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Tumor Necrosis Factor-α in Rats Following Transient Focal Cerebral Ischemia Reperfusion and Its Relation to Oxidative Stress

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Abstract **Background**: The role of TNF-α in ischemic/reperfusion (I/R) is still controversial. The aim of this study was to assess TNF-α in rats subjected to transient cerebral I/R and to correlate their levels with the resulting neurological deficits and oxidative stress biomarkers malondialdehyde and total antioxidant capacity (TAC). **Material and Method:** Experimental procedures were performed on 30 adult male Wistar rats. Divided into two groups fifteen rats in each, test group subjected to transient focal cerebral I/R by occlusion of the left common carotid artery (CCA) for 30 minutes followed by reperfusion for 24-hours. A control group underwent the surgery at the same neck region without occlusion of the CCA. Neurobehavioral assessments were evaluated. TNF-α was measured using ELISA method. Malondialdehyde and TAC were estimated colorimetry. **Results**: In the test group TNF-α and Malondialdehyde concentration in both serum and brain tissue were significantly higher than control group (P =0.000). In contrast, the serum and brain tissue levels of TAC in the test group was significantly lower compared to the sham operated rats (P = 0.000). The brain tissue and serum level of TNF-α were correlated negatively with neurological deficit and TAC and positively with Malondialdehyde (P = *0.000*). **Conclusion:** the present study revealed a potential injurious role of TNF-α in rats subjected to cerebral I/R and demonstrated a direct relationship between TNF-α and oxidative stress biomarkers and the consequent neurological deficits.

Keywords: *cerebral, ischemia/reperfusion, TNF-α, malondialdehyde and total antioxidant capacity*

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1. Introduction

Acute ischemic stroke has serious clinical, social, and economic consequences and necessitates a significant effort from both basic scientists and clinicians to understand the complex underlying pathophysiological mechanisms [1]. However, The pathophysiology regarding ischemia/reperfusion (I/R) injury is still obscured; through involvement of oxidative stress and inflammatory mediators [2]. Reactive oxygen species (ROS), derived from hypoxia and reoxygenation during transient focal cerebral I/R, results in extensive damage to lipids, proteins, DNA and other components of organisms. The role of ROS species has been implicated in the pathogenesis of oxidative stress-related diseases, such as stroke. [3] Brain tissue is not well equipped with antioxidant defenses, so ROS and other free radicals, released by inflammatory cells, threaten tissue viability approximately to the ischemic zone [4].

Oxidative stress induced peroxidation of cell membrane lipids and malondialdehyde (MDA) is frequently used as an indicator of lipid peroxidation and correlated well with the size of ischemic stroke as well as the clinical outcome [5]. Furthermore, estimation of the total antioxidant capacity (TAC) is supposed to be a useful measure of the availability of the antioxidants present to guard against oxidative cell damage [6,7].

An inflammatory cytokine tumor necrosis factor-α (TNF-α) assumed to augment or depress cellular survival through activation of receptor-mediated signal transduction [8]. Both injurious and beneficial roles of TNF-α have been reported in the pathogenesis of cerebral I/R. This explained by diverse molecular switches and dynamic changes in signaling of TNF-α through it receptors [9]. The aim of this work was to evaluate potential role of TNF-α in cerebral ischemia and to determine its relation to oxidative stress in rats subjected to transient cerebral ischemia reperfusion.

2. Materials and Methods

2.1. Animals

The studies were approved by the Ethical Committee of the University of Alexandria, and the investigations

conform to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85–23, revised 1996). 30 Male Wistar rats, weighing 150-250 g were selected and preserved at a constant temperature of 22±2°C with a fixed 12:12-h light-dark cycle. Nutritionally balanced pellets and water were freely available. The animals were divided into two groups (15 rats in each group): test and control

2.2. Cerebral Ischemia Induction

The animals were fasted overnight prior to surgery with free access to tap water. Anesthesia was induced by ether inhalation and maintained by thiopental sodium (2.5mg/kg) [10]. Body temperature was kept constant at 36.5±0.5˚C using warm pad. A longitudinal cervical incision (2cm) was made lateral to the midline and the common carotid artery (CCA) was carefully dissected. In the test group (ischemia/reperfusion) (n=15) ischemia was induced by placing non traumatic microvascular clip on the left CCA just prior to its bifurcation [11]. During ischemia rats were monitored for body temperature and respiration pattern. The vascular occlusion was maintained for 30 minutes, and then the clips were removed to resume blood flow to the ischemic region [12]. The incisions were sutured, and then the animal was allowed to recover from anesthesia, and returned to a warm cage for recuperation during reperfusion period for 24 hours.

In the control group (sham-operation) (n = 15), the rats underwent the surgery at the neck region without occlusion of CCA. The number of animals presented for each group is the number of rats that survived during 24-hour reperfusion period. The collected data of the animals that died during 24 hours reperfusion period were excluded.

2.3. Neurological and Behavioral Evaluation

Neurobehavioral tests of all experimental groups were assessed daily to determine the effect of ischemic injury on them. Neurobehavioral evaluations were performed three times: the day before surgery, one hours after the surgery and before scarify day. The neurobehavioral study consisted of the following six tests: spontaneous activity, symmetry in the movement of the four limbs, forepaw outstretching, climbing, body proprioception and response to vibrissae touch. The score given to each rat at the end of the evaluation is the summation of all six individual test scores. The minimum neurological score was 3 and the maximum was 18 [13].

2.4. Laboratory Investigations

At the end of experimental period, the rats were sacrificed by decapitation. Brains were rapidly removed from the skull and washed with cold saline and stored at −20°C for further analysis. A small part of each brain from the affected hemisphere were dissected in to approximately 1-2 mm pieces and they were homogenized in 7 ml of ice-cold extraction buffer contain: (Triton X-100: 1%, MgSO4: 10 mmol/l, EDTA: 1 mmol/l, Dithiothreitol: 1 mmol/l, NaCl: 0.5 mol/l, Protease inhibitor cocktail: 1%, and 20 mmol/l HEPES (pH 7.5) [14]. The homogenate was centrifuged; the supernatant was taken and stored at −20°C before used. A modification of the method of Lowry was used for the determination of protein in the brain homogenate [15]. Brain tissue and serum level of TNF-α were measured using ELISA kits [16] and TAC concentrations were measured colorimetrically while Satoh method was used to measure serum and brain homogenate MDA levels [17].

3. Data Analysis

Data were expressed as mean ± S.E.M. Differences among groups were evaluated by independent student t-test and the relationships between TNF-α, MDA, TAC and the resulting neurological deficit of rats subjected to ischemia reperfusion were assessed using bivariate correlations. P<0.05 was selected for acceptance of statistical significance.

4. Results

As shown in Figure 1, rats subjected to ischemia reperfusion demonstrated a significant decreased in the neurological deficit (M±SD= 12.798±0.689) compared to sham operated rats (17.50±0.707, P < 0.001). Rats in test group revealed a significant increase in brain tissue of TNF-α and MDA (110.36±6.178 pg/mg protein, 8.56±0.658 nmol/mg protein respectively) as compared to control group (4.9±0.797 pg/mg protein, 3.24±0.226 nmol/mg protein respectively, P = 0.000). While the brain TAC level of rats exposed to ischemia reperfusion (0.0186±0.00373 mmol/mg protein) was significantly lower compared to the sham operated rats (0.070±0.0085 mmol/mg protein, P = 0.000) (Figure 2). In Figure 3, the concentrations of serum TNF-α and MDA in rats' subjected to ischemia reperfusion (734.76±108.82 pg/ml, 14.88±1.14 nmol/mL, respectively) were significantly higher compared to sham operated rats (37.18±10.183 pg/ml, 5.43±0.44 nmol/mL, respectively, P = 0.000). In contrast, the level of serum TAC of rats in test group (1.21±0.169 mM/L) was significantly lower compared to the sham operated rats (2.52±0.062 mM/L, P = 0.000).

The relationships between brain tissue and serum of TNF-α, MDA, and TAC levels and neurological deficit of rats subjected to ischemia reperfusion are given in Table 1.

Table 1. Correlations between TNF and MDA, TAC and Neurological deficit

		The Biomarker	Correlation Coefficient	*P*
Brain tissue of TNF-α	Tissue Level	MDA(nmol/ml)	0.988	.000
		TAC (mM/L)	-0.973	.000
		Neurological deficit	-0.967	.000
Serum of TNF-α	Serum Level	MDA(nmol/ml)	0.954	.000
		TAC (mM/L)	-0.976	.000
		Neurological deficit	-0.933	.000

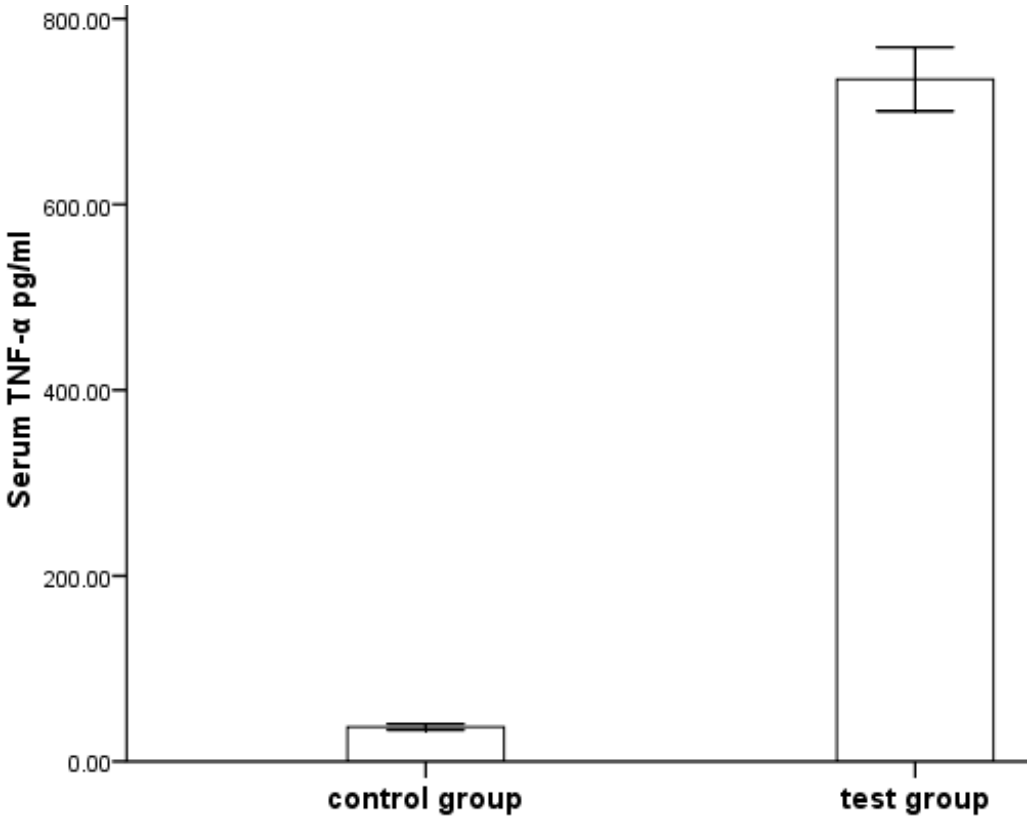

Figure 1. Neurological deficit in **control** (sham operated) and **test** (ischemia reperfusion) rats. (15 rats in each group, data are expressed as mean± SEM)

* p<0.05 significant differences between test and control group

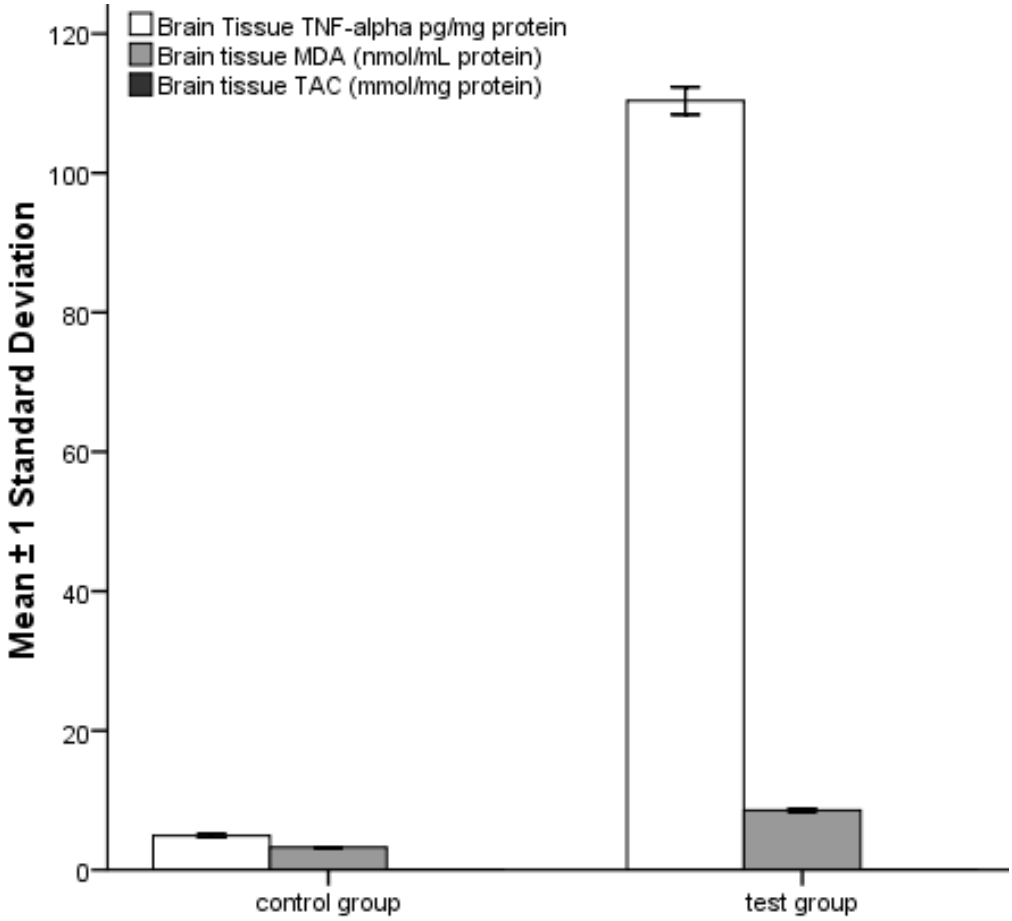

Figure 2. Brain tissue level of TNF-α, MDA and TAC in **control** (sham operated) and **test** (ischemia reperfusion) rats. (15 rats in each group, data are expressed as mean± SEM)

* p<0.05 significant differences between test and control group

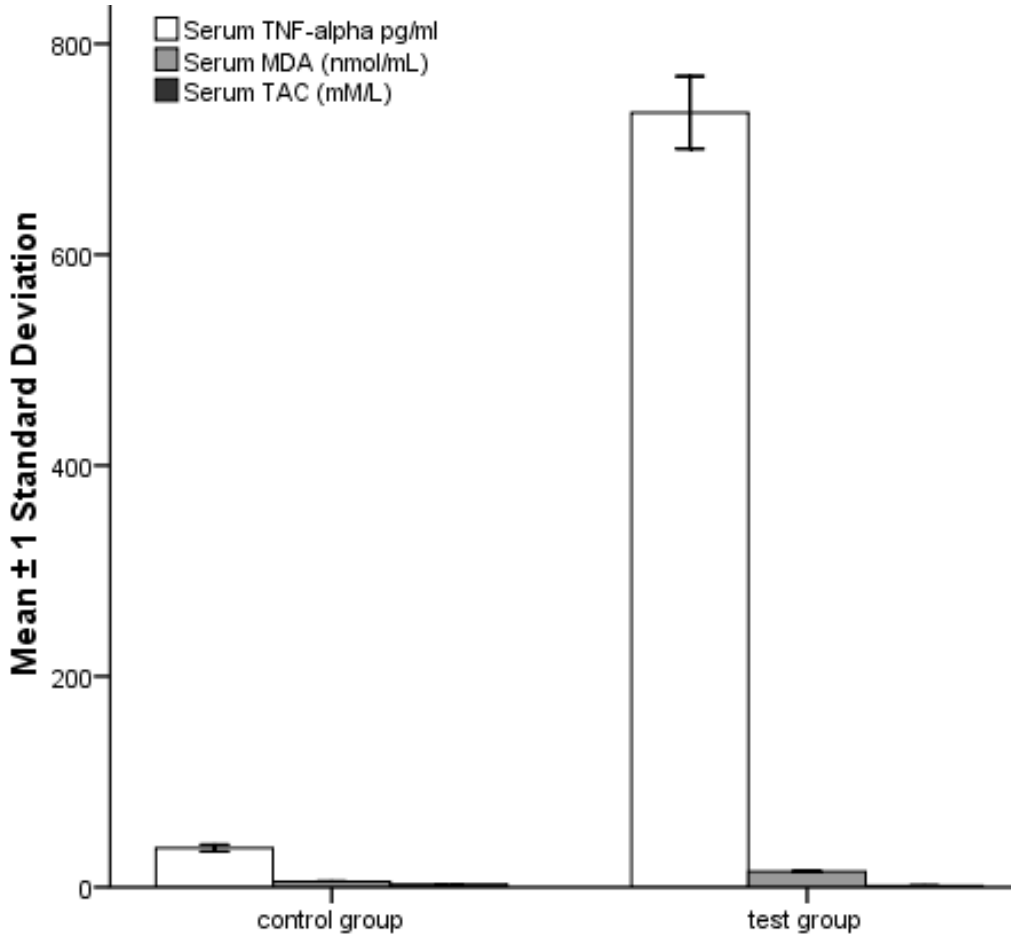

Figure 3. Serum level of TNF-α, MDA and TAC in **control** (sham operated) and **test** (ischemia reperfusion) rats. (15 rats in each group, data are expressed as mean± SEM)

* p<0.05 significant differences between test and control group

5. Discussion

In the present study, it was observed that rats subjected to cerebral ischemia for 30 min followed by reperfusion for 24 hours had a significantly higher serum and brain tissue levels of TNF-α and MDA compared to sham-operated rats. In contrast, TAC was significantly lower in the test group. Interestingly, the current data showed a highly significant negative correlation between both serum and brain tissue level of TNF-α, neurological deficit and MDA, with one more positive correlation between TNF-α and TAC. These findings further support the injurious role of TNF-α and its association with oxidative stress and contribution to neuronal damage in our focal cerebral ischemic model.

The current data are comparable with previous studies conducted to evaluate the role of TNF-α in rat's transient focal and global cerebral ischemia [18,19]. Nevertheless the role of TNF-α during ischemia/reperfusion has not been fully elucidated, with the potential of both beneficial and/or deleterious outcome [9,20].

In accordance to the current finding, numerous researches demonstrated that oxidative stress biomarkers are elevated with reduced levels of antioxidants in the cerebral vasculature during ischemia/reperfusion with contribution to post-ischemic endothelial dysfunction [21,22,23]. Zhang *et al* revealed that a significant increased in MDA level and reduced antioxidant enzymes in rats with cerebral ischemia reperfusion [24]. A similar results obtained by Liu *et al* in different model of cerebral ischemia [25]. Moreover, previous reports repeatedly suggest that a significant attenuation in TAC level take place in cerebral ischemia reperfusion [26].

One of the first indications that TNF-α is an important mediator of stroke is the correlation of its expression with stroke damage. Berti *et al* were in agreement with our finding that TNF-α is increased 3 h after transient middle cerebral artery occlusion (MCAO) and persists for 24 h in the affected hemisphere, this associated with increased level of interleukin (IL)-1b, IL-6, E-selectin, and intercellular adhesion molecule-1 [27]. Furthermore, Haddad *et al* gained similar finding in another different model of transient focal cerebral ischemia [28]. These propositions were further supported by Clausen *et al*, who demonstrated that mice exposed to permanent MCAO exhibited a significantly higher level of TNF-α activity measured at 6, 12 and 24 hours after cerebral ischemia. Moreover, they proved that TNF-α was expressed in largely isolated populations of microglia and macrophages in the ischemic brain [29].

At some point in cerebral ischemia, TNF-α produced by brain parenchymal cells may be beneficial for stroke recovery. Sairanen *et al* reported that astrocytes show vigorous TNF-α immunoreactivity at day 17–18, lagging behind neuronal expression which peaks 2–3 days after human stroke. This may suggest a role of TNF-α in tissue regeneration [30]. Further, Tarkowski *et al* proposed that the level of TNF-α in the CSF of stroke patients correlates

with anti-apoptotic (bcl-2) expression, indicating that TNF-α may not be entirely detrimental to recovery [31]. This contradictory finding is largely attributed to diverse activation of TNF-α receptors (TNFR1 or 2) and to it differential downstream pathway activated during cerebral ischemia [29,32].

In conclusion, the present study demonstrated that serum and brain tissue of TNF-α were significantly higher in rats subjected to I/R, with a significant correlation between TNF-α and oxidative stress biomarkers (MDA and TAC) and the consequent neurological deficits. This added further evidence to the potential injurious role of TNF-α in transient focal cerebral ischemia/reperfusion in rats.

Competing Interest

On behalf of all authors, the corresponding author states that there is no conflict of interest

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References

[1] Harrer JU, Seet RC. Warfarin treatment and thrombolysis in acute stroke: Are the procrastinators right? Neurology. 2012 May; 2012.

[2] Wong CH, Crack PJ. Modulation of neuro-inflammation and vascular response by oxidative stress following cerebral ischemia-reperfusion injury. Curr Med Chem. 2008; 15(1): 1-14.

[3] Zhang L, Zhang ZG, Liu XS, Hozeska-Solgot A, Chopp M. The PI3K/Akt pathway mediates the neuroprotective effect of atorvastatin in extending thrombolytic therapy after embolic stroke in the rat. Arterioscler Thromb Vasc Biol. 2007 Nov; 27(11): 2470-5.

[4] Simao F, Matte A, Matte C, Soares FM, Wyse AT, Netto CA, et al. Resveratrol prevents oxidative stress and inhibition of Na(+)K(+)-ATPase activity induced by transient global cerebral ischemia in rats. J Nutr Biochem. 2011 2011; 22(10): 921-8.

[5] Cherubini A, Ruggiero C, Polidori MC, Mecocci P. Potential markers of oxidative stress in stroke. Free Radic Biol Med. 2005 Oct 1; 39(7): 841-52.

[6] Gariballa SE, Hutchin TP, Sinclair AJ. Antioxidant capacity after acute ischaemic stroke. QJM. 2002 Oct; 95(10): 685-90.

[7] Ryan M, Grayson L, Clarke DJ. The total antioxidant capacity of human serum measured using enhanced chemiluminescence is almost completely accounted for by urate. Ann Clin Biochem. 1997 Nov; 34 (Pt 6): 688-9.

[8] Emsley HC, Tyrrell PJ. Inflammation and infection in clinical stroke. J Cereb Blood Flow Metab. 2002 Dec; 22(12): 1399-419.

[9] Watters O, O'Connor JJ. A role for tumor necrosis factor-alpha in ischemia and ischemic preconditioning. J Neuroinflammation. 8: 87.

[10] Keefer LK, Garland WA, Oldfield NF, Swagzdis JE, Mico BA. Inhibition of N-nitrosodimethylamine metabolism in rats by ether anesthesia. Cancer Res. 1985 Nov; 45(11 Pt 1): 5457-60.

[11] Renolleau S, Aggoun-Zouaoui D, Ben-Ari Y, Charriaut-Marlangue C. A model of transient unilateral focal ischemia with reperfusion in the P7 neonatal rat: morphological changes indicative of apoptosis. Stroke. 1998 Jul; 29(7): 1454-60; discussion 61.

[12] Kuluz JW, Prado RJ, Dietrich WD, Schleien CL, Watson BD. The effect of nitric oxide synthase inhibition on infarct volume after reversible focal cerebral ischemia in conscious rats. Stroke. 1993 Dec; 24(12): 2023-9.

[13] Furuya K, Zhu L, Kawahara N, Abe O, Kirino T. Differences in infarct evolution between lipopolysaccharide-induced tolerant and nontolerant conditions to focal cerebral ischemia. J Neurosurg. 2005 Oct; 103(4): 715-23.

[14] Star RA. Treatment of acute renal failure. Kidney Int. 1998 Dec; 54(6): 1817-31.

[15] Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem. 1951 Nov; 193(1): 265-75.

[16] Leist M, Gantner F, Naumann H, Bluethmann H, Vogt K, Brigelius-Flohe R, et al. Tumor necrosis factor-induced apoptosis during the poisoning of mice with hepatotoxins. Gastroenterology. 1997 Mar; 112(3): 923-34.

[17] Satoh K. Serum lipid peroxide in cerebrovascular disorders determined by a new colorimetric method. Clin Chim Acta. 1978 Nov 15; 90(1): 37-43.

[18] Hosomi N, Ban CR, Naya T, Takahashi T, Guo P, Song XY, et al. Tumor necrosis factor-alpha neutralization reduced cerebral edema through inhibition of matrix metalloproteinase production after transient focal cerebral ischemia. J Cereb Blood Flow Metab. 2005 Aug; 25(8): 959-67.

[19] Murakami Y, Saito K, Hara A, Zhu Y, Sudo K, Niwa M, et al. Increases in tumor necrosis factor-alpha following transient global cerebral ischemia do not contribute to neuron death in mouse hippocampus. J Neurochem. 2005 Jun; 93(6): 1616-22.

[20] Sriram K, O'Callaghan JP. Divergent roles for tumor necrosis factor-alpha in the brain. J Neuroimmune Pharmacol. 2007 Jun; 2(2): 140-53.

[21] Ferretti G, Bacchetti T, Masciangelo S, Nanetti L, Mazzanti L, Silvestrini M, et al. Lipid peroxidation in stroke patients. Clin Chem Lab Med. 2008; 46(1): 113-7.

[22] Lu Q, Xia N, Xu H, Guo L, Wenzel P, Daiber A, et al. Betulinic acid protects against cerebral ischemia-reperfusion injury in mice by reducing oxidative and nitrosative stress. Nitric Oxide. 2011 Apr 30; 24(3): 132-8.

[23] Liu Y, Liu W, Sun X, Li R, Sun Q, Cai J, et al. Hydrogen saline offers neuroprotection by reducing oxidative stress in a focal cerebral ischemia-reperfusion rat model. Med Gas Res. 2011; 1(1): 15.

[24] Zhang JY, Jr., Si YL, Liao J, Yan GT, Deng ZH, Xue H, et al. Leptin administration alleviates ischemic brain injury in mice by reducing oxidative stress and subsequent neuronal apoptosis. J Trauma Acute Care Surg. 2012 2012; 72(4): 982-91.

[25] Liu Y, Liu W, Sun X, Li R, Sun Q, Cai J, et al. Hydrogen saline offers neuroprotection by reducing oxidative stress in a focal cerebral ischemia-reperfusion rat model. Med Gas Res. 1(1): 15.

[26] Jung HW, Mahesh R, Bae HS, Kim YH, Kang JS, Park YK. The antioxidant effects of Joongpoongtang 05 on brain injury after transient focal cerebral ischemia in rats. J Nat Med. Apr; 65(2): 322-9.

[27] 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 Sep; 22(9): 1068-79.

[28] Haddad M, Rhinn H, Bloquel C, Coqueran B, Szabo C, Plotkine M, et al. Anti-inflammatory effects of PJ34, a poly(ADP-ribose) polymerase inhibitor, in transient focal cerebral ischemia in mice. Br J Pharmacol. 2006 Sep; 149(1): 23-30.

[29] Clausen BH, Lambertsen KL, Babcock AA, Holm TH, Dagnaes-Hansen F, Finsen B. Interleukin-1beta and tumor necrosis factor-alpha are expressed by different subsets of microglia and macrophages after ischemic stroke in mice. J Neuroinflammation. 2008; 5: 46.

[30] Sairanen T, Carpen O, Karjalainen-Lindsberg ML, Paetau A, Turpeinen U, Kaste M, et al. Evolution of cerebral tumor necrosis factor-alpha production during human ischemic stroke. Stroke. 2001 Aug; 32(8): 1750-8.

[31] Tarkowski E, Rosengren L, Blomstrand C, Wikkelso C, Jensen C, Ekholm S, et al. Intrathecal release of pro- and anti-inflammatory cytokines during stroke. Clin Exp Immunol. 1997 Dec; 110(3): 492-9.

[32] Ekdahl CT, Kokaia Z, Lindvall O. Brain inflammation and adult neurogenesis: the dual role of microglia. Neuroscience. 2009 Feb 6; 158(3): 1021-9.