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Ameliorative effect of *Nelumbo nucifera* seeds and wheat grass formulated feed against tannery effluent toxicity and stress in freshwater fish *Oreochromis mossambicus*

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ABSTRACT

Amelioration of tannery effluent toxicity and stress revival was analysed through an approach by preparing a formulated feed with feed components comprising *Nelumbo nucifera* seeds and Wheatgrass as major ingredients in an enhanced formulation. The formulated feed was fed to fingerlings of *Oreochromis mossambicus* exposed to tannery effluent in laboratory confinement to observe its effect on growth and protective effect from toxic nature of the effluent. This study aimed to assess the effect of the sublethal concentration of tannery effluent over an exposure period of 45 days. The $LC_{50}$was analysed for the tannery effluent to select the suitable sublethal concentration. The body weight differences was be determined, the muscle tissue was analysed for biomolecular constituents by Fourier Transform Infrared spectroscopy (FT-IR) analysis. Alterations were observed in body weight and muscle tissue biochemical constituents in fishes exposed to the effluent fed on normal diet and formulated feed.

***Key words**: Oreochromis mossambicus, Tannery effluent, Nelumbo nucifera (lotus) seeds, Wheat grass powder, Muscle, FT-IR analysis, Biomolecules*

Introduction

The problem of water and soil pollution due to tanneries is a serious environmental threat especially in the developing countries. In India, there are more than 2,500 tannery units, scattered all over the country, with an annual capacity of processing 0.7 million tons of hides and skins (Rajamani, 1995; Ram *et al*., 1997). Leather processing in a tannery generally comprises three categories: pre- treatment of skin/ hide, chrome or vegetable tanning of skin/hide (tanning operation) and finishing operations (Stoop, 2003; Thanikaivelan *et al*., 2004). Nearly 30m^3 of wastewater is generated during processing of one tonne of raw skin/hide (Suthanthararajan *et al*., 2004).

These wastewaters contains high chemical oxygen demand (COD), color, sodium sulphide, nitrate, chloride, chromium and suspended solids (SS) (Szpyrkowicz *et al*., 2001). The colored wastewaters hamper light penetration (Malaviya and Singh, 2011), whereas high COD resulted in decreased dissolved oxygen in the aquatic ecosystem (Raj *et al*., 1996). Additionally, high concentrations of dissolved solids make the possible discharge of tannery wastewaters into water bodies problematic, as they cause eutrophication and other adverse environmental effects and also affecting aquatic organisms also has ecosystem-wide consequences (Khanipour *et al*., 2018).

Tannery wastewaters are characterized by strongly alkaline , high oxygen demand, high content of salts and nutrients (Bajza and Vrcek, 2001; Abdel Baki *et al.,* 2011). Among all the contaminants, chromium is the one which are directly or indirectly released into aquatic ecosystem (Gheju, 2011). Accumulation of heavy metals in aquatic organisms especially fish depend on ecological conditions, physical, chemical and biological factors of water, physiological state of the organism (Khanipour *et al.,* 2018). Metals interact with proteins enzymes and hinder their biochemical and physiological activities (Kaoud and Mekawy, 2011).

The aquaculture industry, which has been growing rapidly for many years, is still projected to continue growing faster than most other industries for the foreseeable future (Reyahi-Khoram *et al*., 2016). Improved ecologically integrated aquaculture technology, with a tank-based integrated technique for bio-remediation of effluents using the algae and other plant based formulations as a feed are also under trials (Al-Hafedh *et al*., 2012)

Nelumbo nucifera (N.nucifera) seeds is known for its nutritive value. Lotus seeds are used in folk medicines to treat tissue inflammation, cancer, diuretics and skin diseases (Mukherjee *et al*., 2010). Lotus seeds are good source of protein and essential minerals (Ibrahim and El-Eraqy, 1996). The lotus seed contained high amount of potassium, calcium, magnesium and sodium besides small amount of copper, zinc, manganese and iron which are beneficial to human health (Sridhar and Bhat, 2007). It has been reported to have exhibited significant antioxidant activities (Rai *et al.*, 2006). The major phytochemicals present in lotus seeds are alkaloids are dauricine, lotusine, nuciferine, pronuciferine, liensinine, isoliensinine, roemerine, nelumbine and neferine (Tomita *et al*., 1961). Lotus seeds have been reported to possess rich antimicrobial properties (Mukherjee, 2002).

Wheatgrass powder is used as animal food as well as a nutrient supplement for human throughout the world. It is generally rich in essential nutrients such as amino acids, protein, lipids, vitamins, enzymes and minerals. Wheat grass is one of the green foods that contain 70% chlorophyll (Devi *et al.,* 2015). It is reported that wheatgrass powder used as feed ingredients for rearing of magur fry, gave better growth and survival rate (Islam *et al*., 2017).

Fourier Transform Infrared (FT-IR) spectroscopy technique has become an independent and advance modality in terms of high sensitivity in detecting changes in the functional groups belonging to the specific components of tissue such as proteins, lipids, carbohydrates and nucleic acids (Karthikeyan and Easwaran, 2013). The technique because of high sensitivity, is capable of providing a strong insight on the structural and functional changes induced by various factors (Staniszewska *et al*., 2014; Sher Ali Khan *et al*., 2017).

In this pilot study an approach has been made to assess the combination of lotus seed powder along with wheat grass, feed in alleviating tannery effluent metal toxicity in fishes which remains unexplored. Fish muscle was chosen as target organ for assessing the effect of tannery effluent that reflects the concentration of metals in water where the fish species live, and for its importance in human diet. Tilapia is considered as the suitable species for culture because of its tolerance to adverse environmental conditions, and fast growth, so the fish was chosen as experimental model for the present study.

Materials and Methods

Collection of tannery effluent: Samples were collected from Common Effluent Treatment Plant (CETP) at Pallavaram in Chennai which treats around 3000 m^3/day of wastewater. The effluent was collected at a fixed point when the discharges from all the stages of processing are released together. The samples were collected during the month of March 2021. The raw effluent was collected in different polyethylene containers of 20 litrescapacity and stored in dark at room temperature till further use.

Experimental animals: Healthy juveniles fishes comprising fingerlings of *Oreochromis mossambicus* weighing about 4.0 to 4.5 g approximately and 5.5 – 7.5 cms in length was procured from a fish rearing pond at Pazhaverkadu village, Tiruvallur District and were brought to the laboratory in polythene bags containing aerated water. This fish was selected for the study due hardy nature and was able to tolerate derelict water conditions. They are highly edible food fish with nutritive value.

Determination of lethal concentration (LC_{50}): The LC_{50} value of tannery wastewater was analyzed through a static renewal bioassay technique. Preliminary screening was carried out to determine the testing chemical (Solbe, 1975). The effluent was mixed with tap water in appropriate dilution to get wide range of concentration. A range of 7 concentrations (50, 40, 30, 25, 20, 15, 10%/l of water) were elected for lethal dose studies. The mortality in each concentration were noted for 24, 48, 72 and 96 hours exposure. The LC_{50}value (96 hour) was found to be at 20% concentration, for fingerlings of *Oreochromis mossambicus.* From this 50% of sublethal concentration was selected for the present study.

Feed composition and diet formulation: The feed used in this experiment was prepared as given in the table below. The diets were prepared by the method of Jayaram and Shetty (1981) as follows.

The control and experimental feed was subjected to proximate analysis.

Experimental Design : Freshwater acclimated fish fingerlings of *Oreochromis mossambicus* were divided into three groups consisting of 10 fishes in each group.

Group I (Control) :

Fishes were fed with normal feed and maintained in dechlorinated toxicant free water.

Group II (Experimental group-I) :

Fishes fed with normal feed and maintained in tannery effluent

Group III (Experimental group-II) :

Fishes were maintained in tannery effluent and fed with formulated feed

All the groups of fishes were maintained in 30 L of water. Group-II and Group-III fishes were exposed to sublethal concentration (10%) of the tannery wastewater for a period of 45 days. No mortality was recorded during the period of study. Fishes were handled with a clean hand net. Fishes were distributed evenly in each tub with two replication for each group.

The experimental tubs were maintained promptly with the renewal of effluent water daily in group- II and III. The tubs were aerated with air stones attached to an air compressor to saturate oxygen. About 75% of the water in the tubs was changed daily. The fishes were fed with groundnut cake feed and experimental diet twice a day daily initially. Control and Experimental group-I fishes were fed with groundnut cake feed, Experimental group-II were fed with the formulated feed. Left over feed if any was removed by siphoning, two hours post feeding to reduce contamination with food remains. Faecal residues were removed daily through suction. The body weight of the fishes were recorded once in 10 days. Sampling was done and the quantity of feed given was re-adjusted, after each sampling, based on the weight recorded.

At the end of the experiment, fishes were euthanized by decapitation of cervical region and muscle tissues were carefully removed, washed twice in ice cold physiological saline (0.9 N) solution and weighed. Tissue samples were homogenized for two minutes in ice cold Tris buffer (0.1 M, pH 7.4; 1:10, w/v) using a glass homogenizer. The muscle tissue was dried in an hot air oven to remove moisture content and grounded to fine powder for FT-IR analysis.

Analysis of physico-chemical characteristics of tannery effluent : The physico-chemical characteristics of raw tannery effluent were analysed for color, pH, total solids, total dissolved solids (TDS), total suspended solids (TSS), chemical oxygen demand (COD), biochemical oxygen demand (BOD),

Table 1. Composition of formulated feed (in percentage) for control and experimental fishes (Ingredients refer to every 100 gm of feed prepared)

S. No.	Feed Ingredients	Control diet	Experimental diet (Formulated feed)
1	Groundnut fish feed	100	-
2.	*Nelumbo nucifera* seed powder	-	20
3	Wheatgrass powder	-	25
4	Shrimp meal	-	15
5	Wheat flour		10
6	Rice bran	-	10
7	Yellow Corn flour	-	13
	Soya flour		05
8	Vitamin- mineral mixture	-	2
	Total	**100**	**100**

total chromium, hexavalent chromium and trivalent chromium following the standard methods as given by APHA, (1998).

FT-IR analysis: FTIR Analysis was performed by the method of Ozaki and Kanouchi, (1989). Different radiation sources, optical systems and detectors are needed for the different regions which permits the separation of closely spaced absorption bands. Accurate measurements of band positions and intensities and high scanning speeds for a given resolution level. FTIR spectra of the powdered muscle tissue analysed were collected using a Bruker alpha Fourier Transform infrared attenuated total reflection spectrometer(FTIR- ATRS) using Zinc selenide (ZnSe) crystals in the range of 550 cm^{-1} to 4000 cm^{-1}

Results

Protective effect of *N. nucifera* seed powder and commercial wheat grass formulation against tannery effluent stress in fingerlings of *Oreochromis mossambicus* was assessed in muscle tissue after 45 days diet regime. The physico-chemical parameters of raw tannery effluent were analysed for colour, pH, total solids, total dissolved solids (TDS), total suspended solids (TSS), chemical oxygen demand (COD), biochemical oxygen demand (BOD) and content of chromium. All the above characteristics were found to be higher in our study in the tannery effluent (Table 2).

The experimental feed composition show an increase in carbohydrate, protein and fat when analysed for their proximate composition (Table 3). Antioxidant nutrients β-carotene, Vitamin E and ascorbic acid were reported to be higher in the formulated feed than the control feed (Table 4).

Table 3. Proximate composition of control feed and combination of *N. nucifera* seeds and wheat grass formulated experimental feed

Proximate analysis (% dry weight)	**Control feed**	**Experimental feed**
Carbohydrates	19.91	24.66
Protein	29.22	31.67
Fat	8.72	25.20
Fibre	4.34	3.34
Ash	4.16	9.40
Moisture	9.18	6.01
Energy	312.5	440.8

When compared to control, experimental group–I fishes maintained in tannery wastewater show reduction in final body weight. A significant increase was obtained in the final body weight of the fishes maintained in tannery water fed on formulated diet. An average body weight gain of 4.26 g/fish and was observed in experimental diet fed group-II fishes. The observations were statistically significant

Table 4. Content of β-carotene, total carotenoids, vitamin E and ascorbic acid in control feed and combination of *N. nucifera* seeds and wheat grass formulated experimental feed

Antioxidants	Control feed	Experimental feed
Total carotenoids	21.33	1834.5
β- carotene	14.25	434.5
Vitamin E	25.98	144.5
Ascorbic acid	12.33	19.6

Values of β-carotene and Total carotenoids expressed as µg/100 g dry weight; Values of Vitamin E and Vitamin C expressed as mg/100 g dry weight

Table 2. Physico-chemical characteristics of tannery effluent (All values are expressed as mg/l)

Physico-chemical characteristics	Tannery effluent (Untreated)	Tolerance limits for treated outlet as per TNPCB
Colour	Black	-
pH at 25^{0}C	8.50	5.5 to 9.0
Total suspended solids (TSS)	904.9	100
Total dissolved solids (TDS)	4934	2100
Biological oxygen demand (BOD) at 27^{0}C	783	30
Chemical oxygen demand (COD)	1580	250
Sulphates (SO_4)	1990	1000
Chlorides (Cl)	1684	1000
Total chromium (Cr)	25.33	2
Hexavalent chromium (Cr^{6+})	19.45	0.01
Trivalent chromium (Cr^{3+})	5.56	0.05

Table 5. Protective effect of *N. nucifera* seeds and wheat grassformulation on body weight difference against tannery effluent stress in fingerlings of *Oreochromis mossambicus*

Experimental groups	Body weight (g)			Paired sample 't' test		
	Initial	Final	Paired sample test difference	t-value	df	2-tailed significance
Control group (Toxicant free water- Normal feed)	4.02± 0.68	6.24±0.27	2.22±0.76	6.452	4	0.003
Experimental group-I (Tannery effluent- treated with Normal feed)	4.68±0.46	5.46±0.01	2.18±0.01	439.557	4	0.001
Experimental group-II (Tannery effluent treated with Formulated feed)	4.30±0.68	8.56±0.42	4.26±0.01	17.02	4	0.001

Values are Mean ± SD (n=5) observations

($p < 0.05$) in both the experimental groups according to paired sample't' test when compared with control group (Table 5).

The normal FTIR spectra of the muscle tissues of control, tannery exposed fishes given normal feed and tannery effluent maintained in formulated feed in the region of 4000-400 cm^{-1} is demonstrated. The attributes frequency values and detailed band assignment of the spectra of the sample are presented in (Table 6). In the present study, the band observed at ~ 3293 cm^{-1} is due to OH/NH stretching vibration of water/primary amine. The band at 2964 cm^{-1} corresponds to CH_3 symmetric stretching and the band at 2876 cm^{-1} CH_2 symmetric stretching of lipids. The band at 1652 and 1541 cm^{-1} are reported as amide I and II vibrations individually of structural proteins. The intensity of amide I band decreased altogether in both treatments of muscle tissues and the frequency of this band moved to the higher value in experimental group-II. The band at 1044 cm^{-1} corresponds to carbohydrates contents of muscle tissues.

The intensity of I_{1044} represents the carbohydrates compositions and the increase/decrease in intensity corresponds to change in carbohydrates. The peak intensity ratio of I_{1541}/I_{1652} which corresponds to change in protein contents. The intensity of lipids I_{2876} was used to find quatitative composition changes in lipids. Decrease or increase in carbohydrate, protein and fat contents of muscle tissue in control, tannery treated fishes and tannery treated with formulated feed is presented in (Table 7; Fig. 1).

Table 6. Protective effect *N. nucifera* seeds and Wheatgrass formulation on FT-IR analysis of muscle tissue against tannery effluent stress in fingerlings of *Oreochromis mossambicus*

Frequency	Assignments
3293	Amide A NH Stretching
2964	CH_3 Asymmetric Stretching Lipid
2876	CH_2 Symmetric Stretch mainly lipids
1658	Amide I Proteins
1542	Amide II Proteins
1044	Carbohydrates

(Tentative frequency assignment and their functional group of muscle tissue)

Discussion

Several studies have been carried out on the effect of wastewater effluents on the biochemical aspects of fish and other aquatic animals, but very few on it amelioration through dietary sources. Considering the commercial value of *Oreochromis mossambicus*, the present study was designed to obtain information about the effect of tannery effluent and to improve its effect through attenuation with dietary combination of *N. nucifera,* lotus seed powder and wheatgrass formulation. Both components used in the formulation has been considered as supplement in human and animal food.

The LC_{50} value (96 hour) was found to be 20% concentration for *Oreochromis mossambicus* maintained in tannery effluent, from this 50% concentration was selected which was fixed at 10 % for the present study. Navraj and Yasmin (2012) reported LC_{50} value as 7% in adult fishes of *Oreochromis mossambicus* exposed to 96 h tannery industry wastewater. Srivastava *et al*. (2007) also confirmed that 1 to 10% of tannery wastewaters is reported as safe sublethal concentration.

Physico-chemical data of the tannery effluent denotes highly polluted nature. The pH seems to be most important parameter. In the present study pH

(Tentative frequency assignment and their functional group of muscle tissue)

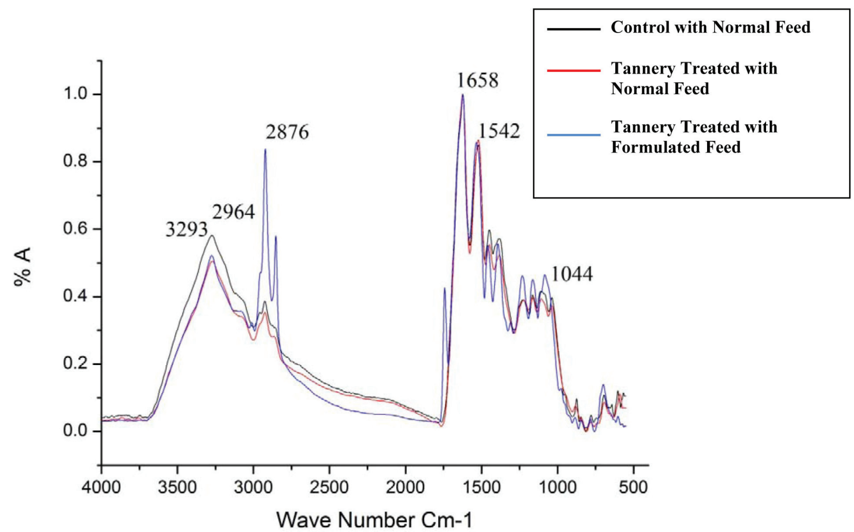

Fig. 1. Protective effect of *N. nucifera* seeds and Wheatgrass formulation on FTIR analysis of muscle tissue against tannery effluent stress in fingerlings of *Oreochromis mossambicus*

of the raw, untreated effluent was found to be alkaline. The pH is the principle factor governing the concentration of the effluent. The alkaline nature of the effluent may be due the presence of high concentration of soluble metals (Malaviya and Rathore, 2007). The high values of total suspended solids (TSS), total dissolved solids (TDS), biochemical oxygen demand (BOD), chemical oxygen demand (COD) are the main indicators of it being heavily polluted due to industrial tannery processing. High level of TSS present in the tannery effluent could be attributed to their accumulation during the process of finished leather. The increase in sulphates and chlorides could be due the use of various inorganic chemicals in the industrial process. The higher levels of chromium may be because of the use of chromic acid during tanning process which is found to be a matter of concern because this metal is known to be toxic (Sahu, 2007). The values are beyond the tolerance limit. Further the settling of suspended particles leads to various damages like clogging of gills and respiratory surface of fishes (Padmini and Sudha, 2004).

In the present study combination of *N. nucifera* seed powder formulation, wheat grass powder and shrimp meal were combined and used as experimental feed. Normal fish feed was used as control feed. When compared to control feed the formulated experimental feed was found to contain increased carbohydrate, protein and fat content along with antioxidant nutrients like total carotenoids, β-carotene, Vitamin E and ascorbic acid.

Wheatgrass is the powdered form of young grass wheat plant. It is rich in iron, vitamin A, B-complex vitamins B_1 and folate. It is good source of antioxidant phytochemicals like carotenoids, chlorophyll

Table 7. Protective effect of *N. nucifera* seeds and Wheatgrass formulation on FTIR analysis - Spectral peaks / intensity ratios of muscle tissue biomolecules content against tannery effluent stress in fingerlings of *Oreochromis mossambicus*.

Experimental Groups	I_{1044} Carbohydrate	I_{1541}/I_{1652} Protein	I_{2876} Lipid
Control –Normal feed	0.42	0.82	0.38
Tannery effluent - Treated with Normal feed	0.38	0.78	0.34
Tannery effluent- Treated with Formulated feed	0.47	0.87	0.84

Intensity ratio - **I** which corresponds to change in carbohydrates, protein and lipid contents and tocopherols. Minerals like calcium, magnesium and sodium , are present along with dietary fibers and carbohydrates (Mujoriya and Bodla, 2011). Wheat grass powder is a good detoxifying agent (Islam *et al*., 2017).

An increase in body weight and gain in length was observed in fishes maintained in tannery effluent fed on formulated feed. It is suggested that the formulated feed may have stimulated the production of enzymes that transport fats in fish for growth instead of storage. β-carotene inthe formulated feed firmly maintains the mucous membrane and thereby prevents the entry of toxic elements into the body. Wheatgrass powder as a feed ingredient, had significant impact on growth and survival of Asian catfish fry (Islam *et al*., 2017). Chlorophyll contents in *N. nucifera* seed powder and wheat grass can actasa cleansing and detoxifying factor against toxic substances (Nath *et al*., 2014). The present study shows dietary supplementation of *N. nucifera* seed powder and wheat grass reduced toxicity of the effluent. Reports indicate that *Spirulina plantensis* reduced the metal toxicity in mercuric chloride exposed *Labeo rohita*, improved weight gain and growth rate significantly in a short period of time (Shelke and Wani, 2015).

FTIR spectroscopy provides information about the amount and chemical and physical nature of the groups in close vicinity (Ceylan *et al*., 2012). This technique qualitatively and quantitatively evaluates shifts in peak positions, changes in bandwidths and band intensities to obtain structural and functional information. The IR spectra of muscle tissue are composed of several bands of various functional groups having a place with lipids, proteins and carbohydrates. The intensity of I_{1044} represents the carbohydrates compositions and the increase/decrease in intensity corresponds to change in carbohydrates. The amide absorptions are viewed as to protein conformation; consequently an increase or a decrease in the ratio of the intensities of the band ~1540 cm^{-1} (amide II) and ~1650 cm^{-1} (amide I) could be ascribed to change in the composition of the whole protein I_{1541}/I_{1652}. The intensity of lipids I_{2876} was used to find quantitative composition changes in lipids.

The frequency and intensity changes are observed due to tannery effluents. The observations from the present study showed that, these effluents at sublethal concentration altered the biochemical composition (carbohydrates, protein and lipid) in the muscle tissues, due to utilization of biochemical energy to counteract the toxic stress caused due to effluents.

Karthikeyan (2014) hypothesized that the amide I and II regions shifts result in conformational changes in the existing set of proteins corresponding to the secondary structural protein change. The toxic effect of heavy metals is evidenced by the overall decrease in protein content in fish muscle tissue. The changes in FT-IR band intensity ratios and band shifts, particularly amide II/I, suggest conformational changes in the existing set of proteins or the appearance of an altered set (Karthikeyan and Mani, 2014).

Muscle carbohydrates decrease in tannery effluents treated groups. A fall in glycogen level demonstrates its quick use to meet the improved vitality requests in tannery treated animals. This might be because of utilization of carbohydrates to meet the stress under toxicity. Consequently supplementation of formulated feed results in significant increase in carbohydrates composition. Chromium can block the energy production and normal cell signaling. The decrease in proteins observed in the present study might be due to their utilization in cell repair and tissue organization with the formation of lipoproteins. Further the depletion of tissue proteins may be due to impaired or low rate of protein synthesis under tannery effluents stress. The depletion of protein fraction in muscle tissues may have been due to their degredation and possible utilization for metabolic purposes. It may be due to their utilization in the formation of mucoproteins which are eliminated in the form of mucous. The results revealed that toxicity due to tannery effluent influences metabolic pathways of *Oreochromis mossambicus* and the biochemical studies utilizing FTIR can fill in as incredible biomarkers of tannery effluent contamination. Similar results were observed in *Cirrhinus mrigala* and *Oreochromis mossambicus* treated with heavy metals and pyrethroids respectively (Karthikeyan *et al*., 2012; Velmurugan *et al*., 2018). It is observed from that decrease in muscle fat was observed due to tannery treatment and the increase in fat noted after supplementation with feed treatment. This decrease in fat may be due to utilization of energy to meet the toxicity stress.

FT-IR spectra revealed significant differences in absorbance intensities between the control, nickel and mercury intoxicated muscle tissue, thus reflecting an alteration on the major biochemical constituents, such as lipids, proteins and nucleic acids of the muscle tissue of *Lates calcarifer*. The molecular changes may retard the growth of species . Further, it is observed that sublethal nickel and mercury exposure causes some alteration in protein profile with a decrease in α-helix and an increase in random coil structure.

Lotus seed possess hepatoprotective, free radical scavenging properties and antifertility properties (Sohn *et al*., 2003). Yen *et al*. (2006) reported the free radical scavenging and protective effects of lotus seed extracts against reactive nitrogen, sodium nitroprusside (SNP), peroxynitrite induced cytotoxicity and DNA damage in macrophage. Recent studies have shown that lotus seed skin contains a high concentration of proanthocyanidins that have multi-functions, such as antioxidation and anti-inflammation effects (Tao Li *et al*., 2019). These phytochemical compounds found to exhibit antioxidant properties by directly scavenging reactive oxygen species and reported to exert effects on the interface against excessive oxidative stress and ROS via modulation of Nrf_2 and NF-βB pathways (Gessner *et al*., 2017). It is reported that lotusseed show highest concentration of chromium. These results confirmed previous findings in which lotus plant has been identified as a good metal dominant hyper-tolerant type of aquatic plant. Lotus can accumulate metals such as lead and chromium in its various parts (Ashraf *et al.*, 2010).

The cultured fish can be protected against untreated effluent, pesticide or fungicide stress by supplementing natural antioxidants such as vitamin E, β-carotene and ascorbic acid at a higher level than what is normally required in the fish diet (Khan, 2015). Wheatgrass is highly nutritive and a source of vitamins, minerals, protein, lipid, enzymes and non-enzymatic antioxidant (Chouhan and Mogra, 2014; Devi *et al*., 2015). The hepatoprotective effect of *Aloe vera* juice supplementation with arsenic increased the secretion of bile and detoxifies the toxins by maintaining the liver functions. *Aloe vera* juice has an effective hepato protective and tissue protective property against arsenic and chromium toxicity in *Labeo rohita* (Zodape, 2010a,b). The results of present study also suggests similar mechanism of action in the tissues of *Oreochromis mossambicus* exposed to tannery effluent and fed with formulated feed.

Lotus seeds and wheat grass have potential nutraceutical advantage, blending its seed flour with other legumes which are nutritionally rich like soybean or millet varieties will be of immense value to develop low cost proteinaceous healthy value added food supplements to aquaculture industry and also to combat the stress of toxic conditions. Bioprospecting of lotus seeds and wheat grass combination hold promising future as an alternate protein supplement and potential pharmaceutical source which could be used as an experimental trial in cultivable fishes. Detailed reports on the toxic effects on long-term consumption of lotus seeds, wheat grass and their products are scarce, further experiments are required to substantiate its effects. Although nutraceutical value of lotus seeds has been well established, further exploration of value-added compounds might be beneficial in promotion of its benefits in fish growth and monitoring health.

Conclusion

N. nucifera seed powder and Wheatgrass formulation in *Oreochromis mossambicus* fingerlings against tannery effluent stress in the present study has reduced the toxic effect when diet supplementation was received. The formulated diet rich in protein and antioxidants has minimized toxicity attributed to free radical scavenging and its inclusion in fish diets would protect fish from tissue damage by inhibition of the formations of reactive oxygen derivatives. Formulated feed supplemented in combination helps the fish to survive in adverse environmental condition and can be substituted as a fish feed ingredient for better growth and high survival rate of the fish in the future along with the higher protein percentage.

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