Pathological potential effects of Alpha-Lipoic acid in preventing cataract Diabetic retinopathy in albino male mice

Bassim M. Jwad¹, Anas. A. Humadi*,² Bushra I. AL-Kaisei³

¹ Department of pathology and poultry disease, College of Veterinary Medicine, University of Baghdad, Baghdad, Iraq.
² Department of pathology and poultry disease, College of Veterinary Medicine, University of Diyala, Diyala, Iraq.

Corresponding author*Email : anashumady@yahoo.com (Humadi)

ABSTRACT:-

Alpha-lipoic acid (α-LA) is prevention complication of diabetes mellitus its potent antioxidant by prevention diabetic retinopathy, 30 albino male mice randomly equally divided into 3 groups 1st group (control) , 2nd group daily injected for 7 days I/P (100)mg/kg streptozotocin, 3rd group daily injected for 7 days I/P (100) mg/kg streptozotocin with α-LA (400)mg/kg for 90 days, the fasting glucose concentration showed increased in 2nd group in 30 & 90 days, while the fasting insulin concentration and glutathione concentration showed decreased in 2nd group in 30 & 90 days, the most pathological lesions showed in 2nd group in 90 days included cataract, disappear and atrophy of retina layer, necrosis, increased eosinophilia lens epithelium and finally multiple atheromatous artery.

Key word: Diabetic retinopathy, Albino mice, Alpha-lipoic acid

How to cite this article: Jwad BM, Humadi AA, Al-Kaisei (2020): Pathological potential effects of alpha-lipoic acid in preventing cataract diabetic retinopathy in albino male mice, Ann Trop Med & Public Health; 23(S9): SP23915. DOI: http://doi.org/10.36295/ASRO.2020.23915

INTRODUCTION-

Diabetic retinopathy (DR) consider as chronic complications, most common occur in diabetes syndrome, and blindness in industrialization countries that occur after five years of diabetes mellitus type 1 and reach to about (60%) after 10 years with retinopathy disorder [1]. Retinopathy in early stages cause changes in vascular characterized by microvascular complications due to change in blood flow of retina and retinal changes [2]. These changes may included macular edema, oxidative stress and information have important role in the progression and development ocular complications [3, 4, 5]. Chronic hyperglycemia by increase regenerate Reactive Oxygen Species(ROS) mostly superoxide [6 and 7]. Inactivated glyceraldehyde-3-phosphate dehydrogenase enzymes (GAPDHE)of glycolysis leading to endothelial cells damage [8, 9 and 10]. Alpha lipoic acid (ALA or α-LA)known as (R)-5-(1,2-Dithiolan-3-yl)pentanoic acid, also called (Thioctic acid or 6,8-Dithiooctanoicacid),have formula (C₈H₁₄O₂S₂)it's reduced from (Dihydro-lipolic acid DHLA) and oxidized form (Alpha-lipoic acid) that present in animals and vegetable tissue especially Tomatoes, Broccoli and spinach [9]. Microangiopathy characterized mostly by alter the vasodilate ability ofcapillaries in injury response [11, 12]. ALA act as antioxidant effects and regenerate endogenous oxidized antioxidant [13].
MATERIALS AND METHODS

Experimental Animals: -

Populations of study composed of 30 albino male mice, age (8-11 week) with (25-30 gram) in weight, which obtained from department of biology, College of Science/University of Baghdad, Iraq. Housing in animals house department of Pathology at College of Veterinary Medicine/University of Baghdad, Iraq. All animals were divided equally after 15 days from adaptation, into three groups, 1st group represented as control group that includes (10 mice) were daily feeding normal mice pellets. 2nd group also include (10 mice) was known as (Diabetic group) were injected I/P with (100 mg/kg bw) as single dose from freshly Streptozotocin (STZ) for 7 days. While 3rd group contains (10 mice) represented as a (Diabetic and α-LA) group daily I/P injection single dose with (100 mg/kg bw) from freshly Streptozotocin (STZ) for 7 days with daily administration (400 mg/kg bw) lipoic acid via stomach tube for 90 day [ 14 ].

Blood Samples: -

Samples of blood were collected at day (30 and 90) of experiment, through blood drawn via cardiac puncher technique directly from mice heart after anesthesia by inhalation of ether, then put in class tube, and centrifuged for 15 minutes at 300 rpm., however serums isolated and keep in freezer in (− 20 °C) for parameters analysis. All parameters were done during (30 and 90 days) from experiment, whereas the histopathological examination for eye tissue samples were done at (day 90).

Biochemical Analysis: -

a) Fasting plasma glucose for (12 hours), and the Post Prandial Plasma glucose (PPG) (mg/dl) after three hours fasting feeding mice on normal pellets.

b) Fasting insulin (µU) level were determined by Enzyme Linked ImmunoSorbent Assay (ELISA) through use (DRG kit).

c) Serum glutathione concentration (SGC) (U/mg/Hb/min), via use especial kits and the analyzing reading was occur by using spectrophotometer at 412 nm.

Histopathological Examination: -

Histopathological examination of experimental animals were done by anesthetized albino male mice with chloroform and sacrificed by withdraw of blood from heart at days 90, eyes longitudinal opening made for histopathological technique, the eyes dislocated and excision by longitudinal under ethics protocol and fixation for 72 hours in neutral buffered formalin 10%, after that routine processing of eye tissue done according to [ 15 ].

Statistical analysis: -

All the grouped data were statistically read by SPSS program, Version 17 software (2010). Testing methods including one way ANOVA for comparisons among groups. P values of less than <0.05 were considered statistical significance. All data were expressed as means ± standard error (se) [ 16 ].

RESULTS:

http://doi.org/10.36295/ASRO.2020.239915
Biochemical Results:

Fasting blood glucose concentration (mg/dl) showed significant increased in diabetic group (2nd group) at day 30 and 90 (241.5 ± 77.19, 219.67 ± 65.74) respectively, when compared with diabetic and α-LA group and control group, while diabetic and α-LA group revealed significant decreased in blood glucose concentration (mg/dl) at day 30 and 90 (198.5 ± 43.90, 197.43 ± 46.81) respectively when compared with diabetic group, table (1).

Table (1): Changes in fasting blood glucose concentration (mg/dl) of diabetics and α-lipoic acid (α-LA) administrated mice.

<table>
<thead>
<tr>
<th>Group</th>
<th>Day 30</th>
<th>Day 90</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>165.50 ± 41.72</td>
<td>172.33 ± 44.75</td>
</tr>
<tr>
<td>Diabetics</td>
<td>241.5 ± 77.19</td>
<td>219.67 ± 65.74</td>
</tr>
<tr>
<td>Diabetics &amp; α-LA</td>
<td>198.5 ± 43.90</td>
<td>197.43 ± 46.81</td>
</tr>
</tbody>
</table>

Values are expressed as means±SE with different letters are significantly different ($P<0.05$).

Post-prandial glucose (mg/dl) indicated significant increase in diabetic group at day 30 and 90 (354.95 ± 60.17, 397.33 ± 65.68) when compared with diabetics and α-LA group and control group, while diabetics and α-LA group showed significant decreased in post-prandial glucose (mg/dl) at day 30 and 90 when compared with diabetic group (188.5 ± 63.90, 177.73 ± 63.87) respectively, table (2).

Table (2): Changes in post-prandial glucose (mg/dl) of diabetics and α-lipoic acid (α-LA) administrated mice.

<table>
<thead>
<tr>
<th>Group</th>
<th>Day 30</th>
<th>Day 90</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>210.40 ± 71.01</td>
<td>212.55 ± 71.70</td>
</tr>
</tbody>
</table>
Values are expressed as means±SE with different letters are significantly different ($P<0.05$). The changes in fasting plasma insulin concentration (μU) showed significantly increased in diabetics and α-LA group at day 30 and 90 (6.15 ± 3.59, 6.85 ± 3.90) when compared with diabetic group (5.30 ± 4.10, 5.30 ± 4.20) respectively, table (3).

Table (3): Changes in fasting plasma insulin concentration(μU) of diabetics and α-lipoic acid (α-LA) administrated mice.

<table>
<thead>
<tr>
<th>Group</th>
<th>Day 30</th>
<th>Day 90</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>8.05 ± 2.74</td>
<td>8.75 ± 4.15</td>
</tr>
<tr>
<td>Diabetics</td>
<td>5.30 ± 4.10</td>
<td>5.30 ± 4.20</td>
</tr>
<tr>
<td>Diabetics &amp; α-LA</td>
<td>6.15 ± 3.59</td>
<td>6.85 ± 3.90</td>
</tr>
</tbody>
</table>

Values are expressed as means±SE with different letters are significantly different ($P<0.05$). The changes in glutathione concentration (U/mg/Hb/min) showed significant decreased of diabetic group at day 30 and 90 (5.21 ± 4.13, 5.90 ± 4.28) respectively when compared with diabetics and α-LA group (6.00 ± 4.70, 6.65 ± 4.10) and control group, table (4).

Table (4): Changes in glutathione concentration(U/mg/Hb/min) of diabetics and α-lipoic acid (α-LA) administrated mice.
<table>
<thead>
<tr>
<th>Group</th>
<th>Control</th>
<th>Diabetics</th>
<th>Diabetics &amp; α-LA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>8.20 ± 2.74</td>
<td>5.21 ± 4.13</td>
<td>6.00 ± 4.70</td>
</tr>
<tr>
<td></td>
<td>A</td>
<td>C</td>
<td>B</td>
</tr>
<tr>
<td></td>
<td>8.85 ± 4.00</td>
<td>5.90 ± 4.28</td>
<td>6.65 ± 4.10</td>
</tr>
<tr>
<td></td>
<td>A</td>
<td>C</td>
<td>B</td>
</tr>
</tbody>
</table>

Values are expressed as means±SE with different letters are significantly different (P<0.05).

**Histopathological Changes :-**

The results of control group and (diabetics & α-LA) group recorded no important pathological lesion, while diabetics group at (day 90) showed cataract characterized by severe swelling of epithelial lining cells (Balloning space) with scattered the lens (figure1). In other sections showed disappear of retina layers and other nuclear layer associated with necrosis and increased eosinophilia of lens epithelium (figure 2 and 3). Atrophy of retinal layers with few disorganization ganglionic cells; also othersections of retina show hyperchromatic of ganglia cells and disorganization with dilated arteries mostly atherosclerotic lesion (figure4 and 5). From another section, area of diffuse irregular hemorrhage and retina hyperplasia with hyperchromatic neural cells (figure6). Other sections of retina show narrowing and atrophy with cystic dilation of epithelium (figure7 and 8). Hemosiderin pigment associated with mononuclear cells infiltration mostly lymphocytes and macrophages in retina (figure9). Thrombosis were recorded in large blood vessel that attached to optic blood vessels and calcified in optic area (figure10). As well as there is multiple atheromatous artery, characterized by thickening of tunica intima and media with narrowing of the lumen, pseudo capillary (B.V.) and thrombosis (cholesterol cleft) (figure11).
Figure 1: Histopathological section of eye show severe swelling of lens epithelium like balloons appearance (a) edema that scattered in lens (b) (X40 H&E stain).

Figure 2: Histopathological section of eye show retina layer were disappeared of outer nuclear layer (a) necrosis with increase eosinophilia of lens (b) (X40 H&E stain).
Figure 3: Histopathological section of eye show narrowing with absence of rod and corneal layers (a) cataract with necrosis dark eosinophilic lens epithelium (b) (X40 H&E stain).

Figure 4: Histopathological section of eye show retinal atrophy (a) disorganization of ganglion cells (b) (X40 H&E stain).
Figure 5: Histopathological section of eye show disorganization of retina with hyperchromatic ganglion cells (a), dilated a retina with atherosclerosis lesion (b) (X40 H&E stain).

Figure 6: Histopathological section of eye show retina with hyperplasia (a) hyperchromatic neural cells (b) extensive hemorrhage (c) (X40 H&E stain).
Figure 7: Histopathological section of eye show narrowing and atrophy of retina (a) cystic dilation (b) (X40 H&E stain).

Figure 8: Histopathological section of eye show necrosis of ganglion cells (a) neuronal edema (b) retina epithelium detachment (c) (X40 H&E stain).
Figure 9: Histopathological section of eye show severe atrophy (a) cystic dilation of retina (b) hemosiderin pigments (c) infiltration of MNCs mainly lymphocyte (d) (X40 H&E stain).

Figure 10: Histopathological section of eye show atherosclerosis with thrombosis (a) calcification (b) thickening of tunica intima (c) and media (d) infiltration of inflammatory cells (e) (X40 H&E stain).
Figure 11: Histopathological section of retina show thickening of tunica intima and media (a) narrowing of the lumen (b) pseudo blood vessels (c) thrombosis (d) (X40 H&E stain).

DISCUSSION:

Biochemical Analysis: -

The decreased in fasting glucose at diabetic and α-LA group because α-LA helps regulate glucose metabolism in insulin resistant animals [17, 18, 19] and α-LA effects stereoisomers on glucose metabolism in insulin resistant skeletal muscle and activation of some steps in insulin signaling pathway [20], also the α-LA works against insulin resistance by increasing the permeability of cell membrane which is decreased and prevents the uptake of glucose [21]. The high doses of alpha lipoic acid can improve glucose utilization in individuals with type 2 diabetes by increasing glucose uptake in skeletal muscles as well as by enhancing insulin stimulated glucose disposal [22, 23].

The α-LA increased glutathione concentration compared with diabetic and control groups because that α-LA have effectively chelate toxic substance (directly and indirectly) and strongly support the chelation of metals by its ability to increase glutathione level inside cells, also glutathione and its associated enzyme play important role in the ability of body to excrete. Wide variety of toxin from free radicals of diabetes, while the α-LA increase intrinsic activity of glutathione similarly to insulin and may medicated by P38 mitogen activated protein kinase [24 and 25].

Histopathological examination: -

Ocular damage within retina which recorded in diabetic group were occurrence as a result of oxidative stress of diabetes and severe inflammation play important role in prognosis of diabetic retinopathy[11, 12]. Retinal barriers damage mainly happen due to white blood cells attachment to vascular epithelium as a result of oxidative stress that's causing adhesion molecule-1 and adhesion intracellular molecule-1 [26, 27, 28]. Generates a reactive Oxygen Species (ROS) in retinal eyes tissue revealed high oxygen mainly superoxide, inactivated glyceraldehyde-3-phosphate dehydrogenase (G3PDH) important enzymes during diabetes[6, 5].
Diabetic and alpha lipoic acid group showed no clear histopathological lesion in eye, microangiopathy characterized by impaired ability to response to injury by vasodilated mostly due to thickening basement membrane causing endothelial dysfunction [5]. Alpha lipoic acid prevent retina and corneal impaired due to antioxidant action to plasma membrane by resentment endogenous antioxidant [2, 9]. From the other hand diabetic and alpha lipoic acid group have essential effects in mitochondrial bioenergetics reactions as antioxidant to managing diabetic retinopathy and vascular diabetes [29, 13].

**CONCLUSIONS**

Alpha-lipoic acid is naturally thiol and acting as biological antioxidant and can regulates the transcription of anti-inflammatory pathway and prevents cataract through treatment of diabetic complications.

**REFERENCES:**


