Carbohydrate Polymers 88 (2012) 112–117

Contents lists available at SciVerse ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

# Response surface optimized extraction of carbohydrate compound from *Folium Ginkgo* and its bioactivity

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ARTICLE INFO

*Article history:*
Received 28 October 2011
Received in revised form
17 November 2011
Accepted 22 November 2011
Available online 1 December 2011

*Keywords:*
RSM
Optimization
Extraction
*Folium Ginkgo*
Focal cerebral ischemia/reperfusion
Oxidative injury

ABSTRACT

This study aims to investigate the optimization of carbohydrate extraction from *Folium Ginkgo*. It was found that the optimum conditions for producing carbohydrate compound using response surface methodology (RSM) included a water/solid of 4 and an extraction temperature of 95 °C for 120 min. Fraction-I was a heteropolysaccharide and consisted of L-rhamnose, L-arabinose, D-mannose, D-glucose, and D-galactose, with the molar ratio of 1:1.7:2.2:1.8:7.0. Fraction-II also was a heteropolysaccharide and consisted of L-rhamnose, D-mannose, D-glucose and D-galactose, with the molar ratio of 1.8:2.1:1.4:4.3. Then, protective effect of carbohydrate extraction from *Folium Ginkgo* on oxidative injury in focal cerebral ischemia–reperfusion animals was evaluated in a cerebral ischemia model. Experimental evidence has shown that carbohydrate extraction from *Folium Ginkgo* have a protective effect against ischemic brain injury.



## 1. Introduction

*Ginkgo biloba* (*Folium Ginkgo*) is among the most sold medicinal plants of this world. Fossils of plants quite similar to *G. biloba* date back to 180 million years ago and thus it has been called a living fossil (Del Tredici, 1991, 2000; Melzheimer & Lichius, 2000). Most of the sales concern special extracts from the leaves which have been standardised for their content of terpene trilactones and flavonol glycosides. The extracts are mainly used for the improvement of the blood circulation, both peripherally and centrally (DeFeudis, 1998). In spite of unfounded claims, Ginkgo's introduction in Traditional Chinese Medicine is relatively recent. It does not occur in the oldest Chinese Herbal, Shen Nong Ben Cao Jing (2800 BC) (Del Tredici, 1991) and the use of seeds against cough, asthma, enuresis, alcohol misuse, pyogenic skin infections and warm infections in the intestinal tract is first mentioned in the great herbal Pen Ts'ao Kang Mu of 1596 by Li Shih-chen (Li, 1956). The prescription of leaf extracts for the improvement of the blood circulation, both peripherally and centrally, started in the 1960s in Germany (Drieu & Jaggy, 2000). Currently the medical indications are intermittent claudication, decreased mental vitality at old age (forgetfulness, early dementia, concentration problems) and tinnitus.

Recent reports have implied that oxygen free radicals are mediators of tissue injuries in an ischemia–reperfusion sequence (Maupoil & Rodnette, 1988). The principal sources of the free radicals and the mechanisms by which these oxygen species damage brain tissue have not been identified (Siesjö, 1992). Excessive production of reactive oxygen species such as superoxide anion, hydroxyl radical, and hydrogen peroxide, and nitric oxide (NO) has been shown to play a critical role in the development of ischemia–reperfusion injury (Dimagl et al., 1995; Tominaga, Sato, Ohnishi, & Ohnishi, 1993). Those reactive oxygen species may induce cerebral damage either directly through interacting and destroying cellular proteins, lipids and DNA, or indirectly by affecting normal cellular signaling pathways and gene regulation (Traystman, Kirsch, & Koehler, 1991). Reactive oxygen species can be scavenged by endogenous antioxidants, including superoxide dismutase (SOD) that catalyzes the dismutation of the superoxide anion, glutathione peroxidase (GSH-PX) and catalase (CAT) that mediate the breakdown of hydrogen peroxide (Ginsberg, 1995; Hall, 1994).

*Abbreviations:* RSM, response surface methodology; NO, nitric oxide; MDA, malondialdehyde; SOD, superoxide dismutase; GSH-PX, glutathione peroxidase; CAT, catalase.

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doi:10.1016/j.carbpol.2011.11.074

**Table 1**
Coded and actual levels of independent variables for experimental design.

| Variable | Coded level of variables | | | | |
|---|---|---|---|---|---|
| | −1.68 | −1 | 0 | 1 | 1.68 |
| Ratio of water to solid ($X_1$) | 3 | 4 | 5 | 6 | 7 |
| Extraction time (min) ($X_2$) | 67 | 80 | 100 | 120 | 133 |
| Extraction temperature (°C) ($X_3$) | 79 | 83 | 90 | 97 | 100 |

This study was conducted to optimize the extraction process of carbohydrate extraction from *Folium Ginkgo* using response surface methodology (RSM) by evaluating the effects of ratio of water to solid, extraction time and extraction temperature as independent variables. Then, protective effect of carbohydrate extraction from *Folium Ginkgo* again oxidative injury in cerebral IR animals was studied.

## 2. Materials and methods

### 2.1. Materials

*Folium Ginkgo* was collected by hand at the end of July 2010. The *Folium Ginkgo* was then sun-dried, and stored under refrigeration.

### 2.2. Extraction process of carbohydrate compound from Folium Ginkgo

Carbohydrate compound was extracted by immersing 0.5 g dry *Folium Ginkgo* powders (60 mesh) in water at a selected ratio, then heating in water bath at selected temperature for various periods of time. The supernatant was collected for the determination of polysaccharides yield.

### 2.3. Experimental design for aqueous extraction

The process conditions for carbohydrate compound of *Folium Ginkgo* were optimized by central composite design. Response surface methodology (RSM) was used to optimize the extraction process to yield the highest amount of carbohydrate compound of *Folium Ginkgo* by controlling the process variables: ratio of water to solid (v/v), extraction time (min) and extraction temperature (°C).

A multiple regression analysis was done to obtain the coefficients and the equation could be used to predict the response. The degree of experiments chosen for this study was Box-Behnken, a fractional factorial design for three independent variables. It was applicable once the critical variables had been identified. A five-level three-factor factorial design was adopted to optimize the extraction conditions, as shown in Table 1. In the model given in Eq. (1), interactions higher than first order had been neglected. A total of 20 experiments were necessary to estimate of the full model.

$$R = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{33} X_3^2 \quad (1)$$

where $R$ was the estimate response, $\beta_0$ was the constant, $\beta_1$, $\beta_2$ and $\beta_3$ were linear coefficients, $\beta_{12}$, $\beta_{13}$ and $\beta_{23}$ were interaction coefficients between the three factors, $\beta_{11}$, $\beta_{22}$ and $\beta_{33}$ were quadratic coefficients.

### 2.4. Purification of carbohydrate compound from Folium Ginkgo

The resulting polysaccharides (50–60 rag) were applied onto a Sephacryl S-500 column (1.5 cm × 40 cm) and eluted with 0.1 M NaCl; the elution was monitored by the reaction with phenol in concentrated sulfuric acid. In each case, the elution curves contained a single peak, which had an elution volume of 40–45 mL (the free volume of the column was 25 mL).

### 2.5. Analysis of monosaccharides

Twenty milligrams of FGC-I and FGC-II was respectively hydrolyzed with 2 mol $L^{-1}$ $H_2SO_4$ (5 mL) for 8 h at 110 °C in a sealed glass tube. After removal of the residual acid with $BaCO_3$, the hydrolysates were analyzed by the HPLC/ELSD system. The chromatograph was fitted with an Agilent ZORBAX carbohydrate analysis column (Agilent, California, US), eluted with acetonitrile–water, 3:1 (v/v) at flow rate of 1.0 mL $min^{-1}$. Results were compared with the following monosaccharide standards: D-glucose, L-rhamnose, D-xylose, D-galactose, D-mannose and L-arabinose (Sigma).

### 2.6. Pharmacological experiment

#### 2.6.1. Animals

All experimental animal procedures were conducted with the approval of the Ethics Committee of HeBei Medical University of China. Male Wister rats (body weight 265–330 g) were housed under conditions of controlled temperature (22 ± 2 °C) and constant humidity, with 12 h light/dark cycle (light on 07:00–19:00). Food and water were available ad libitum.

#### 2.6.2. Experimental protocol

Four groups (model, three medicine-treatment groups), each of 12 animals, received a dietary intervention (10–11.00 h daily) for 30 days. A model control received gastric gavage with daily distilled water. The other three groups received 200, 400 and 600 mg/kg/day carbohydrate extraction from *Folium Ginkgo* for 30 days; Two hours after the last dose, rats were anesthetized with chloral hydrate (400 mg/kg i.p.). Under an operation microscope, right common carotid artery (CCA) and external carotid artery (ECA) were exposed. The internal carotid artery (ICA) was also dissected to the level of petrygopalatine artery. Afterwards, a silk thread was placed loosely around the ECA stump, CCA and ECA were occluded permanently and ICA temporarily using a microvascular clip. Then a small incision was made on ECA, and a nylon thread (3–0) was inserted through. While holding the thread around ECA tightly to prevent bleeding, the microvascular clip on ICA was removed, and the nylon thread was carefully and slowly pushed forward through ICA until a light resistance was felt. Such resistance was indication that tip of nylon thread was wedged at the beginning of anterior cerebral artery (20–22 mm from CCA bifurcation), resulting in occlusion of the middle cerebral artery (Vakili, Nekoeian, & Dehghani, 2006). At 1 h after induction ischemia, the filament was slowly removed. Animals were then recovered from anesthesia, and kept in single cages for 24 h. In addition, another sham control group (12 animals) was managed according to the model control protocol, but underwent surgery without induction ischemia. Subsequently, animals sacrificed, and their brains were removed for determination of infarct volumes. Rectal temperature was measured by a thermometer and maintained at 37 ± 0.5 °C throughout the experiment using an electrical blanket.

#### 2.6.3. Infarct volume analysis

Twenty-four hours after cerebral infarction, the animals were anesthetized with chloral hydrate and sacrificed by rapid decapitation and the brain was removed. After inspection of the anatomy of the surgery, the brain was immersed in cold saline solution for 10 min, and sectioned into standard coronal slices (each 2-mm thick) using a brain matrix slicer (Jacobowitz Systems, Zivic-Miller Laboratories Inc., Allison Park, PA, USA). Slices were placed in vital dye 2,3,5-triphenyltetrazolium chloride (2% TTC, Sigma Co.,

St. Louis, MO, USA) at 37 °C in the dark for 30 min. Staining was followed by fixation with 10% formalin at room temperature overnight. The outline of the right and left cerebral hemispheres as well as that of the infarct tissue, were clearly visible by the lack of TTC staining (Chen, Hsu, Hogan, Maricq, & Balentine, 1986), and was outlined on the posterior surface of each slice using an image analyzer (Color image scanner, Epson GT-9000) connected to an image analysis system (AIS software, Imaging Research Inc., Ontario, Canada) and run on a personal computer (AMD K6-2 3D 400). Whole brain infarct volume was calculated as the sum of the infarct areas per slice multiplied by the slice thickness. Both the surgeon and the image analyst were blinded to the treatment.

*2.6.4. Measurement of CAT, SOD, GPx activities and MDA level in brain homogenates*

Brain tissues were thawed, weighed and homogenized with Tris–HCl (5 mmol/L containing 2 mmol/L EDTA, pH 7.4). Homogenates were centrifuged (1000 × *g*, 15 min, 4 °C) and the supernatant was used immediately for the assays of CAT, SOD, GSH-Px activities and NO, MDA levels in brain homogenates following the commercial kits instructions.

*2.6.5. Single-labeling immunohistochemistry*

Histological assessment for active caspase-3 following focal ischemia was performed on fresh frozen brain slices. In brief, sections were incubated with A92790K neo-epitope polyclonal rabbit active caspase-3 antibody (Simpson et al., 2001) in phosphate-buffered saline (PBS) containing 0.3% Triton X-100 for 24 h at 4 °C in a humidified chamber. After washing, the tissue was incubated with Cy3-conjugated donkey anti-rabbit IgG (1:1000; Jackson Immunoresearch, West Grove, PA, USA) for 3 h at room temperature, and then mounted with Vectashield mounting medium for fluorescence (Vector Laboratories, Burlingame, CA, USA). To confirm the specificity of A92790K, a monoclonal antibody that recognizes the active form of caspase-3 (1:1000; Pharmingen Canada, Mississauga, ON, Canada) was used on serial brain sections in the same manner. A rabbit polyclonal antibody E82684K or ΔC-APP (a C-terminal cleavage product of APP generated by caspase-3) (1:5000; Merck Frosst, Kirkland, QC, Canada), was used to further solidify the activity of caspase-3 in tissue sections (Gervais et al., 1999). For all experiments, the contralateral side of the brain was used as an intrinsic negative control since it is unaffected by ischemia during the surgery. Immunohistochemistry was also performed without incubation with the primary antibody as an experimental negative control. This allowed us to determine the experimental signal to noise ratio.

*2.6.6. Statistical analysis*

The results are expressed as means ± S.E.M. Data were analyzed using unpaired Student's *t*-test. *P* values of <0.05 were considered to be statistically significant.

## 3. Result and discussion

### 3.1. Extraction optimization and chemical analysis

The responses obtained from the experimental design set (Table 2) were subjected to multiple nonlinear regression using the software Design-Expert 7.1.3 Trial (State-Ease, Inc., Minneapolis, MN, USA), to obtain the coefficients of the quadratic polynomial model. The quality of the fitted model was expressed by the coefficient of determination $R^2$, and its statistical significance was checked by an *F*-test.

Experimental results of the 3-factors, 5-level central composite design are presented in Table 1. All the coefficients of linear ($X_1$, $X_2$, $X_3$), quadratic ($X_{11}$, $X_{22}$, $X_{33}$) and interaction were calculated for

**Table 2**
The central composite experimental design and experiment data for hot water extraction of *Folium Ginkgo*.

| Run | $X_1$ | $X_2$ | $X_3$ | Extraction yield (%) |
|---|---|---|---|---|
| 1 | −1.00 | −1.00 | −1.00 | 3.29 |
| 2 | 1.00 | −1.00 | −1.00 | 3.52 |
| 3 | −1.00 | 1.00 | −1.00 | 3.26 |
| 4 | 1.00 | 1.00 | −1.00 | 3.52 |
| 5 | −1.00 | −1.00 | 1.00 | 3.31 |
| 6 | 1.00 | −1.00 | 1.00 | 3.68 |
| 7 | −1.00 | 1.00 | 1.00 | 3.21 |
| 8 | 1.00 | 1.00 | 1.00 | 3.54 |
| 9 | −1.68 | 0.00 | 0.00 | 3.12 |
| 10 | 1.68 | 0.00 | 0.00 | 3.61 |
| 11 | 0.00 | −1.68 | 0.00 | 3.44 |
| 12 | 0.00 | 1.68 | 0.00 | 3.48 |
| 13 | 0.00 | 0.00 | −1.68 | 3.41 |
| 14 | 0.00 | 0.00 | 1.68 | 3.5 |
| 15 | 0.00 | 0.00 | 0.00 | 3.76 |
| 16 | 0.00 | 0.00 | 0.00 | 3.83 |
| 17 | 0.00 | 0.00 | 0.00 | 3.8 |
| 18 | 0.00 | 0.00 | 0.00 | 3.79 |
| 19 | 0.00 | 0.00 | 0.00 | 3.79 |
| 20 | 0.00 | 0.00 | 0.00 | 3.81 |
| Std. Dev. | 0.033 | *R*-squared | | 0.9883 |
| Mean | 3.53 | Adj *R*-squared | | 0.9777 |
| C.V. % | 0.94 | Pred *R*-squared | | 0.9279 |
| PRESS | 0.067 | Adeq precision | | 28.735 |

significant differences using *t*-statistic test and the estimated coefficients of each model are presented in Table 1. To develop the fitted response surface model equations, all insignificant terms ($P \geq 0.05$) were eliminated and the fitted models are shown in Table 1. The values of $R^2$ suggested that the quadratic models could explain most of variabilities in the observed data. Thus, the analysis of variance showed that predicted response surface models were statistically significant ($P < 0.01$).

In addition, when one factor was fixed as the optimal value calculated from above CCRD experiment, effects of another both factors on the extraction yield were shown by the contour optimizer plots. The effects of ratio of water to solid ($X_1$) and extraction time ($X_2$) on the extraction yield ($R$) are reflected in Fig. 1A. With the increase of $X_1$ and $X_2$, $R$ sharply mounted up, and then achieved saturated value when the extraction was conducted for 12 min, and then did not rise any more. The relationship between ratio of water to solid ($X_1$) and extraction temperature ($X_3$) is illustrated in Fig. 1B. The contour plot indicated that increases of $X_1$ and $X_3$ benefit to the extraction yield. However, the changes of $X_2$ had more significant effects on $R$ than those of $X_3$. As $X_3$ increased from 4/1 to 6/1, it could hardly make any promotion on $R$. As for $X_2$, $Y$ went up corresponsive with the increase of $X_2$, and reached the highest level. Similarly, the effects of extraction time ($X_2$) and extraction temperature ($X_3$) on extraction yield are reflected in Fig. 1C. $X_3$ was not as significant as $X_2$ in response for $Y$. Therefore, it could be concluded that the extraction time and extraction temperature played prominent roles in getting high extraction efficiency.

The crude carbohydrate compound was separated and sequentially purified through a Hiload 26/60 Superdex-200 column, giving two big overlapping elution peaks: FGC-I and FGC-II (eluted with water) (Fig. 2), as detected by the phenol–sulfuric acid assay. FGC-I and FGC-II were collected for further identification of structure and monosaccharide compositions.

The monosaccharide compositions of the two fractions were analyzed by HPLC/ELSD. Compared with the monosaccharide standards, FGC-I was a heteropolysaccharide and consisted of L-rhamnose, L-arabinose, D-mannose, D-glucose, and D-galactose, with the molar ratio of 1:1.7:2.2:1.8:7.0. FGC-II was also a heteropolysaccharide and consisted of L-rhamnose, D-mannose, D-glucose and D-galactose, with the molar ratio of 1.8:2.1:1.4:4.3.

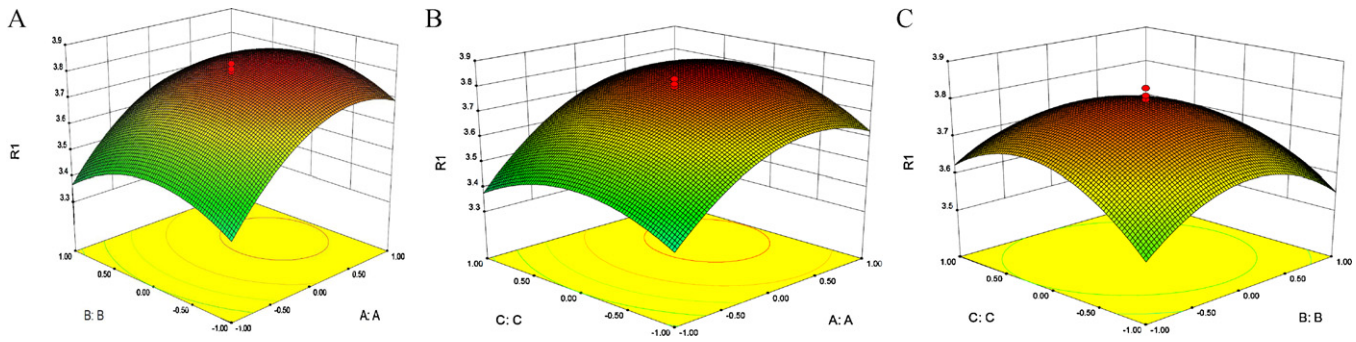


**Fig. 1.** Response surfaces showing the effect of ratio of water to solid ($X_1$, A) and extraction time ($X_2$, B) (A); the effect of ratio of water to solid ($X_1$, A) and extraction temperature ($X_3$, C) (B); the effect of extraction time ($X_2$, B) and extraction temperature ($X_3$, C) (C).

### 3.2. *Effect of carbohydrate extraction from Folium Ginkgo on oxidative injury of cerebral IR animals*

Excessive $O_2^-$ generation after ischemia–reperfusion was associated with early inflammation, oxidative stress, and endothelial activation in the brain and plasma, which might enhance the ischemia–reperfusion injury. Furthermore, it is indicated that the $O_2^-$ values including $\Delta$ and $Q$, might be predictive indicators of early inflammation, oxidative stress, and endothelial activation in the cerebral ischemia–reperfusion pathophysiology. Similar to previous studies, this study found an increase in MDA levels in cerebral I/R (Ozerol et al., 2009; Shi et al., 2011). On the basis of the results obtained from the present study, effect of carbohydrate extraction from *Folium Ginkgo* on brain tissue MDA level in animals was evaluated. The animals were treated with carbohydrate compound at three doses (200, 400, 600 mg/kg body weight) for 30 days. The MDA level for experimental animals is depicted in Fig. 3. The MDA level in brain tissue of model control animals was significantly increased compared to sham control animals. By contrast, the brain tissue MDA levels of animals treated with carbohydrate extraction from *Folium Ginkgo* (200, 400, 600 mg/kg body weight) dose-dependently significantly was reduced compared to the model control ones (Fig. 3).

On the basis of the results obtained from the present study, effect of carbohydrate extraction from *Folium Ginkgo* on brain tissue SOD, CAT and GSH-Px activities in animals was evaluated. The animals were treated with carbohydrate compound at three doses (200, 400, 600 mg/kg body weight) for 30 days. The SOD, CAT and GSH-Px activities for experimental animals are depicted in Figs. 4 and 5. The SOD, CAT and GSH-Px activities in brain tissue of model control animals were significantly reduced compared to sham control animals. By contrast, the brain tissue MDA levels of animals treated with carbohydrate extraction from *Folium Ginkgo* (200, 400, 600 mg/kg body weight) dose-dependently significantly were increased compared to the model control ones (Figs. 4 and 5).

TOS measurements provide a sensitive, novel index of oxidative stress (Alp, Selek, Alp, Taskin, & Kocyigit, 2010). It is well known that oxidative metabolism is essential for the survival of

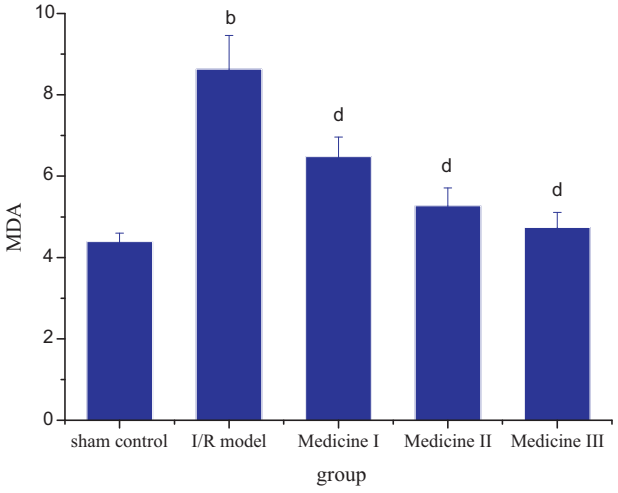


**Fig. 3.** Malondialdehyde (MDA) content in brain tissue of vehicle (distilled water) and carbohydrate compound-treated IR animals.

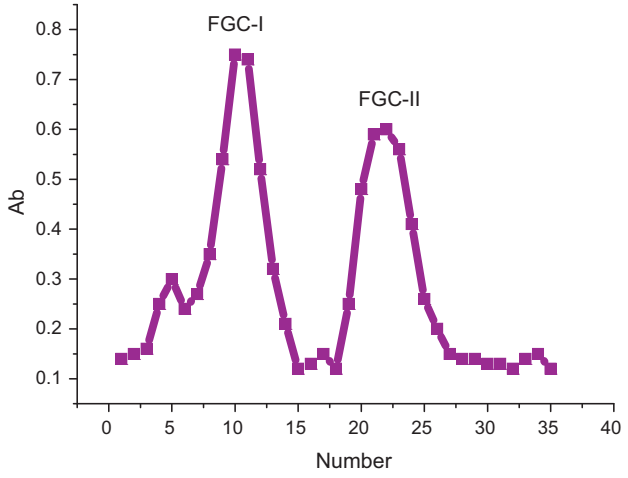


**Fig. 2.** Separation and purification of carbohydrate compound from *Folium Ginkgo*.

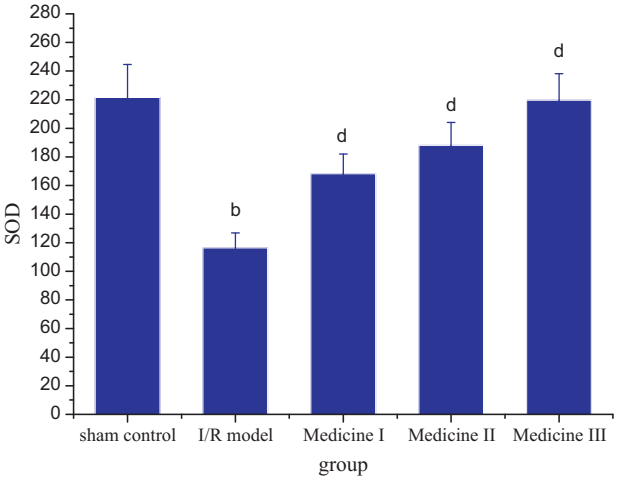


**Fig. 4.** SOD activity in brain tissue of vehicle (distilled water) and carbohydrate compound-treated IR animals.

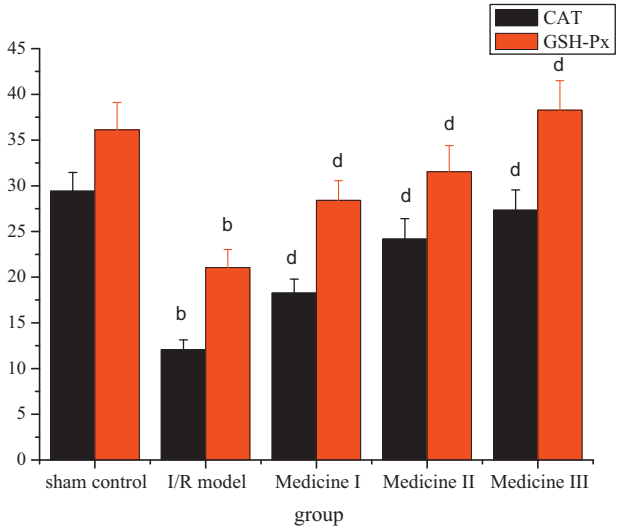


**Fig. 5.** CAT and GSH-Px activities in brain tissue of vehicle (distilled water) and carbohydrate compound-treated IR animals.

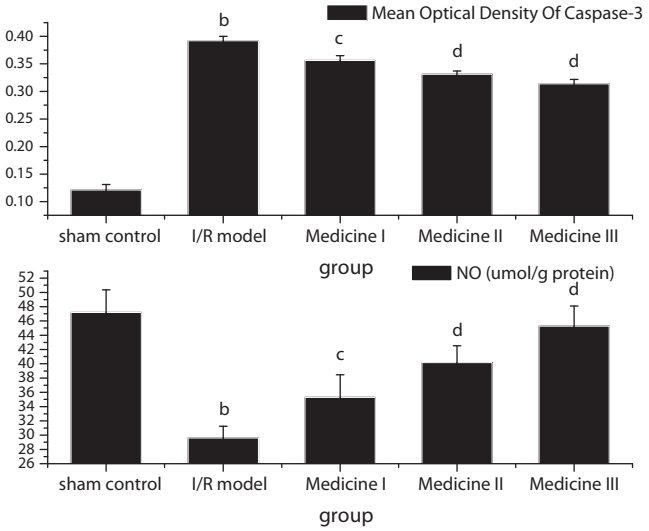


**Fig. 6.** Caspase-3 and NO content in brain tissue of vehicle (distilled water) and carbohydrate compound-treated IR animals.

brain, but is also associated with the generation of reactive oxygen species. Normally there is a balance between the reactive oxygen species generation and the endogenous antioxidant systems, involving the cooperative action of SOD, catalase, GSH-Px and GSH. SOD, the primary line of defense against tissue damage caused by reactive oxygen species, catalyzes the dismutation of superoxide anion to hydrogen peroxide and prevents the formation of the hydroxyl radical. The overproduction of ROS can be detoxified by enzymatic and non-enzymatic endogenous antioxidants – such as glutathione, bilirubin, uric acid, SOD, catalase, and glutathione peroxidase – causing their cellular stores to be depleted (Maes, Galecki, Chang, & Berk, 2011). Decreased TAC and SOD activity in the rats with I/R may be secondary to depleted antioxidant stores against increased oxidative stress. Because of increased oxidants and decreased antioxidants, the oxidant–antioxidant balance demonstrated an increase in oxidative status in rats with I/R. Catalase and GSH-Px are generally regarded as the second line of defense by dismutating peroxide into water and molecular oxygen (Li, Jiang, Zhang, Mu, & Liu, 2008). GSH, an endogenous antioxidant found in all animals, can directly react with reactive oxygen species or act as a cofactor with the enzyme glutathione peroxidase to detoxify hydrogen peroxide and lipid peroxides in tissues (Jagetia, Rajanikant, Rao, & Baliga, 2003). During ischemia/reperfusion, sudden bursts of reactive oxygen species cannot be handled by the endogenous antioxidant systems and the accumulation of reactive oxygen species leads to cellular membrane, protein and DNA oxidative damage (Thiyagarajan & Sharma, 2004; Zheng, Liu, Wang, & Xu, 2007).

The carbohydrate compound-mediated improvements in oxidative stress and inflammatory responses were further defined by evaluating brain tissue NO and caspase-3. Brain tissue NO level was significantly lower (Fig. 6) in model control animals, whereas brain tissue caspase-3 was significantly higher than those in sham control ones, supporting that brain are vulnerable to ROS-mediated insults. Carbohydrate compound at three levels (200, 400 and 600 mg/kg b.w.) normalized the NO and caspase-3 to those of sham animals.

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 (Khan, Elango, Ansari, Singh, & Singh, 2007; Whaley-Connell et al., 2009). This study shows that cerebral I/R leads to significantly lower NO levels in the brain. In contrast, carbohydrate extraction from *Folium Ginkgo* pretreatments prevented decreasing of NO levels in cerebral I/R. Caspase-3 was one key factors in brain ischemia. While caspase-3 is an affirmative deteriorative factor in brain ischemia. It activates the apoptosis program which finally results in DNA fragmentation and cell loss (Manabat et al., 2003). Furthermore, the competition between BDNF and caspase-3 can determine the fate of cell undergoing ischemia injury. It was reported that the reduction of infarct size and brain edema by BDNF was greatly associated with the reduction of DNA fragmentation and apoptotic signals predominantly in the caspase-3 cascades (Kitagawa et al., 1998). A recent study showed the expression of caspase-3 is blocked when increasing the expression of BDNF (Han et al., 2000).

A number of herbs have demonstrated the protective effect of improved neurological outcome and suppressed infarct size following cerebral ischemic injury. Oral grape polyphenol extract intake may confer protection against I/R injury and emphasize that early intervention may be an effective therapeutic measure for ameliorating brain injury in stroke (Wang et al., 2009). Yan et al. (2004) reported that radix puerariae extract ameliorated the abnormal symptoms caused by cerebral ischemia reperfusion, which may throw new lights on the treatment of poststroke depression. Liang, Liu, Wang, Song, and Ji (2011) reported that leonurus heterophyllus extract may be used for treatment of ischemic stroke as a

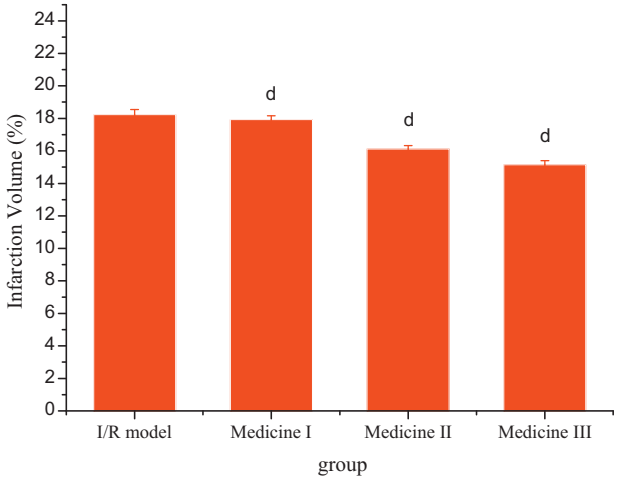


**Fig. 7.** Infarct volume in brain tissue of vehicle (distilled water) and carbohydrate compound-treated IR animals.

neuroprotective agent. Mukherjee, Nazeer Ahamed, Kumar, Mukherjee, and Houghton (2007) reported that the biflavone rich fraction from *Araucaria bidwillii* was found to protect rat brain against I/R induced oxidative stress, and attributable to its antioxidant properties.

In the present study, the brain tissue infarction volume was used to determine the protective effect of carbohydrate extraction from *Folium Ginkgo*. Different doses of carbohydrate compound significantly decreased the content of brain tissue infarction volume in a dose-dependent manner compared with that of untreated model control group (Fig. 7).

In conclusion, carbohydrate compound can reduce oxidative injury and cause reduction of infarct volume following cerebral artery ischemia and reperfusion injury. In this respect carbohydrate extraction from *Folium Ginkgo* might be the choice of therapeutic agent for patients with focal cerebral ischemia.

## References


Alp, R., Selek, S., Alp, S. I., Taskin, A., & Kocyigit, A. (2010). Oxidative and antioxidative balance in patients of migraine. *European Review for Medical and Pharmacological Sciences*, *14*, 877–882.

Chen, S. T., Hsu, C. Y., Hogan, E. L., Maricq, H., & Balentine, J. D. (1986). A model of focal ischemic stroke in the rat: Reproducible extensive cortical infarction. *Stroke*, *17*, 738–743.

DeFeudis, F. V. (1998). *Ginkgo biloba extract (EGb 761). From chemistry to the clinic*. Wiesbaden: Ullstein Medical.

Del Tredici, P. (1991). *Arnoldia-Boston*, *51*, 2.

Del Tredici, P. (2000). In T. A. van Beek (Ed.), *Ginkgo biloba* (p. 7). Amsterdam: Harwood.

Dimagl, U., Lindauer, U., Them, A., Schreiber, S., Pfister, H., Koedel, U., et al. (1995). Global cerebral ischemia in the rat: Online monitoring of oxygen free radical production using chemiluminescence in vivo. *Journal of Cerebral Blood Flow & Metabolism*, *15*, 925–940.

Drieu, K., & Jaggy, H. (2000). In T. A. van Beek (Ed.), *Ginkgo biloba* (p. 265). Amsterdam: Harwood.

Gervais, F. G., Xu, D., Robertson, G. S., Vaillancourt, J. P., Zhu, Y., Huang, J., et al. (1999). Involvement of caspases in proteolytic cleavage of Alzheimer's amyloid-beta precursor protein and amyloidogenic A beta peptide formation. *Cell*, *97*, 395–406.

Ginsberg, M. D. (1995). Neuroprotection in brain ischemia—An update. Parts 1 and 2. *Neuroscientist*, *1*, 95–103, 164–175.

Hall, E. D. (1994). Free radicals in central nervous system injury. In C. A. Rice-Evans, & R. H. Burdon (Eds.), *Free radical damage and its control* (pp. 217–238). Amsterdam: Elsevier.

Han, B. H., D'Costa, A., Back, S. A., Parsadanian, M., Patel, S., Shah, A. R., et al. (2000). BDNF blocks caspase-3 activation in neonatal hypoxia–ischemia. *Neurobiology of Disease*, *7*, 38–53.

Jagetia, G. C., Rajanikant, G. K., Rao, S. K., & Baliga, M. S. (2003). Alteration in the glutathione, glutathione peroxidase, superoxide dismutase and lipid peroxidation by ascorbic acid in the skin of mice exposed to fractionated γ radiation. *Clinica Chimica Acta*, *332*, 111–121.

Khan, M., Elango, C., Ansari, M. A., Singh, I., & Singh, A. K. (2007). Caffeic acid phenethyl ester reduces neurovascular inflammation and protects rat brain following transient focal cerebral ischemia. *Journal of Neurochemistry*, *102*, 365–377.

Kitagawa, H., Hayashi, T., Mitsumoto, Y., Koga, N., Itoyama, Y., & Abe, K. (1998). Reduction of ischemic brain injury by topical application of glial cell line-derived neurotrophic factor after permanent middle cerebral artery occlusion in rats. *Stroke*, *29*, 1417–1422.

Li, H.-L. (1956). *Morris Arboretum Bulletin*, *7*, 3.

Li, Y. H., Jiang, B., Zhang, T., Mu, W. M., & Liu, J. (2008). Antioxidant and free radical-scavenging activities of chickpea protein hydrolysate (CPH). *Food Chemistry*, *106*, 444–450.

Liang, H., Liu, P., Wang, Y. S., Song, S. L., & Ji, A. G. (2011). Protective effects of alkaloid extract from Leonurus heterophyllus on cerebral ischemia reperfusion injury by middle cerebral ischemic injury (MCAO) in rats. *Phytomedicine*, *18*, 811–818.

Maes, M., Galecki, P., Chang, Y. S., & Berk, M. (2011). A review on the oxidative and nitrosative stress (O&NS) pathways in major depression and their possible contribution to the (neuro) degenerative processes in that illness. *Progress in Neuro-Psychopharmacology & Biological Psychiatry*, *35*, 676–692.

Manabat, C., Han, B. H., Wendland, M., Derugin, N., Fox, C. K., Choi, J., et al. (2003). Reperfusion differentially induces caspase-3 activation in ischemic core and penumbra after stroke in immature brain. *Stroke*, *34*, 207–213.

Maupoil, V., & Rodnette, L. (1988). Evaluation of free radical and lipid peroxide formation during global ischemia and reperfusion in isolated rat heart. *Cardiovascular Drugs and Therapy*, *2*, 615–621.

Melzheimer, V., & Lichius, J. J. (2000). T. A. van Beek (Ed.), *Ginkgo biloba* (p. 25). Amsterdam: Harwood.

Mukherjee, P. K., Nazeer Ahamed, K. F. H., Kumar, V., Mukherjee, K., & Houghton, P. J. (2007). Protective effect of biflavones from Araucaria bidwillii Hook in rat cerebral ischemia/reperfusion induced oxidative stress. *Behavioural Brain Research*, *178*, 221–228.

Ozerol, E., Bilgic, S., Iraz, M., Cigli, A., Ilhan, A., & Akyol, O. (2009). The protective effect of erdosteine on short-term global brain ischemia/reperfusion injury in rats. *Progress in Neuro-Psychopharmacology & Biological Psychiatry*, *33*, 20–24.

Shi, L. L., Chen, B. N., Gao, M., Zhang, H. A., Li, Y. J., Wang, L., et al. (2011). The characteristics of therapeutic effect of pinocembrin in transient global brain ischemia/reperfusion rats. *Life Science*, *88*, 521–528.

Siesjö, B. K. (1992). Pathophysiology and treatment of focal cerebral ischemia. Part II. Mechanism of damage and treatment. *Journal of Neurosurgery*, *77*, 337–354.

Simpson, M. T. W., MacLaurin, J. G., Xu, D. G., Ferguson, K. L., Vanderluit, J. L., Davoli, M. A., et al. (2001). Caspase 3 deficiency rescues peripheral nervous system defect in retinoblastoma nullizygous mice. *The Journal of Neuroscience*, *21*, 7089–7098.

Thiyagarajan, M., & Sharma, S. S. (2004). Neuroprotective effect of curcumin in middle cerebral artery occlusion induced focal cerebral ischemia in rats. *Life Science*, *74*, 969–985.

Tominaga, T., Sato, S., Ohnishi, T., & Ohnishi, S. (1993). Potentiation of nitric oxide formation following bilateral carotid artery occlusion and focal cerebral ischemia in the rat: In vivo detection of the nitric oxide radical by electron paramagnetic spin trapping. *Brain Research*, *614*, 342–346.

Traystman, R. J., Kirsch, J. R., & Koehler, R. C. (1991). Oxygen radical mechanisms of brain injury following ischemia and reperfusion. *Journal of Applied Physiology*, *71*, 1185–1195.

Vakili, A., Nekoeian, A. A., & Dehghani, G. A. (2006). Aminoguanidine reduces infarct volume and improve neurological dysfunction in rat model focal cerebral ischemia. *DARU Journal of Pharmaceutical Sciences*, *14*, 31–36.

Wang, Q., Sun, A. Y., Simonyi, A., Miller, D. K., Smith, R. E., Luchtefeld, R. G., et al. (2009). Oral administration of grape polyphenol extract ameliorates cerebral ischemia/reperfusion-induced neuronal damage and behavioral deficits in gerbils: Comparison of pre- and post-ischemic administration. *The Journal of Nutritional Biochemistry*, *20*, 369–377.

Whaley-Connell, A., Habibi, J., Johnson, M., Tilmon, R., Rehmer, N., Rehmer, J., et al. (2009). Nebivolol reduces proteinuria and renal NADPH oxidase-generated reactive oxygen species in the transgenic Ren2 rat. *American Journal of Nephrology*, *30*, 354–360.

Yan, B., Wang, D.-Y., Xing, D.-M., Ding, Y., Wang, R.-F., Lei, F., et al. (2004). The antidepressant effect of ethanol extract of radix puerariae in mice exposed to cerebral ischemia reperfusion. *Pharmacology Biochemistry and Behavior*, *78*, 319–325.

Zheng, Y. Q., Liu, J. X., Wang, J. N., & Xu, L. (2007). Effects of crocin on reperfusion-induced oxidative/nitrative injury to cerebral microvessels after global cerebral ischemia. *Brain Research*, *1138*, 86–94.