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Biochemical Abnormalities in Brain Tissues during Acute Brain Stroke Induced in Wistar Rats

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Brain stroke is the rapid loss of brain function due to disturbance in the blood supply to the brain. So, it is important to test the biochemical abnormalities and oxidative mechanisms of focal cerebral ischemia induced in left common carotid artery occlusion (LCCAO).The rats were divided into five groups. Group 1 (Control normal), group 2, 3, 4 and 5 were subjected to occlusion for ½, 1, 4 and 6 h occlusions, respectively. LCCAO was retracted to allow reperfusion of ischemic region. Blood samples and tissue specimens from brain were collected four times after reperfusion, zero time, 1, 3 and 24 h, respectively. Moreover, the changes in blood and brain biomarkers in ischemia/reperfusion injury were assayed. The results showed that LCCA occlusion in male rats significantly increased the levels of brain L-MDA, lactate, sodium, antioxidant enzymes, AChE, LDH and plasma fibrinogen. Also brain stroke significantly decreased the levels of brain nitric oxide, CAT and serum CK and C-RP as compared to control rats. Moreover, reperfusion of LCCAO significantly increased levels of brain L-MDA, antioxidant enzymes and serum C-RP. While, results showed a significant decrease in brain nitric oxide, sodium, AchE, serum CK as well as fluctuation in brain LDH, lactate and plasma fibrinogen levels as compared to the zero hour post occlusion. In conclusion, the results provide *in vivo* evidence that brain ischemia has harmful effect on brain energy metabolism, through induction of oxidative stress via production and rapid increase in the generation of reactive oxygen species, lipid peroxidation and alteration in antioxidant defenses.

Key words: Brain stroke, biochemical changes, oxidative stress

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INTRODUCTION

Stroke is the third leading cause of mortality in Egypt behind cancer and myocardial infarction (Barc *et al*., 2004). As reported, stroke (cerebrovascular accident) is a rapid loss of brain function due to disturbance in blood supply to brain due to ischemia (lack of blood flow) caused by occurrence of thrombosis or arterial embolism and hemorrhage (Sims and Muyderman, 2010). Ischemia leads to inability to move one or more limbs on one side of body, inability to understand, formulate speech and see one side of the visual field. There are two major types of strock: ischemic and hemorrhagic. Ischemic strokes are caused by interruption of the blood supply while hemorrhagic strokes results from rupture of a blood vessel or an abnormal vascular structure (Sims and Muyderman, 2010). Among the risk factors ranging from old age, hypertension, transient ischemic attack, diabetes, high cholesterol, cigarette smoking and atrial fibrillation, high blood pressure remains the most important and modifiable risk factor (Donnan *et al*., 2008).

Brain cellular death is caused by insufficiency in uptake of oxygen that maintains oxidative metabolism induced in acute cerebral infarction. The brain is especially prone to free radical damage for several reasons. It is very rich in polyunsaturated fatty acids which are particularly vulnerable to free radical induced peroxidation, but also has a low content of antioxidant enzymes, such as catalase and glutathione peroxidase (Cherubini *et al*., 2005). Due to numerous polyunsaturated fatty acids and low content of antioxidant enzymes in the brain, it is prone to free radical damage (Cherubini *et al*., 2005). NO may have a more deleterious effect during ischemia (Sims and Anderson, 2002). Restoration of blood flow could have negative consequences such as generation of free oxygen radicals. Superoxide and hydroxyl (-OH) react with unsaturated lipids of membranes, resulting in the generation of lipid peroxide radicals, lipid hydroperoxides and fragmentation products such as L-malondialdehyde (L-MDA) (Cano *et al*., 2003).

Furthermore, middle cerebral artery occlusion followed by reperfusion is a model of focal ischemia in rats which resembles that of human ischemic stroke and has been associated with generation of free radicals (Yavuz *et al*., 1997). For that, the aim of the present study was to evaluate the biochemical alterations in some blood and brain biomarkers in Left Common Carotid Artery Occlusion (LCCAO), model of brain stroke, followed by reperfusion, induced focal cerebral ischemia in male rats.

MATERIALS AND METHODS

Seventy adult white male albino rats aged 10-11 weeks and weighing 220-250 g were used for the study. Rats were housed in separated metal cages and kept at constant environmental and nutritional conditions throughout the period of experiment. The animals were allowed free access to standard dry rat diet and tap water *ad libitum*.

Induction of Brain stroke (production of cerebral ischemia): Cerebral ischemia was induced by using the model of Left common carotid artery occlusion. Surgical procedure of Left common carotid artery occlusion: Animals were fasted overnight with free access to water and anesthetized with an i.p. injection of sodium thiopental (1 g dissolved in 25 mL of normal saline) at the dose of 0.1 mL/100 g body weight. A median incision was performed in the skin of the ventral part of the neck and the subcutaneous adipose tissue was dissected away from thyroid gland. The omohyoid muscle was cut through a median incision. The common carotid artery was visualized and exposed separating the surrounding tissue. In used groups, the left common carotid artery was ligated by means of 9-0 silk suture firmly tied around the vessel just below the bifurcation. Skin incision was sutured immediately with stitches after the ischemia procedures were completed. Anesthesia was discontinued at the end of the surgical procedure. The animals awakened after about 10-15 min and were returned into their cages with free access to water and food for experimental period (Plaschke *et al*., 2008).

Experimental design: The rats were divided into five groups. Group 1 (Control normal group): contains 6 rats without occlusion. The remaining 4 groups were 16 rats per each. As group 2 subjected to ½ h occluded group (LCCA), group 3 subjected to 1 h occlusion, group 4 subjected to 4 h occlusion and group 5 was subjected to 6 h occlusion.

Ischemia reperfusion: After left common carotid artery occlusion in each group, reperfusion was allowed by retracting the thread. The filament was slowly retracted to allow the reperfusion of the ischemic region and the animals were then returned to their cages for a period of 24 h after reperfusion (end of the reperfusion periods). Blood samples were collected at 0, 1, 3 and 24 h post reperfusion.

Sampling and serum and or plasma extraction: Blood samples and brain tissue specimens were taken from all occluded animal groups four times at the onset of reperfusion (zero time, 1, 3 and 24 h). However, in control rats group without occlusion the blood and brain samples were collected only one time from 6 rats. Blood samples for serum separation were collected by ocular vein puncture at the end of each experimental period in dry,

clean and screw capped tubes and serum were separated by centrifugation at 2500 rpm for 15 min. Serum was kept in a deep freeze at -20°C until biochemical assays. Another blood samples were collected in clean sterile tubes contained trisodium citrate 3.8% as anticoagulant (1 vol. anticoagulant/9 vol. blood) for plasma separation and used directly for fibrinogen determination.

Brain specimens: Rats were killed by decapitation. The brain specimen quickly removed, cleaned by rinsing with cold saline and stored at -20°C. Briefly, brain tissues was minced into small pieces, homogenized with ice cold 0.05 M potassium phosphate buffer (pH 7.4) to make 10% homogenates. The homogenates were centrifuged at 10.000 rpm (round per minute) for 15 min at 4°C. The supernatant was used for subsequent biochemical analyses.

Biochemical analysis: Serum and brain L-malondialdehyde, brain nitric oxide, glutathione peroxidase, Catalase, Superoxide dismutase, Acetylcholinesterase, Lactate dehydrogenase, Lactate and sodium concentrations were determined according to the methods described by Ohkawa *et al.* (1979), Moshage *et al.* (1995), Gross *et al.* (1967), Xu *et al.* (1997), Paoletti *et al.* (1986), Augustinsson *et al.* (1978), Quistorff and Clayson (1973), Noll (1988) and Maruna (1958), respectively. Serum C-Reactive Protein concentration and Creatine kinase activity were determined by the methods of Tietz (1995) and Rosano *et al.* (1976), respectively. Plasma fibrinogen was estimated as described by Thomson *et al.* (1974).

Statistical analysis: The obtained data were statistically analyzed by one-way analysis of variance (ANOVA) followed by the Duncan multiple test. All analyses were performed using the statistical package for social science. Values of $p<0.05$ were considered to be significant.

RESULTS

This study was aimed to test the changes in blood and brain biomarkers that may be affected during the incidence of brain stroke in Wistar rats. The results are outlined in down paragraphs.

Biochemical changes in brain and blood parameters in control and left common carotid artery (LCCA) occluded rats: The results in Table 1 show a significant increase in brain and blood MDA (86.44±1.85 and 82.94±0.84, respectively relative to control values (51.17±6.79) 1 h occlusion. Moreover, a significant increase in lipid peroxidation 1 h following occlusion together with AchE, GPX, LDH and CK has been recorded. Coinciding with recorded findings in Table 1, the changes in blood and brain MDA together with NO following reperfusion showed an overall increase in their levels 1, 3 and 24 h after reperfusion in ½ and 1 h occluded groups (Table 2).

Changes in brain glutathione peroxidase (GPX), catalase (CAT) and superoxide dismutase (SOD): Table 3 shows an increase in GPx levels at 1 h (65.89±2.25), 3 h (90.94±3.00) and (24 90.94±3.00) h after reperfusion in ½ and 1 h occluded groups. Catalase activity was decreased in ½ h occlusion group at 1, 3 and 24 h post perfusion (122.81±6.66, 223.29±6.48 and 115.25±4.9). Moreover, a significant increase in brain SOD activity was noticed in ½ and 1 h occlusion groups compared to zero hr. Ischemic animals, ½ and 24 h occlusion group showed a decrease in SOD activity at 1 h (44.30±1.96) compared to ½ h reperfusion. Also, a significant increase in CAT activity in brain at ½ h occlusion at 1, 3 and 24 h post perfusion.

Changes in activities of brain acetylecholine esterase (AchE), lactate dehydrogenase (LDH) and serum creatine kinase (CK): As seen in Table 4, a significant decrease in brain AchE was observed in ischemic rats 1 h occlusion group at 1, 3 and 24 h post reperfusion (18.00±2.12, 15.75±1.31 and 12.00±0.40, respectively) and only at 1 and 3 in 6 h occluded group (16.00±2.61 and 14.50±0.64). However, it increased in 4 h occluded group at 1 and 24 h post reperfusion when compared to zero hr. Regarding to lactate dehydrogenase, LDH was decreased significantly at 1, 3 and 24 h post reperfusion in ½ h occlusion (279.25±35.79, 287.00±12.74 and 278.00±1.41, respectively) and in 1 h occlusion group at 3 h post reperfusion (251.00±18.36). However, there is an increase in 4 h occlusion group at 3 h post reperfusion and in 6 h occlusion group at 1 and 3 h post reperfusion. Also, there is overall increase in CK activity in ½ h occlusion group at 1 and 3 post reperfusion compared to zero hr. In 4 h occlusion, CK decreased at 3 and 24 h post reperfusion (102.25±13.60 and 74.00±7.77, respectively).

Changes in brain lactate and sodium levels: As seen in Table 5, a significant increase in lactate concentration was seen in ½ h occlusion group at 3 and 24 h post reperfusion (12.02±1.27 and 11.88±0.29, respectively) and in 1 h occlusion group at 3 h after reperfusion (12.84±0.19) while in 4 h occlusion group it decreased at 1 h post reperfusion (4.79±0.67). On the same line, LCCA occlusion significantly increased Na ½ h occlusion at 1.3 h post reperfusion (179.37±2.50 and 179.17±2.51, respectively) and in 6 h occlusion at 3 h post reperfusion (169.57±1.16).

Table 1: Biochemical abnormalities in some brain and blood parameters in control and rats subjected to left common carotid artery occlusion

Animal groups	Brain MDA (nmol/g)	Blood MDA (nmol/L)	NO (mmol g^{-1})	GPX (nmol g^{-1})	CAT (mmol g^{-1})	SOD (IU g^{-1})	AchE (nmol g^{-1})	LDH (IU g^{-1})	CK (IU L^{-1})	Lactate (mmol g^{-1})	Na (nmol g^{-1})	Fibrinogen (mg dL^{-1})	CRP (mg dL^{-1})
Control	51.17±6.79^{b}	55.92±5.68^{b}	20.04±1.75^{a}	25.47±2.40^{c}	68.36±5.47^{a}	6.361.37^{bc}	5.75 ±1.31^{b}	247.50±16.79^{c}	145.75±14.61^{a}	8.12±0.2^{bc}	158.40±1.58^{c}	183.25±2.05^{b}	2.41±0.16^{ab}
½ h occlusion	52.17±3.62^{b}	77.10±9.10^{a}	9.50±1.60^{b}	52.70±10.52^{ab}	44.48±4.03^{a}	4.212.41^{a}	8.00±3.34^{ab}	403.00±11.45^{a}	90.33±2.01^{c}	9.39±0.60^{b}	170.75±1.79^{b}	180.75±5.61^{b}	1.90±0.29^{bc}
1 h occlusion	86.44±1.85^{a}	82.94±0.84^{a}	19.68±1.27^{a}	58.86±2.1^{a}	68.47±10.64^{a}	8.171.64^{a}	23.66 ±2.01^{a}	358.33±7.64^{b}	107.00±8.52^{bc}	10.86±0.63^{a}	184.50±2.13^{a}	198.66±0.84^{a}	2.47±0.06^{a}
4 h occlusion	53.44±4.877^{b}	58.46±4.86^{b}	18.83±1.01^{a}	49.24±1.94^{ab}	60.55±11.51^{a}	3.703.40^{c}	3.00±1.08^{b}	241.66±7.53^{c}	138.00±8.72^{ab}	7.87±0.39^{c}	154.11±3.46^{c}	189.55±5.7^{ab}	1.262±0.14^{d}
6 h occlusion	52.02±5.75^{b}	82.01±6.83^{a}	14.33±3.21^{ab}	33.56±8.20^{bc}	58.13±5.97^{a}	3.352.50^{ab}	23.50±0.64^{a}	257.33±12.88^{c}	155.75±15.60^{a}	8.23±0.09^{bc}	158.19±3.17^{c}	192.19±0.91^{ab}	1.65±0.12^{cd}

Data are presented as (Mean±SE), SE: Standard error for 16 rats per each group, The abbreviations used are MDA: L-malondialdehyde), No: Nitric oxide, GPX: Glutathione peroxidase, CAT: Catalase, SOD: Superoxide dismutase, AchE: Acetylecholinesterase, LDH: Lactate dehydrogenase, CK: creatine Kinase, Na: Sodium) and CRP: C-reactive protein. Mean values with different superscript letters in the same column are significantly different at ($p<0.05$)

Table 2: Changes in brain L-malondialdehyde and Nitric oxide concentrations after 0, 1, 3 and 24 h reperfusion in wistar rats

Time post-reperfusion	L-malondialdehyde in brain (nmol g^{-1})				Serum L-malondialdehyde (nmol L^{-1})				Nitric oxide (mmol g^{-1})			
	½ h occlusion	1 h occlusion	4 h occlusion	6 h occlusion	½ h occlusion	1 h occlusion	4 h occlusion	6 h occlusion	½ h occlusion	1 h occlusion	4 h occlusion	6 h occlusion
0 h	52.17±3.62^{b}	86.44±1.85^{a}	53.44±4.877^{a}	52.02±5.75^{a}	77.10±9.100^{b}	82.94±0.84^{b}	58.46±4.860^{a}	82.01±6.830^{b}	9.50±1.600^{b}	19.66±1.27^{b}	18.83±1.01^{a}	14.33±3.21^{a}
1 h	90.36±4.58^{a}	103.81±19.78^{a}	68.75±9.91^{a}	55.01±3.35^{a}	145.39±7.42^{a}	133.92±15.92^{a}	62.70±1.750^{a}	106.42±2.38^{a}	22.20±0.811^{a}	25.37±0.75^{a}	16.44±0.91^{ab}	6.56±1.23^{b}
3 h	78.80±6.85^{a}	86.11±5.10^{a}	59.61±4.03^{a}	69.44±9.71^{a}	132±17.490^{a}	127.53±10.20^{a}	59.33±8.540^{a}	61.16±9.000^{c}	19.42±2.350^{a}	18.24±1.56^{b}	15.61±0.61^{b}	2.53±0.42^{b}
24 h	81.61±1.78^{a}	97.40±9.88^{a}	69.04±7.48^{a}	71.22±14.9^{a}	80.11±6.060^{b}	133.55±18.49^{a}	65.34±1.150^{a}	77.78±3.610^{bc}	21.36±1.070^{a}	16.62±0.755^{b}	18.82±0.93^{a}	7.83±0.67^{b}

Data are presented as (Mean±SE), SE: Standard error for 16 rats per each group, Mean values with different superscript letters in the same column are significantly different at ($p<0.05$)

Table 3: Changes in brain glutathione peroxidase, catalase and superoxide dismutase activities after 0, 1, 3 and 24 h reperfusion in rats

Time post-Reperfusion	Glutathione peroxidase (nmol g^{-1})				Catalase (mmol g^{-1})				Superoxide dismutase (U g^{-1})			
	½ h occlusion	1h occlusion	4 h occlusion	6 h occlusion	½ h occlusion	1h occlusion	4 h occlusion	6 h occlusion	½ h occlusion	1h occlusion	4 h occlusion	6 h occlusion
0 h	52.07±10.52^{b}	58.86±2.14^{c}	49.24±1.94^{a}	33.56±8.20^{a}	44.48±4.030^{b}	68.47±10.64^{b}	60.55±11.51^{b}	58.13±5.79^{c}	44.21±2.41^{b}	48.175±1.64^{a}	33.70±3.40^{a}	43.35±2.50^{ab}
1 hour	68.94±3.300^{ab}	65.89±2.25^{bc}	29.25±4.95^{b}	33.22±6.75^{a}	122.81±6.66^{a}	124.81±6.660^{a}	46.79±5.180^{b}	124.43±4.02^{a}	47.95±2.28^{ab}	44.30±1.960^{a}	38.38±3.38^{a}	36.61±0.76^{c}
3 h	64.66±6.040^{ab}	90.94±3.00^{a}	34.82±0.90^{ab}	49.44±2.91^{a}	223.29±6.48^{a}	137.46±4.450^{c}	49.31±3.970^{b}	86.88±3.132^{b}	50.66±5.26^{ab}	42.60±7.430^{a}	36.42±1.55^{a}	40.00±0.38^{bc}
24 h	80.51±3.910^{a}	73.82±5.74^{b}	41.97^{a}±8.00^{b}	37.39±2.47^{a}	115.25±4.90^{a}	111.12±7.560^{a}	87.26±5.660^{a}	108.43±14.17^{ab}	55.95±1.07^{a}	52.66±5.160^{a}	35.81±2.06^{a}	47.150±2.78^{a}

Data are presented as (Mean±SE), SE: Standard error for 16 rats per each group, Mean values with different superscript letters in the same column are significantly different at ($p<0.05$)

Table 4. Changes in brain acetylcholinesterase, lactate dehydrogenase and serum creatine kinase activities after 0, 1, 3 and 24 h reperfusion in rats

Time post-Reperfusion	Acetylcholinesterase (nmol g^{-1})				Lactate dehydrogenase (IU g^{-1})				Creatine kinase (IU L^{-1})			
	½ h occlusion	1h occlusion	4 h occlusion	6 h occlusion	½ h occlusion	1h occlusion	4 h occlusion	6 h occlusion	½ h occlusion	1h occlusion	4 h occlusion	6 h occlusion
0 h	18.00±3.34^{a}	23.66±2.01^{a}	13.00±1.08^{c}	23.50±0.64 ^{a}	403.00±11.45^{a}	358.33±15.28^{ab}	241.66±7.530^{b}	257.33±12.88^{b}	90.33±2.010^{b}	107.00±8.520^{a}	138.00±8.727^{a}	155.75±15.60^{a}
1 h	14.00±1.47^{a}	18.00±2.12^{b}	24.25±1.37^{a}	16.00±2.61^{b}	279.25±35.79^{b}	392.25±64.50^{a}	264.00±11.58^{b}	378.25±20.41^{a}	144.75±13.76^{a}	105.75±14.14^{a}	121.25^{a}±5.121^{b}	160.50±6.51^{a}
3 h	14.25±1.37^{a}	15.75±1.31^{bc}	18.00±2.12^{b,c}	14.50±0.64^{b}	287.00±12.74^{b}	251.00±18.36^{c}	358.00±36.83^{a}	391.28±7.580^{a}	160.33±8.980^{a}	123.00±5.640^{a}	102.25±13.60^{bc}	119.00±25.74^{a}
24 h	13.00±1.08^{a}	12.00±0.40^{c}	9.250±2.56^{ab}	18.00±2.79^{ab}	278.00±1.410^{b}	305.75±50.67^{b}	247.25±9.490^{b}	295.00±29.02^{b}	88.00±0.400^{b}	128.50±12.14^{a}	74.00±7.770^{c}	63.00±5.11^{b}

Data are presented as (Mean±SE), SE: Standard error for 16 rats per each group, Mean values with different superscript letters in the same column are significantly different at ($p<0.05$)

Table 5: Changes in brain lactate and sodium concentrations after 0, 1, 3 and 24 h reperfusion in rats

Time post-reperfusion	Lactate (mmol g^{-1})				Sodium (nmol g^{-1})			
	½ h occlusion	1 h occlusion	4 h occlusion	6 h occlusion	½ h occlusion	1 h occlusion	4 h occlusion	6 h occlusion
0 h	9.39±0.60^{b}	10.86±0.63^{b}	7.87±0.39^{a}	8.23±0.09^{a}	170.75±1.79^{b}	184.52±2.13^{a}	154.11±3.46^{a}	158.19±3.17^{b}
1 h	11.00±0.25^{ab}	10.76±0.17^{b}	4.79±0.67^{b}	7.96±0.26^{a}	179.37±2.50^{a}	176.84±4.22^{a}	156.65±5.30^{a}	169.57±1.16^{a}
3 h	12.02±1.27^{a}	12.84±0.19^{a}	6.93±0.62^{a}	8.69±0.52^{a}	179.17±2.51^{a}	168.88±1.00^{b}	154.35±1.87^{a}	158.20±0.97^{b}
24 h	11.88±0.29^{a}	11.37±0.43^{b}	7.35±0.67^{a}	7.75±0.73^{a}	73.97±2.68^{ab}	163.48±2.64^{c}	152.45±2.49^{a}	157.92±3.15^{b}

Data are presented as (Mean±SE). SE: Standard error for 16 rats per each group. Mean values with different superscript letters in the same column are significantly different at ($p<0.05$)

Table 6: Changes in plasma fibrinogen and serum C-reactive protein concentrations after 0, 1, 3 and 24 h reperfusion in rats

Time post-reperfusion	Fibrinogen (mg dL^{-1})				C-reactive protein (mg mL^{-1})			
	½ h occlusion	1 h occlusion	4 h occlusion	6 h occlusion	½ h occlusion	1 h occlusion	4 h occlusion	6 h occlusion
0 h	180.75±5.61^{b}	198.66±0.84^{a}	189.50±5.780^{a}	192.00±0.91^{a}	1.90±0.29^{bc}	2.47±0.06^{a}	1.26±0.14^{a}	1.65±0.12^{bc}
1 h	191.50±5.33^{b}	190.75±6.57^{a}	181.75±6.780^{a}	195.00±2.04^{a}	1.38±0.14^{c}	2.99±0.34^{a}	1.42±0.17^{a}	1.32±0.18^{c}
3 h	193.25±4.98^{b}	199.00±7.77^{a}	200.50±11.27	188.50±2.90^{a}	2.71±0.23^{b}	2.46±0.35^{a}	1.28±0.07^{a}	2.06±0.14^{ab}
24 h	210.75±3.98^{a}	188.25±2.98^{a}	194.25±4.760^{a}	191.75±2.46^{a}	3.68±0.43^{a}	2.25±0.09^{a}	1.43±0.02^{a}	2.24±0.17^{a}

Data are presented as (Mean±SE). SE: Standard error for 16 rats per each group. Mean values with different superscript letters in the same column are significantly different at ($p<0.05$)

However, in ischemic brain damage, Na decreased in 1hocclusion at 3 and 24 h post reperfusion (168.88±1.0 and 163.48±2.64, respectively).

Changes in plasma fibrinogen and C-reactive protein in re-perfused rats: Finally, as shown in Table 6, ½ h occlusion group significantly increased plasma fibrinogen at 24 h perfusion (210.75±3.98) when compared to zero h. Regarding C-reactive protein, a significant increase in C-reactive protein in ½ and 6 h occlusion groups at 24 h post reperfusion (3.68±0.43 and 2.24±0.17, respectively).

DISCUSSION

Stroke is the third most common cause of death in industrialized countries and is a major cause of severe physical disability (De Freitas and Bogousslavsky, 2001). Ischemic stroke accounts for 70-80% of all strokes. Cerebral infarction may be due to primary thrombosis in an artery or occlusion of a vessel by an embolus (Sudlow and Warlow, 1997). These results are in agreement with the findings of Polidori *et al.* (2002) and Cano *et al.* (2003) which showed that products of lipid peroxidation such as L-malondialdehyde (L-MDA) were found to be increased in subjects with thrombotic or cardio embolic ischemic stroke than in controls (Cherubini *et al.*, 2000). Also, Candelario-Jalil *et al.* (2001) reported that initial increase in lipid peroxidation persists for several days (1 h-4 days) in cerebral ischemia of hippocampus and 1-24 h in cortex ischemia. During the reperfusion period after ischemia, increased oxygen supply results in overproduction of Reactive Oxygen Species (ROS) which are involved in the process of cell death. Reactive Oxygen Species (ROS), such as superoxide anions, hydroxyl free radicals, hydrogen peroxide and nitric oxide are produced as a consequence of metabolic reactions and central nervous system activity (Lancelot *et al.*, 1995). These reactive species are directly involved in oxidative damage of cellular macromolecules such as nucleic acids, proteins and lipids in ischemic tissues which can lead to cell death (Chan, 2001). In fact, the release of ROS and lipid peroxidation can be detected as a markers early during exposure to Ischemia/Reperfusion (I/R),, e.g., 1-3 h, an interval in which there was no sign of neuronal death (Collino *et al.*, 2006). Superoxide and hydroxyl radical cause severe cell membrane damage by inducing lipid peroxidation (Bromont *et al.*, 1989).

Inducible Nitric Oxide (NO) synthase is up-regulated after I/R which results in excessive NO production. This excess NO reacts with superoxide to form peroxynitrite, a powerful radical that can induce neuronal death after cerebral ischemia (Iadecola and Alexander, 2001). Nitrite is the major end-product of nitric oxide which was undertaken to monitor NO production during ischemia. These results are nearly similar to those reported by Simao *et al.* (2011) who reported that, NO content significantly increased 1 h after transient global cerebral ischemia in cortex and hippocampus when compared to sham rats. After 24 h of cerebral injury, NO content returned to basal level. Also, Khan *et al.* (2007), demonstrated that, ischemia and reperfusion of the brain induces an increase in the production of superoxide and other ROS that may directly or indirectly decrease in the bioavailability of NO. The NO concentration during and after cerebral ischemia was characterized by a decline of NO during ischemia and increased above basal levels during reperfusion (Heeba and EL-Hanafy, 2012). The

decrease in NO found during ischemia is consistent with the requirement of oxygen for the formation of NO and L-citrulline from L-arginine (Matheis *et al*., 1992; Willmot *et al*., 2005). NO is beneficial as a messenger or modulator, but in conditions such as oxidative stress, it is potentially toxic. The toxic effects of NO may be attributed to peroxynitrite ($ONOO^{\bullet}$-) which is a reaction product of NO with superoxide ($O_2^{\bullet}$-) (Sekhon *et al*., 2003). NO synthase activity and NO release are greatly increased in the acutely ischemic brain (Kader *et al*., 1993; Simao *et al*., 2011).

The enzymatic antioxidant activity of the tissue affected by I/R is particularly important for the primary endogenous defense against the ROS induced injury. SOD, CAT and glutathione GSH-Px are endogenous antioxidants play an important role in the prevention of oxidative damage (Akyol *et al*., 2002). In the presence of excess oxygen species, inactivation of detoxification systems and degradation of antioxidants, endogenous antioxidative defenses are highly effective (Chan, 2001). Numerous antioxidants and scavengers of ROS have been tested and many have shown neuroprotective effects (Ergun *et al*., 2002). These results are nearly similar to those reported by Simao *et al*. (2011) which showed that, After 1 h and 24 h of reperfusion, the hippocampus of ischemic rats showed a significant increase in SOD and GPx activities, whereas there was a significant decrease in catalase. In the present study, SOD and GPx activities increased in ischemic rats. The increase in GPx activity may be a compensatory response for an increase in endogenous H_2O_2 production in ischemic brain because ROS formation promotes the oxidation of fatty acids with resulting H_2O_2 formation (Keller *et al*., 1998; Imam and Ali, 2000). The increase in SOD activity could be due to increase in production of superoxide and H2O2 that act as inducers of tissue SOD production. Increase in both SOD and GPx activities may thus be an adaptive response for increased oxidative stress in the brain tissue. GPx are known to be inactivated by oxidant radicals, as observed in the present study and some earlier studies (Alexandrova *et al*., 2004). Thus, the antioxidant enzyme activity of the tissue affected by ischemia-reperfusion is particularly important as the primary endogenous defense against free radicals induced injury through SOD, CAT and GPx. SOD metabolizes superoxide anion to hydrogen peroxide, whereas CAT and GPx breakdown hydrogen peroxide ending the cycle of superoxide neutralization (Michiels *et al*., 1994).

The key role that regulates the cholinergic system in normal brain functions and in memory disturbances of several pathological process has been well documented (Bartus *et al*., 1982). Nevertheless, no consensus has been reached as to whether the levels of the cholinergic enzyme acetylcholinesterase (AChE) are consistently affected in ischemic brain. The level of AChE activity in spinal cord and different brain areas following ischemia has been reported to decrease or increase (Malatova *et al*., 1999), or remain unchanged (Goldberg *et al*., 1985). These results are nearly similar to those reported by Hu *et al*. (2009) who demonstrated that AChE activity was increased at 0.5 h after MCAO and remained elevated for at least 28 days in the ischemic brain. AChE hydrolyzes acetylcholine and this plays a vital role in terminating neurotransmission at cholinergic synapses. Interestingly, the neuronal injury associated with ischemia is accompanied by changes in the cholinergic system (Hou *et al*., 2008). Preliminary pharmacological studies showed that AChE inhibition improves neuronal survival in distinct animal models of cerebral ischemia (Saez-Valero *et al*., 1999). Although changes in AChE isoforms following ischemia are evident, more work is required to elucidate the role of AChE in ischemia-induced brain damage.

It must be noted that significant alterations in LDH have been observed after a transient exposure of brain slices to oxygen/glucose deprivation (Moro *et al*., 2000). An increase in the concentration of LDH without elevation of the concentrations of other enzymes in the cerebrospinal fluid (CSF) was reported. LDH was found to be the most sensitive CSF enzyme in indicating cerebrovascular events. A significant increased level has been demonstrated in patients with hemorrhagic infarcts, mild and moderate strokes (Donnan *et al*., 1983). Similarly, Vaagenes *et al*. (1986) investigated LDH concentrations in patients with stroke and an increase in LDH concentration was observed in the CSF of stroke patients. So, we can speculate that LDH measurements in CSF can be used and that was supported by findings of (Donnan *et al*., 1983).

Creatine Kinase (CK) is a crucial enzyme for high energy consuming tissues like the brain. Creatine kinase works as a buffering system of cellular ATP levels, playing a central role in energy metabolism (Wolosker *et al*., 1996). It is also known that a decrease in CK activity is associated with neurodegenerative pathways that result in neuronal death in brain ischemia (Tomimoto *et al*., 1993), neurodegenerative diseases, bipolar disorder and other pathological states (Schuck *et al*., 2007). It is also known that CK is very sensitive to free radicals, especially by the oxidation of thiol groups of its structure (Wolosker *et al*., 1996). Moreover, it is well described, that inhibition of this enzyme is implicated in the pathogenesis of a number of diseases, especially in brain (Schlattner and Wallimann,

2000). D'Esposito (2007) showed that, CK was inhibited in prefrontal cortex, cerebral cortex and hippocampus, brain areas that are crucial for cognitive processes. Moreover, inhibition of CK was prevented by antioxidants. In this context, we also speculate that oxidative stress may be involved in the mechanism of CK activity inhibition as recorded by Sener *et al.* (2007). The changes in lactic acid, pyruvate and their ratios have been used as important biochemical markers of cerebral ischemia in experimental animals and clinical studies (Ronne-Engstrom *et al.*, 1995). Our results are nearly similar to those reported by Barc *et al.* (2004) who showed that, five minutes of ischemia without reperfusion produced an increase of lactate levels in all cerebral regions especially in striatum reflecting an ischemic damage and an oxidative stress. Moreover, acidosis within the central nervous system is critical in the pathophysiology of ischemia. Neuronal damage following ischemia appears to be a consequence of a drop in brain pH, cellular ATP level and glucose. Acidosis is more severe when cerebral blood flow and ATP levels are reduced. The subsequent anaerobic metabolism of glucose leads to the production of lactate and H^+ (Siesjo *et al.*, 1993).

Tissue sodium concentration has been investigated as a means of detecting infarcted tissue following ischemia, because the distribution and concentration of tissue sodium is related to the function of the Na^+K^+-ATPase (Jung *et al.*, 2007). Na^+K^+-ATPase is the enzyme, located to the cytoplasmic membrane responsible for the active transport of sodium and potassium ions in the nervous system, maintaining the ionic gradient necessary for neuronal excitability and regulation of neuronal cell volume. It has been demonstrated that this enzyme is susceptible to free radical attack and there are some reports showing that Na^+K^+-ATPase activity is decreased after cerebral ischemia and in various chronic neurodegenerative disorders (Lees, 1993). Also, Simao *et al.* (2011) reported that, ischemia significantly reduces Na^+K^+-ATPase activity in the cortex and hippocampus after 1 h when compared to sham groups and that is coincided with our results. An elevated level of plasma fibrinogen is reported to be a risk factor for the occurrence of cerebral infarction and cardiovascular diseases (Kannel *et al.*, 1987). Elevated levels of plasma fibrinogen were reported to play a role in atherogenesis by facilitating mechanical vascular wall injury and platelet-vessel wall interaction (Grotta *et al.*, 1989), parallel to that Tsuda *et al.* (1997) reported that plasma fibrinogen levels were lower in silent cerebral infarctions than in chronic cerebral infarctions and patients with more advanced grades of silent cerebral infarction showed higher levels of plasma fibrinogen than those with less advanced grades.

As known, stroke triggers marked inflammatory reaction that involves local cellular activation in the brain and production of a multitude of inflammatory mediators, including cytokines, chemokines, proteases, reactive oxygen species and vascular adhesion molecules (Wang *et al.*, 2007). Elevated CRP levels may affect coagulation through the important role of tissue factor expression (Cermak *et al.*, 1993; Feinberg *et al.*, 1996). Tracy (1998) showed that, a strong association between fibrinogen and CRP, suggesting that the effects of higher CRP levels are independent from fibrinogen. Further studies should reveal whether CRP contributes to tissue damage and clinical complications in cerebrovascular disease (Di Napoli *et al.*, 2001). Most studies have focused on the induction and involvement of the inflammatory reaction after the ischemic episode (Wang *et al.*, 2007) but there are extensive data supporting the contribution of inflammation in multiple phases of stroke, including as modifier and possible biomarker of susceptibility, proximate trigger, post-ischemic mediator of brain damage and putative predictor of prognosis and recurrent cerebrovascular events (Muir *et al.*, 2007). In particular, there is emerging evidence that inflammatory events outside the brain occurring prior to, during and after stroke markedly influence stroke susceptibility and outcome (Urra *et al.*, 2009).

CONCLUSION

In brief, this study provides *in vivo* evidence that, brain ischemia followed by reperfusion has harmful effect on brain energy metabolism and induced oxidative stress via production and rapid increase in the generation of reactive oxygen species, rise in lipid peroxidation and disrupted enzymatic antioxidant defenses against overproduction of free radicals during focal cerebral ischemia/reperfusion.

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