

## ORIGINAL ARTICLE

# Protective role of homoeopathic medicines on cerebral ischaemia in animals

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### ABSTRACT

**Objective:** Cerebral ischaemia is the third leading cause of death after cancer and myocardial infarction. The protective effect of some homoeopathic drugs has been studied in the Middle Cerebral Artery Occlusion (MCAO) model of rat.

**Materials and Methods:** The rats were pretreated with 200C potency once daily for 5 days orally (1 drop or 21 µl) and post treated after 24 hr of MCAO with 30C potency three times a day for 5 days orally (1 drop or 21 µl) with homoeopathic medicines *Crotalus*, *Phosphorus*, *Arnica* and *Crocus*.

**Results:** The content of antioxidants, Thiobarbituric Acid Reactive Substances (TBARS) was elevated significantly whereas the level of Glutathione (GSH) was depleted significantly in the MCAO of rats as compared to the sham group. The activities of antioxidant enzymes, Glutathione Peroxidase (GPx), Glutathione Reductase (GR) and Glutathione-S-Transferase were decreased significantly in MCAO group as compared to sham group. The medicines used on rats (pre and post treated in potencies of 200C and 30C respectively) have protected the activities of these enzymes significantly when compared with the animals of MCAO group.

**Conclusion:** The study has shown that the homoeopathic drugs have protected most of the studied parameters significantly but further studies are required to comment on the mechanism and reproducibility of homoeopathic drugs.

**Keywords:** Antioxidants, *Arnica montana*, Cerebral ischaemia, *Crotalus horridus*, Homoeopathy, Stroke

### INTRODUCTION

Stroke is the third leading cause of mortality worldwide. More than two-thirds of stroke deaths occur in the developing world.<sup>[1]</sup> Present demographic trends suggest that the Indian population will survive through the peak years of stroke occurrence (55-65 yr) and the degree of the residual morbidity will pose a major medical problem. Community surveys from many regions

of India show a crude prevalence rate for strokes presumed to be of vascular origin in the range of 200 per 100,000 persons.

Middle Cerebral Artery Occlusion (MCAO) models have been suggested to be of particular relevance because this is the vessel most commonly affected in stroke victims.<sup>[2]</sup> More recently, MCAO technique have been incorporated with reperfusion and thereby, it is proposed more closely to the clinical

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situations. This view is given credence by the fact that clinically the MCA is the primary site of many strokes and because reperfusion frequently occurs as a result of recanalisation.

Metabolic studies of ischaemic brain have consistently shown a rapid decrease of high energy intermediates, increased lactate and a shift towards reduction of the mitochondrial respiratory chain metabolites.<sup>[3]</sup> As a final strategy to prevent death, the cells in ischaemic area initiate anaerobic glycolysis. However the energy obtained with anaerobic glycolysis can not totally compensate for the energy shortage. It provides a very small fraction of the energy, needed for the neuronal survival. Shortage of energy interrupts the activity of the cellular ion pumps and therefore, the intracellular calcium and the extracellular potassium concentrations increase, within 1–2 min after ischaemia. Thereafter the extracellular concentrations of neurotransmitters increase, in particular glutamate and dopamine, and oedema occurs. To some extent this early damage is reversible. Continuation of the ischaemic conditions however rapidly leads to extensive irreversible damage resulting from a so-called ischaemic cascade.

Cerebral ischaemia is a complex event and it enhances the formation of reactive oxygen species (ROS) in the brain,<sup>[4]</sup> which leads to cell death in hippocampus, striatum and different cortical areas.<sup>[5]</sup> Cytotoxic effects of ROS alter several fundamental physiopathological steps, which lead to neuronal death.<sup>[6]</sup> Brain being more vulnerable to ROS<sup>[7]</sup> caused a series of metabolic<sup>[8]</sup> and enzymatic changes,<sup>[5]</sup> which promote delayed cell death.<sup>[9]</sup> Therefore, changes in antioxidant enzymes activity may play a pivotal role to switch on and off the cascade of events that kill cells at their late stages.<sup>[10,11]</sup>

Glutathione (GSH) plays a central role within the finely tuned network of antioxidant systems that respond to the oxidative insult through its peroxide scavenging functions via Glutathione-S-Transferase (GST) and Glutathione Peroxidase (GPx). It efficiently removes  $H_2O_2$ , maintaining protein thiol pools in their appropriate redox state in the cytosol and mitochondria, thus playing a significant neuroprotective role during energy stress.<sup>[12]</sup> Lipid peroxidation is an important implication in ischaemia-reperfusion,<sup>[11,13]</sup> which increases the

formation of conjugated dienes<sup>[14]</sup> as well as the formation of malondialdehyde content.<sup>[15]</sup>

Superoxide dismutase (SOD), catalase and GPx are enzymes responsible for the degradation of  $O_2\bullet$  radical and  $H_2O_2$  content. GPx participates in the pathways responsible for detoxification of lipid peroxy radicals. The function of GST includes detoxification of 4-hydroxynon-2-enal, reactive product of lipid peroxidation and the reduction of lipid hydro peroxides.<sup>[16]</sup>

Homoeopathic medicines are used clinically for after effects of cerebral vascular accidents and injuries but no animal experimentation has been carried out to evaluate their efficacy in standard laboratory conditions. Study was therefore undertaken in collaboration with Central Council for Research in Homoeopathy, New Delhi, India to assess the protective role of homoeopathic medicines on cerebral ischaemia in animals.

## MATERIALS AND METHODS

The following homoeopathic medicines were selected for the treatment or slow down of cerebral ischaemia as suggested by Central Council for Research in Homoeopathy.

Homoeopathic drugs/short name	200C potency one time orally (1 drop or 21 µl), 5 days before MCAO	30C potency 3 times a day orally (1 drop or 21 µl), for 5 days after 24 hr of MCAO
<i>Arnica montana</i> (Ar)	200C	30C
<i>Crocus sativus</i> (Cr)	200C	30C
<i>Crotalus horridus</i> (C)	200C	30C
<i>Phosphorus</i> (P)	200C	30C

MCAO: Middle Cerebral Artery Occlusion

### Animals and drug administration

Wistar albino male rats weighing 250–300 g were used for the study. The animals were divided into four groups for each drug and each group had eight animals. Group 1 was vehicle operated sham, Group 2 was MCAO, Group 3 was MCAO + drug and Group 4 was drug only. They were kept in separate cages under standard laboratory condition with 12 hr light-dark period at standard rat diet and water *ad-libitum*.

The animals of MCAO group were pretreated with 200C potency of the selected drug orally (1 drop or 21 µl), one time for 5 days for one set of study. After 5 days, MCAO was developed and the same drug of

30C potency was given 3 times a day orally (1 drop or 21  $\mu$ l), for 5 days. Thereafter, the animals were sacrificed and their brain was dissected out quickly to separate hippocampus and frontal cortex for biochemical evaluation.

### Surgical Procedure

Focal brain Ischaemia was induced by the intraluminal suture, MCAO method as recently described by us.<sup>[11]</sup> The animals were anaesthetized by choral hydrate 400mg/kg of body weight. The right Common Carotid Artery (CCA), Internal Carotid Artery (ICA) and External Carotid Artery (ECA) was exposed through a midline incision. The ECA was ligated at three sites and cut between two ligatures closer to the head. The cut end was pulled down and a nick was made close to the proximal ligature. Through the nick, a monofilament (4–0) of nylon stiffened with wax was introduced into the ICA. The monofilament was advanced into the ICA 17–19mm beyond the carotid bifurcation. Mild resistance indicates that the monofilament is properly lodged into the Anterior Cerebral Artery (ACA) and thus block the blood flow to the Middle Cerebral Artery (MCA). The neck incision was then sutured and the animal was allowed to recover. After 2 hours of occlusion the monofilament was retracted to allow the reperfusion.

### BIOCHEMICAL STUDIES

**Tissue preparation:** On sixth day of MCAO, animals were sacrificed and brains were taken out quickly and kept on ice. Frontal cortex and hippocampus was dissected out and homogenized in phosphate buffer (10mM, pH 7.0) to give 5% homogenate and centrifuged at 800 g for 5 min at 4°C. The supernatant was used for the TBARS and supernatant was taken in another centrifuge tube to centrifuge at 10,500  $\times$  g for 20 min at 4°C. The supernatant called Post Mitochondrial Supernatant (PMS) was used for another biochemical study and stored at -70°C when not used immediately.

### ASSAYS OF NON ENZYMATIC ANTIOXIDANTS

#### Estimation of reduced glutathione

Reduced glutathione was assayed by the method of Jollow *et al.*<sup>[17]</sup> One ml PMS (5%) was precipitated with 1.0 ml sulfosalicylic acid (4%). The samples was kept at 4°C for 1 hr and then subjected to centrifugation at 1200 g for 15 min at 4°C. The assay mixture was contained 0.1ml filtered aliquot,

2.7 ml phosphate buffer (0.1M, pH 7.4) and 0.2ml DTNB (0.4% in phosphate buffer 0.1 M, pH 7.4) in a total volume of 3.0ml. The yellow colour developed was read immediately at the OD 412nm.

#### Assay of lipid peroxidation

The procedure of Utley *et al.*<sup>[18]</sup> as modified by us<sup>[19]</sup> was used for the estimation of the rate of lipid peroxidation (LPO). 0.5ml of homogenate (5% in child KCl) was pipetted in a test tube (15  $\times$  100 mm) and incubated at 37  $\pm$  1°C in a metabolic shaker (120 rpm/min) for 60 min. Another 0.5 ml of the same homogenate was pipetted in a centrifuge tube and placed at 0°C. After 1 hr of incubation, 0.5 ml 5% chilled TCA followed by 0.5 ml of 0.67% TBA was added to each vial and mixed after each addition. The aliquot from each vial was transferred to centrifuge tube and centrifuged at 3,500 rpm for 15 min. Thereafter, supernatant was transferred to another tube and placed in the boiling water bath. After 10 min, the test tubes were cooled and the absorbance of the colour was read at the OD 535 nm.

### ASSAYS OF ENZYMATIC ANTIOXIDANTS

#### Estimation of Glutathione-S-transferase activity

GST activity was measured by the method of Habig *et al.*<sup>[20]</sup> The reaction mixture consisted of 1.425 ml phosphate buffer (0.1M, pH 7.4), 0.2 ml reduced glutathione (1mM), 0.025 ml CDNB (1-Chloro - 2,4 dinitrobenzene) (1mM) and 0.3 ml PMS in a total volume of 2.0 ml. The changes in absorbance was recorded at the OD 340 nm and enzyme activity was calculated as nmol CDNB conjugate formed/min/mg protein using a molar extinction coefficient of  $9.6 \times 10^3 \text{ M}^{-1} \text{ cm}^{-1}$ .

#### Glutathione peroxidase activity

Glutathione peroxidase activity was estimated according to the procedure described by Mohandas *et al.*<sup>[21]</sup> The reaction mixture consisted of 1.44 ml phosphate buffer (0.05M, pH 7.0), 0.1 ml of EDTA (1 mM), 0.1ml of sodium azide (1 mM), 0.05 ml of glutathione reductase (1 eu/ml), 0.10ml of glutathione (1 mM), 0.10ml of NADPH (Nicotinamide Adenine Dinucleotide Phosphate reduced), (0.2 mM), 0.01 ml of hydrogen peroxide (0.25 mM) and 0.1ml of PMS in a final volume of 2 ml. The disappearance of NADPH at the OD 340 nm was being recorded at room temperature. The enzyme activity was calculated as nmol NADPH oxidized/min/mg/protein by using molar extinction coefficient  $6.22 \times 10^3 \text{ M}^{-1} \text{ cm}^{-1}$ .

### Glutathione reductase activity

Glutathione reductase activity was assayed by the method of Carlberg and Mannervik<sup>[22]</sup> (1975), as modified by Mohandas *et al.*<sup>[21]</sup> The assay system consisted of 1.65ml phosphate buffer (0.1 M, pH 7.6), 0.1 ml NADPH (0.1mM), 0.1ml EDTA (0.5mM) and 0.05 ml oxidized glutathione (1 mM) and 0.1 ml of PMS in a total volume of 2ml. The enzyme activity was quantified at room temperature by measuring the disappearance of NADPH at the OD 340 nm and was calculated as nmol NADPH oxidized/min/mg protein using molar extinction coefficient of  $6.22 \times 10^3 \text{M}^{-1} \text{cm}^{-1}$ .

Estimation of protein: Protein was estimated by the method of Lowry *et al.*<sup>[23]</sup>

### Statistics

Results are expressed as Mean  $\pm$  SEM of eight animals in each group. One way ANOVA with Tukey – Kramer *post hoc* analysis was used to analyze differences between the groups. Significance was ascertained at  $P < 0.05$ .

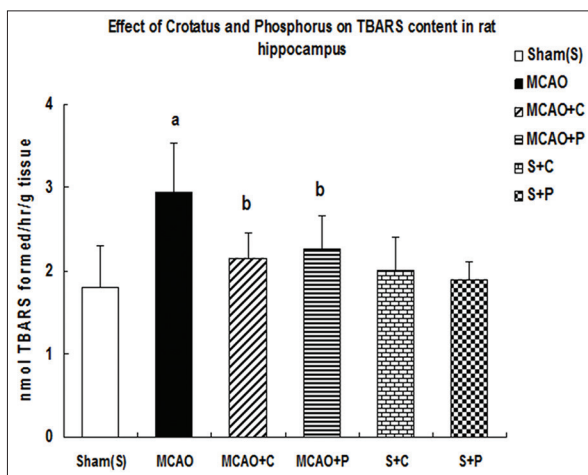
### RESULTS

The pretreatment with 200C potency for 5 days once daily and post treatment with 30C potency three times a day for 5 days of *Crotalus* and *Phosphorus* has significantly protected the increased level of TBARS in MCAO + C and MCAO + P group animals when compared with MCAO group animals [Figure 1]. No significant alteration was observed in sham group pretreated with 200C potency and post treated with 30C of *Crotalus* and *Phosphorus* in S + C and S + P group animals when compared with S group animals.

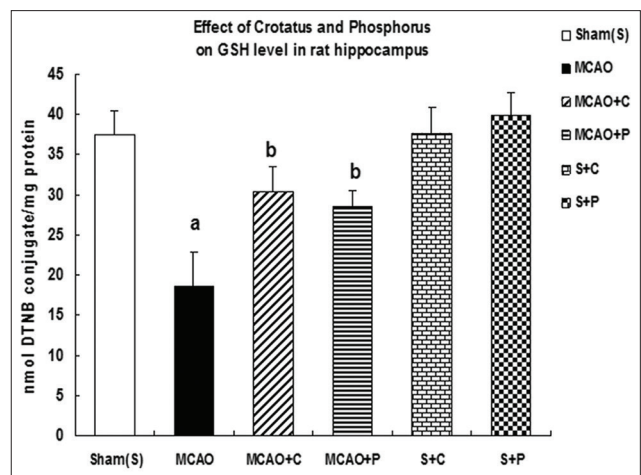
Figure 2 shows the effect of *Crotalus* and *Phosphorus* on GSH in MCAO group and its protection. The GSH content was depleted significantly in MCAO group as compared to S group. The pretreatment of MCAO group with 200C potency for 5 days once daily and post treatment with 30C potency for 5 days daily three times a day with *Crotalus* and *Phosphorus* has protected GSH content significantly in hippocampus. No significant alteration was observed in sham group pretreated and posttreated with 200C and 30C potency of *Crotalus* and *Phosphorus* respectively in S + C and S + P group.

Table 1 shows the protective effect of *Crotalus* and *Phosphorus* on the activities of GPx, GR and GST in MCAO group. The activities of GPx, GR and GST were depleted significantly in MCAO group. The pretreatment of MCAO group with C200 potency of *Crotalus* and *Phosphorus* and post treatment with 30C potency of *Crotalus* and *Phosphorus* have protected GPx, GR and GST activities significantly. No significant alteration was observed in the activities of GPx and GST in sham group pretreated with 200C potency and post treated with 30C potency of *Crotalus* and *Phosphorus*. The GR activity was depleted significantly in S group pretreated with 200C potency and post treated with 30C potency of *Crotalus* but it was not significant with *Phosphorus*.

Figure 3 shows the effect of *Arnica* and *Crocus* on TBARS in MCAO group and its protection by these homoeopathic drugs. The TBARS content was elevated significantly in MCAO group. The pretreatment of MCAO group with 200C potency and post treatment with 30C potency of *Arnica* and



**Figure 1:** Effect of *Crotalus* and *Phosphorus* on lipid peroxidation in hippocampus. Values are expressed as mean  $\pm$  S.E. <sup>a</sup> $P < 0.01$  sham vs. MCAO, <sup>b</sup> $P < 0.01$  MCAO vs. MCAO + C and MCAO + P



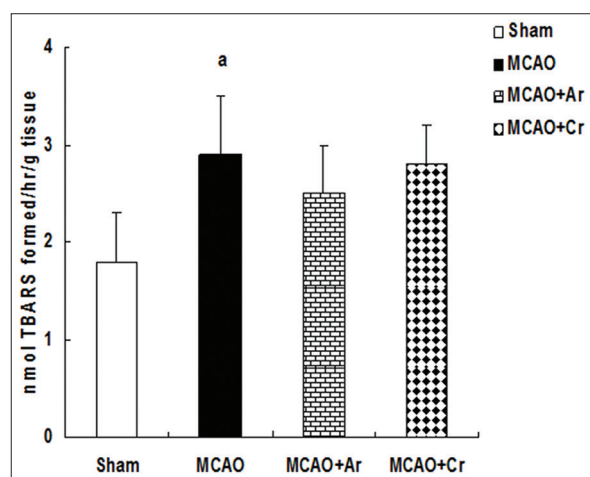
**Figure 2:** Effect of *Crotalus* and *Phosphorus* on GSH in hippocampus. Values are expressed as mean  $\pm$  S.E. <sup>a</sup> $P < 0.01$ , sham vs. MCAO, <sup>b</sup> $P < 0.01$  MCAO vs MCAO + C and MCAO + P



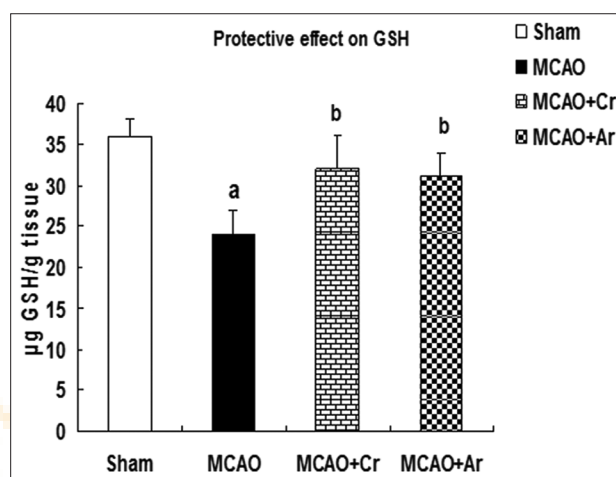
**Table 1: Effect of *Crotalus* (C) and *Phosphorus* (P) on the activities of antioxidant enzymes (GPx, GR and GST)**

Antioxidant enzymes	Treatment					
	Sham	MCAO (%)	MCAO+C (%)	MCAO+P (%)	S+C (%)	S+P (%)
GPx (nmol NADPH oxidized/min/mg protein)	450±45.1	300±40.12 <sup>a</sup> (-33.33)	460±46.6 <sup>c</sup> (+53.33)	410±42.5 <sup>e</sup> (+36.66)	510±48.3 (+13.33)	440±44.2 (+2.22)
GST (nmol CDNB conjugate formed/min/g protein)	799±50.02	608±45.02 <sup>a</sup> (-23.90)	790±43.01 <sup>b</sup> (+16.17)	760±44.21 (+11.76)	780±49.03 (-2.37)	900±55.04 (+12.64)
GR (nmol NADPH oxidized/min/mg protein)	498±56.23	360±56.02 <sup>a</sup> (-27.71)	449±55.23 <sup>b</sup> (+24.72)	406±48.06 (+12.77)	311±50.12 <sup>d</sup> (-37.55)	499±48.06 (+0.20)

Values are expressed as mean±S.E. <sup>a</sup>P<0.01 sham vs MCAO; <sup>b</sup>P<0.05 MCAO vs MCAO+C, MCAO+P, <sup>c</sup>P<0.001 MCAO vs MCAO+C, MCAO+P and <sup>d</sup>P<0.001 S vs S+C; MCAO: Middle cerebral ischaemia occlusion; GPx: Glutathione peroxidase; GST: Glutathione-s-transferase; GR: Glutathione reductase; CDNB: 1-Chloro-2,4-dinitrobenzene; NADPH: Nicotinamide adenine dinucleotide Phosphate reduced



**Figure 3:** Effect of *Arnica* and *Crocus* on lipid peroxidation in hippocampus. Values are expressed as mean ± S.E. <sup>a</sup>P < 0.05 sham vs. MCAO, <sup>b</sup>P < 0.05 MCAO vs MCAO + Cr and MCAO + Ar



**Figure 4:** Effect of *Arnica* and *Crocus* on GSH in hippocampus. Values are expressed as mean ± S.E. <sup>a</sup>P < 0.05 sham vs. MCAO, <sup>b</sup>P < 0.05 MCAO vs MCAO + Cr and MCAO + Ar

*Crocus* has protected TBARS level significantly as compared to MCAO group.

Figure 4 shows the protective effect of *Arnica* and *Crocus* on the content of GSH in MCAO group. The GSH content was depleted significantly in MCAO group. The pretreatment of MCAO group with 200C for 5 days once daily and post treatment with 30C potency of *Arnica* and *Crocus* for 5 days, 3 times a day has protected GSH content significantly when compared with MCAO group.

Table 2 shows the protective effect of *Crocus* and *Arnica* on the activities of GPx, GR and GST in MCAO group. The activities of GPx, GR and GST were depleted significantly in MCAO group. The pretreatment of MCAO group with 200C potency of *Crocus* and *Arnica* and post treatment with 30C potency of *Crocus* and *Arnica* have protected GPx, GR and GST activities significantly.

Table 3 shows the comparative study of homoeopathic drugs, *Arnica* and *Crocus* with alcoholic extract of

*Crocus sativus* and aqueous extract of the Garlic on the prevention of cerebral ischaemia. The homoeopathic drugs (*Arnica* and *Crocus*) have shown the equal protective effect as shown by the herbal extract of *Crocus sativus* and Garlic. The protection of the herbal extract (*Crocus sativus*) was slightly better on the contents of LPO and GR (Glutathione Reductase) but the homoeopathic medicine, *Arnica* has shown better protection on the activity of GPx as compared to the herbal extract of *Crocus sativus*. The aqueous extract of Garlic has shown better protection on LPO, GSH, GST and GR as compared to the homoeopathic drugs (*Arnica* and *Crocus*).

## DISCUSSION AND CONCLUSION

There is no any evidence or report of homoeopathic medicine used on animal model of cerebral ischaemia. The data of our study has shown that the *Arnica* and *Crotalus* were more efficacious in the prevention of cerebral ischaemia than *Crocus* and *Phosphorus*. The increased level of lipid peroxidation in rat brain in the present study after

ischaemia and reperfusion observed after motor performance may be due to hyperexcitability of neurons in the early post-ischaemic period caused by excessive accumulation of glutamate in the extracellular fluid<sup>[24,25]</sup> which can induce excessive activation of N-methyl-D-aspartate or 2-amino-3-hydroxy-5-isoxazole propionate/kainate receptors resulting in accumulation of intracellular fluid and sodium and calcium ions<sup>[26]</sup> which induced generation of lipid peroxide and free radicals.

In cerebral ischaemia, free radicals have been implicated as important pathogenic factors in their pathogenesis. Hypothetically, the mechanisms by which the free radicals are generated are complex and depend on multiple interacting factors: (a) shortage of O<sub>2</sub><sup>•</sup> at the cytochrome oxidase step may give rise to leakage of partially reduced oxygen species; or (b) a rapid fall in cellular ATP, due to diminished aerobic oxidation, may result in the alteration of ionic transport with cytosolic calcium overload. Free radicals generated by the disorder, attack the membrane phospholipids causing their peroxidation, which is the

fundamental aspect of free radical damage in brain due to high lipid content.<sup>[27]</sup> The peroxidative processes in the brain are surely contributory to the inactivation of membrane-bound enzymes, since phospholipids are important for optimum activity of many enzymes. On the other hand, increased catabolism during early training appears to be the main mechanism underlying the generation of free radicals and lipid peroxidation after motor performance in the brain of cerebral ischaemic rats.

It was also noticeable that the elevation of Malonaldehyde (MDA) level after 22 hrs of reperfusion was accompanied by significant depletion in brain GSH after ischaemic brain injury.<sup>[28]</sup> GSH also plays a crucial role in the regulation of expression of several redox-sensitive antioxidant and anti-inflammatory genes<sup>[29]</sup> processes that are aggravated especially post-ischaemic insult as a result of reperfusion of white blood cells to the injured area.<sup>[30]</sup> As a result, there is a rapid loss of reducing equivalents of the cell, and hence an onset of oxidative stress. The oxidative stress further leads to the up-regulation of expression of a wide variety of pro-inflammatory cytokines, including adhesion molecules, all of which contribute to tissue injury and apoptosis/necrosis.<sup>[31,32]</sup> Therefore, maintenance of the GSH pool and other antioxidant levels is critical to cell survival and adaptation to the ischaemic injury.<sup>[33]</sup> In response to the battery of free radicals generated during ischaemia, the cells initially neutralize the oxidative challenge *via* GSH-mediated antioxidant mechanisms.<sup>[34,35]</sup> However, a rapid decline in the levels of GSH, soon follows, which ultimately leads to tissue injury.

Thus, GSH depletion in cerebral ischaemia would increase the susceptibility of plasma membranes towards peroxide attacks. However, the main cause of GSH loss during oxidative stress in brain ischaemia is the formation of protein glutathione

**Table 2: Effect of *Crocus* and *Arnica* on the activities of antioxidants enzymes (GPx, GR and GST)**

Antioxidant enzymes	Treatment			
	Sham	MCAO (%)	MCAO+Ar (%)	MCAO+Cr (%)
GPx (nmol NADPH oxidized/min/mg protein)	445±45.12	305±32.02* (-31.46)	500±43.55 <sup>###</sup> (+63.93)	370±40.12# (+21.31%)
GST (nmol CDNB conjugate formed/min/g protein)	785±55.44	611±50.42* (-22.16)	780±53.4 <sup>###</sup> (+27.66)	730±51.4# (+19.47%)
GR (nmol NADPH oxidized/min/mg protein)	485±50.47	352±44.37* (-27.42)	419±47.21# (+19.03)	550±44.47 <sup>###</sup> (+56.25%)

Values are expressed as mean±S.E. P<0.001 sham vs MCAO; \*P<0.05 MCAO vs MCAO+Cr, MCAO+Ar and <sup>###</sup>P<0.001 MCAO vs MCAO+Cr, MCAO+Ar; MCAO: Middle cerebral ischaemia occlusion; GPx: Glutathione peroxidase; GST: Glutathione-s-transferase; GR: Glutathione reductase; CDNB: 1-Chloro - 2,4 dinitrobenzene; NADPH: Nicotinamide adenine dinucleotide Phosphate reduced

**Table 3: Effect of cerebral ischaemia on lipid peroxidation, GSH and GSH dependent enzymes and their comparison with the earlier published work on the herbal extracts**

Antioxidants	Percentage change against sham value					
	Sham	MCAO	MCAO+Ar	MCAO+C	MCAO+Cr (ref. 34)	MCAO+AGE (ref. 35)
LPO	100	+161.11	-86.21	-96.56	-112.88	-113.56
GSH	100	66.67	+129.16	133.33	+134.78	171.43
GPx	100	68.54	+163.93	+121.31	+127.79	+183.79
GST	100	78.84	+127.66	+119.47	+131.77	+188.73
GR	100	72.58	+119.03	+156.25	+135.14	+204.60

Ar: *Arnica*; C: *Crotalus*; Cr: *Crocus sativus*; AGE: Aqueous garlic extract; MCAO: Middle cerebral ischaemia occlusion; GPx: Glutathione peroxidase; GST: Glutathione-s-transferase; GR: Glutathione reductase; GSH: Glutathione LPO: Lipid peroxidation

mixed disulfides and loss of protein thiols.<sup>[36]</sup> The loss of GSH and formation of protein glutathione mixed disulfides in the brain result in the various membrane dysfunctions, such as inhibition of Na<sup>+</sup>, K<sup>+</sup>-ATPase.<sup>[37]</sup> This enzyme is essential for cellular excitability and is very susceptible to free radical reaction and lipid peroxidation because it is embedded in the cell membrane and requires phospholipids for maintenance of its activity.<sup>[38,39,40]</sup>

Our data indicate that restoration of glutathione content and its dependent enzymes, which maintains energy metabolism, may reduce the deleterious effect of motor performance after acute cerebral ischaemia and support the potential clinical utility of homoeopathic medicines in treating acute cerebro-vascular diseases in humans. This suggests that the antioxidant property of homoeopathic medicines acted as a possible mechanism in protecting the neurons, possibly by increasing the endogenous defensive capacity of the brain to combat oxidative stress induced by ischaemia/reperfusion. Thus, homoeopathic medicines may be considered as a potential candidate in the armamentarium of drugs for prophylactic treatment in patients who are prone to stroke.

The comparative study of *Arnica* and *Crocus sativus* with aqueous extract of garlic from the medicinal plant has shown approximately equal effect on the contents of anti-oxidants and activity of anti-oxidant enzymes. The comparative study is quite interesting and none has reported before this. On the basis of comparative study with homoeopathic medicine and herbal extracts we have hypothesised that the homoeopathic medicines will be better to cure the neurodegenerative disorder as compared to the extract of medicinal herbal plants having less or no side effect. A further detail and comparative study taking two homoeopathic drugs and their herbal extracts will give a better understanding between the homoeopathic medicines and Ayurveda or Unani medicines for the cure of neurodegenerative disorders.

This is the first study of homoeopathic medicines on the cerebral ischaemia in animal model. Though the homoeopathic drugs have protected most of the studied parameters significantly but further studies are required to comment on mechanism and reproducibility.

Schizophrenia and bipolar disorder are associated with lowered glutathione. Accruing data suggest that oxidative stress may be a factor underlying the pathophysiology of Bipolar Disorder (BD), Major

Depressive Disorder (MDD), and Schizophrenia (SCZ). Glutathione (GSH) is the major free radical scavenger in the brain. Diminished GSH levels elevate cellular vulnerability towards oxidative stress; characterized by accumulating reactive oxygen species. Replenishment of glutathione using *N*-acetyl cysteine has been shown to reduce symptoms of both disorders. The findings also open up the new vistas of assessing the role of these medicines in above psychiatric disorders.

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## REFERENCES

1. Bonita R. Epidemiology of stroke. *Lancet* 1992; 339:342-4.
2. Mohr JP, Gautier JC, Hier D, Stein RW. In: Barnett HJ, Stein BM, Mohr JP, Yastu FM, editors. *Stroke: Pathophysiology, Diagnosis and Management*. 1<sup>st</sup> edition. Vol. 1. Churchill Livingstone; 1986. p. 377-450.
3. Goldberg ND, Passonneau JV, Lowry OH. Effects of changes in brain metabolism on the levels of citric acid cycle intermediates. *J Biol Chem* 1966;241:3997-4003.
4. Li L, Shen YM, Yang XS, Wu WL, Wang BG, Chen ZH, et al. Effects of spiramine T on antioxidant enzymatic activities and nitric oxide production in cerebral ischemia-reperfusion gerbils. *Brain Res* 2002; 944:205-9.
5. Larsson E, Lindvall O, Kokala Z. Stereological assessment of vulnerability of immunocytochemically identified striatal and hippocampal neurons after global cerebral ischemia in rats. *Brain Res* 2001;913:117-32.
6. Cao W, Carney JM, Duchon A, Floyd RA, Chevion M. Oxygen free radical involvement in ischemia and reperfusion injury to brain. *Neurosci Lett* 1998;88:233-8.
7. Nelson CW, Wei EP, Povlishock JK, Kontos HA, Moskowitz MA. Oxygen radicals in cerebral ischemia. *Am J Physiol* 1992; 263:H1356-62.
8. Sims NR. Energy metabolism and selective neuronal vulnerability following global cerebral ischemia. *Neurochem Res* 1992;17:923-31.
9. Won MH, Kang T, Park S, Jeon G, Kim Y, Seo JH, et al. The alterations of N-Methyl-D-aspartate receptor expressions and oxidative DNA damage in the CA1 area at the early time after ischemia-reperfusion insult. *Neurosci Lett* 2001;301:139-42.
10. Homi HM, Frietas JJ, Curi R, Velasco IT, Bonfim A, Junior S. Changes in superoxide dismutase and catalase activities of rat brain regions during early global transient ischemia/reperfusion. *Neurosci Lett* 2002;333:37-40.
11. Salim S, Ahmad M, Zafar KS, Ahmad AS, Islam F. Protective effect of Nardostachys Jatamansi in rat cerebral ischemia. *Pharmacol Biochem Behav* 2003;74:481-6.
12. Zeevalk GD, Bernard LP, Nicklas WJ. Role of oxidative stress and glutathione system in loss of dopamine neurons due to impairment of energy metabolism. *J Neurochem* 1998;70:1421-30.
13. Comporti M. Lipid Peroxidation, In: Poli G, Albano E, Dianzani MU,

- editors. An overview: Free Radicals: From Basic Science to Medicine. 1<sup>st</sup> ed. Basel: Birkhauser Verlag; 1993:65-79.
14. Watson BD, Busto R, Goldberg WJ, Santiso M, Yoshida S, Ginsberg MD. Lipid Peroxidation *in vivo* induced by reversible global ischemia in rat brain. *J Neurochem* 1984;42:268-74.
  15. Vagnozzi R, Lazzarino G, Tavazzi B, Di Pierro D, Siragusa P, Giuffre R, Giardina B. Incomplete cerebral ischemia in the rat promotes increase of the tissue and plasma malondialdehyde. *Biol Trace Elem Res* 1995;47:241-6.
  16. Nanduri B, Hayden JB, Awasthi YC, Zimniak P. Amino acid residue O4 in an alpha-class glutathione S-transferase is essential for the high selectivity and specificity of the enzyme for 4-hydroxynonenol. *Arch Biochem Biophys* 1996; 335:305-10.
  17. Jollow DJ, Mitchell JR, Zampaghone N, Gillette JR. Bromobenzene-induced liver necrosis. Protective role of glutathione and evidence for 3,4-bromobenzene oxide as the hepatotoxic intermediate. *Pharmacology* 1974;11:151-69.
  18. Utley HC, Bernheim F, Hochslein P. Effect of sulfhydryl reagent on peroxidation in microsome. *Arch Biochem Biophys* 1967;260:521-31.
  19. Islam F, Zia S, Sayeed I, Zafar KS, Ahmad AS. Selenium-induced alteration of lipids, lipid peroxidation, and thiol group in circadian rhythm center of rat. *Biol Trace Elem Res* 2002; 90:203-14.
  20. Habig WH, Pabst M, Jakoby WB. Glutathione-S-Transferase. The first enzymatic step in mercapturic acid formation. *J Biol Chem* 1974;249:7130-9.
  21. Mohandas J, Marshall JJ, Duggin GG, Horvath JS, Tiller D. Differential distribution of glutathione and glutathione related enzymes in rabbit kidneys: Possible implication in analgesic neuropathy. *Cancer Res* 1984;44:5086-91.
  22. Carlberg I, Mannervik B. Purification and characterization of the flavoenzyme glutathione reductase from rat liver. *J Biol Chem* 1975; 250:5475-80.
  23. Lowry OH, Rosenbrough NJ, Farr AL, Randall RJ. Protein measurement with the folin phenol reagent. *J Biol Chem* 1951;193:265-75.
  24. Choi DW. Glutamate neurotoxicity and disease of the nervous system. *Neuron* 1988;1:623-34.
  25. Benveniste H. The excitotoxin hypothesis in relation to cerebral ischemia. *Cerebrovasc Brain Metab Rev* 1991;3:213-45.
  26. Coyle JT, Puttfarcken P. Oxidative stress, glutamate, and neurodegenerative. *Science* 1993;262:689-95.
  27. Siesjö BK. Calcium in the brain under physiological and pathological conditions. *Eur Neurol* 1990;30:3-9.
  28. Sinha K, Chaudhary G, Gupta YK. Protective effect of resveratrol against oxidative stress in middle cerebral artery occlusion model of stroke in rats. *Life Sci* 2002;71:655-65.
  29. Hall ED. Free radicals in central nervous system injury. In: Rice-Evans, CA, Burdon RH, editors. *Free Radical Damage and Its Control*. 1<sup>st</sup> edition. New York: Elsevier; 1991. p. 217-38.
  30. Lovine WG. Glutathione, lipid peroxidation and regulation of cytochrome P-450 activity. *Life Sci* 1982;31:779-84.
  31. Rahman I, Bel A, Mulier B, Lawson MF, Harrison DJ, Macnee W, et al. Transcriptional regulation of gamma-glutamylcysteine synthetase-heavy subunit by oxidants in human alveolar epithelial cells. *Biochem Biophys Res Commun* 1996;229:832-7.
  32. Marcheselli VL, Hong S, Lukiw WJ, Tian XH, Gronert K, Musto A, et al. Novel docosanoids inhibit brain ischemia-reperfusion-mediated leukocyte infiltration and pro-inflammatory gene expression. *J Biol Chem* 2003;278:43807-17.
  33. Frijns CJ, Kappelle LJ. Inflammatory cell adhesion molecules in ischemic cerebrovascular disease. *Stroke* 2002;33:2115-22.
  34. Berti R, Williams AJ, Moffett JR, Hale SL, Velarde LC, Elliott PJ, et al. Quantitative real-time RT-PCR analysis of inflammatory gene expression associated with ischemia-reperfusion brain injury. *J Cereb Blood Flow Metab* 2002; 22:1068-79.
  35. Zimmermann C, Winnefeld K, Streck S, Roskos M, Haberl RL. Antioxidant status in acute stroke patients and patients at stroke risk. *Eur Neurol* 2004;51:157-61.
  36. Ravindranath V, Reed DJ. Glutathione depletion and formation of glutathione-protein mixed disulphide following exposure of brain mitochondria to oxidative stress. *Biochem Biophys Res Commun* 1990;169:1075-9.
  37. Reed DJ. Glutathione: Toxicological implications. *Annu Rev Pharmacol Toxicol* 1990;30:603-31.
  38. Cooper AJ, Pulsinelli WA, Duffy TE. Glutathione and ascorbate during ischemia and post ischemic reperfusion in rat brain. *J Neurochem* 1980;35:1242-5.
  39. Furui T, Tanaka I, Iwata K. Alterations in Na(+)-K(+)-ATPase activity and beta-endorphin content in acute ischemic brain with and without naloxone treatment. *J Neurosurg* 1990;72:458-62.
  40. Ildan F, Polat S, Gocer AI, Oner A, Isbir T, Mete UO, et al. The effect of the pretreatment of intravenous high dose methylprednisolone on Na<sup>+</sup>, K<sup>+</sup>/Mg<sup>2+</sup>-ATPase and lipid peroxidation and early ultrastructural findings following middle cerebral artery occlusion in the rat. *Acta Neurochir (Wien)* 1996;138:338-45.

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## जंतुओं में प्रमस्तिष्कीय स्थानिक अरक्तता में होम्योपैथिक औषधियों की सुरक्षात्मक भूमिका

### सार

**उद्देश्य:** कर्क रोग एवं हृद्पेशी रोधगलन के बाद प्रमस्तिष्कीय स्थानिक अरक्तता, मृत्यु का तीसरा सबसे बड़ा कारण है। कुछ होम्योपैथिक औषधियों के सुरक्षात्मक प्रभाव का अध्ययन मूषक के मध्य प्रमस्तिष्कीय स्थानिक अरक्तता अधिरोधन (मिडिल सेरेब्रल इस्कीमिया ऑक्लूज़न, एमसीएओ) निदर्श में किया गया है।

**विधियाँ:** मूषक जिन्हें कि होम्योपैथिक दवाओं (क्रोटेलस, फास्फोरस आर्निका व क्रोकस) की 200सी पोटेन्सी से पूर्व उपचारित किया गया था व एमसीएओ के 24 घंटे के बाद इन्हीं दवाओं को 30सी पोटेन्सी से उपचारित किया गया।

**परिणाम:** छद्म समूह की तुलना में मूषकों के एमसीएओ में प्रतिऑक्सीकारकों, थायोबार्बीच्युरिक अम्ल अभिक्रियाशील पदार्थों (टीबीएआरएस) की मात्रा में उल्लेखनीय वृद्धि हुई थी, जबकि अपचयित ग्लूटाथायोन (जीएसएच) का स्तर उल्लेखनीय रूप से घट गया था। क्रोटेलस, फॉस्फोरस, अर्निका और क्रोकस की सी200 शक्ति से प्रतिदिन 1 बार, 5 दिनों के लिए मुख मार्ग (1 बूंद या 21 माइक्रोलीटर) से पूर्व-उपचारित तथा एमसीएओ के 24 घंटों बाद सी30 शक्ति से प्रतिदिन तीन बार, 5 दिनों के लिए मुख मार्ग (1 बूंद या 21 माइक्रोलीटर) से पश्च-उपचारित मूषकों में टीबीएआरएस तथा जीएसएच की मात्रा उल्लेखनीय रूप से सुरक्षित रही है। छद्म समूह की तुलना में एमसीएओ समूह में प्रतिऑक्सीकारी एंजाइमों, ग्लूटाथायोन परॉक्सीडेज़ (जीपीएक्स), ग्लूटाथायोन रिडक्टेज़ (जीआर) एवं ग्लूटाथायोन-एस-ट्रांसफरेज़ की गतिविधियां उल्लेखनीय रूप से घट गई थीं। पूर्व. एवं पश्च.उपचारित शक्तियों, नामतः सी200 और सी30 ने एमसीएओ समूह के जंतुओं के साथ तुलना करने पर, इन एंजाइमों की गतिविधियों की उल्लेखनीय स्तर तक सुरक्षा की है।

**निष्कर्ष:** अध्ययन ने दर्शाया है कि होम्योपैथिक औषधियों ने अधिकांश अध्ययनाधीन प्राचलों की उल्लेखनीय स्तर तक सुरक्षा की है, परंतु होम्योपैथिक औषधियों की क्रियाविधि एवं पुनरुत्पादनीयता पर टिप्पणी के लिए और अध्ययन आवश्यक हैं।

