

# Neuroprotective Role of *Bacopa monnieri* Extract in Epilepsy and Effect of Glucose Supplementation During Hypoxia: Glutamate Receptor Gene Expression

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Accepted: 13 September 2007 / Published online: 18 October 2007  
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**Abstract** The experiments were designed to study the glutamate gene expression during epilepsy in adult and hypoxic insult to brain during the neonatal period and the therapeutic role of neuroprotective supplements. We investigated the role of metabotropic glutamate-8 receptor (mGluR8) gene expression in cerebellum during epilepsy and neuroprotective role of *Bacopa monnieri* extract in epilepsy. We also studied the effect of NMDA receptor 1 (NMDAR1) gene expression during neonatal hypoxia and therapeutic role of glucose, oxygen and epinephrine supplementation. During epilepsy a significant down-regulation ( $P < 0.01$ ) of mGluR8 gene expression was observed which was up-regulated ( $P < 0.05$ ) near control level after *B. monnieri* treatment which is supported by Morris water maze experiment. In hypoxic neonates we observed up-regulation ( $P < 0.001$ ) of the NMDAR1 gene expression whereas glucose and glucose + oxygen was able to significantly reverse ( $P < 0.001$ ) the gene expression to near control level when compared to hypoxia and epinephrine treatment which was supported by open field test. Our results showed that *B. monnieri* treatment to epileptic rats significantly brought the reversal of the down-regulated mgluR8 gene expression toward control level. In neonatal rats, hypoxia induced expressional and functional changes in the NMDAR1 receptors of neuronal cells which is corrected by supplementation of glucose alone or glucose followed by oxygen during the resuscitation to prevent the glutamate related neuronal damage. Thus, the results suggest the clinical

significance of corrective measures for epileptic and hypoxic management.

**Keywords** Glutamate gene expression · Metabotropic glutamate receptor 8 (mGluR8) · NMDAR1 · Neurodegeneration · Epilepsy · Hypoxia · Seizures · *Bacopa monnieri* · Glucose · Oxygen · Epinephrine · Cerebellum · Cerebral cortex · CT value · Morris water maze test and open field test

## Introduction

Neurologic depression occurs following organic brain insult, such as trauma or infection. Survivors of traumatic brain injury usually manifests neurobehavioral changes which include cognitive or memory impairment, apathy, aggressiveness, mood disorders [1] and impaired self-awareness, which can obscure perceptions and affect the self-reporting of depressive symptoms. Neuronal damage in brain regions can result in disrupting neuronal circuits directly by disrupting neurotransmitter systems such as noradrenalin, serotonin, dopamine, and acetylcholine [2]. Such disruption forms a part of the secondary injury cascade that has been well described following brain injury [3] leading to neuronal damage. Glutamate cytotoxicity contributes to neuronal degeneration in many central nervous system (CNS) diseases, such as epilepsy and ischemia. Glutamate is essential for synaptic communication in the CNS, but inadequate regulation of extracellular glutamate can lead to neurodegenerative disorders. The established model for glutamate-induced neuronal cell death is excitotoxicity [4], a mechanism in which excessive cell depolarization leads to apoptosis

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or necrosis. This pathological condition is caused by prolonged stimulation of glutamate receptor subtypes, followed by both intracellular  $\text{Ca}^{2+}$  overload and activation of specific genes, resulting in synthesis of enzymes involved in cell stress response [5]. Elevation of intracellular calcium can lead to cell death [6]. This excitotoxicity model is significant during prolonged and augmented excitation in acute traumatic events such as ischemia [7], stroke [8], and epilepsy [9].

Temporal lobe epilepsy (TLE) is one of the most common forms of intractable epilepsy. Pilocarpine treatment which is characterized by generalized convulsive *status epilepticus* (SE) in rodents well represents the characteristic neuropathological findings in the hippocampus of TLE patients [10, 11]. After a latent period, adult rats exhibit spontaneous recurrent seizures during the remainder of their life. Activation of presynaptic metabotropic glutamate receptors (mGluRs) leads to a powerful inhibition of glutamate release from many synaptic terminals throughout the CNS. mGluRs as autoreceptors are believed to provide a negative feedback system that prevents potentially toxic accumulation of glutamate in the extracellular space during synchronous synaptic activity such as epileptic seizures [12]. In this study we analyzed the function of metabotropic glutamate-8 receptor (mGluR8) gene expression in epileptic and *Bacopa monnieri* treated epileptic rats. *B. monnieri* (Brahmi) is recommended in formulations for the management of a range of mental conditions including anxiety, poor cognition, lack of concentration, and epilepsy. Pharmacologically, it is understood that Brahmi has an unusual combination of constituents that are beneficial in mental inefficiency and illnesses and useful in the management of convulsive disorders like epilepsy.

Hypoxia also leads to free radical and excitotoxic neurotransmitter release, which cause further neuronal damage to these systems [2]. Hypoxia and ischemia induce changes in NMDA receptor expression and function in the developing brain [13–16]. Open-field testing has proven useful for evaluation of the effects of drugs on behavior. The present work is to understand the alterations of glutamate receptors gene expression in the cerebellum of pilocarpine induced epileptic rats and cerebral cortex of hypoxic neonates. The neuroprotective role of *Bacopa monnieri* in epileptic rats and of glucose, oxygen, and epinephrine supplementation in hypoxic neonates was also studied. Our experimental evidence showed that in epilepsy, *B. monnieri* treatment brought the reversal of the down-regulated mGluR8 gene expression. Hypoxia induced NMDAR1 receptor expression of neuronal cell which is corrected by supplementation of glucose alone or glucose followed by oxygen.

## Experimental procedure

### Biochemicals and their sources

Biochemicals used in the present study were purchased from Sigma Chemical Co., St. Louis, CA, USA. All other reagents were of analytical grade purchased locally. Tri-reagent kit was purchased from MRC, IN, USA. Real-Time PCR Taqman probe assays on demand were purchased from Applied Biosystems, Foster City, CA, USA.

Wistar rats were purchased from Amrita Institute of Medical Sciences, Cochin and used for all experiments. They were housed in separate cages under 12-h light and 12-h dark periods and were maintained on standard food pellets and water *ad libitum*. The adult rats of epileptic experiment were sacrificed by decapitation after 15 days treatment with *B. monnieri* extract. All groups of hypoxic neonatal rat were weighed and sacrificed by decapitation after a week. The cerebellum and cerebral cortex was dissected out quickly over ice according to the procedure of Glowinski and Iversen [17]. All animal care and procedures were in accordance with Institutional and National Institute of Health guidelines.

### Plant material and preparation of extract

Specimen of *B. monnieri* (L.) Pennel were collected from Cochin University area and were taxonomically identified and authenticated by Mr. K. P. Joseph, Head, Dept. of Botany (retd.), St. Peter's College, Kollenchery and voucher specimens was deposited at a herbarium (No: MNCB3) of Center for Neuroscience, Cochin University of Science and Technology, Cochin. Whole *B. monnieri* plant was mixed with 100 ml of distilled water and homogenized well. The homogenate was filtered through cheese cloth. This crude whole plant extract was used to study the anti-epileptic effect in pilocarpine induced temporal lobe epilepsy. Fresh, whole *B. monnieri* plant (6–8 months old) was collected and washed. Leaves, roots and stems of *B. monnieri* plant were cut into small pieces and dried in shade. About 100 g fresh plant dried in shade yielded ~15 g powder. Homogenate was extracted at required concentration (150 mg fresh plant/kg body weight) by dissolving 225 mg of dried powder in 80 ml distilled water and used to study the anti-epileptic effect in pilocarpine induced temporal lobe epilepsy. Bacoside A is the active ingredient present in *B. monnieri* extract which facilitates memory. About 100 g of plant material contains ~0.456 g of Bacoside A [18].

### Induction of epilepsy in adult rats

Adult male Wistar rats, weighing 250–300 g, were housed for 1–2 weeks before experiments were performed. Epilepsy was induced by injecting rats with pilocarpine (350 mg/kg i.p.), preceded by 30 min with atropine (1 mg/kg i.p.) to reduce peripheral pilocarpine effects. Within 20–40 min after the pilocarpine injection, essentially all the animals developed SE. Control animals were given saline injection. Behavioral observation continued for 5 h after pilocarpine injection. SE was allowed to continue for 1 h and then control and experimental animals were treated with diazepam (4 mg/kg i.p.). Animals recovered from this initial treatment within 2–3 days and were observed for the next 3 weeks. Twenty-four days after pilocarpine treatment, the rats were continuously video monitored for 72 h. The behavior and seizures were captured with a CCD camera and a Pinnacle PCTV capturing software card. One trained technician, blind to all experimental conditions, viewed all videos. Seizure activity was rated according to Racine Scale [19]. Seizures were assessed by viewing behavioral postures (i.e., lordosis, straight tail, jumping/running, forelimb clonus and/or rearing) during fast forward observation of the videos. Experimental rats which showed continuous recurrent seizures were used for the further experiments. Experimental rats were divided into three groups: (1) Control (C) (2) Epileptic (E), and (3) Epileptic rats treated with *B. monnieri* (E + B). Bacopa treated rats were given extract of *B. monnieri* orally in the dosage 150 mg/kg body/day for 15 days.

### Induction of acute hypoxia in neonatal rats

Wistar neonatal rats of 4-days old (body weight,  $6.06 \pm 0.45$  g) were used for the experiments and were grouped into seven as follows: (a) Control neonatal rats were given atmospheric air (20.9% oxygen) for 30 min (C); (b) Hypoxia was induced by placing the neonatal rats in a hypoxic chamber provided with 2.6% oxygen for 30 min (Hx); (c) Hypoxic neonatal rats were injected 10% dextrose (500 mg/kg body wt) intra-peritoneally (i.p.) immediately after induction of hypoxia (Hx + G). (d) Hypoxic neonatal rats were supplied with 100% oxygen for 30 min immediately after induction of hypoxia (Hx + O); (e) Hypoxic neonatal rats were injected 10% dextrose (500 mg/kg body wt) i.p. immediately after induction of hypoxia and then treated with 100% oxygen for 30 min (Hx + G + O); (f) Hypoxic neonatal rats were injected epinephrine (0.1  $\mu$ g/kg body wt) i.p. immediately after induction of hypoxia and then treated with 100% oxygen for 30 min (Hx + E + O); (g) Hypoxic neonatal rats, 10% dextrose (500 mg/kg body wt) and epinephrine (0.1  $\mu$ g/kg

body wt) were injected i.p. immediately after induction of hypoxia and then treated with 100% oxygen for 30 min (Hx + G + E + O). The experimental animals were maintained in the room temperature for 1 week.

### Morris water maze-time spent in each quadrant

Water maze experiment was conducted during post-treatment. The custom-constructed water maze pool measured 100 cm in diameter by 50 cm in depth and was filled with water to a depth of 35 cm. A 10 cm diameter white platform was located 1.5 cm below the surface of the water. Nontoxic white paint was added to the water to visually obscure the location of the platform. The pool had been divided into four quadrants of homogeneous size and the platform was located in the center of one of the quadrants, halfway between the center and the wall of the pool. All swim latencies were recorded with a manual stopwatch, a technique routinely employed [20]. The water maze task consisted of 15 sessions conducted once daily over 15 successive days. Each session consisted of four trials separated by  $\sim 60$  s. Rats were placed manually into the pool, facing the pool wall in the center of one of the quadrants that did not contain the platform. For any given rat, the location of the platform remained fixed across all trials and all sessions. The time spent in each quadrant to find the platform was recorded as the time from release into the pool until the rat had reached the platform. A maximum of 60 s was allowed for each trial. Rats not reaching the platform within 60 s were guided to the platform and a score of 60 s was recorded for each of these experimenter-terminated trials. The rat was allowed to remain on the platform for the duration of the inter-trial interval. A 60-s probe test to determine the time spent in the platform quadrant after removing the platform from pool was conducted on the 13th, 14th, and 15th day of the study (24 h after the last hidden platform session). Rats were released into the pool in the quadrant opposite to that previously associated with the escape platform. A manual time-sampling procedure (one measurement per second) was utilized to record the swimming bias of the rat in each of the four quadrants of the pool.

### Open-field test: resting response in hypoxia induced neonatal rats: effects of glucose, oxygen and epinephrine

General activity in the open-field, simultaneous measurement of locomotion, rearing, and stereotyped head movements of the experimental animals were measured. Rodents naturally avoid bright light and open spaces. When

placed in a bright lit open-field, rats tend to remain in the periphery of the apparatus or against the walls. Evaluation of open-field behavior was done between 0800 and 1600 h. Extraneous noise was minimized during testing and the observer was the only person present. The observer hand-carried the rat from its cage to the open field. At the beginning of testing, the rat was placed in the center of an open-field enclosure constructed of Plexiglas cage, 41 cm (L) × 41 cm (W) × 38 cm (H) with four walls and floor but no ceiling. The rats were observed for 5 min after being placed in the open field and their behavior were evaluated and recorded every 6 s. During testing the observer sat as far from the open field as possible so as not to disturb the rat and made no sudden movements or noises.

#### Open-field behavior: scoring criteria

Animal movements in the open-field were measured for 5 min. Each trial began with placing the animal in the center of the open-field (to maximize the initial fear response). At the beginning of each trial, the animal was briefly covered by a cardboard box of similar size to the animal's body length and width. When the box was lifted, the animal was allowed to freely ambulate. After each trial, the animal was returned to its home cage, which was placed within the testing room. In order to minimize interference with the animal's behavior, the experimenter remained at the same location in the room during all trials [21]. Data were collected as an indication of activity in the center and periphery of the arena. The data were subsequently expressed with specific parameters [22].

#### Real-Time PCR assay

RNA was isolated from the brain regions using Tri reagent. Total cDNA synthesis was performed using ABI PRISM cDNA Archive kit. Real-Time PCR assays were performed in 96-well plates in ABI 7300 Real-Time PCR instrument (Applied Biosystems). PCR analyses were conducted with gene-specific primers and fluorescently labeled Taq for mGluR8 and NMDAR1 (designed by Applied Biosystems). Endogenous control ( $\beta$ -actin) was labeled with a report dye (VIC). All reagents were purchased from Applied Biosystems.

The thermocycling profile conditions were as follows: 50°C—2 min—Activation, 95°C—10 min—Initial Denaturation, 95°C—15 s—Denaturation 40 cycles, 50°C—30 s—Annealing, 60°C—1 min—Final Extension.

The  $\Delta\Delta CT$  method of relative quantification was used to determine the fold change in expression. This was done by first normalizing the resulting threshold cycle (CT) values

of the target mRNAs to the CT-values of the internal control  $\beta$ -actin in the same samples ( $\Delta CT = CT_{\text{Target}} - CT_{\beta\text{-actin}}$ ). It was further normalized with the control ( $\Delta\Delta CT = \Delta CT - CT_{\text{Control}}$ ). The fold change in expression was then obtained ( $2^{-\Delta\Delta CT}$ ).

#### Statistical methods

Latency values in the Morris water maze and Open field test were analyzed by a three-way ANOVA within dependent factors for treatments, days and trials (repeated measures). Relative quantification software was used for analyzing Real-Time PCR results.

#### Results

##### (1) Real-Time PCR analysis of mGluR8 in post-treated epileptic rats

Real-Time PCR analysis showed that the mGluR8 mRNA significantly decreased ( $P < 0.01$ ) in E when compared to C and it reversed ( $P < 0.05$ ) to near control level in (E + B) (Table 1, Fig. 1).

##### (2) Morris water maze experiment in the control and epileptic rats

Time spent in the platform quadrant of the E showed a significant decrease ( $P < 0.01$ ) when compared to C. *B. monnieri* treatment ( $P < 0.01$ ) reversed the time spent in the platform quadrant to near control level in (E + B) (Table 2, Fig. 2) when compared to E.

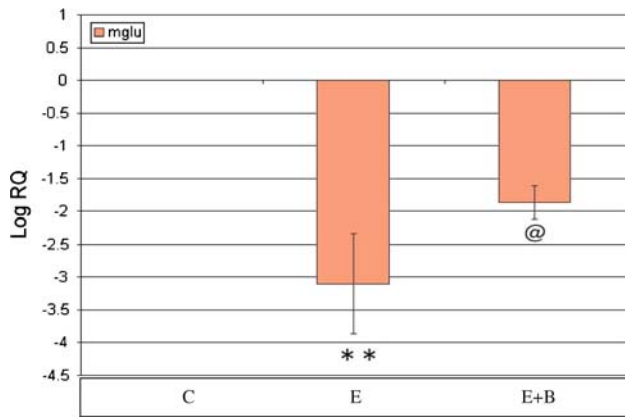
**Table 1** Real-Time PCR amplification of the mGluR8 mRNA from the cerebellum of control and epileptic groups of rats

Experimental group	Log RQ-value
C	0
E	$-3.099 \pm 0.07^{**}$
E + B	$-1.862 \pm 0.25^*$

Values are Mean  $\pm$  SEM of 4–6 separate experiments. Each experimental group contains eight groups of rats. Relative Quantification values and standard deviations are shown in the table. The relative ratios of mRNA levels were calculated using the  $\Delta\Delta CT$  method normalized with  $\beta$ -actin CT-value as the internal control and Control CT-value as the calibrator. Control rats (C), Epileptic rats (E), Epileptic + *Bacopa monnieri* treated rats (E + B)

\*\*  $P < 0.01$  when compared to Control (C)

\*  $P < 0.05$  when compared to Epileptic (E)



**Fig. 1** Real-Time PCR amplification of the mGluR8 mRNA from the cerebellum of control and epileptic groups of rats. Values are mean ± SEM of 4–6 separate experiments. Each experimental group contains eight groups of rats. Relative Quantification values and standard deviations are shown in the table. The relative ratios of mRNA levels were calculated using the  $\Delta\Delta CT$  method normalized with  $\beta$ -actin CT-value as the internal control and Control CT-value as the calibrator. \*\* $P < 0.01$  when compared to C; @ $P < 0.05$  when compared to E

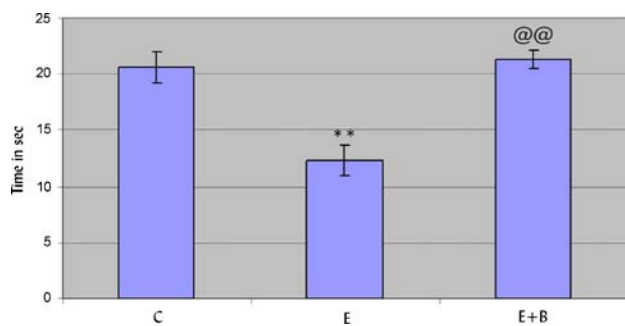
**Table 2** Time spent in platform quadrant by the control and epileptic group of rats in Morris water maze experiment

Experimental group	Log RQ-value
C	20.6 ± 1.45
E	12.3 ± 1.30**
E + B	21.3 ± 0.88*

Values are mean ± SEM of 4–6 separate experiments. Each experimental group contains eight groups of rats. Control rats (C), Epileptic rats (E), Epileptic + *Bacopa monnieri* treated (E + B)

\*\*  $P < 0.01$  when compared to Control (C)

\*  $P < 0.01$  when compared to Epileptic (E)



**Fig. 2** Time spent in platform quadrant by the control and epileptic group of rats in Morris water maze experiment. Values are mean ± SEM of 4–6 separate experiments. Each experimental group contains eight groups of rats. \*\* $P < 0.01$  when compared to C; @@ $P < 0.01$  when compared to E

(3) Real-Time PCR analysis of glutamate NMDAR1 receptor in control and hypoxic neonates

NMDA receptor 1 subunit of glutamate receptor mRNA showed a significant increase ( $P < 0.001$ ) in Hx, Hx + O, Hx + E + O, Hx + G, Hx + G + O, and Hx + G + E + O compared to C, whereas Hx ( $P < 0.001$ ), Hx + O ( $P < 0.001$ ), Hx + E + O ( $P < 0.01$ ), Hx + G + E + O ( $P < 0.001$ ), showed a significant increase when compared to Hx + G + O. A significant increase ( $P < 0.001$ ) was seen in Hx, Hx + O, Hx + E + O, and Hx + G + E + O ( $P < 0.001$ ) when compared to Hx + G. Glucose treatment alone (Hx + G) and glucose with oxygen (Hx + G + O) showed a trend toward control in regulating the over-expression of NMDAR1 compared to other experimental groups. Hx + O showed a significantly ( $P < 0.001$ ) increased expression compared to the other groups of experimental neonatal rats (Table 3, Fig. 3).

(4) Resting time in open field test by control and hypoxic groups of neonatal rats

A significant increase in the resting time were observed in Hx ( $P < 0.01$ ), Hx + O ( $P < 0.001$ ), Hx + E + O ( $P < 0.05$ ) and Hx + G + E + O ( $P < 0.01$ ) compared to

**Table 3** Real-time PCR amplification of the NMDA R1 mRNA from the cerebral cortex of control and hypoxic groups of neonatal rats

Animal status	RQ-value
C	0
Hx	0.19 ± 0.02*, **, ***
Hx + G	0.07 ± 0.02*, †
Hx + O	0.31 ± 0.02*, **, ***
Hx + G + O	0.07 ± 0.02*, †
Hx + E + O	0.09 ± 0.04*, ††, ***
Hx + G + E + O	0.14 ± 0.01*, **, ***

Values are mean ± SEM of 4–6 separate experiments. Each experimental group contains eight groups of rats. Relative Quantification values and standard deviations are shown in the table. The relative ratios of mRNA levels were calculated using the  $\Delta\Delta CT$  method normalized with  $\beta$ -actin CT-value as the internal control and Control CT-value as the calibrator. Control rats C, Hypoxic rats Hx, Hypoxic rats glucose treated Hx + G, Hypoxic rats oxygen treated Hx + O, Hypoxic rats glucose and oxygen treated Hx + G + O, Hypoxic rats epinephrine and oxygen treated Hx + E + O, Hypoxic rats glucose, epinephrine and oxygen treated Hx + G + E + O

\*  $P < 0.001$  when compared to C

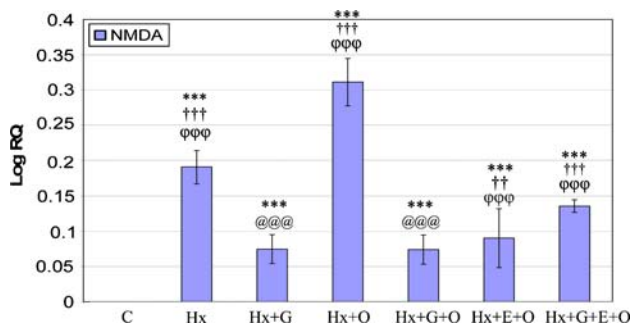
†  $P < 0.001$  when compared to Hx

\*\*\*  $P < 0.001$  when compared to Hx + G

††  $P < 0.01$  when compared to Hx + G + O

\*\*  $P < 0.001$  when compared to Hx + G + O





**Fig. 3** Real-time PCR amplification of the NMDA R1 mRNA from the cerebral cortex of control and hypoxic groups of neonatal rats. Values are mean  $\pm$  SEM of 4–6 separate experiments. Each experimental group contains eight groups of rats. Relative Quantification values and standard deviations are shown in the table. The relative ratios of mRNA levels were calculated using the  $\Delta\Delta CT$  method normalized with  $\beta$ -actin CT-value as the internal control and Control CT-value as the calibrator. \*\*\* $P$  < 0.001 compared to C; @@@ $P$  < 0.001 when compared to Hx; φφφ $P$  < 0.001 compared to Hx + G; †† $P$  < 0.01, ††† $P$  < 0.001 compared to Hx + G + O

**Table 4** Open field test-resting time by control and hypoxic groups of neonatal rats

Animal status	Resting Time (seconds)
C	137.33 $\pm$ 6.74
Hx	206.67 $\pm$ 4.41**, ††
Hx + G	166.67 $\pm$ 8.81
Hx + O	245.33 $\pm$ 7.42***, †††, #
Hx + G + O	128.33 $\pm$ 26.82
Hx + E + O	192.67 $\pm$ 28.96*, †
Hx + G + E + O	207.33 $\pm$ 8.19**, ††

Values are mean  $\pm$  SEM of 4–6 separate experiments. Each experimental group contains eight groups of rats. Control rats C, Hypoxic rats Hx, Hypoxic rats glucose treated Hx + G, Hypoxic rats oxygen treated Hx + O. Hypoxic rats glucose and oxygen treated Hx + G + O, Hypoxic rats epinephrine and oxygen treated Hx + E + O, hypoxic rats glucose, epinephrine and oxygen treated Hx + G + E + O

\*  $P$  < 0.05 compared to C

\*\*  $P$  < 0.01 compared to C

\*\*\*  $P$  < 0.001 compared to C

#  $P$  < 0.01 compared to Hx + G

†  $P$  < 0.05

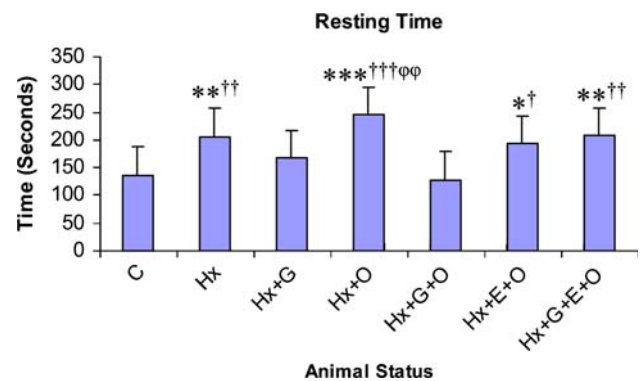
††  $P$  < 0.01

†††  $P$  < 0.001 compared to Hx + G + O

C, Hx + G and Hx + G + O showed no significant change in the behavior compared to C (Table 4, Fig. 4).

## Discussion

In the present study, we have demonstrated that long-term increase in stress/anxiety in rats occurs after brain injury



**Fig. 4** Open field test-resting time by control and hypoxic groups of neonatal rats. Values are Mean  $\pm$  SEM of 4–6 separate experiments. Each experimental group contains eight groups of rats. \* $P$  < 0.05, \*\* $P$  < 0.01, \*\*\* $P$  < 0.001 compared to C; φφφ $P$  < 0.01 compared to Hx + G ; † $P$  < 0.05, †† $P$  < 0.01, ††† $P$  < 0.001 compared to Hx + G + O

during epilepsy and hypoxia. Earlier studies have established that rhythmic output from the cerebellum contributes to the maintenance of generalized seizure [23]. Increased glutamate dehydrogenase and decreased glutamate decarboxylase results in the accumulation of glutamate in rat cerebellum [24]. Activation of mGluR8 in pilocarpine induced epileptic rats [12] has been reported to have anti-convulsant effect [25–27]. Consistent with these reports our results also show a down-regulation of the mGluR8 gene expression during epileptic condition. Treatment with *B. monnieri* extract caused a reversal to near control level.

Water maze experiment was conducted 27 days after pilocarpine injection. Place navigation in the Morris water maze consists of two distinct components: declarative place representations and procedural learning [28]. The procedural aspects include learning to inhibit inborn non-adaptive behavior, such as swimming along the wall [29, 30], while selecting appropriate behavioral strategies, such as swimming across the pool or uniformly searching its surface. Other procedural components involved skills such as improved distance and angle judgment that are a necessary prerequisite for the cognitive demands of the task. The hippocampal formation is critical for computing place representations but is believed to be dispensable for procedural memories [31]. Previous reports suggest that declarative memory is also seriously impaired by pilocarpine-induced SE [20]. Persinger et al. [32] described the deterioration of the declarative and non-declarative form of memory after seizures induced by a systemic injection of lithium pilocarpine. Our results suggest that mGluR8 gene expressions functional balance play an important role in the pathophysiology of pilocarpine induced temporal lobe epilepsy in rats and *B. monnieri* extracts shows a regulatory effect on mgluR8 gene expression in epilepsy. Treatment with crude [33] and alcoholic extract of *B. monnieri* plant

has shown enhanced learning and cognition—facilitating abilities [34]. *B. monnieri* extract has also been reported to have anti-oxidant property in brain [35] and enhance protein kinase activity and 5-HT levels in the hippocampus [36] which is most vulnerable area in epileptic brain damage. It is reported that the *B. monnieri* extract reversed the cognitive deficits in Alzheimer's disease [37]. Several pharmacological studies on the extract of *B. monnieri* have attributed the nootropic activity of the plant to the presence of two major saponins Bacoside A and B [38, 39]. Of these two, Bacoside A has been reported to chiefly facilitate memory [40] and has anxiolytic activity [18]. The decreased expression of mGluR8 was due to vulnerability to increased glutamate toxicity. Treatment with *B. monnieri* extract shows a therapeutic effect which is evident from our result, has immense clinical significance in the therapeutic management of epilepsy.

Hypoxia during the first week of life can induce neuronal death in vulnerable brain regions usually associated with an impairment of cognitive function that can be detected later in life [41]. Our Real-Time PCR results showed an increased expression of the NR1 subunit of NMDA receptors in Hx, Hx + O, Hx + E + O, Hx + G + E + O. NMDA receptor complex in rats subjected to neonatal hypoxia and the toxicity showed a decrease in NMDA receptor binding few hours after hypoxia and a functional defect of NMDA receptors at an older age [14]. Post-natal hypoxia increases NMDA receptor affinity for the antagonist MK-801 in the piglet cortex [13]. Hypoxia-induced generation of nitric oxide (NO) free radical has been reported to cause nitration of a number of cerebral proteins including the NMDA receptor which is a potential mechanism of hypoxia-induced modification of the NMDA receptor resulting in neuronal injury [42]. The up-regulated gene expression was brought back to near control level by the supplementation of glucose alone and glucose with oxygen to the sufferers of hypoxic insult during early neonatal period. The increased glucose entering the neurons is reported to protect neurons from glutamate neurotoxicity [43], stroke or seizure [44] and mitochondrial toxins [45]. On supplementation of 100% oxygen alone to hypoxic rats, there was an over activation of the NMDAR1 gene expression. The supplementation of 100% oxygen generates reactive oxygen species (ROS) which have been well documented as causative mediators of excitotoxicity [46–48]. Both free radicals and glutamate has been suggested to be involved in tandem in the neurotoxicity induced by hypoxia, whereas glutamate alone is involved in ischemic neurotoxicity [49]. NMDA neurotoxicity and oxidative stress have also been well documented as mechanisms underlying hypoxic–ischemic brain injuries. Administration of epinephrine to hypoxic neonates was found to be toxic in our studies. No studies have been

reported that demonstrates definite evidence of epinephrine-induced neurotoxicity during resuscitation program in hypoxic insult. During anesthesia, addition of epinephrine to tetracaine has shown to increase its neurotoxicity, which sustained the increase of glutamate concentrations in the cerebrospinal fluid. Epinephrine itself might decrease uptake of glutamate. Large concentrations of glutamate at the synapse of the dorsal horn activate glutamate receptors of the secondary neurons. The persistent activation of glutamate receptors might cause central sensitization [50], neuronal injury [51] or both. This differential expression of the glutamate NMDAR1 subunit correlates with the peculiar behavioral profile of the experimental rats in open field test. Abnormal behavior in 1-month old hypoxic rats was assessed using the open field test, which is a well-characterized rodent model of anxiety. Locomotion in an open field is considered to reflect general or exploratory activity, whereas a decrease in the overall mobility and ambulation are considered to represent increased anxiety in the animals [52]. Our results demonstrated a significant reduction in resting time after glucose supplementation with and without oxygen. The post-natal exposure to hypoxia specifically modified the behavior of the 1-month old neonatal rats, which showed an attention deficit and an increase in anxiety. Moreover, NMDA has also been reported to have anxiogenic-like profile in elevated plus-maze [53].

Both epileptic and hypoxic model have proved to be relevant for the study of glutamate receptors pathophysiology arising due to neurodegenerative disorders. In both epilepsy and hypoxia, modifications in the activity of receptor gene expression could contribute to the long-term altered responses to stress. In epilepsy, *B. monnieri* treatment significantly brought the reversal of the down-regulated mGluR8 gene expression toward control level. The glutamatergic system, predominantly the NMDA receptor, has important functions in the neonatal period in neurodevelopment. It was also evident that rats subjected to hypoxia during the early neonatal life develop behavioral symptoms suggesting an enhanced vulnerability to excitotoxic damage after exposure to hypoxia. Our experimental evidence shows that hypoxia, induces expressional and functional changes in the NMDA receptor of neuronal cell which can be corrected by supplementation of glucose alone or glucose followed by oxygen during the resuscitation program to prevent the glutamate related neuronal death occurring in hypoxic neonates. These corrective measures when brought to clinical practice have enormous significance for neuroprotection against epileptic and hypoxic neurodegeneration.

**Acknowledgment** This work was supported by research grants from DBT, DST, ICMR, Govt. of India and KSCSTE, Govt. of Kerala to Dr. C. S. Paulose. Finla Chathu thanks Cochin University of

Science and Technology for Junior Research Fellowship. Reas Khan thanks KSCSTE, Govt. of Kerala for Junior Research Fellowship.

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