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NEUROPROTECTIVE ACTIVITY OF SOLASODINE FROM *SOLANUM XANTHOCARPUM* FRUITS AGAINST CEREBRAL ISCHEMIA/REPERFUSION – INDUCED OXIDATIVE STRESS IN RAT

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Keywords:

Cerebrovascular diseases, Solasodine, Ischemia/reperfusion, Neuroprotection, Oxidative stress

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ABSTRACT: Ethnopharmacological Relevance: Traditional herb *Solanum xanthocarpum* contains solasodine as the chief constituent of fruits. Since analgesic, anti-inflammatory, anticonvulsant effects of fruits are reported; study was designed to rationalize the use of solasodine as neuro-protective with its mechanism of action. **Aim of Study:** Cerebrovascular Diseases (CVD) includes devastating disorders like ischemic stroke, hemorrhagic stroke, and cerebrovascular anomalies. Worldwide, around 15 million people suffer a stroke every year. With about 5 million deaths, stroke is the second most common cause of death and a major cause of long-term disability. This study was carried out to evaluate the neuroprotective activity of solasodine from *Solanum xanthocarpum* against global cerebral ischemia/reperfusion induced brain injury in rats. **Materials and Methods:** Neuroprotective activity was carried out by global cerebral ischemia on Wistar albino rats, distributed into 6 groups (n=8). Rats were subjected to bilateral common carotid artery (BCCA) occlusion for 30 min followed by 60 minutes reperfusion. The antioxidant enzymatic and non-enzymatic levels, cerebral infarction area, and histopathology were evaluated. **Results:** Solasodine showed dose-dependent neuroprotective activity by a significant decrease in lipid peroxidation (LPO) and increase in the superoxide dismutase (SOD), catalase (CAT), glutathione (GSH), and total thiol levels in solasodine (30 mg/kg and 60 mg/kg, i.p.) treated groups as compared to ischemia/reperfusion group. Moreover, cerebral infarction area and histopathology revealed protection against ischemic brain damage in solasodine treated groups. **Conclusion:** Solasodine showed potent neuro-protective activity against cerebral ischemia/reperfusion-induced brain injury in rats and may prove to be a useful adjunct in the treatment of stroke.

INTRODUCTION: According to the World Health Organization (WHO), stroke is rapidly developing loss of brain function(s) due to disturbance in the blood supply to the brain [1].

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The time dimension was later included in the definition - rapidly developed clinical signs of focal or global disturbance of cerebral function, lasting more than 24 h or until death, with no apparent non-vascular cause [2]. Ischemia is defined as a reduction in blood flow sufficient to alter normal cellular function. Brain tissue is highly sensitive to ischemia, such that even brief ischemic periods to a neuron can initiate a complex sequence of events that ultimately may culminate in cellular death.

Different brain regions have a varying threshold for ischemic cell damage, with the white matter being more resilient than grey matter. The brain is amongst the most vulnerable tissue of the body to ischemia due to its high oxygen demand, partly needed for nerve impulse propagation and chemical neurotransmission. The human brain represents only 2% of the total body weight but uses 15% of the cardiac output, 25% of the total oxygen consumption of the body at rest; 75 L of molecular oxygen, and 120g glucose daily [3]. Cerebrovascular Diseases (CVD) include some of the most common devastating disorders such as ischemic stroke, hemorrhagic stroke, and cerebrovascular anomalies [4].

Worldwide, around 15 million people have a stroke every year, and with about 5 million deaths, stroke is the second most common cause of death and a major cause of long-term disability. It is estimated that about 25% of people older than 85 years will develop a stroke [5]. In the last decade, the cumulative incidence of stroke ranges from 105 to 152/100,000 persons per year, and the crude prevalence of stroke ranges from 44.29 to 559/100,000 persons for India in different regions, indicating higher values than those of high-income countries [6]. In addition, certain populations of neurons are selectively more vulnerable to ischemia; for example, in the hippocampus, CA-1 pyramidal neurons are highly susceptible to ischemia, whereas dentate granule neurons are more resistant. Early restoration of blood flow remains the treatment of choice for limiting brain injury following stroke [7]. Transient cerebral global ischemia causes delayed neuronal cell death in the hippocampal CA-1 region at 4-7 days after an ischemic insult with the significant role of p63 gene [8]. The major pathobiological mechanisms of ischemia and reperfusion (I/R) injury include excitotoxicity, oxidative stress, inflammation and apoptosis [9]. During the reperfusion period after ischemia, increased oxygen supply results in overproduction in reactive oxygen species (ROS), which are involved in the process of cell death. ROS such as superoxide anions, hydroxyl free radicals, hydrogen peroxide, and nitric oxide are produced as a consequence of metabolic reactions and central nervous system (CNS) activity [10]. These reactive oxygen species are directly involved in oxidative damage of cellular macromolecules such as nucleic acids, proteins, and lipids in ischemic tissues, which can lead to cell death [11]. In fact, the release of ROS and increased lipid peroxidation can be detected early after I/R, where there is no sign of neuronal death [12]. Superoxide and hydroxyl radical cause severe cell membrane damage by inducing lipid peroxidation [13]. Inducible nitric oxide (iNOS) synthase is up-regulated after I/R, which results in excessive NO production. This excess NO reacts with superoxide to form peroxynitrite, a powerful radical that can induce neuronal death after cerebral ischemia [14]. The enzymatic antioxidant activity of the tissue affected by I/R is particularly important for the primary endogenous defense against the ROS induced injury. Superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px) are an endogenous antioxidant which plays an important role in the prevention of oxidative damage [15]. In the presence of excess oxygen species, inactivation of detoxification systems, and degradation of antioxidants, endogenous ant oxidative defenses are highly effective [16]. From the oxidative hypothesis of ischemia-induced cell death, there is an increasing interest in focusing on natural products that may ameliorate Delayed Neuronal Death (DND) due to cerebral ischemia. Numerous antioxidants and scavengers of ROS have not been tested, and many have shown neuroprotective effect [17]. In fact, many antioxidants have shown a neuroprotective effect in I/R-induced cerebral injury [18-20].

Several drugs like recombinant tissue plasminogen activator (rt-PA), calcium stabilizing agents, anti-excitotoxic agents, antioxidants, anti-apoptotic agents, and anti-inflammatory agents are used but it has been reported that rt-PA can exacerbate excitotoxic neuronal death [21].

Solanum xanthocarpum (*S. xanthocarpum*) Schard. & Wendl. (family: Solanceae) commonly-known as Yellow Berried Nightshade (syn - Kantakari), is a prickly diffuse bright green perennial herb, woody at the base, 2-3 m height found throughout India, mostly in dry places as a weed on roadsides and wastelands [22]. The fruits are glabrous, globular berries with green and white stripes when young but yellow when mature [22]. The fruits are known for several traditional medicines uses like anthelmintic, antipyretic, laxative, anti-inflammatory, urinary bladder, antiasthamatic, aphrodisiac activities,

and enlargement of the liver [22]. The stem, flowers, and fruits are prescribed for relief in a burning sensation in feet accompanied by vesicular eruptions [23]. The fruits are reported to contain several steroidal alkaloids like reported to contain several steroidal alkaloids like solanacarpine, solanacarpidine, solasodine, solasonine, solamargine and other constituents like caffeic acid, coumarins like aesculetin and aesculin, steroids carpesterol, diosgenin, campesterol, daucosterol, and triterpenes like cycloartanol and cycloartenol. Solasodine is present in a number of Solanum species (Solanaceae) such as *Solanum khasianum*, *Solanum xanthocarpum*, *Solanum nigrum*, *Solanum gracile*, *Solanum laciniatum etc*. [23]

To the best of our knowledge, there were no any scientific reports available in support of its traditional claim of neuroprotective potential. Therefore, the present study was designed to demon-strate the neuroprotective effect of Solasodine against cerebral ischemia/reperfusion-induced oxidative stress in Wistar albino rats.

MATERIALS AND METHODS:

Collection and Identification of Plant Material: *Solanum xanthocarpum* fruits were collected from Bardoli, Gujarat, during the month of January 2013, identified and authenticated by the botanist, The Patidar Gin Science College, Bardoli, India. Authentification No.: Authen. / 02 / 2013.

Isolation of solasodine: 100 g. dried berries powder of *S. xanthocarpum* was refluxed with 400 ml of absolute ethanol (99%) for 1 h. The extract was decanted, and the process was repeated with another 400 ml absolute ethanol. The total ethanol extract (800 ml) was then concentrated. The concentration of the extract was performed by distillation of ethanol. The ethanol recovered after distillation was reused for the extraction of solasodine from another batch of dried berries. To 100 ml concentrated ethanol extract 3-5 ml of concentrated hydrochloric acid was added, and the mixture was refluxed for 6 h extract was then cooled. After cooling, a white precipitate was obtained, which was filtered, washed with ethanol, and dried. The crude solasodine – hydrochloride thus obtained was added to boiling water (80 ml) and 10% NH_4OH under constant stirring. The mixture was boiled by refluxing for 1 h. The precipitate was cooled, filtered, washed with distilled water, and dried to yield crude solasodine. Crude solasodine was purified by dissolving it in acetone and filtered through Whatman filter paper using activated charcoal and further purified with repeated recrystallization from methanol. White crystals of pure solasodine were recovered. Confirmation of isolated solasodine was carried out by determining melting point, infrared spectro-scopy, and HPTLC.

Animals: Healthy, 8-10 weeks old (200-250 g) male rats (*Rattus norvegicus)* of Wistar strain were obtained from the central animal house, Maliba Pharmacy College. The animals were housed under standard conditions, maintained on a 12 h light/ dark cycle, and had free access to food and water *ad libitum.* The animals were acclimatized to the laboratory environment 1 h before the experiments. Animals were randomly distributed into groups of 8 animals each. All the protocols were approved by the Institutional Animal Ethical Committee (IAEC) (Protocol No. MPC/IAEC/03/2013), and experiments were conducted according to the guidelines of the Committee for the Purpose of Control and Supervision of Experiment on Animals, Ministry of Environment and Forest, Animal Welfare Division, Government of India, New Delhi, India.

Selection of Solasodine Dose: Reported LD_{50} of solasodine was found to be 1500 mg/kg [24] for the assessment of neuroprotective activity, two dose levels were chosen in such a way that the high dose was approximately $1/25^{th}$ of the maximum dose (*i.e*., $1/25^{th}$ of 1500 mg/kg body weight- 60 mg/kg body weight) and a low dose which was 50% of $1/25^{th}$ dose (*i.e.,* 30 mg/kg body weight).

Experimental Protocol for Global Cerebral Ischemia/Reperfusion-induced Oxidative Stress: Animals were divided into six groups of eight rats each and fed with drug or vehicle for 7 days prior to the experiment and treated as follow:

Group I: 1% SCMC (1 ml/kg, i.p), no ischemia.

Group II: 1% SCMC (1 ml/kg, i.p), bilateral Common Carotid Artery (BCCA) occlusion for 30 min.

Group III: 1% SCMC (1 ml/kg, i.p), BCCA occlusion for 30 min followed by 60 min reperfusion.

Group IV: Solasodine (30 mg/kg, i.p), BCA occlusion for 30 min followed by 60 min reperfusion.

Group V: Solasodine (60 mg/kg, i.p), BCA occlusion for 30 min followed by 60 min reperfusion.

Group VI: Quercetin (25 mg/kg, i.p), BCA occlusion for 30 min followed by 60 min reperfusion.

Induction of Ischemia: Animals of group II to VI were subjected to bilateral carotid artery occlusion [25] under ketamine anesthesia (45mg/kg, i.p.) and xylazine (10mg/kg, i.p.) cocktail injected intraperitoneally. Animals were placed on the back; both carotid arteries were exposed and occluded by atraumatic clamps. The temperature was maintained around 37 ± 0.5 °C throughout the surgical procedure, and artificial ventilation (95% O_2 and 5% CO_2) provided with artificial respirator.

Preparation of Post-mitochondrial Supernatant: The following decapitation, the brain was isolated and washed in cooled 0.9% saline, kept on ice and subsequently blotted on filter paper, then weighed and homogenized as 10% (w/v) in cold phosphate buffer (0.05 M, pH 7.4). The homogenates were centrifuged at 10000 rpm for 10 min at 4 °C, and post-mitochondrial supernatant (PMS) was kept on ice until assayed [19].

Biochemical Estimation:

Lipid Peroxidation (LPO): Thiobarbituric acid reactive substances (TBARS) in the homogenates were estimated by using standard protocol [26]. Briefly, the 0.5 ml of 10% homogenate was incubated with 15% Trichloroacetic Acid (TCA), 0.375% thiobarbituric acid (TBA) and 5N Hydrochloric acid (HCl) at 95 °C for 15 min, the mixture was cooled, centrifuged, and absorbance of the supernatant measured at 512 nm against appropriate blank. The amount of lipid peroxidation was determined by us g $\varepsilon = 1.56 \times 10^5\ M^{-1}\ cm^{-1}$ and expressed as TBARS nmoles/mg of protein [27].

Superoxide Dismutase (SOD): SOD activity was determined based on the ability of SOD to inhibit the auto-oxidation of epinephrine to adrenochrome at alkaline pH [28]. Briefly, 25 µl of the supernatant obtained from the centrifuged brain homogenate was added to a mixture of 0.1 mM epinephrine in carbonate buffer (pH 10.2) in a total volume of 1 ml and the formation of adrenochrome was measured at 295 nm. The SOD activity (U/mg of protein) was calculated by using the standard plot.

Catalase (CAT): CAT activity was assayed by the method of Claiborne (1985) [29]. Briefly, the assay mixture consisted of 1.95 ml phosphate buffer (0.05 M, pH 7), 1 ml H_2O_2 (0.019 M), and 0.05 ml homogenate (10%, w/v) in a total volume of 3 ml. Changes in absorbance were recorded at 240 nm. Catalase activity was calculated in terms of nM H_2O_2 consumed/min/mg protein.

Total Thiols: This assay is based on the principle of formation of a relatively stable yellow color by sulfhydryl groups with DTNB.[30] Briefly, 0.2 ml of brain homogenate was mixed with phosphate buffer (pH 8), 40 µl of 10 mM DTNB, and 3.16 ml of methanol. This mixture was incubated for 10 min and the absorbance was measured at 412 nm against appropriate blanks. The total thiols content was calculated by using $\varepsilon = 13.6 \times 10^3\ M^{-1}\ cm^{-1}$.[31]

Glutathione (GSH): GSH was estimated in brain homogenates [31]. Briefly, 5% tissue homogenate was prepared in 20 mM EDTA, pH 4.7, and 100 µl of the homogenate or pure GSH was added to 0.2 M Tris-EDTA buffer (1.0 ml, pH 8.2) and 20 mM EDTA, pH 4.7 (0.9 ml) followed by 20 µl of Ellman's reagent (10mmol/l DTNB in methanol). After 30 min of incubation at room temperature, absorbance was read at 412 nm. Samples were centrifuged before the absorbance of the supernatants was measured [32].

Total Protein: The total protein content of 10% brain homogenates were determined by using the modified method of Lowry *et al*., (1951) by using span diagnostic kit.

Measurement of Infarction Area: The infarction area was measured by 2,3,5-triphenyltetrazolium chloride (TTC) staining method [43]. Following ischemia or reperfusion after varied durations of ischemia, animals were decapitated, and the brains were isolated. After the brains were placed in cold saline, four coronal brain slices (2 mm thick) were made. Then the slices were incubated in phosphate buffered saline (pH 7.4) containing 2% 2,3,5-triphenyltetrazolium chloride (TTC) at 37 °C for 10 min and then kept in neutral-buffered formalin overnight. The images of the TTC-stained sections were acquired by scanning with a high-resolution

scanner (Hewlett-Packard Scanjet 6100C/T). Then the cerebral infarction area was observed, calculated, and compared between various treatment groups and negative control groups. Infarction area was calculated by using Scion (Scion image for windows, Release Beta 4.0.2).

Histopathology: For the histopathological study, the animals were deeply anesthetized with ketamine (45 mg/kg, i.p.). Following decapitation, the brains were isolated and fixed in 10% formalin and sent to histology services (Matrushree Diagnostic Lab, Navsari) for hematoxylene and eosin staining. Same histology slides were subjected to microscopic examination for the presence of congestion of blood vessels, neutrophil infiltration, lymphocytic proliferation, and neuronal necrosis.

Statistical Analysis: All data are presented as mean ± S.E.M. The statistical significance of the difference between groups for the various treatments were determined by one way analysis of variance (ANOVA) followed by Tukey's multiple range test. $P<0.05$ was considered statistically significant as compared to control.

RESULTS:

Phytochemistry:

Yield of Solasodine Isolated from *S. xanthocarpum*: The percentage yield of solasodine from *Solanum xanthocarpum* fruits powder (100 g) was found to be 1.0 % w/w (1 g).

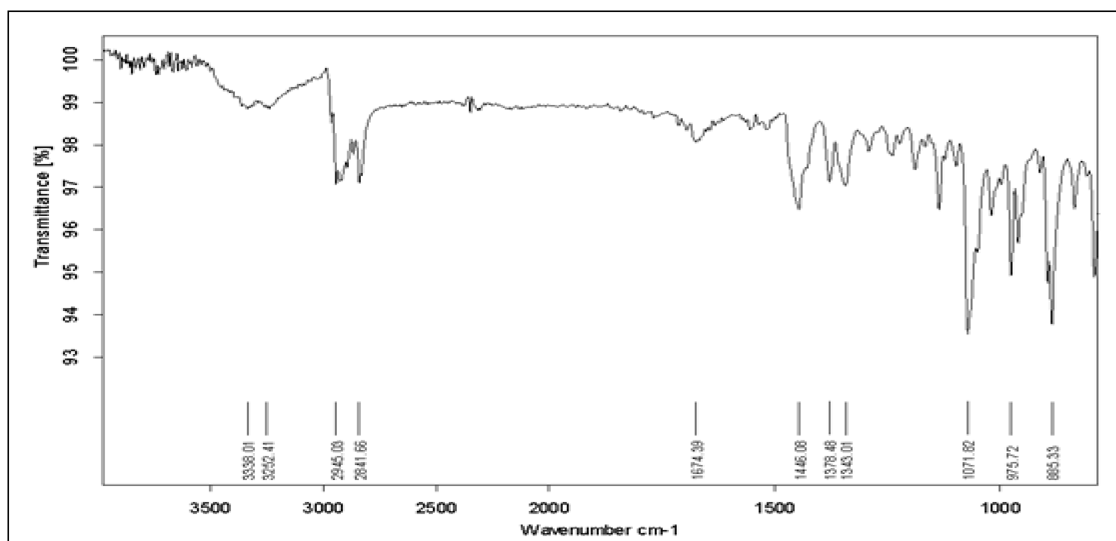
Confirmation of Solasodine isolated from *S. xanthocarpum:*

Melting Point: The melting point of isolated solasodine was found to be 202-204 °C, which was similar to that of standard solasodine 200-202 °C.

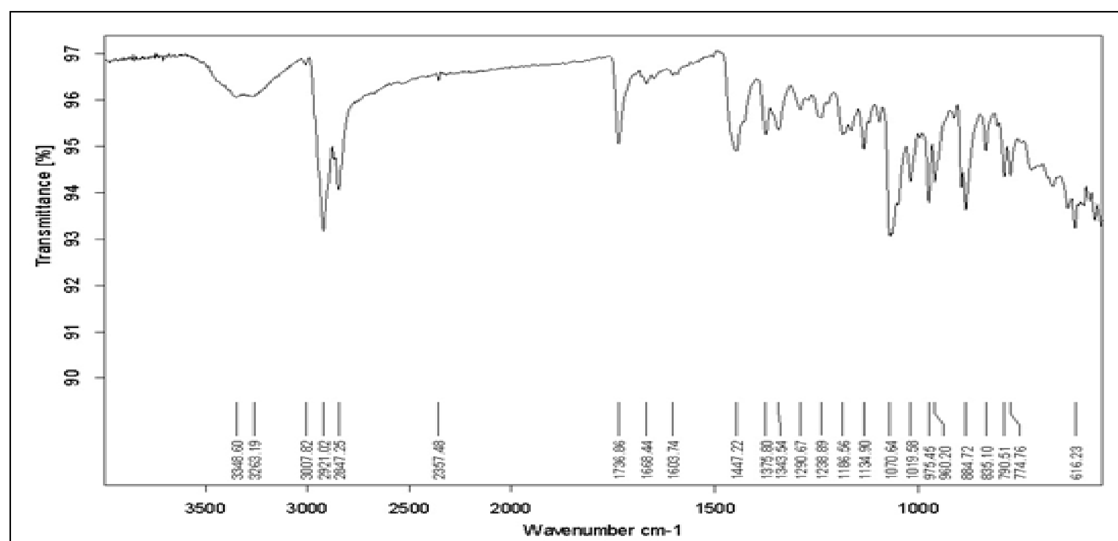
Infrared Spectroscopy: Isolated solasodine was analyzed by FTIR, and resultant spectra were compared with that of standard solasodine. The comparision shows that FTIR spectrum of isolated solasodine was similar to that of standard solasodine **Table 1** and **Fig. 1**.

TABLE 1: COMPARISON BETWEEN PEAKS OF STANDARD AND ISOLATED SOLASODINE

Standard Solasodine Peaks (cm^{-1})	Isolated Solasodine Peaks (cm^{-1})
885.33	884.72
975.72	975.45
1071.82	1070.64
1343.01	1343.54
1446.08	1447.22
1674.39	1668.39
2841.66	2847.25
2945.03	2921.02
3252.41	3263.19
3338.01	3348.60



FTIR SPECTRA OF STANDARD SOLASODINE



FTIR SPECTRA OF ISOLATED SOLASODINE

FIG. 1: FTIR SPECTRA OF STANDARD AND ISOLATED SOLASODINE

High-Performance Thin Layer Chromatography: **Fig. 2** shows the thin layer chromatographic fingerprint of the standard solasodine and isolated solasodine. R_f value of the fingerprint is given in below table **Table 2**.

TABLE 2: LIST OF R_f VALUES

Tracks	R_f value
Track 1 (Standard solasodine 20µg)	0.40
Track 2 (Isolated solasodine 20µg)	0.41
Track 3 (Isolated solasodine 40µg)	0.39
Track 4 (Isolated solasodine 60µg)	0.39

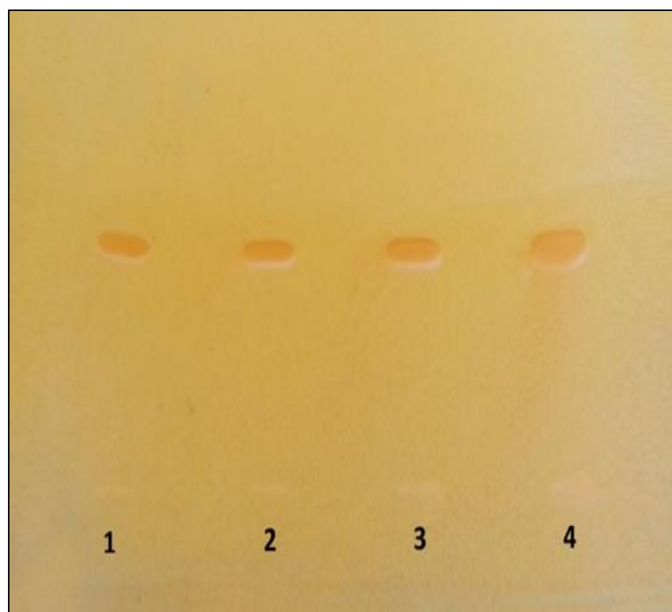


FIG. 2: THIN LAYER CHROMATOGRAPHIC FINGER-PRINT OF THE STANDARD SOLASODINE AND ISOLATED SOLASODINE FROM *S. XANTHOCARPUM*

Track 1: Standard Solasodine 20 µg.
Track 2: Isolated solasodine 20 µg
Track 3: Isolated solasodine 40 µg
Track 4: Isolated solasodine 60 µg

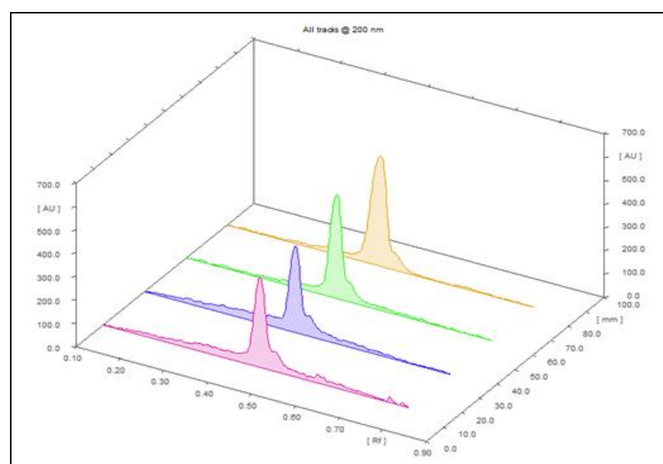


FIG. 3: 3D CHROMATOGRAM OF ALL TRACKS SCANNED AT 200 nm OF STANDARD AND ISOLATED SOLASODINE

Biochemical Estimation: The results showed in **Table 3** revealed the neuroprotective activity of solasodine. The animals from BCCA occluded ischemic groups (SCMC + I) exhibited a significant increase in LPO levels, whereas a significant decrease in SOD and non-enzymatic parameters GSH and total thiols. These levels were further augmented in animals of the Ischemia/Reperfusion (SCMC + I/R) group.

The animals from solasodine treated groups had shown significant protection by reducing the elevated levels of LPO ($p<0.001$) and marked increased in SOD ($p<0.001$), GSH ($p<0.001$), and total thiol ($p<0.001$) levels as compared to I/R-treated group (SCMC + I/R) whereas catalase ($p<0.01$) level as compared to normal.

TABLE 3: EFFECT OF SOLASODINE ON BIOCHEMICAL PARAMETERS IN RATS

Group no.	Treatment	LPO (nmoles/mg of protein)	SOD (U/mg of protein)	Catalase (U/mg of protein)	GSH (nmoles/mg of protein)	Total thiols (µmoles/mg of protein)
I	Normal (SHAM)	6.29 ± 0.39	67.99 ± 3.85	0.042 ± 0.004	12.10 ± 1.04	18.12 ± 1.85
II	SCMC + Ischemia	29.22 ± 4.37**	43.57 ± 5.24***	0.028 ± 0.005	6.90 ± 0.36**	11.48 ± 0.60**
III	SCMC + I/R	53.60 ± 3.59***	34.54 ± 3.30***	0.019 ± 0.002	2.88 ± 0.44***	4.74 ± 0.50***
IV	Solasodine 30 mg/kg + I/R	21.92 ± 3.77###	53.97 ± 2.83##	0.028 ± 0.006	7.47 ± 0.65#	11.78 ± 1.57##
V	Solasodine 60 mg/kg + I/R	15.95 ± 1.83###	68.26 ± 1.04###	0.058 ± 0.006##	10.79 ± 0.66###	15.34 ± 0.77###
VI	Quercetin 25 mg/kg + I/R	9.48 ± 1.43###	71.00 ± 0.97###	0.064 ± 0.008###	67.99 ± 3.85###	13.22 ± 1.08###

Values are expressed as mean ± SEM (n = 6), One-way Analysis of Variance (ANOVA) followed by tukey's test: ***p* < 0.01 and *** *p* < 0.001 *vs*. normal and #*p* < 0.05, ##*p* < 0.01 and ###*p* < 0.001 *vs*. 1.0% w/v SCMC+ I/R. I/R – Ischemia/Reperfusion, SCMC- Sodium Carboxy Methyl Cellulose

Measurement of Cerebral Infarction Area: The cerebral infarction area as shown in **Fig. 4** revealed decrease in solasodine treated groups as compared to negative control group (SCMC + I/R group) especially in caudal and rostral side of hippocampus.

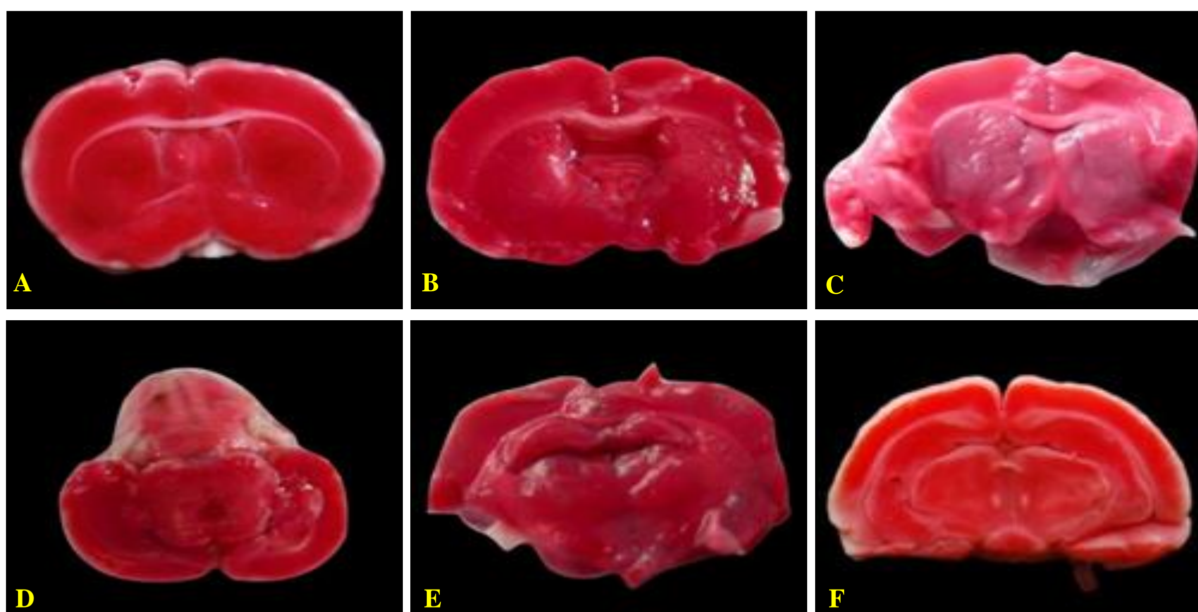


FIG. 4: NEUROPROTECTIVE EFFECT OF SOLASODINE AGAINST GLOBAL CEREBRAL ISCHEMIA/ REPERFUSION DAMAGE IN RATS EVALUATED BY 2, 3, 5-TRIPHENYLTETRAZOLIUM CHLORIDE (TTC) STAINING

Brain coronal sections were prepared (2 mm thickness) and then each section was stained with TTC. Sham (A)-Sham-operated animal; Ischemia (B)- 1.0% w/v SCMC + ischemia for 30 min; I/R (C)- 1.0% w/v SCMC + ischemia for 30 min followed by 60 min reperfusion (I/R); D and E- 30 mg/kg and 60 mg/kg + I/R of solasodine respectively. F - Quercetin 25 mg/kg + I/R; A large infarction area observed mainly in the caudal and rostral side of hippocampus in the damaged brain of ischemia/reperfusion treated rats (I/R) whereas the infarction was markedly reduced in the rat brains treated with D and E of solasodine and also in F (Q-25) (n=6).

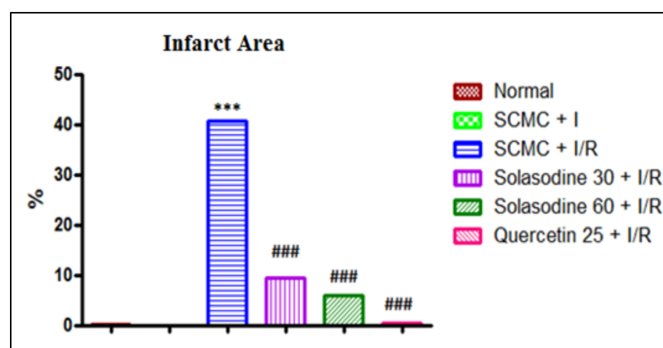


FIG. 5: MEASUREMENT OF CEREBRAL INFARCTION AREA

Sham operated rat brain showed infarction of about 0.24%. The group 1% w/v Sodium Carboxy Methyl

Cellulose (SCMC) + ischemia (I/R) (negative control) showed infarction area of 40.70 % (p<0.001) compared with normal group. The drug treatment groups Solasodine 30 mg/kg + I/R, Solasodine 60 mg/kg + I/R and Quercetin + I/R showed a significant decrease in infarction as 9.34% (p<0.001), 5.89% (p<0.001), and 0.50% (p<0.001) respectively compared with negative control group **Fig. 4** and **5**.

Histopathology:

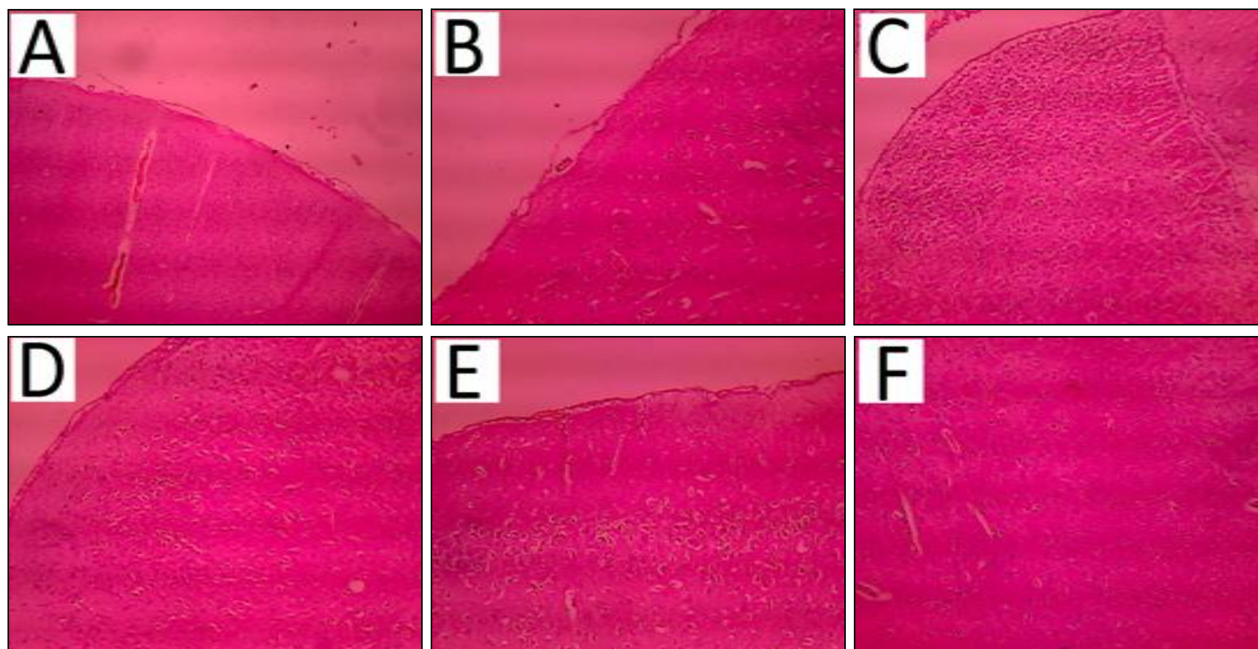


FIG. B6: NEUROPROTECTIVE EFFECT OF SOLASODINE AGAINST GLOBAL CEREBRAL ISCHEMIA/ REPERFUSION DAMAGE IN RATS

Photographs of brain sections from different treatment groups with Haemotoxylin & Eosin, staining (10x). Plates; A- normal (Sham); B- ischemia; C- I/R; D- Quercetin (25 mg/kg, i.p) + I/R; E and F- 30 mg/kg + I/R, and 60 mg/kg + I/R of solasodine respectively. Ischemia (B) caused marked congestion of blood vessels and neutrophil infiltration. These effects were further augmented by reperfusion *i.e.* lymphocytic proliferation and neuronal necrosis (C). There is a significant reversal of damage observed in solasodine treated groups (E and F) and also in Quercetin treated group (D). The reversal was marked as the values were mostly comparable with 1.0% v/w SCMC treated rats without ischemia (A).

DISCUSSION: The present study was designed to evaluate neuroprotective activity of solasodine against cerebral ischemia/reperfusion-induced brain injury in rats. Experimental models of stroke have been developed in animals to mimic the events of human cerebral ischemia. It is well documented that transient global cerebral ischemia results in neurological abnormality. Therefore, global cerebral ischemia of short duration followed by reperfusion has been employed in the present study. Quercetin has been reported to scavenge superoxide radicals in the brain cortex during reperfusion [33].

Oxygen is essential for aerobic life but is also a precursor to the formation of harmful ROS [34]. Oxidative insults, whether over-excitation, excessive release of glutamate or ATP caused by stroke, ischemia or inflammation, may initiate various signaling cascade leading to apoptotic cell death and neuronal damage [35]. Free radical involvement in the development of I/R-induced cerebral injury is well investigated, [36] among which, $O_2^{\bullet-}$ and $OH^{\bullet}$ are potent inducers of lipid peroxidation [37]. Excessive production of ROS can cause cellular damage and subsequent cell death because ROS may oxidize vital cellular components such as lipids, proteins, and DNA, [38] and alter several signaling pathways that ultimately promote cellular damage and death during cerebral I/R. In addition, ROS produces malondialdehyde (MDA), an end

product of lipid peroxidation. MDA reacts with TBA to TBARS [39]. Therefore, MDA was estimated using TBARS assay to estimate the extent of ROS. Potential neuroprotective activity of solasodine was showed by reducing the elevated LPO, and severity of oxidative damage in brain tissue was significantly reduced by increasing the levels of antioxidant enzymes SOD, CAT, and non-enzymatic markers like GSH and total thiols during ischemia/reperfusion-induced oxidative stress.

Free radicals in the living organisms are generated both enzymatically and non-enzymatically, leading to the formation of ROS. Among all the ROS generated, OH^- and $ONOO^-$ are the most potent and can cause damage proteins, lipids, and nucleic acids, resulting in the inactivation of some enzyme activities and disruption of ion homeostasis, and modification of the genetic apparatus and apoptotic death. The overproduction of free radicals can be detoxified by endogenous antioxidants, causing depletion of cellular stores of the latter [40]. During ischemia, glutamate can be released mainly through two different mechanisms. Either through the Ca^{2+} dependant and vesicular mode or through the reversed operation of glutamate transporters, NMDA receptors or G-protein pathway [41].

The reversed uptake mechanism and the neuronal glutamate-pool are believed to dominate during ischemia [42]. Physiologically, SOD reacts with $O_2^{\bullet -}$ to form H_2O_2; CAT and GSH are involved in the detoxification of H_2O_2 to form H_2O andO_2; GSH, which is considered the most prevalent and important intracellular non-protein thiol, has a crucial role as a free radical scavenger. Here, in control groups, GSH content and SOD activity were significantly reduced. Pre-treatment with solaso-dine significantly prevented BCAO induced decline in GSH content and SOD activity.

LPO, GSH, SOD, Catalase and Total thiols were estimated as an index to assess the severity of oxidative damage in the brain tissue, and also the effect of solasodine on the reversal of the damage produced by BCAo. All these parameters were markedly reversed and restored to near-normal levels in the groups pretreated with solasodine.

Glutathione is considered a central component in the antioxidant defenses of cells, acts both to directly detoxify reactive oxygen species and as a substrate for various peroxidases. Dysfunction of the glutathione system has been implicated in a number of neurodegenerative diseases [43] and is a potential contributor to oxidative damage following temporary ischemia. The antioxidant status of the tissue affected by ischemia/reperfusion is of great importance for the primary endogenous defense against free radical-induced injury. In particular, evidence exists that the SOD activity in serum is reduced in stroke patients, and the replacement of antioxidant activity could be beneficial in the acute treatment of cerebral ischemia [44].

Oxidative stress is the result of a redox imbalance between the generation of ROS and the compen-satory response from the endogenous antioxidant network. In the present study, solasodine pre-treatment was found to elevate the activity of SOD in ischemic brain. The increase in SOD activity could be due to its induction by increased production of superoxide and H_2O_2. SOD and glutathione peroxidase were thought to be two dominant enzymes acting as free radical scavengers that could prevent tissue damage caused by peroxidase reactions, and in the impairment of GSH status is the precipitating event in stroke. We looked into glutathione level after ischemic stroke. GSH is the major antioxidant in the brain. It scavenges free radicals, reduces peroxides, and can be conjugated with electrophilic compounds, thereby providing cells with multiple defenses against both ROS and their by-products.

Catalase (CAT) is a common enzyme found in all living organisms exposed to oxygen, where it has functions to catalyze the decomposition of hydrogen peroxide to water and oxygen. The decrease in the level of CAT was noted in the brain of the ischemic rats. That indicates the participation of superoxide radical, which is known to produce highly toxic hydroxyl radical through its reaction with H_2O_2. Overproduction of H_2O_2 can be inactivated by the catalase enzyme. The dose of 30 mg/kg of solasodine was ineffective to alters the CAT level in ischemic brain; this may be explained by insufficient concentration and antioxidative capacity to defend adequately against oxygen free radicals generated in the brain during ischemia.

Triphenyl tetrazolium chloride (TTC) staining has been employed in the present study to determine

the area of infarction in brain tissue. TTC is a water-soluble dye that is reduced to formazone by the enzyme succinate dehydrogenase and cofactor NAD, present in mitochondria and stain viable tissue deep red in color. Ischemic tissue with damaged mitochondria remains unstained [20]. Pretreatment of solasodine before global cerebral ischemia have markedly attenuated ischemia and reperfusion-induced cerebral infarct size. Significant reduction in infarct area indicates the neuroprotective potential of solasodine in global cerebral ischemia.

Brain neurons are highly susceptible to ischemia and reperfusion-induced injury [20]. Ischemia evoked marked congestion of blood vessels and neutrophil infiltration and these effects were further augmented in reperfusion-induced injury [19]. Section of brain showing ischemia induced changes like neuronal necrosis, leukocytes infiltration and blood vessel congestion with marked edema in the area of capsule cortex in ischemic control group. Pre-treatment of solasodine was attenuated ischemia-induced changes in dose-dependent manner.

In addition to it, pretreatment of solasodine was initiated with the evidence that the limitations of the *in-vivo* studies, over *in-vitro*, regarding the neuroprotection by free radical scavengers in particular, superoxide dismutase and catalase, the catalytic scavengers for superoxide anions or hydrogen peroxide, respectively. Modest protective effects had been observed when treatment is administered before ischemia, but little to no protection in a delayed-treatment protocol [45].

Therefore, search for agents providing protection against lipid peroxidation and enhancing antioxidant enzyme defense system should be considered a rational approach for therapy of cerebrovascular ailments. In spite of a relatively short period of ischemia (30 min), we have observed in our study a significantly higher extent of damage in the ischemia/reperfusion control group in comparison with the group being treated by solasodine. Natural products with such properties constitute an ideal choice for maximum therapeutic effect with minimal risk of iatrogenic adverse effects.

We observed direct free radical scavenging activity responsible for the antioxidant action of the solasodine. Further studies are required to pursue the interesting lead emerging from the present results to exploit the full therapeutic potential of the solasodine in cerebrovascular diseases.

CONCLUSION: In conclusion, the results showed that the solasodine has a potent neuroprotective action against the global cerebral ischemia/ reperfusion-induced oxidative damage in brain. There was a significant increase in the protective antioxidant enzyme levels (SOD & Catalase) and non-enzymatic antioxidant (GSH & total thiols) as well as a significant decrease in the lipid per-oxidation observed in the solasodine treated experimental animals.

The histopathological studies also revealed the reversal of damage in the solasodine treated animals. The protective action of solasodine was further proved by the significant reduction in the cerebral infarction area observed in the coronal brain sections. The mechanisms by which solasodine normalized the cerebral damage and stress is probably the free radical scavenging. As evidenced by the results of the present study, it seems, solasodine enhanced the defense mechanism, thereby reducing the damage produced by global cerebral ischemia/reperfusion injury.

This study suggests that solasodine may prove to be a useful adjunct in the treatment of stroke.

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