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Effects of *in ovo* Administration of Zinc Oxide Nanoparticles and Vitamin C on Hatchability Performance and Redox Status in Day Old Kadaknath Hatchlings

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ABSTRACT

Background: In poultry industry, hatcheries play a vital role in connecting the poultry production chain and are expected in the productive performance, with an impact on company profits. The use of *in ovo* feeding support poultry embryonic development and offers the production efficiency and welfare of commercial poultry.

Methods: This study investigated the impact of *in ovo* administration of normal saline, Zinc oxide nanoparticles and Vitamin C on hatchability, chick growth and redox status in Kadaknath hatchlings. Zinc oxide nanostructures were synthesized by chemical method and characterized for size determination. A total of 150 fertile eggs of the Kadaknath poultry breed were divided into five groups (T_0 to T_4) and treated with *in ovo* administration of 200 µl each of normal saline, zinc oxide nanoparticles (5 and 10 ppm) and Vitamin C respectively on the 18th day of incubation through air sac into amniotic fluid.

Result: Rod shaped nanostructures ranging from 45 to 98 nm were synthesized and showed sharp peak positioned at 436.59 cm-1. Zinc nano composite 5ppm and vitamin C administration had significantly ($p<0.05$) improved hatchability, hatch weight, chick weight and egg weight ratio and antioxidant status.

Key words: Kadaknath, Nanoparticles, Vitamin C, Zinc.

INTRODUCTION

The hatchery performance in poultry sector play a vital role in connecting the poultry production chain with an impact on company profits (Desha *et al*. 2015). Kadaknath is the most popular native chicken breed in the Jhabua district of Madhya Pradesh, India. Geographical Indication Registry and Intellectual Property India awarded Geographical Indication (GI) Tag to this chicken breed on 30 July 2018 (GI Journal, 2018). This breed is experiencing higher demand due to its unique characteristics and perceived health benefits of its meat and eggs (Haunshiand Prince, 2021). *In ovo* administration of nanominerals, acting as bioactive agents and carriers of nutrients may be seen as a new method of nano nutrition, providing poultry embryos with bioactive compounds and/or with an additional quantity of nutrients and energy (Palouj *et al*., 2021). Antioxidant capability at hatching time is considered to be an important determinant of chick viability and prevent the damaging effects of free radicals and toxic products of their metabolism (Dominguez *et al*. 2019).Thus, the use of *in ovo* feeding of antioxidant constituents may be of immense benefit to the embryo in the pre-hatch growth phases (Asa *et al*. 2022). Thus, the objective of this study was to investigate the effect of *in ovo* administration of Nano zinc and vitamin C on hatchability performances and antioxidant status in day old Kadaknath hatchlings.

MATERIALS AND METHODS

The present study was conducted from April, 2021 May, 2022 at the Department of Veterinary Physiology and Biochemistry and Poultry unit, College of Veterinary Science and A.H., DSVCKV, Anjora, Durg (C.G.). The research was intended to use Zinc Oxide nanoparticles (ZnONPs) and Vitamin C on hatchability, redox status, in Kadaknath hatchlings.

Synthesis and characterization of zinc oxide nanoparticles (ZnONPs)

The ZnONPs were synthesized by using zinc nitrate hexahydrate ($Zn(NO_3)_2.6H_2O$) and sodium hydroxide (NaOH)

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precursors, calcined at 400°C as per method described by Fiedor-Tobola *et al*. (2018) and characterized by Fourier-transform infrared spectroscopy (FTIR) (Bruker, Vertex 80 FTIR System Germany, IIT Madras) and Scanning Electron Microscopy (SEM) (ZeissEVO18, IIT Delhi).

Experimental design

A total of 250 embryonated eggs of 8-9 month-old Kadaknath hens, laid on the same day represented the same weight class was procured from Poultry Unit, College of Veterinary Science and A.H., Anjora, Durg (Chhattisgarh)-491001 India. The basal diets of for breeder birds were formulated as per ICAR (2013) with nutrient specifications for poultry (Metabolizable energy 2700 Kcal/Kg). The eggs were disinfected, weighed, numbered and incubated with the temperature and relative humidity at 37.8°C and 55% respectively. On the18th day of incubation (18 DOI) infertile eggs and eggs containing dead in-shell embryos were removed. A total of 150 embryonated eggs were individually weighed and randomly assigned to 5 groups with 3 replicates in each and 10 eggs per replicate. The T1 was kept as healthy (Negative) control (without injection), the T2, T3, T4 and T5 received *in ovo* treatment (200 µl) of normal saline (positive control), zinc oxide nanoparticle 5 ppm (ZnONP5 ppm solution in normal saline), zinc oxide nano particle 10 ppm (ZnONP10 ppm solution in normal saline) and Vitamin C (Mankind Pharma Ltd, 150 mg/1.5 ml) respectively through the air sac into amniotic fluid using a 21-gauge needle and eggs were sealed with sterile tape and placed in an incubator. On 21 DOI and 22 DOI, the number of eggs hatched was recorded and all hatchlings were weighed.

Hatchability and growth performance of chicks

Per cent hatchability, hatch weight of chicks and chick weight to egg weight ratio per cent was determined:

Per cent hatchability =

$$\frac{\text{Number of hatched egg}}{\text{Total number of fertile eggs set for hatchability}} \times 100$$

Chick weight to egg weight ratio per cent =

$$\frac{\text{Chick weight (gm)}}{\text{Egg weight (gm)}} \times 100$$

Sample collection and biochemical analysis

Blood samples were collected from randomly selected day-old hatchlings per group (3 per replicate) and hemolysate was prepared as per method described by Huntsman, (1975) to estimate lipid peroxidation and antioxidant status. The haemoglobin (Hb) content of the erythrocytes hemolysate was determined by the cyanmethemoglobin method.

Evaluation of oxidative stress and antioxidant balance

The LPO concentration in the erythrocytes was determined as per method describe by Utley *et al*., (1967) and expressed in nmol MDA/mg of Hb. Reduced glutathione (GSH) assay was performed according to Prins and Loos (1969) and determined as µmol GSH/mg of Hb. The activity of superoxide dismutase (SOD) was measured by using nitroblue tetrazolium and was expressed as in Unit/mg of Hb. (Marklund and Marklund, 1974).The activity of catalase (CAT) in Unit/mg of Hb was assessed as described by Cohen *et al*. (1970).

Statistical analysis

The collected data were subjected to statistical analysis using the general linear model of one-way analysis of variance - ANOVA. The significance of differences between the groups was evaluated using Tukey's test (IBM SPSS Statistics version 26.0).The level of significance was set at $p \leq 0.05$. Simplifying the visualization of these results, depicted interleaved bars±SEM.

RESULTS AND DISCUSSION

Characterization of zinc oxide nanoparticles (ZnONPs)

The FT-IR spectra of chemically synthesized ZnONPs in the range of 4000-400 cm^{-1} (Fig 1a). The observed peaks of the FT-IR spectrum depends on the size and morphology of ZnONP were referred from previous works of literature in order to confirm the findings.

The prepared ZnONPs showed infra-red (IR) peaks at 436 cm^{-1}, 1384.25 cm^{-1} and 3447.98 cm^{-1}. The peak positioned at 436.59 cm^{-1} is attributed to the Zn-O stretching bonds. Ismail *et al*. (2018) revealed that IR broad absorption feature positioned at 443.96 cm^{-1} confirms the stretching vibration of Zn-O.

The absorption peak of hydroxyl group of zinc hydroxide is at 1384 cm^{-1}, in the spectrum was recorded due to C=O stretching. The adsorbed free CO_2 from the air might be responsible for the band at 1384 cm^{-1} (Nejati *et al*. 2007). The bands in the region of 1700-3300 cm^{-1} were lost due to the removal of water molecules.

The SEM image in Fig 1b clearly depicted ZnONPs size ranging from 45 to 98 nm. Nearly rod shaped nanoparticles was recorded with an average size of 68 nm. These results were consistent with the previously reported study of Kumar *et al*. (2022).

Hatchability performance

Hatchability in negative control group was recorded 73.3% whereas 66.7% was observed in normal saline injected group (T_1). Decreased hatchability in positive control group may be due to the injection process. *In ovo* administration of ZnONPs 5 ppm and Vitamin C had around 72% and 76.7% hatchability respectively (Fig 2a). Palouj *et al*. (2021) reported reduces hatchability and increased embryonic mortality with *in ovo* injection of ZnONP.

Significant reduction ($P<0.05$) of hatchability (56%) was observed in ZnONP 10 ppm treatment (T3) as compared to positive control group (T_1). Jose *et al*. (2018) reported reduction in hatchability due to high levels of zinc, results in imbalance of amnion minerals content that interfered with embryogenesis during incubation or due to the toxicity of zinc nano-form due to its high availability.

Maximum hatchability was recorded in group T_4 (vitamin C treatment) as compared to other studied groups. Zhang *et al*. (2019) also suggested that increase concentrations of L-Ascorbic acid (L-AA) may improve the hatchability as well as the post hatch performance of chickens. Zhu *et al*. (2019) suggested that vitamin C, as a cofactor of hydroxylase, promoting gluconeogenesis and enabling embryos to adapt the environment of incubation and pip of the eggshell.

Non significant increased chick weight and chick's weight to egg weight ratio was observed in T_2 group (P=0.004) where as significant increased (P<0.05) T_3 group as compared to positive control group (T_1). Significant decrease (P<0.05) effect on chick growth was observed in T_4 (Fig 2b, 2c). The positive effect of ZnONPs 5 ppm supplementation on growth performance was in line with the findings of Torrs and Korvar, (2018) who recorded the important role zinc in the metabolism of energy, nucleic acids, lipid and protein. *In ovo* supplementation of ZnONP 5 ppm solution might be considered as an emerging alternative feed supplement for poultry with a claim of having a greater bioavailability and growth promoter ability. Soltani *et al*. (2019) reported improvement in growth performance after *in ovo* supplementation of ascorbic acid, should be explained by its antioxidant role.

Oxidative stress and antioxidant status

The perturbation of reactive oxygen species (ROS) and antioxidant balance is often due to an increase in ROS production or/and a depression of the antioxidant system at the time of hatching (Bacau *et al*. 2021).

Lipid Peroxidation

The malondialdehyde (MDA) concentration in the chickens hatched from the positive control group (T_1) increased (18.29%) significantly (P<0.05) as compared to negative control group (T_0). *In ovo* injected ZnONP 5 ppm (T_2) and Vitamin C (T_4) groups had significant decreased malonaldehyde levels, 21.02% and 25.08% respectively whereas ZnONP 10 ppm treatment recorded a significant increased (P<0.05) levels as compared to the positive control group (Fig 3a).

Our findings are consistent with Zhang *et al*. (2019), they also observed that Zn administration can substantially enhance resistance against oxidative stress in developing embryos and hatchlings.

In our study, *in ovo* administration of vitamin C significantly reduced the lipid peroxidation levels, which fell into line with the research results of Carr and Maggini,

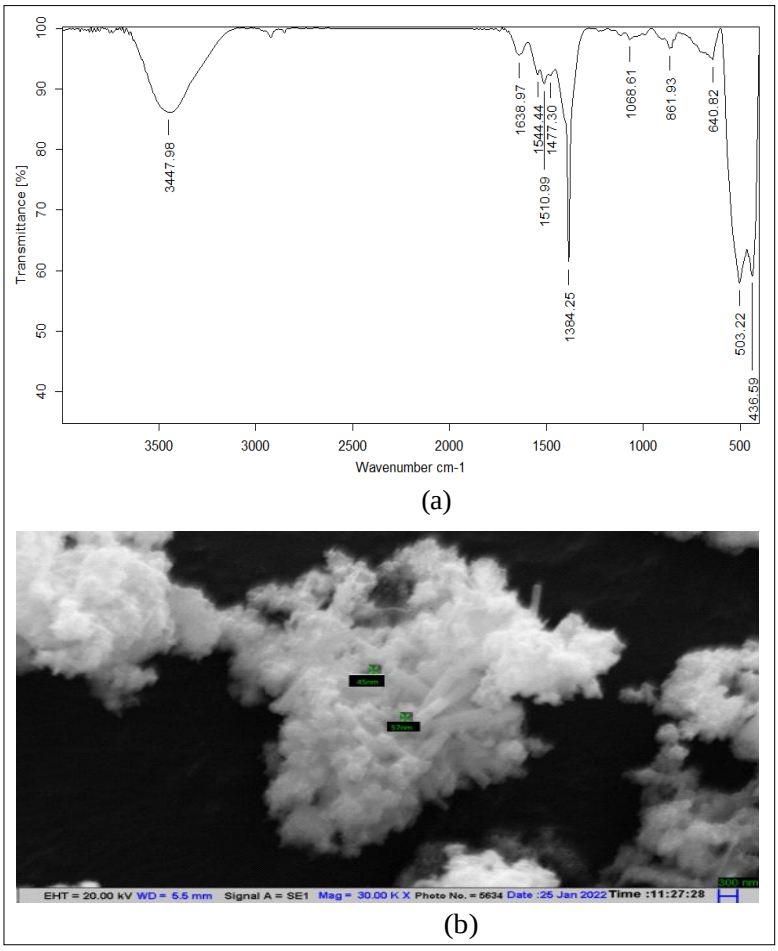

Fig 1: Characterization of ZnO nanoparticle (a) FTIR (b) scanning electron microscopy.

(2017). El-Senousey, *et al*. (2018) recorded that the *in ovo* injection of L-AA at various levels increase antioxidant activity and reduces malonaldehyde levels in blood.

Antioxidant status

Reduced glutathione (GSH) participates in various cellular reactions, scavenges free radicals and maintains redox balance of the cell (Balk *et al*. 2009). Significantly reduced (P=0.001) GSH was observed in T_1 as compare to the negative control group (T0). Significantly increased GSH levels, 51.25% and 75.0% was recorded in the group treated with ZnONP 5 ppm and Vitamin C respectively whereas significantly reduced (P<0.05) GSH level was reported in group treated with ZnONP 10 ppm (Fig 3b). These results obtained are consistent with the findings of Micheletti *et al*. (2001) who revealed that zinc supplementation prevented formation of free-oxygen radicals. Jang *et al*. (2017) also observed that dietary supplementation of vitamin C significantly increased total antioxidant status and decreased serum lipid perioxidation in broiler birds.

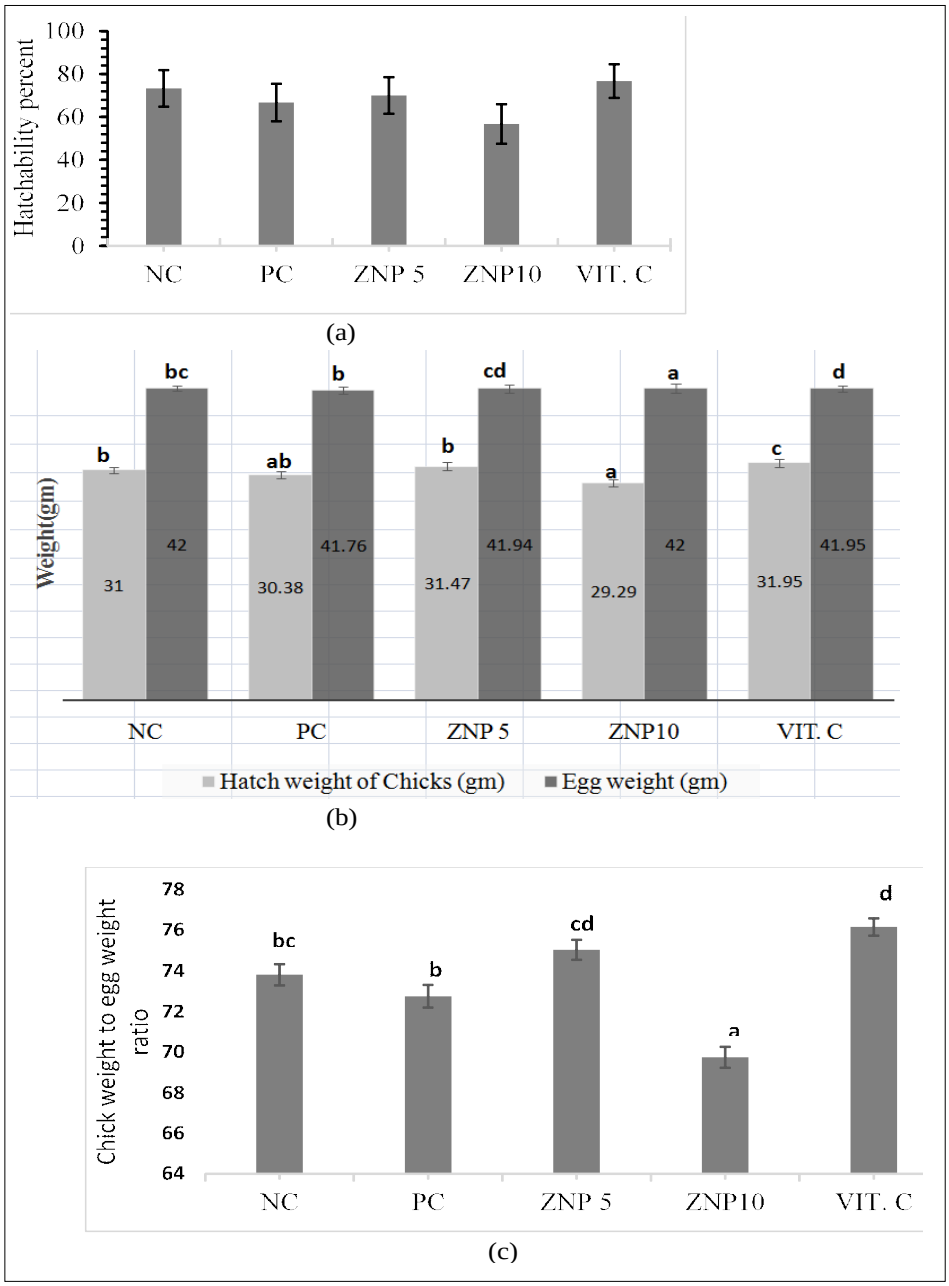

Fig 2: Effect of *in ovo* administration (18DOI) of normal saline, ZnONP and vitamin Con (a): Hatchability percentage; (b): Egg weight and hatch weight of chicks; (c): Chick weight to egg weight ratio, of day old Kadaknath chicks. NC= Negative control (No Injection), PC= Positive control (Normal saline treated), ZNP5= ZnONPs5ppm, ZNP 10= ZnONPs 10ppm, VIT. C= Vitamin C.

Fig 3c presented the activity of antioxidant enzymes, Superoxide Dismutase (SOD: E.C. 1.15.1.1) and Catalase (CAT: E.C. 1.11.1.6) in the experimental study. SOD, as an important vitagene is the main driving force in cell/ body adaptation to various stress condition. During embryonic development of the chicken SOD plays a crucial role as an integral part of the antioxidant network (Surai, 2016). Significant decreased (P<0.05) activity of SOD in Positive control group (T_1) as compared with T_0 group. *In ovo* administration of ZnONP 5ppm and Vitamin C reported significant increased (P=0.001) SOD activity, 25% and 43.44% respectively whereas significant reduced SOD activity was observed in T_3 as compared to all other treatment groups.

Zinc influences oxidative processes and is also necessary for the structure and function of Cu-ZnSOD, protects tissues from the oxidative lesion (Zhang *et al*. 2019). In fact, due to increased antioxidant activity as a result of *in ovo* administration of Nano-ZnO 5 ppm, free radicals can be efficiently scavenged and thus the chick embryo was less exposed to oxidative damage during hatching. The increased SOD activity in vitamin C treated groups might be due to the antioxidant action exerted by the enzyme in response to the oxidative stress in Kadaknath hatchlings.

CAT is one of the most important antioxidant enzymes present in almost all aerobic organisms, breaks down two hydrogen peroxide molecules into one molecule of oxygen (Rodríguez-Ruiz *et al*. 2019). Significant decreased (P<0.05)

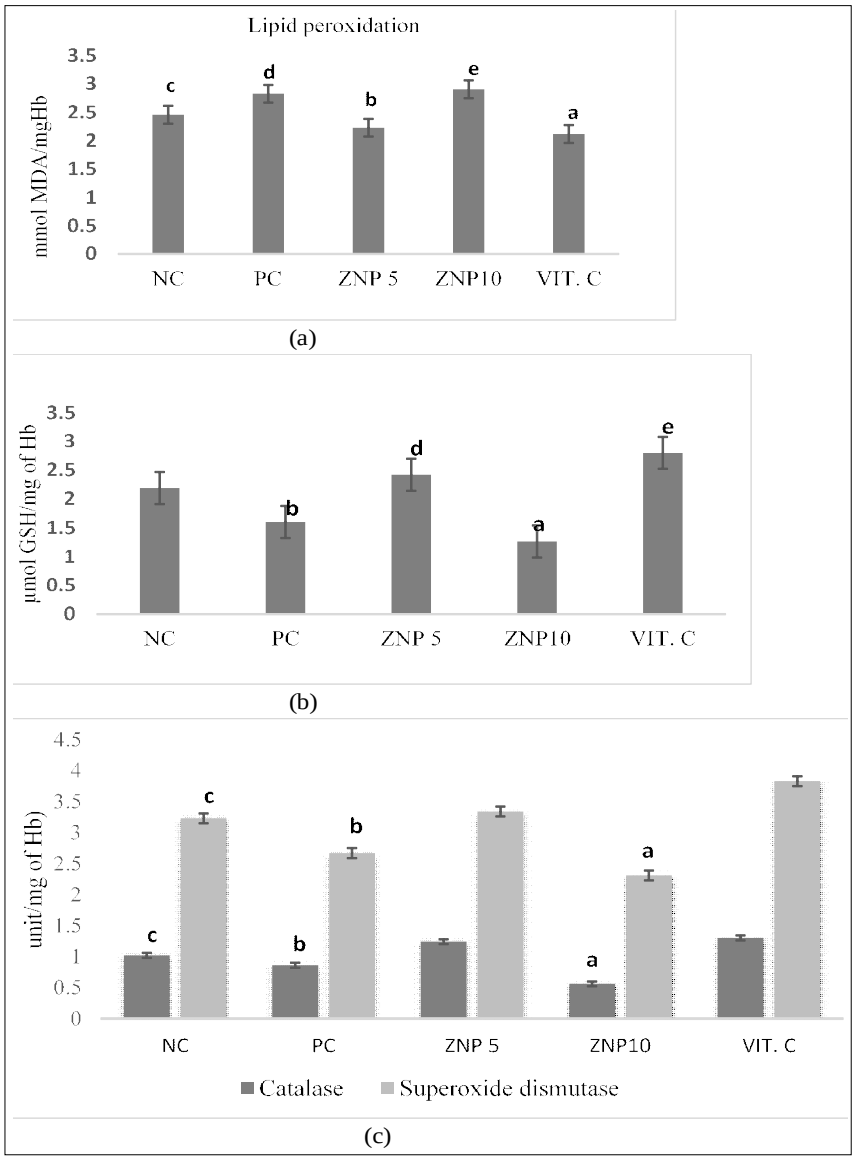

Fig 3: Effect of *in ovo* administration of Kadaknath eggs (18 DOI) with normal saline, ZnONP (5 and 10ppm) and vitamin C on (a): Lipid peroxidation; (b): Reduced Glutathione; (c): Antioxidant enzymes, in day old Kadaknath hatchling. NC= Negative control (No injection), PC= Positive control (Normal saline treated), ZNP5= ZnONPs5ppm, ZNP 10= ZnONPs10ppm, VIT. C =Vitamin C.

catalase activity in T_1 group as compared to negative control group (T_0). ZnONP 5ppm (T_2) and Vitamin C (T_4) treatment had a significant increased catalase (P=0.001) activity as compared to positive control group. ZnONp 10 ppm (T_3) treated group showed significant reduced (P<0.05) catalase activity. Naeem *et al*. (2022) also speculated significantly increased catalase activity after *in ovo* injection of antioxidants in broiler chickens. Min *et al*. (2018) suggested that the high antioxidant activity in the newly hatched chickens can have a positive effect on the growth performance of chickens.

Therefore, the author suggested that the activity of ZnONP 5 ppm and vitamin C following *in ovo* injection could perhaps because of its ability to scavenge ROS and alleviating oxidative stress responses.

CONCLUSION

The results of the present study showed that *in ovo* administration of Nano-ZnO 5 ppm(45 to 98 nm size) and vitamin C on the 18th day of incubation substantially enhanced antioxidant capacity in hatchlings of old Kadaknath hatchlings.

Conflict of interest: None.

The authors declare that they have no competing interests.

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