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Innovative assessment to modulate the toxic effects of CuO-nanoparticles using *Trigonella foenum-graecum* methanol seed extract in *Oreochromis mossambicus*

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Abstract

Trigonella foenum-graecum has diverse medicinal properties therefore; the present study was aimed to investigate the ameliorative effects of the *Trigonella foenum-graecum* methanolic seed extract (T-MSE) against the CuO nanoparticles (NPs) induced toxicity in *Oreochromis mossambicus*. For this purpose, 100 *O. mossambicus* of 30-45g weight were randomly distributed into 5 groups having 10 fish in each group in duplicates namely control (without any treatment), positive control (treated with waterborne CuO-NPs @ 0.12mg/l), G_1, G_2, and G_3 were treated with waterborne CuO-NPs @ 0.12mg/l plus 16 or 32 or 52 mg/l of T-MSE, respectively for 56 days. Blood sampling was done at three intervals at 7th, 28th and 56th day of exposure. It was found that T-MSE remarkably ameliorated the toxic effects of CuO-NPs in G_3 with high T-MSE dose (52 mg/l) in the hematology of fish sampled at 28th and 56th day of exposure while, at 7th day of exposure less improvement was observed as compared with positive control group. It was also observed that the toxic effect of CuO-NPs in G_3 was less ameliorated at 28th day of exposure. There were significant differences in T-MSE treated groups (G_1, G_2 and G_3) having most prominent shielding effects of T-MSE in G_3 at 28th and 56th day of exposure in *O. mossambicus* compared with the positive control, G1 and G2 groups (p<0.05). It was concluded that T-MSE had prominent ameliorative effects on hematology against the toxic effects of water-borne CuO nanoparticles in *O. mossambicus*.

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Introduction

Various metallic-NPs have been used in numerous applications due to their exponentially enhancing demand due to distinctive characteristics which collectively make them unique compared to their bulk materials (Remya *et al.*, 2017). The key factor providing to these metallic nanoparticles, is the large active surface area of these particles. The present era is the era of nanotechnology as it has revolutionized the world by raising the efficiency and durability of the nano-raised products (Raza *et al.*, 2016). Nanoparticles are widely used in different industries as in agriculture, food, energy, mechanical and electronics (Raza *et al.*, 2016). Metallic nanoparticles are consisting of versatile class of materials consisting of pure metal nanoparticles e.g., iron, gold, silver, cobalt and nickel with their compounds such as oxides, sulfides, oxides, phosphates, chlorides and fluorides (Subramanian *et al.*, 2015). Their exciting large surface area, suitable physicochemical properties and unique size and shape make these metallic nanoparticles potential candidates for photography, manufacturing magnetic ferrofluids, catalysis, opto-electronic applications and photonics (Cristea *et al.*, 2017; Chow *et al.*, 2018).

Metallic oxide nanoparticles have become the matter of alarming situation to be addressed to take bio-safety actions due to their drastic effects on the health of living organisms (Wang *et al*, 2015; Wu *et al.*, 2018). Among the various nanoparticles being used commercially, CuO-NPs are among the most frequently used in various fields (Mercado *et al.*, 2019). Their potential toxicity to living organisms has attracted considerable attention with increase of their commercial demands (Morgan *et al.*, 2018). Most of *in vivo* studies have reported that CuO nanoparticles have ability to accumulate in most of the organs of living animals as in liver, kidneys, brain, heart, intestine, blood and even skin and muscles which can be studied by their responses to these exposures. This is worrying as the metallic-NPs have ability to enter the human body and other exposed animals due to their large aspect-to-size ratio and more reactive surfaces that enable these particles to penetrate across the biological barriers and induce stress in various cells of host bodies (Ahmed *et al*, 2016; Noureen *et al*, 2019). Due to accumulation in various organs, these particles have ability to induce changes in the normal structure and function of various organs because these particles have ability to generate reactive oxygen species in the exposed cells (Ahmed *et al.*, 2016).

Aquatic animals are at greater risk of CuO-NPs exposures most likely because the aquatic bodies are the ultimate sink for the accumulation of both Copper nanoparticles and various other micro-pollutants (Noureen *et al.*, 2019). It is therefore, vital to assess the bioaccumulation and toxicity of CuO nanoparticles in various organs of the exposed organisms to investigate their fate and proper management in the exposed environments to solve real-world problems. In the view of above facts *O. mossambicus* was used as a potential bio-indicator of quality of water, due to its sensitivity to frequent changes in the aquatic ecosystem. Various medicinal plants are well-known for their ameliorative and antioxidant traits. These plants are being used as shielding agents against different toxic compounds (Hamid *et al.*, 2013; Kusumaa *et al.*, 2014). These medicinal plants are capable of therapeutic source in fish as well (Hamid *et al.*, 2013). These plants are major sources of various phytochemicals including flavonoids, vitamins, terpenoids, carotenoids, lignin, saponins, curcumins and sterols etc. (Kusumaa *et al.*, 2012). Even the World Health Organization (WHO) has encouraged the usage of these medicinal plants to minimize the toxic effects of different toxicants. More than 80% of world's population is depending on the traditional medicinal plants for the primary healthcare needs to overcome the toxic effects of various toxicants being living factories of endless bio-active compounds (Hamid *et al.*, 2013). These bio-active compounds have anti-mutagenic and anti-carcinogenic properties which make them valuable to prevent certain types of disorders like cancer by mitigating the toxic effects of various toxicants as reported by different epidemiological and experimental studies (Hassan *et al.*, 2013).

Trigonella foenum-graecum (Fenugreek) of family Fabaceae is a leguminous herb which is being cultivated in Asia and North Africa countries. Basically, seeds of this herb are used as spice in foods for thousands of years. Besides, imparting the flavor, fenugreek also modifies the texture of the food. The bulk of the Trigonella constitute the dietary fiber composed of 30% of soluble and 20% of insoluble fractions (Galactomannan). Bitterness of seed is due to the oils, steroids, saponins and alkaloids. Several health benefits of fenugreek have been experimentally validated in both animals and human trials in recent decades (Srinivasan *et al.*, 2019). These herbs are potential source of various bio-active phytochemicals with medicinal values (Prajapati *et al.*, 2102). Based on its extra-ordinary physico-chemical properties, it has been selected for the present study for its ameliorative effects to cover up the toxic effects induced by the exposure of CuO-NPs in *O. mossambicus* (El-Sayed andYussef, 2019). Seed extract of *T. foenum-graecum* is the best source of dietary protein for consumption by both animals and human. Its important properties are anti-pyretic, anti- inflammatory, anti-microbial and anti-bacterial (Hamid *et al.*, 2013). In the light of the literature cited above the present study was designed to assess the ameliorative effects of *Trigonella foenum-graecum* against water-borne CuO nanoparticles induced toxicity on various hematological parameters of *O. mossambicus* at different durations of exposure and doses.

Materials and methods

Fish procurement, acclimatization and water parameters

Oreochromis mossambicus (Tilapia) was procured from Manawan, Fisheries Complex, Lahore, Punjab, Pakistan. They were safely transferred to Fish research laboratory at Department of Zoology Government College University, Faisalabad. Fish husbandry was done prior to the start of experiment and acclimatized in the cemented rectangular tank for two weeks. During acclimatization fish were provided with regular commercial fish feed on daily basis. Each glass tank was supplied with recirculating water filter, where fish were kept under normal photoperiods (12 hrs of light and 12 hrs of darkness) and 27±2ºC temperature while dissolved oxygen and pH were maintained at 6.5-7.4 mg/l, and 6.7-7.2, respectively. Ammonia (NH_3) concentration, total hardness and total dissolved solids were maintained as 0.5-0.7 ppm, 47-52ppm and 6.5-7.8ppm, respectively.

Ethical approval

Prior to the start of experiment, study was approved by the Ethics Committee on Animal Experimentation of Government College University, Faisalabad (GCUF) Pakistan. Experimental fish received proper care and husbandry in compliance to Animal Ethics Committee's guidelines.

Procurement of CuO-NPs and other chemicals

CuO-NPs (<50 nm) were purchased from Sigma-Aldrich (CAS # 1317.38-0 79.55) and other chemicals used in the experiments were of high quality molecular and analytical grade.

Selection of sub lethal dose of CuO-NPs

The sub-lethal dose of CuO-NPs (0.12 mg/l) was selected from our previous research (the part of the same project, unpublished data).

Characterization of the CuO-NPs

The CuO-NPs were characterized by scanning electron microscopy (SEM) and X-ray diffraction (XRD).

Preparation of Trigonella foenum-graecum Methanol Seed Extract (T-MSE)

The dried seeds of *Trigonella foenum-graecum* were purchased from a local authenticated homeo-store. Their taxonomic status was also confirmed from the Department of Botany at Government College University, Faisalabad, Pakistan. The seeds were ground into fine powder using a grinder (Renker, Model: GMO 1 grinder). Trigonella seed extract was prepared using manual method following the standard protocols (Khan *et al.*, 2012; Islam *et al.*, 2019). Extraction is the method to separate out those phytochemicals which are medicinally active parts of the plants by using any pre-dominantly organic solvent by adopting standard method. Maceration was done which involved soaking seeds of plants (2kg fine powdered form of seed) in an airtight beaker in Methanol (2.5l) allowing it for 15 days with frequent agitation and further addition of methanol to keep it soaked. This process intended to soften and to break the seed's cell walls to release all soluble phytochemicals in soluble form. Left it for exhaustive extraction for 15 days at room temperature until the residue in a subsequent extraction become ≥ 10% of the actual material. After 15 days of the stay, the extract was obtained using Soxhlet apparatus. The obtained T-MSE was 55.23g which was preserved in dry air-tight bottle for further use in experiments (Islam *et al.*, 2019).

Experimental plan

For the present study, 100 fish were randomly distributed into five groups in duplicate (10 fish in each group) *viz.*, control, positive control and three treatment groups (G_1-G_3). The control group was without any treatment, positive control was exposed to sub-lethal dose of CuO-NPs (0.12 mg/l) while all other groups were exposed to sub-lethal dose of CuO-NPs (0.12 mg/l) plus various doses of T-MSE i.e., G_1 (16mg/l), G_2 (34mg/l) and G_3 (52mg/l), respectively (Table 1).

The doses of both CuO nanoparticles and T-MSE were repeated on alternate days with regular change of water keeping the physical factors constant as given by the OECD (Organization for Economic Cooperation and Development) for acute fish test criteria (OECD, 2000; Deepa *et al.*, 2018). No mortality was observed during the whole experimental period. Exposure was continued for 56 days. Sampling was done at three intervals after 7th, 28th and 56th days.

Preparation of exposure materials

For the preparation of the exposure medium, the protocol given by Noureen *et al.*, 2019 was followed with some modifications. The required amount of the CuO-NPs in powder form was weighed and placed in polypropylene tubes and dispersed in the deionized/ ultra-pure water (Millipore, 18.2 M cm resistance and unbuffered) with 0.1 ml acetic acid as solvent. To homogenize well, the suspension was shaken well on vortex (2000 rpm for 10 minutes), then it was sonicated (100W, 40kHz) for 1hr to disperse the material in the tanks. The solution was made fresh before dosing every time as dose was given on alternate day. Ultrasonication was used for the preparation of well-homogenized mixture which is an accepted technique for dispersing the highly aggregated or entangled nanoparticles samples as it enhances the reactivity as it dispersed well in the aquarium. Plant seed extract doses were also made in the same way without using any organic solvent.

Sampling

Sampling was done at three intervals as 7th, 28th and 56th day of exposure. Clove oil (100µg/l) was used to anesthetize the fish and blood was collected from the caudal vein. Ethylene-Diamine-Tetra-Acetic Acid (EDTA) was used as coagulant. All hematological parameters were analyzed by using haematology autoanalyser (Shanghaic Drawell Scientific Instrument Co., Ltd and DW-TEK5000 Automated Blood Hematology Analyzer).

Statistical analysis

The data were statistically analyzed by means of Minitab 17 software through ANOVA in general linear model to find out the effect of CuO-NPs on different parameter of hematology. Tukey's test was performed to compare the means of different groups at $p < 0.05$.

Results

Characterization of CuO-NPs

Scanning Electron Microscopy (SEM) revealed that CuO-NPs were of uniform spheres with 99% purity of the particles. XRD spectrum of the CuO-NPs measured the size of CuO-NPs as <90 nm.

Ameliorative potential of Trigonella foenum-graecum

The alterations in various hematological parameters (erythrocytes, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, MCH concentration, white blood cells (leukocytes), neutrophils, monocytes, lymphocytes and basophils) were studied in all groups (control, positive Control, G_1, G_2 & G_3) to assess the CuO-NPs induced toxicity and amelioration by *Trigonella foenum-graecum* seed extract. At 7th day of exposure, no significant change was observed in all hematological parameters compared with the positive control group ($p<0.05$) in all groups (Table 2), whereas 28 and 56th days of exposure showed alterations in hematological parameters in treated groups compared with the control and positive control groups (Table 3 ad 4).

Table 1. Division of fish into different treatment groups.

Sr. #	Groups	Doses (Treatment)		*Oreochromis mossambicus* (n=100)	
		CuO-NPs (mg/l)	T-MSE (mg/l)	No. of fish (in Duplicate)	
1	Control	0.00	0.00	10	10
2	Positive Control	0.12	0.00	10	10
3	G1	0.12	16	10	10
4	G2	0.12	32	10	10
5	G3	0.12	52	10	10

Erythrocytes, hemoglobin and hematocrit generally increased in a dose-dependent manner after 28 and 56 days exposure compared with positive control (Table 3 ad 4). Mean Corpuscular Volume (fl) generally increased in a dose-dependent manner at day 56. Mean corpuscular hemoglobin (pg) and mean corpuscular hemoglobin concentration (g/dl) generally increased in a dose-dependent manner at day 56. Leucocytes (10^3/µl), neutrophils (%), lymphocytes (%), and monocytes (%) generally decreased in a dose-dependent manner at different exposure times compared to the positive control. The amelioration by *Trigonella foenum-graecum* was observed in group G_3, in all hematological parameters.

Table 2. Mean ±SE of various haemato-immunological parameters in different groups of *O. mossambicus* at 7th Day of exposure.

Parameters	Groups / Doses (mg/l)				
	C_1 (Control) 0.00	C_2 CuO-NPs 0.12	G_1 (CuO-NPs + *Trigonella foenum-graecum*) (0.12 + 18.00)	G_2 (CuO-NPs + *Trigonella foenum-graecum*) (0.12 + 26.00)	G_3 CuO-NPs + *Trigonella foenum-graecum* (0.12 + 52.00)
Erythrocytes (10^6/µl)	$2.83±0.005^A$	$1.93±0.009^C$	$1.97±0.004^B$	$1.99±0.007^B$	$2.05±0.007^B$
Hemoglobin (g/dl)	$10.44±0.008^A$	$8.43±0.01^C$	$8.44±0.007^C$	$8.39±0.014^C$	$8.92±0.027^B$
Hematocrit (%)	$35.22±0.124^A$	$24.18±0.43^D$	$26.03±0.17^C$	$26.15±0.46^C$	$29.56±0.40^B$
Mean Corpuscular Volume (fl)	$128.76±0.26^C$	$145.70±0.86^A$	$133.37±0.63^B$	$132.52±0.30^B$	$130.81±0.61^B$
Mean Corpuscular Hemoglobin (pg)	$74.90±0.14^A$	$55.77±0.31^D$	$64.85±0.62^C$	$65.91±0.18^C$	$69.45±0.42^B$
MCH Concentration (g/dL)	$47.44±0.66^A$	$37.70±0.65^B$	$37.91±0.28^B$	$38.14±0.40^B$	$37.95±0.25^B$
Leucocytes (10^3/µl)	$50.92±0.52^D$	$56.57±0.32^A$	$57.12±0.32^{AB}$	$54.94±0.21A^B$	$51.68±0.33^C$
Neutrophils (%)	$44.71±0.70^B$	$47.26±0.20^A$	$46.02±0.75^A$	$45.52±0.84^A$	$43.72±0.48^B$
Lymphocytes (%)	$25.10±0.41^A$	$26.41±0.85^A$	$25.54±0.23^A$	$25.51±0.39^A$	$25.01±0.21^A$
Monocytes (%)	$7.15±0.35^A$	$7.92±0.30^A$	$7.39±0.39^A$	$7.37±0.49^A$	$7.12±0.25^A$

(Mean values sharing different letters in rows were significantly different at $p<0.05$).

Discussion

In the present study, the effects of combined treatments of CuO-NPs (Copper oxide nanoparticles) and T-MSE (*Trigonella* methanol seed extract) were studied on the hematological parameters and their amelioration was evaluated at different dose-concentrations (CuO-NPs 0.12 mg/l in all groups except control and T-MSE as G1 16mg/l, G2 32mg/l and G3 52mg/l) at three intervals (7^{th}, 28^{th} and 56^{th} day).

Table 3. Mean ±SE of various haemato-immunological parameters in different groups of *O. mossambicus* after 28 days of exposure.

Parameters	Groups / Doses (mg/l)				
	C_1 (Control) 0.00	C_2 Positive control CuO-NPs 0.12	G_1 (CuO-NPs + *Trigonella foenum-graecum*) (0.12 + 18.00)	G_2 (CuO-NPs + *Trigonella foenum-graecum*) (0.12 + 26.00)	G_3 CuO-NPs + *Trigonella foenum-graecum* (0.12 + 52.00)
Erythrocytes(10^6/µl)	2.81 ± 0.004^{A}	1.72 ± 0.014^{C}	1.74 ± 0.009^{C}	1.76 ± 0.004^{C}	2.13 ± 0.007^{B}
Hemoglobin(g/dL)	10.24 ± 0.07^{A}	6.50 ± 0.05^{C}	6.69 ± 0.05^{C}	7.21 ± 0.05^{B}	9.52 ± 0.01^{A}
Hematocrit (%)	34.54 ± 0.10^{A}	20.54 ± 0.28^{D}	20.69 ± 0.23^{D}	22.12 ± 0.30^{C}	28.15 ± 0.29^{B}
Mean Corpuscular Volume (fl)	133.29 ± 0.44^{D}	163.74 ± 0.86^{A}	155.35 ± 0.75^{B}	140.02 ± 0.55^{C}	132.02 ± 0.73^{D}
Mean Corpuscular Hemoglobin (pg)	75.69 ± 0.32^{A}	45.41 ± 0.83^{C}	47.12 ± 0.58^{C}	49.09 ± 0.83^{C}	66.13 ± 0.72^{B}
MCH Concentration(g/dL)	48.39 ± 0.40^{A}	33.85 ± 0.36^{C}	34.55 ± 0.32^{C}	34.61 ± 0.50^{C}	46.47 ± 0.62^{B}
Leucocytes (10^3/µl)	51.30 ± 0.34^{C}	58.43 ± 0.32^{A}	58.51 ± 0.59^{A}	56.53 ± 0.65^{B}	51.01 ± 0.83^{C}
Neutrophils (%)	45.11 ± 0.43^{D}	59.51 ± 0.67^{A}	57.34 ± 0.59^{B}	55.92 ± 0.69^{C}	43.60 ± 0.75^{E}
Lymphocytes (%)	24.98 ± 0.54^{C}	29.41 ± 0.68^{A}	29.18 ± 0.26^{A}	27.86 ± 0.35^{B}	23.70 ± 0.61^{C}
Monocytes (%)	7.20 ± 027^{C}	14.65 ± 0.62^{A}	11.58 ± 0.43^{B}	10.58 ± 0.52^{B}	6.34 ± 0.33^{D}

(Mean values sharing different letters in rows were significantly different at $p<0.05$).

The hematological parameters are reliable tool to monitor the fish health in response of any toxicant exposure (Hoseini andNodeh, 2013), nutritional manipulation (Hoseini *et al.*, 2018) and toxicant exposure (Mazandarani and Hoseini, 2017). The present study showed that CuO-NPs exposure led to the anemia in the *O. mossambicus* even in combination with medicinal plant seed extract.

The anemic condition was observed in the groups treated with lowest (16mg/l) and medium dose (32mg/l) of *Trigonella* methanol seed extract (T-MSE) dominating the toxic effects of CuO-NPs. These results agreed with the results of the Hoseini *et al.* (2019) who reported the decrease in the red blood cells (erythrocytes) and hemoglobin (Hb) values as toxic response of the ammonia in the fish hematology parameters. This anemia might be due to the destruction of the RBCs (Red Blood cells) and because of the presence of the free radicals. The current study is in coordination with the study of Akbary *et al.* (2018) who treated gray mullet (*Mugil cephalus*) with CuO-NPs for 21 days and reported the decrease in the RBCs and increase in the leucocytes, monocytes and neutrophils.

In the current study, the ameliorative effect was observed in the group exposed to the high-dose (52mg/l) of *Trigonella* seed extract, in combined treatments (CuO-NPs and T-MSE), suppressing the toxic impact of the CuO-NPs. These results were in correlation with the study of Hoseini *et al.* (2019) who reported the ameliorative effects of Menthol to mitigate the toxic effects of the ammonia in the fish by improving the red blood cell count and reducing the white blood cells. The current study results are in coordination with the study of Akbary *et al.*, (2018) who treated gray mullet (*Mugil cephalus*) with CuO-NPs for 21 days and reported the decrease in the RBCs and increase in the leucocytes, monocytes and neutrophils. The hemoglobin (Hb) concentration is generally an accurate reflection of the extent to which the circulatory red mass is reduced which may result in the anemic condition.

Table 4. Mean ±SE of various haemato-immunological parameters in different groups of *O. mossambicus* after 56 days of exposure.

Parameters	Groups / Doses (mg/L)				
	C_1 (Control) 0.00	C_2 CuO-NPs Potential toxic dose 0.12	G_1 (CuO-NPs + *Trigonella foenum-graecum*) (0.12 + 18.00)	G_2 (CuO-NPs + *Trigonella foenum-graecum*) (0.12 + 26.00)	G_3 CuO-NPs + *Trigonella foenum-graecum* (0.12 + 52.00)
Erythrocytes (10^6/µl)	2.95±0.01^C	1.62±0.014^A	1.74±0.01^A	1.84±0.01^B	2.83±0.01^C
Hemoglobin(g/dL)	10.46±0.01^C	5.50±0.05^A	6.84±0.01^A	7.57±0.11^B	10.12±0.03^C
Hematocrit (%)	35.05±0.25^B	19.44±0.28^A	20.6±0.5^A	22.04±0.34^A	34.78±0.39^B
Mean Corpuscular Volume (fl)	123.07±0.34^A	173.74±0.86^C	159.5±1.01^C	148.75±1.11^B	132.52±0.69^A
Mean Corpuscular Hemoglobin (pg)	75.84±0.23^C	41.12±0.83^A	47.77±0.49^B	45.8±0.41^A	69.67±0.37^C
MCH Concentration (g/dL)	48.5±0.28^A	30.45±0.36^A	33.96±0.32^B	34.8±0.25^B	43.03±0.37^C
Leucocytes (10^3/µl)	51.07±0.32^A	64.13±0.32^C	58.53±0.38^C	55.74±0.71^B	52.19±0.4^A
Neutrophils (%)	45.93±0.17^A	59.51±0.67^B	57.59±0.59^C	52.42±0.92^B	46.96±0.45^A
Lymphocytes (%)	25.72±0.26^A	29.41±0.68^B	28.07±0.74^B	26.73±0.59^{AB}	26.84±0.55^{AB}
Monocytes (%)	7.13±0.18^A	14.65±0.62^C	12.43±0.36^B	11.65±0.19^A	7.08±0.37^A

(Mean values sharing different letters in rows were significantly different at $p<0.05$).

The decrease in the hemoglobin in current study suggested that combined treatment of CuO-NPs and T-MSE interfered with erythropoiesis (Suresh *et al.*, 2012). The observed results are in closer agreement to the study of Suresh *et al.* (2012), who reported similar changes after the administration of the delta-methrin in the experimental animal.

The decrease in erythrocyte cells may be due to decreased life span of RBCs due to toxicant or may be due to suppressed activity of bone marrow stem cells (Alkaladi *et al.*, 2015). Respiratory restrictions caused by CuO led to decrease in RBC's due to gill damage which hinders the oxygen capturing capacity also confirmed by gill histological abnormalities reported in this study (Khabbazi *et al.* 2015; Abdel-Khalek *et al.*, 2015). Reduced values of Hb and Hct may be due to dysfunction in hematopoietic process results in heavy metals toxicity (Lavanya *et al.* 2011). Copper diminishes the power of hemoglobin to attract oxygen molecules by making red blood cell fragile and damage cells by swelling.

Leucocytes defend an organism against foreign pathogens or toxic particles. Increased in leucocytes is the indication of foreign particles entry into the body. CuO nano particles also stimulate the defense system. Increase in the leucocytes, monocytes and lymphocytes in this study declared that the defense system of *O. mossambicus* has been stimulated by CuO-NPs. These results are correlating with the results of the Noureen *et al.*, (2018) who reported significant increase in leucocytes of *Cyprinus carpio* exposed to CuO nano particles. The increase in leucocytes is probably due to the response of immune system against the stress conditions induced by Cu-NPs.

These results agree with the results of the study of Jahanbakhshi *et al.* (2015) who reported a significant rise in neutrophils and monocytes in the *Rutilus rutilus* and similarly, the results of present study are related with the study of Kaviani *et al.* (2019), who also reported significant increase in the neutrophils in Caspian trout treated with CuO Nano Particles.

Subhashini *et al.* (2011) and Patel and Dhanabal (2013), reported the effectiveness of *T. foenum-graecum* extract against free radical medicinal diseases which are corresponding with the results of current study revealing the ameliorative effects to the altered values of hematological parameters due to induced toxic response of CuO-NPs. The ameliorative results of our research work using *T. foenum-graecum* seed extract of reducing the induced toxicity effects of CuO-NPs in all the blood parameters correlate with the study of Goyal *et al.* (2016), who studied the most of the herbs including

Trigonella which showed antioxidant and immune-modulatory activities due to which it becomes an viable novel approach for the treatment of immunological disorders and to protect animals against any kind of oxidative stress against any toxic exposure. Kumar *et al.* (2019) reported about the phytochemical analysis of the *T. foenum-graecum* seed. The bioactive compounds containing vitamins, minerals, amino-acids, fiber and protein declaring the Trigonella as therapeutic plant providing safe management of various stress responses in animals.

The MCH (Mean corpuscular Hemoglobin) refers to the average amount of hemoglobin present in the red blood cells. MCH is an erythrocyte index. MCH concentration (g/dl) is a measure of the concentration of hemoglobin in a given volume of packed red blood cell. The MCV, MCH, MCHC values decreased in the group exposing to both CuO-NPs (0.12mg/l) and TSE (18+26mg/l) respectively as a response of the toxicity of CuO-NPs with maximum decrease at 28th day. These findings of the present study are correlated with the results of Noureen *et al.* (2018) who reported a decrease in the levels of MCV, MCH and MCHC with sharp changes on maximum dose exposure in the *C. carpio*.

The leucocytes are the important components of immune system originated from bone-merrow which circulate in the blood against any type of infection. In the present study, the levels of Leucocytes increased under the exposure of CuO-NPs. These leucocytes are produced under stress conditions to cope with the stress situation. Neutrophils and Monocytes are phagocytic in nature to engulf bacteria. Lymphocytes are immune cells in the cellular and humoral systems. Monocytes play a significant role against any inflammation as these are important part of the immune system of the living animal. These results of increasing the monocytes under stress condition are related to the present study are in correlation with the results of the Khabbazi *et al.* (2015), who reported an increase in the percentage of white blood cells on increasing the duration of the exposure of CuO-NPs in trout. They revealed the fact that this increase in white blood cells is due to the dysfunction in the hematological organs including kidney and liver.

Conclusion

It was concluded that water-borne CuO-NPs is toxic for fish. The extent of the toxicity of CuO-NPs was dependent on both the dose and duration of the exposure. Ameliorative effects of T-MSE were prominent against toxic effects of the CuO-NPs by repairing the alterations in the erythrocyte cells improving the anemic condition.

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