Sindhological Studies, ISSN: 1032-6759, 2016 (1): 19-25

Role of Alpha Lipoic Acid on Metabolic Hepatosteatosis: An Experimental Biochemical & Microscopic Study

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ABSTRACT: Aim of work: Metabolic hepatosteatosis is a common serious prevalent condition in KSA. Thus, our study investigates alpha lipoic acid (ALA) effects on hepatic lipid accumulation in severely fatty rats and secondarily on blood lipid profile to provide a protective medicine. Material and methods: Sixty male Zucker rats were selectively used half of which (Lean) weighed 200±25g & others (Fatty) weighed 375±30g. Fatty animals were allowed free access to food and water for one week before experiment. The animals were divided into lean control (group I), lean ALA – treated (group II), fatty control (group III) and fatty ALA – treated (group IV) (fifteen animals per each group). ALA was taken orally (20 mg/kg/day) for six months. Animals were sacrificed and weighed (BW). Their liver was weighed (LW) and its portion was sliced to study its lipid content. Right tibia length (TL) was measured and LW: TL ratio was calculated. Results: Fatty rats (ZF) had shorter TL but their BW was higher than in lean ones. Also, ZF rats showed high LW: TL ratio. ALA therapy didn't change BW& TL and reduced LW and LW: TL ratio in ZF rats while it didn't affect these parameters in lean (ZL) rats. ZF rats exhibited significant hepatosteatosis evidenced by excessive liver TG and TC contents and microscopically by large cytoplasmic vacuoles. Interestingly, 6-months' ALA therapy in ZF caused both diminished liver triglycerides (not TC) component and its lipid vacuoles. Conclusion: Our findings support effectiveness of ALA therapy in excessive hepatosteatosis and in hyperlipidemia via improving abnormal lipid metabolism.
Key words: Alpha lipoic acid- Fatty liver – Abnormal fat metabolism- Serum Chemistry – Histopathology.

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) represented a spectrum of disease ranging from hepatocellular steatosis through steatohepatitis to fibrosis and irreversible cirrhosis (Charlton, 2004). Also, it was considered as part of the metabolic syndrome affecting adults and children and commonly associated with visceral adiposity, dyslipidaemia, hyperglycaemia and hypertension (Lazo and Clark, 2008). Its importance was delivered from its high prevalence of up to 31% in the general worldwide population (Brunt, 2004). Metabolic hepatosteatosis was clinically important disorder in Saudi Arabia with estimated prevalence of 7% to 10% (Al-hamoudi et al, 2012). Hepatic steatosis was also commonly seen in patients with chronic hepatitis C virus (HCV) infection and had an increased prevalence in women with polycystic ovary syndrome when it was usually associated with inflammatory reaction (Hwang and Lee, 2011). Initial theories for the pathogenesis of NASH were based on 2-hit hypothesis; the first hit was hepatic triglyceride accumulation or steatosis that increased the susceptibility of liver to injury mediated by second hit exemplified by inflammatory cytokines/adipokines, mitochondrial dysfunction and oxidative stress which in turn could lead to steatohepatitis and/or fibrosis (Day, 2006).

However, free fatty acids (FFA) had been evidenced to play in directly progressing liver injury and could directly cause toxicity by increasing oxidative stress and by activation of inflammatory pathways (Feldstein, 2004). An increasing clinical and experimental evidences showed a greater production of oxygen free radicals (OFR) that could result in the known complications of non-alcoholic fatty liver (Robertson, 2004; Bashan et al., 2009).

The potential therapeutic goal in management of hyperglycemia as well as obese with hepatosteatosis had related to achieve reduction in formation and activating power of these OFR (Campión et al., 2006; Venditti et al., 2007). Exogenous antioxidant intake decreased the amount and functions of OFR; therefore, providing anti-oxidative effect in the liver cells (Ihara et al, 1999). Alpha lipoic acid had an anti-oxidation character and was being a natural component that served as significant metabolic cofactor. It could essentially reduce many OFR (Trujillo & Radi, 2002). Furthermore, it removed the metallic ions and reformed a variety of certain

antioxidants such as glutathione, ascorbic acid and tocopherol (Bast & Haenen, 2003; Petersen et al., 2008). Alpha lipoic acid, as well, had a powerful oxidative protecting renal and hepatic potential against cellular damage mediated by OFR (Morakinyo et al., 2012). In addition, alpha lipoic acid application has been reported to be an improving therapeutic agent regarding endogenous insulin sensitivity (Evans et al., 2002; Kamenova, 2006), therefore, preventing the incidence of hyperglycemic complications (Chang et al., 2007). The oxidative stress, defined by the disturbance between defensive antioxidant ability and formation of OFR, had been supposed to be concerned with the pathoetiology of many disorders including the cellular and subcellular membranous fat peroxidation that resulted in an intensive membranous destruction affecting its structure and therefore its function (Matsumoto et al., 1999). Thus, cellular lipids peroxidation might cause cellular damage seen with several stress oxidant-mediated conditions (Olivenza et al., 2000).

It was advised to use large amounts of food antioxidants to overcome these stress associating oxidant-mediated disorders and thus removing and avoiding the harmful effects of OFR (Zaidi & Banu, 2004). Lipoic acid added to antioxidant-rich foods could be absorbed and traversed the blood brain barrier. Moreover, lipoic acid (LA) could act as a helping factor for several mitochondrial multi-enzyme complexes used for energy production (Morikawa et al., 2001).

Our work investigates ameliorating the actions of lipoic acid on rat hepatosteatosis since obesity-mediated fatty liver could lead to serious worse effects and represented a considerable prevalence rate in Saudi Arabia. In addition, it provides a safe protective therapy for this health problem that may affect the personal performance and reflect on the entire world economy**.**

MATERIAL AND METHODS

Grouping of Animals and Therapeutic Administration

Albino male rats used in this study were brought from animal house, Jeddah, KSA. Half of them selectively weighed 200±25 (Lean) & others weighed 375±30g (Fatty**)**. The rats were resided in a standard laboratory conditions for seven days at room temperature, in 50-60% moistness and for twelve hours in light and similar hours in darkness. The animals received clean water as well as standard feeding. One week before starting this work, we allowed fatty animals for free feeding (on diet rich in animal fats and omega-6/omega-9-containing plant oils to induce hepatosteatosis) and drinking water. The used planning coincided with that of Biomedical Research in United States National Institute of Health Guidelines for Care (NIH, 1985).

Four groups of rats were studied each of which involved fifteen animals; lean control group (Group I; LC) received distilled water (0.5 ml), ALA managed lean group (II; LA), fatty control group (III; FC) received distilled water (0.5 ml) and ALA managed fatty group (IV; FA). Daily administration of distilled water or ALA was performed through an oral cannula for duration of six months. Alpha lipoic acid (ALA) was taken orally in a dose of 20 mg/kg/day dissolved in distilled water.

The triglycerides (TG) and total cholesterol (TC) kits were bought commercially from Wako; Osaka, Japan whileas alpha lipoic acid powder was bought from Sigma, China.

Experimental Design

Depending upon the body weight, both serum TG and TC were measured prior to and after ALA treatment before sacrificing animals at the end of this study. The body weight (BW) was assessed every two weeks throughout the experiment alongside with serum lipids and right tibia length (TL). The rats' cervical vertebrae were dislocated after administration halothane anesthesia and then, the animals were weighed at the end of this work before sacrificing. Then, liver was removed & drained with iced saline. We used a filter paper to get rid of extra water found on the liver's outer surface and then the heaviness of liver (Hepatic Weight; HW) was measured. To analyze total cholesterol (TC) and triglycerides (TG) liver contents (described as milligrams per gram of hepatic tissue and declared in grams as total quantity per liver) after animal sacrificing, a part of liver was sliced, frozen in nitrogenous fluid and kept in minus eighty ◦C. Also, the ratio between each TL value and liver weight was evaluated. The hepatic lipid contents were detected; as mentioned previously every two weeks throughout the study, in the centrifugated superficial fluid after homogenization and extraction of lipids from the processed hepatic piece using isopropanol in a concentration of 1ml per 50 mg (Li et al., 2008).

Histopathological Preparation Technique

Small liver tissue pieces were fixed in ten percent formaldehyde to preserve tissue in as natural state as possible and prevent postmortem autolysis and putrefaction, then, the tissue was processed to remove water from it (tissue dehydration), then, it was embedded in waxy paraffin to make solid tissue blocks that were sectioned by a microtome into tissue slices of 5 µm thickness. Finally the sliced sections were put on glass slides, stained with ordinary hematoxylin (Hx.) and eosin (E.) stains, covered with glass covers using Canada balsam and examined by light microscopy for assessment of the cases.

Data Studying

The informative results were described in mean values ± Standard deviation (SD). We used one way variance analysis to achieve significant differences between four animal sets. The statistical probability value less than 0.05 (or less than 0.01) was significant value. Also, we performed SPSS statistical version 8 software package for our finding values.

RESULTS

Zucker fatty rats displayed elevated serum total cholesterol and triglycerides at the beginning of this study while the serum levels of lipids were within normal in Zucker lean animals (Table 1). The Liver weight (LW; expressed in grams) at the end of the experiment (24th week) showed the following means ± standard deviations in the studied four groups respectively; 19.5± 1.11, 21± 0.93, 38.5± 1.21and 24±0.83. The hepatic total cholesterol (TC) content (expressed in mg/gm of liver tissue) at the end of the experiment (24th week) showed the following means ± standard deviations in the studied four groups respectively; 6.02 ± 0.41, 6.01± 0.23, 9.37±0.24 and 5.92± 0.38. The hepatic total triglycerides (TG) content (expressed in mg/gm of liver tissue) at the end of the experiment (24th week) showed the following means ± standard deviations in the studied four groups respectively; 2.06 ± 0.14, 1.99± 0.44, 4.45±0.38 and 2.02 ± 0.29. Moreover, at the end of this experiment, alpha lipoic acid (ALA) - treated Zucker fatty rats showed an improvement in the serum total cholesterol and triglycerides than control Zucker fatty rats whose serum lipid values were still elevated (Table 1). Zucker fatty (ZF) whether control (FC) or ALA-treated (FA) rats had shorter TL, however, the animals' body weights (B.Ws.) were greater than the lean ones whether control (LC) or ALA-received (LA) rats (Table 2). Fatty rats displayed liver enlargement evidenced by its higher weight in addition to greater liver weight: TL relationship. ALA therapy didn't alter either the body weight or tibial length; however, it diminished both in ZF animals (Table 2). ZF animals demonstrated excessive hepatosteatosis evidenced by excessively higher liver triglycerides quantity as well as microscopically seen fatty droplets that appeared as large vacuoles occupying hepatocytic cytoplasm and pushing their nuclei to the periphery against the cell membrane giving signet-ring appearance (Fig1). In addition, there was an increased hepatic TC content. Interestingly, ZF rats treated with ALA showed surprising diminution in both liver triglycerides quantity as well as intracytoplasmic lipid vacuoles although the therapy revealed insignificant change in liver triglycerides component. Moreover, ALA management displayed trivial results in lean animals.

Table 1: Changes of serum total cholesterol (TC) & triglycerides (TG) values during the study:

Week No;	Serum TC (mmol/l) GI (n=15)	GII (n=15)	GIII* (n=15)	GIV** (n=15)	Serum TG (mmol/l) GI (n=15)	GII (n=15)	GIII* (n=15)	GIV** (n=15)
Day 0	4.66±1.12	4.35±0.13	5.12 ± 0.52	5.16 ± 0.23	0.94±0.12	0.96± 0.05	1.67±0.23	1.62±0.14
Week 2	4.52±1.02	4.43±0.23	5.68 ± 0.45	5.65±0.29	0.81±0.21	0.87±0.17	1.78±0.21	1.69±0.17
Week 4	4.55±0.19	4.30±0.24	5.79± 0.17	5.74±1.03	1.07±0.27	1.02±0.15	1.93±0.15	1.88±0.12
Week 6	4.61±1.01	4.15±0.15	5.87± 0.34	5.82±1.11	1.10±0.25	1.05±0.08	1.98±0.11	1.79±0.23
Week 8	4.67±0.23	4.43±0.53	5.99± 0.22	5.89±0.27	0.95±0.12	0.91±0.14	2.09±0.3	1.86±0.18
Week 10	4.51±0.41	4.35±1.11	5.97± 0.14	5.90±1.11	1.09±0.19	1.06±0.08	2.16±0.14	1.81±0.44
Week 12	4.23± 0.3	4.15±0.16	6.13± 0.32	6.09±0.14	1.05±0.23	1.01±0.05	2.19±0.07	1.79±0.13
Week 14	4.69±0.21	4.27±0.34	6.27± 0.3	6.15±0.43	0.97±0.2	0.93±0.02	2.23±0.13	1.76±0.15
Week 16	4.73±0.26	4.31±0.32	6.29 ± 0.14	6.24±0.19	0.91±0.14	0.88±0.11	2.28±0.08	1.85±0.11
Week 18	4.81±0.15	4.22±0.19	6.31± 0.37	5.87±0.64	0.83±0.07	0.79±0.1	2.41±0.21	1.99±0.23
Week 20	4.64±0.34	4.61±1.03	6.36 ±1.02	5.62±1.05	1.05±0.16	0.98±0.08	2.54±0.45	2.02±0.38
Week 22	4.54±0.29	4.47±1.08	6.47± 0.91	5.24±0.19	0.75±0.05	0.72±0.02	2.88±0.42	1.92±0.24
Week 24	4.55±0.31	4.38±0.51	*6.68 ± 0.17*	*4.89±0.27*	0.69±0.3	0.65±0.03	*4.32±0.27*	*1.56±0.19*

The serum TC and TG values in each group are represented as Means ± Standard deviation. Significant p values ($p < 0.05$) were detected between (Group III*) and (GIV**) notably at the 24th week of the experiment (Bold Italic values). Group I (LC; lean control); Group II (LA; lean animals treated with ALA); Group

III (FC; fatty control); Group IV (FA; fatty animals treated with ALA); Day 0 = The first day of performance of the experiment. Normal serum TC was < 5.17 mmol/l and normal TG was < 1.7 mmol/l.

Table 2: Changes of body weight (BW) and Tibia length (TL) values during the study:

Week No;	Body Weight (gm)				Tibia Length (mm)			
	GI* (n=15)	GII* (n=15)	GIII** (n=15)	GIV** (n=15)	GI* (n=15)	GII* (n=15)	GIII** (n=15)	GIV** (n=15)
Day 0	188 ±1.28	186± 0.5	353± 1.12	357±1.43	21± 0.64	21 ± 0.33	19.3± 0.22	19.2± 0.24
Week 2	214± 0.96	215±1.17	394± 1.33	397± 1.21	26± 0.31	27 ± 0.28	18.5± 0.57	18.3± 0.13
Week 4	211± 1.02	213± 0.94	399±0.82	363±0.92	24± 0.17	25 ± 0.39	18.3± 0.44	18.2± 0.19
Week 6	214± 1.31	212± 0.66	403±0.71	352±1.11	26± 0.54	24 ± 0.22	18.2± 0.52	18.0± 0.21
Week 8	215± 1.15	212± 0.66	411±1.28	345±0.89	27± 0.37	24 ± 0.22	18.2± 0.46	18.0± 0.18
Week 10	213± 0.85	210± 0.52	414±1.14	337±1.24	25± 0.81	23 ± 0.57	18.1± 0.61	18.0± 0.22
Week 12	217± 0.46	209±0.74	417±0.63	321±1.06	28± 0.72	23 ± 0.48	18.1± 0.32	17.8± 0.31
Week 14	212± 1.21	206±1.41	421±1.33	312±1.15	24± 0.29	22 ± 0.86	18.0 ± 0.59	17.8± 0.43
Week 16	218± 0.91	205±0.81	424±1.16	310±0.94	28± 0.16	22 ± 0.34	18.0 ± 0.42	17.7± 0.66
Week 20	216± 1.17	205±0.64	426±0.84	301±1.28	27± 0.36	22 ± 0.34	18.0 ± 0.33	17.7± 0.71
Week 22	219±1.12	208±1.21	431±0.41	293±0.47	28.5± 0.68	23 ± 0.82	17.9± 0.85	17.6± 0.44
Week 24	221± 1.37	217± 1.4	434± 1.26	284± 1.22	28.5± 0.44	28 ± 0.71	17.5± 0.66	17.5± 0.27

The body weight and Tibia length values in each group are represented as Means ± Standard deviation. Significant p values ($p < 0.05$) were detected between (GI *and GIII **) and (GII*and GIV**) respectively. Group I (LC; lean control); Group II (LA; lean animals treated with ALA); Group III (FC; fatty control); Group IV (FA; fatty animals treated with ALA); Day 0 = The first day of performance of the experiment. Normal Tibia lengths according to the standard animal body weights of 175-225 gm were 20-30 mm.

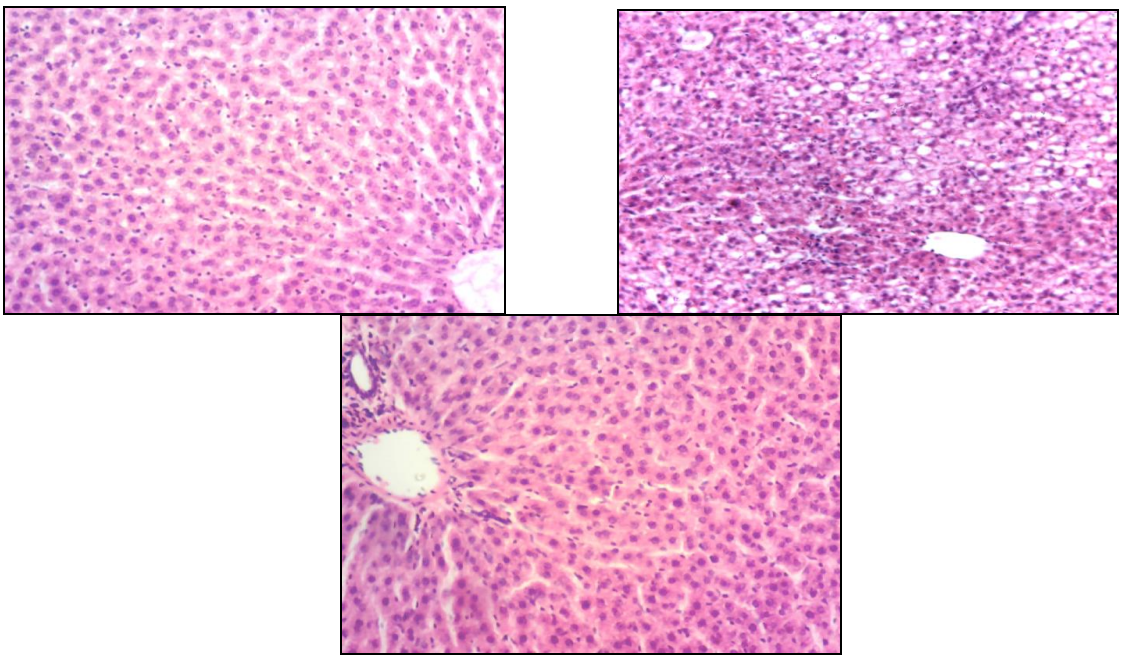

Figure 1: Hepatic tissue photomicrographs of ZL (group I; upper left) showing no intrahepatocytic fatty accumulation, that of ZF (group III ; upper right) with signet ring appearance denoting intracellular fatty vacuoles deposition and that of ALA-treated ZF (group IV) (lower middle; showing an absence of intrahepatocytic fatty deposition (Hx.& E. magnification power x100).

DISCUSSION

In our work, fatty animals (ZF) demonstrated shorter TL and greater body weights regarding the lean ones. Also, ZF animals displayed hepatomegaly evidenced by its higher weight and its greater weight: TL ratio. Alpha lipoic acid (ALA) therapy didn't alter body weight and tibial length although it diminished both hepatic weight and weight: TL ratio in ZF groups. Moreover, ALA didn't influence the mentioned variables in lean groups. Fatty animals revealed marked hepatosteatosis indicated by significantly higher triglycerides as well as total cholesterol liver contents together with fatty droplets visualized by light microscopy. Six- months ALA therapy of fatty animals diminished triglycerides' quantities of fatty livers and improved hepatocytes' intracytoplasmic fatty vacuoles, however, this long-term therapy didn't affect total cholesterol quantities of the fatty livers. ALA management demonstrated trivial and insignificant influences in lean animal groups. Improvement of serum TC and TG had been encountered in ALA-treated ZF rats but not in control ZF animals. The serum lipids of ZL animals were within normal values. Our results explained the antioxidant action of alpha lipoic acid through multiple mechanisms including blockage the cellular and subcellular lipid peroxidation, activation of the hepatic expressed genes related to oxidation of fatty acids and expression of antioxidant enzymes with subsequent decreased expression of sterol regulatory element binding protein-1 and acetyl COA carboxylase as well as subsequent increased expression of glucose transporter -4. These findings are consistent with those of Morakinyo et al (2013). Several researches performed on animals with insulin-resistance revealed a significant lowered hepatic triglycerides' component as well as fatness by peroxisome proliferator activated–alpha (PPAR – α)gene agonists (Guerre-Millo et al., 2000). This gene's agonist had normalized obese hepatosteatosis (Ye et al., 2003) and severely enhances alcoholic hepatosteatosis as well as rats' hepatic triglycerides overloading (Fischer et al., 2003). Moreover, fenofibrate, as an agonist for PPAR- α gene, intensely diminished hepatic triglyceride content that was associated with lowered plasma triglyceride level (Lee et al., 2004). Moreover, Morakinyo et al. (2013) and Akpinar et al. (2008) discovered lowering of the accumulated cardiac TG together with a decrease in the highly expressed certain messenger ribonucleic acids such as PPAR- α and acyl CoA oxidase (ACO) by hearts of diabetic fatty rats following ALA therapy. These findings may be caused by the hypolipidemic effect of ALA that had been displayed in our group IV animals. Furthermore, Akpinar et al. (2008) explained an improvement of mRNA expression of certain genes including the PPAR- α and ACO ones by ZF rats' livers with ALA treatment. So, these findings may suggest that ALA gave better quality for hepatosteatosis in ZF rats through activation of hepatic expressed genes related to oxidation of fatty acids.

The hepatic accumulation of fatty acids has been related to their uptake and synthesis as well as triglycerides' degradation. PPAR-ϒ may activate the hepatic expression of gene included in fatty acids uptake and storage together with their synthesis (Loviscach et al., 2000). In contrast, there was a lowered hepatic expression of PPAR-ϒ factor (Chou et al., 2002). Another factor termed SREBP-1 played a significant role in hepatic fat synthesis via activation of genes associated with hepatic lipogenesis. Alpha lipoic acid and gallic acid were found to increase the PPAR- ϒ protein and mRNA expression in human mature phagocytes (Horton et al., 2002). On contrary, Morakinyo et al, (2012) displayed insignificant change in hepatic PPAR-ϒ-mediated expression of genes. They also found that SREBP- 1 mRNAs didn't vary in fatty rats relatively to the lean ones. Therefore, it has not been evidenced that ALA affected the expression of genes mediating the uptake and formation of fatty acids and also mediating the breakdown of triglycerides.

Interestingly, Shay et al. (2009) reported that ALA administration didn't enhance but lowered the weight of ZF livers, although ALA had no effect on the weight of ZL livers. They, as well, discovered potential antioxidant activity of ALA and its hepatoprotective character. In addition, Shaw et al., (2010) emphasized hepatoprotective antioxidant and anti-inflammatory effects of ALA in acute liver injury induced by chemicals as well as in chronic hepatic fibrosis and cirrhosis.

This work concluded that ALA improved obese hepatosteatosis and secondarily blood lipid profile in the obese rats. Therefore, this work is potentially significant to provide these findings in several clinical trials to elicit a protective safe drug used in the prevention and/or treatment of obesity-associated hepatosteatosis via changing of an unusual fat metabolic state.

RECOMMENDATION

Several future researches will be needed to clarify ALA working mechanism regarding its metabolic effects and to detect the relation of its antioxidant and anti-inflammatory properties with diminution of the fatty livers' weight.

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