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ORIGINAL ARTICLE

Targeted Delivery of Curcumin Using MgONPs and Solid Lipid Nanoparticles: Attenuates Aluminum-Induced Neurotoxicity in Albino Rats

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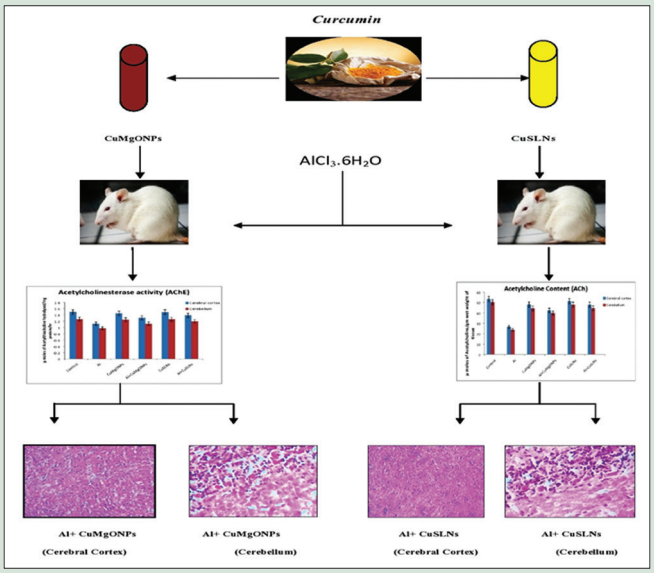
ABSTRACT

Background: Aluminum is a potent environmental toxin and its increasing entry exposes human beings to its neurotoxicity. **Objective:** The authors investigated the efficacy of delivery systems (magnesium oxide nanoparticles [MgONPs] and solid lipid nanoparticles [SLNs]) for curcumin in protecting aluminum-induced neurotoxicity in albino rats. **Materials and Methods:** Albino rats were divided into six groups (n = 6), Group I served as control; Group II Al treated; Group III curcumin loaded MgONPs (CuMgONPs); Group IV Al + CuMgONPs; Group V curcumin loaded SLNs (CuSLNs); Group VI Al + CuSLNs. After the treatment period, i.e., 30 days the animals were sacrificed and biochemical tests were performed to assess the cholinergic damage followed by histological observation of brain regions (cerebral cortex and cerebellum). **Results:** In cholinergic analyses, it was observed that aluminum-induced alterations in ACh and acetylcholine esterase were reversed with concomitant administration of CuMgONPs and CuSLNs. The therapeutic potential of CuMgONPs and CuSLNs was also observed in histological analyses of brain regions treated with aluminum. Among these two drug delivery systems, CuSLNs administration was found to be more potential compared to the CuMgONPs in treating aluminum induced neurotoxicity. **Conclusions:** By using drug delivery systems, MgONPs and SLNs, the problem of low bioavailability of curcumin can be controlled. This will be a promising agent to treat neurodegenerative disorders such as Alzheimer's disease with the potentiality to cross the blood–brain barrier. However, CuSLNs has showed a more protective effect on altered cholinergic system and tissue damage compared to CuMgONPs.

Key words: Acetylcholine and acetylcholine esterase, curcumin, curcumin loaded magnesium oxide nanoparticles, curcumin loaded solid lipid nanoparticles, histological analyses

SUMMARY

- Aluminum induced alterations of neurotransmitters (Ach and AchE) and tissue damage were reduced by curcumin loaded magnesium oxide nanoparticles (CuMgONPs) and curcumin loaded solid lipid nanoparticles (CuSLNs)
- Bioavailability of Curcumin through drug delivery systems of magnesium oxide nanoparticles (MgONPs) and solid lipid nanoparticles (SLNs) was increased with small particle size with good stability
- Aluminum induced neurotoxicity was reduced by targeting curcumin by MgONPs and SLNs
- Among the two drug delivery systems, CuSLNs were found to be more protective compared to CuMgONPs.



Abbreviations Used: CuMgONPs: Curcumin loaded magnesium oxide nanoparticles; CuSLNs: Curcumin loaded solid lipid nanoparticles; Ach: Acetylcholine; AChE: Acetylcholine esterase.

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INTRODUCTION

Aluminum (Al) is a potent cholinotoxin,[1] which disrupts the central cholinergic system that plays an important role in learning and memory functions.[2-7] Progressive neurodegenerative dysfunction of the central cholinergic system causes a reduction in the levels of acetylcholine (ACh) and ACh transferase (AChT), which are the main histological hallmarks observed in AD brains.[8] Otaibi *et al.* 2018 reported that Al has been associated with the manifestation of dementia and causes cognitive neuro decline through long-term exposure in adults.[9]

Curcumin is a biologically active substance, isolated in 1815 from the rhizome of *Curcuma longa,*[10] by Vogel and Pelletier and structurally

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characterized by Milobedzka in 1910[11], it was synthesized and confirmed in 1913 which was grouped under polyphenol compound by Lampe[12], and in 1910 it was extensively used in East Asia as a medicine to treat liver and gall-bladder diseases, inflammatory, rheumatic conditions and anorexia, etc.[13] and also curcumin plays a pharmacological role in treating various disorders such as autoimmune, cardiovascular, neoplastic, pulmonary and neurodegenerative disorders.[14-17] Curcumin is a more potent scavenger than vitamin E[18] that fights against NO-based free radicals[19,20] and protects cell membrane[21,22] with the neuroprotective events such as anti-inflammatory, antioxidant and immunomodulatory properties.[21,23] It was reported that curcumin regulates the β-amyloid accumulation[24,25] and restores the damaged neurotransmitters (ACh and AChE)[26-28] in mouse brain against aluminum-induced cognitive dysfunction. Regardless of the remedial perspective of curcumin, the drawbacks of its usage include rapid metabolism, low gastrointestinal absorption are the factors that are minimizing the curcumin bioavailability, and clinical efficacy.

Nanotechnology has become a very promising field to the pharmaceutical and medical fields through drug release systems. By reducing the phytomedicine (Curcumin) to its nanosize, alters the surface properties and enhances the bioavailability of phytomedicine. The efficacy of nano curcumin can be enhanced by encapsulating into nanocomposites as drug delivery vehicles for effective delivery of curcumin to the targeted tissue by a synergistic mechanism. The present study explores the use of inorganic magnesium oxide nanoparticles (MgONPs) and organic solid lipid nanoparticles (SLNs) as nanocarriers, with the outcome of comparative studies were carried out between both the drug vehicles for effective delivery of curcumin.

MgONPs are odorless, non-toxic white powder forms of nanoparticles that are used for hydrophobic Curcumin delivery because of its biocompatibility, biodegradability, and relatively low cost. Owing to the significant importance of carrier materials, magnesium the fourth most abundant essential mineral in the body, with excellent biocompatibility than other nanocarriers. It has been widely used as an antacid, antibacterial agent, an approved drug, and food additive with high drug loading capacity that acts as a safe nanocarrier for curcumin delivery.[29] This can be obtained mainly by precipitation of magnesium hydroxide,[30,31] Sol-gel method,[32,33] and sonication method.[34]

SLNs are organic nanocarriers typically exists in the range of 50–1000 nm in size and consist of a biocompatible and biodegradable solid lipid core with entrapped hydrophobic or lipophilic drug and an external surfactant shell that can be sustained in various physiological systems. These are developed as a substitute to colloidal drug delivery systems such as polymeric nanoparticles and liposomes[35] to overcome difficulties such as cytotoxicity, drug leakage, and high production cost. Moreover, SLNs possess desired features such as low toxicity, large surface area, prolonged drug release, superior cellular uptake, improve solubility and bioavailability of hydrophilic and lipophilic bioactive drugs[36,37] through controlled and targeted drug delivery.[38] To increase the solubility, stability, and bio-distribution of SLNs, PEG was used which provides a hydrophilic stearic barrier around SLN by attaching covalently to the nanoparticle surface thereby resulting in formulations with increased stability during storage and applications[39] and also with increasing the circulation half-life without affecting its activity during transportation and absorption into the body.

In the present study, we report the use of MgONPs and SLNs for efficient delivery of curcumin against Al-induced neurotoxicity in albino rats.

MATERIALS AND METHODS

Chemicals and reagents

Curcumin (SRL Scientifics), $MgCl_2$, and NaOH from Merck and Hi-media, stearic acid from Sd Fine Chem., Ltd., Mumbai, flaxseed oil form a local market of Tirupati, Tween 80 (polysorbate 80) and Polyethylene glycol (PEG 4000), Acetyl thiocholine iodide, Aluminum Chloride ($AlCl_3.6H_2O$) were purchased from Hi-media and Sigma Aldrich companies of India

Synthesis of curcumin loaded magnesium oxide nanoparticles

For the Synthesis of CuMgONPs, the optimized concentrations were taken as per our previous report.[40] About 2.5 mg of optimized concentration of curcumin was dissolved in ethanol as it is insoluble in water. This curcumin solution was added to the aqueous 25 mM $MgCl_2$ hexahydrate solution and NaOH was added dropwise under continuous stirring at room temperature, the addition of NaOH was stopped at pH 12 for reduced particle size according to our previous reports. The complex formation was depicted by color change from yellow to reddish-brown/orange color based on the pH of the solution. The solution generates $CuMg(OH)_2$, which acts as a precursor of CuMgO, was centrifuged at 10,000 rpm for 10 min. The collected precipitation was washed with ethanol to remove impurities and formed pellet was calcinated at 100°C–300°C, which gives the reddish-brown powder form of CuMgONPs.

Formulation of curcumin loaded solid lipid nanoparticles by high-speed homogenization

The solid lipid (stearic acid) was melted at a temperature >75°C and curcumin was dissolved in the melted lipid with continuous stirring maintaining the same temperature as the lipid or organic process. The aqueous phase containing surfactant and co-surfactant was also heated to 75°C and gradually applied to the lipid process with continuous stirring, The two-phase system is then homogenized at 20,000 rpm for 30 min[41] using a high-speed homogenizer. To yield CuSLNs, the homogenized suspension was freeze-dried. All formulations were prepared in triplicates to obtain optimum concentrations that have been standardized through preliminary experiments. The finalized formulation with good stability and particle size has been used for the targeted delivery of curcumin.

Experimental design for animal studies

Experimental model – Wistar strain male albino rat.

Selection and procurement of experimental animals

Male Wistar strain albino rats were selected for the current investigation. Healthy adult albino rats of 12-month age group (360 ± 5 days and weight 250 ± 15 g) were procured from certified vendors (Sri Venkateswara Traders, Bengaluru, India) for this study. The animals were acclimatized in the lab for 7 days. They were housed in polypropylene cages (47 cm × 34 cm × 20 cm) containing sterile paddy husk as bedding and maintained at 22°C–25°C regulated temperature with a light/dark cycle (12 h/12 h). The rats were fed with standard rat chow (Sai Durga Feed and Foods, India) and water *ad libitum*. Animals were equally randomized into groups; each group contains six animals for investigation.

Experimental design

Experimental protocols were approved by the institutional ethical committee (CPCSEA Registration Number: 1677/PO/Re/S/2016/CPCSEA/dt. 6/05/16.

Treatment period: 30 days.

Route of administration: Oral administration ($AlCl_3$, CuMgONPs, and CuSLNs).

Group-I: Control: Rats in this group were administered with saline (0.9%) solution.

Group-II: Al treated group: The animals were treated with Aluminum Chloride ($AlCl_3$) (100 mg/kg bwt/day).

Group-III: CuMgONPs administered group: Curcumin loaded MgONPs were administered (0.5 mg/kg bwt/day) to the rats.

Group-IV: Al + CuMgONPs treated group: Aluminum Chloride ($AlCl_3$) + Curcumin loaded MgONPs were concomitantly administered to the animals (100 and 0.5 mg/kg bwt/day).

Group-V: CuSLNs administered group: The rats were administered with Curcumin loaded SLNs (50 mg/kg bwt/day).

Group-VI: Al + CuSLNs treated group: Aluminum Chloride ($AlCl_3$) + Curcumin loaded SLNs were administered simultaneously to this group (100 and 50 mg/kg bwt/day).

Isolation of brain regions and preparation of tissue homogenates

After completion of the treatment period, i.e., 30 days, the animals were sacrificed by cervical dislocation, and the brains were rapidly dissected out on ice plate. Tissue homogenates of (cerebral cortex, cerebellum) were prepared by adding 50 mM phosphate buffer containing 0.1 mM EDTA to brain regions and homogenized using homogenizer at 10,000 rpm for 20 min, and resultant supernatant was used for further biochemical analyses.

Biochemical assays

Cholinergic analyses

Cholinergic dysfunction was estimated in terms of ACh content and acetylcholinesterase (AChE) activity.

Acetylcholine content

ACh content was estimated by the method of Hestrin as given by Augustinson.[42,43] Each brain tissue was boiled in hot water-bath for 10 min to arrest the AChE activity. Tissues were homogenized in 2.0 mL of distilled water and 1.0 mL of alkaline hydroxylamine hydrochloride was added, followed by 1.0 mL of 1:1 hydrochloric acid solution. It was mixed vigorously and centrifuged. Supernatant was collected, and 0.5 mL of 0.37 M ferric chloride was added. The brown color developed was read at 540 nm. The ACh content was expressed as µ moles of ACh/g wet weight of tissue.

Acetylcholinesterase activity

AChE activity was assayed by the method of (Ellman and Courtney 1961).[44] The reaction mixture contains 270 µ moles of sodium phosphate buffer (pH 8.0), 10 µ moles of DTNB, 1.5 µ moles of acetyl thiocholine iodide, and 100 µ moles of 2% brain homogenate. The initial absorbance of the reaction mixture was recorded at 412 nm in a UV-vis spectrophotometer before the addition of acetyl thiocholine iodide. After 15 min incubation at room temperature, the yellow color developed was read at 412 nm. A molar extinction coefficient of 4.12×10^{-3} was used to calculate the enzyme activity. The AChE enzyme activity was expressed as µ moles of acetylthiocholinehydrolyzed/mg protein/hr.

Histopathology

Histopathological observation of brain regions was performed in both control and treated groups by using a Phase contrast microscope (Humason, 1972).[45] Brain regions were rinsed with a physiological saline solution (0.9% NaCl) to get rid of blood and debris adhering to the tissues and fixed in 10% formalin for 24 h. All the fixative traces were removed through washing under running tap water. After dehydrating through a graded series of alcohols, the tissues were cleared in methyl benzoate, embedded in paraffin wax. Sections were cut at 6 m thickness and stained with hematoxylin[46] and counterstained with eosin (dissolved in 95% alcohol). After dehydration and clearing, sections were mounted with DPX and observed under microscope.

Statistical analyses

Mean ± percent changes, ANOVA[47] and Bonferroni *post hoc* test for comparisons were performed using SPSS package programming techniques on "Intel Core 2 Duo Processor" of personnel computer. All values of "*t*" <5% and 1% levels ($P < 0.05$, $P < 0.01$ and $P < 0.000$) were considered statistically significant.

RESULTS

Acetylcholine content

In the present study, obtained results in Al treated group showed that ACh levels were decreased in the cerebral cortex (−50.65%); cerebellum (−52.5%). ACh decline was more pronounced in cerebellum compared to cortex region. Whereas synchronous administration of CuMgONPs with Al treatment showed increased ACh levels, cerebral cortex (12.22%); cerebellum (25.54%) compared to Al alone treated group. Co-administration of Al + CuSLNs has ameliorated the effect of Al with more significant refinement of ACh levels, cerebral cortex (31.59%); cerebellum (41.78%) compared to Al + CuMgONPs treated group. Effect of CuMgONPs alone administered group cerebral cortex (29.27%); cerebellum (33.52) and CuSLNs alone administered group cerebral cortex (43.16%); cerebellum (50.65%) have showed normal range of ACh levels in brain regions of albino rats which were almost similar to control group. From the above results, it was found that significant refinement of ACh content was observed in Al + CuSLNs treated group compared to the Al + CuMgONPs group, as shown in Figure 1.

Acetylcholinesterase levels

AChE levels of the Al administered group showed a reduction in AChE levels, cerebral cortex (−31.56%); cerebellum (−28.55%) compared to control. Whereas co-administration of CuMgONPs along with Al has showed increased AChE levels, cerebral cortex (19.54%); cerebellum (16.65%) compared to Al alone treated rats. Al + CuSLNs group has showed significant refinement of AChE levels, cerebral cortex (24.39%), cerebellum (21.74%) compared to Al + CuMgONPs treated group. CuMgONPs alone administered group cerebral cortex (28.75%), cerebellum (26.03%) and CuSLNs alone treated group

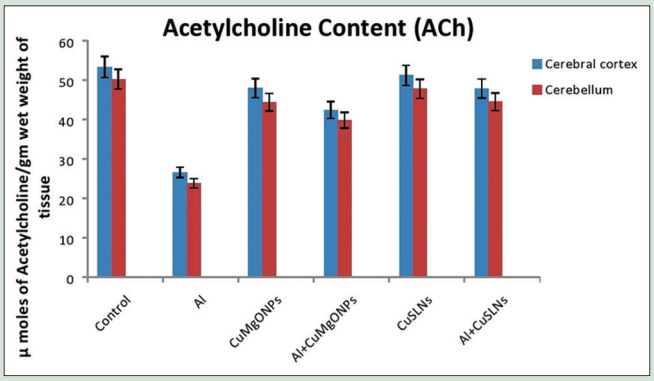

Figure 1: Effect of curcumin loaded magnesium oxide nanoparticles and curcumin loaded solid lipid nanoparticles on acetylcholine content in different brain regions (cerebral cortex: Cerebellum) of albino rats exposed to Al

cerebral cortex (30.69%), cerebellum (28.34%) have showed the normal AChE levels compared to control group. It was clearly demonstrated that from the above results, CuSLNs has effectively recovered the AChE levels in Al + CuSLNs group compared to Al + CuMgONPs treated group, as shown in Figure 2.

Histopathological examination of brain regions of albino rats:

Cerebral cortex

Histopathological examination of cerebral cortex region of control group rats showed intact neurons (NEU) and normal architecture. Al treated group showed degenerative changes and vacuolization along with pericellular edema with distorted neural cell bodies were observed Whereas co-administration of Al + CuMgONPs, showed reduced vacuolization (RV), perineuronal edema and gliosis compared to Al treated group. Whereas co-administration of Al + CuSLNs group showed noticeable improvements with normal neurons and intact nucleus and astrocytes with no vacuolization and degenerated neurons could be observed. CuMgONPs and CuSLNs alone administered groups have shown regular morphological exterior which is almost similar to control group as shown in Figure 3.

Cerebellum

Histology of cerebellum exhibited normal middle Purkinje cell layer (MPKCL) in the control group. Al treated group showed degenerative changes in MPKCL with vacuolization (V). Whereas Co-administration of CuMgONPs with Al, restored the degenerative changes of Purkinje cell layer and granular layer (GL) compared to Al treated group. Al + CuSLNs treated showed more improvement in the architecture of cerebellum with reduced degenerative changes of MPKCL and RV between the cells present in GL. Both CuMgONPs and CuSLNs alone treated groups showed normal GL and MPKCL as in similar to the control group, as shown in Figure 4.

From the above histopathological studies, it was observed that Al caused degenerative changes and vacuolization in neurons were reduced by Synchronous administration of CuMgONPs (Al + CuMgONPs) and CuSLNs with Al (Al + CuSLNs) reduced the degenerative changes and vacuolization in all brain regions. But the neuroprotective effect of CuSLNs was more in restoring the degenerative changes compared to CuMgONPs against Al induced neurotoxicity.

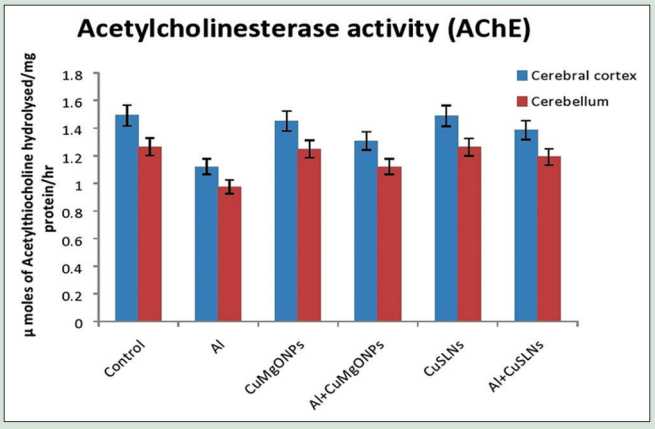

Figure 2: Effect of curcumin loaded magnesium oxide nanoparticles and curcumin loaded solid lipid nanoparticles on acetylcholine esterase activity in brain regions (cerebral cortex: Cerebellum) of albino rats exposed to Al

DISCUSSION

Alzheimer's disease (AD) is an age-associated progressive neurodegenerative disorder observed in Patients with AD due to exposure to toxic metal like aluminum present in the atmosphere. It affects brain development depending on the route of intake, time, and level of exposure[48] and causes dysfunction of the central cholinergic system that plays an important role in cognitive, learning, and memory functions.[2,3] The enzyme, AChE, is one of the major components of the cholinergic system in the central and peripheral nervous system which acts on ACh, a neurotransmitter associated with learning and memory, is degraded by the enzyme AChE, terminating neurotransmission at cholinergic synapses and also plays a role during morphogenesis in the onset of neurodegenerative diseases.[49]

In the present study, ACh levels were also decreased with Al exposure compared to control, which demonstrated that significant neocortical deficits in the enzyme responsible for the synthesis of ACh and ChAT (choline acetyltransferase) would lead to the terminated physiological action of the neurotransmitter[50,51] and also the metabolism of acetyl-coA was disturbed by Al exposure which leads to a reduction in the formation of ACh.[52,53]

In the present study, decreased AChE levels were observed in Al-intoxicated albino rats as Al (III) causes disturbances in cholinergic neurotransmission by interacting with the peripheral sites and secondary structure of AChE, finally decreasing its activity. AChE activity measured in the present study was observed to be decreased after 30 days of Al exposure, these results are in agreement with previous reports of decreased AChE activity Lakshmi *et al.* due to the biphasic effect of Al on AChE activity, shows increased activity observed up to 14 days followed by inactivation of AChE activity at 60 days of Al treatment causing the direct result of losing cholinergic input.[54-56]

Previous reports suggested natural polyphenolics are used to ameliorate the altered cholinergic levels where curcumin acts as a potent antioxidant with anti-inflammatory effect and a thus may have a role as neuroprotectant used for prevention and treatment of AD, because of its potential compound of curcumin has the penetrating ability on the central nervous system[57,58] and attenuates the neuropathological changes in the hippocampus and inhibited apoptosis.[59] Besides the efficiency of curcumin in treating a wide variety of human diseases,[17] low aqueous solubility, rapid metabolism, weak gastrointestinal absorption, alkaline pH degradation are the factors that minimize the bioavailability of curcumin which limited its use in treatments.

With the progression of nanomedicine and advancement of using natural products which can provide feasible and interesting solutions to discover the new drug design challenges, that serve as an inspiration for drug discovery with desired physicochemical properties at the nanoscale level and their targeted release[60-62] using several drug delivery systems have been studied, to increase the drug specificity, diagnostic accuracy, reduced toxicity and increased bioavailability in the organism.[63] There are several drug delivery systems successfully employed in recent times, for the successful delivery of drugs to its target sites. Hence the Nano based drug delivery systems are currently been studied that will facilitate the modified release of the active ingredients in the body.

Nanotechnology provided a way for hydrophobic drugs through nanocarrier mediated drug delivery with the use of inorganic (CuMgONPs) because of its biocompatibility, biodegradability and relatively low cost with high drug encapsulation with minimum drug loss during circulation are desirable features of inorganic nanoparticles, and organic-based (CuSLNs) nanocarriers have become one of the alternatives to enhance the bioavailability and clinical effectiveness of curcumin.

According to the findings of previous studies, the toxicity of NPs toward micro-organisms is increased as the concentration of MgO increased.

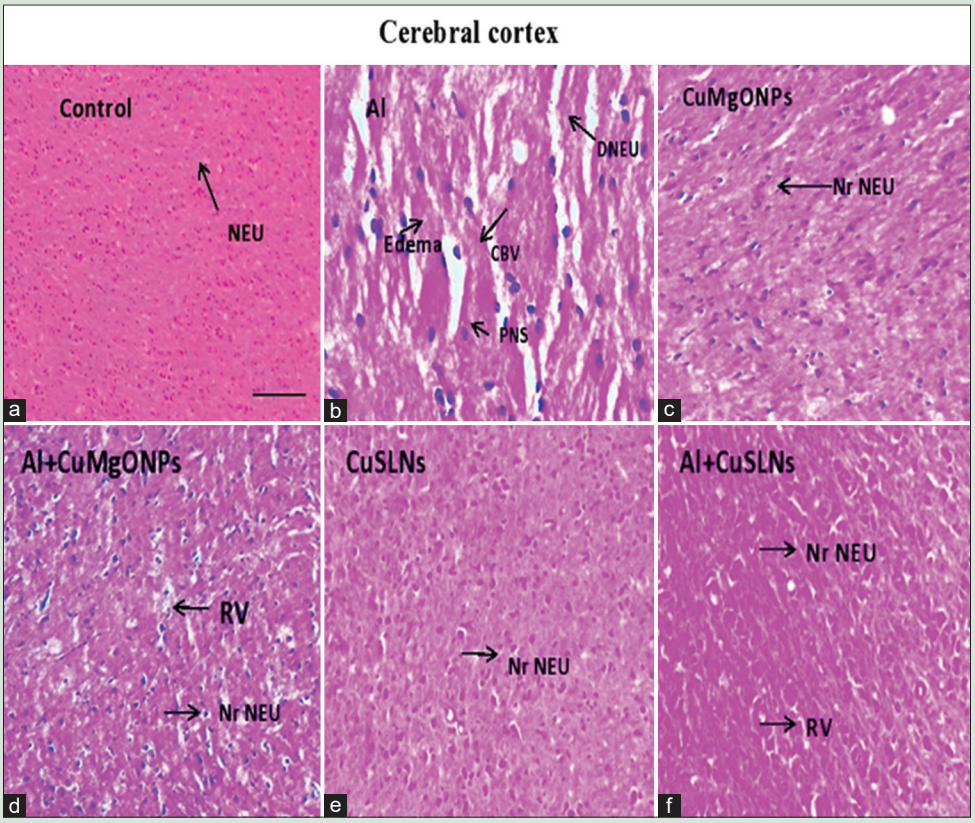

Figure 3: Histology micrographs of Cerebral cortex, after h and e staining showing normal neurons (NR NEU), congestion of blood vessels peri nuclear spaces, reduced vacuolization observed in Al treated and (curcumin loaded magnesium oxide nanoparticles) and (curcumin loaded solid lipid nanoparticles) treated groups with (a) control (b) Al treated group showing degenerative changes in the normal neurons (NR NEU), congestion of blood vessels and vacuolization (v) around the neurons and pericellularedema (c) curcumin loaded magnesium oxide nanoparticles showing normal morphology; (d) Al + CuMgONPs also showed reduced vacuolization with restored neurons, (e) curcumin loaded solid lipid nanoparticles showing intact neurons with normal exterior. (f) Al + CuSLNs has showed appreciable improvements with normal neurons and intact nucleus with no spongiosis. Scale bar = 100 μm is same for all the micrographs

[64-66] In contrast, Ge *et al.* reported the noncytotoxicity of MgO NPs in low concentrations below 200 μg/ml was confirmed by other researchers.[67] Lai *et al.* showed that the treatment of brain cancerous cells with MgO NPs did not show any effect on the survival rate until concentrations were higher than 50 μg/mL.[68] Considering all the previous reports beneficial or adverse effects of MgONPs were dependent on particle composition, particle concentration, particle size surface area of NPs,[69] duration of the exposure time[70] and nature of the biological target and compactness of them[71] are important factors to portrait MgONPs as toxic or safe products. By previous *in vitro* findings and *in vivo* toxicity investigation, MgO nanoparticles at concentrations lower than 250 μg/ml were found to be safe concentration. In the present study, we have used the 500 μg/ml of CuMgONPs has proved the effective delivery observed with significant elevation in the degree of ACh and AChE levels. ACh of cerebral cortex (12.22%); cerebellum (25.54%), AChE levels of cerebral cortex (19.54%); cerebellum (16.65%) by Al + CuMgONPs treatment has showed significance of ($P < 0.000$) compared to Al alone treated group. So balancing the cholinergic levels by CuMgONPs has a good correlation with the increase of total antioxidant levels and toxic effects of increased concentration of MgONps above 200 μg/ml are reversed by loading with curcumin which is a potent antioxidant and neuroprotective agent.

The use of MgONPs acts as Curcumin carrier because of its antiapoptotic, antioxidative, and antidiabetic effects in rat pancreatic islets[72] and Mg in its metal form has neuroprotective effects and reduced LPO after spinal cord injury in rats.[73] Curcumin loaded SLNs (CuSLNs) have been used as suitable drug delivery systems for enhancing the bioavailability of curcumin in agreement with the previous reports of Kakkar *et al.*[74]

In the present study, we have made use of an organic-based drug carrier to compare with the inorganic drug carrier system to investigate through which system effective delivery of the hydrophobic curcumin taking place. To overcome poor pharmacokinetics of curcumin, we proposed the formulation of curcumin loaded SLNs (CuSLNs) with an ability to penetrate the blood-brain-barrier (BBB) because of its lipidic nature and small average particle size enhances the bioavailability of Curcumin with 32–155 times from developed CuSLNs[74,75] by bypassing the liver which is a major site of curcumin degradation such that attains long circulation time. The presence of surfactant (Tween 80) on the surface of CuSLNs provides a high affinity between lipid particles and intestinal membrane, which improves the absorption by the GI tract.

In the present study, the Al + CuSLNs (50 mg/kg) treated group showed enhancing the declined levels of ACh of the cerebral cortex (31.59%); cerebellum (41.78%), and AChE levels of the cerebral cortex (24.39%), cerebellum (21.74%) concerning Al treated group with a significance of ($P < 0.000$) compared to Al treated group.

However, CuMgONPs and CuSLNs co-administration with Al showed significant improvement in the overall histoarchitecture of both the

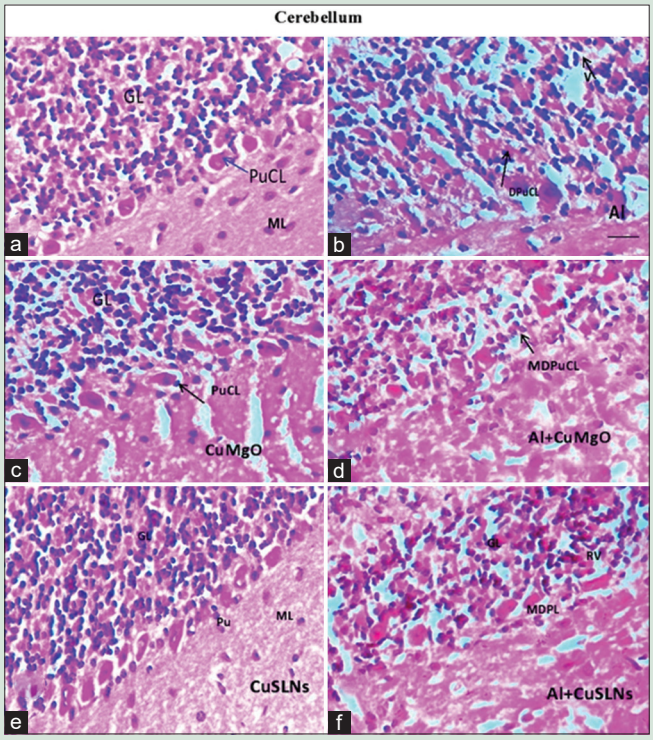

Figure 4: Histology micrographs of Cerebellum stained with H and E, of (a) control group showing middle Purkinje cell layer; (b) Al showed degenerative changes of Purkinje cell layer; (c) curcumin loaded magnesium oxide nanoparticles treated group showing normal neuroglial cells and middle Purkinje cell layer with vacuolisation observed in molecular layer; (d) Al + CuMgONPs treated group showing cerebellum with mild degenerative granular layer with reduced vacuolization with normal purkinje cell layer and molecular layers than Al treated group. (e) Curcumin loaded solid lipid nanoparticles treated group showing normal appearance of Purkinje and molecular layers resembling the control group (f) Al + CuSLNs showing noticeable improvements in restoring the damage compared to Al + CuMgONPs treated group. Scale bar = 100 µm is same for all the micrographs at ×40

cerebral cortex and cerebellum may be accredited to the neuroprotective role of curcumin.

Among Al + CuMgONPs and Al + CuSLNs, Curcumin administered in its highly bioavailable from CuSLNs completely reversed the induced alterations compared to CuMgONPs because of the presence of flaxseed oil a richest plant-based source of antioxidants with long-chain omega-3 fatty acids in the form of DHA in the formulation of CuSLNs exerts a neuroprotective effect on synapse supporting electrical signaling which helps nerve cells to release neurotransmitters that promotes learning and memory, regulate the level of reactive oxygen species by activation of cellular antioxidant enzymes.[76,77]

CONCLUSION

The present study indicates that Al caused significant neurocholinergic damage, which was observed by ACh, and AChE activities and tissue damage by histology studies of the cerebral cortex and cerebellum of albino rats compared to control. CuMgONPs and CuSLNs alone treated groups did not show any alterations in cholinergic levels, which are almost near to the control group. Al + CuSLNs treated group has shown more refinement in declined ACh and AChE levels because of its small size and lipidic nature, which is more compatible with the BBB compared to Al + CuMgONPs treated group.

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Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Gulya K, Rakonczay Z, Kása P. Cholinotoxic effects of aluminum in rat brain. J Neurochem 1990;54:1020-6.
2. Carew TJ, Hawkins RD, Kandel ER. Differential classical conditioning of a defensive withdrawal reflex in Aplysia californica. Science 1983;219:397-400.
3. Atri A, Sherman S, Norman KA, Kirchhoff BA, Hasselmo ME, Stern CE. Blockade of central cholinergic receptors impairs new learning and increases proactive interference in a word paired-associated memory task. Behav Neurosci 2004;118:223-36.
4. Eidi A, Eidi M, Mahmoodi G, Oryan S. Effect of vitamin E on memory retention in rats: Possible involvement of cholinergic system. Eur Neuropsychopharmacol 2006;16:101-6.
5. Gupta R, Gupta L, Mediratta P, Bhattacharya SK. Effect of resveratrol on scopolamine-induced cognitive impairments in mice. Pharmacol Rep 2012;64:438-44.
6. Kruk M, Tendera K, Bioela G. Memory-related effect of cholinergic receptor ligands in mice as measured by elevated plus maze test. Pharmacol Rep 2011;63:1372-82.
7. Power AE, Vazdarjanova A, McGaugh JL. Muscarinic cholinergic influences in memory consolidation. Neurobiol Learn Mem 2003;80:178-93.
8. Hamaguchi T, Ono K, Yamada M. Review: Curcumin and Alzheimer's disease. CNS Neurosci Ther 2010;16:285-97.
9. Otaibi SS, Arafah MM, Sharma B, Alhomida AS, Siddiqi NJ. Synergistic Effect of Quercetin and-Lipoic Acid on Aluminium Chloride Induced Neurotoxicity in Rats. Journal of Toxicology, 2018: 1-8.
10. Shehzad A, Wahid F, Lee YS. Curcumin in cancer chemoprevention: Molecular targets, pharmacokinetics, bioavailability, and clinical trials. Arch Pharm (Weinheim) 2010;343:489-99.
11. Lampe V, Milobedzka J, Kostanecki V. Structure of curcumin.BerDtschChemGes 1910;43:2163-70.
12. Lampe V, Milobedeska J. Studien über Curcumin. Ber Dtsch Chem Ges. 1913; 46: 2235-40.
13. Mishra S, Palanivelu K. The effect of curcumin (turmeric) on Alzheimer's disease: An overview. Ann Indian Acad Neurol 2008;11:13-9.
14. Cole GM, Teter B, Frautschy SA. Neuroprotective effects of curcumin. Adv Exp Med Biol 2007;595:197-212.
15. Garcia-Alloza M, Borrelli LA, Rozkalne A, Hyman BT, Bacskai BJ. Curcumin labels amyloid pathology *in vivo*, disrupts existing plaques, and partially restores distorted neurites in an Alzheimer mouse model. J Neurochem 2007;102:1095-104.
16. Ma QL, Yang F, Rosario ER, Ubeda OJ, Beech W, Gant DJ, *et al*. Amyloid oligomers induce phosphorylation of tau and inactivation of insulin receptor substrate via c jun n-terminal kinase signaling: Suppression by omega-3 fatty acids and curcumin. J Neurosci 2009;29:9078-89.
17. Sugiyama Y, Kawakishi S, Osawa T. Involvement of the beta-diketone moiety in the antioxidative mechanism of tetrahydrocurcumin. Biochem Pharmacol 1996;52:519-25.
18. Zhao BL, Li XJ, He RJ, Cheng SJ, Xin WJ. Scavenging effects of green tea and natural antioxidants on active oxygen radicals. Cell Biophys 1989;14:175-85.
19. Brouet I, Ohshima H. Curcumin, an anti-tumor promoter and anti-inflammatory agent inhibits induction of nitric oxide synthase in activated macrophages. Biochem Biophys Res Commun 1995;206:533-40.
20. Sreejayan , Rao MN. Nitric oxide scavenging by curcuminoids. J Pharm Pharmacol 1997;49:105-7.
21. Menon VP, Sudheer AR. Antioxidant and anti-inflammatory properties of curcumin. Adv Exp Med Biol 2007;595:105-25.
22. Wright JS. Predicting the antioxidant activity of curcumin and curcuminoids. J MolStruct 2002;591:207-17.
23. Yadav VS, Mishra KP, Singh DP, Mehrotra S, Singh VK. Immunomodulatory effects of curcumin. Immunopharmacol Immunotoxicol 2005;27:485-97.

24. Aggarwal BB, Sung B. Pharmacological basis for the role of curcumin in chronic diseases: An age-old spice with modern targets. Trends Pharmacol Sci 2009;30:85-94.
25. Yang F, Lim GP, Begum AN, Ubeda OJ, Simmons MR, Ambegaokar SS, *et al*. Curcumin inhibits formation of amyloid _ oligomers and fibrils, binds plaques and reduces amyloid *in vivo*. J Biol Chem 2005;280:5892-901.
26. Dai J, Buijs RM, Kamphorst W, Swaab DF. Impaired axonal transport of cortical neurons in Alzheimer's disease is associated with neuropathological changes. Brain Res 2002;948:138-44.
27. Gibson GE, Peterson C. Aging decreases oxidative metabolism and the release and synthesis of acetylcholine. J Neurochem 1981;37:978-84.
28. Zatta P, Ibn-Lkhayat M, Zambenedetti P, Kilyen M, Kiss T. *In vivo* and *in vitro* effects of aluminium on the activity of mouse brain acetylcholinesterase. Brain Res Bull 2002;59:41-5.
29. Guo M, Muhammad F, Wang A, Qi W, Wang N, Guo Y, *et al*. Magnesium hydroxide nanoplates: A pH-responsive platform for hydrophobic anticancer drug delivery. J Mater Chem B 2013;1:5273-8.
30. Aramendía MA, Borau V, Jiménez C, Marinas JM, Porras A, Urbano FJ. Synthesis and characterization of various MgO and related systems. J Mater Chem 1996;6:1943-9.
31. Xu BQ, Wei JM, Wang HY, Sun KQ, Zhu QM. Nano-MgO: Novel preparation and application as support of Ni catalyst for CO_2 reforming of methane. Catal Today 2001;68:217-25.
32. Choi H, Hwang S. Sol-gel-derived magnesium oxide precursor for thin-film fabrication. J Mater Res 2000;15:842-5.
33. LópezT, Gómez R, Navarrete J, López-Salinas E. Evidence for Lewis and Brønsted acid sites on MgO obtained by sol-gel. J Sol Gel Sci Tech 1998;13:1043-7.
34. Tang ZX, Shi LE. Preparation of nano-MgO using ultrasonic method and its characteristics. Eclét Quím 2008;33:15-20.
35. Mardhiah Adib Z, Ghanbarzadeh S, Kouhsoltani M, Yari Khosroshahi A, Hamishehkar H. The effect of particle size on the deposition of solid lipid nanoparticles in different skin layers: A histological study. Adv Pharm Bull 2016;6:31-6.
36. Manjunath K, Reddy JS, Venkateswarlu V. Solid lipid nanoparticles as drug delivery systems. Methods Find Exp Clin Pharmacol 2005;27:127-44.
37. Ekambaram P, Sathali A, Priyanka K. Solid lipid nanoparticles: A review. Sci Rev Chem Commun 2012;2:80-102.
38. Ramteke KH, Joshi SA, Dhole SN. Solid lipid nanoparticle: A review. IOSR J Pharm 2012;2:34-44.
39. Üner M, Yener G. Importance of solid lipid nanoparticles (SLN) in various administration routes and future perspectives. Int J Nanomed 2007;2:289-300.
40. John Sushma N, Prathyusha D, Swathi G, Madhavi T, Deva Prasad Raju B, Mallikarjuna K, *et al*. Facile approach to synthesize magnesium oxide nanoparticles by using Clitoriaternatea-characterization and *in vitro* antioxidant studies. Appl Nanosci 2016;6:437-44.
41. Triplett DM, Rathman FJ. Optimization of β- carotene loaded solid lipid nanoparticles preparation using a high shear homogenization technique. J Nanoparticle Res 2009;11:601-14.
42. Hestrin S. The reaction of acetylcholine and other carboxylic acid derivatives with hydroxylamine, and its analytical application. J Biol Chem 1949;180:249-61.
43. Augustinson KB. Cholinesterases and anticholinesterase agents G.B. Koelle: Springer Verlag Berlin; 1957. p. 89-128.
44. Ellman GL, Courtney KD. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem Pharmacol 1961;7:88-90.
45. Humason, G.L. Animal Tissue Techniques. W.H. Freeman and Company, San Francisco 1972; 3rd edn: 307–308.
46. Harris HF. On the rapid conversion of haematoxylin into haematein in staining reactions. J Appl Microsc 1900;3:777-80.
47. Steel RG, Torrie JH. Principles and Procedures of Statistics. New York: McGraw-Hill Book Company Inc.; 1960.
48. Yokel RA, Namara PJ. Aluminium toxicokinetics: An updated mini review. Pharmacol Toxicol 2001;88:159-67.
49. Reyes AE, Perez DR, Alvarez A, Garrido J, Gentry MK, Doctor BP, *et al*. A monoclonal antibody against acetylcholinesterase inhibits the formation of amyloid fibrils induced by the enzyme. Biochem Biophys Res Commun 1997;232:652-5.
50. Bowen DM, Smith CB, White P. Neurotransmitter-related enzymes and indicates of hypoxia in senile dementia and other abiotrophies. Brain 1976;99:459-96.
51. Davies P, Maloney AJ. Selective loss of central cholinergic neurons in Alzheimer's disease. Lancet 1976;2:1403.
52. Szutowicz A, Bielarczyk H, Kisielevski Y, Jankowska A, Madziar B, Tomaszewicz M. Effects of aluminum and calcium on acetyl-CoA metabolism in rat brain mitochondria. J Neurochem 1998;71:2447-53.
53. Ravi SM, Prabhu BM, Raju TR, Bindu PN. Long-term effects of postnatal aluminium exposure on acetylcholinesterase activity and biogenic amine neurotransmitters in rat brain. Indian J Physiol Pharmacol 2000;44:473-8.
54. Kumar S. Aluminium-induced changes in the rat brain serotonin system. Food Chem Toxicol 2002;40:1875-80.
55. Kumar S. Aluminium-induced biphasic effect. Med Hypotheses 1999;52:557-9.
56. Lakshmi BV, Sudhakar M, Aniska M. Neuroprotective role of hydroalcoholic extract of Vitisvinifera against aluminiuminduced oxidative stress in rat brain. Neurotoxicology 2014;41:73-9.
57. Ringman JM, Frautschy SA, Cole GM, Masterman DL, Cummings JL. A potential role of the curry spice curcumin in Alzheimer's disease. Curr Alzheimer Res 2005;2:131-6.
58. Yadav RS, Sankhwar ML, Shukla RK, Chandra R, Pant AB, Islam F, *et al*. Attenuation of arsenic neurotoxicity by curcumin in rats. Toxicol Appl Pharmacol 2009;240:367-76.
59. Pan R, Qiu S, Lu DX, Dong J. Curcumin improves learning and memory ability and its neuroprotective mechanism in mice. Chin Med J (Engl) 2008;121:832-9.
60. Rodrigues T, Reker D, Schneider P, Schneider G. Counting on natural products for drug design. Nat Chem 2016;8:531-41.
61. Prachayasittikul V, Worachartcheewan A, Shoombuatong W, Songtawee N, Simeon S, Prachayasittikul V, *et al*. Computer-aided drug design of bioactive natural products. Curr Top Med Chem 2015;15:1780-800.
62. Chen G, Roy I, Yang C, Prasad PN. Nanochemistry and nanomedicine for nanoparticle-based diagnostics and therapy. Chem Rev 2016;116:2826-85.
63. Mignani S, El Kazzouli S, Bousmina M, Majoral JP. Expand classical drug administration ways by emerging routes using dendrimer drug delivery systems: A concise overview. Adv Drug Deliv Rev 2013;65:1316-30.
64. Krishnamoorthy K, Manivannan G, Kim SJ, Jeyasubramanian K, Premanathan M. Antibacterial activity of MgO nanoparticles based on lipid peroxidation by oxygen vacancy. J Nanopart Res 2012;14:1-10.
65. Stoimenov PK, Klinger RL, Marchin GL, Klabunde KJ. Metal oxide nanoparticles as bactericidal agents. Langmuir 2002;18:6679-86.
66. Jin T, He Y. Antibacterial activities of magnesium oxide (MgO) nanoparticles against foodborne pathogens. J Nanopart Res 2011;13:6877-85.
67. Ge S, Wang G, Shen Y, Zhang Q, Jia D, Wang H, *et al*. Cytotoxic effects of MgO nanoparticles onhuman umbilical vein endothelial cells *in vitro*. IET Nanotechnol 2011;5:36.
68. Lai JC, Lai MB, Jandhyam S, Dukhande VV, Bhushan A, Daniels CK, *et al*. Exposure to titaniumdioxide and other metallic oxide nanoparticles induces cytotoxicityon human neural cells and fibroblasts. Int J Nanomedicine 2008;3:533-45.
69. Arora S, Rajwade JM, Paknikar KM. Nanotoxicology and *in vitro* studies: The need of the hour. Toxicol Appl Pharmacol 2012;258:151-65.
70. Fukui H, Horie M, Endoh S, Kato H, Fujita K, Nishio K, *et al*. Association of zinc ion release and oxidative stress induced by intratracheal instillation of ZnO nanoparticles to rat lung. Chem Biol Interact 2012;198:29-37.
71. Chalkidou A, Simeonidis K, Angelakeris M, Samaras T, Martinez-Boubeta C, Balcells L, *et al*. *In vitro* application of Fe/MgO nanoparticles as magnetically mediated hyperthermia agents for cancer treatment. J MagnMagn Mater 2011;323:775-80.
72. Moeini-Nodeh S, Rahimifard M, Baeeri M, Abdollahi M. Functional improvement in rats' pancreatic islets using magnesium oxide nanoparticles through antiapoptotic and antioxidant pathways. Biol Trace Elem Res 2017;175:146-55.
73. Süzer T, Coskun E, Islekel H, Tahta K. Neuroprotective effect of magnesium on lipid peroxidation and axonal function after experimental spinal cord injury. Spinal Cord 1999;37:480-4.
74. Kakkar V, Singh S, Singla D, Kaur IP. Exploring solid lipid nanoparticles to enhance the oral bioavailability of curcumin. Mol Nutr Food Res 2011;55:495-503.
75. Kakkar V, Singh S, Singla D, Sahwney S, ChauhanAS, Singh G, Kaur IP. Pharmacokinetic applicability of a validated liquid chromatography tandem mass spectroscopy method for orally administered curcumin loaded solid lipid nanoparticles to rats. J Chromatogr B 2010;878:3427-31.
76. Yavin E, Brand A, Green P. Docosahexaenoic acid abundance in the brain: A biodevice to combat oxidative stress. Nutr Neurosci 2002;5:149-57.
77. Bechoua S, Dubois M, Dominguez Z, Goncalves A, Némoz G, Lagarde M, *et al*. Protective effect of docosahexaenoic acid against hydrogen peroxide-induced oxidative stress in human lymphocytes. Biochem Pharmacol 1999;57:1021-30.