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Assessment of ameliorative effect of *Trigonella foenum-graecum* against CuO-NPs induced toxicity in *Oreochromis mossambicus*

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Abstract: The current study assessed the ameliorative effect of *Trigonella foenum graceum* extract against copper oxide nanoparticles (CuO-NPs) induced toxicity in *Oreochromis mossambicus*. For this purpose 100 healthy fish weighing 20±2.34g were randomly divided into five different groups in duplicates and designated as control (C) no treatment, positive control (G*) treated with 0.12mg/L of CuO-NPs, experimental co-treated groups G1, G2 and G3 were treated with *Trigonella foenum-graecum* extract @ 18, 26 and 52mg/L along with 0.12 mg/L of CuO-NPs, respectively. In this study significant (P<0.05) changes were observed in the antioxidant activity of enzymes and histological alterations in the liver and intestine of fish in G*, G1 and G2 groups while a good ameliorative response of *Trigonella foenum-graecum* was observed in G3. Dose dependent alterations in glutathione, lipid peroxides, catalase, and malondialdehyde as well as histological architecture of liver and intestine were observed in treated groups, where more alterations were observed in positive control and low dose treated groups of *Trigonella foenum-graecum*. Moreover, more ameliorative effect was observed in high dose of *Trigonella foenum-graecum* treated group (G3). This study is novel as no previous data is available on the amelioration of *Trigonella foenum-graecum* extract against CuO-NPs induced toxicity in fish.

Keywords: Oxidative stress, histology, *Trigonella foenum-graceum*, *Oreochromis mossambicus*, CuO-NPs.

INTRODUCTION

Trigonella foenum-graecum is a leguminous herb belonging to the family Fabaceae and is locally known as Fenugreek or Methi. Fenugreek's seeds are known due to its multifarious medicinal uses. In Pakistan two common species are *foenum-graecum* and *corniculata* (Lal and Meena, 2018). Fenugreek is originated in South-Europe, Mediterranean area and Western Asia. This herb has been cultivated in Argentina, Egypt, India, Pakistan, Morocco, Southern France, Turkey, Spain and China (Basu *et al.,* 2019). Since thousands years, the seeds of this herbs have been used as spice in foods. The seeds of this plant contain saponins, proteins and dietary fibers of both soluble and insoluble nature (Srinivasan, 2006; Fatima *et al*., 2018). Seeds of *Trigonella foenum-graecum* with major component of trigonelline are beneficial for many ailments (Makwana *et al.,* 2016). Some other major components of seed are glycoside-D, Saponins and trigofoenoside-A (Conti *et al.,* 2010). The leaf of this plant contains glycosides, alkaloids and phenols (Ozbek *et al.,* 2003). The bitter taste of this plant is due to oils, steroidal saponins and alkaloids. This plant is effective against anorexia and it is a carminative, gastric stimulant, and galactagogue (Srinivasan, 2006). Multi pharmaceutical uses of *Trigonella foenum-graecum* have been investigated and found effective as antiobesitic (Kumar and Bhandari, 2015), antidiabetic (Ismail and Yaheya, 2009), anti-inflammatory (Ahmadiani *et al.,* 2001), antibacterial (Premanath *et al.,* 2011), anticancer (El Bairi *et al.,* 2017), antihyperlipidemic (Khlifi *et al.,* 2016), antifungal (Omezzine *et al.,* 2017) and antioxidant including improving health (Dixit *et al.,* 2005).

The applications of nanomaterial are increasing day by day and expecting to expand more during the next decade. The diverse applications of nanomaterial are found in gas sensors, photovoltaic cells, imaging contrasts agents and semiconductors. Hundreds of commercial products are made by metal oxide nanoparticles (Fahmy and Cormier, 2009; Dasgupta *et al.,* 2017). A wide range of applications are also listed in daily used products such as cosmetics, environmental remediation, biomedicine, electronics and material sciences (Jańczewski *et al.,* 2011, Kahru and Dubourguier, 2010, Ramos *et al.,* 2017). Nanotechnology also helps in energy production, food production, water purification, agriculture, chemicals, textiles and decontamination (De Marchi *et al.,* 2017, Vajargah *et al.,* 2018, Yalsuyi and Vajargah, 2017). Along with the benefits of nanotechnology on the other hand nanoparticles production and applications affect the environmental biota due to their release in to the environment from several industries (Moore, 2006; Bhatt and Tripathi, 2011; Martínez *et al*., 2021). Many pollutants migrate from one place to another by using water as a vehicle and this behavior of pollutants especially copper nanoparticles as toxic nature is necessary to study their fate and eco-toxic effects (Keller *et al*., 2017).The domestic applications of these nanoparticles also pose an additional risk of toxicity to aquatic ecosystems (Besha *et al*., 2020). The toxicity of nanoparticles was reported over the last decade but a few studies were performed to assess the environmental hazards of these nano-metal oxides in aquatic ecosystem

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(Zhang *et al.,* 2019). Waterborne copper oxide nanoparticles induce physiological impairments, oxidative stress and tissue pathological damages in fish (Abdel - Khalek *et al*., 2016; Hoseini *et al*., 2016). Therefore, the current study was designed to investigate the ameliorative effects of *Trigonella foenum-graceum* seed extract against CuO-NPs induced toxicity in *Oreochromis mossambicus*. *Oreochromis mossambicus* was selected as bio-indicator of aquatic life due to its eurythermal and euryhaline nature and easy to manage.

MATERIALS AND METHODS

Fish Procurement, Acclimatization and Physico-chemical Parameters

A fish husbandry was done before the start of experiment to maintain the health of fish by maintaining the water quality and the environment of stock aquaria, placed in the Department of Zoology, Government College University Faisalabad (GCUF), Pakistan. *Oreochromis mossambicus* of similar body weight (20-25g) were procured from the fisheries complex Lahore, Pakistan and transported in plastic containers with continuous aeration to the GCUF fish laboratory and acclimatized in a tank with 100 L capacity for two weeks prior to the experiment. The un-chlorinated tap water was used for the experiment and physicochemical parameters of water were determined. During acclimatization and experimental period water temperature was maintained at 25±1ºC, while dissolved oxygen and pH were 6.5-7.4 mg/l, and 6.7-7.2, respectively. NH_3 concentration, total hardness and total dissolved solids were 0.4-0.6 ppm, 47-52 ppm and 6.5-7.8 ppt, respectively. The fish were fed twice daily with commercial fish feed (Oryza Organics Private Ltd Hong Kong, having crude protein 30%, crude fat 6% and crude fiber 5%) @ 4% body weight. The experimental tanks were semistatic and about 80% water was changed every day and dead fish as well as any fish showing any unusual symptoms were excluded. All procedures performed in this study involving fish handling were in accordance with the research ethical standards with the approval of the Ethical Committee of Government College University Faisalabad on Animal Experimentation (Ref. No. GCUF/ERC/121/17, dated 30-03-2017).

Chemicals

The high quality analytical and molecular grade chemicals were used in the study. Copper Oxide Nano Particles (CAS 1314-13-2) were imported from USA manufactured by Sigma-Aldrich. The size of nanoparticles was <50nm and 99.9% pure (According to labeled information on the product).

Procurement of Plant Material and Extract Preparation

The seeds of *Trigonella foenum graecum* (5kg) were purchased from local super market (Al-Fateh) of Faisalabad city, identified by the expert and crushed to fine powder to prepare methanolic extract through Soxhlet apparatus by following the method of DeCastro and Priego-Capote (2010). Briefly, the powder seeds were filled in porous cellulose thimble and inserted into column of soxhlet apparatus. In round bottom flask analytical grade methanol was used according to need and fixed it on electric isomantle. The temperature was setup at 150 ºC and process of extraction was started. Condenser of apparatus recycles the solvent to extract the beneficial compounds of *Trigonella foenum graecum.* After the process of soxhlet extraction, solvent and extracted material was put into rotary evaporator to evaporate the solvent and separated the extract. A dried concentrated extract was measured (approx. 150 grams) and stored in air tight container for further use at room temperature.

Determination of LC_{50} values of Trigonella foenum-graecum

After acclimatization period (2 weeks), fish were transferred to small glass aquaria for LC50 determination. The concentrations used for *Trigonella foenum graecum* extract were 0, 250, 500 and 1000 mg/L. The exposure period was 96 h; with the same temperature, dissolved oxygen and pH as in the acclimatization period. The dead fish was recorded in each concentration to estimate the LC_{50} value via probit analysis.

Suspension preparation

The suspension of copper oxide nanoparticles (CuO-NPs) was prepared in distilled water mixed with one or two drops of Acetic acid to increase its solubility and ultrasonicated (100 W, 40 kHz) for 2 hours to ensure maximum dispersion in water. The suspension was freshly prepared before dosing.

Experimental Setup

The fish (n=100) were randomly allocated in glass aquaria (40x70x26 cm) in duplicate groups (each 10 fish/aquarium). Fish were then divided in to five groups i.e., control group without any treatment, positive control group treated with only CuO-NPs @ 0.12 mg/L and three experimental groups G1, G2 and G3 co-treated with *Trigonella foenum graecum* extract @ 18, 26 and 52 mg/L along with CuO-NPs @ 0.12 mg/L, respectively for 56 days (table1). The rationale of selecting the doses of *Trigonella foenum-graecum* was based on its LC_{50} value ($1/10^{th}$, $1/20^{th}$ and $1/30^{th}$), while dose of CuO-NPs was selected on the basis of our pilot study for toxicity trial. The conditions of the experiments were as those of acclimatization period and water were constantly (every day) checked for pH, temperature and dissolved oxygen. Water was changed daily, and fish were fed 40 minutes before water change.

Fish sampling

After 56 days of trial, fish were dissected and liver and intestinal tissues were isolated, cleaned and processed for

further investigation of histology and oxidative stress analysis.

Histological protocol

After experimental phase fish were picked from aquariums and immediately anesthetized and dissected to separate liver and intestine. The organs were washed with normal saline and preserved in 10% formalin. After proper fixation in formalin, tissues were run in automatic tissue processor following the steps of dehydration, clearing and filtration. In dehydration process tissues were shifted into different grades of methanol as 70, 80, 90 and 100% followed by same concentrations of xylene for clearing process. In tissue processor final step of filtration was done in molten paraffin wax. The timing of each step was setup in analyzer to run the protocol automatically for each grade. The embedding process was performed manually by using steel molds to prepare blocks. Tissues were sectioned (5µm) through manual microtome (Histoline MR-2258). The sections were stained by hematoxylin and eosin. At first sections were dipped in hematoxylin and then in eosin to give contrasting effects to tissues. At the end sections were dehydrated by using methanol and mounted by using canada balsam. Photomicrographs were taken from prepared slides to mark the abnormalities caused by CuO-NPs and curative effect of *Trigonella foenum-graceum* extract.

Oxidative stress determination

Homogenate preparation

Liver and intestine were homogenized in tissue homogenizer in buffer (0.1 M phosphate buffer pH 7). After centrifugation at 10,000 rpm for 10 min supernatant was collected for further anti-oxidant analysis.

Estimation of Lipid peroxidation (LPO)

Lipid peroxidation (LPO) was estimated following the method of Jiang *et al*., 1992. For LPO estimation, 0.1ml of tissue homogenate was treated with 0.9ml of fox reagent (88 mg of butylated hydroxytoulene, 7.6mg of xylenol orange and 9.8 mg of ammonium iron sulphate which were added to 90 ml of methanol and 10 ml 250 mM sulpheric acid) and incubated at 37°C for 30 minutes. The colour developed was then read at 560 nm and LPO was expressed as mM /g of tissue.

Estimation of malondialdehyde

Malondialdehyde (MDA) was measured by following the method of Ohkawa *et al*. (1979). Briefly, 0.2ml of homogenized tissue (10% w/v), 1.5ml of acetic acid (20 %, pH 3.5) along with 0.2 ml (0.8%) of sodium dodecyl sulphate were mixed. Then, distilled water was added to make volume up to 4ml and heated in water bath (DIN EN 60529-IP20) for one hour (at 95ºC, using glass balls). Falcon tubes (Nest; Germany) containing tissue sample were then cooled with tap water, afterward 5ml of solution of pyridine and n-butanol (1:15) was mixed and 1 ml of distilled water was added and solution was shaken efficiently on vortex mixer for 10 minutes. Absorbance of upper organic layer was read (532nm) using spectrophotometer following centrifugation at 4000 rpm for 10 minutes. The amount of MDA was measured as nm/g of the respective tissues.

Estimation of catalase

The catalase estimation was done following Aebi (1974). To estimate the value of catalase, 50µl of homogenate supernatant was treated with 1.95ml of phosphate buffer (50mM) keeping its pH at 7.0. Then, 1.0ml of hydrogen peroxide (30mM) was added in it and variations in the absorbance at 240nm were noted for 30 seconds and second reading was taken after 15 seconds. Catalase activity was expressed as Unit per ml.

Estimation of glutathione

To estimate the reduce glutathione (GSH) in treated and controlled samples the 500 µl of homogenate tissue was mixed with 400 µl of water along with 100µl of tri-chloroacetic acid (50%) to precipitate and then it was centrifuged for 10 minutes at 1000rpm. Mixture was made with 500 µl of supernatant, 100µl of 0.001M DTNP (5' 5'-dithio-bisnitrobenzoic acid known as Ellman's reagent) and 2.0 ml of 0.2M Tris-EDTA buffer with pH 8.9. It was placed at room temperature for further 5 minutes and readings were noted using spectrophotometer (U-2800) at 412nm and measured the GSH as µM/g of the tissue following Sedlak and Lindsay (1968).

STATISTICAL ANALYSIS

The data were statistically analyzed by Minitab17 software using the General Liner Model (ANOVA). Data of all the parameters regarding control and CuO-NPs treatments were expressed as mean+SEM. Tukey's test was used for the comparison of means. *P*-value less than 0.05 were considered to be statistically significant. Probit analysis was used to calculate the LC_{50} of *Trigonella foenum-graceum* by Minitab17 software.

RESULTS

LC_{50} (Trigonella foenum-graceum)

The 96hrs LC_{50} for *Trigonella foenum-graecum* (TFG) extract was calculated as 520.43 mg/L (fig. 1).

Oxidative stress

The oxidative stress markers like glutathione (GSH), malondialdehyde (MDA) and lipid peroxides (LPO) of both liver and intestine were increased significantly in positive control group (*G) as a result of CuO-NPs induced toxicity. Whereas, treatments with *Trigonella foenum-graceum* helped in the restoration of normal activities of enzymes. Experimental groups showed increased values of enzymes in G*, G1 and G2, respectively while, G3 showed no significant difference

as compared to control. Catalase (CAT) was significantly decreased in similar pattern as above mentioned groups and similarly reported with no significant activity in G3 as compared to control group (table 2 & 3).

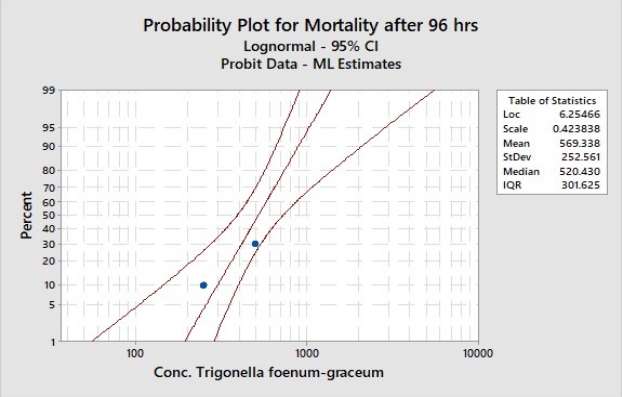


Fig. 1: Showing 96 hours LC_{50} for *Trigonella foenum-graecum* in *Oreochromis mossambicus*.

Histological observations

Histological analysis is an important sensitive tool to specify modification in any tissue or organ under stress. In the current study the histology of liver and intestine was carried out to evaluate the toxicological impact of CuO-NPs and ameliorative effect of *Trigonella foenum-graceum*.

A high frequency of histological alteration was observed in G* group (positive control which was exposed to potentially toxic administration of CuO-NPs). Decrease in histological alterations was observed in G1 and G2 groups in a dose dependent manner of *Trigonella foenum-graceum* administration. Group G3 (treated with high dose of *Trigonella foenum-graceum* extract) showed minimal histological abnormalities in both liver and intestine as compared to control group (without any treatment). Some of the major histological alterations observed in liver were pyknotic nuclei, vacuolation, congestion, haemorrhage and atrophy of hepatocytes while histoarchitecture of control group remained normal. Histological alterations in intestine showed hyperplasia, vacuolation, erosion of villi, expansion in villi, necrosis and congestion in G*, G1 and G2 whereas in G3 and control groups normal histology was observed (fig. 2).

DISCUSSION

Trigonella foenum-graceum grabs attention towards the beneficial effects for health across the globe and can be used as leafy vegetable (Yadav and Baquer, 2014; Bahmani *et al*., 2016). The pods of this plant contain hard brown seeds on maturity which are utilized for medicinal purpose (Srinivasan, 2006). In traditional system of medicine fenugreek was reported beneficial against various digestive and mucosal problems (Passano, 1995; Wani and kumar 2018). Some well-known pharmacological properties of *Trigonella foenum-graceum* are antioxidative (Kenny *et al.,* 2013), antidiabetic (Kumar *et al.,* 2005; Geberemeskel *et al*., 2019), anti-inflammatory (Liu *et al.,* 2012; El-Taib *et al*., 2020), antipyretic (Ahmadiani *et al.,* 2001), antitumor and immunomodulatory (Bin-Hafeez *et al.,* 2003). Amelioration through *Trigonella foenum-graceum* against CuO-NPs or other metal oxide nanoparticles toxicity for fish is not well documented in previous literature so in this discussion most comparisons are made in comparison with rats and other models representing the uniqueness of study.

In this study the oxidative stress was induced by CuO-NPs and best demonstrated in group (G*), only treated with CuO-NPs. *Trigonella foenum graceum* was reported good ameliorant only at high dose treated group (G3). Group G1 and G2 were reported with altered values of oxidative stress enzymes because no or little amelioration was observed in these groups. In this study catalase (CAT) was decreased in liver and intestine due to effect of CuO-NPs but amelioration was observed in G3 after 56 days of *Trigonella foenum-graceum* extract administration. A previous study conducted by Kumar and Bhandari (2013) on rats reported that CAT was decreased in liver due to effect of Monosodium glutamate and restored to normal levels by using extract of *Trigonella foenum-graceum*. These ameliorative findings are in good agreement with this study. The amelioration observed may be due to the anti-oxidant potential of *Trigonella foenum-graceum* extract. CAT was also decreased similarly in the intestine and amelioration was also observed in similar manner as in liver tissues. A recent study of Pradeepkiran *et al.,* 2020, reported that antioxidant enzymes were improved by using *Trigonella foenum-graceum* extract in diabetic rats including catalase also well support catalase findings of this study.

Jafarinejad *et al.,* 2020 investigated that cells have defensive mechanism to protect themselves from oxidative damage. Reactive oxygen species damage the polyunsaturated lipids and produce malondialdehyde (MDA). MDA is also an important biomarker used to explore the health condition of biological membranes (Khosravi-Katuli *et al.,* 2018). Glutathione reductase (GSH) removes metals and toxic electrophiles to protect cells from toxic oxidative damage (Srikanth *et al.,* 2013). Oxidative stress damages cellular membrane by an autocatalytic mechanism of Lipid peroxidation (LPO), which is involved in this mechanism (Asayama and Kato, 1990). Destructions in cells lead to production of free radicles and results in cell death (Messarah *et al.,* 2010). Present study investigated the increase in GSH, MDA and LPO as a result of CuO-NPs toxicity and restoration to normal values by the administration of *Trigonella foenum-graceum* extract in both intestine and liver at

Table 1: Grouping of control and experimental fish.

Groups	Treatments	
	Copper oxide Nanoparticles (mg/L)	*Trigonella foenum-graecum* (mg/L)
C	0.00	0.00
G*	0.12	0.00
G1	0.12	18.00
G2	0.12	26.00
G3	0.12	52.00

Table 2: Mean ± SE of oxidative stress markers (glutathione, malondialdehyde, catalase and lipid peroxidase) showing ameliorative effect of *Trigonella foenum-graecum* against CuO-NPs induced toxicity in liver tissues of *Oreochromis mossambicus* after 56 days of exposure.

Days / Parameters	Groups				
	C	G*	G1	G2	G3
Glutathione (µM/g)	481.22 ± 0.26^{d}	713.8 ± 0.39^{a}	703.3 ± 0.49^{b}	647.46 ± 0.45^{c}	482.69 ± 0.21^{d}
Malondialdehyde (nM/g)	134.32 ± 0.39^{d}	304.92 ± 0.31^{a}	278.24 ± 0.31^{b}	226.68 ± 0.41^{c}	135.37 ± 0.38^{d}
Catalase (Unit/ml)	346.56 ± 0.21^{a}	198.94 ± 0.44^{d}	227.58 ± 0.53^{c}	312.61 ± 0.31^{b}	346.01 ± 0.28^{a}
Lipid Peroxidase (mM/100g)	17.09 ± 0.21^{d}	29.12 ± 0.34^{a}	22.23 ± 0.19^{b}	20.87 ± 0.22^{c}	17.27 ± 0.15^{d}

Table 3: Mean ± SE of oxidative stress markers (glutathione, malondialdehyde, catalase and lipid peroxidase) showing ameliorative effect of *Trigonella foenum-graecum* against CuO-NPs induced toxicity in intestine tissues of *Oreochromis mossambicus* after 56 days of exposure.

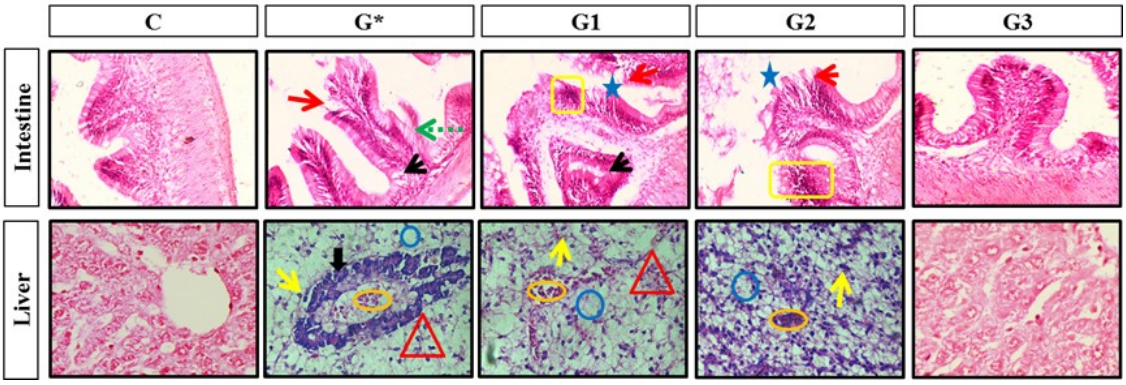
Days / Parameters	Groups				
	C	G*	G1	G2	G3
Glutathione (µM/g)	413 ± 0.29^{d}	853.14 ± 0.66^{a}	714.32 ± 0.49^{b}	678.76 ± 0.82^{c}	414.96 ± 0.15^{d}
Malondialdehyde (nM/g)	8.43 ± 0.1^{d}	152.88 ± 0.29^{a}	91.6 ± 0.31^{b}	86.65 ± 0.22^{c}	8.45 ± 0.1^{d}
Catalase (Unit/ml)	289.84 ± 0.18^{a}	187.75 ± 0.46^{d}	215.83 ± 0.56^{c}	232.32 ± 0.46^{b}	288.22 ± 0.28^{a}
Lipid Peroxidase (mM/100g)	7.77 ± 0.09^{d}	23.57 ± 0.25^{a}	17.72 ± 0.2^{b}	15.39 ± 0.32^{c}	8.05 ± 0.05^{d}

Mean values having different alphabets (Superscript) in rows are significantly different ($P<0.05$).

higher concentration for longer duration. Previous studies (Xue *et al.,* 2011, Chaturvedi *et al.,* 2013, Kumar and Bhandari, 2013, Abdel-Daim *et al.,* 2014) reported the use of *Trigonella foenum-graceum* against oxidative stress induced by different toxic agents. Above mention studies represent good similarities with the findings of present study.

The Histological changes in intestine were reported high in groups G*, G1 and G2 but minimum in G3. Some intestinal histological changes observed in response to toxic action of CuO-NPs are vacuolation, hyperplasia, erosion of villi, necrosis, expansion in villi and congestion. These above mentioned histological changes could be due to dominated toxic effect of CuO-NPs that suppress the beneficial effects of *Trigonella foenum graceum*. In G3 the beneficial effects of *Trigonella foenum-graceum* remain dominated and this plant helps in remedial process after 56 days of exposure. A previous study conducted by (Kheirandish *et al.,* 2011) explained protective effect of *Trigonella foenum-graceum* (fenugreek) seed extract for intestinal ischemia/ reperfusion injury in rats. The results of Kheirandish *et al.* (2011) showed that fenugreek seeds extract protects the intestinal mucosa from injury that well supports our study.

The present study showed different histological abnormalities in liver such as vacuolation, pyknotic nuclei, haemorrhage, congestion and atrophy of hepatocytes. The vacuolations could be due to excessive accumulation of fat in the cytoplasm (Sun *et al.,* 2011). CuO-NPs elicited inflammation response in liver cells in time and dose dependent response (Yahya *et al.,* 2019). The particle concentration, composition and exposure time respond to induce hepatocyto-toxicity (Yahya *et al.,* 2019). A previous study conducted by Rosioru *et al.* (2010) used fenugreek to ameliorate hepatic toxicity in rats due to ethanol induced toxicity. Another study reported damage in liver by using CCl_4 was well recovered by using methanol extract of fenugreek seeds that was close to recovery by using silymarin used as standard drug for liver (Das, 2014). Kaviarasan *et al.* (2006) revealed fenugreek extract as hepatoprotective against ethanol-induced toxicity and apoptosis in liver cells. Said *et al.,* 2011 investigated the hepatoprotective effects of fenugreek seed extracts against carbon tetrachloride induced liver toxicity in rats and reported that fenugreek seed extract showed highly hepatoprotective effect against CCl_4 and helps in regeneration of hepatocytes may well reflect the picture of amelioration as our histological findings. Another study



Intestine Photomicrographs: Significant alterations observed in intestinal histology of positive control group and experimental group were observed as Hyperplasia (Green Dotted Arrow); Vacuolation (Black Arrow); Necrosis (Blue Asterisks); Erosion of villi (Red Arrow) and Congestion (Yellow square).
Liver Photomicrographs: Significant alterations observed in liver histology of positive control group and experimental group were observed as Vacuolation Blue (Circle inside); Atrophy of hepatocytes (Red Triangle inside), Pyknotic nuclei (Yellow Arrow); Congestion (Thick Arrow) and Haemorrhage (Orange Oval).

Fig. 2: Photomicrographs of intestine and liver sections (400X) in control and treated groups i.e., Control (C=without any treatment), Positive control (G*=treated with only potentially toxic dose CuO-NPs @ 0.12mg/L) and experimental groups G1, G2 and G3 co-treated with CuO-NPs @ 0.12mg/L along with *Trigonella foenum-graecum* @ 18, 26 and 52mg/L, respectively. Control group shows the normal architecture, Positive control shows high alterations, G1 and G2 shows significant alterations, while G3 shows minimal alterations in intestine and liver sections.

of Mbarki *et al*., 2017 reported the protective role of *Trigonella foenum-graceum* against CCL_4 toxicity in liver investigated through liver histology, which is in line with the present investigations.

CONCLUSION

This study concluded that *Tigonella foenum-graecum* ameliorated the CuO-NPs induced toxicity in *Oreochromis mossambicus*. The hepato-intestinal toxicity and oxidative stress induced by CuO-NPs showed reversal response by the administration of methanolic extract of *Tigonella foenum-graecum* at high dose exposed for 56 days. The rich load of antioxidants in *Trigonella foenum-graceum* showed their good response and based upon this study this plant has been recommended for the safe use against CuO-NPs and other metal oxide nanomarticles induced toxicity reversal. Further studies are recommended to explore more information about the action of its active ingredients at molecular level using different animal models.

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