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COMPARATIVE NEUROPROTECTIVE POTENTIALS OF THE EXTRACTS OF *TALINUM TRIANGULARE* AGAINST NITROPRUSSIDE-INDUCED DAMAGES IN ALBINO RATS

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ABSTRACT: The inhibitory effects of ethyl acetate, aqueous and ethanolic extracts of *Talinum triangulare* were well observed in the brain homogenate of Swiss albino rats against sodium nitroprusside induced-peroxidation by thiobarbituric acid reactive species (TBARS) *in-vitro*. All the extracts showed significant inhibitory abilities against sodium-nitroprusside (SNP) induced lipid peroxidation in the brain homogenate when incubated with 60mM sodium nitroprusside. In the current work, aqueous extract of *Talinum triangulare* seems to possess higher antioxidant contents with the fact that it shows the highest inhibition potential against SNP-induced peroxidation in the brain tissue homogenate from the results, thereby characterize the ability to protect the brain cell and as well able to prevent nitrosative stress induction at considerable measure in the brain.

INTRODUCTION: Plants are highly rich in antioxidants that are chemically low molecular mass products which eliminate reactive nitrogen species (RNS) and reactive oxygen species (ROS) [1, 2]. *Talinum triangulare* is an herbaceous perennial plant that is a native of tropical America to Mexico and one of the most important vegetables, an erect herb with swollen roots and succulent stems [3].

The consumption of dietary antioxidants from vegetables and fruits is beneficial in preventing common neurodegenerative diseases including; Parkinson's and Alzheimer's diseases [4].

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Sodium nitroprusside in an aqueous medium at physiological pH 7.0 spontaneously generates NO radical, which interacts with oxygen to produce nitrite ions in nitrosative stress state. Scavengers of NO° compete with oxygen, leading to a reduction in the production of NO radicals. Nitrosative stress represents the bodies' imbalance in the production and the elimination of reactive oxygen and nitrogen species and various reducing or antioxidant chemical systems of the body which destroy reactive intermediates that prevent or repair the likely impairment [5].

The resulting reactive nitrogen oxide species and di-nitrogen tri-oxide (N_2O_3) target specific functional factors such as thiols, lysine active sites, and zinc fingers and is dependent upon both the rates of production as well as consumption of NO in the system. Recent studies suggest that NO can affect different neuroprotective pathways through *S*-nitrosylation, since oxidative and nitrosative

stress plays an important role in neurodegenerative disorder development. Mitochondrial complex I dysfunction and increased indices of oxidative stress play major roles in marking Parkinson disease [6, 7]. Therefore, countering ROS and RNS-mediated toxicity would be an essential and integral part of neurodegenerative disease therapy [8].

Nitric oxide has long been recognized as a signaling molecule for vasodilation and neurotransmission. During signal transduction, NO activates soluble guanylate cyclase (sGC) to generate cGMP which acts as a second messenger to induce vasodilation [9, 10, 11]. Excessive stimulation of N-methyl-d-aspartate receptor (NMDAR) can as well leads to activation of neuronal nitric oxide synthase (nNOS) which produces an excess amount of nitric oxide (NO). Nitric oxide can react with O_2^- to form the more reactive peroxynitrite ($ONOO^-$) which is the major cause of neurodegenerative nitrosative-stress [12, 13].

The current study relates the effects of three different extracts of *Talinum triangulare* against neurodegenerative activities of nitroprusside-induced stress in the brain tissue of albino rats.

MATERIALS AND METHODS:

Plant Materials and Preparation: Freshwater leaves, *Talinum triangulare* were bought in Ado-Ekiti, Ekiti State, Nigeria. A sample was taken to the Department of Plant Science in Ekiti State University, Ado-Ekiti, Ekiti State, Nigeria for identification, it was identified by a taxonomist in the department and allotted to it the Herbarium number UHAE 2013/76, after proper taxonomic investigations from the database.

Chemicals: Chemicals and reagents used such as thiobarbituric acid (TBA)were all sourced from BDH Chemicals Ltd., (Poole, England), Tris-HCl buffer, Sodium dodecyl sulfate (SDS), $FeSO_4$ (Iron II sulphate). All other chemicals were of analytical grades and prepared using sterilized distilled water.

Preparation of Sample:

Preparation of 70% Ethanolic Extracts: The leaves (edible) parts of the plant were air-dried in a ventilated place at an ambient temperature of 30 ± 2 °C for 15 days pulverized using a laboratory blender, and the fine powders obtained stored at moderate temperature until further use. 120g of the powdered sample was weighed and used for the extraction with a 1litre solvent combination of 70% ethanol for 72 h. The crude extract was later subjected to bioassay analyses. From the stock solution, different concentrations were obtained.

Preparation of Ethyl Acetate Extract: 5 g ethanolic extract was reconstituted in 50ml of distilled water; to this was added 25ml of petroleum ether (Pet. ether). This was mixed thoroughly, turned into separating funnels (250 ml) and left to stand 30 min. The extract has very minimal and very low fat. The aqueous top layer was removed and to the fraction was added 33.3 ml of ethyl acetate to wash. This procedure was repeated for the second time, and the total mixture measured up to 310 ml and evaporated to dryness using rotary evaporator cum water bath to obtain a total yield of 2 g. The crude extract was later subjected to bioassay analyses. From the stock solution, different concentrations were obtained respectively.

Preparation of Aqueous Extract: 50 g of the powdered sample was extracted in 500 ml distilled water for 48 h. The crude extract was later subjected to bioassay analyses. From the stock solution, different concentrations were obtained respectively. These were stored until further use. The percentage yield of the extracts was calculated as follows.

% yield = Extract obtained in mg × 100 / Crude plant sample in mg

Determination of Lipid Peroxidation in Tissues by Thiobarbituric Reactive Acid Species (TBARS) *in - vitro* Assay: The lipid peroxidation assay was carried out using the method described by Puntel *et al*., 2005. The rats were killed by cervical dislocation. Liver and brain tissues were quickly removed and placed on ice. One gram of tissues were homogenized in cold 0.1M Tris-HCl buffer pH7.4 (1:10w/v) in a Teflon homogenizer. The homogenates were centrifuged for 10 min at 3000g to yield a pellet that was discarded and the supernatant was used for the assay. The supernatant with or without 50 µl of the freshly prepared pro-oxidant (sodium nitroprusside), different concentrations of the plant extracts and an appropriate volume of distilled water which gives a

total volume of 300 µl were pre-incubated at 37 ºC for 1 h. The color reaction which is the second phase was carried out by adding 200 µl of 8.1% sodium dodecyl sulphate (SDS), 500 µl of 1.33M acetic acid (pH 3.4) and 500 µl of 0.6% TBA respectively and the reaction mixture was incubated at 97 ºC for 1 h. The absorbance was read at 532 nm.

RESULTS:

TABLE 1: INHIBITORY EFFECT OF ETHANOLIC EXTRACT OF *TALINUM TRIANGULARE* (TT) ON SODIUM NITROPRUSSIDE-INDUCED LIPID PEROXIDATION IN TISSUE HOMOGENATES

Treatment Groups	Conc. (µg/ml)	% Inhibition in Brain	Log. Equation (r^2)	IC_{50} (µg/ml)
Normal	-	36.03 ± 7.67	Y= 7.4991In(x)+26.628	0.83 ± 0.04
SN + TT	3.33	26.33 ± 8.45	r^2 = 0.9638	
SN + TT	2.67	33.07 ± 9.22		
SN + TT	1.33	33.77 ± 7.81		
SN + TT	0.67	36.57 ± 13.88		
SN + TT	0.33	39.30 ± 17.48		

*****Results are expressed as means of three experiments in duplicate ± standard deviation, TT- *Talinum triagulare*.

TABLE 2: THE INHIBITORY EFFECT OF ETHYL ACETATE EXTRACT OF *TALINUM TRIANGULARE* (TT) ON SODIUM NITROPRUSSIDE-INDUCED LIPID PEROXIDATION IN TISSUE HOMOGENATES

Treatment Groups	Conc. (µg/ml)	% Inhibition in Brain	Log. Equation (r^2)	IC_{50} (µg/ml)
Normal	-	51.57 ± 6.64	Y= -1.703In(x)+38.729	0.48 ± 0.22
SN + TT	3.33	40.43 ± 12.32	r^2 = 0.043	
SN + TT	2.67	30.90 ± 7.24		
SN + TT	1.33	41.53 ± 6.33		
SN + TT	0.67	40.70 ± 2.40		
SN + TT	0.333	1.93 ± 7.82		

*****Results are expressed as means of three experiments in duplicate ± standard deviation, TT- *Talinum triangulare*

TABLE 3: THE INHIBITORY EFFECT OF AQUEOUS EXTRACT OF *TALINUM TRIANGULARE* ON SODIUM NITROPRUSSIDE-INDUCED LIPID PEROXIDATION IN TISSUE HOMOGENATES

Treatment Groups	Conc. (µg/ml)	% Inhibition in Brain	Log. Equation (r^2)	IC_{50} (µg/ml)
Normal	-	49.07 ± 7.08	Y= -0.638In(x)+40.561	0.30 ± 0.07
SN + TT	3.33	35.93 ± 10.18	r^2 = 0.0109	
SN + TT	2.67	41.03 ± 8.84		
SN + TT	1.334	5.13 ± 16.61		
SN + TT	0.67	41.43 ± 8.06		
SN + TT	0.33	36.23 ± 6.47		

***** Results are expressed as means of three experiments in duplicate ± standard deviation, TT- *Talinum triangulare*

DISCUSSION: Tables above show the inhibitory effects of ethanolic, ethyl acetate and aqueous extracts of *Talinum triangulare* (TT) on sodium-nitroprusside (SNP) induced peroxidation in the tissue homogenates of albino rats *in-vitro*. The inhibitory abilities of the extracts of *Talinum triangulare* when incubated with sodium nitroprusside in the brain homogenate are shown in **Table 1-3** above, sodium-nitroprusside (SNP) is a component of antihypertensive xenobiotic substance, causes cytotoxicity through the release of cyanide and NO [14]. Whereas, NO is a complete neuronal messenger in the central nervous system and acts freely; it may also cause neuronal impairment synergistically with other reactive oxygen species (ROS), forming peroxynitrite ($ONOO^-$), an effective oxidizing and nitrating species [15]. This compound can cause impairment of most cellular components, including proteins, DNA and membranes phospholipid [16]. However, the current study shows the inhibitory capability of ethanolic, ethyl acetate and aqueous extracts of *Talinum triangulare* against nitrosative-induced stress. As shown from the tables, the three extracts are potent enough to inhibit SNP-induced cellular impairment by the inhibition of peroxidation on the cell. From **Table 1**, it is indicated that ethanolic extract has the highest inhibitions of 39.3 and

36.6% against SNP-induced peroxidation in the brain homogenate at very low concentrations of 0.33 and 0.67 mg/ml respectively with IC_{50} of 0.83 ± 0.04. At 0.33 mg/ml, it showed a significant increase in inhibitory ability that is higher than that of the normal. While in **Table 2**, ethyl acetate showed inconsistency in the range of inhibition when incubated with SNP in the brain homogenate of the Swiss albino rats, higher inhibitions were observed at 3.33 mg/ml, 1.33 and 0.67mg/ml against SNP-induced peroxidation, with inhibition values of 40.4, 41.5 and 40.7% respectively, with the highest inhibition achieved at 1.33 mg/ml compared to that of the normal.

Inhibitory effect of aqueous extract of *Talinum triangulare* is shown in **Table 3** above, against SNP-induced peroxidation in brain homogenate, combating generation of free NO radical which can cause nitrosative stress in the tissues. From the table 3, 3.33, 1.33 and 0.67 mg/ml concentrations caused greater inhibition potentials against SNP-induced peroxidation in the brain homogenate at 41, 45.1 and 41.4% respectively, with the highest inhibition observed at 1.33 mg/ml with 41.4% percentage inhibition compared to that of the normal.

Aqueous extract of *Talinum triangulare* in this study showed the highest ability to scavenge NO radical generated in the *in-vitro* assay (thiobarbituric acid reactive species, TBARS) by nitroprusside. With these observations, obviously, aqueous extract of *Talinum triangulare* seems to possess higher antioxidant contents with the fact that it showed the highest inhibitory potential against SNP-induced peroxidation in the brain tissue homogenate, thereby characterize the ability to protect the brain cell and as well to able to prevent nitrosative stress induction at considerable measure in the brain.

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