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Toxicity Studies of Aqueous and Ethanolic Extracts of Fermented Seeds of *Parkia biglobosa* (Mimosaceae) in Rats

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Abstract: *Parkia biglobosa* is a medicinal plant that is used in the traditional treatment of various pathologies. The fermented seeds of this plant « néré » are used commonly in the treatment of high blood pressure. In order to establish the safety of these, acute and subacute toxicity tests were performed. For this purpose, hematological and biochemical parameters were determined in rats after repeated dosing of 1000, 2000, 3000, 4000 and 5000 mg/kg body weight (bw) of the aqueous and ethanolic extracts of the fermented seeds of *P. biglobosa* for 28 days. The resulting LD_{50} is greater than 5000 mg/kg bw and the extracts have no effect on most of the measured blood parameters. This study revealed that extracts of the fermented seeds of *Parkia biglobosa* are non-toxic at all doses tested and have immunostimulant and analgesic activity.

Keywords: *Parkia biglobosa*, Toxicity, Blood Parameters, High Blood Pressure

Introduction

Parkia is a pantropical genus of the family Fabaceae (Aubréville, 1950 ; Arbonnier, 2002) which includes 34 species distributed in South America (18 species), Asia (12 species) and Africa (4 species including one in Madagascar) (Hopkins, 1986 ; Luckow and Hopkins, 1995 ; Luckow, 2005). *P. biglobosa* is a species of this genus and is commonly used in medicine and traditional pharmacopoeia (Ouédraogo, 1995). All parts of this plant are used as a main recipe or in combination with other plants for the care of various ailments (Bonnah *et al.*, 1998). In fact, the different parts of P. biglobosa are used for the treatment of dermatoses, hypertension and haemorrhage (Odetola, 2006 ; Kanko, 2000 ; Kouadio, 2000 ; Tringali, 2000). Thus, the leaves are used to treat eye infections, skin lesions and leprosy (Erakhrumen *et al.*, 2010). They are also involved in the care of febrile states (Aubréville, 1950). Bark is used for the treatment of trypanosomiasis, ulcer, fever and for the care of wounds (Osho and Lajide, 2012). Roots are used to treat infertility, hypertension, stomach upset and are also used as an anti-poison (Lawal *et al*, 2010, Erakhrumen *et al*, 2010). In addition, the fermented seeds of *P. biglobosa* serve as food condiments for various peoples. These fermented seeds are called « *afitin* »in Benin (Azopkota *et al.*, 2011), « *dawa-dawa or iru* » in Nigeria, « *nététu* »in Senegal and «*soumbala* »in Mali (N'Dir *et al.*, 1994). In Côte d'Ivoire, this condiment is called in malinké « *soumara* » and is considered to have antihypertensive properties according to its consumers. The aim of this work is to study the toxicity of aqueous and ethanolic extracts of fermented seeds of *P. biglobosa* in rat.

MATERIALS AND METHODS

Materials

Plant Materials

According to N'Dir *et al*., (1994), the fermented seeds of Parkia Biglobosa (soumara) were obtained by essential stages. The first cooking of raw seeds is long. It lasts 15 to 24 hours and its purpose is to soften the seminal integument. After this first essential step, the seeds are dehulled and washed with water and then undergo a second cooking time shorter than the first (one to two hours of time). Finally, the almonds are dewatered, sorted and fermented at the bottom of a canary covered at 28 to 40 ° C for three days. The fermented seeds are finally recovered and dried in the sun to obtain the soumara.

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Experimental animals

Male and female albino white rats of Wistar strains weighing between 180 g and 250 g were used for the study.

Methods

Preparation of plant extracts

Two (02) samples of 500 g of *soumara* were dried at room temperature in the laboratory, then crushed and powdered in a mortar. Subsequently, 250 g of soumara powder were dissolved in 500 mL of distilled water and then homogenized with magnetic stirring for 24 hours at 25°C using a magnetic stirrer. The homogenate obtained was filtered successively twice on hydrophilic cotton and then once on Whatmann filter paper. The filtrate obtained is evaporated using a Med Center Venticell type oven at 50 ° C. to give a powder which constitutes the aqueous extract. The same operation was carried out using instead of distilled water of ethanol 70%. However, the volume of the hydro-alcoholic filtrate obtained is first reduced using a Buchi-type rotary evaporator at a temperature of 60 ° C. Then, the remainder of the filtrate is evaporated using a Med Center Venticell type oven at 50 ° C. to give a powder which represents the 70% ethanol extract. All the vegetable raw extracts thus formed are stored in the refrigerator until 'to their use for different tests.

Acute toxicity

The acute toxicity study was performed on Wistar rats. The experiment was conducted sequentially according to OECD guideline 423 (2001) with predefined doses of 1000, 3000 and 5000 mg/kg bw. Each stage of the experiment required the use of three rats. 12 rats with comparable weights are divided into 4 lots of 3 rats. The animals were acclimatized at least 5 days to laboratory conditions before the start of the experiment. They were fasted all night before administering the different doses, but they had access to water. Acute toxicity was assessed by increasing oral doses of the extracts. The different lots of rats received respectively 1000, 3000 and 5000 mg/kg bw at a rate of 1 mL/rat. The experimental protocol was as follows: first, the first batch of rats received the single dose of 1000 mg/kg bw. Then, they were observed with special attention during the first hours after the force-feeding then during the 24 hours. The observation of the animals continued for 14 days. The absence of mortality observed with the animals of the first batch allowed to always administer by gavage the higher dose which is 3000 mg/kg bw in the second batch. And finally, the last batch received the limit dose of 5000 mg/kg bw. The control group received distilled water.

Subacute toxicity

OECD Guideline 407 (2006) for the testing of chemicals, with some modifications, was used for this study. The test substance is administered orally (gavage) at different dose levels to several batches of animals at a dose level per batch for a period of 28 days. 33 healthy adult rats were randomly assigned to 11 batches of three rats. They were placed in the cages at least five days before the start of the study, to allow them to acclimatize to laboratory conditions. They received by gavage for 28 days the doses of 1000, 2000, 3000, 4000 and 5000 mg / kg bw of the aqueous and ethanolic extracts. The volume of extract administered every three days in a single dose was 1 mL per 100 g bw. The control group received distilled water. During the 28 days of treatment, the animals were observed daily for clinical signs (lack of appetite, convulsions, drowsiness, etc.).

Before the start of the experiment, all the rats were weighed and then twice a week during the treatment period to determine the impact of the extracts on the weight growth of the animals.

After 28 days of gavage, the rats were anesthetized with ether, the blood is taken early in the morning by the technique of amputation at the end of the tail (5 mm from the tip) previously disinfected with 96º ethanol and heated to cause vasodilation.

Hematologic analysis was performed using a Sysmex KX-21 Automated Hematology Analyzer (Canada) according to the manufacturer's instruction manual with appropriate reagents (kits).

For assays of the biochemical parameters, the contents in the dry tubes were centrifuged with a centrifuge at 3000 rpm for 5 minutes. The serum obtained is collected and stored at -20ºC for the analysis of serum markers of the kidney, liver and heart using the COBAS INTEGRA 400 plus analyzer (France). The protocol for each assay was pre-established and then incorporated into the device during assays according to the manufacturer's instruction manual with the appropriate reagents (kits).

Statistical analysis

The statistical analysis of all the results expressed in means accompanied by the standard deviations (Mean ± ET), was carried out according to the analysis of the variances (ANOVA ONE WAY). When there are more than two samples to be analyzed, an appropriate analysis of variance (ANOVA) should be used. One-way (univariate) ANOVA followed by Tukey was chosen. Differences between averages were determined using the Tukey Multiple Comparison Test using Graph Pad Prism 5.0 (Microsoft U.S.A). These differences are considered significant when $p < 0.05$.

RESULTS

Acute toxicity

At the dose of 1000 mg/kg bw, the rats showed no signs of toxicity after administration of the extracts. There is no change in mobility, behavior, appetite. No mortality was observed. These observations were made after three hours and for 14 days after administration of the product. At the dose of 3000 mg / kg bw, the rats drag the forelegs. They are tired with a feeling of thirst. This state is very accentuated with the ethanolic extract. However, there were no deaths. After heavy water consumption, the signs mentioned disappear. At a dose of 5000 mg / kg bw, the animals are very exhausted and weakened but return to normal two to three days later.

Subacute toxicity

Effect on weight

Aqueous and ethanolic extracts did not significantly affect weight gain in rats fed during the 28 day period.

Effect on hematological parameters

The results of the blood count show that at doses of 3000, 4000 and 5000 mg / kg bw, ethanolic and aqueous extracts lead to a significant increase ($P < 0.05$) in white blood cells, platelets and total lymphocyte levels in treated animals compared to control animals. However, no significant variation was observed for red blood cells (RBCs) and afferents (Hb, HCTE, VGM, and MCHC) (Tables 1 and 2).

Effect on biochemical parameters

The results indicate that aqueous and ethanolic extracts do not significantly influence most biochemical parameters except creatinine and urea. In fact, at doses ranging from 2000 to 5000 mg/kg bw, these extracts significantly modify the concentrations of urea and creatinine in the blood. At the dose of 5000 mg / kg bw with the ethanolic extract, the concentrations rose from 0.33 g/l for urea to 1.67 g/l, respectively. The concentration of creatinine has also increased from 21.90 g/l to 51.68 g/l. In contrast, with the aqueous extract at this same dose (5000 mg/kg bw), the concentrations of urea and creatinine were respectively 1.10 g/l and 48.58 g/l (Tables 3 and 4).

Discussion

The acute toxicity study revealed that gavage administration of aqueous and ethanolic extracts resulted in no mortality in animals up to the limit dose of 5000 mg/kg bw. Studies of *Parkia biglobosa* bark extracts yielded similar results (Builders *et al.*, 2012). According to OECD test Guideline 423, the aqueous and ethanol extracts of fermented seeds of *P. biglobosa* have a lethal dose of 50 (LD_{50}) greater than 5000 mg/kg bw. The aqueous and ethanolic extracts of this condiment are therefore classified as non-toxic substances orally according to the classification of Hodge and Sterner (1943). This method does not allow a precise value of the LD_{50} to be defined, but it serves as a suggestion for the classification of the crude extract on the basis of the prediction of the dose at which the animals must survive (Roopashree *et al.*, 2009). Clinical signs of feverishness observed at high doses of the extracts during acute toxicity are not due to toxicity of the extracts but rather signs related to hypotension. According to the study by Coulibaly et al (2017), the fermented seeds of *P. biglobosa* have a hypotensive effect. Hypotension would therefore be responsible for elevating serum urea and creatinine levels at high doses of the extracts in the subacute toxicity study. In fact, creatinine and urea are essentially eliminated from the blood by glomerular filtration, which is itself dependent on the arterial pressure in the capillaries. A decrease in this pressure of 10 mmHg causes a significant decrease in plasma volume by the glomerulus, and therefore a decrease in glomerular filtration (Doumbia *et al.*, 2007).

The subacute toxicity study revealed that hepatic function was well preserved during the 28 days of oral administration of the extracts, indicated by serum AST and ALT levels that are comparable to the control values. These enzymes are the markers of the liver, their activities increase in cases of hepatic toxicity (Rahman *et al.*, 2001 ; Hilaly *et al.*, 2004). Also, the determination of the plasma total protein level can provide information on the capacity of synthesis of the liver (Rasekh *et al.*, 2008). Thus, plasma protein levels unchanged in the treated groups compared to the control group therefore suggest an absence of any abnormality in the liver's synthetic capacity.

The evaluation of the hematological parameters showed that the extracts at the different doses had no significant effect on the red blood cells and the afferent indices (Hb, MCHC, MCV and HCTE) during the experimental period, which indicates that There was no destruction, no problems with maturity, and no change in red blood cell production rate (erythropoiesis) (Udut *et al.*, 2005). It also shows that the extracts do not have the potential to stimulate erythropoiesis which is the humoral regulator of red blood cell production (Sanchez-Elsner *et al.*, 2004). However, the extracts at doses of 3000 to 5000 mg/kg bw had significant effects on the numbers of white blood cells, blood platelets and the lymphocyte count while there were no infections.

These extracts therefore seem to have the possibility of stimulating the immune system. Studies by Yapo et al., (2011) also showed the immunostimulatory

effect of aqueous extracts of *P. biglobosa* leaves. The extracts tested also have analgesic effects because the animals manage to withstand hypotension despite the high doses administered and do not die. Kouadio et al. (2000) also showed the analgesic and anti-inflammatory effects of alcoholic extracts of *P. biglobosa*.

Glucose levels did not change significantly during treatment compared to the control group, suggesting that the extracts did not affect the blood glucose control system.

Also, the extracts did not significantly alter the serum values of HDL (high density lipoprotein), LDL (low density lipoprotein), total cholesterol and triglycerides. However, a slight decrease in LDL levels and a slight increase in HDL levels are observed compared to the control group. The increase in HDL, also known as good cholesterol, is a protective factor for the heart muscle (HAS, 2005b) but also has a beneficial effect against cardiovascular complications such as atherosclerosis. Néré seeds could therefore prevent cardiovascular complications.

Table 1 : Effect of ethanolic extract of *P. biglobosa* fermented seeds on hematological parameters in rat

Hematological parameters	Control	Eth 1000	Eth 2000	Eth 3000	Eth 4000	Eth 5000
WBC (x 10^3/µL)	8,715 ± 0,27	8,600 ± 0,07^a	8,945 ± 0,01^a	9,485 ± 0,02^b	9,840 ± 0,04^b	10,175 ± 0,05^b
RBC (x 10^6/µL)	7,375 ± 0,26	7,505 ± 0,28^a	7,37 ± 0,28^a	7,370 ± 0,08^a	7,255 ± 0,22^a	7,380 ± 0,21^a
PLTS (x 10^3/µL)	648,5 ± 10,50	683,0 ± 2,00^a	729,5 ± 3,00^b	768 ± 15,50^b	783,5 ± 18,50^b	791 ± 10,00^b
LYM (%)	12,34 ± 0,21	12,62 ± 0,04^a	12,99 ± 0,16^a	13,54 ± 0,02^b	13,86 ± 0,04^b	14,03 ± 0,08^b
MCV (Fl/cell)	52,64 ± 3,49	54,29 ± 4,17^a	53,52 ± 3,08^a	56,38 ± 3,38^a	56.32 ± 1,38^a	56 ,62 ± 0,49^a
HCTE (%)	28,23 ± 0,25	28,79 ± 0,56^a	29,47 ± 0,04^a	29,52± 0,02^a	29,81 ± 0,35^a	28,62 ± 0,05^a
Hb (g/dL)	13,39 ± 0,86	13,68 ± 0,56^a	14,91± 0,50^b	13,70 ± 0,22^a	14,29 ± 0,15^b	14 ,27 ± 0,56
MCHC (g/dL)	32,82 ± 0,67	32,66 ± 0,48^a	33,57 ± 0,25^a	33,55 ± 0,32^a	33,13 ± 0,30^a	33,65 ± 0,10^a

Data are expressed as mean ± SEM; (n = 3); a: non-significant difference (p> 0.05) compared to the rats of the control group and b: significant difference (p <0.05) compared to the rats of the control group. Comparisons are made on the lines. WBC: White blood cells (x10^3/µL); RBC: Red blood cells (x10^6/µL); PLTS: Platelets (x10^3/µL); LYM: Lymphocyte (%), MCV: Mean cell volume (FL/cell), HCTE: Hematocrit (%); Hb: Hemoglobin (g/dL), MCHC: Mean corpuscular hemoglobin concentration (g/dL).

Table 2 : Effect of aqueous extract of *P. biglobosa* fermented seeds on hematological parameters in rat

hematological parameters	Control	Aqx 1000	Aqx 2000	Aqx 3000	Aqx 4000	Aqx 5000
WBC (x 10^3/µL)	8,715 ± 0,27	8,505 ± 0,39^a	8,609 ± 0,33^a	9,301 ± 0,01^b	9,570 ± 0,04^b	9,221 ± 0,17^b
RBC (x 10^6/µL)	7,375 ± 0,26	7,41 ± 0,02^a	7,68 ± 0,07^a	7,60 ± 0,03^a	7,60 ± 0,21^a	7,66 ± 0,32^a
PLTS (x 10^3/µL)	648,5 ± 10,50	649,11 ± 6,5^a	667,01 ± 3,3^a	788,12 ± 4,9^b	761,13 ± 7,1^b	765,3 ± 4,5^b
LYM (%)	12,34 ± 0,21	12,55 ± 0,05^a	12,77 ± 0,27^a	12,93 ± 0,11^a	13,57 ± 0,08^b	13,38 ± 0,14^b
MCV (Fl/cell)	52,64 ± 3,49	57,66 ± 5,10^a	52,50 ± 4,21^a	58,01 ± 2,28^a	53,74 ± 5,77^a	55,88 ± 1,01^a
HCTE (%)	28,23 ± 0,25	29,71 ± 0,41^a	29,85 ± 0,52^a	29,55 ± 0,33^a	28,91 ± 0,35^a	29,13 ± 0,17^a
Hb (g/dL)	13,39 ± 0,86	13,57 ± 0,23^a	13,69 ± 0,78^a	13,42 ± 0,49^a	13,63 ± 0,37^a	13,58 ± 0,88^a
MCHC (g/dL)	32,82 ± 0,67	32,93 ± 0,18^a	32,73 ± 0,34^a	32,86 ± 0,74^a	32,89 ± 0,61^a	33,29 ± 0,39^a

Data are expressed as mean ± SEM; (n = 3); a: non-significant difference (p> 0.05) compared to the rats of the control group and b: significant difference (p <0.05) compared to the rats of the control group. Comparisons are made on the lines. WBC: White blood cells (x10^3/µL); RBC: Red blood cells (x10^6/µL); PLTS: Platelets (x10^3/µL); LYM: Lymphocyte (%), MCV: Mean cell volume (FL/cell), HCTE: Hematocrit (%); Hb: Hemoglobin (g/dL), MCHC: Mean corpuscular hemoglobin concentration (g/dL).

Table 3 : Effect of ethanolic extract of *P. biglobosa* fermented seeds on biochemical parameters in rat

biochemical parameters	Control	Eth 1000	Eth 2000	Eth 3000	Eth 4000	Eth 5000
UREA (g/L)	0,3300 ± 0,04	0,3400 ± 0,05^a	0,60 ± 0,14^b	1,01 ± 0,02^b	1,20 ± 0,05^b	1,67 ± 0,01^b
CREATININE (g/L)	21,90 ± 0,25	23,11 ± 0,48^a	27,96 ±0,74^b	46,25 ± 0,18^b	49,85 ± 0,28^b	51,68 ± 0,99^b
GLY (g/L)	0,6650 ± 0,02	0,6800 ± 0,01^a	0,690 ± 0,03^a	0,70 ± 0,02^a	0,690 ± 0,010^a	0,665 ± 0,01^a
LDH (UI/L)	140,0 ± 1,00	138,5 ± 0,50^a	139,0 ± 1,50^a	139,5 ± 0,7^a	137,5 ± 0,7^a	137,0 ± 1,50^a
HDL (mmol/L)	1,490 ± 0,030	1,505 ± 0,13^a	1,498± 0,01^a	1,570 ± 0,04^a	1,533 ± 0,02^a	1,520 ± 0,01^a
LDL (mmol/L)	2,160 ± 0,02	2,145 ± 0,02^a	2,165 ± 0,02^a	2,155 ± 0,02^a	2,180 ± 0,01^a	2,165 ± 0,03^a
CHOL (mmol/L)	2,555 ± 0,03	2,495 ± 0,09^a	2,635 ± 0,21^a	2,595 ± 0,02^a	2,525 ± 0,25^a	2,525 ± 0,02^a
TG (mmol/L)	1,140 ± 0,02	1,125 ± 0,02^a	1,175± 0,01^a	1,150 ± 0,04^a	1,185 ± 0,01^a	1,165 ± 0,02^a
ALT (U/L)	59,84 ± 2,11	61,67 ± 2,16^a	62,20 ± 2,93^a	57,73 ± 4,27^a	62,58 ± 4,44^a	60,85 ± 5,12^a
AST (U/L)	193,43 ± 4,33	197,68 ± 3,13^a	193,34 ± 5,10^a	194,25 ± 5,21^a	195,11 ± 7,14^a	196,25 ± 5,29^a
TP (g/L)	68,55 ± 1,21	68,67 ± 1,32^a	69,02 ± 1,23^a	67,79 ± 1,87^a	69,35 ± 2,25^a	67,88 ± 2,01^a

Data are expressed as mean ± SEM; (n = 3); (a: non-significant difference (p> 0.05) compared to the rats of the control group and b: significant difference (p> 0.05) compared to the control group). Comparisons are made on the lines. GLY : Blood Glucose (g/L), LDH: Lactate dehydrogenase (mmol/L); HDL: High density lipoproteins

(mmol/L); LDL: Low density lipoproteins (mmol/L); CHOL : Cholesterol (mmol/L), TG: Triglycerides (mmol/l), ALT: Alanine transaminase (U/L); AST: aspartate transaminase (U/L); TP : Total protein (g/L).

Table 4 : Effect of aqueous extract of *P. biglobosa* fermented seeds on biochemical parameters in rat

biochemical parameters	Control	Aqx 1000	Aqx 2000	Aqx 3000	Aqx 4000	Aqx 5000
UREA (g/L)	0,3300 ± 0,04	0,3500 ± 0,07^a	0,4900 ± 0,21^b	0,6200 ± 0,0^b	0,9500 ± 0,33^b	1,10 ± 0,17^b
CREATININE (g/L)	21,90 ± 0,25	22,13 ± 0,45^a	23,59 ± 0,27^a	39,55 ± 0,31^b	43,87 ± 0,52^b	48,58 ± 0,18^b
GLY (g/L)	0,6650 ± 0,02	0,680 ± 0,03^a	0,6667 ± 0,04^a	0,6803 ± 0,05^a	0,6807 ± 0,07^a	0,6803 ± 0,03^a
LDH (UI/L)	140,0 ± 1,00	138,7 ± 0,60^a	139,2 ± 1,25^a	138,5 ± 0,2^a	139,5 ± 0,6^a	138,6 ± 0,3^a
HDL (mmol/L)	1,490 ± 0,030	1,523 ± 0,22^a	1,505 ± 0,53^a	1,568 ± 0,22^a	1,517 ± 0,51^a	1,511 ± 0,29^a
LDL (mmol/L)	2,160 ± 0,02	2,153 ± 0,05^a	2,158 ± 0,06^a	2,155 ± 0,03^a	2,162 ± 0,7^a	2,159 ± 0,06^a
CHOL (mmol/L)	2,555 ± 0,03	2,498 ± 0,05^a	2,502 ± 0,07^a	2,591 ± 0,09^a	2,488 ± 0,04^a	2,503 ± 0,03^a
TG (mmol/L)	1,140 ± 0,02	1,135 ± 0,03^a	1,135 ± 0,02^a	1,145 ± 0,06^a	1,147 ± 0,05^a	1,147 ± 0,07^a
ALT (U/L)	59,84 ± 2,11	58,85 ± 3,16^a	60,20 ± 1,34^a	59,65 ± 1,55^a	61,45 ± 2,04^a	68,66 ± 2,23^a
AST (U/L)	193,43 ± 4,33	194,33 ± 6,32^a	197,87 ± 4,75^a	195,15 ± 3,62^a	198,53 ± 2,13^a	196,91 ± 7,23^a
TP (g/L)	68,55 ± 1,21	69,45 ± 1,72^a	67,89 ± 2,38^a	69,33 ± 1,12^a	69,26 ± 1,66^a	68,90 ± 1,59^a

Data are expressed as mean ± SEM; (n = 3); (a: non-significant difference (p> 0.05) compared to the rats of the control group and b: significant difference (p> 0.05) compared to the control group). Comparisons are made on the lines. GLY : Blood Glucose (g/L), LDH: Lactate dehydrogenase (mmol/L); HDL: High density lipoproteins (mmol/L); LDL: Low density lipoproteins (mmol/L); CHOL : Cholesterol (mmol/L), TG: Triglycerides (mmol/l), ALT: Alanine transaminase (U/L); AST: aspartate transaminase (U/L); TP : Total protein (g/L).

Conclusion

Acute and subacute toxicity tests of aqueous and ethanolic extracts of *Parkia biglobosa* fermented seeds by the oral route showed no toxic effect on animals at the tested doses. Indeed, no case of poisoning is known for this condiment from consumers. This result seems to be in favor of its use in the treatment of high blood pressure. Also, this condiment has an immunostimulant and analgesic effect, which could be beneficial for consumers.

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