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Antioxidant Properties of Galangin with β-cyclodextrin: An *in Vitro and in Vivo*

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Abstract

Galangin, a non-toxic phytochemical, is known to have a variety of therapeutic uses. This study looked into the role of inclusion complexes of galangin/β-cyclodextrin in increasing antioxidant activity over pure galangin. The role of this inclusion complex in increasing antioxidant activity in comparison to pure galangin. In this study, hydrogen peroxide assays were used in vitro. Galangin demonstrated concentration-dependent scavenging action in the 2-50 µg mL^{-1} range, with the highest level of activity possible 92.00% at 50 µg mL^{-1}. In pure galangin, a decrease of 85.00% was observed. The ferric thiocyanate lipoperoxidation method was clarified by using galangin and galangin/β-cyclodextrin and demonstrated concentration-dependent suppress lipid peroxidation in the 2-50 µg mL^{-1} range, at 50 µg mL^{-1}, the highest level of activity was 70.00%. A 60.00% decrease in pure galangin was observed. Xanthine oxidase activity using uric acid was given. The co-incubation of galangin and galangin/β-cyclodextrin inhibited xanthine oxidase activity in a concentration-dependent manner in 2-50 µg mL^{-1} range, at 50 µg mL^{-1}, the highest level of activity was 90.00 %. Pure galangin showed an 82.00 % decrease. There were no significant differences in absolute weight of mice organs and hematological parameters between pure galangin and galangin/ β-cyclodextrin when used concentration 80 mg kg^{-1}, compared to control group. According to the findings, galangin combined with β-cyclodextrin has excellent properties as a therapeutic agent and food supplement.

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1. Introduction

Flavonoids are one type of natural product that is very important. They are a type of bioactive phytochemical found in a wide variety of beverages, fruits, and vegetables. Flavonoids come in a variety of forms, such as flavones,

flavonones, flavonols, iso-flavonoids, and anthocyanins [1, 2, 3]. Most flavonoids modulate a variety of enzymes and receptors in signaling pathways involved in cellular differences, capillary density, various drug tolerance reflections, infection, tumors, and cell death [4, 5]. According to phytochemical studies, flavonoids, glycosides, and diarylheptanoids are the three major chemical constituents of *A. officinarum*. Further pharmacological research found that *A. officinarum* was found to have anti-inflammatory, antioxidant, antibacterial, antiparasitic, and anticancer properties. Flavonoids, in particular, have attracted a lot of attention due to their significant antioxidant activity [6]. Galangin (3,5,7-trihydroxyflavone), a flavonoid natural ingredient polyphenol, is found in propolis and the rhizomes of Alpinia officinarum Hance.Galangin is a flavonoid with limited medicinal benefits due to its poor pharmacokinetic properties, solubility in water, and lack of integration in physical media. The medicinal advantages of galangin as an antitumor drug may thus be enhanced by developing nanoparticular delivery methods [7]. Cancer is characterized by uncontrolled cell proliferation, with tumors forming when cells grow to become an abnormal cell mass [8, 9, 10]. Galangin is also abundant in honeysuckle bees, which yield a substance called propolis, which is derived from various crops' toothpaste and contains the following ingredients: quercetin (2%), chrysin (3.8%), and galangin (9%). Galangin has radical-scavenging, anti-oxidative, anti-clastogenic, anti-mutagenic, and biochemical enzyme altering characteristics [11]. Galangin has been shown to have a large selection of anti-inflammatory, antiviral, and antimicrobial influences among the bioactive components [7]. Galangin has been shown to have anticancer properties against a variety of cancers [12]. The molecular structure and biological activities of galangin are shown in Figure 1 [13].

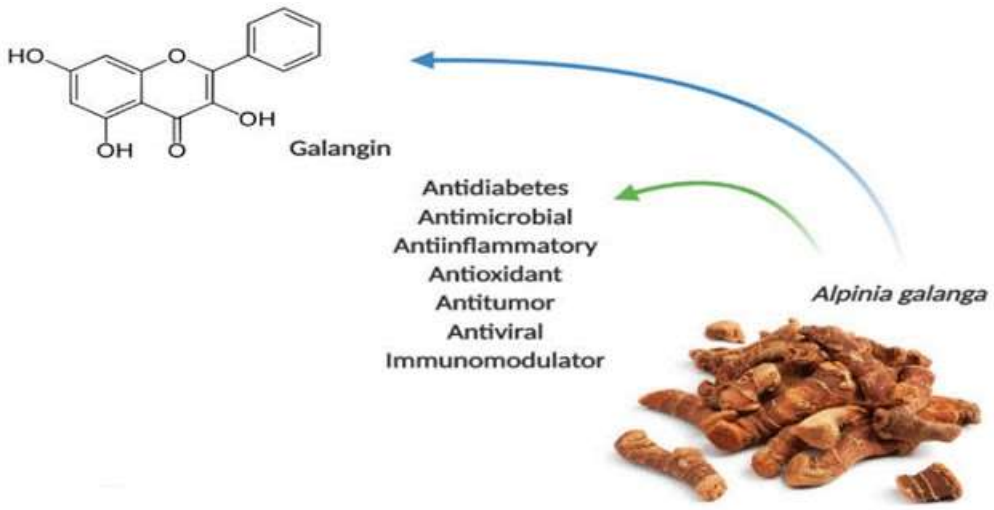

Figure 1: Molecular structure and biological activities of galangin.

Galangin prevents nuclear factor-jB activation, which suppresses inflammatory responses in collagen-induced joint pain and ovalbumin-induced inflammatory processes. [14, 15]. The flavonol group includes galangin, which has an unsaturated C ring between C2 and C3, which is hydroxylated at position C3 and oxidized at position C4 [16]. Galangin is an enzymatic modulator with three carbons on its carbon rings that can reduce chemical cytotoxic effects. In previous studies, galangin is a blocker of the aryl hydrocarbon binding site. At non-toxic concentrations, they've also been discovered to have bioactivities in organisms [17]. Galangin's absorption and delivery methods, on the other hand, are lacking due to its extremely poor aqueous solubility. Galangin is miscible in ethanolic water despite being insoluble or barely soluble in water [18]. Although cyclodextrin-based hydrogels have been shown to form a covalent bond with galangin to enhance its solubility in water [19], their strong affinity ability and poor water viscosity potential may limit their functionality as galangin drug carriers [20]. Glucopyranose side-chain oligosaccharides with a water-insoluble body cavity and a water-soluble external part are known as cyclodextrins [21]. Cyclodextrins are primarily used as complex compounds to increase the aqueous absorption and stability of drugs that are poorly water-soluble [22]. Gene delivery researchers are now experimenting with cyclodextrin molecules in the hopes of finding the best carrier for therapeutic nucleic acids [23]. Increased drug solubility and stability [24], improved drug absorption [25], masking odors and tastes, controlling drug release profiles [26], reducing local and systemic toxicity [27], and improving drug permeability across biological barriers [28] are some of the related applications. The purpose of the study was to see how effective pure galangin and galangin formulated

with β-cyclodextrin were at reducing oxidative stress. The mouse model was often used to look into the case of toxicity in mice's whole blood.

2. Protocol for Experimental Work

2.1. Detergents and Chemicals

Galangin (GAL), β-cyclodextrin (β-CD), both these ingredients from Biotech Co., China, Ethanol (Lobacheme, India), and 1-diphenyl-2-picrylhydrazyl (DPPH), lipoperoxidation, xanthine oxidase, each one of any of those ingredients Sigma Chemical Co. in the United States was used to make this acquisition.

2.2. Antioxidant Activity (DPPH)

DPPH was used to determine the extract's antioxidative activity [29]. The Hydroxyl radical has a dark purple color in addition to its potential to scavenge valence electrons and radicals, which can be seen in a UV spectrophotometer as an absorption coefficient at 517 nm when the pale yellow non-radical form is obtained. In a cuvette, equal volumes (0.5 mL) of DPPH (60 M) and pure galangin and galangin / β-cyclodextrin (2, 5, and 50 µg mL^{-1}) were mixed and allowed to stand for 30 minutes at room temperature. At 517 nm, the absorbance was measured in a UV/VIS Lambda-19 spectrophotometer. The majority of DPPH scavenging efficacy was computed using the formula:

$$\textbf{Anti} - \textbf{Oxidant Capacity}\ (\%) = \frac{\textbf{Ac}-\textbf{As}}{\textbf{Ac}} \times \textbf{100} \quad (1)$$

The peak of pure galangin and galangin/β-cyclodextrin sample solvent is represented by Ac, as is the peak of DPPH.

2.3. Induced Lipoperoxidation

The ferric thiocyanate method was used to assess the antioxidant capacity of pure galangin and galangin/β-cyclodextrin at different concentrations (2, 5, and 50 µg mL^{-1}) to inhibit linoleic acid peroxidation [30]. In a test tube, 5 volumes of 0.02 M linoleic acid surfactant and 4 quantities of 0.2 M phosphate buffer (pH 7.0) have been mixed with a dilution of the sample in distilled water. To allow faster lipid oxidation, the substance was left confused at 4 ◦C for 8 days. The lipid oxidation value was measured using the absorbance at 500 nm after the ferric chloride and thiocyanate treatments were added, and three were measured to have an average value.

2.4. Xanthine Oxidase Activity

The creation of uric acid at 292 nm was used to spectrophotometrically measure xanthine oxidase activity [31]. The assay mixture contained 50 mM phosphate buffer pH 7.8, 25 mM xanthine solution, and 24 mU of xanthine oxidase in a final volume of 1 ml (specific activity 1 U mg^{-1} of protein). To calculate regression lines, different concentrations of pure galangin and galangin / β-cyclodextrin (2, 5, and 50 µg mL^{-1}) were added to samples before the enzyme. The results were calculated using the percentage of inhibitor enzyme activity.

2.5. Laboratory Animals and Mice Blood Collecting

The mice used were Swiss albino male mice that weighed 22–25 g and were 4–5 weeks old. The Iraqi Center for Cancer and Medical Genetic Research at the University of Al-Mustansiriyah in Baghdad, Iraq, generously provided mice for this study. The mice were kept in a 12:12 hr light/dark cycle with a consistent supply of water and food at 24 ℃ and a humidity level of 55 percent at 24 ℃. The Animal Care and Ethics Committee at the University of Technology, Baghdad, Iraq's Biotechnology Division, Applied Sciences Department, approved all procedures. The organs of mice (kidneys, liver, spleen, lungs, thymus and heart) were excised and weighed. Mice blood samples were taken fresh from twenty-one mice from three groups and divided into tubes containing the anticoagulant agent (heparin).

2.5.1. Hematological Evaluation

According to Jasper [32], white blood cell (WBC), lymphocyte and monocytes counts,hematological parameters such as red blood cell (RBC), serum hemoglobin content, hematocrit, mean corpuscular hemoglobin (MCH), mean corpuscular volume (MCV), were measured. A hematological auto tester was used to determine various hematological parameters during the blood picture.

2.6. Statistical Analysis

SPSS statistical software (Version/18.0; SPSS Inc., Chicago, IL) was used to analyze the submitted data. The analysis of variance (ANOVA) was used to see if there were any substantial differences between the study means. A 0.05 p-value was tested for significance.

3. Results and Discussion

3.1. Antioxidant Capacity Assay

In Figure 2, the antioxidant properties of pure galangin and galangin / β-cyclodextrin at three different concentrations are depicted. According to the galangin / β-cyclodextrin mixture used, it had a higher antioxidative capacity than pure galangin. The findings showed that pure galangin decreases the level of DPPH free radicals in its natural state by 85.00% at 50 µg mL^{-1}, and that it outperforms other concentrations. Galangin/β-cyclodextrin achieved 90.00% at the same concentration. The higher activity of galangin/β-cyclodextrin is due to the increased dissolvability of galangin with native β-cyclodextrin in scavenging DPPH radicals. When flavonoids are made into nanoparticles, their biological efficiency is increased. The radical-scavenging method of DPPH (1,1-diphenyl-2-picrylhydrazyl) is based on electrochemical reactions that result in a rich purplish color in ethyl alcohol [33, 34]. These colors change to brighten yellow free radical damage that is constant at 25°C. They turn colorless to light yellow when they combine with a chemical reaction that occurs as a hydrogen donor. The amount of reduction in the reaction is roughly equal to the absorbance quantity of the antioxidant capability.

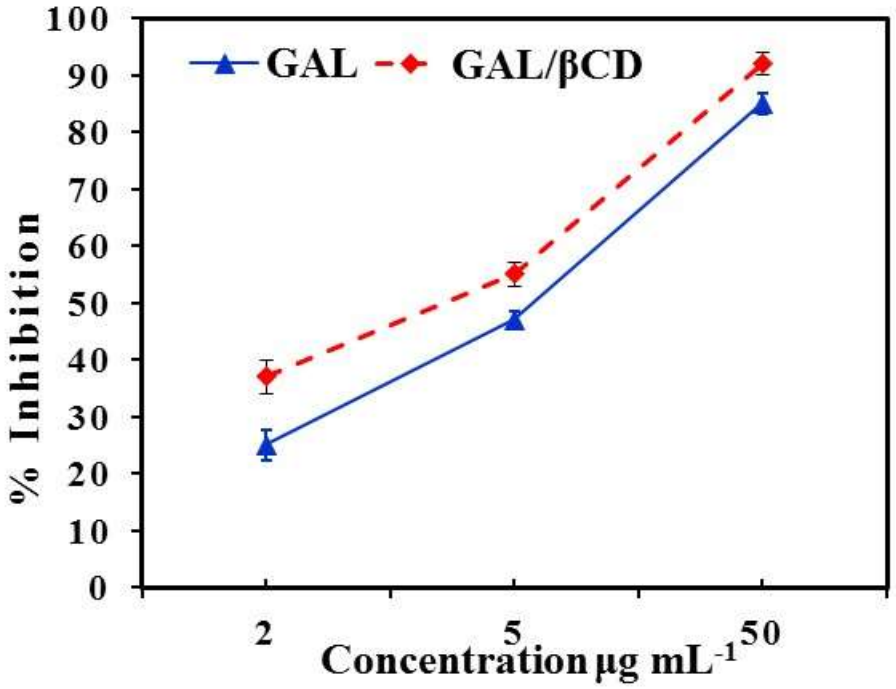

Figure 2: Antioxidant capacity against DPPH of pure galangin (Blue color) and galangin / β-cyclodextrin (Red color) using three different concentrations.

This elevation is due to the rise in the large space of the ground, surface charge, and catalytic reaction. Propolis is by far the most powerful antioxidant of all the honey bee compounds. All those are polyphenols, including galangin, which is a flavonoid. Propolis compounds reduce the production of reactive oxygen species (ROS) by activating the receptor-associated element (ARE), which is responsible for the production of antioxidative enzymes. This antioxidant action mechanism has also been proven for all of the selected representative markers. It is undeniably due to a combination of polyphenols, and the combination will vary depending on the manner of propolis preparation [35]. The DPPH radical assay, which measures antioxidant scavenging ability, is commonly used to assess the antioxidant property. Solvents of varying polarities were used to fractionate the extract of A. officinarum. On the other hand, galangin and chemically related compounds, demonstrated strong antioxidant properties against DPPH [36].This could explain why galangin / β-cyclodextrin had the best ability to scavenge

DPPH. The findings indicate that converting galangin to galangin/ β-cyclodextrin form can significantly boost its antioxidant potential.

3.2. Induced Lipoperoxidation

As shown in Figure 3, galangin and galangin/β-cyclodextrin had good inhibition activity on the oxidation process. The anti-oxidative activity of pure galangin and galangin/β-cyclodextrin at three different concentrations. It was inhibited with 70.00% galangin / β-cyclodextrin and 60.00% with pure galangin at a concentration of 50 µgmL^{-1}. The figure shows that galangin combined with native β-cyclodextrin suppressed lipid peroxidation more effectively than galangin alone, with concentration-dependent effects. The antioxidant capacity of the extracts did not follow the same pattern as the DPPH assay using this strategy. The Linoleic Acid Model System was used to test the antioxidant activity. A good antioxidant should be able to operate as a chain splitter while also inhibiting oxidative destruction during this period [37]. Iron is an essential component in vertebrate metabolism due to its unrivaled adaptability as a biologic activator. Iron, on the other hand, plays a major role in the creation of extremely harmful reactive oxygen, which eventually causes lipid peroxide loss of vital cell structures when not properly insulated or existing in excess. Butylated hydroxytoluene, a very well-string antioxidants that prevents the development of the peroxidation process, nearly completely blocked O_2 absorption generated by ferrous ions [38, 39, 40]. This experiment was important to study galangin and galangin/β-cyclodextrin to know their effect as an anti-inflammatory and in programmed cell death.

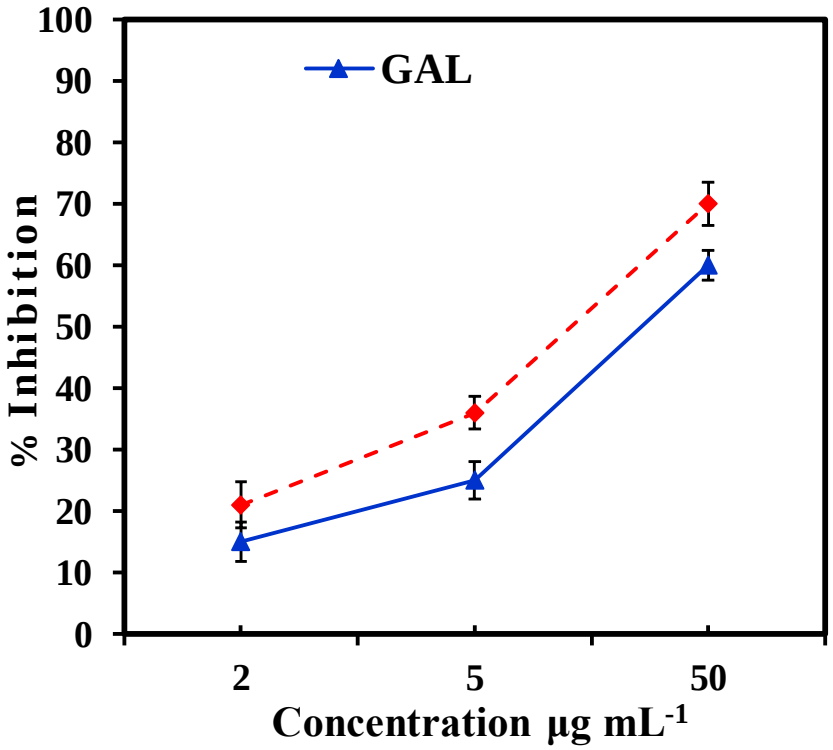

Figure 3: The antioxidant activity of pure galangin (Blue color) and galangin / β-cyclodextrin (Red color) as measured by linoleic acid model system at three different concentrations.

3.3. Xanthine Oxidase Activity

The influence of pure galangin and galangin / β-cyclodextrin on xanthine oxidase activity at various concentrations was demonstrated in Figure 4. The addition of galangin / β-cyclodextrin at 50 µgmL^{-1} resulted in an amount of the drug inhibitory activity of xanthine oxidase activity of 90.00%, but the addition of pure galangin alone resulted in a lower effect of 82.00 %. So, higher concentrations inhibited the activity of xanthine oxidase more effectively than smaller doses. The Uric Acid system and antioxidant activity. Flavonoids have been shown to possess high activity for inhibition of xanthine oxidase, and then decrease uric acid levels in serum. The inhibitory effects of various dietary flavonoids on xanthine oxidase were tested in vitro . As a result, the catechol composition of the B ring, which provides flavonoids with their antioxidative capability, was not linked to XO suppression. These findings suggest that a planar flavanone molecule, instead of a nonplanar flavanone formation, is required for XO

inhibition [41]. Several flavonoids were identified as some of the most powerful inhibitors of XO in vitro, many of which were discovered for the first time [42]. XO is an enzyme that has been widely found in a range of mammalian tissues. In comparison to those in bigger vessels, endothelial cells in microvascular pathways are the most numerous suppliers of the enzyme amongst cellular structures. Because the enzymatic process that transports electrons from xanthine oxidase to uric acid is also accompanied by a reduction of oxygen into superoxide radicals, XO is thought to be important in the pathophysiology of oxidant-induced capillary alterations and cell damage [43].

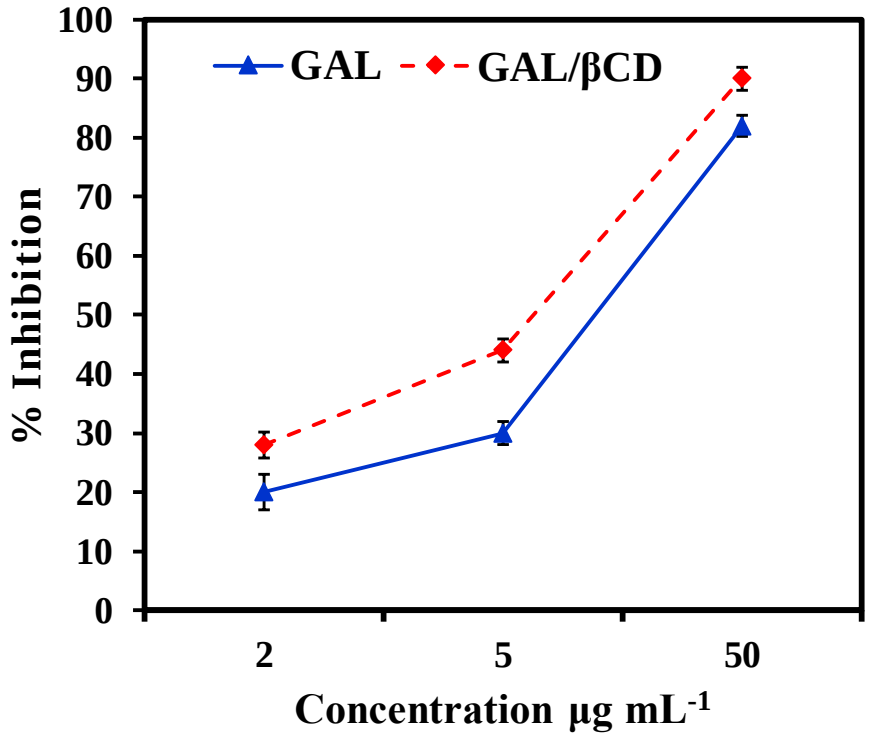

Figure 4: Effect of pure galangin (Blue color) and galangin /β-cyclodextrin (Red color) on xanthin oxidase activity at three different concentrations.

3.4. Absolute Weight of Mice Organs

The absolute weight of organs (kidneys, liver, spleen, lungs, thymus, and heart) was evaluated after orally administering pure galangin and galangin / β-cyclodextrin at doses of 80 mg kg^{-1}, as indicated in Table 1. In a previous study on hesperidine, similar findings were observed and showed no significant differences in body weight between the treated mice and the control mice. Furthermore, there were no differences in clinical symptoms or behavior between the treated and untreated mice. There was no evidence of toxicity inside the animals, and no unfavorable behavioral responses were seen [44]. However, statistically, no significant differences ($p \geq 0.05$) were detected after 14 days. In both the treated and control groups, the absolute (g) of all separated organs such as liver, kidneys, lungs, spleen, and heart remained normal, indicating that the aqueous extract was nontoxic in these critical organs. Organ weight is a key measure of a person's or animal's physiological and pathological status. The principal organs impacted by toxicant-induced metabolic reactions are the cardiac, hepatic, renal, spleen, and lungs [45].

Table 1: Absolute weight of mice organs after treatment with pure galangin and galangin / β-cyclodextrin for 14 days.

Organs	Controls	Galangin	GAL/βCD
		80 mg kg^{-1}	80 mg kg^{-1}
Kidneys (gm)	0.460± 0.02	0.462± 0.04	0.466± 0.04
Liver (gm)	1.650± 0.08	1.646± 0.07	1.640± 0.05
Spleen (gm)	0.310± 0.02	0.315± 0.03	0.305± 0.05

Lungs (gm)	0.372± 0.02	0.380± 0.02	0.375± 0.10
Thymus (gm)	0.272± 0.03	0.270± 0.02	0.277± 0.06
Heart (gm)	0.212± 0.01	0.217± 0.04	0.218± 0.02

3.5. Blood Parameters Assay

Table 2 explains the systems in the body of all groups and demonstrates hematological parameters. The medications pure galangin and galangin / β-cyclodextrin were tested on mice to see if they caused death, and they were considered safe, non-toxic and no mortality appeared in the end. According to our findings, the median qualities of hematological parameters were altered in mice drinking galangin and galangin / β-cyclodextrin at a dose of 80 mg kg^{-1} body weight. When WBCs are an indicator of the health of the immune system in mice, RBCs, and HGB, it is crucial to know the availability of the natural quantity of oxygen in the mice's blood, and PLT plays a part in monitoring the health status of the mice's blood, which is free of clots and fluidity. When all these parameters were treated with pure galangin and galangin / β-cyclodextrin, no significant changes ($p \geq 0.05$) were seen in WBCs, RBCs, HGB, HCT, and PLT, indicating levels of inhibitory effects when compared to untreated samples. Besides that, these particles are non-inflammatory, non-toxic, non-immunogenic, non-thrombogenic, and can be used to treat a wide range of compounds, including medications, proteins, and nucleic acids [46]. Blood factor evaluation is critical for risk assessment, and alterations in the hematological component have a stronger predictive value for human toxicity when material from animal experiments is translated. Hematological characteristics could be used to determine whether foreign chemicals, such as plant extracts, drugs, or other compounds, have protective or harmful effects on the blood cellular constituents of animals. Because granulocytes and monocytes, as well as humoral elements like agglutinin-, lysozyme-, and metalloid-binding proteins, are thought to be the important ingredients of the generic immune system, any external molecule can influence it [47]. However, the current study's findings show that the ability to prepare inclusion complexes has excellent blood compatibility at small doses and have the potential to be used in a variety of applications, including the therapeutic agent.

Table 2: Hematological parameters of male mice submitted to treatment with pure galangin and galangin / β-cyclodextrin for 14 days.

Blood Picture	**Controls**	**Galangin**	**GAL/βCD**
		80 mg kg^{-1}	**80 mg kg^{-1}**
WBC	7.45±0.35	7.80±1.40	7.15±0.45
LYM%	64.30±0.70	63.85±5.25	61.05±4.75
MON%	4.30±0.20	4.15±3.45	4.65±0.03
GRA%	1.40±0.50	1.30±1.80	1.80±1.70
RBC	5.11±0.48	5.64±0.05	5.27±0.13
HGB	11.30±0.40	10.40±0.50	11.90±0.20
HCT	34.85±3.15	35.40±0.10	35.25±0.05
MCV	49.00±1.00	46.50±0.50	48.50±0.50
MCH	14.55±0.45	14.90±0.70	15.00±0.20
MCHC	29.80±1.60	32.25±1.25	30.95±0.65
PLT	423.00±90	492.00±175	454.5±336.5

RBC: Red blood cells (10^{12}/l); HGB: Hemoglobin (g/dl); HCT: Hematocrit (%); MCV: Mean corpuscular volume (fl); MCH: Mean corpuscular hemoglobin (pg); MCHC: Mean corpuscular hemoglobin concentration (g/dl); WBC: White blood cells (10^9/l); LYM%: Lymphocytes (10^9/l); MON%: Monocytes (%); GRA%: Granulocytes (10^9/l); PLT: Platelets (10^9/l).

4. Conclusions

The study revealed that using the precipitation method, galangin and galangin/β-cyclodextrin were prepared successfully. In addition, tests were conducted to demonstrate the novel construct's bio-viability, and it was found to be anti-oxidant beneficial, secure, and excellent biocompatibility for mice (RBC), with no toxic effects, adverse outcomes, or abnormal movements in the delivered mice.

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Conflict of Interest
Regarding the publication of this manuscript, the researchers have no potential biases.

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