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CLINICAL & BASIC RESEARCH

Oxidative Stress and C-Reactive Protein in Patients with Cerebrovascular Accident (Ischaemic Stroke)

The role of *Ginkgo biloba* extract

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جهد الأكسدة والبروتين التفاعلي (ج) في مرضى الحوادث الوعائية الدماغية (السكتة الاقفارية)

تأثير خلاصة الجنكوبالوبا

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الملخص: الهدف: التأكد من وجود علامات جهد الأكسدة والاستجابة الالتهابية في مصل دم مرضى السكتة الاقفارية بقياس مستوى المالونديليهايد وحالة مضادات الأكسدة الكليّة والبروتين التفاعلي عالي الحساسية (ج) في مصل دم المرضى في المرحلة المبكرة التالية للسكتة الاقفارية وتحديد دور العلاج بخلاصة الجنكوبالوبا في تصحيح علامات جهد الأكسدة والاستجابة الالتهابية. **الطريقة**: أجريت هذه الدراسة في مستشفى ابن سينا في مدينة الموصل بالعراق. ضمّت هذه الدراسة واحداً وثلاثين مريضا بالسكتة الاقفارية وثلاثين شخصا من الأصحاء كمجموعة ضابطة. تم تقسيم المرضى إلى **مجموعتين**: المجموعة الأولى (15 مريضا) تمّ علاجهم بالطريقة التقليدية، والمجموعة الثانية (16 مريضا) عولجوا بالطريقة التقليدية إضافة إلى الجنكوبالوبا (1500 ملغم/ يوميا) لمده شهر. تمّ قياس مستوى المالونديليهايد وحالة مضادات الأكسدة الكليّة والبروتين التفاعلي عالي الحساسية (ج) من عينات دم المرضى والمجموعة الضابطة قبل العلاج وبعده. النتائج . هناك زيادة معتدّة في مستوى المالونديليهايد والبروتين التفاعلي عالي الحساسية (ج) عند المرضى مقارنة بالمجموعة الضابطة ($P \leq 0.001$)، وانخفاضا معندًا في مستوى مضادات الأكسدة الكليّة في المرضى. كما أظهرت المجموعة الأولى والثانية من المرضى انخفاضا معتدًا في مستوى المالونديليهايد والبروتين التفاعلي عالي الحساسية (ج)، مع ارتفاع معتدّ في مستوى حالة مضادات الأكسدة الكليّة بالمقارنة مع مستويات ما قبل العلاج. لم يكن هنالك اختلافا معتدًا في مستوى المالونديايهايد في مصل الدم بين مرضى المجموعة الأولى والثانية بينما كان هناك ارتفاعا معتدًا في حالة مضادات الأكسدة الكليّة وانخفاضا معتدًا في مستوى البروتين التفاعلي علي الحساسية نوع (ج) في مرضى المجموعة الثانية. **الخلاصة**: يُصاحب السكتة الاقفارية الحادة جهد أكسدة مع استجابة التهابية في الفترة المبكرة التالية للحالة الاقفارية،، والجنكوبالوبا كان له دور فعال في تقليل ضرر جهد الأكسدة والاستجابة الالتهابية.

مفتاح الكلمات: حادثة وعائية دماغية، البروتين التفاعلي (ج)، الجنكوبالوبا، حالة اقفارية، ،سكتة، مالوندليهايد، جهد الأكسدة ،حالة مضادات الأكسدة، العراق.

ABSTRACT: ***Objectives:*** This study aimed to investigate the presence of oxidative stress and inflammation in ischaemic stroke patients by measuring malondialdehyde (MDA), total antioxidant status (TAS), and highly-sensitivity C-reactive protein (hsCRP) in the early post-ischaemic period, and to determine the role of *Ginkgo biloba* therapy in correcting the markers of oxidative stress and inflammation. ***Methods:*** This study was conducted at Ibn Seena Hospital, Mosul City, Iraq and included 31 cerebrovascular accident (CVA) patients and 30 healthy controls. Ischaemic stroke patients were divided into two groups: group I (n = 15) received conventional therapy; group II (n = 16) received conventional therapy with *G. biloba* (1500 mg/day) for 30 days. Blood samples were obtained from patients and controls before treatment and assays done of serum levels of MDA, TAS, and hsCRP. For CVA patients, a post-treatment blood sample was taken and the same parameters reassessed. ***Results:*** Compared with the controls, patients' serum levels of MDA, and hsCRP were significantly higher ($P \leq 0.001$) and TAS significantly lower. Group I and II patients reported a significant reduction in serum levels of MDA and hsCRP and a significant increase in serum levels of TAS, in comparison with pre-treatment levels. There was no significant difference ($P = 0.19$) in serum MDA levels between groups I and II, whereas, serum TAS levels were significantly higher ($P \leq 0.01$) and hsCRP significantly lower ($P \leq 0.01$) in group II. ***Conclusion:*** Acute stroke is associated with oxidative stress and inflammatory response in the early period. *G. biloba* plays a potential role in reducing oxidative damage and

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inflammatory response.

Keywords: Cerebrovascular accident; C-reactive protein; *Ginkgo biloba*; Ischaemia; Stroke; Malondialdehyde; Oxidative stress; Antioxidant; Iraq.

Advances in Knowledge

- *Ischaemic stroke is associated with oxidative stress and inflammatory responses in the early post-ischaemic period.*

Applications to Patient Care

- *Ginkgo biloba therapy plays a potential role in reducing oxidative damage and inflammatory response in early post-ischaemic stroke patients.*

Stroke, or cerebrovascular accident (CVA), is the third leading cause of death after cardiovascular diseases and cancer.[1] In fact, it is the second-leading cause of mortality and disease among adults over 60 years of age worldwide.[2] Approximately 80% of strokes are ischaemic in origin, since they result either from thrombus *in situ* or an embolism of distant origin.[3]

Cerebral ischaemia initiates a cascade of cellular and molecular events that lead to brain damage, with one such event being post-ischaemic inflammation.[4] Focal cerebral ischaemia is associated with a local inflammatory reaction that contributes to tissue damage.[5] Microglial cells in particular become activated and provoke tissue injury by releasing pro-inflammatory mediators and reactive oxygen species (ROS).[6,7] When oxygen supply is limited during ischaemia, a calcium influx may activate phospholipase C, which results in a breakdown of membrane phospholipids, or may convert xanthine dehydrogenase to xanthine oxidase in the cerebral blood vessels leading to the formation of superoxide radicals and hydrogen peroxide.[8]

ROS causes oxidative damage that may affect lipids, proteins, nucleic acids and other molecules. Quantification of lipid peroxidation end products is considered to be a measure of whole-body oxidative damage. Serum malondialdehyde (MDA), a marker of lipid peroxidation, is the most abundant aldehyde generated by the attack of free radicals on polyunsaturated fatty acids of cell membranes; its measurement provides information of oxidative injury *in vivo*.[9] The impact of free radicals may also be obtained by comparisons of antioxidant concentrations, because serious damage by free radicals implies insufficiency of the body's multilevel defence systems against radicals.[10]Measurement of the total antioxidant capacity (TAC) of biological fluids, however, is regarded as more physiologically representative in certain settings than individual antioxidants, and is believed to be a useful measure of how much the antioxidants present can protect against oxidative damage to membranes and other cellular components.[11]

C-reactive protein (CRP) has been the most extensively studied marker of inflammation. It is a novel plasma marker of atherothrombotic disease.[12] C-reactive protein (CRP) is produced not only by the liver but also in atherosclerotic lesions by vascular smooth muscle cells and macrophages in response to stimulation by the 'pro-inflammatory' cytokine interleukin-6 (IL-6).[13,14] Elevated plasma levels of CRP are not disease specific but are sensitive markers which are produced in response to tissue injury, infectious agents, and inflammation.[12] Various cross-sectional studies support the notion that CRP may be a marker for stroke and post-stroke status.[15,16] Several studies support the role of CRP in the prediction of ischaemic stroke risk and outcome, as well as the possible role of inflammation before and after stroke.[17,18]As the methods traditionally employed to measure CRP do not have good sensitivity, measurement of highly-sensitivity C-reactive protein (hsCRP) is recommended to evaluate atherothrombotic disease, which usually presents with lower CRP levels than the other inflammatory processes.[14]

Ginkgo biloba extract (EGb 761) is known to have neuroprotective properties in diseases associated with free radical generation. Extensive studies on *G. biloba* extracts showed their ability to protect brain neurons from oxidative stress[19] and to inhibit apoptosis in cell culture.[20] The *G. biloba* extracts that are currently used for medicinal purposes contain 24% flavonoid glycosides (quercetin, kaempferol, isorhamnetin) and 6% terpene lactones (ginkgolides A, B, C, M, J and bilobalides).[21] The EGb 761 components eliminate free radicals

Table 1: Concentrations of malondialdehyde (MDA), total antioxidant status (TAS), and highly-sensitivity C-reactive protein (hsCRP) in patients with acute ischaemic stroke before therapy and in healthy controls

Parameters	Control (n = 30)	Ischaemic stroke patients before therapy(n = 31)
MDA (µmol/L)	1.03 ± 0.17	2.11 ± 0.28***
TAS (mmol/L)	1.85 ± 0.12	1.06 ± 0.13***
hsCRP (mg/L)	0.53± 0.09	1.79 ± 0.18***

*Notes: Results are expressed as mean ± SD; *** Significant difference from control at P ≤0.001*

such as the hydroxyl radical and the superoxide anion.[22] Quercetin is a powerful antioxidant in the flavonoid family due to its molecular configuration, which is capable of eliminating free radicals.[23] The pharmacologically active terpene lactones selectively inhibit the platelet-activating factor, preventing thrombus formation. Bilobalide is reported to possess neuroprotective properties.[24]

The aim of the present study was to investigate the presence of oxidative stress and inflammation in serum samples of ischaemic stroke patients by measuring MDA concentrations, total antioxidant status (TAS), and hsCRP in the early post-ischaemic period, and to determine the role of *G. biloba* therapy in correcting the markers of oxidative stress and inflammation in question.

Methods

This double-blind randomised study was conducted in the Ibn Seena Hospital, Department of Neurology, in Mosul City, from January 2009 to April 2011. Approval was obtained from the ethical committee of the main health centre in Nineveh in Mosul City and the College of Medicine University of Mosul, Iraq. Our study included 31 CVA hypertensive patients (26 males and 5 females) suffering from ischaemic stroke, aged 69.03 ± 2.96 years and 30 healthy control subjects aged 69.40 ± 2.69 years.

All patients included in this study were initially diagnosed as having CVA, or acute ischaemic stroke. All had problems with anterior circulation, a diagnosis made on the basis of full physical and neurological examinations by a neurologist. The diagnoses were then confirmed by either a magnetic resonance imaging (MRI) or computed tomography (CT) scan of the brain. Vascular risk factors including hypertension or diabetes mellitus, and smoking and alcohol habits were recorded. Patients with haemorrhagic stroke, intracranial tumour, or other neurological diseases, infection, inflammation, liver disease, and renal failure were excluded. For controls, the criteria were as follows: age ≥60 years, healthy subjects, non-smokers, and not taking vitamin supplements.

Ischaemic stroke patients were divided into two groups: group I (n = 15 of a possible 18 patients) included patients with ischaemic stroke who received conventional therapy (aspirin, rosuvastatin and lisinopril), and group II (n = 16 of a possible 28 patients) included patients who received conventional therapy with *G. biloba* (1500 mg/day) for 30 days. The dose was decided as a safe increment after previous promising results with 500 and 1000 mg/day.

Blood samples were initially obtained from all CVA patients within 48–120 hours of their accidents, and before starting treatment. They were also taken from the controls. Assays of the serums MDA, TAS and hsCRP were done at the Department of Pharmacology in the College of Medicine at the University of Mosul. For the patient group, another blood sample was taken after treatment with either conventional therapy or conventional therapy with *G. biloba*, and the parameters reassessed.

MDA was measured by the method outlined by Buege and Aust where MDA reacts with thiobarbituric acid (TBA) to yield a red-coloured product.[25] The absorbance of a 3 ml coloured layer was measured at 535 nm spectrophotometrically. TAS was measured by peroxidase/H_2O_2/ABTS colorimetric assay using commercial kits from Randox Laboratories, Belfast, UK. CRP was measured using the BioCheck hsCRP ELISA Kit (BioCheck, Inc., Foster City, California, USA).

Data were expressed as means ± standard deviation (SD). Statistical comparisons were performed using the Student's t-test between patients before therapy and controls, and the one-way analysis of variance (ANOVA). The Dunnett test was used to compare groups of patients. Linear regression analysis and Pearson correlation coefficients (r) were performed to determine the relationships between parameters. All statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) for Windows (Version 11.5, Chicago, Illinois, USA). A *P* value of

Table 2: Concentrations of malondialdehyde (MDA), total antioxidant status (TAS), and highly-sensitivity C-reactive protein (hsCRP) in patients with acute ischaemic stroke before therapy and after therapy

Parameters	Group I (n = 15)		Group II (n = 16)	
	Before therapy	After conventional therapy	Before therapy	After Ginkgo biloba+ conventional therapy (n = 16)
MDA (µmol/L)	2.12 ± 0.26	1.98 ± 0.24***	2.10 ± 0.31	1.86 ± 0.23***
TAS (mmol/L)	1.07 ± 0.12	1.15 ± 0.12**	1.05 ± 0.14	1.31 ± 0.15*** δδ
hsCRP (mg/L)	1.80 ± 0.17	1.69 ± 0.20**	1.78 ± 0.19	1.42 ±0.24*** δδ

*Notes: Results are expressed as mean ± SD; ** Significant difference compared to before therapy at P ≤0.01 and *** at P ≤ 0.001; δδ Significant difference compared to after conventional therapy at P ≤0.01*

<0.05 was considered statistically significant.

Results

The serum levels of MDA, TAS and hsCRP from healthy subjects and patients with acute ischaemic stroke before starting drug therapy are shown in Table 1. The serum levels of MDA and hsCRP were found to be significantly higher ($P \leq 0.001$) and TAS were significantly lower in ischaemic stroke patients in the early post-ischaemic period (before starting therapy) in comparison to the controls [Table 1].

Table 2 shows the serum levels of MDA, TAS, and hsCRP in the two groups of ischaemic stroke patients before and after therapy. Patients in group I reported a significant reduction in serum levels of MDA ($P \leq 0.001$) and hsCRP ($P \leq 0.01$), and a significant increase ($P \leq 0.01$) in serum levels of TAS after treatment with conventional therapy in comparison with their levels before therapy.

Also, patients in group II reported a significant reduction in serum levels of MDA ($P \leq 0.001$) and hsCRP ($P \leq 0.001$) and a significant increase in serum levels of TAS ($P \leq 0.001$) after treatment with *G. biloba* together with conventional therapy in comparison with their levels before therapy.

By comparing the serum levels of MDA, TAS, and hsCRP between the two groups of ischaemic stroke patients after therapy, no significant differences ($P = 0.19$) were reported in serum MDA levels between group I, who received conventional therapy, and group II, who received *G. biloba* with conventional therapy, whereas the serum levels of TAS were found to be significantly higher ($P \leq 0.01$) and hsCRP were significantly lower ($P \leq 0.01$) in group II who received *G. biloba* with conventional therapy in comparison with group I who received conventional therapy alone [Table 2].

Regarding correlations between different biochemical parameters, Figures 1 to 3 show the relationships between MDA, TAS, and hsCRP in patients with acute ischaemic stroke. A significant negative correlation (r = -0.418, $P = 0.001$) was observed between MDA and TAS in the serum samples of patients with acute ischaemic stroke. MDA had a significant positive correlation with hsCRP (r = 0.729, $P \leq 0.001$), and there was a significant negative correlation (r = -0.602, $P = <0.001$) between TAS and hsCRP in the serum samples of ischaemic stroke patients.

Discussion

There is strong indirect evidence that free radical production appears to be an important mechanism of brain injury after exposure to ischaemia and reperfusion.[26] Free radicals in biological samples are difficult to measure because they are extremely reactive and have a short half-life. Therefore, particularly in human studies, indirect approaches have been used to demonstrate free radical production during cerebral ischaemia by measuring the products of free radical reaction with other molecules, such as lipids, proteins, and deoxyribonucleic acid (DNA), and the level or activity of antioxidant molecules.[27,28] ROS causes impairment of cellular membrane stability and cell death by lipid peroxidation.[29] MDA is the end product of the lipid peroxidation process.[30] An increase in free radicals causes overproduction of MDA, which is commonly known as a marker of oxidative stress.[31]

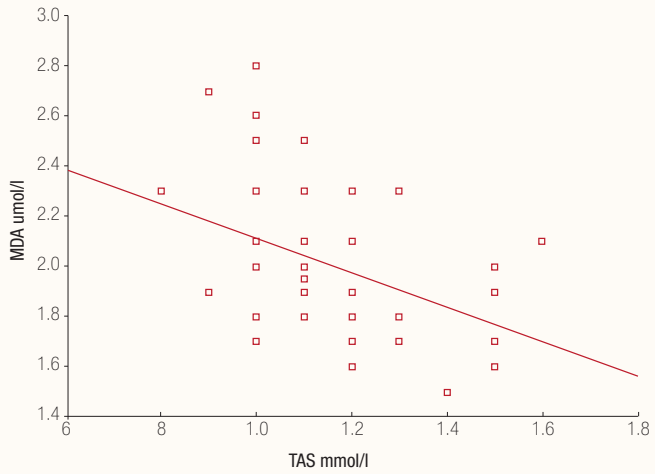

Figure 1: The relationship between malondialdehyde (MDA) and total antioxidant status (TAS) in patients with acute ischaemic stroke (r = -0.418, *P* = 0.001)

In the present study, it was observed that ischaemic stroke patients in the early post-ischaemic period (before starting therapy) had significantly higher levels of serum MDA and hsCRP, and significantly lower TAS than controls. These findings provide evidence for the presence of oxidative stress and inflammation in ischaemic stroke patients.

There are several possible reasons for increased lipid peroxidation in cases of ischaemic stroke. First, the brain's cellular membranes are very rich in polyunsaturated fatty acid side chains, which are especially sensitive to free radical attack. Additionally, they have a low content of antioxidant enzymes, such as catalase and glutathione peroxidase, while the brain contains a significant amount of iron, despite the fact that its iron binding capacity is not very high. Iron ions are known to stimulate free radical generation.[32,33] Lower TAS was accounted for by an increased use of endogenous antioxidants to fight free radicals and oxidative stress during ischaemic stroke.[34]

Our findings are in accordance with several studies which have been done to evaluate oxidative stress, antioxidant status, and markers of inflammation in ischaemic stroke patients.[5-8] Most of these studies have shown enhanced levels of oxidative stress which are markers of inflammation, and reduced levels of antioxidants;[5,10] however, some studies have reported controversial and conflicting results with regard to the levels of antioxidant enzymes.[35]

Studies evaluating markers of oxidative stress in patients in the early period of ischaemic stroke revealed an increase in the blood, cerebrospinal fluid, or salivary concentrations of lipid peroxides, protein carbonyl, homocysteine, nitric oxide, and of MDA, conjugated dienes, and other TBA-reactive molecules at the onset of stroke.[8,10,33,36–39] Some studies showed persistently elevated MDA concentrations 6 months after strokes.[40]

Evaluations of antioxidants in blood, urine, or cerebrospinal fluid of ischaemic stroke patients revealed lower plasma vitamin C, E, vitamin A, and uric acid, lower glutathione peroxidase (GPX) activity, decreased glutathione (GSH) concentration, and a decreased total plasma antioxidant capacity.[8,10,32,34,35,37,41]

Data associated with superoxide dismutase (SOD) activity after acute ischaemic stroke are controversial. SOD activity of patients with acute ischaemic stroke was reported to be reduced in the serum and increased in the cerebrospinal fluid (CSF), increased in both CSF and serum, or remained unchanged.[32,24,43]

Several prospective studies have shown that an elevated serum CRP concentration is a strong predictor of cardiovascular events, including stroke. CRP plays an important role as a marker of outcome and may determine the degree of recovery for stroke patients.

In their study, Sánchez-Moreno *et al.* found that ischaemic stroke patients had significantly

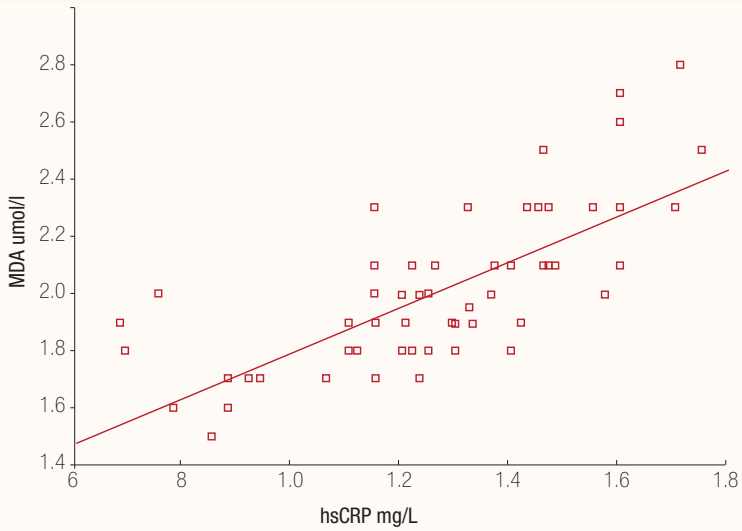

Figure 2: The relationship between malondialdehyde (MDA) and highly-sensitivity C-reactive protein (hsCRP) in patients with acute ischaemic stroke (r = 0.729, *P* = <0.001)

elevated markers of inflammation, marked by CRP, intracellular adhesion molecule-1 (ICAM-1), and chemokine monocyte chemottractant protein-1 (MCP-1), and that elevated CRP concentrations were associated with a two-fold increase in the risk of ischaemic stroke. Winbeck *et al.* observed that an increase in CRP levels between 12 and 24 hours after the onset of symptoms predicts an unfavorable outcome and is associated with an increase in the incidence of cerebrovascular events. Mishra *et al.* observed an increase in hsCRP levels in stroke patients and that the increased levels were correlated with larger infarct, severe neurological deficit, and worse outcomes.[12]

Our study showed a highly significant negative correlation between serum MDA levels and TAS in ischaemic stroke patients, which suggests increased utilisation by ROS as an important contributing factor to the lower concentrations of antioxidants in ischaemic stroke patients.

In the present study, we have investigated the relationship between markers of oxidative stress and markers of inflammation in ischaemic stroke patients. Ischaemic stroke patients with higher serum hsCRP concentrations, indicative of greater inflammatory response, also had higher serum MDA levels and lower TAS than those with lower serum hsCRP concentrations. This can be observed in the figures, which show that serum hsCRP levels positively correlate with lipid peroxidation products and negatively correlate with TAS.

Our findings are in accordance with Sánchez-Moreno *et al.*'s results in which they found that CRP were inversely associated with concentrations of antioxidant vitamins C and E, and positively associated with markers of oxidative stress (8-isoprostanes).[41]

This study also showed that patients in groups I and II reported a significant reduction in serum levels of MDA and hsCRP and a significant increase in serum levels of TAS after treatment in comparison with their levels before therapy.

Interestingly, we did not observe any statistically significant differences in serum MDA levels between group I, who received conventional therapy, and group II, who received *G. biloba* with conventional therapy. In fact, the serum levels of TAS were found to be significantly higher, while those of hsCRP were significantly lower in group II who received *G. biloba* with conventional therapy, in comparison with group I who received conventional therapy alone.

Higher TAS in the serum of ischaemic stroke patients who received *G. biloba* together with conventional therapy (group II) was most likely caused by a lower use of endogenous antioxidants because of supplementary antioxidant effects of flavonoids glycosides.[23].

Antioxidant treatment may be an efficient therapeutic option for cardiac embolisms and macroangiopathic strokes, contributing to an improvement of neurological deficits and the functional status of the patients through the reduction of oxidative stress following ischaemia

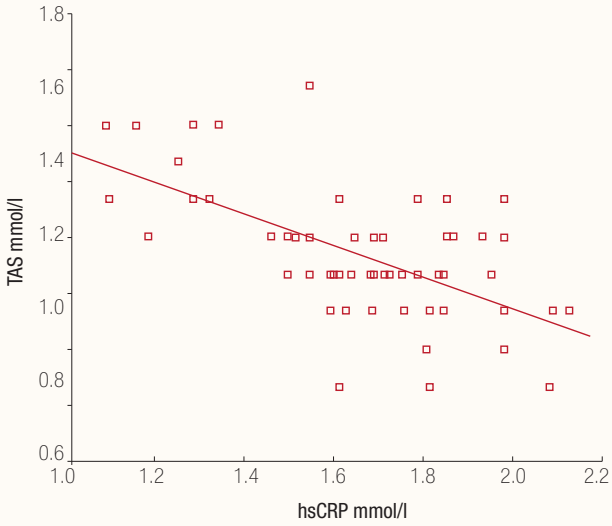

Figure 3: The relationship between total antioxidant status (TAS) and highly-sensitivity C-reactive protein (hsCRP) in patients with acute ischaemic stroke (r = 0.602, P = <0.001)

and/or reperfusion.[48]

It has been reported in many studies that *G. biloba* improved tissue damage in various organs through its antioxidant effect. Zeybek *et al.* reported that *G. biloba* significantly decreased MDA levels and histopathological scores of the pancreatitis in rats.[49] Bridi *et al.* reported that *G. biloba* had antioxidant activity in the hippocampus, striatum, and substantia nigra of rats.[50] EGb 761 has an antioxidant effect as a free radical scavenger, a relaxing effect on vascular walls, an ameliorating effect on blood flow and microcirculation, and a stimulating effect on neurotransmitters. Besides a direct scavenging effect on ROS, *G. biloba* exerts an anti-inflammatory effect on inflammatory cells by suppressing the production of ROS and nitrogen species.[51]

Several studies have shown that EGb 761 could protect cultured neurons against damage induced by peroxynitrate and hydrogen peroxide.[19,51] Zhang *et al.* demonstrated that total ginkgolides (TG) (a terpenoid constituent of EGb 761) protected cultured rat cortical neurons from oxidative damages induced by hydrogen peroxide (H_2O_2).[52]

EGb 761 extract, with free radical scavenging activity, has been shown to reduce the size of cerebral infarction and improve neurological behaviour in rats with permanent and transient mid-cerebral artery occlusion (MCAo).[53,54]

Saleem *et al.* showed that the standardised EGb761 significantly improved the outcome in mice after cerebral ischaemia and reperfusion in terms of neurobehavioral function and infarct size without affecting physiological parameters.[55]

Our findings suggest a potential role of *G. biloba* in acute ischaemic stroke, and the findings are important in view of the fact that stroke is, at present, the third leading cause of death worldwide.[1] The mechanisms by which *G. biloba* normalise the cerebral damage, and reduce oxidative stress and inflammation, can probably be attributed to the antioxidant effects of flavonoids combined with the anti-inflammatory properties of the terpenoids bilobalide and ginkgolides A, B, C, M, J, and the terpenoids antagonists of platelet-activating factor (PAF), making this natural extract plausible to use in the treatment of ischaemic stroke, which is characterised by both oxidative damage and inflammation.[56]

One of the limitations of this study is that it does not relate the biochemical changes to the clinical evaluation and outcome prognosis.

Conclusion

From this study, we conclude that acute ischaemic stroke is associated with oxidative stress and inflammatory response as indicated by increased lipid peroxidation products (MDA), reduced TAS and elevated levels of hsCRP in the early post-ischaemic period, and that *G. biloba* therapy has a potential role in reducing oxidative damage and inflammatory response in ischaemic stroke patients.

CONFLICT OF INTEREST

No funding was received for this study and the authors declared no conflict of interest.

References

1. Thorvaldsen P, Kuulasmaa K, Rajakangas A, Rastenyte D, Sarti C, Wilhelmsen L. Stroke trends in the WHO MONICA project. Stroke 1997; 28:500–6.
2. World Health Organization. The World Health Report (2003): Shaping the Future. Geneva: World Health Organization, 2003.
3. Kidd PM. Integrated brain restoration after ischemic stroke – medical management, risk factors, nutrients, and other interventions for managing inflammation and enhancing brain plasticity. Altern Med Rev 2009; 14:14–35.
4. Lo EH, Dalkara T, Moskowitz MA. Mechanisms, challenges and opportunities in stroke. Nat Rev Neurosci 2003; 4:399–415.
5. Iadecola C, Cho S, Feuerstein GZ, Hallenbeck J. Cerebral Ischemia and Inflammation. In: Wolf P, Ed. Stroke: Pathophysiology, Diagnosis, and Management. New York: Churchill Livingstone, 2004. Pp. 883–94.
6. Giulian D. Reactive Microglia and Ischemic Injury. In: Weir, B, Ed. Primer on Cerebrovascular Diseases. San Diego: Academic, 1997. Pp. 117–124.
7. Schwartz M. Macrophages and microglia in central nervous system injury: are they helpful or harmful? J Cereb Blood Flow Metab 2003; 23:385–94.
8. Sudha K, Rao AV, Rao SN, Rao A. Oxidative damage and plasma antioxidants in cerebrovascular accident. Indian J Physiol Pharmacol 2004; 48:489–92.
9. López E, Illnait J, Molina V, Oyárzabal A, Fernández L, Pérez Y, et al. Effects of D-002 (beeswax alcohols) on lipid peroxidation in middle-aged and older subjects. Lat Am J Pharm 2008; 27:695–703.
10. Gariballa SE, Hutchin TP, Sinclair AJ. Antioxidant capacity after acute ischemic stroke. 2002; 95:685–90.
11. 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; 34:688–9.
12. Mishra PT, Chandra R, Saxena SK, Verma S, Jain R, Bhuyan A. H sensitivity C-reactive protein (hsCRP) level in cerebrovascular accident (stroke). JIACM 2010; 11:204–7.
13. Elkind MS. Inflammation, atherosclerosis, and stroke. Neurologist 2006; 12:140–8.
14. Ramos AM, Pellanda LC, Gus I, Portal VL. Inflammatory markers of cardiovascular disease in the elderly. Arq Bras Cardiol 2009; 92:221–8.
15. Di Napoli M, Di Gianfilippa G, Sollecito A, Bocola V. C-reactive protein and outcome after first-ever ischemic stroke. Stroke 2000; 31:238–9.
16. Ford ES, Giles WH. Serum C-reactive protein and self-reported stroke: findings from the Third National Health and Nutrition Examination Survey. Arterioscler Thromb Vasc Biol 2000; 20:1052–6.
17. Ridker PM, Hennekens CH, Buring JE, Rifai N. C-reactive protein and other markers of inflammation in the prediction of cardiovascular disease in women. N Engl J Med 2000; 342:836–43.
18. Winbeck K, Kukla C, Poppert H, Klingelhofer J, Conrad B, Sander D. Elevated C-reactive protein is associated with an increased intima to media thickness of the common carotid artery. Cerebrovasc Dis 2002; 13:57–63.
19. Oyama Y, Chikahisa L, Ueha T, Kanemaru K, Noda K. Ginkgo biloba extract protects brain neurons against oxidative stress induced by hydrogen peroxide. Brain Res 1996; 712:349–52.
20. Xin W, Wei T, Chen C, Ni Y, Zhao B, Hou J. Mechanisms of apoptosis in rat cerebellar granule cells induced by hydroxyl radicals and the effect of EGb761 and its constituents. Toxicology 2000; 148:103–10.
21. da Silva GGP, Zanoni JN, Buttow NC. Neuroprotective action of Ginkgo biloba on the enteric nervous system of diabetic rats. World J Gastroenterol 2011; 17: 898–905.
22. Ni Y, Zhao B, Hou J, Xin W. Preventive effect of Ginkgo biloba extract on apoptosis in rat cerebellar neuronal cells induced by hydroxyl radicals. Neurosci Lett 1996; 214:115–8.
23. Boots AW, Haenen GR, Bast A. Health effects of quercetin: from antioxidant to nutraceutical. Eur J Pharmacol 2008; 585:325–37.
24. Smith PF, MacLennan CL, Darlington J. The neuroprotective properties of the Ginkgo biloba leaf: a review of the possible relationship to platelet-activating factor (PAF). J Ethnopharmacol 1996; 50:131–9.
25. Buege JA, Aust SD. Thiobarbuturic acid assay. Methods Enzymol 1978; 52:306–7.
26. Traystamn RJ, Kirsch JR, Koehler RC. Oxygen radical mechanism of brain injury following ischaemia and reperfusion. J Appl Physiol 1991; 71:1185–95.
27. White BC, Daya A, DeGracia DJ, O'Neil BJ, Skjaerlund JM, Trumble S. Fluorescent histochemical localization of lipid peroxidation during brain reperfusion following cardiac arrest. Acta Neuropathol (Berl) 1993; 86:1–9.
28. Alexandrova ML, Bochev PG. Oxidative stress during the chronic phase after stroke. Free Radic Biol Med 2005; 39:297–316.
29. Flohe L, Beckmann R, Giertz H, Loschen G. Oxygen-Centered Free Radicals as Mediators of Inflammation.

In: Sies H, Ed. Oxidative Stress. London: Academic Press, 1985. Pp. 403–36.

30. Marnett LJ. Oxy radicals, lipid peroxidation and DNA damage. Toxicology 2002; 27:181–2, 219–22.
31. Gawel S, Wardas M, Niedworok E, Wardas P. Malondialdehyde (MDA) as a lipid peroxidation marker. Wiad Lek 2004; 57:453–5.
32. Yildirim A, Kotan D, Yildirim S, Aygûl R, Akçay F. Increased lipid peroxidation and decreased antioxidant response in serum and cerebrospinal fluid in acute ischemic stroke. Turk J Med Sci 2007; 37:75–81.
33. Aygül R, Kotan D, Yıldırım A, Ulvi H, Akçay F. Plasma and cerebrospinal fluid homocysteine, nitric oxide and malondialdehyde in acute ischemic stroke: possible role of free radicals in the development of brain injury. Eur J Gen Med 2008; 5:57–63.
34. Leionen JS, Ahonen JP, Lonrot K, Jehkonen M, Dastidar P, Molnar G, Alho H. Low plasma antioxidant activity is associated with high lesion volume and neurological impairment in stroke. Stroke 2000; 31:33–9.
35. Cherubini A, Polidori MC, Bregnachi M, Pezzuto S, Cecchetti RBS, Ingegni T, et al. Antioxidant profile and early outcome in stroke patients. Stroke 2000; 31:2295-2300.
36. Santos MD, Valles J, Aznar J, Vilches J. Determination of plasma malondialdehyde-like material and its clinical application in stroke patients. J Clin Pathol 1980; 33:973–6.
37. El Kossi MMH, Zakhary MM. Oxidative stress in the context of acute cerebrovascular stroke. Stroke 2000; 31:1889–92.
38. Al-Rawi NH. Salivary lipid peroxidation and lipid profile levels in patients with recent ischemic stroke. J Int Dent Med Res 2010; 3:57–64.
39. Jawalekar SL, Kulkarni UJ, Surve VT, Deshmukh YA. Status of lipid profile, MDA and protein carbonyl in patients with cardiovascular diseases. Arch Appl Sci Res 2010; 2:8–14.
40. Bir LS, Demir S, Rota S, Koseoglu M. Increased serum malodialdehyde levels in chronic stage of ischemic stroke. Tohoku J Exp Med 2006; 208:33–9.
41. Sánchez-Moreno C, Dashe JF, Scott T, Thaler D, Folstein MF, Martin A. Decreased levels of plasma vitamin C and increased concentrations of inflammatory and oxidative stress markers after stroke. Stroke 2004; 35:163–8.
42. Gruener N, Gross B, Gozlan O, Barak M. Increase in superoxide dismutase after cerebrovascular accident. Life Sci 1994; 54:711–13.
43. Adachi T, Nakamura M, Yamada H, Futenma A, Kato K, Hirano K. Quantitative and qualitative changes of extracellular-superoxide dismutase in patients with various diseases. Clin Chim Acta 1994; 229:123–31.
44. Ridker PM, Cushman M, Stampfer MJ, Tracy RP, Hennekens CH. Inflammation, aspirin, and the risk of cardiovascular disease in apparently healthy men. N Engl J Med 1997; 336:973–9.
45. Koenig W, Pepys MB. C-reactive protein risk prediction: low specificity, high sensitivity. Ann Intern Med 2002; 136:550–2.
46. Barone FC, Feuerstein GZ. Inflammatory mediators and stroke: new opportunities for novel therapeutics. J Cereb Blood Flow Metab 1999; 19:819–34.
47. Winbeck K, Poppert H, Etgen T, Conrad B, Sander D. Prognostic relevance of early serial C-reactive protein measurements after first ischemic stroke. Stroke 2002; 33:2459–64.
48. Jurcau A. The role of antioxidant treatment in acute ischemic stroke: a clinical study. Romanian J Neurol 2007; 6:181–8.
49. Zeybek N, Gorgulu S, Yagci G, Serdar M, Simsek A, Kaymakcioglu N, et al. The effect of Gingko biloba extract (EGb 761) on experimental acute pancreatitis. J Surg Res 2003; 15:286–93.
50. Bridi R, Crosseti FP, Steffen VM, Henriques AT. The antioxidant activity of standardized extract of Gingko biloba (EGb 761) in rats. Phytother Res 2001; 15:449.
51. Chen C, Wei TT, Gao ZH, Zhao BL, Hou JW, Xu HB, et al. Different effects of the constituents of EGb 761 on apoptosis in rat cerebellar granule cells induced by hydroxyl radicals. Biochem Mol Biol Int 1999; 47:397–405.
52. Zhang Y, Yu Q, You S, Sheng L. Ginkgolies protects the cultured rat cortical cells against metabolic, excitotoxic and oxidative insults. J Health Sci 2004; 50:348–55.
53. Morioka T, Kalehua AN, Streit WJ. The microglial reaction in the rat dorsal hippocampus following transient forebrain. J Cereb Blood Flow Metab 1991; 11:966–73.
54. Korematsu K, Goto S, Nagahiro S, Ushio Y. Microglial response to transient focal cerebral ischemia: an immunochemical study on the rat cerebral cortex using anti-phosphotysine antibody. J Cereb Blood Flow Metab 1994; 14:825–30.
55. Saleem S, Zhuang H, Biswal S. Ginkgo biloba extract neuroprotective action is dependent on heme oxygenase 1 in ischemic reperfusion brain injury. Stroke 2008; 39:3389–96.
56. Butterfield DA, Castegna A, Pocernich CB, Drake J, Scapagnini G, Calabrese V. Nutritional approaches to combat oxidative stress in Alzeimer's disease. J Nutr Biochem 2002; 13:444–61.