Biosafety Evaluation of methanol extract of Stem Bark of*Lonchocarpusgriffonianus* (Baill.) Dunn (Fabaceae) in Sprague-Dawley rats

Abstracts

Aim: Due to hazards associated with specific medicinal plant species, the biosafety evaluation of medicinal plants is crucial for guaranteeing both their safe use and regulatory compliance. Assessing the biosafety profile of the methanol extract of *L. griffonianus* stem bark (LGME) is imperative. This research seeks to preserve both the environment and public health by filling in the gaps in the biosafety reports regarding theplant's stem bark's safety for possible usage.

Methodology: Adult maleSprague-Dawley ratsweighing 180 ± 1.63 gwere given oral doses of LGME at 100, 200, and 400 mg/kg daily for 28 days. Hematological parameters such as hemoglobin (Hb), pack cell volume (PCV), white blood cell (WBC), red blood cell (RBC), hematocrit (Hct), lymphocytes (Lym) and granulocytes (Gran) were measured. Biochemical parameters such as kidney function tests (urea, creatinine, sodium, potassium, chloride and bicarbonate), and liver function tests (alkaline phosphate (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin (TBil), conjugated bilirubin (CB), total protein (TP) and albumin (Alb) were examined. Kidney and liver histological investigations were performed to evaluate any indications of organ damage.

Results: No significant ($p<0.05$) alterations in the hematological markers in rats were observed. There were no discernible alterations in the liver and kidney function markers. Liver and kidney histological architecture revealed no severe injuries.

Conclusion: The current data indicate that the toxicity of the *L. griffonianus* methanol extract is lowin Sprague-Dawley rats. These results offer crucial details regarding the toxicity and safety profile of *L. griffonianus*, a plant used in traditional medicinefor the treatment of benign prostatic hyperplasia.

Keywords: biosafety, hematological parameters, kidney function test, liver function test, *L. griffonianus*, Fabaceae

Introduction

Species of plants with medicinal benefits have been known for ages to elicit both therapeutic and toxic responses. These plants are known to contain myriad bioactive compounds at near pharmaceutical doses [1]. Recently, there has been an awakened interest globally in using herbal remedies. Abuse and misuse of these remedies are rising because of the false belief that medicinal plants are safe due to their natural origin[2,3]. Sometimes, the unregulated use of these products has resulted in the occurrence of hepatic, renal and haemopoietic damage.

Fabaceae is a vast family of medicinal plants, and some members have shown nutritional values with the expression of proteins in their leaves and seeds. Numerous plants in this family are used in ethnomedicine to treat several ailments, with some displaying significant toxicity levels [4]. *Abrusprecatorius* (Fabaceae), used in ethnomedicine to treat diarrhea, has been reported to be one of the most toxic plants due to a poisonous lectin known as Abrin [5]. Toxicosis by Abrin could lead to multi-organ damage, including liver failure[6]. *Phaseolus vulgaris* (Fabaceae), an edible bean-producing plant, leaf is used to treat conditions such as diabetes mellitus, microbial infections and painful conditions [7]. Phytohemagglutinin found in the plant destroys erythrocytes and leucocytes in humans. [8]. Therefore, biosafety assessment of medicinal plants is essential for regulatory purposes and also to evaluate the safety of medicinal plant species. Biosafety analysis is expected to prevent unforeseen hazardous occurrences and provide information on the safety status of herbal medicines to eliminate doubt in the minds of consumers and prescribers.

Lonchocarpusgriffonianus(Baill) Dunn (Fabaceae) is usually a tree or shrub of about 10 - 20 m tall with compound leaves having three to five pairs of leaflets about 5- 10 cm long and 2 to 4 cm

wide. The lilac to violet flowers have a strong scent, up to one and a half in length and appear with the new leaves in the dry season. The plant grows around water banks. It is distributed from Nigeria to Angola [9,10]. The stem bark of the plant is used to treat benign prostatic hyperplasia (BPH) in Akwa Ibom. The leaves are used to treat pharynx pulmonary disorders, while its root and stem bark are remedies against stomach aches, infertility, amenorrhea, inflammatory disorders, Benign prostatic hyperplasia and enlarged scrotum in neonates [11]. Biosafety reports on the plant's stem bark are scanty; thus, there is a need to determine the biosafety profile of the methanolic extract of *L. Griffonianus* stem bark.

2Methods

2.1Collection of plant material

Lonchocarpusgriffonianus (LG) stem was collected in Akwa Ibom State, Nigeria. The plant was authenticated by Prof. H. A. Akinnibosun of the Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin, Benin City, Edo State. Specimen number UBH-L611 was allocated to a voucher specimen deposited in the Department's herbarium.

2.2Preparation of plant extract

One kilograms of LG stem bark was dried for four weeks in the shade, powdered using a mechanical mill, and extracted with methanol (99.5%) using a Soxhlet apparatus with a heating mantle set at 67º C. The*L. griffonianus* methanol extract (LGME) was concentrated *in vacuo* at 35℃, weighed, stored in a labelled bottle, and maintained at 4℃ in a refrigerator until needed [12].

2.3 Experimental animals

Thirty-two Sprague-Dawley male rats weighing 180 ± 1.63 g were obtained from the Animal House unit of the Department of Biochemistry, University of Benin, Nigeria. Animals were allowed fourteen (14) days of acclimatization before the commencement of the experiment. The animals were housed under standard environmental factors. They were allowed free access to standard pellets and water *ad libitum*.

2.4 Biosafety experimental design

Thirty-two Sprague-Dawley male rats were randomly assigned to 4 groups (n=8), and extract (LGME) was administered using the following regime for 28 days: Group 1 served as the standard control with access to feed and water *ad libitum*, while groups 2-4 received LGME at 100, 200 and 400 mg/kg, p.o. respectively. They were fasted overnight on the 28th day. Then, on the 29th day, the animals were sacrificed under anesthesia, and their blood was collected via the abdominal aorta for biochemical analysis. Their vital organs (liver and kidney) were collected for histopathology.

2.4.1 Hematological evaluation

Blood samples were analyzed using an automated hematology analyzer. The parameters analyzed included hemoglobin (Hb), pack cell volume (PCV), white blood cell (WBC) count, red blood cell (RBC) count, hematocrit (Hct), lymphocytes (Lym) and granulocytes (Gran).

2.4.2 Biochemical evaluation

Blood was centrifuged at 2000 rpm for 5 minutes to obtain the serum. Serum was analyzed for the levels of alkaline phosphate (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatinine, urea, sodium, potassium, chloride, bicarbonate, total bilirubin (TBil), conjugated bilirubin (CB), total protein (TP) and albumin (Alb).

2.4.3 Histological examination of the animal's vital organs

The harvested organs (kidney and liver) were fixed in 10% buffered forma-saline. They were embedded in paraffin wax blocks and sliced into four μm thick sections. The cut sections were transferred to glass slides and stained with routine hematoxylin and eosin stains. The stained sections were observed under a light microscope at magnifications of 400X. Photomicrographs of the observed sections were taken through the use of Amscope.

2.5 Statistical analysis

Values are expressed as mean ± SEM (n=5). Differences between groups were analyzed using analysis of variance (ANOVA) followed by Dunnett's post hoc test at a 95% confidence interval using GraphPad Prism version 6.01.

3.0 Results

Table 1 Effect of LGME on haematological profile in male Sprague-Dawley rats

	control	100 mg/kg	200 mg/kg	400 mg/kg
WBC (10^3/ μL)	8.18 ± 0.32	13.10 ± 0.51	5.96 ± 0.68	4.62 ± 0.33
RBC (106/ mL)	5.62 ± 0.26	06.23 ± 0.40	5.16 ± 0.51	6.61 ± 0.26
Lym (%)	91.60 ± 0.68	91.30 ± 1.60	93.30 ± 0.66	81.00 ± 7.29
Hb (g/dL)	12.30 ± 0.00	17.00 ± 0.00	11.10 ± 0.00	13.60 ± 0.00
Hct (%)	35.80 ± 1.15	42.60 ± 1.84	34.80 ± 3.41	40.30 ± 1.55
Lym (103/ mL)	7.40 ± 0.27	12.00 ± 0.50	6.02 ± 0.64	4.36 ± 0.32
Gran (%)	0.18 ± 0.02	0.20 ± 0.00	0.16 ± 0.03	0.12 ± 0.04

The values are expressed as mean ± SEM. Data are non-significant at $p<0.05$. n=5

Table 2 Effect of LGME on kidney function of male Sprague-Dawley rats

Parameters	Control	100 mg/kg	200 mg/kg	400 mg/kg
Urea (mg/dL)	31.00±5.71	22.60±2.94	35.80±4.75	21.60±1.54
Creatinine (mg/dl)	0.98±0.10	1.08±0.11	1.14±0.08	0.70±0.03
Sodium (mMol/L)	139±0.95	139±1.36	142±2.05	135±1.22
Potassium (mMol/L)	3.82±0.10	3.80±0.45	4.12±0.08	3.92±0.06
Chloride (mMol/L)	106±1.32	103±1.16	108±1.63	104±0.87
Bicarbonate (mMol/L)	19.80±0.58	22.20±0.66	22.00±0.89	20.60±0.93

The values are expressed as mean ± SEM. Data are non-significant at $p<0.05$. n=5

Table 3 Effect of LGME on liver function of male Sprague-Dawley rats

Parameters	Control	100 mg/kg	200 mg/kg	400 mg/kg
ALP	90.40±2.42	84.20±3.92	76.80±4.72	76.00±4.38
AST	24.20±2.60	18.40±1.12	25.20±0.97	25.40±1.89
ALT	22.20±2.15	11.80±0.58	22.20±0.80	19.60±3.04
ALB	3.96±0.07	3.96±0.05	3.88±0.04	4.04±0.02
TBil	1.00±0.07	2.20±0.55	0.90±0.07	0.86±0.07
CB	0.28±0.04	0.36±0.09	0.26±0.02	0.24±0.02
TP	6.92±0.20	6.72±0.19	6.36±0.08	7.14±0.06

The values are expressed as mean ± SEM. Data are non-significant at $p<0.05$. n=5

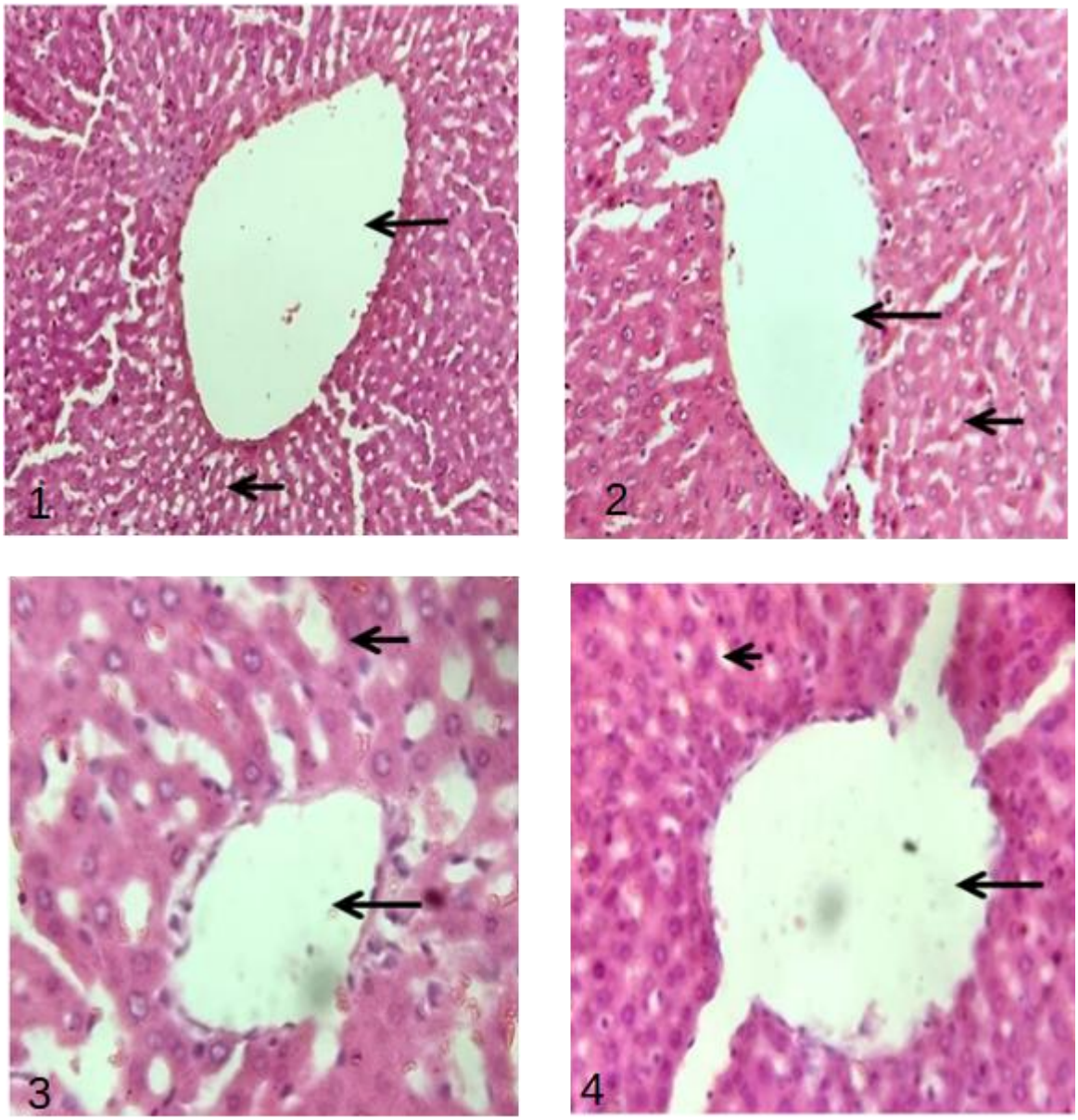

Figure 1: Effect of LGME on the rat liver. **(1**) Thestandard control group reveals visible centriole (long arrow) and well-fenestrated sinusoids and hepatocytes (short arrow), **(2)** The 100 mg/kg group reveals visibly dilated centriole (long arrow) and well-fenestrated sinusoids and hepatocytes with vacuolated nucleus (short arrow) **(3)** The 200 mg/kg reveals visible centriole surrounded with mild mononuclear cells (long arrow) and well-fenestrated sinusoids and hepatocytes with visible steatosis (short arrow) **(4)** The 400 mg/kg reveals visible centriole (long arrow) and well-fenestrated sinusoids and hepatocytes with mild steatosis (short arrow). 400X magnification

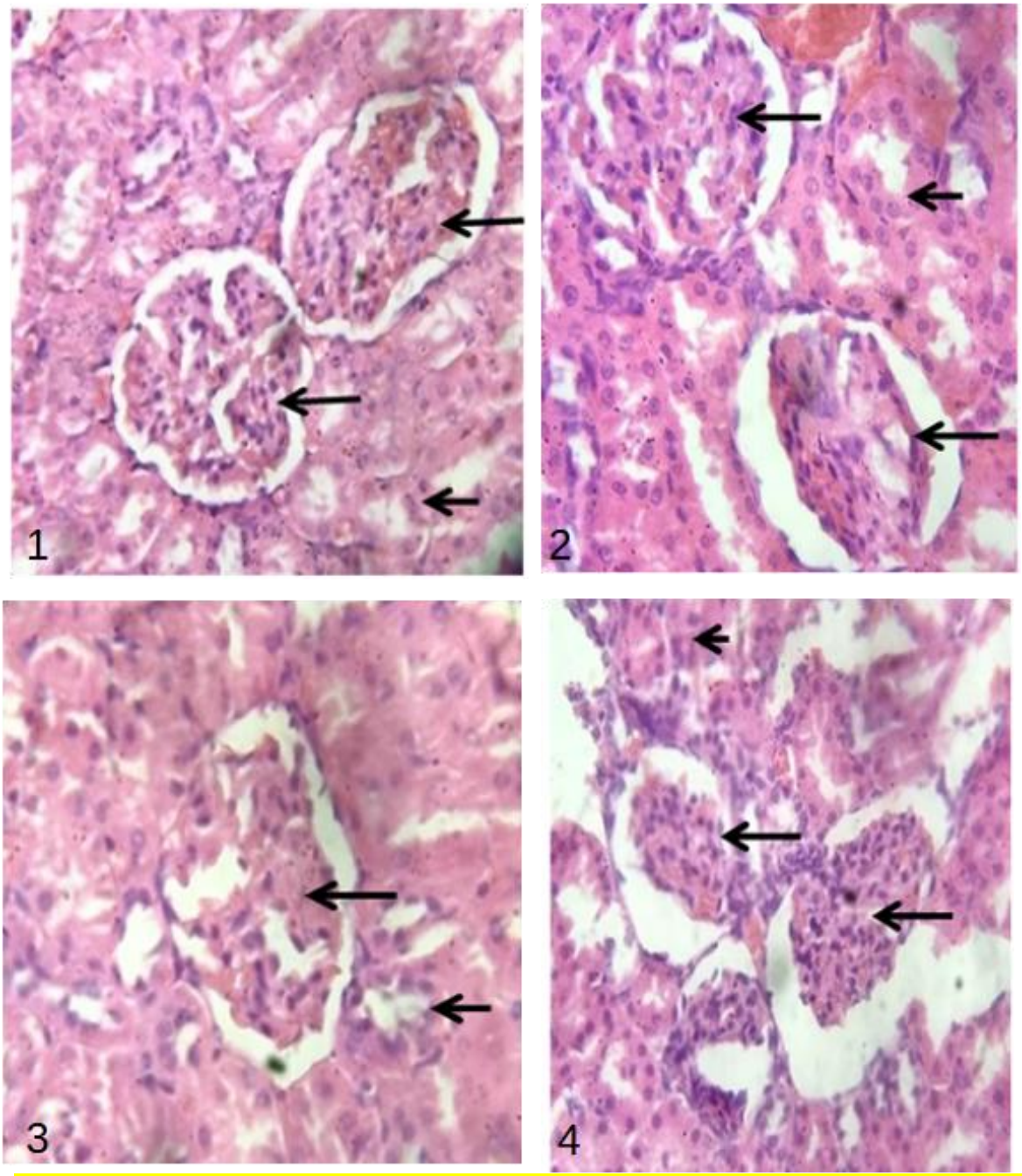

Figure 2: Effect of LGME on the rat kidney. **(1)** The standard control group reveals prominent renal corpuscle with glomerulus (long arrow) with tubules (short arrow) and interstitial. **(2)** The 100 mg/kg group reveals prominent renal corpuscle with glomerulus (long arrow) with tubules (short arrow) and interstitial. **(3)** The 200 mg/kg reveals a prominent renal corpuscle with glomerulus (long arrow) with tubules (short arrow) and interstitial that is not looking very prominent. **(4)** The 400 mg/kg reveals unremarkable renal corpuscle with atrophied glomerulus (long arrow) with a less prominent tubules (short arrow) and interstitial. 400X magnification

4.0 Discussion

Plant extracts contain several phytoconstituents capable of affecting several physiological parameters and components in man. These secondary metabolites could elicit pharmacological or toxicological responses[13,14]. Thus, the effects of LGME on blood components, liver function and kidney function of Sprague-Dawley rats were examined in this study.

The degree of an extract's lethal effect on an animal's blood can be ascertained by assessing hematological parameters. The WBC count can independently predict all-cause mortality, as evidenced by data from several studies[15–17], which can also reveal the degree of systemic inflammation. It is frequently included in regular clinical examinations to assess the level of injury suffered by the internal milieu. WBC count in the treated groups (100-400 mg/kg) was not significantly different ($p=.05$) compared to the control. The result shows that the oral extract given for 28 days did not exert a toxic effect on WBC components (Table 1).

The RBC is the most significant functional iron compartment in the body. Iron is an essential nutrient that is crucial to numerous metabolic processes. Maintaining average concentrations of iron-containing proteins, including transferrin, ferritin, and hemoglobin, is necessary for aerobic metabolism as they facilitate oxygen transport, storage, and utilization. Iron content is measurable in hematological parameters such as RBC count, hemoglobin content and packed cell volume, also known as hematocrit[18]. The administration of graded doses for 28 days did not significantly ($p<0.05$) alter the number of RBCs, hemoglobin contents and hematocrit in the experimental animals. The result means that the extract did not exert a toxic effect on the RBCs at the administered dose for 28 days (Table 1).

Lymphocytes consist of T-lymphocytes, B-lymphocytes, granulocytes, and natural killer cells, which have a variety of functions. These cells produce antibodies, control the immune response, and directly destroy pathogens through cell-mediated means[19]. The LGME given to the experimental animals orally did not significantly ($p<0.05$) alter the lymphocyte's cellular components when compared to the control (Table 1).

Serum creatinine and urea are essential markers used to assess the functional level of the kidney. The administered extract did not significantly ($p<0.05$) alter the urea. Creatinine and other kidney function marker levels in the serum of Sprague-Dawley rats after 28 days of administration of LGME at graded doses (100-400 mg/kg) (Table 2).

No significant ($p<0.05$) alterations in the serum levels of liver function parameters were measured from the experimental animals' serum compared to the standard control. These results signify the non-toxic nature of the extract at the administered dose for 28 days (Table 3).

The histological presentation of the liver in the standard control group reveals visible centriole, fenestrated sinusoids and normal hepatocytes. The treated groups show minor histo-morphological changes, such as dilated centrioles and hepatocytes with a vacuolated nucleus and mild steatosis reversible upon treatment discontinuation (Figure 1). The histological aspects of the kidneys in the control group reveal prominent renal corpuscle with normal glomerulus, tubules and interstitial. The treated groups disclose prominent renal corpuscles with glomeruli, tubules, and interstitials that are not prominent, including atrophied glomeruli in the 400 mg/kg group. These slight changes reflect the transient impact these vital organs receive as a part of the physiological transformation and elimination of the ingested compounds from the system (Figure 2).

Conclusion

Findings in the study revealed that the methanol stem bark extract of the plant at oral doses of up to 400 mg/kg did not adversely affect the experimental animals. It was observed that none of the hematological, biochemical, or histological parameters investigated in this study were significantly altered. Moreover, no fatality linked to the administered extract was recorded during the investigation. These indicate that the extract is safe at the administered dose on the experimental animals but requires further safety evaluations.

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Competing interest: Authors have declared that no competing interests exist.

Ethical approval: All authors now declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the Ethical Committee, Faculty of Pharmacy, University of Benin, Edo state, Nigeria. (EC/FP/022/15)

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