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Original Article

EVALUATION OF SAFETY PROFILE AND CEREBROPROTECTIVE POTENTIAL OF DIMETHYL FUMARATE (DMF) AGAINST ISCHEMIA AND REPERFUSION INDUCED CEREBRAL INJURY IN WISTAR RATS

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ABSTRACT

Objective: Dimethyl fumarate (DMF) is the methyl ester of fumaric acid and used in the treatment of psoriasis, multiple sclerosis, etc. The present study was carried out to reveal safety as well as to find out the cerebro-protective potential of DMF against ischemia and reperfusion-induced cerebral injury in Wistar rats.

Methods: Acute and sub-acute toxicity tests were performed in mice at various increasing doses of DMF as per OECD guidelines 420 and 407 respectively. The cerebral ischemia-reperfusion injury was induced by bilateral common carotid artery (BCCA) occlusion for 1 h and reperfusion for 5 h in Wistar rats. The DMF was administered orally at doses 10 mg/kg and 20 mg/kg for the assessment of its cerebro-protective potential by evaluating % infarction and % protection of cerebral tissue using TTC staining method.

Results: The present study revealed that DMF is a safe drug as mortality was not observed in acute toxicity study up to 2000 mg/kg on oral administration, at the same time in sub-acute toxicity test DMF has showed no alteration in various parameters except a decrease in lymphocyte count. The DMF has shown a reduction in the % infarction. DMF at doses 10 mg/kg and 20 mg/kg has imparted 27.96±1.55% and 58.98±2.3% protection against ischemia and reperfusion-induced cerebral injury in Wistar rats.

Conclusion: The DMF is the relatively safe drug with significant cerebro-protective potential.

Keywords: Toxicity test, Cerebroprotective, DMF, Lymphocytopenia

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INTRODUCTION

The stroke is one of the leading causes of death worldwide;it has been stated that most of the stroke patients are dying and some become permanently disabled due to loss of vision and speech, paralysis, etc. [1].Thus treatment of stroke is very important part of the life of the stroke patients. There are mainly two types of the stroke, ischemic stroke and hemorrhagic stroke. The ischemic stroke is the most common type of stroke. It is characterized by the sudden loss of blood circulation to an area of the brain, leading to corresponding loss of neurologic function. Acute ischemic stroke is caused by thrombotic or embolic occlusion of a cerebral artery and is more common than hemorrhagic stroke [2]. The interruption of the blood supply to the brain leads to a stroke usually because of a blood vessel bursts or is blocked by a clot, this resulting to cerebral ischemia. The probable mechanism behind the cerebral ischemia may be micro-embolism to the brain vessels, stenosis of cerebral artery and decrease in systemic blood pressure, thromboembolism of large blood vessels, decreased cardiac output, etc. The cerebral ischemia leads to brain cell necrosis i.e. damage to brain cells due to lack of oxygen and nutrients [3]. The ischemic stroke can be treated by recanalization of the blood vessels with thrombolytic agents, anticoagulants, antiplatelet agents, etc. or with surgery but it leads to the complication called as ischemia-reperfusion injury. It is rare but significant pathological condition requires proper care and treatment, it occurs due to cerebral hyperperfusion or reperfusion [4]. It has been observed that the traditional drugs used in the treatment of stroke are having some limitations, adverse effects and contraindications too, hence it reinforces this area for further research. There should be novel drugs for the treatment of stroke having a protective effect on the cerebral cells so that some complications can be minimised in the clinical care of stroke patient. DMF is useful for the treatment of multiple sclerosis [5, 6] and also in Germany for the therapy of severe psoriasis. Furthermore, the clinical efficacy of DMF to reduce inflammation in multiple sclerosis has been demonstrated [7]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 through activation of the nuclear 1 factor (erythroid-derived 2)–like 2 (Nrf2) antioxidant response pathway[5, 8].In the present study, we used DMF as a test drug against ischemia and reperfusion-induced cerebral injury by bilateral common carotid artery occlusion for 1 h and 5 h reperfusion in Wistar rats for the assessment of its cerebro-protective effect on cerebral infarction.

MATERIALS AND METHODS

Animals

In the present study healthy adult male, Swiss albino mice (25-30 g) and Albino Wistar strain rats (250 g to 300 g) were procured from Albino research centre, Hyderabad. Animals were housed in clean and transparent polypropylene cages with three animals in each cage and maintained at 25-27°C with 12:12 h light-dark cycle for a period of 7 d 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. Maintenance and handling of animals were done as per CPCSEA guidelines; the prior permission for the study was obtained from institutional animal ethics committee (IAEC) (Regd. No. 516/01/A/CPCSEA).

Drugs and chemicals

Dimethyl fumarate (Sigma Aldrich, USA.), sodium cmc (Desai Chemicals, Vishakhapatnam.), 2, 3, 5-triphenyl-tetrazolium chloride (TTC): (Sigma Aldrich, USA.), glucose kit (Excel Diagnostics, India.), cholesterol kit (AGAPPE Diagnostics Ltd., India.), hdl cholesterol kit (AGAPPE Diagnostics Ltd., India.), triglyceride kit (Excel Diagnostics, India.), creatinine kit (Excel Diagnostics, India.), uric acid kit (Excel Diagnostics, India.), sgpt kit (Excel Diagnostics, India.), sgot kit

(AGAPPE Diagnostics Ltd., India.), urea kit (Excel Diagnostics, India.), total protein kit (AGAPPE Diagnostics Ltd., India.), bilirubin kit (AGAPPE Diagnostics Ltd., India.).

Instruments used

Semiautoanalyzer (MISPA UNO), centrifuge (REMI), incubator (REMI), acto-photometer (Laboratory Enterprises), rota-rod (Laboratory Enterprises), fully automated hemolyzed (SYSMEX).

Acute oral toxicity test (sighting study with various doses) of DMF as per OECD guidelines 420, 2001

The mice were stabilised in the animal house for two weeks and maintained at standard laboratory conditions with free access to food and water, 12 h light 12 h dark cycle. The animals were selected randomly and divided into five groups each group consists of ten mice. All animals were weighed and the drug was administered orally according to their body weight. The DMF was given at the various doses i.e. 5 mg/kg, 50 mg/kg, 300 mg/kg, 2000 mg/kg and 5000 mg/kg to Group I, Group II, Group III, Group IV and Group V respectively. After drug administration, all groups were kept under observation for 6 h and further for 15 d to observe mortality in any group.

Sub-acute repeated dose oral toxicity test for 28 d as per OECD guidelines 407, 2008

The mice were stabilized in the animal house for two weeks and maintained at standard laboratory conditions with free access to food and water, 12 h light and 12 h dark cycle. The mice were selected randomly and placed in one group i.e. Group VI, consists of ten mice.

All animals were weighed and a single dose of DMF i.e. 1000 mg/kg was given orally for 28 d. After 28 d treatment with DMF, the various parameters were estimated in all the Groups i.e. mortality, body weight, locomotor activity by acto-photometer, muscle strength by rotarod, other parameters like blood glucose level, total cholesterol, HDL, LDL, VLDL, triglycerides, creatinine, urea, uric acid, SGOT, SGPT, total protein and bilirubin by semi-autoanalyzer and hematological parameters by fully automated hematolyzer. In the present study, all the parameters were estimated before and after the drug administration i.e. on 1st day and on 28th day.

Evaluation of cerebro-protective potential of DMF against ischemia and reperfusion-induced cerebral injury in Wistar rats

The present study was carried out to evaluate, cerebro-protective potential of DMF against 1 h bilateral common carotid artery occlusion-induced ischemia followed by 5 h reperfusion-induced cerebral injury in Wistar rats. The effects of DMF at the dose of 10 mg/kg and 20 mg/kg on oral administration were observed in acute study i.e. single dose pre-administration against ischemia-reperfusion induced cerebral injury. The extent of infarction was assessed by measuring the percentage cerebral infarction by TTC staining.

Induction of ischemia-reperfusion injury in rat brain

The cerebral ischemia-reperfusion injury was induced by following modified method of Jingtao [9]. The test animals were anaesthetized with intra-peritoneal administration of thiopentone sodium at a dose of 40 mg/kg. 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 cotton thread was passed from each carotid artery. The induction of ischemia was done by occluding both the carotid arteries (Bilateral Common Carotid Artery Occlusion i.e. BCCAO) 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. During the BCCAO experimental animals were observed for the maintenance of dilated pupils, the absence of corneal reflex when exposed to strong light and maintenance of rectal temperature at 37 °C. The animal which did not match such criteria and showing convulsions was discarded from the study [9].

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 a normal group without surgery, i.e. bilateral common carotid artery (BCCA) occlusion and drug treatment. Group II served as sham control received only surgery without common carotid artery occlusion and drug treatment. Group III served as disease control received bilateral common carotid artery (BCCA) occlusion for 1 h and 5 h reperfusion without drug treatment. Group IV and Group V were served as test groups received drug treatment with DMF (single dose) at doses of 10 mg/kg and 20 mg/kg respectively (30 min before reperfusion) and BCCA occlusion for 1 h and 5 h reperfusion. The percentage of infarction was measured in all the groups as described below.

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 aluminium foil and kept at-4 °C for 5 min. The frozen brain was sliced into uniform sections of 0.1 cm thickness.

The slices were incubated in 1 % 2, 3, 5-Triphenyl tetrazolium chloride (TTC) dissolved in phosphate buffered saline having pH 7.4 at 37 °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 [10]. Pale necrotic infarcted tissue was separated, weighed and percentage infarction was calculated.

Statistical analysis

The results were expressed as mean±standard error mean (mean±SEM), differences in results were determined by One Way ANOVA and individual groups were compared by Dunnett's test at 95% confidence interval. Differences with $P<0.05$ were considered statistically significant. The statistical analysis was performed by using Graphpad Prism Software (Version 5.02).

RESULTS

Toxicity study

In acute toxicity study no animal was died after oral administration of DMF at doses of 5 mg/kg, 50 mg/kg, 300 mg/kg, 2000 mg/kg, but all ten mice were died at dose of 5000 mg/kg of DMF four animals died on 2nd day, two animals died on 3rd day, three animals died on 4th day, one animal died on 7th day.

Similarly, in the sub-acute toxicity study, there was no mortality observed in Group VI and results of other parameters are given below in the table No. 1.

After daily oral administration of DMF at a dose of 1000 mg/kg in Group VI the results of other parameters are given below.

Table1: It consist of results of evaluation of various parameters in Group VI before and after administration of DMF (1000 mg/kg daily once orally) for 28 d

Parameters	On 1st Day	On 28thDay
Body Weight	34.81±1.04	34.63±0.81
Locomotor Activity (sec)	73.80±5.15	79.90±5.18
Muscle Streanth (sec)	148.9±25.02	150.3±21.29
Glucose mg/dl	98.83±5.16	93.5±5.90
Choletserol mg/dl	83.33±2.81	83.17±1.77
TGL mg/dl	100.8±6.80	94.67±7.15
HDL mg/dl	15.67±0.49	17.50±0.76
LDL mg/dl	48.50±2.49	46.73±1.55
VLDL mg/dl	20.17±1.36	18.93±1.43
CRE mg/dl	0.68±0.05	0.73±0.05
UA mg/dl	6.80±0.40	6.45±0.30
SGPT UL	59.67±2.45	53.33±4.08
SGOT UL	57.0±3.96	55.67±5.45
UREA mg/dl	53.67±2.17	51.83±2.81
Total Protein mg/dl	5.73±0.17	6.16±0.31
Bilirubin mg/dl	1.05±0.11	1.08±0.06
WBC $\times 10^3/\mu l$	7.0±0.72	5.45±0.77
RBC WBC $\times 10^6/\mu l$	7.79±0.52	7.63±0.22
Platelet WBC $\times 10^3/\mu l$	383.7±12.24	381.5±11.77
Hemoglobin g/dl	14.92±0.56	14.93±0.7
Lymphocyte %	64.33±1.66	44.17±1.92

Cerebro protective activity

The percentage cerebral cell infarction was calculated in all the groups mentioned above as per the protocol which is discussed earlier materials and methods. 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 the Group-IV and Group-V (i.e. treated groups with DMF at doses of 10 mg/kg and 20 mg/kg respectively) percentage, cerebral infarction was significantly decreased as compared to I/R control Group-III. The treated groups with DMF have protected the cerebral cells from the ischemia-reperfusion injury when compared with I/R control group i.e. Group III. The results are shown in table No. 2.

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

$$\% \text{ Protection in cerebral infarction} = \frac{\text{Control (Higher value)} - \text{Sample (Lower value)}}{\text{Control (Higher value)}} \times 100$$

Table 2: Effect of single dose treatment of DMF on ischemia and reperfusion-induced cerebral injury in wistar rats

Groups (n=6)	% Infarction (mean±SEM)	% Protection
Group-I (Normal)	0	-
Group-II (Sham Control)	10.94±1.59	-
Group-III (I/R Control)	62.19±2.27	-
Group-IV (Treated with 10 mg/kg DMF)	44.80±0.96**	27.96±1.55
Group-V(Treated with 20 mg/kg DMF)	25.51±1.43**	58.98±2.3

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

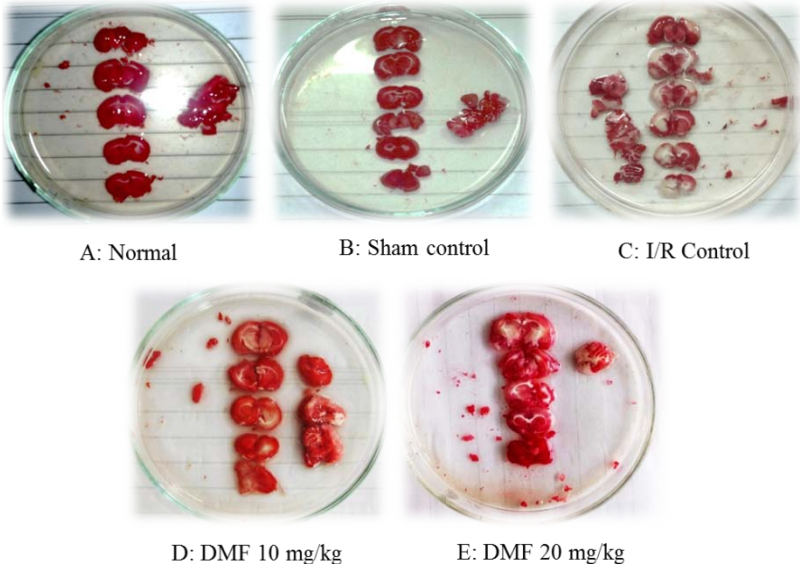

Fig.1: Single dose administration of DMF 30 min before reperfusion against ischemia and reperfusion-induced cerebral injury in Wistar rats

DISCUSSION

The results of acute toxicity test in the present study shown that DMF is safe at doses of 5 mg/kg, 50 mg/kg, 300 mg/kg, and 2000 mg/kg. The dose of DMF above 2000 mg/kg leading to toxicity, it was clearly observed in Group V that all the animals were died within seven days of drug administration at a dose of 5000 mg/kg. There was no significant difference observed in the body weight, locomotor activity, muscle strength, also in the various parameters like blood glucose level, total cholesterol, HDL, LDL, VLDL, triglycerides, creatinine, urea, uric acid, SGOT, SGPT, total protein, bilirubin and in the hematological parameters of Group VI animals on 1st day and on 28th day of DMF administration (daily at a dose of 1000 mg/kg orally), except Lymphocyte count. The results have shown that the lymphocyte count was decreased significantly on 28th day as compared to 1st day of DMF administration. Lymphocytopenia might be the adverse effect of the DMF at a dose of 1000 mg/kg when given orally but it needs further research to confirm the same. The cerebral ischemia-reperfusion-induced infarction has been developed in many animal models. In the present study, partial global cerebral ischemia was achieved by bilateral common carotid artery 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 [9].In this study, the extent of ischemia-reperfusion induced cerebral injury was measured in terms of percentage cerebral infarction by using TTC as a staining agent to differentiate infarcted tissue from non-necrotic normal tissue [11].Results of this study shown that there is significant variation in all the groups, i.e., Even though, 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 a significant increase in destruction and partial staining indicating necrosis in cerebral tissue, due to ischemia and reperfusion injury. Thus, here it was confirmed that bilateral common carotid artery occlusion for 1 h and reperfusion for 5 h model in Wistar rats established successfully. Infarction volume in the brain is an important parameter in the assessment of cerebro-protective activity against cerebral ischemia-reperfusion injury. Hence, the measurement of cerebral infarction was carried out in terms of percentage cerebral infarction in the present study. The results cited above has shown maximum percentage infarction found in I/R control group, after 1 h occlusion 5 h reperfusion of BCCA in Wistar rats. At the same time treatment with DMF at doses of 10 m/kg and 20 mg/kg has shown a significant reduction in the percentage infarction of cerebral tissue. 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 [12, 13]. Results of this study are also in accordance with the previous research work done by Gaur *et al.* and Raghavendra *et al.* in the similar experimental models [14, 15]. The cerebro-protective effect of DMF in the present study was supporting the previous studies on the 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 [5, 6]. As DMF has reduced the percentage infarction in ischemia-reperfusion-induced cerebral infarction, the present study proposes that DMF having a significant cerebro-protective effect.

CONCLUSION

The present study concludes that DMF is a relatively safe drug, as no toxic effect was observed up to dose 2000 mg/kg on oral administration. This information can be useful for the dose adjustment of DMF as well as as election of dose for further research on DMF. As per the results cited above it was observed that the DMF not affecting the vital processes in the mice adversely at a dose of 1000 mg/kg, after performing 28 d repeated dose toxicity test. Further, it includes only adverse effect was found to be lymphocytopenia on oral administration of DMF. The DMF has shown significant cerebro-protective potential against ischemia-reperfusion induced cerebral injury, by reducing the infarct size in brain tissue in Wistar rats. Further research is required for the establishment of the exact mechanism of action of its cerebro-protective effect and the exact toxic effect on various vital organs in the body by performing the histopathological study, which will support the safety profile of DMF in its clinical use.

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CONFLICT OF INTERESTS

Declared none

REFERENCES

1. Thomas J, Fitzpatrick L. Acute ischemic stroke review.J Neurosci Nursing 2008;40:69.
2. Jauch EC. Guidelines for the early management of patients with acute ischemic stroke a guideline for healthcare professionals from the American Heart Association/American Stroke Association. Stroke 2013;44:870-947.
3. Turner, White. Ischemic stroke: pathophysiology and principle of localisation. Neurol Board Rev Manual 2009;13:1-14.
4. Sundt TM.Correlation of cerebral blood flow and electro-encephalographic changes during carotid endarterectomy: with results of surgery and hemodynamics of cerebral ischemia. Mayo Clin Proc 1981;56:533-43.
5. Linker RA, Lee DH, Ryan S. Fumaric acid esters exert neuroprotective effects in neuroinflammation via activation of the Nrf2 antioxidant pathway. Brain 2011;134:678-92.
6. Gilgun-Sherki Y, Melamed E, Offen D. The role of oxidative stress in the pathogenesis of multiple sclerosis: the need for effective antioxidant therapy. J Neurol 2004;251:261-8.
7. Kappos L. Efficacy and safety of oral fumarate in patients with relapsing-remitting multiple sclerosis: a multicentre, randomised, double-blind, placebo-controlled phase IIb study. Lancet 2008;372:1463-72.
8. Scannevin RH, Chollate S, Jung MY. Fumarates promote cytoprotection of central nervous system cells against oxidative stress via the Nrf2 pathway. J Pharmacol Exp Ther 2012;341:274-84.
9. Jingtao J, Sato S, Yamanaka N. Changes in cerebral blood flow and blood brain barrier in the gerbil hippocampal CA1 region following repeated brief cerebral ischemia. Med Electron Microsc 1999;32:175–83.
10. Chintamani Narasinh Joshi, Swatantra Kumar Jain, Puvvada Sri Ramachandra Murthy. An optimised triphenyl tetrazolium chloride method for identification of cerebral infarcts. J Brainresprot 2003;12:001.
11. Angela B, Krisztina M, ZsoltJ.Use of TTC staining for the evaluation of tissue injury in the early phases of reperfusion after focal cerebral ischemia in rats. Brain Res 2006;1116:159-65.
12. Jiang J, Wang W, Sun YJ. Neuroprotective effect of curcumin on focal cerebral ischemic rats by preventingblood-brain barrier damage. Eur J Pharmacol 2007;561:54-62.
13. Orsu P, Murthy BV, Akula A. Cerebroprotective potential of resveratrol through anti-oxidant and anti-inflammatory mechanisms in rats. JNeural Transmission 2013;120:1217-23.
14. Gaur V, Aggarwal A, Kumar A. Protective effect of naringin against ischemic reperfusion cerebral injury: possible neurobehavioral, biochemical and cellular alterations in rat brain. Eur J Pharmacol 2009;616:147-54.
15. Raghavendra M, Maiti R, Kumar S. Role of *Centella asiatica* on cerebral postischemic reperfusion and long term hypo perfusion in rats. Int J Green Pharm 2009;3:88-96.

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