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Effects of Dimethoate Exposure on Locomotor Activity and Anxiety-Like Behavior in Female Wistar Rat

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Abstract

Developmental exposure to organophosphate insecticide is well known to induce neurobehavioral impairments, at late period. The present study aims to investigate the effects of subchronic exposure to Dimethoate, on locomotors skills and anxiety like behavior among wistar rat. Two groups of female's rats are used. The intoxicated group receives daily, during five weeks, by intragastric gavage, a dose of Dimethoate dissolved in corn oil (100 mg/kg body weight). The control group receives only the corn oil. Spontaneous locomotors activity is evaluated using the Open Field test (OF) and anxiety-like behavior is measured using Elevated Plus-Maze (EPM). Dimethoate induced significant impairment of spontaneous locomotors activities, which is reflected by high decrease of number of squares crossed (SC) in OF. Females exposed to Dimethoate develop further anxiety-like response, expressed by significant reductions of the time spent in open arm of Elevated Plus-Maze.

Keywords

Acetylcholinesterase Activity, Neurotoxicity, Dimethoate, Neurobehavior, Organophosphate Insecticides

1. Introduction

Organophosphate pesticides (OP) are widely used in agriculture pest control, in

order to improve quality of human food. However, their general persistence in crops products and in environment is considered as hazardous for the public health [1] [2] [3]. The acute effects of people intoxication especially in suicides attempts, occupational accident case and food contamination are well studied in epidemiology [4] [5]. The Dimethote insecticide is one of the most used OP in United States and throughout the world. Dimethote is used in agriculture, veterinary practice and as ectoparasiticide applied against human body lice [6].

In 1985, the WHO indicated that the Dimethoate degrades in another even more toxic pesticide, the Omethoate; the proportion of Omethoate in the total residues can affect 50 percent after five weeks.

The acute neurotoxic mechanism of action of Dimethoate is typically cholinergique. It involves an inhibition of the Acetylcholinesterase (AChE) of the neuronal tissue through its active metabolic shape, the Omethoate which turns out to be 10 times as toxic as the Dimethoate (WHO, on 2003).

The Omethoate rapidly binds to the hydroxyl group of the active site of AChE, and Undergoes a double displacement reaction involving the serum hydroxyl groups And dimethylphosphorylated from AChE. Thus, phosphorylated AChE is stable and irreversible.

Inhibition of AChE causes an accumulation of acetylcholine released in the synaptic cleft. As a result, hyperstimulation of the nicotinic and muscarinic receptors is induced. Thus, the passage of nerve information is disrupted [7].

Furthermore, the oxidative stress caused by the peroxydation of lipids and favored by the Dimethoate is considered as a second mechanism of toxicity of this organophosphate [8] [9] [10].

Although the acute and sub chronic physical effects of Dimethoate exposure are well documented [11] [12], there are a limited number of studies describing the neurobehavioral deficits caused by this OP.

The current study aims to investigate the effects of exposure to sub toxic doses of Dimethoate, on locomotors skills and anxiety like-behavior in Wistar rats.

2. Materials and Methods

2.1. Chemical

Dimethoate was obtained from commercial grade: Dimethoate 50 (active ingredients 500 g by liter). The Dimethoate concentration (50% purity) in commercial grade was diluted in corn oil.

2.2. Animals and Treatment

The effect of Dimethoate was tested in both male and female's rats. The results are statistically sexe-independent, in this article we chose to illustrate the results of female's rats.

Sixty Wistar female's rats, 4 months of age were obtained from a local breeding colony of Faculty of Sciences, Kenitra-Morocco. Rats were kept under stan-

dard condition, 12 h light/12 dark cycle, 20˚C ± 2˚C and 50% - 70% humidity). They had access to commercial diet (ALF SAHEL-Casablanca, Morocco) and tap water ad libitum. After 2 weeks of acclimation, rats were randomly divided in two groups of treatment. Then, thirty rats received by intragastric gavage incremental doses of Dimethoate insecticide dissolved in corn oil; 100 mg/kg of body weight per day and control group (thirty rats) was given corn oil daily. 100 mg/kg was the maximum tolerated dose. The duration of the intoxication test was five weeks.

2.3. Physical Parameter Measurements

Physical signs of toxicity and body weight were daily recorded during treatment.

2.4. Behavioral Assessment

2.4.1. Open-Field Test (OF)

To assess possible effects of Dimethoate on spontaneous locomotors activity and the ability to response to a novel environment rats were evaluated in open-field test during 5 min. Apparatus consisted to top open wooden bow (100 × 100 × 40) covered by a white consistent plastic. The floor of the arena were divided into 25 squares unit by black lines and lit in the center with halogen lamps of 60 W installed in the ceiling [13] [14]. The frequencies of line crossing with the four paws, the time spent in center of open-field and number of rearing in exploratory activity (anxiety level), were recorded by video camera positioned above the OF.

2.4.2. Elevated Plus-Maze (EPM)

To measure the degree of anxiety-related behavior, we use the elevated plus-maze. The apparatus is made of wood and consisted to two enclosed arm (29 × 5 × 15) and two open arms (29 × 2.5 × 15), placed at a right angle crossing in a common central platform (5 × 5). The central platform is illuminated with halogen lamps of 60 W offer rat an aversive condition spatial. Each animal is placed onto platform facing the open arm and the following behaviors are recorded during 5 min. The time spent in each arm and the numbers of entries in open and close arm are scored from video sequence. The level anxiety of rat is assessed by the time spent on the open arm divided by total time, and the number of open-arm entries divided by total number of arm entries [15]. The spontaneous locomotor activity is evaluated by the number of total entries in the arms of the EPM.

2.4.3. Statistical Analysis

All data are expressed as means ± S.E.M (Standard Error of Mean). Repeat measured and one-way analyses of variance (ANOVA) are used to analyze difference on body weight and behavioral scores respectively between groups. Post hoc comparisons are made using Tukeys' HDS test. Statistical significant is assumed at $p < 0.05$.

3. Results

3.1. General Physical Observations

Signs of systematic toxicity such as the loss of weight, the decrease of food grip were recorded. Dimethoate exposure at dosage 100 mg/kg (b.w) induced toxicity to female's rats. Repeated measure ANOVA shows a significant difference in body weight. Tukey post-hoc analysis revealed that body weight loss is significantly important ($p < 0.001$) Figure 1.

3.2. Open-Field Behavior

3.2.1. The Effect on the Number of Squares Crossed in OF

The Dimethoate significantly altered spontaneous locomotor activity in female's rats. The one-way ANOVA showed a significant reduction in the number of squares crossed by the treated groups $p < 0.001$, compared to the controls as shown in Figure 2.

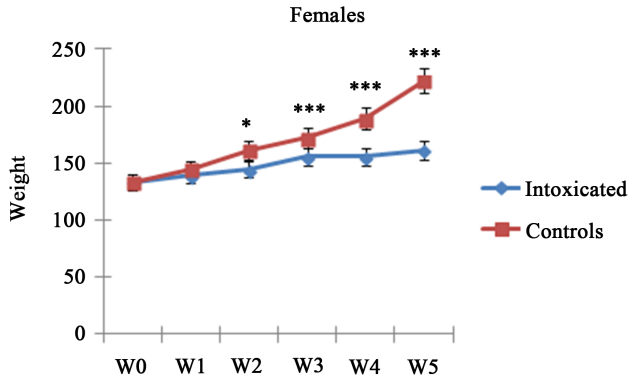

Figure 1. Effect of the Dimethoate on the body weight to female's rats Number of errors on average ± standard error averages (SEM). *$p < 0.05$, ***$p < 0.001$, comparison between intoxicated groups and control groups.

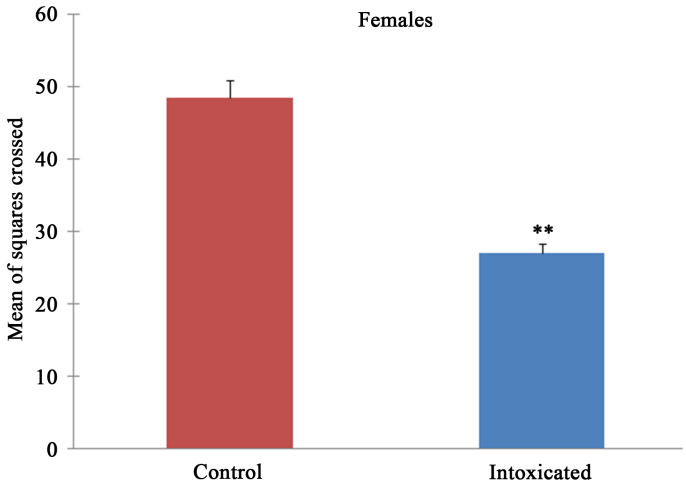

Figure 2. Effects of exposure to Dimethoate on spontaneous locomotors activity in female's rats. The number of total squares crossed expressed on average ± mean standard error (SEM). ***$p < 0.001$, comparison between intoxicated groups and controls.

3.2.2. The Effect on the Number of Elevations in OF

The number of elevations was increased in the control rats in comparison with the Dimethoate poisoned rats. This difference is highly significant between the two groups ($p < 0.001$) Figure 3.

3.2.3. The Effect on the Time Spent in the Center of the OF

The results show a decrease in the time spent in the center of the open field in intoxicated rats. These results are statistically significant $p < 0.01$ Figure 4.

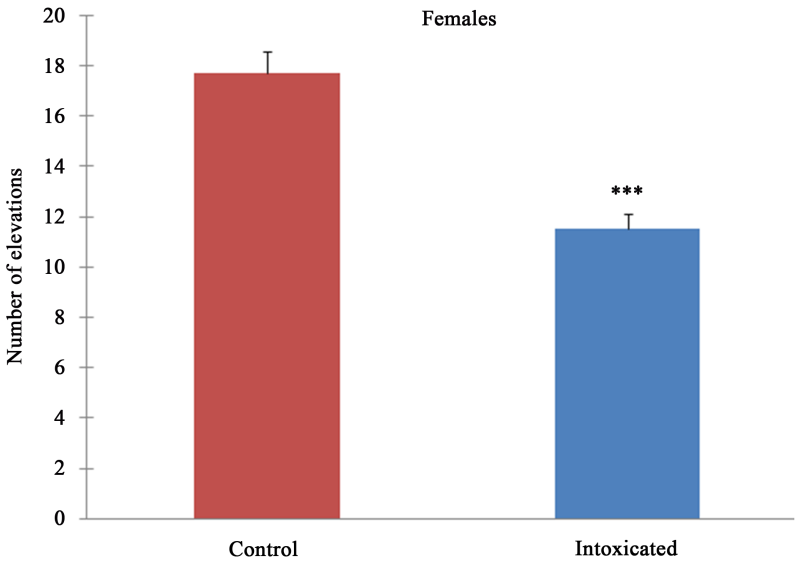

Figure 3. Effects of exposure to Dimethoate on the exploratory activity of female's rats in the Open Field. The number of elevations expressed as mean ± mean standard error (SEM). ***$p < 0.001$, comparison between intoxicated groups and controls.

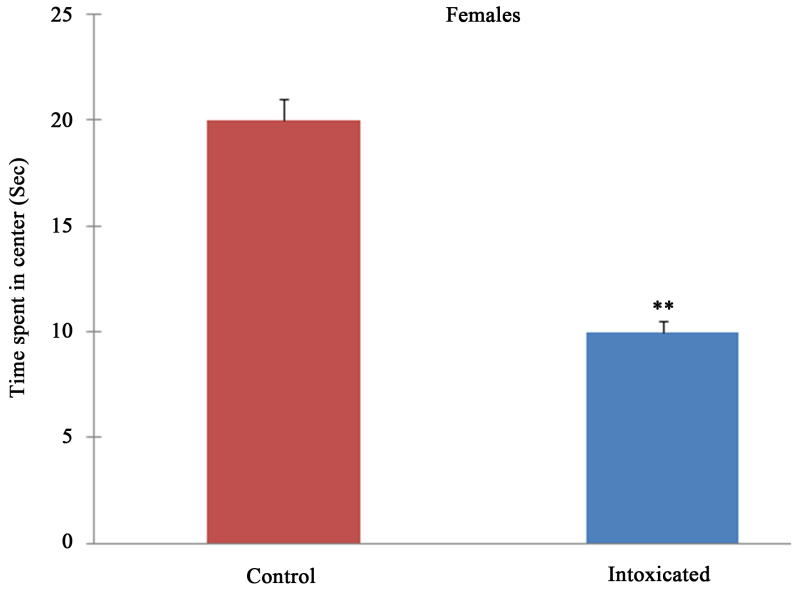

Figure 4. Effects of exposure to Dimethoate on the anxiety level of female's rats in the Open Field. The time spent in center expressed as mean ± mean standard error (SEM). ***$p < 0.01$, comparison between intoxicated groups and controls.

3.3. Anxiety-Like Behaviors Assessment

3.3.1. The Effect on the Number of Entries in Open Arms Case of Gavage

Figure 5 shows that the number of open arms entries is significantly important in rats that are poisoned with Dimethoate. Therefore, there is a very significant difference between the two studied groups $p < 0.001$.

3.3.2. The Effect on Time Spent in Open Arms

Figure 6 shows that the time spent on open arm is also very important in the intoxicated females compared to the controls. This difference is very significant between the two groups, $p < 0.001$.

3.3.3. The Effect on Total Entries in All Arms

Figure 7 shows a reduction in the total number of entries in intoxicated rats. This reduction is statistically significant, $p < 0.001$.

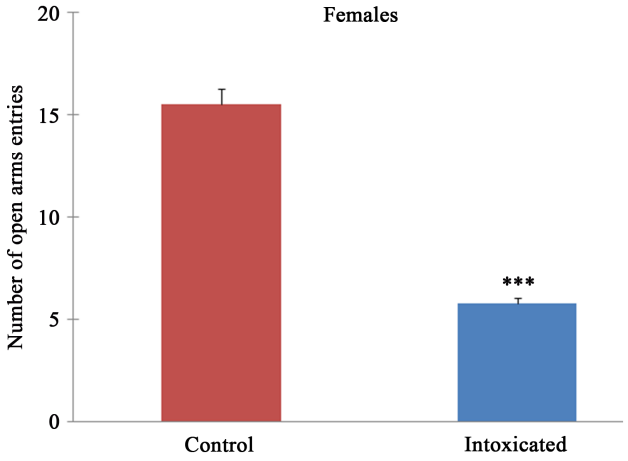

Figure 5. Effect of exposure to Dimethoate on the anxiety level of female's rats number of open arms entries is expressed on average ± mean standard error (SEM). ***$p < 0.001$, comparison between intoxicated groups and controls.

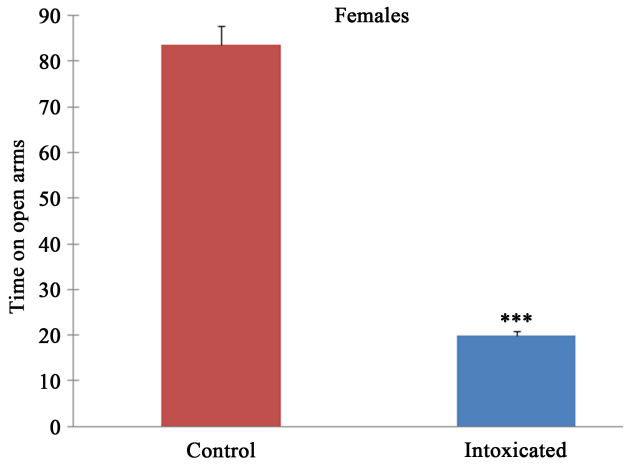

Figure 6. Effect of exposure to Dimethoate on the anxiety level of female's rats the time spent on open arms is expressed as an average ± mean standard error (SEM). ***$p < 0.001$, comparison between intoxicated groups and controls.

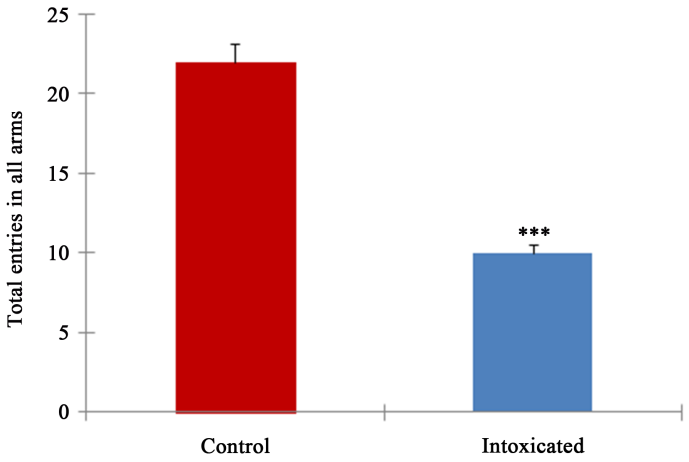

Figure 7. Effect of exposure to Dimethoate on spontaneous locomotors activity in female's rats total entries is expressed as an average ± mean standard error (SEM). ***$p <$ 0.001, comparison between intoxicated groups and controls.

4. Discussion

Organophosphorus insecticides (OPs) are widely used to improve agricultural productivity. However, the persistence of their residues in agricultural products and in the environment causes serious problems of environmental pollution and potentials risks on the health [1]. Concerning the effects of OPs on the brain and Behavior in the animal model, most studies have been conducted on subtoxic doses of compounds of high toxicity such as chlorpyrifos (CFP), Methyl parathion (MP) [16], [17] and Diazinon (DZN) [18]. Indeed, these studies have focused mainly on the effects of developmental exposure [19]. However, studies of effects on the nervous system when exposure occurs at the advanced stage of development (Young subjects, adults, the elderly…) remain rare, but this type of exposure reflects a certain reality because manipulation of insecticides in agricultural practice is made by older subjects. It is in this context that joins this experimental study on the Dimethoate, an organophosphorus widely used in agriculture. We will discuss our results in relation to those obtained with potentially dangerous OPs.

4.1. Systemic Effects of Chronic Exposure to Dimethoate

In the present study, cases of systemic toxicity such as convulsion, salivation and muscle weakness were observed (data not shown). A significant decrease in weight was also recorded **Figure 1**. These results are in perfect agreement with Jallouli's work on mice intoxicated by Dimethoate [20].

Another study of developmental toxicity to Malathion also demonstrated a low body weight gain in young rats at stage (GD 6-PND 45) [21]. From a pathophysiological point of view, the low body weight gain in the exposed groups could be explained by the effect of overexpression of ACh molecules which increases gastric motility and decreases intestinal absorption [22]. Indeed, the parasympathetic nervous system stimulates the processes of digestion, through the

NT ACh. However, the excess of this NT due to the inhibition of AChE by the OP could be at the origin of the dysfunction of this process [23].

4.2. Effects of Dimethoate on Locomotors and Exploratory Activity

In this study, locomotor and exploratory activities were highlighted by two parameters calculated at the OF level; the total tiles traversed and the number of elevations (EL) carried out by the rat. Locomotor activity was also evaluated at the EPM level by the overall number of entries in the different regions of the labyrinth. This parameter constitutes an additional index of locomotion of the animals.

These activities, locomotors and exploratory, are greatly decreased in the intoxicated rats compared to the control rats. Indeed, the reduction in the number of SC and EL correlates positively with the awakening deficit and with the increase of the emotional response or the alteration of the locomotor activity [24]. Our results are consistent with those of a previous study that showed impaired motor function in animals exposed to Dimethoate [25].

The mechanism of action leading to these different effects could be due to the inhibition of acetylcholinesterase (AChE) by Dimethoate. Indeed, previous studies have reported a significant alteration in locomotor activity associated with a very high inhibition rate of AChE in a species of arthropod, Folsomia candida, exposed to subtoxic doses of Dimethoate [26]. However, to better understand the basic process.

It would be preferable to place ourselves in the context of our study (study on the animal model). The long-term developmental exposure (GD 6-PND 45) to Malathion induced a very significant inhibition of AChE associated with altered locomotors activity and high anxiety behavior [21], [27].

Indeed, it has been shown that the OPs pesticides can cause neurobehavioral alterations during development [28] [29], and also a persistent deficiency in cholinergic synapses [30].

Indeed, it has been shown that during brain development, ACh and cholinergic projections are heavily involved in the process of proliferation, migration, synaptogenesis and normal neurons cytoarchitectonique organization [31]. Thus, disruption of the cholinergic transmission induced subsequently inhibition of AChE and therefore an impaired development of the motor system and a motor coordination deficit [32].

It is well known that inhibition of cerebral AChE by OPs leads to an accumulation of acetylcholine in synapses; consequently hyperactivity in the cholinergic pathways.

During the postnatal period, animals exposed to AChE inhibitors are able to develop various behavioral disorders such as motor development disorders and coordination deficit [33] [34]. In addition, in adult rats, inhibition of AChE could interfere with normal mechanisms at the neuromuscular junction [35] [36].

Another study suggested that the action of Dimethoate might be inhibiting the Na + K + ATPase pump. Indeed, this enzyme is responsible for the active transport of sodium and potassium ions in the nervous system thus maintaining the ionic gradient necessary for neuronal excitability and for regulating the volume of the neuronal cell. This enzyme is present in high concentrations in cerebral cell membranes, and consumes 40% - 50% of the ATP generated in this tissue [37]. However, inhibition of the activity of this enzyme could induce membrane depolarization leading to suppression of excitation and neuronal transmission [38], [39]. This was demonstrated by the work of Acker [40], which reported that Malathion inhibited Na + K + ATPase activity in the cerebral cortex of adult rats, an event that is implicated in the neurotoxicity induced by this compound.

4.3. Effects of Dimethoate on Anxiety Behavior

The sub-chronic exposure to Dimethoate is anxiogenic. This result was highlighted at the level of the open field. Indeed, a highly significant alteration of the exploratory activity and time spent in the center of the open field were recorded. The results show a significant decrease in the time spent in the center of the open field in intoxicated rats **Figure 4**.

This altered level of anxiety is confirmed at the level of the EPM; an ethologically validated test for the evaluation of anxiety in the animal model [41]. Our results show that both the time spent in the open arms and the number of entries in the open arms are significantly reduced in the treated rats compared to the control rats **Figure 5** and **Figure 6**.

To our knowledge, no studies had demonstrated the effects of Dimethoate on the anxiety level of adult rats. However, our results reinforce the idea that exposure to organophosphates with moderate toxicity has an anxiogenic effect.

An earlier study reported that adult female's rats showed an altered level of anxiety after being exposed to acute (50, 100, 250 mg/kg ip) and subchronic (25, 50, 100 mg/kg ip) intoxication of Malathion [42].

However, this study is contrary to that of Valvassori, which has not detected any anxiogenic effect with these doses [43].

It is true that contradictions exist for the interpretation of OPs on the level of anxiety; this could be related to several factors. It is suggested that the level at which poisoning occurs, the duration of exposure and the degree of toxicity of the OPs should be considered. Indeed, a previous study showed that exposure to malathion during the critical brain period (GD 6-PND45) induced a highly significant alteration in anxiety level in rats of both sexes with more pronounced effects in female [21]. In addition, short-term exposure (4 days) at a dose of 1 mg/kg CPF resulted in a persistent increase in anxiety in female mice for the majority of postnatal periods tested (PND 1 - 4, PND 5 - 8 and PND 13 - 16). However, for this same dose (1 mg/kg CPF), a 15-day exposure period (PND 9 - 12) had not led to any change in the anxiety. Furthermore, with a slightly higher dose (3 mg/kg) of CPF, a marked decrease in anxiety level was noted [44].

In order to understand the mechanisms of action responsible for increasing the level of anxiety induced by Dimethoate, it is important to understand the affected systems.

That can be possible due to interaction between Dimethoate and estrogenic activity disruption in brain sexual differentiation. Exact neurochemistry mechanisms remain ubiquitous and unclear [45]. Moreover, immunohistological study in cerebellum and cerebral cortex of rats exposed can further contributed to elucidate these disorders.

However, the common effects of organophosphate insecticides on serotoninergic system can be a main explanation of high level of anxiety expressed in females [18].

As mentioned above, the main mechanism of action of OPs including Dimethoate is the irreversible inhibition of AChE, resulting in hyperstimulation of the cholinergic system [7], [46], [47]. Indeed, the work of [21], showed an alteration of the cholinergic system of the hippocampus of intoxicated rats, but it has been shown that the cholinergic system plays a modulatory role in the control of the level of anxiety [48], [49].

5. Conclusions

This study revealed that chronic exposure to a subtoxic dose of Dimethoate, is likely to affect behavioral functions in female's rats.

Our results showed that subchronic exposure by gavage with Dimethoate induces a significant anxiogenic effect and altered locomotors activity. These effects are probably due to a disruption of the cholinergic system.

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