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(RESEARCH ARTICLE)

NEUROCHEMICAL EFFECTS OF *CULCASIA FALCIFOLIA* ETHANOL LEAF EXTRACT ON IN AN EXPERIMENTAL MODEL OF SODIUM NITRITE-INDUCED HYPOXIA

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ABSTRACT

Aim: The present study investigated the effects of the ethanol leaf extract of *Culcasia falcifolia* on selected neurochemical parameters in sodium nitrite-induced hypoxic rats.

Methods: Thirty Wistar rats were divided into five groups: normal control, hypoxic control, standard drug-treated, and extract-treated groups receiving 200 and 400 mg/kg body weight, respectively. Hypoxia was induced by sodium nitrite administration for 14 days. At the end of the experimental period, brain tissue was collected to determine acetylcholinesterase (AChE) activity, glutamate concentration, and dopamine levels. The data were analyzed using one-way analysis of variance followed by Dunnett's test.

Results: Sodium nitrite-induced hypoxia increased brain AChE activity from 17.05 ± 0.20 to 21.21 ± 0.27 and glutamate levels from 76.01 ± 0.49 to 88.13 ± 0.32 µmol/g tissue, while dopamine levels decreased from 645.88 ± 1.00 to 524.79 ± 1.10 pg/mg tissue. Treatment with *C. falcifolia* extract at 200 and 400 mg/kg reduced AChE activity to 14.19 ± 0.19 and 15.28 ± 0.16, respectively, and glutamate levels to 69.16 ± 0.85 and 71.13 ± 0.47 µmol/g tissue. Dopamine levels increased to 559.15 ± 1.09 and 587.30 ± 1.09 pg/mg tissue, respectively. These changes were reported as statistically significant ($p < 0.01$).

Conclusion: The ethanol leaf extract of *C. falcifolia* modulated AChE activity, glutamate concentrations, and dopamine levels in sodium nitrite-induced hypoxic rats. These preliminary findings suggest potential neuroprotective effects and support further investigation into the mechanisms underlying its activity.

Keywords: Hypoxia, sodium nitrite, Wistar rats, Acetylcholinesterase, Glutamate, Dopamine, *Culcasia falcifolia*, Neurochemical alterations.

1 INTRODUCTION

The physiological systems are regulated by the nervous system and the neurotransmitters. The brain consumes about 20% of the total oxygen supply of the body for its normal function. Thus the brain is an extremely sensitive tissue to changes in arterial blood oxygen levels. The enzymes associated with the synthesis of several neurotransmitters require molecular oxygen for their activity. Consequently, hypoxia (i.e., reduced oxygen availability) resulting from perturbations in O_2 homeostasis can profoundly impact neurotransmitter synthesis resulting in altered neuronal functions and consequently impact physiological systems (Kumar, 2011). During hypoxia, changes will occur in the diffusion parameters of the extracellular space and extracellular concentration of energy–related metabolites and glutamate in rat cortex. Hypoxia in rat cortex has shown decrease in extracellular space volume and increase in tortuosity with in few minutes following cardiac arrest (Holingan and Jain, 2006). According to Zedan, $NaNO_2$ administration consequences on oxidative stress, ischemia, inflammation, hypoxia and reduced metabolic energy, resulted in brain and liver injury. Hypoxia damages hepatic, respiratory, digestive, nervous, urinary and endocrine systems. The highest degree of oxidative damage usually occurs in organs like brain, heart and skeletal muscles, since these organs are composed primarily of post mitotic cells. the central nervous system shows increased susceptibility to oxidative stress because of its high oxygen consumption rate (20% of the total oxygen inhaled by the body) that accounts for the increase in generation of oxygen free radicals and reactive oxygen substances (Srinivasan, 2002). Neurotransmitters play essential roles in neuronal communication, and changes in their concentrations or metabolism may contribute to neurological dysfunction during hypoxic conditions. Glutamate is the principal excitatory neurotransmitter in the mammalian central nervous system. Under hypoxic or ischemic conditions, impaired energy metabolism and disturbances in ion gradients may promote excessive extracellular glutamate accumulation and excitotoxic neuronal injury (Dugan & Choi, 1994). Dopamine is a catecholamine neurotransmitter involved in motor control, motivation, reward processing, and other neurological functions. Its synthesis and metabolism are influenced by oxygen availability and cellular metabolic conditions. Hypoxia may alter dopaminergic neurotransmission through changes in neurotransmitter synthesis, release, and neuronal regulation (Kumar, 2011). The AChE activity has been shown to be increased with in and around hypoxic brain. The calcium influx followed by oxidative stress is involved in the increase in activity of AChE induced by sodium nitrite, decreasing cell membrane order and ultimately leading to the exposure of more active sites of the enzyme. Increasing in AChE activity and ROS production indicates that it can be possible to ameliorate cholinergic function by inhibiting sodium nitrite, which induces increase in AChE activity (Muralidharan et al. 2009).

The present study was designed to investigate the effects of the ethanol leaf extract of *Culcasia falcifolia* at doses of 200 and 400 mg/kg on neurochemical parameters in sodium nitrite-treated Wistar rats. AChE activity, brain glutamate levels, and dopamine levels.

2 MATERIALS AND METHODS

2.1 Plant Material and Authentication

The leaves of *Culcasia falcifolia* were collected from along the river Kingwal in Kaptildil, Nandi County, Rift Valley, Kenya. The leaves were identified and authenticated from the National Herbarium, Kenya (voucher number PS 22/05). The leaves were thoroughly washed with tap water to remove dust and other unwanted materials accumulated on the leaves from their natural environment. The dust free leaves were allowed to dry under shade in the laboratory for two weeks. The dried leaves were ground to powder using laboratory electric blender to obtain a powder. The powder was further passed through a sieve to obtain finer particles. The leaf sample was stored in a clean dry labeled glass beaker until needed for extract preparation and analysis.

2.2 Extract preparation

The leaf sample was mixed and macerated with absolute ethanol at a 1:20 ratio (100 g in 1 liter of solvent) for 7 days. Then the extract was filtered through Whatman No 1 filter paper followed by evaporation of the supernatant using the BUCHI Switzerland rotary evaporator to remove the ethanol and to obtain concentrated, oily extract. The crude extract was stored in sterile universal glass bottle until further use.

2.3 Experimental animals

Colony inbred strains of Wister rats of either sex weighing 200-220g were used for the pharmacological studies. The animals were kept under standard conditions (day/night rhythm) 8.00 am to 8.00 pm, 22 ± 1^0C room temperature, in polypropylene cages. The animals were fed by standard pelleted diet (Hindustan Lever Pvt Ltd., Bangalore) and water ad libitum. The animals were housed for one week in polypropylene cages prior to the experiments to acclimatize to laboratory conditions. It is randomly distributed into four different groups with six animals in each group under identical conditions throughout the experiments.

2.4 Experimental design

Thirty Wister rats were divided into five groups of six animals each as follows

- Group I- served as control and received normal saline (Normal control)
- Group II- served as hypoxic rats and received sodium nitrite water for 14 days. (Hypoxic control)
- Group III- animals received standard drug Dextromethorphan (10 mg/kg; p. o) and sodium nitrite water for 14 days. (Standard drug control)
- Group IV- animals received extract (200 mg/ kg; p. o) suspended in 1% gum acacia and sodium nitrite water for 14 days. (Extract 200 mg/kg; p. o)
- Group V- animals received extract (400 mg/kg; p. o) suspended in 1% gum acacia and sodium nitrite water for 14 days. (Extract 400 mg/kg; p. o)

2.5 Induction of hypoxia

Hypoxia was induced by allowing the rats to drink sodium nitrite water (30mg/kg body weight of sodium nitrate was dissolved in normal water) by gavage (5ml/kg in dosage volume) for 14 days except control group which were allowed to drink normal water (Nyakas et al. 1994).

2.6 Collection and preparation of brain tissue

At the end of the 14 days, the animals were sacrificed by decapitation. Brains were immediately carefully removed and washed in 0.9% cold normal saline two times and placed into a sterile cooled glass plate. Then the brains were separated and cut into pieces using small surgical scissors. The cut pieces were homogenized with motor driven Teflon coated homogenizer with 5 mL of ice-cold 0.1 M Tris-HCl buffer pH 7.4 to get 10% homogenate. The homogenate was centrifuged at 10,000 rpm for 20 min at 5˚C in order to separate the two phases. The overlaying organic phase was removed and the supernatant was collected.

2.7 Estimation of acetylcholinesterase activity (AChE)

To 20 mg of brain tissue per mL of phosphate buffer (0.1 M; pH 8) was homogenized in a potter-elvehjem homogenizer. A 0.4 mL aliquot of brain homogenate was added to a cuvette containing 2.6 mL of 0.1 M phosphate buffer. 100 µL of dithio-bis-nitro benzoic acid reagent was added to the cuvette and the absorbance was measured at 412 nm. 20 µL of acetylcholine chloride was added. A change in the absorbance was recorded and the change in absorbance/min was calculated. It was expressed in µ mol/min/g tissue (Ellman et al., 1961).

2.8 Estimation of brain glutamate levels

Weighed quantity of the brain portion was homogenized with 2 parts by weight of perchloric acid and centrifuged for 10 min at 3,000 rpm. 3 mL of supernatant fluid was adjusted to pH 9.0 with 1 mL phosphate solution. It was allowed to stand for 10 min in an ice bath and then filtered through fluted filter paper. Absorbance was measured at 340 nm. Similarly, a blank reading at 340 nm was measured. The level of glutamate was expressed as µmol/g tissue (Bernt and Bergmeyer, 1965)

2.9 Estimation of dopamine levels in brain tissue

To 0.02ml of the aqueous phase 0.005 mL of 0.4 M hydrochloric acid and 0.01 mL EDTA/sodium acetate buffer (pH 6.9) were added, followed by 0.01 mL iodine solution (0.1 M in ethanol) for oxidation. The reaction was stopped after 2 min by addition of 0.01 mL Na_2SO_3 in 5 M NaOH (0.5 g Na_2SO_3 in 2 mL H_2O + 18 mL 5 M NaOH). Acetic acid (0.01 mL, 10 M) was added 1.5 min later. The solution was then heated to 100°C for 6 min. when the sample again reached room temperature, excitation and emission spectra were read in the microcuvette. The readings were taken at 330-375 nm (Schlumpf et al., 1974).

2.10 Statistical analysis

All experimental data are presented as mean ± S.E.M of six animals in each group. The statistical analysis was carried out using one-way analysis of variance (ANOVA), followed by a Dunnett's t-test for multiple-comparison test. Difference in the values at P values < 0.05, $p<0.01$, $p<0.001$ were considered as significant.

3 RESULTS AND DISCUSSION

3.1 Effect ethanol leaf extract of *culcasia falcifolia* on Acetyl cholinesterase activity

The effect of ethanol leaf extract of *culcasia falcifolia* on Acetyl cholinesterase activity in sodium nitrate-induced hypoxic rats are shown in table 1. Induction of hypoxia significantly ($p<0.01$) increased the AChE activity when compared with control group. treatment with the standard drug reduced AChE activity. In the extract-treated groups, (200 and 400 mg/kg) there was a significant ($p<0.01$) reduction in enzyme levels when compared with the hypoxic group. The results indicate that the extract at both doses lowers AChE activity in induced hypoxic rats. These findings are comparable to the findings reported by Muralidharan et al (2009) who investigated the cerebro-protective effects of *Glycyrrhiza glabra* root extract in sodium nitrate induced hypoxic rats.

Table 1: Effect ethanol leaf extract of *culcasia falcifolia* on Acetyl cholinesterase activity

Group	Micromole/min/mg protein sec
I (Normal control) normal saline 5 ml/kg; p. o	17.05±0.20
II (Hypoxic group) $NANO_2$ 30mg/kg; p. o	21.21 ±0.27**
III (Standard drug) 10 mg/kg + $NANO_2$; p. o	16.13 ±0.21**
IV (CF Extract) 200mg/kg + $NANO_2$; p. o	14.19 ±0.19**
V (CF Extract) 400mg/kg + $NANO_2$; p. o	15.28 ±0.16**

Values are expressed as mean ±SEM (n=5); **P<0.01 when compared with control group; **p<0.01 when compared with hypoxic control group; Symbol represents the statistical significance done by ANOVA, followed by Dunnet's "t" test.

3.2 Effect ethanol leaf extract of *Culcasia falcifolia* on glutamate level

The effect of ethanol leaf extract of *Culcasia falcifolia* on glutamate level in sodium nitrate-induced hypoxic rats are shown in table 2. Sodium nitrate induced hypoxia increased the glutamate level 88.13 ±0.32. This shows that hypoxia causes disturbance in glutamate homeostasis. During cerebral hypoxia, reduced oxygen availability can impair energy-dependent processes involved in maintaining neuronal ion gradients and glutamate uptake. Consequently, extracellular glutamate may accumulate and contribute to excitotoxic neuronal damage (Dugan & Choi, 1994). Administration of *C. falcifolia* ethanol leaf extract at 200 and 400 mg/kg reduced glutamate levels to (69.16 ± 0.85) and (71.13 ± 0.47) µmol/g, respectively. These results indicate that the extract may influence glutamate regulation in sodium nitrate-induced hypoxia rats. Fathima et al. (2018) also reported that *Bixa orellana* leaf extract attenuated biochemical alterations, including elevated glutamate levels, in sodium nitrite-induced hypoxic rats. Glutamate is an excitatory neurotransmitter in brain primarily produced from glutamine by the action of phosphate-activated glutaminase, a mitochondrial enzyme. Hypoxia causes increase in glutaminase activity and glutamate levels in dorsal and ventral brainstem regions in rats (Kumar, 2011).

Table 2: Effect ethanol leaf extract of *culcasia falcifolia* on glutamate levels

Group	Micromoles/gm
I (Normal control) normal saline 5 ml/kg; p. o	76.01 ±0.49
II (Hypoxic group) $NANO_2$; 30mg/kg; p. o	88.13 ±0.32**
III (Standard drug) 10 mg/kg + $NANO_2$; p. o	74.33 ±0.59**
IV (CF Extract) 200mg/kg + $NANO_2$; p. o	69.16 ±0.85**
V (CF Extract) 400mg/kg + $NANO_2$; p. o	71.13 ±0.47**

Values are expressed as mean ±SEM (n=5); **P<0.01 when compared with control group; **p<0.01 when compared with hypoxic control group; Symbol represents the statistical significance done by ANOVA, followed by Dunnet's "t" test.

3.3 Effect ethanol leaf extract of *culcasia falcifolia* on dopamine levels

The effect of ethanol leaf extract of *culcasia falcifolia* on dopamine level in sodium nitrate-induced hypoxic rats are shown in table 3. Sodium nitrate induced hypoxia decrease in dopamine level. Treatment with standard drug increased the dopamine level. The results show that the administration of the extract at 200 and 400 mg/kg significantly increased the reduced dopamine level ($p<0.01$).

Table 3: Effect ethanol leaf extract of *culcasia falcifolia* on dopamine levels

Group	Picogram/mg tissue
I (Normal control) normal saline 5 ml/kg; p. o	645.88±1.00
II (Hypoxic group) $NANO_2$; 30mg/kg; p. o	524.79 ±1.10**
III (Standard drug) 10 mg/kg + $NANO_2$; p. o	605.73 ±1.30**
IV (AGMF Extract) 200mg/kg + $NANO_2$; p. o	559.15 ±1.09**
V (AGMF Extract) 400mg/kg + $NANO_2$; p. o	1.09**

Values are expressed as mean ±SEM (n=5); **P<0.01 when compared with control group; **p<0.01 when compared with hypoxic control group; Symbol represents the statistical significance done by ANOVA, followed by Dunnet's "t" test.

4 CONCLUSION

The findings of the present study suggest that the ethanol leaf extract of *Culcasia falcifolia* shows anti-hypoxic effects induced by sodium nitrite and this effect may be mediated by its anti-oxidant properties. The results provide preliminary evidence regarding the influence of the ethanol leaf extract of *Culcasia falcifolia* on cholinergic enzyme activity and glutamate and dopaminergic neurochemical measures.

STATEMENTS ON COMPLIANCE WITH ETHICAL STANDARDS

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Statement of ethical approval

Animal experiments were conducted in accordance with the institutional and international ethical guidelines. Ethical approval was obtained from the Institutional scientific research ethical committee of the University of Eastern Africa, Baraton.

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