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Original Article

Neuroprotective Potential of Hericium Erinaceus aqueous extract utilization in ischemia-reperfusion injury related middle cerebral artery occlusion stroke model.

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Abstract

Background: Globally, stroke is the elusive basis of sensory and motor impairments in humans. Multiple herbal compounds have been shown in studies to have positive therapeutic potential when used in conjunction with pharmaceutical therapies for the treatment and primary prevention of ischemic stroke. Current research investigation is planned to determine the neuro-protective potential of Hericium Erinaceus (HE) aqueous extract utilization in pre and post-middle cerebral artery occlusion (MCAO) model of ischemic–reperfusion injury.

Methodology: Fifty-four Wistar albino rats (200-250 gms; 10-12 weeks) were divided into four experimental groups (n=9). Group I (control); Group II (sham); Group III (MCAO) MCAO for 20-30 min then 24 hours of reperfusion; Group IV was split into three groups as subgroup I (300 mg/kg BWT of AEHE given for 7 days), subgroup II (300 mg/kg BWT of AEHE given for 7 days and then MCAO), subgroup III (at the 4th hour of MCAO induction 300 mg/kg of AEHE was given). Blood and cerebral, hepatic, and renal tissue samples were preserved and evaluated for modifications in plasma lipids levels, liver-kidney levels, C - reactive protein, blood glucose, and tissue antioxidant levels. A histopathological study was done over the selected tissues.

Results: MCAO induction significantly alters the reno-hepatic profiles, CRP levels, and animal tissue antioxidant (CAT, SOD, and GSH) enzymes levels. However, HE-extract in both subgroups (Pre-MCAO HE and Post-MCAO HE) has significantly reduced lipid panel, glucose levels, and reno-hepatic parameters significantly. HE has effectively regulated antioxidant enzymes in cerebral, hepatic, and renal homogenates. In Pre-MCAO HE and Post-MCAO HE groups, the histopathological architecture of tissues is preserved.

Conclusion: Daily dietary consumption of HE extract in calculated quantity significantly reduces the biochemical changes related to MCAO in a rat stroke model in ischemia-reperfusion injury.

Keywords

Ischemia-Reperfusion Injury, Rats, Hericium Erinaceus.

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Introduction

Ischemic stroke is a disabling polyetiologic disorder that severely impacts patients and society with anticipation of increased prevalence in the upcoming years[1]. Asia has 60% of the world's population, and the mortality rate due to stroke is greater in Asian countries relative to Western countries[2]. Venketasurbramanian study estimated 250/100,000 persons per year stroke cases in the Pakistani population[3]. Ischemic strokes are more common and constitute 80% of world stroke incidence[1].

Ischemic stroke associated with the underlying pathological mechanism of atherosclerosis increases systemic oxidative stress but also promotes inflammatory and tissue-damaging phenomenon[4]. Once ischemia begins, neuronal cells undergo excitotoxicity, and increased calcium influx leads to mitochondrial dysfunction, which triggers cell death pathways[5]. Malfunctioning of the mitochondrial oxidative respiratory chain generates reactive oxygen species, which are not neutralized by antioxidant mechanisms in the cell4. Reversal from ischemic neuronal injury in ischemic stroke can be achieved by combining pharmacological i.e. thrombolysis, and non-pharmacological i.e. neuroprotective and dietary modifications[6]. Anti-oxidative and anti-inflammatory pathways are influenced by natural chemicals extracted from plants, herbs, or mushrooms to prevent neurological impairment[7].

Hericium Erinaceus, also known as lion's mane mushroom, is edible and belongs to Phylum Basidiomycota. This mushroom has long been used in Chinese medications, mostly grown in East Asian countries[8]. Recent research has demonstrated that it potentiates antioxidant8, hypolipidemic[8], and nerve growth properties that can help prevent ischemic injury to neurons[9]. The main active ingredients of this mushroom are diterpenoids, i.e., Eranicines and Hericinones, which have neuroprotective potential[10]. The current study aims to determine the neuroprotective and antioxidant potential of the aqueous extract of HE in the MCAO rat model of stroke ischemia-reperfusion damage.

Methodology

Animals

Fifty-four female albino Wistar rats (200-250 gms; 10-12 weeks) bought from the International Center of Chemical and Biological Sciences, animal care and housing facility, Pakistan, were acclimatized initially as one animal per cage under controlled environmental conditions in a well-ventilated animal house of Department of Physiology (University of Karachi, Pakistan) for 7 days. During the study duration, the physical condition of rats was closely monitored, and they were given a standard laboratory diet and water ad libitum.

He extracts

HE mushrooms were bought from Vita Agrotech Sdn. Bhd in Tanjung Sepat, Selangor, Malaysia. In 50 ml of distilled water, 50 gm of fruiting bodies of mushrooms were boiled, cooled for 30 minutes, then filtered and stored at 4ºC. The fresh extract was then given to the animals[11].

AEHE dose

AEHE (300 mg/kg bwt) was given orally for 7 days[11] and then dissected the next day.

Experimental Protocol

Rats were split into four experimental groups, and the treatment groups were further separated into three subgroups.

Control Group: (n=9) includes control animals.

Sham Group: (n=9) Midline neck incision was given to expose the common carotid artery and its branches without ligation. After the procedure incision site was sutured.

MCAO Group: (n=9) Animals were anesthetized with ketamine and xylazine (80:10 mg/kg); in the cervical region midline incision was given, then soft tissues retracted, and to interrupt the blood supply, monofilament nylon suture was inserted in the middle cerebral artery near common carotid artery and advanced to the internal carotid artery. Heating lamps were used to maintain body temperature at 37 ± 2ºC. After 20-30 minutes

filament was withdrawn, and circulation was permitted to resume[12].

Hericium Erinaceus Treated Groups (n=27)

Subgroup I: HE Treated Group (n=9):

Animals were given AEHE (300 mg/kg bwt) orally for 7 days[11] and were dissected the next day.

Subgroup II: Pre MCAO HE group (n=9):

AEHE (300 mg/kg bwt) was given orally for 7 days[11], and MCAO was induced the next day.

Subgroup III: Post MCAO HE group (n=9):

At the 4th hour of MCAO induction[13] AEHE (300 mg/kg bwt) was given orally to the animals.

Sampling, after 24 hours of the MCAO procedure, animals were executed for the collection of blood samples and organs (brain, liver, and kidney).

Homogenization of tissues

Cerebral tissues were homogenized with cold TBS (150mMNaCl, 50Mm TRIS, Ph-7) and centrifuged at 4ºC for 15 minutes[14]. Liver tissues were homogenized with cold KCl solution (1.17%) and then centrifuged for twenty minutes at 4ºC[15]. Kidney tissue was homogenized by mixing with 100 mmol KCl Buffer and then at 4ºC centrifuged for 60 min[15]. Resultant floating fluids were collected and preserved at -80ºC till further analysis[14,15].

Biochemical Analysis

Lipid Profile Estimation: Plasma TC (CHOD-PAP method), TG (GPO-PAP method), and HDL-C (Phosphotungstate precipitation method) levels were measured using an enzymatic kit (Global, UK). LDL-C and VLDL-C levels were estimated by Friedewald's and Bairaktari formula[23], respectively. A formula from Umeshchandra[16] calculated the Atherogenic plasma index.

Liver Function Test: Serum AST, ALT, and ALP levels were analyzed by the kinetic method using an enzymatic kit (Erba diagnostic, Germany).

Kidney Profile: Plasma urea level was measured with an enzymatic kit (Erba Diagnostic, Germany), serum uric acid level was measured with a uric acid assay kit (Abcam, UK), and Jaffe's method enzymatic kit (Biogene Diagnostics, USA) was used for serum creatinine measurement.

Blood urea nitrogen (BUN) level was estimated with the mathematical formula of Chris[17]. A bio latex kit using particle enhanced turbidimetric immunoassay method (Conformidad Europea, Spain) was used to measure the CRP level. And plasma glucose level was estimated with an enzymatic kit (GOD-POD method) (Global, UK). Tissue CAT level in prepared homogenates was measured with the method of Sinha (1972). Tissue SOD level was measured with the method of Kono (1978). Tissue GSH level was measured with Carlberg and Mannervik (1985) method.

Statistical Analysis

SPSS software 25.0.0.0 was used for data management. Values were given in Mean ± Standard Error of Mean. Statistically, ANOVA was used for the assessment of differences between different groups. The conventional significance level was $P<0.05$.

Results

AEHE treatment effects on biochemical analysis

a. Effect of AEHE on lipid profile

In animals of the MCAO group, no significant ($p>0.05$) alteration in lipid profile levels in comparison to the control and sham groups was seen (Table I). With pretreatment of AEHE in Pre MCAO HE group, a significant decline in TC ($p<0.005$), TG ($p<0.05$), LDL ($p<0.01$), VLDL ($p<0.05$), and non-significant change in HDL levels in comparison to the sham group were seen (Table 1).

Animals of the Post-MCAO HE-treated group showed non-significant ($p>0.05$) changes in plasma lipid profile levels compared to animals of Sham, MCAO, and HE-treated groups. However, changes in plasma lipid profile levels of Post MCAO

HE animals compared to Pre MCAO HE animals are significant ($p<0.05$). Administration of AEHE in healthy animals of the HE-treated group has shown non-significant management of plasma lipid profile level ($p>0.05$).

Table 1: Biochemical estimation in different experimental groups.

Parameters	Control (n=9)	Sham (n=9)	MCAO (n=9)	HE treated (n=9)	Pre MCAO HE (n=9)	Post MCAO HE (n=9)
TC (mg/dl)	123.5±9.7	132.1±4.9^{-}	126.4±2.5$^{-/-}$	115.3±5.5$^{-/-/-}$	102.9±2.8$^{-/***/*/-}$	126.8±1.0$^{-/-/-/-/*}$
TG (mg/dl)	59.8±3.0	63.9±2.0^{-}	60.9±1.1$^{-/-}$	58.4±2.1$^{-/-/-}$	50.8±2.4$^{-/***/-/-}$	62.4±1.8$^{-/-/-/-/*}$
LDL (mg/dl)	64.2±8.7	70.8±6.1^{-}	69.0±2.9$^{-/-}$	57.7±4.2$^{-/-/-}$	47.6±1.2$^{-/**/*/-}$	67.6±1.1$^{-/-/-/-/*}$
VLDL (mg/dl)	11.9±0.6	12.7±0.4^{-}	12.1±0.2$^{-/-}$	11.6±0.4$^{-/-/-}$	10.1±0.4$^{-/**/-/-}$	12.4±0.3$^{-/-/-/-/*}$
HDL (mg/dl)	47.3±2.2	48.3±2.8^{-}	45.1±0.5$^{-/-}$	45.8±1.6$^{-/-/-}$	45.0±2.2$^{-/-/-/-}$	46.7±1.0$^{-/-/-/-/-}$
AIP	0.10±0.01	0.12±0.01^{-}	0.13±0.01$^{-/-}$	0.10±0.01$^{-/-/-}$	0.05±0.005$^{-/*/*/-}$	0.12±0.01$^{-/-/-/-/*}$
ALT(IU/L)	18.09±2.5	20.46±1.6^{-}	23.75±0.9$^{-/-}$	22.83±1.8$^{-/-/-}$	18.36±0.4$^{-/-/-/-}$	23.29±0.8$^{-/-/-/-/-}$
AST (IU/L)	34.37±1.6	37.29±3.2^{-}	42.03±1.9$^{-/-}$	42.54±1.7$^{*/-/-}$	26.65±1.1$^{*/***/***/***}$	41.99±0.4$^{*/-/-/-/***}$
ALP(U/L)	48.06±2.3	53.47±3.7^{-}	54.96±1.7$^{-/-}$	52.77±1.2$^{-/-/-}$	35.04±1.6$^{***/***/***/***}$	53.90±2.6$^{-/-/-/-/***}$
Urea (mg/dl)	33.46±1.2	36.41±0.4^{-}	50.68±2.4$^{***/***}$	32.18±0.9$^{-/-/***}$	25.67±0.9$^{*/***/***/-}$	34.63±3.2$^{-/-/***/-/**}$
Uric acid (mg/dl)	2.55±0.01	2.71±0.02^{-}	4.25±0.07$^{***/***}$	2.46±0.05$^{-/-/***}$	3.68±0.09$^{***/***/**/***}$	3.92±0.14$^{***/***/-/***/-}$
Creatinine(mg/dl)	0.36±0.04	0.32±0.02^{-}	0.47±0.03$^{*/***}$	0.26±0.02$^{-/-/***}$	0.35±0.01$^{-/-/*/-}$	0.32±0.02$^{-/-/***/-/-}$
BUN(mg/dl)	16.85±0.82	17.22±0.33^{-}	24.91±0.79$^{***/***}$	16.46±0.48$^{-/-/***}$	9.6±0.29$^{***/***/***/***}$	16.56±0.84$^{-/-/***/-/***}$
Glucose(mg/dl)	113.30±5.8	113.71±4.2^{-}	108.47±8.3$^{-/-}$	116.03±4.4$^{-/-/-}$	103.3±3.9$^{-/-/-/-}$	113.52±4.1$^{-/-/-/-/-}$
C-RP (mg/dl)	9.24±0.44	9.65±0.32^{-}	11.33±0.77$^{*/-}$	9.76±0.32$^{-/-/-}$	9.59±0.28$^{-/-/-/-}$	10.29±0.56$^{-/-/-/-/-}$

Significance level P<0.005***, P<0.05**, P<0.01*, - non-significant; compared with control/ sham/ MCAO/ HE/ Pre MCAO HE/ Post MCAO HE.

b. Effect of AEHE on Hepatic profile

Serum ALP, ALT, and AST levels were elevated ($p>0.05$) in animals of the MCAO group compared to the animals of the sham and control groups. AEHE treatment in animals of the HE-treated group has shown a slight decrease ($p>0.05$) in the levels of these enzymes compared with animals of the Sham group (Table 1).

A significant decrease ($p<0.005$) was noted in serum AST and ALP levels in Pre MCAO HE animals compared with animals of control, sham, MCAO, and HE-treated groups. Serum ALT levels were decreased non-significantly in Pre MCAO HE animals. In animals of the Post MCAO HE group, a non-significant change in serum enzyme (ALT, AST, ALP) levels were seen compared with all other experimental groups. Still, these values were significantly ($p<0.005$) high when compared to animals of the Pre MCAO HE treated group (Table 1).

c. Effect of AEHE on Renal profile

Plasma urea, uric acid, creatinine, and BUN levels raised significantly ($p<0.05$) in MCAO group animals. AEHE intake in Pre and Post-MCAO HE group animals has significantly ($p<0.005$) reduced urea, creatinine, uric acid, and BUN levels compared with MCAO group animals. AEHE administration in animals of the HE-treated group maintains plasma levels of urea, uric acid, creatinine, and BUN close to animals of the control and sham groups (Table 1).

d. Effect of AEHE on Glucose and C-RP levels

Plasma glucose levels changed non-significantly ($p>0.05$) in MCAO, HE treated, and Pre- MCAO HE groups and stayed the same in sham and post-MCAO HE groups in comparison to the control group (Table 1). CRP levels were increased significantly ($p<0.05$) in the MCAO group in comparison to the control group, whereas non-significant changes, were noted in sham, HE

treated, Pre-MCAO HE, and Post-MCAO HE groups compared to the control group (Table 1)

AEHE treatment effects on tissues (brain, liver, kidney)

a. Effect of AEHE on Brain Antioxidants level

Changes in different parameters of brain tissue are shown in table 2. A significant increase in brain weight was observed in the MCAO group ($p<0.005$) in comparison to the control group. In contrast, a significant decrease in brain weight was noted in the HE group ($P<0.005$) compared to the MCAO group. Pre-MCAO HE group has shown a significant ($p<0.05$) increase in brain weight in comparison to control ($p<0.005$) and HE-treated ($p<0.005$) groups. No significant changes were noted in total protein levels in brain tissue compared to different experimental groups.

Tissue catalase levels decreased significantly in the MCAO ($p<0.05$) and Pre-MCAO HE ($p<0.005$) groups in comparison to sham and MCAO groups, respectively. In the HE-treated group, CAT level increased significantly compared to the control and MCAO group. In the Post-MCAO HE group, catalase levels decreased significantly in comparison to the Sham ($p<0.05$), HE-treated, and Pre-MCAO HE ($p<0.005$) groups (Table 2).

Tissue SOD levels decreased significantly in comparison to the control and sham groups. In HE-treated, Pre, and Post-MCAO HE groups, SOD levels increased significantly compared to the MCAO group. In contrast, it decreased significantly in the Post-MCAO HE group compared to control, HE-treated, and Pre-MCAO HE groups (Table 2).

Tissue GSH levels increased significantly ($P<0.01$) in Pre and Post-MCAO HE groups compared to the MCAO group, whereas it changed non-significantly in comparison among other experimental groups (Table 2).

b. Effects of AEHE on liver

Changes in different parameters of liver tissue are shown in table 3. Non-significant changes were seen in the weight of the liver among different animal groups.

Liver tissue protein levels decreased significantly in the MCAO group in comparison to the control group ($p<0.005$), however significant increase in tissue protein levels was noted in HE-treated ($p<0.05$) and Pre- MCAO HE ($p<0.005$) groups.

Tissue catalase levels decreased significantly in the MCAO group compared to the sham group. In contrast, they increased significantly in HE-treated and Pre-MCAO HE groups compared to the MCAO group. In the Post-MCAO HE group, CAT levels decreased significantly compared to the HE-treated group (Table 3).

Tissue SOD level decreased significantly ($p<0.005$) in the MCAO group compared to control and sham groups, whereas a significant ($p<0.005$) increase was noted in HE-treated and Pre- MCAO HE groups in comparison to the MCAO group. In the Post-MCAO HE group, SOD levels decreased significantly compared to HE-treated and Pre-MCAO HE groups (Table 3).

Tissue GSH level decreased significantly ($P<0.05$) in the MCAO group compared to the control and sham groups. A significant increase was seen in HE-treated and Pre-MCAO HE groups compared to the MCAO group. In the Post-MCAO HE group, a significant decrease was present compared to the control, sham, HE-treated, and Pre-MCAO HE groups (Table 3).

c. Effect of AEHE on kidney tissue

Changes in different parameters of kidney tissue are shown in table 4. Non-significant changes were observed in kidney weight in comparison among different groups. Tissue protein levels in the MCAO group decreased significantly ($p<0.05$) compared to the control and sham groups. In contrast, they increased significantly in the Pre-MCAO HE group ($p<0.01$) compared to the MCAO group. In Pre and Post-MCAO HE groups' tissue protein levels changed significantly compared to experimental groups.

Tissue CAT level increased significantly in the MCAO group in comparison to the control. In the HE-treated group, a significant increase was observed compared to the MCAO group. In contrast, in Pre and Post-MCAO HE groups significant decrease was noted in comparison to the HE-treated group (Table 4).

Tissue SOD decreased significantly in the MCAO group compared to the sham and control groups. In contrast, these levels increased significantly in the HE-treated group compared to the MCAO group. Superoxide dismutase levels increased significantly in the Pre-MCAO HE group (P<0.05) compared to the MCAO group. In the Post-MCAO HE group, SOD levels decreased significantly compared to the control group (Table 4).

Tissue GSH levels decreased significantly in animals of the MCAO group compared to the control and sham groups. In contrast, these levels raised significantly in the HE-treated group compared to the MCAO group. GSH levels increased significantly in the Pre-MCAO HE group (P<0.005) compared to the MCAO group.

In the Post-MCAO HE group, GSH levels decreased significantly compared to control, sham, HE-treated, and Pre-MCAO HE groups (Table 4).

Table 2: Brain weight, tissue proteins, and antioxidant enzymes levels.

		Body Weight (g)	Total proteins (g/dl)	Catalase (µmol/gm)	Superoxide dismutase (U/g of tissue)	Glutathione (U/g of tissue)
Control	**n=9**	1.54±0.067	4.45±0.055	20.35±0.232	65.15±0.715	0.073±0.001
Sham	**n=9**	1.59±0.026^{-}	4.51±0.084^{-}	20.78±0.251^{-}	63.84±0.761^{-}	0.074±0.002^{-}
MCAO	**n=9**	1.89±0.026$^{***/***}$	4.66±0.076$^{-/-}$	18.33±0.317$^{-/*}$	56.67±0.744$^{***/***}$	0.065±0.004$^{-/-}$
HE treated	**n=9**	1.53±0.009$^{-/-/***}$	4.64±0.096$^{-/-/-}$	22.35±0.627$^{*/-/***}$	63.91±0.635$^{-/-/***}$	0.073±0.001$^{-/-/-}$
Pre MCAO HE	**n=9**	1.82±0.026$^{***/***/-/***}$	4.48±0.067$^{-/-/-/-}$	21.17±0.298$^{-/-/***/-}$	64.59±0.335$^{-/-/***/-}$	0.077±0.001$^{-/-/**/-}$
Post MCAO HE	**n=9**	1.91±0.0248$^{***/***/-/***/-}$	4.75±0.088$^{-/-/-/-/-}$	18.63±0.254$^{-/*/-/***/***}$	61.25±0.384$^{**/-/***/*/***}$	0.076±0.001$^{-/-/**/-/-}$

Significance level P<0.005***, P<0.05**, P<0.01*, - non-significant; compare with sham/ MCAO/ HE/ Pre MCAO HE/ Post MCAO HE

Table 3: Liver weight, tissue protein, and antioxidant enzymes levels.

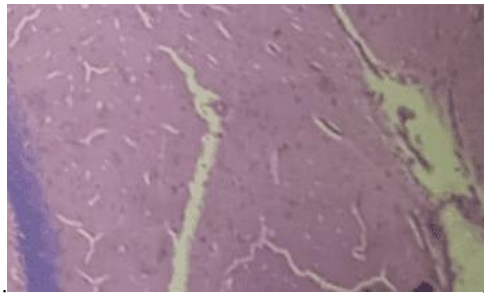
		Weight (gm)	Tissue proteins (g/dl)	Catalase (µmol/gm)	Superoxide dismutase (U/g of tissue)	Glutathione (U/g of tissue)
Control	**n=9**	4.58±0.09	5.43±0.106	19.62±0.28	63.32±0.81	0.085±0.0014
Sham	**n=9**	4.76±0.08^{-}	5.55±0.074^{-}	19.88±0.21^{-}	63.33±0.48^{-}	0.086±0.0015^{-}
MCAO	**n=9**	4.45±0.12$^{-/-}$	4.23±0.034$^{***/***}$	17.27±0.93$^{-/*}$	58.04±1.96$^{***/***}$	0.068±0.0013$^{***/***}$
HE treated	**n=9**	4.56±0.02$^{-/-/-}$	5.30±0.056$^{-/-/***}$	21.63±0.79$^{-/-/***}$	63.75±0.58$^{-/-/***}$	0.084±0.0005$^{-/-/***}$
Pre MCAO HE	**n=9**	4.45±0.06$^{-/-/-/-}$	5.02±0.127$^{-/*/***/-}$	20.09±0.28$^{-/-/*/-}$	63.94±0.54$^{-/-/***/-}$	0.082±0.0013$^{-/-/***/-}$
Post MCAO HE	**n=9**	4.61±0.02$^{-/-/-/-/-}$	4.74±0.119$^{***/***/*/**/-}$	18.23±0.41$^{-/-/-/***/-}$	60.17±0.29$^{-/-/-/*/*}$	0.073±0.0028$^{***/***/-/***/**}$

Note: Significance was declared as *P<0.05, **P<0.01, ***P<0.005, - non-significant; compared with control/ sham/ MCAO/ HE/ Pre MCAO HE/ Post MCAO HE.

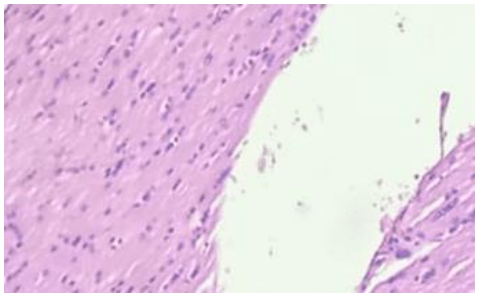
Table 4: Kidney weight, tissue protein, and antioxidant enzymes levels.

		Lt. Kidney Wt. (gm)	Rt. Kidney Wt. (gm)	Tissue proteins (g/dl)	Catalase µmol/gm	Superoxide Dismutase (U/g of tissue)	Glutathione (U/g of tissue)
Control	**n=9**	0.538±0.008	0.586±0.01	4.83±0.15	24.26±0.76	65.92±1.7	0.073±0.003
Sham	**n=9**	0.532±0.01^{-}	0.576±0.008^{-}	4.63±0.02^{-}	22.82±0.43^{-}	64.38±0.32^{-}	0.079±0.002^{-}
MCAO	**n=9**	0.545±0.007^{-} /-	0.585±0.001$^{-/-}$	3.89±0.03$^{***/***}$	20.36±0.36$^{***/-}$	59.99±0.29$^{***/*}$	0.047±0.002$^{***/***}$
HE treated	**n=9**	0.519±0.003^{-} /-/-	0.555±0.006$^{-/-}$ /-	4.68±0.06$^{-/-/***}$	25.13±0.35^{-} /*/***	64.43±0.49$^{-/-/*}$	0.077±0.002$^{-/-/***}$
Pre MCAO HE	**n=9**	0.532±0.006^{-} /-/-/-	0.566±0.007$^{-/-}$ /-/-	4.33±0.08$^{***/-/**/*}$	22.66±0.49$^{-/-/-}$ /*	63.91±0.51$^{-/-/*/-}$	0.075±0.002$^{-/-/***/-}$
Post MCAO HE	**n=9**	0.519±0.004^{-} /-/-/-/-	0.549±0.004$^{*/-}$ /*/-/-	4.29±0.05$^{***/*/**/**/-}$	21.09±0.33$^{***/-}$ /-/***/-	61.34±0.38$^{***/-}$ /-/-/-	0.051±0.002$^{***/***/-}$ /***/***

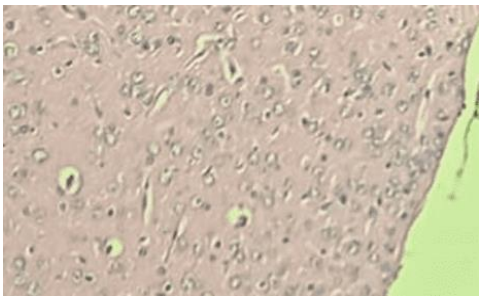
Note: Significance was declared as P<0.005***, P<0.05**, P<0.01*, - non-significant; compared with control/ sham/ MCAO/ HE/ Pre MCAO HE/ Post MCAO HE.
MCAO = Middle cerebral artery occlusion; HE = Hericium Erinaceus



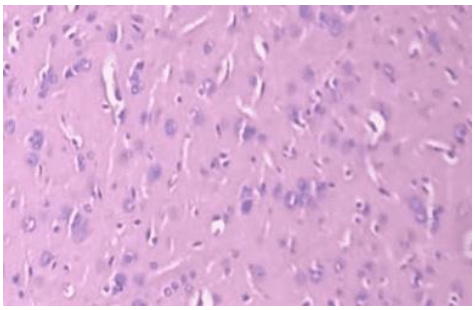
a. Control cerebral histology



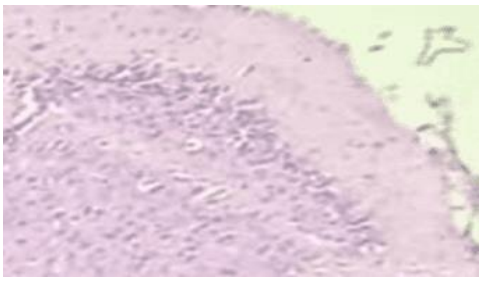
b. Sham group cerebral



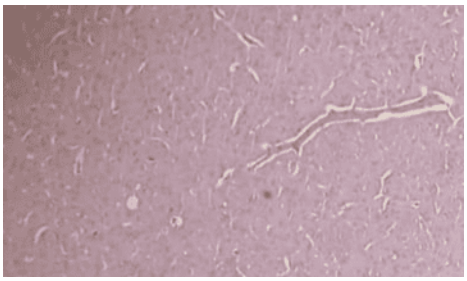
c. MCAO group cerebral histology



d. Pre-MCAO HE group cerebral histology



e. Post-MCAO HE group cerebral histology



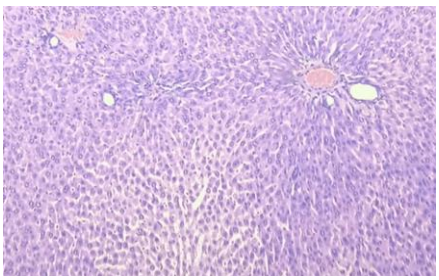
f. HE treated group cerebral histology

Figure 1 (a-f): Histology of cerebral tissue in different groups.

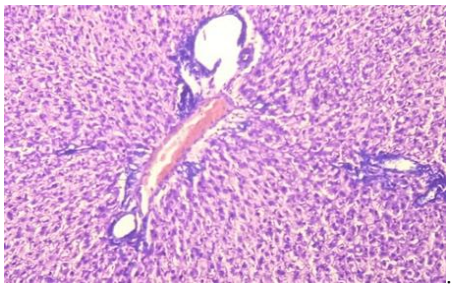
Table 5: Cerebral tissue Analysis.

	Normal tissue architecture	Preserved Neuronal Architecture	Neuronal Degeneration	Inflammatory cell infiltration	Interstitial edema
Control	-	-	-	-	-
Sham	-	+	_	+	-
MCAO	+	+	++	++	+
Pre MCAO HE	+	-	+	+	+
Post MCAO HE	+	+	++	++	+
HE treated	-	-	-	-	-

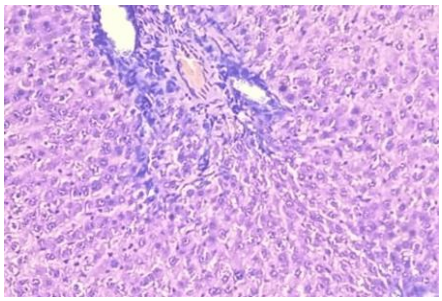
Scale: - No change, +Minor, ++Moderate & +++Maximal changes



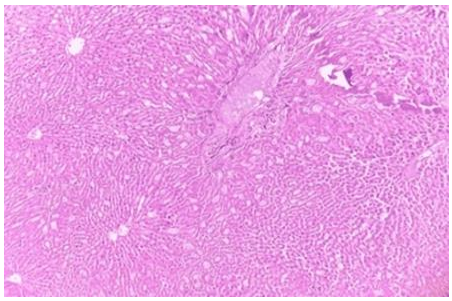
a.Control group Liver histology



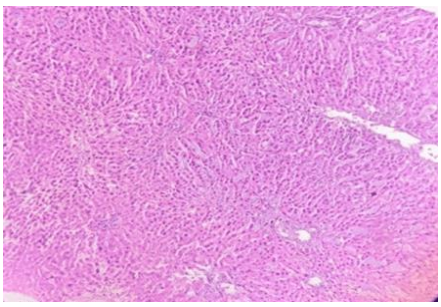
b.Sham group liver histology



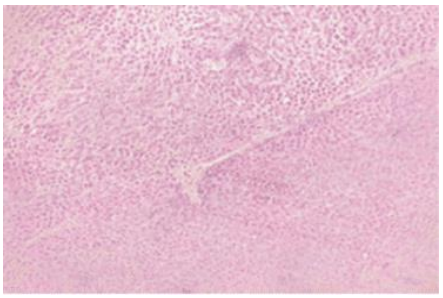
c. MCAO group liver histology



d. Pre-MCAO HE group liver histology



e. Post-MCAO HE group liver histology



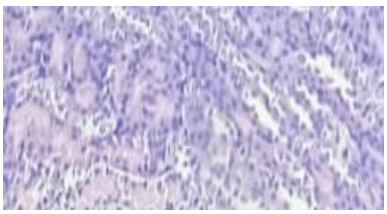
f. HE treated group liver histology

Figure 2 (a-f): Histology of liver tissue in different groups.

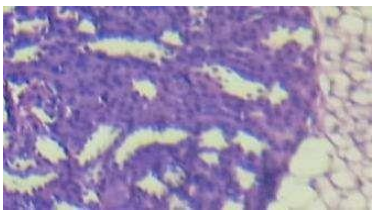
Table 6: Liver Tissue Analysis

	Tissue Color	Texture	Enlargement	Inflammatory infiltrates	Fibrotic Changes
Control	Reddish brown	Normal	-	-	-
Sham	Reddish brown	Normal	-	-	-
MCAO	Reddish brown	Normal	Enlarged	++	+
Pre MCAO HE	Reddish brown	Normal	-	+	-
Post MCAO HE	Reddish brown	Normal	Enlarged	++	++
HE treated	Reddish brown	Normal	-	-	-

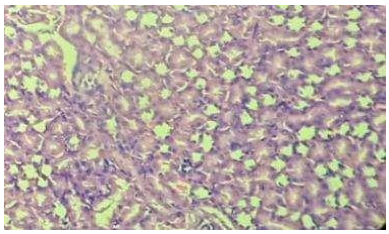
Scale: - No change, + minor, ++ moderate & +++ maximal changes



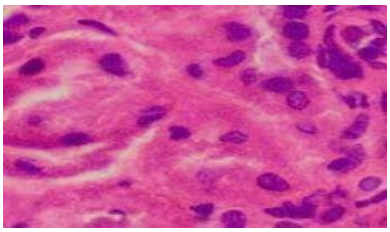
a. Control Kidney histology



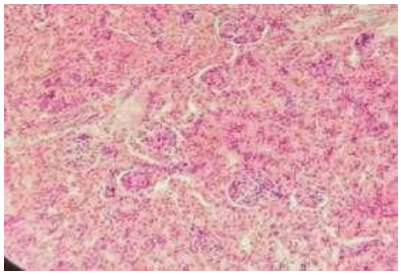
b. Sham group Kidney histology



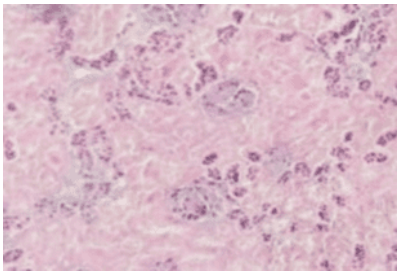
c. MCAO group kidney histology



d. Pre-MCAO HE group kidney histology



e. Post-MCAO HE group kidney histology



f. HE treated group kidney histology

Figure 3(a-f) Histology of kidney tissue in different groups

Table 7: Kidney Tissue Analysis

	Tissue Color	Texture	Enlargement	Inflammation	Phagocytic cell infiltration
Control	Reddish brown	Normal	-	-	-
Sham	Reddish brown	Normal	-	-	-
MCAO	Reddish brown	Normal	+	++	++
Pre MCAO HE	Reddish brown	Normal	-	-	-
Post MCAO HE	Reddish brown	Normal	-	+	+
HE treated	Reddish brown	Normal	-	-	-

Scale: - No change, + minor, ++ moderate & +++ maximal changes

Discussion

Despite the medicinal advancement of the present era, the demand for herbal medicines is continuously increasing worldwide. One of the main reasons for this is that herbal remedies are more affordable and cause fewer side effects[18]. The treatment strategies available for ischemic stroke are the use of thrombolytic agents[19,20], and endovascular thrombectomy[20], but large groups of patients still failed to recover from neurological deficits. These patients with neurological dysfunctions are then treated with herbal medications[20].

Impaired cerebral circulation due to cerebral vessel obstruction is the hallmark phenomenon of ischemic stroke. Vascular obstruction causes brain hypoxia that causes acute metabolic stress and begins an ischemic cascade, ultimately resulting in neuronal cell death in the ischemic core. Reperfusion can lessen the severity of tissue damage in the ischemic region[21]. However, the ischemia/reperfusion phenomenon produces various consequences through the production of reactive oxygen species, which increases the rate of oxidative tissue impairment and initiates the inflammatory response[22].

300mg AEHE, when given orally for 7 days, non-significant hypolipidemic effects are seen, whereas in the Pre-MCAO HE group significant decline in TC levels in comparison with the MCAO group was seen, which is consistent with the study of Liang et al., who reported a significant decline in plasma TC levels in streptozotocin induced-diabetic model of Wistar rats fed with AEHE[23]. In our study, HDL levels remained normal in the HE-treated, Pre, and Post-MCAO HE groups, consistent with the results of a study conducted on a hyperlipidemia model of rats given ethanol extract of HE a rise in HDL-C levels[24].

In our study, hepatic parameters remained maintained, whereas a significant fall in AST and

ALP levels was seen in the Pre-MCAO HE-treated group compared to the MCAO group. Our results are consistent with the study on hepatotoxicity in mice given with HE extract, which showed a reduction in AST levels[25]. Zhang et Al. reported the hepatoprotective action of polysaccharides of HE via the strong in-vitro antioxidant action, which was estimated by the decline in the hepatic biomarkers level[26].

In our study, urea, creatinine, uric acid, and BUN levels changed significantly in HE Treated, Pre, and Post-MCAO HE groups compared to the MCAO group. No difference in urea level was seen in the rodent model study fed with distinctive doses of HE compared to the control group[27]. The mice model of ischemia-reperfusion injury pretreated with HE polysaccharides via gavage for 15 days has shown a significant decline in creatinine levels compared to the no pretreatment group[28]. The study on streptozotocin-induced diabetes mellitus in rats given polysaccharide extract of HE has shown a significant decline in creatinine and BUN levels in the treatment groups[29].

Tissue antioxidant enzymes greatly aid the cell defense system against ischemia and reperfusion damage. Numerous antioxidant enzymes, including CAT, SOD, and GSH, catalyze the process of free radicals neutralization and aid in protecting cells from oxidative stress[30]. In the present study, CAT, SOD, and GSH levels raised significantly in the brain and liver in HE-treated and Pre-MCAO HE groups compared with the MCAO group. In contrast, a significant increase was seen in SOD and GSH levels in liver tissue. Ethanol extract of HE has shown neuroprotective action on H2O2 treated neuronal cells by increasing their viability and tissue antioxidant enzyme levels, i.e., CAT, and GSH, along with a significant drop in ROS levels[31].

Conclusion

The present study has shown HE has effectively controlled systemic oxidative stress and positively impacted neuronal injury. It also maintained the plasma lipid profile and reno-hepatic parameters near baseline values. Results have shown that HE can be effectively used for the risk management and treatment of ischemic reperfusion injury.

Conflicts of Interest

No conflict of interest.

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