International Journal of Health Sciences
Available online at www.sciencescholar.us
E-ISSN: 2550-696X
Int. J. of Health Sci.
DOI: 10.53730

How to Cite:
Ogbuabor, A. O., Igwe, M. C., & Aniagolu, M. O. (2025). Profile of complete blood count of rats treated with extract of roasted dioscorea rotundata poir (white yam) tuber bark flake. *International Journal of Health Sciences*, *9*(S1), 708–719. https://doi.org/10.53730/ijhs.v9nS1.15844

Profile of complete blood count of rats treated with extract of roasted dioscorea rotundata poir (white yam) tuber bark flake

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***Abstract*---**Roasting of yam (*dioscorea rotundata poir*) tuber is an ancient practice in many localities. Although roasted yam bark flake has been associated with potential health risks, roasting of yam remains common among many communities. This study was conducted to investigate the effect of extract of roasted yam bark flake on the complete blood count of treated rats. 150mg/kg body weight of extracts of roasted yam (dioscoR) and boiled yam (dioscoB) were administered to the rats for 21 days. The complete blood counts were determined using hematology analyzer (Mindray 530 BC, China). Data was analyzed using statistical package for social science version 20.0 (IBM statistics Armok, NY, USA) and presented as mean±SD with $p<0.05$ considered significant. Rats treated with dioscoR revealed significant decrease in the complete blood count parameters involving the red blood cell, hemoglobin, hematocrit, mean cell volume, mean cell hemoglobin, mean cell hemoglobin concentration, lymphocyte count but an increase in the red blood cell distribution width, white

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Submitted: 27 September 2025, Revised: 09 November 2025, Accepted: 18 December 2025

blood cell, neutrophil, eosinophil, monocyte, basophil, platelet, plateletcrit, mean platelet volume, platelet distribution width and the platelet large cell ratio compared to the controls. This finding suggests that the consumption of roasted yam bark flake may pose a toxicological risk to health.

***Keywords*---**Complete blood count, Platelet Parameters, Red Blood Cell, Roasted yam, White Blood Cell.

1. Introduction

Dioscorea rotundata poir (White Yam) tuber is a major stable food in the tropics and sub-tropics (Lasekan & Teoh, 2019). It is commonly consumed in different forms including roasted, fried, boiled or pounded into dough after boiling (Adegunwa et al, 2011; Lasekan & Teoh, 2019; Quintana et al, 2023). Roasted yam is popular among many regions due to its characteristics toasting aroma that aids its palatability (Lasekan & Teoh, 2019). Roasting of yam is achieved by heating the yam tuber in an open flame with constant turning until it turns into a uniform dark brown cake (bark flake) with characteristics aroma (Olayaki et al, 2007; Lasekan & Teoh, 2019). Studies have reported significant changes in the nutrient and anti-nutrient compositions of *dioscorea rotundata poir* tuber due to roasting as well as its potential health risks (Adegunwa et al, 2011; Aderibigbe et al, 2017; Lasekan & Teoh, 2019; Adeniji et al, 2020; Nweze et al, 2024).

The complete blood count (CBC) also referred to full blood count (FBC) or haemogram is a routine diagnostic test which provides an overview of an individual's health status (Farkas, 2020; Erhabor et al, 2021; Liu et al, 2023; Bagudo & Abdulrahman, 2024). It evaluates the total numbers and morphological characteristics of the three cellular subsets in the blood namely red blood cells (erythrocytes), white blood cells (leukocytes or leucocytes) and platelets (thrombocytes) (May et al, 2019; Seo & Leo, 2022; Huang et al, 2024).

Previous study had reported the effect of roasted *dioscorea rotundata poir* tuber extract on the red blood cell parameters without reporting its effect on the other subsets of the complete blood count such as the white blood cell and platelet parameters. The present study was therefore designed to investigate the effect of *dioscorea rotundata poir* tuber bark flake extract on the complete blood count involving the red blood cell parameters, white blood cell and platelet parameters which is currently lacking in literature

2. Materials and Methods

2.1 Ethical Clearance

Ethical clearance for the study was obtained from the Animal Research Ethics Committee of the Faculty of Allied Health Sciences, Enugu State University of Science and Technology with assigned number FAHS/EC/2025/001

2.2 Plant Material
Healthy *dioscorea rotundata poir* tubers in their maximum physiological development stage (10-11months) corresponding to the harvest period were purchased from local farmers at Eke Agbani, Nkanu West Local Government Area, Enugu State, Nigeria.

2.3 Preparation of Yam Bark flaked Extracts
Both the ethanolic extracts of boiled *dioscorea rotundata poir* tuber (DioscoB) and roasted dioscorea rotundata poir tuber bark flake (DioscoR) was prepared by the crude extraction method.

2.3.1 DioscoB Extract: The tubers were peeled, cut into small portions, boiled in clean water in an aluminium pot. They were air-dried and blended into powder with an electronic blender. 500g of the powder was suspended in 3500 ml of ethanol at room temperature. The mixture was sieved through a Whatman No.1 filter paper and the filtrate was poured into a wide container for total evaporation to dryness to get a dried solid product (crude extract). 10g of the crude extract was reconstituted with 100ml of distilled water and the concentration was stored in aliquot bottles at 4^{o}C for use.
2.3.2 DioscoR Extract: The tubers were cut into small portions without peeling. They were roasted until the back turned into a characteristic brown cake (bark flake) with crack-like aroma and then ground into powder with an electronic blender. 500g of the powdered bark flake was suspended in 3500 ml of ethanol at room temperature. The mixture was sieved through a Whatman No.1 filter paper and the filtrate was poured into a wide container for total evaporation to dryness to get a dried solid product (crude extract). 10g of the crude extract was reconstituted with 100ml of distilled water and the concentration was stored in aliquot bottles at 4^{o}C for use.
2.3.3 Determination of Dose for Extract Administration
The administered dose was calculated from the stock and given as milligram per kilogram body weight using the relation (Oboma et al, 2025): RV/O where R = required concentration, V = required volume and O = original concentration. Based on 10g/100ml of stock solution of the extract and weight of 160g for each rat, 2.4ml (150mg/kg) of the extract was administered to the test rats.

2.4 Phytochemical Screening
Both the qualitative and quantitative tests were carried for the phytochemical screening of the extracts.

2.4.1 Qualitative Tests
The presence of different secondary metabolites in each of the extract was determined by appropriate biochemical tests (Sheikh et al, 2013).
Detection of flavonoids (lead acetate test): One gram (1g) of the test extract was treated with few five drops of lead acetate solution. Formation of yellow color precipitate indicates the presence of flavonoids.
Detection of alkaloids (Wagner's test): One gram (1g) of test extract was dissolved in dilute hydrochloric acid and filtered. The filtrate was treated with Wagner's reagent (iodine in potassium iodide). Formation of brown/reddish precipitate indicates the presence of alkaloids.

Detection of Saponins (froth test): One gram (1g) of test extract was diluted with distilled water to 20ml and was followed with shaking in a graduated cylinder for 15minutes. Formation of 1cm layer of foam indicates the presence of saponin.
Detection of tannins (ferric chloride test): Three milliliters of distilled water and five drops of ferric chloride solution were added to three milliliters of the aqueous extract. The formation of green color precipitate indicates the presence of tannins.
Detection of steroids (salkowaski's test): One milligram (1mg) of the crude extract was put in a test tube and dissolved with 10ml chloroform and then equal volume of concentrated sulphuric acid was added to the test tube by sides. The turning of the upper layer in the test tube into red and sulphuric acid layer into yellow with green fluorescence shows the presence of steroids.
Detection of terpenoids (Salkowski's test): Two milliliters (2ml) of chloroform was added to 5ml of extract and mixed with three milliliters of sulphuric acid. Formation of reddish brown color indicates the presence of terpenoids.
Detection of phenols (ferric chloride test): One gram of test extract was treated with four drops of ferric chloride solution. Formation of bluish black color indicates the presence of phenols.

2.4.2 Quantitative Tests
The quantitative values of the bioactive substances were determined by measurement of the color development for the different tests using a spectrophotometer (Multiskan FC; Thermo Fischer, Scientific, USA) (Oladeji et al, 2014).
Detection of total flavonoids: The total flavonoids content of the test extract was determined by using aluminum chloride solution. The test extract was mixed with distilled water and sodium nitride solution. After 6 minutes, aluminum chloride solution was added and allowed to stand for 6minutes. Sodium hydroxide solution was added to the mixture. Immediately distilled water was added to bring it to the final volume and the mixture was extensively mixed and allowed to stand for another 15minutes. Optical density of the mixture was recorded at 510mm. Rutin was used as a standard compound for the evaluation of total flavonoids. The total flavonoids were calculated using the standard curve and expressed as rutin equivalent in mg/g of the test extract.

Detection of total alkaloids: Half milliliter (0.5ml) of the test extract, 5ml of phosphate buffer (PH 4.7) and 5ml of bromocresol green were added in a separatory funnel and it was shaken vigorously with 5ml of chloroform. The chloroform layer at bottom was carefully removed and its absorbance was measured at 470nm in the presence of blank without adding the extract. The alkaloid concentration was determined as atropine equivalents (mg of AE/g of test extract) with reference to the standard curve of atropine.

Detection of total saponin: The test extract was dissolved in 80% methanol, two milliliters of vanillin in ethanol was added mixed well and 2ml of 72% sulphuric acid solution was added, mixed well and heated on a water bath at 60% for 10minutes. Absorbance was measured at 544 nm against reagent blank. Diosgenin was used as a standard material and compared the assay with Diosgenin equivalents.

Detection of total tannins: Five hundred milligrams of the sample was weighed into a 50ml plastic bottle. 50ml of distilled water was added and shaken for 1hour in a mechanical shaker. This was filtered into a 50ml volumetric flask and made up to the mark with distilled water. Then 5ml of the filtrate was pipetted into a test tube and mixed with 2ml of 0.1M FeCl3 in 0.1HCL and 0.008M potassium ferrocyanide. Absorbance was measured at 120 mm within 10min.

Detection of total Steroids: To 1ml each of standard cycloartenol solutions, blank and test extract in separate 10ml volumetric flasks, was added 2ml of 4M sulphuric acid and then 2ml of 0.5%(v/v) of iron (111) chloride. This was followed by the addition of 0.5ml of 0.5ml (w/v) of potassium hexacyanoferrate (111) solution. After each addition, the contents of the various flasks were thoroughly shaken. The respective volumetric flasks with the mixture contained therein were subsequently heated for 30min in a thermostatic water bath maintained at 70^{o}C. During the heating stage, the flasks were shaken at intervals of 5min for 30secs. At the end of the heating period, the flasks and their respective contents were cooled at room temperature. Thereafter, the content of each flask was made up to 10ml mark with distilled water subsequently, absorbance reading was taken at 780nm using a spectrophotometer. The respective steroids content of each extract were calculated using value obtained by extrapolation from the standard graph of cycloartenol.

Detection of total terpenoids: The extract (1g) was macerated with 50ml of ethanol and filtered. To the filtrate, 2.5ml of 5% aqueous phosphomolybdic acid solution was added and 2.5ml of concentrated H2SO4 was gradually added and mixed. The mixture was left to stand for 30 minutes and then made up to 12.5ml with ethanol. The absorbance was measured at 700nm.

Detection of total phenols: Two hundred microliter of the test extract, 800ml of Folin -Ciocalteu reagent mixture and 2ml of 7.5% sodium carbonate were mixed. The total content was diluted to 7 volumes with distilled water. The tubes were kept for 2 hours and allowed to incubate in the dark. The absorbance was measured at 765 nm. Gallic acid dilutions were used as standard solutions. The results of phenols are expressed in terms of Gallic acid in mg/ml of extract.

2.5 Animal Handling

Wistar rats weighing 160-200g were used for the study. The animals were allowed for an acclimatization period of two weeks. They were housed in cages at room temperature of 25-28^{o}C and moisture control under a naturally illuminated environment of 12:12 hour's dark/light, fed on standard rat pellet and water ad libitium in line with the rules of the National Institute of Health Guide for the care and use of Laboratory Animals.

2.6 Acute Toxicity Testing

The Lorke technique was used to determine the LD50 of the extracts (Lorke, 1983). This was conducted in two phases using 24 rats (12 rats for each extract). In the first phase, 3 groups of 3 rats in each cage were administered 100,500 and 1000mg/kg of the aqueous extracts orally. Rats were observed for signs of

toxicity and mortality within 24 hours with particular attention during the first 4 hours of the experiment. The second phase was followed in similar conditions by the administration of 2000, 3000 and 5000mg/kg to the next 3 groups of one rat in each cage to detect the signs of toxicity and mortality during 24 and 72 hours respectively.

2.7 Experimental Protocol

We adopted the post-test random protocol. Animals were randomly distributed into three groups each consisting of five rats. Group 1 served as the control which were fed with only water and rat pellet, Group 2 was fed with 2.4ml of the DioscoB extract while Group 3 were fed with 2.4ml of the DioscoR extract by oral gavage daily for 21days. Animals were fast overnight on the day 21 and blood samples collected via the inferior vena cava under chloroform anesthesia into ethylene diamine tetra acetic acid tubes for determination of the complete blood count.

2.8 Determination of the Complete Blood Count

The complete blood count was determined using the automated hematology analyzer (Mindray, 530 BC, China). The machine was allowed to boot for 30 minutes while the blood samples were gently mixed using a vortex mixer and aspirated by the machine by letting the machine sample probe into the blood sample bottle and pressing the probe button. Approximately 20ul of the sample was aspirated by the machine. The values of the complete blood parameters involving the red blood cell count, hemoglobin, hematocrit, mean cell volume, mean cell hemoglobin, mean cell hemoglobin concentration were displayed in the screen after 30 seconds and print out copy of the results released immediately on the thermal printing paper.

2.9 Data Analysis

The data obtained was analyzed using statistical package for social sciences (SPSS) version 20.0 and all data were expressed as mean ± deviation (SD). Comparisons were determined using t-test, one way analysis of variance (ANOVA) and Bonferonni multiple Post hoc test. Difference between groups were considered significant at p- value less than or equal to 0.05 ($p<0.05$).

3. Results and Discussion

Our health and well-being depend on the food we eat as most diseases could be prevented with dietary supplements/proper nutrition while some diseases could develop as a consequence of bad diets (Adeniji et al, 2020). The present study was designed to investigate the effect of extract of roasted *dioscorea rotundata poir* tuber extract on the complete blood count of treated rats.

3.1 Acute Toxicity Effect of the Extracts

The acute toxicity test carried out on both the extract of boiled *dioscorea rotundata poir* tuber (dioscoB) and extract of roasted *dioscorea rotundata poir*

tuber bark flake (dioscoR) did not yield any signs of toxicity in the rats up to a dose of 5000mg/kg after 24 hours (Tables 1 and 2). We therefore, adopted an LD50 of more than 5000mg/kg body weight. This shows that both dioscoB and dioscoR extracts may be relatively safe at high doses.

Table 1: The 24-hour Acute Toxicity Test (LD50) of low doses of the extracts (phase 1)

Extracts	Concentration (mg/kg body weight)	Mortality/number of rats
DioscoB	100	0/3
	500	0/3
	1000	0/3
DioscoR	100	0/3
	500	0/3
	1000	0/3

Number of deaths per group = 0, Number of rats per group = 3, DioscoB- extract of boiled *discorea rotundata* tuber, DioscoR- extract of roasted *dioscorea rotundata* tuber

Table 2: The 24-hour Acute Toxicity Test (LD50) of high doses of the extracts (phase 2)

Extracts	Concentration (mg/kg body weight)	Mortality/number of rats
DioscoB	2000	0/1
	3000	0/1
	5000	0/1
DioscoR	2000	0/1
	3000	0/1
	5000	0/1

Number of deaths per group = 0, Number of rats per group = 3, DioscoB- extract of boiled *discorea rotundata* tuber, DioscoR- extract of roasted *dioscorea rotundata* tuber bark flake

3.2 Qualitative and Quantitative Phytochemical Composition of the Extracts

The extracts revealed presence of bioactive substances including phenols, tannins, saponins and alkaloids but an absence of flavonoids, terpenoids and glycosides (Table 3). The mean values of the secondary metabolites for the DioscoB extract were significantly higher compared to those of the DioscoR extract (Table 5). This finding agrees with the findings of similar studies which reported that raosting of yam tubers significantly reduces its nutrient contents (Adegunwa et al, 2011; Ukom et al, 2014).

Table 3: The Phytochemical Composition of the Extracts (Qualitative Test)

Constituents	DioscoB	DioscoR
Phenol	+++	+
Tannins	+++	+
Saponins	+++	+
Alkaloids	+++	+
Flavonoids	-	-
Terpenoids	-	-
Glycosides	-	-

Key**:** (-) absent, (+) present, (++) moderately present, (+++) abundantly present, DioscoB- extract of boiled *dioscorea rotundata poir* tuber, DioscoR- extract of roasted *dioscorea rotundata poir* tuber bark flake

Table 4: The Phytochemical Composition of the Extracts (Quantitative Test)

Constituents	DioscoB	DioscoR	t-value	p-value
Phenol	14.3 ± 0.8	2.1 ± 0.5	2.064	0.002*
Tannins	10.9 ± 0.2	1.6 ± 0.7	0.520	0.007*
Saponins	5.2 ± 1.6	0.8 ± 0.1	0.816	0.001*
Alkaloids	11.6 ± 2.0	2.7 ± 1.3	1.649	0.010*
Flavonoids	-			
Terpenoids	-			
Glycosides	-			

Data are presented as mean ± SE, values represent a triplicate measurement, $p<0.05$ was considered significant*, DioscoB-extract of boiled *dioscorea rotundata poir* tuber, DioscoR- extracts of roasted *discorea rotundata poir* tuber bark flake

3.3 Effects of the Extracts on the Red Blood Cell Parameters

Table 5 shows the effect of the extracts on the red blood cell parameters involving the red blood cell count, hemoglobin, hematocrit, red blood cell distribution width, mean cell volume, mean cell hemoglobin and mean cell hemoglobin concentration. The rats treated with the extract of roasted *dioscorea rotundata poir* tuber bark flake (DioscR) revealed significant decrease ($p<0.05$) in the parameters compared to those treated with the extract of boiled *dioscorea rotundata poir* tuber (DioscB) and the controls. This finding agrees with the findings of a similar study which reported a significant decrease in the red blood cell count, hemoglobin and hematocrits of rats treated with the extracts of roasted *dioscorea rotundata poir* tuber bark flake compared to those treated with the extracts of boiled *dioscorea rotundata poir* tuber (Olayaki et al, 2007). This may be attributed to the decreased content of the bioactive nutrients such as phenols, tannins, saponins and alkaloids in the DioscoR as reported in Table 4. A change in the values of red blood cell parameters gives diagnostic insights into many diseases (Barve et al, 2015).

Table 5: Effect of the Extract on the Red Blood Cell Parameters

Parameters	Group 1 (Control)	Group 2 (150mg/kg of DioscoB)	Group 3 (150mg/kg of DioscoR)	f-value	t-value
RBC ($x10^{12}$/l)	5.80 ± 0.21	6.72 ± 0.34	2.3 ± 0.29	0.713	0.004*
HGB (g/dl)	12.22 ± 1.10	13. 40 ± 0.86	6.6 ± 2.9	0.303	0.001*
HCT (%)	36.18 ± 2.4	39.11 ± 0.55	31.26±1.74	1.296	0.002*
RDW (%)	14.50 ± 1.82	14.23 ± 0.69	23.75±2.30	0.273	0.007*
MCV (fl)	58.18 ± 2.40	61.10 ± 1.20	46.03±2.78	0.644	0.003*
MCH (pg)	22.13±0.56	21.90 ± 1.77	17.12±0.20	0.594	0.001*
MCHC(g/dl)	34.09 ± 2.07	36.12 ± 1.24	22.15±1.97	5.119	0.003*

Data are presented as mean ± SD, $p<0.05$ was considered significant*, RBC- red blood cell, HGB- hemoglobin, HCT- hematocrit, RDW- red blood cell distribution width, MCV- mean cell volume, MCH- mean cell hemoglobin, MCHC- mean cell hemoglobin concentration, DioscoB- extract of boiled *dioscorea rotundata poir* tuber, DioscoR- extract of roasted *dioscorea rotundata poir* tuber

3.4 Effect of the Extracts on the White Blood Cell Parameters

Table 6 shows the effect of the extracts on the white blood cell parameters involving the white blood cell count, neutrophil, lymphocyte, eosinophil, monocyte and basophil counts. The rats treated with the extract of roasted *dioscorea rotundata poir* tuber (DioscR) revealed significant increase ($p<0.05$) in the white blood cell parameters with the exception of the lymphocyte which revealed a significant decrease compared to those treated with the extract of boiled *dioscorea rotundata* poir tuber (DioscB) and the controls. Abnormal numbers of white blood cell parameters signal an immune reaction to the diet which may precede the development of a myriad of diseases (Sorsa, 2018; Famous et al, 2022; Tara et al, 2022; Sul et al, 2024).

Table 6: Effect of the Extracts on the White Blood Cell Parameters

Parameters	Group 1 (Control)	Group 2 (150mg/kg of DioscoB)	Group 3 (150mg/kg of DioscoR)	f-value	t-value
WBC **($x10^9$/l)**	6.2 ± 0.9	5.8 ± 1.5	11.9 ± 2.3	0.084	0.001*
Neutrophil (%)	51.4 ± 2.7	52.7 ± 1.9	71.05 ± 1.3	0.106	0.008*
Lymphocyte (%)	38.6 ± 0.1	38.24±0.4	20.93 ± 2.2	1.482	0.000*
Eosinophil (%)	0.1 ± 1.1	0.2 ± 0.4	3.30 ± 1.6	0.051	0.009*
Monocyte (%)	0.1 ± 0.6	0.3 ± 0.1	2.1 ± 0.8	0.419	0.010*
Basophil (%)	0.2 ± 0.1	0.7 ± 0.4	1.6 ± 0.1	0.630	0.001*

Data are presented as mean ± SD, $p<0.05$ was considered significant*, WBC- white blood cell count, DioscoB- extract of boiled *dioscorea rotundata poir* tuber, DioscoR- extract of roasted *dioscorea rotundata poir* tuber bark flake

3.5 Effect of the Extracts on the Platelet Parameters

Table 7 shows the effect of the extracts on the platelet parameters involving the platelet count, mean platelet volume, platelet distribution width, plateletcrit and the platelet large cell ratio. The rats treated with the extract of roasted *dioscorea rotundata poir* tuber bark flake (DioscR) revealed significant increase ($p<0.05$) in the parameters compared to those treated with the extract of boiled *dioscorea rotundata poir* tuber (DioscB) and the controls. Abnormal numbers of platelet parameters give insight on an individual's health by characterizing diseases associated with thrombocytopenia (decrease in platelet parameters) and thrombocytosis (increased in platelet parameters) (Wongsaengsak et al,2019, Yoon et al, 2024). The increase in the platelet parameters for the rats treated with the extracts of *dioscorea rotundata poir* tuber bark flake may be a reflection of a systemic insult.

Table 7: Effect of the Extracts on the Platelet Parameters

Parameters	Group 1 (Control)	Group 2 (150mg/kg of DioscoB)	Group 3 (150mg/kg of DioscoR)	f-value	t-value
PLT ($x10^9$/l)	2.56 ± 18.4	2.52 ± 6.9	311 ± 10.7	2.080	0.003*
MPV (fl)	7.12 ± 0.36	7.70 ± 0.21	13.9 ± 0.44	0.988	0.001*
PDW (fl)	8.00 ± 0.5	8.24 ± 1.30	15.02 ± 2.60	0.117	0.002*
PCT (%)	0.30 ± 0.1	0.28 ± 0.1	1.32 ± 0.84	1.149	0.000*
P-LCR (%)	9.66 ± 2.4	10.04 ±3.20	17.11 ± 1.90	1.825	0.002*

Data are presented as mean ± SD, $p<0.05$ was considered significant*, PLT – platelet count, MPV – mean platelet volume, PDW – platelet distribution width, PCT – plateletcrit, P-LCR – platelet large cell ratio, DioscoB- extract of boiled *dioscorea rotundata poir* tuber, DioscoR- extracts of roastedd *dioscorea rotundata poir* tuber bark flake

4. Conclusion

The results of the present study revealed significant alterations in the red blood cell, white blood cell and platelet parameters of rats treated with the extracts of roasted *dioscorea rotundata poir tuber* bark flake. This suggests that the consumption of roasted *dioscorea rotundata poir* tuber bark flake may pose toxicological risk to health.

Acknowledgments
The author wishes to acknowledge the research assistance provided by Dr. Mrs. Milicent Nkiruka Amadi

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