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Original Article

ACUTE TOXICITY EVALUATION OF ETHANOL EXTRACT OF INDONESIAN VELVET BEANS

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ABSTRACT

Objective: This research was conducted to determine the acute toxicity of ethanol extract of velvet beans (*Mucuna pruriens)* from Indonesia. Extract of Indonesian *Mucuna pruriens* seeds showed antiparkinson activity due to the presence of L-DOPA inadequate levels. However, research on the toxicity level of *Mucuna pruriens* seeds from Indonesia is still limited. Acute toxicity data are needed to make *Mucuna pruriens* as standardized herbal medicine for Parkinson disease.

Methods: The dried seed of *Mucuna pruriens* was extracted by ethanol and suspended with tragacanth to make several dosas of ethanol extract of *Mucuna pruriens* seeds. Determination of acute toxicity was performed on six groups, each consisting of five *Wistar* rats. One group was used as a control group; other groups were given ethanol extract of *Mucuna pruriens* seeds orally at a dose of 5 mg/kg, 50 mg/kg, 300 mg/kg, 2000 mg/kg, and 5000 mg/kg body weight. Symptoms of toxicity, including death, were observed daily for 14 d. At the end of the observation, the surviving *Wistar* rats were autopsied and the brain, heart, liver, lungs, stomach, spleen, kidneys, and ovaries were weighed to give relative organ weight.

Results: There was no mortality observed in all groups. The *Wistar* rats gained weight within the normal range. The relative organ weights in all groups generally did not show a significant difference. However, the significant differences (P<0.05) were seen in the liver for all treatment groups compared to the control group.

Conclusion: The ethanol extract of *Mucuna pruriens* seeds from Indonesia administered orally has LD_{50}>5000 mg/kg, thus it could be regarded as safe or non-toxic. However, this extract may be potentially toxic to the liver.

Keywords: *Mucuna pruriens*, Velvet beans, Indonesia, Acute, Toxicity, Oral

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INTRODUCTION

Velvet beans *(Mucuna pruriens*) is a tropical leguminous plant that belongs to *Mucuna* genus, the Fabaceae family. This plant is a native of Africa and tropical Asia and has been widely cultivated. The plant itself is an annual shrub, can grow up to 15 meters, the young is covered with fuzzy hair, but it will be lost when the plant is getting older [1, 2].

Traditionally, the seed of *Mucuna pruriens* is used as an aphrodisiac and believed to overcome problems regarding fertility, impotence, spermatorrhea, and sterility [3]. The seed is also used as a diuretic, nerve tonic, anti-inflammatory, and stimulant due to its antidepressant activity, and used in the treatment of Parkinson's disease [4]. The pod was reported as anthelmintic, while root was used in the treatment of nervous disorders and blood purification. In many countries, this plant is also used in the treatment of cholesterol, diabetes, hypertension, paralysis, rheumatism, cancer, tumors, tuberculosis, asthma, cholera, diarrhea, dysentery, irritation, burns, cough, muscle pain, and many others [5–7]. Various studies reported the bioactivity of *Mucuna pruriens*, such as increasing the sexual activity of normal male rats, hepatoprotective activity with increasing the *in vivo* antioxidants [8], antidiabetic activity [9–12], and as a potential antiparkinson from the seeds [13, 14].

The results of the phytochemical study showed that *Mucuna pruriens* contains saponins, tannins, anthraquinones, steroids, terpenoids, alkaloids, flavonoids, resins, and glycoside-containing compounds [5, 15–17]. The seed of *Mucuna pruriens* is known contains L-DOPA, a precursor to the neurotransmitters dopamine. L-DOPA is able to cross the blood-brain barrier and help to increase the lack of dopamine levels in the brain of people with Parkinson's. The use of the seed extract in Parkinson's treatment becomes reasonable due to the presence of L-DOPA. However, the concentration of L-DOPA in the seed of *Mucuna pruriens* was different depend on the regions of the plant's origin [4, 6, 18, 19]. The concentration of L-DOPA also would be different depending on cooking method. Boiling method is commonly used to produce extracts of medicinal plants. However, research showed that boiling method could reduce the concentration of L-DOPA in the seeds greatly. Whereas roasting method is better because reduce the concentration of L-DOPA slightly [20].

The seed of *Mucuna pruriens* from Indonesia were reported to contain 7.56-13.9% of L-DOPA and potentially as an antiparkinson. This was supported by the research that showed the ethanol extract of *Mucuna pruriens* seeds from Indonesia can reduce the symptoms of catalepsy at a dose of 200 mg/kg body weight [16, 21]. The extract of *Mucuna pruriens* use in the treatment of Parkinson's disease have a variation of the number of doses, thus there should be data relating to the toxicity that might be incurred. The LD_{50} of *Mucuna pruriens* suspension administered orally was to be possible greater than 1600 mg/kg body weight. It was also reported that this suspension did not cause significant differences in most haematological parameters [22]. The methanol extract of *Mucuna pruriens* seeds did not cause any mortality of the animals in acute toxicity studies even at a dose of 2000 mg/kg [17, 23]. Whereas the ethanol extract of *Mucuna pruriens* leaves caused the signs of mortality at 2000 mg/kg [24]. In another report, extract of *Mucuna pruriens* seeds that extracted by hot water and tested in adult albino male rats of the *Wistar* strain has the LD_{50} as 1300 mg/kg [25]. Eze (2012) reported that acute toxicity of the ethanol extract of *Mucuna pruriens* leaves administered by oral route was found to be 2154 mg/kg body weight [11].

Plants used in traditional medicine is expected to have low toxicity because it is often used in the long-term [26]. For that, there must be research that could reveal data of the toxicity of the plants used in traditional medicine, including *Mucuna pruriens* from Indonesia. Therefore, this study was conducted to determine the level of acute toxicity of ethanol extract of *Mucuna pruriens* seeds from Indonesia.

MATERIALS AND METHODS

Sample preparation

The sample used in this study was the seed of *Mucuna pruriens* collected from Yogyakarta, Indonesia (7 °48′5″S 110 °21′52″E). The plant was authenticated in School of Life Sciences, Bandung Institute of Technology, Indonesia with 468/11. CO2.2/PL/2017 voucher specimen number.

The seed was dried and ground to obtain a powder. *Mucuna pruriens* seeds powder was extracted by maceration with ethanol-water at room temperature and then evaporated to obtain ethanol extract. The ethanol extract was suspended with tragacanth.

Chemicals

Tragacanth, ethanol, distilled water, ether were obtained from local suppliers with technical and pro analyst's quality.

Determination of acute toxicity

Determination of acute toxicity was performed by observation of the *Wistar* rats that were given ethanol extract *Mucuna pruriens* for 14 d [23, 27]. A total of thirty healthy *Wistar* rats were divided into six groups, each group contains five rats. One group was used as a control group and five other groups were treated with the suspension of ethanol extract of *Mucuna pruriens* seeds with each dose of 5 mg/kg, 50 mg/kg, 300 mg/kg, 2000 mg/kg, and 5000 mg/kg body weight (group I-V) administered by oral. The *Wistar* rats in all groups were observed daily for 14 d to determine the symptoms of toxicity, including death. Body weight, mortality/ viability, and clinical signs were recorded. At the end of the observation, the surviving *Wistar* rats were autopsied and examined macroscopically. The brain, heart, liver, lungs, stomach, spleen, kidneys, and ovaries were dried with absorbent paper and weighed immediately. Organ weight was compared to its body weight to give the relative organ weight.

Statistical analysis

The statistical analysis was carried out using statistical package for social sciences (SPSS-computer package). Average body weight and relative organ weight were expressed as mean±SD. Values in all groups were compared using the one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison tests.

RESULTS

There were no signs of mortality in all groups of rats. In general, the rats gained weight as seen in fig. 1. The average weight gains for all groups were within the normal range. There was no any significant difference in average weight gains for all treatment groups (fig. 1). It showed that all treatment groups had similar weight gains. However, a significant difference (P<0.05) was seen when the extract given at a dose of 50 mg/kg (P=0.006). The average body weight on all groups could be seen in table 1.

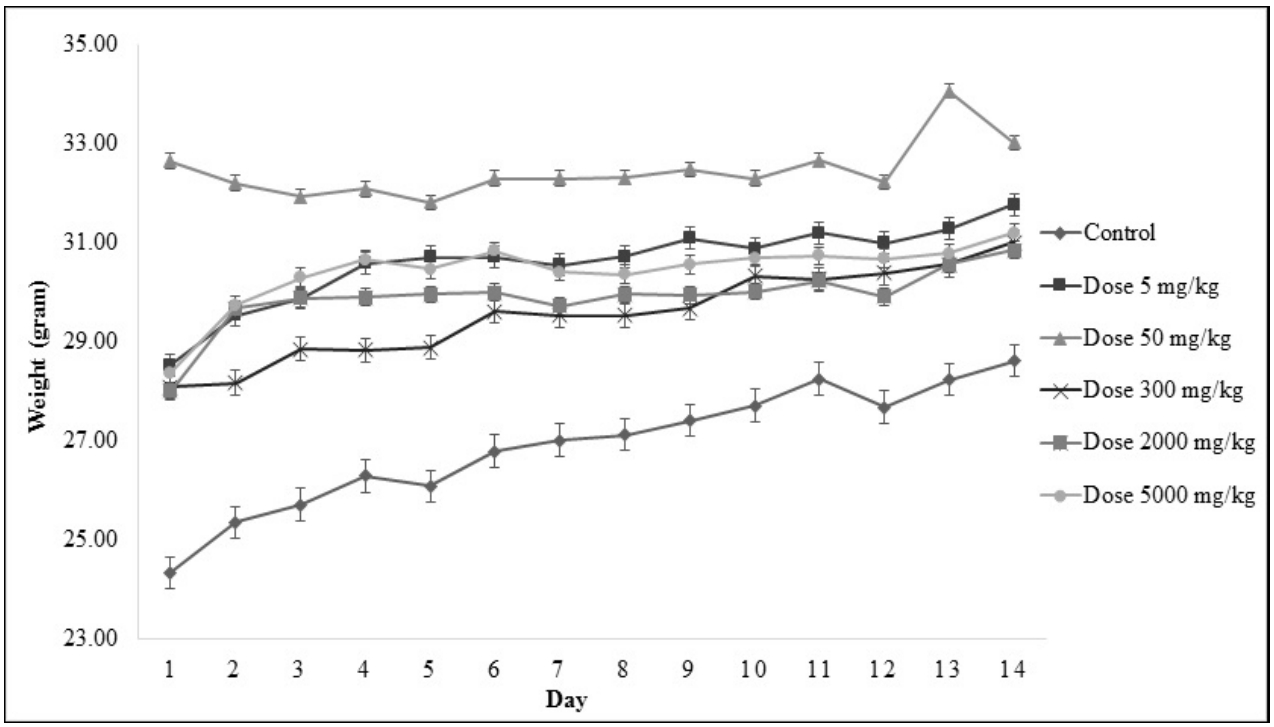

Fig. 1: The average body weight of *Wistar* rats after receiving the ethanol extract of *Mucuna pruriens* seeds for 14 d. *P values<0.05 were considered significant using one-way ANOVA followed by Tukey's multiple comparison tests

Table 1: Average body weight of *Wistar* rats after receiving the ethanol extract of *Mucuna pruriens* seeds at 7 and 14 d

Days	Average of body weight (gram)					
	Control	Dose 5 mg/kg	Dose 50 mg/kg	Dose 300 mg/kg	Dose 2000 mg/kg	Dose 5000 mg/kg
7	25.92±3.33	30.06±0.48 (P=0.122)	**32.17±3.57* (P=0.006)**	28.84±2.26 (P=0.440)	29.57±1.91 (P=0.215)	30.10±1.85 (P=0.116)
14	27.85±4.31	31.12±0.48 (P=0.338)	32.71±2.32 (P=.053)	30.24±2.66 (P=0.663)	30.19±1.85 (P=0.679)	30.70±1.75 (P=0.484)

Values are expressed as mean±SD (n=5 for each group). *P values<0.05 were considered significant using one-way ANOVA followed by Tukey's multiple comparison tests. Asterisks denote significant difference compared to control.

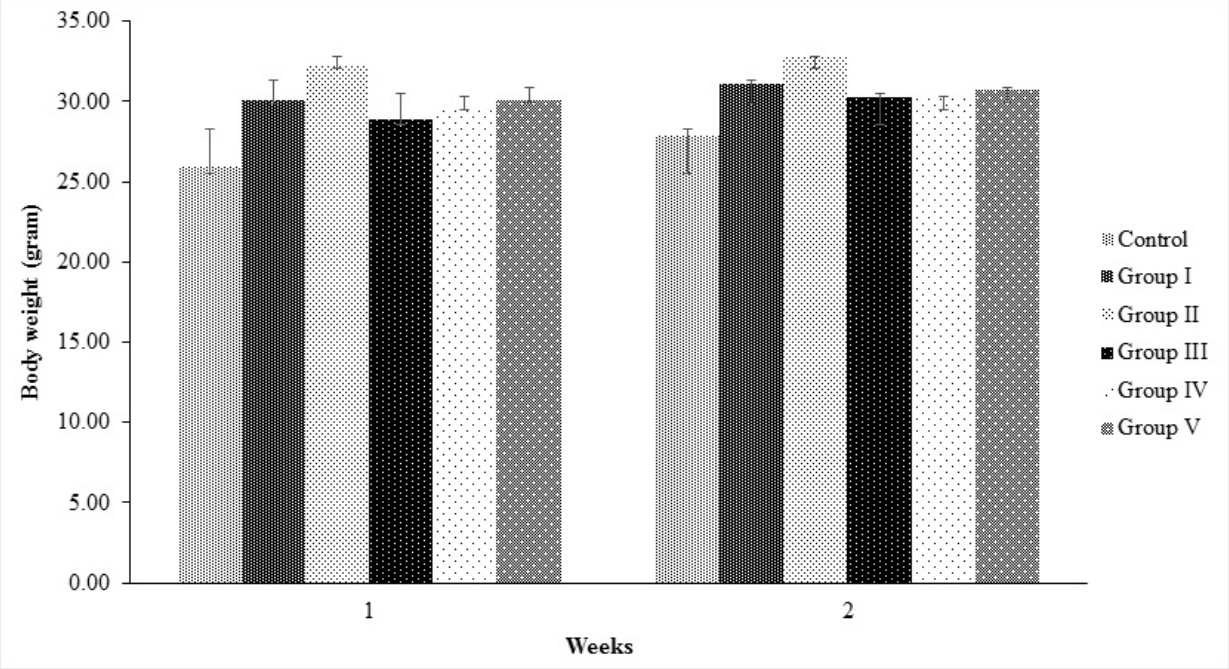

Fig. 2: Average body weight of rats after receiving the ethanol extract of *Mucuna pruriens* seeds. **P* values<0.05 were considered significant using one-way ANOVA followed by Tukey's multiple comparison tests. Asterisks denote significant difference compared to control

In this study, the relative organ weights in all treatment groups generally did not show a significant difference compared with control group. The ethanol extract of *Mucuna pruriens* seeds at a dose of 5000 mg/kg had no adverse effect on the tested *Wistar* rats up to 14 d. Significant differences ($P<0.05$) were seen in the liver of the treatment groups compared with the control group. There were no significant differences for the relative organ weights within the treatment group. The relative organ weights could be seen in table 2.

Table 2: The relative organ weights of the rats after receiving ethanol extract of *Mucuna pruriens* seeds for 14 d

Organ	Average of relative organ weights (%)					
	Control	Dose 5 mg/kg	Dose 50 mg/kg	Dose 300 mg/kg	Dose 2000 mg/kg	Dose 5000 mg/kg
Brain	1.33±0.09	1.15±0.11 (*P*=0.147)	1.18±0.12 (*P*=0.278)	1.13±0.10 (*P*=0.085)	1.20±0.14 (*P*=0.389)	1.17±0.11 (*P*=0.242)
Heart	0.43±0.06	0.39±0.03 (*P*=0.959)	0.41±0.12 (*P*=0.996)	0.39±0.05 (*P*=0.927)	0.42±0.05 (*P*=0.999)	0.42±0.11 (*P*=0.999)
Liver	6.37±0.67	5.14±0.33* (*P*=0.003)	5.38±0.55* (*P*=0.020)	5.25±0.39* (*P*=0.006)	4.80±0.29* (*P*=0.000)	4.76±0.31* (*P*=0.000)
Lungs	0.66±0.11	0.55±0.15 (*P*=0.782)	0.62±0.09 (*P*=0.997)	0.62±0.16 (*P*=0.998)	0.65±0.09 (*P*=1.000)	0.59±0.14 (*P*=0.967)
Stomach	2.75±0.73	1.89±0.23 (*P*=0.262)	2.13±0.47 (*P*=0.593)	2.44±1.08 (*P*=0.965)	1.75±0.38 (*P*=0.138)	1.65±0.34 (*P*=0.080)
Spleen	0.84±0.13	0.61±0.12 (*P*=0.345)	0.90±0.22 (*P*=0.985)	0.64±0.24 (*P*=0.501)	0.59±0.12 (*P*=0.258)	0.54±0.15 (*P*=0.092)
Kidneys	1.16±0.13	1.04±0.08 (*P*=0.652)	1.19±0.16 (*P*=0.998)	1.06±0.05 (*P*=0.788)	1.05±0.09 (*P*=0.699)	1.02±0.17 (*P*=0.460)
Ovaries	1.15±0.38	1.18±0.26 (*P*=1.000)	1.33±0.36 (*P*=0.943)	1.24±0.26 (*P*=0.997)	1.41±0.28 (*P*=0.788)	1.36±0.29 (*P*=0.891)

Values are expressed as mean±SD (n=5 for each group). **P* values<0.05 were considered significant using one-way ANOVA followed by Tukey's multiple comparison tests. Asterisks denote significant difference compared to control.

DISCUSSION

As one of the efforts in the treatment of various diseases, many people use various plants as a medicine. This has been done for centuries throughout the world. One of the plants that used in the treatment of various diseases, including in Indonesia, is *Mucuna pruriens*. Various studies related to phytochemical, bioactivity, and toxicity have been done. However, the study related to the level of toxicity of *Mucuna pruriens* from Indonesia is still limited. Therefore, this study was conducted to determine the level of acute toxicity of ethanol extracts of *Mucuna pruriens* seeds on *Wistar* rats for 14 d. From the study, the acute toxicity data of the plants may be useful as (a) basis for classification and labeling; (b) preliminary information about mode of toxic substances action; (c) foundation to assist in determining new compound dose; (d) basis in dose determination for animal studies; and (e) basis in determination of LD_{50} values that would provide an overview of the many potential drug activities [28].

Acute toxicity is defined as the emergence of adverse effects within a short time after administration of a single dose or multiple doses within 24 h [27, 29, 30]. The purpose of this testing is to obtain the information about the chemical effects on biological activity, thus it

would be known whether the effect of the substance is very toxic, toxic, less toxic, or whether the toxic effect is not significant. The observation was conducted by measuring the body weight and the organ weight of the rats after receiving the extract of *Mucuna pruriens* seeds for 14 d. Autopsy and macroscopically examination for any pathological changes were performed to all survive rats [27].

After being exposed to substances, there would be changes in body weights and internal organ weights. It would reflect the toxicity if the body weights lose more than 10% of the initial body weights, and it would be considered as statistically significant [31]. There were no mortality, no clinical signs of toxicity, and the average of the body weight was in normal range, thus LD_{50} value for ethanol extract of *Mucuna pruriens* seeds from Indonesia was found to be greater than 5000 mg/kg and this plant could be considered as quite safely to use as herbal medicine. This is in line with [23] that showed the methanol extract of *Mucuna pruriens* seeds from India did not cause mortality in animals even the extract given at 2000 mg/kg body weight. The animals also had gained body weight within the normal range [23]. The *Mucuna pruriens* that extracted by ethanol-water and administered by oral, given up to 10.000 mg/kg did not cause any visible signs of toxicity and mortality. When the ethanol-water extract of *Mucuna pruriens* administered by intraperitoneal injection, it was estimated to give LD_{50} at about 1500 mg/kg [32]. In another case, the ethanol extract of *Mucuna pruriens* leaves from India caused the signs of mortality at a dose of 2000 mg/kg [24]. It is caused by the difference of the compounds contain in each part of the plant and the difference of the region of the plant origin that would influence the composition of compounds.

A significant difference (P<0.05) was seen when the extract administered at dose 50 mg/kg (P=0.006). The significant difference might indicate that the extract might have dose-dependent effects on weight gains. *Mucuna pruriens* seeds used to stimulate the growth of hormone and the build of muscle. It is also might be contributed to weight gains. However, it was observed in certain dose only, not in all of the dosas gave the effect of significant weight gains. This dose-dependent effects could be happen, similar to dose-dependent effects on weight gains when the ethanol extract of *Mucuna pruriens* seeds administered to diabetic rats [10] and dose and time-dependent effects on sexual behavior when the ethanol extract of *Mucuna pruriens* seeds administered to normal male rats [3].

The organ weight is an important indicator of the physiological and pathological status of the animals. The main reason for using the relative organ weights is to enhance the detection of the level of drug effects on the body by reducing the degree of variability in the experimental groups. The relative organ weight is the basic data to confirm whether the organ weight is exposed to the injury or not. The heart, liver, kidney, spleen and lungs are the organs that usually affected by the metabolic reaction caused by toxicant [31]. Some cell types within the brain, heart, and kidneys are also particularly susceptible to the effects of toxic [33]. Generally, the relative organ weights in this study did not show a significant difference, thus it could be said that the ethanol extract of *Mucuna pruriens* seeds up to a dose of 5000 mg/kg has no adverse effect on *Wistar* rats tested for 14 d. However, the significant differences (P<0.05) were seen in the relative organ weights of the liver in all the treatment groups compared with the control group. This indicated that there was a toxic effect from the ethanol extract of *Mucuna pruriens* seeds on the liver of the rats after receiving the ethanol extract of *Mucuna pruriens* seeds.

An indication of toxic effects on the liver is similar to [34] who reported the aqueous extract of *Mucuna pruriens* leaves from Nigeria was dangerous to the liver along with the increase of the alanine transaminase (ALT) and aspartate transaminase (AST) [34]. ALT and AST are enzymes that catalyze the aminotransferase, where increasing the concentration of aminotransferase may indicate an injury to the liver. For more specific, an increase of ALT levels indicated liver damage. The magnitude of the change in aminotransferase levels could be classified into three levels. Changes in mild level are achieved when the magnitude of the change is less than 5 times upper reference limit, while the moderate changes achieved when the change is 5-10 times upper reference limit. Changes in severe level are indicated by the change occurred more than 10 times upper reference limit [35]. Accordingly, the determination of levels of ALT and AST is required as advanced research to ensure the effect of the ethanol extract of *Mucuna pruriens* seeds in the liver. However, in other reports related to *Mucuna pruriens* extracts showed hepatoprotective activity such as the ethanol-water extract of *Mucuna pruriens* leaves from Nigeria [8], and the ethanol extract of *Mucuna pruriens* seeds from India [9, 37]. These findings clarify that the content and composition of compounds in plants depending on the part of the plant, a region of origin, and solvents used to extract.

Based on the recommendations of the Organization for Economic Co-operation and Development (OECD) related to chemical labelling and classification of acute systemic toxicity level, the ethanol extract of *Mucuna pruriens* seeds could be classified in the fifth class where the value of LD_{50} is predicted greater than 5000 mg/kg. This is a class with the lowest toxicity level. Based on the study conducted by Kennedy, a substance with an LD_{50} value greater than 5000 mg/kg orally is considered safe and practically non-toxic [37].

CONCLUSION

In this acute toxicity study of ethanol extract of *Mucuna pruriens* seeds from Indonesia administered orally for 14 d, showed no signs of toxicity or death in all *Wistar* rats, although the extract given at high dose. The rats have gained weight within the normal range. This showed that the ethanol extract of *Mucuna pruriens* seeds from Indonesia administered orally has LD_{50} value might be greater than 5000 mg/kg body weight. The relative organ weights for all groups generally did not show a significant difference. The significant differences (P<0.05) were found in the liver in all treatment groups compared with the control group. These findings suggest that the ethanol extract of *Mucuna pruriens* seeds from Indonesia are considered safe or non-toxic, but has a potential toxicity to the liver. This finding requires further research related to the parameters of liver damage.

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CONFLICT OF INTERESTS

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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