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ORIGINAL ARTICLE

Attenuation of uremia by orally feeding alpha-lipoic acid on acetaminophen induced uremic rats

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Abstract Uremia means excess nitrogenous waste products in the blood & their toxic effects. An acute acetaminophen (paracetamol, *N*-acetyl *p*-aminophenol; APAP) overdose may result into potentially fatal hepatic and renal necrosis in humans and experimental animals. The aims of this present study were to investigate the protective effect of alpha-lipoic acid (ALA) on oxidative stress & uremia on male albino rats induced by acetaminophen. The study was performed by 24 albino male Wister strain rats which were randomly divided into four groups: Group I, control – receives normal food and water, Groups II, III & IV receive acetaminophen interperitoneally at the dose of 500 mg/kg/day for 10 days, from 11th day Groups III & IV were treated with ALA at the dose of 5 mg & 10 mg/100 g/day for 15 days, respectively. After 25 days of treatment, it was observed that there was a significant increase in plasma urea, creatinine, sodium and malondialdehyde (MDA) levels ($p < 0.05$) but a significant decrease in super oxide dismutase (SOD) & catalase activity & potassium level in uremic group is compared with control group & there was a significant increase in SOD & catalase ($p < 0.05$) & a significant decrease in serum urea, creatinine & Na and MDA ($p < 0.05$) in Group III & Group IV is compared with Group II & significant changes were observed in high ALA dose group. In conclusion it was observed that the ALA has nephroprotective activities by biochemical observations against acetaminophen induced uremic rats.

1. Introduction

At present kidney diseases appear to be a major problem across the globe. As per global and regional overview, 17,83,000 patients are suffering from End Stage Renal Disease of which dialysis has been given to 13,71,000 patients and kidney has been transplanted in 4,12,000 patients suffering from renal failure, live under particularly pro-oxidative conditions causing uremia (Moeller et al., 2004; Toborek et al., 1992). Uremia is a potentially fatal condition that demands immediate treatment

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(Toborek et al., 1992). Treatment for uremia includes kidney transplant and dialysis (Tsuchiya and Sato, 1990), which are very expensive, time-consuming, complicated techniques and are not free from side effects. There is great urgency for a nonconventional, affordable therapy for patients who cannot afford expensive dialysis or kidney transplant to keep them alive. Many studies have been showed that herbal therapy may be the sort out of this global problem of uremia (Das et al., 2010a,b). Acetaminophen is a commonly used antipyretic agent which, in high doses, causes renal tubular damage and uraemia. An acute acetaminophen (paracetamol, *N*-acetyl *p*-aminophenol; APAP) overdose may result into potentially fatal hepatic and renal necrosis in humans and experimental animals (Jones and Vale, 1993). Acetaminophen induced oxidative stress results in lipoperoxidation, protein thiol oxidation, mitochondrial endoplasmic reticulum injury, altered homeotaxis and irreversible DNA damage characterized by protein adduct formation (Sies, 1993). 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 (Murugavel and Pari, 2004). Recent evidence established that alpha-lipoic acid co-treatment significantly inhibited the levels of lipid hydroperoxide, protein carbonyl contents & stimulated anti-oxidants enzyme activities like SOD, GPx and GST & CAT. The observed increase in total anti-oxidants & anti-oxidant enzyme levels with alpha-lipoic acid treatment to lead ingesting rats could be due the anti-oxidant effects of alpha-lipoic acid (Caylak et al., 2008). The aim of this study was to search out the possible effect of alpha-lipoic acid on acetaminophen induced uremic rats.

2. Materials & methods

2.1. Selection of animals and care

The study was conducted on 24 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 °C) 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 (Olert et al., 1993).

2.2. Grouping of animals & 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) & feed normal diet & water ad libitum.

Group II or acetaminophen induced uremic – Six animals were randomly placed in cage with normal diet & 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 & treated similarly as uremic group & 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 15 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 & 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 15 days at daily 10.00 A.M. before giving the food.

2.3. Animals sacrificed & plasma & organ collection

This experimental design was continued for 25 days. After 25 days, the animals were sacrificed & blood was collected from the aorta after which the kidneys were collected for different biochemical analysis.

2.4. Methods of measuring the concerned parameters

2.4.1. Blood uremia profile

2.4.1.1. Biochemical estimation of blood urea (Burtis and Ashwood, 1999). The collected blood was centrifuged and plasma fraction was separated. Urea level of plasma was measured by commercially available standard Blood Urea Kit (Merck, Japan) by Semiautoanalyzer (Merck, Japan) by standard protocol for photometric determination of urea according to the Urease GLDH method (kinetic UV test).

2.4.1.2. Biochemical estimation of blood creatinine (Sabbagh et al., 1988). The collected blood was centrifuged and plasma fraction was separated. The plasma creatinine level was measured by commercially available standard Blood Urea Kit (Merck, Japan) by Semiautoanalyzer (Merck, Japan) by standard protocol for phtotometric determination of creatinine based on Jaffe kinetic method without de-proteinization.

2.4.2. Anti-oxidant enzymes

2.4.2.1. Biochemical assay of catalase activity (Beers and Sizer, 1952). Catalase activity (CAT) was measured biochemically. For the evaluation of CAT in plasma, the collected blood was centrifuged and the plasma fraction was separated. The kidneys were homogenized separately in 0.05 M Tris hydrochloric acid (HCl) buffer solution (pH – 7.0) at a tissue concentration of 50 mg/mL. These homogenates were centrifuged separately at 10,000*g* at 4 °C for 10 min. Following this, 0.5 mL of hydrogen peroxide (H_2O_2) and 2.5 mL of distilled water were mixed and reading of absorbance was noted using a spectrophotometric cuvette at 240 nm. Forty microliters of tissue supernatant and plasma were added separately and six subsequent readings were noted at 30 s intervals.

2.4.2.2. Biochemical assay of superoxide dismutase (SOD) (Marklund and Marklund, 1974). The kidneys were homogenized in ice-cold 100 mM Tris–cocodylate buffer to give a tissue concentration of 50 mg/mL and centrifuged at 10,000*g* for 20 min at 4 °C. The SOD activity of the supernatant was estimated by measuring the percentage of inhibition of the pyragallol autooxidation by SOD. The buffer was 50 mM Tris (pH – 8.2) containing 50 mM cocodylic acid (pH – 8.2), 1 mM ethylene diamine tetra acetic acid (EDTA) and 10 mM hydrochloric acid (HCl). In a spectrophotometric cuvette, 2 mL of buffer, 100 μL of 2 mM pyragallol and 10 μL of

Table 1 Anti-uremic & anti-oxidative effect of alpha-lipoic acid at two different doses (50 & 100 mg/kg of body weight) on body growth on acetaminophen induced uremic male albino rats. Data are expressed as mean ± S.E. ($n = 8$). ANOVA followed by multiple two-tail t-test and data with different superscripts (a, b, c, d) in a specific vertical column differ from each other significantly ($p < 0.05$).

Group	Initial body weight (g)	Final body weight (g)	Increases or decreases in body weight (g %)	Kidney somatic index (g)
I	99.16 ± 3.83	121 ± 5.16[a]	21.63	0.70 ± 0.05[a]
II	98.33 ± 4.34	100.17 ± 3.56[b]	1.75	0.62 ± 0.01[a]
III	97.5 ± 6.77	112.5 ± 7.4722[c]	14.74	0.77 ± 0.02[ab]
IV	100 ± 4.08	100.83 ± 3.837[b]	1.126	0.92 ± 0.03[c]

Group I – control, Group II – acetaminophen induced uremia, Groups III and IV – acetaminophen + co-administration of ALA at the dose 50 & 100 mg/100 g of body wt., respectively.

Table 2 Anti-uremic & anti-oxidative effect of alpha-lipoic acid at two different doses (50 & 100 mg/kg of body weight) on plasma and kidney SOD & catalase on acetaminophen induced uremic male albino rats. Data are expressed as mean ± S.E. ($n = 8$). ANOVA followed by multiple two-tail t-test and data with different superscripts (a, b, c, d) in a specific vertical column differ from each other significantly ($p < 0.05$).

	Groups	Plasma		Kidney
	Catalase (m mol of H_2O_2 consumption/dL of plasma/min)	SOD (m mol of H_2O_2 consumption/dL of plasma/min)	Catalase (m mol of H_2O_2 consumption/mg of tissue/min)	SOD (m mol of H_2O_2 consumption/mg of tissue/min)
I	0.711 ± 0.04[a]	0.65 ± 0.04[a]	0.57 ± 0.05[a]	0.89 ± 0.14[a]
II	0.343 ± 0.02[b]	0.09 ± 0.01[b]	0.22 ± 0.03[b]	0.20 ± 0.13[b]
III	1.44 ± 0.04[c]	0.44 ± 0.12[c]	0.42 ± 0.02[c]	0.55 ± 0.13[c]
IV	2.69 ± 0.18[d]	0.60 ± 0.09[a]	0.79 ± 0.09[d]	0.62 ± 0.14[a]

Group I – control, Group II – acetaminophen induced uremia, Groups III & IV – acetaminophen + co-administration of ALA at the dose 50 & 100 mg/kg of body wt., respectively.

Table 3 Anti-uremic & anti-oxidative effect of alpha-lipoic acid at two different doses (50 & 10 mg/kg of body weight) on plasma & kidney MDA on acetaminophen induced uremic male albino rats. Data are expressed as mean ± S.E. ($n = 8$). ANOVA followed by multiple two-tail t-test and data with different superscripts (a, b, c, d) in a specific vertical column differ from each other significantly ($p < 0.05$).

Group	Plasma	Kidney
	MDA (n mol/dL of plasma)	MDA (n mol/mg of tissue)
I	25.80 ± 1.34[a]	40.27 ± 1.69[a]
II	45.78 ± 1.58[b]	98.82 ± 3.79[b]
III	38.14 ± 1.14[c]	74.30 ± 2.68[c]
IV	28.15 ± 1.85[a]	61.75 ± 2.10[d]

Group I – control, Group II – acetaminophen induced uremia, Groups III & IV – acetaminophen + co-administration of ALA at the dose 50 & 100 mg/kg body wt., respectively.

supernatant were poured and the absorbance was noted in spectrophotometer at 420 nm for 3 min. One unit of SOD was defined as the enzyme activity that inhibited the autooxidation of pyragallol by 50%.

2.4.3. Oxidative stress marker

2.4.3.1. Biochemical estimation of MDA (Ohkawa et al., 1979). The kidneys were homogenized separately at a tissue concentration of 50 mg/mL in 0.1 M of ice-cold phosphate buffer (pH = 7.4) and the homogenates and blood samples were separately centrifuged at 10,000 g at 4 °C for 5 min. Supernatant and plasma were used for the estimation of MDA and CD. For the measurement of MDA, 0.5 mL homogenate and plasma were mixed separately with 0.5 mL normal saline and 2 mL of TBA-TCA mixture (0.392 g of TBA in 75 mL of 0.25 N HCl with 15 g of TCA, with the final volume of the mixture being made up to 100 mL with ethanol) and, then boiled at 100 °C for 10 min. The mixture was then cooled at room temperature and centrifuged at 4000g for 10 min. The whole supernatant and plasma was transferred into a spectrophotometer cuvette and read at 535 nm. Calibration was performed by using the acid hydrolysis of 1, 1, 3, 3 tetra-methoxy propane, as a standard. The MDA present within the sample was calculated by using the extinction coefficient of 1.56×105 M/cm and expressed as the unit of nM/mg of tissue or nM/mL of plasma.

2.4.4. Electrolytes marker

2.4.4.1. Biochemical estimation of serum Na & K (Sunderman, 1959). Sodium is precipitated as a triple salt with magnesium & uranyl acetate. The excess of uranyl ions is reacted with ferrocyanide in an acidic medium to develop a brownish color. The intensity of the color produced is inversely proportional to the concentration of sodium in the sample. Potassium reacts with sodium tetra phenyl boron in a specially prepared buffer to form a colloidal suspension. The amount of the turbidity produced is directly proportional to the concentration of potassium in the sample.

2.5. Statistical analysis

The values were expressed as mean ± S.E. Data were analyzed using one-way ANOVA followed by t-test. p value < 0.05 was considered as significant.

Table 4 Anti-uremic & anti-oxidative effect of alpha-lipoic acid at two different doses (50 & 100 mg/kg of body weight) on plasma urea & creatinine conc. on acetaminophen induced uremic male albino rats. Data are expressed as mean ± S.E. ($n = 8$). ANOVA followed by multiple two-tail *t*-test and data with different superscripts (a, b, c, d) in a specific vertical column differ from each other significantly ($p < 0.05$).

Groups	Urea (mg/dL of blood plasma)	Creatinine (mg/dL of blood plasma)
I	11.16 ± 0.93^{a}	1.11 ± 0.22^{a}
II	60.61 ± 3.12^{b}	7.30 ± 1.44^{b}
III	34.17 ± 2.23^{c}	3.30 ± 1.17^{c}
IV	43.69 ± 2.94^{d}	4.95 ± 0.67^{d}

Group I – control, Group II – acetaminophen induced uremia, Groups III & IV – acetaminophen + co-administration of ALA at the dose 50 & 100 mg/kg of body wt., respectively.

Table 5 Anti-uremic & anti-oxidative effect of alpha-lipoic acid at two different doses (50 & 100 mg/kg of body weight) on plasma sodium & potassium conc. on acetaminophen induced uremic male albino rats. Data are expressed as mean ± S.E. ($n = 8$). ANOVA followed by multiple two-tail *t*-test and data with different superscripts (a, b, c, d) in a specific vertical column differ from each other significantly ($p < 0.05$).

Groups	Sodium (mmol/L)	Potassium (mmol/L)
I	123.95 ± 4.68^{a}	5.58 ± 0.59^{a}
II	273.12 ± 6.46^{b}	2.88 ± 0.29^{b}
III	170.9 ± 13.07^{a}	4.09 ± 0.36^{a}
IV	209.16 ± 13.92^{d}	6.32 ± 0.50^{a}

Group I – control, Group II – acetaminophen induced uremia, Groups III & IV – acetaminophen + co-administration of ALA at the dose 50 & 100 mg/kg of body wt., respectively.

3. Results

3.1. Body weight & somatic indices of kidneys

Body weight increased at the end of experiment in Groups I, III compared to their initial body weight (Table 1). In Groups II & IV rats, the percentage in the increase in body growth was dramatically less than the other groups due to acetaminophen induced oxidative stress (Table 2).

3.2. Serum urea & creatinine

Serum urea & creatinine levels were significantly higher in uremic rats treated with acetaminophen alone than rats in Groups I, III & IV ($p < 0.05$, Table 5). ALA in addition to acetaminophen treatment group caused decreases in serum urea & creatinine levels when compared with control.

3.3. Activities of catalase & SOD

In Groups I, III & IV, CAT activities in blood & kidney were elevated, compared to Group II. After acetaminophen treatment (Group II), the activity of this enzyme in blood & kidney was decreased significantly, compared with Group I. There was a significant protection in catalase activity after co-administration of ALA (Groups III & IV), compared with the animals only treated with acetaminophen (Group II) (Table 3). Administration of ALA to acetaminophen treated animals (Groups III & IV) & Group I resulted in significant elevation in the activity of SOD in blood & kidney, compared to the animals in Group II ($p < 0.05$, Table 3). The activity of this enzyme was decreased significantly in blood & kidney, compared to Group II, in comparison with Group I ($p < 0.05$). Acetaminophen treatment followed by the co-administration of ALA to the animals in Groups III & IV resulted in a significant restoration of the SOD activity, compared with Group II, & the values were resettled to the control group.

3.4. Quantification of MDA

Quantities of MDA was increased significantly in blood & kidney of Group II animals, compared to Groups I, III & IV ($p < 0.05$, Table 4). In the acetaminophen treated rats (Group II), 10 days of acetaminophen injection resulted in a significant elevation in the values of both parameters in blood & kidney. However, in Groups III & IV co-administration of ALA significantly decreased MDA quantities & these values were resettled to the control level.

3.5. Alteration of electrolytes on plasma

In uremia, levels of sodium and potassium are one of the important parameters for electrolyte imbalance. Due to excess accumulation of waste products in body, sodium level is significantly increased in uremic group & potassium level is lower. After biochemical estimation there is a significant ($p < 0.05$) high plasma sodium level in uremic group compared to other groups and a significant ($p < 0.05$) low plasma potassium level in uremic (Group II) group compared to control (Group I).

4. Discussion

Acetaminophen over dose is often linked to many metabolic disorders including serum electrolyte, urea & creatinine derangements. Increased concentration of serum urea & creatinine are considered for investigating drug induced nephrotoxicity in animals & man (Ohkawa et al., 1979). Blood urea nitrogen is found in the liver protein that is derived from diet or tissue source & is normally excreted in the urine. In renal disease, the serum urea accumulates because the rate of serum urea production exceeds the rate of clearance (Mayne, 1994). Creatinine on the other hand is mostly derived from endogenous source by tissue creatinine breakdown. Elevation of urea and creatinine levels in the serum was taken as the index of nephrotoxicity (Anwar et al., 1999; Bennit et al., 1982). In this study, acetaminophen induced nephrotoxicity showed a significant ($p < 0.05$) increase in the plasma urea and creatinine concentrations in Group II (acetaminophen induced) rats when compared to Group I (control group). Moreover, oral administration of ALA significantly ($p < 0.05$) decreased plasma urea and creatinine in Groups

III & IV when compared to Group II. Recent studies have shown that oxidation stress is highly present in patients with renal disease (Vaziri, 2004). It is 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 & oxidized thiol group accumulation, with depletion of reduced thiol group, which are particularly important as part of anti-oxidant defence. Previous studies have clearly demonstrated that acute acetaminophen overdose increases the lipid peroxidation and suppresses the anti-oxidant defence mechanisms in renal tissues (Abdel-Zaher et al., 2007). Several investigations have shown that acetaminophen induced nephrotoxicity is associated with lipid peroxidation. This is ascribed to a free radical mediated chain reaction that damages cell membranes & MDA is a good indicator of the degree of lipid peroxidation. Alpha-lipoic acid is characterized by high reactivity towards reactive oxygen species & its capability of increasing tissue levels of anti-oxidant enzymes (Packer et al., 1995; Shay et al., 2008). It has been demonstrated that lipoic acid reduces oxidative stress in healthy adults & diabetic patients by decreasing significantly lipid hydroperoxide formation (Packer et al., 1995; Smith et al., 2004). The protective action of alpha-lipoic acid against lipid peroxidation as a factor modifying membrane organization may be due alpha-lipoic acid's ability to scavenge the free radicals, which are produced during the peroxidation of lipids (EI-Sokkary et al., 2003). However in the acetaminophen treated animals the MDA levels are increased significantly, when compared to control rats. On administration of the ALA, levels of MDA decreased significantly ($p < 0.05$) when compared to acetaminophen induced rats (Group II). Oxidative stress and lipid peroxidation are early events related to radicals generated during the hepatic metabolism of acetaminophen. Also the generation of reactive oxygen species has been proposed as a mechanism by which many chemicals can induce nephrotoxicity (Somani et al., 2000). During kidney injury, superoxide radicals are generated at the site of damage, which modulate SOD and CAT, resulting in the loss of activity and accumulation of superoxide radical, which damages kidney. SOD and CAT are the most important enzymes involved in ameliorating the effects of oxygen metabolism (Pande and Flora, 2002). The present study also demonstrated that acute acetaminophen overdose resulted in a significant decrease in the SOD and CAT activities in Group II, when compared with control groups but co-administration of ALA in acetaminophen treated groups (Groups III & IV) can show a significant increase ($p < 0.05$) in SOD and CAT activities in blood & kidney & this anti-oxidant enzymes level is significantly higher in high dose of ALA treated group (Group IV).

5. Conclusion

In conclusion, alpha-lipoic acid had shown a significant anti-uremic activity & anti-oxidative properties on acetaminophen induced uremia & increases anti-oxidative properties on acetaminophen treated uremic rats.

Conflict of interest

The authors declare no conflict of interest.

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