



E-ISSN: 2320-7078  
P-ISSN: 2349-6800  
JEZS 2016; 4(5): 738-747  
© 2016 JEZS  
Received: 12-07-2016  
Accepted: 13-08-2016

**Moattar Raza Rizvi**

Assistant Professor (Medical Physiology), Department of Nursing, College of Applied Medical Science, Majmaah University, Majmaah, Kingdom of Saudi Arabia

**Mohammad Al-Mansour**

Assistant Professor, Family Medicine, College of Medicine, Majmaah University, Majmaah, Kingdom of Saudi Arabia

**Mohammad Al-Rukban**

Professor & Consultant of Family Medicine, College of Medicine, King Saud University, Kingdom of Saudi Arabia

**Nasser Ali Jarallah**

Vice Dean of Academic Affairs, Applied Science College, AlMaarefa Colleges, Riyadh, Kingdom of Saudi Arabia

**Raidah Saleem Al-Baradie**

Associate Professor, Pediatric Neurologist/Epileptologist, KFUH, Al-Khobar, Kingdom of Saudi Arabia

**Khalid Tohami**

Assistant Professor, Community Medicine, Department of Public Health & Community Medicine, College of Medicine, Majmaah University, Majmaah KSA

**Waqas Sami**

Lecturer (Biostatistics), Department of Public Health & Community Medicine, College of Medicine, Majmaah University, Majmaah

**Kashif Hanif**

Scientist, Division of Pharmacology, CSIR, Central Drug Research Institute (CDRI), Lucknow, India

**Raid Al-Baradie**

Associate Professor (Pathology), Department of Medical Laboratories, College of Applied Medical Science, Majmaah University, Majmaah, Kingdom of Saudi Arabia

**Correspondence****Salma Batool**

Department of Zoology, University of Gujrat Hafiz Hayat Campus, Gujrat, Pakistan

## Role of natural herbs - *Matricaria recutita* and *Artemisia absinthium* on infarct volume, hemispheric swelling and functional impairments following focal cerebral ischemia in rats

**Moattar Raza Rizvi, Mohammad Al-Mansour, Mohammad Al-Rukban, Nasser Jarallah, Raidah Saleem Al-Baradie, Khalid Tohami, Waqas Sami, Kashif Hanif, Raid Al-Baradie**

**Abstract**

Stroke ranks as the third largest cause of death worldwide after ischemic heart disease and cancer. Despite recent advances in antithrombotic treatment, post-stroke disability has significant economic and social burden. Thus, the problem of finding a safe and effective acute neuroprotective treatment for stroke remains urgent. The present study evaluates neuroprotective and antioxidant action of two natural herbs - *Matricaria recutita* and *Artemisia absinthium* following middle cerebral artery occlusion (MCAo) in rats. Thirty-six male rats were randomly selected into 6 groups (n=6 per group) as follows- Group 1: Sham-operated rat without MCAo, Group 2: Ischemic control following MCAo, Group 3: *M. recutita* (200 mg/kg) + MCAo, Group 4: *M. recutita* (400 mg/kg) + MCAo, Group 5: *A. Absinthium* (200 mg/kg) + MCAo, Group 6: *A. Absinthium* (400 mg/kg) + MCAo. The effect of extract on the volume of infarct, hemisphere swelling, neurobehavioral score, grip strength and oxidative stress [levels of thiobarbituric acid reactive substances (TBARS), superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione-S-transferase (GST) and reduced glutathione (GSH)] were estimated following middle cerebral artery occlusion (MCAo). *Matricaria recutita* pretreatment at dose of 400 mg/kg showed significant neuroprotective effect on infarct area, infarct volume, hemispheric swelling, neurobehavioral score and grip strength test following cerebral ischemia in rats. *Artemisia absinthium* could not produce any significant neuroprotection except for significant reduction in hemisphere swelling at a dose of 400 mg/kg. *Matricaria recutita* also showed significant decrease in lipid peroxidation (LPO) and increase in the superoxide dismutase (SOD) activities, catalase (CAT), reduced glutathione (GSH), total thiol and protein levels in group 3 and 4 (*Matricaria recutita* treated MCAo animals) as compared with sham operated and MCAo group ( $P<0.001$ ). There was no remarkable change in the oxidative stress parameters in group 5 and group 6 (MCAo rats pretreated with *Artemisia absinthium*). The present study for the first time shows potent neuroprotective activity of the methanol extract of *Matricaria recutita* in rats following cerebral ischemia through middle cerebral artery occlusion.

**Keywords:** *Matricaria recutita*, chamomile, neuroprotective, oxidative stress, Middle Cerebral Artery (MCAo) occlusion

**Introduction**

Cerebral stroke has been found to be the second most common cause of death and disability globally. About 80% of ischemic strokes are focal in nature and only 20% are due to intracerebral bleeding. As per WHO, stroke and other cerebrovascular diseases are responsible for approximately 5.7 million deaths each year. In the United States alone, it is estimated that 780,000 symptomatic strokes are detected each year, which may be accompanied by a further 11 million asymptomatic strokes [1]. The need to reduce this burden should target the appropriate use of existing therapies and identification of future ones is imperative. Currently, the only interventions to improve functional recovery after stroke are rehabilitative strategies, which have had limited success, and genetically engineered tissue plasminogen activators (tPAs), which are still the only medications approved by the FDA and come with serious medical risks and limitations [2]. Thus, the problem of finding a safe and effective acute neuroprotective treatment for stroke remains urgent. Herbs have been used for treating diseases for centuries, and many natural neuroprotective compounds from plants had been discovered [3]. Treatment of stroke by Traditional Chinese Medicine has a wealth of clinical experience and theoretical basis, and a large number of effective clinical prescriptions have been accumulated [4].

*Matricaria recutita* L. is a well-known medicinal plant that is suggested as being carminative, analgesic, and anticonvulsant in traditional medicine [5]. A depressive activity of a lyophilized infusion of flowers of *M. recutita* on the central nervous system have been demonstrated [6]. The flowers of this plant have been used as demulcent, anti-inflammatory, and analgesic, anxiolytic, and sedative in Iranian folk medicine [7]. The pharmacological profile of apigenin, and flavonoid isolated from this plant, was studied and the results have shown that this compound may act on benzodiazepine receptors in the central nervous system [8].

*Artemisia absinthium* L., commonly known as wormwood belongs to the Asteraceae family and grows as a perennial herb with fibrous roots. Wormwood is used as stomachic, antiseptic, antispasmodic, carminative, cholagogue, febrifuge and anthelmintic [9]. Traditionally, dried flowers of arnica are used, as tincture or ointment, for topically treatment of skin bruises, contusions and pain [10-11]. The extracts of the plant have shown to inhibit strong antimicrobial activity [12].

The aim of this study was to investigate the neuroprotective effect of hydro-methanolic extract of *Matricaria recutita* and *Artemisia absinthium* on hemispheric swelling, cerebral infarction, and functional deficits induced by MCAo in the rat. Further, we explored the neuroprotective activity of herbal extract on oxidative stress. Finally, we examined whether *Matricaria recutita* and *Artemisia absinthium* could protect against cerebral-ischemic injury by enhancing the antioxidant defence capacity.

## Materials and Methods

### Drugs and chemicals

Trichloroacetic acid (TCA), 2-thiobarbituric acid (TBA) were purchased from Sigma-Aldrich Co., St. Louis, MO, USA. 2,3,5-triphenyltetrazolium chloride (TTC) was purchased from HiMedia, Mumbai. All other chemicals were of the highest purity.

### Preparation of plant extracts

#### *Matricaria recutita*

Seventy grams of powder of dried aerial parts of *M. recutita* was extracted with 80% aqueous methanol by percolation for (72 h). The extract was filtered, and the residue was concentrated by a rotary evaporator apparatus (45 °C) and then dried at room temperature. The weight of the dried extract

was 6 g, corresponding to an 8.6% yield. The residue was dissolved in normal saline to obtain the desired concentration (200 and 400 mg/10 mL) [13-14].

#### *Artemisia absinthium*

*A. absinthium* leaves were washed thoroughly in tap water to remove adhering mud particles, rinsed in distilled water, drained, and dried in a hot air oven at 40±2 °C for 2 days. The dried leaves were finely powdered. The dried powder was extracted with 5x2 L methanol for 24 h. After removal of the solvent in vacuum, the crude extract was suspended in 0.5 L distilled water and extracted with 0.2 L portions of ethyl acetate until the extracts were nearly colorless. Solvents were removed in vacuo, and extracts were obtained respectively [15].

### Sample Size Calculation for the Model of Stroke

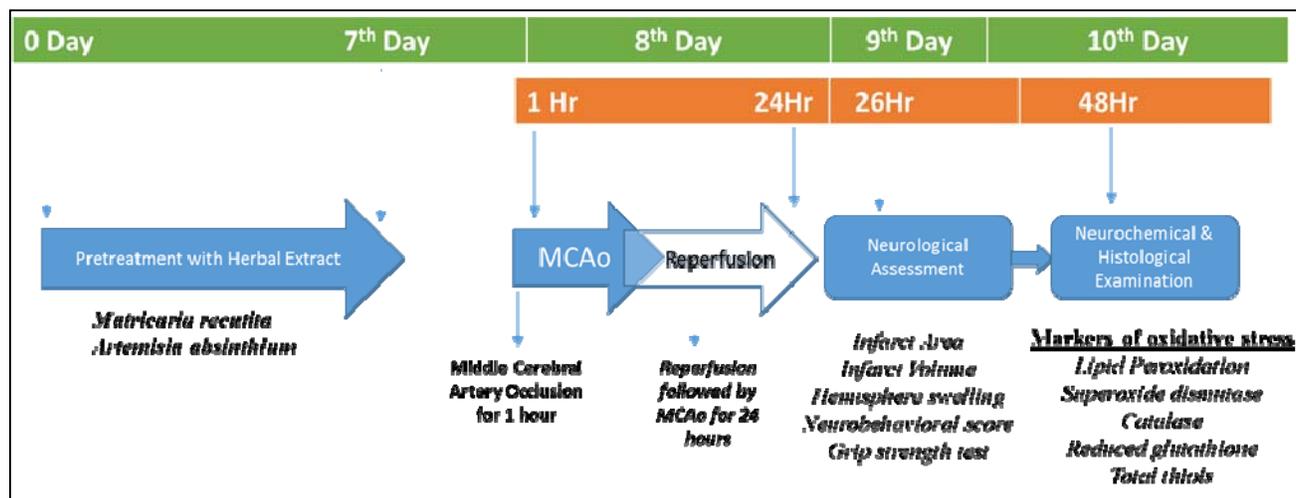
Initial experiments with the model used here suggested that mortality rates of ~50% and ~20% likely would be encountered with sham vs. MCAo. Power analysis was performed (Power and Precision; release 3.2) for a two-sample proportion with these rates. It was found that group sizes of 25-40 would yield 61-82% power of rejecting the null hypothesis ( $\alpha 0.05$ ; two-tailed).

### Experimental subjects

Male Sprague-Dawley rats (200-300g) were housed 5 per cage and had free access to water and food. They were maintained at a constant temperature (22±2 °C) and humidity (55±10%), with a 12-h light/dark cycle (lights on 07:30-19:30 h). Animals were randomized into six groups of eight animals and all rats were acclimatized to the laboratory for at least 1 h before testing and were used for one experiment only. Approved protocols met the International Guidelines: Principle of Laboratory Animal Care (NIH publication no. 85-23, revised 1985) requires that investigators take all necessary steps to minimize the number of animals needed for statistical analysis and any unnecessary pain and discomfort caused by the experimental procedures.

### Experimental Design

All the animals were subjected to two-hours MCAo followed by a 24-hr reperfusion and were divided in following six groups each containing at least 6 (n = 6) animals.



**Group 1:** Sham-operated rat without MCAo.

**Group 2:** Rats having MCAo without administration of any drug (Ischemic control).

**Group 3:** Receives 10 ml of *Matricaria recutita* extract 200 mg/kg alone for 7 days (p.o.), on 8<sup>th</sup> day middle cerebral artery occlusion for 1 hour followed by 24 hr reperfusion.

**Group 4:** Receives 10 ml of *Matricaria recutita* extract 400 mg/kg alone for 7 days (p.o.), on 8<sup>th</sup> day middle cerebral artery occlusion for 1 hour followed by 24 hr reperfusion.

**Group 5:** Receives 10 ml of *Artemisia absinthium* extract 200 mg/kg alone for 7 days (p.o.), on 8<sup>th</sup> day middle cerebral artery occlusion for 1 hour followed by 24 hr reperfusion.

**Group 6:** Receives 10 ml of *Artemisia absinthium* extract 400 mg/kg alone for 7 days (p.o.), on 8<sup>th</sup> day middle cerebral artery occlusion for 1 hour followed by 24 hr reperfusion.

Three rats in each group were used for histological analysis on 9<sup>th</sup> day and 26 hr after MCAo. In the case of the sham-operated and vehicle-treated MCAo groups, an amount of vehicle equivalent to drug treatment conditions was administered in the same manner.

**Middle Cerebral Artery Occlusion (MCAO)-Induced Focal Cerebral Ischemia in Rats** Adult (8-10 week-old) Sprague Dawley rats were anesthetized with chloral hydrate (300 mg/kg) and placed in a supine position on a preheated operating table with thermostatically controlled temperature to maintain at  $37 \pm 0.5$  °C during the surgery. Focal cerebral ischemia was induced in the male rats by the middle cerebral artery occlusion (MCAo) using a modification of the intraluminal technique [16-17].

In brief, after the midline incision of the ventral cervical skin, the left common carotid was exposed and was traced anteriorly to reveal the external carotid artery (ECA) and internal carotid artery (ICA). A 4.5 cm long 3-0 nylon suture (Ethicon, Johnsons & Johnsons Ltd, Mumbai) with rounded tip was introduced into the ICA through a small nick in the ECA and gently advanced about two-centimetres from the ICA origin until the resistance was felt, which blocked the origin of middle cerebral artery (MCA). The suture was retracted after one-hour to allow reperfusion. In sham-operated rats, a similar surgical procedure was performed except for the insertion of suture. The experimental animals were allowed to recover from anesthesia and then transferred into an animal house, with temperature maintained at  $37 \pm 0.5$  °C, and were allowed food and water *ad libitum*.

Criteria for inclusion of rats from the study group was based on laser-Doppler flowmetry (LDF) measurement of cerebral blood flow (CBF). LDF is an established, practical, and reliable system for monitoring changes in CBF due to induction of focal cerebral ischemia [18]. To ensure relative uniformity of the ischemic insult, animals with mean ischemic CBF >30% (measured over a 5-minute period after occlusion) of baseline CBF were excluded from the cohort. This procedure results in more edema and more uniform infarcts, reducing experimental variability.

#### Neurobehavioral assessment

The assessment of neurological deficit was performed by a blind observer on a 10-point scale after 24 h of reperfusion in the all groups of rats [16, 17]. The neurological deficit was scored as follows: Flexion (failure to fully extend left forepaw) = 1; Circling (movement in circles in a clockwise, contralateral direction) = 2; Hemiparesis (failure to scale a vertical grid due to loss of grip) = 3; Non-spontaneity (no

spontaneous movement observed) = 4. The neurobehavioral scores obtained after testing on each scale were averaged to denote the degree of neurological deficit. Animals were sacrificed after 48 h of reperfusion for biochemical and histological studies.

#### Assessment of Grip Strength

Forelimb grip strength in rats was determined 24 h after surgery using a grip-strength meter (Columbus Instruments, Columbus, OH, USA). We used an electronic digital force gauge that measured the peak force exerted by the action of the animal while gripping the sensor bar. While being drawn back along a straight line leading away from the sensor, the animal released its grip at some point and the gauge then recorded the maximum force attained at the time of release. The digital reading (in Kg units) of three successive trials was obtained for each rat, averaged, and used for data analysis.

#### Analysis of brain infarct volume

The rats were sacrificed for histology at 48 h after occlusion with an overdose (75 mg/kg) of Nembutal sodium solution and their brains were extracted and evaluated for hemispheric swelling and infarct volume. The brains were carefully removed and placed in chilled saline, and then sliced into 7 serial coronal sections of 2mm thickness using a rat brain matrix (Harvard Apparatus) starting at 3mm posterior to the anterior pole. After sectioning, the slices were stained with 2% 2, 3, 5-triphenyltetrazoliumchloride (TTC; Sigma Aldrich, St. Louis, MO, USA) in saline and kept for 15 min at 37 °C.

Both hemispheres of each stained coronal section was scanned using a high resolution scanner (Epson Perfection 2400 Photo), and further evaluated by digital image analysis (Image Pro System, Media Cybernetics, Silver Spring, MD, USA). Unstained areas (pale color) were defined as ischemic lesions. The areas of infarction and total areas of both hemispheres was calculated for each brain slice.

An edema index was calculated by dividing the total volume of the left hemisphere by the total volume of the right hemisphere. The actual infarct volume adjusted for edema was calculated by dividing the infarct volume by the edema index.

Infarct volumes was expressed as percentage of contralateral hemisphere. The infarct volume percentage was calculated thus: (contralateral hemisphere volume - volume of nonischemic ipsilateral hemisphere) / contralateral hemisphere volume  $\times$  100%. These techniques have been used repeatedly in the literature to measure and evaluate stroke outcome in experimental preparations [19, 20].

#### Neurochemical Assays Preparation of Tissue Homogenate

Cortical cortexes were collected after sacrificing the rats at 48 hours after MCAo. A 10% (w/v) homogenate was prepared ice-cold phosphate buffered saline (0.1 M, pH 7.4) using Teflon glass homogenizer and the supernatant was obtained after centrifugation at 10,000 r.p.m. at 4 °C for 15 min and the pellet was discarded. Neurochemical assays were conducted in accordance with the specification of medical kits. The supernatant obtained was used for the neurochemical assays for quantification of antioxidant levels like GSH, CAT, LPO, TT, SOD, total protein levels in accordance with the specification of medical kits [21].

#### Lipid Peroxidation (LPO)

Briefly, the reaction mixture contained 0.1 ml of brain regions homogenate/ mitochondria (1mg protein), 1.5 ml of 20%

acetic acid (pH 3.5), 1.5 ml of 0.8% thiobarbituric acid (0.8% w/v) and 0.2 ml SDS. Following these additions, tubes were mixed and heated at 95 °C for one hour on a water bath and cooled under tap water before mixing 1 ml of distilled water and 5ml mixture of n-butanol and pyridine (15:1). The mixture was centrifuged at 2200g for 10 min. The amount of MDA/TBARS formed was measured by the absorbance of upper organic layer at a wave length of 532 nm. The results are expressed as nmol MDA/mg protein. The absorbance of the clear pink color supernatant was measured at 532 nm against appropriate blank. The amount of lipid peroxidation was determined by using molar extinction coefficient  $1.56 \times 10^5 \text{ M}^{-1} \text{ cm}^{-1}$  and the results were expressed as nMoles MDA/g of protein [22].

#### Catalase (CAT)

In brief, the incubation mixture contained in a final volume of 2.0 ml, 0.1 ml of diluted homogenate, 1.0 ml of phosphate buffer and 0.4 ml of distilled water to which 0.5 ml of  $\text{H}_2\text{O}_2$  solution was added to initiate the reaction, while the  $\text{H}_2\text{O}_2$  solution was left out in control tubes. After incubating for 1 min at 37 °C the reaction was stopped by addition of 2 ml of potassium dichromate acetic acid reagent. The samples were kept in boiling water bath for 15 minutes, finally cooled and the absorbance measured at 570 nm against control. The catalase content was calculated by using molar extinction coefficient =  $58.03 \times 10^{-3} \text{ M}^{-1} \text{ cm}^{-1}$  and the values are expressed as nMoles/mg protein [23].

#### Reduced Glutathione (GSH)

The assay is based on the principle of Ellman's reaction. The sulfhydryl group of glutathione reacts with DTNB (5,5-dithiobis-2-nitrobenzoic acid) and produces a yellow colored 5-thio- 2nitrobenzoic acid (TNB). Measurement of the absorbance of TNB at 412 nm provides an accurate estimation of glutathione in a sample. Briefly, 0.5 ml of homogenate is mixed with 0.1 ml of 25% TCA to precipitate proteins and centrifuged at 4000rpm for 5 min. Then 0.3 ml of the supernatant was mixed with 0.5 ml of 0.1M phosphate buffer (pH 7.4) and 0.2 ml of 10 mM DTNB. This mixture was incubated for 10 min and the absorbance was measured at 412 nm against appropriate blanks. The glutathione content was calculated by using extension coefficient  $13.6 \times 10^3 \text{ M}^{-1} \text{ cm}^{-1}$ . The values are expressed as nMoles/mg protein<sup>24</sup>.

#### Super Oxide Dismutase (SOD)

The assay mixture contained 0.1ml of sample, 1.2ml of

sodium pyrophosphate buffer (pH 8.3, 0.052M), 0.1 ml of phenazine methosulphate (186 $\mu\text{m}$ ), 0.3 ml of nitro blue tetrazolium (300 $\mu\text{m}$ ), 0.2 ml of NADH (750 $\mu\text{M}$ ). Reaction was started by addition of NADH. After incubation at 30 °C for 90 secs, the reaction was stopped by the addition of 1 ml of glacial acetic acid. The mixture was allowed to stand for 10 min. The color intensity of the chromogen was measured at 560 nm against blank and concentration of SOD was expressed as units/min/mg of protein [25, 26].

#### Total Thiols (TT)

The assay is based on the formation of a relatively stable yellow product when sulphhydryl groups react with DTNB. Briefly, 0.2 ml of brain tissue supernatant was mixed with 0.36 ml of 0.1 M phosphate buffer (pH= 7.4), 40  $\mu\text{l}$  of 10 mM DTNB and 1.4 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 molar extinction coefficient =  $13.6 \times 10^3 \text{ M}^{-1} \text{ cm}^{-1}$  and the values are expressed as nMoles/mg protein [27].

#### Total Protein

The total protein of brain tissue was determined by Biuret method in ERBA diagnostic kit [28]. Total protein (g/dl) = Absorbance of test/Absorbance of standard concentration of standard (g/dl).

#### Statistical Analysis

SPSS 21.0 for Windows (SPSS Inc.) was used to analyze the data. Results are presented as the mean  $\pm$  S.D. and statistical differences between groups were analyzed by ANOVA followed by the Tukey's HSD Post-hoc test. Differences were considered statistically significant if P values were less than 0.05.

#### Results

##### Effects of *Matricaria recutita* and *Artemisia absinthium* on Neurobehavioral score

*Matricaria recutita* of 200 and 400 mg/kg p.o. (per os) decreased the neurobehavioral score as compared to the vehicle-treated MCAo group. But *Matricaria recutita* (400 mg/kg) showed statistical difference as compared with the MCAo group. There was no significant decrease in the neurobehavioral score following *Artemisia absinthium* of 200 and 400 mg/kg p.o. Data are expressed as means  $\pm$  S.D., \* $P < 0.05$ , \*\* $P < 0.01$  vs. vehicle-treated MCAo group, n = 6.

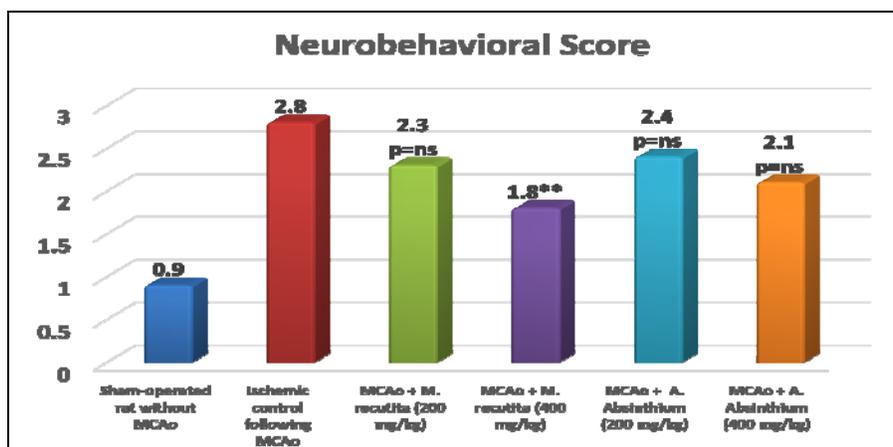


Fig 1: Neuroprotective effects of *Matricaria recutita* and *Artemisia absinthium* on Neurobehavioral score. Neurobehavioral score was measured at 24 h after MCAo.

**Neuroprotective effects of *Matricaria recutita* and *Artemisia absinthium* on Grip-Strength Test**

The grip strength in the sham-operated group was 0.963±0.11 kg units. A significant decrease in the grip strength was observed in the MCAo group, as compared to the sham rats ( $P<0.001$ ). *Matricaria recutita* at higher dose of 400 mg/kg p.o. significantly improved the grip strength whereas this improvement did not reach to a level of significant with 200 mg/kg of *Matricaria recutita*. Further, *Artemisia absinthium* of 200 and 400 mg/kg p.o. slightly improved the grip strength but this increase in grip strength was not significant (Table 1).

**Table 1:** Neuroprotective effects of *Matricaria recutita* and *Artemisia absinthium* on Grip Strength test (kg units)

Groups	Grip Strength (Kg Units)	p value
Group 1: Sham-operated rat without MCAo	0.96±0.11	
Group 2: Ischemic control following MCAo	0.64±0.08	0.0001
Group 3: <i>M. recutita</i> (200 mg/kg)+MCAo	0.77±0.07	0.11
Group 4: <i>M. recutita</i> (400 mg/kg) + MCAo	0.81±0.10	0.0001
Group 5: <i>A. Absinthium</i> (200 mg/kg) + MCAo	0.69±0.08	0.879
Group 6: <i>A. Absinthium</i> (400 mg/kg) + MCAo.	0.72±0.06	0.512

**Effects of *Matricaria recutita* and *Artemisia absinthium* on cerebral Infarct Size**

Representative brain slices showed that normal brain tissues were stained deep red, whereas the infarct tissues were not stained by (Triphenyl tetrazolium chloride) TTC. TTC-staining results showed that MCAo significantly caused serious brain infarct area. *Matricaria recutita* treatment resulted in significant decrease with 400 mg/kg dose ( $p<0.001$ ) but not at 200 mg/kg dose ( $p = ns$ ) (Fig 2). In *Artemisia absinthium* treated group, there was no significant change in infarct area of brain both with 200 mg/kg and 400 mg/kg dose.



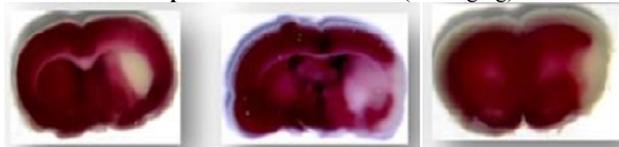
**Group 1:** Sham-operated rat without MCAo



**Group 2:** Ischemic control following MCAo



**Group 3:** MCAo + *M. recutita* (200 mg/kg)



**Group 4:** MCAo + *M. recutita* (400 mg/kg)



**Group 5:** MCAo + *A. Absinthium* (200 mg/kg)

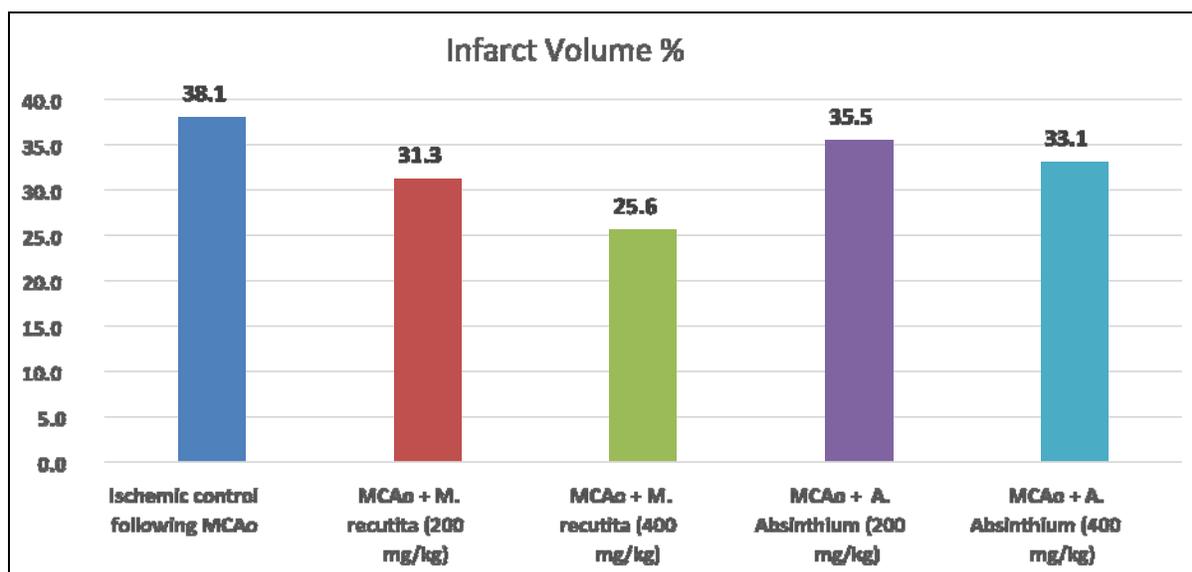


**Group 6:** MCAo + *A. Absinthium* (400 mg/kg)

Figure 2. Neuroprotective effects of *Matricaria recutita* and *Artemisia absinthium* on percentage of brain infarct area in MCAO rats. Representative coronal brain sections stained with 2% TTC from the vehicle-treated MCAo group, and groups treated with *Matricaria recutita* 200 and 400 mg/kg and *Artemisia absinthium* 200 and 400 mg/kg, respectively. *Matricaria recutita* brains showed significantly ( $p<0.05$ ) decreased infarct area % when compared with vehicle-treated MCAo brains. \* $P<0.05$ , ANOVA followed by Tukey's HSD Post-hoc test,  $n = 6$  for all groups.

**Effects of *Matricaria recutita* and *Artemisia absinthium* on Infarct Volume**

There was significant ( $p<0.001$ ) reduction in the infarct volume (% contralateral hemisphere) of *Matricaria recutita* treated rats at a dose of 400 mg/kg (25.60 ± 2.88%) however, at lower dose of 200 mg/kg this decrease was not significant (31.25±2.14%) as compared to ischemic control following MCAo (38.05 ± 5.32%) (Figure1). In addition, there was no significant decrease in infarct volume in *Artemisia absinthium* treated group following MCAo 200mg/kg, (35.47±3.60%) and 400 mg/kg (33.10±2.70%) as compared to sham-operated control.

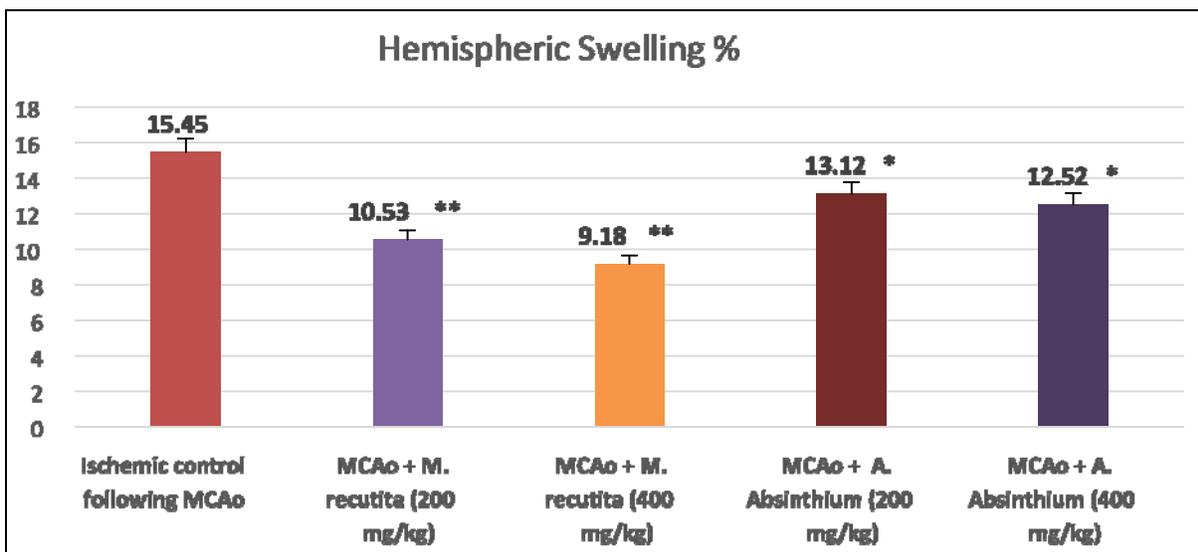


**Fig 3:** Neuroprotective effects of *Matricaria recutita* and *Artemisia absinthium* on percentage of brain infarct volume in MCAO rats. Quantitative analysis of the percentage of brain infarct volume. *Matricaria recutita* of 200 and 400 mg/kg p.o. diminished the percentage of brain infarct volume in MCAo model. There is a statistical difference compared with the MCAo group. Data are expressed as means $\pm$ S.D, \* $p$ <0.05, \*\* $p$ <0.001 vs. vehicle-treated MCAo group, n = 6.

### Hemispheric Swelling

Figure graphs the effects of *Matricaria recutita* and *Artemisia absinthium* on hemispheric swelling. Treatments with *Matricaria recutita* significantly ( $p$ <0.001) decreased hemispheric swelling 32% at a dose of 200 mg/kg and 41% at dose of 400 mg/kg compared to the ischemic control

following MCAo. *Artemisia absinthium* also resulted in hemispheric swelling both at dose of 200 mg/kg (10.53 $\pm$ 1.19%) and 400 mg/kg (9.18 $\pm$ 0.77 %) compared with 15.45 $\pm$ 1.70 percentage in vehicle treated ischemic control group.



**Fig 4:** Neuroprotective effects of *Matricaria recutita* and *Artemisia absinthium* on hemispheric swelling following MCAo. *Matricaria recutita* brains showed significant decreased hemispheric swelling (%) with a dose of 200mg/kg and 400 mg/kg when compared ischemic control brains following MCAo. \* $p$ <0.05, ANOVA followed by Tukey's HSD Post-hoc test, n = 6 for all groups. *Artemisia absinthium* treatment although resulted in decrease in hemisphere swelling but it did not reach to a level of significance.

### Antioxidant Effects of *Matricaria recutita* and *Artemisia absinthium*

MCAo-induced antioxidant enzyme changes are shown in Table 2 (Figure 5). As shown in Table 2, brain lipid peroxidation levels were significantly increased ( $P$ <0.001) in terms of TBARS in MCAo animals as compared with sham-operated group. The *Matricaria recutita* treated group showed a reduced levels of lipid peroxidation at 200 and 400 mg/kg dose as compared with MCAo group. In addition, *Artemisia*

*absinthium* treated rats also showed significant decrease in the levels of lipid peroxidation at both 200mg/kg and 400 mg/kg dose.

At 24 h after the MCAo, catalase, superoxide dismutase activities, reduced glutathione level, total thiols and protein level were much lower in the MCAo group than in the sham-operated group, which implied that oxidative stress had occurred. *Matricaria recutita* treated rats at a dose of 400 mg/kg significantly increased catalase, superoxide dismutase

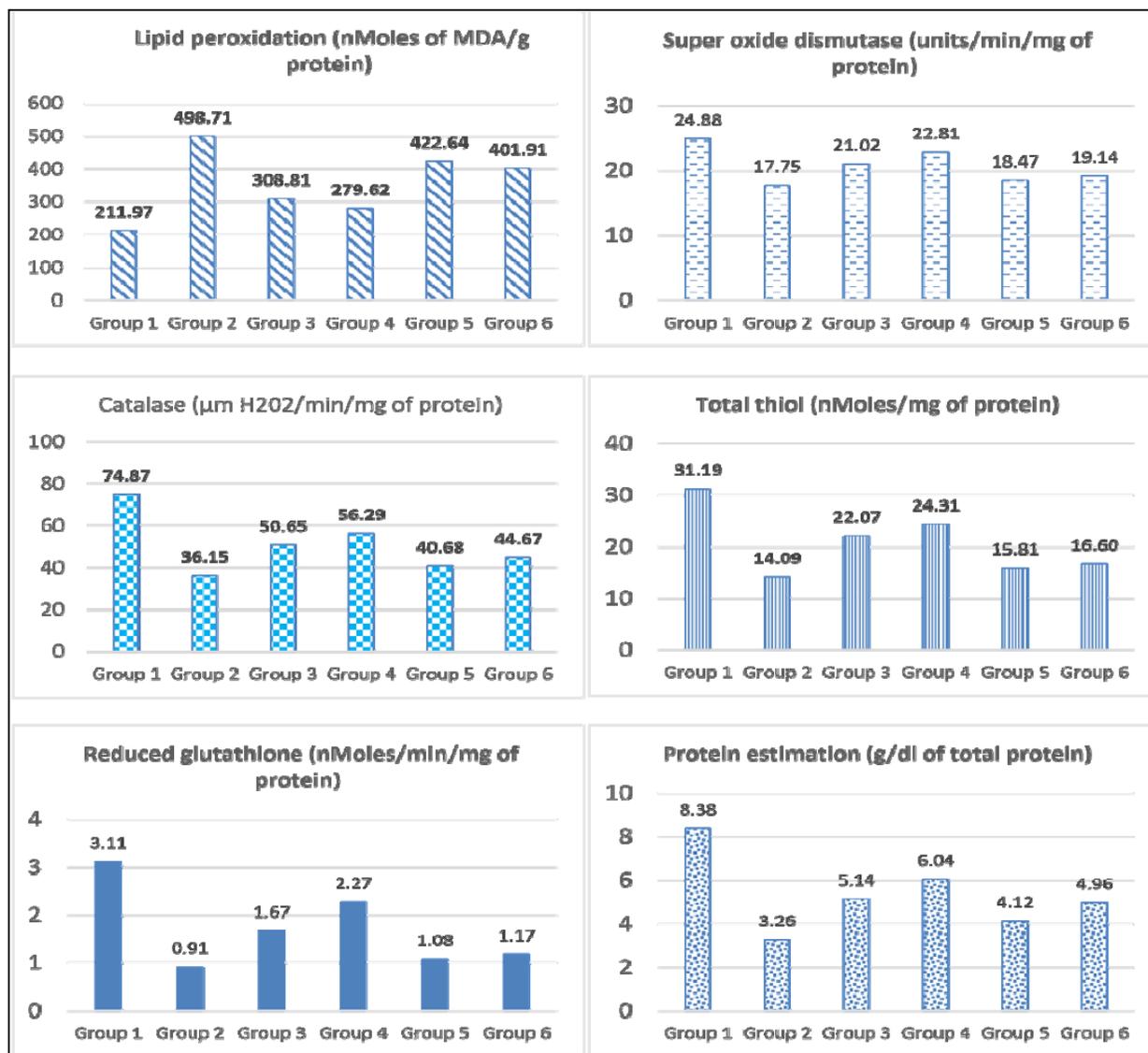
activities, reduced glutathione level, total thiols and protein level compared with the MCAo group, which implied that *Matricaria recutita* significantly suppressed oxidative stress in ischemic brain cortex. On the other hand, it was noticed that *Matricaria recutita* at 200 mg/kg dose could not produce a significant increase on marker of oxidative stress. In

addition, *Artemisia absinthium* treatment at higher dose of 400 mg/kg significantly resulted in improvement on catalase only but not superoxide dismutase, reduced glutathione, total thiol and protein level. There was no significant change in different biomarkers of oxidative stress in *Artemisia absinthium* treated MCAo group (200mg/kg and 400mg/kg).

**Table 2:** Effect of *Matricaria recutita* and *Artemisia absinthium* on oxidative stress in rats subjected to global cerebral ischemia (MCAo) followed by reperfusion

Treatment	Lipid peroxidation (nMoles of MDA/g protein)	Catalase ( $\mu\text{m H}_2\text{O}_2/\text{min}/\text{mg}$ of protein)	Reduced glutathione (nMoles/min/mg of protein)	Super oxide dismutase (units/min/mg of protein)	Total thiol (nMoles/mg of protein)	Protein estimation (g/dl of total protein)
Group 1	211.97 $\pm$ 7.91 <sup>a</sup>	74.87 $\pm$ 5.94 <sup>a</sup>	3.11 $\pm$ 0.48 <sup>a</sup>	24.88 $\pm$ 6.11 <sup>b</sup>	31.19 $\pm$ 5.20 <sup>a</sup>	8.38 $\pm$ 1.47 <sup>a</sup>
Group 2	498.71 $\pm$ 11.22	36.15 $\pm$ 4.37	0.91 $\pm$ 0.29	17.75 $\pm$ 4.09	14.09 $\pm$ 2.91	3.26 $\pm$ 0.30
Group 3	308.81 $\pm$ 8.63 <sup>a</sup>	50.65 $\pm$ 2.66 <sup>a</sup>	1.67 $\pm$ 0.30 <sup>b</sup>	21.02 $\pm$ 1.87 <sup>ns</sup>	22.07 $\pm$ 3.65 <sup>c</sup>	5.14 $\pm$ 1.65 <sup>ns</sup>
Group 4	279.62 $\pm$ 13.79 <sup>a</sup>	56.29 $\pm$ 2.94 <sup>a</sup>	2.27 $\pm$ 0.16 <sup>a</sup>	22.81 $\pm$ 2.75(b)	24.31 $\pm$ 3.01 <sup>a</sup>	6.04 $\pm$ 3.14 <sup>c</sup>
Group 5	422.64 $\pm$ 17.32 <sup>a</sup>	40.68 $\pm$ 3.64 <sup>ns</sup>	1.08 $\pm$ 0.12 <sup>ns</sup>	18.47 $\pm$ 3.75 <sup>ns</sup>	15.81 $\pm$ 3.62 <sup>ns</sup>	4.12 $\pm$ 1.23 <sup>ns</sup>
Group 6	401.91 $\pm$ 14.62 <sup>a</sup>	44.67 $\pm$ 3.01 <sup>c</sup>	1.17 $\pm$ 0.13 <sup>ns</sup>	19.14 $\pm$ 3.38 <sup>ns</sup>	16.60 $\pm$ 2.96 <sup>ns</sup>	4.96 $\pm$ 1.79 <sup>ns</sup>

Group 1: Sham-operated rat without MCAo; Group 2: Ischemic control following MCAo; Group 3: MCAo + *M. recutita* (200 mg/kg); Group 4: MCAo + *M. recutita* (400 mg/kg) Group 5: MCAo + *A. Absinthium* (200 mg/kg); Group 6: MCAo + *A. Absinthium* (400 mg/kg). Values are expressed as mean  $\pm$  SD., (n = 6). <sup>a</sup>P<0.001, <sup>b</sup>P<0.01, <sup>c</sup>P<0.05 when compared to ischemic control rats by one-way analysis of variance (ANOVA), followed by Tukey's HSD Post-hoc test.



**Fig 5:** Antioxidant effects of *Matricaria recutita* and *Artemisia absinthium* in MCAo rats. After the MCAo 24 h, brain cortices were quickly removed for determination of lipid peroxidation, catalase, superoxide dismutase activities, reduced glutathione level, total thiols and protein levels with methods as described in the assay kits. All data were obtained from three independent experiments. Error bars represent mean $\pm$ SD significantly different from the corresponding control (\*\*P<0.05 vs. control; ##P<0.05 vs MCAo model).

## Discussion

Ischemic stroke is one of the leading cause of death worldwide and is a devastating cerebrovascular event. Stroke sufferers may present with various disabilities, including hemiplegia, dysesthesia, ataxia and even visual impairment. Two relevant mechanisms promoting these complications are extensive blood-brain barrier (BBB) disruption [28] and cerebral edema [29]. After ischemic onset, interruption of oxygen and glucose supply leads to cell death cascades, which consequently results in BBB breakdown and cerebral edema. Increased BBB permeability contributes to vasogenic edema, causing intravascular fluid to move to the surrounding brain parenchyma. This detrimental edema further reduces blood flow supplying the neurons, causing irreversible apoptosis [31]. Extensive interactions among these three components become a vicious cycle, which accelerates brain damage.

Despite recent advances in antithrombotic treatment, poststroke disability has significant economic and social burden. As brain has limited capacity to regenerate, there is the need to develop therapeutic strategies to enhance neuroprotection and repair. Currently, the only interventions to improve functional recovery after stroke are rehabilitative strategies, which have had limited success, and genetically engineered tissue plasminogen activators (tPAs), which are still the only medications approved by the FDA and have poor availability in the developing countries and very narrow time window for its intervention. Autologous stem-cell transplantation has been tried but has limited due to unproven efficacy and lack of available facility widespread [32]. Thus, the problem of finding a safe and effective acute neuroprotective treatment after stroke remains urgent.

Traditional plant medicines are useful alternative source of novel drugs containing various pharmacological activities. Zingiberaceae, the Ginger family, includes many important medicinal plants and is a rich source of biologically active compounds [33].

Middle Cerebral Artery Occlusion (MCAo) is most commonly model used to induce experimental focal cerebral ischemia [34]. The advantages of the MCAo model are its reproducibility and ease of reperfusion. Besides, the type of ischemic injury observed is similar to that found in human [35]. Therefore, MCAo model has been employed in the present study to determine the actions of *Matricaria recutita* and *Artemisia absinthium*.

*Matricaria recutita* L. (Chamomile) has been used medicinally for thousands of years, and is widely used in Europe. It is a popular treatment for numerous ailments, including sleep disorders, anxiety, digestion, intestinal conditions, skin infections, inflammation (including eczema), wound healing, infantile colic, teething pains and diaper rash. In the United States, chamomile is best known as an ingredient in herbal tea preparations advertised for mild sedative effects [36]. However, these uses have not been thoroughly studied in humans, and there is limited scientific evidence about safety and effectiveness. The major flavonoid of chamomile, apigenin, was shown to inhibit the in vitro neurotoxicity of amyloid  $\beta$ -protein (A $\beta$ ), a neurotoxin associated with the development of Alzheimer's disease [37]. However, there is no work has been done to prove scientific applicability in neuroprotective.

*Artemisia absinthium* Linn. (Asteraceae), commonly known as wormwood, is an aromatic, perennial undershrub growing naturally in Europe, North America, and Asia. Traditionally, wormwood has been used as an antiseptic, antispasmodic, anticancer, febrifuge, cardiac stimulant, for the restoration of

declining mental function and inflammation of the liver, and to improve memory [38, 39]. Pharmacological reports revealed that *A. absinthium* enhance the cognitive ability as evidenced by its nicotinic and muscarinic receptor activity in homogenates of human cerebral cortical membranes [39]. Hexane-, chloroform-, and water-soluble extracts of the plant exhibited antipyretic activity against subcutaneous yeast injections in rabbits. Moreover, it has been reported that methanol extract of this plant enhanced neurite outgrowth induced by nerve growth factor and PC12D cells [40]. Recently, the authors have shown that *A. absinthium* exhibited significant neuroprotective effects against ischemia and reperfusion insult in rats [41].

The brain injury induced by MCAo leads to neurobehavioral impairment. The neurobehavioral assessment showed a neurologic deficit after MCAo injury. Surprisingly, treatment of rats with *Matricaria recutita* at a dose of 400 mg/kg for 7 days after MCAo improved the neurological deficit scores compared to the vehicle treated group. Unfortunately, the low and *Matricaria recutita* did not produce the significant changes on this parameter. One possible explanation for this phenomenon might be due the *Matricaria recutita* at a low dose might possibly fail to raise the concentration of active ingredient in the extract to the therapeutic level. On the other hand, the increasing a dose of the crude extract of *Matricaria recutita* might also increase the concentration of some ingredients which masked the effect of the active ingredient. Furthermore, the MCAo in rat model cause cerebral cell death, resulting in local infarction. The infarct area represents the degree of brain injury [42]. In the present study, treatment or rats with *Matricaria recutita* at a dose of 400 mg/kg for 7 days before MCAo showed markedly the reduction in infarction volume.

Accumulating lines of evidence reported that oxidative stresses such as generation of damaging reactive oxygen species would lead to cell death under ischemic condition [43]. Oxidative stresses such as generation of damaging reactive oxygen species has been proven in relation to pathogenesis of cerebral ischemia [44]. Furthermore, oxidative stress involves redox signalling to molecular mediators of inflammation pathways, which induce further cell damage [45]. Increasing evidence has indicated that ischemia, which occurs due to blockage of the Middle Cerebral Artery or due to recirculation of blood flow, causes oxidative stress that may potentiate ischemic injury [44]. In recent times, growing evidences support participation of phenolic antioxidants from traditional plant medicines to possess as neuroprotective agents against cerebral ischemia [46, 47]. In the present study, the treatment with *Matricaria recutita* resulted in reduced levels of lipid peroxidation, and increased level of activities of catalase and superoxide dismutase, total thiols and protein levels) significantly. However, *Artemisia absinthium* showed some evidence of neuroprotective and antioxidative effect on cerebral ischemia but this effect did not reach to any level of significance.

These encouraging results may have future clinical importance because of the increase using of herbal supplements by the general population.

## Conclusion

In conclusion, our data demonstrated that out of *Matricaria recutita* and *Artemisia absinthium* extracts, *Matricaria recutita* resulted in decreased infarct area, infarct volume and hemispheric swelling at higher dose of 400 mg/kg. It also improved the oxidative stress found in the MCAo rat model.

Based on these findings, we recommend that the traditional use of *Matricaria recutita* and not *Artemisia absinthium* could help us in alleviating the consequences of stroke. Further studies will determine which compounds from these plant extracts are responsible for their protective properties.

#### Disclosure/conflict of interest

The authors declare no conflict of interest.

#### Acknowledgments

This research was entirely supported by the Sheikh Abdullah Tuwajiri Stroke Chair (SATSC), under Majmaah University. We would like to thank Dr. Raid Saleem Al-Baradie, Vice Dean for postgraduate research studies of College of Applied Medical Science for his unconditional support.

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