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EVALUATION OF THE TOXIC POTENTIAL OF GRAMOXONE HERBICIDE ON THE WEIGHT AND LIVER OF ALBINO RATS.

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ABSTRACT

This research was carried out to evaluate the effect of diluted gramoxone herbicide at a dilution factor of 10^4 in a 10-fold serial dilution in graded doses of 0.25, 0.5 and 0.75 mg/kg b.w on male and female rats. Forty male and female rats of weights 79g to 119g were divided into group A, B, C and D. Each group contains 5 males and 5 females. Control group A and 0.25,0.5 and 0.75 mg/kg b.w orally treated rats in group B, C, and D were fed with pelletized feeds and water *ad libitum* for 28 days respectively. The rats were allowed to starve a day and weighed prior to sacrifice. Blood samples were collected in EDTA bottles. Result showed a decrease in AST, ALT, ALP in all treated groups in male rats, a decrease in AST in all treated groups, in ALT in group B and C ($p>0.05$) but an increase in ALT in group D and an increase in ALP in all treated group in female rats ($p<0.05$) compared with control. TP showed a decrease in all treated group in male rats ($p<0.05$) measured with control. Albumin decreased/increased in group B/C in male rats, increased in group C and equal values in group B and D in male and female rats compared with control. There was an increased in weight in all treated groups and mild inflammatory effects on the liver tissue in group B and D. The results further registered gramoxone herbicide having paraquat as active ingredient toxic to the liver even at a much diluted state and at low doses.

Key words: gramoxone herbicide, liver enzyme, histopathology, paraquat , weight

INTRODUCTION

Gramoxone herbicide which has Paraquat (1,1-dimethyl-4-4-bipiridinium dichloride, PQ) as its active ingredient, is a non-selective quick-acting contact herbicide that is widely used in crop cultivation and conservation tillage systems worldwide (Richa *et. al.,* 2002). Paraquat has different trade names such as gramoxone, Crisquat, Dextrone X and Esgram in nearly 100 industrialized and developing countries in the world (Edo, 2022). The use of herbicide in farming operation improves yield of crops by killing the plants that compete with agricultural crops, forest and forage grasses for light and nutrient and these competitors are called weeds (Richard, 2007). As generally known, herbicide are used mostly by commercial farmers to enhance productivity and for easy way of controlling weeds. However, this is contrary to the reason the local peasant farmers use of herbicides in Okpe local government area and other peasant farmers in other local government area in Delta State, Nigeria. The use of herbicide generally by local farmers is to avoid the strenuous and energy consuming physical method of removing weeds from the farm with the use of hoes and cutlasses. Weeds are unwanted plants that compete with the needed crops for nutrients. The use of gramoxone herbicide especially by local farmers without person protective equipment (PPE) in any concentration may lead to liver diseases, which provokes great concern to human health (Kim *et al.,* 2019).

The mechanism of most hepatotoxicity-inducing pollutants is the formation of reactive free oxygen radicals (ROS) that cause oxidative stress and lipid peroxidation. A cumulative effect of these events became evident in the form of damage to the membrane of hepatocytes leading to

swelling, degeneration, necrosis, and fibrosis of hepatocytes (Chohan *et al.,* 2010; Mehmetçik *et al*.,2008)

The liver is the major organ of the body. Its functions include secretion of bile, metabolism of bilirubin, vascular and hematologic storage, metabolism of fat, protein, carbohydrate, storage of minerals and vitamins and detoxification of material. The liver also synthesizes nonessential amino acids and serum enzymes such as aspartate aminotransferase (ASP), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) (McCance and Huether,2002). Paraquat has high gastrointestinal absorption rate and very poisonous, it harms the liver, kidney, and the lungs by letting out free radicals. (Okolonkwo *et al.*2023.)

Several researches have published on the effect of gramoxone herbicide (paraquat as active ingredient) on hepatotoxicity (Han *et al.,* 2014; *Liu et al*., 2018), lung toxicity (Dinis-Oliveira *et. al*., 2008; Li *et al.,* 2017), nephrotoxicity (Kim *et al*., 2009), neurotoxicity (Baltazar *et al.*,2014,), immuno-toxicity (Khilji *et al.,* 2011), and reproductive toxicity (Debe *et al.,* 2007 andAkintunde and Abdulrahman 2019). Exposure to pesticides have been claimed to induce genotoxicity and oxidative stress in mammals (Morgan *et al.* 2019). The researcher deemed it fit to further evaluate how toxic gramoxne herbicide that paraquat is the active ingredient when diluted in 10-fold serial dilution, using a 10^4 dilution factor of the diluent and administered at minute doses of exposure.

MATERIALS AND METHODS

Experimental animals

All procedure adopted in the experiment in regard to animal handling complied with the International Guideline for the care and use of Laboratory Animals (IGCULA) as state by the Ethical Committee, Faculty of

Science University of Port Harcourt, River State.

A total of 40 male and female albino rats weighing 78 -119 were used for the experiment. The animals were procured in the animal house of the Department of Animal and Environmental Biology, University of Port Harcourt, River State under standard laboratory conditions (12 h light/dark cycle, temperature, humidity). The animals were acclimatized for one week because they were taken to a separate room from where they were purchased for the experiment. During this period the animals were fed with pelletized feeds and water ad libitum.

Serial dilution of the gramoxone herbicide

This is a series of dilutions usually twofold or tenfold used to determine the titer or concentration of a substance in solution as described by Eugene *et al* 2009). A tenfold serial dilution was carried out and dilution factor of 10^4 was selected for the treatment.

Experimental design

A total of Forty (40) male and female albino rats of variable weights 79-119g were randomly grouped into group A (control) and B, C and D which were the treated groups. Each group contained 10 rats (male and female rats. Group B (male and female) was administered 0.25mg/kg body weight dose, group C (male and female) was administered 0.5mg/kg body weight dose and group D (male and female) was administered 0.75mg/kg body weight dose by oral gavage respectively. The doses were administered for 28 days to the animals and the animals were starved for one day prior to their sacrificing.

At the end of the experiment after 28 days, the animals were sulphurated in a mild ether anesthesia. Blood samples were collected from the animals by sacrificing and the blood was dispersed into ethylene

diaminetetraacetic acid (EDTA) tubes for liver enzymes analysis. As for hepatocyte histological studies, the liver was preserved in 10% formadehyde.

Aspartate aminotransferase (AST) analysis

The analysis of Aspartate aminotransferase (AST) activity was carried out using the method of (Reitman and Frankel, 1957) with the commercially available test kit (Randox Laboratories Ltd, UK). 0.1 ml each of serum and distilled H_2O were delivered into test tubes labeled Sample and Blank respectively. In tube 1 and 2, 0.5 ml of AST reagent I was added, completely mixed and incubated at 37°C for 30 min. Then, 0.5 ml of AST reagent II was added to all test tubes, mixed and incubated at 25°C for 20 min. At the end, 5.0 ml NaOH was added to the test tubes and mixed. Absorbance was taken at 510 nm against reagent blank after 5 min.

Alanine aminotransferase (ALT) analysis

The method of Reitman and Frankel (1957) was employed using commercially available test kit (Randox Laboratories Ltd, UK). 0.1 ml of serum and distilled water (dH_2O) was delivered into test tubes labeled Sample and Blank respectively. Then, 0.5 ml of ALT reagent I was added into both tubes, mixed and incubated at 37°C for 30 min. Afterwards 0.5 ml of ALT reagent II was added to all test tubes, mixed and allowed to stand for 20 min at 25°C. Finally, 5.0 ml NaOH was added to the tubes, mixed and absorbance read against the reagent blank at 510 nm after 5 min and the activity calculated.

Alkaline phosphatase (ALP) analysis

ALP activity was assayed with the aid of a commercial test kit (Randox Laboratories Ltd, UK) according to the method described by Englehardt (1970). 0.01 ml sample was added into labelled test tube. Then, 0.50 ml ALP reagent (containing diethanolamine buffer, magnesium chloride and p-

nitrophenylphosphate) was added to the tube and stirred evenly. Absorbance was read against water blank at 405 nm and the activity determined.

Weight determination

The animals were weighed prior to the treatment and after the treatment to ascertain if there was change in weight. The initial weight (W1) and final weight (W2) and the difference- in- weight, DW3 were recorded using digital electrical weighing balance. For effective weighing the rats were anesthetized with chloroform before weighing.

Statistical analysis

The statistical analysis was done using ANOVA and student t-test of computer based excel package.

RESULTS

Biochemically, only aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) of the liver were examined. Total protein and albumin were also analysed.

Table 1. Male liver enzyme results given below

Group	AST µ/l	ALT µ/l	ALP µ/l	Total protein g/l	Albumin g/l
A (control)	100.00±2.42^{a}	33.40±6.92^{a}	48.20±2.88^{a}	95.00±1.78^{a}	37.20±0.76^{a}
B (0.25mg/kg)	88.80±7.75^{b}	23.40±3.35^{b}	41.80±1.93^{b}	86.00±2.28^{b}	35.20±0.96^{c}
C (0.5mg/kg)	64.20±1.77^{c}	13.40±0.97^{c}	41.20±2.51^{b}	86.00±4.25^{b}	38.60±0.92^{d}
D (0.75mg/kg)	82.00±4.78^{d}	11.00±0.71^{d}	29.80±3.10^{c}	88.40±5.00^{c}	37.20±0.37^{b}

Values are expressed as mean ± SEM of significance difference $p>0.05$

There was a general decrease of aspartate aminotransferase (AST) in all doses when compared with the control.

In Alanine aminotransferase (ALT), there was a significant decrease in all doses compared with the control.

Alkaline phosphatase (ALP) showed a decrease in value in doses 0.25mg/kg and 0.5mg/kg and 0.75mg/kg compared with the control.

Table 2. Female liver enzyme

Group	ASTµ/l	ALTµ/l	ALPµ/l	Total Protein g/l	Albumin g/l
A(control)	79.20±2.70[a]	11.00±0.77[a]	25.00±1.89[a]	88.40±1.80[a]	36.40±0.40[a]
B(0.25mg/kg)	69.00±6.53[b]	6.40±0.97[b]	27.60±2.37[b]	85.40±1.72[b]	36.80±0.80[b]
C(0.5mg/kg)	63.00±3.28[c]	8.00±1.26[c]	27.00±2.44[b]	83.60±1.63[c]	40.00±2.16[c]
D(0.75mg/kg)	72.00±4.53[d]	14.20±0.86[d]	36.40±4.74[c]	85.64±1.63[b]	36.00±0.31[b]

Values are expressed as mean ± SEM of significance difference p>0.05

The biochemical indices analyzed were aspartate aminotransferase (ASP), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) respectively. Also analyzed were total protein and albumin. There was a decrease in aspartate aminotransferase (AST) in all treated groups compared with the control. Alanine aminotransferase (ALT) experienced a significant increase in 0.75mg/kg dose and a decrease in 0.25mg/kg and 0.5mg/kg doses when compared with the control. Alkaline phosphatase (ALP) had an increase in

0.25mg/kg and 0.5mg/kg and 0.75mg/kg respectively compared with the control.

Table 3. Weight of rats before and after treatment (male)

Control group A male			Group B (0.25mg/kg)			Group C (0.5mg/kg)			Group D (0.75 mg/kg)		
W2	W1	DW	W2	W1	DW	W2	W1	DW	W2	W1	DW
164	116	48	160	102	58	162	100	62	134	96	38
213	78	135	161	103	58	157	102	55	126	95	31
119	75	44	136	90	46	145	92	53	132	91	41
136	79	57	127	78	49	158	90	68	138	82	56
166	100	66	169	89	80	140	86	54	142	102	40

Note: W2 =final weight, W1= initial weight and DW= difference in weight

The table above displayed the raw data of the weight of the male rats before and after treatment. The result showed that the weights were not affected by the different doses administered in the different treatment groups. There were great increase in weights of the entire animals both control and treated groups.

Table 4. Weight of rats before and after treatment (female)

Control group A	Group B (0.25mg/kg)	Group C (0.5mg/kg)	Group D (0.75mg/kg)

female											
W2	W1	DW	W2	W1	DW	W2	W1	DW	W2	W1	DW
139	95	44	123	83	40	157	116	41	148	93	55
138	97	41	143	98	45	137	92	45	150	94	56
160	83	77	116	75	41	108	78	30	151	103	48
143	97	46	147	81	66	132	88	44	150	82	68
183	119	64	190	100	90	142	109	33	176	106	70

Note: W2 =final weight, W1= initial weight and DW= difference in weight

The table below displayed the raw data of the weights of the female rats before and after treatment. The result showed that the weights were not affected by the different doses administered in the different treatment groups. There were great increase in weights of the entire animals both control and treated groups.

Histopathology of the Control and the Treated Male and Female Rats to Indicate Whether there are Inflammations on the Liver due to the Oral Exposure of the 10-Fold Serial Dilution of Gramoxone Herbicide.

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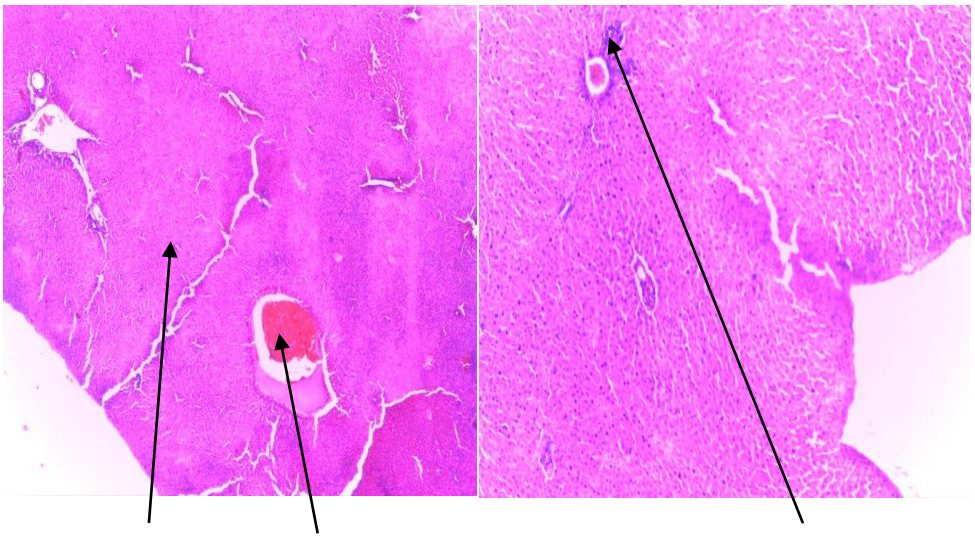

Plate 1. Male control H&E X100 control slide shows normal histology

Plate 2. Group B male H&E × 200

Histologic slide shows mild periportal

inflammation at 0.25mg/kg bw in group B

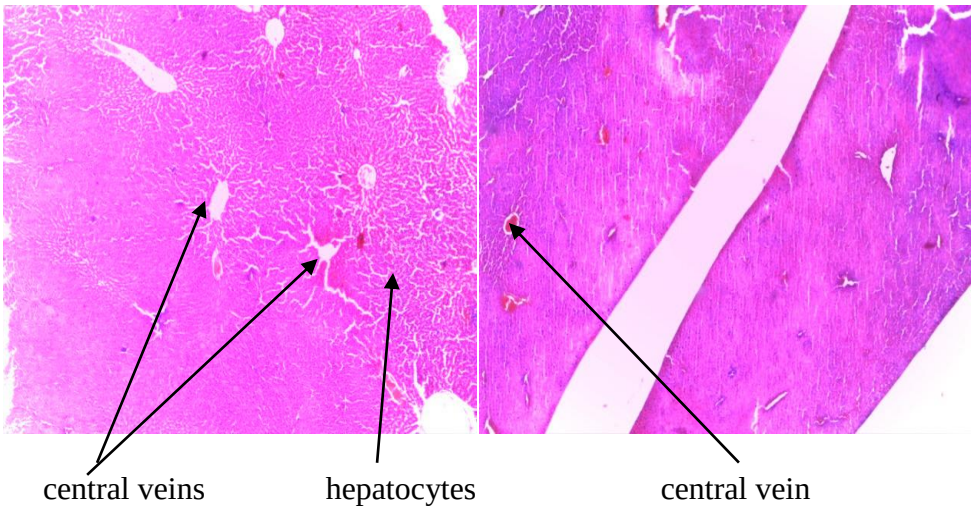

Plate 3: Group C male H&E × 200

Slide shows no obvious histologic change

at 0.5mg/kg bw in group C

Plate 4: Group D male H&E × 200

Slide shows no obvious histologic

change at 0.75mg/kg bw in group D

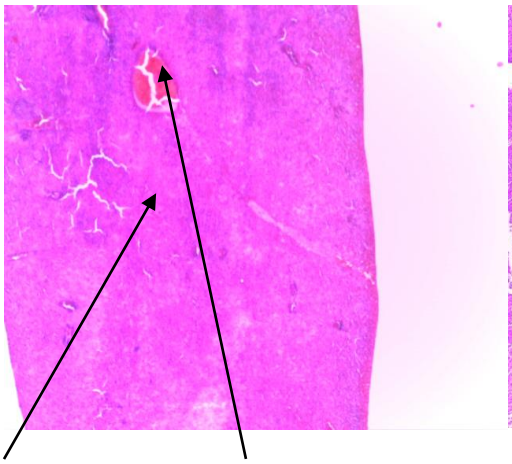

Plate 5. Female control group H&E X 100 Control slide shows normal histology of the liver tissue

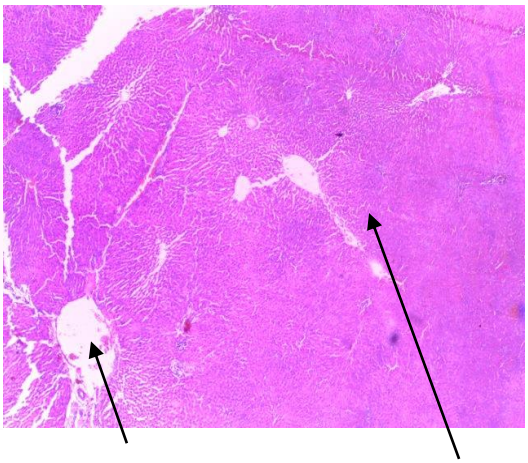

Plate 6: Group B female H&E X 200 Slide shows no obvious histologic change at 0.25mg/kg bw in group B

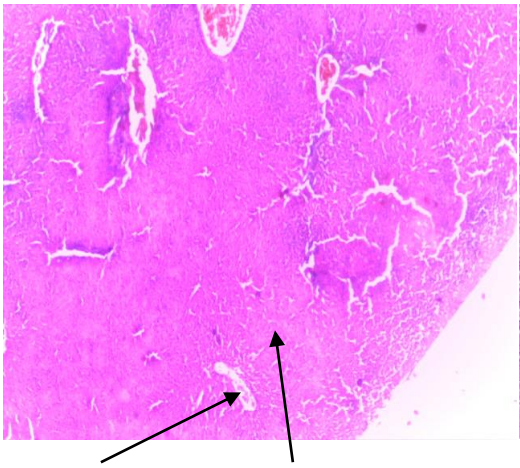

Plate 7: Group C female H&E X 200 Slide shows no obvious histologic change at 0.5 mg/kg bw in group C

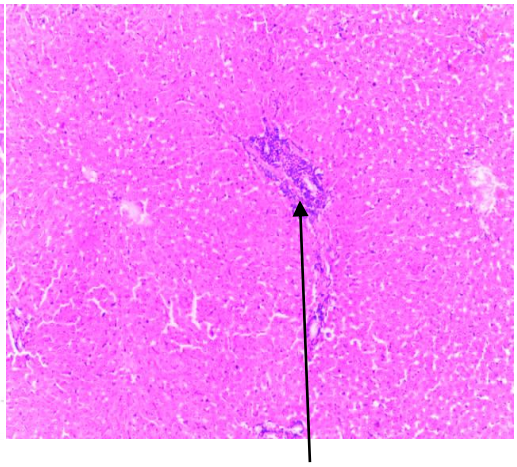

Plate 8: Group D female H&E X 200 Slide shows periportal inflammation at 0.75mg/kg bw in group D

DISCUSSION

Gramoxone herbicide (paraquat as active ingredient) is use to control weeds. The essence of this research is to further examine

the toxic potency of gramoxone herbicide and to add to scientific record that at 10fold serial dilution, using a dilution factor of 10^4 and exposing humans and animals to sublethal doses of 0.25, 0.5 and 0.75mg/kg bw could still have effects on animals and human on a long term exposure. This is supported by the findings of (Ujowundu *et al.,* 2018) which states that paraquat at sublethal dose of 1.5 mg/kg bw has hepatoxic effect on the liver. In the aspartate aminotransferase (AST) male and female, alanine aminotransferase (ALT) male and femalein group B and C and alkaline phosphatase (ALP) showed a significant decrease compared with the control which was contrary to the normal increase in liver enzymes activities when exposed to paraquat that did not blended with the findings of (Novaes *et.al.,* 2012) and others. ALT in treated group D and ALP in all treated group in the female registered an increase which correspond to the study of (Lalruatfela *et.al* 2014). This result from the female actually intensified the fact that paraquat diluted at 10-fold and exposed at minute doses cause hepatotoxicity of the liver. Long time exposure at minute dose will manifest to chronic condition which might result to other complication of other organs of the body. The effects on the liver enzymes at very low doses of this research supported the findings of (Okonkwo *et.al* 2021) when they exposed albino rats to minute doses of different concentration.

The total protein of the male and female rats treated groups were found to be significantly reduced compared with the control due to paraquat exposure. This result was in line with the findings of (Mohammadi-Bardbori and Ghazi-Khansari, 2008 and Shekoufeh *et. al.,* 2017) that reported a decreased in total protein levels in paraquat toxicity may be due to free radical-mediated membrane damage. A decrease in total protein is a clinical sign of liver injury and this was

indicated in the mild inflammation of the liver tissue in group B male and group D female rats. This corresponded with outcome of (Lalruatfela *et al.,* 2014) studies that showed significant decrease in total protein in paraquat exposed groups

Albumin in group B and C treated male and female rats experienced a decrease and equal level with the control group which corresponded with the finding of (Attia and Nasr 2009) that reported reduction in plasma albumin and globulin levels during paraquat toxication in rats.

There was an increment in the weight of all the animals treated compared with the control. Most of the treated animal's weights surpass that of the control

The fact that the weights of the animals in this research were not affected by the diluted, minute doses and 28 days period of exposure should not be used to undermine the toxic potential of gramoxone herbicide on the rats. This may be seen as a decisive symptom in shading the clinical implication of the effect the gramxone herbicide (paraquat as active ingredient) will cause to rats and other animals including human beings. The increase in weight of the rats in this research indirectly compliment the studies of (Edo 2022; Konradsen *et.al*., 2023 and Kumar *et.al* 2013) were they recorded weight reduction which was attributed to reduced feed and low water consumption in accordance with gastrointestinal track toxic activity of paraquat dichloride and free radical oxidative damage in several vital organs on the subcellular level. The implication of this is that 10-fold dilution and minute doses as used in this research will not cause weight reduction in albino rats.

Inflammation is a biochemical and cellular process that tends to defend injured tissues against infection, repair tissue and healing (Kathrynl and Sue 2002). The fact that there was no observable effects on the liver tissue

administered with 0.5 and 0.75 mg/kg body weight at 10 – fold serial dilution in the male rats and 0.25 and 0.5 mg/kg body weight in the female rats which is in support of the low dose 0.01 mg PQ/kg/day has no histopathological or functional disturbances as publish by (Bamdad *et .al.,* 2011). However, there was mild inflammation of the liver tissue of the male rats exposed to 0.25mg/kg and tissue of the liver exposed to 0.75 of the female. Thus, gramoxone herbicide no matter it dose has effect on the liver. This research further strengthened the reason for banning the herbicide because it has no safe use tolerance level as documented by (Narayanan P. 2011). The mild inflammation observed in the liver tissues exposed to 0.25mg/kg bw in group B male and 0.75mg/kg bw in group D female indicated hepatocyte injury.

CONCLUSION

The outcome of this findings further contributed that gramoxone herbicide has great potency to cause liver damage even when diluted in ten-fold and exposed to minute dose of the diluted gramoxone herbicide. Therefore, farmers and wild animals exposed to such diluted doses are prone to have chronic effect on their livers and other visceral organs.

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