RESEARCH ARTICLE

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DL-α-Lipoic Acid Attenuates Acute Aniline Induced Splenic Toxicity in Rats: A Biochemical and Histoarchitecture Study

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ABSTRACT

Introduction: Present study was designed to evaluate the protective effects of DL-α-Lipoic acid in aniline induced spleen toxicity in rats.

Material and Methods: Splenic toxicity was induced in rats by administration of aniline (30mg/kg/p.o) for a period of 7 days. Treatment groups received DL-α-Lipoic acid (40 mg/kg/day, p.o) for 7 days after aniline administration for 7 days i.e the study protocol was for 14 days. At the end of treatment period various serum and tissue parameters were evaluated. Rats administered with Aniline showed a significant alteration in general parameters such as organ weight, body weight, water intake, feed consumption and faecal matter content. It also alters hematological parameters (RBC, WBC and Hemoglobin content), biochemical parameters (total iron content, lipid peroxidation, reduced glutathione and nitric oxide content) and histopathology of spleen. **Results:** Treatment with DL-α-Lipoic acid for 7 days post aniline administeration showed significant recovery in aniline induced splenic toxicity. **Conclusion:** The present result showed that involvement of oxidative and nitrasative stress in aniline induced splenic toxicity and DL-α-Lipoic acid protects the rats from the toxicity which might be due to its strong antioxidant property.

Key words: aniline; spleen toxicity; DL-α-Lipoic acid, antioxidants.

INTRODUCTION

Aniline is widely used in manufacturing of various products such as a toxic aromatic amine, widely used industrial chemical, particularly in the perfumes, pigments, varnishes, dyes and resins. Exposure to aniline is reported to produce spleen toxicity. Other clinical symptom of aniline toxicity includes cyanosis, weakness, dizziness, headache, stupor, loss of co-ordination and coma (within 1 to 3 hrs) after ingestion [1]. The main effect of short term exposure to aniline is the formation of methaemoglobin and Heinz body by the action of the metabolites phenylhydroxylamine and aminophenol [2].

DL-α-lipoic acid is a disulfide compound that is a cofactor in vital energy production reaction in the body. DL-α-lipoic acid has solubility in both aqueous and fat region of the body hence it is often termed as "Universal antioxidant" [3]. It possesses wide range of pharmacological activities such as antidiabetic, metal chelation, anti atherosclerotic and nephroprotective [4-7] One of the mechanisms of Aniline toxicity is the production of free radicals and lipoic acid acts as potent antioxidant. Based on the above information present study was design to screen liopic acid in aniline induced spleen toxicity by assessing some biochemical parameters and histoarchitecture of spleen.

MATERIAL AND METHODS:

Drugs and Chemicals

Aniline, DL--α-lipoic acid, 2, 2 dipyridyl, 5, 5 dithiobis (2-nitrobenzoic acid) and N-(1-Napthyl) ethylenediamine dihydrochloride were purchased from HiMedia lab. Pvt. Ltd. Mumbai. All the other chemicals used in the study were of analytical grade and procured from standard supplier.

Animals

Wistar rats of either sex (200-250g) were used in the study. The animals were procured from Lachmi biotech, Pvt. Ltd, Pune. The animals were maintained under standard laboratory conditions. The experimental protocol was approved by Institutional Animal Ethic Committee (IAEC) of SSDJ College of pharmacy, Neminagar, Chandwad. (Approval No. SSDJ/IAEC/2012/026).

Experimental Protocol

The study protocol was for 14 days. Animals were divided into different groups (n=6). Group I: served as normal control and received 1% CMC, orally as vehicle. Group II: rats received Aniline (30mg/kg/p.o) for 7 days and vehicle for next 7 days. Group III: rats received Aniline (30mg/kg/p.o) for 7 days and α-lipoic acid (40mg/kg/p.o) for next 7 days. Group IV: rats received vehicle for 7 days and α-lipoic acid (40mg/kg/p.o) for next seven days.

Biochemical Evaluation

General parameters like body weight, liver and spleen weight, water intake, feed intake, faecal matter content were studied in between and at the end of study.

At the end of treatment period blood was withdrawal by retro orbital plexus and serum was separated. Blood sample was used for the estimation of hemoglobin (sahli's haemometer method), RBC and WBC count using haemocytometer [8, 9], serum

sample was used for the estimation of iron content [10], protein content, SGOT and SGPT (Span Diagnostic kit). Spleen and liver was quickly transferred to ice-cold Tris hydrochloric buffered saline (pH 7.4). The organs were cross-chopped, homogenized and centrifuged. (10,000 rpm at 0^0C for 15 minutes using). The clear supernatant was used for the determination of lipid peroxidation, reduced glutathione level and nitric oxide in spleen.

Histopathology study:

After decapitation, spleen was rapidly dissected out and washed immediately with normal saline and fixed in 10% buffered formalin. Small sections of tissues were cut and stained with haematoxyline & eosin (H&E) for general morphological evaluation. It was carried out from (Safline histology and specialty center. Sr. No.48, Katraj Kondhawa Road, Near Micro Engg. Gokhul Nagar, Pune, 411098).

Statistical analysis

All the values are presented as mean ± S.E.M. Statistical significance between more than two groups was tested using one-way ANOVA followed by the Tukey's multiple comparison test as appropriate using computer based fitting program (Prism 5). Differences were considered to be statistical significant when $p<0.05$.

RESULTS:

Effects of DL-α-LA on body weight, feed consumption, water intake, faecal matter, weight of spleen and liver.

Rats post-treated with DL-α-LA for 7 days followed by aniline (30mg/kg/p.o) upto 7 days showed decrease in feed consumption. A significant decrease in body weight, water intake and faecal matter content was observed after 7 & 14 days of DL-α-LA treatment. Weight of spleen and liver was found to be significantly increased in aniline treatment where as post-treated group showed significant improvement in the weight of spleen and liver (Table 1).

Effect of DL-α-LA on RBC, WBC and haemoglobin level.

It was found that aniline showed significant ($p<0.001$) decrease in RBC as compared to normal control rats. However the WBC count was found to be slightly changed as compare to normal control. Rats treated with DL-α-lipoic acid (40mg/kg, p.o.) significantly ($p<0.001$) showed increased in the RBC count as compare to aniline treated rats (Table: 2).

Aniline (30mg/kg/p.o) was administered via drinking water for 7days before treatment of DL-α-LA (40mg/kg/p.o). Hemoglobin level in aniline treated rats was significantly ($p<0.001$) decrease as compare to normal rats at the intervals of 7 and 14 days. At day 14 there was a significant increased in the level of hemoglobin than control rat (Figure 1).

Effect of DL-α-LA on serum total protein and total Iron content:

Rats treated with aniline (30mg/kg/p.o) upto 7 days showed significant decrease in total protein and iron content as compared to control rat after 7 days. Treatment with DL-α-LA (30mg/kg/p.o) for 7 days showed significant increased in the level of total protein and serum iron content as compared to aniline treated rats.(Figure 2 & 3)

Effect of DL-α-LA on tissue lipid peroxide, reduced glutathione and serum nitric oxide levels.

Rats treated with aniline showed significant ($p<0.001$) increased in LPO level in spleen & liver as compare to normal control rats. However the level of reduced glutathione and nitric oxide content were significantly reduced. Treatment with DL-α-LA (40mg/kg, p.o.) for 7 days after aniline treatment showed significant ($p<0.001$) decreased in LPO and a significant increased in reduced glutathione and nitric oxide content (Table 3).

DISCUSSION:

Exposure to aniline produces substantial increases in total iron content and oxidative stress in rats and thereby develops spleen toxicity. [11] Present study demonstrates the splenoprotective effects of DL-α-Lipoic acid against aniline-induced spleen toxicity. In the present study spleen toxicity was induced by administration of Aniline (30 mg/kg p.o) and it was confirmed by significant changes in hemoglobin and iron content in rats. Spleen toxicity was developed after 7 days of aniline administration and marked changes in the, body weight, feed consumption, water intake, faecal matter and blood parameters such as hemoglobin level, RBC and WBC count, bleeding time, clotting time, total iron content and total protein were observed. The above changes are in line with previous report of Khan *et.al.,* 1999 [12]. Significant decreased in body weight, feed consumption, water intake and faecal matter in aniline treated rats might be due to toxicity of aniline which decreased the food consumption which can directly co related to decreased body weight.

The changes observed in the blood parameters (RBC and WBC count) were rather expected and consistent with those of earliest reports [13]. The changes in the blood parameters were closely associated with simultaneous enlargement in the spleen size (splenomegaly) which appears to be due to excessive deposition of chemically damaged erythrocytes [12]. In the present study aniline administered rats display a significant increased in iron load and decreased in protein contents. In the present study markers of oxidative stress such as lipid peroxidation, glutathione and nitric oxide were evaluated. Aniline induced rats showed a significant increased in lipid peroxidation and nitric oxide content and decreased in GSH level in spleen and liver. The increased in the lipid peroxidation and protein oxidation are closely associated with the accumulation of iron in the spleen. Iron deposition in the spleen may result in the formation of ROS, which can react with and damaged protein, nucleic acid, and lipids, particularly the fatty acid component of membrane phospholipids leading to

the development of oxidative stress which results in lipid peroxidation [12].

Treatment with DL-α-Lipoic acid reverse the changes in body weight, feed consumption, water intake, faecal matter, and also showed the increase in hemoglobin, RBC, total protein and decrease in total iron content. The splenoprotective effects of DL-α-Lipoic acid might be due to the strong antioxidant/free radical scavenging activity and reduced iron overload capacity of [14]. The total nitric oxide, an indicator of nitrosative stress, is increased in the experimental model of aniline induced toxicity. DL-α-Lipoic acid treatment showed the attenuation of increased Lipid and nitric oxide level which might be due to its ROS inhibitory potential as well as potent free radical scavenging activity.

The biochemical changes are always supported with histolopathological alteration. The histopathological changes in the aniline treated rat spleen include vascular congestion, increased red pulp cellularity, and accumulation of damaged red blood cells. These were closely associated with dramatically increased iron deposition in the red pulp of the spleen. The increased iron deposition can lead to develop fibrotic lesions in the aniline treated rats presumably due to iron mediated production of ROS which might act as a stimulus for increased collagen production in splenic tissue leading to fibrosis [13]. Treatment with DL-α-Lipoic acid decreased the congestion of red pulp and accumulation of ruptured RBCs which might be due to scavenging ROS in mitochondrial redox cell and decreases the level of oxidative stress in spleen toxicity. In conclusion: DL-α-Lipoic acid attenuates the aniline induced spleen toxicity by virtue of its strong antioxidant activity.

Parameters	Days	Control	Aniline	Lipoic acid	ANL + LA
Body Weight	0 Day	205.5 ± 1.44	238.7 ± 0.66	233.5 ± 3.77	233.3 ± 5.69
	7 Day	210.7 ± 1.96	225.7 ± 0.17	237.5 ± 4.25	219.3 ± 5.91
	14 Day	215.8 ± 2.33	227.3 ± 0.15 * (↓11.4 g)	244.8 ± 4.92	229.8 ±5.73 (↓3.5 g)
Feed Consume (g)	0 Day	23.83 ± 0.33	28.00 ± 1.04	21.67 ± 3.11	21.5 ± 3.27
	7 Day	25.24 ± 0.71	20.51 ± 1.20	24.57 ± 3.54	14.3 ± 2.05
	14 Day	26.98 ± 0.68	19.96 ± 0.96 (↓8.04 g)	27.98 ± 3.58	25.17 ± 6.11
Water Intake (ml)	0 Day	32.83 ± 1.64	37.33 ± 0.66	31.17 ± 0.60	33.33 ± 2.96
	7 Day	32.86 ± 2.75	29.16 ± 0.82	33.23 ± 0.28	25.44 ± 1.55
	14 Day	35.51 ± 0.85	28.75 ± 0.38 ** (↓8.58 ml)	36.38 ± 0.32	31.25 ± 0.57# (↓2.08 ml)
Faecal Matter (g)	0 Day	10.50 ± 0.28	10.50 ± 0.28	7.33 ± 0.60	11.33 ± 1.16
	7 Day	9.94 ± 0.82	6.84 ± 0.41	7.77 ± 0.61	7.533 ± 1.17
	14 Day	11.23 ± 0.44	6.193± 0.24 **(↓4.30 g)	8.99 ± 0.72 ##	8.793± 0.39 ##(↓2.53 g)
Wt. of Spleen (g)	3.61 ± 0.28		7.89 ± 0.37	3.23 ± 0.32	3.84 ± 0.31
Wt. of Liver (g)	10.90 ± 0.81		11.96 ± 0.36	10.36 ± 0.79	10.98 ± 0.38

Table 1: Effect of DL-α-Lipoic acid on body weight, organ weight, water, feed intake and faecal matter content in aniline induced spleen toxicity.
Values are presented as mean ± SEM (n=6). One way ANOVA followed by Tukey's multiple comparison test. **p<0.05, **p<0.01, **p<0.001* compared to control rats *#p<0.05, ##p<0.01, ###p<0.001* compared to aniline treated group.

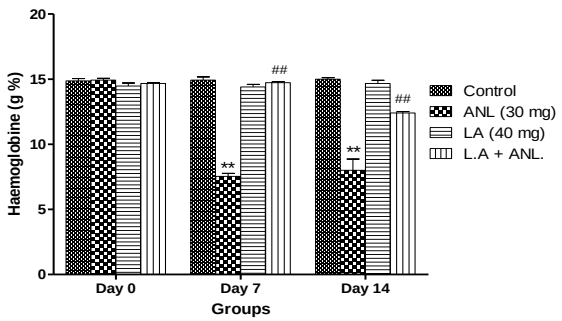

Figure 1: Effect of DL-α-Lipoic acid on haemoglobine content in aniline induced spleen toxicity.
Values are presented as mean ± SEM (n=6). One way ANOVA followed by Tukey's multiple comparison test. **p<0.05, **p<0.01, **p<0.001* compared to control rats *#p<0.05, ##p<0.01, ###p<0.001* compared to aniline treated group.

Parameters	RBC's (x10⁶/µL)			WBC's (x106/µL)		
	0 Day	7 Day	14 Day	0 Day	7 Day	14 Day
Control	10.24± 0.33	10.27± 0.27	10.45± 0.23	7.64 ± 0.13	7.58± 0.25	7.85 ± 0.06
ANL	10.43 ± 0.19	6.28±0.05 **	6.88± 0.25**	7.62 ± 0.11	8.07± 0.21	7.72 ± 0.34
LA	8.93 ± 0.12	8.90± 0.08 ##	8.91± 0.03 ##	7.61 ± 0.11	7.65± 0.13	7.76 ±0.14
ANL+ LA	9.96 ± 0.13	6.68± 0.34	8.82± 0.41 ##	7.76 ± 0.23	8.21± 0.19	7.88 ± 0.12

Table 2: Effect of DL-α-Lipoic acid on RBC and WBC content in aniline induced spleen toxicity.
Values are presented as mean ± SEM (n=6). One way ANOVA followed by Tukey's multiple comparison test. **p<0.05, **p<0.01, **p<0.001* compared to control rats *#p<0.05, ##p<0.01, ###p<0.001* compared to aniline treated group.

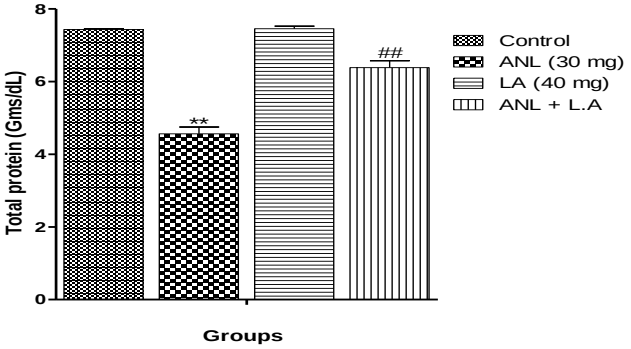

Figure 2: Effect of DL-α-Lipoic acid on total protein content in aniline induced spleen toxicity.

Values are presented as mean ± SEM (n=6). One way ANOVA followed by Tukey's multiple comparison test. **p<0.05, **p<0.01, **p<0.001* compared to control rats *#p<0.05, ##p<0.01, ###p<0.001* compared to aniline treated group.

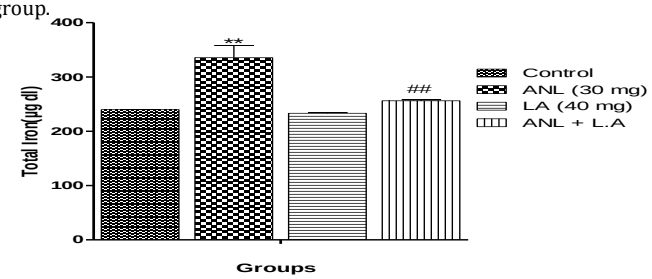

Figure 3: Effect of DL-α-Lipoic acid on total iron content in aniline induced spleen toxicity.

Values are presented as mean ± SEM (n=6). One way ANOVA followed by Tukey's multiple comparison test. **p<0.05, **p<0.01, **p<0.001* compared to control rats *#p<0.05, ##p<0.01, ###p<0.001* compared to aniline treated group.

Parameters	Groups			
	Control	ANL (30)	L.A (40)	ANL+LA
LPO (Spleen)	0.84 ± 0.00	6.68 ± 0.13**	1.00 ± 0.03	1.50 ± 0.02 ##
LPO (liver)	1.17 ± 0.02	3.10 ± 0.16 **	1.19 ± 0.00	0.88 ± 0.04 ##
GSH (Spleen)	1.58 ± 0.19	1.28 ± 0.03 **	1.32 ± 0.25	1.84 ± 0.04 ##
GSH (liver)	3.08 ± 0.31	2.17 ± 0.05*	2.88 ± 0.20	16.75 ± 0.92 ##
NO (Nitrite)	6.29 ± 0.35	79.17 ± 3.48 **	7.03 ± 0.20	30.63 ± 3.71 ##

Table 3: Effect of DL-α-Lipoic acid on LPO, GSH and NO content in aniline induced spleen toxicity.

Values are presented as mean ± SEM (n=6). One way ANOVA followed by Tukey's multiple comparison test. **p<0.05, **p<0.01, **p<0.001* compared to control rats *#p<0.05, ##p<0.01, ###p<0.001* compared to aniline treated group.

LPO (nmole of MDA/g of tissue), GSH (µg/g of tissue), NO (nmol/L)

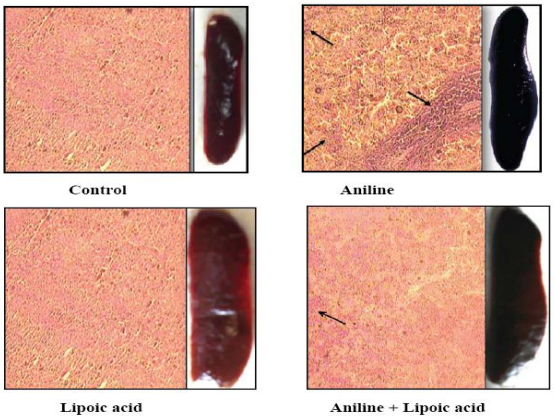

Figure. 4: Represent the histopathology of spleen stained with hematoxyline-eosin staining.

Control section shows the histopathology of spleen tissue of normal control rats showing the normal spleen red pulp. Aniline (30mg/kg/p.o): Shows the section of aniline treated animals. Section showing multiple areas of sinusoidal congestion and accumulation of red blood cells (arrow). Normal DL-α-Lipoic acid (40mg/kg/p.o) Shows section of normal treatment with DL-α-Lipoic acid (40 mg/kg/p.o) for seven day. Section showing the normal spleen red pulp and no change in the spleen architecture Aniline + Lipoic acid treatment: Shows section of post treatment of rat with aniline (30 mg/kg/p.o) for seven days then treatment of DL-α-Lipoic acid (40 mg/kg/p.o) for seven days. Section showing the decrease in sinusoidal congestion and decreased or less accumulation of ruptured red blood cell (arrow).

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