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BIOCHEMICAL STUDIES ON LIPOIC ACID IN RELATION TO AGING

Omayma, A.R.[a], **Dowidar, M.F.**[b] and **Dina, M.I.**[a]

[a] *Biochemistry Department, Fac. Vet. Med., Benha University,* [b] *Biochemistry Department, Fac. Vet. Med., Zagazig University,*

A B S T R A C T

This study was carried out to spot the light on the effectiveness of lipoic acid as a powerful antioxidant in delaying aging process and its possible defense role by reducing the oxidative damage of free radicals. Experiments were applied in 60 rats which divided in to: young; 4-month-old (n=30) and aged; 24-month-old (n=30) groups. Animals were subdivided into four groups, each of which consisted of 15 animals. Group I; young control rats, Group II; young rats supplemented with lipoic acid, Group III; aged control rats, and Group IV; aged rats supplemented with lipoic acid. Lipoic acid was administered at a dose of 40mg/kg for 30 days. Liver samples were obtained to estimate the activity of some hepatic antioxidant enzymes as (SOD, CAT, GSHPX and GSHR). Results revealed that lipoic Acid induced a significant increase of hepatic antioxidant enzymes in young and aged rats in comparison with control groups.

KEY WORDS: Antioxidant enzymes, Lipoic acid, Oxidative damage, Rat

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1. I N T R O D U C T I O N

The free radical theory of aging hypothesizes that oxygen-derived free radicals are responsible for the age-related damage at the cellular and tissue levels. In a normal situation, a balanced-equilibrium exists among oxidants, antioxidants and biomolecules [4]. Co-supplementation of lipoic acid has a beneficial effect in reversing the age-related abnormalities seen in aging. This effect was associated with the decrease in free radical production and rise in antioxidant levels by lipoic acid, thereby lowering oxidative stress [2].

Oxidative stress is one of the factors responsible for the damaging effect of free oxygen radicals in cells and tissue [3]. Alpha-lipoic acid has been called a "universal antioxidant" because it is both water- and fat-soluble, and thus can penetrate tissues composed mainly of fat, such as the nervous system, as well as those made mainly of water, such as the heart, to protect them from free-radical damage. Alpha-lipoic acid also helps the body use other antioxidants, such as vitamin E, vitamin C, and glutathione, more efficiently [22].

The antioxidant defenses consist of low molecular mass antioxidants such as vitamin C and vitamin E and enzymes e.g. SOD, CAT and GSH-px, their function is to act as a coordinated and balanced system to protect tissues and body fluids from damage by ROS/RNS/RCS whether produced physiological or as response to inflammation, infection or disease [5].

Aging significantly decreased levels of the antioxidant enzymes as super oxide dismutase (SOD) and concurrent treatment with alpha lipoic acid significantly reversed the oxidative effects related to aging [15].

Catalase works closely with superoxide dismutase to prevent free radical damage to the body. SOD converts the dangerous

superoxide radical to hydrogen peroxide, which catalase converts to harmless water and oxygen. Catalases are some of the most efficient enzymes found in cells; each catalase molecule can convert millions of hydrogen peroxide molecules every second [16].

Oxygen free radicals have been proposed to be involved in the process of aging. Catalase (CAT) are important for anti-oxidative defense, where the activity of catalase and its mRNA level which investigated in hepatic tissues increased with age and exhibited higher levels at 6 and 12 months but decreased thereafter, that due to the increase of oxidative potential and the loss of proper antioxidant defense in the rats appear to be highly involved in the aging process. But after supplementation with lipoic acid it showed a highly significantly increasing in both the activity and gene expression of catalase enzyme [12].

The activities of GPx and GR were found to be decreased in aged rats when compared with young rats. Supplementation of lipoic acid to aged rats significantly increased the GSH levels thereby increasing the activity of GPx, GR, and G6PDH in liver of aged rats. In conclusion, so suggests that supplementation of lipoic acid to aged rats improves the glutathione redox system [17].

Glutathione peroxidase (GSH-px) catalyzes the reduction of hydrogen peroxide to water and oxygen at the expense of glutathione (GSH). Therefore the increase in GSH-px activity indicates that more oxidized glutathione (GSSH) is reduced to GSH. This recycling of glutathione is hypothesized to be due to ALA [15].

2. MATERIAL AND METHODS

2.1. *Material*:

2.1.1. *Animals*:

2.1.1.1. *Animal selection and grouping*:

Sixty male albino rats were used in the present experiment and divided in to four groups according to age:

Group (A): (4-month-old) young control (n=15).

Group (B): (4-month-old) young rats supplemented with lipoic acid (n=15).

Group (C): (24-month-old) aged control (n=15).

Group (D): (24-month-old) aged rats supplemented with lipoic acid (n=15).

2.1.1.2. *Animal management and housing*

Animals were housed in cages at 24±2ºC, under a 12:12h light –dark cycle, and provided with free access of food and water. Animals from all groups were kept under similar environmental conditions of temperature, illumination, acoustic noise, and ventilation, and received the same diet during the course of the experiment. Food and water were kept in special open containers in cages. Cleaning and changing water and food was done for all animals twice daily.

2.1.2. *Dosage*:

Lipoic acid (40 mg/kg body weight/day) dissolved in physiological saline were administrated orally for 30 days. Control animals received physiological saline alone.

2.1.3. *Sampling*

A part used for preparation of tissue homogenate with 0.9% saline using electrical homogenizer, centrifuged at 3000 rpm for 15 minutes, the resulting supernatant were collected and used for estimation of antioxidant enzymes activities as Super oxide dismutase, catalase, glutathione peroxidase and glutathione reductase [25]. Livers from animals were preserved at -20ºC until performing the investigations.

2.2. *Methods*:

2.2.1. *Determination of superoxide dismutase activity in liver homogenate*.

Hepatic superoxide dismutase was determined spectrophotometerically using ready-made kits [21].

2.2.2. *Determination of catalase activity in liver homogenate*.
Hepatic catalase was determined spectrophotometerically using ready-made kits [1].

2.2.3. *Determination of glutathione peroxidase activity in liver homogenate*.
Hepatic glutathione peroxidase was determined spectrophotometerically using ready-made kits [7].

2.2.4. *Determination of glutathione reductase activity in liver homogenate*.
Hepatic glutathione reductase was determined spectrophotometerically using ready-made kits [23].

2.3. *Statistical analysis*
The results are expressed as mean (±S.D.). Differences between groups were assessed by one-way ANOVA analysis using the SPSS software package for windows.

3. RESULTS

From the data presented in table 1 and fig.1, it is noticed that the hepatic superoxide dismutase (SOD) enzyme activity was significantly increased in young rats that administrated Lipoic Acid compared with the young control group and other groups, also there was a significantly increased in aged rats that administrated Lipoic Acid compared with the aged control group, which indicates that the administration of Lipoic Acid to rats leads to increase of hepatic Superoxide dismutase SOD.
From the data presented in table 2 and fig. 2, it is noticed that the hepatic Catalase (CAT) enzyme activity was significantly increased in young rats that administrated Lipoic Acid compared with the young control group and other groups, also there was a significantly increased in aged rats that administrated Lipoic Acid compared with the aged control group, which indicates that the administration of Lipoic Acid to rats leads to increase of hepatic Catalase CAT.
From data shown in table 3 and fig. 3, it is noticed that the hepatic Glutathione Peroxidase (GPX) enzyme activity was significantly increased in young rats that administrated Lipoic Acid compared with the young control group and other groups,

Table 1 Effect of lipoic acid on Superoxide dismutase (SOD) activity in young and aged rat liver (U/gm tissue)

Group	SOD activity (U/gm tissue) in rats liver		
	n	Mean± S.E	Rang.
Control (young)	15	2.90±0.24	2.66-3.15
Lipoic acid(young)	15	3.54±0.14	3.40-3.69
Control(Aged)	15	1.99±0.41	1.58-2.41
Lipoic acid(Aged)	15	2.51±0.30	2.21-2.81

Means within the same row carrying different superscripts are significant at ($p \leq 0.05$).

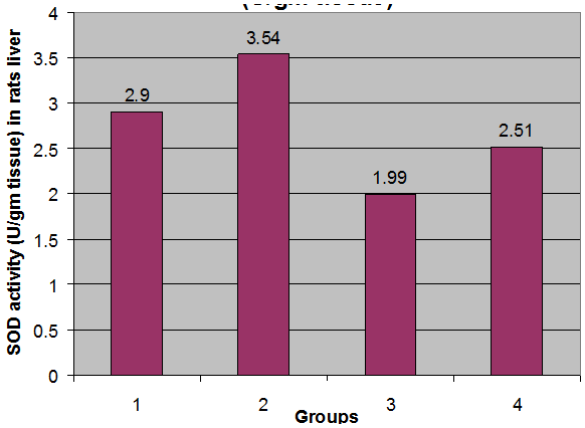

Fig. 1 Effect of lipoic acid on Superoxide dismutase (SOD) activity in young and aged rat liver (U/gm tissue)

Table 2 Effect of lipoic acid on Catalase (CAT) activity in young and aged rat liver (U/g tissue)

	CAT activity (U/g tissue) in rats liver			
	n	Mini.	Max.	Mean± S.E
Control (young)	15	0.77	0.84	0.80±0.035
Lipoic Acid(young)	15	0.84	0.96	0.90±0.06
Control (Aged)	15	0.65	0.74	0.69±0.045
Lipoic Acid (Aged)	15	0.74	0.82	0.78±0.04

Means within the same row carrying different superscripts are significant at ($p \leq 0.05$).

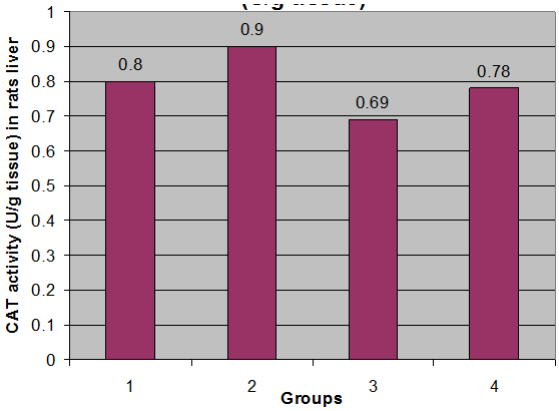

Fig. 2 Effect of lipoic acid on Catalase (CAT) activity in young and aged rat liver (U/g tissue)

Also there was a significantly increased in aged rats that administrated Lipoic Acid compared with the aged control group, which indicates that the administration of Lipoic Acid to rats leads to increase of hepatic Glutathione Peroxidase GPX.

Hepatic Glutathione Reductase (GRD) enzyme activity was significantly increased in young rats that administrated Lipoic Acid compared with the young control group and other groups (table 4 and fig. 4). Also there was a significantly increased in aged rats that administrated Lipoic Acid compared with the aged control group, which indicates that the administration of Lipoic Acid to rats leads to increase of hepatic Glutathione Reductase GRD.

4. DISCUSSION

Hepatic antioxidant enzymes (Superoxide dismutase, Catalase, Glutathione peroxidase and Glutathione reductase) activities were significantly increased after administration of Lipoic Acid for 30 days in young and aged rats in comparison with control groups. Administration of DL-α-lipoic acid, a thiol antioxidant to the aged rats, led to a time-dependent reduction in hydroxyl radicals and elevation in the activities/level of glutathione systems. Hence it can be suggested that lipoate, a dithiol prevents the oxidation of reduced glutathione and protects its related enzymes from peroxidative damage [9].

Table 3 Effect of lipoic acid on Glutathione Peroxidase (GPX) activity in young and aged rat liver (mU/ml)

Group	GPX activity (mU/ml) in rats liver			
	N	Mini.	Max.	Mean± S.E
Control (young)	15	77.80	116.72	97.26±19.46
Lipoic Acid (young)	15	116.72	175.08	145.9±29.18
Control (Aged)	15	19.45	58.36	38.90±19.45
Lipoic Acid (Aged)	15	58.36	97.26	77.81±19.45

Means within the same row carrying different superscripts are significant at ($p \leq 0.05$).

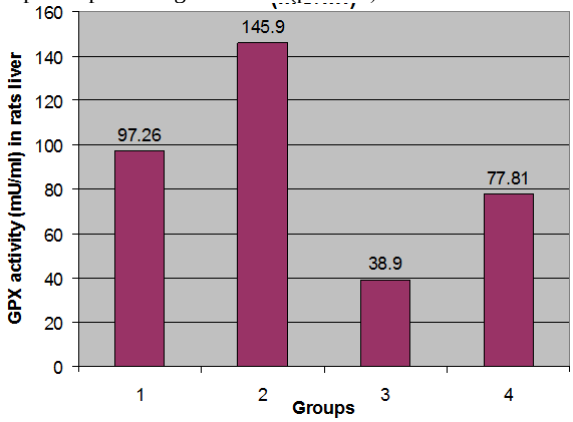

Fig. 3 Effect of lipoic acid on Glutathione Peroxidase (GPX) activity in young and aged rat liver (mU/ml)

Table 4 Effect of lipoic acid on Glutathione Reductase (GRD) activity in young and aged rat liver (U/L)

Group	GRD activity (U/L) in rats liver			
	N	Mini.	Max.	Mean± S.E
Control (young)	15	60.28	76.36	68.32±8.04
Lipoic Acid (young)	15	80.38	92.40	86.39±6.01
Control (Aged)	15	32.15	56.26	44.20±12.05
Lipoic Acid (Aged)	15	52.24	64.34	58.29±6.05

Means within the same row carrying different superscripts are significant at ($p \leq 0.05$).

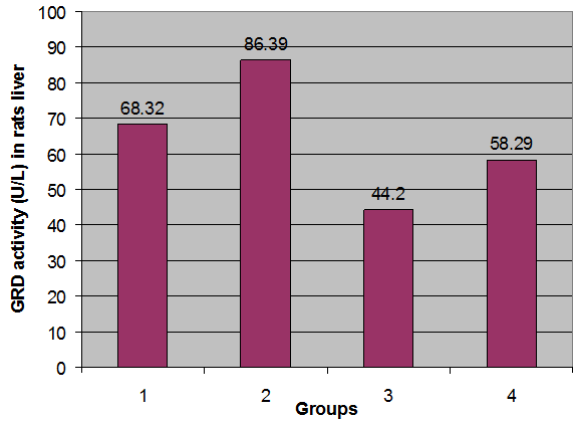

Fig. 4 Effect of lipoic acid on Glutathione Reductase (GRD) activity in young and aged rat liver (U/L)

During aging, mitochondria decay rats of oxidant production increase and oxidative damage to important biomolcules increase and may in part be responsible for aging as well as age-associated degenerative diseases such as cancer and atherosclerosis. It is important to understand whether the cellular distribution and bioavailability of key antioxidants have become altered with age [26]. By aging which results in increasing in free radical activity in living organisms which leads to form of excessive amounts of active oxygen forms. They induce damage to DNA, change enzymes activity, gene expression, and affect membrane structure and function [14].

Moreover, DL-α-lipoic acid treated aged rats showed a decrease in the level of lipid peroxides and an increase in the antioxidant status. The results of this study provide evidence that DL-α-lipoic acid treatment can improve antioxidants during aging and minimize the age-associated disorders in which free radicals are the major cause [27].

Oxygen is an essential component of living organisms. The generation of reactive oxygen species such as superoxide anion, hydrogen peroxide, hydroxyl radicals, and singlet oxygen is inevitable in aerobic metabolism of the body. Reactive oxygen species cause lipid oxidation, protein oxidation, DNA strand break and base modification, and modulation of gene expression. The body protects itself from the potential damages of reactive oxygen species. Its first line of defense is superoxide dismutase, glutathione peroxidase, and catalase. Scientists have indicated that antioxidant as lipoic acid supplied from daily diets quench the reactive oxygen species or are required as cofactors for antioxidant enzymes. Lipoic acid plays significant roles in the prevention of a number of age-related diseases and is essential for healthy aging [24].

The antioxidant enzymes are those including glutathione redox cycle (glutathione peroxidase, glutathione reductase) as well as SOD and catalase. All these enzymes have been regarded as the primary defense system against oxidative stress; they exert their work by eliminating reactive oxygen species and other hydroperoxides during normal cellular metabolism [6, 18].

In endogenous antioxidant systems SOD is widely distributed and plays a critical role within mammalian organism. SOD has a pivotal role against damaging effect from superoxide radical [24].

Superoxide dismutase (SOD) and glutathione peroxidase (GPx) are important enzymes in the body. SOD catalyses the conversion of superoxide free radical to hydrogen peroxide and water. GPx continues where SOD leaves off by catalyzing the reduction of hydrogen peroxide to water at the expense of glutathione. Previous studies have shown that aging reduces the activity of SOD and GPx rendering the body more vulnerable to oxidative damage [15].

A decrease in SOD activity results in accumulation of superoxide anion radicals in blood and liver. And we can conclude that a decrease in SOD activity may be due to aging effect [16].

Oxidative stress is a possible aging-accelerating factor. During the aging process, tissues are damaged to some extent due to the oxidative processes primarily caused by reactive oxygen species. In particular, superoxide anion radicals are believed to be the major cause for such oxidative damages of living tissues. Among various antioxidative mechanisms in the body, SOD is thought to be one of the major enzymes which protects against tissue damage caused by the potentially cytotoxic reactivates of radicals. It is therefore possible that the decreases in SOD activities with age may further accelerate the aging process [20].

Hydrogen superoxide is then eliminated with CAT. As hydrogen superoxide is a product of SOD study it is also a strong inhibitor of this enzyme. As CAT was the

first antioxidant. Enzyme to be characterized and catalyses the two stages conversion of hydrogen peroxide to water and oxygen and sharing this function with GSH-px [25].

Glutathione is the most abundant non thiol protein in the mammalian cells [10].It plays a vital role in annihilating O_2 toxicity by interrupting the reaction lead to formation O_2^- in its reduced form it metabolizes H_2O_2 and OH^- [13]. As glutathione plays an important role in intracellular protection against the toxic compounds, ROS and other free radicals also glutathione protects liver microsomes against ROS [25].

The activity of antioxidant enzyme superoxide dismutase (SOD) was highly significantly increased in hepatic tissue of young and aged rats that administrated lipoic acid when compared with the young and aged control groups. Super oxide dismutase activity in liver was increased in aged rats after treatment with α-lipoic acid, which has been shown to have substantial antioxidant properties [2].

Superoxide dismutase (SOD) activity was determined in liver at two different age groups (4 months; 24 months) and it was founded that, the activity of superoxide dismutase (SOD) decrease significantly for the aged liver, but after administration of lipoic acid, the activity of SOD increased significantly. The results indicate that, the liver antioxidative defense decrease with age but its reversible after treatment with lipoic acid as effective antioxidant [19] due to the fact that superoxide dismutase (SOD) has an important role in free radical detoxification of the liver, the age-related decrease in the activity of these enzymes might predispose this tissue to increased free radical damage, but after treatment with lipoic acid it noticed that, there was a significantly increased in activity of SOD enzyme [20].

The activity of antioxidant enzyme catalase (CAT) was highly significantly increased in hepatic tissue of young rats that administrated lipoic acid when compared with the young control group also there was a significantly increased in aged rats hepatic tissues after supplemented on lipoic acid when compared with control aged group. The decrease in enzymatic activity of catalase (CAT) was related to aging decrease and the genetic expression of this enzyme. On the other hand, the activity of this enzyme was brought back to normal levels with co-administration of ALA [15].

The activity antioxidant enzyme glutathione peroxidase (GSH-px) was highly significantly increased in hepatic tissue of young and aged rats that administrated lipoic acid when compared with the control groups. There was a significant reduction in the activity levels of antioxidants as glutathione peroxidase (GSH-px) in both middle-aged and aged rats which is reversible with co-supplementation of lipoid acid which improved the antioxidant status by increasing the activity and gene expression of glutathione peroxidase (GSH-px) [28].

Several studies provided evidence that α-lipoic acid supplementation decreases oxidative stress and restores reduced levels of other antioxidants *in vivo*. However, there is also evidence indicating that α-lipoic acid and dihydrolipoic acid may exert prooxidant properties *in vitro*. A-lipoic acid and dihydrolipoic acid were shown to promote the production of glutathione peroxidase (GSH-px) in rat liver mitochondria [8].

The activity of antioxidant enzyme glutathione reductase (GSH-R) was highly significantly increased in hepatic tissue of young rats that administrated lipoic acid when compared with the young control group also there was a significantly increased in aged rats hepatic tissues after supplemented on lipoic acid when compared with control aged group. In aged rats, activities of glutathione reductase (GSH-R) and the level of glutathione were low, whereas the level of hydroxyl radical was higher than in the young ones. Administration of DL-α-lipoic acid, a thiol

antioxidant to the aged rats, led to a time-dependent reduction in hydroxyl radicals and elevation in the activities/level of glutathione systems and antioxidant enzymes as glutathione reductase (GSH-R). Hence it can be suggested that lipoate, a dithiol prevents the oxidation of reduced glutathione and protects its related enzymes from per-oxidative damage [11].

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مجلة بنها للعلوم الطبية البيطرية
BENHA UNIVERSITY
FACULTY OF VETERINARY MEDICINE

الدراسات الكيميائية على حمض الليبويك وعلاقته بالتقدم فى العمر

اميمة ابو زيد[1]، محمد دويدار[2]، دينا محمد محمد إسماعيل[1]

قسم الكيمياء الحيوية– كلية الطب البيطرى–جامعة بنها، [2] قسم الكيمياء الحيوية– كلية الطب البيطرى–جامعة الزقازيق

الملخص العربى

أجرى هدا البحث لإلقاء الضوء على الدور الفعال لحمض الليبويك باعتباره من مضادات الأكسدة وما له من تأثير قوى فى تأجيل عملية التقدم فى العمر وكدلك دوره الدفاعى المحتمل فى التقليل من عملية الأكسدة الضارة وتراكم عناصر الأكسدة بالخلايا. تم استخدام عدد 60 فأر حيث تم تقسيمهم إلى 30 فأر عمر حديث(4 أشهر) و30 فأر متقدم فى العمر (24شهر)، ثم تم تقسيمهم إلى 4 مجموعات. المجموعة الأولى: 15 فأر عمر حديث (4شهور) تم تغديتها على حمض اليبويك 40 مجم/كجم لمدة30 يوم. المجموعة الثانية: 15 فأر عمر حديث (4شهور) مجموعة ضابطة. المجموعة الثالثة: 15 فأر متقدمة فى العمر (24 شهر) تم تغديتها على حمض اليبويك 40 مجم/كجم لمدة 30 يوم. المجموعة الرابعة: 15 فأر متقدمة فى العمر (24 شهر) مجموعة ضابطة. تم أخد عينات من الكبد لدراسة نشاطية بعض الأنزيمات المضادة للأكسدة مثل: سوبر أوكسيد ديزميوتيز، كتاليز، جلوتاثيون بيرواوكسيديز، جلوتاثيون ريداكتيز. وكان تأثير حمض الليبويك واضحا على الأنزيمات من خلال زيادة نشاطية تلك الأنزيمات فى المجموعات التى تناولت حمض الليبويك سواء فى المجموعة الأولى دات العمر الحديث او المجموعة الثالثة المتقدمة فى العمر بالمقارنة بالنتائج التى حصلنا عليها بالمجموعات الضابطة.

(مجلة بنها للعلوم الطبية البيطرية: عدد 22 (2)، ديسمبر 2011: 221– 229)