

Lead nitrate induced oxidative dysfunctions in *Cyperinus carpio*: Role of *Typha latifolia*Irfan Ullah¹, *Munawar Saleem Ahmad¹, * Rahmat Ali Khan²¹ Department of Zoology, Hazara University Mansehra, KPK, Pakistan² Department of Biotechnology, University of Science and Technology Bannu, KPK, Pakistan
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Abstract: *Typha latifolia* is traditionally used for the treatment of cancer, heart problems and skin diseases in Pakistan. The present project was therefore arranged to study the protective effects of methanol extracts of *Typha latifolia* (TLME) ethno pharmacologically against lead nitrate induced hepatic common carp. To study the protective effects of TLME, 40 *Cyperinus carpio* were equally divided into 4 groups (10 rats). Group 1 was given normal diet (control). Group II was treated with lead nitrate (100 ppm) alone while Group III and IV were given 250 ppm and 500 ppm of TLME (for a week) along with lead nitrate (100 ppm). After a round of the clock of the last treatment fish samples were weighed, anesthetized with tricaine methane sulphonate (0.2 g/L of distilled water) and the peripheral blood on mid-ventral line was collected. After that fish were euthanized by anesthetizing overdose. Operculum and peritoneal cavity were dissected and liver were collected. Liver was placed in -20 °C freezers for further enzymatic analysis. Administration of lead nitrate altered the level antioxidant enzymes viz; Catalase (CAT), peroxidase (POD), reduced glutathione assay (GSH), lipid per oxidation (TBARS), super oxide dismutase (SOD), glutathione-S-transferase (GST), glutathione reductase (GR) and glutathione peroxidase (GPX). Supplement of TLME ameliorated the effects of lead nitrate improved antioxidant enzymes and lipid per oxidation contents (TBARS). The results revealed that TLME effectively protect liver against the lead nitrate induced oxidative damage in rats, through antioxidant and free radical scavenging effects.

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Key words: Lead nitrate; Liver; Antioxidant; Lipid peroxidation; Common carp

1. Introduction

Hepatotoxicity is considered the most widespread pathology worldwide, representing up to 83% of all cases. Hepatitis, viral infection, food additives, alcohol, toxins, toxic industrial chemicals, air and water pollutants are the major risk factors of liver toxicity (Jemal et al., 2007). Lead Nitrate (Pb) is one of the most toxic heavy metals which pollute the aquatic environment. Its concentration is getting high due to its rapid use in industries. In fishes the accumulation of lead occurs in different body organs such as liver, kidney, gills, spleen and muscles and causes toxic effects (Cicik et al., 2004). Due to the delicate structure of gills, lead damages it (Adewole et al., 2007). It has been studied that lead nitrate causes blood cell injury in fishes (Ellis, 2003), gastrointestinal problems and anemia (Rogers et al., 2009), neurological disorders upon long time exposure on fish. It causes hyperglycemia, impaired hearing behavior, darkening of caudal region and eventual spinal curvatures (Hodson et al., 2010). *Typha latifolia* L. is a monocotyledonous flowering plant belongs to family Typhaceae, which is commonly known as bulrush or reedmace in britin English and Della in in local Pashto. It is among the first wetland plants which make colonies of newly exposed wet mud, with its abundant wind dispersed

seeds (Valko et al., 2004). *Typha latifolia* is traditionally used for the treatment of diarrhea, whooping cough, abdominal pain, dysuria and vaginitis (Yeo, 2004). The present project was therefore arranged to study the protective effects of methanol extracts of *Typha latifolia* (TLME) ethno pharmacologically against lead nitrate induced hepatic common carp.

2. Materials and Methods**Plant collection and Preparation of crude extract**

Typha latifolia at maturity was collected from District Bannu (Pakistan). Plants were identified and a specimen was submitted at Herbarium of Pakistan, University of Science and Technology Bannu, KPK, Pakistan. Aerial parts of plant were shade dried at room temperature, chopped, grinded mechanically of mesh size 1 mm. 1 kg powder of *Typha latifolia* was extracted in 2L methanol to get crude methanolic extract, evaporated under reduced pressure in rotary evaporator, dried and stored at 4°C for *in vivo* studies.

Animals and Treatment

To study the protective effects of TLME, 40 *Cyperinus carpio* were equally divided into 4 groups (10 rats). Group 1 was given normal diet (control). Group II was treated with lead nitrate (100 ppm) alone while Group III and IV were given 250 ppm and 500

ppm of TLME (for a week) along with lead nitrate (100 ppm). After a round of the clock of the last treatment fish samples were weighed, anesthetized with tricaine methane sulphonate (0.2 g/L of distilled water) and the peripheral blood on mid-ventral line was collected. After that fish were euthanized by anesthetizing overdose. Operculum and peritoneal cavity were dissected and liver were collected. Liver was placed in -20 °C freezers for further enzymatic analysis. The study protocol was approved by Ethical committee of University of Science and Technology Bannu for laboratory animal feed and care.

Assessment of antioxidant status

50 mg of liver tissue was homogenized in 10 volume of 100 mmol KH_2PO_4 buffer containing 1 mmol EDTA (pH 7.4) and centrifuged at $12,000 \times g$ for 30 min at 4°C. The supernatant was collected and used for determination of antioxidant status.

Catalase assay (CAT)

CAT activities were determined by the method of Chance and Maehly (Chance B, Maehly, 1955) with some modifications. The reaction solution of CAT activities contained: 2.5 ml of 50 mmol phosphate buffer (pH 5.0), 0.4 ml of 5.9 mmol H_2O_2 and 0.1 ml enzyme extract. Changes in absorbance of the reaction solution at 240 nm were determined after one min. One unit of CAT activity was defined as an absorbance change of 0.01 as units/min.

Superoxide dismutase assay (SOD)

SOD activity of liver tissues was estimated by the method of Kakkar et al., (1984). Reaction mixture of this method contained: 0.1 ml of phenazine methosulphate (186 μmol), 1.2 ml of sodium pyrophosphate buffer (0.052 mmol; pH 7.0), 0.3 ml of supernatant after centrifugation ($1500 \times g$ for 10 min followed by $10000 \times g$ for 15 min) of lung homogenate was added to the reaction mixture. Enzyme reaction was initiated by adding 0.2 ml of NADH (780 μmol) and stopped after 1 min by adding 1 ml of glacial acetic acid. Amount of chromogen formed was measured by recording color intensity at 560 nm. Results are expressed in units/mg protein.

Glutathione-S-transferase assay (GST)

Glutathione-S-transferase activity was assayed by the method of Habig et al., (1974). The reaction mixture consisted of 1.475 ml phosphate buffer (0.1 mol, pH 6.5), 0.2 ml reduced glutathione (1 mmol), 0.025 ml (CDNB; 1 mmol) and 0.3 ml of tissue homogenate in a total volume of 2.0 ml. The changes in the absorbance were recorded at 340 nm and enzymes activity was calculated as nmol CDNB conjugate formed/min/mg protein using a molar extinction coefficient of $9.6 \times 10^3 \text{ M}^{-1}\text{cm}^{-1}$.

Glutathione reductase assay (GSR)

Glutathione reductase activity was determined by method of Carlberg and Mannervik (1975). The

reaction mixture consisted of 1.65 ml phosphate buffer: (0.1 mol; pH 7.6), 0.1 ml EDTA (0.5 mmol), 0.05 ml oxidized glutathione (1 mmol), 0.1 ml NADPH (0.1 mmol) and 0.1 ml of homogenate in a total volume of 2 ml. Enzyme activity was quantitated at 25°C by measuring disappearance of NADPH at 340 nm and was calculated as nmol NADPH oxidized/min/mg protein using molar extinction coefficient of $6.22 \times 10^3 \text{ M}^{-1}\text{cm}^{-1}$.

Glutathione peroxidase assay (GSH-px)

Glutathione peroxidase activity was assayed by the method of Mohandas et al. (1984). The reaction mixture consisted of 1.49 ml phosphate buffer (0.1 mol; pH 7.4), 0.1 ml EDTA (1 mmol), 0.1 ml sodium azide (1 mmol), 0.05 ml glutathione reductase (1 IU/ml), 0.05 ml GSH (1 mmol), 0.1 ml NADPH (0.2 mmol), 0.01 ml H_2O_2 (0.25 mmol) and 0.1 ml of homogenate in a total volume of 2 ml. The disappearance of NADPH at 340 nm was recorded at 25°C. Enzyme activity was calculated as nmol NADPH oxidized/min/mg protein using molar extinction coefficient of $6.22 \times 10^3 \text{ M}^{-1}\text{cm}^{-1}$.

Reduced glutathione assay (GSH)

Reduced glutathione was estimated by the method of Jollow et al., (1974). 1.0 ml sample of homogenate was precipitated with 1.0 ml of (4%) sulfosalicylic acid. The samples were kept at 4°C for 1 h and then centrifuged at $1200 \times g$ for 20 min at 4°C. The total volume of 3.0 ml assay mixture contained: 0.1 ml filtered aliquot, 2.7 ml phosphate buffer (0.1 mol; pH 7.4) and 0.2 ml DTNB (100 mmol). The yellow color developed was read immediately at 412 nm on a SmartSpec™ plus Spectrophotometer. It was expressed as $\mu\text{mol GSH/g tissue}$.

Estimation of lipid peroxidation assay (TBARS)

The assay for lipid peroxidation was carried out following by the method of Iqbal et al. (1996). The reaction mixture in a total volume of 1.0 ml contained: 0.58 ml phosphate buffer (0.1 mol; pH 7.4), 0.2 ml homogenate sample, 0.2 ml ascorbic acid (100 mmol), and 0.02 ml ferric chloride (100 mmol). The reaction mixture was incubated at 37°C in a shaking water bath for 1 h. The reaction was stopped by addition of 1.0 ml 10% trichloroacetic acid. Following addition of 1.0 ml 0.67% thiobarbituric acid, all the tubes were placed in boiling water bath for 20 min and then shifted to crushed ice-bath before centrifuging at $2500 \times g$ for 10 min. The amount of malonaldehyde formed in each of the samples was assessed by measuring optical density of the supernatant at 535 nm using spectrophotometer against a reagent blank. The results were expressed as nmol TBARS/min/mg tissue protein at 37°C using molar extinction coefficient of $1.56 \times 10^5 \text{ M}^{-1}\text{cm}^{-1}$.

Statistical analysis

To determine the treatment effects one way

analysis of variance was carried by computer software SPSS 13.0. Level of significance among the various treatments was determined by LSD at 0.05% level of probability.

3. Results

Effect of TLME on liver tissue homogenate CAT, POD and SOD

Changes in antioxidant enzymes viz; CAT, POD and SOD are shown in Table 1. Administration of Pb (NO₃)₂ significantly ($P < 0.01$) increased the amount of protein while decreased the activities of antioxidant enzymes like CAT, POD and SOD as compared to control group. Co-administration of TLME significantly erased the toxicity of Pb (NO₃)₂ in hepatic homogenate in a dose dependent way towards the normal level.

Table 1. Effect of TLME on tissue homogenate protein and antioxidant enzymes

	Protein			SOD
	(µg/mg	CAT	POD	(U/mg
Sample	tissue)	(U/min)	(U/min)	protein)
Control	6.8±0.9++	1.6±0.3++	0.8±0.1++	2.2±0.4++
100 ppm Pb (NO ₃) ₂	4.7±0.7**	0.8±0.2**	0.6±0.5**	1.6±0.3**
250 ppm TLME+100 ppm Pb(NO ₃) ₂	6.6±0.9+	1.2±0.5++	0.7±0.2+	2.3±0.2++
500 ppm TLME+100 ppm Pb(NO ₃) ₂	6.6±0.8++	1.5±0.1++	1.3±0.1++	2.4±0.5++

Mean ±SE (n=6 number)

** indicate significance from the control group at $P < 0.01$ probability level

++ indicate significance from the lead nitrate group at $P < 0.01$ probability level

Effect of fractions on GSH-Px, GSR, GST activity

Changes in different enzyme activity levels viz; GSH-Px, GSR and GST are shown in Table 2. Pb (NO₃)₂ -treatment to fishes significantly ($P < 0.01$) reduced the activity of GST, GSR and GSH-Px as

compared to the control group. Treatments of Pb (NO₃)₂ -intoxicated common carps with TLME markedly increased the levels of GSH-Px, GSR and GST in a dose-dependent manner.

Table 2. Effect of TLME on GST, GR and GPx

Sample	GST (nM/min/mg protein)	GSR (nM/min/mg protein)	GSH-Px(nM/min/mg protein)
Control	99.5±7.0++	84.7±5.5++	66.05±5.4
100 ppm Pb (NO ₃) ₂	43.8±5.2**	75.0±4.7**	54.75±8.0
250 ppm TLME+100 ppm Pb	66.7±6.0++	76.8±6.4	55.47±8.4
500 ppm TLME+100 ppm Pb(NO ₃) ₂	110.4±4.1++	113.7±5.0++	60.87±7.0

Mean ±SE (n=6 number)

** indicate significance from the control group at $P < 0.01$ probability level

++ indicate significance from the lead nitrate group at $P < 0.01$ probability level

Effect of TLME on GSH and TBARS

Table 3. Effect of TLME on GSH and TBARS

Sample	GSH (M/g tissue)	TBARS (nM/min/mg protein)
Control	1.10±0.04++	0.5±0.03++
100 ppm Pb (NO ₃) ₂	0.08±0.01**	0.2±0.02**
250 ppm TLME +100 ppm Pb	1.09±0.02++	0.2±0.04
500 ppm TLME +100 ppm Pb(NO ₃) ₂	1.25±0.03++	0.4±0.01++

Mean ±SE (n=6 number)

** indicate significance from the control group at $P < 0.01$ probability level

++ indicate significance from the lead nitrate group at $P < 0.01$ probability level

Treatment of Pb (NO₃)₂ significantly decreased ($P < 0.01$) the GSH contents while increased ($P < 0.01$) the TBARS contents as compared to the control group (Table 3). Co-treatment of fishes treated with Pb (NO₃)₂ with TLME showed the ameliorating effects significantly ($P < 0.01$) on the TBARS and GSH contents towards the normal levels.

4. Discussion

Reactive oxygen species (ROS) are extremely reactive molecules resulting from the metabolism of oxygen causes degenerative disorders and cancer (Jia et al., 2002; Khan et al., 2011). Pb (NO₃)₂ is metabolized by cytochrome P450 into free radicals leading to accumulation of lipid peroxidation products that causes hepatic injuries (Fahmy et al., 2009). Medicinal plant composed of different amounts of antioxidants which play main role in controlling of various pathological condition including oxidative stress, cancer, cardiovascular diseases, liver diseases and lipids peroxidation (Martin et al., 1993; Sai et al., 1994; Van Gijssel et al., 1997; Aleynick et al., 1997; Finkel and Holbrook, 2000). Free radicals cause the peroxidation of the polyenoic lipids of the endoplasmic reticulum and generated secondary free radicals derived from these lipids. This destructive lipid peroxidation leads to breakdown of membrane structure and function (Singh et al., 1993; Al-Shabanah et al., 2000; Shenoy et al., 2001). Pb (NO₃)₂ -treatment significantly decreased the activities of antioxidant enzymes viz; CAT, POD, SOD, GSHpx, GSR and GST as well as lipid peroxidation. Co-administration of TLME markedly erased the toxicity of Pb (NO₃)₂ and the activities of antioxidant enzymes towards the normal range in this experiment. Similar result has been documented in various studies (Lin et al., 2007; Sreelatha et al., 2009; Khan et al., 2010). Pb (NO₃)₂ induces lipid peroxidation and increased the TBARS contents in liver cells. TBARS is a major reactive aldehyde resulting during the peroxidation of polyunsaturated fatty acids (PUFA) a useful indicator showing tissue damages including a series of chain reactions (Sahreem et al., 2011; Khan, 2012). Administration of TLME significantly recovered the TBARS contents near to control rats as was revealed by other plant extracts (Ohkawa et al., 1979; Manna et al., 2006; Khan, 2012).

Conclusion

TLME regulated the activities of antioxidant enzymes. The exact mechanism of TLME action has not fully elucidated. Although its action and efficacy seems to be its effectiveness is due to the presence of bioactive constituents.

Competing Interest

The authors declare that they have no competing interests.

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