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Research Article
Neuroprotective Effects of *Melissa officinalis* on Oxygen and Glucose Deficiency Induced Damage in Rat's Brain Cortex Slices

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Abstract

Background and Objective: Ischemia is a stern decline or absolute obstruction in blood, flowing to various parts of the body. This pathophysiological episode causes cerebral mutilation, a protuberant feature of stroke, which is the 3rd leading cause of demise after cancer and heart attack globally. The principal objective of this work was to understand the sights of neuroprotection provided by *M. Officinalis* against OGD-R in rat's brain cortex slices. **Materials and Methods:** Mitochondrial viability assays were performed via the colorimetric 3(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) method. After 2 h of oxygen and glucose deprivation (OGD) followed by 1 h of reperfusion, only viable slices showed the ability to trim down MTT into a purple "Formazan" product that was soluble in dimethyl sulfoxide (DMSO). Absorbance was measured at 570 and 630 nm and the net absorbance (A570-A630) was taken as an index of cell viability. **Results:** The results of the present investigation demonstrated that oxygen-glucose deprivation (OGD) followed by re-oxygenation led to cell damage/death via an amplifying ROS/free radicals production in rat's brain cortex slices compared with control after 2 h OGD followed by 1h reperfusion. *Melissa officinalis* at a concentration of 40 μg mL^{-1} displayed potential role in neuro-protection against OGD, followed by re-oxygenation in mitochondrial viability assays *in vitro*. In addition, *Melissa officinalis* declined or slow down the production of free radicals in the supernatant and slices homogenate of cortex at the end of 2 h OGD followed by 1 h reperfusion. Furthermore, higher concentrations of *Melissa officinalis* slightly showed neurotoxicity for cortex slices which might be attributed to its pro-oxidant outcome. **Conclusion:** The results obtained during this study offer evidence for neuroprotective properties of *M. officinalis* against *in vitro* ischemia in rat's cortex slices. *Melissa officinalis* could be considered as a therapeutic agent in the prevention of neuronal cell death in Ischemia induced by oxygen and glucose deprivation of cortex slices, strengthening further investigations to define the actual component for its use in human. Furthermore, *in vivo* ischemic models are now in progress to confirm and better characterize its neuroprotection.

Key words: Oxidative stress, brain ischemia, neuroprotection, cortex slices, OGDR

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Human brain is predominantly susceptible to ischemic stroke. Complete blockage of blood flow to the human brain (for 5 min) can cause the demise/death of susceptible neurons in more than a few regions of the brain, whereas to kill cardiac myocytes or kidney cells, 20-40 min of ischemia is sufficient. The high-flying susceptibility of brain tissue to ischemic damage shows its sky-scraping metabolic rate. It is to be noted that the human brain represents 2.5% of total body weight and accounts for almost 25% of basal metabolism and having a metabolic rate which is 3.5 times elevated than the brains of other primates[1]. Ischemic stroke is associated with long lasting disabilities and accounts for the 3rd major cause of demises worldwide[2]. The OGD (Oxygen and glucose deprivation) in brain tissues is mostly under consideration as an *in vitro* model of cerebral ischemia[3-6] that permits for the valuation of neuronal collapse. Moreover, this replica system helps in the study of the effects of putative protective compounds in a cerebral structure that is particularly prone to ischemic affront in a tissue preparation that maintains the cellular structural design studied *in situ*[7]. Na^+/K^+ electrochemical gradient collapse is resulted due to reduced ATP levels which are because of oxygen and glucose scarcity in OGD. Soon after OGD, which is also termed as the re-oxygenation stage, when oxygen levels comes to normal, ischemic affront can be provoked by the additionally generated ROS (reactive oxygen species), most often in the mitochondrial electron transport chain and in inflammation[8]. As a result of this, transporters of glutamate activate to work in a reverse track, resulting in a release of excessive glutamate, which in turn resulting in the overstimulation of the NMDA (N-methyl-D-aspartate) receptor[9].

Melissa officinalis (lemon or common balm) belongs to family Lamiaceae. The plant's extract has been incorporated as traditional medicine for a long time in Iran[10]. Indigestion, anemia, palpitation and mood disorders can also be treated using extract of the plant[11]. Various nervous disorders including the reduction of excitability, anxiety, stress and sleep disturbance can also be treated using plant's extract[12]. The administration of *M. officinalis* extract in AD patients can perk up symptoms of disease[13]. However, the mechanism and constituents involved in its neuroprotective properties are not well known. It is claimed that the main effective components of this plant are polyphenols and terpenoids. Hence the main objective of this work was to understand the sights of neuroprotection provided by *M. officinalis* against OGD-R in rat's brain cortex slices.

MATERIALS AND METHODS

Place and duration of study: The study was carried out in the research laboratory of the Department of Biotechnology, University of Science and Technology, Bannu from January, 2017 to October, 2017.

Plant's collection and extraction: *Melissa officinalis* was obtained in powdered form from local market of District Bannu, KPK, Pakistan. The powder (100 g) was soaked at room temperature in 70% ethanol and extracted for a week. At the end of 7th day, the ethanolic extract was filtered using Whatman filter paper 1. The solvent was fully evaporated using rotavap under reduced pressure. The concentrated extract was reserved for further use.

Animals and chemicals: Male wister rats having weight 270-320 g with an age from 2.5-3.5 months obtained from NIH Islamabad, Pakistan. The rats were kept in cages with free access to foods and water in a room having controlled temperature (22±3°C) and in 12 h light/dark cycle. The procedures were planned in order to lessen torment and limit the number of animals to be used. All the chemicals used in this work were of analytical grade (Sigma Chemicals).

Animal ethics: The protocol of this study was in accordance with the guidelines of the Brazilian Association for Laboratory Animal Science (COBEA). The permission was granted by the Departmental Ethical Committee of the university through letter No Biotech 0119/2017.

Oxygen and glucose deprivation and treatment: Animals were sacrificed by decapitating just before starting the experiment. The cortex was speedily separated from the whole brain and kept in an artificial cerebrospinal fluid (aCSF) containing (in mM): 20 NaCl, 0.5 KCl, 35 $NaHCO_3$, 1.5 $CaCl_2$, 1.3 $MgCl_2$, 1.25 Na_2HPO_4, 10 D-glucose (PH 7.4). McIlwain tissue chopper (Campden instruments) was incorporated to make cortex slices. After that, cortex slices were alienated into two equal sets i.e., control and OGD and pre-treated for 30 min (in the absence or presence of *Melissa officinalis*, 1-100 μg mL^{-1}) in an artificial cerebrospinal fluid (aCSF). Control and OGD experiments were run parallel using three slices of the same animal in each plate. After pre-treatment, the medium in the OGD-groups was replaced with another aCSF free glucose. To copy ischemic conditions, OGD slices were incubated at 37°C in a chamber containing an anaerobic gas mixture (95% Argon, 5% CO_2) for 2 h (OGD period).

Whereas control slices were incubated for 2 h at 37°C with 95% O_2/5% CO_2. After OGD period, the medium from both control and OGD-groups was replaced with a medium with glucose. Slices were incubated for 1 h (reperfusion) in an incubator in the presence or absence of *Melissa officinalis* (1-100 μg mL^{-1}) as indicated.

Assessment of mitochondrial viability (MTT assays): Cellular viability assay (as assessed by mitochondrial activity) was performed by the colorimetric 3(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT, Sigma Chemical) method. After 2 h OGD followed by 1 h reperfusion, cortex slices were incubated in the presence of 45 μg mL^{-1} MTT at 37°C for a period of 45 min. Only viable slices showed the ability to reduce MTT into a purple Formazan product that was soluble in dimethyl sulfoxide (DMSO).This is because the active mitochondrial dehydrogenase in living cells causes reduction and cleavage/breakage of soluble yellow MTT dye to purple formazan. Absorbance was measured at 570 and 630 nm and the net A570-A630 was taken as cell viability index.

ROS measurement: The free radicals formation was evaluated by the addition of 2'-7'-Dichlorofluorescein diacetate (DCFH-DA, Sigma Aldrich) using as a probe. At the end of 2 h OGD followed by 1 h reperfusion, 5 μM DCFH-DA was added to supernatants and incubated for an additional 1 h at 37°C. Samples readings were taken after the additional incubation time by measuring the formation o f the fluorescence product DCF using excitation and emission wavelengths of 488 and 525 nm, respectively (spectrofluoro photometer, ParkinElmar LS45). All the working procedures were carried out in dark. On the other hand, the slices of each sample were homogenized and an aliquot was used to quantify intracellular ROS production[14].

Statistical analysis: Values are expressed as Mean±SEM (standard error of mean). Statistical analysis was done by one-way ANOVA followed by Duncan's multiple range tests. The results were considered statistically significant for $p<0.05$.

RESULTS

The results of the present study shows that when cortex slices are exposed to OGD, marked changes are observed both in cellular viability and free radicals level. The changes in cellular viability are due to a decline in mitochondrial activity as compared to non-OGD control slices. *Melissa officinalis* displayed noticeable protective effect against OGD-induced cellular death at a concentration 40 μg mL^{-1} in rat cortex slices

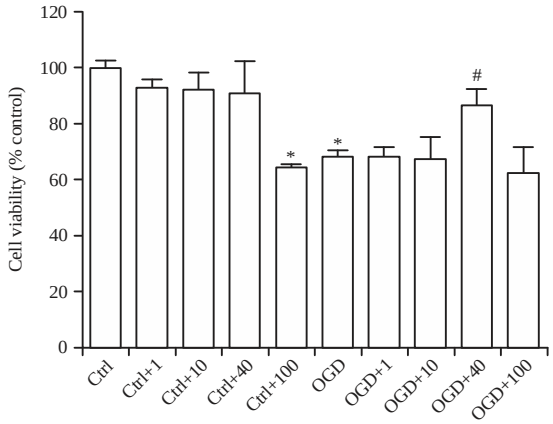

Fig. 1: Effect of different concentrations of *M. officinalis* on cellular viability

*Values significantly different from those of non-OGD group *$p<0.001$ ctrl vs OGD and Ctrl+100. #Values significantly different between OGD group as determined by one-way ANOVA followed by Duncan's test #$p<0.05$ OGD vs OGD+40 ($p = 0.045$)

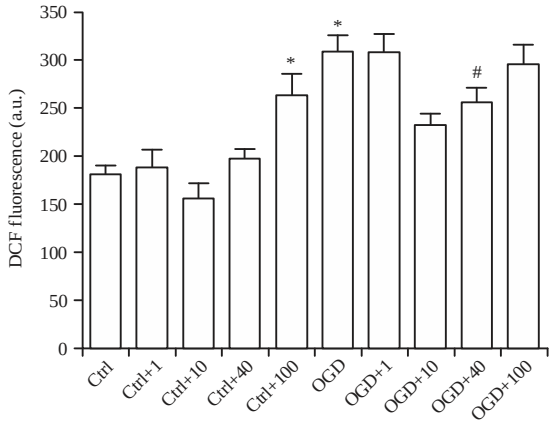

Fig. 2: Effect of methanol extract of *M. officinalis* on brain's cortex slices of rats, exposed to oxygen and glucose deprivation (OGD) for 2 and 1h reperfusion. Results are expressed in atomic unit

Each column represents the Mean±SEM,*$p<0.01$ ctrl vs OGD, #$p<0.01$ OGD vs OGD+10, *$p<0.05$ = 0.03 Ctrl vs Ctrl 100

(Fig. 1). The neuroprotection in this case is observed at higher doses suggest that the extract sample is retained in extra and intracellular compartments of the cells. It should be remembered that the extract is composed of a no of compounds of out of which only a few might be responsible for the activity observed. The effective doses of the compounds must be lower than the doses of the extract used in this activity.

In control slices, *Melissa officinalis* had increased cellular injury at 100 μg mL^{-1} as mentioned in Fig. 2. Total ROS were measured in supernatant of cortex slices at the end of 2 h

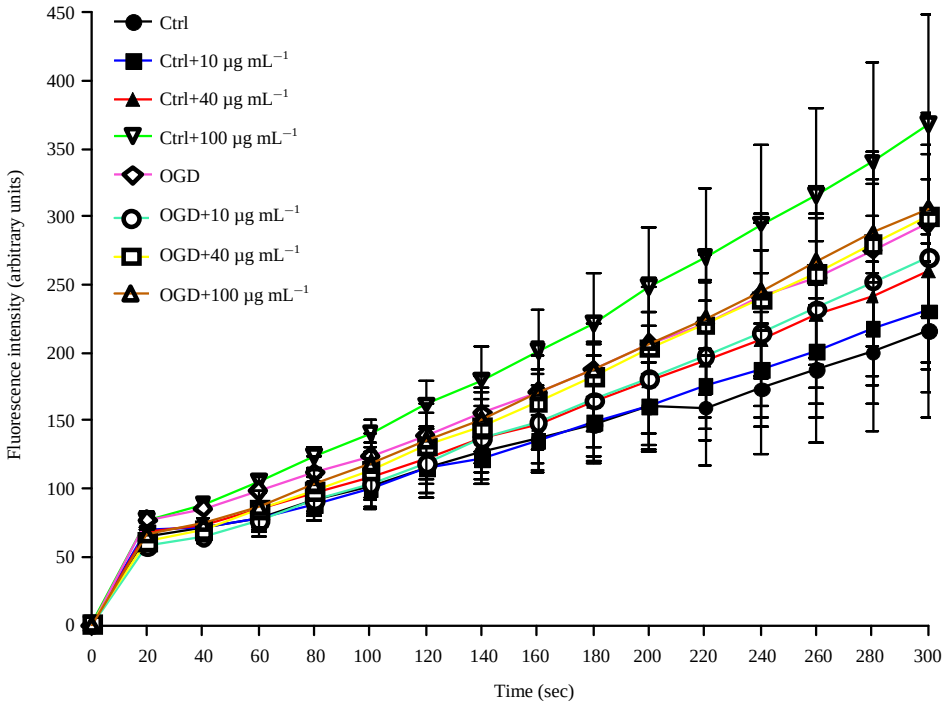

Fig. 3: Effect of *M. officinalis* extract on OGD-induced ROS production in rat cortex slices homogenates. Cortex slices homogenates (0.5 mg mL^{-1}) were incubated in a medium containing a CSF (see material and methods). 5 µM DCFH-DA was added in the medium

Data are expressed as Mean±SEM of four independent experiments. Data of ROS levels are presented as fluorescence intensity emission

OGD followed by 1 h reperfusion. The results showed that *Melissa officinalis* at a concentration of 40 µg mL^{-1} protect the release of free ROS in supernatant of cortex slices (Fig. 2) at the end of 2 h OGD followed by 1 h reperfusion. The free radicals scavenging activity of lemon balm is due to the presence of flavonoids, such as quercitrin, apigenin and phenolic acids. Furthermore, same type of protection at a concentration of 40 µg mL^{-1} of *Melissa officinalis* was observed in the homogenate of cortex slices (Fig. 3). However, *Melissa officinalis* had not showed any significant effect on the control groups slices homogenate of cortex (Fig. 3).

DISCUSSION

Stroke is the most common fatal disorder worldwide and ischemic stroke accounts for more than 80% of registered cases. By restoring blood flow within a short span of time can make up the situation. However, many reports have shown that reperfusion may aggravate the ischemic insult resulting in brain cell's death. This is due to the formation of free radicals that escape local defense system of the body that ultimate causes change in mitochondrial membrane and DNA, jeopardizing cell viability. At present, various clinical measures, to protect neurons from ischemic damage, focus on various aspects including acidosis, increase in the intracellular calcium and enhanced excitatory and inhibitory amino acids and changes in cytokines etc. However, the exact mechanism of ischemic insult is still unknown and requires further studies.

It is believed that reactive free radical species play an important role in brain injury and neurodegenerative diseases, such as Parkinson, Alzheimer, hypoxia and ischemia. In addition, cortex is considered as an important part of brain, most vulnerable to oxidative stress[14], therefore, cortex slice *in vitro* ischemic model is widely used and provide mechanism of protection against neuronal cell damage or death. In the present study, the authors selected cortex slices to mimic *in vitro* ischemic conditions.

Natural products are full of medicinally important compounds having neuroprotective properties[15]. Literature studies revealed that ROS can be scavenged through utilizing natural antioxidant compounds present in medicinal plants and has a potential role against OGD[15]. In the present study *Melissa officinalis* prevent or slowdown cortex slices injury in ischemia. The exposure of cortex slices to an *in vitro* ischemic event (OGD) followed by re-oxygenation significantly affected cellular viability, by causing a decline in mitochondrial activity in comparison to control (non-OGD) slices. In addition, incubation of rat's cortex slices with *Melissa officinalis* at a

concentration 40 μg mL^{-1} significantly protected the cell death compared with control (OGD) while in non-OGD groups *Melissa officinalis* had not showed any significant effect in cortex slices. At higher concentration (100 μg mL^{-1}), it's become neurotoxic for slices, which could be due to a pro-oxidant effect[15]. The protective effects of lemon balm have been reported in PC12 cell line that is considered a useful model in neuroscience because of its phenotypic characters with sympathetic neurons. The protective effects of this plant in these cells mean that it might prevent oxidative stress in neurons[16]. The exact mechanism through which the plant reduced free radical production (OGD induced) remains a matter for future investigation.

Literature review showed that in culture of cortical neuronal cell, hypoxia induced cell injury is reduced by the essential oil of the plant. Furthermore, the plant possibly can decrease cell injury due to transitory hippocampal ischemia in rat's model via reduction of lipid peroxidation and HIF-1(hypoxia-inducible factor), though, no effect on the pro-inflammatory cytokines was noticed[17].

Oxygen and glucose deprivation followed by re-oxygenation led to an increase of ROS production (expressed by the amount of DCF formed) both in supernatant and slices homogenate of cortex slices when compared to control (non OGD). The effects of these ROS production were imitated in supernatant and slices homogenate of cortex by *Melissa officinalis* extract. Taking together, these results indicate that *Melissa officinalis* at low concentration, has a role of neuroprotection against OGD, particularly at 40 μg mL^{-1}. In addition, its start to be a pro-oxidant to cortex slices at concentration 100 μg mL^{-1}.

Oxygen and glucose deprivation followed by re-oxygenation led to an increase of ROS production (expressed by the amount of DCF formed) both in supernatant and slices homogenate of cortex when compared to control (non OGD). The effects of these ROS production were imitated in supernatant and slices homogenate of cortex by *Melissa officinalis* extract. Taking together, these results indicate that *Melissa officinalis*, at low concentration, has a role of neuroprotection against OGD, particularly at 40 μg mL^{-1}. In addition, it starts to be a pro-oxidant to cortex slices at concentration 100 μg mL^{-1}.

CONCLUSION AND FUTURE RECOMMENDATION

It is concluded that the results obtained offer evidence for neuroprotective properties of *M. officinalis* against *in vitro* ischemia in rat cortex slices. *Melissa officinalis* could be considered as a therapeutic agent in the prevention of neuronal cell death in Ischemia induced by oxygen and glucose deprivation of cortex slices, strengthening further investigations to define the actual component for its use in human. Furthermore, *in vivo* ischemic models are now in progress to confirm and better characterize its neuroprotection.

There were some limitations while conducting this study, e.g., limited data was available about the protective effects of *Melissa officinalis* on rat's brain cortex slices etc. This remained a significant obstacle in relating our results with previously available data. With this study, data will be available for researchers to relate their results with our and conduct further studies on this plant for future. Further, isolation of secondary metabolites, which are involved in the plant's neuroprotective effects is recommended.

SIGNIFICANCE STATEMENT

The neuroprotective effects of *M. officinalis* on rat's brain cortex slices are investigated and are being reported for the 1st time. Nobody has reported its protective effects on rat's brain cortex or hippocampal slices before our investigation. This study discovers the protective effect of *M. officinalis* on oxygen and glucose deprivation induced damage in rat's brain cortex slices that can be beneficial for providing good evidence about the plant as a promising source in treatment or prevention and management of neurode generative diseases. This study will help the researchers to uncover the active compounds within the mentioned plant for designing new drugs for the treatment of debilitating neuro-developmental disorders. Thus, a new theory on these active compounds may be arrived at.

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REFERENCES

1. Kalogeris, T., C.P. Baines, M. Krenz and R.J. Korthuis, 2012. Cell biology of ischemia/reperfusion injury. Int. Rev. Cell Mol. Biol., 298: 229-317.
2. Savitz, S.I. and H.P. Mattle, 2013. Advances in stroke: Emerging therapies. Stroke, 44: 314-315.
3. Cho, S., A. Wood and M.R. Bowlby, 2007. Brain slices as models for neurodegenerative disease and screening platforms to identify novel therapeutics. Curr. Neuropharmacol., 5: 19-33.

4. Molz, S., H. Decker, T. Dal-Cim, C. Cremonez, F.M. Cordova, R.B. Leal and C.I. Tasca, 2008. Glutamate-induced toxicity in hippocampal slices involves apoptotic features and $p38^{MAPK}$ signaling. Neurochem. Res., 33: 27-36.
5. Morrison, S.F., K. Nakamura and C.J. Madden, 2008. Central control of thermogenesis in mammals. Exp. Physiol., 93: 773-797.
6. Oleskovicz, S.P.B., W.C. Martins, R.B. Leal and C.I. Tasca, 2008. Mechanism of guanosine-induced neuroprotection in rat hippocampal slices submitted to oxygen-glucose deprivation. Neurochem. Int., 52: 411-418.
7. Dal-Cim, T., W.C. Martins, A.R.S. Santos and C.I. Tasca, 2011. Guanosine is neuroprotective against oxygen/glucose deprivation in hippocampal slices via large conductance Ca^{2+}-activated K^+ channels, phosphatidilinositol-3 kinase/protein kinase B pathway activation and glutamate uptake. Neuroscience, 183: 212-220.
8. Kostandy, B.B., 2012. The role of glutamate in neuronal ischemic injury: The role of spark in fire. Neurol. Sci., 33: 223-237.
9. Lai, T.W., W.C. Shyu and Y.T. Wang, 2011. Stroke intervention pathways: NMDA receptors and beyond. Trends Mol. Med., 17: 266-275.
10. Ross, I.A., 2010. Medicinal Plants of the World, Volume 3: Chemical Constituents, Traditional and Modern Medicinal Uses. Humana Press, New Jersey, Pages: 298.
11. Naghibi, F., M. Mosaddegh, M.M. Motamed and A. Ghorbani, 2005. Labiatae family in folk medicine in Iran: From ethnobotany to pharmacology. Iran. J. Pharm. Res., 4: 63-79.
12. Dobetsberger, C. and G. Buchbauer, 2011. Actions of essential oils on the central nervous system: An updated review. Flavour Fragrance J., 26: 300-316.
13. Kennedy, D.O. and E.L. Wightman, 2011. Herbal extracts and phytochemicals: Plant secondary metabolites and the enhancement of human brain function. Adv. Nutr.: Int. Rev. J., 2: 32-50.
14. Intlekofer, K.A. and C.W. Cotman, 2013. Exercise counteracts declining hippocampal function in aging and Alzheimer's disease. Neurobiol. Dis., 57: 47-55.
15. Konrath, E.L., K. Santin, M. Nassif, A. Latini, A. Henriques and C. Salbego, 2008. Antioxidant and pro-oxidant properties of boldine on hippocampal slices exposed to oxygen-glucose deprivation *in vitro*. Neurotoxicology, 29: 1136-1140.
16. Lopez, V., S. Martin, M.P. Gomez-Serranillos, M.E. Carretero, A.K. Jager and M.I. Calvo, 2009. Neuroprotective and neurological properties of *Melissa officinalis*. Neurochem. Res., 34: 1955-1961.
17. Bayat, M., A.A. Tameh, M.H. Ghahremani, M. Akbari, S.E. Mehr, M. Khanavi and G. Hassanzadeh, 2012. Neuroprotective properties of *Melissa officinalis* after hypoxic-ischemic injury both *in vitro* and *in vivo*. DARU J. Pharm. Sci., Vol. 20, No. 1. 10.1186/2008-2231-20-42.