Sys Rev Pharm 2019;10(1):206-213
A multifaceted review journal in the field of pharmacy
E-ISSN 0976-2779 P-ISSN 0975-8453

DMF Ameliorating Cerebral Ischemia/ Reperfusion Injury in Male Rats

Laith M Abbas Al-Huseini [1] , Rihab H Al-Mudhaffer [2] , Saif M Hassan [3] , Najah R Hadi [3]

[1] Department of Pharmacology and Therapeutics, Faculty of Medicine, University of Al-Qadisiyah, Iraq

[2] Director of Middle Euphrates Unit for Cancer Research, Faculty of Medicine, University of Kufa, Iraq

[3] Department of Pharmacology and Therapeutics, Faculty of Medicine, University of Kufa, Iraq

[1] Correspondence should be addressed. Department of Pharmacology and Therapeutics, College of Medicine, University of Al-Qadisiyah, P. O. Box 80, Diwaniyah, Iraq. E-mail: laith.abbas@qu.edu.iq

Article History: Submitted: 14.12.2018 Revised: 25.02.2019 Accepted: 10.03.2019

ABSTRACT

Cerebral I/R causes oxygen deprivation to the brain tissue and generation of reactive oxygen species (ROS) which can stimulate immune responses, leading to inflammatory cells activation and infiltration. Methods: Rats were subjected to general anesthesia during which an occlusion of bilateral common carotid arteries for half hour then reperfusion for one hour with and without pretreatment with Dimethyl fumarate (DMF) at 50 mg/kg intraperitoneal (i.p) 24 hr. before ischemia.HO-1, TLR2, and TLR4 antibodies were assessment by IHC technique while ELISA type used to measure Nrf2, NF-κB and MDA in brain tissue levels. Levels of nuclear Nrf2 were increased significantly (P<0.05) in the DMF group. The levels of NF-κB and MDA were decreased significantly (P<0.05).

Histological findings including: hemorrhage, dark neurons, and necrosis were reduced in DMF groups in addition to the reduction in the infarction area. Neuroprotective effect of DMF against cerebral I/R injury probably occurred via up-regulation of nuclear Nrf2 and down-regulation of inflammatory and oxidative stress.

Keyword: Cerebral I/R, DMF, Nrf2, MDA, NF-κB, oxidative stress

Correspondance:
Laith M Abbas Al-Huseini
Department of Pharmacology and Therapeutics, Faculty of Medicine
University of Al-Qadisiyah, Iraq
Email: laith.abbas@qu.edu.iq

DOI: 10.5530/srp.2019.1.36

INTRODUCTION

Stroke is considered as one of the major health problem worldwide which can cause increased rates of death [1]. The World Health Organization (WHO) has estimated that cerebral ischemia caused death accounted for 9.7% globally and more than 85% in countries with the low-middle economic state [2] while decrease to 42% in countries with the high-economic state. The principal systems of reperfusion damage incorporate oxidative stress, leukocyte invasion, mitochondrial instruments, platelet activation and aggregation, supplement actuation, and blood-brain-barrier (BBB) interruption, which at last prompt cerebrum edema or hemorrhagic change and in the long run causing huge neuron death and neurological dysfunctions. ROS and the recruitment of leukocytes in post-ischemic micro vessels is often accompanying by the accumulation of platelets. Platelets then bind to its surface receptors such as P and E-selectin, glycoproteins, and the fibronectin receptor [3]. This binding can lead to up-regulation of selections and ICAM through the release of proinflammatory mediators from platelet. Platelets are strongly attracting to and activating the sub endothelial molecules such as collagen and Von Will brand factor. Reestablishing ATP levels during reperfusion, as an outcome, permits dynamic take-up of Ca^{+2} by the mitochondria, bringing about huge Ca^{+2} over-burden and pulverization of the mitochondrial content. High level of Ca^{+2} in the mitochondria causes opening mitochondrial permeabilitytransition pore (MPTP) [4] which causes mitochondrial swelling, inner membrane depolarization, oxidative stress generationsuch as ROS, RNS, $O_2^{-\bullet}$ and hydrogenperoxide (H_2O_2), and they in the long run prompt oxidative stress. ROS causes lipid oxidation, membrane damage, distribute DNA and cell processes. In addition to the distribution the balance of the BBB that permitting the penetration of neutrophils and different cells [5]. Nrf2 is a transcription-factor that considered as a master regulator of oxidative stress due to its role in the drug metabolism especially in phase II drug metabolism or conjugation of xenobiotics. Nrf2 is a member of the cap'n'collar (CNC) basic-leucine-zipper transcription-factor which regulates the basal or stress-inducible expression of genes components of glutathione and thioredoxin-antioxidant systems. As a result, it plays an important role in the adaptation of cells to ROS and xenobiotics [6]. GSH synthesis is under the control of two enzymes which are γ-glut amyl cysteine ligase and glutathione synthesize [7]. NQO1 catalyzes the reduction and detoxification quinines and $O_2^{-\bullet}$ scavenger. Nrf2 is the major regulatory system of GSH via controlling and modulator release of γ-glut amyl cysteine ligase. Dimethyl fumarate (DMF), a fumarate ester, is a recently FDA approved drug for the treatment of multiple sclerosis (MS) and psoriasis under market name Ticfedra [8]. DMF is a potent anti-oxidant and anti-

inflammatory agent which has a mechanism of action via activation of Nrf2 pathway. Thereby it causes modulation of the glutathione system and enhancement of cellular response to oxidative stress. Following administration, DMF can not be detected in the plasma because it undergoes rapid hydrolysis to its active metabolite, monomethyl fumarate, before entering the systemic circulation [9]. In the current study, we evaluate the role of DMF in ameliorating damage caused by cerebral I/R injury.

MATERIALS AND METHODS

Twenty-four Albino male rats weight ranged between 250-350 g were obtained from animal house, Faculty of Science, University of Zakho. The rats were kept in the animal house at Faculty of Medicine, University of Kufa in a well-ventilating room under a 12hr/12hr cycle (light/dark) at temperature 25±2 °C and they were given standard animal diet and free access to water [18]. The experiment was approved by University of Kufa-Animal Care and Research Committee, and the investigation according to the Laboratory Animals Guide Care.

Study groups

Rats were divided into four groups after one week of acclimatization, as follows: Sham group: Rats were subjected to general anesthesia without occlusion of the bilateral common carotid arteries. Control group (ischemic-reperfused): Rats were subjected to general anesthesia followed by occlusion of bilateral common carotid arteries (BCCAO) for half an hour then reperfusion for one hour but not receive any drug [10]. Vehicle group: Rats were subjected to the same surgical procedure as in control group but received the vehicle of drugs, DSMO, intraperitoneal (i.p) 24 hr. before ischemia. Dimethyl fumarate group: Rats were subjected to the surgical procedure as control group plus DMF at 50 mg/kg intraperitoneal (i.p) 24 hr. before ischemia [11].

Drug Preparation: DMF (Cat No. 242926) was purchased from Sigma Aldrich, (Germany) and dissolved in stock solution (29 mg/ 1 ml DMSO plus enough distill water).

Induction of global brain ischemia: Rats were subjected to general anesthesia via injection of ketamine and xylazine i.p at 100 mg/kg and 10 mg/kg respectively [12], when rats became unconscious, an incision on the neck was made and carotid arteries were seen then by using vascular clamps blocked the arteries on both sides (left and right) at same time as possible for 30 min, after 30 min the clamps were removed from the arteries to begin reperfusion for 1 hr to induce global cerebral ischemia/ reperfusion injury. After 1 hr of reperfusion, rats were decapitated, brains were removed quickly and cold in pre-cold PBS solution, then transfer to freezer for 10 min, after that each brain was cut to three slices, one for histopathological and immune-histopathological studies, second for TTC staining, and third for ELISA study [13].

Assessment of cerebral infarction by using of TTC stain: Rats were sacrificed after 30 min BCCAO and 60 min reperfusion. Brains were rapidly removed and frozen at –20°C for 10 min with cold PBS. Brain tissues were an assessment by 2, 3, 5 Triphenyl tetrazolium chloride stain (TTC) M. wt. = 334.8 was purchased from Direvo industrial biochemical staining that immediately prepared by dissolved it in PBS at 0.2 % (w/ v) concentration before slicing of the brains. After brains cuts into coronal slices, it immersed in TTC stain at 37°C for 30 min in a glass Petri dish with aluminum foil covering to prevent the effect of light on TTC stain followed by immersion in PBS-10% par formaldehyde. Infarct volume was measured by using digital imaging (digital camera) and image analysis software (image J system). The infarct area was measured by Swanson's method [14]. The total volumes of both white area (infarction area) and red area (valid area) were measured, and the infarct percentage (I) was calculated as:

$$\%I = \frac{\text{white area}}{\text{total area}} \times 100\%$$

Histopathological assessment: At the end of reperfusion, brain tissues were put in formalin and immersed in a paraffin wax, cut 6 µM thick sections, then stained by hematoxylin-eosin (H and E) and seen under a microscope. The pathological scoring scale used in this study as following: Normal (0): there is no edema, RBCs, or eosinophilic neurons, slight (1): there is either edema or eosinophilic neurons. Moderate (2): there are edema, eosinophilic neurons, and a little number of RBCs. Severe (3): there are edema, eosinophilic neurons, RBC, and necrosis.

SAMPLES PREPARATION

Tissue preparation for MDA and NF-κB measurement: The brain tissues were cut into very small pieces under cold condition followed by homogenized with homogenization solution which contains PBS, cocktail inhibitor and Triton X100 for 20 min by using a ultrasonic liquid processor under cold condition followed by centrifuging at 2,000 - 3,000 r.p.m for 20 min at 4°C and stored at –80°C for future analysis [15].

Cytoplasmic and Nuclear extraction of Nrf2: The extraction and isolation of Nrf2 from tissue samples were performed according to (Cat no. ab221978) was purchased from Beyotime biotechnology, China.

Measurement levels of Nrf2, MDA, and NF-κB through ELISA technique: The collected supernatants obtained from all groups were used for measuring of Nrf2 at cytoplasmic and nucleus level, in addition to MDA, and NF-κB levels by ELISA (Nanjing Pars Biochem CO., Ltd, China) according to manufacturer protocol.

Measurement levels of HO-1, TLR2, and TLR4 through immunohistochemistry: At end of reperfusion, brain tissues cut into 6 µM thick sections, dew axing, and

immersed in xylene to be followed by dehydration using different concentration of alcohol 100%, 95%, 90%, and 70%. Break down the crosslink between formalin and antigen, we used retrieval buffer in water bath and then blocked the peroxides effect by using blocking agent. Incubation the brain tissues with primary antibody purchased from Cosabio, USA followed by secondary antibody and finally stained by using DAB and hematoxylin solution and see under microscope.

Statistical analysis: The data are expressed as mean ±standard error of the mean (SEM). One-way analysis of variance (ANOVA) followed by Turkey's post hoc; a P value of ≤ 0.05 was considered to be statistically significant. Analyses were done by using SPSS software update version

RESULTS

DMF reduced infarction area percentages: Analysis of the infarct area for the whole brain in all groups has been measured by software (image J). DMF has clearly reduced the white (infracted) area in comparison to control group (data not shown). A photographic analysis showed that DMF reduced infarction area percent significantly ($p<0.05$) as compared to control group (13.81±1.81% vs. 43.15±7.72%) as seen in Table (1).

DMF reduced cytoplasmic level of Nrf2 in brain tissue: Cerebral I/R injury represents an acute stage of oxidative stress, therefore we expect that there will be an enhancement in cytoprotective mechanisms in the brain tissue including translocation of Nrf2 form the cytoplasm to the nucleus. Our results showed that CI/ RI reduced cytoplasmic levels of Nrf2 as compared to sham group (49.23±1.30 vs. 73.41±2.11 mg/ ml) while DMF caused significant ($p<0.05$) further reduction in the cytoplasm level of Nrf2 as compared to control group (36.77±0.86 vs. 49.23±1.30 mg/ ml) as seen in Table (2).

DMF increased nuclear level of Nrf2 in brain tissue: We found that DMF elevated the level of Nrf2 in nucleus significantly ($p<0.05$) as compared to control group (41.03±0.56 vs. 31.49±0.43 mg/ ml) as shown in Table (3).

DMF didn't cause expression of HO-1: Cerebral I/R injury didn't cause any changes in HO-1 level. We analyzed the HO-1 level in brain tissue for all groups via the immunohistochemistry technique as shown in Figure (1).

DMF reduced MDA level: We found that MDA level of DMF group was reduced significantly ($p<0.05$) as compared to control group (0.54±0.03 vs.1.22±0.05 µmole/ml) as shown in Table (4).

DMF reduced level of NF-κB: Cerebral I/ R injury caused elevation in NF-κB level. DMF reduced level of NF-κB significantly ($p<0.05$) as compared to control group (114.73±5.92 vs. 166.18±7.73 mg/ml) as shown in Table (5).

DMF inhibited the expression of TLRs: An IHC study showed that control and vehicle groups expressed TLR4 and TLR2 with weak-moderate intensity and few positive cells while DMF inhibits expression of them in brain tissues, we found insignificant difference ($p<0.05$) between DMF group and sham group as shown in Figure (2).

DMF reduced brain damage scores: Cerebral I/ R injury caused brain tissue damage which can be classified as: mild, moderate, or severe damage. A histopathological examination, as shown in Figure (3), revealed elevated brain tissue damage scores significantly ($p<0.05$) in control and vehicle groups when compared to sham group with significant improvement ($p<0.05$) in DMF group.

DISCUSSION

BCCAO is one of several models to study the brain ischemic stroke in rats [16] it caused increase in oxidative stress, inflammatory responses, cell death, and neurological damage within minutes after the onset of ischemia. In the present study, we suggested that pretreatment rats with DMF (50 mg/kg) ameliorates BCCAO-induced cerebral ischemia-reperfusion injury after 30 min cerebral ischemia and 60 min reperfusion in an experimental rat model by increasing Nrf2 nuclear level, reduced infarct volume, decreased MDA content, modulation inflammation response by reducing level of NF-κB, TLR2, and TLR4. Cerebral I/ R injury causes dysfunction and death of neurons in the brain tissues due to elevated inflammatory and oxidative mediators. Nrf2 is a transcription factor has a regulatory role in cell survival by up regulation of anti-oxidative and cytoprotective genes [17]. In our study, we found that minimal infarction area percent was shown in sham group that an indicated there are minimum necrotic cells in non-ischemic brain slices and we noted increase significantly in untreated groups after induction of BCCAO when compared to sham group that indicated increased of necrotic cell due to down regulate Na/k -ATPase pump, increase oxidative stress and inflammatory response, while pre-treatment with Nrf2 activator drugs 24 hr. before ischemia reduced the infarction area percent significantly due to the increase of the nuclear level of Nrf2 to induce anti-oxidant genes and also reduce inflammatory markers and this result suppose our suggestion, Ruihe Lin et al. (2016) who study the effect of DMF on middle cerebral artery occlusion and they found that DMF reduced the size of infarction about 30 % [18].

DMF increases accumulation of nuclear Nrf2 level of brain tissue: In our experiment, we found that maximum cytoplasmic and minimal nuclear level of Nrf2 was shown in sham group which indicated a basal level of protein in non-ischemic brain and we noted that cytoplasmic level decreased while nuclear level increased significantly in untreated groups after induction of BCCAO when compared to sham group that indicated Nrf2 released from its complex and translocated to nucleus due to increase in

stress and inflammation. Pretreatment with DMF reduced cytoplasm level and increased nuclear level of Nrf2 as compared to control group which indicated more Nrf2 accumulation in nucleus. Several authors studied the effect of Nrf2 activator on the CI/ RI but no study of DMF on this model. Chen et al. shown that BCCAO didn't increase the nuclear level of Nrf2 and reported that endogenous Nrf2 protein insufficient to protect neurons against I/R while administration of rifampicin significantly decreased cytoplasm and elevated nuclear Nrf2 levels in the nucleus. Keita Yamauchi et al. (2016) found that treatment of mice with RS9 (derivative of Bardoxolone) elevated the level of Nrf2 after focal ischemia. Wang H et al. (2019) who studied the effect of swertiamain on same our model, they found that swertiamain reduced the cytoplasmic level of Nrf2 and elevated its nuclear level [19]. Cerebral I/R injury induce oxidative stress which may result in tissue damage, abnormal lipid metabolism and cellular membranes disturbance by lipid per oxidation while pre-treated with Nrf2 activators before ischemia decreased per oxidation. We found that sham group has low MDA level and we noticed increased in MDA level in control group after induction of cerebral I/ R that indicated MDA generated to increase stress. Pretreatment with DMF reduced MDA level significantly when compared to control group which indicated reduction in oxidative stress and brain damage. Several studies showed that MDA level increased in control group while pretreatment different Nrf2 activators reduced its level [20]. Yang Yao et al. (2016) found that when mice undergo left middle cerebral artery occlusion and treated with DMF, MDA level was reduced significantly as compared to control. These results support our finding of Nrf2 induced brain cells protection of membrane per oxidation. In our study, we found that HO-1 does not express after CI/ RI according to immunohistochemistry staining while the positive control (normal human spleen) showed high expression of HO-1. We proposed that the time of administration of Nrf2 activators before I/R was not enough to induce expression of HO-1.Jie Han et al. (2014) who studied Epigallocatechin Gallate (Nrf2 activators) and found HO-1 increased in treated groups significantly after 2hr. ischemia and 24 hr. reperfusion [21]. Other author found HO-1 increased after 3 days of ischemia and continue over 48 or more. Activation of Nrf2 inhibits NF-κB translocated and binding to its promoters. Nrf2 inhibits factors could activate NF-κB including TNF-α, IL-1, ROS, and oxidative stress which be elevated after 10-30 min. after cerebral ischemia. In our experiment, we found that low NF-κB level was shown in sham group and its level increased significantly in control group after induction of BCCAO that indicated NF-κB over expression. Pretreatment with DMF 24 hr. before ischemia reduced NF-κB level significantly when compared to control group which indicated accumulation of Nrf2 protein in nucleus causing reduction in brain inflammation. Keita Yamauchi et al. (2016) suggested Nrf2 activator (RS9) decreased the level of the NF-κB reflecting the anti-inflammatory mechanism of RS9 [22]. In our study, we found that pretreatment with DMF inhibits TLR2 and TLR4 expression after 30 min ischemia and 1 hr. reperfusion while the control and vehicle groups show weak-moderate intensity with few positive cells. These receptors are component of innate immune system and inhibition of their expression by pharmacological Nrf2 activator reduced the inflammation response. Hyakkoku K et al. (2010), who studied the expression of TLR4 in MCAO, found that the number of positive cells increases in control group after ischemia-reperfusion. Tissue kallikrein (Nrf2 activator) reduced TLR4 cerebral I/R expression and this reduction was better seen when tissue kallikrein administered immediately than that of 12 hr. after reperfusion. Cerebral I/ R injury cause interruption in blood-brain barrier, edema formation, and induce pyknotic and dark eosinophilic neurons [23-24]. In our experiment, we found that low damage score was shown in sham group which indicated a basal score in non-ischemic brain slices and we noted that the damage scores increased significantly in control group after induction of BCCAO. While pretreatment by DMF reduced damage scores when compared to control group which indicated activation of Nrf2 protein reduces brain damages such as hemorrhage and necrosis. Chandrasekhar et al. (2010) who noticed that microscopic examination of control group brain tissue showed congestion of blood vessels and neurotic necrosis features after BCCAO. Our results elucidated the neuroprotective effect of DMF in improving brain tissue damage induced by cerebral I/ R injury through its ability to increase nuclear Nrf2 translocation and thereby down-regulation of inflammatory and oxidative stress markers which eventually reduced brain tissue damage.

The Authors declare that there is no conflict of interest.

Funding: This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors Ethical approval: approved by University of Kufa-Animal Care and Research Committee, and the investigation according to the Laboratory Animals Guide Care.

REFERENCES

1. Tran J, Mirzaei M, Anderson L, Leeder SR. The epidemiology of stroke in the Middle East and North Africa. Journal of the Neurological Sciences [Internet]. Elsevier BV; 2010 Aug;295(1-2):38–40. Available from: http://dx.doi.org/10.1016/j.jns.2010.05.016
2. Lopez AD, Mathers CD, Ezzati M, Jamison DT, Murray CJ. Global and regional burden of disease and risk factors, 2001: systematic analysis of population health data. The Lancet [Internet]. Elsevier BV; 2006 May;367(9524):1747–57. Available from: http://dx.doi.org/10.1016/s0140-6736(06)68770-9
3. Feigin VL, Forouzanfar MH, Krishnamurthi R, Mensah GA, Connor M, Bennett DA, et al. Global and regional burden of stroke during 1990–2010: findings from the Global Burden of Disease Study 2010. The Lancet

[Internet]. Elsevier BV; 2014 Jan;383(9913):245–55. Available from: http://dx.doi.org/10.1016/s0140-6736(13)61953-4

4. Sumii T, Lo EH. Involvement of Matrix Metalloproteinase in Thrombolysis-Associated Hemorrhagic Transformation After Embolic Focal Ischemia in Rats. Stroke [Internet]. Ovid Technologies (Wolters Kluwer Health); 2002 Mar;33(3):831–6. Available from: http://dx.doi.org/10.1161/hs0302.104542
5. Rodrigues SF, Granger DN. Role of blood cells in ischaemia-reperfusion induced endothelial barrier failure. Cardiovascular Research [Internet]. Oxford University Press (OUP); 2010 Mar 17;87(2):291–9. Available from: http://dx.doi.org/10.1093/cvr/cvq090
6. Weisman H, Bartow T, Leppo M, et al. Recombinant soluble CR1 suppressed complement activation, inflammation, and necrosis associated with reperfusion of ischemic myocardium. *Transactions of the Association of American Physicians.* 1990; 103: 64-72.
7. Piantadosi CA, Zhang J. Mitochondrial Generation of Reactive Oxygen Species After Brain Ischemia in the Rat. Stroke [Internet]. Ovid Technologies (Wolters Kluwer Health); 1996 Feb;27(2):327–32. Available from: http://dx.doi.org/10.1161/01.str.27.2.327
8. Lee M-C, Velayutham M, Komatsu T, Hille R, Zweier JL. Measurement and Characterization of Superoxide Generation from Xanthine Dehydrogenase: A Redox-Regulated Pathway of Radical Generation in Ischemic Tissues. Biochemistry [Internet]. American Chemical Society (ACS); 2014 Oct 6;53(41):6615–23. Available from: http://dx.doi.org/10.1021/bi500582r
9. Chan PH. Reactive Oxygen Radicals in Signaling and Damage in the Ischemic Brain. Journal of Cerebral Blood Flow & Metabolism [Internet]. SAGE Publications; 2001 Jan;21(1):2–14. Available from: http://dx.doi.org/10.1097/00004647-200101000-00002
10. Tebay LE, Robertson H, Durant ST, Vitale SR, Penning TM, Dinkova-Kostova AT, et al. Mechanisms of activation of the transcription factor Nrf2 by redox stressors, nutrient cues, and energy status and the pathways through which it attenuates degenerative disease. Free Radical Biology and Medicine [Internet]. Elsevier BV; 2015 Nov;88:108–46. Available from: http://dx.doi.org/10.1016/j.freeradbiomed.2015.06.021
11. Rahman I, Antonicelli F, MacNee W. Molecular Mechanism of the Regulation of Glutathione Synthesis by Tumor Necrosis Factor-α and Dexamethasone in Human Alveolar Epithelial Cells. Journal of Biological Chemistry [Internet]. American Society for Biochemistry & Molecular Biology (ASBMB); 1999 Feb 19;274(8):5088–96. Available from: http://dx.doi.org/10.1074/jbc.274.8.5088
12. Siegel D, Gustafson DL, Dehn DL, Han JY, Boonchoong P, Berliner LJ, et al. NAD(P)H:Quinone Oxidoreductase 1: Role as a Superoxide Scavenger. Molecular Pharmacology [Internet]. American Society for Pharmacology & Experimental Therapeutics (ASPET); 2004 Apr 20;65(5):1238–47. Available from: http://dx.doi.org/10.1124/mol.65.5.1238
13. Correa F, Ljunggren E, Mallard C, Nilsson M, Weber SG, Sandberg M. The Nrf2-inducible antioxidant defense in astrocytes can be both up- and down-regulated by activated microglia:Involvement of p38 MAPK. Glia [Internet]. Wiley; 2011 Feb 23;59(5):785–99. Available from: http://dx.doi.org/10.1002/glia.21151
14. Correa F, Ljunggren E, Mallard C, Nilsson M, Weber SG, Sandberg M. The Nrf2-inducible antioxidant defense in astrocytes can be both up- and down-regulated by activated microglia:Involvement of p38 MAPK. Glia [Internet]. Wiley; 2011 Feb 23;59(5):785–99. Available from: http://dx.doi.org/10.1002/glia.21151
15. Fox R. The New England Journal of Medicine publishes pivotal data demonstrating efficacy and safety of oral BG-12 (dimethyl fumarate) in multiple sclerosis. *Can J Neurosci Nurs.* 2012; 34: 7-11.
16. Gold R, Linker RA, Stangel M. Fumaric acid and its esters: An emerging treatment for multiple sclerosis with antioxidative mechanism of action. Clinical Immunology [Internet]. Elsevier BV; 2012 Jan;142(1):44–8. Available from: http://dx.doi.org/10.1016/j.clim.2011.02.017
17. Dibbert S, Clement B, Skak-Nielsen T, Mrowietz U, Rostami-Yazdi M. Detection of fumarate–glutathione adducts in the portal vein blood of rats: evidence for rapid dimethylfumarate metabolism. Archives of Dermatological Research [Internet]. Springer Science and Business Media LLC; 2013 Mar 25;305(5):447–51. Available from: http://dx.doi.org/10.1007/s00403-013-1332-y
18. Cada DJ, Levien TL, Baker DE. Dimethyl Fumarate. Hospital Pharmacy [Internet]. SAGE Publications; 2013 Aug;48(8):668–79. Available from: http://dx.doi.org/10.1310/hpj4808-668
19. Lee YT, Laxton V, Lin HY, Chan YWF, Fitzgerald-Smith S, To TLO, et al. Animal models of atherosclerosis. Biomedical Reports [Internet]. Spandidos Publications; 2017 Jan 17;6(3):259–66. Available from: http://dx.doi.org/10.3892/br.2017.843
20. Obadia N, Lessa MA, Daliry A, Silvares RR, Gomes F, Tibiriçá E, et al. Cerebral microvascular dysfunction in metabolic syndrome is exacerbated by ischemia–reperfusion injury. BMC Neuroscience [Internet]. Springer Nature; 2017 Sep 8;18(1). Available from: http://dx.doi.org/10.1186/s12868-017-0384-x
21. Walsh J, Jenkins RE, Wong M, Olayanju A, Powell H, Copple I, et al. Identification and quantification of the basal and inducible Nrf2-dependent proteomes in mouse liver: Biochemical, pharmacological and toxicological implications. Journal of Proteomics [Internet]. Elsevier BV; 2014 Aug;108:171–87. Available from: http://dx.doi.org/10.1016/j.jprot.2014.05.007
22. Cho H, Hartsock MJ, Xu Z, He M, Duh EJ. Monomethyl fumarate promotes Nrf2-dependent neuroprotection in retinal ischemia-reperfusion. Journal of Neuroinflammation [Internet]. Springer Science and

Business Media LLC; 2015 Dec;12(1). Available from: http://dx.doi.org/10.1186/s12974-015-0452-z

23. Terao S, Yilmaz G, Stokes KY, Russell J, Ishikawa M, Kawase T, et al. Blood Cell-Derived RANTES Mediates Cerebral Microvascular Dysfunction, Inflammation, and Tissue Injury After Focal Ischemia–Reperfusion. Stroke [Internet]. Ovid Technologies (Wolters Kluwer Health); 2008 Sep;39(9):2560–70. Available from: http://dx.doi.org/10.1161/strokeaha.107.513150 .
24. Tang H, Tang Y, Li N, Shi Q, Guo J, Shang E, et al. Neuroprotective effects of scutellarin and scutellarein on repeatedly cerebral ischemia–reperfusion in rats. Pharmacology Biochemistry and Behavior [Internet]. Elsevier BV; 2014 Mar;118:51–9. Available from: http://dx.doi.org/10.1016/j.pbb.2014.01.003

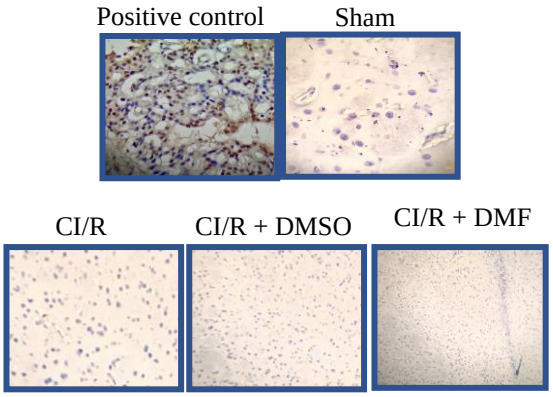

Figure 1: HO-1 expression for all experimental groups. Absence of HO-1 expression by Immunohistochemistry Staining was seen in all experimental groups. A positive control, using normal spleen tissue, was used to test the validity of the antibody (X 10×40).

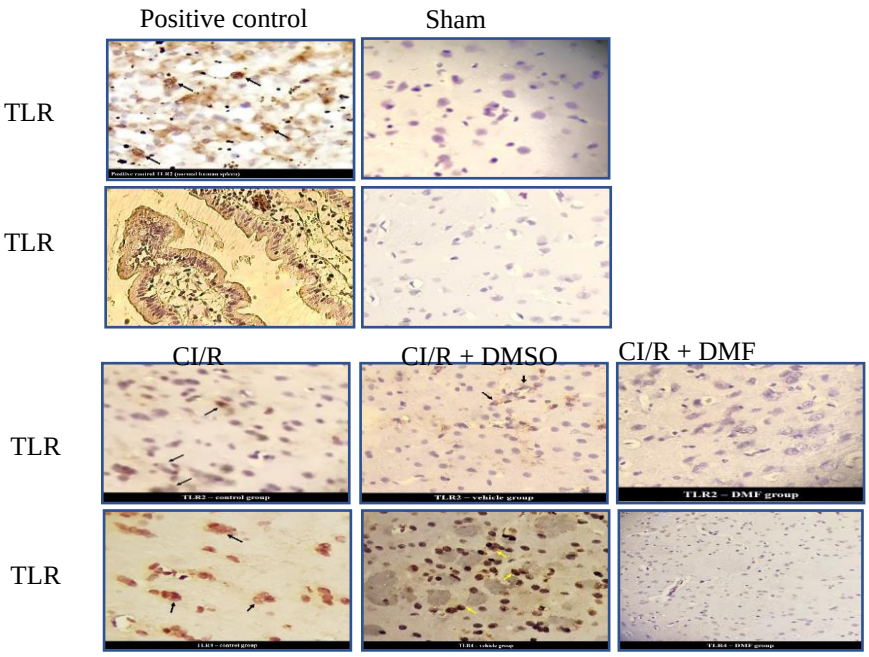

Figure 2: TLR2 and TLR4 expression in all experimental groups. Immunohistochemistry Staining showed control and vehicle groups with 10-15 positive cells while for DMF group <10% positive cells with moderate intensity. A positive control, using normal spleen tissue was used to test the validity of the antibody. (X 10×40).

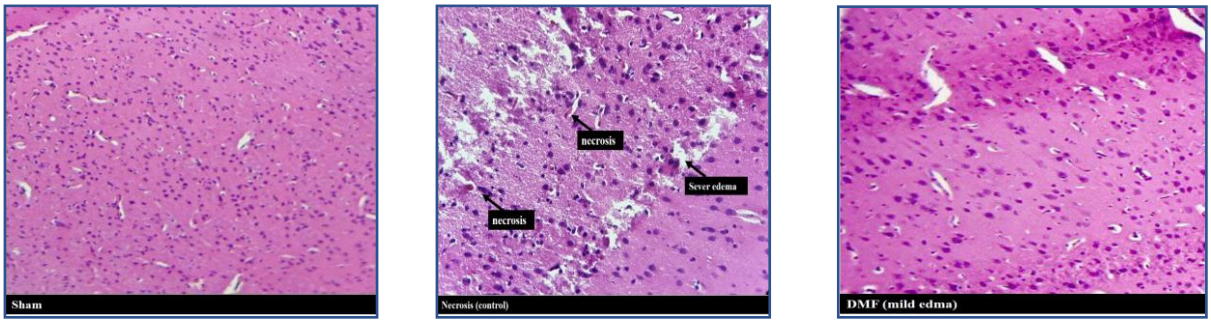

Figure 3: Histological scores of brain tissue damage

Group	Mean ± SEM	95% C I		P value
		Lower	Upper	
Sham	0.59 ± 0.09%	0.17%	0.61%	
Control	43.15 ± 7.72%	23.30%	63.01%	# P< 0.05
DMSO (vehicle)	42.18 ± 2.11%	36.75%	47.62%	# P< 0.05
DMF	13.81 ± 1.81%	9.14%	18.47%	* P< 0.05

Table (1): Percentages of infracted areas for all experimental groups
Data expressed by one-way ANOVA. # vs sham, * vs control.

Group	Mean ± SEM	95% CI		P value
		Lower	Upper	
Sham	73.41 ± 2.11	67.99	78.83	
Control	49.23 ± 1.30	45.88	52.57	# P< 0.05
DMSO (vehicle)	47.54 ± 1.43	43.87	51.22	# P< 0.05
DMF	36.77 ± 0.86	34.56	38.97	* P< 0.05

Table (2): Cytoplasm level (ng/ml) of Nrf2 in all experimental groups
Data expressed by one-way ANOVA.* vs sham, # vs control.

Group	Mean ± SEM	95% CI		P value
		Lower	Upper	
Sham	22.67 ± 0.72	20.82	24.52	
Control	31.49 ± 0.43	30.40	32.59	# P< 0.05
DMSO (vehicle)	31.66 ± 0.64	30.01	33.30	# P< 0.05
DMF	41.03 ± 0.56	39.60	42.46	* P< 0.05

Table (3): Nuclear level (ng/ml) of Nrf2 in all experimental groups
Data expressed by one-way ANOVA. * vs sham, # vs control.

Group	Mean ± SEM	95% C I		P value
		Lower	Upper	
Sham	0.44 ±0.03	0.35	0.52	
Control	1.22 ± 0.05	1.09	1.36	# P< 0.05
DMSO (vehicle)	1.23 ± 0.05	1.11	1.35	# P< 0.05
DMF	0.54 ± 0.03	0.48	0.61	* P< 0.05

Table (4): MDA levels (µmole/ml) for all experimental groups
Data expressed by one way ANOVA.# vs sham, * vs control.

Group	Mean ± SEM	95% C I		P value
		Lower	Upper	
Sham	108.62 ± 32.17	74.86	142.39	
Control	166.18 ± 7.73	158.07	174.28	#P< 0.05
DMSO (Vehicle)	159.80 ± 22.68	136.01	183.60	#P< 0.05
DMF	114.73 ± 5.92	108.52	120.95	*P< 0.05

Table (5): NF-κB levels (ng/ml) for all experimental groups
Data expressed by one-way ANOVA. # vs sham, * vs control.