

Evaluation of valoneic acid and the cytostasis properties of *lagerstroemia speciosa* ethanolic leaf extract on the accumulation of MPS in the complete blood count of the albino rats

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Abstract

Microplastics, also referred to as "microbeads," are a versatile kind of plastic that have numerous uses. The most widely used herbicide is glyphosate, which is regarded as non-toxic. However, excessive usage of it on agricultural land has contaminated the rivers and soils. These days, food, water, and soil all contain glyphosate residues. Glyphosate, a harmful substance, is quickly eliminated from humans and can be found in blood and urine, especially in exposed workers. It has severe acute and long-term toxicological consequences as a result. Medicinal plants, rich in bioactive phytochemicals, are crucial for preventing and treating diseases due to their curative efficacy. *Lagerstroemia speciosa*, a plant belonging to the *Lythraceae* family, is referred to as the "Pride of India". A combination derived from *L. speciosa* that includes one or more chemicals chosen from the gallo-tannins, ellagitannins, and valoneic acid dilactones group. The *Lythraceae* family also includes the slightly acidic compound valoneic acid dilactone. The proposed study includes an evaluation of the valoneic acid and cytostasis effects of *L. speciosa* ethanolic leaf extract against MPs-glyphosate-induced albino rats blood profiles. The experimental design includes thirty rats who were randomly divided into five groups of six animals dosing up to 28 days: The first was the control group, which only used normal saline. In the II group, Glyphosate received p.o for 28 days, the group III receives Glyphosate with valoneic acid for 28 days, the IV and V groups are recovery groups, receives low and high concentration of LELE received 200mg/kg/day, p.o for 28 days. The samples of blood are used to evaluate Hematological parameters like RBC, WBC, and platelets.

Keywords: Microplastics; *Lagerstroemia speciosa*; Valoneic acid; Glyphosate; Red blood cells and Albino rats.

1. Introduction

A naturally occurring phenolic chemical is called valoneic acid commonly found in cloves, common walnut, Japanese walnut, the heartwood of *Shorea laevifolia* and other oak species, and a wide range of plant sources [1]. Valoneic acid, a naturally occurring phenolic chemical, forms hydrolysable tannins from plant sources by hydrolyzing stronger tannin molecules. It shares structure similarities with gallic acid [2]. It demonstrates antioxidant activity, which aids in defending cells against oxidative stress and harm brought on by free radicals. Additionally, it has demonstrated anti-inflammatory effects that may suppress pro-inflammatory mediators and enzymes [3]. Valoneic acid has also proven to be an effective antibacterial agent against a variety of microbes. Despite the fact that valoneic acid has been researched for its phytochemical properties and possible health advantages, its precise pharmacological effects and therapeutic uses are still not fully understood [4].

Plastics consist of small monomers polymerized with supplements of additives, such as stabilizers, plasticizers, and pigments [5]. The Ministry of Commerce & Industry, Government of India, the study reveals that during December 2023, India exported plastics worth USD 1,115 million, an increase of 12.7% from USD 989 million in December 2022.

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Microplastics (100 nm - 5 mm) and Nano plastics (1-100 nm) are collectively referred to as Micro and Nano plastics (MNPs). These particles are known to be resistant to degradation, prone to migration, small in size, and possess strong adsorption properties. They are ubiquitous in human living environments. Paper cups often feature an interior lining made of a hydrophobic film primarily composed of polyethylene (PE), with occasional use of copolymer alternative. The biological significance and harmful effects of exposure are included in toxicology.

Glyphosate is an herbicide used in agriculture, forestry, lawn care, and gardening to kill weeds and grasses by inhibiting an enzyme essential for plant growth. It can enter the food web through accidental consumption by organisms that misidentify them as food, because of their small size, leading to cumulative impacts on predators higher up the food chain through bio-magnification [6]. Residues of glyphosate and plant products mixed with water are taken up by animals and humans are largely (60-70%) excreted in their faeces and urine [7-13]. Glyphosate was detected in the urine of up to 96% of farm animals, with a maximum of 164 µg/L [14-16].

The use of cytostatic agents, such as isotretinoin, thalidomide, tamoxifen, and celecoxib, has been investigated in recent years in clinical trials, often combined with cytotoxic drugs. Isotretinoin (13-cis-retinoic acid) is a vitamin A derivative primarily utilized for treating acne. At higher doses, it has demonstrated efficacy in certain contexts. Tamoxifen is an antiestrogenic agent known for its protein kinase C inhibitory properties at high doses. In brain tumor trials, single-agent tamoxifen has shown minimal to modest efficacy [18].

Lagerstroemia speciosa, native to tropical and subtropical Asia, is known as the "Pride of India" and is commonly planted as an ornamental tree in gardens and parks [19]. *L. speciosa* leaves were extracted with aqueous acetone, causing the isolation of seven ellagitannins, ellagic acid, ellagic acid sulphate and four methyl ellagic acid derivatives, including corosolic acid, gallic acid, 4-hydroxybenzoic acid, 3-O-methyl protocatechuic acid, caffeic acid, p-coumaric acid, kaempferol, quercetin and isoquercitrin [20]. Phytochemicals comprising > 100,000 structures are spatiotemporally accumulated in different subcellular compartments of the plants [3]. A composition derived from *L. speciosa* comprising one or more compounds selected from the group consisting of gallotannins, ellagitannins and valoneic acid dilactone [21].

The study investigates the impact of MP concentrations on hematological changes in liver and kidney function, as a proxy for the health effects of microplastics.

2. Materials and methods

2.1. Collection and authentication of experimental plants

The leaves of *L. speciosa* were collected from the PG Girls Hostel, Government Arts College (Autonomous), Coimbatore District, Tamil Nadu, India. The *L. speciosa* was identified and authenticated at the Botanical Survey of India, Tamil Nadu Agricultural University, Coimbatore-03 (No. BSI/SRC/5/23/2020/Tech/53).

2.2. Experimental Plant Extract

The collected samples of *L. speciosa* were observed carefully for any kind of disease or infection; the clean samples from those were isolated for the experiment. The selected plant parts were to be cleaned of dust and any other particles stuck to them. The samples were then kept under the shade at room temperature ($27 \pm 2^\circ\text{C}$) for about 2 weeks until they were completely dry. The dried leaves were powdered with the help of a mixer grinder. Then, 100g of the powder was soaked in 1000 ml of ethanol solvent, stored in an airtight bottle, and kept for 4 days with periodic shaking. The extract was then filtered using Whatman No. 1 filter paper and kept in Petri dishes to dry at room temperature [22]. The 500mg of ethanolic extracts of leaves were given orally after being evenly suspended in 1% carboxymethyl cellulose (CMC).

2.3. Phytochemical Analysis

Qualitative phytochemical analysis of the leaf ethanolic extracts of *L. speciosa* was carried out according to the methodologies of Horbone [23], Trease and Evans [24], and Pavithran and Sujatha [25]. The main group of chemical constituents was identified and listed in the (Table 1).

2.4. Studies on Acute Oral Toxicity

Glyphosate is low in toxicity to rats when ingested. The acute oral LD₅₀ in rats is greater than 4320 mg/kg. A 14-day toxicological investigation was conducted on three groups of six mice, monitoring their behaviour and mortality rates, and continued until safe.

2.5. Experimental Animal

The experiment involved a Wistar strain of healthy adult male albino rats, housed in standard metal cages, maintained at $22\pm 1^\circ\text{C}$, and approved by the Institutional Animal Ethics Committee, KMCH College of Pharmacy, Coimbatore (Approval No. KMCRET/ ReRc/ Ph.D./ 26/ 2021)

2.6. Chemical Purchased

Glyphosate is a non-selective, broad-spectrum systemic roundup herbicide and crop desiccant; it was purchased from Sigma-Aldrich Chemical Company. It is an organophosphorus compound, specifically a phosphonate, which acts by inhibiting the plant enzyme 5-enolpyruvyl shikimate-3-phosphate synthase. The chemical supplier of the medication valoneic acid dilactone (VAD) or valoneic acid (VA) was Sigma-Aldrich. Hydrolysable tannins like mallojaponin contain valoneic acid dilactone, which is one of their constituents.

2.7. Drug Preparation

A disposable syringe of suitable size was used to deliver a single dosage of glyphosate (MPs) orally to each rat. The different doses were as follows: Considering the body weight, the concentration was changed. 10 milliliters per kilogram of body weight was the maximum volume. In order to guarantee a constant volume at all dosage levels, the concentration was changed to limit test volume variability.

2.8. Experimental Design

The first was the control group, which only used normal saline. In the II group, Glyphosate received p.o for 28 days, the group III receives Glyphosate with valoneic acid for 28 days, the IV and V groups are recovery groups, receives low and high concentration of LELE received 250 and 500 mg/kg/day, p.o for 28 days. The animals were given ketamine hydrochloride anesthesia after the treatment period, and blood was collected from the retro-orbital sinus by capillary centrifugation tube for hematological parameters that contains EDTA. The hematological parameters using the following methods: differential cell count, MCV, MCHC, Hematocrit, MCH, platelet count, and percentages of RBC, WBC, and Hb.

2.9. Hematological parameters

The samples of blood are used to evaluate Hematological parameters like RBC, WBC, and platelets.

2.9.1. Enumeration of Red Blood Cells [26]

Blood and RBC diluting fluid were mixed, transferred to a counting chamber, and allowed to settle for 2 minutes. RBCs were counted uniformly in larger corner squares using a 45X objective, and expressed as the number of cells $\times 10^{12}/\text{l}$.

2.9.2. Enumeration of White Blood Cells [27]

A blood sample was mixed with WBC dilution fluid, shaken, and transferred to a counting room. The liquid remained liquid for two minutes, and WBCs were counted uniformly using a 10X objective, expressing the cells per 10 mm.

2.9.3. Enumeration of Hb

Hemoglobin (Hb) was estimated using whole blood. The remaining parameters were measured in serum. All of the above biochemical parameters were estimated using semi-autoanalyzer (Photometer 5010 V5+, Germany) with enzymatic kits procured from Piramal Healthcare Limited, Lab Diagnostic Division, Mumbai, India.

2.10. Identification of Glyphosate Residues

Glyphosate Residues (GR) were qualitatively identified in kidney and liver segments, of varying sizes were sliced and immersed in a 30% hydrogen peroxide solution. These sections were then heated to 70°C for two hours in a hot water bath. The herbicidal residue of glyphosate was identified in the tissue segments. A methodology for characterizing the microplastics was performed using light and transmission electron microscopy at Bharathiar University [28].

2.11. Statistical Analysis

Fundamental statistics, homogeneity of variance, one-way ANOVA, and Tukey and Dunnett tests were used to analyze raw data and compare treatments and control groups.

3. Results

3.1. Phytochemical Analysis

The qualitative phytochemical investigation of *L. speciosa* leaf revealed that it contained steroids, terpenoids, glycosides, polyphenolic compounds, amino acids, saponins, alkaloids, flavonoids, reducing sugars, tannins, and many other active metabolites.

Table 1 Phytochemical Analysis of Ethanolic Leaf Extract of *Lagerstroemia speciosa*

S. No	Phytoconstituents	Leaf extract
1	Alkaloids	++
2	Flavonoids	++
3	Saponins	+++
4	Phenols	+++
5	Tannins	+++
6	Protein and Amino acids	+++
7	Reducing sugar	+
8	Steroids	++
9	Glycosides	+
10	Phytosterols	++
11	Quinones	+
12	Coumarins	++

'+' indicates the presence of Phytoconstituents; '++' indicates the Phytoconstituents present in a moderate level; '+++' indicates the Phytoconstituents present abundantly

3.2. Acute Toxicity Rate

High-dose males exhibited higher liver weight, cataracts, lens abnormalities, and lower urine pH, while low-dose and mid-dose groups lacked side effects.

3.3. Hematological Parameters and biochemical parameters

In the treated groups, the concentration of 10 µg/ml GRs affected neutrophils and lymphocytes, the concentration of 10 µg/ml GRs significantly affected RBCs, HBs, HTs, and monocytes, these concentrations had highly significant effects on neutrophils, lymphocytes and the N/L ratio (Table 2).

Table 2 Hematological Analysis

Group	Control	Glyphosate	Glyphosate +V A	Glyphosate+ Leaf Extract Low Dose	Glyphosate + Leaf Extract High Dose
RBC (X10 ³ /µL)	3.32±1.1	5.57±0.323 ^{ns}	4.96±0.353 ^{ns}	5.39±0.483 ^{ns}	2.29±0.895 ^{ns}
WBC (X10 ³ /µL)	10.1±0.662	13.2±0.868 ^{**}	14.2±0.573 ^{***}	12.1±0.472 ^{ns}	12.6±0.237 [*]
HB (g/dl)	14.5±0.88	15±1.1	13.4±0.571	14.8±0.54	13.8±0.431
PCV (%)	46.1±2.59	45.4±2.45	40.4±1.09	44.4±1.44	41.7±1.52
Polymorphs (%)	5.67±0.882	9.17±1.25	3.5±1.2	6.17±0.601	6.17±0.703

Lymphocytes (%)	83.3±1.54	80.7±1.33	87.7±1.28	83.2±1.33	81.7±1.17
Monocytes (%)	3±0.365	4.33±0.558	4.5±0.428	3.67±0.76	3.17±0.401
Eosinophils (%)	4.17±0.703	4.67±0.422	4.67±0.843	4.83±0.477	4.67±0.667
MCH (Pg)	25.7±0.779	24.2±1.2	24.3±0.738	24.8±0.612	25.6±1.5

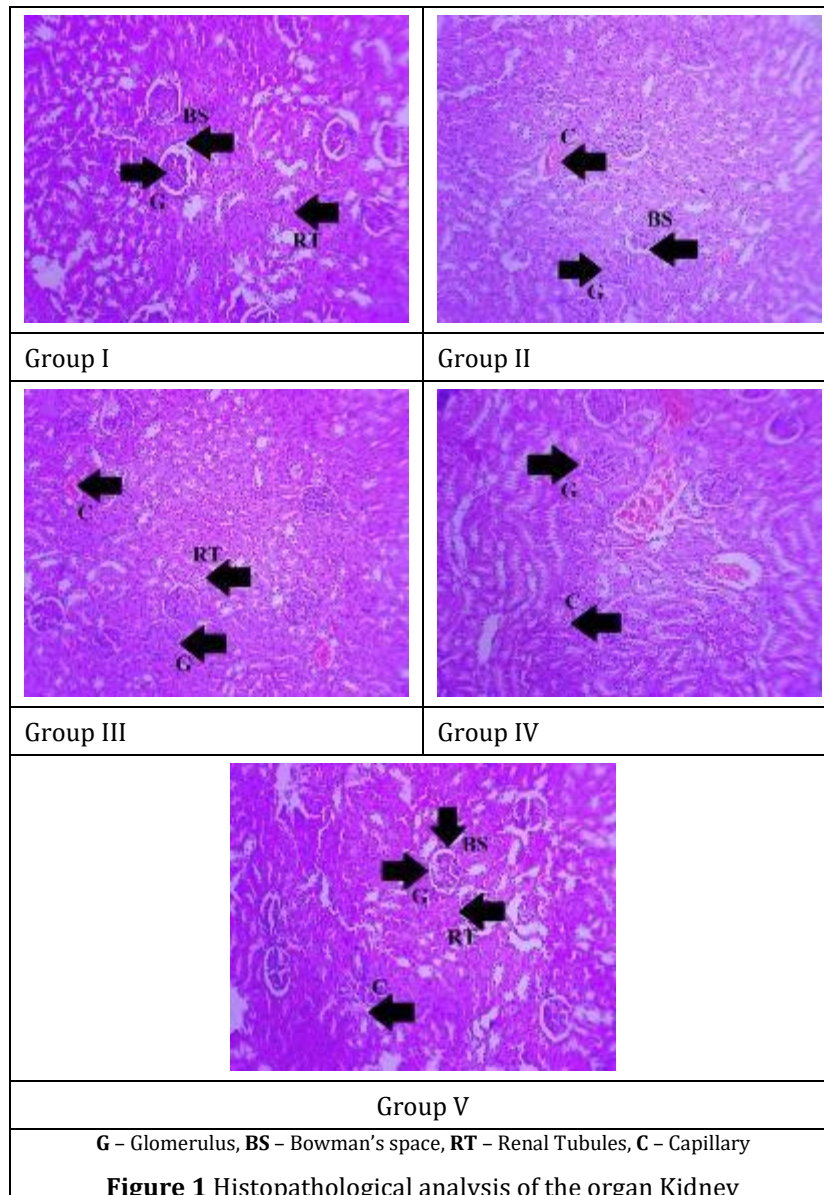
Values are expressed as the mean ± SEM. Statistical significance (P) calculated by one- way ANOVA followed by Dunnett's ^cP<0.001, ^bP<0.01, ^aP<0.05 calculated by comparing treated group with control group

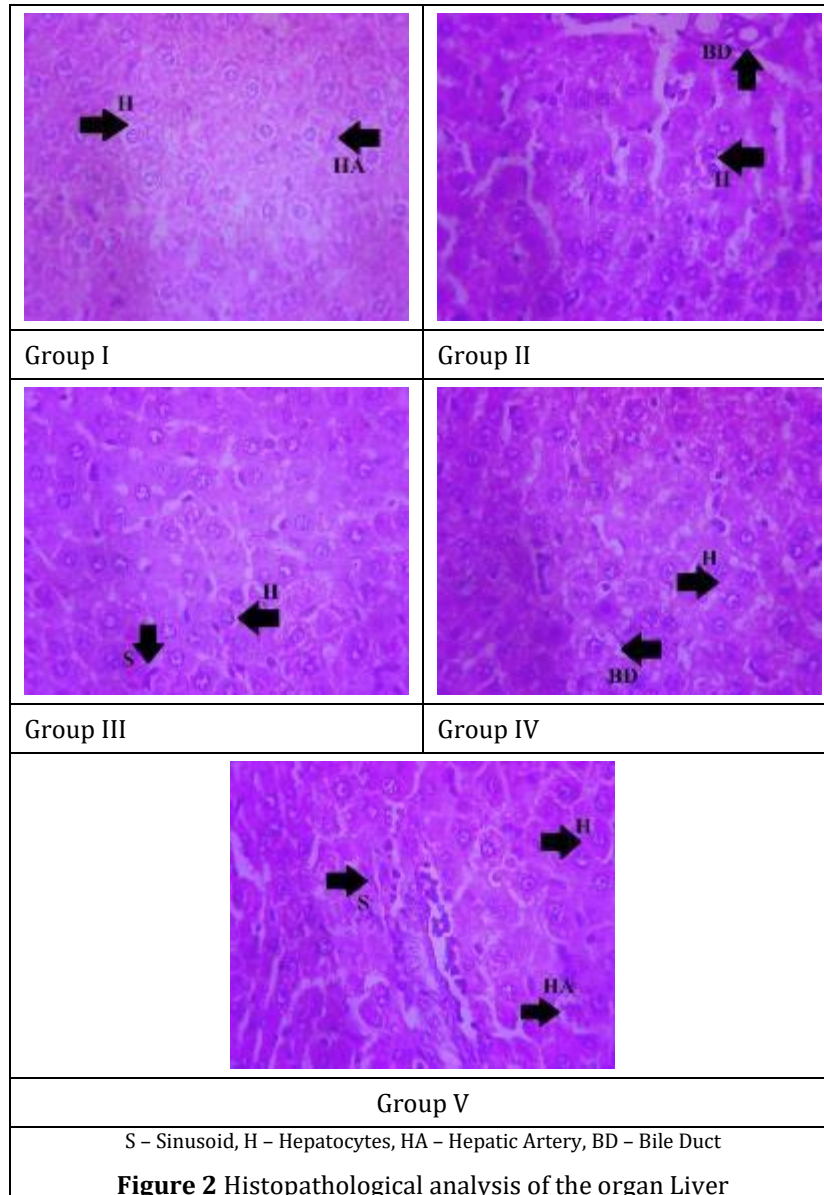
3.4. Identification of Glyphosate Residues

The Glyphosate Residues had irregular forms, as shown by the images captured with a light microscope at a ×40 magnification. The treated groups had higher gut Glyphosate Residues (GR) counts than the control group. GRs were not discovered after the recovery period, in contrast to the exposure groups.

3.5. Histopathological Changes

Mucosal epithelial cells were deteriorated along with the degeneration of hepatic cells. Glomerular degeneration, mononuclear cells infiltration and tubular necrosis were noticed in the kidneys of the rats (Figure 1 and 2).





4. Discussion

A number of harmful effects that might result in fatal illnesses include vascular endothelial damage, thrombosis, decreased immunity, hemolysis, plasma protein denaturation, and blood coagulation. The primary mechanisms of toxicity include inflammation, oxidative stress, cytokine changes, protein corona formation, and cyto and genotoxicity. The transport functions of plasma proteins are impaired by MNPs as they alter their secondary structure.

Micro- and Nano plastics (MNPs) are bound to find their way into the human body. They penetrate the gut epithelium of the digestive system, the lung by the respiratory tract, and the cutaneous layer of the skin barrier and enter blood circulation through ingestion, inhalation, and dermal contact. Numerous reports have been published regarding their toxicity to tissues and organs.

Exposure to toxic substances negatively affects the blood's oxygen-carrying capacity and blood-electrolyte balance, resulting in a decrease in cell size due to the exosmosis of RBCs [29]. Hematological, biochemical, and antioxidant alterations recorded in the animal after exposure to MPs are attributed to their toxic effects; however, little is known about the damage mechanism inside the cells and tissues [28], [30]. Eryptosis and Erythron profiles (poikilocytosis and nuclear abnormalities) of erythrocytes are valuable biomarkers of the toxicity of different chemicals, pharmaceutical residues, and ultraviolet radiation [31-38].

The hematological properties, such as red blood cell (RBC), hematocrit (Ht), and hemoglobin (Hb), are vital indicators for the evaluation of the health status after being exposed to different environmental stresses, bacterial infections, and chemical toxicity [39], [40]. Xu *et al.* [5] reported that the two types of polystyrene nanoparticles (PS-NP25 and PS-NP70) caused an immense increase in apoptosis in human lung epithelial cells.

A complete blood count (CBC) is a standard medical examination that detects and identifies red blood cells, which transfer oxygen and carbon dioxide between cells. The control group shows the RBC ($X10^3/\mu\text{L}$) 3.32 ± 1.1 Group II $5.57\pm 0.323^{\text{ns}}$. The negative group shows the highest count of RBCs compared to the control group because the RBCs are harmed by inflammatory mediators and cytokines, which hasten their clearance. The production of inflammatory mediators and cytokines occurs during the majority of infections. These could disrupt the membrane, change the shape, trigger immunologic clearance, or use other methods to impact RBCs.

A number of RBC alterations have been observed in the groups exposed to MPs, including teardrop-like cells [41], helmet cells (HE), stomatocytes (ST), sickle cells (SI), schistocytes (SC), folded cells (FC) [42], boat-shaped cells (BO), ovalocytes (OV), and echinocytes (EC) [43]. The recovery group shows Group IV $5.39\pm 0.483^{\text{ns}}$ and Group V $2.29\pm 0.895^{\text{ns}}$. The decreased levels of red blood cells and hemoglobin may be caused by a combined extract because of the elevated levels of pro-inflammatory cytokines that cause the reticulo-endothelial system, gastrointestinal tract, and liver to retain iron, which in turn inhibits the production of erythroid precursors [44]. The decreased count of WBC in Group IV and V $12.1\pm 0.472^{\text{ns}}$ & $12.6\pm 0.237^*$. The study's observed considerable drop in WBC could be explained by the suppression of the hemopoietic system, which lowers WBC production [45] and the toxicant's bioconcentration in the kidney and liver [46].

According to Hussein *et al.* [47], GR group variations in HB (15 ± 1.1), PCV (45.4 ± 2.45), MCH (24.2 ± 1.2), values may indicate that macrocytic anemia, which can cause very slow erythroblast production in bone marrow [48], causes these erythroblasts to grow in size and shape and have fragile membranes called megaloblasts, which are indicative of pernicious anemia.

5. Conclusion

The human body is exposed to microplastics through ingestion of food containing microplastics, inhalation of microplastics in the air and by dermal contact of these particles, contained in products, textiles or in the dust [49]. Modelling of polystyrene behavior leads to the estimation of human threshold concentrations in the range of $5.1 - 53.3 \text{ mg g}^{-1}$ of body weight [50]. Microplastics, due to increasing plastic consumption and persistence, interact with tissues on their surface, potentially leading to inflammatory lesions in high concentrations or individual susceptibilities. Valoneic acid is primarily found in the myrtle, *Fabaceae*, *Lytharaceae*, and *Rosaceae* plant families, with various plant sources used to separate it. A compound combination developed from *Lagerstroemia speciosa* that includes one or more compounds chosen from the group that includes ellagitannins, gallotannins, and valoneic acid dilactone. Valoneic acid is a naturally occurring phenolic component that is frequently found in a variety of plant sources. Its classification as a hydrolysable tannin implies that it was formed by hydrolyzing the bigger tannin molecules [51]. Tannins, especially those that have been hydrolyzed, have been used as astringents, tightening and compressing tissues to treat skin conditions, heal wounds, and stop bleeding. A cytostatic agent is a chemical that, without causing death to the cells, inhibits or stops their growth. One of the primary phytocomponents of the *Lytharaceae* family is tannin. The recovery group's cytostasis impact is caused by tannins.

Compliance with ethical standards

"The experiment involving the Wistar strain of healthy adult male albino rats adhered to ethical standards. Ethical approval for the study was obtained from the Institutional Animal Ethics Committee, KMCH College of Pharmacy, Coimbatore (Approval No. KMCRET/ReRc/Ph. D/26/2021)."

Disclosure of conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

Statement of ethical approval

Ethical approval for the use of animals in this study was obtained from the Institutional Animal Ethics Committee, KMCH College of Pharmacy, Coimbatore (Approval No. KMCRET/ReRc/Ph. D/26/2021).

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