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STUDIES ON CEREBROPROTECTIVE ACTIVITY AND ESTABLISHMENT OF PROBABLE MECHANISM OF ACTION OF DIMETHYL FUMARATE (DMF) AGAINST THE ISCHEMIA AND REPERFUSION INDUCED CEREBRAL INJURY IN WISTAR RATS

Ketan Vinayakrao Hatware[1, 2*], Akula Annapurna[1], Sanjay Sharma[2] & Sravani Karri[2]

[*1]AU College of Pharmaceutical Sciences, Andhra University, Vishakhapatnam, India.
[2]School of Pharmacy and Technology Management, SVKM's NMIMS, Shirpur, Maharashtra, India..

Keywords: *Ischemia reperfusion injury(IRI), Cerebroprotective, Dimethyl-fumarate (DMF), Inflammation, Oxidative Stress.*

Abstract

In the present study two months prior oral treatment of Dimethyl fumarate (DMF) at doses 10 mg/kg and 20 mg/kg was studied on ischemia and reperfusion induced cerebral injury in Wistar rats. The ischemia reperfusion injury (IRI) was induced by bilateral common carotid artery (BCCA) occlusion for 1 h and reperfusion for 5 h. The % infarction of cerebral cells was measured by staining frozen brain slices of 0.1 cm thickness with freshly prepared 2, 3, 5-Triphenyl tetrazolium chloride (TTC) solution (1%). At end of the study various inflammatory as well as oxidative stress parameters were analyzed such as tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), interleukin-10 (IL-10), c-reactive protein (CRP), superoxide dismutase (SOD), catalase (CAT), malondialdehyde (MDA). The DMF has shown significant protection against IRI by reducing the % infarction of cerebral cells. DMF has reduced the levels of pro-inflammatory markers i.e. TNF-α, IL-6 and CRP while increases the levels of anti-inflammatory marker i.e. IL-10. Similarly DMF has shown antioxidant activity by increasing the levels of SOD, CAT and decreasing levels of MDA in Wistar rats. This study concluded that the DMF is having significant cerebroprotective potential and anti-inflammatory as well as antioxidant activity.

Introduction

Stroke is one of the most complicated pathological condition leading to serious consequences in the patients suffering from it. The consequences of a stroke are depending on injured part of brain. A severe stroke can cause sudden death of an individual[1]. A stroke is caused by the interruption of the blood supply to the brain or cerebral cells, usually because a blood vessel ruptured or is blocked by a thrombus or embolus. This decreases the supply of oxygen and nutrients, causing damage to the brain tissue. The probable mechanism behind the cerebral ischemia may be microembolism to the brain vessels, stenosis of cerebral artery and decrease in systemic blood pressure, thromboembolism of large blood vessels, decreased cardiac output. The cerebral ischemia leads to brain cell necrosis i.e. damage to brain cells due to lack of oxygen and blood[2].It has been postulated that, there are complex as well as multifactorial mechanisms of ischemic stroke like atherosclerosis, thromboembolism, cardiac or vasculopathic disorders etc; among this atherogenic thrombosis is the common cause for occlusion of blood vessels which leads to ischemic stroke[3]. Acute ischemic stroke is caused by thrombotic or embolic blockade of a cerebral artery and is more common than hemorrhagic stroke[4].

Ischemia reperfusion injury is rare but significant pathological condition requires proper clinical care, it occurs due to cerebral hyper perfusion or reperfusion, the hyperperfusion implies excessive flow, while the reperfusion suggests normalization of flow[5, 6] both are leading to cerebral injury. Therefore, some authors prefer to address this subject as

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reperfusion syndrome[6]. The studies have shown that reperfusion injury is involved directly in the potentiation of stroke damage. Markers of the inflammatory response, including cytokine release and leukocyte adhesion, has shown important role in these harmful effects. The blood-brain barrier (BBB) is damaged in ischemia reperfusion injury [7-9].

The neutrophils, macrophages, cytokines and chemokines are inflammatory mediators that act to dilate blood vessels, increase vascular permeability, increase blood flow, and destroy invading damaged cells. However, the inflammatory response can cause more damage to the cerebral cells rather than imparting good favorable response to it in case of cerebral ischemia and reperfusion[10]. As neutrophils firmly adhered to vascular endothelium, they migrate from the vasculature into the surrounding brain tissue. Neutrophil products (reactive oxygen species (ROS), proteases, and cytokines) accelerate the damage to the blood brain barrier, vascular endothelium, and eventually brain parenchyma[11]. Myeloperoxidase (MPO) expression in neutrophils and macrophages/microglia, which has often been used as a histopathological marker for inflammation, generates ROS such as hypochloride and super oxide anion radical (O^2) and causing further tissue damage[12].

Owing to low oxygen supply during ischemia, moderate levels of ROS generation may occur, most probably from a mitochondrial source. The depletion of energy and decreased pH during ischemia leads to failure of electron transport chain (ETC), resulting in the generation of ROS, creating an oxidation state, which worsen with intracellular Ca^{++} overload upon reperfusion, causes massive generation of ROS[13]. The generation of ROS during reperfusion may occur from a different cellular sources including; mitochondrial dysfunction, arachidonic acid metabolism, catecholamine oxidation, neutrophil activity, activation of nitric oxide synthase, xanthine oxidase activity[14]. It has been assumed that ROS induced oxidative stress is a major part in the pathogenesis of reperfusion injury. ROS is responsible for cell death by inducing peroxidative damage of lipids, proteins and nucleic acids. It has been suggested that ROS are involved in ever fundamental physiological step that contributes to neuronal death and thus are regarded as an important target in getting an effective stroke therapy[15].

The most important cytokines associated with inflammation in ischemia/reperfusion are tumor necrosis factor-α (TNF-α), the interleukins (IL); IL-1β, IL-6, IL-20, IL-10 and transforming growth factor (TGF)-β. Among these TNF-α, IL-1β and IL-6 are potent inflammatory cytokines, found to have a central role in ischemia-reperfusion induced inflammation[16]. All three cytokines have been demonstrated to enhance brain damage in an experimental model of ischemia-reperfusion in rodents and also their levels have been found to be increased in cerebro spinal fluid (CSF) and blood after ischemic stroke in humans[17], supporting their role in ischemia-reperfusion injury. Accumulating evidence is available, proving oxidative stress as the important mediator of cerebral ischemia-reperfusion injury. Significant levels of superoxide anion and hydroxy1 radicals have been detected after reperfusion[18, 19] and a substantial decline in catalase activity and SOD activity has been observed at the reperfusion onset[20]. Oxidative stress results in numerous pathological consequents, including membrane damage by lipid peroxidation, induction of inflammatory responses, mitochondrial dysfunction, induces apoptotic pathway leading to cell death[18, 21]. Therefore, down regulating the oxidative stress can minimize reperfusion injury and potentiate the effectiveness of reperfusion therapy.

DMF the investigational drug in the present study is useful for the treatment of multiple sclerosis[22, 23], for the therapy of severe psoriasisin Germany, furthermore the clinical efficacy of DMF to reduce inflammation in multiple sclerosis has been demonstrated by Kappos L et al[24]. DMF is the methyl ester of fumaric acid, has been shown to have beneficial effects in preclinical models of neuroinflammation, neurodegeneration, and toxic oxidative stress, which appear to be mediated predominately through activation of the nuclear 1 factor (erythroid- derived 2)–like 2 (Nrf2) antioxidant response pathway, the primary cellular defense against the cytotoxic effects of oxidative stress[22, 25]. Our findings in the previous research work proved that the DMF is relatively safe drug and having cerebroprotective activity against ischemia and reperfusion induced cerebral injury in BCCA ligation model in Wistar rats[26]. The present study is the extension of the previous findings for the establishment of its probable

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mechanism for its cerebroprotective activity by evaluating the anti-inflammatory and anti-oxidant activities in the same experimental model.

Materials and Methods

Animals

In the present study healthy adult male Albino Wistar strain rats (250 g to 300 g) were procured from Albino research center, Hyderabad. Animals were housed in clean and transparent polypropylene cages with three animals in each cage and maintained at 25-27 ^{0}C with 12:12 h light-dark cycle for a period of 7 days prior to the study. They were fed ad libitum regular grain chow (Rayan's Biotechnologies Pvt. Ltd, Hyderabad). The experimental protocol has been approved by Institutional Animal Ethics Committee (IAEC). Maintenance and handling of animals were done as per CPCSCA guidelines; the prior permission for the study was obtained from IAEC (Regd. No. 516/01/A/CPCSEA).

Drugs and Chemicals

Dimethyl fumarate (Sigma Aldrich, USA.), 2, 3, 5- triphenyl-tetrazolium chloride (TTC): (Sigma Aldrich, USA). ELISA kit for TNF-α (DIACLONE, France.), Interleukin-6 and Interleukin-10(BIOSPES, China.), C - reactive protein estimation kit(AGAPPE Diagnostics Ltd., India.), diagnostic kits for SOD, catalase and MDA (Biodignostics, Egypt).

Instruments Used

Semiautoanalyzer (MISPA UNO), centrifuge (REMI), incubator (REMI), ELISA reader (MediBiotronics), etc.

Evaluation of cerebroprotective potential of DMF against ischemia and reperfusion induced cerebral injury in Wistar rats

The present study was carried out to evaluate, cerebroprotective potential of DMF against 1 h BCCA occlusion induced ischemia followed by 5 h reperfusion induced cerebral injury in Wistar rats. The effects of two months pretreatment of DMF at the dose of 10 mg/kg and 20 mg/kg (per oral), were observed against ischemia reperfusion induced cerebral injury. The extent of infarction was assessed by measuring the percentage cerebral infarction using freshly prepared 1% TTC solution staining.

Experimental Protocol

The rats were selected randomly and divided into five groups each group consists of six rats.

Group I	:	Normal
Group II	:	Sham control
Group III	:	I/R control
Group IV	:	Treated with 10 mg/kg DMF
Group V	:	Treated with 20 mg/kg DMF

Group I served as normal group without surgery, i.e. BCCA occlusion and drug treatment. Group II served as sham control received only surgery without BCCA occlusion and drug treatment. Group III served as disease control received BCCA occlusion for 1 h and 5 hreperfusion without drug treatment. Group IV and Group V were served as test groups received drug treatment with DMF at doses 10 mg/kg and 20 mg/kg respectively for two months (30 min before reperfusion on last day of the treatment of two months) and BCCA occlusion for 1 hour and 5 hours reperfusion. The percentage of infarction was measured in all the groups as described below. Similarly the same experimental protocol was followed for other parameters estimations in the study like inflammatory parameters (TNF-α, IL-6, IL-10 and CRP), antioxidant (SOD, CAT and MDA) as well as histopathological changes in the Wistar rats.

Induction of ischemia reperfusion injury in rat brain

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The cerebral ischemia reperfusion injury was induced by following modified method of Jingtaoet al.[27]. The test animals were anaesthetized with thiopentone sodium at a dose 40 mg/kg intraperitoneally. The carotid arteries were exposed over by giving midline incision and dissection was made between the sternocleidomastoid and the sternobyoid muscle parallel to the trachea. Each carotid artery was made free from its adventitial sheath and vagus nerve, which was carefully separated and maintained. A silk thread was passed from each carotid artery. The induction of ischemia was done by occluding BCCA occlusion for 1 h. After 1 h occlusion the knots of both the carotid arteries were released and blood flow was allowed i.e. reperfusion, for 5 h[27].

The measurement of percentage infarction after cerebral ischemia reperfusion injury

After 1 h occlusion and 5 h reperfusion, animals were sacrificed by cervical dislocation method and brain was isolated immediately. The removed brain was washed carefully with ice cold saline solution. The brain was wrapped in aluminum foil and kept at -4^{0}C for 5 minutes. The frozen brain was sliced into uniform sections of 0.1 cm thickness. The slices were incubated in 1 % TTC solution dissolved in phosphate buffered saline having pH 7.4 at 37^{0}C for 30 min. TTC is converted to red formazone pigment by nicotinamide adenine dinucleotide (NAD) and dehydrogenase present in living cells. Hence viable cells were stained deep red. As infarcted cells deficient to these enzymes, thus remained unstained[28]. Pale necrotic infarcted tissue was separated, weighed and percentage infarction was calculated.

Evaluation of anti-inflammatory activity of DMF by estimating inflammatory biomarkers in ischemia and reperfusion induced cerebral injury in Wistar rats

In the present study, same experimental protocol mentioned above (2.4 and 2.4.1) was used for evaluation of pro-inflammatory biomarkers like TNF-α, Interleukin 6 and C- reactive protein as well as anti-inflammatory biomarker like Interleukin 10. At the end of study blood sample was collected from retro-orbital plexus of rat, after 1 h occlusion and 5 h reperfusion to cerebral cells. Serum was separated from the blood by centrifugation for 10 min at 4000 rpm. All mention parameters were estimated according to the procedure given in the respective diagnostic kit.

Evaluation of antioxidant activity of DMF in ischemia and reperfusion induced cerebral injury in Wistar rats

The same experimental protocol mentioned above (2.4 and 2.4.1) was used for evaluation of antioxidant role of DMF by estimating the antioxidant enzymes like superoxide dismutase (SOD), catalase (CAT) and end product of lipid peroxidation i.e. malondialdehyde (MDA) levels in the rat brain tissue. These oxidative parameters were estimated according to the procedure mentioned in their respective diagnostic kits.

Preparation of brain tissue Sample for assessment of antioxidant parameters

All samples were prepared before the reconstitution of reagents given in the estimation kit.The tissue was perfused with a PBS (phosphate buffered saline) solution, pH 7.4, containing 0.16 mg/ml heparin, before dissection to remove any red blood cells.The tissue was homogenized in 5 – 10 ml cold buffer (i.e. 100 mM potassium phosphate, pH 7.0, containing 2 mM EDTA) per gram tissue.Then kept for centrifugation at 4,000 rpm for 15 minutes at 4 ^{0}C.The supernatant was collected.0.5 ml of ice-cold extraction reagent was added to 1.0 ml of supernatant in glass test tube.Mixed for at least 30 seconds.Kept for centrifugation at 4000 rpm and 4 ^{0}C for 10 minutes.The aqueous upper layer was collected and kept at 0-4 ^{0}C for immediate assay.

Statistical Analysis

The results were expressed as mean ± standard error mean (mean ± SEM). Differences in infarct size, TNF-α, IL6, IL10, CRP, SOD, Catalase, and MDA were determined by One Way ANOVA. Individual groups were compared by Dunnett's test of significance. Differences with $P<0.05$ were considered statistically significant. The statistical analysis was performed by using Graphpad Prism Software (Version 5.02).

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Results

Evaluation of cerebroprotective potential of Dimethyl Fumarate (DMF) against ischemia and reperfusion induced cerebral injury in Wistar rats

In the present study evaluation of the protective effect of Dimethyl Fumarate (DMF) was carried out against ischemia reperfusion injury induced by bilateral common carotid artery occlusion (1 hour) and reperfusion (5 hours) in Wistar rats at doses 10 mg/kg and 20 mg/kg (Orally) by measuring the extent of cerebral cell infarction. The extent of infarction was assessed by using 2, 3, 5- triphenyl-tetrazolium chloride (TTC) as a staining agent. The results are given below,

Formula for calculations of % protection of cerebral cells infarction after ischemia reperfusion injury in rats is as follows,

% Protection in cerebral infarction =(Control – Sample) / Control x100

Table No. 1: Effect of daily treatment for two months of DMF (at doses of 10 mg/kg as well as 20 mg/kg) on ischemia and reperfusion induced cerebral injury in Wistar rats.

Groups (n=6)	% Infarction (Mean±SEM)	% Protection
Normal	**0**	**-**
Sham Control	**10.07±1.60**	**-**
I/R Control	**62.09±2.14**	**-**
Treated with 10mg/kg DMF	**37.32±1.81****	**39.89±2.91**
Treated with 20mg/kg DMF	**16.79±1.34****	**72.96±2.16**

** P<0.05 (Significant) compared treated groups with I/R control group using one way ANOVA followed by Dunnett's test at 95% confidence interval.

Figure No. 1: Effect of daily treatment for two months of DMF (at doses of 10 mg/kg and 20 mg/kg) on ischemia and reperfusion induced cerebral injury in Wistar rats.

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The percentage cerebral cell infarction was calculated in all the groups mentioned above as per the protocol which is discussed earlier. In the present study the Group-III (I/R Control) has shown significant cerebral infarction when compared to all other groups. The percentage infarction observed in Group-I (Normal) and Group-II (Sham Control) was almost equal and negligible. Similarly in both the treated groupsi.e. in Group-IV and Group-V percentage cerebral infarction was significantlydecreased as compared to I/R control Group-III. The results are shown in Table No. 1.

Evaluation of anti-inflammatory activity of DMF by estimating inflammatory biomarkers in ischemia and reperfusion induced cerebral injury in Wistar rats

The levels of TNF-α, IL-6, IL-10 and CRP were estimated in serum samples of various groups. Group III was served as I/R control group has shown significant increase in the levels of TNF-α, IL-6 and CRP (pro-inflammatory biomarkers) when compared to Normal and Sham Control Groups. Whereas in test groups i.e. Group IV and Group V pretreated for two months with DMF at doses 10 mg/kg and 20 mg/kg respectively has shown significant reduction in TNF-α, IL-6 and CRP level as compared to Group III (I/R control group). Whereas anti-inflammatory biomarkers IL-10 levels are decreased in Group IIIwhen compared to Group I and Group II. At the same time in group IV and Group V IL-10 levels were increased significantly. The results are given in Table No. 2.

Table No. 2: The measurement of TNF-α, IL-6, IL-10 and CRP in ischemia and reperfusion induced cerebral injury in Wistar rats, after two months treatment of DMF at dose 10 mg/kg and 20 mg/kg.

Parameter (Mean±SEM)	Group I Normal	Group II Sham Control	Group III I/R control	Group IV Treated with DMF 10mg/kg	Group V Treated with DMF 20mg/kg
TNF-α (Pg/ml)	12.65±0.63	16.43±0.36	48.69±4.03	19.63±0.90**	14.52±1.41**
IL-6 (Pg/ml)	248.6±14.63	289.7±11.30	490.2±12.49	277.1±17.04**	234.8±8.00**
IL-10 (Pg/ml)	83.56±3.57	79.74±2.35	35.45±1.65	56.62±2.28**	83.99±2.72**
CRP (mg/ml)	0.41±0.03	0.58±0.03	3.35±0.27	0.83±0.12**	0.45±0.04**

** P<0.05 (Significant) compared treated groups with I/R control group using one way ANOVA followed by Dunnett's test at 95% confidence interval.

Evaluation of antioxidant activity of DMF in ischemia and reperfusion induced cerebral injury in Wistar rats

The antioxidant activity of DMF was evaluated by estimation of SOD, CAT and MDA in brain tissue. In the present study SOD and CAT levels were decreased significantly while MDA levels increased significantly in I/R control group i.e. Group III when compared to sham control group i.e. Group II. The levels of SOD and CAT were increased and levels of MDA were decreased significantly in pretreated groups i.e. Group IV and Group V with DMF for two months at doses 10m/kg and 20mg/kg respectively. The antioxidant activity of DMF in this study was observed. Results are given in Table No. 3.

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Table No. 3: The levels of SOD, CAT and MDA estimation in ischemia and reperfusion induced cerebral injury in Wistar rats, after two months treatment of DMF at doses of 10 mg/kg and 20 mg/kg.

Groups (n=6)	SOD U/g tissue	Catalase U/g tissue	MDA nM/g tissue
Group I	**27.85±1.35**	**29.40±0.98**	**1.06±0.08**
Group II	**21.24±1.76**	**20.72±1.01**	**1.24±0.14**
Group III	**7.87±0.42**	**9.88±0.41**	**7.89±0.61**
Group IV	**15.42±1.12****	**17.15±1.01****	**4.74±0.34****
Group V	**20.44±1.03****	**24.72±1.30****	**3.06±0.16****

** P<0.05 (Significant) compared treated groups with I/R control group using one way ANOVA followed by Dunnett's test at 95% confidence interval.

Discussion and Conclusion

The cerebral ischemia reperfusion induced infarction has been developed in many animal models. In the present study, partial global cerebral ischemia was achieved by BCCA occlusion for 1 h followed by 5 h reperfusion. Induction of partial ischemia without affecting the collateral circulation reflects the event occurs during transient ischemic attacks and clinical cerebral infarction[27]. In this study, the extent of ischemia reperfusion induced cerebral injury was measured in terms of percentage cerebral infarction using TTC as a staining agent to differentiate infarcted tissue from non-necrotic normal tissue[29]. Results of the present study shown that there is significant variation in all the groups i.e. Group I and Group II has shown uniform and dark stained cerebral tissue indicating non-necrotic and live tissues. However, Group- I was completely normal when compared to Group II which is sham control. Whereas I/R control Group III has shown significant increase in destruction and partial staining indicating necrosis in cerebral tissue, due to ischemia and reperfusion injury. Thus, here it was confirmed that, BCCA occlusion (1 h) and reperfusion (5 h) model established successfully. From the results it has been demonstrated that two months pretreatment of DMF has imparted protection against ischemia reperfusion induced cerebral infarction. The findings of this study shown correlation with the earlier work carried out on natural herbal plant extract or compounds have been demonstrated their protective effect against ischemia reperfusion injury induced cerebral infarction[30, 31]. However the results of the present study are in accordance with the previous research work done by Gaur et al. and Raghavendra et al.; in the similar experimental models[32, 33]. The cerebroprotective effect of DMF in the present study was supporting the previous studies on neuroprotective effect of DMF in the treatment of multiple sclerosis, It has been reported that DMF controls inflammation and oxidative stress are central pathologic factors in multiple sclerosis [22, 23]. As DMF has reduced the percentage infarction in ischemia reperfusion induced cerebral infarction, the present study propose that Dimethyl Fumarate having significant cerebroprotective effect.

It has been proved that the TNF-α, IL-6 and CRP levels are increasing in ischemia and reperfusion induced cerebral injury. The experimental model in the present study has shown significant elevation in the concentration of TNF-α, IL-6 and CRP in I/R control group (Group III) when compared to sham control group (Group II). This was demonstrating the role of TNF-α, IL-6 and CRP induced inflammation in cerebral ischemia reperfusion injury. The results of this study are in accordance with previous findings of Clausen et al., that the expression of TNF-α and IL-1β alters after ischemic stroke in mice[34] and Lentsch et al. 1999 i.e. increased proinflammatory cytokine (TNF-α, IL-1β and IL-6) have been observed in ischemic cortex, 1 h after middle cerebral artery (MCA) occlusion in experimental models of brain ischemia[35]. At the same time Grau AJ and, Rost NS et al. suggested that the elevated plasma levels of CRP are not disease specific but are sensitive markers produced in response to the tissue injury, infectious agents, immunologic stimuli, and inflammation[36, 37]. It has been proved that during cerebral ischemia reperfusion injury the levels of pro-inflammatory biomarkers are increasing while anti-inflammatory biomarkers like

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IL-10 are decreasing. The experimental model in present study has shown significant decrease in the concentration of interleukin-10 (IL-10) in I/R control group (Group III) when compared to sham control group (Group II). The results have been indicating the correlation of anti-inflammatory IL-10 and inflammation in cerebral ischemia reperfusion injury. In Group I and Group II the concentration of IL-10 was more, in these groups there were no inflammation and ischemia reperfusion induced injury. Thus the results of this study are in accordance with previous findings of Ahmed M. A. et al. has been reported that Pomegranate extract protects against cerebral ischemia and reperfusion induced injury by increasing the levels of Interleukin-10 and cerebral ATP production in brain[38]. Spera et al. proved that both central and systemic administration of IL-10 to rats reduced infarct size in ischemia-reperfusion model[39]. Though IL-10 is having anti-inflammatory and neuroprotective potential, the mechanism of action is still unclear. The current hypothesis of the pharmacodynamic effect of fumaric acid esters (FAEs) is based on the concept that DMF and monomethyl fumarate (MMF) influences pro-inflammatory signal transduction pathways through modulation of the intracellular redox system[40, 41] and suppression of not only the production of T helper type-1and pro-inflammatory mediators such as TNF-α and IFN-γ but enhancement of the formation of cytokines with anti-inflammatory properties such as IL-10 and IL-1RA[42, 43]. Wilms H. et al. stated that pretreatment with DMF decreased synthesis of the proinflammatory mediators like TNF-α, IL-1β and IL-6 at the RNA level in activated microglia and astrocyte *in vitro*[44].

The results of the present study supports the hypothesis mentioned above regarding mechanism of action of Dimethyl Fumarate. It has been demonstrated that pretreated groups i.e. Group IV and Group V with DMF at doses of 10 mg/kg and 20 mg/kg respectively has significantly reduced the levels of TNF-α, IL-6, and CRP while anti-inflammatory IL-10 levels were significantly increased when compared to Group III (I/R control group). The results from Group V pretreated with higher dose of DMF were similar to the results of Group I which served as normal. Thus anti-inflammatory action of DMF has been proved through inhibition of TNF-α, IL-6, CRP and enhancement of anti-inflammatory IL-10 levels.

In a normal/healthy state, persistently produced reactive oxygen species (ROS) are detoxified by endogenous antioxidant systems. Among these SOD and CAT provides primary defense against ROS. SOD and CAT together involved in the defense mechanism against ROS induced damage to the tissue. The excessive consumption of antioxidants occurs due to increased production of ROS, inactivation of detoxification systems and failure to adequately replenish antioxidants in the ischemic brain tissue. These events are leading to the depletion of cellular antioxidant enzymes during ischemia-reperfusion. Therefore, the measurement of the endogenous antioxidant enzymes i.e. SOD and CAT has been used indirectly to estimate the amount of oxidative stress [45]. Many experimental studies have demonstrated decreased SOD and CAT levels in cerebral ischemia-reperfusion injury as indirect evidence of oxidative stress. It has been demonstrated that lower serum SOD levels correlate negatively with infract volume in stroke patients [46]. Exogenous administration of different forms of SOD and catalase has been proved to reduce brain injury by ischemia-reperfusion in experimental animal models[47-49]. Further evidence for the important role of SOD in the defense of ROS damage in reperfusion injury came from transgenic animal experiments. Mice over expressing SOD were highly resistant to reperfusion injury[50]. PrabhakarOrsu et al. stated that the Resveratrol produced significant dose-dependent reduction in percent cerebral infarct volume. The Resveratrol has shown a significant reduction in oxidative stress marker like malondialdehyde, and in contrast there was a significant increase in anti-oxidants markers like superoxide dismutase and catalase levels. Resveratrol showed significant cerebroprotective action mediated by anti-oxidant mechanism[51].

Malondialdehyde, a stable product of lipid peroxidation, is used as indirect marker in the evaluation of oxidative damage. Higher levels of MDA indicate higher amounts of free radicals. Sakamoto et al. demonstrated that, there is a positive correlation between the free radicals production and lipid peroxidation during ischemia reperfusion injury in the rat brain[52]. There is a clinical evidence of the higher levels of MDA in stroke patient[53]. MDA levels were found to be increased significantly in the brain in parallel to significant increase in infarct size in I/R control group i.e. Group III when compared to sham control group i.e. Group II. This was indicating the induction of oxidative stress and subsequent lipid peroxidation successfully in the present rat model of ischemia and reperfusion induced

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cerebral injury in Wistar rats. This has been supported by Gaur et al., 2009, that the levels of MDA were elevated in similar experimental model[32]. In the present study SOD and CAT levels were decreased significantly while MDA levels were increased significantly in I/R control group i.e. Group III when compared to sham control group i.e. Group II. The levels of SOD and CAT were increased and levels of MDA were decreased significantly in pretreated groups i.e. Group IV and Group V with Dimethyl Fumarate for two months at doses 10 mg/kg and 20 mg/kg respectively. The antioxidant activity of DMFwas proved in this study.DMF is an antioxidant agent has already shown therapeutic potential by activating nuclear factor (erythroid derived 2)-like 2 (Nrf2) and thus induced a cascade of cytoprotective effect and antioxidant pathways in previous studies[23, 25, 54-57]. In the present study antioxidant property of DMF has been proved by further mechanism by estimating SOD, Catalase and MDA levels in rats against ischemia reperfusion induced cerebral infarction.The observations of the present study concluded that the DMF has significant cerebroprotective potential with probable mechanisms may include its anti-inflammatory and anti-oxidant activities.Histopathological evaluations is required after ischemia reperfusion induced cerebral injury for further confirmation of cerebroprotective potential of DMF.

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REFERENCES

1. *Peter Soros and Vladimir Hachinski, (2012).Cardiovascular and neurological causes of sudden death after ischemic stroke. The Lancet Neurology Volume 11, Issue 2, 179 – 188.*
2. *Turner and White, (2009).Ischemic stroke: Pathophysiology and principle of localization, Neurology board review manual, volume 13, part 1, 1-14.*
3. *Berliner JA, Navab M, Fogelman AM, Frank JS, Demer LL, Edwards PA, Watson AD, Lusis AJ (1995). Atherosclerosis: basic mechanisms. Oxidation, inflammation, and genetics. Circulation 91: 2488–2496.*
4. *Edward C Jauch, Helmi L Lutsep, (2015).Ischemic strokeemedicine.medscape.com/article/1916852.*
5. *Sundt TM Jr, Sharbrough FW, Piepgras DG, Kearns TP, Messick JM Jr, O'Fallon WM. (1981). Correlation of cerebral blood flow and electroencephalographic changes during carotid endarterectomy: with results of surgery and hemodynamics of cerebral ischemia. Mayo Clin Proc. 56(9):533-43.*
6. *Karapanayiotides T, Meuli R, Devuyst G, Piechowski-Jozwiak B, Dewarrat A, Ruchat P, (2005).Postcarotidendarterectomyhyperperfusion or reperfusion syndrome. Stroke 36(1):21-6.*
7. *Piepgras DG, Morgan MK, Sundt TM Jr, Yanagihara T, Mussman LM. (1988).Intracerebral hemorrhage after carotid endarterectomy. J Neurosurg. 68(4):532-6.*
8. *Abou-Chebl A, Yadav JS, Reginelli JP, Bajzer C, Bhatt D, Krieger DW(2004).Intracranial hemorrhage and hyperperfusion syndrome following carotid artery stenting: risk factors, prevention, and treatment. J Am CollCardiol.43(9):1596-601.*
9. *Wagner WH, Cossman DV, Farber A, Levin PM, Cohen JL(2005).Hyperperfusion syndrome after carotid endarterectomy. Ann Vasc Surg. 19(4):479-86.*
10. *Barone FC, Feuerstein GZ(1999).Inflammatory mediators and stroke: new opportunities for novel therapeutics. J Cereb Blood Flow Metab. 19; 819-834.*
11. *Del Zoppo GJ, SchemidSchonbein GW, Mori E (1991).Polymorphonuclear leukocytes occlude capillaries following middle cerebral artery occlusion and reperfusion in baboons. Stroke. 22:1276-1283.*
12. *Breckwoldt MO, Chen JW, Stangenberg L (2008).Tracking the inflammatory response in stroke in vivo by sensing the enzyme myeloperoxidase. Proceedings of the National Academy of Sciences of the United States of America. 105(47); 584-589.*
13. *Chan PH. (2001).Reactive oxygen radicals in signalling and damage in the ischemic brain. J Cereb Blood Flow Metab, 2; 2-14.*
14. *Fiskum G, Rosenthl RE, Vereczki V (2004).Protection against ischemic brain injury by inhibition of mitochondrial oxidative stress. J of Bione and Biomemb, 36(4): 347-352.*

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15. *Kirsch JR, Phelam AM, Lange DG and TraystmanRJ(1987).Reperfusion induced free radical formation following global ischemia. Ped Res. 21:202A.*
16. *Barone FC, Arvin B, White RF (1997).Tumor necrosis factor-α: a mediator of focal ischemic brain injury. Stroke. 2: 1233-1244.*
17. *Whiteley W, Jackson C, Lewis S, Lowe G, Rumley A, Sandercock P, Wardlaw J, Dennis M, Sudlow C. (2009).Inflammatory markers and poor outcome after stroke: a prospective cohort study and systematic review of interleukin-6. PLoS Med. 6(9):e1000145.*
18. *Fabin RH, DeWitt DS, and Kent TA. (1995).In vivo detection of superoxide anion production by the brain using a cytochrome c electrode. J Cereb Blood FlwoMetab. 15; 242-247.*
19. *Lancelot E, Callebert J, Revaud ML, (1995).Detection of hydroxyl radicals in rat striatum dring transient focal cerebral ischemia: possible implication in tissue damage. NeurosciLett. 197: 85-88.*
20. *Lerouet D, Beray-Berthat V, Palmier B. (2002).Changes in oxidative stress, iNOS activity and neutrophil infiltration in severe transient focal cerebral ischemia in rats. Brain Res 958: 166-175.*
21. *Granger DN, Hiwarth ME, Parks DA. (1986).Ischemia-reperfusion injury: role of oxygen-derived free radicals. Actaphysiol Scand. 548: 47-63.*
22. *Linker RA, Lee DH, Ryan S, (2011).Fumaric acid esters exert neuroprotective effects in neuroinflammation via activation of the Nrf2 antioxidant pathway. Brain 134:678-92.*
23. *Gilgun-Sherki Y, Melamed E, Offen D. (2004).The role of oxidative stress in the pathogenesis of multiple sclerosis: the need for effective antioxidant therapy. J Neurol 251:261-8.*
24. *Kappos L, Gold R, Miller DH, Macmanus DG, Havrdova E, Limmroth V, et al. (2008). Efficacy and safety of oral fumarate in patients with relapsing-remitting multiple sclerosis: a multicentre, randomised, double-blind, placebo-controlled phase IIb study. Lancet 372:1463-1472.*
25. *Scannevin RH, Chollate S, and Jung MY, (2012).Fumarates promote cytoprotection of central nervous system cells against oxidative stress via the Nrf2 pathway. J PharmacolExpTher 341:274-84.*
26. *Ketan V H, Annapurna A, (2016). Evaluation of safety profile and cerebroprotective potential of dimethyl fumarate (dmf) against ischemia and reperfusion induced cerebral injury in wistar rats. Int J Pharm PharmSci, Vol 9, Issue 2, 241-254.*
27. *Jingtao J, Sato S, Yamanaka N, (1999).Changes in cerebral blood flow and blood brain barrier in the gerbil hippo-campal CA1 region following repeated brief cerebral ischemia. Med Electron Microsc, 32, 175–183.*
28. *ChintamaniNarasinh Joshi, Swatantra Kumar Jain, Puvvada Sri Ramachandra Murthy, (2003). An optimized triphenyl tetrazolium chloride method for identification of cerebral infarcts. doi.org 10.1016 j.brainresprot; 12:001.*
29. *Angela B, Krisztina M, ZsoltJ et al., (2006). Use of TTC staining for the evaluation of tissue injury in the early phases of reperfusion after focal cerebral ischemia in rats. Brain Research. 1116(1): 159-165.*
30. *Jiang J., Wang W., Sun YJ et al. (2007).Neuroprotective effect of curcumin on focal cerebral ischemic rats by prevening blood brain barrier damage. Eur. J Pharmacol. 561(1-3):54-62.*
31. *Annapurna A., Prabhakar O., and Jayarami Reddy M., (2012).Studies on the cerebroprotective potential of reseveratrol against reperfusion induced cerebral infarction in rats. ICMBP Singapore. 28-29.*
32. *Gaur V, Aggarwal A, and Kumar A., (2009).Protective effect of naringin against ischemic reperfusion cerebral injury: possible neurobehavioral, biochemical and cellular alterations in rat brain. Eur J Pharmacol. 616(1-3): 147-154.*
33. *Raghavendra M., Maiti R., Kumar S. (2009). Role of centellaasiatica on cerebral post ischemic reperfusion and long term hypoperfusion in rats. Int. J. Green Pharm. 3: 88-96.*
34. *Clausen BH. Lambersten KL. Babcock AA. (2008). Interleukin -1beta and tumor necrosis factor alpha are expressed by different subsets of microglia and macrophages after ischemia stroke in mice. J Neuroinflammation 5:46.*
35. *Lentsch AB., YoshidomeH., Warner RL. (1999). Secretory leukocyte protease inhibitor in mice regulates local and remote organ inflammatory injury induced by hepatic ischemia/reperfusion. Gastroenterology 117:953-61.*

Volume 4 (Issue 5): May 2017 ISSN: 2394-9414
DOI- 10.5281/zenodo.582772 Impact Factor- 3.109

36. *Grau AJ. (1997). Infection, inflammation, and cerebrovascular ischemia. Neurology 49 (suppl 4): S47–S51.*
37. *Rost NS, Wolf PA, Kase CS, Kelly-Hayes M, Silbershatz H, Massaro JM, D'Agostino RB, Franzblau C, Wilson PWF (2001). Plasma concentration of C-reactive protein and risk of ischemic stroke and transient ischemic attack: the Framingham Study. Stroke 32: 2575–2579.*
38. *Ahmed MA, Morsy EM, Ahmed AA. (2014).Pomegrannate extract protects against cerebral ischemia/reperfusion injury and preserves brain DNA integrity in rats. Life Sci. 110(2): 61-69.*
39. *Spera PA, Ellison, JA, Feuerstein GZ, Barone FC, (1998). IL-10 reduces rat brain injury following focal stroke. NeurosciLett 251, 189–192.*
40. *Altmeyer PJ, Matthes U, Pawlak F, Hoffmann K, Frosch PJ, Ruppert P, (1994).Antipsoriatic effect of fumaric acid derivates: Results of a multicenter double-blind study in 100 patients. J Am AcadDermatol 30 (6):977-81.*
41. *Mrowietz U, Christophers E, Altmeyer P. (1997). Treatment of psoriasis with fumaric acid esters: Results of a prospective multicentre study. German Multicentre Study. Br J Dermatol 138:456-60.*
42. *Asadullah K, Schmid H, Friedrich M, Randow F, Volk HD, Sterry W, (1997). Influence of monomethyl fumarate on monocytic cytokine formation--explanation for adverse and therapeutic. Arch Dermatol Res 289(11):623-30.*
43. *Litjens NH, Nibbering PH, Barrois AJ, Zomerdijk TP, Van Den Oudenrijn AC, Noz KC, (2003). Beneficial effects of fumarate therapy in psoriasis vulgaris patients coincide with downregulation of type 1 cytokines. Br J Dermatol 148:444-51.*
44. *Wilms H, Sievers J, Rickert U, Rostami-Yazdi M, Mrowietz U, Lucius R. (2010).Dimethylfumarate inhibits microglial and astrocytic inflammation by suppressing the synthesis of nitric oxide, IL-1β, TNF-α and IL-6 in an in-vitro model of brain inflammation. Journal of neuroinflammation 7(1):1.*
45. *LeinonenJS.,Ahonen P., Loennrot K., (2000). Plasma antioxidant capacity is associated with high lesion volume and neurological impairment in stroke. Stroke 31(9):33-39.*
46. *Spranger M., Krempien S, Schwab S., (1997). Superoxide dismutase activity in serum of patients with acute cerebral ischemic injury; correlation with clinical course and infarct size. Stroke 28(12): 2425-2428.*
47. *Liu TH., Beckman JS., Freeman BA., (1989). Polyethylene glycol- conjugated superoxide dismutase and catalase reduce ischemic brain injury. Am J Physiol 256:589-593.*
48. *He YY., Hsu CY., Ezrin AM., and Miller MS., (1993). Polyethylene glycol-conjugated superoxide dismutase in focal cerebral ischemia –reperfusion . Am J Pysiol 265: 252-256.*
49. *Francis JW.,Ren JM., Warren L. (1997). Post ischemic infusion of Cu/Zn superoxide dismutase or SOD: Tet451 reduces cerebral infarction following focal ischemia/reperfusion in rats. ExpNeurol 146: 435-443.*
50. *Saito A., Hayashi T., Okuno S., (2003). Overexpression of copper/zinc superoxide dismutase in transgenic mice protects against neuronal cell death after transient focal ischemia by blocking activation of the bad cell death signaling pathway. J Neurosci 23: 1710-1718.*
51. *PrabhakarOrsu, B V S N Mruthy, A. Annapurna, (2013). Cerebroprotective potential of resveratrol through antioxidant and anti-inflammatory mechanisms in rats. Journal of Neural TransmissionVolume 120, Issue 8,pp 1217-1223.*
52. *Sakamoto A., Ohnishi ST., Ohnishi T., and Ogawa R., (1991). Relationship between free radical production and lipid peroxidation during ischemia reperfusion injury in the rat brain. Brain Res 554(1-2): 186-192.*
53. *Cherubini A., Polidori MC, Bregnocchi m., (2000). Antioxidant profile and early outcome in stroke patients. Stroke 31(10): 2295-2300.*
54. *Duffy S., So A., Murphy T.H. (1998). Activation of endogenous antioxidant defenses in neuronal cells prevents free radical-mediated damage. Journal of Neurochemistry71(1):69–77.*
55. *Lin S.X. (2011). The anti-inflammatory effects of dimethyl fumarate in astrocytes involve glutathione and haem oxygenase-1. ASN NEURO3(2) 21382015.*
56. *Haider L. (2011) Oxidative damage in multiple sclerosis lesions. Brain134(7):1914–1924.*
57. *Van Horssen J. (2008). Severe oxidative damage in multiple sclerosis lesions coincides with enhanced antioxidant enzyme expression. Free Radical Biology and Medicine 45(12):1729–1737.*