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miRNA-124 loaded chitosan as novel therapy to induce neuroprotective and neurogenesis for improving brain revitalization after ischemic stroke

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ABSTRACT
Stroke is a world leading cause of death and disability in the field of neurology. Ischemic stroke occurs from the obstruction of blood flow to the brain and accounts for 85% of all strokes. Currently, the initial management of stroke to reduce the mortality rate is well known, resulting in increasing number of stroke survivor over the years. However, lack of appropriate treatment for post-stroke recovery lead to prolonged disability that will produce a negative impact, in particular for the productive-aged survivor. Researchers found that miRNA-124 has a lot of beneficial effect on the ischemic brain. miRNA-124 will upregulate the growth factor substances and down-regulate the TNF-α and other cytotoxic substances and increase the number of M2 microglia which is important to promote angiogenesis and matrix remodeling. Expression of miRNA-124 will also lead to differentiation and migration of neuro-progenitor cells to the lesion site while reducing the formation of the glial scar. Furthermore, chitosan derived from the extraction of shells, shrimp, and crabs, have been reported for its various advantages such as anti-infection, anti-tumor and also as carrier-mediated transported across the blood–brain barrier. Administration of miRNA-124 loaded chitosan by intranasal route will improve the drug delivery into neuron by provides moiety for cell penetration and as affinity agent towards neuronal tissues. Based on those points, the combination of chitosan and miRNA-124 may be a potential therapy to improve revitalization and reduce disability after stroke ischemic.

Keyword: miRNA-124, chitosan, ischemic stroke, neuroprotective, neurogenesis

INTRODUCTION
Stroke is one of the leading causes of death and disability in Indonesia especially in the field of neurology that is still increasing over the years. A stroke occurs due to problems with the blood supply to the brain either the blood supply is blocked or a blood vessel within the brain ruptures, causing irreversible damage to the brain tissue. Arterial occlusions or ischemic stroke account for 85% of all strokes, while primary intracerebral bleeding has a relatively low incidence of approximately 15%. Ischemic stroke resulted from an obstruction in an intracranial blood vessel or sudden loss of blood circulation to particular brain area, which drastically reduces their access to oxygen and glucose causing the corresponding loss of neurologic function. These blockages could be due to thrombotic or embolic occlusion of a cerebral artery or resulting from fatty deposits within the arteries called plaque atherosclerosis.

Several studies have reported that in the past 20 years there has been a global increase in stroke burden. WHO also estimates an increase in the number of stroke patients in some European countries by 1.1 million per year in 2000 to 1.5 million per year by 2025. Meanwhile, according to Riskesdas data (2007), stroke is the highest cause of death in Indonesia compared to another disease that is equal to 15.4%. Ghani et al. (2015) demonstrated that the prevalence of stroke in Indonesia was 8.3% in 2007 and increased to 12.1% in 2013.

Nowadays, ischemic stroke is the leading cause of disability in adults. Over a third (41%) of ischemic stroke survivors in England, Wales and Northern Ireland were discharged from hospital requiring help with activities of daily living. Most surviving patients are left with physical weakness and often suffer from pain and spasticity which cause them to rely on other people’s continuous support. Unfortunately, the prevalence of ischemic stroke survivor’s disabilities is still increasing over the years, especially on productive-aged survivor. The longer time of recovery will bring the negative impact to their working and social participation in such an age.

Currently, the initial management of stroke has been good especially for the ischemic stroke aims at dissolving the blood clot and attempt to establish revascularization such as fibrinolytic therapy,
antiplatelet agent and many else. However, the management for post-stroke recovery was not well developed yet. Even more, in developing country, longer time to revascularize also contribute to the patient prognostic and complicated post-stroke management. Therefore, research that conducted new breakthrough to accelerate the recovery and avoid the long-term disability need to be encouraged.

Recently, some research found that miRNA-124 has a lot of beneficial effect on the ischemic brain. It could prevent the death of neurons that are still in the penumbra, direct the progenitor cells differentiate and migrate to the infarct region, and help to produce VEGF and factors that support angiogenesis and neurogenesis but its expression was diminished on the ischemic brain tissue. In the other hand, chitosan derived from the extraction of shells, shrimp, crabs, and other crustaceans, has been reported for its various advantages such a carrier-mediated transported across the blood–brain barrier, anti-infection, anti-tumor and also could decreasing the non-HDL levels in circulations that will help to reduce the atherosclerosis plaque. Based on those points, the combination of chitosan and miRNA-124 could be a potential therapy to induce neuroprotective and neurogenesis for improving brain revitalization and reduce disability after ischemic stroke.

METHOD

The writing methodology used was the literature review. The source of study consists of relevant journals from the search engines www.pubmed.com, scholar.google.com, and proquest.com. Writers searched with keywords miRNA-124, “chitosan”, “ischemic stroke” “neuroprotective”, and “neurogenesis”. The inclusion criteria were all ischemic stroke patient, miRNA-124, and chitosan. The reference materials should not exceed the last ten years. From 60 journals that were reviewed, 40 were found suitable as references for this paper. The collected information noted and analyzed for validity and reliability, interpreted and compiled into one scientific literature review.

DISCUSSION

Pathogenesis of Ischemic Stroke

Since stroke is primarily a disease of vascular occlusion, it is not surprising that the size of the subsequent infarction can be predicted precisely by the severity and duration of the local cerebral blood flow (CBF) reduction. Inadequate blood flow in a single brain artery is often compensated by an efficient collateral system, particularly between the carotid and vertebral arteries via the circle of Willis or between major arteries supplying the cerebral hemispheres. However, variations in the circle of Willis such as atherosclerosis and other arterial lesions obtained may disrupt the collateral flow, increasing the chances of blockage from one artery to develop brain ischemia. The obstruction could be caused by blood clots, emboli, thrombus, and also lipid accumulation.

The most upstream consequence of cerebral ischemia fundamentally is composed of an energetic problem, the adenosine triphosphate (ATP) insufficient synthesis occurs, causing total ATP levels to drop and lactate acidosis resulting in ionic imbalance and neurotransmitter release and reuptake inhibition. In this case, glutamate neurotransmitter will be excessively released, which is the primary excitotoxic neurotransmitter. Glutamate will bind to ionotropic NMDA and AMPA receptors (iGluRs), directly or indirectly leads to a further increase in the intracellular Ca concentration and also promote an excessive sodium and water influx with joint cell swelling, edema and shrinking of extracellular space. Massive calcium influx will activate a catabolic process mediated by proteases, lipases, and nucleases causing damage cell structures which are the components of the cytoskeleton, membrane, and DNA of the cell itself.

High calcium and sodium, activation of enzymes such as cyclooxygenase and nitric oxide synthase, and mitochondrial dysfunction in the ischemic cell will stimulate the production of excessive mitochondrial oxygen radical. These reactive oxygen species (ROS) directly damage proteins, nucleic acid, lipids, and carbohydrates. The disruption of cellular calcium homeostasis, energy failure, and free radicals will disrupt the function of neuronal mitochondria. Therefore, the mitochondrial permeability transition pore (MTP) will be formed from the mitochondria. The resulting MTP will cause impending ATP production (through loss of mitochondrial potential), burst of free oxygen radicals, mitochondrial swelling, and also release of the pro-apoptotic molecules.

Inflammation also has an important role in this pathway especially in the reperfusion of cerebral blood flow causing secondary injury. Microglia has a pivotal role in the inflammatory phase in the ischemic cerebral cell. Microglia will activate and accumulates at the lesion site and in the penumbra at this phase. Activation of microglia will produce cytotoxic substances such as TNFα, Prostaglandin E2 (PGE2), Interferon gamma (IFN-g) and other cytotoxic substances, this called M1 microglia or pro-inflammatory microglia.
Thus, these cytokines will induce proliferation of the astrocyte which astrocyte becomes hypertrophy and also induce external pathway of the apoptotic cell of the neuron. In the other hands, activation of microglia will also have a significant role in promoting angiogenesis and matrix remodeling such as insulin-like growth factor (IGF)-1, TGF-b, and VEGF, this called M2 or anti-inflammatory microglia. However, under ischemic stroke, the activated microglia are mostly M1 with only small number transient M2.11

The apoptotic cells are characterized by the activation of caspase proteases that may catalyze the destruction of the cell, its compartments, and its structures. Based on the mechanism explained above, apoptotic process is divided into two, such as extrinsic pathway (activation of Fas-receptor by TNF-a) and intrinsic pathway (intracellular Ca, glutamate, ROS, and DNA damage).4,5,7 Both route will directly or indirectly lead to the activation of caspases 1,3,8,9 by damaging the mitochondrial membranes. Caspase 1 involved in the activation of cytokines and link inflammation of apoptosis, caspase 8,9 are the initiator cascade or at the beginning of the signaling cascade while caspase 3 has a central part in the apoptotic signaling cascade. Cytochrome c also act as a central mediator in the activation of the caspase cascade.8,12 The release of cytochrome c depends on the BCL-2 proteins (anti-apoptotic) activation. There are pro-apoptotic (Bax, bid, bad, etc.) and anti-apoptotic (BCL-2 and BCL-XL) proteins.12 The release of cytochrome c is triggered by activation of bad and Bax from the MTP and damage mitochondrial membrane by free radicals release. The overexpression of cytochrome c and other pro-apoptotic protein will suppress the release of the BCL-2 or BCL-XL, which further supporting the apoptotic process of the neuron cell (Figure 1).11,12

Once the mechanism of neuron apoptotic finished, the formation process of the glial scar occurs occupying the space of dead neuron. The glial scar consists predominately of reactive astrocytes, microglia, infiltrating immune cells and extracellular matrix molecules, especially CSPGs.11,13 The pro-inflammatory cytokines produced by the M1 activated microglial also can induce the proliferation of astrocyte. Thus, the astrocyte will become hypertrophic. Reactive astrocytes could inhibit axonal outgrowth and cellular migration by secreting CSPG and other inhibitory molecules of neurogenesis such as tenascins and collagen.14 Furthermore, CSPG potentially suppresses the migration and proliferation of the oligodendrocyte precursor cells (OPCs) that will lead to the failure of remyelination of the regenerated axons. Not only producing CSPG, but the reactive astrocytes will also induce IL-1 and IL-6 release, which could activate more M1 microglia.13 This complicated process will be repeated and could disrupt the neuronal repairs and neurogenesis process.10,13,14

Overview of miRNA-124

miRNAs are (ncRNA) non-coding RNA that consist of 18-25 nucleotides, and play roles as a master controller of the transcription and translation of protein expression. It represents approximately 98% of the transcriptional output in the eukaryotic genome. They could modulate protein expression by binding to complementary or partially complementary target mRNAs.15 They worked as a regulator, play important roles in the initiation and progression of several diseases. They regulate gene expression at multiple levels, which are mRNA degradation, translational and transcriptional repression, and also mRNA sequestration.16 There are many types of miRNA, but it is found that miRNA-124 is the most abundant miRNA in the CNS and proved to play important roles in neural development and differentiation.17-19 miRNA-124 is a sub-ventricular zone (SVZ) neuronal differentiation determinant and SVZ is the largest neuronal differentiation determinant.5 Besides the effect on neuronal differentiation, they also contribute to control of neurite outgrowth during neuronal differentiation.17 During the prenatal period, the expression of miRNA-124 is increasing and reach its peak level by the end of the fetal development and remains high in the postnatal brain. miRNA-124 is widely expressed in post-mitotic neurons in all CNS regions and strikingly increasing with the exit of neuroblast from the cell cycle during normal

Figure 1  Increase expression of BCL-2 suppress the expression of Bax, inhibit cytochrome c release, and inhibit the migration of AIF thus suppressing the apoptosis of the neuron
neuronal differentiation; therefore, this molecule may play important roles in neurogenesis and neural function. They are targeting hundreds of mRNA transcript, and many of the regulated genes have been validated as miRNA target. Ectopic over-expression of miRNA-124 in non-neural HeLa cells leads to the repression of non-neural mRNAs and shift their expression profile towards the neuronal phenotype.17,18

Overview of Chitosan

Chitosan [(1→4)-2-amino-2-deoxy-D-glucopyranosyl] is a cationic polysaccharide obtained from chitin deacetylation.20 It is made by treating chitin shells of shrimp, crabs and other crustaceans with an alkaline substance, like sodium hydroxide.21 Depending on the source and preparation procedure, its molecular weight may range from 300 to over 1000 kDa with a degree of deacetylation from 30 to 95%. In general, chitosan is non-toxic and biodegradable within living tissues, and also provoke a minimal foreign body reaction with little or no fibrous encapsulation.22,23 Lately, there is much pharmacological research related to chitosan because chitosan has shown particularly promising results due to the polyelectrolytes intrinsic properties including low toxicity, excellent biocompatibility, good delivery ability for hydrophilic molecules, high absorption capacity, complete biodegradability, anti-infection, even anti-tumor effect.24 Chitosan has been extensively examined in the pharmaceutical industry for its potential in the development of controlled release drug delivery systems and can be applied as a bioadhesive, transmucosal drug transport, vaccine delivery, DNA delivery and many else.7 Chitosan is also suitable for drug delivery to the brain as a carrier-mediated transported across blood-brain barrier (BBB). The BBB represents an effective shield for the delivery of neuroactive agents to the central nervous system (CNS), and it makes the treatment of many CNS diseases difficult to achieve.24,7 Chitosan also have the mucoadhesive ability which is an advantage for the intranasal delivery drug. As a mucoadhesive agent that could improve the distribution of the drug into neuron by provides moiety for cell penetration and as affinity towards neuronal tissues.25 Furthermore, chitosan also has been demonstrated to be a functional lipid-lowering agent.7 Some studies indicated that chitosan had beneficial lipid-regulating effects in animal and humans.9 The administration of chitosan might increases HDL cholesterol and reduces LDL cholesterol and also promoted reverse cholesterol transport (RCT) that will be very useful against atherosclerosis.7,26 A high non-HDL level in circulation is one of the most important factors in inducing atherosclerosis diseases due to oxidative non-HDL particles which are prone to uptake by macrophages in the subendothelial space and lead to the formation of foam cells in the endothelial walls.7,25 Thus, an effective lipid-lowering therapy may attenuate the atherosclerosis disease by decreasing the non-HDL levels in circulation.25,26

Reconstruction and Administration

Low Molecular Weight (LMW) 10KD of chitosan is prepared, which has a viscosity of 5~20cP and a deacetylation of 80.0% polyethylene glycol monomethyl ether mPEG which its M.W is 2,000, sodium tripolyphosphate (TPP), and glacial acetic acid. The preparation of LMW also needs a 1339.63 M.W. of trans-activated tran-cription (TAT) peptide (NH2RKKRRQRRR) and mechano growth factor (MGF) peptide.

Figure 2  Expression of miRNA-124 in resting microglia polarizing the microglia into the M2 type activated microglia thus will reduce the inflammation and increase angiogenesis

Figure 3  Expression of miRNA-124 suppress the expression of SOX9 and JAG-1 mostly, deactivated NOTCH pathway, inhibit expression of STAT3, and reducing number of M1 activated microglia thus will result in reducing size of glial scar and inducing neurogenesis
miRNA-124 is chemically synthesized and covalently linked to a 3′-biotin moiety using three different linkers: C6, TEG, TEG-phosphate. The biotin-tagged miRNA-124 is premixed with 200 μL of TPP and dropped into 800 μL of CS-PEG-TAT/ MGF polymer solution, under a constant magnetic stirring at 80 rpm for an hour, then concentrate it into 0.5 mg/kg dose and administered through intranasal route.

As the miRNA-124 loaded chitosan administered by intranasal route, chitosan which is positively charged will easily bind to the nasal mucosa which is negatively charged. Chitosan also facilitates the drug transport by opening the tight junctions between epithelial cells. There are two transport mechanism, intracellular route, and extracellular route. The intracellular transport is a relatively slow process, while the extracellular transport provides rapid entrance of drug into the brain within minutes of intranasal administration. There are two extracellular routes. First, it can cross the gaps between the olfactory neurons which are later transported into the olfactory bulb in the brain. Second, it can be transported along the trigeminal nerve to bypass BBB and enter the brain.

After reaching the olfactory bulb region, it may enter another part of neuron in brain by diffusion which is also facilitated by perivascular pump.

**Mechanism of Action**

a. **miRNA-124 Loaded Chitosan induces Neuroprotection Effect during Ischemic Stroke**

As miRNA-124 packed chitosan enters the area of the penumbra in the brain, TAT peptide, which is tagged to the chitosan, will provide moiety to facilitate cell penetration. Another peptide tagged to the chitosan, the MGF peptide, promote affinity toward neurons. Therefore, it will be phagocytozed by neuron around the lesion. The expression of miRNA-124 in the brain tissue has been proven to prevent apoptosis of the neuron under ischemic condition. The exogenous miRNA-124 will increase the expression of the anti-apoptotic protein from B-cell lymphoma 2 (BCL 2) family group, especially the BCL-2 and BCL-XL. Expression of BCL-2 protein will inhibit the BAX-mediated cytochrome c release. This inhibition will lead to suppression of apoptosis.
caspase activation. Expression of BCL-2 will also inhibit translocation of apoptosis-inducing factor (AIF) from mitochondria to the nucleus. Therefore, the overall outcome of this inhibition cascade by BCL-2 is the termination of the intrinsic apoptotic pathway and increasing the survival of the neuronal cell during ischemic condition.17,31–35

There are two types of activated microglia during the ischemic stroke in the brain. The M1 activated microglia are continuing source of cytotoxic substances, such as Tumour Necrotizing Factor alpha (TNF-α), Prostaglandin E2 (PGE2), Interferon gamma (IFN-γ) and other cytotoxic substances. On the other hand, the M2 activated microglia is important to promote angiogenesis and matrix remodeling because the M2 activated microglia produces brain-derived growth factor (BDNF), insulin-like growth factor (IGF1).3,6,7 However, under ischemic stroke, the activated microglia are mostly M1 with only small number transient M2. Furthermore, the M1 microglia numbers are increased rapidly during ischemic condition and maintained up to one month after stroke. Meanwhile, the M2 microglia numbers only transiently increased and back to normal level by seven days after ischemic neuronal injury.36

As miRNA-124 loaded chitosan phagocytosed by the microglia in the brain, the expression of miRNA-124 in the activated microglia will promoting polarizing effects of the microglia to become the M2 type (Figure 2). Therefore, miRNA-124 will increase the number of M2 microglia, upregulating the growth factor substances and downregulating the TNF-α and other cytotoxic substances. Survival of the neuronal cell will be increased along with the decreased external apoptotic pathway through activation of death receptor by TNF-α.15,32,36

b. *Loaded Chitosan induce Neurogenesis*

Neuro remodeling by neurogenesis after stroke has been proven exist, even though it is subtle, especially after brain ischemia.6 Exogenous miRNA-124 will cause expression of miRNA-124 in the brain thus will promote the differentiation of the neural progenitor into neural lineage thus will increase the neurogenesis. The expression of miRNA-124 in the neuron at the subventricular zone (SVZ) will target the SRY-box transcription factor (SOX9) and Jagged-1 (JAG1). As the neuron phagocytoses it, the miRNA-124 will inhibit the expression of SOX9 and JAG1 which will lead to differentiation and migration of neuroprogenitor cell into glial lineage instead of neural lineage.17,37,38 Expression of miRNA-124 also reduces the PTBP1, SCP1, Ephrin-B1, and BAF53a levels which are leading to the neuroprogenitor cells to differentiate into nervous lineage.17,38

Another barrier for the neurogenesis after brain ischemia is the excessive formation of the glial scar which is occupying the space of dead neuron. Glial scar formation is involving the reactive astrocyte, activated microglia, fibroblast, endothelial cells, infiltrating immune cells and the extracellular matrix surrounding the lesion area. Inflammation is the critical step to begin the glial scar formation. Microglia, especially the M1 activated microglia, releases cytokines (IL-6, TNF-α, IFN-γ). Those cytokines are the inducer of the astrocyte proliferation.36 The astrocyte is not only proliferating but also become hypertrophy due to increasing number of intermediate filaments, such as GFAP and vimentin. Thus, the reactive astrocyte is produced physical wall and produce inhibitory proteoglycans (CSGPs, tenascins, and collagen) which will overcome the axonal regeneration. CSGPs is also capable of suppressing the oligodendrocyte precursor cells (OPCs) migration and differentiation which will lead to the failure of remyelination of the regenerated axons. The reactive astrocyte also releases some cytokines (IL-1 and IL-6) which in returns will exacerbate the inflammation and activating more microglia into M1 type. Thus, modulation of the glial scar formation is crucial for neuronal repairs and neurogenesis.36,39 As explained in the section above, the expression of miRNA-124 will polarize the activated microglia into M2 type, thus will reduce the activation of astrocyte proliferation due to cytokines produced by the M1 activated microglia. Expression of miRNA-124 also capable of inhibiting the translation of signal transducer and activator of transcription 3 (STAT3) which could lead to reduced astrocytic lineage differentiation (Figure 3).5,17,38 Therefore, the expression of miRNA-124 will lead to differentiation and migration of neuroprogenitor cells to the lesion site while minimizing the formation of glial scar by reactive astrocyte.

**Benefit and Limitation**

*a. Benefit*

Ischemic stroke is representing a significant cerebrovascular problem in public health. The ischemic condition will lead to an apoptotic chain within the neuron in the brain. These dead neurons later cannot have replaced new neurons due to the weak intrinsic ability of neurogenesis after stroke.6 This limited neurogenesis ability is due to exacerbation of inflammation which will induce the apoptosis of the neurons and induce excessive proliferation of the astrocyte to give rise to glial scar.32,35,36

Research of Yang Sun et al, have shown that the BCL-2 and BCL-XL protein level in the mice with middle cerebral artery occlusion (MCAO) is 1.5 times higher after the injection of miRNA-124.
On the other hand, the proapoptotic protein level (Bax and Bad) is reduced after injection of the miRNA-124 to the MCAO mice. Expression of miRNA-124 has been proved further polarizing the activated microglia form M1 type to M2 type. The M2 activated microglia will reduce the inflammation and inhibit the apoptotic pathway of the neuron cell during ischemic condition thus will increase the survival of the neuronal cell. The research by Somayyeh Hamzei et al. revealed that rising number of M2 has a positive correlation with number of neurons in the brain of MCAO mice. The size of the glial scar is also reduced in MCAO mice after injected with miRNA-124 (Figure 4).

Expression of miRNA-124 also inhibits the expression of Sox9 and JAG-1 which in return will induce the differentiation of neuroprogenitor cells into the neuronal lineage and induces migration of the neuroprogenitor cells to the ischemic area to take over the dead neuron. The research from Jialei Yang et al. proves that injection of miRNA-124 to ischemic mice will increase the number of DCX marker, which is the marker for immature neuronal, in the ischemic brain region (Figure 5).

The intranasal route is novel drug delivery method bypassing blood brain barrier (BBB) which provides rapid drug absorption, ease administration, non-invasive, rapid onset of action, and can improve the bio-availability of the drug. Chitosan is a simple delivery system which is a linear disaccharide made of D-glucosamine and N-acetyl-D-glucosamine and derived from the de-acetylation of the naturally abundant chitin that mostly found in crustacean exoskeletons. Chitosan is a positively charged molecule which typically interacts with negatively charged miRNA to form nanoparticles. Chitosan is a mucoadhesive agent which is an advantage for intranasal delivery. TAT and MGF protein tagged to chitosan will improve the distribution of the miRNA-124 loaded chitosan to neurons. TAT provides moiety for cell penetration, while MGF is used for its affinity towards neuronal tissues. Therefore, intranasal administration of TAT/MGF tagged PEGylated miRNA-124 loaded chitosan is the novel treatment which able to be diffused rapidly into the brain and give both the neuroprotection and induces neurogenesis for the revitalization of the patient after ischemic stroke.

b. Limitations

The optimum dosage, pharmacokinetic profile and the adverse effect of the miRNA-124 loaded chitosan are still unknown. Therefore, more research is needed to profiling the safety of miRNA-124 loaded chitosan which is administered by intranasal route into the patient with ischemic stroke.

CONCLUSION

In conclusion, miRNA-124 loaded chitosan is used as a new option to accelerate the recovery and avoid the disability. miRNA-124 loaded chitosan induces neuroprotection and suppress apoptosis through the expression of Bcl-2 and reduce the expression of Bax, and inhibit the migration of AIF. They also induce neurogenesis and lessen the size of glial scar through the suppression SOX9 and JAG-1, deactivated NOTCH pathway, inhibit expression of STAT 3, and reducing the number of M1 activated microglia. The intranasal route is an ideal choice for the administration of this drug because it is rapid and directly crossing the gaps between olfactory neuron, transport to trigeminal nerve to bypass BBB and enter the brain within minutes, non-invasive, and can improve the bioavailability of the drug. Due to those therapeutical effects, miRNA-124 loaded chitosan could be a novel therapy after ischemic stroke.

SUGGESTION

Further study of clinical trials should be carried out to support the findings of optimum dosage, pharmacokinetic profiles, and the adverse effect of miRNA-124 loaded chitosan.

REFERENCES

