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Research Article

Attenuation of neuronal damage by mangiferin in experimentally induced cerebral ischemia in rats

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ABSTRACT

The objective of the current study was to evaluate the attenuation of neuronal damage by mangiferin. Neuronal damage in rats was induced by occlusion of carotid arteries. Experimental outcomes showed that the method adopted significantly produced infarction in brain and induced oxidative stress. This could be because of cellular seepage and damage of the membrane of neurons. However, groups treated with mangiferin in different doses have significantly protected neuronal damage and showed effect on acetyl cholinesterase enzyme level, lipid peroxidase, reduced glutathione and improvement in total protein, suggesting neuroprotective influence of mangiferin. This might be due to facilitated scavenging effect on free radicals. In addition, histopathological studies also support the inhibition of damage of architecture of brain with mangiferin treatment hence; adjuvant therapy by the administration of mangiferin might be useful in treating stroke.

Keywords: acetyl cholinesterase; carotid artery occlusion; cerebral ischemia; lipid peroxidase; mangiferin; Neuroprotective

INTRODUCTION

Neurological problems are commonly associated with brain ischemia, hypertensive disorders and brain hemorrhage. Studies showed that stroke is a major cause of death which is associated with or without long term disability and according to WHO, around 15 million people suffer due to stroke throughout world. Five million deaths and additional five million permanent disabilities are noted globally. (WHO Monica, 1988).Pathologically, when blood flow to the brain is affected produces hypoxia results in to brain damage that is very similar to damage of due to anaerobic metabolism, finally causes neuronal death. Energy failure, stress due to oxygen free radicals, inflammation, apoptosis like events occurred in brain stroke. Oxygen deficiency caused brain infarctions are not detectable until 6-12 hours, but long term brain hypoxia produces discoloration and change in the brain tissue caused by infiltration and edema. Brain tissue is vulnerable for oxidative damage owing to its oxygen dependent metabolism and high lipid content. Alzheimer, bipolar disorder, Parkinson, epilepsy, shock and schizophrenia like neurodegenerative diseases are the problems of immense clinical importance that are mainly associated with oxidative stress and lipid peroxidation.

Adjuvant therapy using antioxidants has gained importance because reperfusion of the blood after cerebral ischemia further adds to the complications of stoke by releasing various mediators such as pro-inflammatory cytokines and generated free radicals. Various naturally occurring antioxidants are available as food supplements and could be used effectively in treating brain ischemic conditions as these agents reduces pro-inflammatory cytokines and generation of free radicals. A decreased oxidative stress condition resembles reperfusion of the blood preventing neuronal cell death. Cerebral ischemia is further accompanied by inflammation which can deteriorate the neuronal injury (del Zoppo Get al., 2000; Allan SM& Rothwell NJ, 2001; Iadecola C & Alexander M, 2001).

Figure 1: Chemical structure of Mangiferin

Mangiferin is a naturally occurring agent has been successfully isolated and chemical structure showed in Figure 1. It is polyphenol compound (Cunha-Vaz J, 1979) isolated from *Anemarrhena asphodeloides* (United States Health and Human Services, 2001) and *Mangifera indica* (Andreu GP *et al*., 2005). Mangiferin has

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got antioxidant property (Lai L *et al*., 2003), anti-apoptotic (Severi JA *et al*., 2009) immunomodulating (Rajendran P *et al*., 2008) and anti-diabetic potential (Campos-Esparza MR *et al*., 2009). This prompted to undertake a putative role of mangiferin in treating certain CNS disorder like shock.

MATERIALS AND METHODS

Chemicals

Bovine serum albumin, DTNB, TBA, ethanol, sodium tartrate, tween 80, n-hexane, TCA, Ellman's reagent, and other chemicals purchased from Himedia Laboratories, Mumbai, India.

Animals

Rats used in the experiments were obtained from Venkateshwara Enterprises; Karnataka, India which were weighing between 180-200 g. Animals were kept in a separate animal house at S.E.T's College of Pharmacy, Dharwad, India. Standard environmental conditions (temperature range 23 ± 2°C) with around 50 % humidity were maintained at animal house during study. Rats were feed with pellets and water ad libitum. For the present study, clearance from Institutional Animal Ethical Committee (IAEC) of SET's College of Pharmacy, Dharwad, India was obtained.

Induction of cerebral ischemia

Pentobarbitone (50 mg/kg BW) was dosed to experimental animals for induction of anesthesia during experimental procedures. The carotid arteries of rats were opened by making an incision at the midline region of neck and sternum. Cerebral ischemia was produced by clipping both arteries using aneurysm clips. After 10 min of occlusion, the aneurysm clips were detached for reperfusion of blood. After experimental procedure the surgical opening was closed by suturing with sterile suture followed by disinfection using ethanol. Animals were then relocated separately to their home cages for recovery (Masaya *et al.,* 1999).

Measurement of cerebral infarct size

Measurement of size of cerebral infarct was done under anesthesia and then brain was removed. Uniform slices of brain were made of 1 mm thickness and submerged in solution of triphenyl tetrazolium chloride (1%) at 37°C prepared in 0.2 M phosphate buffer of pH 8.5 for about 20 min. Further, slices were soaked in fixing solution of a 10% formaldehyde for five min. TTC is converted to red pigment of formaldehyde thereby stained the viable cells with deep red colour. Dull yellow colored infarcted cells were remained unstained due lack of essential enzymes and cofactor. Tissue pieces were retained on the plate and a transparent 100 squares/1cm^2 plastic grid was placed over it. Calculation of infarcted area as a percentage of total volume of brain to the actual measurement of red color zone was carried out. Edema of the brain was calculated by weighing the whole brain. Water present in the brain contributed for the edema as compared to control group (Chandrashekhar *et al*. 2010).

Biochemical parameters

Various biochemical parameters and estimation of brain protein assay were carried out. Brain protein content estimation was carried out by the method of Lowry *et al*. (Tietz 1986). Estimation of lipid peroxidation is carried out using thiobarbituric acid-reactive substances (TBARS). Malondialdehyde (MDA) was identified as the end product of peroxidation of various lipids that are readily reacts with reagent to get red light absorbency at 535 nm. (Ohkawa et al., 1979).

The superoxide dismutase (SOD) was estimated on inhibitory effect of SOD on tetrazolium reduction by anions of super oxide present in the solution. (Dalla *et al.,* 2009). Glutathione peroxidase (GSH-Px) estimation is done using tissue homogenate. Samples were mixed with 50 mM phosphate buffer, pH 7.4 and to which a mixture of EDTA (5mM), sodium azide (0.01 ml of 1.125 M), 0.1 ml of 0.15 M glutathione, glutathione reductase (2.4 units, 10 µl) and 0.1 ml of 8.4 mM. After 10 min H_2O_2 solution (0.1 ml of 2.2 mM) was add and decrease in absorbance at 340 nm was measured for 4 min. (Dalla *et al*., 2009). Estimation of activity of acetyl cholinesterase of whole brain was done as per standard procedure using and calculated using following formula. (Kiran *et al*., 2015).

$$R = 5.74 \times 10^{-4} \times \frac{A}{CO}$$

Where,

R = Rate in moles of substrate hydrolyzed /minute /gm of brain tissue

A = Change in absorbance / min.

CO = Original concentration of the tissue (mg/ml)

Histopathological Examination

Brain slices were dipped in 10% formalin used for histopathological analysis to observe neuronal damage. Hematoxylin and eosin were used as staining agents and slides were examined for infarct cells by microscopy. This study was carried out at Jeevan Lab Pvt Ltd. (Belgaum, India). (Oisson *et al,* 2003).

Statistical analysis

Results obtained were expressed as average with standard error mean. Differences and significance among data were calculated using one-way ANOVA.

RESULTS

In the present study, carotid artery occlusion method was used to produce ischemia. Screening of mangiferin for neuroprotection was done by measurement of various biochemical parameters and brain infarcts size.

Table 1: Results of total protein, lipid peroxidase (LPO), reduced glutathione (GSH) and acetyl cholinesterase (AChE) activities of various groups

S.No	Groups	Total Protein	LPO (µmols/g)	GSH (µmols/g)	AChE (µmols/min/mg)	Water Contents (%)	Infarct size (mm)
1	Normal (Vehicle)	3.01±0.015	0.56±0.031	3.025±0.012	6.89±0.233	65.08±2.120	-------
2	Sham (Surgery)	1.60±0.040	0.28±0.013	3.74±0.065	6.83±0.140	74.37±1.210	22.11±1.30
3	Cerebral ischemia group	1.41±0.012##	0.71±0.030 ###	1.12±0.072 ###	10.31±0.144 ###	80.82±1.110 ##	80.19±2.07 ###
4	Mangiferin (100 mg/kg)	1.85±0.040	0.53±0.011	1.89±0.156*	8.79±0.533 *	75.51±1.132	69.3±0.45 **
5	Mangiferin (200 mg/kg)	2.87±0.019 **	0.48±0.140 **	2.87±0.023 ***	7.08±0.324 ***	68.53±0.069 **	38.18±2.11 ***

The average values are presented ± standard error mean (n=6) *P< 0.05, **P< 0.01, ***P< 0.001. Significant differences between Sham group and Ischemia group are defined with #, significant differences between Surgery group and Treatment groups are defined with *

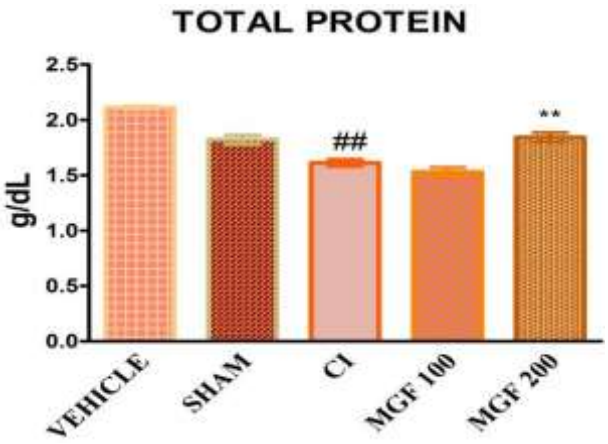

Figure 2: Effect of Mangiferin on Total Protein content in various groups

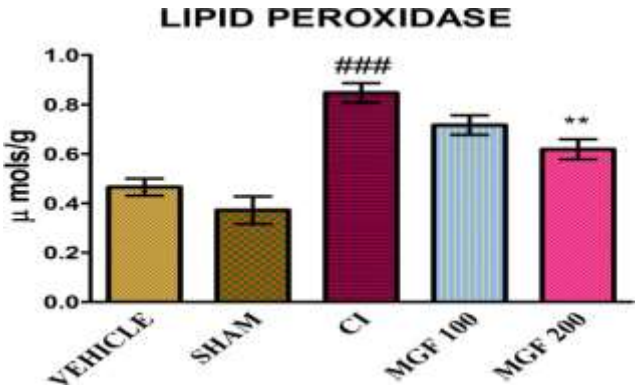

Figure 3: Effect of Mangiferin on Lipid Peroxidase (LPO) activity in various groups

Effect of mangiferin on cerebral ischemia induced brain edema and infarction

Water content in the brain tissue was 65.08±2.120, 74.37±1.210, 80.82±1.11, 75.51±1.132 and 68.53±0.069 respectively for normal, sham, cerebral ischemia group, mangiferin 100 and mangiferin 200 mg/kg treated groups. This indicates that significant accumulation of water in brain as well as infarct size was found in inducing group as compared sham group. Groups treated with test drug mangiferin has

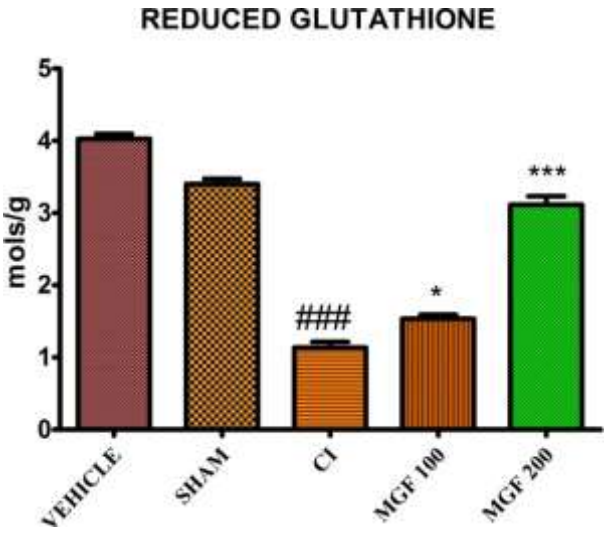

Figure 4: Effect of Mangiferin (100 and 200 mg/kg) on Reduced Glutathione (GSH) activity in various groups

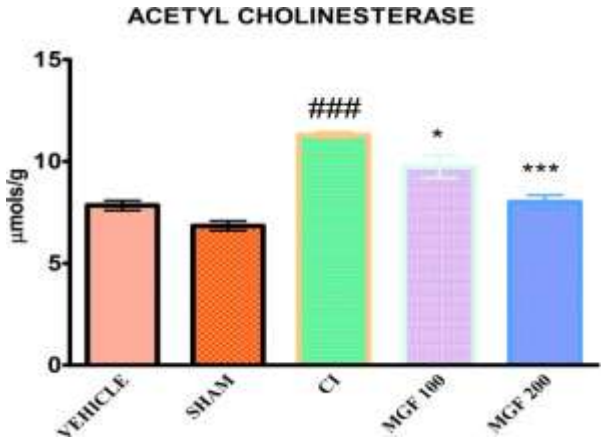

Figure 5: Effect of Mangiferin (100 and 200 mg/kg) on Acetyl cholinesterase (AChE) activity in various groups.

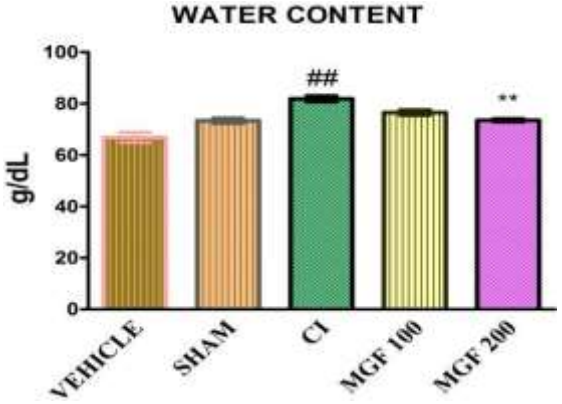

Figure 6: Effect of Mangiferin (100 and 200 mg/kg) on Water content of brain in BCCAO induced global cerebral ischemia.

shown significant reduction in water content and shows protection against cerebral ischemia induced brain edema. The infarction area also reduced in groups treated with mangiferin (Table 1).

Effect of mangiferin on antioxidant activity

Antioxidant results and acetyl cholinesterase activity are presented in Table 1. It was observed that LPO and acetyl cholinesterase enzyme activity were found to be more as compared to induced group. Similarly, there was attenuation was observed with mangiferin treatment. Comparatively lower antioxidant activity was found in ischemia groups as indicated by low levels of GSH and total protein as compared to sham group.These antioxidant enzyme levels were improved with treatment with mangiferin indicating improved

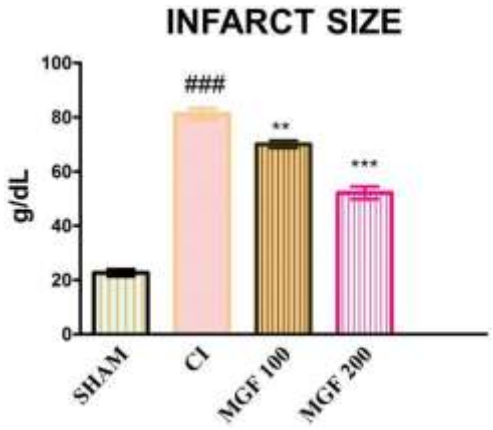

Figure 7: Effect of Mangiferin (100 and 200 mg/kg) on infarct size of brain in BCCAO induced global cerebral ischemia

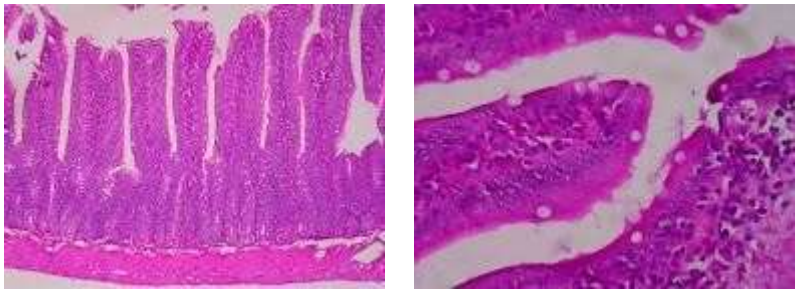

Figure 8: Histoarchitecture of brain in control group (A) and induced group (B)

antioxidant enzyme activity against brain ischemia (Figure 2to7).

Histopathological Examination

Histoarchitecture of brain in control, induced group and groups treated with Mangiferin are observed and only histoarchitecture of brain in control and induced group are depicted as Figure 8 A & B respectively. Examination of histoarchitecture of brain showed cerebral edema with moderate cerebral congestion. There was mild neutrophilic infiltration observed and beta amyloid precursor protein immune reactivity has been seen in cerebral ischemia induced group. It may be noted that mangiferin potentially protects against brain injury due to bilateral common carotid artery occlusion for 10 min.

DISCUSSION

The model selected in the present study (i.e., global cerebral ischemia in rat model). resembles ischemic stroke that produced by decreased oxygen supply as well as edema formation. Intracerebral bleeding stroke is called as hemorrhagic stroke that accounted for 85% of all strokes (Beal et al., 2010). On other hand other category of strokes are dependent on the activation of certain specific genes which subsequently leads to apoptosis (Dirnagl *et al.,* 1999; Lipton *et al.,* 1999; Zheng *et al.,* 2004). There are several evidences indicated oxidative stress and apoptosis are linked in the pathophysiology of ischemic stroke. There are three major categories of stroke: subarachnoid hemorrhage, intracerebral hemorrhage and ischemic stroke. Cerebral ischemia causes considerable reductions in cerebral blood flow (CBF) as the result of cardiac arrest. Occlusion of cerebral and extra cerebral vessel s supplying nervous tissues produces lack of blood supply in brain which leads to deprivations in oxygen, glucose delivery and accumulation of potentially toxic substances. If reperfusion is initiated immediately, metabolic and ionic homeostasis can be mentioned so that cells remain less affected. The earliest neuronal injury consisted in increasing cytoplasm eosinophilia, with the nucleus appearing shrunken and darkly basophilic, or developing clumped chromatin condensations. In the later stages, the cytoplasm was uniformly structure less, and the nucleus showed advanced degeneration and appeared homogeneous. Finally, the neurons are disintegrated resulting in eosinophilic debris. The neuronal debris were dispersed throughout the neuropil and scattered eccentrically from the remainder of the dead neurons. Later on, all these remains were phagocytized by macrophages (also called “foamy” macrophages in respect to their fine vacuolated cytoplasm that accumulates myelin debris).

Impaired antioxidant defenses are predicted to contribute in the pathophysiology of neurodegenerative disorders. Altered AchE and increased lipid peroxidation, measured by the thiobarbituric acid reactive substances (TBARS/MDA), are increased. Besides, Mangiferin has the potential of complexing iron and it can also defense against lipid peroxidation produced

byFe^{2+}citrate. The neurotoxin 6-hydroxydopamine (6-OHDA) is well-known to cause neuronal death by a free radical-mediated mechanism as well as by inhibiting the mitochondrial complex I and IV. As Mangiferin has a varied spectrum of pharmacological activity as well as antioxidant and anti-inflammatory activity correspondingly accruing evidence also suggests that Mangiferin is neuroprotective.

There is a close relationship between compromised mitochondrial function and neurodegenerative disorders which is one of over 50 associated disorders triggered by mitochondrial dysfunction. Mangiferin potentiates the enzymes in the Krebs cycle and increase ATP levels. Since Mangiferin is responsible for diminishing oxidative stress, apparently it can also decrease inflammation. For example Mangiferin prevents respiratory eruption in neutrophils of rat. Furthermore, Mangiferin subdues prostaglandin (PG) endo peroxide synthase 2 expression and thus decreases synthesis of PGE2 in rat microglia cells.

CONCLUSION

Stroke is caused by ischemia in turn it leads to various consequences such as, accrual of noxious substances, insufficiency in Oxygen and glucose delivery which are essential for ATP production. Altered levels antioxidant enzymes are responsible for cell death. Mangiferin being potential antioxidant protects neuronal cell s from oxidative cell damage and also stimulates enzymes involved in TCA cycle. Hence it can be concluded that mangiferin can be used for the treatment of stroke.

CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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