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Research Article
Modulatory Efficiency of LP/LF Nano-Combination on Neurochemical and Behavioural Retardations in the Brain of Induced-Epileptic Rats

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Abstract

Background and Objective: Epilepsy is one of the normal neurological problems that came about because of strange electrical movements and prompt serious and far-reaching cell misfortune in the mind. This study aimed to investigate if a nano-Chitosan formulation loaded with bovine milk lactoperoxidase (LPO) and lactoferrin (LF) could prevent Lithium Chloride/Pilocarpine-induced epilepsy in rats or not. **Materials and Methods:** Adult male rats (200-250 g) were partitioned into four groups (8 animals each) as follows: Group (1) Normal rats served as control group and received saline orally, group (2) Normal rats ingested with a daily oral dose of LPO and LF-NPS formulation at 50 mg kg^{-1}, group (3) Pilocarpine-induced epileptic rats and group (4) Epilepsy-modeled rats were treated with LPO+LF NPs (50 mg/kg/day, orally) for 6 weeks. **Results:** The results revealed that the administration of LPO+LF-NPs markedly improved the induced-epilepsy disorders, this was monitored from the significant reduction in the values of caspase-3, TNF-α, IL-1β, $CD4^+$, MDA and NO coupled with remarkable raise in AchE-ase, dopamine, serotonin, SOD and GPx, CAT and GSH values in both brain regions. **Conclusion:** This study supported the anti-epilepsy features of LPO+LF-NPS against Lithium Chloride/Pilocarpine-induced epilepsy in rats through the improvement of the immune response, reduction of inflammation and restoration of the impaired oxidative stress status.

Key words: Epilepsy, lithium chloride/pilocarpine, LPO and LF, nano-formulation, biogenic amines, oxidative stress markers

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Epilepsy is a persistent sickness portrayed clinically by repetitive and unusual seizures[1] because of uncontrolled neuronal hyperactivity. A new enormous scope epidemiological review of 196 nations and districts all over the planet observed that there were 45.9 million individuals with epilepsy in 2016, with the most elevated rate in youngsters matured 5-9 years[1]. Severe status epilepticus or recurrent seizures can cause cognitive decline, impair quality of life and increase the risks of injury and sudden death. The most common treatments for epilepsy are oral antiepileptic drugs (AEDs). However, about 30% of children are resistant to currently available AEDs[2].

Although many studies have attempted to develop new antiepileptic drugs that could be effective in terminating SE and preventing its long-term consequences[2], more than a third of patients with epilepsy suffer from uncontrolled seizures that lead to substantially diminished quality of life[3].

Researchers revealed that lithium treatment showed anti-convulsant, neuro-protective and anti-manic against neurodegeneration[4,5].

The most convenient model of temporal lobe epilepsy is the pilocarpine model in rats. Pilocarpine, a muscarinic receptor agonist, is utilized to cause SE, which is then followed by its neuro-pathological characteristics, such as neuronal death, reactive gliosis and remodelling of synaptic circuitry. In combination with pilocarpine, lithium pre-treatment potentiates the epileptogenic action of pilocarpine and allows a reduction of the pilocarpine dose required to elicit SE[6].

Alpha-lactalbumin (α-LA), lactoperoxidase (LPO) and lactoferrin (LF) are the most major active proteins in whey[7]. Lactoperoxidase is a key whey enzyme that oxidizes halides and pseudohalides with hydrogen peroxide to produce biocidal small molecules. Hydrogen peroxide is harmful to the epithelium, its concentration must be carefully monitored. On the other hand, LPO has antioxidant properties and can destroy carcinogens, its tumoricidal properties have only been reported in a few other studies[8]. LF is an iron-binding protein with a wide range of biological functions including antibacterial, antioxidant, anti-inflammatory and anti-cancer potentials[8].

The fundamental need of a biomaterial is to be non-toxic and biocompatible. Furthermore, some nano-materials are viewed as broken cell parts and begin cell debasement through autophagy, a commonplace outcome of maternal poisonousness[9]. Whereas, the physical and chemical characteristics of bovine milk lactoperoxidase-lactoferrin with chitosan nano-formulation belong to the effective nanoscale of drug delivery, therefore, the goal of this study was to investigate if this nano-combination could exert its therapeutic efficiency against lithium chloride/pilocarpine (Li/Pc)-induced epilepsy in adult male rat.

MATERIALS AND METHODS

Study area: This study was carried out in the period from January to July, 2022 at the Medical Physiology Department, National Research Centre, Egypt.

Chemicals: Lithium chloride, chloral hydrate, pilocarpine (Sigma, USA), Bovine lactoferrin (LF) and lactoperoxidase (LPO) were purified from bovine skim milk according to the previous methods[10,11]. Both purified LF and LPO were characterized for their purity and activity according to the previous studies[12,13].

Preparation of LF and LPO loaded chitosan nano-combinations: Nanoparticles of chitosan were synthesized using an ionic gelation method according to Anitha *et al.*[14] with a slight modification as described by the Abu-Serie and El-Fakharany[11]. Chitosan solution was synthesized by dissolving 2 mg mL^{-1} chitosan in an aqueous solution of 0.1% acetic acid with continuous mixing until dissolving, 1 N NaOH was added until the PH reached 5.5. So, at that point, both LF and LPO (at centralization of 0.5 mg mL^{-1}) were added to more than 1 hr (to the concentration of 0.5 mg mL^{-1}) chitosan with continuous mixing at 4°C. Then, dextran sodium sulfate was added as a cross-linker until the synthesis of NPs. The NPs were centrifuged at 14000 rpm for 30 min at 4°C, the hastened NPs were washed two times by the use of phosphate buffer saline (PBS) and lyophilized until needed.

Characterization of the prepared nano-combination

Surface charge and particle size characteristics: The zeta potential and hydrodynamic size of the prepared nano-combination were determined by Zetasizer Nano ZS (Malvern Instruments, Worcestershire). The investigations were made at a 532 nm frequency at 25°C, at a fixed scattering angle detection of 11° and 90°.

Analysis of Scanning Electron Microscopy (SEM): The surface morphology and Shape of the prepared nano-combinations were analyzed by SEM (JEOL, JSM-6460LV, Japan) at 20 kV.

Analysis of Transmission Electron Microscopy (TEM): The size and morphology of the prepared nano-combinations were determined by TEM (JEOL, JEM-1230, Japan) and visualized at 120 kV.

Experimental modelling: This work was conducted on 32 adult male Wistar albino rats (200-250 g) obtained from the Animal Colony, National Research Centre, Egypt. Before starting the experiment, the animals were habituated in plastic cages for one week for acclimation. Regular water and standard rat pellets were generally accessible. All animals received human care in compliance with the standard institutional criteria for the care and use of experimental animals according to the NRC ethical committee.

Induction of epileptic model: Rats were treated with lithium chloride (3 mol kg^{-1}), then followed with intraperitoneal administration of pilocarpine (30 mg kg^{-1}) after 18-20 hrs, Seizures were segregated according to Racine[15]. The 0 levels were characterized by no symptoms, the 1 level was portrayed by facial clonus. Level II: Grade I plus rhythmic nods, Grade III: Embraced not only Grade II but also forelimb myoclonus, Grade IV: Comprehended Grade III plus upright hind limb, Grade V: A burst of seizures and loss of normal position. To decline symptoms of a seizure, an intraperitoneal dose of chloral hydrate 200 mg kg^{-1} 1 hr later was given, rats carried grade IV characters and more were identified as epileptic model[14].

Animals' grouping: Epileptic and normal rats were partitioned into four groups as follows: Group 1: Rats ingested orally with saline, and served as control, group 2: Rats ingested orally with LPO/LF-NPs combination (50 mg/kg/day) for 28 days, group 3: Epilepsy-induced rats and group 4: Epileptic-rats orally treated with LPO/LF-NPs combination (50 mg/kg/day) for 28 days.

Behavioural tests

Open-field test: In this test, each animal was placed at the bottom of the right corner of the device was carried out. Each animal was placed at the bottom of the right corner of the device and the behaviours of locomotion, rearing, self-grooming and central latency were noticed for 5 min according to the method of Zimcikova *et al.*[16].

Tissue sampling: Finally, sera samples were separated after sacrificing the animals. Each factor brain was anatomized to expose different regions of the cortex and the hippocampus, then frozen at -80°C until analyzed. A 10% w/v tissue homogenates of the hippocampus and the cortex of the brain's right half were prepared (ultrasonically) in Tris-HCl buffer (pH 7.4) for the the determination of oxidative stress markers, inflammatory cytokines, acetylcholinesterase and biogenic amines.

Detection of acetylcholinesterase activity: Acetylcholinesterase (AchE) activity was determined according to the modified method of Islam *et al.*[17].

Detection of biogenic amines: The levels of brain biogenic amines such as dopamine, and serotonin were estimated by the HPLC technique according to the method of the Garabadu *et al.*[18].

Detection of oxidative stress markers: GSH, SOD, CAT and NO levels were estimated using kits purchased from Biodiagnostic Co., Giza, Egypt. Lipid peroxidation end product (MDA) level was investigated according to the method described by Mourad *et al.*[19].

Cytokines and apoptotic markers: Tumour Necrosis factor alpha (TNF-α), Interleukin-1 beta (IL-1β), caspase-3 and CD4 levels were estimated using rats' ELISA kits purchased from Sino Gene Clon Biotech Co, Hang Zhou, China.

Statistical analysis: One-way Analysis of Variance (ANOVA) followed by a *post hoc* test (Duncan's) at $p \leq 0.05$ according to Steel *et al.*[20], was performed using a Statistical Analysis System (SAS) program software, Copyright (c) 1998 by SAS Institute Inc., Cary, NC, USA.

RESULTS

Synthesis and characterization of loaded lactoperoxidase and lactoferrin-chitosan-nano-combinations: The size of lactoferrin and lactoperoxidase-loaded NPS was estimated to be 336.7 nm with a zeta potential measurement of around 47.28 mV (Fig. 1a-b). The morphology of LF and LPO-loaded chitosan NPs using SEM and TEM was demonstrated in Fig. 1c-d, respectively.

Unfortunately, pilocarpine-induced Status Epilepticus (SE) showed a marked drop in the levels of dopamine, serotonin and acetylcholinesterase (AChE-ase) in both cortex and hippocampus brain regions. Contrarily in a favourable manner, the treatment of SE-rats with LPO/LF-NPs combination recorded a significant improvement in values of biogenic amines and AchE-ase activity in the concerned brain areas towards the normal group (Table 1).

(a)

Sample name: 2101044660_ES3

Comments:
Operator:
Temperature: 20.0°C
Start time: 05 June, 21 12:22:40 pm
Auto SDP: Yes
Angle: 11.1°
Run time (manual): 300 sec
Sample time (auto): 224 us
Prescale (auto): 512
21 CRR part 11 compliant? NO

SOM/SOP name: Defaultsomsom
Diluent: Water
End time: 05 Jan, 21 12:39:30 pm
Diluent visoosity/R1: 1.002 cP/1.333
90.0°
3000 sec
5.5 us
32

Unimodal results summary

Angle	Mean (nm)	P.I.	Off. coef (m^2/sec)	Counts/sec	Baseline error	Overflow
11.1°	471.8	-6.797	9.08e-13	1.11e+06	0.17%	0
90.0°	278.9	0.236	1.54e-12	1.51e+06	0.01%	0

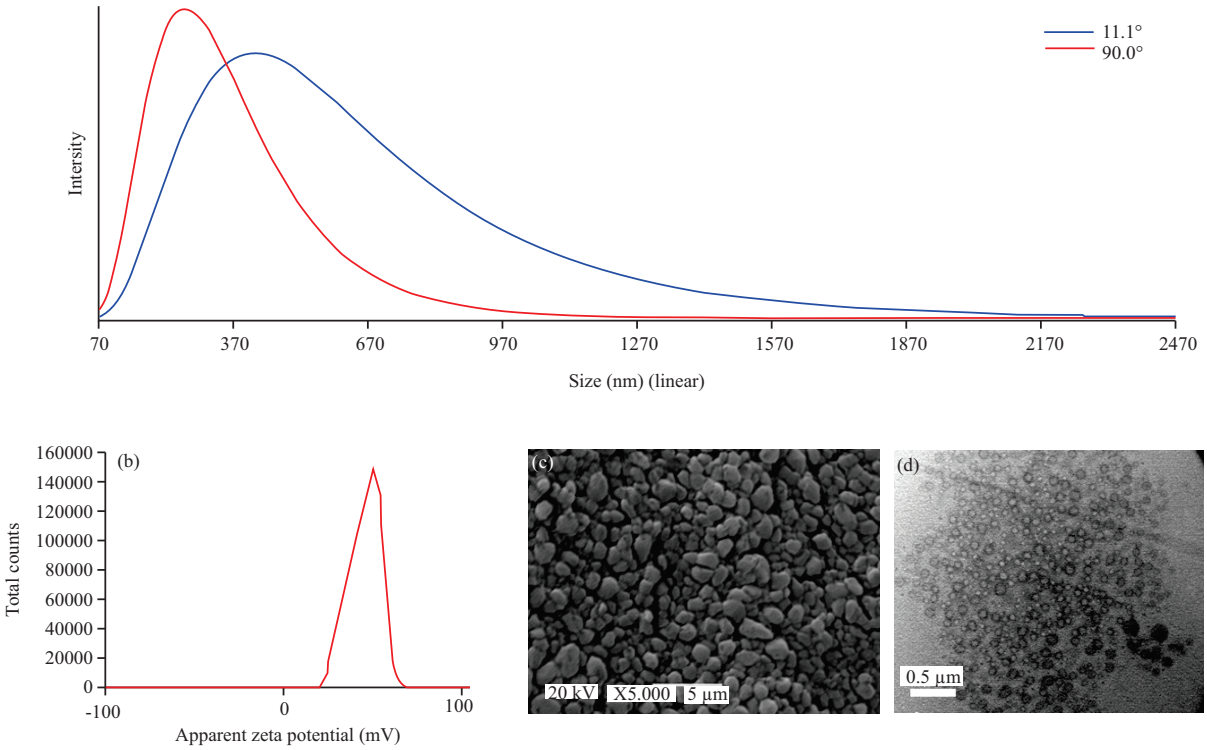

Fig. 1(a-d): Identical characterization of the prepared LF coated LPO-loaded chitosan Nps, (a) Distribution analysis of nanoparticle size, (b) Investigation of zeta potential presenting surface charge distribution of Nps, (c) SEM analysis of the typical morphological shape of LF-coated LPO-loaded chitosan Nps and (d) TEM analysis of LF-coated LPO-loaded chitosan Nps

All images are representative of three different experiments

Table 2 showed the effect of LPO/LF-NPs on the oxidative status of the brain regions of SE-rats. Treatment of SE-rats with LPO/LF-NPs combination showed a significant drop in the oxidative markers (MDA and NO) matched with a significant elevation in the antioxidant battery (GSH, SOD, CAT and GPx) in both cortex and hippocampus in compare to the epilepsy group.

Dislike, the immune-inflammatory and apoptotic markers (TNF-α, IL-1β, $CD4^+$ and caspase-3) recorded a significant rise in both brain regions of the SE animals group. Favourably, post-treatment of SE-rats with LPO/LF-NPs combination markedly down-regulated the mentioned inflammatory and apoptotic markers near to the normal group (Table 3).

Table 1: Biogenic amines level and ACh-ase activity of brain cortex and hippocampus of control, epileptic and LPO/LF-NPS combination-treated rats

Parameters	Control	Nano-combination	Epilepsy	Epilepsy+Nano-combination
Cortex				
ACh-ase (μmol/min/g)	10460±65.4	10832±54.8	6154±23.5*	8482±28.9#
Dopamine (pg g^{-1})	1250±7.89	1245±8.22	753±6.51*	1081±6.87#
Serotonin (pg g^{-1})	986±5.5	999±6.01	606±6.87*	949±6.33#
Hippocampus				
ACh-ase (μmol/min/g)	15423±89	15690±77	9864±61*	13067±74#
Dopamine (pg g^{-1})	578±9.2	563±10.11	336±5.31*	442±5.87#
Serotonin (pg g^{-1})	381±2.17	392±2.4	212±1.47*	308±2.01#

Data are presented as Mean±SEM. The data were subjected to one-way ANOVA followed by a *post hoc* test (Duncan's) at $p \leq 0.05$. Within the same row, symbol and *Significantly different from the control group, while symbol and #Significantly different from the epilepsy group

Table 2: Levels of oxidative stress markers of brain cortex and hippocampus of control, epileptic and LPO/LF-NPS combination-treated rats

Parameters	Control	Nano-complex	Epilepsy	Epilepsy+Nano-combination
Cortex				
MDA (μmol g^{-1})	4249±104	4054±78	7950±89*	5914±71#
NO (nmol g^{-1})	0.88±0.013	0.86±0.014	1.52±0.028*	0.98±0.017#
CAT (μmol/min/g)	6641±53	6457±61	4112±53*	5858±57#
GPx (μmol/min/g)	18.44±1.01	18.93±1.17	13.65±0.97*	16.08±1.21#
SOD (U g^{-1})	2684±65	2784±57	1627±37*	2398±81#
GSH (μmol g^{-1})	5492±61	5601±55	4678±38*	5300±49#
Hippocampus				
MDA (μmol g^{-1})	4224±111	4030±99	6611±71*	5186±52#
NO (nmol g^{-1})	3.13±0.88	3.03±0.71	5.97±0.87*	3.78±0.12#
CAT (μmol/min/g)	5909±43	6022±48	3954±39*	5430±44#
GPx (μmol/min/g)	27.44±0.91	28.45±0.89	19.82±0.64*	24.84±0.79#
SOD (U g^{-1})	3255±58	3299±55	1867±32*	2346±37#
GSH (μmol g^{-1})	16419±72	16617±97	11515±67*	15096±81#

Data are presented as Mean±SEM. The data were subjected to one-way ANOVA followed by a *post hoc* test (Duncan's) at $p \leq 0.05$. Within the same row, symbol *Significantly different from the control group, while, symbol #Significantly different from the epilepsy group

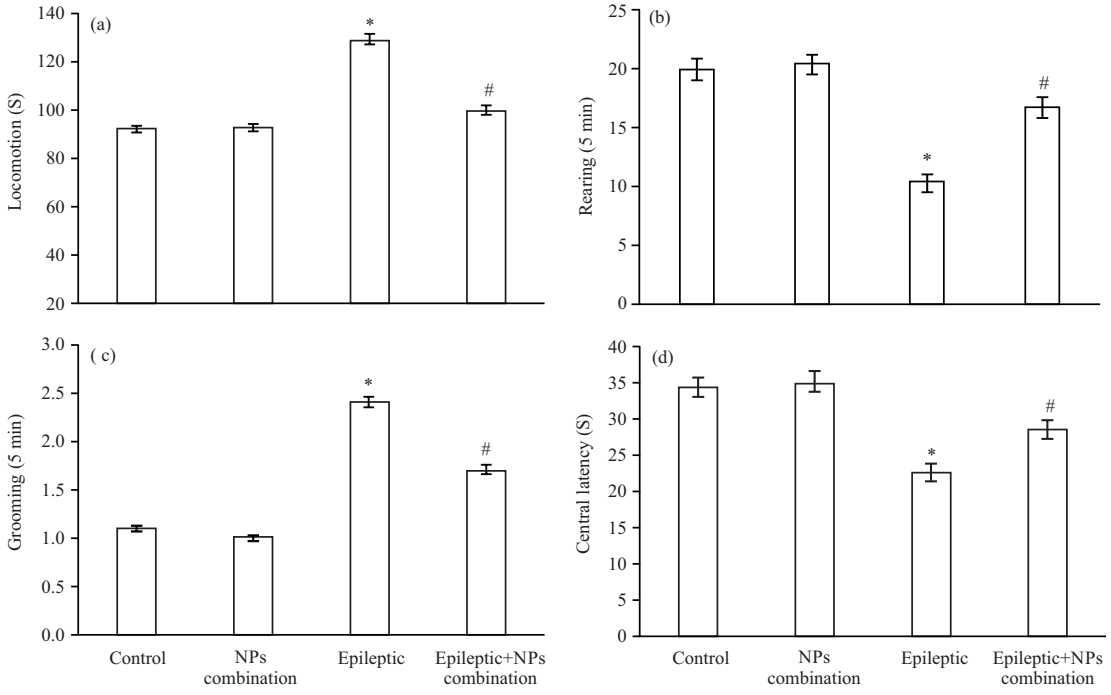

Fig. 2(a-d): Effect of LPO/LF-NPs combination administration on, (a) Locomotion, (b) Rearing, (c) Self-grooming and (d) Central latency of epileptic rats

Symbol *Significantly different from the control group, while symbol and #Significantly different from the epilepsy group

Table 3: Levels of immune-inflammatory and apoptotic markers of brain cortex and hippocampus of control, epileptic and LPO/LF-NPs combination treated rats

Parameters	Control	Nano- combination	Epilepsy	Epilepsy+Nano-combination
Cortex				
TNF-α (ng g^{-1} tissue)	12.35±0.89	11.97±0.66	21.78±1.22*	14.38±1.08#
IL-1β (ng g^{-1} tissue)	119.60±2.87	118.32±2.84	169.89±3.95*	141.79±3.24#
CD4 (U g^{-1} tissue)	42.63±2.41	39.33±2.38	71.36±3.84*	50.04±3.01#
Caspase 3 (pg g^{-1} tissue)	17.68±1.1	17.29±1.08	23.86±2.33*	19.61±2.10#
Hippocampus				
TNF-α (ng g^{-1} tissue)	63.42±3.77	60.47±4.17	106.34±3.89*	87.97±4.1#
IL-1β (ng g^{-1} tissue)	140.24±6.32	134.51±7.01	282.38±7.22*	177.44±5.65#
CD4 (U g^{-1} tissue)	22.31±1.11	21.32±1.32	34.32±1.84*	26.201±1.65#
Caspase 3 (pg g^{-1} tissue)	66.45±2.14	64.29±2.02	119.86±3.45*	82.55±2.99#

Data are presented as Mean±SEM. The data were subjected to one-way ANOVA followed by a *post hoc* test (Duncan's) at $p \leq 0.05$. Within the same row, symbol *Significantly different from the control group, while symbol and #Significantly different from the epilepsy group

Concerning the behavioural tests (locomotion, rearing, self-grooming and central latency), SE-rats showed notable retardation. Interestingly, treatment of SE-rats with LPO/LF-NPS combination resulted in a marked behavioural improvement and recorded values close to that of the control group (Fig. 2a-d).

DISCUSSION

Epilepsy has a complicated, multifaceted and multifactorial pathophysiology. Numerous epilepsy models have been developed to date, including those brought on by pharmacological agents and electrical stimulation, the pathophysiological mechanisms in the lithium chloride/pilocarpine-induced paradigm are distinctive[21].

The term oxidative stress refers to the set of alterations that occurs after the exposure of biological tissues, cells and macromolecules, to an excess of oxidizing agents. Disruption of the cellular oxide reduction balance may lead to severe damage, at both tissue and organ levels, leading to impaired function. It has been well established that, endogenous antioxidants play a pivotal role in antioxidant defense mechanisms against oxidative impairment, reflecting the protective role of biological functions[22].

The brain is more vulnerable to oxidative stress damaging effects than other tissues for several reasons. One reason lies in its high consumption of oxygen[23].

In the present study, an elevation in MDA and NO content coupled with a marked drop in the activity of SOD, GPx, GSH and CAT were observed in the brain cortex and hippocampus of the SE rats, these findings agree with those previously established[24-28]. It was stated that the relationship between status epilepsy and ROS is well known as the epileptiform activity causes excessive free radical production of ROS, a factor believed to be involved in the mechanism leading to cell death and neurodegeneration. Free radicals-mediated reactions aren't the main cause of the development of seizures during epilepsy while being involved in the biochemical sequelae of events leading to seizure-induced cell death[29].

Interestingly, treatment of SE-modeled rats with LPO/LF-NP combination efficiently succeeded in the restoration of the impaired oxidative status in both brain regions. Recently, progress has been made in the study on LPO/LF-NPs combination[10]. As bioactive material, LPO/LF-NPs combination was demonstrated to exhibit many pharmacological effects, including anti-ageing, antitumor, antioxidation, immune enhancement and memory improvement[8,30].

Nitric oxide (NO), a ubiquitous gaseous cellular messenger, plays significant roles in a variety of neurobiological processes, including the process of endothelium-dependent vasodilatation[31,32], neuro-transmission[31] and host-defence mechanisms[32]. The current study showed a marked elevation of NO level in both brain regions of epileptic rats. It was reported that in many brain degenerative processes there is a rapid rise in the level of NO due to hyperactivity of iNOS[32]. In addition, in seizure experimental models, NO rise was attributed to the release of glutamate that in its turn led to the overstimulation of NMDA receptors, ending with prolonged NO elevation. Moreover, the possibility that excessive NMDA receptor activation (with the consequent increase in intraneuronal Ca^{2+} through Ca^{2+}/calmodulin-regulated NOS) enhances the neurotoxicity of glutamate through the further release of NO[33].

Pilocarpine-induced epilepsy caused a valuable drop in the values of dopamine, serotonin and ACh-ase in both brain areas, this finding agrees with previous studies[33,34] although the mechanism of neuronal apoptosis post seizures has not yet been fully elucidated, it may be related to one or more of the following pathways: (1) Seizures lead to neurotransmitter changes in the brain, release considerable excitatory amino acid glutamine, which can open channels for Na^+, Cl^- and water molecules, causing neuronal swelling followed by neuronal apoptosis, (2) Glutamine also acts on postsynaptic

NMDA and non-NMDA receptors, so Ca^{2+} enters cells, which can increase intracellular Ca^{2+} concentration, activate voltage-dependent calcium channels and ultimately cause neuronal degeneration, (3) Glutamine increase and calcium ion inflow open the permeable pores of mitochondrial membrane, leading to mitochondrial damage via releasing cytochrome c and (4) seizures affect the regulation of neuronal apoptosis-related gene expressions and signaling pathways as P53-gene and caspase-3 were associated with neuronal apoptosis[34]. Also, it was verified that neuronal damage was related to the MAPK-signaling pathway after seizures[34,35].

Treatment of epileptogenic-modelled rats with LPO/LF chitosan nano-combination resulted in a significant improvement of biogenic amines level and ACh-ase activity in the brain cortex and hippocampus. This favourable improvement may be attributed to the excessive block and/or stabilization of free radicals generation. It was demonstrated that LPO-LF nanoparticles have been claimed to improve and strengthen the body systems through their antioxidant properties that in its turn restore[35,36]. One of the mechanisms included in the initiation and progression of epileptogenesis is oxidative stress (d).

The increase in TNF-α, IL-1β, $CD4^+$ and the caspase-3 level was observed in brain hippocampal and cortical tissues of epileptic rats, this finding runs following studies of researchers[37,38] that interpreted this elevation due to an inflammatory response that causes neuronal damage, which accelerates the development of neurodegenerative diseases and additional production of inflammatory molecules, reactive oxygen species and other mediators[39]. Many studies have shown the relevance of neuroinflammation in the pathophysiology of epilepsy[40,41]. It has been demonstrated that TNF-α regulates glutamate receptor transport through TNF-receptor-1 to induce enhanced excitatory synaptic transmission[41].

Interestingly, this study also showed that LPO/LF-NPs succeeded efficiently in down-regulation of the inflammatory response and neuronal apoptosis in the hippocampus and cortex tissue of epileptic rats. Several mechanisms are involved, either singular or combined, in the LPO/LF-NPs immunomodulating activity: It was reported that LPO/LF-NPs act on B-cells to promote the maturation of T-cell precursors into T-helper cells and induce the differentiation of immature B-cells into antigen-presenting cells[42], it has been suggested that LPO/LF-NPs may play a role in T-cell activation through modulation of dendritic cell function[43], the anti-inflammatory effect is probably due to the inhibition of production of pro-inflammatory cytokines, this could occur through the translocation of LPO/LF-NPs to the nucleus, where it blocks NF-kB activation pathway.

The SE-induced epileptogenesis provided evidence for alterations in thalamic glucose metabolism and septal 5-HT1A receptor binding in rats with epilepsy, these metabolic and molecular alterations correlated with neurobehavioral, physiological and biochemical alterations[44].

In the present study, induced epilepsy resulted in a significant increase the grooming and locomotor activity in the open-field test. Previous studies have shown that epileptic seizures increased dopamine-mediated behaviour, such as locomotion, in rats and mice[36]. The extracellular concentration of 3,4-dihydroxyphenylacetic acid (a dopamine metabolite), was markedly increased in EZ rats' brains[37], this report interprets the decreased dopamine level in our study and suggests dopaminergic system elevation which seems responsible for the locomotor hyperactivity.

The LP/LF nano-combination potentially restored the behavioural alteration that occurred post-induced seizures, this effect is possibly due to the preservation of the hippocampal GABA-receptor, suggesting inhibitory action of the nano-combination on the neuronal excitotoxicity.

CONCLUSION

This study concluded that LPO/LF-NPs possess efficient potential to ameliorate and regenerate the neuro-chemical and behavioural disorders that occurred in the brain hippocampus and cortex of epileptic rats. This effect could take place through several mechanisms such as stabilization and preventive production of free radicals, immunomodulation via inhibition of inflammatory factors expression and/or antioxidant behaviour. These effects provide a new therapeutic strategy for epilepsy management in the future.

SIGNIFICANCE STATEMENT

This study discovered that LPO/LF-NPs can be beneficial for improving the neurochemical and behavioural retardations occurred in the brain's hippocampus and cortex, this study will help the researchers to uncover the critical areas of LPO/LF-NPs that many researchers were not able to explore.

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REFERENCES

1. Beghi, E., 2020. The epidemiology of epilepsy. Neuroepidemiology, 54: 185-191.
2. Franco, V., J.A. French and E. Perucca, 2016. Challenges in the clinical development of new antiepileptic drugs. Pharmacol. Res., 103: 95-104.
3. Banach, M., B. Piskorska, S.J. Czuczwar and K.K. Borowicz, 2011. Nitric oxide, epileptic seizures, and action of antiepileptic drugs. CNS Neurol. Disord. Drug Targets, 10: 808-819.
4. Honar, H., K. Riazi, H. Homayoun, S. Demehri and M. Dehghani *et al*., 2004. Lithium inhibits the modulatory effects of morphine on susceptibility to pentylenetetrazole-induced clonic seizure in mice: Involvement of a nitric oxide pathway. Brain Res., 1029: 48-55.
5. Licht, R.W., 2012. Lithium: Still a major option in the management of bipolar disorder. CNS Neurosci. Ther., 18: 219-226.
6. Vezzani, A., 2009. Pilocarpine-induced seizures revisited: What does the model mimic? Epilepsy Curr., 9: 146-148.
7. Nadeem, A., J. Sanborn, D.L. Gettel, H.C.S. James and A. Rydström *et al*., 2015. Protein receptor-independent plasma membrane remodeling by HAMLET: A tumoricidal protein-lipid complex. Sci. Rep., Vol. 5. 10.1038/srep16432.
8. Wang, J., M. Bi, H. Liu, N. Song and J. Xie, 2015. The protective effect of lactoferrin on ventral mesencephalon neurons against MPP^+ is not connected with its iron binding ability. Sci. Rep., Vol. 5. 10.1038/srep10729.
9. Adair, J.H., M.P. Parette, E.I. Altınoğlu and M. Kester, 2010. Nanoparticulate alternatives for drug delivery. ACS Nano, 4: 4967-4970.
10. Almahdy, O., E.M. El-Fakharany, E. El-Dabaa, T.B. Ng and E.M. Redwan, 2011. Examination of the activity of camel milk casein against hepatitis C virus (genotype-4a) and its apoptotic potential in hepatoma and heLa cell lines. Hepatitis Mon., 11: 724-730.
11. Abu-Serie, M.M. and E.M. El-Fakharany, 2017. Efficiency of novel nanocombinations of bovine milk proteins (lactoperoxidase and lactoferrin) for combating different human cancer cell lines. Sci. Rep., Vol. 7. 10.1038/s41598-017-16962-6.
12. EL-Fakharany, E.M., L. Sánchez, H.A. Al-Mehdar and E.M. Redwan, 2013. Effectiveness of human, camel, bovine and sheep lactoferrin on the hepatitis C virus cellular infectivity: Comparison study. Virol. J., Vol.10. 10.1186/1743-422X-10-199.
13. El-Fakharany, E.M., V.N. Uversky and E.M. Redwan, 2017. Comparative analysis of the antiviral activity of camel, bovine, and human lactoperoxidases against herpes simplex virus type 1. Appl. Biochem. Biotechnol., 182: 294-310.
14. Anitha, A., V.G. Deepagan, V.V.D. Rani, D. Menon, S.V. Nair and R. Jayakumar, 2011. Preparation, characterization, *in vitro* drug release and biological studies of curcumin loaded dextran sulphate-chitosan nanoparticles. Carbohydr. Polym., 84: 1158-1164.
15. Racine, R.J., 1972. Modification of seizure activity by electrical stimulation: II. Motor seizure. Electroencephalography Clin. Neurophysiol., 32: 281-294.
16. Zimcikova, E., J. Simko, I. Karesova, J. Kremlacek and J. Malakova, 2017. Behavioral effects of antiepileptic drugs in rats: Are the effects on mood and behavior detectable in open-field test? Seizure: Eur. J. Epilepsy, 52: 35-40.
17. Islam, M.S., A.M. Al-Majid, E.N. Sholkamy, S. Yousuf and M. Ayaz *et al*., 2022. Synthesis, molecular docking and enzyme inhibitory approaches of some new chalcones engrafted pyrazole as potential antialzheimer, antidiabetic and antioxidant agents. J. Mol. Struct., Vol. 1269. 10.1016/j.molstruc.2022.133843.
18. Garabadu, D., N. Srivastava and Y. Murti, 2019. *Calotropis procera* attenuates chronic unpredictable mild stress-induced depression in experimental animals. Metab. Brain Dis., 34: 1635-1647.
19. Mourad, I.M., N.A. Noor, H.S. Mohammed, H.S.A. Ezz and Y.A. Khadrawy, 2021. A neurochemical and electrophysiological study on the combined effects of caffeine and nicotine in the cortex of rats. Basic Clin. Neurosci., 12: 681-692.
20. Steel, R.G.D., J.H. Torrie and D.A. Dickey, 1997. Principles and Procedures of Statistics: A Biometrical Approach. 3rd Edn., McGraw-Hill Co., New York, USA., ISBN: 9780070610286, Pages: 666.
21. Zhong, M., W.L. Song, Y.C. Xu, Y. Ye and L.Y. Feng, 2015. Paeoniflorin ameliorates ischemic neuronal damage *in vitro* via adenosine A1 receptor-mediated transactivation of epidermal growth factor receptor. Acta Pharmacol. Sin., 36: 298-310.
22. Genchi, G., M.S. Sinicropi, G. Lauria, A. Carocci and A. Catalano, 2020. The effects of cadmium toxicity. Int. J. Environ. Res. Public Health, Vol. 17. 10.3390/ijerph17113782.
23. Sah, R., F. Galeffi, R. Ahrens, G. Jordan and R.D. Schwartz-Bloom, 2002. Modulation of the GABAA-gated chloride channel by reactive oxygen species. J. Neurochem., 80: 383-391.
24. Lin, T.K., S.D. Chen, K.J. Lin and Y.C. Chuang, 2020. Seizure-induced oxidative stress in status epilepticus: Is antioxidant beneficial? Antioxidants, Vol. 9. 10.3390/antiox9111029.
25. Shakeel, S., M.U. Rehman, N. Tabassum, U. Amin and M.U.R. Mir, 2017. Effect of naringenin (A naturally occurring flavanone) against pilocarpine-induced status epilepticus and oxidative stress in mice. Pharmacogn. Mag., 13: 154-160.

26. Shekh-Ahmad, T., A. Lieb, S. Kovac, L. Gola, W.C. Wigley, A.Y. Abramov and M.C. Walker, 2019. Combination antioxidant therapy prevents epileptogenesis and modifies chronic epilepsy. Redox Biol., Vol. 26. 10.1016/j.redox.2019.101278.
27. Parsons, A.L.M., E.M.V. Bucknor, E. Castroflorio, T.R. Soares, P.L. Oliver and D. Rial, 2022. The interconnected mechanisms of oxidative stress and neuroinflammation in epilepsy. Antioxidants, Vol. 11. 10.3390/antiox11010157.
28. Rostamian, S., S.K. Hedayati, S. Khosraviani, E. Aali and Y. Naderi , 2021. Anticonvulsive and antioxidant effects of pioglitazone on pilocarpine-induced seizures in mice, Iran. J. Toxicol., 15: 271-278.
29. Freitas, R.M., V.S. Nascimento, S.M.M. Vasconcelos, F.C.F. Sousa, G.S.B. Viana and M.M.F. Fonteles 2004. Catalase activity in cerebellum, hippocampus, frontal cortex and striatum after status epilepticus induced by pilocarpine in Wistar rats. Neurosci. Lett., 365: 102-105.
30. Elzoghby, A.O., M.A. Abdelmoneem, I.A. Hassanin, M.M.A. Elwakil and M.A. Elnaggar *et al*., 2020. Lactoferrin, a multi-functional glycoprotein: Active therapeutic, drug nanocarrier & targeting ligand. Biomaterials, Vol. 263. 10.1016/j.biomaterials.2020.120355.
31. Kuriyama, K. and S. Ohkuma, 1995. Role of nitric oxide in central synaptic transmission: Effects on neurotransmitter release. Jpn. J. Pharmacol., 69: 1-8.
32. Danek, A., T. Rainer and S.D. Sala, 2022. Ockham's razor, not a barber's weapon but a writer's tool. Brain, 145: 1870-1873.
33. Aseervatham, G.S.B., E. Abbirami, T. Sivasudha and K. Ruckmani, 2020. *Passiflora caerulea* L. fruit extract and its metabolites ameliorate epileptic seizure, cognitive deficit and oxidative stress in pilocarpine-induced epileptic mice. Metab. Brain Dis., 35: 159-173.
34. Yang, Z., J. Wang, C. Yu, P. Xu and J. Zhang *et al*., 2018. Inhibition of p38 MAPK signaling regulates the expression of EAAT2 in the brains of epileptic rats. Front. Neurol., Vol. 9. 10.3389/fneur.2018.00925.
35. Vezzani, A., S. Balosso and T. Ravizza, 2019. Neuroinflammatory pathways as treatment targets and biomarkers in epilepsy. Nat. Rev. Neurol., 15: 459-472.
36. Sogawa, Y., M. Monokoshi, D.C. Silveira, B.H. Cha and M.R. Cilio *et al*., 2001. Timing of cognitive deficits following neonatal seizures: Relationship to histological changes in the hippocampus. Dev. Brain Res., 131: 73-83.
37. Yoshida, K., H. Higuchi, M. Kamata, M. Yoshimoto, T. Shimizua and Y. Hishikawa, 1997. Dopamine releasing response in rat striatum to single and repeated electroconvulsive shock treatment. Prog. Neuro-Psychopharmacol. Biol. Psychiatry, 21: 707-715.
38. Zhang, M., N. Lu, Q. Li, M. Cui and M. Zhang, 2022. Editorial: Drug delivery system based on nanoparticles for inflammation and cancer therapy. Front. Mol. Biosci., Vol. 9. 10.3389/fmolb.2022.938348.
39. Wang, A., Z. Si, X. Li, L. Lu, Y. Pan and J. Liu, 2019. FK506 attenuated pilocarpine-induced epilepsy by reducing inflammation in rats. Front. Neurol., Vol. 10. 10.3389/fneur.2019.00971.
40. Rana, A. and A.E. Musto, 2018. The role of inflammation in the development of epilepsy. J. Neuroinflammation, Vol. 15. 10.1186/s12974-018-1192-7.
41. Patel, D.C., G. Wallis, E.J. Dahle, P.B. McElroy and K.E. Thomson, 2017. Hippocampal TNFα signaling contributes to seizure generation in an infection-induced mouse model of limbic epilepsy. eNeuro, Vol. 4. 10.1523/ENEURO.0105-17.
42. Actor, J.K., S.A. Hwang and M.L. Kruzel, 2009. Lactoferrin as a natural immune modulator. Curr. Pharm. Des., 15: 1956-1973.
43. Hwang, S.A., M.L. Kruzel and J.K. Actor, 2005. Lactoferrin augments BCG vaccine efficacy to generate T helper response and subsequent protection against challenge with virulent *Mycobacterium tuberculosis*. Int. Immunopharmacol., 5: 591-599.
44. di Liberto, V., R.M. van Dijk, M. Brendel, A.M. Waldron and C. Möller *et al*., 2018. Imaging correlates of behavioral impairments: An experimental PET study in the rat pilocarpine epilepsy model. Neurobiol. Dis., 118: 9-21.