Cognitive-Enhancing and Anti-Oxidant Activities of Garlic and Ginger Mixture in Wistar Rats

Abstract

Mental acuity and cognition are intelligent Index which could be threatened by oxidative stress in organisms and the widespread use of ginger and garlic in food is acclaimed to be good anti-oxidant in stressful conditions. The study was designed to examine the relationship between oxidative stress/inflammatory variables and cognition as well as studying comparisons of oxidative stress status and cognition between test groups and controls. A measured quantities (5kg each) of Fresh garlic and ginger were purchased, washed, peeled and blended very finely and the pastes were squished to extract the juices (mixture). 20 male rats were randomized into five groups and treated as follows: group 1(control), group 2(low dose mixture), group 3(high dose mixture), group 4(donepezil), group 5(low dose mixture and donepezil), group 6(high dose mixture and donepezil). After two weeks of treatment, the animals were made to undergo Morris Water Maze task and Elevated Plus Maze task while at the end of four weeks, they were sacrificed and 5ml of blood was collected from the rats for oxidative stress maker evaluation. It was observed that the mixture of garlic and ginger in the administered groups improved significantly the performance potential and cognition in both tasks, although the drug donepezil exhibited more activities but the performance was more attenuated when combined with the mixture. The pattern of oxidative stress status declined significantly with the administration of the mixture indicating strongly, a potent anti-oxidant potential in stress-laden scenarios, the obtained results suggest that the blend of garlic and ginger (GARLGING) (100 and 300 mg/kg) exerts potent anti-amnesic and cognitive enhancing effects through modulation of the antioxidant activity in the hippocampus of the rat model.

Keywords: Mental acuity, Garlic, Ginger, Oxidative stress, Cognition, Donepezil

Introduction

Garlic, Allium sativum L. is a member of the Alliaceae family, has been widely recognized as avaluable spice and a popular remedy for various ailments and physiological disorders. The name garlic may have originated from the Celtic word 'all' meaning pungent. Cultivated practically throughout the world, garlic appears to have originated in central Asia and then spread to China, the Near East, and the Mediterranean region before moving west to Central and Southern Europe, Northern Africa (Egypt) and Mexico (1). The medicinal qualities of garlic were described by Pliny the Elder, Aristophanes, and Galen. Hippocrates, the Father of Medicine, observed that garlic was excellent for curing tumors and is an effective diuretic. Aristotle attributed garlic as a cure for rabies, and the Prophet Mohammad recommended it for treating scorpion stings. In Historia

Naturalis by Pliny (2), garlic was recommended for gastro-intestinal disorders as well as dog and snake bites. Desired medicinal results of garlic are obtained when bulbs are chewed and swallowed or mixed with food and eaten.

Ginger (Zingiber officinale, Roscoe Zingiberaceae) is one of the most widely consumed spices worldwide. From its origin in Southeast Asia and its spread to Europe, it has a long history of use as herbal medicine to treat a variety of ailments, including vomiting, pain, indigestion, and cold-induced syndromes [4, 5]. Although ginger is not widely known for its effectiveness in diabetes prevention and/or treatment, modern pharmacological studies support its potential for treating both the hyperlipidemic and hyperglycemic aspects of diabetes. However there is much less information on the mechanism of action of ginger underlying its effects in cognition. Whilst the chemical composition of Zingiber officinale is well investigated, it is much less clear which of the many components in ginger are responsible for its effects in cognition (6, 7)

Consumers of both garlic and Ginger lay claim on the soothing feelings and cerebral calmness and tranquilization that follow overnight usage of the plants. However there is no direct link between these plants and cognition hence the purpose while this research was carried out.

Materials & Methods

Forty-five healthy and sexually matured male and female albino rats of 12 weeks old weighing between 80-200g were used in this study. The rats were obtained from the Experimental Animal Unit of Department of Human Physiology, University of Port Harcourt, Rivers State. The rats were housed in conventional wire mesh cages under standard laboratory conditions.

The animals were allowed free access to water and feed throughout the period of the experiment. The animal feed was gotten from Rumuosi local market Port Harcourt.

Fruit Preparation

A measured quantities (5kg each) of Fresh garlic and ginger were purchased, washed, peeled and blended very finely and the pastes were squished to extract the juices. 2 litters of each were derived and mixed and preserved in a refrigerator to prevent fermentation and for use whenever needed. A standard laboratory practice protocol was observed when all experiments were conducted. The recommendations for quality standards of biomedical research were noted and implemented.

Drug

Donepezil drug was manufactured by Eisai Medical Research with Approval for use was purchased from a reputable pharmaceutical company in Ibadan, Oyo State and prepared for use.

Table 1. Experimental Design

groups	Treatment	Dosage/Administration
Group 1	Distilled water	1ml/day/4 weeks
Group 2	Garlic/ginger (low dose)	(100+100)mg/kg/4 weeks
Group 3	Garlic/ginger (high dose)	(300+300)mg/kg/4 weeks
Group 4	donepezil drug	50mg/kg/b.w./4 weeks
Group 5	Garlic/ginger+drug (low dose)	(100+100+50)mg/kg/4 weeks
Group 6	Garlic/ginger+drug (high dose)	(300+300+50)mg/kg/4 weeks

Behavioural Assessment and Stress markers quantification

The Morris water maze

The Morris water maze consisted of a circular pool with a white underside and black side surface, a white platform, a camera and a computer. The pool was made of circular galvanized steel pool (1.2 m diameter, 0.5 m height) filled with water (20-22°C). A white platform (8 cm diameter) was placed in the middle of one quadrant and submerged 1 cm

below the surface of the water. A camera situated above the pool was used to capture the mice's swim trace. A computer was used to analysis data. Mice were trained on water maze with four trials for 5 d.

At the begining of 0 day, mice was allowed to freely search platform with four trials. From the first day to the fourth day, the mouse was given four trials, a trial lasted 60 s or until the mouse reached the platform and remained a few seconds. If a mouse didn't reach the platform in 60 s, it meant that its escape latency was 60 s, then the mouse was allowed to rest for 30 s between trials. On the fifth day, the platform was removed and mice were tasked with a probe trial for 2 min [8].

Elevated Plus Maze (EPM)

Practical Steps in the Use EPM

Behavioral responses in the elevated plus maze are easily assessed and quantified by an observer. Briefly, rodents are placed in the intersection of the four arms of the elevated plus maze and their behavior is typically recorded for 5 min. This was based upon the early studies by Montgomery (9) that revealed that rats demonstrated the most robust avoidance responses in the first 5 min after placement in the elevated open alleys. The behaviors that are typically recorded when rodents are in the elevated plus maze are the time spent and entries made on the open and closed arms. Behavior in this task (i.e., activity in the open arms) reflects a conflict between the rodent's preference for protected areas (e.g., closed arms) and their innate motivation to explore novel environments.

Blood Sample Collection and Analysis

The Animals were sacrificed after the fourth week of administration.

Blood samples were collected via cardiac puncture for liver enzymes evaluation. And this Analysis took place at the Research Laboratory of the department of Biochemistry, University of Port Harcourt.

Measurement of Oxidative Stress Markers

Superoxide dismutase (SOD)

Superoxide dismutase (SOD) are a group of metalloenzymes that are found in all living things. Superoxide dismutase activity was determined according to the method of McCord and Fridovich (10). Briefly, 0.01 ml of the brain homogenate was mixed with 0.2 ml of 0.1 M EDTA containing 0.0015 % NaCN, 0.1 ml of 1.5 mM NBT and phosphate buffer with pH 7.8 to a total volume of 2.6 ml. On adding 0.05 ml of riboflavin, the absorbance of the solution was measured against distilled water at 560 nm. All the tubes were illuminated uniformly for 15 minutes and absorbance of the blue color formed was measured again. Percent of inhibition was calculated after comparing absorbance of sample to the absorbance of control (the tube containing no enzyme activity). The volume of the sample required to scavenge 50 % of the generated superoxide anion was considered as 1 unit of enzyme activity and expressed in U/L protein.

Malondialdehyde (MDA)

The level of lipid peroxidation was measured as malondialdehyde (MDA) according to the method of Ohkawa et al. (11). Lipid peroxidation: The level of lipid peroxidation in the tissue was measured as malondialdehyde (MDA) according to the method of Ohkawa et al. (11). Absorbance of the clear supernatant was measured at 532 nm against butanol: pyridine mixture. The MDA level was calculated and is expressed in μmol/L

Catalase (CAT)

CAT is a common and very important antioxidant enzyme which catalyses hydrogen peroxide to water and oxygen. Catalase breaks down two hydrogen peroxide molecules into one molecule of oxygen and two molecules of water in a two-step reaction. Deisseroth (12). Catalase activity was evaluated according to sadauskiene et al (13). The obtained result was expressed in U/ml.

Glutathione peroxidase (GPx):

Glutathione peroxidase activity was determined according to the method of Hafeman et al. (14). The absorbance of the yellow colored complex was measured at 412 nm after incubation for 10 minutes at 37 o C against distilled water.

Statistical Analysis

Data were analyzed using SPSS version 20 and results were presented as Mean ± SEM. Post Hoc test was done using LSD. Level of significance was set at $P \leq 0.05$.

Results

Table 2. Assessment of stress markers in lipid peroxidation levels on exposure to a mixture of Garlic and ginger administration in the brain homogenates of rats

groups	Treatment	Superoxide dismutase (u/ml±sem)	Malonhydehyde (µg/ml±sem)	PROTEIN (g/L±sem)	Glutathione oxidase (µg/ml±sem)	CATALASE (u/g±sem)
Group 1	Distilled water	364.56±49.89	54.54±1.66	10.30±1.49	28.05±4.52	36.20±6.59
Group 2	Garlic/ginger (low dose)	260.52±16.41	67.78±3.68	10.10±1.03	19.43±2.15	18.80±7.83
Group 3	Garlic/ginger (high dose)	262.64±38.67	49.27±10.27	9.65±0.64	25.43±8.17	42.80±12.32
Group 4	donepezil drug	395.36±37.89	65.62±5.74	12.98±1.34	21.89±2.00	42.00±13.92
Group 5	Garlic/ginger+drug (low dose)	455.93±59.15	49.75±10.68	7.80±1.61	22.30±3.58	52.00±14.63
Group 6	Garlic/ginger+drug (high dose)	743.04±225.05	62.86±5.14	7.15±1.61	44.49±10.79	70.00±13.04

Values are presented in mean ± sem. n= 5. $P \leq 0.05$ *means values are statistically significant when compared to the control

Key: Garlic and ginger low dose, (100mg/kg) , Garlic and ginger high dose, (300mg/kg), Donepezil drug (50mg/kg b.w.).

TABLE 3. Pattern of time spent on the open arm of EPM at different trials on exposure to various doses of mixture of garlic and ginger and Donepezil drug of test groups.

GROUPS	ELEVATED PLUS MAZE OPEN ARM (s±sem)			
	TASK 1 (O Minute)	TASK 2 (After 30 Minutes)	TASK 3 (AFTER 60 MINUTES)	% RELATIVE CHANGE IN PERFORMANCE
control	13.60±4.56	10.20±7.57	10.60±4.96	-22.06
Garlic/ginger low dose	10.40±4.22	22.60±8.41	15.40±2.94	48.08
Garlic/ginger high dose	12.00±11.92	38.20±4.02	45.00±19.59	275
donepezil drug	15.00±8.09	39.40±7.20	41.40±16.01	176
Garlic/ginger+drug low	25.00±10.75	39.20±6.18	52.00±16.87	108
Garlic/ginger+drug high	40.00±3.35	48.80±1.63	58.00±8.24	45

Values are presented in mean ± sem. n= 5. $P \leq 0.05$ *means values are statistically significant when compared to the control

Key: Garlic and ginger low dose, (100mg/kg) , Garlic and ginger high dose, (300mg/kg), Donepezil drug (50mg/kg b.w.).

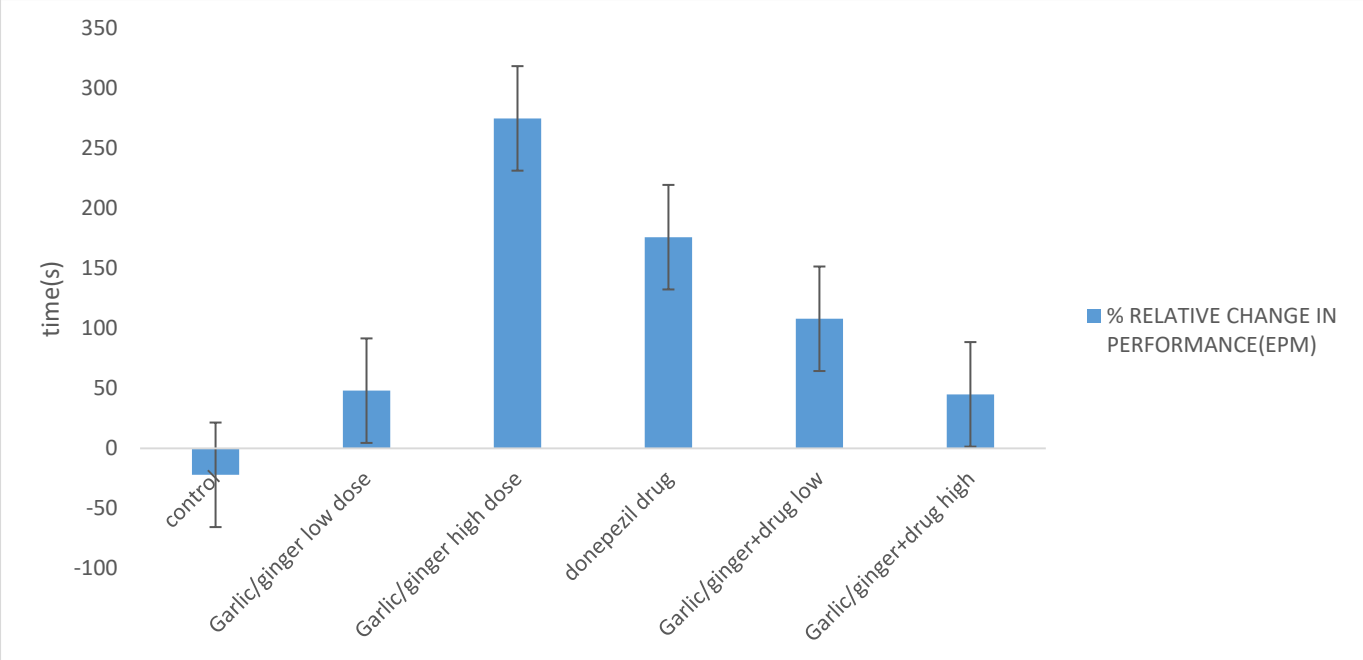

Fig. 1. Relative change in performance during Morris Water Maze (MWM) at 1st trial on exposure to various doses of mixture of garlic and ginger and Donepezil drug of test groups.

TABLE 4. Pattern of time spent during Morris Water Maze (MWM) at different trials on exposure to various doses of mixture of garlic and ginger and Donepezil drug of test groups

GROUPS	MORRIS WATER MAZE TEST (s±sem)			
	TASK 1 (O Minute)	TASK 2 (After 30 Minutes)	TASK 3 (AFTER 60 MINUTES)	% RELATIVE CHANGE IN PERFORMANCE
control	15.60±3.76	21.40±1.72	32.40±2.84	105
Garlic/ginger low dose	10.40±4.98	10.80±2.71	9.40±1.36	-9.62
Garlic/ginger high dose	12.80±12.57	12.60±3.44	5.20±9.50	-59.4
donepezil drug	10.00±4.57	9.80±2.59	8.40±1.91	-16
Garlic/ginger+drug low	12.00±2.00	6.20±2.63	8.40±3.12	-30
Garlic/ginger+drug high	11.00±2.88	6.00±1.64	9.20±3.48	-16.4

Values are presented in mean ± sem. n= 5. P ≤ 0.05 *means values are statistically significant when compared to the control

Key: Garlic and ginger low dose, (100mg/kg) , Garlic and ginger high dose, (300mg/kg), Donepezil drug (50mg/kg b.w.).

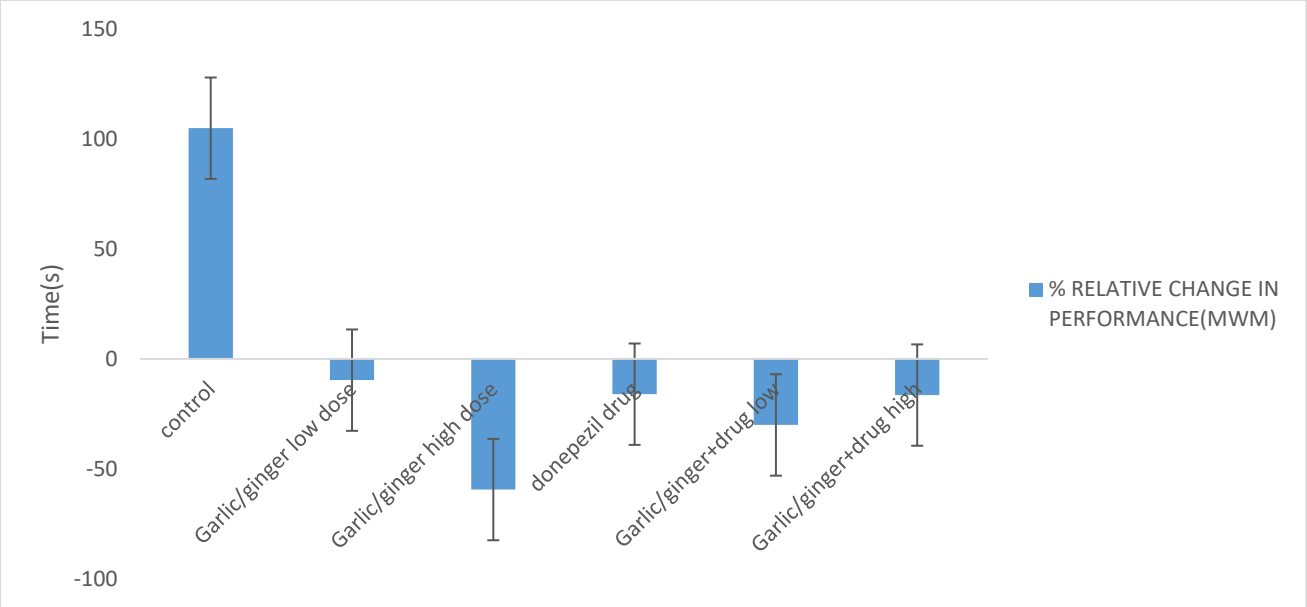

Fig. 2 Relative change in performance during Morris Water Maze (MWM) at 1st trial on exposure to various doses of mixture of garlic and ginger and Donepezil drug of test groups

Discussions

The results of present work revealed that chronic treatment with a mixture of garlic and ginger blend (GARLGING) prevents cognitive deficits in rats. Indeed, the in vitro assays showed a clear antioxidant effect.

Morris Water Maze (MWM) task is used to test spatial reference memory and is widely thought to have relevance for human hippocampal-dependent memory (15-19). Although not as commonly used, reversal learning in the MWM is more difficult than the standard task, and therefore can be used to detect subtle memory deficits (15). Further, because of its more complex nature, reversal learning may be viewed as a task of executive function (20, 21)

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It was evidently shown and demonstrated in the results that animals in the test groups swim more slowly throughout the experiments in all trials of the Morris Water Maze (MWM), when compared to controls especially at higher dose, with no difference from each other, no differences were observed between groups in relation to ambulatory activity in the open field and in the latency to enter in the dark compartment of the inhibitory avoidance apparatus during the training the opposite was the case when the drug Donepezil was introduced.

Probing further, the beneficial effect of GARLGING on the MWM and inhibitory avoidance paradigms are unlikely to be a result of differences in motor abilities. Indeed, because no significant difference was found in grooming time and in explorative tendency in the open field, as well as in the percentage of entries and time spent in the open arms of the elevated plus maze, alterations in the motivational and/or emotional state of the animal, which could have affected performance in both inhibitory avoidance and water maze learning, cannot be overlooked.

Trullas and Skolnick [22] characterized elevated plus-maze behavior on the basis of levels of open arm exploration. Thus, test group 6 (high dose GARLGING PLUS DRUG group) and control group exhibiting low levels of open arm activity were classified as ``high reactive", while those showing high levels of activity in the open arms (e.g., groups 2-5) were labeled ``low reactive." However, while time spent in the open arms was indeed relatively high,

levels of exploratory behaviors and general locomotors activity in the rats were also substantially higher than those observed in laboratory/Swiss mice or those previously observed (23).

As such, rather than displaying low reactivity to the plus-maze, the activities of the test groups including the Donepezil drug group, (group 4), would be more accurately described as one of high reactivity.

From trial 1 through trial 6, there was a consistent and repetitive similar behavior and responses recorded from the test groups (both at low and high doses), and donepezil drug group. This interpretation is supported. Thus, the test group animals made a number of ``jump attempts'' from the open arms, a behavior that was very pronounced and which involved the animal standing on hind-paws on the edges of the open arms and making movements as if to jump, but then failing to do so. A very similar behavior has recently been reported in laboratory rats exposed to an unstable plus-maze apparatus [23, 24] and interpreted as evidence of an intense anxiety (panic-like) state.

In addition, test group animals actually explored the upper ledges of the closed arms, i.e., animals jumped onto the top of the closed arm walls and ambulated along the very narrow ledges. Although time spent on the upper ledges accounted for a relatively small proportion of the test session, it was nonetheless very striking and appeared to further demonstrate the escape motivation of these animals.

Also noticeable was freezing in the plus-maze, a response that was noticeably higher in groups treated with the mixture and the drug at both higher and lower doses. Consonant with this observation, Blanchard et al. [25] found that similar treatment with certain pro-oxidant substances show more movement inhibition than do normal rats in response to potential threat.

One of the important mechanism in the development and progression amnesia is oxidative stress and any substance that will inhibit or ameliorate oxidative stress would be highly beneficial to the organisms. In the present study, GARLING mixture at both high and low doses increased SOD, GPX, and GSH and decreased the MDA and protein carbonyl levels in the rat hippocampal homogenates opposite to what was obtained in the control group that was not treated.

It is well known that oxidative stress always leads to oxidative damage of bio-macromolecules, including lipoprotein within the cellular membranes. Elevated MDA is regarded as a specific indicator of lipid peroxidation during oxidative impairment [25]. In addition, oxidative injury could also destroy the anti-oxidant defense system, such SOD, GSH-PX and CAT. In fact, it was previously found that oxidative brain damage caused by oxidative stress contributed to the serious impairment of learning and memory deficits during aging in rats [26]

It was observed that the mixture worked in opposite fashion to what was observed by Parks et al., that scopolamine administration induced a neurochemical alteration in the brain along with changes in oxidative status of the brain [28]. Thus, scopolamine created an imbalance between antioxidant and oxidant defense systems which may be responsible for observed impairment of memory in rats.

Furthermore, many studies have reported that the scopolamine-induced amnesic rats show similar patterns of memory impairments and oxidative damage with amnestic mild cognitive impairment (MCI) patients [29].

The evidence from the study suggested that the GARLGING mixture could possess potent cognitive effects that may be mediated by improving the brain oxidative status [30]. Consequently, the mixture treatment restored the antioxidants status as evidenced by an

increase of SOD, GPX, and GSH while the levels of MDA (lipid peroxidation) and protein carbonyl significantly decrease which supports its antioxidant property.

From previous works, several natural products with antioxidant proprieties have been reverting the cognitive impairment in aged rats (31-35). Reactive oxygen species (ROS) can be highly damaging to cells due to the oxidation of essential cellular constituents such as lipids, proteins and DNA. The brain is particularly susceptible to oxidation by ROS because of its dependency on aerobic metabolism, large contents of polyunsaturated lipid in the mitochondrial and plasma membranes of brain cells and its low antioxidant defenses, such the antioxidant enzyme catalase (36). The ROS-production mediated protein oxidation can be measured by tyrosine nitration (37), as well as, lipid peroxidation as indicated by malondialdehyde (38).

Conclusions

In summary, the obtained results suggest that the blend of garlic and ginger (GARLGING) (100 and 300 mg/kg) exerts potent anti-amnesic and cognitive enhancing effects through modulation of the antioxidant activity in the hippocampus of the rat model. Therefore, the aqueous extract may possibly be used as a promising natural product for the prevention of memory disorders and Amnesia and ultimately Alzheimer's disease.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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