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Research Article

Modulation of Oxidative Stress Induced Cerebral Ischemia in Wistar Rats by Hydroalcoholic Extract of *Talinum triangulare*

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Abstract

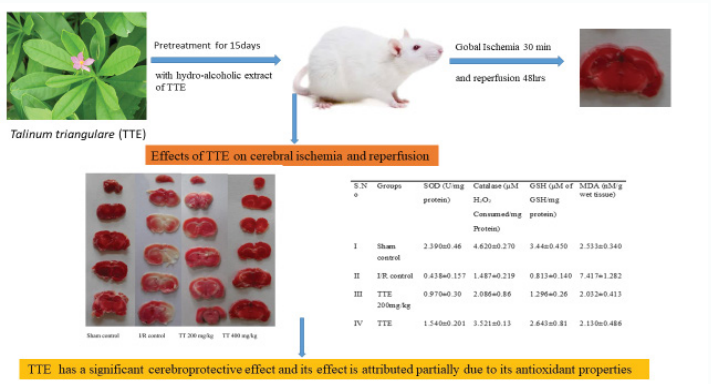
Background: Neuroprotection is one of considerable interest in search of novel therapies to improve ischemic brain damage in the present scenario. The objective of the present investigation is to study the actions of *Talinum triangulare* in cerebral ischemia/reperfusion induced oxidative stress in Wistar rats and *in-vitro* anti-oxidant actions.

Methods: Four different groups of adult Wistar rats are selected for study where group III and group IV are treated for 15 days with TTE (200 mg/kg, 400 mg/kg) earlier to ischemia, on 16th day rats are dissected through global cerebral ischemia by occlusion of unilateral common carotid artery, prior to the dissection animals are sedated with thiopentone and after ischemia induction that 30 min reperfusion was followed. Following the ischemic injury various parameters such as infarct size, SOD, MDA, CAT and GSH levels were assessed after 48 hr of reperfusion.

Results: Phytochemical screening of TTE indicated the presence of alkaloids, flavonoids, glycosides, carbohydrates, saponins, tannins and resins. TTE also showed a significant *in-vitro* anti-oxidant activity. Pre-treatment of TTE for 15 days prior to the ischemic-reperfusion, the results reported a huge reduction in reduction in infarct size, MDA levels and significant increase in SOD, CAT and GSH levels compared to ischemic control rats which was statistically significant.

Conclusion: The present study showed that hydroalcoholic extract of TTE leaves showed a significant *in-vitro* and *in-vivo* anti-oxidant activity. The present investigation reported that *Talinum triangulare* leaves extract showed dose dependant cerebroprotective effect with the selected dose of TTE 200 mg/kg and 400 mg/kg (Pictorial Abstract).

Keywords: ***Talinum triangulare*; Cerebral ischemia reperfusion; Oxidative stress; Cerebroprotection; Anti-oxidant activity**



Pictorial Abstract: TTE has a significant cerebroprotective effect and its effect is attributed partially due to its antioxidant properties.

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Abbreviations

TTE: *Talinum Triangulare* Extract; I/R: Ischemic Reperfusion; GSH: Glutathione; ROS: Reactive Oxygen Species; TTC: Tetrazolium Chloride; DPPH: 2,2-Diphenyl-1-picrylhydrazylradical; DMSO: Dimethyl Sulphoxide; SOD: Superoxide Dismutase, NBT: Nitroblue Tetrazolium; CAT: Catalase, GPx: Glutathione Peroxidase; MDA: Malondialdehyde; DTNB: 5, 5'Dithiobisnitro Benzoic Acid; ANOVA: Analysis of Variane; SEM: Standart Error of Mean

Introduction

Ischemia is the condition that causes acute ischemic stroke, where the cells are deprived of oxygen, glucose and other nutrients which

are essential for the survival of the cells. In recent years the incidence of ischemic stroke was increased due to the growth of the older global population [1]. Stroke is a serious health condition and it is the third driving cause of death in western countries. Pathogenically, various heterogeneous processes involve stroke. Occlusion of the blood vessels is the most common cause of ischemia in the brain, called "cerebral-ischemia" [2].

However, studies revealed that the damage occurred in the ischemic episode, progresses even after the revival of the blood flow [2]. The injury formed after the revival of the blood flow is called "ischemia-reperfusion injury" which can lead to permanent disability in patients [2]. Reperfusion of ischemic tissue causes oxidative stress by an increase in oxygen derived free radicals and inflammatory cells influx in the brain. Free radicals are moreover vital in drawn-out ischemia [3].

Oxidative stress leads to an imbalance between the excess generation of free radicals and impaired action of the body's antioxidant system [2]. After ischemia- reperfusion injury several free radicals generated such as ROS play an important role in neural cell damage due to ischemia [1]. Several endogenous antioxidant defence systems of the brain detoxifies the free radicals produced in the normal course of metabolism protects the brain from oxidative stress. This defence system includes molecular proteins such as Superoxide Dismutase (SOD), Glutathione Peroxidase (GPx), and catalase. Other antioxidants enzymes include reduced glutathione (GSH), α-tocopherol including ascorbic acid detoxifying free radicals produced in normal course of metabolism [4]. However this defence fails in pathological situations like ischemia reperfusion due to overproduction of the ROS than the neutralizing capability of endogenous antioxidants [5]. Transient global ischemia impairs behavioural performance associated with cognitive and motor disorders in rodents and cognition in humans [6,7].

Therefore strengthening of the brain's endogenous antioxidant defence by prophylactic treatment of the natural antioxidant-rich diet is one of the approaches to minimize reperfusion injury. *Talinum triangulare* is commonly known as waterleaf, the beneficial constituents are natural antioxidants such as flavonoids as well as phenolic compounds [8,9]. It is a earthbound herbaceous plant, cultivated as a medicinal as well as food crop in most parts of Asia including Sri Lanka, India as well as in South America and Nigeria [10,11]. Its extract was shown to have antioxidant and immunomodulatoy effects by in vitro studies. In Taiwan, *T. triangulare* has been utilized within the treatment and avoidance of hepatic sickness and cancer in people [12]. Experimental, as well as epidemiological studies demonstrate that plant flavonoids and phenolic compounds reported neuroprotective actions cerebral ischemia-reperfusion injury [13,14]. In the present study we have attempted to assess the cerebroprotective activity of *Talinum triangulare* leaf extract and to establish its antioxidant role in the cerebroprotection in the rodent model.

Methods

Chemicals and reagents

All the chemicals were obtained from Sigma Chemical, St. Louis, Mo, USA, and are of analytical grade. All the bioanalytical kits were procured from Allied Scientific Products, Kolkata, India, such as Malondialdehyde (MDA), Superoxide Dismutase (SOD), Catalase (CAT) and reduced glutathione analysis kits.

Collection, processing and extraction of TTE leaves

SV Government Polytechnic College, Tirupathi, Chittoor (dist). A.P., India was the site of plant material collection and plant material was authenticated by Dr. B Sitaram, Professor/ Senior Consultant, Department of Dravyaguna, S.V. Ayurvedic Medical College, Tirupati (Figure 1).

The collected plant material was shade dried and pulverised to powered form. 500 g of pulverised leaves were subjected to cold maceration in 100 ml of hydro-alcoholic (30:70; Water: Ethanol) mixture for 72.

The solvent elimination was carried by using concentrated filtrate in a water bath; the leftover residue was stored and used when required.

Anti-oxidant activity: *In-vitro* method

2,2-Diphenyl-1-picrylhydrazylradical scavenging assay: DPPH activity of TTE experiment was carried out by standard procedure described by Braca et al. [15] with ascorbic acid set as standard and comparing the TTE activity with that of standard, DPPH activity of TTE was assessed after preparing different concentration of the test sample by dissolving the plant extract in Dimethyl Sulphoxide (DMSO) at different concentrations (15.6 µg/ml to 1000 µg/ml). Absorbance was recorded at 517 nm.

Superoxide radical scavenging: The SOD activity of TTE is estimated by the standard reported method developed by McCord and Fridovich [16,17]. Using UV absorption light at 560 nm, induction of superoxide free radical generation by riboflavin and subsequent reduction by Nitroblue Tetrazolium (NBT) was calculated.

Hydroxy free radical scavenging: Hydroxyl radical scavenging activity was estimated by the method developed by Kunchandy and Rao et al. [18], the $Fe^{+2}H_2O_2$ system generates the hydroxyl radicals which are calculated by using UV absorption at 532 nm.

Selection of animals and toxicology studies: The animals used in the study were procured from Raghavendra enterprises, Bangalore. Wistar rats of both sexes weight range 230 g to 300 g were divided into groups of 6-7 and housed in colony cages at suitable conditions such as an ambient temperature of 25°C ± 2°C and 45% to 55% relative humidity with 12 h light/dark cycle. The rats are also supplied with standard feed pellet chow (Pranav Agro Limited) and water *ad libitum*. The present experimental study protocol was approved by the ethical committee Institutional Animal Ethical Committee of Sri Padmavathi School of pharmacy (Approval No: 1016/a/06 /CPCSEA/04/2012 dt 09-12-2012) CPCSEA New Delhi, Government of India, under the oversight of the Committee for the Purpose of Control and Supervision of Experiments on Animals. Fresh plant materials were collected, air

Figure 1: *Talinum triangulare*.

dried and pulverized leaves were subjected to cold maceration in 100 ml of hydro-alcoholic (30:70; Water: Ethanol) mixture for 72 hrs. The filtrate was processed for solvent extraction in a water bath; the leftover residue was used for experimental study.

Acute toxicity studies: OECD-423 (Organisation for Economic Co-operation and development, 2001) guidelines were followed to perform acute toxicity studies of TTE. The animals were fasted overnight supplied only water, after which the extract was administered orally at the dose level of 2000 mg/kg weight by gastric intubations and animals are kept under observation for 24 hrs for any change in behavioural profile, neurological profile and autonomic profile. After dosing, any change in the animals was noted initially at least once during the first 30 min and during the first 24 hrs for any chances of toxic symptoms or mortality. The acute toxicity studies of the selected plant extracts showed no symptoms of toxicity or behavioural changes at the maximum dose (4000 mg/kg). Hence 1/10th of the maximum dose *viz.* 400 mg/kg b.wt and submaximal dose 200 mg/kg were set as high dose for further studies.

Dose Selection: The doses were selected based on the acute toxicity studies as well as previous experimental studies in animals with *Talinum triangulare*. The acute toxicity studies of the *Talinum triangulare* Leaves Extracts (TTLE) showed no symptoms of toxicity or behavioural changes at the maximum dose (4000 mg/kg). Hence 1/10th of the maximum dose *viz.* 400 mg/kg b.wt and sub-maximal dose 200 mg/kg were set as dose for present studies.

Experimental Protocol

Determination of infarction size

Determination of infarct size in rats was carried out as described in earlier studies [16]. Following 48 hrs of reperfusion brains of rats were isolated by cervical dislocation and frozen for 5 min at -4°C. Thereafter, coronal sections were made to have sections of 1 mm to 2 mm which were drenched in 1% 2,3,5-Triphenyltetrazolium Chloride Solution (TTC) at a temperature of 37°C for 30 min. TTC was converted into red formazone pigment by the enzymes present in viable cells i.e., NAD and dehydrogenase producing living cells deep red colour and un-stained for infracted cells due to exhaustion of these enzymes. The slices of the brain were weighed. The unstained infracted portions were separated from the slices by examination and expressed as percentage of total weight of the brain.

Preparation of brain tissue for evaluating various biochemical parameters

After 48 hr reperfusion, the brain of each animal was isolated and washed with cold saline, after drying the tissue on filter paper, it was homogenized with buffer solution using phosphate buffer 0.1 M prepared at pH 7.4 using homogenizer and centrifuged at a speed of 1000 rpm using cold centrifuge at a temperature of 4°C for 3 min. The supernatant portion was divided into two parts; one part was utilized for estimation of MDA. The left over supernatant was again centrifuged at 12,000 rpm, maintaining the above conditions for15 min which was utilized for the estimation of superoxide dismutase, catalase and reduced glutathione.

Estimation of MDA level

Procedure: Malondialdehyde levels in brain tissue were estimated by the Ohkawa et al. [19] method. To the brain tissue homogenate TBA solution, water and a mixture of butanol and pyridine ((15:1 v/v)) was added and absorbance was measured at 532 nm. Values of MDA are represented as nmol/g tissue with reference of the standard curve.

Estimation of Superoxide Dismutase (SOD)

Procedure: Superoxide Dismutase (SOD) assay was estimated by Kakkar et al. [20] method. Using 0.1 M ice cold phosphate buffer brain tissue was homogenized to produce 10% w/v homogenate which was centrifuged at 12,000 rpm for 15 min for 4°C. To 0.1 ml of supernatant sodium pyrophosphate buffer (pH 8.3), phenazine, methosulphate, nitroblue tetrazolium and NADH was added and incubated at 30°C, to this reaction mixture glacial acetic acid was added to stop the reaction to which glacial acetic acid was added and centrifuged, organic layer was separated and absorbance was measured at 560 nm. The SOD level was represented as Units/mg protein.

Estimation of catalase

Procedure: Catalase assay was performed according to the method developed by Aebi [21]. Ice cold phosphate buffer (0.1M, pH 7.4) was used to homogenize the brain tissue using homogenizer to produce a 10% w/v homogenate. The obtained homogenate was separated by centrifugation at 12,000 rpm at 4°C for 15 min. 0.1 ml of was added to the cuvette containing 1.9 mL of 50 Mm phosphate buffer. To this solution freshly prepared 30 mM H_2O_2 of 1.0 mL was added and change in absorbance was observed at an interval of 30 sec at 240 nm for 3 min. A control was prepared using 0.1 mL of distilled water without homogenate solution. Activity of catalase was expressed as µ moles of H_2O_2 metabolized/mg protein/min.

Determination of glutathione

Glutathione (GSH) levels were assayed according to the method described by Ellman et al. [22]. Mixed solutions of homogenate (w/v) and 10% TCA of equal measures were centrifuged to separate the proteins. 0.01 ml of this supernatant, 2 ml of phosphate buffer (pH 7.4), 0.5 ml of DTNB and 0.4 ml of double distilled water were mixed and absorbance was read at 412 nm within 15 min. The enzyme level was assayed as nM glutathione oxidized/min/mg protein. Reduced glutathione levels were expressed as µ moles of GSH/mg protein [20].

Statistical analysis

All the results were expressed as (Mean ± SEM). Statistical differences between groups were determined by one way ANOVA. Differences between groups were compared using Tukey's test where $p<0.05$ was considered as statistically significant. Statistical analysis was performed using Prism software (Version 5.0).

Results

The hydro alcoholic extract of TTE was evaluated for its *in-vitro* anti-oxidant activity compared with the standard ascorbic acid. The *in-vitro* anti-oxidant effect was represented in the Figure 2 the graph plotted between log concentration and % inhibition of respective free radicals. The present study leaf extract of TTE showed a significant effect in inhibiting DPPH compared with the standard ascorbic acid which was shown in Figure 2. The hydro alcoholic extract of TTE showed a significant effect on inhibiting lipid peroxidation effect which was shown in Figure 2. Similarly the TTE extract also showed a significant effect on inhibiting nitric oxide radical which was also shown in Figure 2.

Potency of TTE extract was evaluated with pre-treatment of extract and evaluated after cerebral ischemia. The *in-vitro* results found that TTE significantly improved the inhibition of free radicals which was also confirmed by *in-vivo* methods.

Effect of TT extract on % infarct size after ischemia-reperfusion

Significant cerebral damage was observed in the I/R control group (47.52 ± 2.45) when compared to the sham control (1.57 ± 0.178) which was represented as percent infarct size after reperfusion shown in Figure 3. The % reduction of infarctions is 38.14 ± 2.13 and 30.54 ± 2.57 for the doses 200 mg/kg and 400 mg/kg of TTE extract, when compared to the I/R control group TTE showed cerebro-protective action. The reduction of infarction is dose dependent and the TTC stained brain sections were represented in Figure 4.

Effect of TT extract on Superoxide Dismutase (SOD) levels (U/mg Protien)

There was a significant decline in superoxide dismutase levels in I/R control rats (0.438 ± 0.157) when compared to sham control rats (2.390 ± 0.46) which was represented in Figure 5. Significant elevation of SOD levels was detected in animals treated with TT extract and the effect is dose dependent as shown in the Figure 5.

Effect of TTE extracts on Catalase (CA) levels (μM H_2O_2 consumed/mg Protein)

The results of CA levels are presented in Figure 5. Catalase levels were significantly decreased in I/R control rats (1.487 ± 0.219) when compared to sham control rats (4.62 ± 0.27). In animals treated with TTE extract showed a significant elevation of catalase levels in a dose dependent manner as shown in Figure 5.

Effect of TTE extract on Malondialdehyde levels (MDA) (nM of MDA/g wet tissue)

MDA is an index of lipid peroxidation. MDA level was significantly increased in I/R control rats (7.417 ± 1.282) when compared to sham control rats (2.533 ± 0.34). Figure 5 show the results of MDA in different groups. Significant reduction of MDA levels was detected in animals treated with TTE extract in a dose dependent manner as shown in the Figure 5.

Effect of TTE extracts on Glutathione (GSH) levels (μM GSH consumed /mg Protien)

The levels of Glutathione were significantly decreased in I/R control rats (0.813 ± 0.14) when compared to sham control rats (3.44 ± 0.45). Significant elevation of GSH levels was reported in groups treated with TTE extract 200mg/kg, 400mg/kg as shown in the Figure 5.

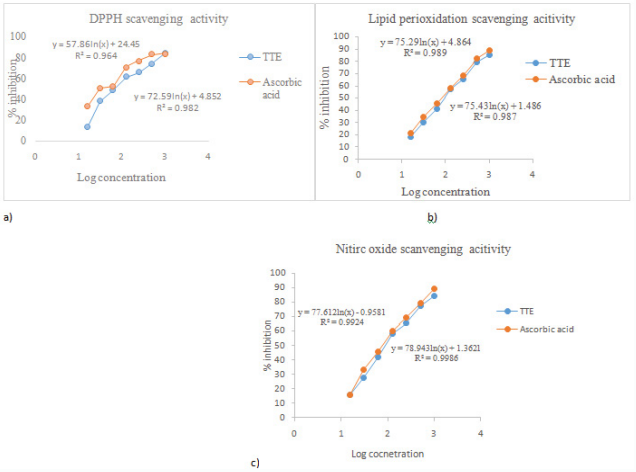

Figure 2: *In-vitro* activity of alcoholic extract of *Talinum triangulare* a) DPPH scavenging activity b) Lipid peroxidation scavenging activity c) Nitric oxide scavenging activity.

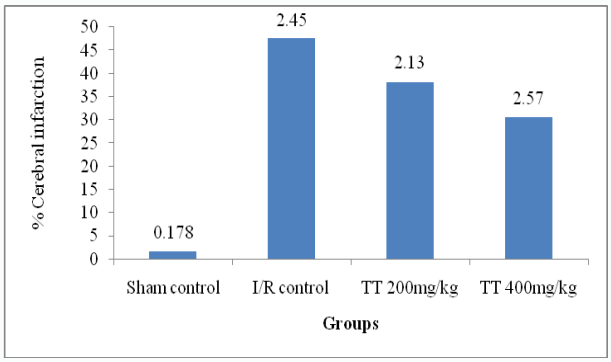

Figure 3: Effect of TTE extract on percentage infraction of rat's brain to follow by reperfusion. The values are expressed as mean±SEM (n=6) and the results are compare with in ischemic com control group.

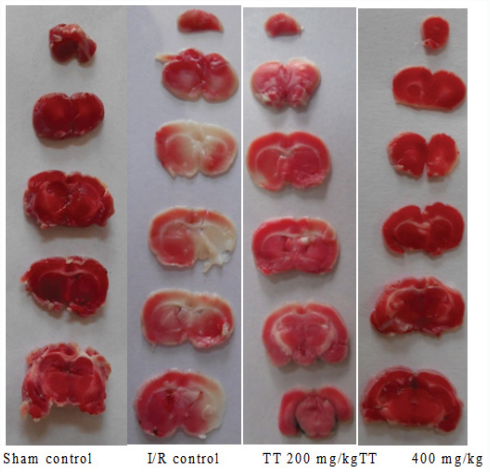

Figure 4: Showing the effect of TTE stained sections of brain tissue in rats subjected to global ischemia followed by reperfusion: I/R group has pale staining with TTC compared to other groups which determines the neural damage.

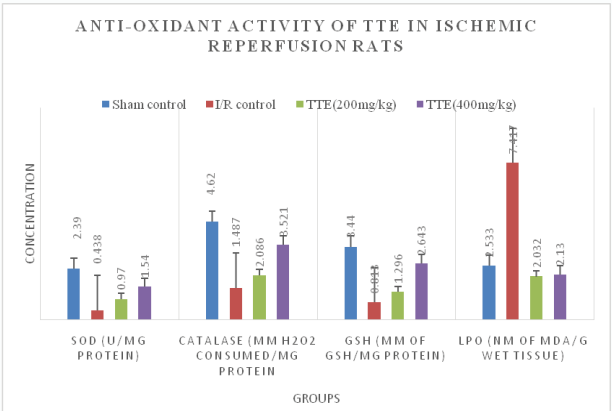

Figure 5: Graph showing cerebral biochemical parameters changes in different groups.

Discussion

New therapeutic agents are developed from compounds extracted and isolated from plants. In this investigation, hydro alcoholic extract of TTE leaves exhibited free radical (DPPH, nitric oxide, lipid peroxide radicals) scavenging activity which may be related to phenolic compounds present in the leaves. As reactive oxygen species are important contributors to several disorders in the body, the hydro alcoholic extract of TTE might be useful for the development of newer and potent natural antioxidants that are useful in preventing oxidative stress related degenerative diseases. In 2015, Liao et al. [23] also reported that stem extracts of TTE showed potent antioxidant activities due to its flavonoid and phenolic compounds. Based on these results, further studies were carried out on this plant for evaluation of *in vivo* cerebroprotective potential.

The main cause for as many as 100 human diseases including cancer worldwide is oxidative stress. Various pathways are responsible for the cause of brain injury in ischemia-reperfusion. Several past studies demonstrate that ROS (oxygen free radicals) play the major character in the pathophysiology of ischemia-reperfusion injury and that they are elevated in cerebral ischemia-reperfusion [21,22]. In this research, the pre-treatment with *Talinum triangulare* leaf extract was evaluated after cerebral ischemia and the extract showed a great capability in reducing I/R injury where *in-vitro* studies showed that TTE has better capability in reducing the expression of free radicals, hence *Talinum triangulare* leaves were tested against cerebral ischemia which causes brain damage due to oxidative stress. The extent of infarction size is an indication of cerebral damage. In contrast, group 1 has significant cerebral damage was observed in I/R control rats, which was observed in Figure 4. The percentage of infraction was significantly diminished in groups treated with the TTE in when correlated to the I/R control rats and the protection was dose dependent [23].

A variety of mechanisms such as glutamatergic excitotoxicity, oxidative stress, cytokine effects and inflammatory injury etc. are responsible for damage in reperfusion. Oxidative stress results from failure of endogenous antioxidant defense [24]. Since the plant extract had shown antioxidant and lipid peroxidation inhibiting properties in *in vitro* studies, the cerebroprotective effect of the *Talinum triangulare* may be due to its antioxidant role [25,26].

The cerebropotective property of the TTE may be attributed to its antioxidant property due to its flavonoid content [27,28]. To investigate the mechanism of protection of TTE extract against the ischemic reperfusion injury, the parameters such as SOD, CAT and GSH were measured in the brain tissue of rats subjected to ischemia-reperfusion [29,30]. MDA is a final product of lipid peroxidation of poly unsaturated fatty acids and it is an indication of lipid peroxidation.

The findings indicate an elevation in tissue MDA levels is in coordinated to increase in infraction size in I/R group relative to the sham control [31,32]. There was a significant reduction in the lipid peroxidation index in TTE treated groups as shown in Figure 5 which was also reported by Olarewaju M. Oluba et al. [33] in his study on STZ-induced diabetic rats.

Mammalian cells have antioxidant defense within it to fight against the ROS but their strength is very limited. Enzymes like SOD inactivate superoxide into hydrogen peroxide, and catalase convert hydrogen peroxide into water and molecular oxygen [27-31]. The reduced GSH is an antioxidant or free radical scavenger which is present in all mammalian cells. Since the antioxidant defence is limited in mammalian cell, they exhaust rapidly when there is surge of ROS which occurs in I/R [33]. Thus, during cerebral ischemia-reperfusion, dose dependent alteration in antioxidant enzyme activity might be responsible for neural death other than ischemia alone as shown in Figures 4 and 5. The treatment with hydro alcoholic extract of *Talinum triangulare* leaves in our present study resulted in elevation of endogenous antioxidant enzymes such as superoxide dismutase and Catalase which suggest increased biochemical protection to scavenge the overproduced reactive oxygen species which was also reported in several past studies (Tables 1-3).

Conclusion

The current study shows an increase in the elevated levels of SOD, CAT as implied from I/R brain homogenate of rats treated with TTE which shows that hydro alcoholic extract of *Talinum triangulare* leaves have a potent cerebroprotective effect which might be due to TTE antioxidant activity.

Acknowledgement

All the authors are thankful to the Faculty of Sri Venkteswara polytechnic college, Tirupathi and Prof. B. Sitaram, Department of Dravyaguna, S.V. Ayurvedic Medical College for their constant support to carry out this research work. The authors also acknowledge the staff of Andhra University for their support in the present research.

Compliance with Ethical Standards

National, international and/or institutional guidelines for the care and use of animals was followed for the present research. An ethical standard of the institution or practice was followed for the experimental procedure in studies involving animals in the present research.

Summary

- The Hydroalcoholic extract of TTE reported cerebroprotective effect in albino rats against oxidative stress induced due to ischemic reperfusion in rats.
- The release of free radicals caused brain injury was significantly attenuated by treatment with hydroalcoholic extract of *Talinum triangulare* 200 mg/kg and 400 mg/kg b.wt in rats.
- The *in vitro* antioxidant properties on TTE were proved by DPPH scavenging activity, hydroxyl radical activity, nitric oxide radical activity.
- Benefits of TTE on oxidative stress induced brain damage was proved by decreased lipid peroxidation and increased SOD, catalase and glutathione in rat brain tissues.
- Hydro alcoholic extract of *Talinum triangulare* leaves in our present study resulted in elevation of SOD and CAT which are endogenous antioxidant enzymes indicates the beneficial effects of TTE in ischemic brain damage.

References

1. Prakash T, Kotresha D, Nedendla RR. Neuroprotective activity of *Wedelia calendulacea* on cerebral ischemia/reperfusion induced oxidative stress in rats. Ind J Pharmacol. 2011;43(6):676-82.
2. Zitnanova I, Siarnik P, Kollar B, Chomova M, Pazderova P, Andrezalova L, et al. Oxidative Stress Markers and their Dynmaic Changes in Patients after acute ischemia stroke. Oxid Med Cell Longev. 2016;2016:9761697.
3. Macdonald RL, Stoodley M. Pathophysiology of Cerebral Ischemia. Neurol Med Chir (Tokyo). 1998;38(1)1-11.

Table 1: *In-vitro* antioxidant activity of TTE at different doses.

Conc. of TTE µg/ml	% inhibition on			Conc. of ascorbic acid µg/ml	% inhibition on		
	Lipid peroxidation free radical	Nitric oxide free radical	DPPH free radical		Superoxide free radical	Hydroxyl free radical	DPPH free radical
15.6	15.63 ± 0.32	18.54 ± 0.22	13.14 ± 2.21	15.6	15.82 ± 0.14	21.33 ± 0.31	33.11 ± 2.51
31.2	27.34 ± 0.13	30.05 ± 0.82	38.05 ± 2.39	31.2	33.42 ± 0.72	34.56 ± 0.42	50.91 ± 1.95
62.5	42.15 ± 0.52	41.63 ± 0.28	49.65 ± 3.36	62.5	46.16 ± 0.52	45.64 ± 0.23	52.70 ± 1.89
125	58.42 ± 0.21	57.26 ± 0.43	61.42 ± 4.02	125	60.58 ± 0.14	58.55 ± 0.44	71.31 ± 2.54
250	65.53 ± 0.54	65.32 ± 0.13	65.58 ± 3.17	250	69.32 ± 0.35	68.87 ± 0.52	77.34 ± 2.87
500	77.67 ± 0.42	79.52 ± 0.33	74.99 ± 2.07	500	79.48 ± 0.62	82.46 ± 0.21	83.14 ± 3.07
1000	84.54 ± 0.23	85.45 ± 0.24	84.70 ± 2.86	1000	89.22 ± 0.75	89.32 ± 0.54	84.45 ± 1.52

All the values are represented as Mean ± SEM.

Table 2: Showing the effect of TTE on cerebral infraction in rats subjected to global ischemia followed by reperfusion in all the four groups.

Group (n=6)	Cerebral infarction	% Reduction of infarction
Sham control	1.57 ± 0.178	-
I/R control	47.52 ± 2.45	-
TT extract 200 mg/kg)	38.14 ± 2.13	19.74
TT extract (400 mg/kg)	30.54 ± 2.57	35.75

P<0.05, all values expressed in mean ± SEM (n=6). I/R indicate ischemia-reperfusion and TTE (Hydro-alcoholic extract of *Talinum triangulare* leaves).

Table 3: Showing the effect of TTE on SOD, Catalase, GSH and MDA levels in rats subjected to global ischemia followed by reperfusion in all the four groups.

S.No	Groups	SOD (U/mg protein)	Catalase (µM H2O2 Consumed/mg Protein)	GSH (µM of GSH/mg protein)	MDA (nM/g wet tissue)
I	Sham control	2.390 ± 0.46	4.620 ± 0.270	3.44 ± 0.450	2.533 ± 0.340
II	I/R control	0.438 ± 0.157	1.487 ± 0.219	0.813 ± 0.140	7.417 ± 1.282
III	TTE200 mg/kg	0.970 ± 0.30	2.086 ± 0.86	1.296 ± 0.26	2.432 ± 0.413
IV	TTE400 mg/kg	1.540 ± 0.201	3.521 ± 0.13	2.643 ± 0.81	2.00 ± 0.486

P<0.05, all values expressed in mean ± SEM (n=6). I/R indicate ischemia-reperfusion, SOD (Superoxide Dismutase), CAT (Catalase), GSH (Reduced Glutathione), LPO (Lipid Peroxidation), MDA (Malondialdehyde) and TTE (Hydro-alcoholic extract of *Talinum triangulare* leaves).

4. Nishigaya K, Yoshida Y, Sasuga M, Nukui H, Ooneda G. Effect of recirculation on exacerbation of ischemic vascular lesions in rat brain. Stroke. 1991;22(5):635-42.

5. Adibhatla RM, Hatcher JF. Phospholipase A2, reactive oxygen species and lipid peroxidation in cerebral ischemia. Free Radic Biol Med. 2006;40(3):376-87.

6. Bora KS, Sharma A. Evaluation of antioxidant and cerebroprotective effect of *Medicago sativa* Linn. against ischemia and reperfusion Insult. Evid Based Complement Alternat Med. 2011;2011:792167.

7. Langdon KD, Granter-Button S, Corbett D. Persistent behavioural impairments and neuroinflammation following global ischemia in the rat. Eur J Neurosci. 2008;28(11): 2310-8.

8. Jokinen H, Kalska H, Mantyla R, Pohjasvaara T, Ylikoski R, Hietanen M, et al. Cognitive profile of subcortical ischaemic vascular disease. J Neurol Neurosurg Psychiatry. 2006;77(1):8-33.

9. Dominguez C, Delgado P, Vilches A, Martín-Gallán P, Ribó M, Santamarina E, et al. Oxidative stress after thrombolysis-induced reperfusion in human stroke. Stroke. 2010;41(4):653-60.

10. Olorunnisola OS, Adetutu A, Afolayan AJ, Owoade AO. Effect of Methanolic Leaf Extract of Talinumtriangulare(Jacq). Willd. on Biochemical Parameters in Diet induced DyslipidemiaWistar Rats. Pharmacogn Mag. 2016;12(48):333-9.

11. Kumar A, Prasa MNV, Sytar O. Lead toxicity, defence and associated indicative biomarkers in Talinumtriangularegrown hydroponically. Chemosphere. 2012;89(9):1056-65.

12. Liang D, Zhou Q, Gong W, Wang Y, Nie Z, He H, et al. Studies on the antioxidant and hepatoprotective activities of polysaccharides from *Talinum triangulare*. J Ethnopharmacol. 2011;136(2):316-21.

13. Pu F, Mishima K, Irie K, Motohashi K, Tanaka Y, Orito K, et al. Neuroprotective effects of quercetin and rutin on spatial memory impairment in an 8-arm radial maze task and neuronal death induced by repeated cerebral ischemia in rats. J Pharmacol Sci. 2007;104(4):329-34.

14. Sun AY, Wang Q, Simonyi A, Sun GY. Botanical phenolics and brain health. Neuromol Med. 2008;10(4):259-74.

15. Braca A, Fico G, Morelli I, Simone FD,Tome F, De Tommasi N. Antioxidant and free radical scavenging activity of flavonol glycosides from different Acontium species. J Ethnopharmacol. 2003;86(1):63-7.

16. McCord JM. Oxygen-derived free radicals in postischemic tissue injury. N Engl J Med. 1985;312(3):159-63.

17. McCord JM, Fridovich I. Superoxide dismutase: an enzymatic function for erythrocuprein (hemocuprien). J Biol Chem. 1969;244(22):6049-55.

18. Kunchandy E, Rao MNA. Oxygen radical scavenging activity of curcumin. Int J Pharm.1990;58(3):237-40.

19. Ohkawa H, Ohishi N, Yagi K. Assay of lipid peroxides in animals tissue by thiobarbituric acid reaction. Anal Biochem. 1979;95(2):351-8.

20. Kakkar P, Das B, Viswanathan PN. A modified spectrophotometric assay of superoxide dismutase. Ind J Biochem Biophys. 1984;21(2):130-2.

21. Aebi H. Catalase. In. Bergmeyer HU ed. Methods in Enzymatic Analysis. Chemic Academic Press. 1974;673-85.

22. Ellman GL. Tissue sulphydryl groups. Arch Biochem Biophys. 1959;82(1):70-7.

23. Liao DY, Chai YC, Wang SH, Chen CW, Tsai MS. Antioxidant activities and contents of flavonoids and phenolic acids of *Talinum triangulare* extracts and their immunomodulatory effects. J Food Drug Anal. 2015;23(2):294-302.

24. Bederson JB, Pitts LH, Tsuji M, Nishimura MC, Davis RL, Bartkowski H. Rat middle cerebral artery occlusion: evaluation of the model and development of a neurologic examination. Stroke. 1986;17(3):472-6.

25. Wang C, Zhang D, MA H, Liu J. Neuroprotective effects of emodin-8-O-beta-D-glucoside *in vivo* and *in vitro*. Eur J Pharmacol. 2007;577(1-3):58-63.

26. Afolabi OB, Oloyede OI. Antioxidant Properties of the Extracts of *TalinumTriangulare* and its Effect on Antioxidant enzymes in Tissue Homogenate of Swiss Albino Rat. Toxicol Int. 2014;21(3):307-13.

27. Li W, Yang S. Targeting oxidative stress for the treatment of ischemic stroke: Upstream and downstream therapeutic strategies. Brain Circ. 2016;2(4):153-63.

28. Woodruff TM, Thundyil J, Tang SC, Sobey CG, Taylor SM, Arumugam TV. Pathophysiology, treatment and animal and cellular models of human ischemic stroke. Molecular Neurodegener. 2011;6(1):11.

29. Amorim APO, de Oliveira MCC, Amorim TA, Echevarria A, Antioxidant, Iron Chelating and Tyrosinase Inhibitory Activities of Extracts from *Talinum triangulare* Leach Stem. Antioxidants (Basel). 2013;2(3):90-9.

30. Hu B, Sun S, Mei G, Chen L, Tong E. Protective effects of Ginkgo biloba extract on rats during cerebral ischemia/reperfusion. Chinese Med J (Engl). 2002;115(9):1316-20.

31. Kumari NK, Panigrahi M, Prakash BP. Changes in endogenous antioxidant enzymes during cerebral ischemia and reperfusion. Neurol Res. 2007;29(8):877-83.

32. Li W, Yang S. Targeting oxidative stress for the treatment of ischemic stroke. Upstream and downstream therapeutic strategies. Brain Circ. 2016;2(4):153-63.

33. Oluba OM, Adebiyi FD, Odutuga AA. Effects of Talinumtriangulare leaf flavonoid extract on streptozotocin-induced hyperglycemia and associated complications in rats. Food Sci Nutr. 2018;7(2):385-94.