

NANO-ENCAPSULATED *TAMARINDUS INDICA* FRUIT PULP EXTRACT ATTENUATES TARTRAZINE-INDUCED HAEMATOLOGICAL AND BIOCHEMICAL ALTERATIONS IN WISTAR RATS

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Abstract: This study examined the protective effects of *Tamarindus indica* fruit pulp extract (TIFPE) and Chitosan-TPP encapsulated *Tamarindus indica* fruit pulp extract (Nano-TIFPE) against tartrazine-induced toxicity by assessing haematological and serum biochemical parameters in Wistar albino rats. A total of 48 rats were randomly assigned to eight groups, each comprising six animals. Groups I, II, III, and IV served as the Normal Control, TIFPE Control, Nano-TIFPE Control, and Vitamin E Control, respectively. Group V received tartrazine, while Groups VI, VII, and VIII were co-administered tartrazine with TIFPE, Nano-TIFPE, and Vitamin E, respectively, over a 28-day period. At the conclusion of the experimental period, blood samples were collected for haematological and serum biochemical analysis. In the tartrazine-treated rats, haematological parameters such as RDW-CV, RDW-SD, WBC, lymphocytes, granulocytes, PLT, PDW, P-LCC, and P-LCR exhibited a significant increase, whereas RBC, Hb, HCT, MCV, MCH, and MCHC showed a significant decrease as compared to the control group. Additionally, serum biochemical parameters, including total protein, albumin, and HDL, were significantly reduced, while ALT, AST, ALP, urea, creatinine, total cholesterol, triglycerides, LDL, and VLDL were significantly elevated in the tartrazine-treated rats compared to the control group. Notably, rats treated with Nano-TIFPE demonstrated significant amelioration when co-administered with tartrazine, exhibiting a more pronounced protective effect than TIFPE co-administration. The nano-encapsulation of TIFPE with chitosan-TPP resulted in enhanced amelioration due to increased bioavailability and biocompatibility, as well as reduced biodegradation, thereby sustaining the effect of TIFPE for a longer duration within the animal body.

Key Words – *Tamarindus indica* fruit pulp, Tartrazine-induced toxicity, Chitosan-TPP nanoparticles, Haematology, Serum biochemical parameters.

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1. Introduction

Food additives are substances introduced into foods during processing to enhance their physical and chemical properties, such as color, texture, flavor, stability, and shelf life (Codex Alimentarius, 1995). Their widespread use is driven by increasing consumer demand for attractive, safe, and convenient food products, as well as the need for efficient food production and distribution. Despite these technological benefits, the safety of certain food additives, particularly synthetic food colourants, remains a subject of concern due to their potential adverse health effects, including allergic reactions, behavioural changes, carcinogenicity, and organ toxicity (Maffini & Vandenberg, 2017; Jain & Mathur, 2015).

Tartrazine (E102; FD&C Yellow No. 5) is a synthetic azo dye widely used as a yellow food colorant across the food, pharmaceutical, cosmetic, and textile industries due to its intense colour, stability, affordability, and minimal flavour impact (Anaji *et al.*, 2024; Barciela *et al.*, 2023). Following ingestion, the azo bond (-N=N-) undergoes reductive cleavage by intestinal microbiota, producing aromatic amine metabolites such as sulfanilic acid and 4-amino-3-carboxy-5-hydroxy-1-(4-sulphophenyl) pyrazole (SCAP), which contribute to its toxicological effects (Pay *et al.*, 2023). The acceptable daily intake (ADI) of tartrazine varies internationally, with EFSA setting it at 0-7.5 mg/kg body weight per day (2009), JECFA increasing it to 0-10 mg/kg (2016), and the FDA confirming a lower

ADI of 5 mg/kg (2011), reflecting differing regulatory assessments worldwide (JECFA, 2002).

Numerous experimental studies have demonstrated that oral administration of tartrazine in experimental animals has been associated with elevated liver enzymes (ALT, AST, and ALP), increased levels of urea, uric acid, creatinine, lipid peroxidation markers, and nitric oxide, along with depletion of endogenous antioxidant defences. Histopathological alterations in the liver and kidneys, together with DNA damage in leukocytes, further indicate its hepatotoxic, nephrotoxic, and genotoxic potential (Khayya *et al.*, 2017; Acharya *et al.*, 2025). *In vitro* studies with tartrazine have confirmed concentration-dependent cytotoxic and mutagenic effects on mammalian cell lines, supporting concerns regarding its biological safety (Santos *et al.*, 2022). Recent evidence also suggests that tartrazine may act as an endocrine-disrupting chemical, interfering with hormonal regulation and contributing to metabolic, developmental, and reproductive disorders (Paramasivam *et al.*, 2024). Further studies have demonstrated that tartrazine induces oxidative stress, inflammation, genotoxicity, and organ toxicity, with increased lipid peroxidation, reduced activities of antioxidant enzymes (CAT, SOD, and GSH-Px), inflammatory responses, intestinal barrier dysfunction, and alterations in gut microbiota in aquatic models (Wu *et al.*, 2021). In mammalian studies, tartrazine administration elevated serum liver and kidney biomarkers, induced DNA damage, and caused histopathological alterations in the liver and kidneys, with concentration-dependent cytotoxic and mutagenic effects reported in human cell lines (Floriano *et al.*, 2018; Baysal *et al.*, 2026; Ameer *et al.*, 2019; Gupta *et al.*, 2019).

Given the growing evidence of tartrazine-induced toxicity, there is increasing attention on natural antioxidants as potential protective agents. *Tamarindus indica* L., a member of the Fabaceae family, is a nutritionally and medicinally important tropical tree widely cultivated in Asia and Africa. The fruit pulp is rich in phenolic compounds, flavonoids, vitamins, organic acids, and essential minerals, contributing to its antioxidant, antimicrobial, anti-inflammatory, and other pharmacological activities (Ferreira *et al.*, 2018; Singh *et al.*, 2025; Okello *et al.*, 2018). Phytochemical studies have identified compounds such as 5-hydroxymethylfurfural and other bioactive constituents responsible for its antioxidant and antimicrobial properties (Fagbemi *et al.*, 2022). Tamarind-derived bioactive compounds possess antioxidant, antibacterial, anti-inflammatory, and anticancer properties, while encapsulation approaches have further improved their stability and bioavailability (Nagar *et al.*, 2022; Meena *et al.*, 2022).

Nanotechnology has emerged as an effective strategy to enhance the stability, bioavailability, and therapeutic efficacy of plant-derived bioactive compounds. The green synthesis of nanoparticles using *Tamarindus indica* fruit pulp extract, along with chitosan and tripolyphosphate (TPP), represents an environmentally friendly approach that eliminates the need for toxic reducing agents and harsh chemical conditions. In this system, tamarind extract acts as a natural reducing and capping agent, while chitosan serves as a biocompatible and biodegradable polymer matrix. TPP functions as a non-toxic ionic crosslinker that interacts with the protonated amino groups of chitosan through ionic gelation, producing stable nanoparticles with controlled size and morphology (Wardani *et al.*, 2017). Chitosan-TPP nanoparticles have gained considerable attention as delivery systems due to their excellent biocompatibility, biodegradability, mucoadhesive properties, and controlled release characteristics. Encapsulating plant extracts within these nanoparticles enhances the stability of phytochemicals, improves bioavailability, and promotes sustained biological activity, thereby increasing their antioxidant, antimicrobial, and anti-inflammatory potential (Kim *et al.*, 2022; Ahmad *et al.*, 2023; Gagliardi *et al.*, 2023; Mariano *et al.*, 2019; Jin *et al.*, 2025; Kravanja *et al.*, 2019; Riezk *et al.*, 2020).

While the antioxidant properties of *Tamarindus indica* and the advantages of chitosan-tripolyphosphate (Chitosan-TPP) based nano-formulations are well-established, there is a paucity of research on the protective efficacy of nano-formulated *Tamarindus indica* fruit pulp extract against tartrazine-induced toxicity. This study, therefore, seeks to evaluate the haematological and serum biochemical changes induced by tartrazine, as well as the ameliorative potential of hydroalcoholic *Tamarindus indica* fruit pulp extract and chitosan-TPP encapsulated *Tamarindus indica* fruit pulp extract in counteracting tartrazine-induced toxicity.

2. MATERIALS AND METHODS

2.1 Experimental Animals: Female Wistar albino rats (*Rattus norvegicus*), weighing [180-220 grams] and aged [8-10 weeks], were obtained from Laboratory Animal Facility (LAF), College of Veterinary and Animal Sciences, Pookode, Kerala Veterinary and Animal Sciences University, Wayanad, Kerala. Before the experiment, the animals were acclimatized for seven days under standard laboratory conditions. They were housed in polypropylene cages with sterilized paddy husk bedding under controlled environmental conditions, including a temperature of $22 \pm 3^\circ\text{C}$, relative humidity of 50–60%, and a 12 h light/12 h dark cycle. The animals were provided with a standard pellet diet and filtered drinking water *ad libitum* throughout the study. Their health and general behaviour were monitored daily. All experimental

procedures were conducted in accordance with the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India, and were approved by the Institutional Animal Ethics Committee (IAEC) of College of Veterinary and Animal Sciences, Pookode, Wayanad, Kerala Vide No. IAEC/COVAS/PKD/25/1/2025.

2.2 Experimental Design and Treatment

Groups	Treatment
1	Distilled water at 5 ml/kg orally for 28 days
2	TIFPE extract at 400 mg/kg ^{&} orally in distilled water for 28 days
3	Nano-TIFPE extract equivalent to 400 mg/kg ^s body weight of TIFPE extract orally in distilled water for 28 days
4	Vitamin E at 300 mg/kg [*] orally in 0.01% Tween 80 for 28 days
5	Tartrazine at 15 mg/kg [#] orally in distilled water for 28 days
6	Tartrazine @ 15 mg/kg and TIFPE extract at 400 mg/kg orally in distilled water for 28 days
7	Tartrazine @ 15 mg/kg and Nano-TIFPE extract equivalent to 400 mg/kg body weight of TIFPE extract orally in distilled water for 28 days
8	Tartrazine @ 15 mg/kg and Vitamin E @ 300 mg/kg in 0.01% Tween 80 and in distilled water orally for 28 days

[&] Komakech *et al.*, 2019, ^{*}Usman *et al.*, 2022, [#] El-Rahman *et al.*, 2024, ^sDandekar *et al.*, 2010

2.3 Preparation of Hydroalcoholic Tamarind Fruit Pulp Extract

The fruit pulps of *Tamarindus indica* were collected and meticulously cleaned to eliminate any adhering dirt and extraneous materials, followed by deseeding. The pulp was then subjected to hydroalcoholic extraction using a solvent mixture of ethanol and distilled water in a 70:30 volume ratio, with a sample-to-solvent ratio of 1:10 (w/v). This extraction process was conducted over a period of 2–3 days, with intermittent agitation to enhance the extraction of bioactive constituents. Post-extraction, the mixture was filtered through Whatman No. 1 filter paper, and the filtrate obtained was concentrated using a rotary evaporator at 40 °C. The concentrated extract was subsequently collected and stored in an airtight container under suitable storage conditions until further analysis (Iskandar *et al.*, 2017).

2.4 Preparation of *Tamarindus Indica* Fruit Pulp extract and its Nano-formulations: *Tamarindus indica* L. fruit pulp hydroalcoholic extracts

nanoparticles were prepared by the ionic gelation method using chitosan as the polymer and sodium tripolyphosphate (TPP) as the cross-linking agent. A 0.1% (w/v) chitosan solution was prepared by dissolving chitosan in 3% (v/v) acetic acid under continuous magnetic stirring until a clear and homogeneous solution was obtained. Separately, a 0.1% (w/v) TPP solution was prepared in distilled water. Tamarind fruit pulp was dissolved in 5% dimethyl sulfoxide (DMSO) to obtain a final concentration of 0.3% (w/v). The tamarind fruit pulp solution was added to the chitosan solution under continuous magnetic stirring to obtain a homogeneous mixture. Subsequently, the 0.1% TPP solution was added dropwise to the chitosan–tamarind mixture under constant stirring to induce ionic cross-linking and facilitate nanoparticle formation. The resulting nanoparticle suspension was stirred continuously until complete formation of the nanoparticles. The nanoparticle suspension was centrifuged at 10,000 rpm for 60 min. The supernatant was discarded, and the nanoparticle pellet was collected and washed with distilled water to remove any unreacted chitosan and TPP. The purified nanoparticles were dried by freeze-drying (or the drying method employed in the study) and stored in airtight containers at 4°C until further characterization and experimental use (Goswami & Chaudhary, 2023)

2.5 Sample Collection for Haematological and Serum Biochemical Analysis:

At the end of the 28-day experimental period, the animals were fasted overnight with free access to drinking water. On the 29th day, the animals were humanely anaesthetized using ketamine and xylazine in accordance with the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). Blood samples were collected immediately from the retro-orbital plexus under aseptic conditions. The collected blood was divided into two portions for subsequent analysis. One portion was transferred into ethylenediaminetetraacetic acid (EDTA)-coated tubes for haematological analysis of WBC (White Blood Cell count), RBC (Red Blood Cell count), Hb (Haemoglobin), HCT (Haematocrit), MCV (Mean Corpuscular Volume), MCH (Mean Corpuscular Haemoglobin), MCHC (Mean Corpuscular Haemoglobin Concentration), RDW-CV (Red Cell Distribution Width–Coefficient of Variation), RDW-SD (Red Cell Distribution Width–Standard Deviation), PLT (Platelet count), MPV (Mean Platelet Volume), PDW (Platelet Distribution Width), PCT (Procalcitonin Test), P-LCC (Platelet Large Cell Count), P-LCR (Platelet Large Cell Ratio), Lymphocyte count, MID (Mid-sized cell count), and Granulocyte count. The remaining blood was collected into plain tubes without anticoagulant for serum separation and were allowed to clot at room temperature and subsequently centrifuged at

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3000 rpm for 10-15 minutes to obtain serum. The separated serum was aspirated and analyzed for serum glucose, TP (Total Protein), TB (Total Bilirubin), ALT (Alanine Aminotransferase), AST (Aspartate Aminotransferase), Creatinine, BUN (Blood Urea Nitrogen), TG (Triglycerides), TC (Total Cholesterol), HDL-C (High-Density Lipoprotein Cholesterol), LDL-C (Low-Density Lipoprotein Cholesterol), CRP (C-Reactive Protein), ACP (Acid Phosphatase), ALP (Alkaline Phosphatase), CK-MB (Creatine Kinase-MB), Ca (Calcium), PCT (Plateletcrit), and P (Phosphorus).
Statistical Analysis: Data are presented as Mean $\pm$ SEM (n=6). Superscripts in a single column indicate significant differences as determined by one-way ANOVA followed by Tukey's multiple comparison test. Comparisons with the control group (Normal Control), tartrazine and tartrazine + TIFPE groups are denoted by *, #, and \$, respectively. The number of superscripts corresponds to the level of significance, with p < 0.05 and p < 0.01 represented by one and two superscripts, respectively.

3. RESULT AND DISCUSSION

3.1 HEMATOLOGY PARAMETERS:

Parameters	NC	TIFPE	N-TIFPE	Vit E	TAR	TIFE + TAR	N-TIFE + TAR	Vit E + TAR
MC	315.	319.	318.	321.	264.3	278.	311.2	318.4
H	4 $\pm$ 3.	57 $\pm$ 3.	76 $\pm$ 3.	45 $\pm$ 3.	1 $\pm$ 3.0	63 $\pm$ 3.	7 $\pm$ 3.2	2 $\pm$ 3.2
C (g/L)	68.	7.	8.	4.	2 ^{**}	0.	6 ^{##}	1 ^{##}
R	13.	14.	13.	13.	16.	16.	14.	13.
D	.6	.0	.6	.9	32	.2	25	98
W	8 $\pm$ 0.	2 $\pm$ 0.	9 $\pm$ 0.	1 $\pm$ 0.	$\pm$ 0.72	8 $\pm$ 0.	$\pm$ 0.59 [#]	$\pm$ 0.61 [#]
-C V (%)	54	63	71	71	*	68	^s	[#]
R	25.	26.	25.	26.	48.	44.	34.	32.
D	.6	.3	.6	.3	66	.3	68	69
W	8 $\pm$ 0.	8 $\pm$ 0.	9 $\pm$ 0.	9 $\pm$ 0.	$\pm$ 0.68	6 $\pm$ 0.	$\pm$ 0.51 [#]	$\pm$ 0.46 ^{##}
-S D (μ m ³)	29	31	32	34	^{**}	62	^s	^{##}
P	397.	392.	396.	396.	504.2	486.	421.3	405.3

T (10 ³ / μ l)	65 $\pm$ 6.5	46 $\pm$ 7.0	54 $\pm$ 7.0	45 $\pm$ 6.5	6 $\pm$ 7.35 ^{**}	35 $\pm$ 7.2	6 $\pm$ 6.86 ^{##}	6 $\pm$ 6.53 ^{##}
M	4.	4.	4.	4.	4.1	4.	3.9	4.0
P	15	18	20	21	5 $\pm$	16	8 $\pm$	8 $\pm$
V (fL)	$\pm$ 0.	$\pm$ 0.	$\pm$ 0.	$\pm$ 0.	0.0	$\pm$ 0.	0.0	0.0
(%)	.08	.07	.09	.09	8	.08	.09	.09
P	9.	9.	10	9.	12.	12	11.	10.
D	98	76	.0	86	69	.3	56	39
W	$\pm$ 0.	$\pm$ 0.	6 $\pm$	$\pm$ 0.	$\pm$ 0.	1 $\pm$	$\pm$ 0.	$\pm$ 0.
(%)	.20	.23	0.18	.27	36*	0.38	31 [#]	39 [#]
P	0.	0.	0.	0.	0.4	0.	0.4	0.4
C	41	49	43	41	0 $\pm$	42	1 $\pm$	1 $\pm$
T	$\pm$ 0.	$\pm$ 0.	$\pm$ 0.	$\pm$ 0.	0.0	$\pm$ 0.	0.0	0.0
(%)	.04	.05	.04	.05	4	.06	.04	.05
P-L	258.	257.	253.	258.	289.6	276.	265.6	261.3
C	39	42	68	39	7 $\pm$	68	9 $\pm$	5 $\pm$
C	$\pm$ 3.	$\pm$ 3.	$\pm$ 3.	$\pm$ 3.	3.3	$\pm$ 3.	3.4	3.4
(10 ³ / μ l)	.39	.34	.31	.37	9 ^{**}	.75 [#]	1 ^{##}	5 ^{##}
P-L	368.	378.	369.	368.	41.25	39.5	37.69	37.02
C	6 $\pm$ 0.	6 $\pm$ 0.	8 $\pm$ 0.	9 $\pm$ 0.	$\pm$ 0.68	6 $\pm$ 0.	$\pm$ 0.86 [#]	$\pm$ 0.52 [#]
(%)	45	51	52	58	^{**}	49	^s	[#]
W	11.	11.	11.	11.	20.	18.	15.	13.
B	.2	.6	.7	.6	36	.5	63	02
C	9 $\pm$ 0.	9 $\pm$ 0.	5 $\pm$ 0.	9 $\pm$ 0.	$\pm$ 0.98	2 $\pm$ 0.	$\pm$ 0.65 [#]	$\pm$ 0.48 ^{##}
(10 ³ / μ l)	38	42	39	51	^{**}	74 [#]	^s	^{##}
Ly	7.	7.	7.	7.	19.	17.	12.	11.
m	86	89	59	82	68	.5	39	02
ph	$\pm$ 0.	$\pm$ 0.	$\pm$ 0.	$\pm$ 0.	$\pm$ 0.	6 $\pm$ 0.	$\pm$ 0.	$\pm$ 0.
oc	.6	.6	.4	.7	75 ^{**}	0.	62 [#]	46 ^{##}
yte (10 ³ / μ l)	27	79	99	44	^{**}	59 [#]	^{##}	^{##}
M	0.	0.	0.	0.	0.2	0.	0.2	0.2
id	25	26	29	25	7 $\pm$	25	7 $\pm$	6 $\pm$
Ce	$\pm$ 0.	$\pm$ 0.	$\pm$ 0.	$\pm$ 0.	0.0	$\pm$ 0.	0.0	0.0
lls (10 ³ / μ l)	.008	.007	.008	.006	.009	.008	.007	.006
Gr	2.	2.	2.	2.	8.1	7.	5.2	4.6
an	69	64	36	39	2 $\pm$	86	1 $\pm$	8 $\pm$
ul	$\pm$ 0.	$\pm$ 0.	$\pm$ 0.	$\pm$ 0.	$\pm$ 0.	$\pm$ 0.	$\pm$ 0.	$\pm$ 0.

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ocyt (10 ³ /μl)	.15	.14	.12	.16	0.46 ^{**}	.31	0.26 ^{#s}	0.21 ^{##}
RBC (10 ⁶ /μl)	6.68 ± 0.19	6.89 ± 0.17	6.76 ± 0.21	6.98 ± 0.15	5.21 ± 0.22 ^{**}	5.75 ± 0.18 [#]	6.18 ± 0.17 ^s	6.23 ± 0.19 ^{##}
Hemoglobin (g/dl)	12.7 ± 0.26	12.3 ± 0.21	12.4 ± 0.19	12.8 ± 0.29	8.52 ± 0.16 ^{**}	9.21 ± 0.18 [#]	11.24 ± 0.19 ^s	12.09 ± 0.17 [#]
Hematocrit (%)	39.6 ± 0.39	38.5 ± 0.41	39.7 ± 0.38	39.8 ± 0.37	21.45 ± 0.34 ^{**}	22.4 ± 0.31	31.26 ± 0.35 ^s	36.25 ± 0.38 ^{##}
MCV (fL)	64.8 ± 0.45	65.8 ± 0.47	68.2 ± 0.48	68.3 ± 0.49	51.28 ± 0.45 ^{**}	55.6 ± 0.49	62.39 ± 0.46 ^s	65.28 ± 0.42 ^{##}
MCH (pg)	21.3 ± 0.26	20.3 ± 0.22	21.3 ± 0.21	22.3 ± 0.23	10.81 ± 0.16 ^{**}	14.6 ± 0.21 [#]	18.36 ± 0.26 ^s	21.38 ± 0.24 ^{##}

NC- Normal Control, TIFPE- Aqueous extract of *Tamarindus indica* Fruit Pulp, N-TIFP- Chitosan-TPP encapsulated aqueous extract of *Tamarindus indica* Fruit Pulp, VitE- Vitamin E, TAR- Tartrazine, TIFPE+TAR - Aqueous extract of *Tamarindus indica* Fruit Pulp + Tartrazine, N-TIFPE + TAR - Chitosan-TPP encapsulated aqueous extract of *Tamarindus indica* Fruit Pulp + Tartrazine, VitE + TAR – Vitamin E + Tartrazine. Values (Mean ± SEM, n=6) bearing superscript in single column are significantly different in One way ANOVA followed by Tukey's multiple comparison test. Comparison with control group (Normal Control), TAR and TIFPE+ TAR groups has shown in *,[#] and ^s accordingly. The number of superscripts indicates the level of significance $p < 0.05$, $p < 0.01$ are represented by one and two superscripts respectively.

Tartrazine exposure significantly decreased RBC count, haemoglobin, haematocrit, MCV, MCH, and MCHC ($p < 0.01$), while increasing RDW-CV and RDW-SD, indicating impaired erythropoiesis and heightened anisocytosis. This is consistent with tartrazine-induced ROS generation compromising erythrocyte membrane integrity and shortening cell lifespan (El-Desoky et al., 2021), alongside broader oxidative damage to haematopoietic tissue (Demircigil et al., 2023). Aqueous TIFPE partially ameliorated these erythrocyte alterations, consistent with its antioxidant bioactives myo-inositol, ascorbic acid derivatives, and phytosterols (Amaravel et al., 2024) and its reported inhibition of NO/iNOS-mediated inflammation (Leya and Anitha, 2019), though limited bioavailability likely explains the incomplete correction. N-TIFPE produced significantly greater restoration of all erythrocyte indices than both TAR and TIFPE + TAR, with most values approaching normal control attributed to chitosan-TPP enhancing phytochemical protection, absorption, and sustained release (Fitri et al., 2026), alongside chitosan's own antioxidant and haematoprotective activity (Mohamed et al., 2024; Almukainzi et al., 2024; Songkro et al., 2022). Tartrazine similarly elevated total WBC, lymphocyte, and granulocyte counts ($p < 0.01$), reflecting its immunomodulatory and genotoxic effects on leukocytes (Zand et al., 2023; Savas et al., 2025). TIFPE ameliorated this leucocytosis, consistent with the immunomodulatory phenolics in tamarind pulp (Amir et al., 2015), while N-TIFP produced a significantly greater reduction in WBC, lymphocytes, and granulocytes than both TAR and TIFPE groups, attributed to improved solubility and cellular uptake (Soliman et al., 2023). Vitamin E similarly restored leukocyte parameters, consistent with its established antioxidant and antigenotoxic protection (Obeid et al., 2020).

Tartrazine also increased platelet count, PDW, P-LCC, and P-LCR, indicating enhanced platelet activation via ROS-mediated membrane and endothelial injury (Tahiroglu and Kara, 2022), while MPV and PCT remained unchanged indicating a selective effect on platelet activation rather than production or biomass (Balta et al., 2019). TIFPE alleviated these platelet disturbances, with N-TIFPE showing greater efficacy, plausibly due to improved phytochemical stability and sustained antioxidant release (Hassan et al., 2025).

Collectively, tartrazine-induced haematotoxicity impaired erythropoiesis, leukocyte activation, and platelet disturbance is consistent with oxidative stress and disrupted antioxidant defence (Golli et al., 2016). TIFPE co-administration offered partial protection, while N-TIFPE co-administration showed superior, near-normal restoration across all three cell lineages, with efficacy comparable to vitamin E, supporting nano-encapsulated tamarind extract as a promising hemoprotective strategy

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against food-additive-induced toxicity (Imam et al., 2021; Zhang et al., 2026).

3.2 SERUM BIOCHEMICAL PARAMETERS:

Parameter	NC	TFPE	N-TFPE	Vit E	TAR	TFPE + TAR	N-TFPE + TAR	Vit E + TAR
Serum glucose (mg/dL)	104.23 ± 2.45	108.56 ± 2.49	102.39 ± 2.39	102.85 ± 2.75	150.36 ± 2.16*	146.38 ± 2.31	129.9 ± 2.29 [#]	117 ± 2.28 ^{##}
Total Protein (g/dL)	7.45 ± 0.15	7.65 ± 0.16	7.69 ± 0.14	7.58 ± 0.15	5.26 ± 0.16*	5.63 ± 0.14	6.58 ± 0.15 [#]	7.32 ± 0.16 ^{##}
Total Bilirubin (mg/dL)	0.026 ± 0.001	0.028 ± 0.002	0.025 ± 0.001	0.028 ± 0.002	0.051 ± 0.002*	0.040 ± 0.001	0.034 ± 0.002 [#]	0.030 ± 0.001 ¹
ALT (IU/L)	45.69 ± 1.26	46.84 ± 1.28	47.29 ± 1.24	46.8 ± 1.27	84.56 ± 1.24**	74.56 ± 1.78 [#]	52.69 ± 1.52 [#]	48.64 ± 1.28 ^{##}
AST (IU/L)	10.68 ± 0.84	11.36 ± 0.76	10.69 ± 0.69	11.24 ± 0.89	19.36 ± 0.2*	17.34 ± 0.87 [#]	13.8 ± 0.6 ^{##}	12.7 ± 0.84 ^{##}
Creatinine (mg/dL)	0.725 ± 0.07	0.719 ± 0.08	0.698 ± 0.07	0.713 ± 0.07	1.42 ± 0.08*	1.02 ± 0.09 [#]	0.95 ± 0.09 [#]	0.842 ± 0.08 ^{##}

dL)								
BUN (mg/dL)	26.58 ± 0.95	27.42 ± 0.96	26.96 ± 0.94	27.14 ± 0.86	39.75 ± 0.99**	35.41 ± 0.95	31.27 ± 0.92 [#]	29.74 ± 0.89 [#]
Triglycerides (mg/dL)	123.56 ± 2.98	127.28 ± 2.94	126.74 ± 2.97	125.28 ± 2.96	71.25 ± 2.21*	85.69 ± 2.28 [#]	105.67 ± 2.68 ^{##}	117 ± 2.69 ^{##}
Total Cholesterol (mg/dL)	75.92 ± 1.24	78.41 ± 1.21	76.84 ± 1.23	78.54 ± 1.21	124.57 ± 1.24*	105.47 ± 1.24 [#]	85.69 ± 1.23 [#]	84.57 ± 1.27 ^{##}
HDLC (mg/dL)	51.26 ± 1.29	50.75 ± 1.31	52.67 ± 1.32	51.69 ± 1.31	35.47 ± 1.29**	41.56 ± 1.32 [#]	47.68 ± 1.31 [#]	48.95 ± 1.28 [#]
LDLC (mg/dL)	70.24 ± 1.21	71.56 ± 1.21	72.47 ± 1.19	70.45 ± 1.18	118.17 ± 1.20*	102.67 ± 1.18 [#]	85.69 ± 1.21 [#]	81.57 ± 1.19 ^{##}
CRP (mg/dL)	10.86 ± 0.36	10.75 ± 0.37	10.96 ± 0.41	10.49 ± 0.42	39.48 ± 0.74**	32.41 ± 0.68 [#]	21.45 ± 0.54 [#]	19.74 ± 0.49 ^{##}
Acid Phosphate	6.35 ± 0.14	6.45 ± 0.13	6.51 ± 0.12	6.38 ± 0.15	11.56 ± 0.14**	9.58 ± 0.13	8.75 ± 0.12 [#]	7.69 ± 0.14 ^{##}

NANO-ENCAPSULATED TAMARINDUS INDICA FRUIT PULP EXTRACT ATTENUATES
TARTRAZINE-INDUCED HAEMATOLOGICAL AND BIOCHEMICAL ALTERATIONS IN WISTAR RATS

(K A U nit s)								
Al ka lin e Ph os ph ata se (K A U nit s)	11 .4 5± 0. 32	11 .6 9± 0. 34	11 .8 7± 0. 31	11 .6 7± 0. 32	18. 67 ±0. 34 **	16 .9 8± 0. 32	13. 69 ±0. 31 [#] s	13. 02 ±0 .30 ##
C K- M B (U /L)	12 .6 8± 0. 38	11 .5 6± 0. 37	13 .5 4± 0. 39	12 .7 8± 0. 35	25. 68 ±0. 41 **	20 .2 4± 0. 40 #	16. 69 ±0. 38 [#] s	13. 47 ±0 .37 ##
Ca lci u m (m g/ dL)	10 .3 8± 0. 32	10 .6 9± 0. 34	11 .2 4± 0. 36	11 .0 2± 0. 32	19. 78 ±0. 36 **	16 .5 7± 0. 34 #	14. 56 ±0. 32 [#]	13. 98 ±0 .30 ##
Ph os ph or us (m g/ dL)	5. 68 ±0 .1 4	5. 96 ±0 .1 5	5. 76 ±0 .1 6	5. 69 ±0 .1 7	12. 47 ±0. 17 **	10 .2 8± 0. 19	7.6 5± 0.1 7 [#] s	6.5 4± 0.1 2 ^{##}

NC- Normal Control, TIFPE- Aqueous extract of *Tamarindus indica* Fruit Pulp, N-TIFP- Chitosan-TPP encapsulated aqueous extract of *Tamarindus indica* Fruit Pulp, VitE- Vitamin E, TAR- Tartrazine, TIFPE+TAR - Aqueous extract of *Tamarindus indica* Fruit Pulp + Tartrazine, N-TIFPE + TAR - Chitosan-TPP encapsulated aqueous extract of *Tamarindus indica* Fruit Pulp + Tartrazine, VitE + TAR – Vitamin E + Tartrazine. Values (Mean ± SEM, n=6) bearing superscript in single column are significantly different in One way ANOVA followed by Tukey's multiple comparison test. Comparison with control group (Normal Control), TAR and TIFPE+ TAR groups has shown in *,# and s accordingly. The number of superscripts indicates the level of significance $p < 0.05$, $p < 0.01$

are represented by one and two superscripts respectively.

Tartrazine exposure induced widespread metabolic and organ-specific derangement, consistent with systemic oxidative stress-mediated toxicity. Serum glucose rose significantly, indicating disrupted glucose homeostasis, likely through oxidative impairment of pancreatic β -cell function and hepatic glycogenolysis, while total protein fell sharply, reflecting compromised hepatic protein synthetic capacity under sustained oxidative burden (Ibrahim et al., 2024).

Hepatic injury was clearly evident: ALT and AST rose markedly, alongside elevated total bilirubin, indicating hepatocellular membrane damage with leakage of cytosolic enzymes and impaired bilirubin conjugation/excretion, a hallmark of azo dye-induced hepatotoxicity driven by ROS-mediated lipid peroxidation of hepatocyte membranes. Concurrently, elevated creatinine and BUN signalled reduced glomerular filtration and renal tubular compromise, extending the oxidative insult beyond the liver to renal architecture (Abd Elaziz et al., 2024).

The lipid profile shifted toward a distinctly atherogenic pattern total cholesterol and LDL-C increased substantially, while HDL-C and triglycerides declined consistent with oxidative modification of circulating lipoproteins and disrupted hepatic lipid metabolism under tartrazine challenge (Dafallah et al., 2015). This dyslipidemic shift was accompanied by a marked rise in CRP, confirming a systemic inflammatory component underlying the biochemical toxicity rather than isolated organ-specific injury. Elevated acid and alkaline phosphatase activities further indicated hepatobiliary and membrane-turnover disturbance, while raised CK-MB pointed to cardiac muscle stress, reinforcing that tartrazine's toxic reach extends to cardiac tissue. The parallel elevation in serum calcium and phosphorus is consistent with disrupted mineral homeostasis secondary to renal compromise and altered bone/parathyroid signalling.

TIFPE co-administration produced a moderate, but incomplete, correction across all these parameters, in line with the antioxidant phytoconstituents of tamarind pulp partially neutralizing tartrazine-derived oxidative stress, yet values remained significantly different from control, pointing to the bioavailability ceiling of the free aqueous extract. N-TIFPE, by contrast, drove significantly greater normalization across every biochemical axis metabolic (glucose, protein), hepatic (ALT, AST, bilirubin, phosphatases), renal (creatinine, BUN), lipid (cholesterol, LDL-C, HDL-C, triglycerides), inflammatory (CRP), cardiac (CK-MB), and mineral (calcium, phosphorus) with most values converging toward those of the Vitamin E-treated group (Lawson 2023., Nguyen and Goycoolea., 2017).

This markedly superior efficacy is consistent with chitosan–TPP encapsulation enhancing the solubility, protection, and systemic availability of tamarind's antioxidant constituents relative to the unencapsulated extract, translating into more effective suppression of tartrazine-induced oxidative organ injury.

Conclusion: Tartrazine administration induced significant haematological and serum biochemical alterations in Wistar rats, indicating adverse effects on physiological and metabolic functions. Treatment with *Tamarindus indica* fruit pulp extract attenuated these alterations, while nano-encapsulated tamarind extract demonstrated higher protective efficacy, with several parameters approaching normal control values. Overall, nano-encapsulated tamarind extract shows promising potential in mitigating tartrazine-induced haematological and biochemical disturbances, highlighting the possible benefits of nano-encapsulation in enhancing the protective effects of tamarind bioactive compounds.

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