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Research Article

Neuronal and Oxidative Damage in the Catfish Brain Alleviated After *Mucuna* Seed Extract Treatment

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ABSTRACT

The neurodegenerative activity of a synthetic detergent sodium dodecyl sulphate (SDS) on brain physiology in Indian native catfish *Heteropneustes fossilis* and the efficacy of methanol extract of *Mucuna pruriens* seeds for alleviating such effects were demonstrated. Fish (n=36, 2 replicates) were exposed to SDS (2.75 mg/l) for 0 (control), 15 and 30 days. After 30-days treatment, methanol extract of *Mucuna* seed was injected for continuous seven days and sampling was done on each alternate odd days (1, 3, 5 and 7 days). Levels of different enzymatic and non-enzymatic antioxidants, Na^+-K^+-ATPase, acetylcholine esterase; monoamine oxidase; nitric oxide were measured in *H. fossilis* brain tissue. 30-days treatment with SDS caused significant decrease in reduced glutathione, catalase, superoxide dismutase, glutathione S-transferase, while glutathione reductase, malondialdehyde level increased significantly ($P<0.05$). Administration of *Mucuna* seed extract (15.5 mg/kg body weight) was found to restore the neurological activity and reduce stress in a time-dependent manner as the biochemical and neurological parameters in fish after 7-day extract administration showed no significant difference ($P>0.05$) compared to those in control without SDS treatment, except for GST and GPx which were unable to return to the basal level.

Keywords: *Mucuna pruriens*, SDS, Stress, Neuronal damage, *Heteropneustes fossilis.*

INTRODUCTION

Different agricultural and industrial wastes enter regularly into the aquatic environment via different routes and many of them induce physiological changes in fish. Some of these pollutants potentially modulate the reactive oxygen species (ROS) formation whereas others act indirectly by binding with cellular thiol molecules and reducing antioxidant functionality[1]. A large scale of tissue and cellular site specific differences in oxidative damage have been documented in fishes collected from different toxicant contaminated sites[2]. Hence, a collective point of view with the appreciation of balance between peroxides formed in tissue level and respective stress protection in biological systems needs to be a significant point to monitor the toxic effects implemented under severe stressful environmental conditions. Neurons within nervous system are comparatively sensitive to reactive oxygen species molecules. Many neurodegenerative disorders have been linked to detrimental consequences caused by oxidative stress and reactive oxygen species[3]. Detergents present in urban or rural usage may reduce dissolved oxygen; alter pH and salinity of respective freshwater bodies, thereby affecting oxygen consumption ability of fishes[4]. However, any potential impact of these synthetic detergents on central nervous system and the mechanism of such detergent induced impairment of nervous system function in fish are still not properly documented except for a recent report in a carp[5,6]. Many plant extracts are reported to be with antioxidant activity and act as a potent scavenger of superoxide radical[6,7]. The Indian medicinal herb, *Mucuna pruriens* has been documented to have anti-depressant potency, libido enhancing effect, antispasmodic, antipyretic, anti-inflammatory activity, and has been used to manage Parkinson's disease[8]. Fishes are susceptible to exposure to various pollutants including synthetic detergents. In the present study, *Heteropneustes fossilis*, a native Indian cat fish was treated with a synthetic detergent, sodium dodecyl sulphate (SDS), to elucidate the neurotoxic effects of any detergent surfactant in a catfish model. Subsequently, the antioxidant defence and neuroprotective efficacies, if any, of *Mucuna* seed extract were evaluated for considering the use of the plant extract as source of new drugs against neurotoxic damage. Therefore, the aim of the study was to measure functional responses of stress physiology as well as documenting brain functions and biochemical responses during exposure to severe household pollutants in aquatic environment.

MATERIALS AND METHODS

Collection of fish

Heteropneustes fossilis (80±3 gm) were collected from the fish farm of West Bengal Government, oxygen packed and transported to the laboratory. In the

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laboratory, the fish was kept in 45 l aerated aquaria maintaining the physicochemical parameters such as water temperature (T=27±2^{0}C), pH (7.2-7.5) and dissolved oxygen (5-5.5 mg/l) at appropriate values.

Experimental design

After 10 days of acclimatization period fish were divided into 2 groups (n=36, 2 replicates). One group was exposed to SDS at the concentration of 2.75 mg/l, while the control group was not exposed to SDS. This sub-lethal concentration (1/10th of the 96 h LC50) for exposure was determined based on the 96 h LC50 value (27.5 mg/l) of SDS for the concerned fish. SDS powder was weighed and thoroughly mixed with running tap water. The exposure solution was renewed completely each day in order to ensure constant concentrations. After15 and 30 days of treatment schedule, brain tissue were collected from 6 fish each from both the control and the treatment groups. Remaining fish from SDS-treatment groups (30 days) were injected intraperitoneally with *Mucuna* extract (15.5 mg/kg body weight) for seven consecutive days and samples were collected from 6 fish each after 1, 3, 5 and 7 days of *Mucuna* treatment. All samples were stored in -20^{0}C for biochemical analysis.

Preparation of Mucuna extract

Mucuna seeds were procured from local plant market, washed in sterile distilled water, air-dried and powdered. Powdered plant materials (250 gm) were extracted with 500 ml methanol in a Soxhlet apparatus and the extracts were evaporated to dryness under pressure at 45ºC using rotary evaporator and stored under nitrogen at -20ºC in amber glass bottle.

Measurement of enzymatic and non-enzymatic antioxidants in brain tissue

Brain tissues were homogenized with Tris buffered saline (10 mM Tris-HCl, 0.1 mM EDTA-Na, 10 mM sucrose,0.8% NaCl, pH 7.4), centrifuged and supernatant was used to measure levels of different enzymatic and non-enzymatic antioxidants such as Malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GRd), glutathione peroxidise (GPx), glutathione S-transferase (GST) according to standard methods described earlier [9]. In brief, the level of MDA equivalents was measured in brain by thiobarbituric acid reactive substances (TBARS) assay with minor modifications. SOD activity was measured following a spectrophotometric method based on assessment of O_2-mediated nitro blue tetrazolium reduction by an aerobic mixture of NADH and PMS. For measurement of CAT activity, absorbance was monitored at 240 nm up to 90 s at 15 s intervals. The assay was validated by treating the tissue homogenates with sodium azide, a known inhibitor of CAT activity[10]. For GPx activity, absorbance was measured at 492 nm against blank (100 µl extra OPD solution instead of sample). The activity of GR was determined by monitoring the glutathione-dependent oxidation of NADPH at 340 nm, in a reaction mixture containing 950 µl of 0.15 mM NADPH, 0.5 mM glutathione, and 3 mM MgCl2 in 50 mM Tris (pH 7.5) and 50 µl extract. The GST activity was spectrophotometrically assessed using glutathione (GSH, 2.4 mM/l) and 1-chloro-2, 4-dinitrobenzen (CDNB, 1 mM/l) as substrate.

Measurement of activity of brain enzymes

Na+-K+-ATPase activity, expressed as µmol Pi liberated/mg protein/h in the brain was measured by liberating PO_4 from a hydrolysis reaction with ATPase[11]. AChE activity in brain homogenates was determined with an AChE kit according to standard method[12]. The activity of 1 U of AChE was defined as the number of hydrolyzed micromoles of acetylthiocholine iodide per min per microgram of protein. The activity of MAO was determined using a detection kit, which assessed the production of benzyl aldehyde from the reaction of MAO and its specific substrate, aniline hydrochloride[13].

Statistical analysis

Two separate principal component analysis (PCA) for all the antioxidant enzymes with two hormones and brain enzymes were done to understand the ordination. First, interspecific patterns of stress were examined using a PCA on the correlation matrix of standardized enzymes and hormones. This analysis allows comparison of stress physiology, brain enzymes and serum hormones within a multivariate morphological space and identification of patterns of correlation among physiological variables. Eigen values of each component of the PCA were considered to interpret results. All data were expressed as Mean ± SD and analyzed by one-way ANOVA. Where F values indicated significance, the means were comparedby a post hoc multiple range test taking $p < 0.05$ as the threshold.

RESULTS

Activity of GRd were observed to significantly ($P<0.05$) increase with the increasing duration of the SDS-treatment, while SOD and CAT activity showed a sharp decrease in their expression after SDS treatment but no significant alteration was noted along with schedule of treatment. Similarly, significant change ($P<0.05$) in the activity of both GPx and GST was monitored in any of the two SDS-treatment groups compared to that in control. Administration of *Mucuna* extract significantly re-established ($P<0.05$) the levels of the SOD, CAT and GRd activity and such significant response was observed after 5 days of *Mucuna* extract treatment (Table 1). However, *Mucuna* treatment was unable to restore the levels of both GPx and GST even after 7 days of treatment. GSH level decreased significantly at 15 days SDS-treatment group compared to that in control. MDA levels were observed to increase with the SDS-treatment, though no significant alteration ($P>0.05$) was observed in the levels of GSH and MDA between 15 and 30 days of SDS treatment. Administration of *Mucuna* extract significantly restored ($P<0.05$) the levels of the GSH and lipid peroxidation and the response was noted after 5 and 7 days of treatment respectively (Table 1).

The activity of AChE, MAO and NO in the brain was significantly inhibited after 15 days of the SDS-administration ($P<0.05$), but the level of inhibition was not duration dependent, as the activity did not reduce significantly ($P>0.05$) after 30 days of treatment than that

after 15 days treatment. Administration of *Mucuna* extract significantly restored ($P<0.05$) the levels of the enzyme activity. The activity of MAO, NO and AchE was observed to increase significantly ($P<0.05$) after 5 days of *Mucuna* treatment (Table 2). There was a marked decrease in the activity of Na^+K^+ATPase enzyme in both the SDS-treatment duration categories than that of the control fish. However, no significant variation ($P>0.05$) was observed for Na^+K^+ATPase activity between 15-days and 30-days SDS-treatment groups. Levels of Na^+K^+ATPase activity started to increase significantly ($P<0.05$) after 3 days of *Mucuna* injection though highest activity was observed after 7 days of treatment (Table 2).

DISCUSSION

Detergents, including the biodegradable ones have been discovered to induces poisonous effects and osmoregulatory imbalances in aquatic lives especially if present in concentration that exceed metabolic demand. Such xenobiotic compounds could be persistent and more mobile in soil and water, hence, it is known to be one of the most common terrestrial and aquatic contaminants[14]. The detergent effluents are also noticed to induce severe damage to such vital organs like the gills, kidney, liver, skin, heart and the brain. Current communication tries to elucidate the levels of oxidative and neurodegenerative damage caused by synthetic detergent SDS in the brain.

Under stress conditions, body mechanisms are altered to combat the effect of the pollutants/stressors in order to stabilize the organism. Oxidative stress is the crucial manifestation of a multi-step pathway, culminating in an imbalance between pro-oxidant and antioxidant defence mechanisms due to the functional detoriation of antioxidants, or the excessive accumulation of super oxide radicals, or both, which leads to tissue damage[15]. It has been demonstrated that exposure to various contaminants like SDS could produce ROS which cause various organ lesions[16]. MDA, a lipid peroxidation marker, was used to assess the levels of oxidative stress. The significant increase in MDA level indicated the generation of super oxide radicals in fish exposed to SDS though the effect didn't depend on the duration of the exposure. Due to the inhibitory effects on oxy-radical formation, the SOD-CAT system provides the primary safeguard against oxygen toxicity[17]. Decrease in the activity of SOD-CAT system was most likely a response to toxicant stress, and failed to neutralize the detrimental consequences of increased super oxide molecule generation[18]. SOD and CAT activities in the catfish tissues were strongly inhibited with long time exposure of toxicant, which could be due to the flux of superoxide radicals, resulting in increase in cellular peroxide molecule[19]. GSH plays a significant role in the cellular antioxidant protection and adjustment processes of the metabolic pathways, GST reduced the levels of xenobiotic substances present the aquatic system and GPx catalyzes the reduction of H_2O_2 along with protection against lipid peroxidation[3]. Together they all constitute a strong line of defence against oxidative stress induced by such type of xenobiotic pollutants. In the present study, GRd were found to be stimulated after SDS treatment, suggesting that the SDS-induced accumulation of ROS has interfered with the antioxidant enzymes and they tried to neutralize the effect of increased ROS generation in the brain of exposed fish, specially the balance between reduced and non-reduced glutathione level. There was a negative correlation was observed between the antioxidant enzymes and MDA (Figure 1, Table 3) indicating SDS toxicity over antioxidative enzymes. This indicated higher level of ROS generation which can be prevented by higher consumption rate. AchE significantly modulates the synaptic transmission at cholinergic synapses by controlling the activity of acetylcholine, whereas MAO also plays a vital role in the metabolism of different neurotransmitters[20]. The AchE and MAO functional detoriation may be due to altered affinity for free-SH groups and consequential inhibition of their function[19]. NO plays an important role in neuronal cell signalling, protection and regulatory effects in different cellular and physiological conditions[21]. Therefore, faulty NO production can be a indicator of developmental neurotoxicity. So, just like as in the old studies[22], SDS toxicity is found with down regulated NO production and uplifted ROS generation which is also documented as causative agent of neurodegenerative disorders[23]. Our study supports the facts that SDS exposure may lead to neuronal functional damage in catfish *H. fossilis* with increased oxidative stress. The inhibition of Na^+-K^+-ATPase activity produces membrane depolarization, leading to the suppression of neuronal and excitatory transmission. Na^+-K^+-ATPase activity shows a moderate negative correlation with MDA level but a strong positive correlation with the neurological parameters was observed (Figure 1, Table 3). The possible reasons may be related to the special physiological characteristics in fish brain. Brain tissue, as the centre of the nervous system in the vertebrate system, contains large amounts of polyunsaturated fatty acids, which are particularly vulnerable to free radical attacks. Earlier, it was reported that tributyltin exposure in kelpfish or SDS exposure in carp caused brain damage, associated with increasing of ROS stress and decreasing of Na^+/K^+-ATPase activity[17,24]. Similarly, here, the SDS-induced inhibition of Na^+-K^+-ATPase after long-term exposure most likely disturbed the Na-K pump, which might be responsible for the SDS neurotoxicity.

Administration of *Mucuna* seed extract for 5 days to SDS-treated fish resulted in a significant ($P<0.05$) decrease in MDA levels, and increase in activities of SOD, CAT, GSH and GRd activities and neurological parameters regulating the brain function compared to those in catfish which were exposed to SDS for 30 days but not to *Mucuna* extract. GPX and GST was found to be unable to restore their activities even after 7 days of *Mucuna* treatment. Possibly the damage to these two enzymes was more and needed more times to recover. Current study on the catfish also indicated that response

Table 1: Effect of *M. pruriens* seed extract on antioxidant parameters in the SDS treated *H. fossilis.*

Indices	Test Groups						
	Control	S1	S2	M1	M3	M5	M7
SOD (U/mg protein)	16.5±0.6^{a}	12.66±0.55^{b}	12.73±0.32^{b}	12.76±0.51^{b}	12.46±0.20^{b}	16.36±0.25^{a}	17.3±0.2^{a}
CAT (U/mg protein)	16.73±0.65^{a}	12.93±0.32^{bc}	12.83±0.40^{bc}	12.46±0.32^{c}	13.63±0.5^{b}	16.73±0.2^{a}	17.4±0.36^{a}
GPx (U/mg protein)	15.03±0.6^{a}	12.6±0.3^{b}	12.36±0.37^{b}	12.53±0.15^{b}	12.8±0.26^{b}	12.3±0.4^{b}	12.76±0.41^{b}
GST (U/mg protein)	25.6±1.1^{a}	22.83±0.87^{b}	19.86±0.75^{cd}	18.96±0.66^{d}	20.83±0.94^{bcd}	20.93±0.25^{bcd}	21.6±1.15^{bc}
GRd (U/mg protein)	9.03±0.40^{b}	12.56±0.72^{a}	12.56±0.35^{a}	12.6±0.85^{a}	11.76±0.47^{a}	10.03±0.5^{b}	9.06±0.25^{b}
GSH (nmol/mg protein)	50.8±0.65^{a}	45.8±0.7^{b}	44.3±1.15^{b}	44.16±0.5^{b}	45.73±1.36^{b}	51.03±0.56^{a}	52.1±0.55^{a}
MDA (U/mg protein)	7.3±0.2^{c}	10.3±0.4^{a}	10.6±0.2^{a}	10.8±0.55^{a}	10.26±0.2^{a}	9.1±0.2^{b}	8.4±0.1^{b}

Notes: SOD - Super Oxide Dismutase, CAT - Catalase, GPx - Glutathione Peroxidase, GST - Glutathione S-transferase, GR - Glutathione reductase, GSH - Reduced Glutathione (nmol/mg protein), MDA -Malondialdehyde; S1 and S2 - 15 and 30 days of SDS treatment; M1, M3, M5 and M7 - Days (1, 3, 5 and 7) of observation after *Mucuna sp.* seed extract treatment. Data are means ± SD; (n = 6). Different alphabets within same row indicate significant variation in mean value.

Table 2: Effect of *M. Pruriens* seed extract on neurological enzyme parameters in the SDS treated *H. fossilis.*

Indices	Test groups						
	Control	S1	S2	M1	M3	M5	M7
MAO(U/mg protein)	13.46±0.07^{a}	10.23±0.12^{c}	10.10±0.18^{c}	10.11±0.05^{c}	10.25±0.07^{c}	12.88±0.05^{b}	13.23±0.3^{ab}
NO(U/mg protein)	1.71±0.07^{a}	1.16±0.03^{c}	1.12±0.03^{c}	1.10±0.07^{c}	1.37±0.04^{b}	1.69±0.04^{a}	1.7±0.01^{a}
AchE(U/mg protein)	6.84±0.07^{a}	4.96±0.05^{c}	5.04±0.09^{c}	5.01±0.08^{c}	5.6±0.07^{b}	6.8±0.08^{a}	6.92±0.1^{a}
Na^{+}-K^{+}ATPase (U/mg protein)	1.71±0.05^{a}	1.08±0.03^{b}	1.05±0.04^{b}	1.08±0.04^{b}	1.12±0.08^{b}	1.7±0.03^{a}	1.76±0.05^{a}

Notes- MAO - Monoamine oxidase, NO - Nitric oxide, AchE- Acetylcholin esterase. The abbreviations used for representing different fish groups are the same as in Table 1. Data are means ± SD; (n = 6). Different alphabets within same row indicate significant variation in mean value.

Table 3: Principal component loadings of PCA with neurological and antioxidant parameters in SDS-treated *H. fossilis.*

	Component	
Parameters	1	2
MAO	.987	-.074
NO	.964	-.121
AchE	.973	-.165
MDA	-.950	-.219
SOD	.954	-.210
CAT	.973	-.150
GPx	.544	.785
GST	.613	.715
GRd	-.957	.053
GSH	.961	-.171
$N^{+}K^{+}$ATPase	.976	-.166

mechanism for different fishes after similar exposure might be different as we observed in our previous studies in the carp[7]. These observations indicate that with its antioxidant property, the plant extract may play a crucial role in maintaining the neurological parameters in the brain and protect the tissue from possible neurotoxic damage. The antioxidant and neuroprotective activity of the extracts may be attributed to the presence of specific phytochemicals such as flavonoids, terpenoids and tannins[25].

CONCLUSION

Detergents which end up in the aquatic environment through indiscriminate use, careless handling, accidental spillage or discharge of untreated effluents into natural water-ways have harmful effects on the fish population and other forms of aquatic life and ultimately humans who make use of the water for various purposes such as drinking, cooking, bathing, washing, etc. The results from this study with respect to manifestation times, overturning times and survival times show that the studied detergents are toxic to fish. Even at the lowest concentration, it could

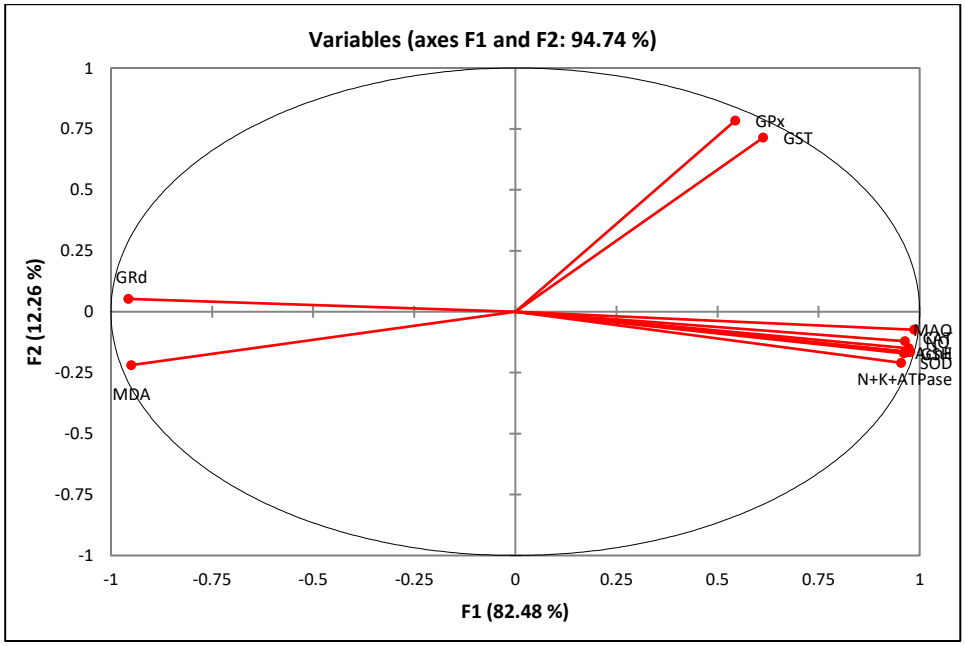

Figure 1: Ordination diagram of PCA of SDS concentrations and all the enzymatic and non-enzymatic parameters measured in the catfish brain after chronic exposure to SDS.

have a significant toxic effect on catfishes and other aquatic lives. For these detergents to have significant toxic effects even at low concentrations, it means that utmost care must be taken in using them in order not to exceed the threshold levels specification and safe limits for effluents discharged into all categories of water bodies. With exposure in aquatic medium, SDS is definitely capable to induce oxidative stress in brain by inhibition of antioxidative enzymes along with functional damage to the enzymes critical for neuronal physiology. *Mucuna* seed extract treatment has showed significant antioxidative and neuronal protective functionality. Thus, the plant might act as a possible natural source of antioxidants and could be useful in therapy of free radical pathologies. However, further study will be necessary to correlate this observation with other aquatic organisms and higher vertebrates, and for clarifying the extent of pharmacological activity of *M. pruriens*.

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DECLARATION OF INTEREST

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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