

Study the preventive effect of aqueous extract of *Gingko biloba* on cerebral damage induced by ischemia/reperfusion in rat

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Abstract:

Background: cerebral damage may occur in a variety of clinical settings and this remains a significant problem. Therefore, it seems possible that the administration aqueous extract of *Gingko biloba* might protect the brain against the ischemia reperfusion injury.

Objectives: The study included knowledge Phytochemical Composition and determine whether *Gingko biloba* in the aqueous extract prevents or decreases I/R brain damage.

Methods: Thirty rats were divided into three groups as control, I/R group and *Gingko biloba* treatment group. Serum was used for the estimation of biochemical parameters: oxidative stress levels of nitric oxide (NO) and lipid peroxidation (LPO) and blood was used for the estimation of enzyme immunoassay: Interleukin-8 (IL-8), tumor necrosis factor Alpha (TNF- α), interleukin-1 β (IL-1 β).

Results: The results showed that the extract contain : glycosides , saponins ,tannins ,phenolic compounds , resins, flavonoids ,alkaloids and proteins.The levels of Oxidative stress in group 3 were significantly lower than those in group 2 as well as the levels of enzyme immunoassay in group 3 were significantly lower than those in group 2.

Conclusion: The present results suggest that *Gingko biloba* treatment protects the rat brain against ischemia-reperfusion brain damage.

Key words: *Gingko biloba*, preventive effect , ischemia/reperfusion , brain damage.

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Introduction:

Ischemia brain is a major cause of disability and death in the human being; it is the primary cause of long-term disability in adult and the third leading cause of death. Therefore, there is a big benefit in the basic mechanisms by which ischemia/reperfusion (IR) cause harm and in the prevention or decreases of the injury. Reactive oxygen species (ROS) generated from the respiratory chain in mitochondria, ischemia-activated xanthine/hypoxanthine oxidase and lipid fatty acid metabolism play an important role in the brain IR process [1]. Because of the high content of oxidative metabolic activity, high rate of polyunsaturated fatty acids, relatively low antioxidant capacity, low retrieve activity and non-replicating nature of the neuronal cells, the brain is very prone to the Injury caused by oxygen radicals [2]. The burst in production of ROS results in damage to lipids, cellular proteins and DNA. In the cerebral circulation system, the fracture in production of ROS reparation the endothelium cell and soft muscle cell, induces vascular permeability changes and blood platelet aggregation, and results in Injury [3].

Gingko biloba extract (GbE) is a kind of Chinese herb, consisting of 6 % terpene lactones (including ginkgolides A, B, C, J and bilobalide) ,70 % other substances (for example proanthocyanidins, organic acids, and sugars, etc)and 24 % flavonoid glycosides. GbE has general protective effects on CNS (central nerve system) and circulation system.

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GbE can remove reactive oxygen species ,decrease lipid peroxidation and facilitate the synthesis and/or let go of epoprostenol[4]. *Gingko biloba* extract is extensively used to get better blood flow in the treatment of vascular dementia, Alzheimer's disease, intermittent claudication, and other vascular disorders [5]. It is reported to have antioxidant ,neuroprotective activity, and antiplatelet [6-8]. *Gingko biloba* extract is used in herbal drug and possibly coadministered to patients using antiplatelet agents such as ticlopidine. Trumbeckaite et al. reported that GbE best the animals heart mitochondrial function [9]. Wu et al. reported that *Gingko biloba* extract enhanced coronary blood flow in healthy elderly adults[10]. Chen et al. reported the effects of *Gingko biloba* extract on cation currents in animal ventricular myocytes [11]. The typical extract of ginkgo sold in the mart is standardized to contain a minimum of 6% terpene lactones (TL), 24% flavone glycosides (FGL) and less than 5 ppm ginkgolic acids [12]. Some of the most salient health benefits attributed to this type of ginkgo extracts are for addressing medical conditions for example intermittent claudication (peripheral artery disease of legs), decreased mental vitality at old age, tinnitus, depression and Alzheimer's disease [13]. The pharmacological activity of the ginkgo extracts is linked to two major groups of phytochemicals, terpene lactones and the flavone glycosides (FGL) [14], as ginkgolic acids are the markers (undesired compounds) for safety reasons such as their cytotoxicity [15].

Materials and Methods:

Fifty gram from leaves were suspended in one liter of distilled water and left for 24 hrs at 35°C with continuous stirring in shaking incubator. Then the mixture was filtered by filter paper, the filtrate was centrifuged for 10 min. at 2500 rpm, and the extract evaporated to dryness at 40°C in the incubator[16,17]. The chemical components of the prepared aqueous extract were detected as shown in table .1. [18]. Thirty male Wistar rats weighting 180-230g were used in this study, Placed under standard conditions. The animals were deprived of food, but not water, for 24 h before surgery. Rats were divided into three groups, Group 1 (sham), Group 2 (I/R), and Group 3 (treated with GbE). All rats were anesthetized with 40-50 mg / kg of thiopental sodium. the extracted was given to the animals in group 3, before ischemia at a dose of 200 mg/kg by intraperitoneal route. We chose the dose of this agent according to reported studies about I/R and *Gingko biloba*. [19] Rats in the I/R group were infused only with saline. Surgical procedure [20]. Biochemical analyses: Oxidative stress indices: The left hemisphere was used to detect ROS according to a previously published method with minor modifications[21]. nitric oxide (NO) measured as total nitrite [22] and lipid peroxidation (LPO) as Thio Barbituric Acid Reactive Substances [23]. IL-8, TNF- α , IL-1 β levels were measured using commercial enzyme immunoassay kits(Endogen, Woburn, MA, USA).

Results

The results in Table.1 showed that the GbE gave positive (tests Iodine test gave Orange ppt, Molish test gave Violet ring, Benedict test gave blue ppt. for glycosides, Folin-Ciocalteu reagent gave blue color for proteins, Fast stirring gave Dense foam for long time, Mercuric Chloride gave White ppt. for saponins, Lead acetate%1 gave Preface yellow ppt. for tannins, (Ethanol + Boiling + D.w.) gave turbidity for resins, for phenolic compounds, Mayer's reagent gave white ppt., Wagner reagent gave Brown ppt., Picric acid gave yellow ppt. for alkaloids and aqueous%1 Ferric chloride gave Green ppt., Ethanol hydroxide alcohol gave yellow ppt. for flavonoids). similar results are also obtained by other studies [24].

Table 1. Phytochemical Composition of aqueous extract of *Gingko biloba*

Phytochemical	Indication in Plant
Glycoside	+++
Proteins	+++
Saponins	+
Phenolic compounds	+++
Tannins	++
Resins	+++
Flavonoids	++
Alkaloid	+++

Key: +++ = Presence of bioactive compound in very high concentration

++ = High concentration

+ = Presence of bioactive compound

- = Absence of bioactive compound

The level of oxidative marker NO: nitric oxide (Figure 1) and LPO: lipid peroxidation (Figure 2) were significantly ($p < 0.001$) higher in (group2) It compared to (group1) while its levels were significantly ($p < 0.001$) lower in *Gingko biloba* treated group (Group 3) compared to I/R group (Group 2).

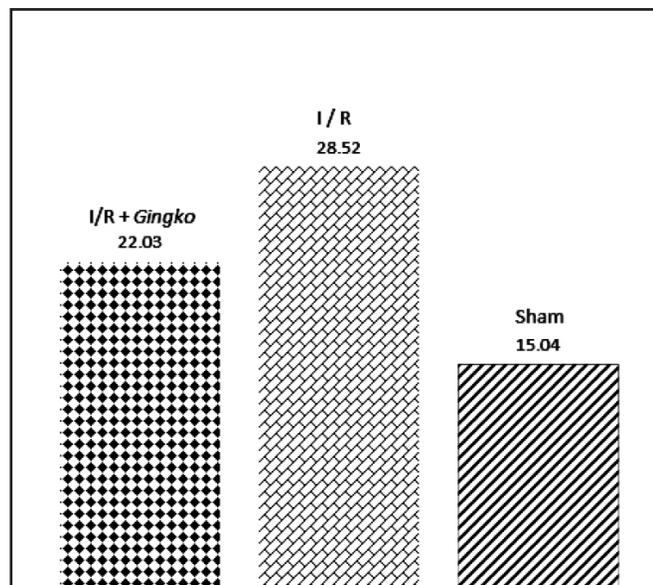


Figure 1. NO (µmol/ml) in sham, I/R and I/R + GbE (n=10, mean± SD)

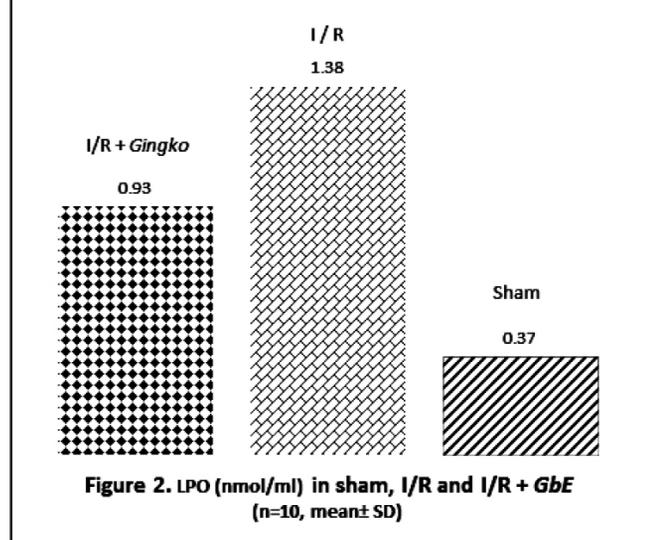


Figure 2. LPO (nmol/ml) in sham, I/R and I/R + GbE (n=10, mean± SD)

Results listed in (Table 2) showed that blood IL-8, TNF- α and IL-1 β levels in treated group (Group 3) were significantly lower ($p < 0.001$) than its levels in I/R group.

Table 2. Effect of *Gingko biloba* on IL-8, TNF- α , IL-1 β levels in rats.

parameter	sham	I/R	I/R + <i>Gingko biloba</i>	P
IL-8 (ng/mL) In blood	0.67 \pm 0.02	1.87 \pm 0.41	1.24 \pm 0.09	0.001
TNF- α (ng/mL) In blood	1.13 \pm 0.08	2.41 \pm 0.23	1.61 \pm 0.05	0.001
IL-1 β (ng/mL) In blood	0.45 \pm 0.07	1.02 \pm 0.04	0.67 \pm 0.08	0.001

interleukin-8 (IL-8), tumor necrosis factor Alpha (TNF- α), interleukin-1 β (IL-1 β)

Discussion:

The present results demonstrated that *Gingko biloba* could protect or decreases ischemic brain damage in a rat. Moreover, it also reduced cognitive deficits induced by ischemia. Brain functions need great amounts of energy in the form of a constant supply of glucose and oxygen. In dementia because of degeneration with neuronal loss and impaired neurotransmission, refuse of intellectual purpose is associated with disturbances in the supply of glucose and oxygen [25] Release of lipid peroxidation and free radicals possibly will occur in these state of affairs with hurtful consequences. Because brain cells contain a high percentage of unsaturated fatty acids in their membranes, they are extremely vulnerable to free radical damage. The oxidation of unsaturated fatty acids in membranes leads to a decrease in membrane fluidity and disruption of membrane role and structure [26]. This cellular damage possibly a main mechanism of age-related functional refuse.[27,28] The mind cell is also very vulnerable to hypoxia. Thus, diminished circulation to the brain sets off a chain reaction that disrupts membrane role and energy production and ultimately leads to cellular death. The diverse compounds found in ginkgo can play a protective role in different stages of the reject of intellectual function by means of several mechanisms of action: vasoregulating activity of arteries, capillaries, and veins (increased blood flow); platelet activating factor (PAF) antagonism; homeostasis of inflammation and Reactive oxygen species (stress); prevention of cell membrane injure caused by Reactive oxygen species(ROS); and neurotransmission modulation.[29,25]

Gingko biloba extract protects hippocampal neurons against cell death induced by beta-amyloid toxicity[30]. The EGb, also exerts a neuroprotective effect against permanent and transient focal cerebral ischemia[31]also, beneficial effects of EGb on different neurodegenerative disorder and peripheral arterial occlusive disease have been reported in clinical trials[30]. as well as, excessive free radical production caused by ischemic damage induces protein oxidation and DNA damage.[33]. in addition the antioxidant function of EGb, we suggest that EGb elicits neuroprotective effect by activation of a survival pathway. A previous study suggests the fact that EGb mediates the Akt signaling pathway[34]. In the present study, blood IL-8, TNF- α , IL-1 β levels were found to significantly increased compared to the sham group. However, treatment with *Gingko biloba* significantly decreased blood IL-8, TNF- α , IL-1 β _ levels in IR rats. This indicated that *Gingko biloba*

could reduce inflammation in ischemia brain tissue.

That study confirms that EGb administration significantly decreases the cerebral infarct volume following focal cerebral ischemia.

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