Innovare Academic Sciences

International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 7, Issue 7, 2015

Original Article

ACUTE DERMAL TOXICITY OF COAL FLY ASH NANOPARTICLES *IN VIVO*

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Received: 24 Apr 2015 Revised and Accepted: 01 Jun 2015

ABSTRACT

Objectives: To study the toxicity effects of coal fly ash nanoparticles-induced acute dermal toxicity in Wistar albino rats.

Methods: Acute dermal toxicity studies for coal fly ash nanoparticles (CFA-NPs) were conducted in Wistar albino rats. A single dose of various concentrations of CFA-NPs or vehicle was applied on the dorsal layer after shaving. Animals were observed for 14 days. Parameters like body weight, feed intake and histopathology were studied.

Results: The CFA-NPs treated rats did not show any abnormal clinical signs. The body weight was not significantly altered when compared with the control group. Treatment with CFA-NPs showed mild to severe histological changes in all organs with increase dose-related manner in concentrations of CFA-NPs treated groups. The acute dermal LD50 of CFA-NPs was found to be greater than 2000 mg/kg body weight (bw) for rats.

Conclusion: Dermal acute treatment with CFA-NPs can induce mild to significant dose-dependent histological changes in biological organs.

Keywords: Coal fly ash nanoparticles, Dermal toxicity, Histopathology.

INTRODUCTION

Acute toxicity refers to adverse effects occurring following a single exposure to a substance within 24 h. Generally it is necessary to identify the lethal dose 50 (LD50) value for environmental and occupational exposure particles, it is due to their intended biological activity and toxic mode of action, as well as daily exposure to the particles via multiple roots such as, dermal and/or inhalation. The purpose of the acute toxicity studies was to obtain information on the biologic activity of a given substance and gain insight into its mechanism of action. Long term studies like sub-acute and sub-chronic studies usually start with a dose-finding exercise under acute conditions. Nanoparticles (NPs) possess dramatically different physicochemical properties compared to fine particles of the same composition. Smaller the size of the particle, the greater is its surface area to volume ratio, and the higher its chemical and biological reactivity [1].

Coal fired boilers are generally used in power stations to generate electricity due to low cost and abundance of this fuel. Combustion of coal generates large amount of particulate matter (PM) in to the atmosphere which are formed by mineral transformation during high-temperature combustion process. Coal fly ash particulate matter (CFA-PM) emissions were controlled by several methods, but these control measures are not completely effective. CFA-PM can still remain a significant fraction of the overall particle exposure for power plant workers and highly impacted communities. The efficiencies of the filtering devices fail within the size of PM matter below 100 nm [2]. These ultra fine particles escapes and get dispersed in air [3]. Human health impacts of CFA (PM 0.1) released in to the atmosphere is the major health concern in developing countries. Especially exposure to combustion derived nanoparticles (CD-NPs) has increased dramatically within the past century. The greater surface areas of ultra fine CD-NPs compared with larger particles with the same chemical compositions make them more environmentally active with respect to biouptake and associated diseases [4-6].

Information is lacking in describing the human health and environmental implications of coal fly ash nanoparticles (CFA-NPs). Data is needed for cutaneous analysis after CFA-NPs exposure that could occur during the work conditions and also for close living communities. Currently, there is no information is available regarding whether nanoparticles can be absorbed through skin and cross the skin's stratum corneum barrier or whether systemically administered particles can accumulate in dermal tissue. The tendency of CFA-NPs to traverse the skin is a primary determinant of its dermatotoxic potential. That is, the nanoparticles should penetrate the uppermost stratum corneum layer in order to gain entrance to the viable epidermis and exert toxicity in the lower cell layers from then to systemic circulation [7]. The nano sized particles are then able to penetrate through the basic biological organs and leads to inflammation of tissues and also alters the cellular redox balance towards oxidation, leading to cell death [8].

Elemental composition of CFA varies with the type of the Coal and combustion. Like other engineered nanoparticles, CFA-NPs agglomerate readily and move into the accumulation mode which decreases the particle number but probably leaves the surface area dose unaffected. NPs has ability to penetrate through the skin and cause inflammation and cell death and also, In the case of insoluble CDNPs, have potential to escape from the site of deposition and translocate to the blood and to other target organs [9]. CFA-NPs emits large amounts of PM into the atmosphere has adverse effects on living organisms when exposed directly to the skin. It is previously reported that CFA-NPs contains several toxic heavy metals present in it [10].

In this report, we have estimated the quantitative relationship between dermal exposure to CFA-NPs and it toxic response. A basic tenant of toxicity is that the degree of harm is directly related to the amount of exposure. There is no information on dermal exposure toxicity of CFA-NPs. Therefore we investigated the potential acute toxicity, providing useful information for assessing the toxicological relevance of CFA-NPs. Tests were carried out according to the OECD guidelines. The dermal exposures to various concentrations of CFA-NPs were extensively studied. The collection and characterization of CFA-NPs were previously done by Sambandam *et al.* (2014) [10]. The same collected CFA-NPs were used in the present study.

MATERIALS AND METHODS

Animal ethics

This study was approved by an Institutional Animal Ethical committee (IAEC) and was executed according to the approved methodology (XV/VELS/PCOL/12/2000/CPSCEA/IAEC/30.10.13)

Experimental conditions

The acute dermal toxicity was conducted as per the OECD guidelines 402 [11]. Wistar albino rats of female sex weighing about 180-250 g were used for experiments. The females used were nulliparous and non-pregnant. Animals were divided in to 6 Groups (n=6) as follows: Group I (control group), Group II (vehicle control), Group III (500 mg/kg bw of CFA-NPs), Group IV (1000 mg/kg bw of CFA-NPs), Group V (1500 mg/kg bw of CFA-NPs) and Group VI (2000 mg/kg bw of CFA-NPs).

Approximately 24 h before the test, the hair coat was removed by closely shaving the dorsal area of trunk of the animals. Care was taken to avoid abrading the skin which may alter its permeability. The various concentrations of CFA-NPs 500, 1000, 1500 and 2000 mg/kg bw were mixed with saline and made in to a paste and applied on the shaved area. Then the skin area was covered pourous gauze dressing and non-irritating tape for a period of 24 h, whereas for control groups, gauze moistened with physiological saline was applied and held in contact with skin similar way as the treatment groups. Animals were observed within 30 min, 4 and 24 h after the removal of films and kept under observation for 14 d. Total individual feed intake, weight of the animals was determined on the day of application of various concentrations of CFA-NPs and weekly thereafter. The animals were observed for changes in skin, eyes and mucous membranes, behavioural patterns, diarrhea, salivation and termors. Mortality was recorded during the course of study. At the end of the study, animals were weighed, sacrificed and subjected to histopathology.

Histopathological study

Animals, in each group were sacrificed after fasting overnight upon termination of their regular feeding. Organs like lung, liver, kidney, spleen and skin were excised and the organs were fixed in 10% formalin saline. After fixing for 24 h the tissues were cleared and embedded to paraffin wax (60-62 °C). Embedded tissue was made in to the paraffin block. Using microtome embedded block was cut in uniform sections of 4-5 µm thickness and stained with hematoxylin and eosin by routine procedures. First dewax with xyline and fix slides in Isopropyl alcohol for 10 min and wash the slides with water. Add hematoxylin for few min and again wash it with water. Dip the slides once in 1% acid alcohol (Alcohol-70 ml, distilled water-30 ml, mix and discard 1.0 ml, add 1 ml of concentrated HCl) and wash it with water followed by Ammonia water (distilled water 50 ml, Liquid ammonia-3 drops). Finally add Eosin and leave it for 1 min and wash it with water and dry and mount. The stained sections were examined for pathological changes at desired magnification.

Statistics

The results for body weight were presented as the mean±standard deviation (SD). Comparisons were made between control and treatment groups using one way ANOVA followed by Dunnett's test using SPSS program (Version 11.5). Values of $P \leq 0.05$ were regarded as statistically significant.

RESULTS

Effects of CFA-NPs-induced acute dermal toxicity in rats

Changes in general behavior, food intake, body weight and histopathology parameters are critical for evaluating the impact of any test compound on the biological system. The acute dermal toxicity for various concentrations of CFA-NPs was tested. There were no appreciable clinical signs throughout the observation period of 14 d. After removal of patch some changes were observed in the treated area of skin, like inflammation and irritation in all treated concentrations of CFA-NPs. There were no changes observed in eyes, mucous membranes and behavioral pattern. There was no mortality throughout the observation period. Both control and the treated group were observed to consume the entire amount of food (10 g/rat/day). Also, there were no significant ($P \geq 0.05$) changes in body weight in the treatment group when compared to the respective control group rats as shown in table 1. The result of acute toxicity study indicates that the LD_{50} of the CFA-NPs is greater than 2000 mg/kg bw.

Histopathological observations in different organs

Liver

The histology of liver sections is shown in fig 1. The liver section of rats treated with various concentrations of CFA-NPs showed some mild to severe damage in all treated concentrations of CFA-NPs when compared to the control. Both the control and vehicle control section showed normal liver architecture. At lower concentration, 500 mg/kg bw of CFA-NPs treated section shows mild central vein dilatation and congestion. In 1000 mg/kg bw of CFA-NPs treated rats the section showed congested central vein dilatation, sinusoidal dilatation and inflammatory infiltration. At 1500 mg/kg bw of CFA-NPs treated group; the section showed congested central vein dilatation and sinusoidal widening. At higher dose of 2000 mg/kg bw of CFA-NPs treated group, the section showed focal bile duct proliferation (Nut-meg liver), increased central vein congestion and inflammatory infiltration. The histopathology of the tissues showed considerable damage in all concentration of CFA-NPs treated groups when compared to the control.

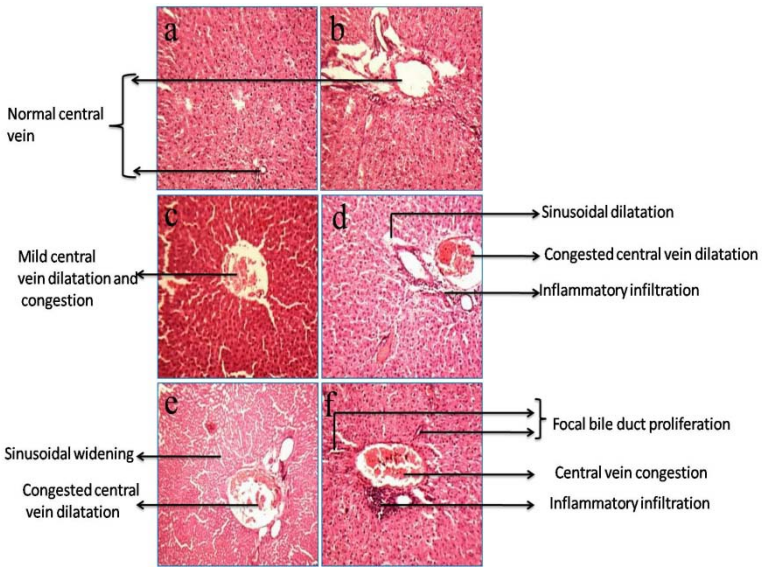

Fig. 1: Liver sections of rats dermally treated with various concentrations of CFA-NPs for 14 days (a-normal control, b-vehicle control, c-500 mg/kg bw of CFA-NPs, d-1000 mg/kg bw of CFA-NPs, e-1500 mg/kg bw of CFA-NPs and f-2000 mg/kg bw of CFA-NPs)

Table 1: Effect of CFA-NPs treatment on body weight (g) in rats during acute dermal toxicity study. None of these values are significant when compared to the control (P ≥ 0.05). Values are expressed as mean±SD

No. of days	Control	Vehicle Control	500 mg/kg bw of CFA-NPs	1000 mg/kg bw of CFA-NPs	1500 mg/kg bw of CFA-NPs	2000 mg/kg bw of CFA-NPs
Day 0	188.00±4.47	192.33±6.28	192.00±6.98	193.83±7.83	194.00±9.71	191.66±7.84
Day 7	192.08±4.34	196.66±6.28	196.75±7.18	198.91±6.74	198.50±9.62	196.33±7.63
Day 14	197.33±3.73	201.75±6.16	201.08±7.28	203.46±7.05	202.91±9.42	200.91±7.67

Kidney

The kidney sections of rats treated with various concentrations of CFA-NPs showed mild to severe tissue damage in all treated concentrations when compared to control was shown in fig. 2. Both the control sections showed normal glomeruli with normal tubules. At lower concentration of 500 mg/kg bw of CFA-NPs treated tissue sections showed thick walled congested blood vessels, mild hyper cellular glomeruli and congestion of distal convoluted tubule. At 1000 mg/kg bw of CFA-NPs treated rats; the section showed congested tubule and mild hyper cellular glomeruli. At higher concentration of 1500 mg/kg bw of CFA-NPs treated sections showed dilated distal convoluted tubule with congestion and increased cellular glomeruli. At 2000 mg/kg bw of CFA-NPs treated kidney sections showed congested tubule, dilated distal convoluted tubule with congestion and increased cellular glomeruli. On the whole, the histopathology of kidney tissues showed mild damages at lower concentration of CFA-NPs and severe damage at higher dose when compared to the control groups.

Spleen

The spleen sections of rats treated with various concentrations of CFA-NPs showed severe damage of tissues in all the treated concentrations when compared to control groups as shown in fig. 3. Both the control sections show normal spleen. At lower concentration (500 mg/kg bw of CFA-NPs) the spleen section showed congested and prominent red pulp and distorted lymphoid follicles.

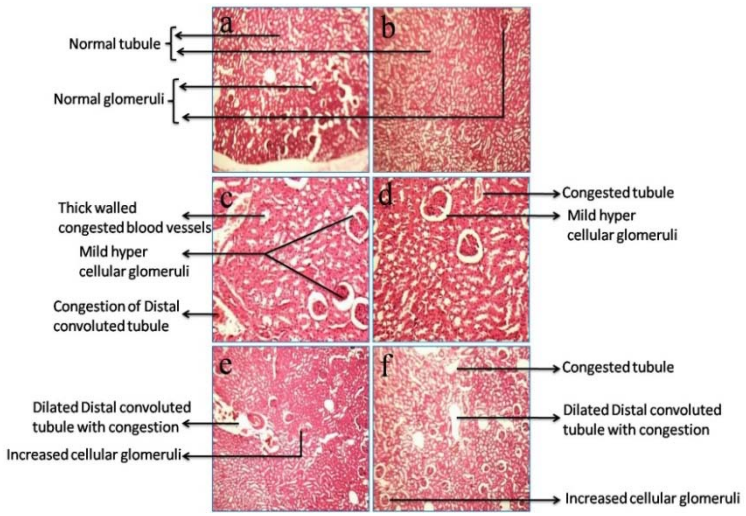

Fig. 2: Kidney sections of rats dermally treated with various concentrations of CFA-NPs for 14 days (a-normal control, b-vehicle control, c-500 mg/kg bw of CFA-NPs, d-1000 mg/kg bw of CFA-NPs, e-1500 mg/kg bw of CFA-NPs and f-2000 mg/kg bw of CFA-NPs)

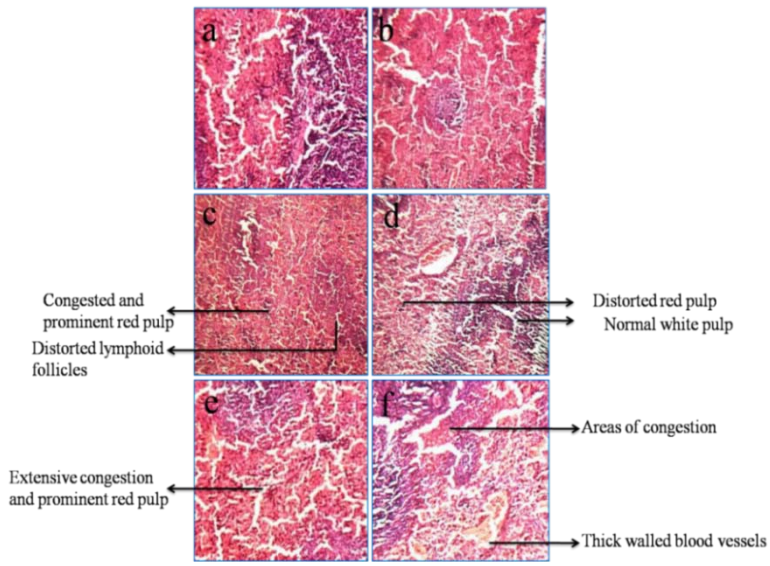

Fig. 3: Spleen sections of rats dermally treated with various concentrations of CFA-NPs for 14 days (a-normal control, b-vehicle control, c-500 mg/kg bw of CFA-NPs, d-1000 mg/kg bw of CFA-NPs, e-1500 mg/kg bw of CFA-NPs and f-2000 mg/kg bw of CFA-NPs)

The rats treated with 1000 mg/kg bw of CFA-NPs showed congested spleen with distorted red pulp and normal white pulp. At 1500 mg/kg bw of CFA-NPs treated spleen section showed extensive congestion and prominent red pulp. At higher dose 2000 mg/kg bw of CFA-NPs treated group showed spleen with areas of congestion and thick walled blood vessels. The histopathology of spleen tissues showed very severe damage in all treated concentration of CFA-NPs. In all sections there was appearance of red pulp; this was not found in the control groups. In all treated concentrations of CFA-NPs there were notable changes found in the tissues when compared to the control.

Skin

The histology of skin sections are shown in fig. 4. The skin sections of rats treated with various concentrations of CFA-NPs showed severe damages.

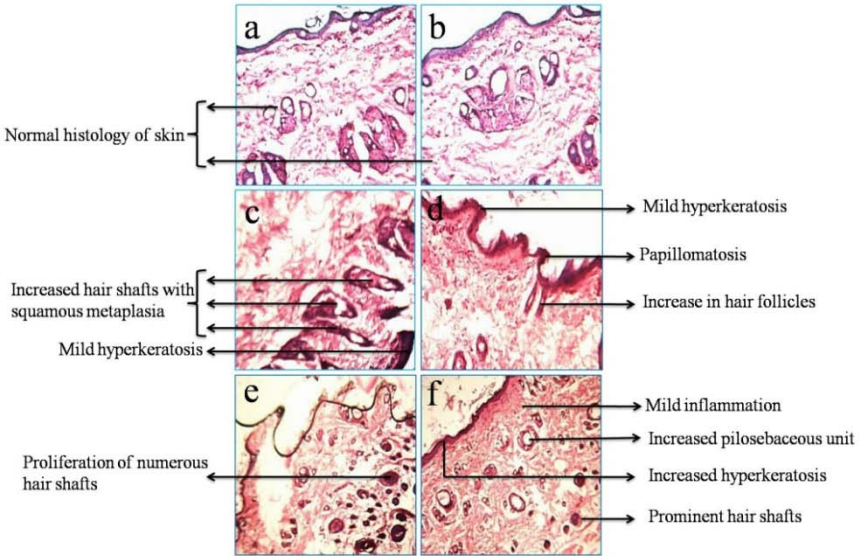

Fig. 4: Skin sections of rats dermally treated with various concentrations of CFA-NPs for 14 days (a-normal control, b-vehicle control, c-500 mg/kg bw of CFA-NPs, d-1000 mg/kg bw of CFA-NPs, e-1500 mg/kg bw of CFA-NPs and f-2000 mg/kg bw of CFA-NPs)

In control groups; the skin section showed normal histology. At lower concentration of 500 mg/kg bw of CFA-NPs treated skin section showed mild hyperkeratosis and increased hair shafts with squamous metaplasia. In 1000 mg/kg bw of CFA-NPs treated skin sections showed mild hyperkeratosis, papillomatosis and increase in hair follicles. In 1500 mg/kg bw of CFA-NPs treated groups section showed proliferation of numerous hair shafts. At higher concentration (2000 mg/kg bw of CFA-NPs) the skin section showed mild inflammation, increased pilosebaceous units, increased hyperkeratosis and prominent hair shafts. Overall, the histopathology of the skin showed severe damage in all treated concentrations of CFA-NPs when compared to the untreated control groups. Since the CFA-NPs is treated dermally; there was significant damage observed in the skin tissues in all treated concentrations of CFA-NPs.

DISCUSSION

A study of acute toxicity by the dermal route and determination of a LD_{50} provides an estimate of the relative toxicity of a substance by the dermal route of exposure and they may serve as a basis for classification and labeling. The LD50 is defined as the statistically derived dose that, when administered in an acute toxicity test, is expected to cause death in 50% of the treated animals in a given period. It is an initial step in establishing a dosage regimen in sub acute, sub chronic and other studies. It may provide information on dermal adsorption and the mode of toxic action of a substance by this route. During acute dermal toxicity studies, no mortality was observed at any stage of the study. The skin inflammation is caused by exposure to CFA-NPs. The skin inflammation was observed in all treated concentrations of CFA-NPs and no changes were observed in control groups. In comparison with control groups, there was no significant changes were observed in body weight of various concentrations of CFA-NPs treated groups and also in vehicle control groups.

Histopathology of tissues revealed mild to marked cellular changes upon dermal administration to various concentrations of CFA-NPs in rats. There were severe histological changes were observed in all the organs, in the treated concentrations of CFA-NPs. The magnitude and manifestation depends on the health status of experimental animals. Histopathological changes in the tissues of animals exposed to various chemical agents have been reported [12,13]. There were no acute dermal toxicity studies reported previously in relation to CFA. In control groups the tissues of liver, kidney, spleen and skin showed normal architectural pattern without any changes. Dermal treatment with various concentrations of CFA-NPs showed mild to severe histological changes in the tissues of liver, kidney, spleen and skin. At higher concentrations; the CFA-NPs dermally treated group organ tissues showed more damage when compared to the lower concentration treated groups. The occurrence of changes in all CFA-NPs treated groups is due to toxic elements present in the CFA-NPs. Changes in the histopathology of the tissues is due to some toxic metals enters through the skin barriers and may be transported through blood, resulting in organ damage. In comparison with other tested organs, the CFA-NPs treated skin showed severe effect. Since the CFA-NPs were treated dermally, there was direct exposure of CFA-NPs in the dermal layers, thus the dermal tissues showed severe histological changes when compared to other organs.

The CFA-NPs are smaller than the size of the skin pores, when CFA-NPs nanomaterials are applied topically to the skin, they must be able to penetrate through the viable epidermal layers and epidermal-dermal junction (basement membrane) to gain access to the capillaries within the papillary layer of the dermis in order to get into the systemic circulation [7]. There is only few information regarding dermally induced NPs toxicity but no literature pertaining to the absorption of CFA-NPs through skin. CFA-NPs have several toxic elemental compositions present in it. Due to the possibility of chemical interactions between the elements, the effects of dermal application of NPs with other NPs are largely unknown. The estimated risk of two or more metal particles is not a simple additive process. Particle surface plays a critical role in toxicity as it makes contact with cells and biological organs. The presence of transition metals [14] on nanoparticles surfaces leads to the generation of ROS and the induction of inflammation in cells [15], reported that the spherical shaped particles with diameter between 750 nm and 6

microns penetrate through the hair follicles of the skin with maximum penetration depth of more than 2.4 mm. NPs can also penetrate through the skin when the skin is flexed. Broken skin facilitates the entry of a wide range of larger particles around 500 nm to 7 nm in diameter [5]. The mechanical deformation is capable of transporting particles through the stratum corneum and into the epidermis and dermis. The dermis has a rich supply of blood and macrophages, lymph vessels, dendritic cells and nerve endings [5]. Therefore, the NPs that penetrate through the stratum corneum and into the epidermis and dermis are potentially available for recognition by the immune system. Tinkle *et al.* (2003) [16] provides compelling evidence that NPs could penetrate the skin in demonstrating epidermal and dermal penetration by fluorescent microspheres (0.5-1.0 μm) in human skin in an *in vitro* flexed skin model.

CONCLUSION

Acute dermal treatment with various concentrations of CFA-NPs from coal-fired power plants may induce mild to significant dose-dependent pathological changes in all the observed biological organs. The LD_{50} value for acute dermal toxicity of CFA-NPs was greater than 2000 mg/kg bw. The CFA-NPs treated animals did not show any abnormal clinical changes or mortality in treated groups. Single exposure to various concentrations of CFA-NPs in rats showed an increased susceptibility to infection in all the organs. Especially, more histological damage was observed on skin tissue; it is due to route of direct exposure to CFA-NPs. Thus, single to CFA-NPs could cause skin inflammation in a matter of minutes or hours. Therefore prolonged exposure to CFA-NPs may cause serious damages to the peoples in the working environment and also to the peoples living close to the Coal fired power plants. Hence, long term exposure studies are warranted in the future.

CONFLICT OF INTERESTS

Declared None

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