

DOI: <https://doi.org/10.71317/kjard.2.10.2026.890>

Kashmir Journal of Academic Research and Development

Journal homepage: <https://rjsaonline.org/index.php/KJARD>

Innovative Assessment to Modulate the Toxic Effects of a Potentially Toxic Dose of CuO Nanoparticles using Trigonella Foenum-Graecum Methanol Seed Extract in Oreochromis Mossambicus

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Email: drshakeelahmadshahid@gmail.com**ARTICLE INFO****ABSTRACT****Received:**

September 07, 2026

Revised:

September 30, 2026

Accepted:

October 11, 2026

Available Online:

October 21, 2026

Keywords:

copper oxide nanoparticles;
Trigonella foenum-graecum;
methanolic seed extract;
Oreochromis mossambicus;
hematology; aquatic toxicology.

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This study evaluated the potential ameliorative effects of methanolic seed extract of *Trigonella foenum-graecum* (T-MSE) on hematological changes associated with exposure to copper oxide nanoparticles (CuO-NPs) in *Oreochromis mossambicus*. Seventy-two fish (reported body mass: 30–45 g) were allocated to four treatment groups, including an untreated control and three groups receiving CuO-NPs at 0.12 mg/L together with T-MSE. The manuscript reports T-MSE doses inconsistently across the abstract, methods, and tables; the correct doses must be confirmed from the original experimental records before submission. Blood samples were collected at three reported intervals (days 7, 28, and 56), although the exposure duration is also reported as 28 days. These timeline details require confirmation. The reported results indicate that hematological changes were limited at the earliest sampling point and became more pronounced at later sampling points in the lower-extract treatment groups. The highest T-MSE treatment generally showed values closer to the control for several reported hematological measures, including erythrocyte count and hemoglobin. These findings are consistent with a possible partial ameliorative effect, but the magnitude and statistical support of that effect should be interpreted only after the treatment doses, sampling schedule, experimental unit, and statistical analysis have been verified. The study highlights the need for controlled follow-up experiments to determine whether T-MSE can mitigate CuO-NP-associated hematological effects in fish.

Introduction

Metallic nanoparticles are used in a range of industrial, agricultural, electronic, and biomedical applications. Their physicochemical properties, including small size and high surface-area-to-volume ratio, can make them behave differently from their bulk counterparts (Ramay et al., 2015; Raza et al., 2016). Metal and metal-oxide nanomaterials are used in applications

such as catalysis, electronics, photonics, and other technologies (Subramanian et al., 2015; Cristea et al., 2017; Chow, 2018). These uses have increased interest in understanding their environmental fate and potential biological effects. Nanomaterials are also investigated for medical and diagnostic applications, including biosensing and drug-delivery technologies (Kuunal et al., 2016). Their antimicrobial properties have led to use in some coatings and consumer products (Molling et al., 2014; Quadros et al., 2013). However, the presence of engineered nanomaterials in products and industrial processes creates opportunities for their release into the environment, making assessment of their safety important.

The increasing use of nanomaterials has raised concerns about possible effects on human and environmental health (Luo et al., 2018). After release, nanoparticles may enter soil and aquatic systems and interact with organisms through routes such as the gills, skin, or ingestion. Their behavior and biological effects can depend on factors including particle size, shape, surface charge, aggregation, and interactions with surrounding molecules (Lee and Ytreberg, 2012; Mattsson et al., 2015; Agnihotri et al., 2014). These factors make it important to characterize exposure materials and evaluate biological responses under clearly defined experimental conditions. Copper oxide nanoparticles (CuO-NPs) are used in several commercial and technological applications. Their potential toxicity has therefore attracted attention in environmental and toxicological research (Wang et al., 2015; Wu and Tang, 2018; Mercado et al., 2019). Experimental studies have reported that copper-based nanomaterials can affect biological tissues and may be associated with oxidative stress and changes in physiological function (Ahmed et al., 2016; Noureen et al., 2019). The extent of these effects may depend on exposure concentration, duration, particle characteristics, and the species under investigation. The behavior of CuO-NPs in biological systems may be influenced by their surface chemistry, aggregation, and interactions with dissolved substances. Because these properties can affect dispersion and bioavailability, the characterization of the material used in an experiment is important. Reports of oxidative stress and other biological responses following nanoparticle exposure support the need to evaluate potential effects in non-target organisms, including aquatic species (Kumar et al., 2019; Pagano and Faggio, 2015). The specific claims and references in this paragraph should be checked against the original sources during reference verification. Aquatic organisms may be exposed to nanoparticles released into water bodies, where they can encounter organisms that are not the intended targets of these materials (Noureen et al., 2019). Fish are commonly used in aquatic toxicology because measurable physiological and hematological responses can provide information about exposure-related stress. *Oreochromis mossambicus* was selected for the present study to investigate hematological responses to CuO-NP exposure and to examine whether methanolic fenugreek seed extract may modify those responses.

Medicinal plants contain diverse compounds, including phenolics, flavonoids, terpenoids, saponins, and other constituents that have been investigated for antioxidant or other biological activities (Hamid et al., 2013; Kusumaa et al., 2014). Plant-derived preparations have therefore been studied as potential modifiers of stress responses in animals, including fish. However, the effects of a plant extract depend on its composition, preparation, dose, and the experimental context; any protective effect should be demonstrated rather than assumed. *Trigonella foenum-graecum* (fenugreek), a member of the Fabaceae family, is cultivated in parts of Asia and North Africa and is commonly used as a food and spice. Its seeds contain dietary fibre and a range of phytochemicals, and published research has examined their potential biological activities (Meghwal and Goswami, 2012; Srinivasan, 2019). On this basis, the present study investigated whether methanolic fenugreek seed extract could modify hematological changes associated with CuO-NP exposure in *O. mossambicus*. The objective of this study was to assess hematological responses in *O. mossambicus* exposed to CuO-NPs and to evaluate the potential ameliorative effects of T-MSE. The original manuscript reports sampling on days 7, 28, and 56; the relationship between the 28-day exposure period and the day-56 sampling point must be clarified by the authors.

Materials and Methods

Fish procurement, acclimatization, and water-quality parameters

Oreochromis mossambicus (tilapia) were obtained from the Manawan Fisheries Complex, Lahore, Punjab, Pakistan, and transferred to the Fish Research Laboratory, Department of Zoology, Government College University Faisalabad, Pakistan. Fish were acclimatized for two weeks before the experiment and fed a commercial diet manufactured by Wudi Taiija Agriculture Development Co. Ltd., China. The reported diet contained 65% crude protein and 10% fat. Water temperature was maintained at 28 °C, with a 12 h light/12 h dark photoperiod. The manuscript reports dissolved oxygen of 6.5–7.4 mg/L, pH 6.7–7.2, ammonia of 0.5–0.7 ppm, total hardness of 47–52 ppm, and total dissolved solids of 6.5–7.8. The units and measurement methods for total dissolved solids should be confirmed.

Ethical approval

The authors report that the in vivo fish experiments were approved by the Animal Ethics Committee of Government College University Faisalabad, Pakistan. The approval/reference number and approval date should be added if available. Fish were reportedly maintained and handled in accordance with the committee's animal-care guidance.

Chemicals

Copper oxide nanoparticles (CuO-NPs; reported nominal size below 50 nm) were purchased from Sigma-Aldrich. The supplier catalogue number and the complete CAS number should be verified against the product documentation; the number in the original manuscript is corrupted.

Selection of CuO-NP concentration

The study used a nominal CuO-NP concentration of 0.12 mg/L, selected on the basis of preliminary work from the same research project that is described in the original manuscript as unpublished. The basis for selecting this concentration should be explained, and any available supporting data should be retained for editorial review.

Characterization of CuO-NPs

The original manuscript states that CuO-NPs were characterized using scanning electron microscopy (SEM), X-ray diffraction (XRD), and zeta-potential analysis. It reports a nominal supplier particle size below 50 nm, an XRD-derived crystallite size of approximately 90 nm, and SEM-visible particles/agglomerates in the micrometre range. These measurements are not necessarily equivalent, but the distinction between primary particle size, crystallite size, and agglomerate size must be stated clearly. The phase assignment in the original XRD description also alternates between CuO and Cu₂O; this must be checked against the raw diffraction pattern and instrument report before publication.

Preparation of *Trigonella foenum-graecum* methanolic seed extract (T-MSE)

Dried *T. foenum-graecum* seeds were purchased from a local supplier. The authors report that the plant material was taxonomically confirmed by the Department of Botany, Government College University Faisalabad, Pakistan. A voucher specimen number should be supplied if one exists.

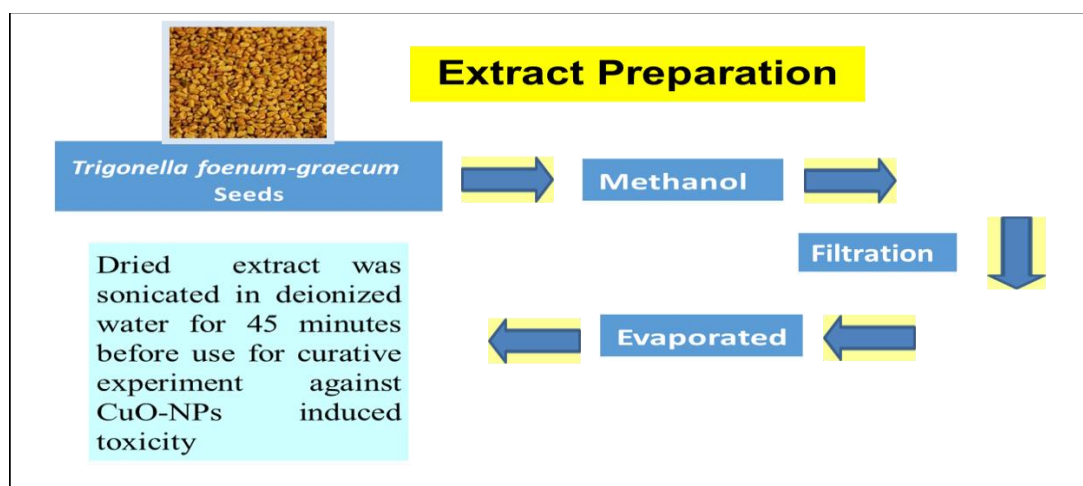


Fig. 1: Diagrammatic presentation of plant seed extract preparation

The seeds were ground to a fine powder using a Renker GMO 1 grinder. The original protocol describes macerating 2 kg of powdered seeds in 2.5 L of methanol in an airtight container for 15 days at room temperature, with periodic agitation and additional methanol as needed to keep the material wet. The extract was subsequently processed using a Soxhlet apparatus. The reported yield of T-MSE was 55.23 g, which was stored in a dry, airtight container for later use. The authors should clarify the sequence of maceration and Soxhlet extraction, the volume of methanol used overall, and whether the solvent was removed before the extract was administered.

Experimental design and treatment groups.

Seventy-two fish were reportedly assigned randomly to four groups, with three replicates per group and six fish per replicate. The control group received no CuO-NPs or T-MSE; the other groups received CuO-NPs at 0.12 mg/L together with different T-MSE concentrations. The treatment concentrations are inconsistent in the source manuscript: the narrative reports 16, 34, and 52 mg/L; the design table reports 16, 32, and 52 mg/L; and the hematology tables report 18, 26, and 52 mg/L. The correct concentrations must be confirmed from the original records before the final manuscript is submitted.

Table 1.

Experimental groups and treatment allocation

Sr #	Groups	Doses (Treatment)		# of fish allocated		
		CuO (mg/l)	T-MSE (mg/l)	<i>Oreochromis mossambicus</i> (Triplicate)		
1	Control	—	—	6	6	6
2	G1	0.12	16	6	6	6
3	G2	0.12	32	6	6	6
4	G3	0.12	52	6	6	6

The original manuscript states that CuO-NPs and T-MSE were administered on alternate days and that water was changed regularly while physical conditions were maintained. It reports no mortality during the experiment and states that exposure continued for 28 days. However, sampling is also reported on day 56. The authors must clarify whether the fish were sampled on day 56 after a 28-day exposure followed by a recovery/observation period, or whether exposure continued for 56 days. The applicable OECD guidance should be cited accurately and should match the experimental design; an acute-test guideline should not be presented as validation of a different exposure design.

Preparation of exposure media

Exposure media were reportedly prepared with reference to Shaw et al. (2012) and Noreen et al. (2019). The required quantity of CuO-NPs was weighed and dispersed in deionized/ultrapure water (reported resistivity: 18.2 MΩ-cm), with 0.1 mL acetic acid added as a dispersing solvent. The suspension was vortexed at 2,000 rpm for 10 min and sonicated at 100 W and 40 kHz for 1 h. Fresh suspensions were prepared before each dosing event. The original manuscript states that T-MSE solutions were prepared similarly but without an organic solvent. The final concentration of acetic acid in the exposure medium, the volume administered per tank, and whether solvent controls were used should be specified.

Sampling and hematological measurements

Sampling was reportedly conducted on days 7, 28, and 56. Fish were anesthetized using clove oil at 100 µg/L, and blood was collected from the caudal vein into tubes containing EDTA as an anticoagulant. Turk's solution and Toisson's solution were used for leukocyte and erythrocyte counts, respectively, according to the original manuscript. Hematocrit was measured using microhematocrit tubes following the cited Dacie and Lewis method. The original text refers to a hemocytometer for hemoglobin detection; this is likely a method-description error and must be checked against the laboratory protocol, with the actual hemoglobin assay and equipment reported accurately.

Statistical analysis

The original manuscript reports analysis using one-way analysis of variance (ANOVA), with results expressed as mean ± standard error (SE), followed by Tukey's multiple-comparison test in Minitab. The authors should report the software version, exact sample size used for each analysis, assumptions/checks, and the statistical model used across treatment groups and sampling times. Because fish were housed in replicate tanks, the tank may be the independent experimental unit; the analysis must avoid treating fish within the same tank as independent replicates. Exact p-values and effect sizes should be included where available.

Results

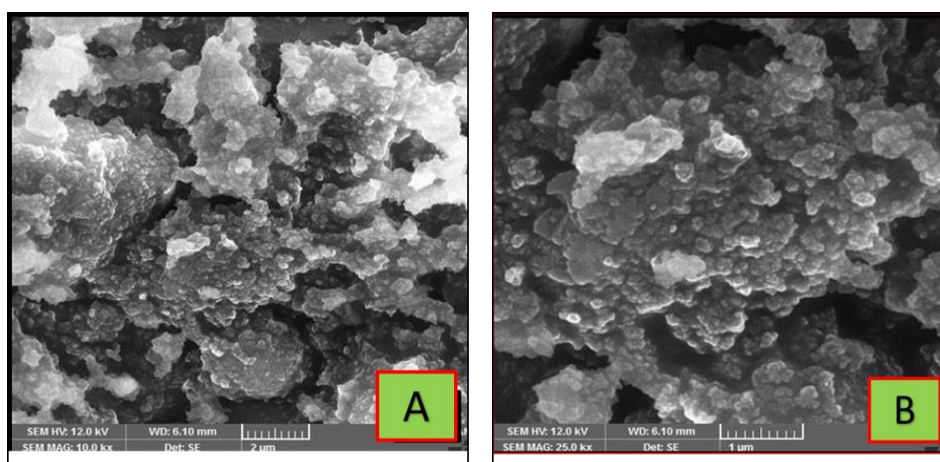


Fig. 2. SEM micrographs of the CuO-NPs at various magnifications as 10.0 kx, 25.0 kx and 50.0 kx

Scanning electron microscopy (SEM)

SEM images were obtained at reported magnifications of 10,000 $\times$, 25,000 $\times$, and 50,000 $\times$. The original description reports irregular particles and agglomerates with apparent dimensions of approximately 1–2 μm and a stated purity of 99%. These observations should be interpreted as the sizes visible in the SEM images unless the primary-particle dimensions were independently measured. The figure caption and text should be aligned with the scale bars and the supplier's characterization data.

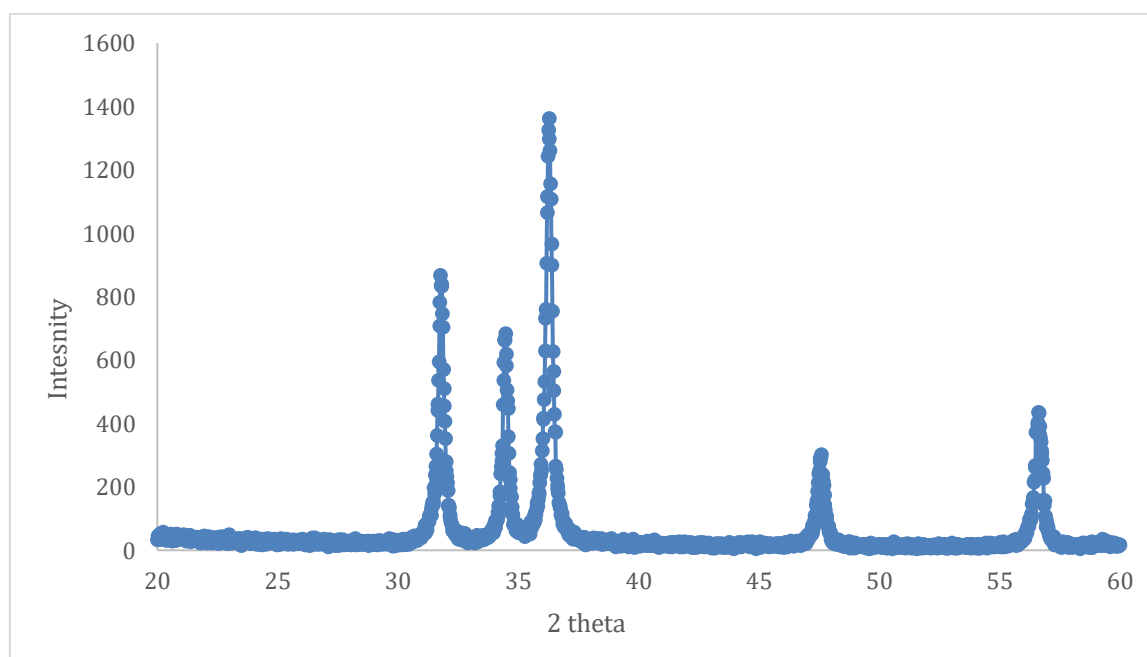


Fig. 3. XRD image of CuO-NPs; XRD analysis show intense peak at 23.5°, 32.5°, 33.5 °, 46.0 °, 59.0 °

The XRD pattern was reportedly collected from dried CuO-NP material over a 2θ range of 10–80° using Cu K α radiation. The original manuscript lists peaks at several angles and describes them as crystalline copper oxide, but it also assigns some peaks to Cu₂O while referring to the material as CuO. The phase identification and peak indexing must be re-evaluated using the raw diffractogram and a suitable reference database before the results are reported as confirmed.

$$D = 0.9\lambda/(\beta \cos \theta)$$

where λ is the X-ray wavelength, β is the full width at half maximum (FWHM) of the selected diffraction peak in radians, and θ is the Bragg angle. The original manuscript estimates a crystallite size of approximately 90 nm using the Scherrer equation. This value should be recalculated from the raw peak data and reported with the selected peak and calculation assumptions.

Hematological responses and potential ameliorative effects of T-MSE

Hematological responses to CuO-NP and T-MSE exposure

The study examined erythrocyte count, hemoglobin, hematocrit, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), leukocyte count, neutrophils, lymphocytes, monocytes, and basophils. According to the original tables and narrative, the earliest sampling point showed few or no statistically significant differences from the control, whereas several hematological parameters differed from the control at later sampling points. Interpretation of these patterns depends on confirmation of the sampling timeline, treatment concentrations, and statistical analysis.

Erythrocyte counts were reported to be lower in the lower-T-MSE groups than in the control at days 28 and 56. At day 56, the table reports erythrocyte counts of $1.94 \pm 0.003 \times 10^6/\mu\text{L}$ in the control, $1.73 \pm 0.003 \times 10^6/\mu\text{L}$ in G1, $1.78 \pm 0.003 \times 10^6/\mu\text{L}$ in G2, and $1.82 \pm 0.004 \times 10^6/\mu\text{L}$ in G3. Hemoglobin was also reported to be lower in the treatment groups than in the control at later sampling points. These numerical values and their significance annotations should be checked against the raw dataset before publication.

The original results describe changes in MCV, MCH, and MCHC at later sampling points. The direction and statistical significance of each parameter should be described individually and should match the corresponding table values; the current narrative contains conflicting descriptions of whether these indices increased or decreased. No direction of change should be inferred where the tabulated values or underlying data do not clearly support it.

The manuscript reports increases in leukocyte count and in the percentages of neutrophils, lymphocytes, and monocytes in some treatment groups at later sampling points. These responses may be consistent with a physiological response to exposure-related stress, but the interpretation should remain cautious and be based on the verified data and statistical comparisons. Basophil results should also be described if they were measured and are included in the tables.

The highest T-MSE treatment group (G3) was reported to show values closer to the control for several hematological parameters than the lower-extract groups. For example, the day-56 erythrocyte count in G3 was $1.82 \pm 0.004 \times 10^6/\mu\text{L}$ compared with $1.94 \pm 0.003 \times 10^6/\mu\text{L}$ in the control. This pattern suggests partial amelioration rather than complete normalization. The interpretation should be retained only if confirmed by the raw data and appropriate statistical comparisons.

Table 1: Hematological parameters of *O. mossambicus* following 7 days of reported treatment with CuO-NPs and T-MSE.

Parameters	Units	Days	Groups / Doses (mg/L)			
			C (Control) 0.00	G1 (CuO-NPs <i>Trigonella</i> <i>foenum- graecum</i>) (0.12 + 18.00)	G2 (CuO-NPs <i>Trigonella</i> <i>foenum- graecum</i>) (0.12 + 26.00)	G3 (CuO-NPs <i>Trigonella</i> <i>foenum- graecum</i>) (0.12 + 52.00)
Erythrocytes	($10^6/\mu\text{L}$)		1.93 $\pm$ 0.005 ^A	1.93 $\pm$ 0.004 ^A	1.92 $\pm$ 0.007 ^A	1.90 $\pm$ 0.007 ^A
Hemoglobin	(g/dL)		8.44 $\pm$ 0.008 ^A	8.44 $\pm$ 0.007 ^A	8.39 $\pm$ 0.014 ^A	8.42 $\pm$ 0.027 ^A
Hematocrit	(%)		26.02 $\pm$ 0.24 ^A	26.03 $\pm$ 0.17 ^A	25.15 $\pm$ 0.46 ^A	25.56 $\pm$ 0.40 ^A
Mean Corpuscular Volume	(fL)		142.76 $\pm$ 0.26 ^A	143.37 $\pm$ 0.63 ^A	142.52 $\pm$ 0.30 ^A	143.81 $\pm$ 0.61 ^A
Mean Corpuscular Hemoglobin	(pg)	07	54.90 $\pm$ 0.74 ^A	54.85 $\pm$ 0.62 ^A	55.91 $\pm$ 0.18 ^A	54.45 $\pm$ 0.42 ^A
MCH Concentration	(g/dL)		37.44 $\pm$ 0.66 ^A	37.88 $\pm$ 0.28 ^A	37.94 $\pm$ 0.40 ^A	37.95 $\pm$ 0.25 ^A
Leucocytes	($10^3/\mu\text{L}$)		50.42 $\pm$ 0.52 ^A	51.61 $\pm$ 0.32 ^{AB}	51.94 $\pm$ 0.21 ^B	51.68 $\pm$ 0.33 ^B
Neutrophils	(%)		44.71 $\pm$ 0.70 ^A	44.52 $\pm$ 0.75 ^A	45.52 $\pm$ 0.84 ^A	43.72 $\pm$ 0.48 ^A
Lymphocytes	(%)		25.10 $\pm$ 0.41 ^A	25.54 $\pm$ 0.23 ^A	25.51 $\pm$ 0.39 ^A	25.75 $\pm$ 0.21 ^A
Monocytes	(%)		7.15 $\pm$ 0.35 ^A	7.39 $\pm$ 0.39 ^A	7.37 $\pm$ 0.49 ^A	7.99 $\pm$ 0.25 ^A

Values are presented as mean $\pm$ SE from three reported replicates. Different superscript letters within a parameter indicate a statistically significant difference at $p < 0.05$, according to the original analysis. Verify the statistical comparisons and letters against the raw data.

Table 2; Hematological parameters of *O. mossambicus* following 28 days of reported treatment with CuO-NPs and T-MSE.

Parameters	Units	Days	Groups / Doses (mg/L)			
			G1 (Control) 0.00	G2 (CuO-NPs <i>Trigonella</i> <i>foenum- graecum</i>) (0.12 + 18.00)	G3 (CuO-NPs <i>Trigonella</i> <i>foenum- graecum</i>) (0.12 + 26.00)	G4 (CuO-NPs <i>Trigonella</i> <i>foenum- graecum</i>) (0.12 + 52.00)
Erythrocytes	(10 ⁶ /μl)	28	1.93±0.004 ^C	1.74±0.009 ^A	1.76±0.004 ^A	1.83±0.007 ^B
Hemoglobin	(g/dL)		8.45±0.07 ^D	6.99±0.05 ^A	7.21±0.05 ^B	7.52±0.01 ^C
Hematocrit	(%)		25.54±0.10 ^B	20.59±0.23 ^A	21.12±0.30 ^A	21.15±0.29 ^A
Mean Corpuscular Volume	(fl)		143.29±0.44 ^A	155.35±0.75 ^C	150.02±0.55 ^B	152.02±0.73 ^B
Mean Corpuscular Hemoglobin	(pg)		55.69±0.32 ^C	43.12±0.58 ^A	45.09±0.83 ^{AB}	46.13±0.72 ^B
MCH Concentration	(g/dL)		38.39±0.40 ^B	31.55±0.32 ^A	31.61±0.50 ^A	32.47±0.62 ^A
Leucocytes	(10 ³ /μl)		51.30±0.34 ^A	57.31±0.59 ^B	57.53±0.65 ^B	56.10±0.83 ^B
Neutrophils	(%)		45.11±0.43 ^A	57.34±0.59 ^C	55.92±0.69 ^{BC}	53.60±0.75 ^B
Lymphocytes	(%)	56	24.98±0.54 ^A	29.38±0.26 ^B	27.86±0.35 ^B	27.70±0.61 ^B
Monocytes	(%)		7.20±0.27 ^A	11.58±0.43 ^B	10.58±0.52 ^B	10.34±0.33 ^B

Values are presented as mean ± SE from three reported replicates. Different superscript letters within a parameter indicate a statistically significant difference at $p < 0.05$, according to the original analysis. Verify the statistical comparisons and letters against the raw data.

Table 3; Hematological parameters of *O. mossambicus* at the reported day-56 sampling point following treatment with CuO-NPs and T-MSE.

Parameters	Units	Days	Groups / Doses (mg/L)			
			C (Control) 0.00	G1 (CuO-NPs <i>Trigonella</i> <i>foenum- graecum</i>) (0.12 + 18.00)	G2 (CuO-NPs <i>Trigonella</i> <i>foenum- graecum</i>) (0.12 + 26.00)	G3 (CuO-NPs <i>Trigonella</i> <i>foenum- graecum</i>) (0.12 + 52.00)
Erythrocytes	(10 ⁶ /μl)	56	1.94±0.003 ^D	1.73±0.003 ^A	1.78±0.003 ^B	1.82±0.004 ^C
Hemoglobin	(g/dL)		8.48±0.004 ^C	6.85±0.003 ^A	6.93±0.007 ^A	7.39±0.04 ^B
Hematocrit	(%)		25.74±0.19 ^C	19.84±0.21 ^A	20.79±0.23 ^B	21.51±0.20 ^B
Mean Corpuscular Volume	(fl)		143.71±0.61 ^A	158.69±0.58 ^C	150.71±0.23 ^B	148.47±0.51 ^B
Mean Corpuscular Hemoglobin	(pg)		56.11±0.38 ^C	42.75±0.27 ^A	43.86±0.61 ^A	47.88±0.22 ^B
MCH Concentration	(g/dL)		38.54±0.28 ^C	30.63±0.20 ^A	30.86±0.22 ^A	32.63±0.25 ^B
Leucocytes	(10 ³ /μl)		51.23±0.25 ^A	58.58±0.28 ^B	57.38±0.32 ^B	55.82±0.35 ^C
Neutrophils	(%)		45.99±0.14 ^A	58.20±0.53 ^B	57.54±0.31 ^B	54.55±0.32 ^C

Lymphocytes	(%)	25.02±0.19 ^A	30.50±0.32 ^B	28.79±0.29 ^B	27.72±0.65 ^C
Monocytes	(%)	7.94±0.17 ^A	12.21±0.24 ^C	10.82±0.31 ^B	10.49±0.15 ^B

Values are presented as mean ± SE from three reported replicates. Different superscript letters within a parameter indicate a statistically significant difference at $p < 0.05$, according to the original analysis. Verify the statistical comparisons and letters against the raw data.

Discussion

Hematological measurements are widely used to assess physiological status in fish and can respond to environmental stressors (Javed and Usmani, 2014; Akbary et al., 2018). The present study examined several red- and white-blood-cell indices in *O. mossambicus* following reported exposure to CuO-NPs with different concentrations of T-MSE. The reported changes at later sampling points suggest that exposure may have been associated with altered hematological status. However, conclusions about exposure duration and dose response must remain provisional until the conflicting treatment concentrations and sampling timeline are resolved. Plant-derived preparations have been investigated as potential modifiers of physiological responses because they contain diverse bioactive constituents (Allaoui et al., 2019; Khorshidian et al., 2016). Fenugreek seeds have been studied for their nutritional composition and potential biological activities (Meghwal and Goswami, 2012; Srinivasan, 2019). In the present study, the group assigned the highest T-MSE concentration was reported to show values closer to the control for some hematological parameters than groups assigned lower concentrations. This pattern is compatible with a possible ameliorative effect, but it does not establish the active compounds, mechanism, or optimal dose of the extract.

Hemoglobin concentration and erythrocyte count are relevant indicators of oxygen-carrying capacity. The source tables report lower hemoglobin and erythrocyte values in some CuO-NP plus T-MSE groups than in the control at later sampling points. Such changes may be consistent with an anemia-like response or altered erythrocyte status, but this interpretation should be supported by the verified data and by consideration of other measures, including hematocrit and erythrocyte indices. The original manuscript cites related fish toxicology studies in discussing these findings; each citation should be checked to ensure that it supports the specific comparison being made. Hematological indices can provide useful information about fish responses to environmental exposure, nutritional changes, and other stressors (Hoseini and Nodeh, 2013; Hoseini et al., 2018; Mazandarani and Hoseini, 2017). The present results should be interpreted in this broader context rather than as evidence of a mechanism specific to CuO-NPs. The absence of a T-MSE-only group, if confirmed, would also limit the ability to distinguish effects of the extract itself from its potential interaction with CuO-NPs. The original discussion proposes that reduced erythrocyte counts may reflect shortened erythrocyte lifespan, altered blood-cell production, or respiratory stress. These are possible explanations, not mechanisms demonstrated by the present experiment. Direct evidence such as blood-cell morphology, tissue histology, oxidative-stress markers, or relevant physiological measurements would be needed to evaluate them. The discussion should therefore avoid attributing the observed changes to a specific organ or process unless that process was measured. The manuscript reports higher leukocyte counts and changes in the proportions of neutrophils, monocytes, and lymphocytes in some treatment groups. These changes may reflect an immune or stress response, but they are not, on their own, proof of infection, inflammation, or organ dysfunction. Similar responses have been described in some fish exposed to copper-based nanoparticles (Noureen et al., 2018; Jahanbakhshi et al., 2015; Kaviani et al., 2019). The exact direction, magnitude, and statistical significance of each response should be stated only after checking the raw dataset and table annotations.

The highest T-MSE treatment was described as showing the greatest apparent improvement in several hematological measures at the later sampling point. Because the values did not consistently return to the control values, the results are more appropriately described as partial amelioration rather than repair or complete protection. In addition, interpretation of a concentration-related trend requires the treatment concentrations to be confirmed and the analysis to account for the true experimental unit. The original manuscript discusses antioxidant and immunomodulatory properties attributed to fenugreek in previous literature (Goyal et al., 2016). These properties provide a rationale for further investigation but were not directly demonstrated by the hematological measurements reported here. Without chemical profiling of the extract and measurements of oxidative stress or related pathways, the study cannot identify which constituents might contribute to the observed pattern or establish a mechanism of action.

MCV, MCH, and MCHC describe different aspects of erythrocyte size and hemoglobin content. The original narrative contains inconsistent descriptions of the direction of change in these indices and refers to different T-MSE concentrations than those listed in the tables. These statements should be reconciled against the raw results before publication. Comparisons with previous work on copper nanoparticle exposure in fish (Noureen et al., 2018) should be retained only where the species, exposure conditions, and reported outcome are accurately represented. Leukocytes and leukocyte subtypes contribute to

immune defence, but changes in their counts can have multiple causes. The current results may indicate a physiological response to the experimental treatments, yet they do not independently demonstrate dysfunction of the kidney, liver, or another organ. Such mechanistic claims would require supporting measurements. Overall, the study offers preliminary observations on hematological responses in *O. mossambicus* and the possible modifying effect of T-MSE; reliable conclusions depend on resolving the data and design inconsistencies identified in the author-verification notes.

Disclosure statement

The authors declare that they have no competing interests.

Acknowledgements

This study was supported by the Higher Education Commission (HEC), Pakistan, under project No. 20-3893/NRPU/R&D/HEC/14/913, entitled "Oxidative Stress and Genotoxic Effects of Waterborne Copper Nanoparticle and Copper Sulphate and the Ameliorating Effects of *Myristica fragrans*, *Moringa oleifera*, and *Trigonella foenum-graecum* in *Oreochromis mossambicus*." The project title and botanical spellings should be checked against the official award documentation.

Conclusion

The present study provides preliminary evidence that exposure to waterborne copper oxide nanoparticles (CuO-NPs) is associated with alterations in several hematological parameters of *Oreochromis mossambicus*, suggesting potential physiological stress in exposed fish. The reported differences in erythrocyte count, hemoglobin concentration, and other blood indices highlight the value of hematological assessment as a tool for investigating the biological responses of aquatic organisms to nanoparticle exposure. These findings underscore the importance of evaluating the potential environmental implications of nanomaterials, particularly in aquatic ecosystems. The treatment group receiving the highest concentration of methanolic *Trigonella foenum-graecum* seed extract (T-MSE) generally exhibited values closer to the control for certain hematological parameters than groups receiving lower extract concentrations. This pattern suggests a possible partial ameliorative effect of T-MSE against CuO-NP-associated hematological alterations. Nevertheless, the observed responses do not demonstrate complete protection, establish a definitive dose-dependent relationship, or identify the biochemical mechanisms responsible for apparent improvement. The potential contribution of fenugreek-derived bioactive compounds therefore remains an important area for further investigation rather than a confirmed protective mechanism.

Future research should incorporate verified treatment concentrations, clearly defined exposure and sampling periods, appropriate extract-only and solvent controls, adequately replicated experimental designs, and statistical analyses based on the correct experimental unit. In addition, integrating hematological measurements with oxidative-stress biomarkers, biochemical assessments, and histopathological examinations could provide a more comprehensive understanding of the biological effects of CuO-NPs and the potential role of T-MSE. Careful characterization of the nanoparticles and chemical profiling of the extract would further strengthen the interpretation and reproducibility of the findings.

Overall, this study offers a useful preliminary basis for further investigating the interaction between nanoparticle exposure and plant-derived preparations in fish. Confirmation through rigorous, independently replicated experiments is essential before drawing firm conclusions about the protective efficacy, optimal concentration, or practical environmental application of fenugreek seed extract. Addressing these research priorities could contribute to a more robust understanding of nanoparticle-associated aquatic stress and support future investigations into scientifically validated, plant-derived approaches to mitigating adverse biological response.

References

- Abdel-Khalek AA, Kadry MA, Badran SR, Marie MA. (2015). Comparative toxicity of copper oxide bulk and nano particles in Nile tilapia; *Oreochromis niloticus*: biochemical and oxidative stress. *The Journal of Basic & Applied Zoology*, 72, 43-57.
- Akbary P, Yarahmadi SS, Jahanbakhshi A. (2018). Hematological, hepatic enzymes' activity and oxidative stress responses of gray mullet (*Mugil cephalus*) after sub-acute exposure to copper oxide. *Environmental Science and Pollution Research*, 25(2), 1800-1808.
- Agnihotri S, Mukherji S, Mukherji S. (2014). Size-controlled silver nanoparticles synthesized over the range 5–100 nm using the same protocol and their antibacterial efficacy. *Royal Society of Chemistry, Advances*, 4(8), 3974-3983. DOI: 10.1039/c3ra44507k

- Alkaladi A, El-Deen NA, Afifi M, Zinadah OA. (2015). Hematological and biochemical investigations on the effect of vitamin E and C on *Oreochromis niloticus* exposed to zinc oxide nanoparticles. Saudi journal of biological sciences, 22(5), 556-563. DOI <https://doi.org/10.26524/nr1921>
- Ahmed S, Saifullah AM, Swami BL, & Ikram S. (2016). Green synthesis of silver nanoparticles using *Azadirachta indica* aqueous leaf extract. Journal of Radiation Research and Applied Sciences, 9(1), 1-7. [Hhps://doi.org/10.1016/j.jrrs](https://doi.org/10.1016/j.jrrs).
- Allaoui AS, Gascón S, Benomar J, Quero J, Osada M, Nasri A. Boualga. 2019. Protein Hydrolysates from Fenugreek (*Trigonella foenum graecum*) as Nutraceutical Molecules in Colon Cancer Treatment. Nutrients. 11(4), 724-732. Doi: 10.3390/nu11040724
- Chow JCL. 2018. Recent Progress in Monte Carlo Simulation on Gold Nanoparticle Radio-sensitization. AIMS Biophysics. 5, 231-244. DOI: 10.3934/biophy.2018.4.231
- Cristea CM, Tertis R, Galatus. 1991. Magnetic Nanoparticles for Antibiotics Detection. Practical Hematology, 7 Churchill Livingstone: London, UK, 1991.
- Cristea CM. Tertis R. Galatus. 2017. Magnetic Nanoparticles for Antibiotics Detection. Nanomaterials 7, 119-122.
- Deepa N, Usha R, Anbarasu A, Anuanandhi K, Karnan P, Elumalai K. (2018). Destaining Potential of Bacterial Lipase enzyme Isolated from *Providencia rettgeri* inhabiting the fresh-water fish *Mystus bleekeri*. World Journal of Pharmaceutical Research, 7, 2101-13. DOI: 10.20959/wjpr20187-11855
- El-Sayed SM, Youssef AM. (2019). Potential application of herbs and spices and their effects in functional dairy products. Heliyon, 5(6). <https://org/10.1016/j.heliyon.2019.e01989>
- Goyal SN, Gupta S, Chatterjee. 2016. Investigating the Therapeutic Potential of *Trigonella foenum-graecum* L. as our Defense Mechanism Against Several Human Diseases. Journal of Toxicology. 2, 1-10. <http://dx.doi.org/10.1155/2016/1250387>
- Hamid NH, Shirzad H. 2013. Journal of Herbs and Medicines Pharmacology, 2(2), 21-22.
- Hassan S M, Al-Aqil A A and Attimarad M 2013 Adv. Medicinal Plant Research. 1(1), 24-28. <https://doi.org/10.1088/1361-6528/aade23>
- Hassan W, Abdullah S, Afzal M, Hussain M. 2018. Assessment of Acute Metals Toxicity in Catla Catla Through Hematological and Biochemical Blood Markers. Pakistan Journal of Agricultural Sciences. 55, 34-38. DOI: 10.21162/PAKJAS/18.6002
- Hoseini SM, Mirghaed AT, Iri Y, Ghelichpour M. (2018). Effects of dietary cineole administration on growth performance, hematological and biochemical parameters of rainbow trout (*Oncorhynchus mykiss*). Aquaculture. 495, 766-772
- Hoseini SM, Mirghaed AT, Iri Y, Ghelichpour M. (2018). Effects of dietary cineole administration on growth performance, hematological and biochemical parameters of rainbow trout (*Oncorhynchus mykiss*). Aquaculture. 495, 766-772. <https://doi.org/10.1016/j.aquaculture.2018.06.073>
- Hoseini SM, Nodeh A. 2013. Changes in blood biochemistry of common carp *Cyprinus carpio* (Linnaeus), following exposure to different concentrations of clove solution. Compound Clinic Pathology. 22, 9-13. DOI 10.1007/s00580-011-1362-9
- Hoseini SM, Yousefi M, Hoseinifar SH, Van Doan, H. (2019). Antioxidant, enzymatic and hematological responses of common carp (*Cyprinus carpio*) fed with myrcene-or menthol-supplemented diets and exposed to ambient ammonia. Aquaculture. 506, 246-255. <https://doi.org/10.1016/j.aquaculture.2019.03.048>
- Islam M, Shehzadi N, Salman M, Zahid F, Qamar S, Danish MZ, Bukhari NI. (2019). Metabolomics and marker-based stability studies of methanol extract of seeds of *Syzygium cumini* L. Pakistan journal of pharmaceutical sciences, 32(2).
- Javed M, Usmani Islam N. 2014. Assessment of Heavy Metals (Cu, Ni, Fe, Co, Mn, Cr, Zn) in Rivulet Water, their Accumulations and Alterations in Hematology of Fish *Channa Punctatus*. African Journal of Biotechnology. 13(3), 492-501. DOI: 10.5897/AJB2013.13131
- Jahanbakhshi, A., Hedayati, A. and Pirbeigi, A. (2015). Determination of acute toxicity and the effects of sub-acute concentrations of CuO-NPs on blood parameters in *Rutilus rutilus*. Nanomed. J., 2(3); 195-202.
- Kaviani EF, Naeemi AS, Salehzadeh A. (2019). Influence of Copper Oxide Nanoparticle on Hematology and Plasma Biochemistry of Caspian Trout (*Salmo trutta caspius*), Following Acute and Chronic Exposure. Pollution, 5(1), 225-234.

- Khabbazi M, Harsij M, Hedayati SAA, Gholipoor H, Gerami MH, Ghafari FH. 2015. Effect of CuO Nanoparticles on some Hematological Indices of Rainbow Trout *Oncorhynchus Mykiss* and their Potential Toxicity. *Nanomedicine Journal*. 2(1), 67-73. DOI: 10.7508/nmj.2015.01.008
- Khabbazi M, Harsij M, Hedayati SAA, Gholipoor H, Gerami MH, Ghafari FH. 2015. Effect of CuO Nanoparticles on some Hematological Indices of Rainbow Trout *Oncorhynchus Mykiss* and their Potential Toxicity. *Nanomedicine Journal*. 2(1), 67-73. DOI: 10.22059/poll.2018.251034.383
- Khan RA, Khan MR, Sahreen S. 2012 Expt. Toxicological Pathology. 64(7-8) 725-731 <https://doi.org/10.1016/j.etp.2011.01.007>
- Khorshidian NMY. Asli AM, Mirzaie AA, Mortazavian AM. 2016. Fenugreek: Potential Applications as a Functional Food and Nutraceutical. *Nutrition Food Sciences Research*. 3, 5-16.
- Kumar SV, Bafana AP, Pawar P, Faltane M, Rahman A, Dahoumane SA, Jeffryes CS. 2019. Optimized Production of Antibacterial Copper Oxide Nanoparticles in a Microwave-Assisted Synthesis Reaction Using Response Surface Methodology. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*. 2(21), 12-25. <https://doi.org/10.1016/j.colsurfa.2019.04.063>
- Kusumaa IW, Arunga MET, Kim SY. 2014. *Food Science and Human Welfare*, 3, 191-196
- Kuunal S.S. Kutti P, Rauwel M, Guha D, Wragg E, Rauwel. 2016. Biocidal Properties Study of Silver Nanoparticles Used for Application in Green Housing. *International Nanotechnology*. 6, 191-197. DOI10.1007/s40089-016-0186-7
- Lee, K. H., & Ytreberg, F. M. (2012). Effect of gold nanoparticle conjugation on peptide dynamics and structure. *Entropy*, 14(4), 630-641., K. H., & Ytreberg, F. M. (2012). Effect of gold nanoparticle conjugation on peptide dynamics and structure. *Entropy*, 14(4), 630-641. <https://doi.org/10.3390/e14040630>
- Lavanya S, Ramesh M, Kavitha C, Malarvizhi A (2011) Hematological, biochemical and ionoregulatory responses of Indian major carp *Catla catla* during chronic sub-lethal exposure to inorganic arsenic. *Chemosphere* 82:977-985. <https://doi.org/10.1016/j.chemosphere.2010.10.071>
- Luo S, Lutkenhaus, JL. H Nasrabadi. 2018. Effect of Nano-Scale Pore Size Distribution on Fluid Phase Behavior of Gas IOR in Shale Reservoirs. In *SPE Improved Oil Recovery Conference*. Society of Petroleum Engineers. <http://doi.org/10.2118/190246-MS>
- Mazandarani M, Hoseini SM, 2017. Anaemia and plasma lipid profile in common carp (*Cyprinus carpio*) exposed to ambient copper sulphate and nano-scale copper oxide. *Aquaculture Research*. 48, 844-852. <https://doi.org/10.1111/are.12928>
- Mathews TJ, Driscoll AK. 2017. Trends in infant mortality in the United States, 2005-2014. <https://europepmc.org/abstract/med/26828319>
- Mercado, N., Bhatt, P., Sutariya, V., Florez, F. L. E., & Pathak, Y. V. (2019). Application of Nanoparticles in Treating Periodontitis: Preclinical and Clinical Overview. In *Surface Modification of Nanoparticles for Targeted Drug Delivery*, 467-480. https://doi.org/10.1007/978-3-030-06115-9_24
- Mattsson KMT. Ekvall L, Hansson S, Linse A, Malmendal Cedervall T. 2015. Altered Behavior, Physiology, and Metabolism in fish Exposed to Polystyrene Nanoparticles. *Environmental Science Technology*. 49, 553-561. <https://doi.org/10.1021/es5053655>
- Meghwal M, Goswami TK. 2012. A Review on the Functional Properties, Nutritional Content, Medicinal Utilization and Potential Application of Fenugreek. *Journal of Food Processing and Technology*. 3, 181-190.
- Mathews, T. J., & Hamilton, B. E. (2016). Mean age of mothers is on the rise: United States, 2000-2014. *NCHS data brief*, (232), 1-8.
- Molling JW, Seezink BEJ, Teunissen I, Muijers-Chen PJ.A. 2014. Comparative Performance of a Panel of Commercially Available Antimicrobial Nanocoatings in Europe. *Nanotechnology and Science Applications*. 7, 97-104. Doi:10.2147/NSA.S70782
- Morgan A, Ibrahim MA, Galal MK, Ogaly HA, Abd-Elsalam RM. 2018. Innovative Perception on Using Tiron to Modulate the Hepatotoxicity Induced by Titanium Dioxide Nanoparticles in Male Rats. *Biomedicine & Pharmacotherapy*. 103, 553-561. <https://doi.org/10.1016/j.biopha.2018.04.064>

- Noureen A, Jabeen F, Tabish TA, Ali M, Iqbal R, Yaqub S, Chaudhry SA. 2019. Histopathological Changes and Antioxidant Responses in Common Carp (*Cyprinus Carpio*) Exposed to Copper Nanoparticles. Drug and chemical toxicology.1-8. <https://doi.org/10.1080/01480545.2019.1606233>
- Noureen A, Jabeen F, Tabish TA, Yaqub S, Ali M, Chaudhry AS. 2018. Assessment of Copper Nanoparticles (Cu-NPs) and Copper (II) Oxide (CuO) Induced Hemato-and Hepatotoxicity in Cyprinus Carpio. Nanotechnology. 29(14), 144003-17. <https://doi.org/10.1088/1361-6528/aaaaa7>
- OECD (2000) Guidance Document on Acute Oral Toxicity. Environmental Health and Safety Monograph Series on Testing and Assessment No.24
- Pagano M, Faggio C. 2015. The use of Erythrocyte Fragility to Assess Xenobiotic Cytotoxicity. Cell biochemistry and function. 33(6), 351-355. <https://doi.org/10.1002/cbf.3135>
- Prajapat UK, Jain D, Kumar S, Sharma N. 2018. Proximate Composition of some Indian Herbs used as Feed Additive in Livestock and Poultry. International Journal of C Science. 6(2), 2105-2106.
- Quadros MR, Pierson N, Tulve R, Willis K, Rogers T, Thomas L. Marr C. 2013. Release of Silver from Nanotechnology-Based Consumer Products for Children. Environmental Science Technology. 47, 8894-8901. <https://doi.org/10.1021/es4015844>
- Raza M, Kanwal Z, Rauf A, Sabri A, Riaz S, Naseem S. 2016. Size-and Shape-Dependent Antibacterial Studies of Silver Nanoparticles Synthesized by Wet Chemical Routes. Nanomaterials. 6(4), 74-82. <http://doi.org/10.1080/01480545.20191606233>
- Suresh M, Mallikarjuna RN, Sharan B, Singh M, Hari Krishna B, Shravya KG. 2012. Hematological changes in chronic renal failure. International Journal of Scientific Research Publications. 2(9):1-4.
- Shaw BJ, Al-Bairuty GA, Handy RD. 2012 Aquatic Toxicology. 116–117 90-10.
- Srinivasan K. 2019. Fenugreek (*Trigonella foenum-graecum* L.) Seeds Used as Functional Food Supplements to Derive Diverse Health Benefits. In *Nonvitamin and Nonmineral Nutritional Supplements*, 217-221. Academic Press. <https://doi.org/10.1016/B978-0-12-812491-8.00031-X>
- Subramanian V, Semenzin E, Hristozov D, Zondervan-van den Beuken E, Linkov I, & Marcomini A. (2015). Review of decision analytic tools for sustainable nanotechnology. *Environment Systems and Decisions*, 35(1), 29-41. <https://doi.org/10.1016/13978-0-12-812491-8-00031-X>
- Wu T. & Tang, M. (2018). Review of the effects of manufactured nanoparticles on mammalian target organs. Journal of Applied Toxicology, 38(1), 25-40. <https://doi.org/10.1002/jat3499>
- Villarreal A, Seoane R, González Torres A, Rosciszewski G, Angelo MF, Rossi A, Ramos, AJ. 2014. S100B protein activates a RAGE-dependent autocrine loop in astrocytes: Implications for its role in the propagation of reactive gliosis. Journal of neurochemistry, 131(2), 190-205. <https://doi.org/10.1111/Sac.12790>
- Wang Yu, Monopoli MP, Sandin P, Mahon E, Salvati A, Dawson, K. A. 2015. Nanomedicine: nanotechnology. Biological Medicines. 11, 313-327. DOI: 10.4172/2157-7439.1000261
- Dioxide Nanoparticles in Male Rats. Biomedicine & Pharmacotherapy. 103, 553-561. <https://doi.org/10.1016/j.biopha.2018.04.064>.



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