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Antioxidant property of *Spirulina* and L*iv-52* against lead induced toxicity in albino rats

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Abstract

Objective: The present study was carried out to evaluate the antioxidant property of *spirulina* and L*iv-52* against lead induced toxicity in albino rats. Materials and methods: The antioxidant property of *spirulina* and L*iv-52* was investigated by using lead acetate to induce toxicity in albino rats. The extent of lipid peroxidaiton in terms of thiobarbituric acid reactive susbstances (TBARS), superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and reduced glutathine (GSH) were assayed in the liver and kidney homogenate. Results: Oral administration of lead (10mg/kg body weight/day) as lead acetate for 30 days resulted in a significant increase ($P<0.01$) in the level thiobarbituric acid reactive substances (TBARS) and a decrease in the level of glutathione (GSH), superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) when compared to normal control. Administration of both *spirulina* and L*iv-52* produced a pronounced protective effect ($P< 0.01$) in respect to these parameters when compared to their individual administration in lead intoxicated rats. Conclusion: The results of the present study suggested the antioxidant and protective efficacy of *spirulina* and L*iv-52* against lead induced toxicity in albino rats.

Key words: Thiobarbituric acid reactive substances (TBARS), Lead intoxicated rats, Protective efficacy.

1.Introduction

Lead is an ubiquitous pollutant in the global ecosystem because of its natural occurance and industrial use. It is one of the most common environmental pollutants known to cause poisoning. Lead is not known to have any necessary biological functions in the body and its presence in the organism has always been considered as a sign of environmental pollution [1].

Biological compounds with antioxidant properties contribute to the protection of cells and tissues against deleterious effects of reactive oxygen species and other free radicals. Protective agents from plant origin with antiperoxidative and antioxidant properties play an important role in protecting the liver against toxicity [2]. Traditional medicines are effective in certain disorders and are based on experience in the use of plant products in amelioration of common diseases. L*iv 52*, an ayurvedic multiherbal formulation is widely used in various hepatic disorders [3,4]. *Spirulina* is a microscopic, multicellular filamentous blue green algae (cyanobacterium). It is also known to have a protective role against toxic effects of various chemicals [5].

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However, the antioxidant and protective effect of *spirulina* and L*iv-52* against lead induced toxicity in respect to antioxidant status in tissues remains unexplored. Therefore, the present study was undertaken to evaluate antioxidant and protective role against lead induced toxicity in rats.

2. Materials & Methods

2.1.Chemicals

Lead acetate, thiobarbituric acid (TBA), nitroblue tetrazolium (NBT), reduced glutathione (GSH), 5,5'- dithio-2-nitrobenzoic acid (DTNB) were purchased from Sigma chemical Co, (St, Louis, MO, USA). Other chemicals used were of analytical grade.

*2.2. L*iv-52 *and* Spirulina *samples*

L*iv-52* tablets (500 mg each) obtained commercially from Himalaya Drug Co.Bangalore, India. Each L*iv–52* tablet is composed of *Capparis spinosa* (65mg), *Cichorium intybus* (65 mg), *Solanum nigrum* (32mg), *Cassia occidentalis* (16mg), *Terminalia arjuna* (32mg), *Achillea millefolium* (16mg), *Tamarix gallica* (16mg). *Spirulina* tablets (100mg each) were obtained commercially from Parrys Neutraceuticals Ltd, Chennai, India.

2.3 Animals

Male albino rats (Wistar strain) weighing 150-175g were obtained from animal breeding centre, P.S.G. Institute of Medical sciences & Research, Coimbatore, Tamilnadu, India. They were housed in KMCH College of Pharmacy, Coimbatore, Tamilnadu, India, in controlled temperature (27±2°C), humidity (55±10%) and light. Animals were fed with standard pellet (Hindustan lever Ltd, India). They were given a week time to get acclimatized with laboratory condition.

2.4 Treatment

After acclimatization the animals were divided into the following groups of six rats each.

Group A : Normal control

Group B : Rats were given lead (10 mg/kg body weight/day) as lead acetate orally for 30 days.

Group C : Rats were treated with *spirulina* (500 mg/kg body weight/day) orally for 30 days.

Group D : Rats were treated with lead acetate (10 mg/kg body weight/day) and *spirulina* (500 mg/ kg body weight) orally for 30 days.

Group E : Rats were given L*iv-52* (500 mg/ kg body weight /day) orally for 30 days

Group F : Rats were treated with lead acetate (10 mg/kg body weight/day) and L*iv 52* (500 mg/kg body weight /day) orally for 30 days.

Group G : Rats were given L*iv-52* (500 mg/kg body weight/day) and *spirulina* (500 mg/kg body weight day) orally for 30 days.

Group H : Rats were treated with lead acetate (10 mg/kg body weight/day), L*iv-52* (500 mg/kg body weight/day) and *spirulina* (500 mg/kg body weight/day) orally for 30 days.

At the end of the experimental period, the rats were deprived of food overnight and sacrificed by light ether anaesthesia. Liver and kidney were removed and cleaned in normal saline. A known weight of these tissues was homogenized (10% w/v) in ice cold phosphate buffer (0.1M, pH 7.4) using potter Elvehjem teflon homogenizer. The homogenate was centrifuged at 5000g at 4°C for 30 minutes and supernatant obtained

was used for the assay of various enzymes. Liver and kidney homogenates were used for the assay of lipid peroxdation (Das *et al*., 1994). Supernatants were used for the assay of superoxide dismutase (Misra ad Fridovich, 1972), catalase (Sinha, 1972), glutathione peroxidase (Rotruck *et al*., 1973) and reduced glutathine (Moron *et al.*, 1979).

2.5 Phytochemical Analysis

Preliminary quantitative measurement of total phenols [11], flavonoids [12], vitamin C [13], vitamin E [14] and glutathione (GSH) [10] was done in *spirulina* and L*iv -52* samples.

2.6 Statistical Analysis

Statistical analysis was performed by one way analysis of variance (ANOVA). Critical difference (CD) was calculated at 1% level and results were expressed as mean ± SD of six rats in each group.Values of $P < 0.01$ were considered significant.

3.Results

Table 1 shows the level of nonenzymic antioxidants in *spirulina* and L*iv-52*. This revealed that *spirulina* and L*iv-52* are good sources of flavonoids, total phenols, vitamin C, vitamin E and reduced GSH.

Table 1. Levels of Nonenzymic antioxidants in *spirulina* and L*iv-52*

Sample	Vit C (mg/g)	Vit E(mg/g)	Red GSH (nm/g)	Total phenols (mg/g)	Flavonoids (mg/g)
L*iv-52*	1.30 ± 0.02	0.838 ± 0.04	87.30 ± 0.32	12.96 ± 0.23	6.92 ± 0.08
Spirulina	0.11 ± 0.01	0.150 ± 0.02	123.80 ± 0.11	9.65 ± 0.18	8.98 ± 0.12

Values are mean ± SEM of triplicates.

Table 2. Effect of *spirulina* and L*iv-52* on hepatic antioxidant systems in lead induced toxicity in rats.

Groups & Treatment	TBARS (nm/mg protein)	SOD U/ mg protein	CAT U/ mg protein	GPx U/ mg protein	GSH (µg/mg protein)
Normal control (A)	0.66 ± 0.03^{a}	6.99 ± 0.18^{a}	70.68 ± 1.12^{a}	12.56 ± 0.05^{a}	5.22 ± 0.06^{a}
Lead treated (B)	1.86 ± 0.05^{b}	3.95 ± 0.22^{b}	39.42 ± 0.75^{b}	8.36 ± 0.04^{d}	70.68 ± 0.04^{d}
Spirulina treated (C)	0.63 ± 0.05^{a}	7.08 ± 0.22^{a}	71.11 ± 0.98^{a}	12.61 ± 0.02^{a}	5.24 ± 0.02^{a}
Lead + *spirulina* (D)	1.03 ± 0.17^{c}	5.99 ± 0.24^{c}	65.16 ± 0.35^{c}	9.93 ± 0.02^{c}	4.55 ± 0.05^{c}
L*iv 52* treated (E)	0.61 ± 0.06^{a}	7.17 ± 0.12^{a}	71.50 ± 1.25^{a}	12.67 ± 0.12^{a}	5.27 ± 0.01^{a}
Lead + L*iv 52* (F)	0.98 ± 0.03^{c}	6.11 ± 0.28^{c}	65.3 ± 1.18^{c}	10.10 ± 0.10^{c}	4.63 ± 0.02^{c}
Spirulina + L*iv 52* (G)	0.59 ± 0.10^{a}	7.22 ± 0.38^{a}	71.62 ± 0.89^{a}	12.73 ± 0.18^{a}	5.31 ± 0.12^{a}
Lead + *spirulina* + L*iv 52*(H)	0.79 ± 0.01^{d}	6.54 ± 0.31^{d}	67.86 ± 1.30^{d}	11.39 ± 0.12^{d}	4.87 ± 0.03^{d}
CD (1%)	0.1287	0.4308	2.105	0.6186	0.1134

Values are expressed as mean ± SD (n = 6).

Values with same superscript did not differ significantly at 1% level.

SOD = Amount of enzyme required to inhibit 50% reduction of nitroblue tetrazolium (NBT)

CAT = µM of H_2O_2 decomposed / min/ mg protein.

GPx = µg of GSH consumed / min / mg protein.

Tables 2 and 3 show the level of thiobarbuturic acid reactive substances (TBARS), superoxide dismutase (SOD), catalase (CAT), glutathione (GSH), glutathione peroxidase (GPx) in liver and kidney of different experimental groups of rats. In rats treated with lead (group B) there was a significant increase ($p<0.01$) in the level of thiobarbutric acid reactive substances (TBARS) and decrease in the level of SOD, CAT, GSH, GPx when compared to normal control (group B). Simultaneous treatment with *spirulina* (Group D) or L*iv-52* (Group F) or both *spirulina* and L*iv-52* (Group H) produced a significant decrease ($P< 0.01$) in the level of thiobarbituric acid reactive substances and increase in the level of SOD, CAT, GSH, GPx when compared to the rats treated with lead alone (Group B).

4. Discussion

A significant increase in the levels of thiobarbituric acid reactive substances (TBARS) in animals treated with lead showed the induction of lipid peroxidation by lead. This might be due to the release of free radicals and membrane damage by lead. Lead is reported to release free radicals thereby stimulating the process of lipid peroxidation. Lipid peroxides have been shown to impair tissue membranes which is a risk factor in variety of diseases [15].

The results of the present study was supported by the findings of Hsu and Guo [16], who have reported that lead induced oxidative stress contributes to the pathogenesis of lead poisoning for disrupting the delicated prooxidant or anti-oxidant balance that exist within mammalian cells.

Endogenous antioxidant enzymes (SOD and CAT) are responsible for preventing and neutralizing the free radical induced damages on tissues [17]. Lead induced decrease in SOD and CAT might be due to the formation of reactive oxygen species by lead [18]. *Spirulina* contains super-oxide dismutase (SOD) that can prevent the cell damage by free radicals [19]. The enzymes SOD and CAT constitute the first line of defense against free radical induced damage and the restoration of these enzyme activity by L*iv-52* and *spirulina* may account for their protective effect.

Table 3 : Effect of *spirulina* and L*iv-52* on renal antioxidant systems in lead induced toxicity in rats.

Groups & Treatment	**TBARS (nm/mg protein)**	**SOD U/ mg protein**	**CAT U/ mg protein**	**GPx U/ mg protein**	**GSH (µg/mg protein)**
Normal control (A)	1.14 ± 0.09^{a}	5.43 ± 0.25^{a}	61.32 ± 0.52^{a}	10.12 ± 0.02^{a}	4.71 ± 0.04^{a}
Lead treated (B)	2.26 ± 0.02^{b}	3.15 ± 0.25^{b}	35.66 ± 0.22^{b}	6.18 ± 0.03^{b}	2.73 ± 0.14^{b}
Spirulina treated (C)	1.12 ± 0.12^{a}	5.49 ± 0.32^{a}	61.94 ± 0.74^{a}	10.17 ± 0.12^{a}	4.75 ± 0.12^{a}
Lead + *spirulina* (D)	1.48 ± 0.14^{c}	4.28 ± 0.31^{c}	57.07 ± 0.30^{c}	7.82 ± 0.02^{c}	3.83 ± 0.10^{c}
L*iv 52* treated (E)	1.09 ± 0.02^{a}	5.56 ± 0.30^{a}	62.27 ± 0.95^{a}	10.20 ± 0.15^{a}	4.81 ± 0.06^{a}
Lead + L*iv 52* (F)	1.41 ± 0.03^{c}	4.52 ± 0.34^{c}	55.01 ± 0.70^{c}	7.95 ± 0.11^{c}	3.91 ± 0.01^{c}
Spirulina + L*iv 52* (G)	1.04 ± 0.04^{a}	5.61 ± 0.10^{a}	62.83 ± 0.35^{a}	10.25 ± 0.2^{a}	4.85 ± 0.02^{a}
Lead+*spirulina*+L*iv 52*(H)	1.26 ± 0.08^{d}	4.96 ± 0.18^{d}	58.27 ± 0.26^{d}	9.37 ± 0.04^{d}	4.32 ± 0.03^{d}
CD (1%)	0.1140	0.3431	2.827	0.6420	0.1631

Values are expressed as mean ± SD (n = 6).
Values with same superscript did not differ significantly at 1% level.
SOD = Amount of enzyme required to inhibit 50% reduction of nitroblue tetrazolium (NBT).
CAT = µM of H_2O_2 decomposed / min/ mg protein.
GPx = µg of GSH consumed / min / mg protein.

Lead induced decrease in glutathione peroxidase (GPx) activity has been reported in blood and brain. Lead decreases the level of GSH in rats which could have resulted in the reduced activity of GPx [20]. This could be probably due to either increased utilisation of GSH by the cells to act as scavengers of free radicals caused by toxic chemical agents or enhanced utilization of GSH by GPx [21] or decreased availability of selenium which leads to inefficient disposal of peroxides and results in elevated lipid peroxidation [22].

The antioxidant property of *spirulina* and L*iv-52* might also be attributed to the presence of antioxidant vitamins (Vitamin E, Vitamin C), flavonoids, phenolic compounds and reduced glutathione (GSH) in these drugs. Vitamin E and vitamin C are potent free radical scavengers and prevent oxidative damage by utilizing the free radicals [23].

Vitamin E is a major lipid soluble antioxidant within the cell membrane where it protects membrane fatty acids from lipid peroixdation [24]. It acts in conjugation with ascorbate and reduced glutathione (GSH). Once the tocopheroxyl radical is formed it migrates to the membrane surface and is reconverted to α - tocopherol by reaction with either ascorbate or GSH. The resulting ascorbate radical can regenerate ascorbate by reaction with GSH [25].

Flavonoids and phenolic compounds have long been recognized as excellent scavengers of superoxide, hydroxyl ion and peroxyl radicals and as potent inhibitors of lipid peroxidation [26]. Suja *et al.*, [27] reported that administration of L*iv 52* reduced the peroxidative effects of hydrogen peroxide and inhibited the deleterious effects of lipid peroxidation by enhanced supply of reduced GSH.

Spirulina is reported to have free radical scavenging property which inhibited microsomal lipid perodation [28]. GSH is a major cellular antioxidant that protects protein thiols and inhibits cellular damage due to oxygen free radicals. It participates directly in the deactivation of hydrogen peroxide and also promotes the formation of reduced forms of ascorbate [29]. The results of the present clearly manifested the antioxidant and protective efficacy of *spirulina* and L*iv-52* against lead induced toxicity in albino rats.

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