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Protective effects of alpha-lipoic acid and coenzyme Q10 on lipopolysaccharide-induced liver injury in rats

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ABSTRACT

Lipopolysaccharide (LPS) is a major cell wall component of gram-negative bacteria known to stimulate the synthesis and secretion of several toxic metabolites, such as reactive oxygen species and cytokines. In this study, the protective effect of alpha-lipoic acid (ALA) and coenzyme Q10 (CoQ10) were evaluated in LPS-induced hepatic injury in rats. To this end, male adult Sprague Dawley rats were divided into five groups; normal control, LPS control where rats were injected with an initial dose of LPS (4 mg/kg; i.p.) on the 1st day of the experiment followed by a challenging dose (2 mg/kg; i.p.) on the 8th day, ALA (50 mg/kg), CoQ10 (10 mg/kg) and ALA plus CoQ10. Treatments continued for 15 days and the last three groups also received LPS. At the end of the study, liver function tests, as well as interleukin-6 (IL-6) were estimated in serum. Liver lipid peroxides (MDA), reduced glutathione (GSH), superoxide dismutase (SOD) and total antioxidant capacity (TAC) were also assessed, in addition to histological examination of liver sections from all groups. The obtained data revealed that LPS markedly elevated activities of serum aminotransferases, alkaline phosphatase and gamma-glutamyl transferase, as well as, total bilirubin and interleukin-6 levels. LPS-treated rats showed an increase in MDA liver content versus decrease in GSH content, SOD activity and TAC. Oral administration of ALA, CoQ10 and their combination ameliorated LPS-induced increases in liver function enzymes and IL-6, coupled by hampering of oxidative biomarkers. This was supported by histopathological evaluation results. In conclusion, administration of ALA, CoQ10 and their combination improved pathological abnormalities in liver tissues and reversed the deleterious effects induced by LPS.

Key words: alpha-lipoic acid, co-enzyme Q10, hepatotoxicity, lipopolysaccharide, oxidative stress.

INTRODUCTION

All gram-negative bacteria have an asymmetric outer membrane, in which the inner and the outer leaflet are formed by phospholipid and lipopolysaccharide (LPS), respectively [1, 2]. LPS are glycolipids with an ability to incite a vigorous inflammatory response [3, 4]. In humans, nanograms of LPS injected into the blood stream can result in all the physiological manifestations of septic shock [5, 6]. LPS binds to proteins with subsequent activation of oxygen free radicals and pro-inflammatory cytokines [7]. The release of these toxic mediators is the contributing factor to most of LPS toxicity in liver and in the systemic circulation [8].

Alpha-lipoic acid (ALA) has multifunctional antioxidant, as well as, anti-inflammatory effects. Therapeutic potential of ALA has been reported in a variety of disorders, including diabetes mellitus, cardiovascular diseases, and cancers [9, 10, 11, 12, 13]. ALA is able to produce its antioxidant effect in aqueous or lipophilic environments. It presents a

highly negative reduction potential, increases the expression of antioxidant enzymes and participates in the recycling of vitamins C and E [14, 15]. Due to these properties, ALA is sometimes called the "universal antioxidant"[16].

Coenzyme Q10 (CoQ10), or ubiquinone, is considered one of the natural antioxidants that can be synthesized endogenously or supplied through food. It exists in the biological membranes of cellular organelles, such as peroxisomes and lysosomes, and is principally located in the inner mitochondrial membrane as part of the electron transport chain, which is responsible for adenosine triphosphate synthesis [17]. CoQ10 is considered a potent lipophilic antioxidant; it acts directly with free radicals or as a reducing agent for regenerating vitamins C and E from their oxidized forms [18]. CoQ10 inhibits the generation of reactive oxygen species (ROS) and lipid peroxidation products [19]. In addition, it exhibits anti-inflammatory properties by reducing the release of pro-inflammatory cytokines [20].

The present study aimed to investigate the hepatic response due to LPS injection and to investigate the possible hepatoprotective effect of ALA and CoQ10 in this model of liver injury.

MATERIALS AND METHODS

1. Animals:
Male Sprague-Dawley rats (weighing 150-200 g) were obtained from the animal house of National Organization for Drug Control and Research (NODCAR, Cairo, Egypt). Rats were kept under standardized conditions throughout the period of investigation (23 ± 1ºC, 55 ± 5 % humidity and a 12-h light: 12-h dark cycle). They were fed standard pellet chow and maintained with free access to water. Animals' procedures were performed in accordance with the Ethics Committee of Faculty of Pharmacy, Cairo University (PT 1388).

2. Drugs:
Lipopolysaccharides, from Escherichia coli, serotype O55:B5 was purchased from Sigma-Aldrich (Germany), ALA was obtained from Eva Pharma (Cairo, Egypt), and CoQ10 was obtained from MEPACO-MEDIFOOD, Arab Company for Pharmaceutical and Medicinal plants (Cairo, Egypt).

3. Experimental design:
Male Sprague-Dawley rats were randomly allocated into five groups, 7 rats each, and treated as follows: Group 1received 0.9 % NaCl (normal control group),groups 2-5 received initial dose of LPS (4 mg/kg; i.p.) on the 1stday followed by a challenging dose (2 mg/kg; i.p.) on the 8thday.Group 2served as LPS control group meanwhile, groups 3-5 received ALA (50 mg/kg), CoQ10 (10 mg/kg)and ALA plusCoQ10, respectively p.o. for 15 days.

4. Preparation of blood samples and tissue homogenate:
Twenty- four hours after the last treatment, blood samples were withdrawn from the retro-orbital vein of each animal, under light ether anesthesia, according to the method of [21]. Blood was allowed to coagulate and then centrifuged at 3000 rpm for 15min. The obtained serum was used for estimation of the chosen biochemical markers. Immediately after blood sampling, all animals were sacrificed by cervical dislocation and the liver tissues were rapidly removed, washed in ice-cooled saline, plotted dry and weighed. The left lobe of each liver was dissected and placed in 10% formalin in saline to be used for histopathological examination. A weighed part of each right lobe was homogenized in ice-cooled saline to prepare 20% w/v homogenateand then centrifuged at 4000 rpm for 5 min at 4°C using a cooling centrifuge. The obtained supernatant was divided into several aliquots for estimation of the chosen parameters.

5. Biochemical markers:
5.1. Determination of serum liver function markers:
Determination of serum activities of AST, ALT and ALP, as well as, TB level were carried out using test reagent kits provided by Biodiagnostic (Cairo, Egypt).Determination of serum GGT activity was carried out using a test reagent kit provided by Chronolab (Barcelona, Spain).

5.2. Determination of serum IL-6 level:
The rat IL-6 ELISA kit Quantikine ® (USA) was used for the estimation of serum IL-6.

5.3. Determination of liver oxidative stress parameters:
Liver MDA content was determined according to the method described by [22]. Meanwhile, liver GSH content was determined using Ellman's reagent according to the method described by [23]. Liver SOD activity was determined according to the method of [24]. Meanwhile, liver TAC was determined according to the method specified by [25].

6. Histopathological studies:
Liver specimens from all animals were dissected immediately after death, and fixed in 10% neutral-buffered formal saline for at least 72 hours. All the specimens were washed in tap water for half an hour and then dehydrated in ascending grades of alcohol, cleared in xylene and embedded in paraffin. Serial sections of 6 µm thick were cut and stained with haematoxylin and eosin [26] for histopathological investigation. Images were captured and processed using Adobe Photoshop version 8.0.

7. Statistical analysis:
In the present study, all results were expressed as mean ± standard error of the mean (SE). Data were analyzed by one-way analysis of variance (ANOVA). Comparisons between different groups were done using Tukey Test. The data were analyzed with GraphPad prism version 5.0 (GraphPad Software, Inc., CA, and USA). Difference was considered significant when *p* value was <0.05.

RESULTS AND DISCUSSION

Lipopolysaccharide has been extensively studied as a major factor contributing to the pathogenesis of gram negative bacterial infection through eliciting a systemic inflammatory response accompanied by severe hepatic injury [3, 4]. The current investigation focused on the decline in hepatic functions associated with LPS and the possible correction by ALA, CoQ10 and their combination.

1-Effects on serum liver function markers:
The present biochemical results revealed that LPS injection significantly elevated serum AST and ALT activities to 167.1 % and 121.8 %, respectively, as compared to normal control group. Meanwhile, both biomarkers were almost restored by oral administration of ALA, CoQ10 and their combination **(Table 1).**

Similarly, ALP, as well as, GGT activities were highly induced to 256.9 and 408.4 % by LPS injection compared to normal control group. However, oral administration of the selected drugs attenuated serum ALP and GGT activities **(Table 1).**

The intraperitoneal injection of LPS enhanced serum TB level to 841.2 % compared to normal control group. While, oral administration of ALA, CoQ10 and their combination significantly reduced serum TB level to 83.2 %, 18.2 % and 27.3 %, respectively as compared to LPS control group **(Table 1).**

The present study revealed that LPS injection showed significant elevation in serum liver functional markers levels (AST, ALT, ALP, GGT, and TB), accompanied by histopathological changes. The elevation in liver enzymes comes in accordance with Debnath et al. [27] and Chiu et al. [28] who have shown that LPS induces hepatic damage and increases the activities of serum aminotransferases. Similarly, Helal [29] reported elevation in serum activities of ALP and GGT beside TB level after LPS injection through its free radical generation mechanism indicating hepatic dysfunction. The hepatic enzymes are cytoplasmic in nature but usually leaked into circulation under hepatocellular damage, thus, causing their levels to rise in serum **[30]**. On the other hand, oral administration of ALA reduced the increase in serum liver functional markers levels related to reduction in oxidative stress evoked due to LPS administration. The present results are in agreement with recent researches [31, 32, 33]. This effect can be explained on the basis that ALA or its reduced form dihydrolipoic acid (DHLA) can prevent lipid peroxidation and protein damage via interaction with vitamins C, E, and glutathione [31, 34]. It was shown that ALA reduces the increased ROS generation and protein oxidation in liver as a result of its potent antioxidant capacity [35].

Table (1): Effects of ALA, CoQ10 or their combination on LPS-induced changes in serum liver function markers

	Groups	Serum AST (U/ml)	Serum ALT (U/ml)	Serum ALP (IU/L)	Serum GGT (U/L)	Serum TB (mg/dl)
	Normal control (saline)	23.83 ± 0.98	36.67 ± 1.09	72.67 ± 3.53	2.38 ± 0.43	0.17 ± 0.01
Lipopolysaccharide (LPS)	**Control (saline)**	39.83 ± 1.25 *	44.67 ± 2.39 *	186.70 ± 3.19 *	9.72 ± 0.84 *	1.43 ± 0.09 *
	Alpha-lipoic acid (50 mg/kg)	27.67 ± 0.67 #	35.67 ± 0.88 #	89.17 ± 7.05 #	2.38 ± 0.53 #	1.19 ± 0.02 * #
	Co-enzyme Q10 (10 mg/kg)	26.33 ± 0.67 #	32.50 ± 0.76 #	83.83 ± 3.86 #	1.79 ± 0.27 #	0.26 ± 0.03 #
	Alpha-lipoic acid +co-enzyme Q10	25.17 ± 1.08 #	35.50 ± 1.41 #	92.33 ± 3.52 * #	1.79 ± 0.60 #	0.39 ± 0.07 #

LPS was injected on day 1(4 mg/kg i.p) and day 8 (2 mg/kg i.p).
Values are represented as means ±SE (n=7).
Statistical analysis was done using one-way ANOVA followed by Tukey Test.
**Significantly different from normal control group at p<0.05.#Significantly different from LPS control group at p<0.05.*

Co-enzyme Q10 resulted in a significant reduction in the serum liver functional markers levels and these results are in a harmony with the data of Baskaran and Sabina [36], who reported that concurrent supplementation of CoQ10 in antitubercular drugs-treated rats showed liver protective effects by reduction of the extent of hepatic damage and restoring near-normal levels of antioxidants.

2- Effects on serum IL-6:

The i.p. injection of LPS significantly exaggerated serum IL-6 level to 321.9 % compared to the normal control group. On the other hand, administration of ALA, CoQ10 and their combination significantly reduced serum IL-6 level to 53.7 %, 46.5 % and 53.1%, respectively as compared to LPS control group **(Figure 1).**

Our data showed that LPS administration in rats induces pronounced inflammatory response evidenced by an increased IL-6 level. The present results find support in a recent study by Chiu et al. [28].

Circulating LPS binds to Toll-like receptor-4 (TLR-4) on hepatic phagocytes and macrophages, leading to their stimulation and subsequently tend to release reactive oxygen species (ROS), reactive nitric species (RNS), as well as, pro-inflammatory cytokines [37]. Additionally, LPS induces the migration of activated polymorphonuclear leukocytes (PMNs) into the liver, which constitutes another source of free radicals [38].

Alpha-lipoic acid is also involved with anti-inflammatory action; independently of its antioxidant activity [16] manifested by reduction in serum IL-6 level accompanied by remarkable improvement in histological structure of liver tissues. Regarding CoQ10, its administration resulted in a significant reduction in the serum liver functional markers levels and IL-6. These results are in harmony with the data of Baskaran and Sabina [37], who referred to CoQ10 hepatoprotective effect by restoring near-normal levels of antioxidants and reducing IL-6 level.

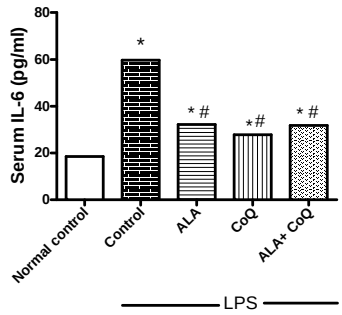

Figure (1): Effects of alpha-lipoic acid (ALA; 50 mg/kg), co-enzyme Q10 (CoQ; 10 mg/kg) or their combination on lipopolysaccharide (LPS)-induced changes in serum interleukin-6 (IL-6) level

LPS was injected on day 1(4 mg/kg i.p) and day 8 (2 mg/kg i.p).
Values are represented as means ± SE (n=7).
Statistical analysis was done using one-way ANOVA followed by Tukey Test.
**Significantly different from normal control group at p<0.05.*
#Significantly different from LPS control group at p<0.05.

3- Effects on oxidative stress parameters:

The present biochemical results revealed that LPS injection significantly elevated liver MDA content to 121.9%. Meanwhile, oral administration of ALA or CoQ10 normalized its content. The combination of ALA with CoQ10 did not alter MDA content as compared to LPS control group **(Figure 2A)**.

In the same context, GSH, SOD and TAC were reduced in LPS rats to 61.8%, 39.9%, and 23.01%, respectively. These effects were normalized after administration of ALA, CoQ10, and their combination **(Figures 2B, C, and D)**. The current study showed that LPS administration also increased liver MDA content, but decreased liver SOD activity, as well as, GSH content [39]. These observations come in accordance with Debnath et al., [27] and Chiu et al., [28], who have shown that LPS induces hepatic damage evidenced by elevation in liver MDA content accompanied by inhibition of liver SOD activity.

Under conditions of oxidative stress, ROS and RNS attack the polyunsaturated fatty acids (PUFAs) of cell membranes causing destabilization, disintegration and alteration in membrane fluidity and permeability, all events which increase the rate of protein degradation and eventually leads to cell lysis [40]. Decomposition products of lipid hydroperoxides such as MDA can interact with protein and nucleic acids, leading to oxidative protein and DNA damage [41].

Oral administration of ALA significantly decreased the formation of liver MDA in LPS-challenged rats. The present results are in agreement with those of other investigators **[31, 32, 33**]. Meanwhile, CoQ10 supplementation showed

an anti-peroxidative effect in the rat liver tissues by significantly decreasing the LPS-induced rise of liver MDA levels. The reduced form of CoQ10 (ubiquinol) is believed to be a powerful lipophilic antioxidant that participates in tocopherol and ascorbate recycling as antioxidants, thus protecting lipids from peroxidation [42]. Accordingly, this might lend a plausible explanation for the rise in TAC. By protecting cells against further oxidation, CoQ10 increases DNA repair rate such effect is likely attributed to the known antioxidant activity of CoQ10 [43].

Moreover, treatment during LPS challenge restored the decline in liver GSH level. This observation comes in accordance with Goraca et al. [32] and El-Feki et al. [33]. The increase of GSH in liver tissue after ALA administration may be due to the direct action of DHLA, which is a potent reducing agent and therefore converts GSSG to GSH [44] or the fact that ALA is able to correct deficient thiol status of the cell by increasing de novo synthesis of cellular GSH by improving cystine utilization [45].

Superoxide dismutase protects the organism against the deleterious effects triggered by the superoxide radical [46], thereby, its reduction allows free radical chain reaction to occur, then, reflected on TAC. Additionally, liver sections exhibited disruption of normal architecture with inflammatory cells parallel with the biochemical findings.

In the present results, the supplementation of ALA to LPS rats caused a marked restoring of liver SOD activity. These results were confirmed by Heibashy et al. [31].

In the present study, concurrent administration of CoQ10 with LPS restored liver GSH content, SOD activity, as well as TAC. These observations come in accordance with Mustafa et al., [47], who reported protective effect of CoQ10 in doxorubicin-treated rats.

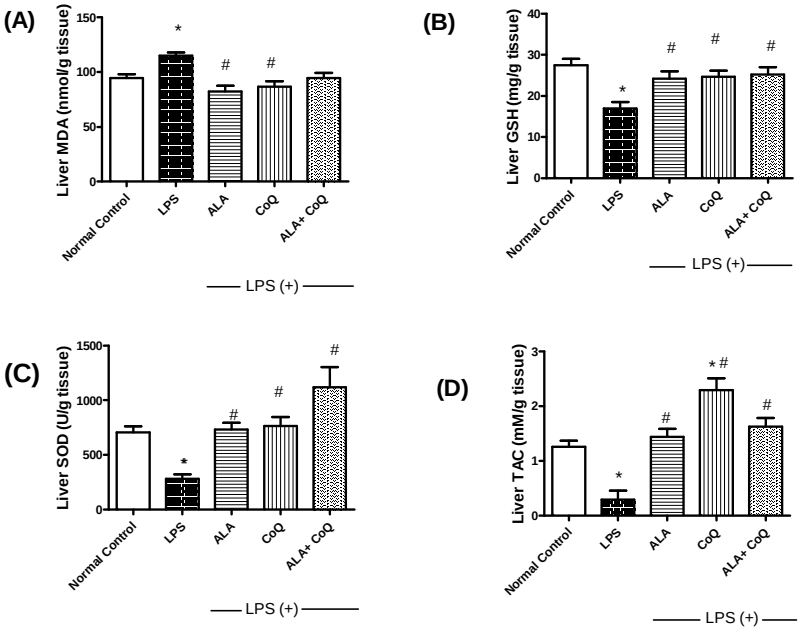

Figure (2): Effects of alpha-lipoic acid (ALA; 50 mg/kg), co-enzyme Q10 (CoQ; 10 mg/kg) or their combination on lipopolysaccharide (LPS)-induced changes in liver contents of : A) malondialdehyde (MDA), B) reduced glutathione (GSH) , as well as, C) superoxide dismutase (SOD) activity and D) total antioxidant capacity (TAC)

LPS was injected on day 1(4 mg/kg i.p) and day 8 (2 mg/kg i.p).
Values are represented as means ± SE (n=7).
Statistical analysis was done using one-way ANOVA followed by Tukey Test.
**Significantly different from normal control group at p<0.05.*
#Significantly different from LPS control group at p<0.05.

4-Effects on histopathological changes in hepatic tissue:

The liver sections of the control group were histologically normal **(Figure 3A).** After LPS administration, liver sections exhibited few foci of inflammatory cells around portal triad (portal traditis), scattered activated Kupffer cells, and congested portal vein (**Figure 3B**).

Microscopical examination showed that rats subjected to LPS hepatotoxicity and treated with ALA displayed normally looking hepatic cells except for dilated and congested central veins and activated Kupffer cells **(Figure 3C)**. While rat that received CoQ10 showed normally looking hepatic tissue with minimal dilated central vein and

normal portal tract **(Figure 3D)**. In addition, combination of ALA and CoQ10 presented normally looking hepatic cells except for moderately dilated central veins with minimal congestion **(Figure 3E).**

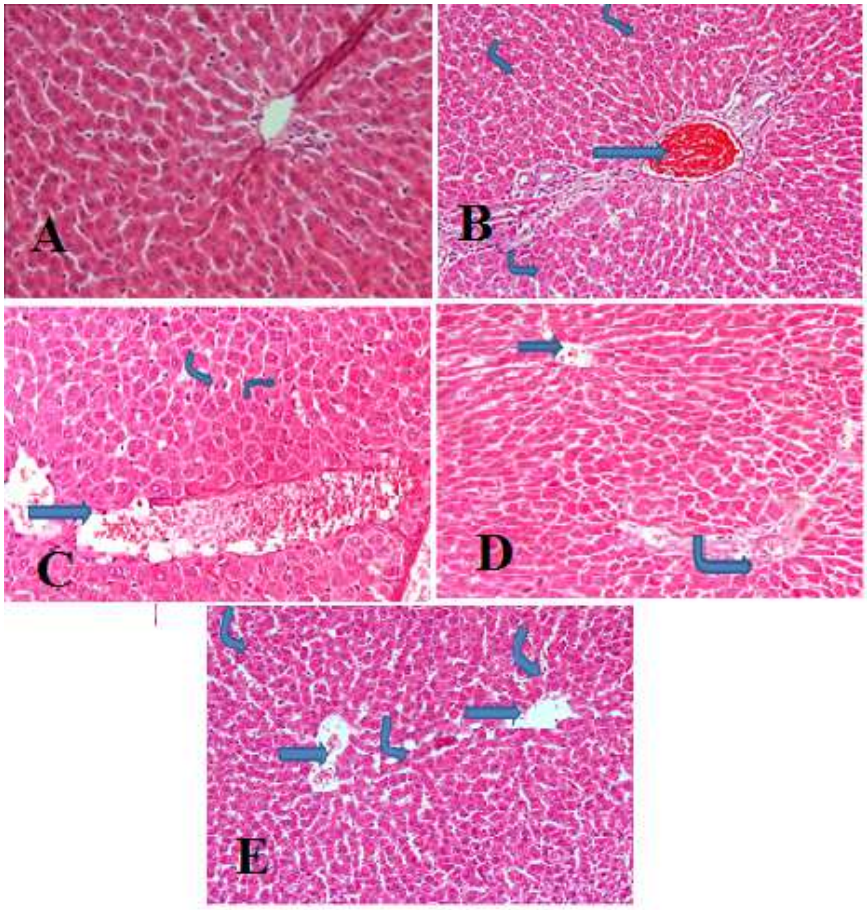

Figure (3): **a photomicrograph of a section of liver tissue of (A): normal rat, (B): LPS control rat, (C): rat receiving LPS and alpha-lipoic acid, (D): rat receiving LPS and co-enzyme Q10 and (E): rat receiving LPS, alpha lipoic acid and co-enzyme Q10 (H & E X 200)**

CONCLUSION

Our results demonstrated that administration of ALA, CoQ10, and their combination can provide new hepatoprotective intervention in LPS-induced liver damage and corroborate preservation of hepatic cells via halting functional enzymes and ameliorating oxidative stress damage, as well as, inflammation. These effects could be due to their antioxidant nature, which include free radical scavenging properties and their antioxidant activities.

REFERENCES

[1] N Ruiz, D Kahne and TJ Silhavy, *Nat Rev Microbiol*, **2009**, 7(9), 677–683.
[2] C Whitfield and MS Trent, *Annu Rev Biochem*, **2014**, 83, 99–128.
[3] GL SU, *Am J Physiol Gastrointest Liver Physiol*, **2002**, 283(2), G256–G265.
[4] F Senga, LYin, H Karasuno, H Ohtaki, T Nakamachi, K Satoh and S Shioda, *J Clin Biochem Nutr*, **2008**, 42(3), 222–227.
[5] HR Michie, KR Manogue, DR Spriggs, A Revhaug, S O'Dwyer, CA Dinarello, et al, N *Engl J Med*, **1988**, 318(23), 1481–1486.
[6] SJ van Deventer, HR Büller, JW ten Cate, LA Aarden, CE Hack and A Sturk, *Blood*, **1990**, 76(12), 2520–2526.
[7] MI Luster, DR Germolec, T Yoshida, F Kayama and M Thompson, *Hepatology*, **1994**, 19(2), 480-488.
[8] T Hartung and A Wendel, *J Biochem Pharmacol*, **1991**, 42(5), 1129-1135.
[9] JL Evans and ID Goldfine, *Diabetes Technol Ther*, **2000**, 2(3), 401–413.
[10] S Sola, MQ Mir, FA Cheema, N Khan-Merchant, RG Menon, S Parthasarathy and BV Khan, *Circulation*, **2005**, 111(3), 343-348.
[11] S Golbidi, M Badran, I Laher, *Front Pharmacol*, **2011**, 2, 69.

[12] B Feuerecker, S Pirsig, C Seidl, M Aichler, A Feuchtinger, G Bruchelt, et al, *Cancer Biol Ther*, **2012**; 13(14): 1425–1435.
[13] MC Castro, ML Massa, G Schinella, JJ Gagliardino and F Francini, *Biochim Biophys Acta*, **2013**, 1830(1), 2226-2232.
[14] D Malińska and K Winiarska, *Postepy Hig Med Dosw*, **2005**, 59, 535-543.
[15] A Gorąca, H Huk-Kolega, A Piechota, P Kleniewska, E Ciejka and B Skibska, *Pharmacol Rep*, **2011**, 63(4), 849-858.
[16] FA Moura, KQ de Andrade, JC Dos Santos, OR Araujo and MO Goulart, *Redox Biol*, **2015**, 6, 617–639.
[17] C da Silva Machado, LM Mendonça, VP Venancio, ML Bianchi and LM Antunes, *Neurotoxicology*, **2013**, 36, 10-16.
[18] MA Jiménez-Santos, IE Juárez-Rojop, CA Tovilla-Zárate, MT Espinosa-García, MA Juárez-Oropeza, T Ramón-Frías and et al, *Lipids Health Dis***, 2014**, 13,22.
[19] FM Sohet, AM Neyrinck, BD Pachikian, FC de Backer, LB Bindels, P Niklowitz and et al, *Biochem Pharmacol*, **2009**, 78(11), 1391-1400.
[20] AA Salama and FK El-Baz, *J Nat Sci Res*, **2013**, 3, 17-24.
[21] DM Cocchetto and TD Bjornsson , *J Pharm Sci*, **1983**, 72, 465-492.
[22] H Ohkawa, N Ohishi and K Yagi, *Anal Biochem*, **1979**, 95(2), 351-358.
[23] E Beutler, O Duron and BM Kelly, *J Lab Clin Med*, **1963**, 61, 882-888.
[24] M Nishikimi, NA Roa and K Yogi, *Biochem Biophys Res Common*, **1972**, 46(2), 849-854.
[25] D Koracevic, G Koracevic, V Djordjevic, S Andrejevic and V Cosic, *J Clin Pathol*, **2001**, 54(5), 356-361.
[26] RAB Drury and EA Wallington, Oxford University Press, **1980**, 41–54.
[27] T Debnath, DH Kim, JE Jo, JJ Lee, HJ Pyo and BO Lim, *Journal of Food Biochemistry*, **2015**, 39, 310–315.
[28] CH Chiu, CC Chang, ST Lin, CC Chyau and RY Peng, *Int J Mol Sci*, **2016** ,17(7).
[29] GK Helal, *Pak J Pharm Sci*, **2010**, 23(2), 131-137.
[30] JE Jensen, SE Stainberg, P Freese and E Marino, *J Digest Disord*, **2004**, 6, 1-3.
[31] MIA Heibashy, GMA Mazen and MI Shahin, *Journal of American Science*, **2013**, 9 (12), 529-538.
[32] A Goraca, H Huk-Kolega, A Kowalczyk and B Skibska, *Postepy Hig Med Dosw*, **2015**, 69, 270-276.
[33] MA El-Feki, HM Amin, AA Abdalla and M Fesal, *Middle East Journal of Applied Sciences*, **2016**, 6(3), 460-467.
[34] Y Tanaka, M Kaibori, H Miki, R Nakatake, K Tokuhara, M Nishizawa, T Okumura and AH Kwon, *journal of surgical research*, **2015**, 193(2), 675-683.
[35] YF Tian, CH Hsieh, YJ Hsieh, YT Chen, YJ Peng and PS Hsieh, *Eur J Clin Invest*, **2012**, 42, 637-648.
[36] UL Baskaran and EP Sabina, *Cell Biol Toxicol* , **2015**, 31, 211–219.
[37] J-H Zhu and XG Lei, *Biochem Biophys Res Commun*, **2011**, 404(1), 559-563.
[38] W Zou, RA Roth, HS Younis, E Malle and PE Ganey, **2011**, *Toxicology*, 290(2-3), 279-286.
[39] V Vetvicka, JM Garcia-Mina, M Proctor and J-C Yvin, *Austin J Clin Pathol*, **2014**, 1(4).
[40] S Bharrhan, K Chopra and P Rishi, *Am J Biomed Sci*, **2010**, 2(1), 51-62.
[41] L Pari and K Shagirtha, *Exp Toxicol Pathol*, **2012**, 64, 513-520.
[42] MA Jiménez-Santos, IE Juárez-Rojop, CA Tovilla-Zárate, MT Espinosa-García, MA Juárez- Oropeza, T Ramón-Frías, et al, *Lipids Health Dis*, **2014**,13,22.
[43] M Tomasetti, R Alleva, B Borghi and AR Collins, *FASEB J*, **2001**, 15(8), 1425–1427.
[44] SC Lu, *Mol Aspects Med*, **2009**, 30(1-2), 42-59.
[45] D Han, G Handelman and L Marcocci, *Biofactors*, **1997**, 6(3), 321-338.
[46] C Ritter, M Andrades and M Frota, *Intensive Care Med*, **2003,** 29(10), 1782-1789.
[47] HN Mustafa, SA El Awdan, GA Hegazy and GA Abdel Jaleel, *Indian J Pharmacol*, **2015**, 47(6), 649–656.