ACETYL-L-CARNITINE AND VITAMIN E PROTECT AGAINST INCREASED RISK OF CEREBRAL ATHEROSCLEROSIS AND HIGH BRAIN LIPIDS: BIOCHEMICAL AND HISTOCHEMICAL EXPERIMENTAL STUDY

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ABSTRACT

Triton is a non-ionic detergent, which produces hyperlipidemia via inhibition of lipoprotein lipase. Oxidative damage takes place in plasma lipids, mainly LDL, leading to atherosclerosis. Complications of atherosclerosis, including cerebral atherosclerosis, constitute a major cause of death. Based on the anti-oxidant and neuroprotective effects of acetyl-L-carnitine (ALC) and vitamin E, this study was designed to evaluate their protective role against triton-induced oxidative stress and atherosclerosis in the brain of adult male rats. The present study was performed on 50 male albino rats, weighed 150±20 g. The animals were randomly assigned to five groups of ten rats each. Group I was used as control. Group II was injected I.V. with triton. Group III was treated with triton and ALC (I.P.). Group IV was treated with triton and vitamin E (orally). Group V was treated with triton and combination of ALC and vitamin E. Triton induced atherosclerosis via increased serum total cholesterol, triacylglycerol, and LDL and total brain lipids. These effects were associated with decline in serum HDL level and total protein concentrations in the brain. Meanwhile, triton increased oxidative stress through an elevation in brain malondialdehyde (MDA) concentrations, associated with depletion of total thiol concentration, total antioxidant capacity and catalase activity. The use of ALC and
vitamin E attenuated the toxic effects of triton on lipid profile, and it improved the antioxidant defense system of the experimental rats. The protective effect of the combination, with its antioxidant and neuroprotective properties, gives additive advantage, which exceeds that of ALC or vitamin E alone.

**KEYWORDS:** triton; atherosclerosis; lipid peroxidation; antioxidant enzymes; vitamin E; acetyl-L-carnitine; brain.

**INTRODUCTION**
Atherosclerosis is a condition where the arteries become narrowed and hardened due to excessive buildup of plaques around their walls. Elevated plasma levels of cholesterol, especially low density lipoprotein (LDL) and triacylglycerol (TAG) associated with oxidative stress and lipid peroxidation are recognized as leading cause of atherosclerosis, stroke and coronary heart diseases.\(^1\)\(^,\)\(^2\) Complications of atherosclerosis constitute a foremost cause of death in many industrialized countries. The main complications of atherosclerosis are associated with occlusion or inadequate blood flow to organs perfused by the affected artery. Blood vessels sometimes become mechanically unstable and dilate, forming aneurysms. These aneurysms may favors the formation of blood clots that break off and occlude vessels downstream, or they may burst and bleed, which may be fatal.\(^3\) Furthermore, cerebral atherosclerosis can cause Alzheimer's disease, cerebral micro-bleeds, transient ischemic attacks and stroke.\(^4\)\(^,\)\(^5\)

Hypercholesterolemia may occur as a toxic response to certain synthetic detergents. The non-ionic detergent, triton WR 1339 (tyloxapol), by virtue of its ability to inhibit lipoprotein lipase, causes increased serum cholesterol and TAG.\(^6\) The levels of serum cholesterol and TAG produced by tyloxapol were very high in relation to cholesterol and oil supplemented diets, which have been commonly used to produce hyperlipidemia in rats.\(^7\)

Oxidative damage takes place in plasma LDL by hydroxyl radicals generated by the metal ions present in the serum due to the alterations in their oxidative states.\(^8\) Free radicals generated by endothelial cells of the arterial wall and activated macrophages are thought to oxidize LDL particles, making them chemo-tactic to attract monocytes.\(^9\)

Over the past decade, a large body of experimental and epidemiologic data has indicated that dietary antioxidants, such as vitamin E, might lessen the risk of atherosclerosis. Vitamin E,
mainly α-tocopherol, is the major fat-soluble antioxidant present in the LDL particle. On average, 5–9 vitamin E molecules are carried by each LDL particle and are believed to protect LDL from oxidative damage.\textsuperscript{10} In addition to the inhibition of oxidative modification of LDL, vitamin E has been identified newly as a constructive modulator of other atherogenic processes at molecular and cellular levels.\textsuperscript{11} As an antioxidant, vitamin E acts as a peroxyl radical scavenger, preventing the propagation of free radicals in tissues, by reacting with them to form a tocopheryl radical.\textsuperscript{12}

Acetyl-L-carnitine (ALC) is an amino acid that is naturally synthesized in the human brain, liver, and kidneys. It is an acetylated form of L-carnitine (LC), which delivers it in the blood by plasma esterases. In most tissues of the body, both LC and ALC are essential co-factors in fatty acid oxidation. Their major biochemical function is to facilitate the transport and metabolism of long-chain fatty acids into the mitochondria for beta-oxidation and energy generation.\textsuperscript{13,14} Acetyl-L-carnitine boosts energy by stimulating the body's burning of TAG as fuel, and sparing the supply of glycogen stored in the liver for heavier exertion.\textsuperscript{15} In the current study, we preferred using ALC to LC because of its powerful antioxidant effect for cell membranes and more efficient passage of the blood-brain barrier.\textsuperscript{16} Hence, it can better support cellular energy especially in the brain and helps prevent brain cell deterioration as a result of cerebral atherosclerosis.\textsuperscript{17} Also, we preferred using both of ALC and vitamin E to reduce the risk of using each one alone in large doses. High doses of vitamin E may be associated with increased risk of bleeding problems, while large doses of ALC may lead to restlessness and insomnia.\textsuperscript{14,18}

The aim of the work was to investigate the protective role of ALC, vitamin E and especially their combination against triton-induced oxidative stress and atherosclerosis in the brains of adult male rats.

**MATERIAL AND METHODS**

**Chemicals**

Triton WR 1339 (Tyloxapol) was purchased from Sigma-Aldrich Co., USA, while ALC was obtained from Mepaco Arab Co. for pharmaceuticals and medicinal plants, Egypt and vitamin E was obtained from EMA pharm, Egypt. All other used chemicals in the experiment were of analytical grade.
Animals
Fifty adult male albino rats were obtained from Animal Farm of the Egyptian Holding Company for Biological Products and Vaccines, Helwan, Egypt. The weights of rats were 150±20 g. Rats were housed in plastic cages and under standard conditions of temperature (23±2 °C) and lighting (12 h light/dark cycles), and they were allowed free access to food and drinking water.

Experimental design
The local ethical committee approved the design of the experiment in accordance with Principles of Laboratory Animal Care. The animals were randomly divided into 5 groups; each group contained 10 rats. The first group was used as control. The animals of Group I (Control group or C) were treated with the vehicles as they were injected with saline intraperitoneally (I.P.) and intravenously (I.V.) via the tail and were also treated orally with corn oil. Group II (triton-treated group or T) animals were injected I.V. with triton WR 1339 via their tails. Group III (triton and ALC-treated group or T+ALC) was treated I.V. with triton and I.P. with ALC. Group IV (triton and vitamin E-treated group or T+E) was treated I.V. with triton and orally with vitamin E. Group V (triton, ALC and vitamin E-treated group or T+ALC+E) was treated with triton and combination of ALC and vitamin E. The duration of the experiment was three successive weeks (three times/week; every other day). Triton was used in a dose of 500 mg/kg BW according to Luiz et al. [8] and ALC was used in a dose of 100 mg/kg BW according to Scafidi et al., [19] while vitamin E was used in a dose of 540 mg/kg BW according to Kailash et al. [20] After an overnight fast, the animals were euthanized, and a blood sample from each rat was collected from the eyes by retro-orbital puncture using blood capillary tubes. Brain samples were immediately washed in cold saline then stored at –20 °C until biochemical assays.

Assay of the biochemical parameters
Lipid profiles such as total cholesterol, TAG, and LDL were assayed using commercial kits (Spinreact, Santa Coloma, Spain). High-density lipoprotein (HDL) was assayed using a commercial kit (Human, Wiesbaden, Germany).

Total lipid content in the rats’ brains were assayed spectrophotometrically by the method described by Folch et al. [21] The brain total protein content of each sample was determined according to Banay-Schwartz et al. [22]
The brain lipid peroxidation end products (malondialdehyde) using was determined using the thiobarbituric acid method.[23]

The content of total thiol (TT) in the brain was determined according to Sedlak and Lindsay method.[24] The brain total antioxidant capacity (TAC) by ferric reducing antioxidant power was determined according to Benzie and Strain.[25] The activity of catalase enzyme was determined by the method of Xu et al.[26]

**Statistical analysis**
Data were expressed as mean ± SE. The comparisons of data were carried out using One Way Analysis of Variance (ANOVA) followed by Tukey-Kramer Multiple Comparisons Test. All analysis utilized SPSS 16.0 statistical package for Windows (SPSS Inc., Chicago, IL, USA). A probability level of less than 0.05 was accepted as statistically significant.

**RESULTS**

**Relative brain weight**
Table (1) shows the relative weight of the brain in the different tested groups. The relative brain weight of the triton-treated rats shows significant decrease compared to the control group. Animals given a combination of ALC and vitamin E show a significant increase in their brain weights.

**Serum lipids**
Serum cholesterol, TAG and LDL increased significantly in triton-treated group, as shown in Table (2). Administration of ALC to triton-treated animals was associated with significant reduction in the levels of the above mentioned parameters, an effect that was more pronounced with administration of either vitamin E alone or a combination of ALC and vitamin E. On the contrary, serum levels of HDL were reduced significantly in triton-treated rats. This effect was partially antagonized in groups given either ALC, vitamin E or a combination of both.

**Brain lipids and proteins**
The brain total lipids increased significantly with triton administration to rats. The use of ALC, vitamin E or combined administration of both to triton-treated animals resulted in significant reduction of their brain total lipids. On the contrary, the brain total proteins were reduced significantly in rats overdosed with triton, but increased significantly when triton-
treated rats were given either ALC, vitamin E or a combination of both, approaching nearly the control levels (Table 3).

**Brain antioxidants**

The brain MDA in triton-treated rats increased significantly compared to the control. Reduction of the elevated MDA brain levels was noticed in groups given ALC, vitamin E or a combination of both besides triton treatment. Also, triton-induced reduction in brain TT was partially corrected through administration of either ALC, vitamin E or an ALC/vitamin E combination. Animals treated with triton besides a combination of ALC and vitamin E showed an elevated brain TAC above the control levels. Administration of triton alone to the animals was associated with a significant reduction in brain TAC compared to the control animals. Significant reduction in brain catalase was seen in triton-treated group. While administration of either ALC or vitamin E induced an elevation of the reduced levels of brain catalase, the most pronounced effect was noticed in animals given ALC/vitamin E combination (Table 4).

**Table 1. Relative brain weight (g/100 g body weight) of male rats treated with triton (T), acetyl-L-carnitine (ALC), vitamin E (E) and their combination.**

<table>
<thead>
<tr>
<th>Organs weight</th>
<th>Control</th>
<th>T</th>
<th>T + ALC</th>
<th>T + E</th>
<th>T + ALC + E</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brain</td>
<td>0.7 ± 0.01</td>
<td>0.57 ± 0.04*</td>
<td>0.64 ± 0.03</td>
<td>0.69 ± 0.02</td>
<td>0.86 ± 0.03*</td>
</tr>
</tbody>
</table>

T: Triton; ALC: acetyl-L-carnitine; E: Vitamin E

*Significant.

**Table 2. Serum lipid profile parameters of male rats treated with triton (T), acetyl-L-carnitine (ALC), vitamin E (E) and their combination.**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>T</th>
<th>T + ALC</th>
<th>T + E</th>
<th>T + ALC + E</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol (mg/dl)</td>
<td>94.7 ± 0.9</td>
<td>630.7 ± 3.2*</td>
<td>305.9 ± 3.2*</td>
<td>280.9 ± 1.8*</td>
<td>269.6 ± 1.5*</td>
</tr>
<tr>
<td>Triacylglycerol (mg/dl)</td>
<td>53.5 ± 1.0</td>
<td>1205.5±8.6*</td>
<td>666.3 ± 3.4*</td>
<td>382.3 ± 5.6*</td>
<td>279.1 ± 2.8*</td>
</tr>
<tr>
<td>HDL (mg/dl)</td>
<td>42.1 ± 0.7</td>
<td>22.9 ± 0.8*</td>
<td>31.0 ± 0.8*</td>
<td>34.6 ± 0.7*</td>
<td>34.7 ± 0.7*</td>
</tr>
<tr>
<td>LDL (mg/dl)</td>
<td>56.9 ± 07</td>
<td>459.6 ± 3.0*</td>
<td>293.3±2.1*</td>
<td>273.3 ± 1.5*</td>
<td>254.4 ± 1.5*</td>
</tr>
</tbody>
</table>

*Significant.

**Table 3. Brain total lipids and total proteins of male rats treated with triton (T), acetyl-L-carnitine (ALC), vitamin E (E) and their combination.**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>T</th>
<th>T + ALC</th>
<th>T + E</th>
<th>T + ALC + E</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total lipids (mg/g tissue)</td>
<td>62 ± 0.3</td>
<td>80 ± 1.0*</td>
<td>73 ± 0.3*</td>
<td>69 ± 0.6*</td>
<td>67 ± 0.6*</td>
</tr>
<tr>
<td>Total proteins (mg/g tissue)</td>
<td>182 ± 5.5</td>
<td>159 ± 1.5*</td>
<td>168 ± 1.5</td>
<td>169 ± 3.3</td>
<td>180 ± 4.2</td>
</tr>
</tbody>
</table>

*Significant.
Table 4. Brain antioxidant parameters of male rats treated with triton (T), acetyl-L-carnitine (ALC), vitamin E (E) and their combination.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>T</th>
<th>T + ALC</th>
<th>T + E</th>
<th>T + ALC + E</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDA (nmole/g tissue)</td>
<td>79 ± 2.2</td>
<td>160 ± 1.1*</td>
<td>153 ± 2.8*</td>
<td>111 ± 2.1*</td>
<td>98 ±4.8*</td>
</tr>
<tr>
<td>TT (mmole/g tissue)</td>
<td>3.9 ± 0.1</td>
<td>2 ± 0.1*</td>
<td>2.2 ± 0.15*</td>
<td>3.0 ± 0.12*</td>
<td>3.6 ± 0.17</td>
</tr>
<tr>
<td>TAC (µmol Fe^{2+}/g tissue)</td>
<td>1.9 ± 0.11</td>
<td>0.8 ± 0.07*</td>
<td>1.2 ± 0.01*</td>
<td>1.8 ± 0.07</td>
<td>2 ± 0.08</td>
</tr>
<tr>
<td>Catalase (mmole/min/g tissue)</td>
<td>49 ± 1.4</td>
<td>26 ± 1.0*</td>
<td>30 ± 1.6*</td>
<td>34 ± 1.7*</td>
<td>39 ± 0.9*</td>
</tr>
</tbody>
</table>

*Significant.

DISCUSSION

The results of the present study made clear the injurious effects of atherosclerosis on the brain through severe hyperlipidemia and oxidative stress. Additionally, it discusses the effective role of ALC, vitamin E and their combination against atherosclerosis and its complications.

Initially, the results of the current work showed that the relative brain weights were decreased in triton-treated animals when compared with the control. This may be attributed to decreased food intake, delay of gastric emptying or poor food palatability due to treatment-related toxicity.\cite{27} Furthermore, triton induces cerebral atherosclerosis and ischemia as well as oxidative stress, which may be responsible for the brain weight reduction.\cite{6,7} For this reason, treatment with antioxidants and free radical scavengers, vitamin E and/or ALC, can decrease the oxidative stress and improve the metabolic process of triton-treated rats. They improved rat food palatability, food intake and consequently the brain relative weight when compared with the control as shown in our results.

It is well known that triton WR1339 prevents lipolysis of TAG-rich lipoproteins by inhibiting lipoprotein lipase, and it blocks the removal of TAG from plasma.\cite{8} The previous consequence is compatible with our results where triton toxicity led to significant increase in the serum levels of cholesterol, TAG and LDL. Besides, the results showed significant decrease in serum HDL in T group compared to the control one, which was also declared by Korolenko et al.\cite{28}

The most important thing to be discussed is the great amelioration of the brain atherosclerosis toxic effects induced by triton via administration of ALC and vitamin E especially as a combination. In atherosclerotic condition the increased level of lipids is responsible for the
higher level of lipid peroxides, which accelerate the incorporation of LDL into arterial smooth muscle cells and the development of atheromatous plaques.\textsuperscript{[29]}

Our records demonstrated that, treatment with ALC and/or vitamin E reduced serum cholesterol, TAG and LDL concentrations when compared with T group, and it is in agreement with the results reported by earlier studies.\textsuperscript{[30-33]} The decreased level of TAG in rats overdosed with triton and supplemented with ALC can be attributed to a preferential stimulation of ALC on saturated fatty acid breakdown in peripheral tissues.\textsuperscript{[30,31]} In addition, according to Richard et al.,\textsuperscript{[34]} ALC administration prevented the development of early atherosclerotic lesions by increasing liver vitamin E, and decreasing the levels of plasma cholesterol and TAG. The study of Ghaffari and Ghiasvand\textsuperscript{[35]} demonstrated that increased dietary vitamin E lowers plasma LDL concentrations, had an inhibitory effect on early atherosclerosis, increased the lag phase of LDL oxidation and decreased the rate of LDL oxidation in the hypercholesterolemic hamsters. Moreover, vitamin E scavenges reactive oxygen species (ROS) and thereby inhibits the proliferative response, LDL oxidative modification, the uptake of modified LDL by macrophages, and their subsequent activation.\textsuperscript{[10, 36]}

Also, administration of ALC, vitamin E and their combination, is able to decrease the extension of the fatty deposits and macrophages infiltration in the arterial wall, which further highlights the role of the oxidative stress in the development of atherosclerosis.\textsuperscript{[20,36]} It is well known that ROS can damage cellular components such as proteins, lipids and DNA, resulting in cell apoptosis and death. Also, they react with membrane lipids, resulting in altered cell membrane fluidity and in the formation of end products, which attack proteins and DNA bases.\textsuperscript{[36]}

In the present study, ROS, probably generated by triton induction, provoked a rise of protein carbonyls products, which are markers of protein oxidative injury. All that explain the decrease of protein content of brain tissues in T group when compared with control one. Administration of ALC, vitamin E or their combination showed enhancement in total protein content, this is due to the role of them in preventing lipid peroxidation and protein oxidation induced by triton due to their antioxidant properties. These results were in agreement with those of Bazotte and Lopes-Bertolini.\textsuperscript{[37]}
It is well known that lipids in the brain are a major target of oxidative stress and MDA has been shown to be reliable markers of lipid peroxidation. Exploring the results of the current study revealed that the oxidative stress markers, catalase enzyme activities, MDA and total thiol concentrations, showed an aggressive oxidative stress in the brain of T group. The increased levels of the brain lipid peroxides in the atherosclerotic rats are in agreement with the results reported by Zhang et al. Administration of ALC, vitamin E or a combination of both attenuated the triton-induced increase in MDA concentration. Acetyl-L-carnitine has an active role in the transport of fatty acids for energy production, thereby lowering the availability of lipids for peroxidation, while vitamin E role may be related to the trapping of the chain-propagating peroxyl radicals.

Oxidative stress has been involved in the pathogenesis of several neurological diseases including cerebral atherosclerosis. Our results showed that supplementation with ALC to the triton-intoxicated rats caused improvement of the oxidative stress parameters. These results suggested that ALC has neuroprotective effect by improving the state of oxidative stress during ischemic injury caused by the triton-induced atherosclerotic changes. Previous studies demonstrated that superoxide dismutase (SOD) has a significant role in neuroprotection in nature. Acetyl-L-carnitine may help SOD stabilization and can lower age-related cholesterol levels by facilitating the transport of fatty acids into the mitochondria. Also, it enables dietary fats to be converted to energy and muscle. Moreover, ALC may be responsible for a reduction in brain glycolytic flow promoting utilization of alternative energy sources, such as lipid substrates or ketone bodies.

Several studies indicate other neuroprotective benefits of ALC, which may be due to its role in maintenance of cellular membrane stability. Furthermore, it improves metabolic function while decreasing oxidative stress, reducing apoptotic cell death and inhibiting the production of platelet-activating factor. This is confirmed by our results, which showed an elevation of total thiol concentration.

The main limitation of the study is that, in addition to the limited sample size, the current study was not designed to evaluate the concomitant pathological changes. Further studies are necessary in order to elucidate whether the current brain histochemical findings are associated with definite cerebral blood vessels histo-pathological changes.
CONCLUSION
The combined treatment by ALC and vitamin E showed a protective role against triton-induced brain atherosclerosis because of their power to correct hyperlipidemia and combat atheroma formation through their antioxidant and neuroprotective properties. The use of both agents revealed a synergistic combination. ALC was superior as a neuroprotective agent, while vitamin E took the upper hand in combating atherosclerosis as strong antioxidant agent. It would be worthwhile to investigate the effect of ALC and vitamin E supplementation especially in combination in a randomized clinical trial with a suitable number of patients.

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REFERENCES


