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Histological and biochemical changes in the eye stalk of *Scylla olivacea* (Herbst, 1796) exposed to Cadmium Nanoparticles

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Abstract
Nanotechnology is an upcoming science which deals with the application of nanoparticles in day to day life. But it also had a impact on the environment in which nanoparticles were released as a contaminant into the aquatic ecosystem. The present study is aimed to determine the impact of cadmium nanoparticle (CdNP) in aquatic system with the help Histological and biochemical changes induced in the eye stalk of mud crab, *Scylla olivacea* (Herbst, 1796) when exposed to Cadmium Nanoparticles (20ppm). The histological results revealed that the CdNP induced necrosis and atrophy of the eye stalk tissues. The biochemical results suggested that treatment of CdNP had reduced the protein, carbohydrate and lipids in exposed crabs which revealed that neurohormonal factors released from eyestalk of crabs regulates carbohydrate, protein and lipid contents. The overall results showed that nanoparticle had a negative impact on the animals exposed to the aquatic system contaminated by the nanoscale materials.

Keywords: Neurohormone, eyestalk, cadmium, toxicity, mud crabs

1. Introduction
Nanotechnology was an emerging technology which had a wide spread of it applications in various fields including food, cosmetics, industries and so on, while it also had its impact on the environment as well as to the organisms belonging to that environment [1, 2]. Those nano particles which were released through the industrial wastes would appear in the aquatic system and affecting the survival of the organisms of that aquatic system. Although trace metals such as cobalt, copper, and selenium are natural components of aquatic systems and essential for a number of physiological functions, however in the form of nano material they can become toxic.

Cadmium (Cd) is one of the most toxic nanomaterials for human; the main source of non-occupational exposure to Cd includes smoking, air, and food and water contaminated by Cd [3]. Cd is a common inorganic contaminant of coastal sediments and waters due to anthropogenic pollution and natural sources [4-6]. It can be accumulated in aquatic animals (eg. crabs, shrimps, oysters and mussels) after entering through different way such as respiratory tract, digestive tract, surface penetration etc., [7, 8, 5, 6]. It is seriously harmful to the growth of aquatic life and survival, resulting in decline of their populations. At the same time, as aquatic food products, those animals exposed to Cd might threaten human health. Cd in water can be absorbed by aquatic organisms via respiratory system, digestive system and body surface without significant excretion [9, 10]. Accumulated Cd was distributed to all organs with the highest proportions of body content being found in the exoskeleton, gills, hepatopancreas, and so on.

Neurosecretory structures (X-organ–sinus gland) in the eyestalk are the most important components of the neuro endocrine system of the stalk eyed crustaceans. In crustaceans, eyestalk hormones are attributed with control of a number of physiological processes namely, somatic changes, blood glucose level, osmoregulation, moulting, reproduction and oxygen consumption [11]. It has been shown that carbohydrates, proteins and lipids in crustaceans are regulated by neurohormonal factors released from eyestalk [12]. In accordance with the previous findings, the present work was aimed to study the histological and biochemical changes in *Scylla olivacea* (herbst, 1796) exposed to Cadmium nanoparticles (CdNP).

2. Materials and Methods
Animal Collection
Fresh samples of *Scylla olivacea* (both male and female crab species) are collected from Pulicate Lake, Tamil Nadu, India. Both crabs are maintained separately in tanks with aerator which was (capacity of 1000 litres) filled with filtered sea water. The sea water was changed periodically and crabs were fed with commercial fish feed. The morphological identification and authentication of species was done by a Scientist from Central Institute of Brackish water Aquaculture (CIBA), Santhome, Chennai, India. The crab was acclimatized for further studies for ten days before the experiment. During the acclimatization period, the specimens were fed twice a day. Naturally aged estuarine water was used after being shifted through a 0.45 mm pore filter and activated charcoal to remove dissolved organic matter and trace metals. Water temperature was maintained within a range (27.5± 0.5°C) as recommended for optimal growth of mud crabs [13].

Acute toxicity test
Young crabs were acclimatized for 14 days and onset of sexual maturity in *S. olivacea* was considered for the toxicity studies. Semistatic toxicological bioassays were carried out for 120

hrs [14-16]. A series of six different concentrations such as 20, 40, 60, 80, 100 and 120 ppm of Cadmium nano particle (Cd NP) suspension of 100nm in size (Sigma and Co) was injected intraperitonially per kg of crab weight (Three replicates of at least 10 animals). The complete absence of movement when the animals are gently touched was utilized to determine the death. Mortality was recorded for every 24 hrs and the experimental conditions (temperature, salinity, and pH) of the toxicity test were similar to those found in the environment during the period. A probit analysis was used to estimate the LD_{50}.

Cadmium nanoparticle treatment

After acclimatization, healthy adult male and female crabs with a homogeneous size (carapace width 14-16cm, weight 200-300g) were selected for control and Cadmium nanoparticle (20 ppm/kg of crab weight) treatment. Mud crab, *S. olivacea* was acclimatized in tanks and the temperature was maintained at 27°C. After standardization, LD_{50} value of 20ppm/kg of body weight was used for assessment of acute toxicity. The acute exposure lasted for 8 days. During the experiment, crabs were fed and dead animals were removed in time.

Light Microscopic analysis

Cadmium nanoparticle treated and control crabs of both male and female *S. olivacea* were taken from the tank, anaesthetized in ice water for five minutes and sacrificed at every 2 day interval up to 8 days. Eye stalk was removed and then fixed by direct immersion in phosphate buffer (0.1 M, pH 7.4) with 4% formaldehyde for 24 h at room temperature. Samples were dehydrated with ethanol and toluene series and embedded in paraffin. Serial sections (4 mm) were mounted on gelatin-coated glass slides and stained with hematoxylin and eosin. Slides were examined with a light microscope (Olympus BX51) and the results were documented. Four sections were analyzed from each tissue.

Biochemical Assays

The buffer soluble protein content was determined by the dye binding method of Bradford [17] with Bovine Serum Albumin fraction V (Sigma chemical Co., USA) as a standard. Total carbohydrate was estimated by the method of Roe [18] and total lipid content was estimated by **t**he gravimetric, chloroform-methanol extraction method of Folch *et al.*, [19].

3. Results

Histology

a) Histological changes in the eyestalk of male *S. olivacea*: Figure 1-4 represents the CdNP induced structural changes in the eyestalk of the male crab, stained by hematoxylin and eosin. In control, the retina is constituted by photoreceptors and pigment glial cells showed normal architecture (Fig. 1A). The photoreceptors project through the zona fasciculata to a group of three successively arranged optic ganglia: the lamina ganglionaris, the external medulla and the internal medulla. Two chiasmas, the external and the internal, connect the lamina ganglionaris to the external medulla and the external medulla to the internal medulla respectively. On day 2, disorganization of corneal cuticle and medulla were observed (Fig. 1B). On day4 and day6, further disorganization of corneal cuticle and formation of corneal cones and constriction of internal medulla (Fig.2B & 3B). On day8, complete disorganization of basement membrane constriction of external medulla was evident (Fig.4B).

b) Histological changes in the eyestalk of female *S. olivacea*: Figure1-4 represents the CdNP induced structural changes in the eyestalk of the female crab. In control, normal structure of retina was evident in pigment glial cells (Fig.1C). On day2, of CdNP exposure there was a prominent disorganization of lamina ganglionaris and external chiasma (Fig. 1D). On day 4 and day 6, further disorganization of corneal cuticle (Fig. 2D & 3D) and day 8, further disorganization of medulla are evident and severe deterioration of basement membrane and complete collapse of external medulla (Fig.4D) was observed.

Effects of CdNP on Total Protein Content of Eye stalk

A time course study in the eye stalk of *S. olivacea* showed an increase in total protein content upon exposure to CdNP than control. Total Protein content started increasing in CdNP treated male crabs on day 2 compared to its control, reaching a peak on day 4 compared to its control (Fig.5a). Similarly, in the case of female also total protein content started increasing on day 2 compared to its control and reached peak compared to its control on day 10 of exposure (Fig.5b).

Effect of CdNP on Total Lipid Content of Eye stalk

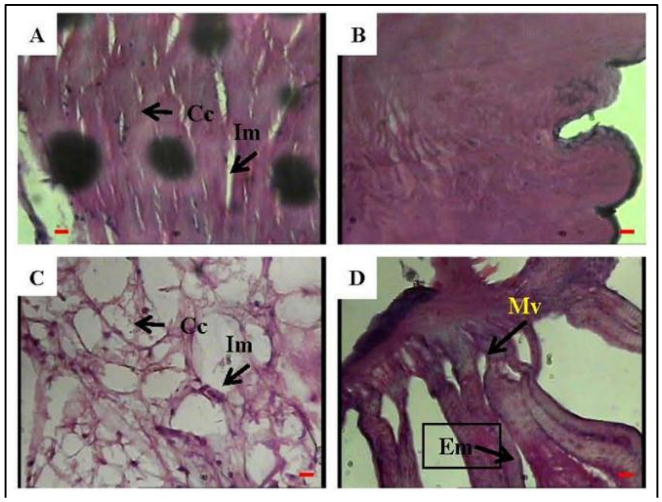
A time course study of eye stalk of *S. olivacea* showed increase in total lipid content upon exposure to CdNP. Total lipid content started increasing in CdNP treated male crabs on day 2 and reached peak on day 4 after exposure (Fig.6a). Similarly, in the case of female also total lipid content started increasing on day 2 and continued to increase up to day 6 compared to their respective controls (Fig.6b).

Effects of CdNP on Total Carbohydrate Content of Eye stalk

Results of total carbohydrate content in the eye stalk of *S. olivacea* exposed to Cadmium nanoparticle (20ppm) were presented in Figure 7a & 7b. In general, CdNP resulted in increased total carbohydrate content in eye stalk than in control crabs. In males, total carbohydrate content started increasing on day 2 and increased up to day 10 (Fig.7a). Similarly, in the case of female also carbohydrate content started increasing on day 2 and decreased further on day 4 onwards.

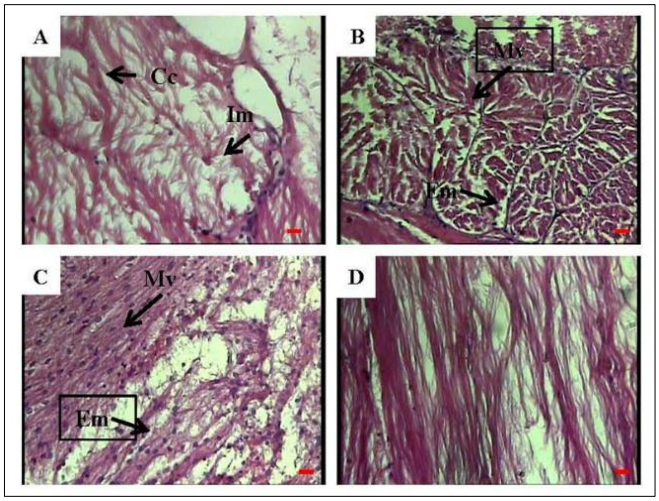
Table 1: Total Protein, carbohydrate and Total Lipid content in the eye stalk of *Scylla olivacea* (Herbst, 1796) exposed to Cadmium Nanoparticles

Days of CdNP exposure	Protein (mg/g of tissue)				Carbohydrate (mg/g of tissue)				Lipid (mg/g of tissue)			
	Male		Female		Male		Female		Male		Female	
	Control	Treated	Control	Treated	Control	Treated	Control	Treated	Control	Treated	Control	Treated
2	8.11	10.15	8.11	9.11	10.11	10.56	9.14	10.21	14.15	15.11	13.72	14.54
4	9.11	11.27	9.14	10.22	10.21	10.61	9.31	9.31	13.76	15.75	13.12	14.16
6	9.16	10.17	8.16	9.22	9.41	10.32	9.41	9.76	13.14	15.15	13.43	14.31
8	8.36	9.23	8.17	9.11	9.16	10.25	9.16	8.15	14.11	14.76	13.77	13.16
10	8.25	9.11	9.21	10.12	9.31	10.21	10.02	9.25	14.65	13.11	13.21	12.45



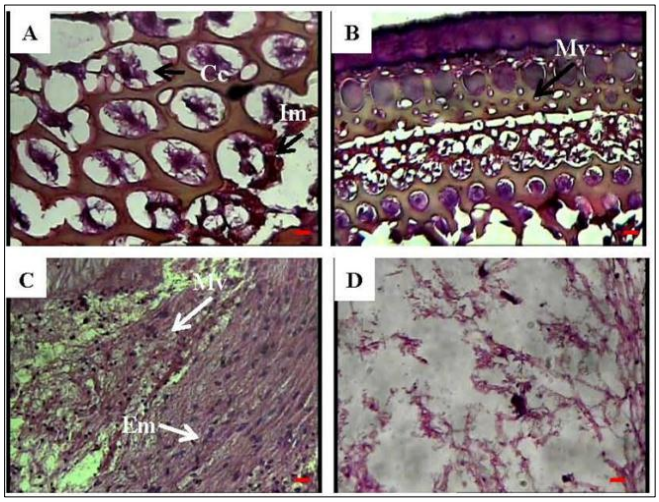
A) Control (Male) B) CdNP treated (Male)
C) Control (Female) D) CdNP treated (Female)

Fig 1: Effects of CdNP (20ppm) on the anatomy of eyestalks in *Scylla olivacea* by light microscope on day 2. Scale bar, 10um. Co-corneal cuticle; cc- crystalline cones; ec-external chiasma; em – exrernal medulla; im-internal medulla.



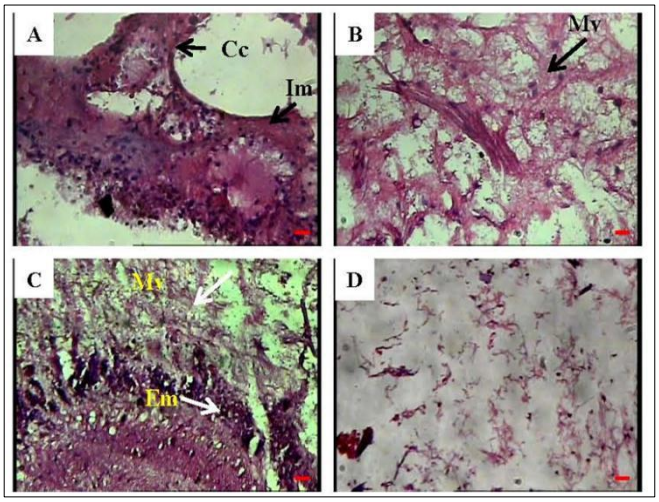
A) Control (Male) B) CdNP treated (Male)
C) Control (Female) D) CdNP treated (Female)

Fig 2: Effects of CdNP (20ppm) on the anatomy of eyestalks in *Scylla olivacea* by light microscope on day 4. Scale bar, 10µm. Co-Corneal Cuticle; Cc- Crystaline cones; Ec-External chiasma; Em-External medulla; Im-Internal medulla



A) Control (Male) B) CdNP treated (Male)
C) Control (Female) D) CdNP treated (Female)

Fig 3: Effects of CdNP (20ppm) on the anatomy of eyestalk in *Scylla olivacea* by light microscope on day 6. Scale bar, 10 µm. Co- Corneal cuticle; Cc- Crystaline cone; Ec-External chiasma; Em- External medulla; Im-Internal medulla.



A) Control (Male) B) CdNP treated (Male)
C) Control (Female) D) CdNP treated (Female)

Fig 4: Effects of CdNP (20ppm) on the anatomy of eyestalks in *Syclla olivacea* by light microscope on day 8. Scale bar, 10 µm. Co- Cprmeal cuticle; Cc- Crystaline Cones; Ec- External Chiasma; Em- External medulla; Im-Internal medulla.

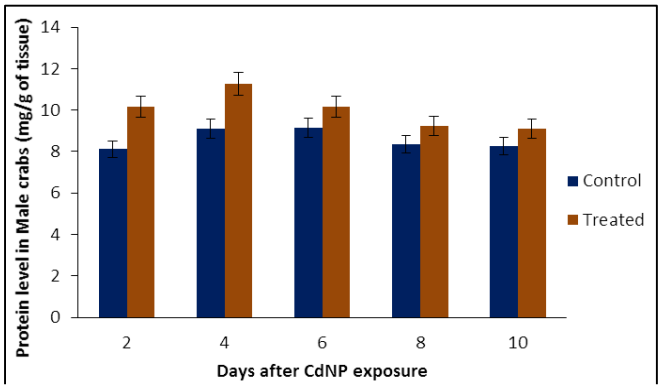


Fig 5a: Total protein content in eye stalk of male *S. olivacea* after exposure of CdNP.

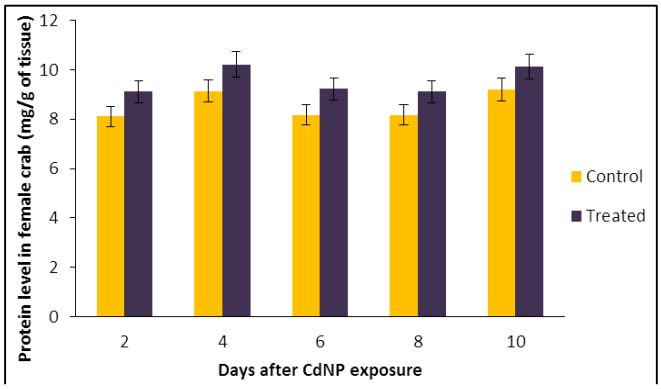


Fig 5b: Total protein content in eye stalk of female *S. olivacea* after exposure of CdNP.

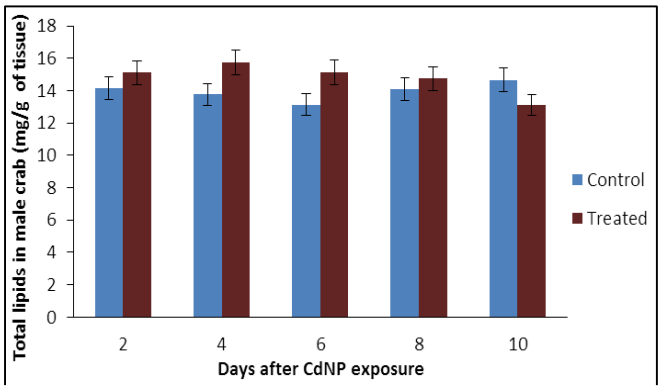


Fig 6a: Total Lipid content in eye stalk of male *S. olivacea* after exposure of CdNP.

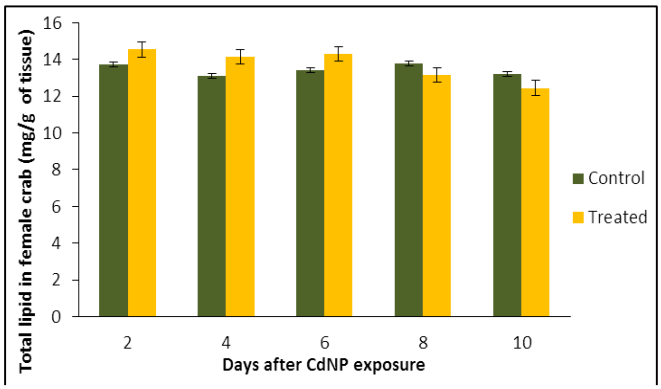


Fig 6b: Total lipid content in eye stalk of female *S. olivacea* after exposure of CdNP.

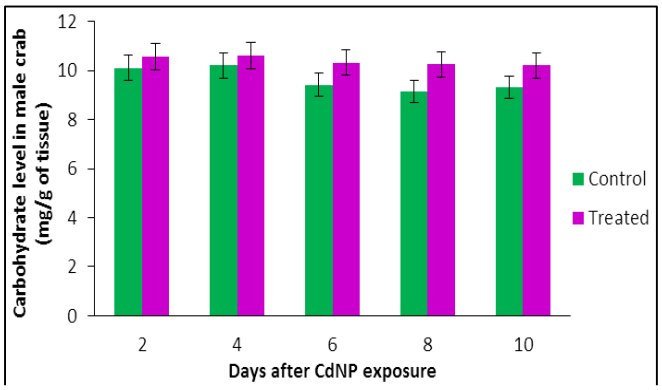


Fig 7a: Total Carbohydrate content in eye stalk of male *S. olivacea* after exposure of CdNP.

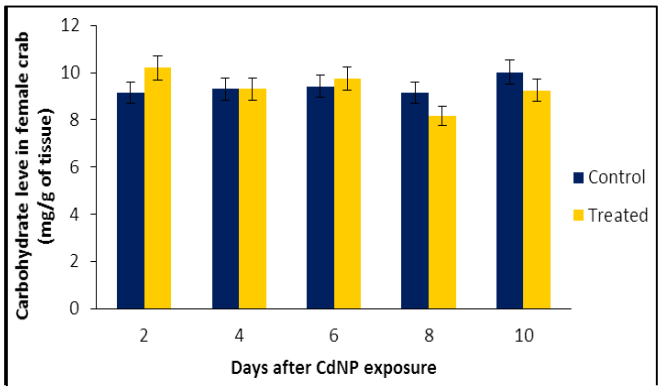


Fig 7b: Total Carbohydrate content in eye stalk of female *S. olivacea* after exposure of CdNP.

4. Discussion

The present study was focused to study the effect of Cadmium nanoparticle (CdNP) on the histology and biochemical changes in mud crab *Scylla olivacea*. Acute toxicity tests revealed that LD50 value for *S. olivacea* was found to be 50ppm/kg body weight of crabs. So, for further studies a dose of 20ppm/kg i.e., 50% value of the LD50 of CdNP was chosen. Exposure of CdNP induces morphological changes and inactivity, slow movement, a low feeding rate, incomplete withdrawal during ecdysis and eventual mortality. The accumulation of toxic compounds within the organism at lethal levels led to histological lesion in the crab, *S.olivacea.* Histopathological studies are also useful in evaluating the potential of CdNP, since trace amount of these chemicals which do not bring animal mortality over a given period, were capable of producing considerable organ damage [20, 21]. The study of micro-anatomy of the specific tissue constitutes an important diagnostic tool to observe the histological effects caused by a pollutant. The histological changes may be the manifestation of sick tissue [22]. In the present investigation disorganization of corneal cuticle and medulla, formation of corneal cones, constriction of internal medulla, complete

disorganization of basement membrane and constriction of external medulla were observed in the eyestalk of mud crab *Scylla olivacea* followed by CdNP exposure.

Neurohormonal factors released from the eyestalk regulate carbohydrates, proteins and lipids in crustaceans [12]. This suggested that eyestalk play a vital role in the regulation of biochemical contents. Enzymes and proteins play important roles in defence, detoxification, and elimination in trace metal contamination, especially as their biochemical changes are the responses to changes in the environment, e.g. existence of contaminants [23]. Thus it is possible to use them as biochemical biomarkers. During the physiological stress there is a great demand for energy to adjust the enhanced metabolic activities. This energy may be obtained from proteins, carbohydrates or lipids. In *Barytelphusa guerini* total protein contents of blood, gills, hepatopancreas and muscles increased initially (24 hours) when exposed to copper sulphate and cadmium sulphate. The results of More [24] showed that in cadmium sulphate exposed animals, total protein contents was increased in blood and muscles while it was decreased in gills and hepatopancreas as compared to control value after 96 hours exposure.

Reddy *et al.* [25] reported that exposure of cadmium caused hyperglycemia which is induced by the hyperglycemic hormone produced from the eyestalk in *Procambarus clarkii*. It was strongly supported by the works of Reddy *et al.* [26] in fresh water crab, *Oziotelphusa senex senex*. Sekar *et al.* [27] reported that eyestalk of fresh water female crab S*piralothelphusa hydrodroma* (Herbst) exposed to textile dye industry effluent showed decline in the protein, carbohydrate and lipid contents. It was supported by the works of Sreenivasan *et al.* [28] in *Spiralothelphusa hydrodroma* (herbst) exposed to Cypermethrin toxicity. Chourpagar and Kulkarni [29], suggested that decrease in the levels of protein, glycogen and lipid in different tissues of *Barytelphusa cunicularis* during the exposure to $HgCl_2$. In accordance with the previous findings, in the present work also eyestalk of mud crab, *Scylla olivacea* exposed CdNP for 10 days shown decline in the levels of protein, carbohydrate and lipid content which would suggest that exposure of animals to the nanoparticles in the aquatic system also had an impact on the histological and biochemical changes like other contaminants such as environmental pollutants, pesticides, agricultural runoff and industrial effeluents.

5. Conclusion

From this research work it was concluded that heavy metals in the form of nanoparticles contaminating aquatic system had a negative impact on the animals exposed to the nanoparticles and thus in turn indirectly affect human health.

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