Anti-inflammatory Efficacy of Berberine Nanomicelle for Improvement of Cerebral Ischemia: Formulation, Characterization and Evaluation in Bilateral Common Carotid Artery Occlusion Rat Model

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Research Article

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Abstract

Backgraound: Berberine (BBR) is a plant alkaloid which possesses anti-inflammatory and anti-oxidant effects with low oral bioavailability. In this study, micelle formulation of BBR was investigated in order to improve therapeutic efficacy and examined its effect on secretion of inflammatory cytokines in cerebral ischemia in animal model.

Material and Methods: Nano formulation was prepared by thin film hydration method, and characterized by particle size, zeta potential, morphology, encapsulation efficacy and drug release in Simulated Gastric Fluid (SGF) and Simulated Intestine Fluid (SIF). Then, rats were pretreated with drug (100 mg/kg) and nano drug (25, 50, 75, 100 mg/kg) for 14 days. Stroke induction was accomplished by Bilateral Common Carotid Artery Occlusion (BCCAO), TNF-α, IL-1ß, and MDA levels were measured in the brain and the anti-inflammatory effect of BBR formulations were examined.

Result and discussion: Micelles were successfully formed with appropriate characteristics and smaller sizes than 20 nm. The PDI, zeta potential, encapsulation efficacy of micelles were 0.227, -22 mV, 81%, respectively. Also, the stability of nano micelles was higher in SGF as compared to SIF.

Conclusion: Our clinical data show that treated groups in different doses (nano BBR 100, 75, 50 mg/kg, and BBR 100 mg/kg) successfully showed decreased levels of the inflammatory factors in cerebral ischemia compared with stroke group and treated group with nano BBR in dose of 25 mg/kg. Nano BBR formulation with lower dose can be a better candidate than conventional BBR formulation to reduce oxidative and inflammatory factors in cerebral ischemia.

Introduction

Many studies have been done on cerebral ischemia and all of them have confirmed its morbidity and mortality worldwide [1]. In fact, cerebral ischemia occurs due to a blockage in the carotid arteries, which results in damage to brain tissue. Results indicate factors such as oxygen free radical and inflammatory cytokines (such as TNF-α, IL-1ß) and MDA as marker of oxidative stress which play an important role in damaging brain tissue and neurons due to cerebral ischemia [2-4]. There is evidence that TNF-a and IL-1B levels in the brain increase many-fold (up to 40 or 60 time) during the first 24 hours after inducing stroke [5]. Therefore, it is necessary to recognize safe compounds with high and effective therapeutic efficacy in reducing inflammation during stroke.

Berberine (BBR), an isoquinoline alkaloid extracted from Coptidis rhizome and Cortex phellodendri, is one of the herbal and safe compounds ($C_{20}H_{18}NO_4^+$) which exhibits anti-oxidative and anti-inflammatory effects on brain diseases such as ischemia [6-9], Alzheimer [10] and tumors [11]. Also, it has been reported that BBR possess effects of antibacterial [12], Antioxidant [13] antiviral [14], and anticancer [15]. However therapeutics effectiveness of BBR is restricted due to its hydrophobic nature and poor water solubility [16]. To overcome to these challenges, we need to a novel formulation in drug delivery system to improve drug solubility and efficiency.

Lately, the hydrophobic drugs encapsulating in micelles as nano carrier have attracted much attention for drug delivery applications [17]. Micelles are amphiphilic colloidal structures, with particle diameters from 5 to 100 nm range. The core of the micelle is formed by the hydrophobic fragments of amphiphilic molecules, whereas micelle's shell consists of hydrophilic fragments of micellar molecules [18]. Micelles possess plentiful advantages for oral drug delivery, such as ability to solubilize hydrophobic drugs due to their unique structure (core-shell) resulting in enhancing drug effectiveness without change or disruption in drug formulation [19-22]. So, design of the drug delivery system based on micelles could show promising performances in the oral administration by providing a high level of therapy.

In this study, we tried focusing on preparation of nano formulation by thin film hydration method and its characterization, then after inducing stroke, levels of inflammation and oxidative factors (TNF-α, IL-1ß, MDA) were measured in brain tissue. Thus, the aim of this study was to preparation and evaluation of BBR and BBR-loaded micelle formulations and survey of its anti-inflammatory effects on cerebral ischemia in rat by Bilateral Common Carotid Artery Occlusion (BCCAO) model.

Materials And Methods

Deoxicholate and BBR were purchased from Sigma. Ketamine hydrochloride and xylazine were from Alfasan (Holland). The chemical used including NaoH, KH2Po4, NaCL, HCL and other materials and solvents were analytical grade. Equipments were used including rotary (Buchi, Switzerland), centrifuge, filter amicon 3000 KD, and spectrophotometer UV-Vis (SPEKOL 1300; Analytik Jena, Germany).

Preparation of BBR-loaded micelle solution

Thin film hydration method was used for preparation of BBR-loaded micelle solution [23]. Briefly, in a round-bottom flask BBR (1% w/w) and deoxicholate (49% w/w) as surfactant, was mixed with minimum amount of methanol as organic solvent. The rotary evaporator was applied for more than 2 h to evaporate solvent under reduced pressure and to deposit thin film. Then, in the hydration phase, deionized water (50% w/w) added to it, and bain-marie was applied to 50 °C, while stirring was done for a 20 min at the same temperature until a clear solution was obtained that was light yellow.

Characterization

Dynamic light scattering was reported size and size distribution, PDI, Zeta potential of nano micelles (Malvern instruments, UK). The transmission electron microscope (TEM) was applied to show the size of nanomicellar formulations (PHILIPSCM 300, Netherlands).

For determination of encapsulation efficiency, an indirect method was used. Briefly, after centrifugation of micelles at 4,000 rpm for 30 minutes, the concentration of BBR in the samples was determined by spectroscopy UV-Vis. The drug encapsulation efficacy was calculated by the following equations [24]:

Drug release study

The Release behavior of BBR from nano micelles was investigated in SGF and SIF. To prepare the SGF, 246 µL HCl and 200 mg NaCl was added to 60 ml deionized water (DW), and pH was adjusted at 2, then the final volume was filled to 100 mL with DW. Also SIF was prepared by dissolving 680 mg of KH_2PO_4 and 61.6 mg of NaOH in 60 mL DW, and pH was adjusted at 6.5. Then the final volume was filled to 100 mL with DW. Samples were diluted in a ratio of 1:10 in SIF and SGF, and incubated at 37 ^{o}C. Then sampling carried out at time points 0, 30 min, 1, 2, 4, 8, 24 h [25]. Then the determination of BBR was accomplished by spectroscopy UV-Vis.

Animal Groups and Ethical considerations

96 adult male wistar-rats, weighting 200-220 g (aged 4-5 weeks) were purchased from Pharmacology School of Tehran University of Medical Sciences. All of them were kept in an animal house under standard laboratory condition at temperature of 22±1 ^{o}C and humidity of 80%, with a typical 12 h light/dark cycle and were allowed to have free access to food and water. All phases of the experiment were approved by the ethics committee of Tehran University of Medical Sciences for the maintenance and application of laboratory animals(IR.TUMS.MEDICINE.REC.1398.593). All attempts were made to minimize the animal suffering. After completing the experiments, the animals were euthanized by carbon dioxide gas. To collect brain tissue samples, the animals underwent spinal cord in complete anesthesia. Animal carcasses were disposed of with hospital waste.

The rats were randomly divided into 8 groups (12 rats in each group) as follow:

The control group

The stroke group

The nano micelle group (without drug)

The pretreated group with BBR (100 mg/kg)

The pretreated group with different doses of nano BBR (100, 75, 50, 25 mg/kg)

The control group hasn’t received any drug and no induction has been made on it. The stroke group Induction has been performed but has not received drug. Also, groups 4-8 were treated orally with BBR and nano-BBR for 14 days with concentrations of 25, 50, 75, 100 mg/kg. For oral administration of berberine hydrochloride, it (approximately 1% of body weight) was dissolved in water [26].

Stroke inducing by BCCAO model

For the inducing of C.I, rats were anesthetized by an intraperitoneal (i.p) injection of 50 mg/kg ketamine and 2-8 mg/kg xylazine. Rats were placed on its back. The animal’s tail and paws were fixed using adhesive tape. A sagittal ventral midline incision (~ 1cm length) was performed, then the bilateral common carotid arteries revealed, and both carotid arteries were carefully separated from the vagal

nervous. Also, it is crucial to avoid any manipulations of the vagal nervous. A 5-0 silk suture loop was made around each carotid artery. Both carotid arteries were occluded for 30 minutes. Then a five minutes reperfusion period was initiated. After the reperfusion the wounds were sutured and with an anti-septic solution were disinfected. Thereafter, rats were returned to their animal cages [27-29].

Biochemical assays

Rats were decapitated under deep anesthesia, the brains were removed, and all tissues were stored at −80°C for later biochemical assays. The animal's brains were homogenized in buffer (TRIS HCL, SDS, DTT, NP40, and glycerol), and centrifuged for 5 min [30], then the levels of IL-1β and TNF-α in serum were detected with ELISA kits, also the activity of MDA (as marker for lipid peroxidation) in serum was measured using the commercial kit according to the manufacturer's instructions.

Data analysis

Data analysis was carried out using GraphPad prism version 7 software. All data were analyzed using one-way analysis of variance (ANOVA) and Tukey post-hoc. Data were presented as Mean ± SEM. Statistically, differences with $p<0.05$ were recognized as significant.

Results

Characterization

BBR-loaded micelle formulation successfully was prepared. The dynamic light scattering was used to find out the three of the main parameters of particle size (based on intensity, volume, and number), PDI, and zeta potential of nano micelles (Fig 1-4). DLS results showed average size about of 12 nm, zeta potential (which represents surface charge) and PDI (which represents the size distribution and system uniformity) were -22 mv and 0.227, respectively. Also size less than 20 nm was confirmed by TEM (Fig 5). The samples were photographed with a Philips cm300 electron microscope (Philips cm300/ 200 k/ Holland). Diluted nanomicelles were dispersed for 20 min and were deposited onto carbon coated coper grid. After drying, the images were taken. The morphology of nanomicelles using TEM revealed that they have spherical-like shapes.

Encapsulation of BBR into nano micelles was measured by using centrifugation method, and parameters were calculated according to equation 1. EE% was calculated as 81%, which suitable for the objective of the current study.

Drug release study

Percent of drug released from nano micelle formulation in SGF and SIF solutions. Drug release study in SGF and SIF were evaluated within 24-hour sampling, but maximum drug residence time in the stomach is 2 h, so the results of the first 2 hours are important for survey of drug behavioral in SGF.

The level of BBR didn't fluctuate much in the first 4 h in SGF. However it was decreased to 71% after 8 h incubation. The stability of nano micelles was higher in SIF as compared to SGF. In SIF, the concentration of BBR decreased from 97% at 0 h to 86% after 8 h. As result shown, this time is well enough for nano micelles to pass the stomach and reach to the intestine, where they can absorb with different mechanisms.

Effects of nano BBR-loaded micelle on TNF-α, IL-1ß, MDA

To evaluate anti-inflammatory effect of berberine, we examined BBR and nano BBR on cytokines and the marker of stress oxidative secreted into brain tissue within cerebral ischemia. Results were shown in (Fig 6-8). After treatment with various concentrations of BBR (100 mg/kg) and nano BBR (25, 50, 75, 100 mg/kg) for 14 days, brain tissue analyzed by ELISA kits to examine whether the treatments affect the release of inflammatory cytokines. According to the results, the level of TNF-α (from 9 pg/mg in nanomicelle group to 6 pg/mg in treated group with nano BBR concentration of 100 mg/kg), IL_1ß (from 6 pg/mg in nanomicelle group to 3.8 pg/mg in treated group with nano BBR concentration of 100 mg/kg), and MDA (from 4.8 pg/mg in nanomicelle group to 3.6 pg/mg in treated groups with nano BBR concentration of 100 and 75 mg/kg) were reduced in a dose-dependent manner in , indicating the inhibited effect by BBR on the secretion of inflammatory cytokines.

Also, our results show that not only there were no significant differences between stroke group and nano micelle group (without drug) in each three parameters, but also significant difference was observed between the stroke group and treatment groups.

It has been accepted that berberine can decrease inflammatory agents-induced IL-1b and TNF-a ensuing inflammation in cerebral ischemia [31]. In present work, studies have shown that Induction of stroke caused a significant increase in TNF-a, IL-1B, and MDA levels compared to the control group ($p < 0.001$). Also observed increase of cytokines and the marker of oxidative stress in treated group (nano BBR 25 mg/kg) and nano micelle (without drug) group compared to the control group ($p<0.001$).

On the other hand, treated groups (BBR 100 mg/kg, nano BBR 100 mg/kg ($p<0.01$), 75 mg/kg, and 50 mg/kg ($p<0.05$)) showed a significant reduction in TNF-α, IL-1B, and MDA levels compared to the stroke group.

Discussion

The aim of this study was to emphasis on 2 main parameters including preparation and survey of anti-inflammatory effect BBR-loaded micelle nano formulation on cerebral ischemia.

First, BBR-loaded micelles successfully were prepared by thin film hydration method. The size of prepared micelles was approximately 12 nm and shows a good distribution. Simoes et al reported this small size of micelles provides enhanced water solubility of hydrophobic drugs, increased absorption of free drug, and they release the drug in target site of the gastrointestinal tract together with high concentration

gradient nearby the epithelium, and etc [32]. For these reasons, small size of micelles has particular importance in oral administration in drug delivery system. Also, zeta potential show that prepared formulation has negative surface charge which makes it, so is stable against aggregation.

The results presented in this study demonstrated that 81% of BBR molecules successfully entrapped within the core of the micelle. To calculate it, we examined centrifuge method for separation of entrapped BBR from the micellar system. Also, we confirmed that centrifuge method compared to other methods (gel chromatography and dialysis method) is the most appropriate for separation of free drug form micellar system [33]. Drug release studies have shown that BBR was released in a declining trend from nano micelle formulations in SGF and SIF, and they are stable for at least 4 hours. Moreover, the BBR release rate from nano micelle formulation was higher in SIF than in SGF.

Second, we compared the effect of BBR and BBR-loaded micelle on TNF-a, IL-1B (inflammations factors), and MDA (as marker of oxidative stress) due to inducing cerebral ischemia in rat. BCCAO model was used to induce stroke into rat's brain. An increase in inflammation cytokines and MDA have been reported after induce stroke [34]. In the present study, the results of treated groups (BBR and BBR-loaded micelle) showed significantly decreased in TNF-α, IL-1ß, MDA levels in comparison with the stroke group. This result confirmed by Zhang et al. that reported BBR could be suppressed the activation of proinflammatory cytokines (TNF-α, IL-1ß) after ischemic stroke [35]. This result indicates the anti-inflammatory properties of berberine. Moreover this property was further observed in the nano formulation.

Generally, we designed a useful nano formulation, prepared with BBR as drug and micelle as carrier, BBR-loaded micelle, by thin film hydration method for reduction of inflammatory effect in cerebral ischemia in rat. The results of the study suggest that prepared nano formulation possess improved anti-inflammatory effects more compared to usual formulation. This effect could be due to the increased aqua solubility in BBR-loaded micelle compared to BBR, resulting in its increased efficiency. Thus, BBR-loaded micelle formulation could be developed as a potential preventive agent on cerebral ischemia.

Declarations

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Authors' contributions

RA and SEM contributed equally to the study conception, design, and preparation of the manuscript. HY, NM, and MRJ contributed to performing the experiments. SEM, MR, and HY supervised the study and

contributed to the critical revision of the manuscript. All the authors read and approved the final manuscript.

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Availability of data and materials

The datasets used and/or analyzed during the current study available from the corresponding author on reasonable request.

Ethics approval and consent to participate

All the experiments were in accordance with the guidelines of Tehran University of Medical Sciences and experiments were approved by the ethics committee of Tehran University of Medical Sciences.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

The ARRIVE guidelines

The study was carried out in compliance with ARRIVE guidelines 2.0 .

References

1. Bejot Y, Daubail B, Giroud M. Epidemiology of stroke and transient ischemic attacks: Current knowledge and perspectives. Rev Neurol 2016; **172**: 59-68.
2. Lee R, Lee M, Wu C, Silva A, Possoit H, Hsieh T, Minagar A, Lin H. Cerebral ischemia and neuroregeneration. Neural Regen Res 2018; **13**: 373–385.
3. Siniscalchi A, Gallellia L, Malferrari G, Pirritano D, Serra R, Santangelo E, Sarro G. Cerebral stroke injury: the role of cytokines and brain inflammation. J Basic Clin Physiol Pharmacol 2014; 25: 131–137.
4. Cheng Ch, Lee Y. Anti-Inflammatory Effects of Traditional Chinese Medicines against Ischemic Injury in In Vivo Models of Cerebral Ischemia. Evid Based Complementary Altern Med 2016; doi: 10.1155/2016/5739434.

5. Lambertsen K L, Biber K, Finsen B. Inflammatory cytokines in experimental and human stroke. J CEREBR BLOOD F MET 2012; 32:1677–1698.
6. Wang Q, Qi J, Hu R, Chen Y, Kijlstra A, Yang P. Effect of Berberine on Proinflammatory Cytokine Production by ARPE-19 Cells following Stimulation with Tumor Necrosis Factor-a. IOVS 2012; 53: 2395-2402.
7. Srivastava Sh, Srivastava M, Misra A, Pandey G, Ravat A. A review on biological and chemical diversity in berberis. EXCLI J 2015; **14**: 247-267
8. Kim M, Kim T, Kim Ch, Shin M, Hong M, Park H, Park S. Berberine Ameliorates Brain Inflammation in Poloxamer 407-Induced Hyperlipidemic Rats. Int Neurourol J 2019; **23**: 102-110
9. Zhang Q, Fu X, Wang J, Yang M, Kong L. Treatment Effects of Ischemic Stroke by Berberine, Baicalin, and Jasminoidin from Huang-Lian-Jie-Du-Decoction (HLJDD) Explored by an Integrated Metabolomics Approach. Oxid. Med. Cell. *Longev* Article 2017; 1-20, doi: 10.1155/2017/9848594.
10. Yuan N, Cai C, Wu M, Su H, Li M, Lu J. Neuroprotective effects of berberine in animal models of Alzheimer's disease: a systematic review of pre-clinical studies. BMC COMPLEM ALTERN M 2019; 19: 109.
11. Tong L, Xie Ch, Wei Y, Qu Y, Liang H, Zhang Y, et al. Antitumor Effects of Berberine on Gliomas via Inactivation of Caspase-1-Mediated IL-1β and IL-18 Release. Front. Oncol 2019; 9: 364.
12. Tan J, Wang J, Yang Ch, Zhu Ch, Guo G, Tang J, Shen H. Antimicrobial characteristics of Berberine against prosthetic joint infection-related Staphylococcus aureus of different multilocus sequence types. BMC COMPLEM ALTERN M 2019; 19: 218.
13. Rajasehar K, Samanta S, Bagoband V, Murugan N, Govindaraju T. Antioxidant Berberine-Derivative Inhibits Multifaceted Amyloid Toxicity. Iscience 2020; 23: 101005.
14. Botwina P, Owczarek K, Rajfur Z, Ochman M, Urlik M, Nowakowska M, et al. Berberine Hampers Influenza A Replication through Inhibition of MAPK/ERK Pathway. Viruses 2020; 12: 344.
15. Wang CY, Bai XY, Wang CH. Traditional Chinese medicine: a treasured natural resource of anticancer drug research and development. Am J Chin Med 2014; 42 :543–59.
16. Chen w, Miao Y, Fan D, Yang Sh, Lin X, Meng L, Tang X. Bioavailability Study of Berberine and the Enhancing Effects of TPGS on Intestinal Absorption in Rats. AAPS Pharm Sci Tech 2011; 12: 705-711.
17. Luo J, Zhang Z, Gong T, Fu Y. One-step self-assembled nanomicelles for improving the oral bioavailability of nimodipine. Int J Nanomedicine 2016; 11: 1051–1065.
18. Parikh A, Kathawala K, Song Y, Zhou X, Garg S. Curcumin-loaded self-nanomicellizing solid dispersion system: part I: development, optimization, characterization, and oral bioavailability. DRUG DELIV TRANSL RE 2018; **8**: 1389-1405.
19. Jin Qu, Cai Y, li S, et al. Edaravone-Encapsulated Agonistic Micelles Rescue Ischemic Brain Tissue by Tuning Blood-Brain Barrier Permeability. Theranostics 2017; **7**: 884-898.

20. Patra J, Das G, Fraceto L, et al. Nano based drug delivery systems: recent developments and future prospects. J Nanobiotechnol 2018; **16**: 71.
21. Gaucher P, Satturwar P, Jones M. Polymeric micelles for oral drug delivery. Eur J Pharm Biopharm 2010; 76: 147-158.
22. Salimi A, Makhmalzadeh B, Esfahani G. Polymeric Micelle as a New Carrier in Oral Drug Delivery Systems. Asian J Pharm. Sci 2017; 11: 704.
23. Ai X, Zhong L, Niu H, He Zh. Thin-film hydration preparation method and stability test of DOX-loaded disulfide-linked polyethylene glycol 5000-lysine-di-tocopherol succinate nanomicelles. Asian J. Pharm. Sci 2014; 9: 244-250.
24. Ebrahimi nik M, Nikouei B, Amin M, Hatamipour M, Teymouri M, Sadeghnia HR, et al. Liposomal formulation of Galbanic acid improved therapeutic efcacy of pegylated liposomal Doxorubicin in mouse colon carcinoma. Sci. Rep 2019; 9: 9527.
25. Hatamipour M, Sahebkar A, Alavizadeh S, Dorri M, Jaafari M. Novel nanomicelle formulation to enhance bioavailability and stability of curcuminoids. Iranian J basic Med Sci 2019; 22: 282-289.
26. Turner PV, Brabb T, Pekow C, Vasbinder MA. Administration of Substances to Laboratory Animals: Routes of Administration and Factors to Consider. JAALAS 2011; 50: 600-613.
27. Partoazar A, Nasoohi S, Rezayat SM, Gilani K, Mehr SE, Amani A, et al. Nanoliposome containing cyclosporine A reduced neuroinflammation responses and improved neurological activities in cerebral ischemia/reperfusion in rat. FUND CLIN PHARMACOL 2017; **31**: 185-93.
28. Farbiszewski R, Bielawski K, Bielawska A, Sobaniec W. Spermine protects in vivo the antioxidant enzymes in transiently hypoperfused rat brain. ACTA NEUROBIOL EXP 1995; 55: 253-8.
29. Chen YF, Wang YW, Huang WS, Lee MM, Wood WG, Leung YM, et al. Trans-Cinnamaldehyde, An Essential Oil in Cinnamon Powder, Ameliorates Cerebral Ischemia-Induced Brain Injury via Inhibition of Neuroinflammation Through Attenuation of iNOS, COX-2 Expression and NFkappa-B Signaling Pathway. Neuromolecular Med 2016; 18: 322-33.
30. Nayak VS, Kumar N, D'souza AS, Nayak SS, Cheruku SP, Pai K. The effects of Mucuna pruriens extract on histopathological and biochemical features in the rat model of ischemia. Neuroreport 2017; 28: 1195-201.
31. Maleki S, Aboutaleb N, Souri F. Berberine confers neuroprotection in coping with focal cerebral ischemia by targeting inflammatory cytokines. J Chem Neuroanat 2018; 87: 54–59.
32. Simo˜es S, Figueiras A, Veiga F, Concheiro A, Alvarez-Lorenzo C. Polymeric micelles for oral drug administration enabling locoregional and systemic treatments. Expert Opin. Drug Deliv 2014; 12: 1-22.
33. Li CH, Li X, Li Sh, Weng Y, Wang K, Zhang T, et al. Development and Validation of a Method for Determination of Encapsulation Efficiency of CPT-11/DSPE-mPEG2000 Nanoparticles. Med chem 2016; 6: 345-348.
34. Wu L, Xiong X, Wu X, Ye Y, Jian Z, Zhi Z, et al. Targeting Oxidative Stress and Inflammation to Prevent Ischemia-Reperfusion Injury. Frontiers in Molecular Neuroscience 2020; 13: 28.

35. Zhang Q, Fu X, Wang J, Yang M, Kong L. Treatment Effects of Ischemic Stroke by Berberine, Baicalin, and Jasminoidin from Huang-Lian-Jie-Du-Decoction (HLJDD) Explored by an Integrated Metabolomics Approach. Oxid. Med. Cell. Longev 2017; doi.org/10.1155/2017/9848594

Figures

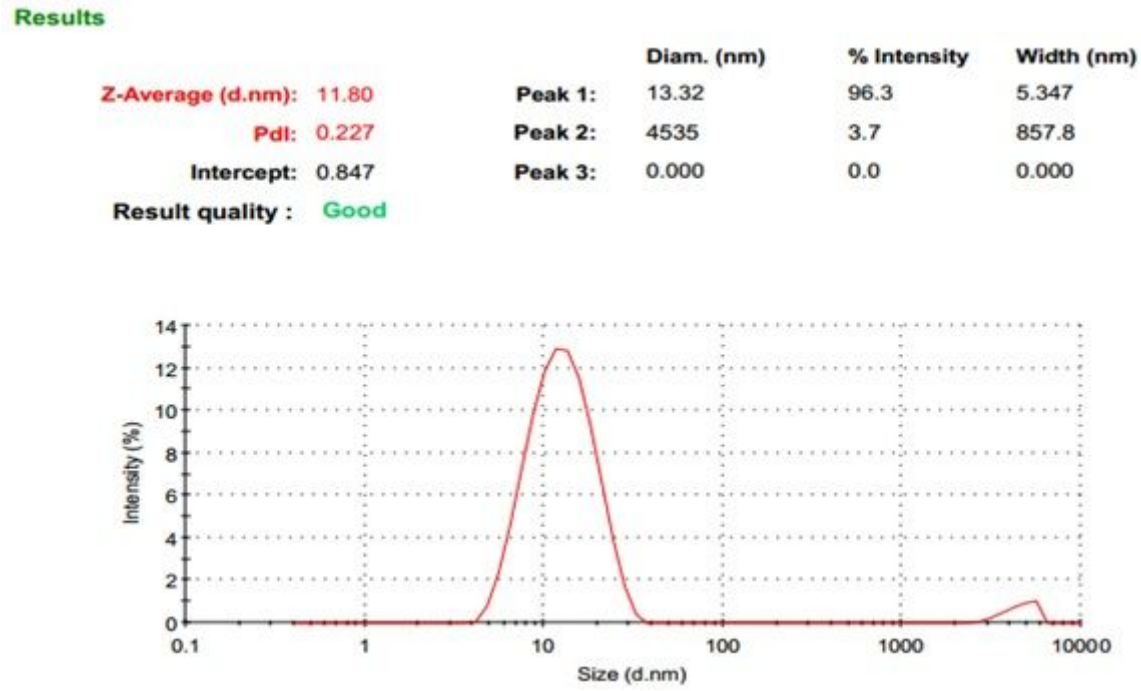

Figure 1

Size distribution diagram based on Intensity

Results

		Diam. (nm)	% Volume	Width (nm)
Z-Average (d.nm): 11.80	Peak 1:	8.575	99.8	3.430
PdI: 0.227	Peak 2:	4790	0.2	945.6
Intercept: 0.847	Peak 3:	0.000	0.0	0.000
Result quality : Good				

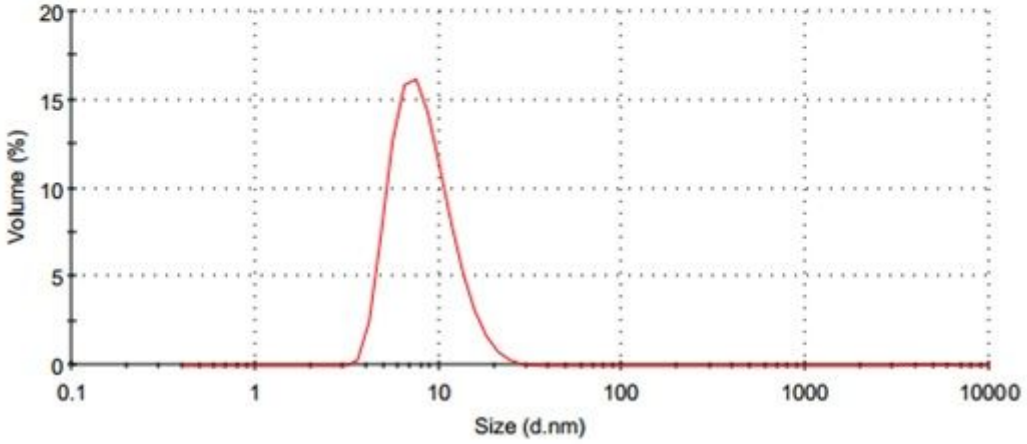

Figure 2

Size distribution diagram based on Volume

Results

		Diam. (nm)	% Number	Width (nm)
Z-Average (d.nm): 11.80	Peak 1:	6.416	100.0	1.946
PdI: 0.227	Peak 2:	0.000	0.0	0.000
Intercept: 0.847	Peak 3:	0.000	0.0	0.000
Result quality : Good				

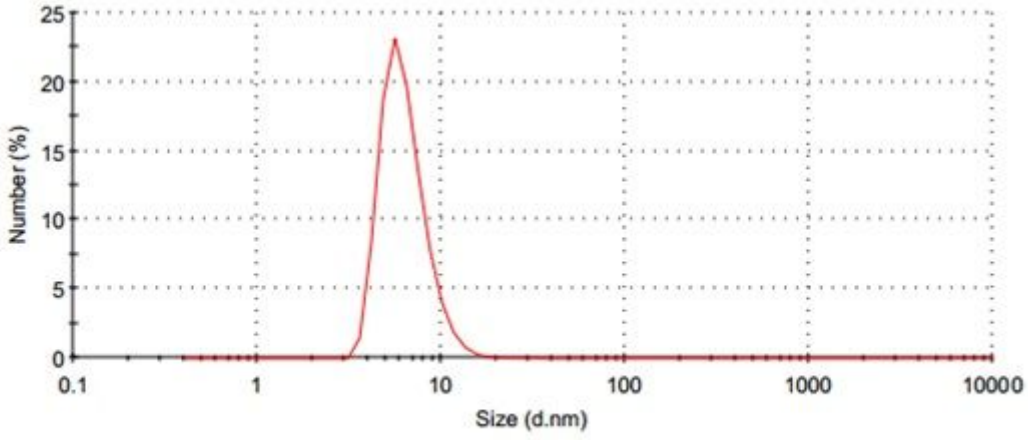

Figure 3

Size distribution diagram based on Number

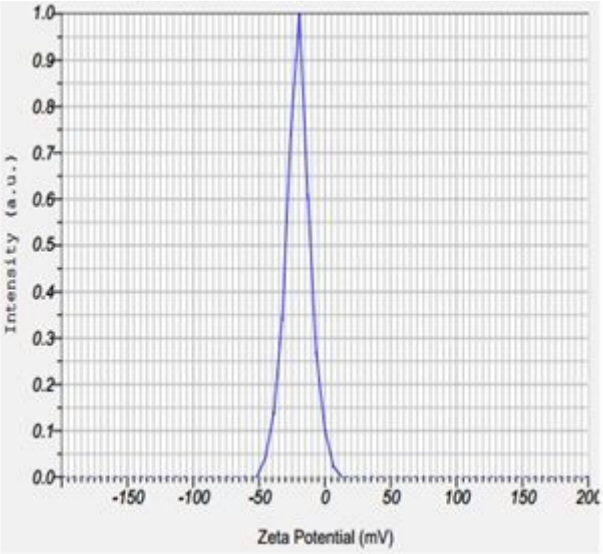

Figure 4

Size distribution Diagram based on Zeta Potential

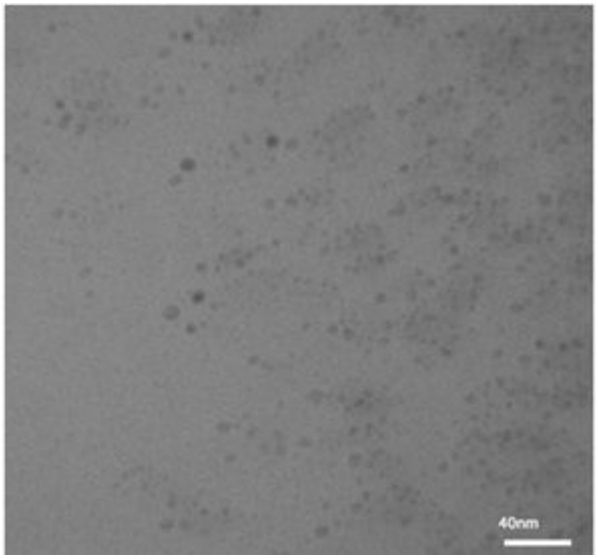

Figure 5

TEM image of BBR-Loaded micelle

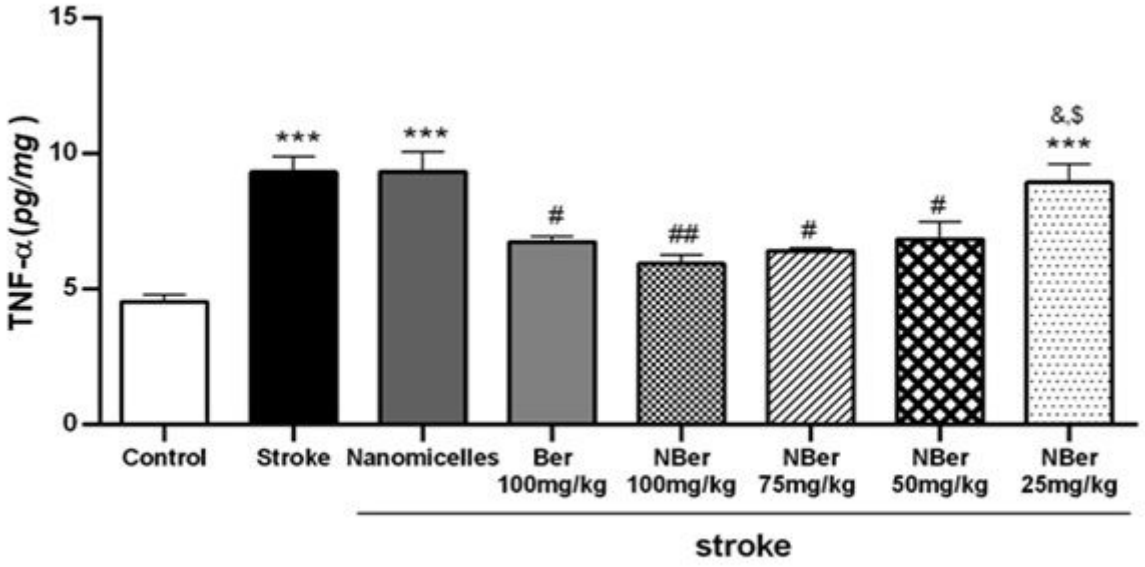

Figure 6

Rate changes of TNF-α in different groups after inducing stroke in rat. Rats were pretreated with BBR (100 mg/kg, oral administration) and BBR-loaded micelle (100, 75, 50, 25 mg/kg) for 14 days before ischemia. ELISA kit was used to detect the level of TNF-α after ischemia. Results show concentrations of BBR 100 mg/kg, and nano BBR 100 mg/kg ($P<0.01$), 75 and 50 mg/kg ($P<0.05$) had more reduction in TNF-α level compared to the stroke group

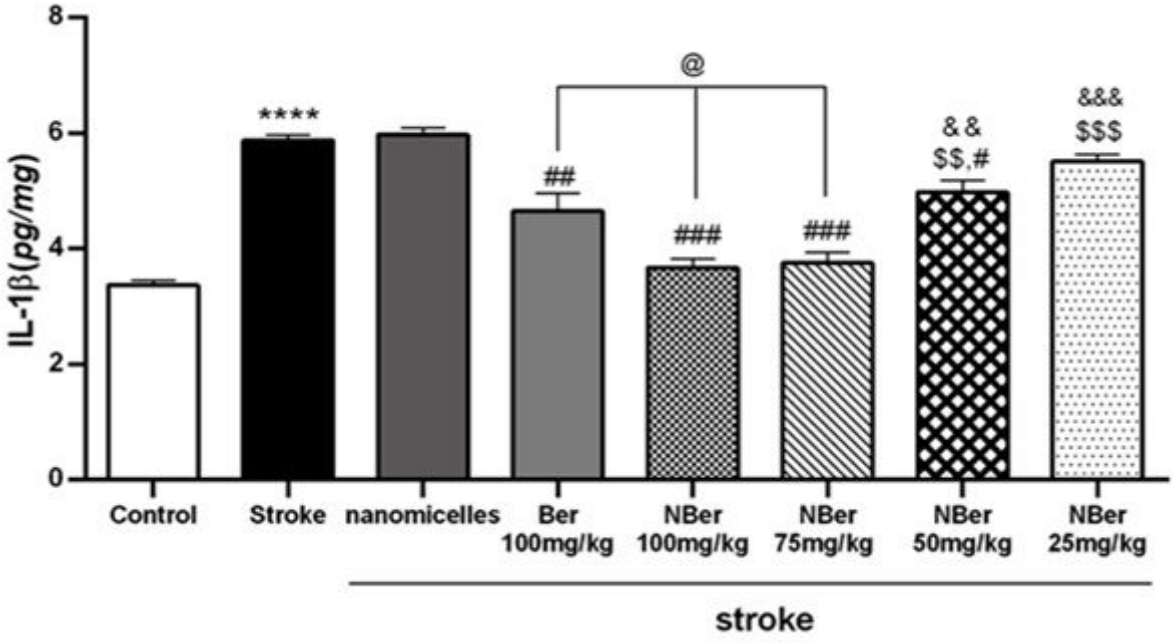

Figure 7

Rate changes of IL-1ß in different groups after inducing stroke in rat. N = 8 for each group. $P<0.01$, $P<0.05$ compared to stroke group. Data are presented increase the level of IL-1ß in treated group (nano

BBR 25mg/kg) and nano micelle (without drug) group compared to the control group (p<0.001)

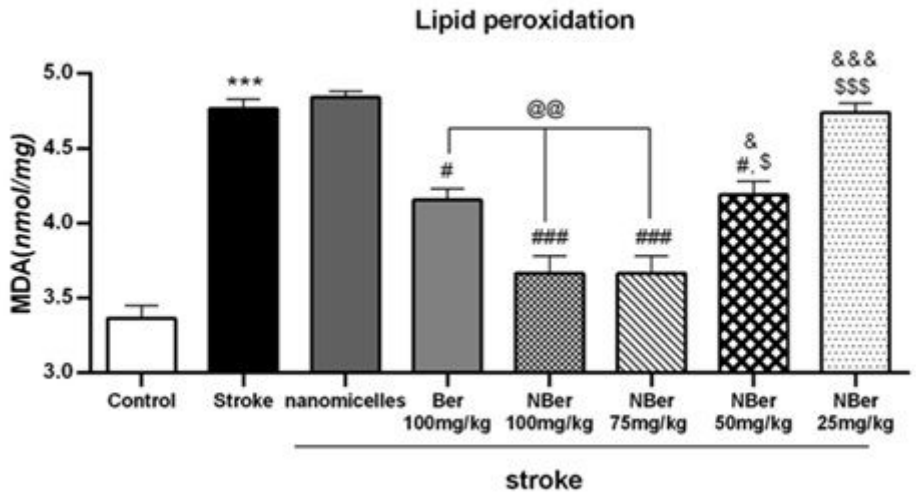

Figure 8

Rate changes of MDA in different groups after inducing stroke in rat

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- dataazadirawdata.xlsx
- paperrawinformation.zip
- SupplmentaryData.pdf