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Original Research Article

Hepatoprotective effects of *Curcuma longa* and *Phyllanthus emblica* co-treatment in polypropylene microplastic-exposed male Wistar rats

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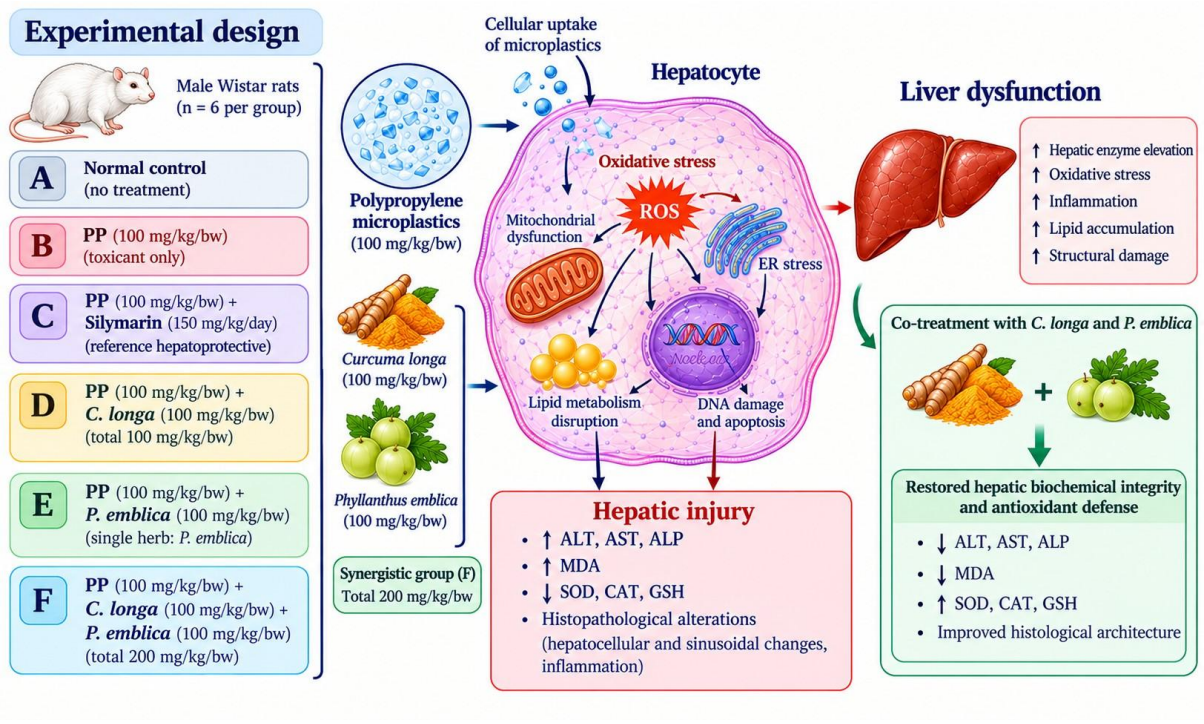
Abstract: Polypropylene microplastics are becoming a contaminant of the environment with potential effects on hepatic function and metabolic homeostasis. The study of *Curcuma longa* and *Phyllanthus emblica* for liver protection may yield valuable insights into the potential of plant-based approaches for maintaining liver health in the context of microplastic exposure. In this study, the effects of individual and combined treatment with *Curcuma longa* and *Phyllanthus emblica* were compared in male albino Wistar rats fed with polypropylene (PP) microplastics on the liver. Normal control (A), PP alone (B), PP with Silymarin (C), PP with *C. longa* (D), PP with *P. emblica* (E), and PP with both botanicals (F) were the six groups of six rats. The protocol used PP at 100 mg/kg/day, silymarin at 150 mg/kg/day and each botanical at 200 mg/kg/day. The mean ± SD of serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and hepatic redox parameters were summarized for n = 6 rats per group. Qualitative assessment of five group coded liver accessions. Mean ALT, AST, and ALP activities were 68.3, 240.8, and 493.3 U/L in B, compared with 32.1, 125.5, and 245.0 U/L in A. Corresponding means in F were 37.4, 152.3, and 302.8 U/L, representing reductions of 45.2%, 36.8%, and 38.6% relative to B. The PP-exposed groups had the best response to the combined botanical treatment, with ALT, AST, and ALP levels reduced by 45.2%, 36.8%, and 38.6%, respectively, compared to PP alone. It also had the highest SOD, CAT and GSH values and the lowest MDA value compared to the other PP-exposed groups, and means that were closer to the normal-control profile. Histopathological examination showed that the treatment-induced changes in the hepatocellular organization, sinusoidal and vascular architecture, and inflammatory features were observed in all experimental groups, which was consistent with the biochemical response. All general behaviour was normal throughout observation. The results suggest that co-treatment with *C. longa* and *P. emblica* may be the most effective treatment for maintaining hepatic biochemical integrity and antioxidant defense in vivo under PP microplastic exposure.

Keywords: *P*olypropylene microplastics, *Microplastic toxicity, Curcuma longa*, *Phyllanthus emblica*, *Hepatoprotection, Oxidative Stress.*

Research Highlights:

- ❖ The exposure to polypropylene microplastics caused liver damage and oxidative stress in male Wistar rats.
- ❖ The combined use of *Curcuma longa* and *Phyllanthus emblica* enhanced the liver enzyme levels and antioxidant status.
- ❖ The combination was the most effective treatment, improving the liver architecture and biochemical parameters.

Graphical Abstract:



1. Introduction

Particles can be released from a broad range of consumer materials, such as polypropylene (PP), which is relevant to the study of oral microplastic exposure. Li et al. [1] found that microplastic leaching was observed from PP infant-feeding bottles during the process of preparing formula, and the leaching amount was affected by the processing conditions. Human biomonitoring offers alternative evidence of exposure: Schwabl et al. [2] detected several polymers, including PP, in stool, while Leslie et al. [3] measured selected plastic polymers in blood, but not PP. These results provide a basis for continued experimental studies specific to

the polymer, but do not prove that a laboratory exposure regimen is equivalent to an exposure regimen in humans.

The liver offers a chance to study biochemical markers in the circulation, as well as the architecture and redox status. Lipidomics and Raman spectroscopy were used to assess the changes in lipid profiles and lipid-droplet characteristics in mice exposed to PP [4]. However, responses depend on the material and exposure conditions. Kim et al. [5] reported that fragmented and weathered PP particles were tolerated in a four-week repeated oral study in Sprague-Dawley rats at doses up to 25 mg/kg/day. Cellular studies likewise indicate that particle size, concentration, dispersion, and polymer identity influence biological responses [6, 7].

Particle characterization is essential to this distinction. Size-dependent oxidative and mitochondrial effects reported for polystyrene particles illustrate the need to measure particle dimensions rather than transfer size–toxicity relationships between polymers [8]. Simulated-digestion experiments also distinguish initial material properties from particle characteristics after gastrointestinal passage [9]. The present exposure material was commercially designated PP, with a nominal size interval of 36–116 μm. Supplier descriptions are retained as nominal properties rather than treated as independently verified composition or size measurements, consistent with the reporting principles described by Cowger et al. [10].

Botanical interventions offer a means of investigating whether biochemical responses to particle exposure are accompanied by changes in liver morphology. *Curcuma longa* and *Phyllanthus emblica* have been studied in experimental hepatic injury, although interpretation depends on plant part, extraction method, administered preparation, dose, and injury model. In carbon tetrachloride-induced injury, purified curcumin was associated with biochemical, antioxidant, and histological responses and changes in Nrf2/HO-1 and TGF-β1/Smad3 signaling [11]. Joshi et al. [12] also assessed the hepatoprotective effect of *C. longa* extract and curcumin in mercuric chloride-induced liver damage, which included oxidative endpoints and

CYP2E1 activity. The studies back a rationale for preparation instead of assuming that the mass of an aqueous botanical preparation equals a dose of purified curcumin.

There is also evidence for *P. emblica* in different preparations and biological contexts. A fruit preparation (aqueous) was used in a high-fat-diet model to modify hepatic steatosis, antioxidant enzyme activities and lipid-regulatory markers [13] and a hydroalcoholic bark extract was investigated in ethanol-induced injury, compared with silymarin [14]. In aged rats, Amla preparations were found to be associated with lower lipid and oxidative-stress indices [15] and in alcohol-induced hepatic injury, *Emblica officinalis* fruit extract was studied [16].

In vitro characterization of authenticated *P. emblica* and *C. longa* powdered materials was performed to compare the phenolic and flavonoid content, radical-scavenging activity and GC–MS profiles [17]. Similarly, the protective effects of ellagic acid reported during polystyrene nanoplastic exposure in rats support investigation of polyphenol-related interventions but cannot be transferred directly to aqueous botanical preparations or PP microparticles [18].

Serum enzymes, hepatic redox measurements, and histopathology describe related but non-equivalent aspects of liver injury. The diagnostic performance of traditional hepatobiliary biomarkers varies with lesion type in rat toxicology studies [19]. An integrated assessment is therefore preferable to interpreting an enzyme reduction as evidence of complete tissue recovery. Combining two botanical preparations also increases total preparation exposure; a greater numerical response does not, by itself, establish pharmacological synergy, which requires a defined reference model of combined action [20].

With research on the biological effects of microplastic exposure and the possible advantages of plant-derived interventions are increasing, the hepatic effects of polypropylene (PP) microplastics and the potential protective role of combined *Curcuma longa* and *Phyllanthus emblica* treatment are still insufficiently characterized. The association between treatment-induced biochemical changes and the corresponding hepatic histopathological changes warrants further investigation. In the current study, it was hypothesized that the combined

treatment of *C. longa* and *P. emblica* would be associated with more favourable hepatic biochemical and histological profiles in PP microplastic-exposed male Wistar rats. The present study was therefore undertaken with the aim to characterize hepatic responses to PP microplastic exposure and to evaluate the effects of individual and combined botanical treatments compared to adequate control and reference-treatment groups. The study aimed to integrate biochemical and histopathological findings to obtain a more holistic view of the potential role of these botanical interventions in modulating microplastic-induced hepatic alterations.

2. Materials and Methods

The exposure material was a specially prepared commercial microplastic sample provided by Green Nano Enterprises, Udaipur, Rajasthan, India and labelled as PP. The protocol called for a nominal particle-size range of 36-116 µm and a PP dose of 100 mg/kg body weight/day.. *C. longa* and *P. emblica* were collected from Chandwaji, Jaipur, Rajasthan, India and botanically authenticated in the University of Rajasthan, Jaipur. The documented preparation sequence included washing, oven drying, powdering and aqueous extraction. The dose of each botanical was indicated as 200 mg/kg/day and the combination as 100 mg/kg/day of each botanical, summing-up to 200 mg/kg/day. The doses are based on the amount of preparation, not the amount of marker compounds. The parts of the plant, voucher accession number, extraction conditions, yields and dose-batch composition must be confirmed from the original preparation records. Patnaik et al. [17] have conducted separate studies of the phytochemicals and GC–MS, which is not considered as characterization of the dosed batches.

The study involved 36 male albino Wistar rats which were grouped into six groups of 6 rats each. Group A was normal control, B was given PP only, C was given PP and silymarin, D was given PP and *C. longa*, E was given PP and *P. emblica* and F was given PP and both botanicals. The group assignments and nominal daily doses are provided below. The serum enzyme and hepatic redox results are from n = 6 rats in each of the six groups. Qualitative histopathology

was available as five group coded accessions (A, B, D, E, and F). The study was approved by the Institutional Animal Ethics Committee (IAEC) of NIMS University, Jaipur, Rajasthan, India (NIMS/NIOP/IAEC/26-02-21). Animals were purchased from Rodent Research India Pvt. Ltd., Dhatrath, Jind, Haryana, India. Shipment documentation indicated an age at dispatch of 4-5 weeks and a body mass of 50-70g. These values do not replace the enrolment or terminal age and body mass data that must be kept on the animal level. The experimental allocation of 36 rats is separate from the 48 male albino rats used for ethical authorization, which were kept for 12 months. The housing protocol consisted of polypropylene cages with 4-6 animals per cage, 12 hours light/dark cycle, temperature of 22 ± 3 °C, relative humidity of 50-60%, standard chow and filtered water ad libitum and seven days of acclimatization. Monitoring plan consisted of body mass, food and water intake, behaviour, coat condition, lethargy, diarrhoea and mortality/moribund status. Proposed humane endpoints were more than 20% body-weight loss or severe lethargy. Qualitative behaviour is reported in Section 3.1, The distinction of protocol specifications and completed observations is reported [21]. Experimental groups and nominal daily doses were as follows: Group A, normal control (PP 0 mg/kg/day; normal feed + water; n = 6); Group B, PP only (PP 100 mg/kg/day; vehicle; n = 6); Group C, PP + silymarin (PP 100 mg/kg/day; silymarin 150 mg/kg/day; n = 6); Group D, PP + *C. longa* (PP 100 mg/kg/day; *C. longa* 200 mg/kg/day; n = 6); Group E, PP + *P. emblica* (PP 100 mg/kg/day; *P. emblica* 200 mg/kg/day; n = 6); and Group F, PP + combined botanicals (PP 100 mg/kg/day; *C. longa* 100 mg/kg/day + *P. emblica* 100 mg/kg/day; n = 6). Dosages are given as mg/kg body weight/day. Total botanical preparation is 200 mg/kg/day in combination. The six rats per group (36 rats total) in the serum enzyme and hepatic redox summaries were representative. PP, polypropylene; vehicle, 0.5% w/v CMC-Na (as per administration protocol).

The administration protocol included fresh suspensions of 0.5% w/v sodium carboxymethylcellulose (CMC-Na) which were prepared just prior to oral gavage once daily, based on body weight. The nominal administration volume was 1 mL/100 g body weight (10

mL/kg); another annotation was 1-2 mL/animal/day. The concentration of the formulation, total volume of gavage and sequence and spacing between agents must be checked against dispensing records. The recommended dose of silymarin was 150 mg/kg/day. The experimental dose of 140 mg per tablet is not the body weight adjusted dose but is a formulation label. The principal protocol called for sampling on day 61 after 60 days of treatment; the other protocol called for 45 days of treatment and sampling on day 46. The terminal-procedure consisted of deep anaesthesia with ketamine (80 mg/kg i.p.) and xylazine (10 mg/kg i.p.), retro-orbital or cardiac blood collection (2–3 mL) and cervical dislocation. Other annotations included: isoflurane, sevoflurane, and retro-orbital collection. Original terminal records are required for the anesthetic used, dose or concentration, route of collection, method of euthanasia and confirmation of death.

The activities of ALT, AST and ALP were reported as IFCC assay and in U/L, with ALT being equivalent to SGPT and AST being equivalent to SGOT. The sample preparation protocol called for a 12 hour fast with water, collection into plain tubes, clotting for 30-45 minutes at room temperature, and centrifugation at 3,000 rpm for 10 minutes at 4 °C. Storage conditions were at −20 °C for short term storage and at −80 °C for longer term storage. Laboratory reference intervals were ALT 20–45 U/L, AST 94–190 U/L, and ALP 140–420 U/L. Concurrently normal-control group A was used for descriptive normalization instead of a reference-interval boundary. Total bilirubin and total protein were protocol-specified, and albumin was included in the wider plan. The biochemical assessment included the following enzymes: hepatic SOD, CAT, GSH, and MDA, and a 10% liver-homogenate preparation was used in the protocol. Data are presented as mean ± SD for six rats per group. The activities of SOD and CAT are expressed in U/mg protein, GSH in μmol/g tissue and MDA in nmol/mg protein as shown in Table 4.

Liver specimens were qualitatively evaluated for hepatocellular organization and degeneration, sinusoidal and vascular changes and infiltration of inflammatory cells. The

protocol for processing involved 3-5 mm thick tissue slices, fixation in 10% neutral buffered formalin for 48 hours, paraffin embedding, 5 μm sectioning, and hematoxylin and eosin (H&E) staining. Confirmation of batch specific processing, anatomical sampling, observer masking and original grading records are required. Detailed microscopy descriptions and overall pathological impressions will be reported separately. Five liver accessions and 19 image fields were available. The comparisons are given in overview and detail in Figures 5 and 6, and in full in Figures 7-11. The original photomicrographs were 1792 × 1374 pixels. Images are kept intact and in their original aspect ratio, and provided composites were resized and lossless encoded without selective cropping, color balancing or contrast adjustment. The qualitative accession level results are therefore separated from the n = 6 biochemical group summaries.

The experimental unit was the rat, not individual analytes, repeated assay readings, or individual histological fields [23]. The levels of serum ALT, AST, ALP and hepatic SOD, CAT, GSH and MDA were summarized as mean ± SD for six rats in each of the group's A–F. Treatment comparisons were based on means of the groups of 6 rats, rather than on individual profiles. X represents a group mean, A the normal-control mean, B the PP-only mean, and T a botanical-treatment mean for each serum analyte. These were the following descriptive expressions used:

Control-normalized activity = X/A.

Absolute difference (U/L) = T − B.

Treatment-to-exposure ratio = T/B.

Relative reduction (%) = 100 × (B − T)/B.

For comparisons of the combination F with an individual-botanical group K (D or E), the absolute difference was F − K and the relative reduction was 100 × (K − F)/K. Absolute differences are expressed in U/L, ratios to two decimal places, and percentages to one decimal place using round-half-up rounding at presentation. Negative differences indicate lower treatment-group mean activities. Tables 2 and 3 contain these descriptive comparisons. Between-group inferential tests. Figures 1–4 retain the means and SDs [24]. The numerical

outcome scope comprises serum enzyme activities and hepatic redox parameters, complemented by qualitative behaviour and liver histopathology. Other planned domains included serial body and liver weights, food and water intake, additional serum analytes, qualitative phytochemical screening, total phenolic and flavonoid assays, tannin and ascorbic-acid assessments, chemical profiling, and DPPH/ABTS radical-scavenging assays.

3. Results

3.1. Animal serum liver profile: measured enzyme activities

Exposure to polypropylene (PP) microplastics increased the mean serum activities of ALT, AST and ALP as compared with the normal-control group (Table 1). The mean activities of ALT, AST, and ALP were 68.3, 240.8 and 493.3 U/L in the PP-only group and 32.1, 125.5 and 245.0 U/L in the normal-control group. These values were 2.13-, 1.92- and 2.01-fold increases over the respective control means. The group with the lowest mean activities among the treatment groups is the combination of the botanicals, where the ALT, AST, and ALP mean activities are 37.4, 152.3, and 302.8 U/L, respectively. In contrast, the means for the silymarin treatment group (C) are 41.0, 162.7, and 326.0 U/L, those of the *Phyllanthus emblica* (E) group are 46.2, 178.2, and 361.2 U/L, and those of the *Curcuma longa* (D) group are 52.3, 197.2, and 395.3 U/L. Consequently, the order of mean activities from highest to lowest is PP-only (B) > *C. longa* (D) > *P. emblica* (E) > silymarin (C) > botanical combination (F) > normal control (A) for the three enzymes. This order is just a description since no statistical analysis is done. These results are detailed in Tables 1 and Figure 1-4.

Table 1. Animal serum liver profile: mean ± SD activities.

Group / condition	Analysed serum n	ALT (U/L)	AST (U/L)	ALP (U/L)
A / Normal control	6	32.1 ± 1.9	125.5 ± 4.9	245.0 ± 9.7
B / PP toxicity control	6	68.3 ± 2.9	240.8 ± 9.8	493.3 ± 15.0
C / Silymarin + PP	6	41.0 ± 2.0	162.7 ± 4.9	326.0 ± 8.4
D / *C. longa* + PP	6	52.3 ± 2.0	197.2 ± 5.4	395.3 ± 10.3
E / *P. emblica* + PP	6	46.2 ± 1.4	178.2 ± 3.9	361.2 ± 7.9
F / *C. longa* + *P. emblica* + PP	6	37.4 ± 1.2	152.3 ± 4.3	302.8 ± 6.6

Numeric entries are mean ± SD in U/L, with n = 6 rats per group.

Assay designation: IFCC. Laboratory reference intervals: ALT, 20–45 U/L; AST, 94–190 U/L; ALP, 140–420 U/L. ALT/SGPT, alanine aminotransferase; AST/SGOT, aspartate aminotransferase; ALP, alkaline phosphatase. Total bilirubin, total protein, and albumin require additional measurements and are not assigned numerical concentrations here.s

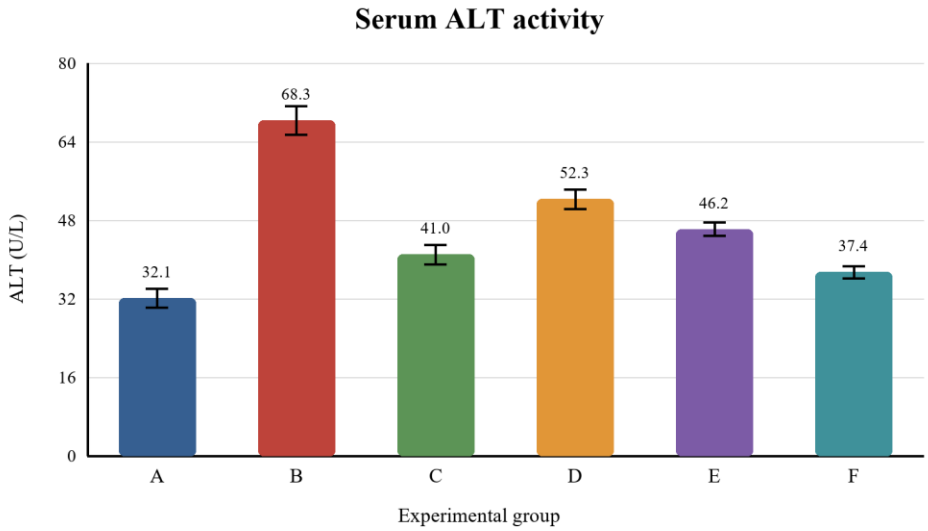


Figure 1. Serum ALT activity across groups A–F.

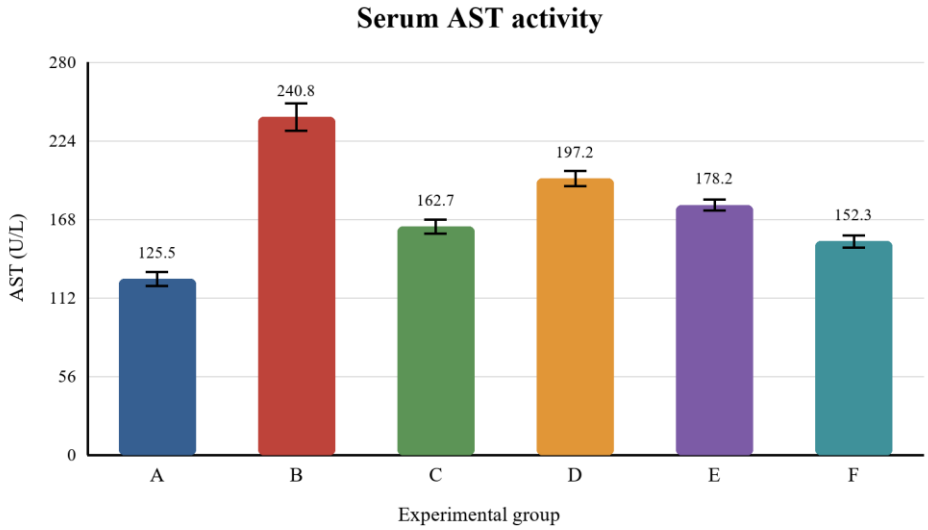


Figure 2. Serum AST activity across groups A–F.

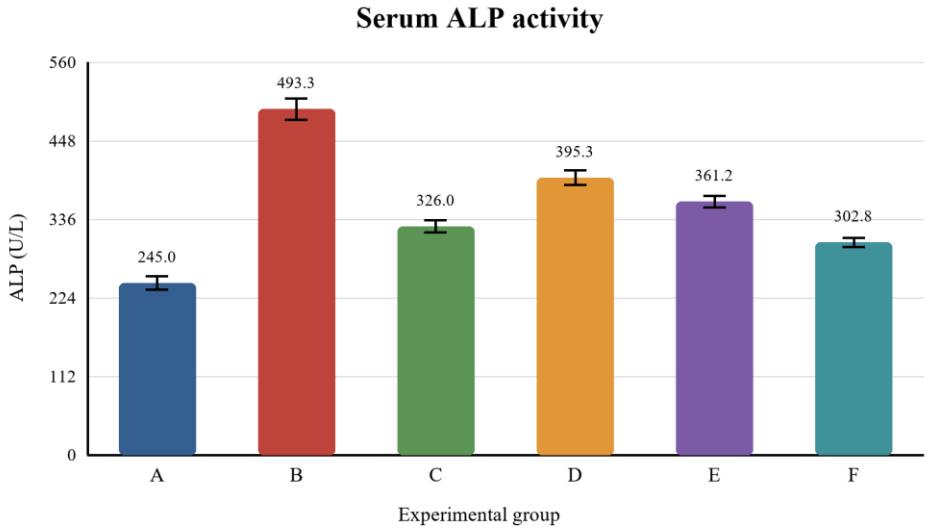


Figure 3. Serum ALP activity across groups A–F.

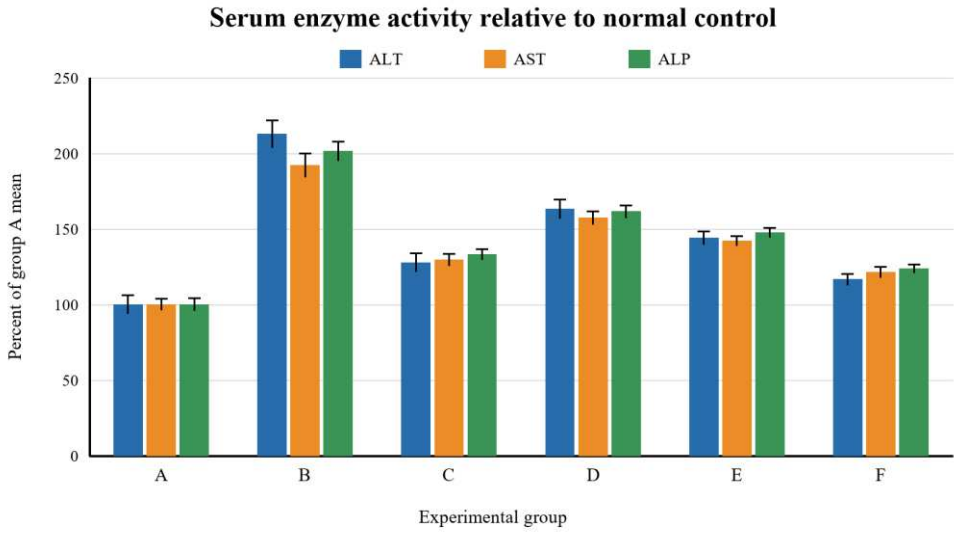


Figure 4. Comparative serum ALT, AST and ALP activities as percentages of the normal-control group A mean.

3.3. Descriptive treatment comparisons

The ALT was 23.4% lower in profile D compared to B and AST was 18.1% lower in profile D compared to B. Profile E showed 32.4%, 26.0%, 26.8% lower ALT, AST and ALP, respectively. Profile F showed 45.2%, 36.8%, 38.6% lower ALT, AST and ALP, respectively (Table 2). The values represent differences between group mean profiles at the endpoint recorded.

The original activity scale differences for ALT, AST and ALP, respectively, were: D-minus-B, -16.0, -43.6, -98.0 U/L; E-minus-B, -22.1, -62.6, -132.1 U/L; and F-minus-B, -30.9, -88.5,

-190.5 U/L. The treatment-to-exposure ratios for F were 0.55, 0.63, 0.61 for ALT, AST and ALP (Table 2). These calculations describe the observed group mean contrasts.

The activities of the combined treatment were approximately 1.17, 1.21, 1.24-fold of the control ALT, AST and ALP, respectively. The values for all analytes in F were lower than the corresponding means in the individual botanical groups. Its ALT was 28.5% lower than D and 19.0% lower than E; its AST was 22.8% lower than D and 14.5% lower than E. Table 3 shows the absolute and relative contrasts; F-profile ALP was 23.4% lower than D and 16.2% lower than E.

Table 2. Absolute and relative liver-enzyme contrasts against polypropylene alone.

Botanical regimen	**Analyte**	**Difference T − B (U/L)**	**Activity ratio T/B**	**Relative reduction (%)**
PP + *C. longa* (D)	ALT	-16.0	0.77	23.4
PP + *C. longa* (D)	AST	-43.6	0.82	18.1
PP + *C. longa* (D)	ALP	-98.0	0.80	19.9
PP + *P. emblica* (E)	ALT	-22.1	0.68	32.4
PP + *P. emblica* (E)	AST	-62.6	0.74	26.0
PP + *P. emblica* (E)	ALP	-132.1	0.73	26.8
PP + combination (F)	ALT	-30.9	0.55	45.2
PP + combination (F)	AST	-88.5	0.63	36.8
PP + combination (F)	ALP	-190.5	0.61	38.6

T denotes the botanical-treatment activity and B the polypropylene-only activity. Absolute difference = T − B (U/L); ratio = T/B; relative reduction = 100 × (B − T)/B. Inputs are the mean activities in Table 1. Ratios and percentages are rounded to two and one decimal places, respectively. Negative differences denote lower treatment activities. Each contrast compares group means and carries no estimate of contrast uncertainty or statistical significance.

Table 3. Absolute and relative contrasts of combination versus individual-botanical activities.

Comparison	Analyte	F activity (U/L)	Comparator activity (U/L)	Difference F − K (U/L)	Relative reduction (%)
F versus D	ALT	37.4	52.3	-14.9	28.5
F versus D	AST	152.3	197.2	-44.9	22.8
F versus D	ALP	302.8	395.3	-92.5	23.4
F versus E	ALT	37.4	46.2	-8.8	19.0
F versus E	AST	152.3	178.2	-25.9	14.5
F versus E	ALP	302.8	361.2	-58.4	16.2

K denotes the single-botanical comparator D or E. Absolute difference = F − K (U/L); relative reduction = 100 × (K − F)/K. Percentages use round-half-up rounding to one decimal place. Inputs are the group mean activities.

3.4. Hepatic antioxidant parameters

The results showed lower SOD, CAT and GSH and higher MDA in the PP-only group than in the normal control. The treatment groups showed values closer to control, with the combination group approaching the control means. Group mean ± SD values are shown in Table 4.

Table 4. Hepatic antioxidant and oxidative-stress parameters, mean ± SD (n = 6 rats per group).

Group	SOD (U/mg protein)	CAT (U/mg protein)	GSH (µmol/g tissue)	MDA (nmol/mg protein)
A	8.43 ± 0.37	62.42 ± 2.32	7.87 ± 0.30	1.82 ± 0.09
B	4.23 ± 0.26	35.80 ± 2.26	4.12 ± 0.25	4.76 ± 0.21
C	7.32 ± 0.29	55.40 ± 2.26	6.93 ± 0.26	2.42 ± 0.14
D	5.92 ± 0.26	46.70 ± 2.07	5.68 ± 0.20	3.31 ± 0.17
E	6.43 ± 0.26	50.80 ± 1.98	6.21 ± 0.21	2.91 ± 0.13
F	7.53 ± 0.26	57.90 ± 1.96	7.19 ± 0.24	2.21 ± 0.13

3.5. Liver histopathology

Control A revealed organized hepatocellular cords around central veins, intact sinusoids and uniform hepatocytes with round nuclei and eosinophilic cytoplasm (Table 5; Figures 5–7). The five pieces of tissue were gray-brown and soft and had a cut surface. They are reported in the Table 5 note and relate to the size of the parts examined and not to the size or mass of the organs. The specimen B (completely polypropylene) had moderate sinusoidal congestion, multifocal scattered small mononuclear-cell infiltrates and mild to moderate hepatocellular

degenerative changes. The pathological impression overall was a mild to moderate degeneration and mild inflammation. The entire B-accession image series is displayed in Figure 8. The *C. longa* specimen D was characterized by mild hepatocellular degeneration, mild sinusoidal congestion and multifocal mononuclear and polymorphonuclear inflammatory-cell infiltration. The overall impression was the presence of mild multifocal hepatitis and degenerative changes (Figure 9). The *P. emblica* specimen E exhibited mild hepatocellular degenerative changes and some marked sinusoidal congestion and congested vessels with fibrin deposition. This resulted in a more prominent vascular description (Figure 10) and mild hepatocellular changes.

The specimen from combined treatment (specimen F) on microscopy demonstrated moderate hepatocellular degeneration, mild sinusoidal congestion and focal infiltration of the mononuclear cells. It showed a mild to moderate hepatocellular degenerative change overall. Table 5 shows the differences between the detailed description of microscopy and the summary impression of the microscopy; Figure 11 displays the full image series of F. Group C is kept for consistency with the experimental design and a validated histopathological evaluation is necessary for this arm.

Table 5. Qualitative liver histopathology across the experimental groups.

Group	Hepatocellular findings	Sinusoidal / vascular findings	Inflammatory features	Overall pathological impression
A	Uniform hepatocytes in organized cords; round nuclei and eosinophilic cytoplasm	Preserved sinusoidal architecture	Preserved tissue architecture; inflammation was not a reported feature	Preserved hepatic architecture; no significant histopathological abnormality
B	mild hepatocellular degenerative changes	Mild sinusoidal congestion	Minimal to mild focal mononuclear inflammatory-cell infiltration	Mild residual hepatocellular injury with substantial preservation of hepatic architecture
C	Mild-to-moderate degenerative changes	Moderate sinusoidal congestion	Multifocal scattered small mononuclear infiltrates	Mild-to-moderate degeneration with mild inflammation
D	Mild degenerative changes	Mild sinusoidal congestion	Multifocal mononuclear and polymorphonuclear infiltration	Mild multifocal hepatitis with degenerative changes
E	Mild degenerative changes	Areas of marked sinusoidal congestion; congested vessels with fibrin deposition	Inflammatory infiltration was not described	Mild hepatocellular degenerative changes
F	Moderate degeneration in the microscopy description	Mild sinusoidal congestion	Focal mononuclear infiltration	Mild-to-moderate hepatocellular degenerative changes

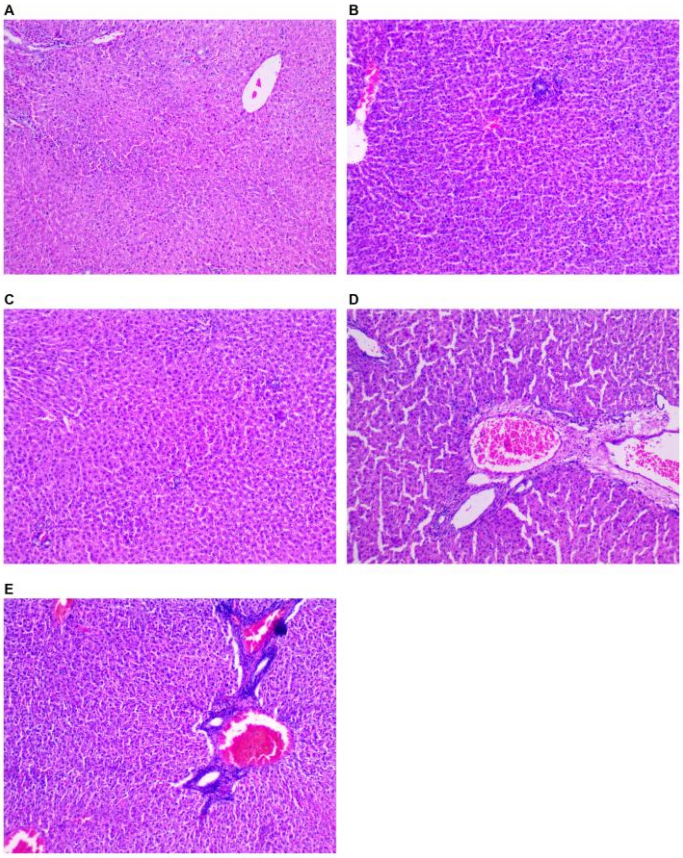


Figure 5. Overview liver fields. Panels A–E represent A (normal control), B (PP only), D (PP + *C. longa*), E (PP + *P. emblica*), and F (PP + combination).

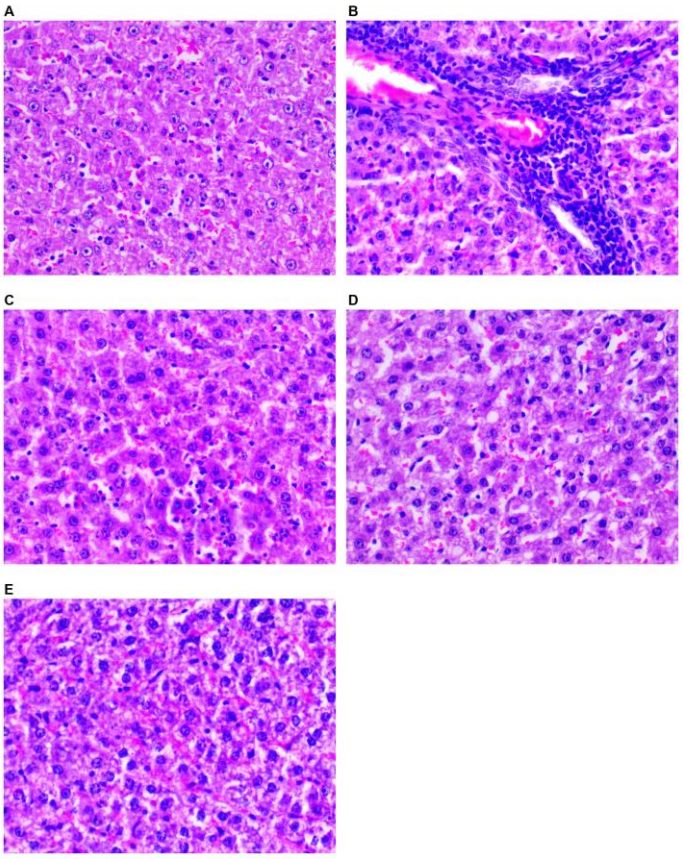
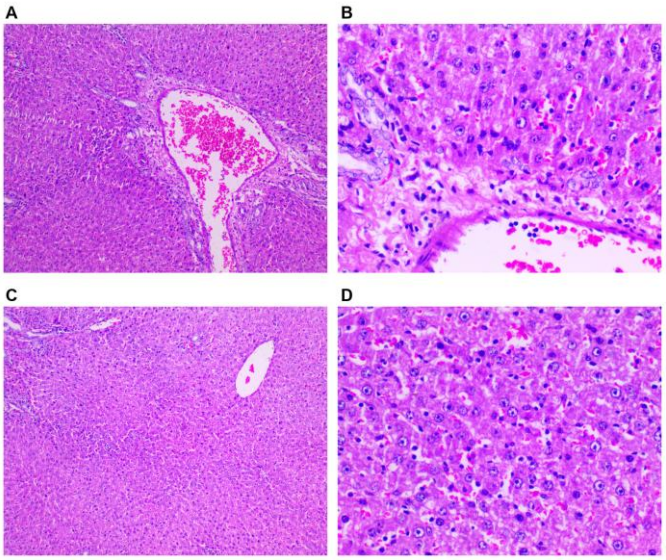
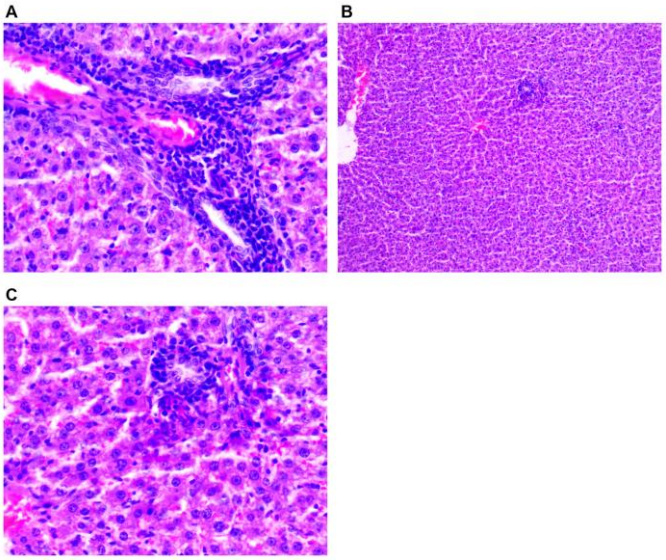


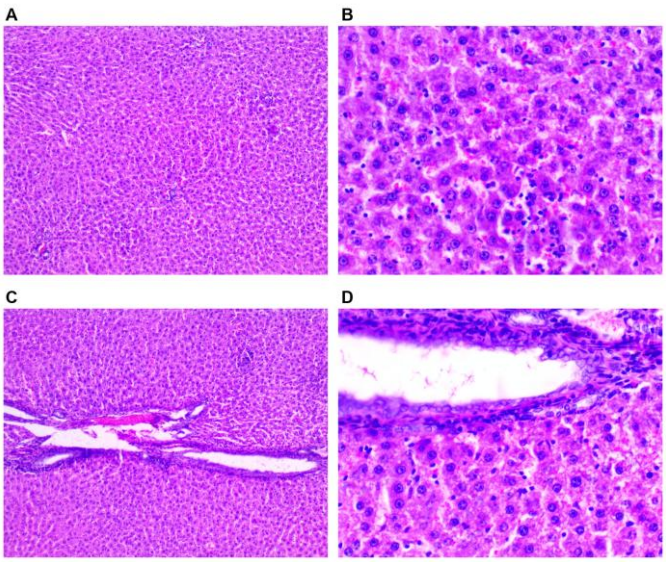
Figure 6. Detail liver fields. Panels A–E follow the group order in Figure 5.



312
313 **Figure 7.** Complete normal-control series.



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315 **Figure 8.** Complete polypropylene-only series, accession. Panels A–C:.



316
317 **Figure 9.** Complete PP + *C. longa* series, accession. Panels A–D.

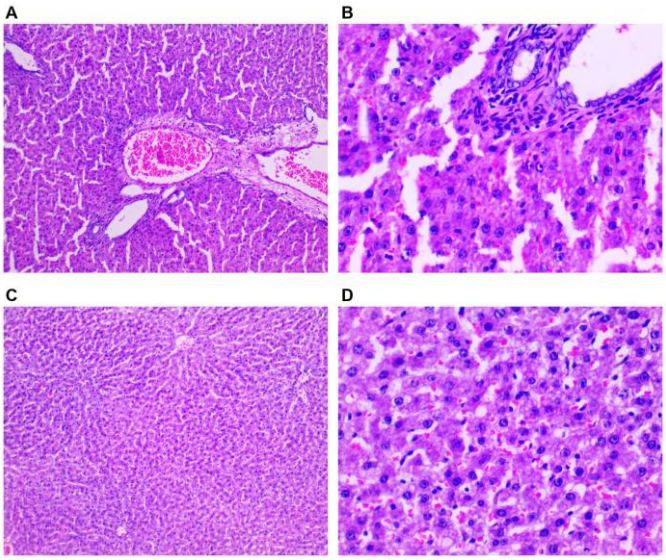


Figure 10. Complete PP + *P. emblica* series, accession. Panels A–D

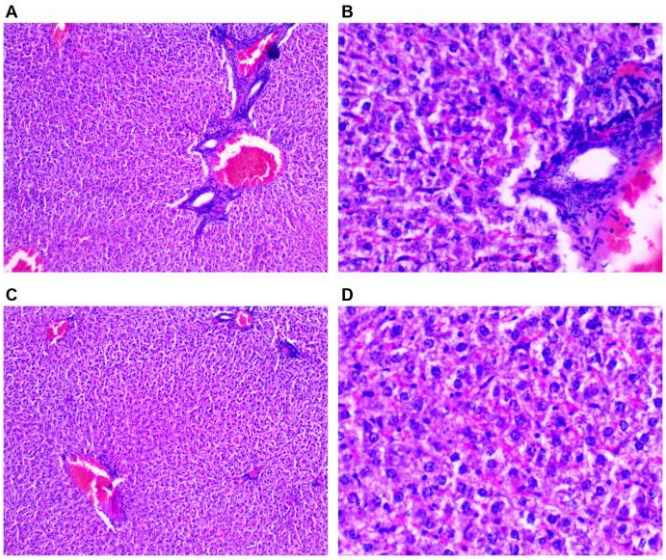


Figure 11. Complete combined-treatment series, accession. Panels A–D

The finalized biochemical results show a consistent treatment-associated pattern across the six-rat groups. PP-only group B had the highest mean serum ALT, AST, and ALP activities, whereas all co-treatment groups had lower means. The combination F showed the largest numerical reductions among the botanical regimens: 45.2% for ALT, 36.8% for AST, and 38.6% for ALP relative to B. Nevertheless, F remained above the concurrent control, at approximately 1.17-, 1.21-, and 1.24-fold the A means, respectively. These findings support a descriptive biochemical difference rather than complete normalization.

4 Discussion

The present study investigated the effect of polypropylene (PP) microplastic exposure on the liver and the ameliorative effect of silymarin, *Curcuma longa*, *Phyllanthus emblica* and the combination of all three on the liver of male albino Wistar rats. The changes in serum hepatic enzymes and antioxidant indices were the highest in the PP-only group (B) and the mean biochemical values were the lowest in the combined botanical group (F) when compared to the normal control (A). The pattern of the treatment was assessed in terms of enzymes, hepatic redox markers, routine observations and liver histopathology. The reported group means are numerical comparisons of the six rats in each biochemical group.

4.1 Observational and behavioural changes

During routine observation, general behaviour was noted to be normal. This was accompanied by a clear hepatic biochemical response in PP-exposed animals, highlighting the importance of organ-specific measurements to characterize the changes associated with micro-plastics. Liu et al. (2023) [4] also reported hepatic lipid changes in mice exposed to PP, but with no apparent external health symptoms. These observations are combined to demonstrate the value of biochemical and tissue analysis in addition to the normal behavioural monitoring.

4.2 Serum biochemical parameters

4.2.1 Alanine aminotransferase (ALT)

ALT activity increased from 32.1 U/L in A to 68.3 U/L in B, corresponding to a 112.8% elevation. The means in the silymarin (C), *Curcuma longa* (D), *Phyllanthus emblica* (E), and combined (F) groups were 41.0, 52.3, 46.2, and 37.4 U/L, respectively. Relative to B, the botanical regimens reduced mean ALT by 23.4% in D, 32.4% in E, and 45.2% in F. The combination yielded the lowest ALT activity among the PP-exposed groups, consistent with attenuation of hepatocellular injury-associated enzyme release. Similar improvement in serum transaminase profiles has been described with *C. longa* or curcumin in mercury-exposed rats

[12], with *P. emblica* extract in ethanol-exposed rats [14], and with silymarin in a polystyrene-microplastic model [38].

4.2.2 Aspartate aminotransferase (AST)

AST increased from 125.5 U/L in A to 240.8 U/L in B, a 91.9% elevation. Mean activities in C, D, E, and F were 162.7, 197.2, 178.2, and 152.3 U/L, respectively. These corresponded to reductions of 32.4%, 18.1%, 26.0%, and 36.8% relative to B. The ordering closely paralleled ALT, with the combined botanical group demonstrating the mean closest to the normal control. Since AST and ALT both increased in B and decreased in the intervention groups, their coordinated pattern provides consistent evidence of a favourable hepatic biochemical response. Comparable aminotransferase findings in experimental liver injury have been reported for *C. longa* and *P. emblica* preparations [12] [14].

4.2.3 Alkaline phosphatase (ALP)

ALP activity rose from 245.0 U/L in A to 493.3 U/L in B, a 101.3% elevation. Values in C, D, E, and F were 326.0, 395.3, 361.2, and 302.8 U/L, respectively. Relative to the PP-only group, the combined treatment showed a 38.6% reduction, compared with 19.9% for *C. longa* and 26.8% for *P. emblica*. The parallel movement of ALP with ALT and AST highlights a coordinated response across the serum hepatic enzyme panel. Joshi et al. (2017) [12] and Chaphalkar et al. (2017) [14] also reported favourable ALP responses following botanical administration in chemical hepatotoxicity models, supporting the direction observed in the present study.

4.3 Hepatic antioxidant and lipid peroxidation parameters

4.3.1 Superoxide dismutase (SOD)

SOD activity decreased from 8.43 U/mg protein in A to 4.23 U/mg protein in B, reflecting a 49.8% reduction in this antioxidant enzyme. The means in C, D, E, and F were 7.32, 5.92, 6.43, and 7.53 U/mg protein, respectively. The combined group showed a 78.0% higher activity than B and the highest activity among PP-exposed groups. As SOD contributes to the removal

of superoxide radicals, this pattern supports preservation of enzymatic antioxidant defence. Peng et al. (2018) [11] found that curcumin increased the activity of SOD in the liver in carbon tetrachloride-induced liver damage model, and Huang et al. (2017) [13] reported that *P. emblica* fruit extract increased the antioxidant enzyme activity in the liver.

4.3.2 Catalase (CAT)

CAT activity declined from 62.42 U/mg protein in A to 35.80 U/mg protein in B. Mean activities increased to 55.40, 46.70, 50.80, and 57.90 U/mg protein in C, D, E, and F, respectively. The combined treatment showed 61.7% increase in CAT activity compared with B. The parallel elevation of CAT and SOD in the intervention groups is consistent with a coordinated antioxidant response, as these enzymes are involved in complementary steps of the detoxification of ROS. Other experimental models of liver injury have shown similar improvements with curcumin [11] and *P. emblica* fruit extract [16].

4.3.3 Reduced glutathione (GSH)

Hepatic GSH decreased from 7.87 µmol/g tissue in A to 4.12 µmol/g tissue in B. Values in C, D, E, and F were 6.93, 5.68, 6.21, and 7.19 µmol/g tissue, respectively. The combined botanical regimen had the highest concentration of GSH compared to the PP-only group with a value of 74.5% higher than the PP-only group. The result suggests that the hepatic reduced-glutathione pool is more preserved, thus providing intracellular antioxidant buffering. Reddy et al. (2010) [16] also found that *P. emblica* fruit extract increased the level of reduced glutathione in the liver, and Peng et al. (2018) [11] demonstrated that curcumin enhanced the content of reduced glutathione in an experimental liver injury model.

4.3.4 Malondialdehyde (MDA)

MDA increased from 1.82 nmol/mg protein in A to 4.76 nmol/mg protein in B, a 161.5% elevation. Values in C, D, E, and F were 2.42, 3.31, 2.91, and 2.21 nmol/mg protein, respectively. Compared with B, MDA was 30.5% lower with *C. longa*, 38.9% lower with *P. emblica*, and 53.6% lower with their combination. The concurrent lower MDA and higher

SOD, CAT, and GSH values in F present a coherent pattern of reduced lipid-peroxidation-related burden and improved antioxidant status. Comparable reductions in lipid-peroxidation indices have been reported in curcumin and *P. emblica* studies [16].

4.4 Histopathological changes

The normal control specimen (A) displayed preserved hepatocellular cords, uniform hepatocytes, and normal sinusoidal organization. Microscopic examination of the PP-only specimen (B) revealed mild to moderate hepatocellular degeneration, moderate sinusoidal congestion, and multifocal mononuclear inflammatory infiltration. These results are a tissue level counterpart to the increase in serum liver enzymes and the change in the hepatic redox profile.

In the *C. longa* specimen (D), there was mild hepatocellular degeneration and mild sinusoidal congestion, with multifocal inflammatory-cell infiltration. In the *P. emblica* specimen (E), hepatocellular degeneration was observed, along with areas of marked sinusoidal congestion and congested vessels with fibrin. The detailed microscopic description of the combined-treatment specimen (F) revealed moderate hepatocellular degeneration, mild sinusoidal congestion and focal mononuclear infiltration. These results are consistent across the specimens examined and show differences in the hepatocellular, vascular and inflammatory characteristics between the experimental conditions. The serum enzyme and antioxidant profile of F is particularly good, and this is an important biochemical aspect to complement the tissue observations.

4.5 Comparative interpretation of treatment groups

4.5.1 Individual botanical responses

Both botanical regimens exhibited lower ALT, AST, ALP and MDA, and higher SOD, CAT and GSH than the PP-only regimen. In all the individual botanical groups, *P. emblica* showed biochemical values that were closer to the normal control than *C. longa*. This is consistent with the experimental reports of antioxidant and hepatoprotective effects of *P. emblica* preparations

in ethanol associated hepatic injury and high-fat-diet models [14] [16]. The *C. longa* response also corroborates the results of enhanced transaminase activities and hepatic antioxidant measures following *C. longa* extract or curcumin supplementation [11] [12].

4.5.2 Combined botanical treatment and silymarin comparison

The mean values of silymarin were more favorable than the botanical groups and were closer to the control. The overall botanical treatment resulted in further improvement numerically for all biochemical parameters measured. Relative to silymarin, group F had 8.8%, 6.4%, and 7.1% lower ALT, AST, and ALP; 2.9%, 4.5%, and 3.8% higher SOD, CAT, and GSH; and 8.7% lower MDA. The uniformity of the direction of these group means for hepatic enzyme activities, antioxidant defence and lipid-peroxidation related measurements indicates the favourable biochemical performance of the combination under the study conditions.

4.5.3 Integrated significance of the combined regimen

The total dose of preparation was 200 mg/kg/day of *C. longa* and 200 mg/kg/day of *P. emblica*, which is a multi-botanical intervention. The PP-exposed groups that received this evaluated regimen showed the highest number of biochemical parameters moving towards the normal-control profile of all the seven parameters. The simultaneous decrease in serum enzyme activities, and increase in SOD, CAT, and GSH, with a decrease in MDA, corroborates its hepatoprotective effect in this model. The results presented here serve as a solid foundation for additional research into the optimization of botanical dose combinations and the molecular hepatic responses of the combinations.

Based on the above study and discussion, the toxicity mechanism of microplastics (MPs) and nanoplastics (NPs) will be influenced by particle size, physicochemical characteristics, and exposure duration. The larger particles are more likely to be excreted in the faeces, but the smaller ones can be taken up by cells or transported via other routes into the lymphatic or blood system and thus reach remote organs such as the liver [46] . As depicted in Fig. 12, Microplastic-induced Hepatotoxicity is a complex process including intestinal barrier

interaction, particle translocation, oxidative stress, organellar dysfunction, inflammation, and metabolic alterations in the liver. After being taken up, part of the ingested microplastics can pass through the intestinal barrier via transcellular pathway, M cell uptake, or via a mechanism mediated by barrier dysfunction. The translocated particles can be transported into either portal circulation or lymphatic vasculature. However, the contribution of both pathways is yet unclear [42] – [43]. Once they reach the liver, cellular stress and gut-liver inflammatory signalling caused by particle uptake may induce ROS generation, lipid peroxidation, mitochondrial dysfunction, altered autophagy, and hepatocellular injury. These complex mechanisms could impair the liver's lipid metabolism and antioxidative capacity, resulting in biochemical and pathological alteration [42], [43], [44]. However, the relative contributions of these mechanisms depend on the characteristics of the particles and the experimental conditions. From the current study, the use of *Curcuma longa* and *Phyllanthus emblica* combination was found to have a positive influence on liver enzymes, antioxidant function, and histological structure in rats exposed to polypropylene microplastics. The results of this study seem to hint at some kind of hepatoprotective effect due to improved redox balance and hepatic function. Future research should include quantifying of particles in tissues, intestinal permeability, inflammatory pathways, and intracellular organelle biomarkers.

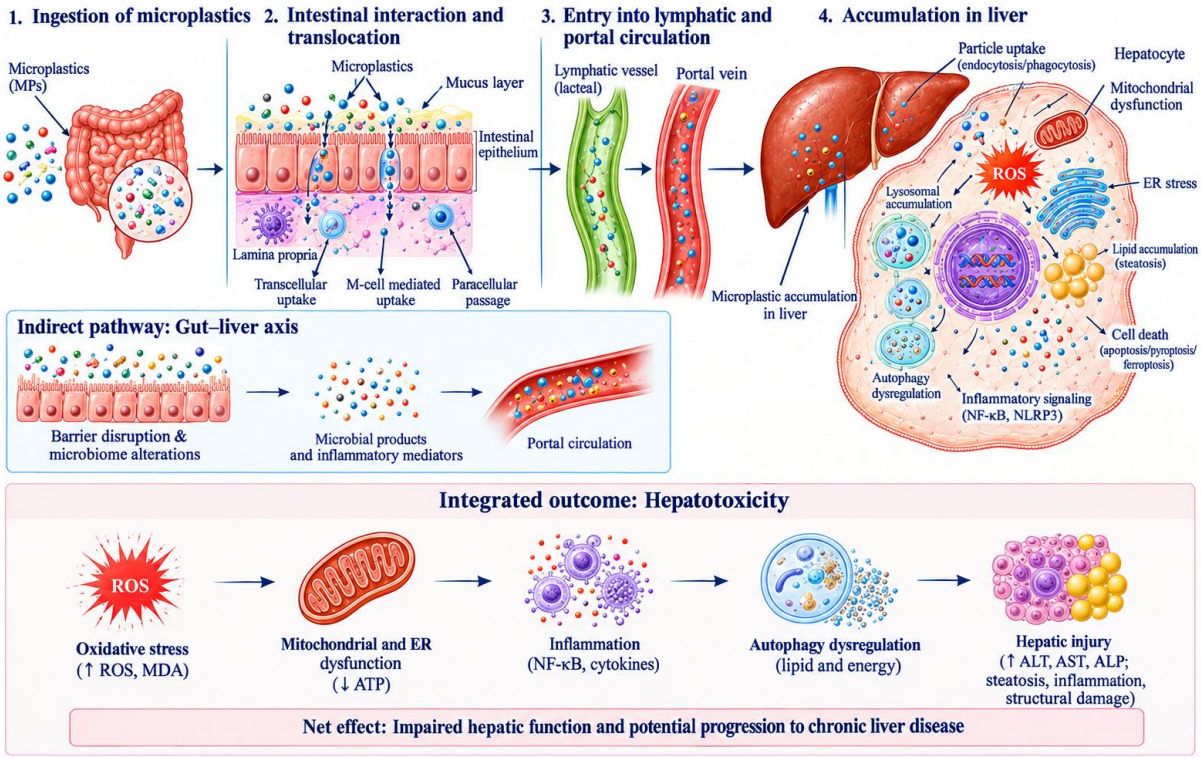


Figure 12. *Mechanisms of Microplastic-induced liver toxicity*

5. Conclusion

The present study characterized the hepatic alterations associated with polypropylene (PP) microplastic exposure and the hepatoprotective responses of *Curcuma longa* and *Phyllanthus emblica*, administered individually and in combination, in male albino Wistar rats. Polypropylene exposure was characterized by elevated serum ALT, AST, and ALP activities, reduced hepatic antioxidant defence, increased lipid peroxidation, and histopathological evidence of hepatocellular degeneration, sinusoidal congestion, and inflammatory infiltration. These hepatic alterations were observed despite the maintenance of normal general behaviour during routine qualitative observations. Administration of both botanical preparations was associated with a more favourable hepatic biochemical profile compared with the polypropylene-only group. In the individual treatments, *P. emblica* had consistently lower values than *C. longa*, and closer to the normal control. In particular, the PP-exposed group with combined botanical regimen had the most favourable biochemical response. The mean values of ALT, AST and ALP activities were 45.2%, 36.8% and 38.6% lower, respectively, than those in the polypropylene-only group (n = 6). This response resulted in an increase of 78.0%, 61.7%

and 74.5% in SOD, CAT and GSH, respectively and a decrease of 53.6% in MDA concentration. The uniformity in the hepatic enzyme activity, antioxidant indices and lipid peroxidation is indicative of a significant reduction in the biochemical disturbance associated with PP in the experimental conditions. The hepatic response was also characterized by histopathological examination, which showed variation in hepatocellular, vascular and inflammatory changes between treatment groups. Overall, these results offer experimental proof of the hepatoprotective effect of *C. longa* and *P. emblica* against PP microplastic-induced alterations in the liver. The overall biochemical response was best for the combined therapy of the treatments evaluated. The study thus suggests that combined *C. longa* and *P. emblica* supplementation could be a promising botanical intervention for further studies, especially in the form of dose-controlled studies with quantitative histopathology and molecular analysis of hepatic injury.

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Author Contributions

B Gyani Priyanka Patnaik, Sanjeevi Ramakrishnan, Dushyant Singh Chauhan, Anuradha Jayaraman and Sandeep Tripathi contributed equally to all stages of the study. They jointly participated in the conceptualization of the research, data curation, visualization, and experimental investigation. All three authors contributed to the preparation of the original

manuscript draft and were involved in project administration. They also collaboratively participated in the writing, review, and critical editing of the manuscript. All authors read and approved the final version of the manuscript.

Conflict of Interest Statement

The authors declare that there are no conflicts of interest related to the publication of this manuscript. The research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Ethics Statement

The experimental project was approved by the Institutional Animal Ethics Committee of Nims University Rajasthan (Jaipur), India (NIMS/NIOP/IAEC/26-02-21).

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