



Effects of α -Lipoic Acid on γ -Radiation and Lindane-Induced Heart Toxicity in Rats

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ABSTRACT

The present study was undertaken to evaluate the possible protective role of α -lipoic acid (α -LA) as a natural antioxidant on lindane and γ -radiation (IR)-induced heart toxicity. A total of thirty-six healthy adult male rats were divided into control, α -LA alone (40 mg/kg b.wt orally daily for 3 weeks), IR (6 Gy; 2Gy/week for 3 weeks), Lindane alone (8.8 mg/kg b.wt. orally daily for 3 weeks), α -LA with IR and α -LA with lindane. Exposure to lindane and IR resulted in significant increases in serum free fatty acids, phospholipids, triglycerides and total cholesterol levels and significant elevation of heart damage markers, serum creatine phosphokinase, aspartate aminotransferase and lactate dehydrogenase activities. Heart toxicity was further confirmed by a significant increase in lipid peroxides and a significant decrease of antioxidants (superoxide dismutase, glutathione peroxidase, and catalase activities and glutathione content) in heart tissues substantiate heart damage. Administration of α -LA produced a significant amelioration in the level of serum lipids and heart damage markers associated with significant amelioration of oxidative stress in heart tissues. In conclusion, α -LA appears to have protective effects against lindane and IR-induced heart toxicity.

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Authors' Contributions

EMKED designed the study, performed the experiments and wrote the article. LMA helped in designing the experiment and preparation of manuscript. SMEA supervised the work, assisted in data interpretation and manuscript preparation.

Key words

α -lipoic acid, γ -rays, Oxidative stress, Lipids

INTRODUCTION

Exposure to environmental hazards including ionizing radiation, dietary factors, drugs and herbicides amongst others has urged scientists to find natural substances to protect human health. The toxic effect of pesticides is not necessarily a result of direct application; some pesticides accumulate into the food to a toxic level and affect the public health (Cantor *et al.*, 1992). Radiation-related disorders are one of the challenging current health problems with far-reaching medical, social and economic consequences. Exposure to ionizing radiation has become inevitable since besides natural sources (cosmic rays and radioactive elements in the earth's crust) the rapid technological advancement in the field of medicine (radiotherapy and radio diagnosis), agriculture, industries, accidental and occupational exposures has increased the risk of exposure to ionizing radiation (Karavidas *et al.*, 2010).

Problems associated with pesticides hazard to man and environment are not confined to the developing countries, but extended to developed nations and still facing some problems in certain locations. Lindane is an organochlorine pesticide (γ -hexachlorocyclohexane)

that persists in the environment, bio-accumulates through food chain and has a risk of causing adverse effects on human health and environment. It is also one of the pesticides approved by World Health Organization (WHO, 2005) for use in public health practices. The main routes of human exposure are food and water contaminated with lindane. Lindane residues have been found in milk, cooking oil and fish (Roy *et al.*, 2005). Although banned currently, lindane had been widely used to control arthropod pests on food crops, timber, and farm animals. Lindane is absorbed through respiratory, digestive and accumulates in fatty tissues in the body. It is reported to damage human hepatic and renal tissues, as well as nervous and immune systems, and induces birth defects, cancer and death (Bano and Bhatt, 2010).

The use of antioxidant rich food or antioxidant food supplements became immensely popular since many diseases have been associated with oxidative stress (Hamza and El-shennawy, 2016). Thus, one of the natural molecules as lipoic acid has received considerable attention (Marangon *et al.*, 1999). Alpha-lipoic acid (α -LA; 1,2-dithiolane-3-pentanoic acid, 6,8-dithio-octanoic acid, thioctic acid) is a strong antioxidant that plays an essential role as a cofactor in the metabolism of all organisms, from microorganisms to humans (Navari-Izzo *et al.*, 2002). α -LA is a naturally occurring compound synthesized in small quantities by most plants and animals. In humans, α -LA is synthesized in the mitochondria from octanoic acid (Jordan and Cronan,

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1997). Huge interest has been garnered in recent times on the antioxidant properties of α -LA and its reduced form dihydrolipoic acid (DHLA). α -LA can be found in dietary sources such as animal liver, spinach, broccoli, and tomatoes (Wollin and Jones, 2003).

The widespread usage of organochlorine compounds, such as lindane has stimulated research to the existence of effects related with their body toxic activity. Determination of serum markers for heart damage such as CPK, and LDH permits a highly sensitive diagnosis of heart toxicity which is commonly elevated following cellular damage. Therefore, the present study was designed to investigate the possible role of α -LA against γ -radiation- and lindane induced- heart toxicity.

MATERIALS AND METHODS

Experimental animals

Male albino rats Sprague Dawley (8 weeks old and 180 ± 20 g body weight), were purchased from the Egyptian Holding Company for Biological Products and Vaccines (Helwan, Cairo, Egypt). The animals were housed under standard laboratory conditions during the experimental period. The rats were provided with tap water and commercial diets. Experimental animals were acclimatized to laboratory conditions for 10 days before commencement of the experiment. Drinking water and food were provided *ad libitum* throughout the study. The animal care and handling was done according to the guidelines set by the World Health Organization, Geneva, Switzerland and according to approval from the ethics committee for animals care at the National Research Centre (ethic No.10-230).

Radiation treatment

Irradiation was carried out using a Canadian Gamma Cell-40 (137Cs), located at the National Center for Radiation Research and Technology, Nasr City, Cairo, Egypt. The animal's whole body was exposed to gamma rays and received 6Gy administered at 2Gy/week at a dose rate 0.5Gy/min.

Lindane treatment

Lindane was obtained from the Central Laboratory of Pesticide, Dokki, Egypt, dissolved in distilled water and administered daily by gavages to rats at a dose of 8.8 mg/kg body weight daily for 3 weeks (Smith, 1991).

α -LA treatment

The animals were orally intubated α -LA purchased from Sigma-Aldrich Chemical Company, St. Louis, MO, USA. α -LA was administered daily to rats by gavages at a dose of 40 mg/kg body weight (Pari and Muruqavel, 2004) 2 h before treatment with pesticide or radiation for

a duration of 3 weeks. α -LA was prepared freshly just before its administration daily.

Experimental design

Animals were divided into 6 groups of six rats each: Group 1 (Control): Normal healthy rats did not receive any treatment. Group 2 (α -LA): Rats given α -LA daily via gavages (40mg/kg b.wt/day) for 3 weeks. Group 3 (IR): Rats whole body gamma irradiated with 6Gy administered at 2Gy/week. Group 4 (lindane): Rats given lindane daily via gavages (8.8mg/kg b.wt/day) for 3 weeks. Group 5 (α -LA+IR): Rats given α -LA daily through the irradiation period. Group 6 (α -LA+ lindane): Rats given α -LA simultaneously with lindane during 3 weeks.

Biochemical analysis

Blood examination is a good way of assessing the health status of animals as it plays a vital role in physiological, nutritional and pathological status of an organism. Six animals from each group were sacrificed on the 21st day and blood was collected. Blood was centrifuged at 1000g for 15 min using a refrigerated centrifuge K3 Centurion Scientific Ltd, London, UK to obtain serum.

Serum lactate dehydrogenase (LDH), aspartate aminotransferase (AST) and creatine phosphokinase (CPK) activities were assayed according to the methods of Yoshida and Freese (1975), Reitman and Frankel *et al.* (1957) and Oliver (1955), respectively. The free fatty acids, triglycerides, total cholesterol levels were determined according to Cox and Pearson (1962), Fossati and Principe (1982), and Charles *et al.* (1974), respectively using a UV/VIS T60 UV/VIS spectrophotometer, PG instruments, London, UK. Phospholipids content was determined according to the procedure described by Nie *et al.* (1993).

Heart homogenates was obtained using a tissue homogenizer. The homogenates (1:10 w/v) were prepared using a 100 mM KCl buffer (pH 7.0) containing EDTA 0.3 mM. All homogenates were centrifuged at 200 x g for 20 min. at 4°C, and the supernatants were used to estimate the level of thiobarbituric acid reactive substances (TBARS) (Yoshioka *et al.*, 1979). Reduced glutathione (GSH) content was determined according to Beutler *et al.* (1963). Superoxide dismutase (SOD) and catalase (CAT) activities were determined according to Minami and Yoshikawa, (1979) and Johansson and Borg (1988), respectively. GSH-Px activity was measured by the method of Paglia and Valentine (1967). The amount of protein in the tissues was determined by using the method of Lowry *et al.* (1951) referring to the albumin as standard.

Table I.- Serum total cholesterol, total triglycerides, free fatty acids and phospholipids in the different animal groups.

Animal groups	Total cholesterol (mg/dl)	Total triglycerides (mg/dl)	Total free fatty acids (mg/dl)	Total phospholipids (mg/dl)
Control	122±0.51	106±0.62	56±0.22	62±0.31
α -lipoic acid	122±0.42	107±0.43	55±0.18	63±0.27
Irradiated	154±0.82 ^a	135±0.86 ^a	110±1.24 ^a	131±0.32 ^a
Lindane	151±0.70 ^a	122±1.01 ^a	112±0.43 ^a	122±0.42 ^a
α -lipoic acid+ Irradiation	123±0.50 ^b	110±0.51 ^b	59±0.31 ^b	66±0.15 ^b
α -lipoic acid + Lindane	125±0.44 ^{cb}	107±0.39 ^{cb}	57±0.22 ^{cb}	63±.14 ^{cb}

a, significant difference in comparing with the control group.

b, significant difference in comparing with the irradiated group.

c, significant difference in comparing with the pesticide treated group.

Data are expressed as mean \pm SE of 6 rats in each group. Values are statically significant at $P < 0.05$.

Table II.- Antioxidant enzymes activity, glutathione content and lipid peroxidation levels in the rat's heart tissue in the different animal groups.

Animal groups	TBARS (nmol/mg protein)	GSH Px (nmol/mg protein)	GSH (nmol/mg protein)	CAT (nmol/mg protein)	SOD (nmol/mg protein)
Control	68±0.16	3.66±0.42	7.84±0.11	120±0.54	3.14±0.12
α -lipoic acid	66±0.15	3.85±0.37	8.64±0.89	122±0.31	2.95±0.09
Irradiated	97±3.62 ^{ac}	1.81±0.24 ^{ac}	2.91±0.21 ^{ac}	44±0.09 ^{ac}	0.89±0.24 ^{ac}
Lindane	82±0.30 ^{ab}	2.12±0.11 ^{ab}	4.86±0.22 ^{ab}	85±0.24 ^{ab}	1.24±0.85 ^{ab}
α -lipoic acid+ Irradiation	70±0.19 ^{bc}	2.93±0.12 ^b	7.51±0.15 ^{bc}	115±0.44 ^{bc}	2.78±0.15 ^{bc}
α -lipoic acid + Lindane	68±0.13 ^{bc}	3.03±0.12 ^{bc}	6.88±0.24 ^{bc}	118±0.29 ^{bc}	3.09±0.6 ^{bc}

For statistical detail see Table I.

Data are expressed as mean \pm SE of 6 rats in each group. Values are statically significant at $P < 0.05$.

Abbreviations: CAT, catalase; GSH, glutathione; GSH Px, glutathione peroxidase; SOD, superoxide dismutase, TBARS, thiobarbituric acid reactive substances.

Statistical analysis

The results are presented as Mean \pm Standard Error (SE) (n=6). Data were analyzed using ANOVA (one-way classification F-test) followed by Duncan (Multiple Range-test). P values were considered significant at $P < 0.05$.

RESULTS

The data obtained in the present study showed that supplementation of rats with α -LA daily for 21 days caused non-significant changes in the serum content of triglycerides, total cholesterol, phospholipids and free fatty acids, LDH, CPK and AST activities. Moreover, non significant changes were recorded in the cardiac TBARS, SOD, catalase and GPx activities (Tables I-III).

The data expressed in Table I showed that serum phospholipids, free fatty acids, total cholesterol and triglyceride levels were significantly increased ($P < 0.05$) in rats administered lindane or irradiated alone. Rats co-treated with α -LA along with lindane showed a

significant decrease ($P < 0.05$) in phospholipids content, free fatty acids, serum cholesterol and triglyceride levels compared to rats treated with lindane or irradiated alone.

The data expressed in Table II revealed that in addition to the classical mechanism of pesticides there is an excess production of free radicals demonstrated by the significant elevation in oxidative stress biomarker malondialdehyde (TBARS) associated with a significant decrease in the activity of antioxidant enzymes SOD, GSH-Px and CAT compared to their relative values in control rats. On the other hand, the consecutive supplementation with α -LA (21 days) to pesticides or radiation induced a significant reduction in heart TBARS level, while the activity of heart SOD, GSH-Px and CAT were increased compared to their respective values in rats not receiving α -LA.

The data expressed in Table III demonstrated that lindane or irradiated rats alone provoked heart toxicity demonstrated by significant increases in LDH, AST and CPK activities. The oral supplementation of α -LA (21 days) to pesticides or radiation induced a significant

reduction in heart TBARS level, while the activity of heart SOD, GSH-Px and CAT were increased compared to their respective values in rats not receiving α -LA.

Table III.- Serum creatine phosphokinase (CPK), aspartate aminotransferase (AST) and lactate dehydrogenase (LDH) activities in the different animal groups.

Animal groups	CPK (U/L)	LDH (U/L)	AST(U/L)
Control	94±9.52	65±0.28	22±0.46
α -lipoic acid	92±8.24	66±0.23	23±0.42
Irradiated	200±6.32a	140±0.40a	40±0.04a
Lindane	190±8.16a	132±0.51a	42±1.15a
α -lipoic acid + Irradiation	146 ±10.80ab	132±0.31a	33±0.07abc
α -lipoic acid + Lindane	132±8.19abc	73±0.33bc	29±0.04abc

For statistical detail see Table I.

Data are expressed as mean±SE of 6 rats in each group. Values are statically significant at $P<0.05$.

DISCUSSION

Lindane, an insecticide, is widely used as a pesticide in agriculture and ectoparasites in human and veterinary medicine. Widespread use of pesticides in agriculture has increased the number of intoxications in mammals. One of the natural molecules known to prevent or retard oxidation is α -LA and, therefore, α -LA/DHLA redox couple has received considerable attention (Marangon *et al.*, 1999). α -LA is readily absorbed from diet and also crosses the blood-brain barrier (BBB) (Harnett *et al.*, 2001).

Heart tissue is rich in polyunsaturated fatty acids and is known for its high oxygen consumption. In addition, the heart has relatively lower levels of antioxidant enzyme activity than the majority of other tissues. Therefore, it is more susceptible to oxidative stress than other tissues. This is because per-oxidative damage of membrane lipids leads to many damages in a cell such as decreases in membrane fluidity, elevated sensitivity to oxidant stress and changes in enzyme activities (Rozenberg *et al.*, 2006). Therefore, the important consequences of chronic stress could be attributed to stress-induced lipid peroxidation.

Lindane is classified by the WHO as moderately hazardous. Human exposure to ionizing radiation has become inevitable with its vast application in diagnosis and industry. Lindane and γ -radiation enhances oxidative stress by interacting with the cell membrane, triggering the generation of reactive oxygen species (ROS) and altering the level of antioxidant molecules. Thus causes

severe physiological dysfunction in various organ systems (Starkov *et al.*, 2004; Bano and Bhatt, 2007). Lindane induced membrane perturbation, causes functional impairment in blood brain barrier, altered glutathione homeostasis and alteration in cytochrome P450 mono-oxygenase enzymes (Abhilash and Singh, 2009).

Cytosolic enzymes CPK, LDH and AST which serve as the diagnostic markers leak out from the damaged tissue to blood stream when cell membrane becomes permeable or rupture. The amount of these cellular enzymes in serum reflects the alterations in plasma membrane integrity and/or permeability. Elevation of these enzymes in the serum has been reported to indicate cellular damage, tissue necrosis, as well as higher risk for cardiovascular diseases, and elevated myocardial infarction (Ioannou *et al.*, 2006).

Hyperlipidemia, including hyper-cholesterolemia and hyper-triglyceridemia, is a major risk factor for the development of cardiovascular diseases (Makni *et al.*, 2008). In the present study, radiation and lindane treatment induce hyperlipidemia and heart toxicity. Hyperlipidemic rats showed significant increase in serum total cholesterol, triglyceride, free fatty acids and phospholipids levels while heart toxicity manifested by significant increases in CPK, AST and LDH activity.

In the present study, there was a pronounced increase in serum cardiac enzymes in rats submitted to radiation and lindane which indicate the severity of damage to myocardial membrane. The high serum activity of LDH, AST and CPK may be attributed to the alterations in dynamic permeability of membranes due to peroxidation (Gaw *et al.*, 2001) or may be attributed to the damage in the heart muscle, rendering the leakage of enzymes into the serum (Brodie *et al.*, 2003). Also, an increased enzyme activity is an indication of the severity of the necrotic damage to myocardial membrane. The amount of cardiac enzymes in serum is proportional to the number of necrotic cells (Geetha and Sanker, 1990). Earlier reports suggested that these enhanced levels of specific marker enzymes might be due to enhanced susceptibility of heart cell membrane to the irradiation and lindane mediated peroxidative damage (Ananya *et al.*, 2005).

Lipids are one of the most susceptible targets of free radicals (Watson, 2006). The hyperlipidaemic state observed in the present study could be attributed to the mobilization of fats from the adipose tissue to the blood stream (Chajek- Shaul *et al.*, 1989), in addition to mitochondrial dysfunction (Madamanchi and Runge, 2007). The increase of cholesterol might be attributed to lipid peroxidation causing damage to the LDL-receptor that mediates the endocytosis of LDL-c as a result,

clearance of LDL-c from the circulation decreases, causing elevated blood cholesterol (Gent and Braakman, 2004). Increased triglycerides after stress might result from inhibition of lipoprotein lipase activity leading to reduction in uptake of triglycerides by adipose cells (Sedlakova *et al.*, 1998). The liver is particularly important in the synthesis and regulation of circulating lipids so the hyperlipidemia state in the present study might be attributed to hepatotoxicity which lindane accumulates in the adipose tissue surrounding the visceral organs which includes liver, muscle and heart (Sharma *et al.*, 2010). In addition, hyperlipidemia can induce oxidative stress in liver (Bolkent *et al.*, 2005).

Treatment of hyperlipidemia disorders can be achieved through diet and/or drug administration (Grundy *et al.*, 2004). Treatment with α -LA to stress rats (radiation and lindane) caused a significant decrease in the serum hyperlipidemic state when compared with stress group. This may be explained on the basis that α -LA increasing the transfer of blood cholesterol to be used in bile synthesis and thus, biliary excretion of cholesterol or bile acids is increased resulting in reduced availability of cholesterol to be incorporated into lipoproteins (An *et al.*, 1997) or might be related to the increase rate of lipolysis by increase of plasma lipase activity at the same periods of decrease plasma triacylglycerols (Bennani-Kabchi *et al.*, 2000).

Also, in the present study, radiation and lindane treatment have provoked oxidative damage in heart tissues demonstrated by significant decreases in the activity of heart SOD, GSH-Px and CAT, while the level of TBARS was increased as compared to the control group. The treatment of rats with α -LA showed an increase in the activity of antioxidant enzymes with a decrease in lipid peroxidation. In accordance with the earlier studies (Arivazhagan *et al.*, 2000; Sahin *et al.*, 2006), α -LA has been observed to be highly effective in protecting stress-induced lipid peroxidation.

Lipid peroxidation is a marker of cellular oxidative damage initiated by ROS. Lindane and γ -radiation enhances oxidative stress by interacting with the cell membrane, triggering the generation of ROS. Also, the result of hyperlipidemia state, recorded in the present study, is consistent to that have been reported previously by Wang *et al.* (2014) who reported that hypercholesterolemia could increase the cholesterol content of platelets, polymorphonuclear leukocytes and endothelial cells; lead to the formation of oxygen free radicals; and accelerate the process of lipid peroxidation and altering the level of antioxidant molecules. Thus causes severe physiological dysfunction in various organ systems (Starkov *et al.*, 2004; Bano and Bhatt, 2007).

In the present study, α -LA administered to rats via

gavages during lindane and radiation period, significantly attenuated radiation-induced oxidative stress in the heart. The decrease of TBARS suggests its free radical scavenging activities. Furthermore, the significant amelioration of SOD, GSHPx and CAT activity suggest its potential effect in enhancing antioxidant defense.

LA is readily absorbed from the diet and across cell membranes. In the cell, LA converts to its reduced form DHLA, a compound that also possesses biological activities (Goraca *et al.*, 2011). α -LA significantly decreased the malondialdehyde (MDA) level via interaction with vitamin C and glutathione (Packer *et al.*, 1995). As relatively small molecule, α -LA antioxidant properties have been reported to be via indirect increase in intracellular and hepatic ascorbate level (Michels *et al.*, 2003) or due to thiol groups of LA and DHLA make them capable of scavenging a variety of ROS (Biewenga *et al.*, 1997). LA and DHLA have been shown to cause regeneration of endogenous antioxidants such as vitamins C and E, and GSH, chelation of metal ions and repair of oxidatively damaged proteins (Bast and Haenen, 2003; Biliska and Wlodek, 2005). Although the precise mechanisms are not completely defined, there is also some evidence that LA exerts lipid-lowering effects (Carrier and Rideout, 2013).

CONCLUSION

In conclusion, α -LA has shown a significant ameliorative value in counteracting IR and lindane induced heart toxicity in rats via scavenging free radicals as well as enhancing the antioxidant system. Because of its ability to act as an antioxidant in fat- and water-soluble tissues in both its oxidized and reduced forms, LA could be used as a potentially effective therapeutic agent in clinical conditions associated with free radical damage.

Statement of conflict of interest

Authors have declared no conflict of interest.

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