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NEUROPROTECTIVE EFFECTS OF CASSIA SIAMIA LEAVES EXTRACT IN CEREBRAL ISCHEMIA REPERFUSION INJURY INDUCED RATS

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ABSTRACT

Cerebral ischemia occurs when a blood clot has occluded a cerebral vessel. It reduces blood flow to a specific brain region, increasing the risk of cell death to that particular area. It can be either caused by thrombosis or embolism. Hence, It results in BBB injury and brain edema and also contribute to brain injury. Thus, the protective effects of *cassia siamea* leaf extract (CSLE) on the structure and function of the BBB were quantified in the present study. CSLE is having many antioxidant compounds such as barakol, vitamin C, Vitamin E, carotenoids, α-tocopherol, xanthophylls, tannins, flavonoid, phenolic acids, and diverse enzyme (superoxide dismutase, catalase, and peroxidase) could be responsible for this activity. we showed that CSLE (450mg/kg) treatment markedly reduced cerebral infarct area and improved neurological function in rats with cerebral ischemia– reperfusion. We also found that CSLE alleviated BBB morphological damage and attenuated increase in BBB permeability after cerebral ischemia–reperfusion. Measurement of NO, leukocytes and neutrophils in the plasma revealed that all were significantly increased in response to ischemia and reperfusion. Measurement of circulating leukocytes, indicative of a systemic inflammatory response, revealed that NO ($p < 0.001$) and the total number of leukocytes ($p < 0.001$) and neutrophils ($p < 0.004$) were significantly elevated 4 hours after ischemia and reperfusion. Administration of L-NA reduced the level of NO in the plasma and did not affect the cell count for leukocytes and neutrophils.

KEYWORDS: Cassia siamea, Cerebral ischemia, Reperfusion injury Brain injury disruption of the blood–brain barrier, resulting in leukocyte transmigration into the surrounding brain tissues.

1. INTRODUCTION

Brain ischemia (or cerebral ischemia, cerebro vascular 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 cerebral infarction / ischemic stroke.[1] It is a sub-type of stroke along with subarachnoid hemorrhage and intracerebral hemorrhage. Ischemia leads to alterations in brain metabolism, reduction in metabolic rates, and energy crisis.[2]

The main symptoms involve impairments in vision, body movement, and speaking. The causes of brain ischemia vary from sickle cell anaemia to congenital heart defects.[3] Symptoms of brain ischemia can include unconsciousness, blindness, problems with coordination, and weakness in the body. Other effects that may result from brain ischemia are stroke, cardiorespiratory arrest, and irreversible brain damage.[4]

An interruption of blood flow to the brain for more than 10 seconds causes unconsciousness, and an interruption in flow for more than a few minutes generally results in irreversible brain damage. In 1974, Hossmann and Zimmermann demonstrated that ischemia induced in mammalian brains for up to an hour can be at least partially recovered.[5] Accordingly, this discovery raised the possibility of intervening after brain ischemia before the damage becomes irreversible.

Central nervous system ischemia – reperfusion injury

Ischemia–reperfusion injury of the central nervous system (CNS) may occur after stroke, traumatic head injury, carotid endarterectomy, aneurysm repair, or deep hypothermic circulatory arrest.[6] CNS I-R injury is characterized by Release of various proteases, lipid-derived mediators, and ROS by leukocytes into the brain tissue irreversibly damages potentially salvageable cells, particularly within the ischemic penumbra.[7]

Disruption of the blood–brain barrier after I-R also results in the development of cerebral edema and increased intracranial pressure. Compounding the cerebral edema is a loss of

cerebral vasoreactivity resulting in a reactive hyperemia. Thus, CNS I-R injury may clinically manifest as significantly worsened sensory, motor, or cognitive functioning, or death.[8]

2. MATERIALS AND METHODS

All animal experiments were undertaken with the approval of the Animal Care and Animal ethical committee.

2.1 Collection of plant material

Leaves, bark, seeds and flowers of *C.siamea* were collected from Harekrishn herbals Kattamuru, E.G dist, Andhra Pradesh, India. The species was authenticated by Dr. P Ramara rao Naidu garu, Ethanobotanical scientist, Vishaka A Colony, Srikakulam.

2.2 Preparation of *Cassia siamea* leaf extraction (CSLE)

The arial parts of *C.siamea*, cleaned with deionized water, oven dried at 50ºC for 48 h and powdered in a grinder. The plant material (100 g) was extracted with different solvents (1500 m L).[9] using Soxhlet apparatus for 24 h at a temperature not exceeding the boiling point of the respective solvents. The obtained extracts were filtered using Whatmann filter paper No. 1 and concentrated under vacuum at 40 C using a rotary vacuum evaporator (Büchi Laboratoriums, Switzerland) to dryness. The extractive values of the extracts were calculated. Separation of active ingredients from leaves of *C.siamea*.[10] Total eight solvents were used for extraction of active ingredients from the leaves of *C.siamea*. Maximum yield was obtained in 95% ethanol and results are summarized in the table. Extraction yield obtained from various aerial parts of Cassia siamea and with respect to different solvents and time.[11] (Table 1).

Table 1: Cassia siamea leaves extraction.

S. No.	Time (hrs)	Methanol	Water	Chlorofom
1.	12	15.96	7.94	4.24
2.	18	21.56	8.59	4.54
3.	24	24.22	9.65	5.38

2.3 Animals grouping

Rats, weighing 150-200 g were used. Animals were maintained in a temperature-controlled colony (20-22°C) and humidity controlled (55 ± 5 %) rooms with a 12-hour light/dark cycle and with free access to food (Rat Feed, S.W.P. LTD.) and water at last date of experiment, animals were fasted for at least 14 hours prior to drug administration. Rats were randomly

divided into four groups each group having five animals (n=5): Group 1: control (c);Group 2: Test (T_1) 150mg/kg; Group 3: Test (T_2) 300mg/kg; Group 4: Test (T_3)450mg/kg.

2.4 Method of drug treatment

CSLE was dissolved homogeneously in dimethyl sulfoxide (DMSO). In the group treated with CSLE, rats were administered appropriate doses of CSLE (150,300,450 mg/kg/day) via the oral route each day for 10 days. Administration was continued until killing at the conclusion of the experiment. Other rats (control) were given the same volume of DMSO.[12]

2.5 Focal ceebral ischemia reperfusion model preparation

The Focal cerebral ischemia was induced by cerebral artery occlusion (CAO). In this, rats were anesthetized with thiopentone sodium (40mg/kg, oral route.). The left common carotid artery (CCA), the right carotid artery (RCA), were exposed and occluded with nylon thread.30 minutes after left and right carotid artery occlusion,[13] rats were re-anesthetized with thiopentone, and reperfusion was initiated by withdrawal of the nylon thread. Core body temperature was maintained at 36.5–37.5 ^{o}C using a rectal probe and heating pad. Physiological before, during, and temperature was maintained at 36.5–37.5 ^{o}C using a rectal probe and heating pad. Physiological before, during, and after surgery did not significantly differ across all groups of carotid artery occlusion rat.[14]

2.6 Measerment of cerebral infarct area

Rats were killed under deep anesthesia 24 or 72 h after reperfusion (n = 5). Brains were removed immediately and sectioned into 2-mm slices. Samples were placed in 2% 2,3,5-triphenyl tetrazolium chloride (TTC; Sigma– Aldrich) stain for 15 min at 37^{o}C.[15] Brain tissue was differentiated according to white-colored infarct area and red– purple non-infarct area. Cerebral infarct areas were measured using by weighing balance. The ratio of infarct area to total brain area was calculated.[16]

2.7 Blood parameters count

Cell counts were performed using routine hemocytometry. Data is expressed as the number of cells per cubic millimeter (mm3).

2.8 Statistical analysis

Data are expressed as mean ± S.E.M. and were analyzed by one-way analysis of variance (ANOVA).

3. RESULTS

3.1 Cerebral Infarct Area

The experiment shows the development of infarction after 4 hours reperfusion injury following by carotid artery occlusion for 30 minutes. Brain tissue was differentiated according to white-colored infarct area and red– purple non-infarct area.

We studied the effect of CSLE on cerebral infarct area by TTC staining. Compared with the group 1 (control), rats treated with 450 mg/kg/day CSLE had markedly reduced cerebral infarct area at 4 hrs after reperfusion. The greatest protection was seen in 450mg/kg/day CSLE- treated rats at 4hrs after reperfusion when compared with the test groups T_1 150mg/kg/day and test groups T_2 300mg/kg/day (Table 2).

3.2 Blood parameters

We found a positive linear correlation between infarct size and the peripheral white blood cell count, specifically the polymorphonuclear leukocyte count. A relationship was also observed for the cerebrospinal fluid protein level, the gamma globulin level, and the cerebrospinal fluid/serum albumin ratio. The correlations observed presumably reflect the extent of tissue injury and secondary inflammatory response in acute cerebral ischemia (Table 3).

4. DISCUSSION

The objective of this article is to show the recent progress in the exploration of *C.siamea* as phytotherapy[17] and to illustrate its potential as a therapeutic agent. With the current information, it is obvious that *C.siamea* has pharmacological functions[18] including antimalaria, antidiabetic, antihypertensive, antioxidant, antitumor, anti- inflammatory, analgesic, antipyretic, anxiolytic, sedative, antibacterial, and antifungal activities. As the current information shows more ninety bioactive compounds were isolated from *C.siamea*. Pharmacological effects of most of these compounds are not yet known. Nevertheless, from the results of studies carried out, it is possible that chromone alkaloids (barakol,[19] cassiarin A), antraquinones (emodin, chrysophanol), and biantraquinones (cassiamin A, cassiamin B) might be useful in the development of new drugs to treat various diseases. However, the present results suggest a possibility that these compounds can be further developed as a potential disease-curing remedy. It must be kept in mind that clinicians should remain cautious until more definitive studies demonstrate the quality and effectiveness of *C.siamea*.[20] For these reasons, extensive pharmacological and chemical experiments, together with human metabolism will be a focus for future studies. Last but not the least, this

review emphasizes the potential of *C.siamea* to be employed in new therapeutic drugs and provide the basis for future researches on the application of transitional medicinal plants.

Brain slices

Seperation of brain infract part

Table 3: Effects of C.siamea extracts on brain.

S. No.	Group	Mean Body Weight (gms)	Mean Dose (mg/kg)	Mean Brain Weight (gms)	Mean Non Infract Part Brain Weight (gms)	Mean Infract Part Brain Weight (gms)
1.	Group 1 (control)	250	300	1.56	0.65	0.91
2.	Group 2 (test t_1)	150	150	1.58	0.92	0.45
3.	Group 3 (test t_2)	180	300	1.58	1.35	0.23
4.	Group 4 (test t_3)	200	450	1.58	1.58	0.11

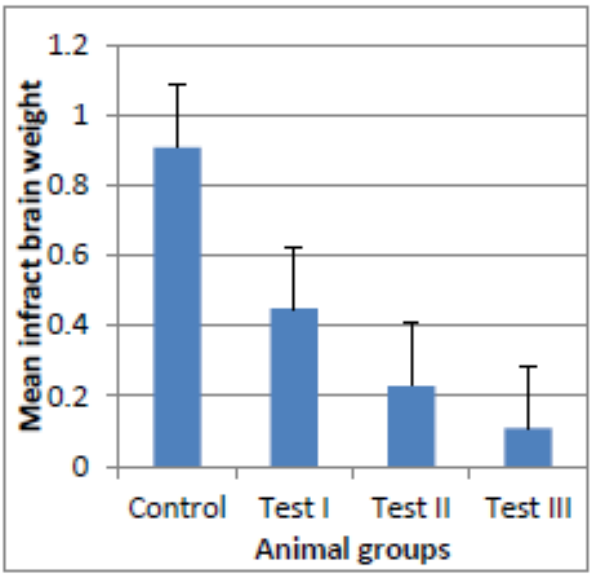

Table 4: Effects of C. Siamea extracts on WBC levels.

Blood Parameters	Normal Values	Group I (Control) 300mg/kg/day	Group II (Test T_1) 150mg/kg/day	Group III (Test T_2) 300mg/kg/day	Group IV (Test T_3) 450 mg/kg/day)
RBC (10^6/Cmm)	6.76-9.75	10.6	10.52	9.42	9.8
WBC (10^3/Cmm)	6.6-9.75	5.42	5.28	5.1	4.2
HgB(gm/dL)	11.5-16.1	16.8	15.2	14.9	15.5
MCV(fL)	48-70	56.1	56.2	52.6	55.8
MCH(Pg)	17.8- 20.9	15.8	15.8	14.9	15.8
MCHC (gm/dL)	40-42	39.2	40.2	40.1	38.3
MPV(fL(μm^3)	6.2-9.8	8.2	8.22	8.23	8.47

A positive linear correlation between infarct size and the peripheral white blood cell count, specifically the polymorphonuclear leukocyte count.

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