



Neuroprotective activity of *Matricaria recutita* Linn against global model of ischemia in rats

V.M. Chandrashekhar^{a,*}, V.L. Ranpariya^a, S. Ganapaty^b, A. Parashar^a, A.A. Muchandi^a

^a Department of Pharmacology, Hanagal Shri Kumareshawr College of Pharmacy, BVVS campus, Bagalkot-587101, Karnataka, India

^b Pharmacognosy and Phytochemistry Division, College of Pharmaceutical Sciences, Andhra University, Visakhapatnam-530003, Andhra Pradesh, India

ARTICLE INFO

Article history:

Received 4 January 2009
Received in revised form 2 December 2009
Accepted 3 December 2009
Available online 16 December 2009

Keywords:

Cerebrovascular diseases
Chamomile
Ischemia/reperfusion
Matricaria recutita L.
Neuroprotection
Oxidative stress

ABSTRACT

Ethnopharmacological relevance: Traditionally, the whole plant is used for various diseases, including neuronal disorders.

Aim of the study: To evaluate the neuroprotective effect of *Matricaria recutita* L. against global cerebral ischemia/reperfusion (I/R) injury-induced oxidative stress in rats.

Materials and methods: Neuroprotective activity was carried out by global cerebral ischemia on Sprague–Dawley rats by bilateral carotid artery (BCA) occlusion for 30 min followed by 60 min reperfusion. The antioxidant enzymatic and non-enzymatic levels were estimated along with cerebral infarction area and histopathological studies.

Results: The *Matricaria recutita* L. methanolic extract showed dose-dependent neuroprotective activity by significant decrease in lipid peroxidation (LPO) and increase in the superoxide dismutase (SOD), catalase (CAT), glutathione (GSH) and total thiol levels in extract treated groups as compared to ischemia/reperfusion group. Cerebral infarction area was significantly reduced in extract treated groups as compared to ischemia/reperfusion group.

Conclusion: The methanolic extract of *Matricaria recutita* L. showed potent neuroprotective activity against global cerebral ischemia/reperfusion injury-induced oxidative stress in rats.

© 2009 Elsevier Ireland Ltd. All rights reserved.

1. Introduction

Cerebrovascular diseases (CVD) include some of the most common devastating disorders such as ischemic stroke, hemorrhagic stroke, cerebrovascular anomalies, etc. They cause two lacks deaths each year and are major cause of disability (Wade et al., 2005). Stroke has been ranked third most common cause of death worldwide and cerebrovascular diseases are considered second most frequent causes of projected deaths in the year 2020 (Anonymous, 1998; Huang and McNamara, 2004). At the present state of knowledge treatment of ischemic brain injury is far from adequate (Norris and Hachinski, 1993). Therefore, search for cerebro-protective agents with minimal risk of iatrogenic effects must, therefore continue. Natural products (specially the medicinal plants) probably represent an ideal source to develop safe and effective agents for management of stroke and deserve scientific probe.

Oxidative stress, which results from an imbalance between the generation and removal of reactive oxygen species (ROS), probably plays an important role in the development of tissue damage

induced by arterial occlusion with subsequent reperfusion (Liu et al., 1989; Kinouchi et al., 1991; Yu et al., 1998). Neuronal death in a stroke is complex event involving failure of metabolic processes, excitotoxicity, loss of calcium homeostasis and oxidative stress, among other factors (Alexi et al., 2000). During ischemic stroke, increased glutamate release leads to increased Ca^{2+} level (Nicholis and Attwell, 1990). The massive Ca^{2+} entry activates enzymes such as proteases, oxidases, phospholipases and endonucleases (Nicotera and Lipton, 1999) that can hydrolyze the DNA molecule and destroy the cytoskeleton (Welch et al., 1997). Glutamate and Ca^{2+} both activates NMDA receptors and G-proteins and leads to phospholipase A_2 activation favors the metabolism of arachidonic acid through lipoxygenases and eicosanoids in turn activate lipid peroxidation. Increased intracellular Ca^{2+} also activates protein kinase C that can modify the function of many ion channels (Picq et al., 1989). Several of these activated intracellular metabolic events lead to the generation of oxygen free radicals, which overcome the antioxidant defenses and provoke oxidative stress (Dugan and Choi, 1999).

Matricaria recutita L. (Asteraceae, commonly known as German chamomile) is one of the most widely used and well-documented medicinal plants in the world (Salaman, 1992). Chamomile is also extensively consumed as a tea or tonic. Chamomile is used both internally and externally to treat an extensive list of conditions.

* Corresponding author. Tel.: +91 9880298342; fax: +91 8354220008.
E-mail address: chandupharm@yahoo.com (V.M. Chandrashekhar).

It is used externally for wounds, ulcers, eczema, gout, skin irritations, neuralgia, sciatica, rheumatic pain, and hemorrhoids (Newall et al., 1996) and internally to treat anxiety, hysteria, nightmares, insomnia and other sleep problems, convulsions and even delirium tremens (Martens, 1995). The main chemical constituents of the German chamomile are terpenoids like α -bisobolol, chamazulene, sesquiterpenes and flavonoids like apigenin, luteolin and quercetin (Newall et al., 1996).

The purpose of the present study is to know the safe and potent neuroprotective effect of German chamomile against global cerebral ischemia/reperfusion injury-induced oxidative stress in Sprague–Dawley rats.

2. Materials and methods

2.1. Drugs and chemicals

Trichloroacetic acid (TCA), 2-thiobarbituric acid (TBA), 5-5'-dithiobis (2-nitrobenzoic acid), glutathione and (\pm)-epinephrine were purchased from Sigma–Aldrich Co., Spruce Street, St. Louis, MO, USA. 2,3,5-Triphenyltetrazolium chloride (TTC) was purchased from Hi-Media, Mumbai. All other chemicals were of the highest purity commercially available.

2.2. Plant material

In the present study, capitulas of *Matricaria recutita* L. were collected from the National Botanical Research Institute (NBRI), Lucknow, India during the month of June 2008. Herbarium was prepared and the specimen was further identified and authenticated in Department of Botany, Basaveshwar Science College, Bagalkot, Karnataka. Voucher specimen (B.Sc./Bot./14/08) was deposited in the herbarium of the same college. All capitulas were dried at room temperature until they were free from moisture. Finally the capitulas were subjected to pulverizer to get coarse powder and then passed through sieve #44 to get uniform powder. The sieved powder was stored in airtight high density polyethylene container before extraction. The powdered capitulas (600 g) were subjected to successive extraction with petroleum ether (40–60 °C) and subsequently with methanol (64–65.5 °C). After the residue extraction, solvent was distilled off and excess solvent was completely removed by using a rotatory flash evaporator to get concentrated, then completely dried in freeze drier and stored in airtight container under refrigeration. The obtained extract (64 g, percentage yield-10.67%) then used for neuroprotective activity.

2.3. Phytochemical screening

Phytochemical screening of the crude extract was carried out employing standard procedures and tests (Trease and Evans, 1989), to reveal the presence of chemical constituents such as terpenoids, flavonoids, tannins, coumarins among others.

2.4. Acute toxicity study

The acute toxicity study was performed as per the method described by Litchfield and Wilcoxon (1949), and LD₅₀ was calculated accordingly. Briefly, the methanolic extract of German chamomile in the dose range of 10–2000 mg/kg was administered orally to different groups of mice ($n=10$). The animals were examined at every 30 min up to a period of 3 h and then, occasionally for additional period of 4 h; finally, overnight mortality was recorded. All tests on rats were performed at three dose levels; 100, 200 and 300 mg/kg of body weight

corresponding to 10, 20 and 30% of LD₅₀ value (1000 mg/kg), respectively.

2.5. Animals

The Sprague–Dawley rats of either sex (200–250 g) were obtained from the central animal house of H.S.K. College of Pharmacy & Research Centre, Bagalkot. The animals were housed at room temperature (25 ± 1 °C) with 50–55% relative humidity and given standard laboratory feed (Amruth, Sangli, Maharashtra) and water *ad libitum*. The study was conducted after obtaining ethics committee clearance from the Institutional Animal Ethical Committee (F. No. HSKCP/IAEC, Clear/2007-08/1-8, dated 28/11/2007). For the present study, animals were randomized into 11 groups of 8 animals each and allowed to acclimatize for 1 week before the experiments.

2.6. Experimental protocol for global ischemia

The Sprague–Dawley rats of either sex (200–250 g) were divided into 11 groups of 8 rats each and fed with drug or vehicle for 10 days prior to the experiment and treated as follow (Shah et al., 2005):

Group-I: normal saline (10 ml/kg, orally), no ischemia.

Group-II: *Matricaria recutita* (chamomile) extract (100 mg/kg, orally), no ischemia.

Group-III: *Matricaria recutita* (chamomile) extract (200 mg/kg, orally), no ischemia.

Group-IV: *Matricaria recutita* (chamomile) extract (300 mg/kg, orally), no ischemia.

Group-V: Quercetin (25 mg/kg, orally), no ischemia.

Group-VI: normal saline (10 ml/kg, orally), bilateral carotid artery (BCA) occlusion for 30 min.

Group-VII: normal saline (10 ml/kg, orally), BCA occlusion for 30 min, followed by reperfusion for 60 min.

Group-VIII: *Matricaria recutita* (chamomile) extract (100 mg/kg, orally), BCA occlusion for 30 min, followed by reperfusion for 60 min.

Group-IX: *Matricaria recutita* (chamomile) extract (200 mg/kg, orally), BCA occlusion for 30 min, followed by reperfusion for 60 min.

Group-X: *Matricaria recutita* (chamomile) extract (300 mg/kg, orally), BCA occlusion for 30 min, followed by reperfusion for 60 min.

Group-XI: Quercetin (25 mg/kg, orally), BCA occlusion for 30 min, followed by reperfusion for 60 min.

2.7. Induction of ischemia

Animals of group VI to XI were subjected to bilateral carotid artery occlusion (Farbiszewski et al., 1995) under ketamine anesthesia (45 mg/kg, i.p.). Animals were placed on the back; both carotid arteries were exposed and occluded by atraumatic clamps. Temperature was maintained around 37 ± 0.5 °C throughout the surgical procedure and artificial ventilation (95% O₂ and 5% CO₂) provided with artificial respirator.

2.8. Preparation of post-mitochondrial supernatant

Following decapitation, the brain was removed and washed in cooled 0.9% saline, kept on ice and subsequently blotted on filter paper, then weighed and homogenized as 10% (w/v) in cold phosphate buffer (0.05 M, pH 7.4). The homogenates were centrifuged at $1000 \times g$ for 10 min at 4 °C (MPW-350R, Korea) and post-mitochondrial supernatant (PMS) was kept on ice until assayed.

2.9. Biochemical estimation

2.9.1. Lipid peroxidation (LPO)

Thiobarbituric acid reactive substances (TBARS) in the homogenate were estimated by using standard protocol (Prabhakar et al., 2006). Briefly, the 0.5 ml of 10% homogenate was incubated with 15% TCA, 0.375% TBA and 5N HCl at 95 °C for 15 min, the mixture was cooled, centrifuged and absorbance of the supernatant measured at 512 nm against appropriate blank. The amount of lipid peroxidation was determined by using $\epsilon = 1.56 \times 10^5 \text{ M}^{-1} \text{ cm}^{-1}$ and expressed as TBARS nmoles/mg of protein (Braugher et al., 1987).

2.9.2. Superoxide dismutase (SOD)

Superoxide dismutase activity was determined based on the ability of SOD to inhibit the auto-oxidation of epinephrine to adrenochrome at alkaline pH (Misra and Fridovich, 1972). Briefly, 25 μl of the supernatant obtained from the centrifuged brain homogenate was added to a mixture of 0.1 mM epinephrine in carbonate buffer (pH 10.2) in a total volume of 1 ml and the formation of adrenochrome was measured at 295 nm. The SOD activity (U/mg of protein) was calculated by using the standard plot.

2.9.3. Catalase (CAT)

Catalase activity was assayed by the method of Claiborne (1985). Briefly, the assay mixture consisted of 1.95 ml phosphate buffer (0.05 M, pH 7.0), 1.0 ml hydrogen peroxide (0.019 M), and 0.05 ml homogenate (10%, w/v) in a total volume of 3.0 ml. Changes in absorbance were recorded at 240 nm. Catalase activity was calculated in terms of nM H_2O_2 consumed/min/mg protein.

2.9.4. Total thiols

This assay is based on the principle of formation of relatively stable yellow color by sulfhydryl groups with DTNB (Moron et al., 1979). Briefly, 0.2 ml of brain homogenate was mixed with phosphate buffer (pH 8), 40 μl of 10 mM DTNB and 3.16 ml of methanol. This mixture was incubated for 10 min and the absorbance was measured at 412 nm against appropriate blanks. The total thiol content was calculated by using $\epsilon = 13.6 \times 10^3 \text{ M}^{-1} \text{ cm}^{-1}$ (Sedlak and Lindsay, 1968).

2.9.5. Glutathione (GSH)

GSH was estimated in various tissues by the method of Sedlak and Lindsay (1968). Briefly, 5% tissue homogenate was prepared in 20 mM EDTA, pH 4.7, and 100 μl of the homogenate or pure GSH was added to 0.2 M Tris–EDTA buffer (1.0 ml, pH 8.2) and 20 mM EDTA, pH 4.7 (0.9 ml) followed by 20 μl of Ellman's reagent (10 mmol/l DTNB in methanol). After 30 min of incubation at room temperature, absorbance was read at 412 nm. Samples were cen-

trifuged before the absorbance of the supernatants was measured (Khyntiam and Prasad, 2003).

2.10. Protein

Protein concentration in all samples was determined by the method of Lowry et al. (1951).

2.11. Measurement of infarction area

The infarction area was measured by 2,3,5-triphenyltetrazolium chloride (TTC) staining method according to Bederson et al. (1986). Following ischemia or reperfusion after varied durations of ischemia, animals were decapitated, and the brains were removed. After the brains were placed briefly in cold saline, four coronal brain slices (2 mm thick) were made. Then the slices were incubated in phosphate buffered saline (pH 7.4) containing 2% 2,3,5-triphenyltetrazolium chloride (TTC) at 37 °C for 10 min and then kept in neutral-buffered formalin overnight. The images of the TTC-stained sections were acquired by scanning with a high-resolution scanner (Hewlett–Packard Scanjet 6100C/T). Then the cerebral infarction area was observed and compared between various treatment groups and negative control group.

2.12. Histopathology

The brains from control and experimental groups were fixed with 10% formalin and embedded in paraffin wax and cut into longitudinal section of 5 μ thickness. The sections were stained with haemotoxylin and eosin dye for histopathological observation.

2.13. Statistical analysis

All the data are presented as means \pm S.E.M. The significance of difference in means between control and treated animals for different parameters was determined by using one-way analysis of variance (ANOVA) followed by multiple comparisons Dunnett's test. A value of $P < 0.05$ was considered statistically significant.

3. Results

3.1. Biochemical estimation

The results showed in Table 1 and Figs. 1–5 revealed potential neuroprotective activity of German chamomile methanolic extract. LPO levels exhibited significant increase and all other enzymatic and non-enzymatic parameters (SOD, CAT, GSH and Total thiols) showed a significant decrease in the BCA-occluded ischemic group (N.S.+I). These levels were further augmented

Table 1

Effect of *Matricaria recutita* L. methanolic extract in global cerebral ischemia/reperfusion-induced oxidative stress in rats.

Groups	Treatment	LPO (nmoles/mg of protein)	SOD (U/mg of protein)	Catalase (U/mg of protein)	GSH (nmoles/mg of protein)	Total thiols ($\mu\text{moles/mg}$ of protein)
I	Normal	12.13 \pm 2.911	171.7 \pm 21.56	0.1508 \pm 0.02223	7.058 \pm 0.6364	18.88 \pm 0.8871
II	100 mg/kg MR	20.61 \pm 2.224 [†]	106.8 \pm 12.91 [†]	0.08268 \pm 0.004997	5.202 \pm 0.4286	15.33 \pm 0.7892 [†]
III	200 mg/kg MR	19.44 \pm 1.322	107.5 \pm 8.720 [†]	0.08915 \pm 0.01985	7.062 \pm 0.6746	18.00 \pm 0.9388
IV	300 mg/kg MR	16.48 \pm 1.281	116.6 \pm 13.45	0.08744 \pm 0.02951	10.03 \pm 0.9453 [†]	17.89 \pm 1.405
V	Quercetin	16.89 \pm 2.153	149.5 \pm 18.38	0.09179 \pm 0.03275	9.259 \pm 0.8319	17.11 \pm 0.5790
VI	N.S. + ischemia	45.94 \pm 4.159 ^{***c}	49.91 \pm 22.32 ^{**}	0.01697 \pm 0.004854 [†]	5.257 \pm 0.2192 [†]	13.71 \pm 1.078 ^{***a}
VII	N.S. + I/R	71.35 \pm 4.566 ^{***}	41.29 \pm 5.812 ^{**}	0.01045 \pm 0.006854 [†]	3.909 \pm 0.3661 ^{***}	9.983 \pm 1.145 ^{***}
VIII	100 mg/kg MR + I/R	41.61 \pm 1.568 ^c	122.6 \pm 31.65	0.1113 \pm 0.01378	7.238 \pm 0.7288 ^c	16.20 \pm 0.9092 ^c
IX	200 mg/kg MR + I/R	27.95 \pm 2.218 ^c	150.4 \pm 20.94 ^b	0.1706 \pm 0.03383 ^b	7.313 \pm 0.4403 ^c	16.58 \pm 0.7089 ^c
X	300 mg/kg MR + I/R	22.33 \pm 5.742 ^c	163.2 \pm 26.58 ^b	0.1459 \pm 0.0179 ^a	7.483 \pm 0.3700 ^c	17.19 \pm 0.4162 ^c
XI	Quercetin + I/R	8.924 \pm 2.413 ^c	212.4 \pm 30.10 ^c	0.2391 \pm 0.06237 ^c	7.153 \pm 0.255 ^c	16.88 \pm 0.5664 ^c

Values are mean \pm S.E.M.; n = 8, one-way analysis of variance (ANOVA) followed by multiple comparison Dunnett test: [†] $P < 0.05$, ^{**} $P < 0.01$ and ^{***} $P < 0.001$ vs. normal and ^a $P < 0.05$, ^b $P < 0.01$ and ^c $P < 0.001$ vs. normal saline + I/R. I/R – ischemia/reperfusion, MR – *Matricaria recutita* L. methanolic extract.

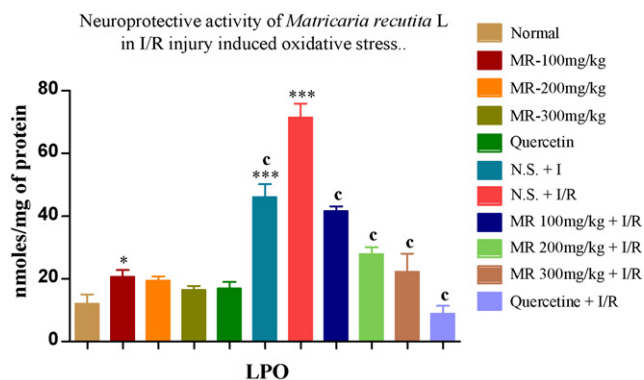


Fig. 1. Estimation of TBARS in the brain homogenates of rats. Statistical significance: one-way analysis of variance (ANOVA), followed by multiple comparison Dunnett test: ^{*} $P < 0.05$, ^{***} $P < 0.001$ vs. normal and ^c $P < 0.001$ vs. N.S. + I/R. N.S. – normal saline, I/R – ischemia/reperfusion, MR – *Matricaria recutita* Linn.

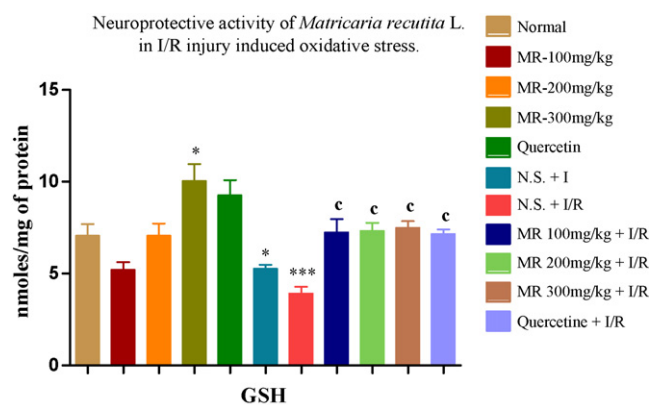


Fig. 4. Estimation of glutathione (GSH) in the brain homogenates of rats. Statistical significance: one-way analysis of variance (ANOVA), followed by multiple comparison Dunnett test: ^{*} $P < 0.05$, ^{***} $P < 0.001$ vs. normal and ^c $P < 0.001$ vs. normal saline + I/R. I/R – ischemia/reperfusion, MR – *Matricaria recutita* Linn.

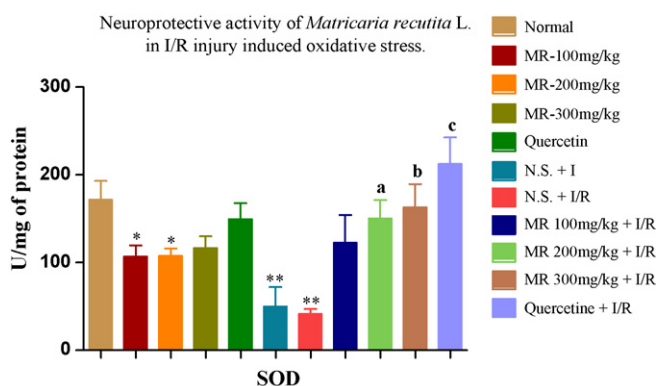


Fig. 2. Estimation of superoxide dismutase (SOD) in the brain homogenates of rats. Statistical significance: one-way analysis of variance (ANOVA), followed by multiple comparison Dunnett test: ^{*} $P < 0.05$, ^{**} $P < 0.01$ vs. normal and ^a $P < 0.05$, ^b $P < 0.01$ and ^c $P < 0.001$ vs. N.S. + I/R. N.S. – normal saline, I/R – ischemia/reperfusion, MR – *Matricaria recutita* Linn.

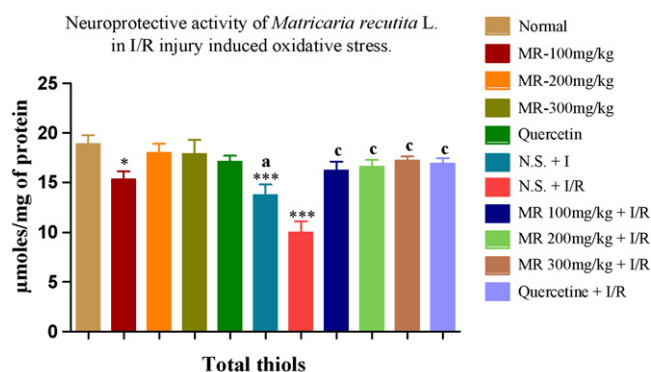


Fig. 5. Estimation of total thiols in the brain homogenates of rats. Statistical significance: one-way analysis of variance (ANOVA), followed by multiple comparison Dunnett test: ^{*} $P < 0.05$, ^{***} $P < 0.001$ vs. normal and ^a $P < 0.05$, ^c $P < 0.001$ vs. N.S. + I/R. N.S. – normal saline, I/R – ischemia/reperfusion, MR – *Matricaria recutita* Linn.

in animals of ischemia/reperfusion (N.S. + I/R) group. The animals from extract treated groups had shown a significant protection by reducing the elevated levels of LPO ($P < 0.001$) and marked increase in SOD ($P < 0.001$), CAT ($P < 0.01$), GSH ($P < 0.001$) and Total thiol ($P < 0.001$) levels as compared ischemia/reperfusion treated group (N.S. + I/R group). The levels of all enzymatic and non-enzymatic parameters were normal in animals treated

with different doses of extract without any ischemia/reperfusion injury.

3.2. Cerebral infarction area

The cerebral infarction area revealed significant decrease in German chamomile treated groups as compared to negative control group especially in caudal and rostral side (Fig. 6).

3.3. Histopathology

As shown in Fig. 7, in-group VI (N.S. + I), ischemia caused marked congestion of blood vessels and neutrophil infiltration (Plate F). These effects were further augmented in group VII (N.S. + I/R), i.e. lymphocytic proliferation and neuronal necrosis (Plate G). There was significant protection of the brain damage observed in the German chamomile treated groups (Plates H–J) and in Quercetin treated group (Plate K). The normal animals and chamomile extract treated animals groups did not showed any pathological changes, when they administered without ischemia (Plates A–E).

4. Discussion

Oxygen is essential for aerobic life, but is also precursor to the formation of harmful ROS (Halliwell, 1991). Oxidative insults, whether over-excitation, excessive release of glutamate or ATP caused by stroke, ischemia or inflammation may initiate various

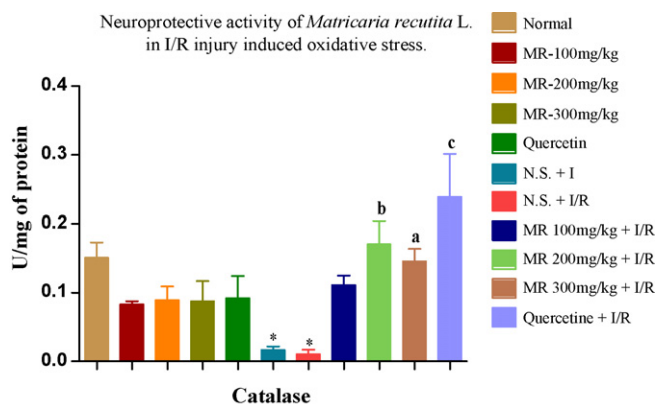


Fig. 3. Estimation of catalase (CAT) in the brain homogenates of rats. Statistical significance: one-way analysis of variance (ANOVA), followed by multiple comparison Dunnett test: ^{*} $P < 0.05$ vs. normal and ^a $P < 0.05$, ^b $P < 0.01$ and ^c $P < 0.001$ vs. N.S. + I/R. N.S. – normal saline, I/R – ischemia/reperfusion, MR – *Matricaria recutita* Linn.

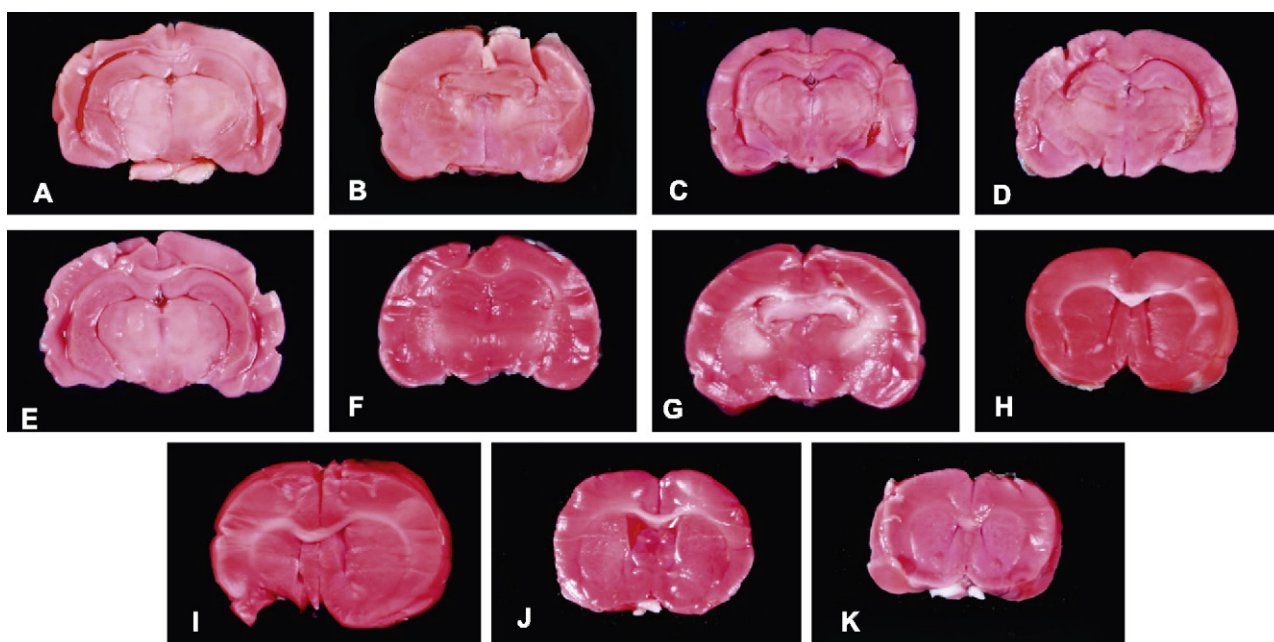


Fig. 6. Neuroprotective effect of *Matricaria recutita* L. methanolic extract against global cerebral ischemia/reperfusion damage in rats evaluated by 2,3,5-triphenyltetrazolium chloride (TTC) staining. Brain coronal sections were prepared (2 mm thickness) and then each section was stained with TTC: (A) normal; (B–D) 100 mg/kg, 200 mg/kg and 300 mg/kg of extract, respectively; (E) quercetin 25 mg/kg; (F) normal saline + ischemia for 30 min; (G) normal saline + ischemia for 30 min followed by 60 min reperfusion (I/R); (H–J) 100 mg/kg + I/R, 200 mg/kg + I/R and 300 mg/kg + I/R, respectively; (K) quercetin 25 mg/kg + I/R. A large infarction area observed mainly in the gray mater and less in brain stem in the damaged brain of ischemia/reperfusion treated rats (G) whereas the infarction was markedly reduced in the rat brains treated with 100 mg/kg (H), 200 mg/kg (I) and 300 mg/kg (J) of extract and also in quercetin treated animals (K); $n = 3$.

signaling cascade leading to apoptotic cell death and neuronal damage (Viglino et al., 1988; Jenner, 1994; Farbiszewski et al., 1995). In the present study, we observed the therapeutic potential of *Matricaria recutita* Linn (German chamomile) for its neuroprotective action on global model of ischemia in which global cerebral ischemia/reperfusion injury induces the oxidative stress and causes neuronal damage in brain. Potential neuroprotective activity of German chamomile were showed by reducing the elevated LPO and severity of oxidative damage in brain tissue was significantly reduced by increasing the levels of antioxidant enzymes SOD, CAT and non-enzymatic markers like GSH and total thiols during ischemia/reperfusion-induced oxidative stress. Free radicals in the living organism are generated both enzymatically and non-enzymatically, leading to the formation of ROS. Among all the ROS generated, OH^- and OONO^- are the most potent and can cause damage to proteins, lipids and nucleic acids, resulting in the inactivation of some enzyme activities, disruption of ion homeostasis and modification of the genetic apparatus and apoptotic death (Albert and Yong-Mei, 1998). The reactive oxygen species of potential importance in cerebral ischemia include superoxide and hydroxyl radicals (Nakashima et al., 1999) and their formation was indicated (Cao et al., 1998) and confirmed (Morimoto et al., 1996) by an increase in hydroxylated salicylate in post-ischemic insult. Glutamate is the principle excitatory neurotransmitter in the brain and an increased glutamatergic transmission has been implied in the pathogenesis of several neurological disorders including cerebral ischemia (Meldrum, 2000). During ischemia, glutamate can be released mainly through two different mechanisms. Either through the Ca^{2+} -dependant and vesicular mode or through the reversed operation of glutamate transporters, NMDA receptors or G-protein pathway (Jabaudon et al., 2000; Rossi et al., 2000). The reversed uptake mechanism and the neuronal glutamate-pool is believed to dominate during ischemia (Ottersen et al., 1996; Danbolt, 2001). There is an increased xanthine oxidase (XO) activity after traumatic brain injury. XO catalyzes the oxidative hydroxylation of

hypoxanthine to xanthine and also the subsequent hydroxylation of xanthine to uric acid, the final two steps of purine metabolism in mammals. After cerebral ischemic events, it is known that XO may catalyze the formation of the superoxide anion in the presence of hypoxanthine. Xanthine degradation by XO leads to the formation of hydrogen peroxide and hydroxyl radicals. The increased XO activity may be responsible for the generation of oxygen free radicals. Additionally, cerebrospinal fluid (CSF), hypoxanthine, xanthine, and uric acid levels reflect glutamate-mediated excitotoxicity in patients with neurological illnesses (Solaroglu et al., 2005). Glutathione is considered a central component in the antioxidant defenses of cells, acts both to directly detoxify reactive oxygen species and as a substrate for various peroxidases. Dysfunction of the glutathione system has been implicated in a number of neurodegenerative diseases (Dringen, 2000; Schulz et al., 2000) and is a potential contributor to oxidative damage following temporary ischemia. The antioxidant status of the tissue affected by ischemia/reperfusion is of great importance for the primary endogenous defense against the free radical-induced injury, was observed in our study in control groups animals, with the increase in LPO and the decrease in protective enzymes levels. In particular, evidence exists that the SOD activity in serum is reduced in stroke patients, and replacement of antioxidant activity could be beneficial in the acute treatment of cerebral ischemia (Spranger et al., 1997).

In addition to it, pretreatment with German chamomile was initiated with the evidence that the limitations of the *in vivo* studies, over *in vitro*, regarding the neuroprotection by free radical scavengers in particular, superoxide dismutase and catalase, the catalytic scavengers for superoxide anions or hydrogen peroxide, respectively, modest protective effects had been observed, when treatment is administered before ischemia, but little to no protection in a delayed-treatment protocol (Tagaya et al., 1992). Therefore, search for agents providing protection against lipid peroxidation and enhancing antioxidant enzyme defense system should, be

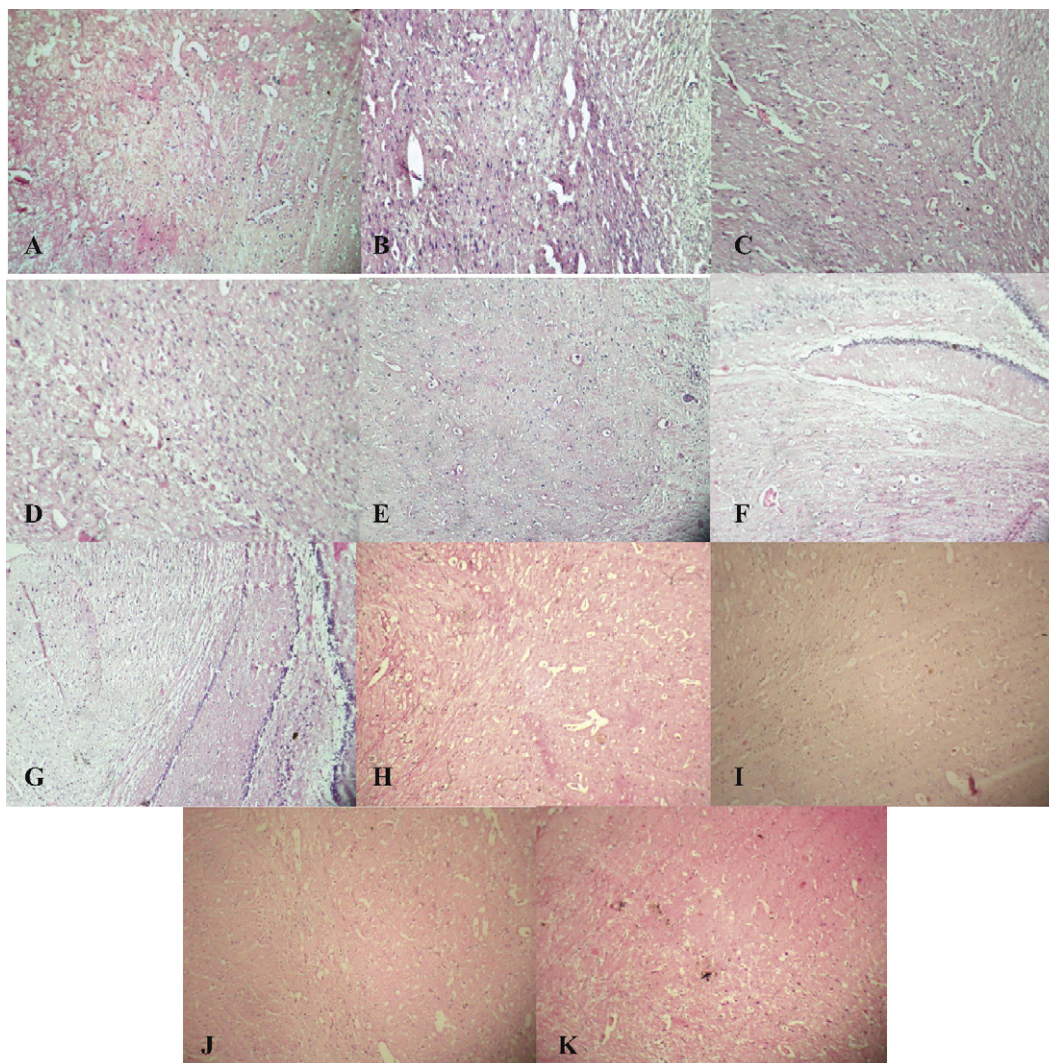


Fig. 7. Neuroprotective effect of *Matricaria recutita* L. methanolic extract against global cerebral ischemia/reperfusion damage in rats. Photographs of brain sections from different treatment groups stained with Haematoxylin & Eosin, 10 \times . Plates: (A) normal; (B–D) 100 mg/kg, 200 mg/kg and 300 mg/kg of extract, respectively; (E) quercetin 25 mg/kg; (F) normal saline + ischemia for 30 min; (G) normal saline + ischemia for 30 min followed by 60 min reperfusion (I/R); (H–J) 100 mg/kg + I/R, 200 mg/kg + I/R and 300 mg/kg + I/R, respectively; (K) quercetin 25 mg/kg + I/R. Ischemia (F) caused marked congestion of blood vessels and neutrophil infiltration. These effects were further augmented by reperfusion, i.e. lymphocytic proliferation and neuronal necrosis (G). There is significant reversal of damage observed in German chamomile treated groups (H–J) and also in quercetin treated group (K). The reversal was marked as the values were mostly comparable with saline and only drug treated rats without ischemia (A–E).

considered a rational approach for therapy of cerebrovascular ailments. In spite of a relatively short period of ischemia (30 min), we have observed in our study a significantly higher extent of damage in the ischemia/reperfusion control group in comparison with the group being treated by German chamomile. Natural products with such properties constitute an ideal choice for maximum therapeutic effect with minimal risk of iatrogenic adverse effects. Our findings, suggests that German chamomile contains flavonoids like apigenin and other phenolic compounds may be responsible for neuroprotective activity mediated through the inhibition of XO oxidase enzyme activity or by decreasing the calcium mobilization, this may leads to decreased glutamate levels or lipid membrane stabilization, because lipid peroxidation produces a progressive loss of membrane fluidity, reduces membrane potential and increased permeability to ions such as calcium. The histopathological studies revealed that in German chamomile treated groups showed significant decreased infiltration and brain damage as comparison to control group. Cerebral infraction area was very less in extract treated groups as compared to control groups. With this German chamomile exhibited marked significant ($P < 0.01$)

protection against BCAA-induced ischemia/reperfusion brain damage.

In conclusion, these findings suggest a potential protective role of German chamomile against the global cerebral ischemia/reperfusion-induced brain injury. Further studies are required to pursue the interesting lead emerging from the present results to exploit the full therapeutic potential of German chamomile as a neuroprotective.

Acknowledgements

We are thankful to Principal and Head, Department of Pharmacology, H.S.K. College of Pharmacy, Bagalkot, Karnataka, India, for providing necessary facilities during the course of this study.

References

- Albert, Y.S., Yong-Mei, C., 1998. Oxidative stress and neurodegenerative disorders. *Journal of Biomedical Science* 5, 401–414.
- Alexi, T., Borlongan, C., Faull, C., Williams, C., Clark, R., Gluckman, P., Hughes, P., 2000. Neuroprotective strategies for basal ganglia degeneration: Parkinson's and Huntington's diseases. *Progress in Neurobiology* 60, 409–470.

- Anonymous, 1998. From what will we die in 2020? (Editorial). *Lancet* 349, 1263–1264.
- Bederson, J.B., Pitts, L.H., Germano, S.M., Nishimura, M.C., Davis, R.L., Bartkowski, H.M., 1986. Evaluation of 2,3,5-triphenyltetrazolium chloride as a stain for detection and quantification of experimental cerebral infarction in rats. *Stroke* 17, 1304–1308.
- Braugher, J.M., Chase, R.L., Pregenzer, J.F., 1987. Oxidation of ferrous iron during peroxidation of various lipid substrates. *Biochimica Biophysica Acta* 921, 457–464.
- Cao, W., Carney, J.M., Duchon, A., Floyd, R.A., Chevion, M., 1998. Oxygen free radical involvement in ischemia and reperfusion injury to brain. *Neuroscience Letters* 88, 233–238.
- Claiborne, A., 1985. Catalase activity. In: Greenwald, R.A. (Ed.), *CRC Hand Book of Methods for Oxygen Radical Research*. CRC Press, Boca Raton, Florida, USA, pp. 283–284.
- Danbolt, N.C., 2001. Glutamate uptake. *Progress in Neurobiology* 65, 1–105.
- Dringen, R., 2000. Metabolism and functions of glutathione in brain. *Progress in Neurobiology* 62, 649–671.
- Dugan, L.L., Choi, D.W., 1999. Hypoxic-ischemic brain injury and oxidative stress. In: Siegel, G.J., Agranoff, B.W., Alber, R.W., Fischer, S.K., Uhler, M.D. (Eds.), *Basic Neurochemistry*. Lippincott-Raven Publishers, Philadelphia, pp. 637–669.
- Farbiszewski, R., Bielawski, K., Bielawska, A., Sobaniec, W., 1995. Spermine protects in vivo the antioxidant enzymes in transiently hypoperfused rat brain. *Acta Neurobiologica Experimentalis* 55, 253–258.
- Halliwell, B., 1991. Reactive oxygen species in living systems: source, biochemistry, and role in human disease. *American Journal of Medicine* 91, 145–225.
- Huang, Y., McNamara, J.O., 2004. Ischemic stroke: “Acidotoxicity” is a perpetrator. *Cell* 118, 665–670.
- Jabaudon, D., Scanziani, M., Gahwiler, B.H., Gerber, U., 2000. Acute decrease in net glutamate uptake during energy deprivation. *Proceedings of the National Academy of Sciences of the United States of America* 97, 5610–5615.
- Jenner, P., 1994. Oxidative damage in neurodegenerating diseases. *Lancet* 344, 796–798.
- Khyriam, D., Prasad, S.B., 2003. Changes in endogenous tissue glutathione level in relation to murine ascites tumor growth and the anticancer activity of cisplatin. *Brazilian Journal of Medical and Biological Research* 36, 53–63.
- Kinouchi, H., Epstein, C.J., Mizui, T., Carlson, E., Chen, S.F., Chan, P.H., 1991. Attenuation of focal cerebral ischemic injury in transgenic mice over expressing CuZn superoxide dismutase. *Proceeding of National Academy of Sciences* 88, 11158–11162.
- Litchfield, J.T., Wilcoxon, F., 1949. A simplified method of evaluating dose–effect experiments. *Journal of Pharmacology and Experimental Therapeutics* 96, 99–113.
- Liu, H., Beckman, J.S., Freeman, B.A., Hogan, E.L., Hsu, C.Y., 1989. Polyethylene glycol-conjugated superoxide dismutase and catalase reduce ischemic brain injury. *American Journal of Physiology* 256, H589–H593.
- Lowry, O.H., Rosebrough, N.J., Fair, A.L., Randall, R.J., 1951. Protein measurement with Folin phenol reagent. *Journal of Biological Chemistry* 193, 265–275.
- Martens, D., 1995. Chamomile: the herb and the remedy. *The Journal of the Chiropractic Academy of Homeopathy* 6, 15–18.
- Meldrum, B.S., 2000. Glutamate as a neurotransmitter in the brain: review of physiology and pathology. *The Journal of Nutrition* 130, 1007–1015.
- Misra, H.P., Fridovich, I., 1972. The role of superoxide anion in the autooxidation of epinephrine and a simple assay for superoxide dismutase. *Journal of Biological Chemistry* 247, 3170–3175.
- Morimoto, T., Globus, M.Y., Busto, R., Martinez, E., Ginsberg, M.D., 1996. Simultaneous measurement of salicylate hydroxylation and glutamate release in the penumbral cortex following transient middle cerebral artery occlusion in rats. *Journal of Cerebral Blood Flow and Metabolism* 16, 92–99.
- Moron, A., De Pierre, J.W., Mannervick, B., 1979. Levels of glutathione, glutathione reductase, glutathione-S-transferase activities in rat liver. *Biochimica et Biophysica Acta* 582, 67–68.
- Nakashima, M., Niwa, M., Iwai, T., Uematsu, T., 1999. Involvement of free radicals in cerebral vascular reperfusion injury evaluated in a transient focal cerebral ischemia model of rat. *Free Radical Biology and Medicine* 26, 722–729.
- Newall, C.A., Anderson, L.A., Phillipson, J.D., 1996. *Herbal Medicines: A Guide For Health-Care Professionals*. Pharmaceutical Press, London.
- Nicholis, D., Attwell, D., 1990. The release and uptake of excitatory amino acids. *Trends in Pharmacological Sciences* 11, 462–468.
- Nicotera, P., Lipton, S., 1999. Excitotoxins in neuronal apoptosis and necrosis. *Journal of Cerebral Blood Flow and Metabolism* 19, 583–591.
- Norris, J.W., Hachinski, V.C., 1993. Stroke prevention: past, present and future. In: Norris, J.W., Hachinski, V.C. (Eds.), *Prevention of Stroke*. Springer-Verlag, New York, pp. 1–15.
- Ottersen, O.P., Laake, J.H., Reichelt, W., Haug, F.M., Torp, R., 1996. Ischemic disruption of glutamate homeostasis in brain: quantitative immunocytochemical analysis. *Journal of Chemical Neuroanatomy* 12, 1–14.
- Picq, M., Dubois, M., Munri-Silem, Y., Prugent, A.F., Pacheco, H., 1989. Flavonoid modulation of protein kinase C activation. *Life Sciences* 44, 1563–1571.
- Prabhakar, K.R., Veerapur, V.P., Vipani, P.K., Priyadarsini, K.I., Rao, B.S.S., Unnikrishnan, M.K., 2006. Evaluation and optimization of radio protective activity of *Coronopus didymus* Linn. in γ -irradiated mice. *International Journal of Radiation Biology* 82, 525–536.
- Rossi, D.J., Oshima, T., Attwell, D., 2000. Glutamate release in severe brain ischemia is mainly by reversed uptake. *Nature* 403, 316–321.
- Salaman, I., 1992. Chamomile: a medicinal plant. *The Herb, Spice, and Medicinal Plant Digest* 10, 1–4.
- Schulz, J.B., Lindenau, J., Seyfried, J., Dichgans, J., 2000. Glutathione, oxidative stress and neurodegeneration. *European Journal of Biochemistry* 267, 4904–4911.
- Sedlak, J., Lindsay, R.H., 1968. Estimation of total, protein-bound, and non-protein sulfhydryl groups in tissue with Ellman’s reagent. *Analytical Biochemistry* 25, 192–205.
- Shah, Z.A., Gilani, R.A., Sharma, P., Vohora, S.B., 2005. Cerebroprotective effect of Korean ginseng tea against global and focal models of ischemia in rats. *Journal of Ethnopharmacology* 101, 299–307.
- Solaroglu, I., Okutan, O., Kaptanoglu, E., Beskonakli, E., Kilinc, K., 2005. Increased xanthine oxidase activity after traumatic brain injury in rats. *Journal of Clinical Neuroscience* 12, 273–275.
- Spranger, M., Krempien, S., Schwab, S., Donneberg, S., Hacke, W., 1997. Superoxide dismutase activity in serum of patients with acute cerebral ischemic injury; correlation with clinical course and infarct size. *Stroke* 28, 2425–2428.
- Tagaya, M., Matsumoto, M., Kitagawa, K., Niinobe, M., Ohtsuki, T., Hata, R., Ogawa, S., Handa, N., Mikoshiba, K., Kamada, T., 1992. Recombinant SOD can attenuate ischemic neuronal damage in gerbils. *Life Sciences* 51, 253–259.
- Trease, G.E., Evans, W.C., 1989. *Pharmacognosy*, 13th ed. Bailliere Tindall, London, pp. 683–684.
- Vigilino, P., Scarpa, M., Rotilio, G., Rigo, A., 1988. A kinetic study of the reactions between H₂O₂ and Cu–Zn superoxide dismutase, evidence for an electrostatic control of the reaction rate. *Biochemistry Biophysics Acta* 952, 77–82.
- Wade, S.S., Johnson, S.C., Easton, J.D., 2005. Cerebrovascular disease. *Principle’s of Internal Medicine*, vol. II. McGraw-Hill, pp. 2372.
- Welch, K.M.A., Kaplan, L.R., Reis, D.J., Siesjo, B.K., Weir, B., 1997. *Primer on Cerebrovascular Diseases*. Academic Press, San Diego, CA, USA.
- Yu, Z.F., Bruce-Keller, A.J., Goodman, Y., Mattson, M.P., 1998. Uric acid protects neurons against excitotoxic and metabolic insults in cell culture, and against focal ischemic brain injury in vivo. *Journal of Neuroscience Research* 53, 613–625.