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Impact of Microplastics and Antioxidant Effects of *Lagerstroemia speciosa* Ethanolic Leaf Extract against Glyphosate Treated Albino Rats

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Abstract

Humans are exposed to plastic debris via drinking water, contact with food packaging, inhalation of particles, or Disposable paper cups are popular for consuming beverages, seafood, and personal care items. Microplastic (MPs) particle accumulation in humans can lead to acute reactions such as haemolysis, cytotoxicity, hypersensitivity, and an unwanted immune response. Antioxidants promote cell oxidation, maintain redox balance, defend physical and metabolic integrity, and limit medication and illness-related harm. *Lagerstroemia speciosa* is a medicinal and ornamental plant that belongs to the family Lythraceae, commonly known as 'Poomaruthu' in Tamil. The study aims to explore the effects of microplastics on glyphosate-treated albino rats as well as the antioxidant properties of *L. speciosa* ethanolic leaf extract. Group II is the negative control group, Groups IV and V receive the leaf extract, and Group III is given valoneic acid treatment. The control group is given a typical diet. The experiments were carried out for 45 days. The animals were sacrificed, their livers were separated, and their organs were dissected in order to conduct antioxidant research. Determined were the chosen parameters, such as SGOT, SGPT, ALP, urea, uric acid, creatinine, and total Bilirubin. The SGOT was highest in Group II (101±14.3^{ns}) and the experimental group V (253±47.4^{ns}) shows the lowest level of ALP. The level of SGPT was higher in the Control Group (60.8±5.683) and lowest in (46.12±5.508^{ns}) Group V. The result showed that *L. speciosa* is a source of valoneic acid with antioxidant capacity. Research validates the antioxidative and immune-stimulating characteristics of naturally occurring polypeptides, which are vital in controlling the body's redox status and medication-induced harm.

***Key words*:** Microplastic (MPs), Antioxidants, *Lagerstroemia speciosa,* Valoneic acid, Polypeptides, Glyphosate

Microplastics are found in many species intended for human consumption, including invertebrates, crustaceans, and fish [1]. Plastic particles are often found concentrated in an organism's digestive tracts, such that bivalves and small fish consumed whole are more likely to expose microplastics to the human diet [2]. Human health effects depend on exposure concentrations. The total microplastic intake from salts is at most 37 particles per individual annually [3]. Microplastics are often categorized into primary and secondary types. Primary microplastics were originally produced to be < 5 mm in size, while secondary microplastics result from the breakdown of larger items. Microbeads in personal care products are an example of primary microplastics [1]. Plastic products are composed of monomers joined to make the polymer structure and additive chemicals. During production, plastic is processed with additives to provide specific properties. Several thousand distinct additives are used, including plasticizers, flame retardants, pigments, antimicrobial agents, heat stabilizers, UV stabilizers, fillers, and flame retardants such as polybrominated diphenyl ethers (PBDEs).

Glyphosate is one of the most extensively used broad-spectrum organophosphorus herbicides [4]. It is a widely used herbicide in agriculture against perennial and annual weeds and in silviculture, domestic gardens, and urban areas [5]. It is an essential component of non-selective and post-emergent herbicides used to protect the crop from grasses, annual broad-leaved weeds, woody plants, etc. [6]. The parent compound was first sold in 1974 under the trade name "Roundup" by Monsanto. Glyphosate-containing products are acutely toxic to animals, including humans. Symptoms include eye and skin irritation, headaches, nausea, numbness, elevated blood pressure, and heart palpitations. The surfactant used in a common glyphosate product (Roundup) is more acutely toxic than glyphosate itself; the combination of the two is yet more toxic. Glyphosate treatment has reduced populations of beneficial insects, birds, and small mammals by destroying vegetation on which they depend for food and shelter [7].

Microplastics, chemical toxicity, and chronic exposure to microplastics may pose risks to human health, especially with increasing direct exposure to plastic and localized chemicals [1]. The toxicity associated with consuming microplastics is likely dependent on size, associated chemicals, and dose. Antioxidants are responsible for the Défense mechanisms of the organism against the disease associated with the attack of free

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radicals [8]. Thus, the intake of plant derived antioxidants is involved in the prevention of degenerative diseases caused by oxidative stress.

Lagerstroemia speciosa (Lythraceae) is a shrub to large tree with multiple trunks or stems diverging from just above the ground level. The common names of *L. speciosa* are giant crape-myrtle, queen's crape-myrtle, banaba plant for Philippines. It is also known as 'Pride of India'. Banaba is widely distributed in Philippines, India and Malaysia [9]. Valoneic acid a naturally occurring phenolic chemical forms hydrolysable tannins from plant sources by hydrolyzing stronger tannin molecules. It has antioxidant and anti-inflammatory properties potentially aiding in managing inflammatory conditions. The structure and chemical properties of valoneic acid are involved in its phytochemistry. It is a derivative of gallic acid, containing a galloyl group attached to a glucose molecule through an ester bond. Depending on the quantity of lactone rings present, valoneic acid can exist in a wide range of forms, including valoneic acid dilactone and valoneic acid trilactone [10]. The study aims to explore the effects of microplastics on glyphosate-treated albino rats as well as the antioxidant properties of *Lagerstroemia speciosa* ethanolic leaf extract.

MATERIALS AND METHODS

Collection and authentication of experimental plant

The leaves of *L. speciosa* were collected from the P. G. Girls Hostel, Government Arts College (Autonomous), Coimbatore District, Tamil Nadu, India. The *Lagerstroemia 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).

Experimental plant extract

The collected samples of *Lagerstroemia 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± 2ºC) 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, and 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 [11].

Qualitative phytochemical analysis

Qualitative phytochemical analysis of the leaf ethanolic extracts of *Lagerstroemia speciosa* was carried out according to the methodologies of Horbone [12] and Trease and Evans [13-14].

GC-MS analysis

The GC-MS analysis was conducted at The South Indian Textile Research Association, Coimbatore. 1µl of sample powder was injected into a Thermo GC –Trace ultra ver: 5.0, Thermo MS DSQ 11. The chromatography was performed using the DB 35- MS capillary standard non- polar column. Helium flow was 1 ml/min. The oven temperature was increased from 70°C /min to 250ºC. Important compounds were identified in the GC-MS analysis of *L. speciosa* ethanolic extracts of leaf [14].

Acute oral toxicity studies

The leaves of ethanolic extracts were orally fed to the three groups of six animals, and they were observed to check for behavioural changes, if any. The toxicological study was done for 14 days to find out the mortality, if any. It was found to be safe, so the experiment was continued.

Experimental animal

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

Experimental design

The MPs (Glyphosate) were induced in experimental animals except in the control group. After induction, the experimental animals were divided into 5 groups of 6 animals each (n=6). Group I, is the control (untreated group). Group II, is the negative control receiving Glyphosate only. Group III, is receiving Glyphosate and treated with Valoneic acid. Group IV and V, received glyphosate and treated with *L. speciosa* ethanolic leaf extracts at low doses (250 mg/kg) and high doses (500 mg/kg).

Group I - Control
Group II - Glyphosate
Group III - Glyphosate with valoneic acid
Group IV - Glyphosate + Leaf extract LD (250 mg/kg)
Group V - Glyphosate + Leaf extract HD (500 mg/kg)

Biochemical parameters

At the end of the experiment, the blood samples, each of 10 ml, were withdrawn from the jugular vein of each animal into vacuum glass tubes containing no anticoagulant. Following standing at room temperature for 20 min, the tubes were centrifuged at 3,000 rpm for 10 min, and the serum samples were stored at -25°C until the biochemical tests were performed.

In vivo antioxidant activity

The study analyzed antioxidants activities like SGOT, SGPT [15], ALP [16], and Urea, Uric acid, Creatinine [17], and total bilirubin [16].

Statistical analysis

One-way ANOVA and Dunnett's test were used to analyze the data, with statistical significance calculated using the mean ± SEM. Data were statistically analyzed using the software GraphPad Prism 5.0 version.

RESULTS AND DISCUSSION

Result - 1: Quality control

The qualitative phytochemical analysis of *L. speciosa* ethanolic leaf extracts revealed the presence of the following phytocompounds (Table 1-2).

Result – II: Acute toxicity studies

Administration of ethanolic leaf, flower, and seed extracts of *L. speciosa* at a dosage of 500 mg/kg to rats throughout an acute toxicity test indicated no toxicity, mortality, or morbidity, and there were also no significant changes in behaviour or gait.

Result – III: Antioxidant activity

The blood samples were collected from the experimental animals. Determined were the chosen parameters, such as

SGOT, SGPT, ALP, urea, uric acid, creatinine, and total Bilirubin. The serum glutamic-oxaloacetic transaminase (SGOT) was highest in Group II (101 ± 14.3 ns) and the experimental group V (253 ± 47.4 ns) shows the lowest level of ALP. The level of SGPT was higher in the Control Group (60.8 ± 5.683) and lowest in (46.12 ± 5.508 ns) Group V. The result showed that *Lagerstroemia speciosa* is a source of valoneic acid with antioxidant capacity.

Table 1 Phytochemical analysis of ethanolic leaf extract of *Lagerstroemia speciosa* [14]

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

Table 2 Compounds identified in the GC-MM analysis of *Lagerstroemia speciosa* [14]

S. No.	Compound name	Molecular formula	Area percent (%)
1	Cholesterol	$C_{27}H_{46}O$	84.29
2	Cholesterol, 7- oxo-	$C_{27}H_{44}O_2$	3.42
3	Lucenin- 2	$C_{27}H_{30}O_{16}$	1.33
4	Hexadecanoic acid, ethyl ester (CAS)	$C_{18}H_{36}O_2$	1.08
5	Betulin	$C_{30}H_{48}O_3$	0.84
6	6-Octadecanoic acid	$C_{18}H_{34}O_2$	0.83
7	1-Hexadecanoic, acetate (CAS)	$C_{18}H_{36}O_2$	0.79
8	1-Octadecanol (CAS)	$C_{18}H_{38}O$	0.73
9	Hexadecanoic acid, methyl ester (CAS)	$C_{17}H_{34}O_2$	0.72
10	Lucenin-2	$C_{27}H_{30}O_{16}$	0.58

Table 3 Antioxidant activity of *Lagerstroemia speciosa* ethanolic leaf extract

Group	Control	Glyphosate	Glyphosate + VA	Glyphosate + L. Ext L.D	Glyphosate + L. Ext H.D
SGOT (u/l)	75±5.18	101±14.3^{ns}	78±5.08^{ns}	79.1±1.64^{ns}	77.8±3.13^{ns}
SGPT (u/l)	60.8±5.683	49.62±4.747^{ns}	50.85±8.576^{ns}	60.1±3.977^{ns}	46.12±5.508^{ns}
ALP (u/l)	289±14.8	292±23.5^{ns}	271±14.2^{ns}	254±7.19^{ns}	253±47.4^{ns}

Values are expressed as the mean ± SEM
Statistical significance (*P*) calculated by one way ANOVA followed by Dunnett's $^{c}P< 0.001$, $^{b}P < 0.01$, $^{a}P < 0.05$ calculated by comparing treated group with control group

Table 4 Activity of urea, uric acid, creatinine and total bilirubin

Group	Control	Glyphosate	Glyphosate + VA	Glyphosate + L. Ext L.D	Glyphosate + L. Ext H.D
Urea (mg/dl)	31.4±3.64	30±2.17^{ns}	32.1±2.09^{ns}	28.7±1.5^{ns}	27.5±0.739^{ns}
Uric acid (mg/dl)	1.38±0.142	0.89±0.153*	1.51±0.0962^{ns}	1.47±0.11^{ns}	1.27±0.112^{ns}
Creatinine (mg/dl)	0.357±0.0196	0.223±0.0296^{ns}	0.3±0.107^{ns}	0.3±0.0297^{ns}	0.335±0.0255^{ns}
Total bilirubin (mg/dl)	0.617±0.0499	0.763±0.0812^{ns}	0.845±0.0302*	0.92±0.0442**	0.653±0.0431^{ns}

Values are expressed as the mean ± SEM
Statistical significance (*P*) calculated by one way ANOVA followed by Dunnett's $^{c}P< 0.001$, $^{b}P < 0.01$, $^{a}P < 0.05$ calculated by comparing treated group with control group

Plants produce bioactive phytocompounds, including phytosterols, polyphenols, indoles, glycosylates, carotenoids, and saponins, which provide antioxidant qualities to food and plants. Blue berries may have the highest antioxidant capacity of all the popular fruits and vegetables. It contains tannins and flavonoids [18-21]. The Ingested microplastics can cause inflammation, DNA damage, and potentially promote cancer. Free radicals in small and controlled quantities are useful in everyday metabolism [22]. They take part in several normal reactions within the body including breathing. These free

radicals are mainly produced during oxygen metabolism within the cells. The problem starts when the production of these free radicals increases and goes out of control. These free radicals attack important macromolecules leading to cell damage and homeostatic disruption [23]. The defence mechanisms against these are inactivating them within the cells soon after production, removing them by scavenging antioxidants and increasing the elimination of material already damaged by free radicals. The term antioxidant refers to chain-breaking compound.

The leftover plastic debris, gradually degrade into minute fragments with a diameter of less than 5 mm, known as MPs. The microplastic concentration in food (Ci) is also a complex metric. Microplastics were also isolated from various processed foods. They were investigated in liquids such as beer [24-25], honey [26-28], and milk [29]. The concentrations ranged from n.d. to several hundred particles per liter [24-29]. The major ways that MNPs enter the human body are through food and drinking water. Human faeces contain a significant amount of MNPs, which indicates that food consumption is the primary route through for MNPs to enter the human body [30-31]. Humans are ideal omnivores, and the enrichment effect of the food chain can add harmful substances to the body besides nutrients, such as MNPs, heavy metals, hazardous organic compounds, etc. Numerous studies have shown that the magnetic nanoparticles (MNPs) from environmental pollution have entered the lower animals and plants in the human food chain [32].

Magnetic nanoparticles (MNPs) were used to treat lung macrophages and alveolar cells in in vitro cell studies, and it was found that these MNPs had toxic effects on lung cells [33-34]. Understanding these mechanisms can help in designing safer magnetic nanoparticles for biomedical applications, ensuring minimal adverse effects on lung cells and improving their therapeutic potential. The toxicity associated with consuming microplastics is likely dependent on size, associated chemicals, and dose. The potential negative effects of microplastics in the human body, focusing on pathways of exposure and toxicity. The phytocompounds of *Lagerstroemia speciosa* contains tannins in the form of quercetin, is quercetin have antioxidant properties.

CONCLUSION

Antioxidants promote cell oxidation, maintaining redox balance, defending physical and metabolic integrity, and limiting medication and illness-related harm Exposure may occur by ingestion, inhalation and dermal contact due to the presence of microplastics in products, foodstuff and air. The plant based natural antioxidants are assumed to counteract the harmful effects of ROS and therefore prevent or treat oxidative stress - related disease.

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