Biomedicine & Pharmacotherapy 80 (2016) 87–94

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Study on cerebroprotective actions of *Clerodendron glandulosum*leaves extract against long term bilateral common carotid artery occlusion in rats

Saritha Surapaneni[a], T. Prakash[a,*], MdAsif Ansari[a], PM. Manjunath[a], D. Kotresha[b], Divakar Goli[a]

[a] *Department of Pharmacology, Acharya & B.M. Reddy College of Pharmacy, Bangalore, 560 107, Karnataka, India*
[b] *Biochemistry, Indian Institute of Science, Bangalore, 560 012. Karnataka, India*

ARTICLE INFO

Article history:
Received 15 September 2015
Received in revised form 20 February 2016
Accepted 22 February 2016

Keywords:
Cerebral ischemia
Infarct size
Oxidative stress
C. glandulosum
Cerebroprotective

ABSTRACT

Stroke is a major cause of death and disability worldwide. The resulting burden on the society continues to grow, with increase in the incidence of stroke. Oxidative stress has been involved in the pathogenesis of several neurological diseases including acute stroke.Focal and global cerebral ischemia represents diseases that are common in the human population.In recent years much attention is being paid towards the exploration of herbal preparation, antioxidant agents and combination therapies including COX-2 inhibitors in experimental model of stroke.Possible effect of a hydroalcoholic leaf extract of *Clerodendron glandulosum*Coleb (*C. glandulosum*)on oxidant-antioxidant status in ischemia-hypoperfusion injury in the rat forebrain has been investigated.Healthy adult male Wistar albino rats were divided into five groups ($n=8$). Group I was served as Sham control (normal saline 1 ml/kg, orally), group II was served hypoperfusion control (normal saline 1 ml/kg, orally), group III, group IV were served as hydroalcoholic extract treated (200 and 400 mg/kg, orally) and group V was treated with Quercetin (10 mg/kg, orally) for 14 days to assess preventive and curative effects of *C. glandulosum*. Flavonoid and phenolic compounds exhibit a broad spectrum of biological activity, including antioxidant. *C. glandulosum* extract (200 and 400 mg/kg, p.o) was administered orally, once daily for a period of 2 weeks after the occlusion of BCCA. After 14th days rats were subjected to behavioral studies. After behavioral studies animals were sacrificed and brain was removed and homogenized. Estimation of Lipid peroxidation (LPO) Myeloperoxidase (MPO), estimation of protein levels and the activities of Superoxide dismutase (SOD), Catalase (CAT), were performed. Infarct size and histopathological changes were observed in treated groups.

1. Introduction

Cerebral ischemia is a condition in which there is insufficient blood flow to the brain to meet metabolic demand. This leads to poor oxygen supply or cerebral hypoxia and thus to the death of brain tissue or stroke [1]. Among stroke patients, 80% suffer from cerebral infarction and 20% from cerebral hemorrhage [2]. Risk factors for stroke include advanced age, hypertension, transient ischemic attack, diabetes; high cholesterol, cigarette smoking and atrial fibrillation.Many antioxidants are reported to reduced reactive oxygen species-mediated reaction and protect neurons from ischemia-reperfusion induced neural loss in animal models of cerebral ischemia. Methanolic extract of *C. glandulosum* leaves extract was assayed for its qualitative and quantitative phytochemical constituents as well as for its exhibits free radical scavenging potential using different *in vitro* assays for hydrogen peroxide and hydroxyl superoxide, DPPH, nitric oxide, peroxynitrite, singlet oxygen and hypochlorous acid radicals. It also exhibits hepatoprotective, anti-obesity, anti-atherosclerosis, prevention of metabolic syndromes, non-alcoholic steatohepatities activity [3]. Traditionally, people across Manipur consume decoction of *C. glandulosum* leaves for treating diabetes, obesity and hypertension. Phytochemical analysis of extract of *C. glandulosum* revealed presence of phenols, steroids, alkaloids, anthraquinones, flavonoids and polyphenols. The hydroalcoholic extract of the leaves showed antioxidant activity [3]. There is considerable evidence which supports the role of reactive oxygen species (ROS) in the pathogenesis of ischemic reperfusion induced oxidative stress in brain were reported [4]. Novel therapeutic neuroprotective strategies support the applications of ROS scavengers and

* Corresponding author.
E-mail address: prakasht@acharya.ac.in (T. Prakash).

http://dx.doi.org/10.1016/j.biopha.2016.02.029
0753-3322/

induction of endogenous antioxidants drugs, such as natural antioxidants, e.g. plant derived polyphenolic compounds, flavonoids, in mono therapy, or as part of an antioxidant formulation for the treatment of neurodegenerative diseases [5]. Hence on the basis of the literature review, and active constituents present in the leaves of *C. glandulosum,* this study was undertaken to study the cerebroprotective effect of hydroalcoholic extract of *C. glandulosum* against bilateral common carotid artery occlusion induced cerebral ischemia in rats.

2. Materials and methods

2.1. Animals

After obtaining permission from Intuitional Animal Ethics Committee (IAEC) with study protocol number (IAEC/ABMRCP/2012-13/01), male albino Wistar rats (150–250 g) were obtained from Bioneeds, Bangalore, Karnataka. All the experiments were performed in accordance with the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). Rats were caged in fully ventilated room. Animals were maintained in 12:12 h light and dark cycle and were housed at temperature of 25 ± 2 °C. They had free access to a standard chow diet (Chow diet contain ingredients such as ground corn, ground oats, alfalfa meal, soybean meal and ground wheat, as well as vitamins, minerals and fat are added to ensure nutritional adequacy) and water *ad libitum*.

2.2. Chemical

Nicotinamide adenine dinucleotide phosphate reduced (NADPH), 1-chloro-2,4-dinitrobenzene (CDNB), thiobarbituric acid (TBA), O-dianisidinedihydrochoride, 5-5-dithiobis-2-nitrobenzoic acid (DTNB), Folin-Ciocalteu phenol reagent, ethylene diamine tetra acetic acid (EDTA), nitrobluetetrazolium (NBT), 2,3,5-triphenyltetrazolium chloride (TTC), Quercetin, were procured from Sigma Aldrich (St. Louis, MO, USA) and SRL, Bombay, India.

The plant was authenticated by Dr.T.H. Brojendra Singh, Institute of Biotech Hub Centre, Takyalapat, Imphal, Manipur. The voucher specimen no-1BT H-OC-01-2013 of the plant was deposited in the department. All other chemicals and reagents used were of analytical grade and procured from approved vendors.

2.3. Preparation of extract

The plant, *C. glandulosum* leaves were procured from Manipur India. The leaves of *C. glandulosum* were shade dried. Dried leaves were then powdered and passed through sieve no: 44. Fine powder was collected; 100 g of fine powder was packed, and the powdered material was defatted with petroleum ether, chloroform solvents. The defatted powder was extracted with hydroalcoholic solvents at 65–70 °C for 24 h in soxhlet column. After extraction the solvent was removed by using vacuum evaporator. Dried extract was stored in bottle wrapped with aluminium foil at 2–8 °C. The chemical constituents of the hydroalcoholic extract of *C. glandulosum* were identified by qualitative analysis [6].

2.3.1. Determination of total flavonoid compound

The total flavonoid compounds in extract were determined by an aliquot (1.5 ml) of hydroalcoholic extract of *C. glandulosum* was added to 6 ml of deionized water and then 0.45 ml of 5% (w/v) $NaNO_2$ and incubated for 6 min. $AlCl_3$ 10% (w/v) of 0.45 ml and 6 ml of 4% (w/v) NaOH was added and the total volume was made upto 15 ml with distilled water. The absorbance was measured at 510 nm by using visible spectrophotometer. The results were expressed as milligram quercetin equivalent/g. The experiments were performed by triplicates [6].

2.3.2. Determination of total phenol compound

Total phenolic compounds present in the hydroalcoholic extract of *C. glandulosum* were determined with the Folin-Ciocalteu reagent method. 1 ml of the sample solution was mixed with 1 ml Folin-Ciocalteu reagent. After 3 min, 35% of 3 ml Na_2CO_3 was added to the mixture followed by the addition of 7 ml of distilled water. The reaction mixture was kept in dark for 90 min and the absorbance was measured at 725 nm. The concentration of the total phenolic compound was determined as milligram gallic acid equivalent/g extract [6].

2.4. Acute toxicity

Acute toxicity studies were carried out by using rats as per Organization for Economic Co-operation and Development (OECD) 425 guidelines and the protocol was approved by Institutional Animal Ethics Committee (IAEC) (Protocol No: IAEC/ABMRCP/2013-14/01). Mice were divided into three groups and three animals in each group. A limit test at dose of 2000 mg/kg body weight was carried out of hydroalcoholic extract. The animals were observed for clinical signs and mortality for 15 days. Body weight was recorded every week. The tested sample was found to be safe and did not produce any mortality after 15 days. [7]

2.5. Experimental design

The study was planned to assess ability of *C. glandulosum* to cure ischemic injury due to cerebral ischemia. Wistar albino rats were randomly divided into five groups containing eight animals each. In Group I (Sham control) cerebral ischemia was not induced. Group II (hypoperfusion control) cerebral ischemia was induced and treated with normal saline (1 ml/kg, p.o) for 14 days. Group III & Group IV were induced cerebral ischemia and treated with *C. glandulosum* (200 & 400 mg/kg, p.o) for 14 days. Group V was induced cerebral ischemia and treated with Quercetin (10 mg/kg, p.o) for 14 days as reference group.

2.5.1. Induction of cerebral ischemia

Surgical technique for induction of cerebral ischemia was adapted from the method of Iwasaki et al. (1989) and rats were anesthetized by giving thiopentone sodium (40 mg/kg, i.p.). Both common carotid arteries were exposed over a midline incision, and a dissection was made between the sternoicleidomastoid and the sternohydoid muscles parallel to the trachea. Each carotid artery was freed from its adventitial sheath and vagus nerve, which was carefully separated and maintained. A cotton thread was passed below each carotid artery. The induction of the ischemia was performed by BCCA occlusion with cotton thread. Temperature was maintained around 37 ± 0.5 °C. The carotid arteries were doubly ligated with 3–0 silk thread and cut in between. The skin was then sutured, animals were kept in the individual cages and applied betadine ointment by sterilized cotton for sutured animals. The dose of *C. glandulosum* was continued up to 14th post surgical day. On the 14th day, 60 min after last dose of *C. glandulosum*, the animals were subjected to behavioral study [8].

2.6. Effect of C. glandulosum on behavioural parameters

2.6.1. Open field test

The locomotor activity was evaluated in an open fieldparadigm. The apparatus consisted of a wooden box (60 × 60 × 30 cm). The floor of the box was divided into 16 squares (15 × 15 cm). The apparatus was illuminated with a 40-W lamp suspended 100 cm