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Research Article

Naringenin Prevents the Zinc Oxide Nanoparticles Induced Toxicity in Swiss Albino Mice

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Keywords

- Toxicity
- Naringenin
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- SGPT
- CK-MB expressions

Abstract

Objective: The present investigations were undertaken to evaluate prevention of toxicity with naringenin treatment in ZnO nano particles induced toxicities in Swiss albino mice.

Methods: The effects of orally administered naringenin on serum creatinine, SGOT &SGPT and CK-MB expressions were observed in ZnO nano toxicities induced groups and treated groups, over a period of 14 days treatment.

Results: ZnO nano toxicities induced groups produced a significant increased serum creatinine, SGOT&SGPT and CK-MB expressions. Treatment with Naringenin, decreased serum creatinine levels, SGPT levels and CK-MB expressions. There was a significant decreased the body weight in ZnO nano toxicities.

Conclusion: Our results suggesting that Naringenin drug therapy is to be beneficial for the treatment of ZnO nano toxicities.

INTRODUCTION

Nanotechnology has wide range of applications. They are using in diagnosis, drug delivery system, food industry, paints, electronics, sports, environmental cleanup, cosmetics and sun screen products. At the same time, they have a high potential interaction with different biological systems like kidney, spleen, liver etc., so it is essential to find out the toxicities of the nano-materials. Different types of nano-particles (NP) were using in different applications, among them, ZnO nano-particles having wide range applications like cosmetics and sunscreen lotions because of their efficient UV-A and UV-B efficient absorption properties without scattering visible light [1]. ZnO nano-particles have the other application to their antimicrobial properties [2,3], they also using in fungicida. in agriculture and anticancer therapy [4,5]. The ZnO nano-particle production day by day increased and also increasing the accidental exposure to humans and animals. Already so many animals' studies were revealed that there was a great toxicity of ZnO nano -particles by in-vitro studies on different biological system like bacteria and mammalian cells [6-8]. The in-vivo studies also conducted to evaluate the organ toxicity and genotoxicities [9].

The evaluation of ZnO NP, there is no proper guidelines, so some committees like committees on toxicity (COT), mutagenicity (COM), and carcinogenicity (COM) gave some guidelines to find out the toxicities of chemical present in food and also gave source guidelines for route of administration for in-vivo studies [10]. In the case of ZnO NP, the route of administration is oral because the accidental contamination with cosmetics and food and also the people who were worked at the area of production. Some NP also enters into the GIT after inhalation [11,12].

Earlier studies shown that there was a greater toxicity with nano particle than micro particles, and they were distributed in to the organs like liver spleen, kidney and heart. [13]. Bioflavonoids have the wide range of activities like antoxidant, antiinflamatory, antidiabetic, and anti alzimer. Narigenin is a natural flavonoid, it has the antioxidant and anti diabetic activities. Therefore present study was undertaken to evaluate the sub acute toxicity of ZnO-nanoparticles and that is prevention with naringenin. If the ZnO NP is accidental exposures were controlled by the daily food containing flavonoids [22].

MATERIALS AND METHODS

ZnO nano particle powder (nanorods) was procured from "International Advanced Research center for Powder Metallurgy and New materials", Hyderabad. The method by which ZnO Nanorods were prepared by flame spray pyrolysis (FSP) and is pre characterized and the characterization details are as follows.

Characterization details of zinc oxide nanoparticles

Characteristics	Details
Shape	Rod shaped
Size	18-20nm
Surface area	$30m^2/g$
Aspect ratio	≈ 3-4

Animals: Thirty male swiss albino mice (30), weighing 20-25g were procured from Sainath agencies, Hyderabad. Mice were housed and cared for under pathogen free condition.Mice were given standard diet and water ad libitumand were housed in a 12h light/dark cycle.In addition mice were acclimatized for 1 week to laboratory environment prior to study.

Preparation of Zinc oxide (ZnO) Nano rods dispersion: Note: right precautionary measures are followed while handling nanoparticles.Lab coat,gloves,nose safety mask and safety glasses were used to prevent likely exposure.

Procedure: Required quantity of ZnO nano-rods powder was weighed with help of high sensitive electronic balance using clean and neat spatula. The powder was transferred into a beaker containing 100 ml drinking water in an air free environment, preventing disposal of nano-particles into working area. Into the beaker the probe of ultrasonicator was immersed such that it is at $1/4^{th}$ of position beaker height. Ultra sonicator was performed for a period of 15 min. From this dispersion, which serves as a stock, and from which the required doses were administered.

Experimental design

Swiss Albino mice were divided into 5 groups (n=6).

Group I serves as a normal control, which receives normal drinking water.

Group II and **III** serve as toxic groups (ZnO NPs 150mg/kg & 300mg/kg).

Group IV and **V** serve as preventive groups (Naringenin 20mg/kg).

Bio chemical estimation

In all the groups blood samples were drawn from retro orbital puncture and centrifuged at 1000 rpm per 15 min, serum was separated and performed the following biochemical parameters therefore Determination of serum creatinine (Scr), Serum creatine phosphokinase isoenzyme (CK-MB) activity, SGPT (ALT) & SGOT (AST), body weight was assessed before and after treatment and end of the study find out the Histopathalogical study of Liver, Kidney, Heart, bone and Testes.

RESULTS

The serum creatinine levels were elevated in toxic groups (ZnO NP, 150 mg/kg and 300 mg/kg groups) after inducing zinc oxide NP. Where as in the treatment group with Naringenin the levels were found that decreased when compared to the induced groups. These results were represented in (Figure 1) The SGPT and SGOT levels were elevated in 150 mg/Kg and 300 mg/kg groups. After the preventive therapy with Naringenin, the SGPT and SGOT levels were found that decrease when compared with the induced groups. These results were expressed in (Figure 2,3).

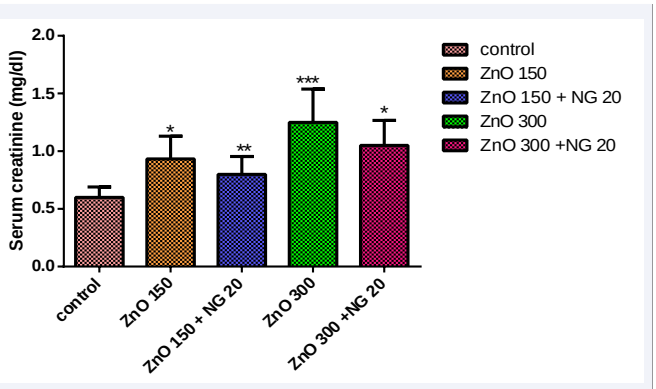

Figure 1 Estimation of serum creatinine levels (Data were expressed in Mean ± SD (n=6) * P< 0.05 Significant, ** P< 0.01, *** P< 0.001 Compared with control, toxic and treatment groups).

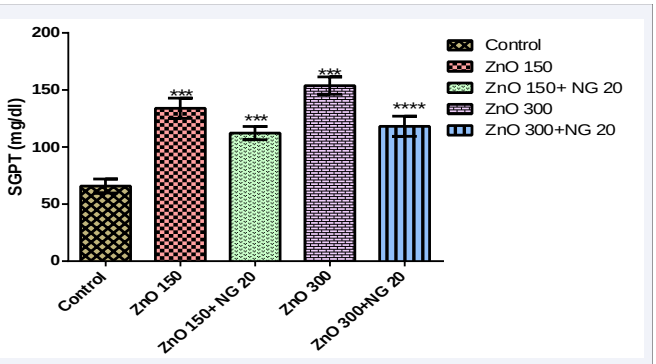

Figure 2 Graph showing the SGPT levels(Data were expressed in Mean ± SD (n=6) *** P< 0.001 Very Highly significant. Compared with control, toxic and treatment groups).

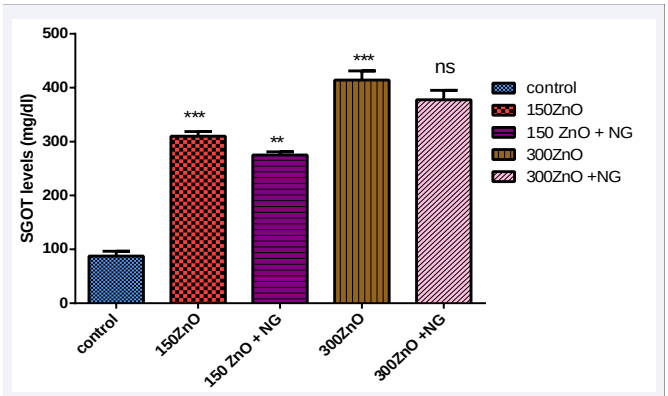

Figure 3 Graph showing the SGOT levels.(Data were expressed in Mean ± SD (n=6) ns Non-significant, ** P< 0.01, *** P< 0.001 Compared with control, toxic and treatment groups.).

The CK-MB levels were expressed more in 150 mg/Kg and 300 mg/kg ZnO NP groups. After the treatment with Naringenin the levels were found to be decrease when compared with the induced groups, these results were expressed in (Table 1). The control group animals were showed that there was no significant

change in body weight. Where as in the other preventive groups like the naringenin there was a significant change in the body weight. The body weights were found to be increased in the preventive group, and the ZnO NP treated groups lost their weight, these results were expressed in (Figure 4).

Histopathological findings in kidney, the normal group kidney section revealed that glomeruli and tubules had normal morphology and no evidence of interstitial lymphocytic infiltration. In toxic control group there was a dilated congested vessels and areas of interstitial haemorrhages were seen. In Naringenin prevention groups the sections were showed less hemorrhagic regions the total histopathology were expressed in (Figure 5) Histopathological findings in heart shows in normal control group the sections revealed that there is no evidence of inflammatory and necrosis in toxic groups chambers are filled with hemorrhage, evidence of necrosis and inflammatory cell infiltration. In Naringenin preventive groups the sections revealed less hemorrhage and inflammation were observed these results showed in (Figure 6).

Histopathological findings in liver, the normal histopatholgy of liver showed that structure of hepatic parenchyma with single cell thick trabeculae dilated congested vessel and no lymhatic infiltration. In toxic groups, hepatic parenchyma were observed with foamy degeneration of hepatocytes and hepatic cell necrosis. In Naringenin preventive groups the section revealed that there is no cell necrosis and parenchyma infiltration, which is present in (Figure 7).

Table 1 : Effect of Naringenin on ZnO NPs induced CK-MB levels in swiss albino mice.

Groups	CK-MB levels (IU) Mean ± SD
Control	614.2± 79.2
ZnO NP 150mg/kg	846.8± 84.6**
ZnO NP 300mg/kg	949.7±136***
ZnO NP 150mg/kg +20mg/kg NG	685.5± 29.4**
ZnO NP 300mg/kg +20mg/kgNG	769.8 ±115.3 *

(Data were expressed in Mean ± SD (n=6) * P< 0.05 Significant, ** P< 0.01, *** P< 0.001, Compared with control, toxic and treatment groups).

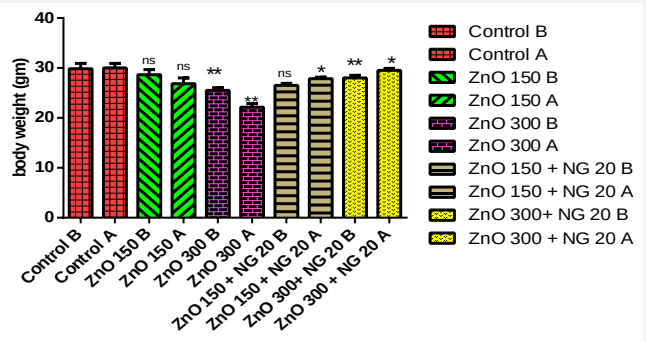

Figure 4 Graph showing the body weight. (Data were expressed in Mean ± SD (n=6) * P< 0.05, ** P< 0.01 , ns-Non- significant. Compared with control, toxic and treatment groups).

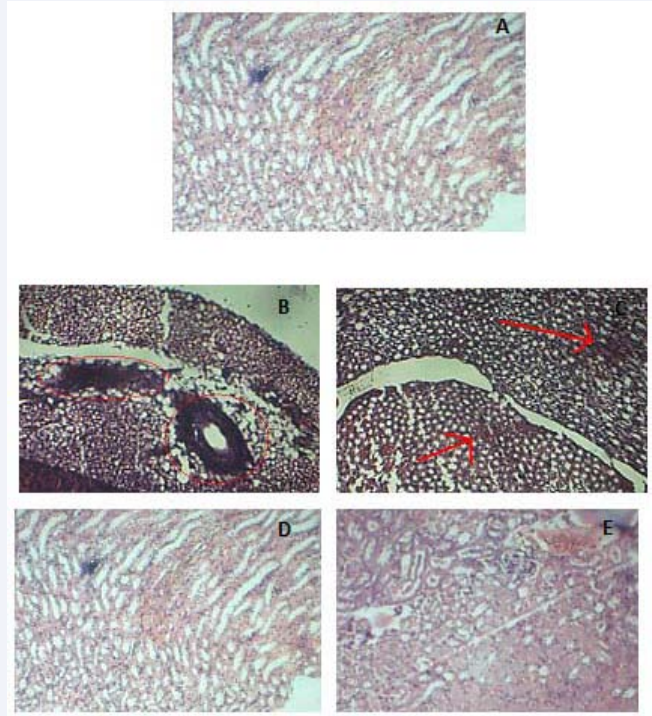

Figure 5 Histopathology of kidney: A) Control B) 150 mg/kg bw ZnO NP C) 300 mg/kg bw ZnO NP D) 150 mg/kg ZnO NP bw + NG E) 300 mg/kg ZnO NP bw + NG.

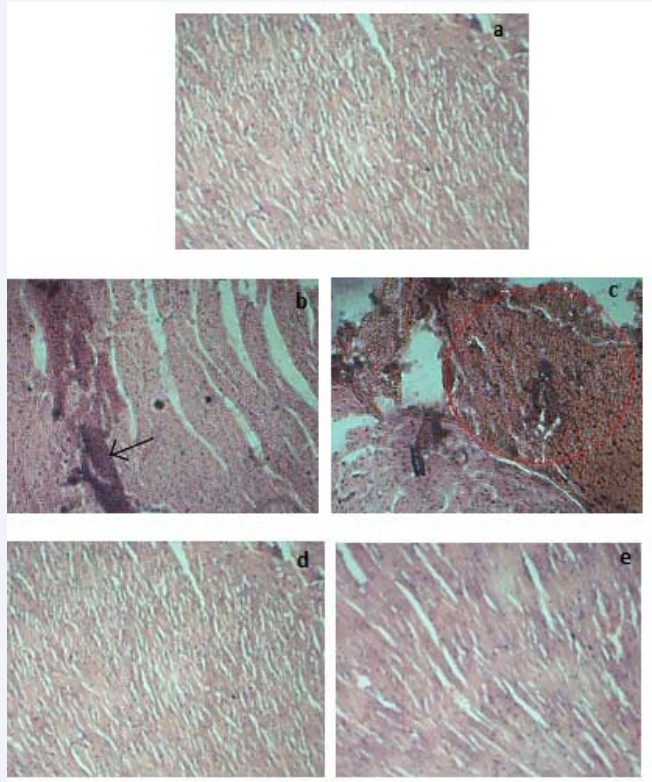

Figure 6 Histopathology of Heart: a) Control b) 150 mg/kg bw ZnO NP c) 300 mg/kg bw ZnO NP, d) 150 mg/kg bw ZnO + NG e) 300 mg/kg bw ZnO + NG.

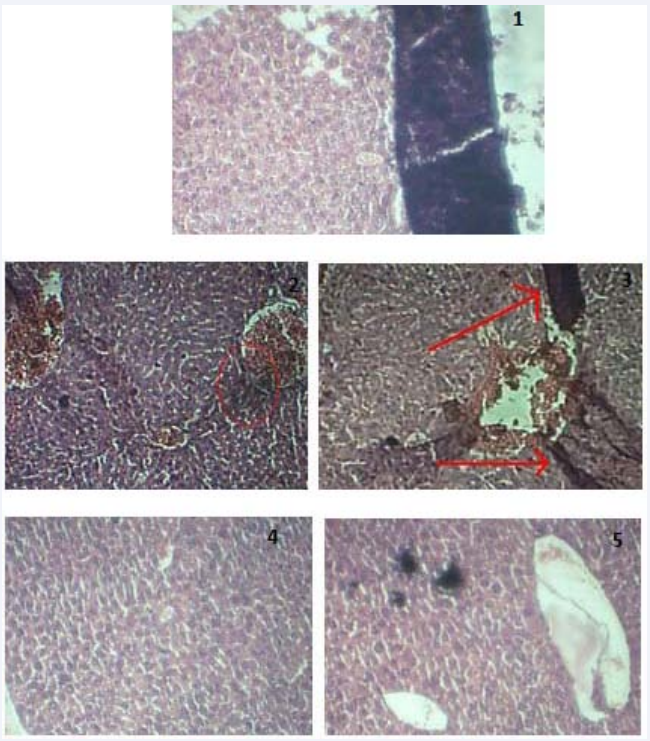

Figure 7 Histopathology of liver: 1) Control 2) 150 mg/kg bw ZnO 3) 300 mg/kg bw ZnO 4) 150 mg/kg bw ZnO + NG 5) 300 mg/kg bw ZnO + NG.

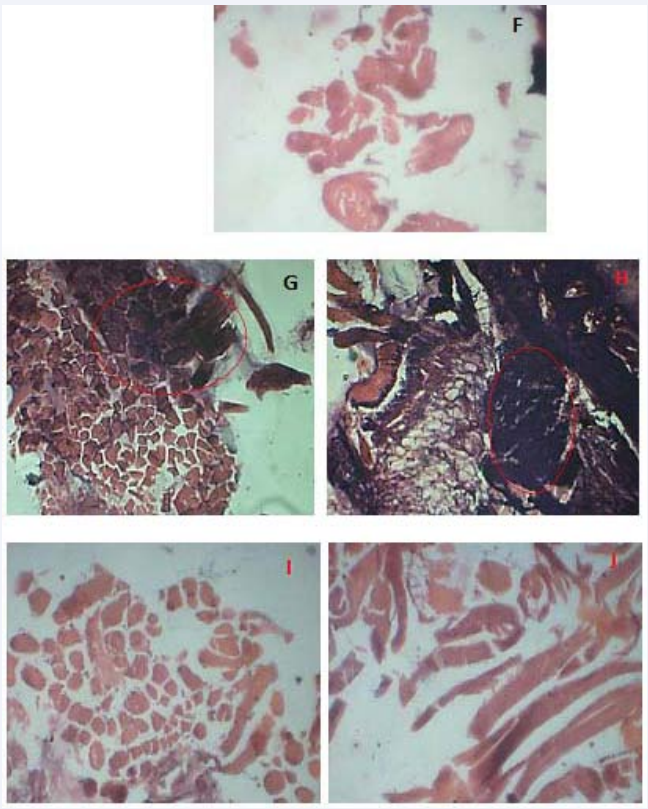

Figure 8 Histopathology of Bone: F) Control G)150 mg/kg bw ZnO H) 300 mg/kg bw ZnO I) 150 mg/kg bw ZnO + NG J) 300 mg/kg bw ZnO + NG.

Histopathological findings in bone results shows that the normal group bone sections revealed that regular bony trabeculae without oesteoblastic lining, skeletal muscle tissue and fragments of cartilage were seen. The toxic group sections revealed that tiny irregular bony trabeculae with focal oesteoblastic lining with areas of hemorrhagic and bundle of skeletal muscle fibres. The preventive group with Naringenin showed that irregular bony trabeculae without oesteoblastic lining and hemorrhage, which were expressed in (Figure 8).

Histopathological findings in testis: shown the normal group sections revealed hat seminiferous tubules showing spermocytic maturation, no interstitial lymphocytic infiltration. The toxic group sections revealed that occasional premature tubule and areas of adipose tisue with lymphocytic infiltration. Preventive group with Naringenin section reveals slight lymphocytic infiltration, which was repressed in (Figure 9).

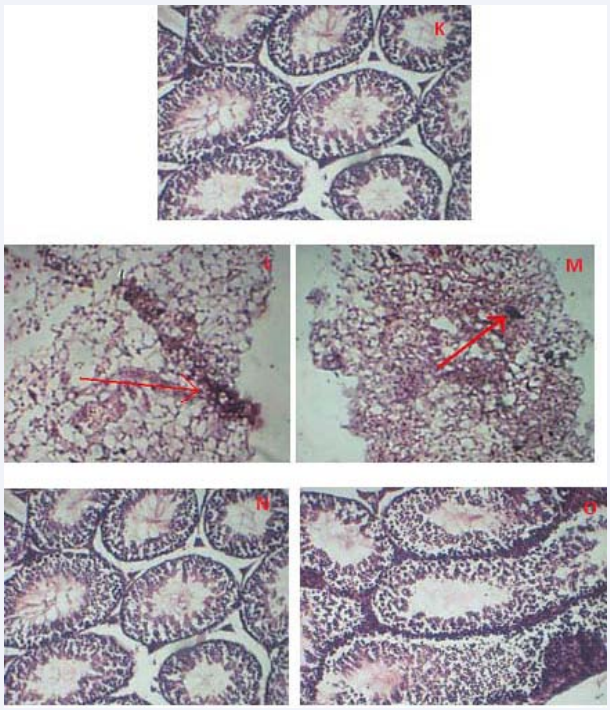

Figure 9 Histopathology of testis: K) Control L)150 mg/kg bw ZnO M) 300 mg/kg ZnO N) 150 mg/kg bw ZnO + NG O) 300 mg/kg bw ZnO + NG.

DISCUSSION

ZnO Nanoparticles (NPs) were used in a variety of different applications including cosmetics, paints, as drug carrier and filling in medical materials [13]. including sunscreens and environmental remediation, direct and indirect release of these nanoparticles (NPs) into aquatic environments via bathing, sewage effluent and other engineering application, said that feeding of ZnO nanoparticles suspension through digestive tract at a dose 0.6mg daily which leads to damage of some primary organs (heart, lung, liver & kidney) of mice [14-15]. In our study we were estimated different biochemical and histopathological

changes which were supported by the previous study results that, the nanoparticles when ingested into the body can be distributed to different regions because of their small size. They can cross the small intestine and further distribute into the blood, brain, lung, heart, kidney, spleen, liver, intestine and stomach [16].

The study explained the mechanisms involved in nano-toxicity, when exposed to light or transition metals, nanoparticles may promote the formation of pro-oxidants which, in turn, destabilizes the delicate balance between the biological system's ability to produce and detoxify the reactive oxygen species (ROS). ROS include free radicals such as the superoxide anion (O2), hydroxyl radicals (OH) and the non-radical hydrogen peroxide (H2O2), which are constantly generated in cells under normal conditions as a consequence of aerobic metabolism. When cells are exposed to any insult (chemical/physical), it results in the production of ROS [17]. Furthermore, oxidative stress activates specific signaling pathways including mutagen activated protein kinase (MAPK) and NF-kB, which together with the depletion of antioxidant defenses that leads to release of pro-inflammatory cytokines. The overall result of this signaling cascade is the triggering of inflammation, a defensive reaction that leads to further ROS release from inflammatory cells (e.g. neutrophils), resulting in a vicious circle of events that is also central to the pathogenic consequences of particle exposure [18].

In our study found that there was a liver toxicity. This was expressed by the elevated levels of SGPT and SGOT, and histopathological changes were occuered [16]. Naringenin has a preventive therapy because naringenin contain antioxidant effect which was supported by the previos reports [19,20]. The altered serum creatinine levels were increased after 14 days oral 150mg/kg and 300 mg/kg of ZnO treatment groups because of renal toxicity [16,21]. The bioflavonoid preventive therapy is significantly reduced the elevated serum creatinine levels in this study. In our study there is elevated CK-MB levels indicated that there was cardio toxicity and there were a necrosis and inflammatory cells in cardiac muscles. Serum creatine phosphokinase isoenzyme (CK-MB) activity was measured kinetically to measure the cardiac toxicity. The cadiotoxicity was prevented by Naringenin, because of antioxidant activity and anti inflammatory activity [22,23]. In our study the ZnO NPs induced toxicities were significantly reduced by Naringenin, which was supported by biochemical and histiopathological studies.

CONCLUSION

In our study ZnO nanoparticles 150 mg/kg and 300 mg/kg showed toxicity, in preventive therapy with Naringenin 20mg/kg showed protective activity. In our study the results support the ZnO nanoparticle toxicity was reduced by bioflavonoid. Naringenin Finally it was concluded that bioflavonoid reduced the nanoparticle induced toxicities.

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