dose of 60 mg/kg. After oral administration of the tested composites of 30mg/kg and 60mg/kg, no adverse effects related to treatment or mortality was observed. Animals treated with nanocomposites and the control group was observed for general behavior over a short period of 24 hours, followed by a longer period of 14 days. Neither group exhibited any drug-related alterations in behavior, breathing, skin condition, or temperature. Only food intake was less in CZ8 60mg/kg, CZ67 60mg/kg and water consumption was high in group who were taking curcumin alone and CZ60mg/kg. Given that the LD50 was found to be greater than 60 mg/kg, the composites appear to be safe at a dose level of 60 mg/kg. Table 2 displays the parameters observed during the acute toxicity study, comparing the effects of the nanocomposites with those of the control group. A confirmation of the test result was made when there is no mortality recorded.

In Table 2, it is observed that animals receiving high doses exhibited slower weight gain compared to those receiving low doses, aligning with previous literature. Ng'uni, T. et al. (2018) demonstrated a similar trend in animals administered higher doses of Galenia africana (Ng'uni, Klaasen, & Fielding, 2018). Our study reinforces these findings, highlighting a consistent decrease in weight gain among animals exposed to higher doses. This observation suggests a dose-dependent relationship, possibly affecting metabolic processes or appetite regulation. The mean values of weight changes in gram during toxicity study is shown in table 3 and figure 1

Observation	CT	CM	CM	CZ8	CZ8	CZ67	CZ67
Doses mg/kg		30	60	30	60	30	60
Body weight	NM	MC	NM	NM	MC	NM	MC
Digestion	NM	NM	NM	NM	NM	NM	NM
Temperature	NM	NM	NM	NM	NM	NM	NM
Food intake	NM	NM	NM	NM	LS	NM	LS
Water intake	NM	NM	IN	NM	NM	NM	IN
Respiration changes	NM	NM	NM	NM	NM	NM	NM
Skin changes	NM	NM	NM	NM	NM	NM	NM
Drowsiness	AB	AB	AB	AB	AB	AB	AB
Eye colour changes	NM	NM	NM	NM	NM	NM	NM
Diarrhea	NP	NP	NP	NP	NP	NP	NP
Tremors	AB	AB	AB	AB	AB	AB	AB

Coma	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Death	AL	AL	AL	AL	AL	AL	AL

* NM=normal, * MC= minor changes, * LS=less, * IN= increased * AB=absent, * AL=alive

Table 2. Appearance andbehavioral observations of toxicity study

Days	CT	CCM	CCM	CZ8	CZ8	CZ67	CZ67
Doses mg/kg	---	30	60	30	60	30	60
1st	177.5±12	186.6±22	192.4±19	166.0±26	162.2±23	178.6±12	176.1±32
4th	190.8±22	190.2±34	206.3±22	172.5±10	166.3±20	184.1±23	178.8±18
7th	198.3±14	196.1±14	220.2±12	184.9±16	164.5±25	192.7±30	178.6±10
10th	208.9±19	206.9±19	228.1±32	192.8±18	176.1±10	196.4±19	184.3±15
14th	224.2±28	214.4±25	238.5±28	200.7±20	184.7±19	216.3±32	188.1±26

Table 3. Mean values of weight changes in gram during toxicity study

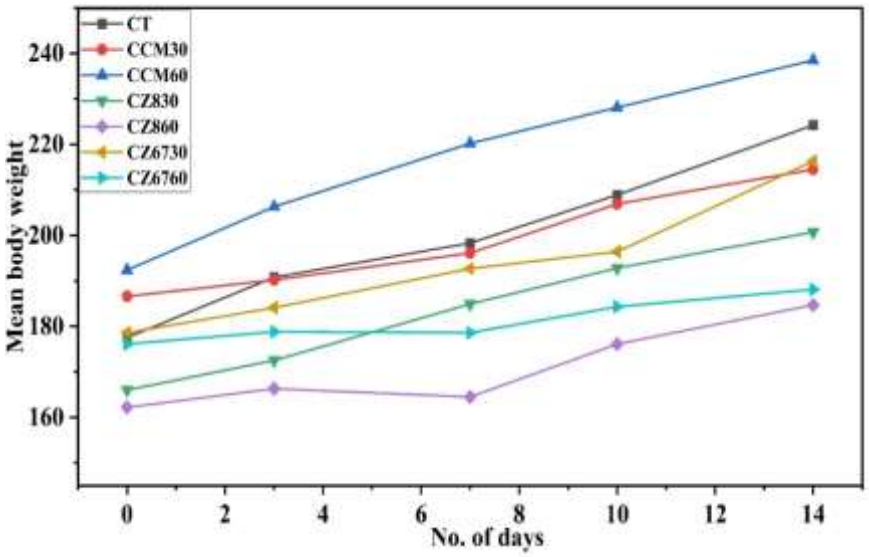

Figure 2. The mean weight of different groups at different days

Table 4. Change in body weight percentage mean±SEM

Days	Doses mg/kg	1st	4th	7th	10th	14th
CT	---	0 ± 0^{a}	7.34 ± 0.7^{b}	4.21 ± 0.3^{c}	5.05 ± 0.3^{ab}	7.69 ± 0.3^{b}
CCM	30	0 ± 0^{a}	2.15 ± 0.2^{a}	3.15 ± 0.4^{c}	5.10 ± 0.4^{ab}	3.88 ± 0.3^{a}
CCM	60	0 ± 0^{a}	7.29 ± 0.6^{b}	6.79 ± 0.6^{d}	3.63 ± 0.3^{ab}	4.38 ± 0.4^{ab}
CZ8	30	0 ± 0^{a}	3.61 ± 0.2^{ab}	6.97 ± 0.5^{d}	4.34 ± 0.2^{ab}	4.16 ± 0.5^{ab}
CZ8	60	0 ± 0^{a}	2.46 ± 0.3^{a}	-1.20 ± 0.2^{a}	7.31 ± 0.5^{c}	4.54 ± 0.4^{ab}
CZ67	30	0 ± 0^{a}	3.37 ± 0.2^{ab}	4.34 ± 0.1^{c}	2.08 ± 0.3^{a}	10.20 ± 0.7^{c}
CZ67	60	0 ± 0^{a}	1.13 ± 0.2^{a}	0 ± 0.0^{b}	3.37 ± 0.2^{ab}	2.17 ± 0.2^{a}

Different alphabet show the significant difference at (P<0.05)

Figure 3. Overall percentage changes in body weight

The ANOVA (Duncan's Post Hoc Test) revealed that the overall means of body weight within groups were significantly different ($P<0.05$). However, repeated measures ANOVA (Dunnett's T3 Post Hoc Test) revealed that the CT and CCM60 group showed significant higher values of body weight percentage while the CCM 30, CZ8 60 and CZ67 60 showed significantly lower values than all other groups while all other groups were non-significant at 4th day.
On the 7th day the group CZ860 showed significantly lower values of body weight than the all groups while CZ6760 showed higher values than CZ860 while low values than the all other groups. The group CCM60 and CZ830 showed significant higher values. The CZ860 showed the significantly higher values of body weight percentage while the CZ6730 showed significant low values compare toall other groups. At the end of the experiment the group CZ6730 showed significantly higher values while the CZ6760 showed significantly low values. The group CCM60, CZ830, CZ860 showed no significant difference to the CT group.
The overall mean of body weight percentage is shown in figure 4.1. which showed that the CT group and CCM60 have no significant difference but both showed no significant difference. The group CZ6730 and CZ830 showed no significant difference to the all group except CZ6760 which showed significantly low values than all the groups. The gradual increase in body weight suggest that it does not affect the vital organs of the rata and their functioning was normal they gain the proper weight.

Histopathology results:
The control group treated solely with distilled water. Histopathological analysis of liver of the group control, CCM 30, CCM 60, CZ8 30 and CZ67 30 reveals intact hexagonal shapes, indicative of normal liver tissue morphology. Hepatic lobules are discernible within the triangular portal area. The cytoplasm appears granulated and hepatocytes exhibit normal characteristics. Most hepatocytes display single spherical nuclei, with some containing either one or two nuclei. Nuclear sizes range from diploid to polyploid, with sinusoids and blood vessels maintaining their normal appearance. Blood cells exhibit typical morphology without vacuolization or degranulation. Endothelial cells appear normal, devoid of apoptotic signs, and infiltrates are absent. Whereas, the histopathology of the liver of CZ8 60 and CZ67 60 exhibits slight alterations in liver tissue morphology, potentially disrupting the characteristic hexagonal shape and causing irregularities in the cellular lining as shown in Figure 3. Table 5 illustrates the severity of various histopathological changes observed in the liver across different groups. In the previous study conducted by Lan et al., 2022 used metal organic framework MOF-74 and find the similar results that no significant change found (Lan et al., 2022)

Table 5. Severity of histopathological changes in the liver among various experimental groups

Pathological Alterations	CT	CCM	CCM	CZ8	CZ8	CZ67	CZ67
Doses mg/kg	00	30	60	30	60	30	60
Nuclear Fragmentation	-	-	-	-	-	-	-
Central vein congestion	-	-	-	-	-	-	-
Disrupted hepatic cord	-	-	-	-	-	-	-
Formation of Vacuoles in Cytoplasm	-	-	-	-	+	-	+
Hepatocytes Necrosis and Hydropic Alterations	-	-	-	-	-	-	-
Condensed Nuclear Chromatin	-	-	-	-	-	-	+
Cytoplasmic vacuolization	-	-	-	-	-	-	-
Nuclear degeneration	-	-	-	-	+	-	++
Hepatocytes degeneration	-	-	-	-	+	-	++

Signs: (-) = Normal, (+) = Mild, (++) = Moderate, (+++) = Severe, (++++) = Very Severe

Histopathological examination of kidney tissue of group control, CCM 30, CCM 60, CZ8 30, CZ8 60CZ8 30 and CZ67 60 revealed intact nephron structure, including Bowman's capsule, glomeruli, and tubules with regular cellular organization. No signs of inflammation, fibrosis, or abnormalities in blood vessel morphology were observed. Overall, the kidneys exhibited healthy tissue morphology without significant pathology. The composites show no lethal effects on kidney tissue at dose

30mg/kg and 60mg/kg as shown in Figure 3. Table 6 illustrates the severity of various histopathological changes observed in the kidney across different groups.

Table 6. Severity of histopathological changes in the kidney among various groups

Pathological Alterations Doses mg/kg	CT	CCM	CCM	CZ8	CZ8	CZ67	CZ67
	00	30	60	30	60	30	60
Expansion of Bowman's Space	-	-	-	-	-	-	-
Congestion	-	-	-	-	-	-	-
Renal Tubular Epithelial Cell Necrosis	-	-	-	-	-	-	-
Nuclear Enlargement	-	-	-	-	-	-	-
Tubular Necrosis	-	-	-	-	-	-	-
Degenerative Alterations in Glomeruli	-	-	-	-	-	-	-
Hemorrhage	-	-	-	-	-	-	-
Edema	-	-	-	-	-	-	-

Signs: (-) = Normal, (+) = Mild, (++) = Moderate, (+++) = Severe, (++++) = Very Severe

Spleen tissues from all groups showed normal structure with well-preserved white and red pulp regions. White pulp exhibited typical lymphoid follicles, while red pulp displayed spleenic cords and sinusoids. Overall spleen architecture, including marginal zone and trabeculae, appeared normal with no abnormalities. Connective tissue elements were distributed normally. Histopathological examination indicated healthy spleen morphology with no toxic effects of test samples observed. See Table 7. for severity of histopathological changes across groups.

Table 7. Severity of histopathological changes in the spleen among various experimental groups

Pathological Alterations	CT	CCM	CCM	CZ8	CZ8	CZ67	CZ67
Doses mg/kg	00	30	60	30	60	30	60
Congestion	-	-	-	-	-	-	-
Depletion of red and white pulp	-	-	-	-	-	-	-
Ceroid formation	-	-	-	-	-	-	-
Depletion of lymphoid follicles	-	-	-	-	-	-	-

Signs (-) = Normal, (+) = Mild, (++) = Moderate, (+++) = Severe, (++++) = Very Severe

The brain tissue of all treated groups, including the normal control, exhibited neurons that appeared normal with no signs of destruction observed. Neuropil is present in typical amounts with no signs of necrosis. Vacuolization is absent, and glial cells appear prominent and healthy without any signs of apoptosis. No signs of hemorrhage, necrosis, inflammation, or apoptosis are observed as shown in Figure 3. Table 8. illustrates the severity of various histopathological changes observed in the brain across different groups.

Table 8. Severity of histopathological changes in the brain among various experimental groups

Pathological Alterations	CT	CCM	CCM	CZ8	CZ8	CZ67	CZ67
Doses mg/kg	00	30	60	30	60	30	60
Congestion	-	-	-	-	-	-	-
Inter-Cellular Swelling	-	-	-	-	-	-	-
Neurons necrosis	-	-	-	-	-	-	-
Neurons atrophy	-	-	-	-	-	-	+
Vacuolization in cytoplasm	-	-	-	-	-	-	-

Signs (-) = Normal, (+) = Mild, (++) = Moderate, (+++) = Severe, (++++) = Very Severe

Histopathological examination of heart tissue of all experimental groups along with normal control, represent characteristic features. Nuclei are centrally located and exhibit a normal shape, contributing to the overall cellular integrity. Blood vessels maintain their typical structure, and cells present a healthy appearance. Cardiac muscle fibers and Purkinje fibers appear normal, devoid of any signs of

hemorrhage, cirrhosis, vacuolization, or abnormalities in morphology. The absence of myofibrosis and necrosis further underscores the normalcy of the cardiac tissue.
Table 9. illustrates the severity of various histopathological changes observed in the heart across different groups.

Table 9. Severity of histopathological changes in the heart among various experimental groups

Pathological Alterations	CT	CCM	CCM	CZ8	CZ8	CZ67	CZ67
Doses mg/kg	00	30	60	30	60	30	60
Neutrophilic myocarditid	-	-	-	-	-	-	-
Inflammatory exudate	-	-	-	-	-	-	-
Cardiac muscles degeneration	-	-	-	-	-	-	-
Myofibrillolysis	-	-	-	-	-	-	-
Edema	-	-	-	-	-	-	-
Muscles disorganization	-	-	-	-	-	-	-
Vacuolization	-	-	-	-	-	-	-

Signs (-) = Normal, (+) = Mild, (++) = Moderate, (+++) = Severe, (++++) = Very Severe

Figure 4. Histological Analysis of Various Organs in Curcumin and CZ8 and CZ67 Composites from Toxicity Study (scale bars, 100 μm for all panels).

The liver function tests (LFTs) and renal function tests (RFTs) of all animal groups subjected to toxicity studies were found to be comparable to those of normal controls. This observation suggests that the experimental interventions did not induce significant hepatotoxic or nephrotoxic effects in the study subjects. Only ALT, AST and ALP levels of CZ67 60 were found raised than all other groups. Simlarly in the previous study conducted by Lan et al., 2022 used metal organic framework MOF-74 and find effect on the liver (Lan et al., 2022). Graphical representation of liver function tests and renal function tests are shown in figure 5 and 6.
The maintenance of normal LFTs and RFTs underscores the safety profile of the interventions assessed in this study. The preservation of liver and kidney function is crucial in evaluating the overall systemic toxicity of any therapeutic or experimental agents. The comparable LFTs and RFTs between the treatment groups and normal controls indicate the absence of adverse effects on these vital organs as shown in Table 10.

Table 10. The mean±S.dev of LFT's and RFT's of toxicology groups

GR	**Doses mg/kg**	**Mean data of biochemical parameters**					
		Creatinine	**Urea**	**AST**	**ALT**	**ALP**	**Bilirubin**
NC	00	20.32±2.05^{a}	32.38±6.37^{a}	75.33±7.81^{a}	39.22±9.12^{a}	115.73±12.88^{a}	0.36±0.02^{a}
CCM	30	22.74±3.61^{a}	36.24±5.44^{a}	82.43±14.70^{a}	44.47±7.38^{a}	117.45±17.23^{a}	0.35±0.03^{a}
CCM	60	19.42±1.89^{a}	29.44±7.26^{a}	81.12±17.92^{a}	41.12±9.66^{a}	110.60±11.24^{a}	0.36±0.07^{a}
CZ8	30	21.39±2.11^{a}	33.62±7.21^{a}	79.71±13.21^{a}	46.98±10.52^{a}	119.37±18.87^{a}	0.38±0.02^{a}
CZ8	60	23.20±2.49^{a}	34.20±8.20^{a}	92.46±15.02^{a}	42.35±6.32^{a}	117.71±23.99^{a}	0.37±0.03^{a}
CZ67	30	24.25±3.10^{a}	30.95±6.56^{a}	80.54±12.81^{a}	42.22±8.73^{a}	104.87±17.48^{a}	0.36±0.06^{a}
CZ67	60	19.15±3.02^{a}	31.54±7.72^{a}	121.30±9.30^{b}	65.92±7.26^{b}	130.52±18.42^{b}	0.44±0.02^{a}

Different alphabet show the significant difference at (P<0.05)

Figure 5. Liver function test parameters of toxicology experimental animals

Figure 6. Kidney function test parameters of toxicology experimental animals

Table 11. present the hematological findings from the toxicity study, comparing the experimental groups treated with CCM, CZ8, and CZ67 at doses of 30mg/kg and 60mg/kg with the normal control

group. Remarkably, the hematological parameters assessed in these groups did not exhibit any significant differences when compared to the normal control group. These results suggest that the administration of Curcumin and its composites at both 30mg/kg and 60mg/kg doses did not induce any hematological abnormalities in the study animals. Similarly the experiment conducted on mice by Deng et al., 2021 treated with ZIF-67 showed no significant effect on the hematology (Deng et al., 2021). In the previous study conducted by Lan et al., 2022, metal organic framework MOF-74 was used and find the similar results that no significant changes found (Lan et al., 2022). The study conducted by Kumari et al., 2023 on rat showed that ZIF-8 not effect the hematology (Kumari et al., 2023).

The maintenance of hematological parameters within normal ranges underscores the safety profile of these formulations, indicating minimal adverse effects on hematopoiesis and blood cell function. Furthermore, the absence of significant differences between the experimental and normal control. groups strengthens the notion of the biocompatibility of curcumin and its composites, even at higher doses. At a limit dose of 60mg/kg, neither composite caused mortality or adverse effects. Body weight gain was lower in high dose groups compared to low dose groups. Histopathological analysis of vital organs showed slight alterations in the liver at the highest dose only. Liver and kidney function tests, as well as hematological parameters, remained normal across all treatment groups. These findings suggest good biocompatibility and safety of curcumin composites CZ8 and CZ67 at doses up to 60mg/kg, warranting further exploration of their therapeutic potential and long-term effects.

Table 11. Mean values of biochemical parameters related to RBC's of toxicology groups

GR	Doses mg/kg	Mean data of biochemical parameters					
		RBCs	**HGbs**	**MCV**	**MCH**	**MCHC**	**Hct**
NC	00	8.2±3.6[a]	32.6±3.6[a]	59.5±7.0[a]	18.2±4.3[a]	20.5±2.3[a]	46.5±4.2[a]
CCM	30	9.9±2.8[a]	27.8±3.3[a]	53.5±8.4[a]	15.9±6.0[a]	21.2±3.1[a]	48.2±5.1[a]
CCM	60	10.7±2.4[a]	30.8±2.0[a]	56.2±6.2[a]	20.9±3.4[a]	24.4±2.9[a]	46.1±6.1[a]
CZ8	30	9.6±1.4[a]	31.5±4.1[a]	53.8±7.1[a]	21.2±2.5[a]	26.8±4.4[a]	45.9±4.6[a]
CZ8	60	9.7±1.4[a]	26.7±3.3[a]	60.1±10.3[a]	17.3±4.9[a]	22.6±3.2[a]	47.8±5.3[a]
CZ67	30	10.3±1.9[a]	28.9±4.2[a]	63.5±7.3[a]	19.5±2.2[a]	19.3±2.7[a]	46.3±7.2[a]
CZ67	60	8.9±1.5[a]	30.4±2.9[a]	61.6±9.5[a]	16.2±3.2[a]	22.3±3.9[a]	44.7±2.5[a]

Different alphabet show the significant difference at (P<0.05)

Table 12. Mean values of biochemical parameters related to WBC's and platelets of toxicology groups

GR	Doses mg/kg	Mean data of biochemical parameters				
		WbCs	**Lymphocyte**	**Monocyte**	**Granulocyte**	**PLT**
NC	00	6.2±1.7[a]	50.2±12.6[a]	2.4±0.6[a]	50.2±12.1[a]	853.3±187.3[a]
CCM	30	6.0±1.2[a]	56.1±16.2[a]	1.9±0.3[a]	45.8±8.9[a]	891.4±121.0[a]
CCM	60	5.9±1.8[a]	57.3±13.3[a]	2.6±0.5[a]	47.6±10.8[a]	881.8±90.7[a]
CZ8	30	5.7±1.7[a]	53.8±10.9[a]	2.1±0.4[a]	52.5±11.9[a]	857.1±61.9[a]
CZ8	60	6.6±1.3[a]	54.5±11.5[a]	2.6±0.8[a]	49.9±9.7[a]	869.8±88.7[a]
CZ67	30	5.8±1.8[a]	55.9±9.2[a]	2.3±0.2[a]	48.3±3.8[a]	866.7±137.5[a]
CZ67	60	6.3±1.2[a]	60.2±14.4[a]	2.6±0.7[a]	51.0±4.5[a]	878.2±122.4[a]

Different alphabet show the significant difference at (P<0.05)

Conclusion

This study successfully assessed the acute toxicity of curcumin composites, CZ8 and CZ67, in male albino Wistar rats. The findings demonstrate that both composites exhibit good biocompatibility and safety at doses up to 60mg/kg. No mortality or adverse effects were observed at the limit dose, and

histopathological analysis revealed minimal alterations only at the highest dose tested. Liver and kidney function tests, along with hematological parameters, remained within normal ranges in all treatment groups. These results are promising for the further development of curcumin composites of ZIF-8 and ZIF-67 as potential therapeutic agents.

Future Aspects

After the successful acute toxicity evaluation, several future research aspects will emerge. A long-term toxicity evaluation of these composites should be conducted for a deeper understanding. It should examine their therapeutic potentials against cancer, arthritis, and other chronic diseases. Moreover, exploring potential synergistic effects with other therapeutic agents may lead to the development of more powerful combination therapies. These avenues promise to further illuminate the potential of these composites for improving health outcomes.

Statement of Conflict of Interest:

There is no conflict of interest among the authors.

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