

Effect of Diet supplementation with Alpha-lipoic acid and brewer's yeast on kidney and liver functions on diabetic rats

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ABSTRACT

Alpha-lipoic acid is an ideal antioxidant used in both the treatment and prevention of various oxidative stress related to diseases. *This study aims to* evaluate the efficiency of alpha-lipoic acid in different doses and their natural animal sources (brewer's yeast) in diabetic rats. Thirty rats weighing approximately 210 ± 10 g were divided into five groups after injection with alloxan diabetic rats were fed on a basal diet, alpha-lipoic acid in different doses and 15% of brewer's yeast. At the end of the experimental period (30 days), the results show that supplementation with 30 and 60 mg /kg B.W/day of alpha-lipoic acid, and 15% of brewer's yeast caused a significant increase in food intake compared with the positive control group. Data show a significant decrease in the level of blood glucose after supplementation with 30 and 60 mg /kg B.W/day of alpha-lipoic acid and brewer's yeast as compared with untreated groups, and a positive correlation between increasing the dose of alpha-lipoic acid and decreasing in blood glucose levels. The levels of these parameters of AST, ALT, and ALP increased in diabetic rats while supplemented with alpha-lipoic acid (30 and 60 mg /kg B.W/day) reduced significantly the levels of AST, ALT and ALP, there was a positive correlation between increasing the dose of alpha-lipoic acid and the decreasing effect. Also, brewer's yeast induced the same reduction effect in these enzymes. Significantly decreased in uric acid, urea nitrogen, and creatinine concentrations for diabetic rats, which supplemented with alpha-lipoic acid (30 and 60 mg /kg B.W/day). Also, the same resulted observed in the group fed on brewer's yeast. Significant increase in reduced superoxide dismutase (SOD) and glutathione (GSH) after supplementation with 30 and 60 mg /kg B.W/day of alpha-lipoic acid for diabetic rats. Brewer's yeast, as sources of alpha-lipoic acid, induced the same effect on GSH, SOD and MDA levels.

CONCLUSION: Diet Supplementation with Alpha-lipoic acid, significant decreased the liver and kidney enzyme activities in diabetic rats.

Keywords: alpha-lipoic acid, Brewer's yeast, diabetic rats, kidney, liver

INTRODUCTION

Diabetes mellitus is a chronic metabolic disorder characterized by fasting hyperglycemia and plasma glucose levels above defined limits during an oral glucose tolerance test. Besides, it is caused by a total lack of insulin “type 1, insulin-dependent diabetes mellitus” or by insulin resistance in the presence of normal elevated plasma insulin level, decreasing the sensitivity or responsiveness tissue to insulin level “type 2, non-insulin-dependent diabetes mellitus” (Birchtmod et al., 2009). Oxidative stress it observed widely in diabetes. Diabetic patients have increased levels of lipid peroxidation products and decreasing levels of protective antioxidants including ascorbic acid, α -tocopherol, and reduced GSH. (Nourooz et al., 2010 and, Bostecea et al., 2012). Alpha-lipoic acid rapidly stimulates glucose uptake by activating the insulin pathway (Winiarska et al., 2017).

Brewer's yeast contains all the essential amino acids, 14 minerals, and 17 vitamins. It is one of the best natural sources of B-complex vitamins. It is also high in minerals including chromium, zinc, iron, phosphorus, and selenium (Yaworsky et al., 2011). Brewer's yeast considers a good source of RNA, an immune-enhancing nucleic acid that maybe help to prevent degenerative diseases and slowing the process. (Griffith, 2014). Therefore, this research aimed to evaluate the effects of orally given Alpha-lipoic acid on different levels and feeding on brewer's yeast (as an animal source) on liver and kidney functions on diabetic rats.

MATERIAL AND METHODS

Materials:

Alpha-lipoic acid was purchased from Sigma Chemical Co. (St. Louis, Mo, USA). Cellulose, Alloxan, Casein, Choline chloride, minerals mixture, DL-methionine, and vitamin mixture obtained from Morgan Co. Cairo, Egypt, and chemical kits used in this study (ALT, ALP, AST, Cr, UN, and uric acid) were purchased from El-Gomhoria Company for chemicals and Drugs, Cairo, Egypt.

Methods:

Experimental design

Thirty Sprague -Dawley Strain male albino rats weighing approximately (310 ± 15 g) used in this study according to **Canadian Council on Animal Care**

(1993). All rats fed the control diet (basal diet) according to (Campbell, 1961) for one week. Rats divided into five groups n= 6 rats with a similar total body. **The first group** (*control negative*) fed on the basal diet, **second group** (control positive) fed on the basal diet, **third group** (diabetic rats) supplemented with (60mg / alpha-lipoic acid /kg B.W/day.), **fourth group** (diabetic rats) supplemented with (30mg/ alpha-lipoic acid/kg B.W/day), **Fifth Group** (diabetic rats) supplemented with 15% of brewer's yeast on basal diet .

Induction of experimental diabetes:

Diabetes millets induced in normal healthy rats via intraperitoneal injection of alloxan 125 mg/kg body weight according to (Desai and Bhide, 1999). After six hours of injection with alloxan, fasting blood samples were collected to estimate serum glucose. Rats considered diabetic with a fasting blood glucose level above 200 mg/dl and used in this study.

Biochemical analysis:

Alkaline phosphatase (ALP), Alanine aminotransferase (ALT), and Aspartate aminotransferase (AST) enzymes measured by the methods described by (Varley et al., 1980 & Kachmar and Moss., 1976), respectively. The superoxide dismutase (SOD) activity was measured by the method of (Marklund; 1974 & Croce and Dunn; 2004). Glutathione peroxidase enzyme (GSH) and malonaldehyde (MDA) were determined according to the methods described by (Rose; 1997). Serum glucose determined by using chemical kits according to (Trinder, 1969). Creatinine was determined according to the kinetic method (Henry, 1974). Urea determined according to the enzymatic method (Petton and Crouch, 1977). Uric Acid determined by an enzymatic colorimetric test using kits according to (While et al., 1979).

Statistical Analysis:

The results were analyzed by using the program (SPSS, 2011), version (18). ANOVA test was used to compare results among groups and $P < 0.05$ was considered to be significant (Artimage and Berry, 1987).

Results and Discussion.

Effect of Diet supplementation with alpha-lipoic acid, brewer's yeast on food intake and body weight gain is present in Table (1).

The results showed a reduction in body weight gain for diabetic control as compared with control negative group and the mean values were -21.43 ± 0.65 and $+18.93 \pm 1.16$ g, respectively. The results of body weight gain indicated that, no significant differences between the effect of treated groups which were supplemented with alpha-lipoic acid at (30 & 60 mg /kg B.W/day) and brewer's yeast it was significant as compared with diabetic control rats. These data agree well with (Nourooz et al., 2010) who reported that Alpha-lipoic acid reduced body weight and increased whole-body energy expenditure.

Table (1): Effect of Diet supplementation with different levels of alpha-lipoic acid, brewer's yeast on food intake and body weight gain (mean+SD).

Groups	Initial body weight (g)	Final body weight (g)	Body weight gain (g)	Final intake (g/day)
Control negative	334.42±18.47 ^a	352.35±19.07 ^a	+18.93±1.16 ^a	18.4±1.09 ^a
Control positive	335.32±3.72 ^a	313.86±4.28 ^b	-21.43±0.65 ^b	15.6±0.61 ^b
Diabetic +LA 30 mg/kg	337.54±7.07 ^a	300.30±5.82 ^{bc}	-34.24±4.47 ^c	21.1±1.61 ^a
Diabetic +LA 60 mg/kg	331.65±6.03 ^a	282.03±1.53 ^c	-48.62±5.28 ^c	23.3±2.13 ^a
Diabetic +BY	333.76±7.74 ^a	301.98±4.06 ^{bc}	-34.78±3.76 ^c	20.9±2.61 ^a
LSD	16.32	15.35	6.22	3.04

(LA) α -lipoic acid, (BY) brewer's yeast (P<0.05)

Effect of Diet supplementation with alpha-lipoic acid, and brewer's yeast on blood glucose levels in Table (2).

The data illustrated that the level of blood glucose was low in control negative group (healthy rats) as compared with all diabetic groups at zero time. As well as, after two weeks, the experimental diabetic rats supplemented with alpha-lipoic acid at 30 and 60 mg /kg B.W/day were caused a significant decreased in the levels of blood glucose which the mean values were 217.52 ± 6.36 and 205.71 ± 3.67 mg/dl. Also, there were decreased values of blood glucose in diabetic which treated with brewer's yeast were 247.10 ± 3.10

mg/dl. At the end of the experiment, there was a positive correlation between this reduction in the levels of blood glucose and the period of supplementation. The levels of blood glucose in diabetic rats supplemented with alpha-lipoic acid at 30 and 60 mg/kg B.W/day, brewer's yeast were 172.58 ± 2.74 , 151.12 ± 2.90 , and 182.55 ± 3.68 mg/dl, respectively. These results agree well with the data of (Sacob *et al.*, 2015) who demonstrated that alpha-lipoic acid administration in humans with type II diabetes enhanced insulin-stimulated whole-body glucose disposal by about 50%. Winiarska *et al.*, (2017) show that the low doses of alpha-lipoic acid (10 mg/kg body weight) did not affect serum glucose concentration. Ferreira *et al.*, (2016) illustrated that a significant decrease in the glucose of serum for diabetic rats administered with 12% of yeast *Saccharomyces*.

Table (2):Effect of Diet supplementation with different levels of alpha-lipoic acid, and brewer's yeast on blood glucose levels (mean±SD).

Groups	Glucose (mg/dl)			
	Zero time	After 2 weeks	After 3 weeks	At the end of experiment
Control negative	144.76 ± 3.52^b	141.34 ± 4.33^f	142.67 ± 3.77^f	142.03 ± 4.79^f
Control positive	288.95 ± 1.24^a	287.35 ± 5.23^a	284.62 ± 3.55^a	285.34 ± 2.57^a
Diabetic +LA 30 mg/kg	290.74 ± 1.91^a	217.52 ± 6.36^d	185.30 ± 4.76^d	172.58 ± 2.74^d
Diabetic +LA 60 mg/kg	288.30 ± 9.50^a	205.71 ± 3.67^c	177.36 ± 5.34^c	151.12 ± 2.90^c
Diabetic +BY	290.26 ± 5.65^a	246.10 ± 3.11^c	212.64 ± 2.78^c	182.55 ± 3.68^c
LSD	8.84	7.77	6.81	5.64

(LA) α -lipoic acid, (BY) brewer's yeast

Least significant differences at $P \leq 0.05$.

Means with the same letter are insignificantly difference.

Effect of Diet supplementation with alpha-lipoic acid, and brewer's yeast on AST, ALT, and ALP are represented in Table (3).

Injected rats with alloxan induced elevation in levels of ALT and the value was 42.57 ± 2.28 U/L as compared with other groups. Supplemented with 60 mg/kg B.W/day of alpha-lipoic acid induced a significant decrease in the levels of ALT compared with diabetic control rats by mean value 22.51 ± 1.22 U/L. These results agree with **Huong and Ide, (2017)** who reported that Alpha-lipoic acid has a positive effect in reducing the levels of AST, ALT and ALP in rats.

Table (3): Effect of Diet supplementation with different levels of alpha -lipoic acid, and brewer's yeast on AST, ALT and ALP (mean+SD).

Groups	AST(U/ L)	ALT(U/ L)	ALP(U/ L)
Control negative	57.45 ± 6.63^{bc}	32.29 ± 2.33^b	58.71 ± 3.67^b
Control positive	75.09 ± 1.62^a	42.57 ± 2.28^a	68.34 ± 4.28^a
Diabetic +LA 30 mg/kg	60.35 ± 2.70^b	28.87 ± 3.56^b	51.25 ± 3.85^c
Diabetic +LA 60 mg/kg	46.27 ± 2.93^d	22.51 ± 1.22^c	42.91 ± 3.68^d
Diabetic +BY	53.60 ± 3.73^{bcd}	31.04 ± 2.08^b	54.36 ± 1.36^{bc}
LSD	6.46	3.99	5.97

(LA) α -lipoic acid, (BY) brewer's yeast

Least significant differences at $P \leq 0.05$.

Means with the same letter are insignificantly difference.

Effect of Diet supplementation with alpha-lipoic acid, and brewer's yeast on uric acid, urea nitrogen and creatinine are given in Table (4)

The data revealed that there was a significant increase in uric acid levels for diabetic rats compare with all other treated groups with a value of 5.26 ± 0.34 mg/dl. After supplementation with alpha-lipoic acid at 30 and 60 mg/kg B.W/day and brewer's yeast, decrease the mean values to 3.76 ± 0.19 , 2.64 ± 0.18 , and 4.06 ± 0.21 mg/dl, respectively as compared with diabetic control rats, the lowest value belonged to the group supplemented with a high dose of alpha-lipoic acid (60 mg/kg B.W/day). Supplementation with alpha-lipoic acid at 30 and 60 mg/kg B.W/day, brewer's yeast significantly decreased in the concentration of urea nitrogen as compared with untreated group. As results demonstrated that, the highest value of creatinine levels recorded for diabetic

control, in which the mean value was 2.07 ± 0.17 mg/dl. There was a significant decrease in creatinine levels, that appeared, after supplementation with alpha-lipoic acid at 30 mg /kg B.W/day, and brewer's yeast recorded mean values 1.6 ± 0.22 and 1.44 ± 0.08 mg/dl, respectively. The highest reduction, which was 0.94 ± 0.17 mg/dl, belonged to diabetic group supplemented with alpha-lipoic acid at 60 mg /kg B.W/day. It could be seen from the previous data that supplementation with alpha-lipoic acid (especially the high dose) caused a marked decline in uric acid, urea nitrogen, and creatinine levels which increased in diabetic rats. Similarly, earlier reports have shown that alpha-lipoic acid is a naturally occurring that has potent antioxidative activity in a wide variety of experimental systems and clinically used to treat diabetic neuropathy (Biewenga et al., 2015). Also, Winiarska et al., (2017) reported that supplementation with a-lipoic acid decreased the serum urea and creatinine concentrations.

Table (4): Effect of Diet supplementation with different levels of alpha -lipoic acid, and brewer's yeast on uric acid, urea nitrogen and creatinine (mean+SD).

Groups	uric acid (mg/dl)	urea nitrogen(mg/dl)	Creatinine(mg/dl)
Control negative	3.81 ± 0.15^b	64.39 ± 3.96^b	1.56 ± 0.12^b
Control positive	5.26 ± 0.34^a	71.44 ± 1.97^a	2.07 ± 0.17^a
Diabetic +LA 30 mg/kg	3.76 ± 0.19^b	58.27 ± 1.50^c	1.6 ± 0.22^{bc}
Diabetic +LA 60 mg/kg	2.64 ± 0.18^d	48.19 ± 1.63^d	0.94 ± 0.17^d
Diabetic +BY	4.06 ± 0.21^b	60.45 ± 2.88^{bc}	1.44 ± 0.08^{bc}
LSD	0.36	4.19	0.27

(LA) α -lipoic acid, (BY) brewer's yeast

Least significant differences at $P \leq 0.05$.

Means with the same letter are insignificantly difference.

Effect of Diet supplementation with alpha -lipoic acid, and brewer's yeast on GSH, MDA, and SOD in Table (5).

The data illustrated that there was a significant increase in the levels of Glutathione peroxidase enzyme (GSH) in diabetic groups supplemented with

alpha-lipoic acid with 30 and 60 mg/kg B.W/day and brewer's yeast as compared to diabetic group. It could be observed that supplementation with alpha-lipoic acid (especially the high dose) has a positive effect on GSH and The superoxide dismutase (SOD) levels by increasing their levels in diabetic rats, which has the lowest values of them, wherein it decreased : malonaldehyde (MDA) levels as it increased in diabetic rats. Brewer's yeast induced the same effect on GSH, SOD, and MDA. These results agree with (**vukkarasu and Anuradha., 2014**) found that the activity of SOD enzymatic antioxidants was significantly lowered in fructose-fed rats (has elevation level in blood glucose) meanwhile treatment of fructose-fed rats with alpha-lipoic acid was significantly increased in SOD levels.

Table (5): Effect of Diet supplementation with different levels of alpha -lipoic acid, and brewer's yeast on GSH, MDA, and SOD in diabetic rats (mean+SD).

Groups	GSH (mg/dl)	MDA(n mol/ml)	SOD (unit /ml)
Control negative	32.22±2.35 ^d	25.91±2.21 ^b	26.42±4.68 ^c
Control positive	26.48±1.61 ^c	31.65±3.83 ^a	23.20±2.27 ^d
Diabetic +LA 30 mg/kg	56.23±2.44 ^c	22.10±1.84 ^{bc}	36.04±2.41 ^b
Diabetic +LA 60 mg/kg	68.36±3.18 ^a	16.44±0,58 ^d	44.49±1.88 ^a
Diabetic +BY	59.04±2.11 ^c	22.81±1.79 ^{bed}	35.14±0.84 ^b
LSD	3.98	3.86	4.53

(LA) alpha -lipoic acid, (BY) brewer's yeast

(GSH): reduced glutathione.

(MDA): malondialdehyde.

(SOD): superoxide dismutase.

(LA) α -lipoic acid, (BY) brewer's yeast

Least significant differences at $P \leq 0.05$.

Means with the same letter are insignificantly difference.

CONCLUSION

To summarize, in this study; the high doses of alpha-lipoic acid significantly lowered the liver enzymes ALT, AST and ALP activity, it also lowered uric acid, urea nitrogen, and creatinine, but significantly increase in the levels of GSH, and SOD, brewer's yeast give the less effective than high doses of alpha-lipoic acid. From this study, it can be concluded that alpha-lipoic acid may protect liver and kidney damage in diabetic.

Ethics approval and consent to participate.

All experiments were carried out according to recommendations of the ethical conditions approved by the Ethics Committee of Ophthalmology Research Center Giza, Egypt, of Experimental Animals, which conformed to the international ethics for handling and care of experimental animals according to the Canadian Council on Animal Care "CCAC" (1993).

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الملخص العربي

تأثير الوجبة المدعمة بحامض الالفا ليبويك والخميرة المجففة على وظائف الكبد والكلى في الفئران المصابة بالسكري

يعتبر حامض الالفا ليبويك من مضادات الأكسدة المستخدمة حالياً في العلاج والوقاية من مختلف الأمراض المتعلقة بزيادة الشقوق الحرة كالشيخوخة ومرض السكر، ولذلك تهدف هذه الدراسة إلى التعرف على التأثير الذي يقوم به حامض الالفا ليبويك بجرعات مختلفة وكذلك الخميرة المجففة كمصدر حيواني غني به على الفئران المصابة بالسكري، حيث تم استخدام ٣٠ فأر من ذكور الألبينو تصل أوزانهم 20 ± 310 جم وتم تقسيم الفئران إلى خمس مجموعات وتم الحقن بالالوكسان ثم تغذية الفئران المصابة بالسكري على الوجبة الاساسية + تركيبات مختلفة من حمض الالفا ليبويك (٦٠، ٣٠) ملجم /كجم من وزن الجسم/اليوم، وعلى الخميرة المجففة كمصدر حيواني غني بالالفا ليبويك (١٥%) استمرت التجربة لمدة (٣٠ يوم) وفي نهاية تلك الفترة تم حساب الزيادة في وزن الفئران وكمية الطعام المتناول لديهم حيث أظهرت النتائج أن التدعيم بحامض الالفا ليبويك بتركيزاته المختلفة وكذلك الخميرة المجففة يؤدي إلى زيادة معنوية في كمية الطعام المتناول مقارنة بالمجموعة الضابطة المصابة بالسكري. أظهرت النتائج حدوث انخفاض معنوي في مستوى جلوكوز الدم عند الفئران المصابة والمعالجة وكان الانخفاض الاكبر في استخدام جرعات مرتفعة من حامض الالفا ليبويك (٦٠ ملجم /كجم من وزن الجسم/اليوم ، كما لوحظ ارتفاع في مستوى نشاط إنزيمات الألانين أمينوترانس فيريز (ALT) والأسبارتات أمينوترانس فيريز (AST) وكذلك الألكالين فوسفاتيز (ALP) في الفئران المصابة بالسكري. بينما أظهرت النتائج انخفاضاً معنوياً في نشاط هذه الإنزيمات في الفئران المدعومه بحامض الالفا ليبويك كما وجد أن زيادة المتناول من حامض الالفالبيويك (٦٠ ملجم/كجم من وزن الجسم/اليوم) يؤدي لزيادة هذا الإنخفاض. كذلك كانت التغذية على الخميره المجففة التأثير الخافض لهذه الإنزيمات. أشارت النتائج إلى حدوث انخفاض معنوي في مستويات اليوريا والكرياتينين وحمض اليوريك في الفئران المصابة بالسكري والمدعمة بحامض الالفا ليبويك (٣٠ و ٦٠ ملجم/كجم من وزن الجسم/اليوم). كذلك التغذية على الخميره المجففة أظهرت أيضاً تأثيرها الخافض لمستويات اليوريا والكرياتينين وحمض اليوريك كما تبين وجود زيادة معنوية في مستويات الجلوتاثيون (GSH) وسوبر أوكسيد دسميوتيز (SOD) في الفئران المصابة بالسكر المدعمة بحامض الالفا ليبويك (٣٠ و ٦٠ ملجم/كجم من وزن الجسم/اليوم) مقارنة بالإنخفاض الملحوظ في مستوياتهم للمجموعات الضابطة الغير مدعمة. بينما أدى هذا التدعيم إلى إنخفاض معنوي في مستويات المالدنيد (MDA) التي كانت في زيادة ملحوظة في مجموعات الفئران الضابطة.

الخلاصة: أظهرت الوجبة المدعمة بحامض الالفا ليبويك أسد انخفاض معنوي في نشاط انزيمات الكبد والكلى في الفئران المصابة بداء السكري.