Research Article

Alpha-Lipoic Acid A Cholesterol Reducer for Targeting Acetaminophen Induced Uremic Male Albino Rat Models

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ABSTRACT

Heart disease and Diabetes possess the first and second leading cause of death in the world and people are suffering from chronic kidney disease are likely more tend to suffer from hypercholesterolemia. Alpha-lipoic acid is unsaturated fatty acids having strong anti-oxidative properties. The aim of this study was to characterize alpha-lipoic acid as a cholesterol reducer for acetaminophen induced uremic rats. The study was performed on thirty male albino rats. Plasma total cholesterol, triglyceride levels, liver enzymes assay and hematologic parameters were performed to investigate its cholesterol reducing properties. In this present study it was observed that receiving acetaminophen intraperitoneally for 10 days significantly decreased Hb level and RBC count compared with control. The present study demonstrated that cholesterol and triglycerides levels were significantly higher in acetaminophen treated group compare to control. Alpha-lipoic acid ameliorates cholesterol and triglyceride levels at high dose due to its anti-oxidative properties. The ALP and ALT were significantly higher in acetaminophen treated group compared with control group. It may be hypothesized that there was a cholesterol reducing properties of alpha-lipoic acid at higher dose.

Keywords: Acetaminophen, Alpha-lipoic acid, Hypercholesterolemia, Triglyceride, Uremia.

INTRODUCTION

Now a days, Heart disease and Diabetes possess the first and second leading cause of death in the world respectively and people are suffering from chronic kidney disease or ESRD are likely more tend to suffer from hypertension and hyperlipidemia. In this connection our research work searching to find out the different anti-uremic and nephroprotective phyto compound from different plant extract such as hydro-methanolic root extract of *Asparagus racemosus*[1], methanolic bark extract of *Terminalia arjuna*[2], methanolic root extract of *Withania somnifera*[3] which had been effective for acetaminophen induced chronic renal failure. Besides this in our laboratory, recent research work on neutraceuticals like Alpha lipoic acid[4] and probiotic[5] therapy had been shown excellence nephroprotective activity against acetaminophen induced renal failure male rats. Acetaminophen is a commonly used antipyretic agent which, in high doses, causes renal tubular damage and showed significant elevation in the levels of cholesterol, triglycerides in both serum and liver tissues. The acetaminophen induced animals showed significant alteration in the activities of lipid metabolizing enzymes like serum lecithin cholesterol acyl transferase (LCAT) and hepatic triglyceride lipase (HTGL).[6] Previous research work suggests that LA ameliorate the lipid peroxidation and the loss of cellular anti-oxidants, thereby protecting the CQ-induced oxidative damage in kidney.[7] Recent evidence established that alpha-lipoic acid co-treatment significantly inhibited the levels of lipid hydroperoxide, protein carbonyl contents and stimulated anti-oxidants enzyme activities like SOD, GPx and GST and CAT. There was increased in total anti-oxidants and anti-oxidant enzyme levels with alpha-lipoic acid treatment to lead ingesting rats could be due the anti-oxidant effects of alpha-lipoic acid[8]. The present study was designed to investigate the cholesterol reducing effect of alpha-lipoic acid on acetaminophen induced uremic rat model.

MATERIALS AND METHODS

Selection of animals and care

The study was conducted on 30 healthy, adult, male albino rats of Wister strain (Supplied from Ghosh animal, animal foods and animal cages Supplier, Kolkata 54) having a body weight of 100 ±15 g. They were acclimatized to laboratory condition for 2 weeks prior to experimentation. Animals were housed three rats/cage in a temperature-controlled room (22± 2^0C) with 12–12 h dark–light cycles (8.00–20.00 h light, 20.00– 8.00 h dark) at a humidity of 50 ±10%. They were provided with standard food and water ad libitum. Animal care was provided according to the Guiding Principle for the Care and Use of Animals.[9]

Drugs and Chemicals

Acetaminophen (paracetamol, *N*-acetyl *p*-aminophenol; APAP) was purchased from AshChemie India. It was administered intraperitoneally with saline water. Alpha-lipoic acid was purchased from Sigma-Aldorich (P) Ltd. All the chemicals used for Bio chemical tests including Urea, Creatinine, Na, K kit and Methanol, K2HPO4, KH2PO4, Pyragallol, Tris, TCA,TBA and other chemicals are collected from Merck Specialities Private Limited Worli. Mumbai, HiMedia Laboratories Pvt. Ltd. Mumbai, India and Crest Biosystems Goa, India.

Grouping of animals and experimental procedure

The rats were divided into four equal groups as follows:

Group I or control – Six animals were subjected to control group. They were housed at room temperature (25± 3°C) and feed normal diet and water ad libitum.

Group II or acetaminophen induced renal failure rats – Six animals were randomly placed in cage with normal diet and injected with acetaminophen at the conc. of 500 mg with de-ionized water 5 mL/kg of body weight/day for 10 days to achieve uremia.

Group III or acetaminophen with treatment by ALA – Six animals were randomly placed in cage with normal diet and treated similarly as uremic group and co-administered with ALA at the dose of 50 mg/0.5 mL de-ionized water/kg body weight/rat, respectively, by forceful feeding for 10 days at daily 10.00 A.M. before giving the food.

Group IV or acetaminophen with treatment by ALA – Six animals were randomly placed in cage treated similarly as uremic group and co-administered with ALA at the dose of 100 mg /0.5 mL de-ionized water/kg body weight/rat, respectively, by forceful feeding for 10 days at daily 10.00 A.M. before giving the food.

Group V or acetaminophen with treatment by ALA – Six animals were randomly placed in cage treated similarly as uremic group and co-administered with ALA at the dose of 200 mg /0.5 mL de-ionized water/kg body weight/rat, respectively, by forceful feeding for 10 days at daily 10.00 A.M. before giving the food.

Animals sacrificed and plasma and organ collection

This experimental design was continued for 24 days. After 24 days, the animals were sacrificed and blood was collected from the aorta after which the kidneys were collected for different biochemical analysis.

Parameters

Hematological study

After the total experimental period, animals were sacrificed by diethyl ether anesthesia. Blood samples were collected by hepatic artery puncture under diethyl ether anesthesia using 21 gauge needles mounted on a 5 ml syringe into heparin coated sample bottles for analysis of hematological parameters like total RBC by haemocytometer and hemoglobin (Hb) by standard kit method (Merck, Japan).

Biochemical analysis

Activities of serum liver enzymes aspartate aminotransferase, alanine aminotransferase (AST and ALT) and alkaline phosphatase were chemically determined.[10]

Hyper cholesterolemic profile

Serum total cholesterol and triglycerides were determined by standard Kit method (Merck, Japan).[10]

RESULTS

Table 1: Effect of different dose of Alpha-lipoic acid on body weight of Acetaminophen induced Hypercholesterolemia & uremic condition in male rats. Group-I – Control; Group-II- Acetaminophen (500mg.kg^{-1} body weight/day) induced hyper cholesterolemic & uremic group Group-III- Acetaminophen + Alpha-lipoic acid (50mg.kg^{-1} body weight/day); Group-IV- Acetaminophen + Alpha-lipoic acid (100mg.kg^{-1} body weight/day); Group-V- Acetaminophen + Alpha-lipoic acid (200mg.kg^{-1} body weight/day)

Groups	Initial body weight [g]	Final body weight [g]	Elevation/diminution in body growth [g%]
I	170.46±0.82^{a}	254.78±1.46^{a}	33.09
II	176.75±0.67^{a}	190.12±3.81^{b}	7.03
III	173.87 ±.41^{a}	192.6±3.7^{b}	9.72
IV	176.91±1.1^{a}	195.93±6.13^{c}	9.70
V	178.31 ±0.91^{a}	233.3 ±9.7^{a}	23.57

Data are expressed as Mean ± SE (n=6). ANOVA followed by multiple two-tail t-test and data with different superscripts (a, b, c, d, e) in a specific vertical column differ from each other significantly (P< 0.05).

Table 2: Effect of different dose of Alpha-lipoic acid on different hematological parameters (RBC & hemoglobin) of acetaminophen induced hypercholesterolemia & uremic condition in male rats. Group-I – Control; Group-II- Acetaminophen (500mg.kg^{-1} body weight/day) induced hyper cholesterolemic & uremic group Group-III- Acetaminophen + Alpha-lipoic acid (50mg.kg^{-1} body weight/day); Group-IV- Acetaminophen + Alpha-lipoic acid (100mg.kg^{-1} body weight/day); Group-V- Acetaminophen + Alpha-lipoic acid (200mg.kg^{-1} body weight/day).

Groups	RBC [cumm×1000000].	Hemoglobin [g %]
I	7.38± 0.35^{a}	6.94 ± 0.24^{a}
II % changes vs. control	4.52 ± 0.33^{b} 38.75↓	4.72 ± 0.26^{b} 31.98↓
III % changes vs. Gr II	5.06 ± 0.35^{b} 10.67↑	6.19 ± 0.29^{a} 23.74↑
IV % changes vs. Gr II	6.14 ± 0.20^{c} 26.38↑	6.38 ± 0.47^{a} 26.01↑
V % changes vs. Gr II	5.83 ± 0.33^{b} 22.46↑	7.20 ±.19^{a} 34.44↑

Data are expressed as Mean ± SE (n=6). ANOVA followed by multiple two-tail t-test and data with different superscripts (a, b, c, d, e) in a specific vertical column differ from each other significantly (P< 0.05).

Table 3: Effect of different dose of Alpha-lipoic acid on plasma ALP and ALT on acetaminophen induced hypercholesterolemia & uremic condition in male rats. Group-I – Control; Group-II- Acetaminophen (500mg.kg^{-1} body weight/day) induced hyper cholesterolemic & uremic group Group-III- Acetaminophen + Alpha-lipoic acid (50mg.kg^{-1} body weight/day); Group-IV- Acetaminophen + Alpha-lipoic acid (100mg.kg^{-1} body weight/day); Group-V- Acetaminophen + Alpha-lipoic acid (200mg.kg^{-1} body weight/day)

Groups	ALP [U/L of plasma]	ALT[U/L of plasma]
I	116.46±3.46^{a}	48.52±1.45^{a}
II % changes vs Control	303.1±19.46^{b} 61.57↑	109.88±4.83^{b} 55.84↑
III % changes vs Gr II	211.01±7.61^{c} 30.38↓	98.36±1.45^{c} 10.48↓
IV % changes vs Gr II	203.2±5.59^{d} 32.95↓	86.45±1.06^{d} 21.32↓
V % changes vs Gr II	186.75±4.23^{e} 38.38↓	57.01±2.82^{a} 48.11↓

Data are expressed as Mean ± SE (n=6). ANOVA followed by multiple two-tail t-test and data with different superscripts (a, b, c, d, e) in a specific vertical column differ from each other significantly (P< 0.05).

Table 4: Effect of different dose of (ALA) on plasma AST on acetaminophen induced hypercholesterolemia & uremic condition in male rats. Group-I – Control; Group-II- Acetaminophen (500mg.kg^{-1} body weight/day) induced hyper cholesterolemic & uremic group Group-III- Acetaminophen + Alpha-lipoic acid (50mg.kg^{-1} body weight/day); Group-IV- Acetaminophen + Alpha-lipoic acid (100mg.kg^{-1} body weight/day); Group-V- Acetaminophen + Alpha-lipoic acid (200mg.kg^{-1} body weight/day)

Groups	AST [U/L of plasma]
I	44.42±0.89^{a}
II : % changes vs Control	83.41±1.71^{b} 46.74↑
III : % changes vs Gr II	76.9±0.66^{c} 7.80↓
IV: % changes vs Gr II	70.48±0.63^{d} 15.50↓
V : % changes vs Gr II	63.75±1.14^{e} 23.57↓

Data are expressed as Mean ± SE (n=6). ANOVA followed by multiple two-tail t-test and data with different superscripts (a, b, c, d, e) in a specific vertical column differ from each other significantly (P< 0.05).

RESULTS AND DISCUSSION

Acetaminophen over dose is often linked to many metabolic disorders including serum electrolyte, urea and creatinine derangements. Increased concentration of serum urea and creatinine are considered for investigating drug induced nephrotoxicity in animals and mans [11]. In this present study it was observed that receiving acetaminophen intraperitoneally for 10 days significantly increased urea (76.66%) & creatinine (71.38%) level in compared with control group. Moreover, oral administration of ALA at three different doses (like 50mg, 100mg and 200mg/kg/day) significantly decreased plasma urea like 9.2%, 10.58% & 52.91% and creatinine level as 39.23%, 43.95%, 62.93% compared with acetaminophen treated group (Table 6). The fundamental function that blood cells perform, together with the susceptibility of this highly proliferative tissue to intoxication by xenobiotics, makes the hematopoietic system unique as a target organ. Erythrocytes, leukocytes and platelets are produced at a turnover rate of about 1 - 3 million per second in a healthy human adult and these values can be distorted in certain physiological and pathological states during hemolytic anemia and suppressive inflammation.[12] The final body weight of acetaminophen treated group were significantly decreased (7%) compared with control group (Table 1). In this present study it was observed that receiving acetaminophen intraperitoneally for 10 days significantly decreased Hb level (31.98%) and RBC count (38.78%) compared with control group. The decreased in Hb was supposed to be due to destruction of RBCs and lowered total RBC counts were due to hemolytic anemia or suppressive inflammation. After administration of alpha-lipoic acid at the different doses (like 50mg, 100mg and 200mg/kg/day) for ten days, Hb levels were significantly higher as 23.74%, 26.01% and 34.44% compared with acetaminophen treated groups. Total RBC counts were improved with alpha-lipoic acid treated group like 10.67%, 26.38% and 22.46% respectively compared with acetaminophen treated groups (Table 2). Oxidative stress plays an important role in the etiology and pathogenesis of many chronic diseases such as hepatotoxicity, nephrotoxicity, atherosclerosis, hypertension and cancers[13]. The present study showed that the activities of ALT, AST and ALP were increased with hepatotoxicity due to oxidative stress. The ALP and ALT were significantly higher as 61.57% and 55.84% in acetaminophen treated group compared with control group (Table 3). Oral - administration of alpha-lipoic acid reduces the ALP levels at the different doses (like 50mg, 100mg and 200mg/kg/day) as 30.38%, 32.95% and 38.38 % respectively compared with acetaminophen treated group and also minimize the ALT levels at the different doses (like 50mg, 100mg and 200mg/kg/day) as 10.48%, 21.32% and 48.11% respectively. Recent studies have shown that oxidation stress is highly present in patients with renal disease.[14] Oral-administration of alpha-lipoic acid reduces the AST (7.80%, 15.50%, 23.57%) levels at the different doses (like 50mg, 100mg and 200mg/kg/day) as 30.38%, 32.95% and 38.38 % respectively compared with acetaminophen treated group (Table 4). It is well known that LDL from uremic patients presents an elevated susceptibility to oxidation. Uremic oxidative stress is characterized from a biochemical point of view as a state of reactive aldehyde and oxidized thiol group accumulation, with depletion of reduced thiol group, which are particularly important as

part of anti-oxidant defense. Previous studies have clearly demonstrated that acute acetaminophen overdose increases the lipid per oxidation and suppresses the anti-oxidant defense mechanisms in renal tissues.[15] Several investigations have shown that acetaminophen induced nephrotoxicity is associated with lipid per oxidation. This is ascribed to a free radical mediated chain reaction that damages cell membranes and MDA is a good indicator of the degree of lipid per oxidation. Alpha-lipoic acid is characterized by high reactivity towards reactive oxygen species and its capability of increasing tissue levels of anti-oxidant enzymes.[16,17] It has been demonstrated that lipoic acid reduces oxidative stress in healthy adults and diabetic patients by decreasing significantly lipid hydro peroxide formation.[18,19] The protective action of alpha-lipoic acid against lipid per oxidation as a factor modifying membrane organization may be due alpha-lipoic acid's ability to scavenge the free radicals, which are produced during the per oxidation of lipids.[20]

Table 5: Effect of different dose of Alpha-lipoic acid on plasma total cholesterol & triglyceride level on acetaminophen induced hypercholesterolemia & uremic condition in male rats. Group-I – Control; Group-II- Acetaminophen (500mg.kg^{-1} body weight/day) induced hyper cholesterolemic & uremic group Group-III- Acetaminophen + Alpha-lipoic acid (50mg.kg^{-1} body weight/day); Group-IV- Acetaminophen + Alpha-lipoic acid (100mg.kg^{-1} body weight/day); Group-V- Acetaminophen + Alpha-lipoic acid (200mg.kg^{-1} body weight/day).

Groups	Plasma Total cholesterol [mg/dl]	Plasma Triglyceride[mg/dl]
I	114.86±0.74^{a}	94.28±2.16^{a}
II % changes vs Control	174.2±1.93^{b} 34.06↑	129.83±2.50^{b} 27.38↑
III % changes vs Gr II	167.88±1.09^{b} 3.62↓	110.76±2.72^{c} 14.68↓
IV % changes vs Gr II	154.78±1.40^{c} 11.14↓	103.01±3.35^{d} 20.65↓
V % changes vs Gr II	136.38±1.84^{d} 21.71↓	95.08±1.40^{a} 26.76↓

Data are expressed as Mean ± SE (n=6). ANOVA followed by multiple two-tail t-test and data with different superscripts (a, b, c, d, e) in a specific vertical column differ from each other significantly (P< 0.05).

The present study also demonstrated that plasma total cholesterol (34.06%) and total triglycerides levels (27.38%) were significantly higher in acetaminophen treated group compare to control group due to oxidative stress (Table 5). Alpha-lipoic acid ameliorates the plasma total cholesterol and triglyceride levels at different doses (like 50mg, 100mg and 200mg/kg/day) due to its anti-oxidative properties. Plasma total cholesterol levels were decreased by different doses (like 50mg, 100mg and 200mg/kg/day) 3.62%, 11.14% and 21.71 % respectively compared with acetaminophen treated group. Alpha-lipoic acid also decreased the triglycerides levels at different doses as (like 50mg, 100mg and 200mg/kg/day) 14.68%, 20.65% and 26.76% respectively and most cholesterol reducing properties had been shown the higher dose of alpha-lipoic acid (200mg/kg /day).

Table 6: Effect of different dose of Alpha-lipoic acid on plasma urea & creatinine level on acetaminophen induced hypercholesterolemia & uremic condition in male rats. Group-I – Control; Group-II- Acetaminophen (500mg.kg^{-1} body weight/day) induced hyper cholesterolemic & uremic group Group-III- Acetaminophen + Alpha-lipoic acid (50mg.kg^{-1} body weight/day); Group-IV- Acetaminophen + Alpha-lipoic acid (100mg.kg^{-1} body weight/day); Group-V- Acetaminophen + Alpha-lipoic acid (200mg.kg^{-1} body weight/day)

Group	Urea (mg/dL of blood plasma)	Creatinine (mg/dL of blood plasma)
I	11.69 ±1.22^{a}	.97 ±0.07^{a}
II % changes vs Control	50.10±2.0^{b} 76.66↑	3.39 ±0.28^{b} 71.38↑
III % changes vs Gr II	31.73±1.85^{c} 9.2↓	2.06±.30^{c} 39.23↓
IV % changes vs Gr II	28.98±3.07^{c} 10.58↓	1.90±0..34^{d} 43.95↓
V % changes vs Gr II	23.59±1.58^{d} 52.91↓	1.04±0.07^{a} 69.32↓

Data are expressed as Mean ± SE (n=6). ANOVA followed by multiple two-tail t-test and data with different superscripts (a, b, c, d, e) in a specific vertical column differ from each other significantly (P< 0.05).

CONCLUSION

In conclusion, the results suggest that oral administration of alpha-lipoic acid at the three different doses of uremic rats improves hepato cardiac functions as well as acts as a cholesterol reducer for targeting the acetaminophen induced uremic male rats. It also observed that the higher dose (200mg/kg/day) is the most effective dose for reducing hypercholesterolemia.

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