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Original Research Article

Scopolamine-Induced Alzheimer's Disease in Wistar Rats: Aqueous *Talinum Triangulare* Potency on the Hippocampal Nissl Bodies and Long-Term Learning and Memory

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ABSTRACT

Alzheimer's disease is a chronic neurodegenerative ailment represented clinically by studying and recalling alteration by pathologies such as intracellular neurofibrillary tangles and extracellular amyloid plaques. The study aimed at elucidating the potency of aqueous extract of *Talinum triangulare* leaves on the hippocampal neurons as well as assessing the long term learning and memory of scopolamine-induced Alzheimer's type rats. Fifty-four Wistar rats (180-200 g) were used for the study; thirty rats were grouped into five groups; A, B, C, D and E while twenty-four rats were used to establish LD_{50}. Alzheimer's type cognitive dysfunction was intraperitoneally (ip) induced with scopolamine hydrobromide (SHB) (1 mg/kg, ip) for seven days in groups B-E prior to the oral administration of the extract (875 and 1750 mg/kg and donepezil (1 mg/kg), followed by the Morris water maze test and histochemical staining process (Cresyl Fast Violet stains). The results revealed that the Nissl bodies were mild to moderately stained indicating protein synthesis. The enhancement of learning and memory were also seen in the treated groups compared to the untreated group. The ameliorative potentials may be attributed to the antioxidative properties of *Talinum triangulare* leaves which restored cells, increased protein synthesis hence, leading to enhanced learning and memory. From the above results, *Talinum triangulare* has ameliorative effect on the hippocampus of scopolamine-induced Alzheimer's type cognitive dysfunction in Wistar rats.

***Keywords*:** Alzheimer's disease, Hippocampus, Learning and Memory, Nissl bodies, *Talinum triangulare*.

Introduction

The neuronal cells encode information, conduct it, and send the information to different neurons or non-neuronal tissues.[21] Destruction of small areas of the brain can severely impair specific function such as speech, ability to move one part of the body as well as the ability to retain memory. This vulnerability of the brain to a small lesion is compounded by its very limited capacity to reconstitute damaged tissues.[5] Learning and memory are complex processes that require coordinated interaction of multiple brain structures. The hippocampus and medial prefrontal cortex are essential for the encoding and retrieval of episodic memories.[44,10,22,29]

The hippocampus is a trilaminar archicortex that lie superior to the subiculum and medial parahippocampal gyrus forming a curve elevation almost 5cm along the floor of the inferior horn of the lateral ventricle.[21] It plays a major role in the formation of new autobiographical and fact memories.[6] This brain region organizes sensory and cognitive experience into a unified long-term memory[30] and is also involve in retrieving the actual memory such as a person's name.[51]

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The hippocampus along with the olfactory bulb is the place in adult in which new neuronal cells can develop throughout life.[63]

The hippocampus is a vulnerable brain region to oxidative stress.[7,17] Oxidative stress is an underlying factor in Alzheimer's disease. Beta peptide has been identified as the main origin of oxidative stress in Alzheimer's disease.[45,46] Beta amyloid-caused cytopoison is antecedent by precipitation of oxidants in the cell which induces lipid peroxidation and death of neurons.[32] Some exogenous agents such as scopolamine are known to disrupt attention, acquisition of new information and the consolidation of memory.[58]

Scopolamine is an antimuscarinic agent that opposes the effects of acetylcholine (ACh) on the muscarinic receptors in the cerebral cortex and the hippocampus.[31,32,58,8] It diminishes cerebral blood flow due to cholinergic hypofunction.[58,59] This pharmacological agent triggers oxidants, causes free oxidant injury and increases brain malondealdehyde (MDA) levels and depletion of antioxidant status in a scopolamine-treated group[23,13,28] and has been proven to elevate the status of amyloid precursor proteins and tau proteins. Scopolamine treatment leads to marked histological distortions in the cerebral cortex including neuronal degeneration[3] and new drugs for Alzheimer's disease were evaluated using scopolamine induced dementia humans and animals.[4]

Alzheimer's disease is a chronic neurodegenerative ailment represented clinically by studying and recalling alteration by pathologies such as intracellular neurofibrillary tangles and extracellular amyloid plaques.[64,35] Neurofibriliary tangles are enriched with hyperphosphorylated microtubules-associated protein tau which can be phosphorylated by multiple protein kinases. Amyloid plaques compromise a heterogeneous population of proteolytically-generated small β-amyloid peptide.[64] Overproduction and precipitation of Aβ in

the brain is the main cause of Alzheimer's disease. The amyloid cascade hypothesis stipulates that neuropoisonous Aβ can start a series of perplex neuron degrading process resulting to synaptic alteration, neurofibrillary tangle production and probably loss of neuron in affected brain areas.[34,24,25,26] Alzheimer's disease affects nearly thirty-six million people worldwide according to estimations in 2010.[48,39,62,55,9] Alzheimer's disease is the main frequent result of dementia in intermediate-to-belated life and has a ravaging influence on public health and society. However, plants such as *Talinium triangulare* leaves with high amount of antioxidants can help to ameliorate the effects of scopolamine hydrobromide in the hippocampus.

Talinum triangulare (Waterleaf) is herbaceous perennial plant that is widely distributed in the world. Its crude protein constituent compares with that in cowpea and others.[43] This annual and perennial plant is usually seen in western Africa as well as western North America. Studies have it that improved dietary intake of waterleaf can reduce the onset of disease. Studies in Nigeria, South America and different areas of Asia revealed that this plant is widely grown as a therapeutic or food crop. Also, reports revealed that the plant is used to treat and prevent hepatic ailments as well as cancer in folk medicine, which increase stamina and also stimulate immune system.[1,18] David *et al*. documented that *Talinum triangulare* has beneficial outcome on the cerebral neurons and can improve mental activities in mice,[11] but did not consider the problems of which they were trying to solve in addition to the basis of extract administration. Since *Talinum triangulare* is known to contain some of the antioxidants, increased intake of this plant may reduce the onset of neurodegenerative disease hence, the need for this study.

Materials and Methods

Animals

Fifty-four adult female and male Wistar rats (180-200 g) were obtained from the University of Calabar. The animals were acclimatized in the animal room in the Department of Anatomical Sciences, for two weeks under standard conditions of temperature (27 – 30^{o}C). The animals were fed with rat chow (Agro Feed Mill Nigeria Limited, Calabar) and allowed access to drinking water *ad libitum*. After acclimatization, thirty rats were randomly grouped into five; each containing six rats designated A, B, C, D and E while twenty-four rats were used to establish LD_{50}. Ethical approval number: FAREC-FBMS 042ANA3719.

Plant extract preparation

Fresh *Talinum triangulare* leaves were obtained from Watt market, Calabar, Cross River State, Nigeria. The *Talinum triangulare* leaves were identified, authenticated and registered by a Botanist Mr Effa A. Effa with voucher number: HERB/BOT/UCC/120 in the Department of Botany, University of Calabar, Calabar. The plant leaves were washed to free debris, chopped into smaller pieces and air-dried in the laboratory. The dried samples were blended into powder (model number: Bravo3JARS Mixer grinder) weighing 1600g and then macerated in 1000 mL of distilled water for twenty-four- hours. The mixture was then filtered using chess cloth and Whatman No.1 filter paper. A solution was obtained and concentrated to a syrupy residue at 40-50^{o}C using man-made vacuum (model number F.NR: 1508.0271) and kept in a cool dry place for later use.

Induction of Alzheimer's type cognitive dysfunction

Alzheimer's type cognitive dysfunction was induced in the adult female and male rats in groups B, C, D and E with intraperitoneal injection (ip) of 1.0 mg/kg of scopolamine hydrobromide (SHB) for seven days.

Determination of LD_{50}

The LD_{50} of the *Talinum triangulare* leaves aqueous extract was established using the modified Lorke's method.[38] Twenty-four adult Wistar rats (180-200g) were used for the first and second phases of the study. These rats were divided into 8 groups of 3 rats each. Three groups were used for each phase. The rats were fasted overnight before administering orally 500, 1000, 2000 and 4000 mg/kg body weight of *Talinum triangulare* leaves aqueous extract to the groups respectively, for the first phase with no signs of toxicity and mortality for seventy-two hours post administration. The second phase of the study was carried out using the remaining groups of animals; and was administered 4500, 5000, 6000 and 7000 mg/kg of aqueous *Talinum triangulare* leaves extract orally. Thereafter, the rats were monitored for signs of toxicity including mortality for 24, 48, 72 hours, one week and two weeks post administration. Hence, no signs of toxicity were observed, LD_{50} was established and the dosage of aqueous extract administration was determined using 12.5% and 25% of the established LD_{50}.[38]

Experimental design

Group A served as the positive control, received animal feed and water *ad libitum*; group B served as the negative control and received 1.0 mg/kg of scopolamine hydrobromide (ip) only; group C received 1.0 mg/kg of SHB (ip) and 1.0 mg/kg of Donepezil (orally), group D received 1.0 mg/kg of SHB (ip) and 875 mg/kg of aqueous *Talinum triangulare* leaves extract (orally) while group E received 1.0 mg/kg of SHB (ip) and 1750 mg/kg of aqueous *Talinum triangulare* extract (orally). The extract and Donepezil were administered for fourteen days. Twenty-four hours after the last administration, the animals were sacrificed, perfused, the brain was removed from the skull and the hippocampus was dissected and processed for histochemical staining

Cresyl fast violet stain

Nissl granules in the hippocampal cells were stained using Goggin *et al.* method for Cresyl Fast violet solution.[20] The paraffin wax was removed from the sections in two changes of xylene for the period of 10 minutes. Each of the section was rehydrated in descending grades of alcohol (100%, 90% and 70%) in two changes for 10 minutes each and rinsed in distilled water. Warm 0.1% Cresyl violet solution was used in staining the sections at a temperature of 37^{o}C. The sections were rinsed immediately in distilled water, differentiated in 95% alcohol for 30 minutes and then dehydrated in 100% alcohol for 10 minutes. They were cleared in xylene for 10 minutes and allowed to dry. Xylene was dropped on the sections for restoration of colour before mounting in DPX. The stained sections were observed under light microscope.[20]

Morris water maze test

Twenty-four hours after the last administration, the experimental animals were subjected to Morris water maze (MWM) test in order to assess spatial learning and memory using Morris method.[40] The rats were trained to use extra maze visual cues to locate an escape platform hidden just below the surface of opaque water. The ability to do this depends on learning and remembering locations. The hidden platform version of MWM is a test of spatial memory sensitive to hippocampal damage while the visible platform version of MWM is a non-hippocampal task, disrupted by dorsal striatum lesion. The testing in Morris water maze lasted for seven days. The animals were trained for six days; the first three days were for acquisition training with the invisible escape platform (North-West quadrant). Day 4 to 6 were the reversal training with the invisible escape platform (South-East quadrant). On the seventh day, a probe trial was conducted with no escape platform.[40]

Statistical analysis

Data obtained were analyzed using analysis of variance (ANOVA) with the statistical package for social science (SPSS) version 22. Multiple comparisons were done using a two-way post hoc test with results expressed as mean ± standard error of mean (SEM) at $p < 0.05$.

Results and Discussion

The Nissl substance in group B are less stained, sparsely populated in group C, moderately stained in group D and densely stained in group E indicating high protein synthesis in group E. Neurobehavioral test using Morris water maze revealed significant decrease in groups A, C, D and E during the acquisition and reversal training days compared to

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the positive group B indicating learning enhancement. During the probe trial day, group A, C and D were significantly different indicating memory enhancement compared to group B and E at the North East quadrant whereas in South West which was the opposite quadrant group E and C were significantly increased indicating memory enhancement while the decrease in group B indicated memory deficit.

Scopolamine hydrobromide-induced cognitive models in rodents is an established method to facilitate research and the production of compounds for Alzheimer's disease and other diseases with negative impact on memory and cognitive functions.[41] The cognitive impairment associated with scopolamine hydrobromide (SHB) is similar to that in AD. After intraperitoneal injection of SHB, the cholinergic neurotransmitter is blocked; resulting to cholinergic malfunction and cognitive alteration in rats.[42] A study revealed that SHB caused recall alteration related with impaired brain oxidative stress level.[19] In this study, rats with SHB-induced recall alterations were used for elucidating the potentials of *Talinum triangulare* leaves.

In this study, histochemical staining of the hippocampal Nissl substance shows mild to moderate staining intensity in the treated experimental groups (Plates 3, 4 and 5). Nissl bodies are granular bodies found in neurons. They are prominent in neurons where they are mostly located in the cell body and dendrites and are responsible for synthesizing proteins. Disappearance of the Nissl granules may be due to exogenous insult or as a result of trauma.[14] Furthermore, it was seen that there was a marked reduction of Nissl stained neurons in the hippocampus of rats treated with 1mg/kg body weight of SHB alone. This research agrees with the work of Davis and Robertson who revealed that chemicals and toxic substance affect Nissl substance thereby influencing their metabolic activity.[12]

The Nissl substance of the animals treated with *Talinum triangulare* showed that those that received 875 mg/kg body weight were mildly stained (plate 4), while the animals that received 1750 mg/kg body weight were moderately stained (Plate 5). Whereas, the group treated with 1 mg/kg body weight of donepezil showed mildly populated Nissl granules (Plate 3). The above results mean that the plant administered at higher dose ratio was able to offer the best protection against the cellular damage by SHB. The observed result revealed dose related pattern of cellular repair with group E exhibiting the most ameliorative potentials from *Talinum triangulare* on the hippocampal pyramidal cells which could enhance learning and memory in line with David *et al.* who suggested that the consumption of *Talinum triangulare* has beneficial results on the cerebral neurons, and could be attributed to the high polyphenols (antioxidants) content in this plant[11] which according to Pharm-Huy *et al.*, may aid to remove the excess free radicals, prevent the cell upon their poisonous effect as well as prevent further damage from the SHB.[47]

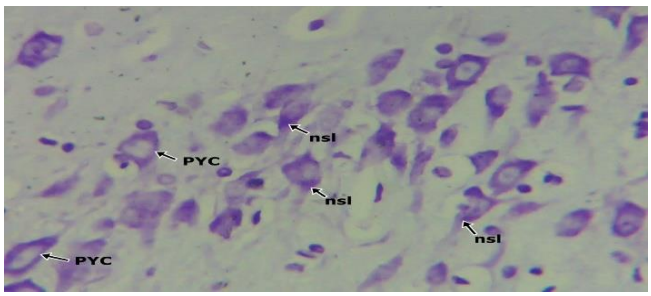

Plate 1: photomicrograph of a section of hippocampus from group A showing deeply stained pyramidal cells with granular cytoplasm.

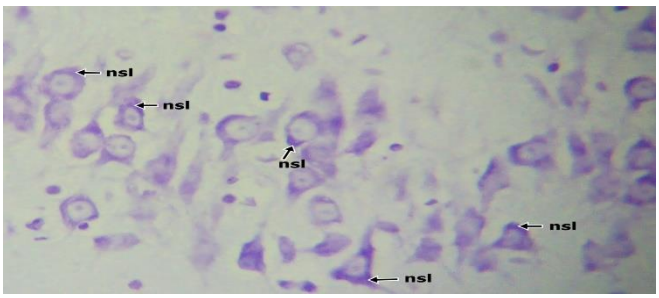

Plate 2: photomicrograph of a section of hippocampus from group B showing less stained Nissl substance.

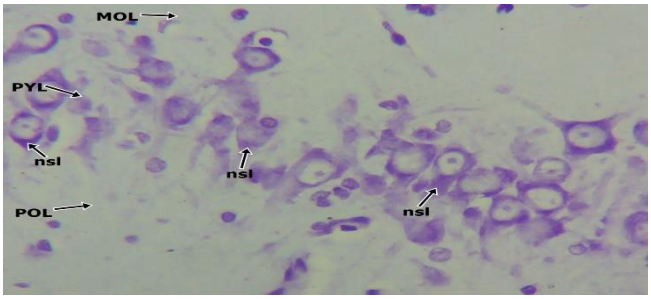

Plate 3: photomicrograph of a section of hippocampus from group C showing abundant Nissl substance within the cytoplasm and more at the periphery of the pyramidal cell body.

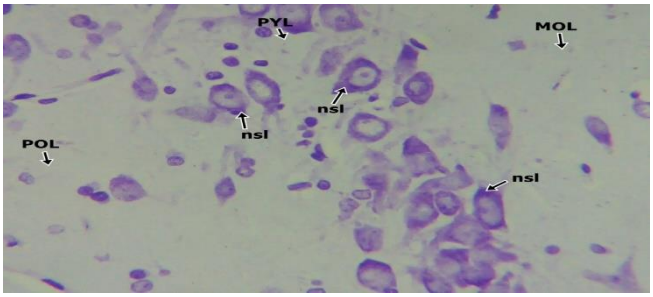

Plate 4: photomicrograph of a section of hippocampus from group D showing evenly distributed moderately stained Nissl granules within their cytoplasm.

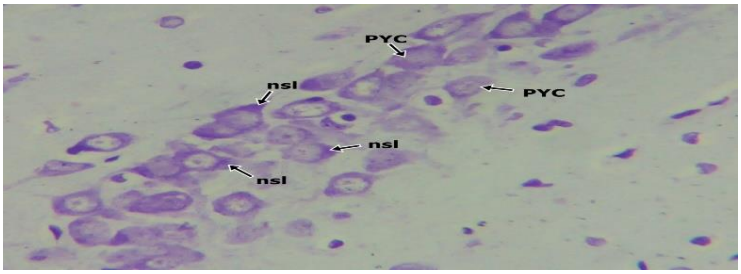

Plate 5: photomicrograph of a section of hippocampus from group E showing evenly distributed densely stained Nissl granules within their cytoplasm.

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Several metamorphoses happening during the loss of neuronal viability have been used as labels for dead cells. They include morphological and histochemical metamorphoses such as the development of basophilia, eosinophilia and loss of Nissl stain, shrinkage of the cell perikarya with the formation of angular-shaped neurons containing microvacuolations, dendritic swellings and accumulation of calcium deposits. The restoration of the staining intensity of the treated groups agrees with Akinola *et al.*, who revealed that neem and bitter leaf excerpt restores Nissl bodies in diabetes induced prefrontal cortex of albino wistar rats.[2]

During the neurobehavioral test, the results revealed a general elevation in escape latency in SHB treated group during the acquisition and reversal training compared to the negative control (Figure 1). This indicates learning impairment induced by SHB. Several literatures abound on scopolamine inducing memory impairment assessed using MWM.[49,36,27] The result in donepezil significantly reversed this effect by decreasing the escape latency time. This is attributed to the nootrophic properties of donepezil (Figure l). Alzheimer's dementia patients have profound deficits in cognitive and social functions, mediated in part by a decline in cholinergic function. Acetylcholinesterase inhibitors (AChEI) are the most commonly prescribed treatment for the cognitive deficits in AD patients.[37] Donepezil is an AChEI and possibly improved scopolamine-induced impairment by inhibiting acetylcholinesterase activity and improve cholinergic neurotransmission in the brain.

Rats in other groups treated with *Talinum triangulare* leaf showed reduced escape latency or swim latency time.

This indicates that aqueous *Talinum triangulare* leaf reversed SHB-induced cognitive impairment (Figure 3). It has been established that scopolamine causes recall deficit associated with attenuation of cholinergic neurotransmission as well as an increase of processes linked with oxidative trouble in the brain.[19] The mechanism of action of this plant in mitigating the deleterious effect of scopolamine might be through its antioxidant properties or acting as acetylcholinesterase inhibitor.

Probe trial in MWM determines whether the animals remembered the hidden platform in the target quadrants. Results revealed SHB treated group (group B) had the least time spent on the target quadrants indicating lack of memory (Figure 3). Probe trial constitutes evidence for spatial memory. Rats with lesions of the hippocampus do poorly in probe test.[56,40] This correlates with Nissl substance result which showed depletion of Nissl bodies in the hippocampus of SHB treated group (Plate 2). Group A, C and D spent more time in North East quadrant compared to group B and E, however, group C and E spent more time at the opposite quadrant indicating memory enhancement (Figure 3). The decrease in group B indicated memory deficits. This result showed that the treated groups showed intact spatial working memory. This means that the extracts possess the potentials that can enhance learning and memory in rats, however, this is in line with Eru *et al*, Rabiei *et al.*, Rubio *et al*, Tromfimiuk *et al*, Sadeyeng *et al* and Saija *et al*, who reported that *Telfairia occidentalis, Hypericum perforandum, Lipidiummeyenii, Prunella vulgaris, Averrhoa carabola* and *Cyprus rotundus* have the potentials to enhance studying and recall, passive avoidance studying and astrogliotic reduction.[13,15,16,50,52,53,61,13,54,57]

Conclusion

The study revealed that *Talinum triangulare* leaf has the potentials to ameliorate the depleted hippocampal Nissl granules and enhancing learning and memory in scopolamine-induced Alzheimer's type cognitive dysfunction rats.

Conflict of Interest

The authors declare no conflict of interest.

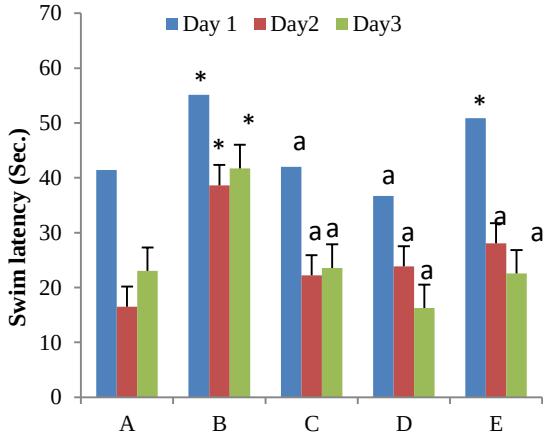

Figure 1: Comparison of swim latency in the different experimental groups during acquisition training on day 1, 2 and 3 of Morris water maze test.
Values are expressed as mean ± SEM, n = 6. * = significantly different control at P < 0.05, a = significantly different scopolamine hydrobromide at P < 0.05.

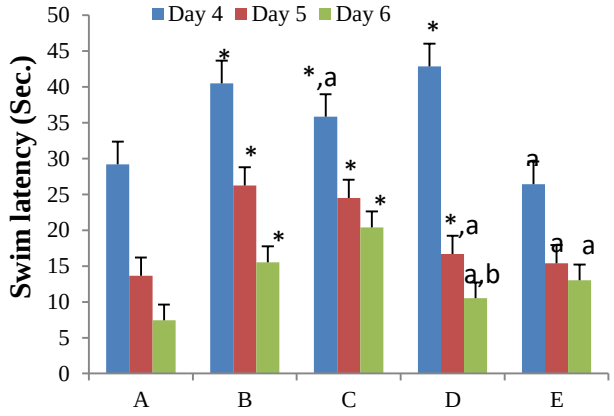

Figure 2: Comparison of swim latency during reversal training on days 4, 5 and 6 of Morris water maze test.
Values are expressed as mean ± SEM, n= 6. * = significantly different control at P < 0.05, a = significantly different scopolamine hydrobromide at P < 0.05, b = significantly different Donepezil at P < 0.05.

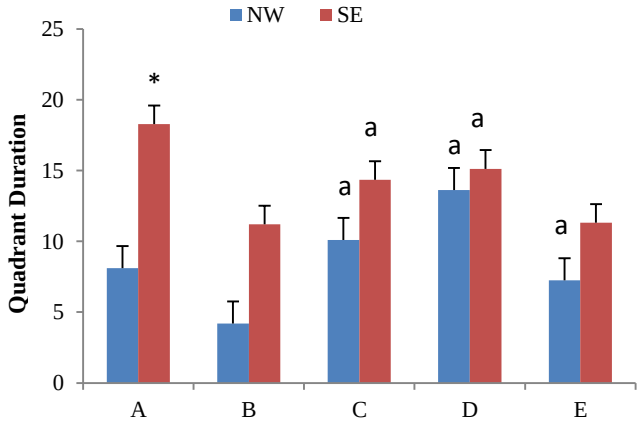

Figure 3: Comparison of swim latency in the different groups during probe trial on day 7 of Morris water maze test.
Values are expressed as mean ± SEM, n= 6. * = significantly different control at P < 0.05, a = significantly different scopolamine hydrobromide at P < 0.05.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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