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In-vitro and *In-vivo* Evaluation of Anti-*trypanosomal* Activity of *Carica papaya* Seed Extracts and Fractions in Albino Wister Rats

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Authors' contributions

This work was carried out in collaboration among all authors. Author MAM designed the study. Author HK wrote the protocol, managed the analyses of the study, performed the statistical analysis, managed the literature searches and wrote the first draft of the manuscript. Author SS made necessary correction during and after the analyses. All authors read and approved the final manuscript.

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ABSTRACT

Aims: This study presents baseline data on the *In-vitro* and *In-vivo* evaluation of anti-trypanosomal activity of *Carica papaya* seed extracts and fractions in Albino Wister rats.
Study Design: Mention the design of the study here.
Place and Duration of Study Sample: Department of Biochemistry, Modibbo Adama University of Technology, Yola, between June 2009 and July 2010.
Methodology: 56 Wister rats of both sexes were randomly divided into 8 groups (I – VIII) of 7 rats each were used for this study. Four concentrations (100, 200, 400 and 800 mg/kg) of different extracts of seed *carica papaya* were screened for trypanocidal activity against Trypanosoma brucei *In vitro* and *In vivo*. The effect of the extracts was evaluated for trypanocidal activity in rats infected and not infected with the parasite. Administration of the extract and the drugs was orally daily for 5 consecutive days from day 7 of post infection. Level of parasitemia and body weight was taken daily for 21 days.

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Results: The extracts inhibited parasite motility and totally eliminated the organisms at the concentrations used *In vitro*. The extract also showed promising in vivo trypanocidal activity. The observed *In vitro* and *In vivo* trypanocidal activities may be due to the presence of bioactive compounds present in the extracts as seen in this study. The extract also improved the observed decreases in haematological parameters of the treated rats, which may be due to their ability to decrease parasite load. The LD_{50} was estimated to be ≥2,000 mg/Kg (v/v) for acute oral toxicity test (because all the rats survived at the end of the 14-day observation period). This is an indication of very low toxicity, implying that the extract could be administered with some degree of safety. A significant decreased ($p<0.05$) were observed in weight of rats at 100 mg/kg, 200 mg/kg 400 mg/kg 800 mg/kg negative control and prophylactic at four to eight days of infections, while significantly increased ($p<0.05$) were observed in weight of rats for berenil control and normal control.
Conclusion: The decrease in weight of rats could be as a result of loss of appetite due to severe fever and also the trypanosome.

Keywords: Trypanocidal; haematological; infectivity test; Carica papaya; albino rats.

1. INTRODUCTION

Trypanosomiasis remains a major health hazard to humans, animals and livestock populations in developing and under developed countries. It is one of the deadliest disease caused by the flagellated haemoprotozoan parasite (Trypanosoma) which is transmitted by infected tse-tse fly *Glossina* species [1,2]. Trypanosome infestation is an important constraint in livestock and animal production especially when the available drugs for its control are toxic and expensive. It is estimated that over 60 million people and 70 million animals are exposed to this infection [1,3] and Trypanosomiasis is ranked among the top 10 global cattle disease affecting livestock production in Sub-Saharan Africa [4]. Chemotherapy is the most widely used means of controlling and treating trypanosomiasis. The few registered trypanocides are toxic and often associated with severe side effects [4]. The economic importance of the disease is often characterized by increasing anemia, induce oxidative stress, inflammation, suppress immune system, poor reproductive performance, poor lactation, weakness, extreme emaciation and eventually death [5-7]. The most pathogenic trypanosomes belong to the *Vivax* group (Duttonella), congolense group (Nannomonas) and the brucei group (Trypanozoon) subgenera. *Trypanosoma brucei gambiense* and *Trypanosoma brucei rhodesiense* cause Human African Trypanosomosis (HAT, sleeping sickness), whereas African Animal Trypanosomosis (AAT) is caused by *Trypanosoma brucei brucei*, *Trypanosoma vivax* and *Trypanosoma congolense* (in cattle, sheep, goats, and dogs), *Trypanosoma equiperdum* (in equidae), *Trypanosoma simiae*(in pigs) and *Trypanosoma evansi*(in camels) [8]. The current chemotherapy in humans and animals relies on, are diminanazene aceturate, berenil, suramin, pentamidine, melasoprol and efforithine, they are widely used means of controlling and treating trypanosomiasis. Each of these drugs has one or more of these challenges: expensive, highly toxic, need parenteral administration and parasites increasing resistance, while some were developed more than 30 years ago [9]. There is urgent need to source for new, cheap and safe alternative chemotherapy against trypanosomiasis from natural origin [10]. Medicinal plants remain a viable option in controlling and treating trypanosomiasis since medicinal plants remain available, mostly affordable and they are known to have therapeutic value. They are used in treating ailments of both human and livestock over the past few years due to its accessibility [11]. Many pharmaceutical companies derive their active chemicals compounds from such plants. The active chemical compound like alkaloids has been reported to inhibit DNA intercalation in combination with protein biosynthesis which is the basic mechanism of action observed to be responsible for anti-trypanosomal effect. The addition of digitonin significantly stimulated the activity of almost all alkaloids against trypanosomes [12,13]. In North Eastern Nigeria, lactating Fulani women consumed *strychnos spinosa* (loganiaceae) to stimulate breast milk production [14]. Serigosterol, 24-hydroperoxy-24-vinyl cholesterol (17) isolate of dichromethane leaf shows *in vitro* antitrypanosomal activities with IC_{50} of values of 7.5 and 3.2μM respectively [14]. Approximately 119 pure chemical substances extracted from higher plants are used as medicine throughout the world [15]. World Health Organization (WHO) encourages

the use of herbal preparations in the treatment of local health problems where the products are easily affordable and already integrated into the people's cultures. Nigeria, located in West Africa on the Gulf of Guinea, has a rich biodiversity. There are many reports documenting the potentials of medicinal plants in Nigeria against several diseases [8]. Common ailments treated by the plants include stomach pain, menstrual pain, tuberculosis, fever, cough, tooth related problems and body pain; the most treated being fever, cough and stomach related pains, the bark, leaves, seed, root and flower of these plants are believed to have anti-bacteria, anti-fungi and anti-parasitic effect [16]. *Carica papaya*, also called as pawpaw is traditionally cultivated for fruit. *Carica papaya* belonging to the genus Carica, family Caricaceae and this family consists of 34 species, one is hybrid from six genera. The six genera as follows: have being reported by many researchers to contain the enzyme papain, chymopapain which is biologically active and has medicinal and nutritional values [17]. All parts of the plant like leaves, fruits, seeds, flowers bark and roots can be used by humans as food and medicinal purposes. This plant has therapeutic uses such as anti-parasitic, anti-amoebic, anti-microbial, anti-fertility activity, anti-ulcerogenic, anti-fungal, antitumor, hypolipidaemic and employ in wound - healing activity, free radical scavenging activity, diuretic activity, uterotonic activity [18]. The aim of this study is to determine the anti-trypanosomal activity of aqueous and methanolic seed extract of *Carica papaya* on *Trypanosoma brucei* infected albino rats.

2. MATERIALS AND METHODS

2.1 Plant Material

Fresh mature seed of *Carica papaya,* was collected in Vom, Jos south local government area of Plateau state. Nigeria. The plant specimen was identified and authenticated in the herbarium unit at Federal College of Forestry, Jos, Nigeria.

2.1.1 Plant extraction

2.1.1.1 Aqueous extraction

600 g of the seed of *Carica papaya* was pulverized and macerated with 30000 ml of distilled water for 72 hours (with daily shaking). After extraction the mixture was filtered, using Whatman filter No. 1 Paper. The filtrate was concentrated by drying at room temperature at 27°C. The dried extract was stored at 4°C before used [19].

2.1.1.2 Methanolic extraction

The pulverized seed of *Carica papaya,* (600 g) was extracted with methanol. The maceration process was performed at room temperature by adding 600 g of the power form to 2600 mls of methanol it was then extracted by cold maceration with daily shaking for three days and was filtered using Whatman filter No.1 Paper. The filtrated was air dried. It was harvested and weight, the dried extracts was preserved in a desiccators [20].

2.1.2 Animals

Albino rats weighing between 80-120 grams of either sex was used for the study. The albino rat was kept in clean wire meshed cages under standard animal condition in accordance with the recommendations in Guide for the Care and Use of Laboratory Animal. The was given standard feed diet and water during the entire period of the experiment.

2.2 Acute Toxicity Study

The mean lethal dose (LD_{50}) was determined using the limit dose test. Six Wistar rats were used for this study. Briefly one rat (No. 1) was administered orally with 2,000 mg/kg of the plant extracts prepared in a 150 mg/ml concentration, with rat No. 2, 3, 4, 5 and 6 given the same dose on day 2, 3, 4, 5 and 6, respectively.

Prior to the experiments, the rats were clinically checked twice a day prior to dosing, and then hourly, during the first 8 hours post-treatment, and three times a day during the rest of the experimental period. Clinical signs such as nasal secretions, salivation, tremors and convulsions, anxiety, respiratory rates, other clinical signs and death were checked.

2.3 Test Organism

Trypanosoma brucei (*F*ederestrian) used for this work was obtained from Nigeria Institute for Trypanosomiasis Research (NITR),Vom, Plateau State, Nigeria. The parasite was maintained in their laboratory by continuous passage in albino rats, until commencement of the work.

Table 1. Acute oral toxicity

Sample	Solubility test	Limit Test (LD_{50}) mg/Kg
CP seed methanol	Sparingly soluble	>2,000
CP seed aqueous	Sparingly soluble	>2,000

2.4 *In vitro* Test for Trypanosome Activity

2 ml of blood was collected in bijou bottles containing the anticoagulant Ethylene Diamine Tetra-acetic Acid (EDTA) from an albino rat heavily infected with *Trypanosoma brucei for* trypanosome suspension preparation. A suspension of trypanosomes was prepared in normal saline and the concentration was adjusted to about 1 x 10^6 organisms per ml. 0.5 ml of the suspension of Ringer's solution and 0.5 ml of suspension of trypanosomes were dispensed into 5 tubes (1-5). 0.5 ml of the extract concentrations 0.005 mg/ml, 0.05 mg/ml, 0.5 mg/ml and 5 mg/ml were added to the first 5 tubes (1-5). The sixth tube was an untreated control (no extract added). The tubes were then incubated at 37°C. The contents of the tubes were each examined at the intervals of 15 minutes i.e. from 0 – 105 minutes, by aspirating small amounts using a Pasteur pipette onto clean slides then covered with cover slips and checking for the presence and motility of the parasites under the microscope (X 40 objective lens). This measure was considered as anti-trypanosomal activity [21].

2.4.1 Infectivity test

About 0.2 ml of 0.005 mg/ml, 0.05 mg/ml, 0.5 mg/ml, 5 mg/ml of various concentration of the mixture of extract, ringer's solution and the blood containing the parasite were taken into a clean 1 ml insulin syringe with a 25-gauge needle and was inoculated into 3 rats each group not previously infected with the trypanosome. Control was also treated same way, but without extract. The rats were observed daily for development of parasitemia for a period of 90 days.

2.4.2 *In vivo* study of the plants extracts

Fifty-six Wister rats of both sexes were randomly divided into 8 groups (I – VIII) of 7 rats each were used for this study. Forty-nine rats were inoculated intraperitoneally (IP) with the parasite inoculum of 10^6 trypanosomes in the whole blood of infected rat. The quantitative estimation of trypanosomes was done by rapid matching method [21]. After detection of the parasitemia, animals in group I, II, III, IV, V, VII and IX were inoculated intraperitoneally (IP). Administration of the extract and the drugs was orally daily for 5 consecutive days from day 7 of post infection. Level of parasitemia and temperature was taken daily for 21 days.

Group I: 100 mg/kg of extract
Group II: 200 mg/ml of extract
Group III: 400 mg/kg of extract
Group IV: 800 mg/kg of extract
Group V: Control infected and treated with diminazene aceturate, Hoechst (Berenil standard drug) at 3.7 mg/per body weight.
Group VI: Normal Control, not infected and not treated with the extract
Group VII: Negative control, infected and not treated with the extract
Group VIII: Not infected but treated with 800 mg/kg of the extract.
Group IX: Treated with 800 mg/kg, before infected with the parasite.

2.4.3 Body weight

The behavioral changing was observed and the body weight of the rats per kg were recorded on daily basis for 21 days, both pre and post infection.

2.5 Statistical Analysis

Values were expressed as mean ± SEM n = 7. Data were subjected to one-way analysis of variance (ANOVA), followed by SPSS version 23. Tukey's comparison *post-hoc* test used to compare the differences between the experimental groups. (GraphPad Software, San Diego, CA, USA). Values of $p<0.05$ was considered significant.

3. RESULTS AND DISCUSSION

3.1 The Mean Lethal Dose (LD_{50}) of Methanolic Extracts the Fractions of *Carica papaya* Seeds

The LD_{50} was estimated to be ≥2,000 mg/Kg (v/v) for acute oral toxicity test (because all the rats survived at the end of the 14-day observation period). This implies that the plant extracts were relatively safe in rats following oral acute exposure. These finding is in agreement

with [22], findings which showed LD_{50} of C. *papaya seed* above 5000 mg/kg of body weight of rat. Biu et al. [6] also reported the lethal dose of aqueous extract *C. papaya leaf* to be safe at 1000 mg/kg.

3.2 The Result of *In vitro* Evaluation of the Effect of Extract and Fractions of *Carica papaya* Seed on *Trypanosoma brucei*

The *in vitro* tests were performed in duplicates. In this *in vitro* studies, decreased and total inhibitor of the parasite motility and complete disintegration of parasite morphology was observed at 30 minutes intervals, at a concentration of 5 mg/ml. Drastic reduction in motility of trypanosomes was observed after 30 minutes at 0.5 mg/ ml, after 75 minutes interval at 0.05 mg/ml of both crude methanol extract, fractions of n-hexane, ethyl acetate and n-butanol of *C. papaya* seed which was taken as a measure of the anti-trypanosomal effect of the crude methanol extract of *C. papaya* seed and fractions of n-hexane, ethyl acetate and n-butanol, these were compared with the control which shows active motility even at 105 minutes intervals. These observation that shown activity even at a very low concentration of 0.005 mg/ml are in confirmed with the works of Ogbole et al. [23] and other workers which stated that natural products from medicinal possess biochemical structures capable of generating radicals that may cause peroxidative damage to trypanothione reductase which is very sensitive to alterations in redox balance. The *in vitro* anti-trypanosomal activity of the methanol crude extract could be attributed either to the solubility of the active ingredient(s) responsible for the observed *in vitro* activity or the variations in the types of phytochemicals compound as revealed by the result of phytochemicals screening. Accumulated evidences suggested that many natural products exhibited their anti-trypanosomal activity by virtue of their interference with the redox balance of the parasites acting either on the respiratory chain or on the cellular defenses against oxidative stress. Eze et al. [24]; Ermias et al. [25], and Ogbole et al. [23]). Alkaloid was also among the phytochemical compound detected in *Carica papaya* seed, which was reported by Wink et al. [26] to interact with neuroreceptors, DNA and other molecular target and inhibit protein biosynthesis. The complete cessation of in motility of trypanosomes in all the fractions could be as a result of the analysis of the composition and results obtained from GC–MS allowed identifying 22 constituents, representing 69.57% of the oil from n-hexane, ethyl acetate and n-butanol fractions. The two main constituents of the essential oil, n-hexadecanoic acid and Oleic Acid may have interfered with glycolysis, these observations confirmed the work of Bero [27] which stated that anti-trypanosomal activities on procyclic forms (Tbb PF) Compounds which were more active on bloodstream forms (BSF) than procyclic forms (PF) of Tbb may act on glycolysis. Indeed, bloodstream forms which have no Krebs cycle or mitochondrial respiratory chain coupled to ATP synthesis are exclusively dependent on glycolysis for their ATP formation. The higher concentration in phenolic content could attributed the inhibitory and disintegration of the morphology of the parasite, this research also in agreement with the work of Amisigo et al. [28] which stated that the phenolic acid which have been link to the numbers and position of hydroxyl groups of the benzene ring inhibitory and altering the morphology of the parasite by forming reactive oxygen intermediate in the parasites.

3.3 Infectivity Evaluation of the Effect of Methanol Extract, N-butanol Fraction, Ethyl Acetate Fraction and N-hexane Fraction of *Carica papaya* Seed on *Trypanosoma brucei*

After intraperitoneal inoculation of 0.1 mls of incubated mixture of *T. brucei* to the rats in control group which contain only *T. brucei* with ringer's solution and methanol extract, n-butanol, fraction, ethyl acetate fraction and n-hexane fraction respectively at a concentration of 0.005 mg/ml, 0.05 mg/ml, 0.5 mg/ml and 5 mg/ml. The presents of *T. brucei* were detected in the blood samples of rats after three days of inoculation at concentration of 0.005 mg/ml n-hexane fraction. Parasitemia was also detected in the control group of the rats. While no presences of *T. brucei* were detected in the blood samples of rats at the various concentrations of 0.005 mg/ml, 0.05 mg/ml, 0.5 mg/ml and 5 mg/ml of both methanol extract, n-butanol fraction and ethyl acetate fraction after 90 days of observation when compared to control groups which shows presences of *T. brucei* after 3 days of infection. Methanol extract, n-butanol and ethyl acetate fraction showed the highest anti-trypanosomal activity against the parasite even at a lower concentration of 0.005 mg/ml when compared to n-hexane fraction which less activity at 0.005 mg/ml. The highest activity exhibited by methanol

extract, n-butanol fraction and ethyl acetate may as a result of higher concentration of phytochemicals compound which reduced the level of parasitemia as compared to n-hexane fraction ((P<0.05) which have lesser concentration of the phytochemicals compound. Albaldrani [29] also reported on the phytochemical screening of the extract which revealed the presence of alkaloids, carbohydrates, triterpenes, steroids, cardiac glycosides, saponins, tannins and flavonoids which significantly (P < 0.05) reduced levels of parasitemia at 250 mg/kg.

3.4 Body Weight of the Rats for both Pre and Post Infected with *T. brucei* and Treated with *Carica papaya* Methanol Extract

The rats in all the groups were weight at 4 days intervals before and during infection period, significantly decreased (p<0.05) were observed in weight of rats at 100 mg/kg, 200 mg/kg 400 mg/kg 800 mg/kg negative control and prophylactic at four to eight days of infections, while significantly increased (p<0.05) were observed in weight of rats for berenil control and normal control. The decrease in weight of rats could be as a result of loss of appetite due to severe fever and also the trypanosome competes for nutrient from the host and also consumption/utilization of host's lipids during a *T. brucei* infection, this could contribute to the weight loss observed in patients with sleeping sickness, cattle with Nagana, and mice infected with *T. brucei* [30]. While the increased in body weight of the berenil and normal was because the animals were eating their normal feed and no sign of trypanosome in them.

Clinical Signs and Symptoms. All the inoculated rats developed clinical trypanosomiasis, which was characterized by reduction in feed intake, fever, loss of hair, weight loss anorexia, dullness, emaciation, posterior paresis and death of some infected rats. All the infected rats developed parasitemia on the 3rd day post infection (p.i.). Peak parasitemia was attained between day 7 p.i. Thereafter, there were fluctuations in the levels of parasitemia which continued until the end of the study.

Table 2. *In vitro* motility effect of methanolic extract of *Carica papaya* seed on *Trypanosoma brucei*

Conc. in mg/ml	0	15	30	45	60	75	90	105 (Mins)
Control	++++	++++	+++	+++	+++	+++	+++	+++
0.005 mg/ml	++++	++++	+++	+++	++	++	+	-
0.05 mg/ml	++++	++++	+++	++	++	+	-	-
0.5 mg/m	++++	+++	+	-	-	-	-	-
5 mg/ml	++++	++	-	-	-	-	-	-

Key: ++++ very active; +++ active; ++ sluggish; + very sluggish; (-); motility not detected

Table 3. *In vitro* motility effect of n-butanol fraction of *Carica papaya* seed on *Trypanosoma brucei*

Conc. in mg/ml	0	15	30	45	60	75	90	105 (Mins)
Control	++++	++++	+++	+++	+++	+++	+++	+++
0.005 mg/ml	++++	++++	+++	+++	++	+	+	-
0.05 mg/ml	++++	++++	+++	++	+	+	-	-
0.5 mg/m	++++	+++	++	+	+	-	-	-
5 mg/ml	+++	+	-	-	-	-	-	-

Key: ++++ very active; +++ active; ++ sluggish; + very sluggish; (-); motility not detected

Table 4. *In vitro* motility effect of ethyl acetate fraction of *Carica papaya* seed on *Trypanosoma brucei*

Conc. in mg/ml	0	15	30	45	60	75	90	105 (Mins)
Control	++++	++++	+++	+++	+++	+++	+++	+++
0.005 mg/ml	++++	++++	+++	+++	++	++	+	-
0.05 mg/ml	++++	++++	+++	+++	++	+	-	-
0.5 mg/m	++++	+++	++	+	+	-	-	-
5 mg/ml	++++	++	-	-	-	-	-	-

Key: ++++ very active; +++ active; ++ sluggish; + very sluggish; (-); motility not detected

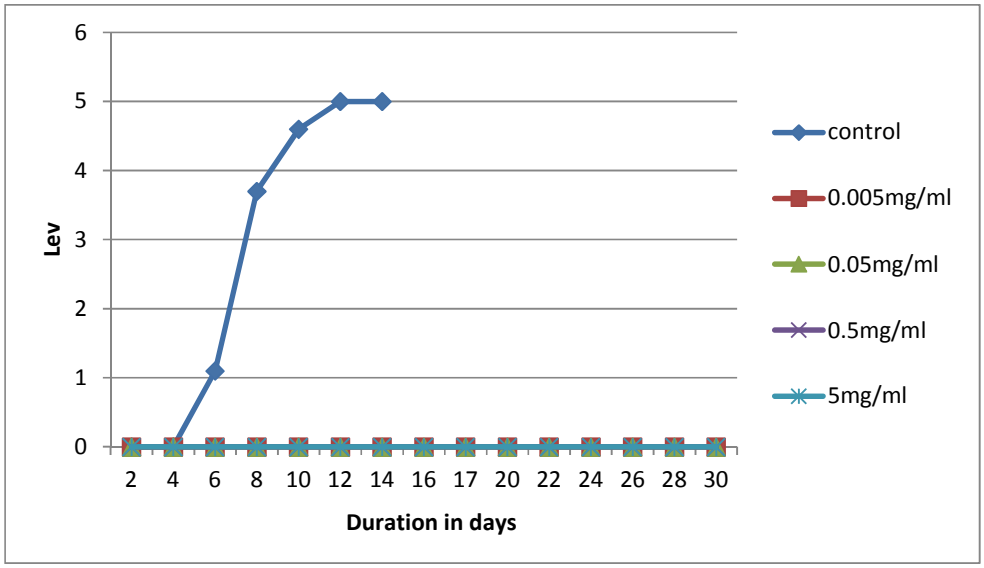

Fig. 1. Infectivity evaluation of the effect of methanol extracts of *Carica papaya* seed on *Trypanosoma brucei*

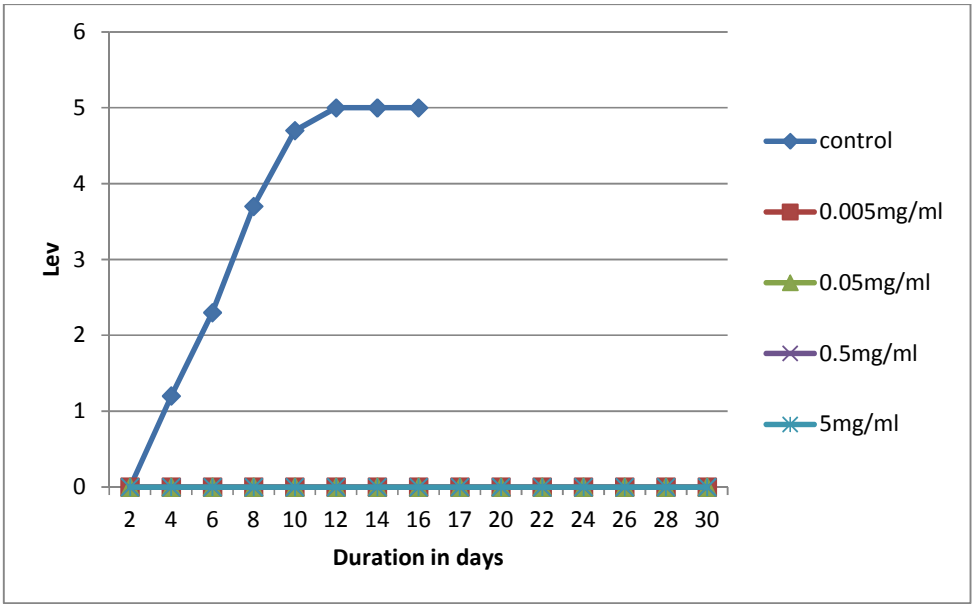

Fig. 2. Infectivity evaluation of the effect of n-butanol fraction of *Carica papaya* seed on *Trypanosoma brucei*

Table 5. *In vitro* motility effect of n-hexane fraction of *Carica papaya* seed on *Trypanosoma brucei*

Conc. in mg/ml	0	15	30	45	60	75	90	105 (Mins)
Control	++++	++++	+++	+++	+++	+++	+++	+++
0.005 mg/ml	++++	++++	+++	+++	++	++	+	-
0.05 mg/ml	++++	++++	+++	+++	++	+	-	-
0.5 mg/m	++++	+++	++	+	+	-	-	-
5 mg/ml	++++	++	-	-	-	-	-	-

Key: ++++ very active; +++ active; ++ sluggish; + very sluggish; (-); motility not detected

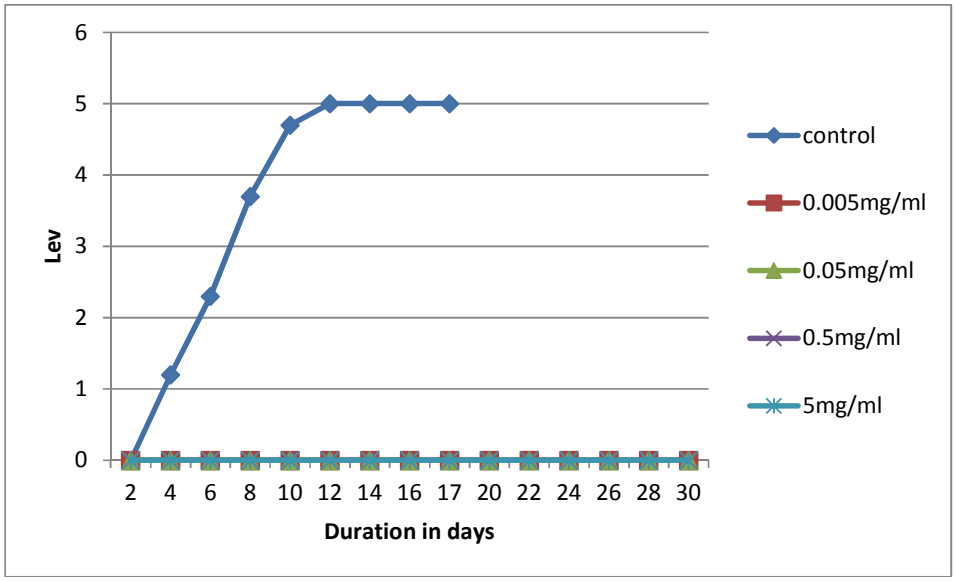

Fig. 3. Infectivity evaluation of the effect of ethyl acetate fraction of *Carica papaya* seed on *Trypanosoma brucei*

3.5 Haematological Parameters of Rat Infected with *T. brucei* and Treated with *Carica papaya* Seed Methanol Extract

Results of haematological parameters were monitor. The haemoglobin concentration, PCV, RBC, WBC and platelet count of infected untreated rats were significantly decreased ($p< 0.05$) when compared with the results of normal control group of not infected untreated, not infected but treated with extract and infected but treated with standard drug. Platelet counts of infected untreated, prophylactic and late stage treated rats show significant decreased when compared to the normal control rats. The reduction in mean value of haematological parameters are consistent with the study [31] which observed decreased in haematological values of pigs infected with *T. brucei*. The drop in haematological values could be as a result of important factor said to be hemolysis, trypanosomiasis can also cause reduction in red cell mass, life span and also on the occurrence of erythrophagocytosis, hemosiderosis and sometimes hyperbilirubinemia [31,32]. The reduction in the mean leucocyte count observed during this infection agrees with findings by Allam [32]. The depression of leucocyte levels could have been a result of immuno-suppression, which usually co-exists with trypanosomiasis infection. Increased in neutrophil percentage were observed in blood sample of rat infected and treated with standard drug, normal control and extract control when compared with the blood sample of rat infected and treated with different concentration of extract and negative control. This finding is in contrast to earlier finding by Chamond et al. [33] which shows reduced in the number of circulatory neutrophils and increased lymphocytes number of mice infected *T. vivax*, this discrepancy could be attributed tothe species or strain of the trypanosomes.

3.6 Erythrocyte Osmotic Fragility of Rat Infected with *T. brucei* and Treated with *Carica papaya* Seed Extract

Osmotic fragility result shows the mean and range of percentage haemolysis at different saline dilution giving 50% haemolysis. The infected not treated control rat had more fragile red cells when compared with rat infected and treated with different concentration of extract, standard drug control rat, normal control not infected not treated rat, extract control rat and prophylactic. This finding is similar to that of Ikede et al. [32] who observed increased in red cell of mice infected with *T. congolense* and *T. brucei brucei* and suggest the presence of some mildly toxic (haemolysin) substance in the plasma of *T. brucei brucei* infected mice. The reduced in haemolysis in red cell of infected and treated rat with different concentration of extract could be as a result of phytochemicals substance

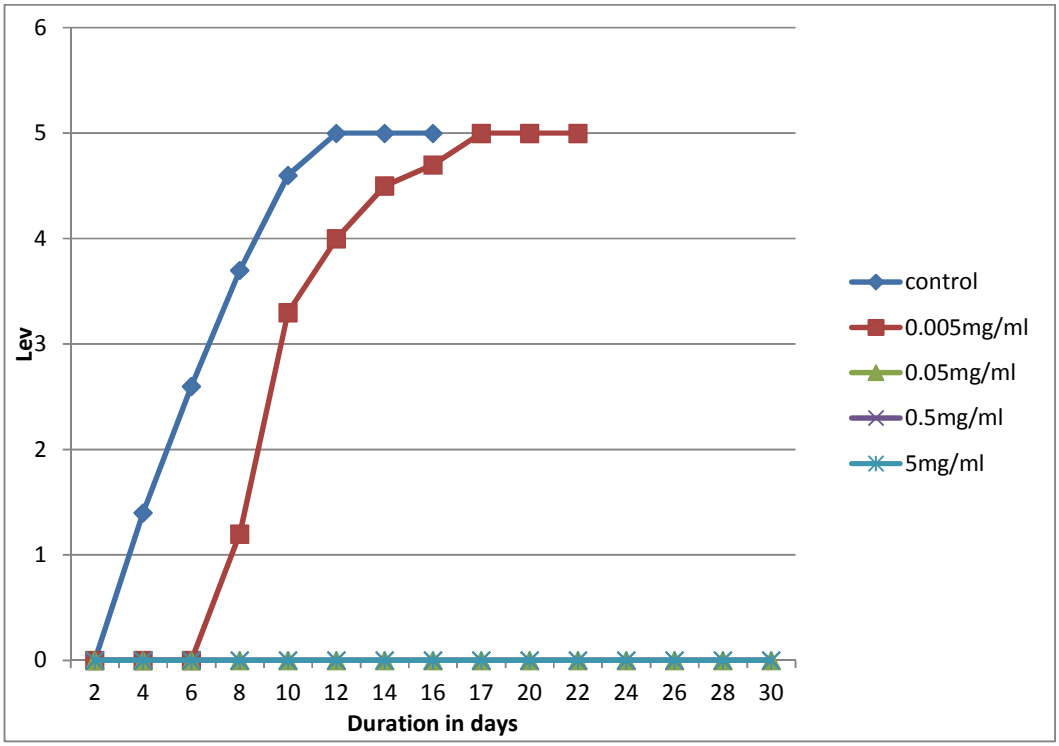

Fig. 4. Infectivity evaluation of the effect of n-hexane of *Carica papaya* seed on *Trypanosoma brucei*

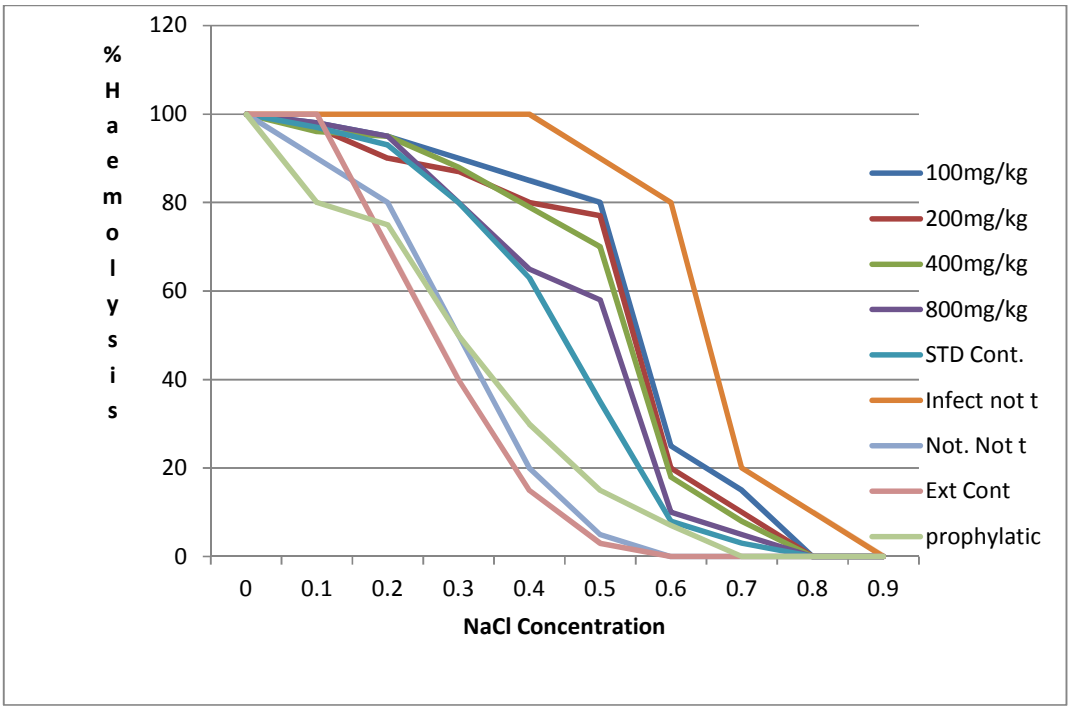

Fig. 5. Erythrocyte osmotic fragility of rat infected with *T. brucei* and treated with *Carica papaya* seed extract

Table 6. The body weight of the rats for both pre and post infected with *T. brucei* and treated with *Carica papaya* methanol extract

Treatment groups	Weight of rats (g) on day		
	Day 1	Day 4	Day 8
100 mg/Kg	90.14±3.10	90.11±09.05	88.80±19.07*
200 mg/Kg	96.41±4.00	91.33±5.89*	89.43±15.31
400 mg/Kg	97.42±6.33	92.20±9.59*	88.40±22.82*
800 mg/kg	98.33±17.63	92.17±16.29	87.50±73.94*
Berenil	101.33±1.63	114.83±5.31*	120.17±7.63*
Normal Cont.	100.50±1.23	115.17±3.76*	121.50±6.03*
Neg. Control	104.50±5.51	85.17±2.86*	71.83±3.49*
Extract Contr	101.00±1.10	120.00±2.10	146.40±41.74*
Prophylactic	107.34±14.91	100.91±11.60	85.00±42.95*

*Values are Mean ± SEM for three determinations n=3. * Significantly ($p<0.05$) lower compared across the days*

Table 7. The Haematological parameters of rat infected with *T. brucei* and treated with *Carica papaya* seed methanol extract

	Red blood cells indices of rats					
Treatment Groups	**RBC (1012/L)**	**HGB (g/dl)**	**HCT (%)**	**MCV (fl)**	**MCH (pg)**	**MCHC (g/dl)**
100 mg/Kg	3.05±0.82*	7.00±1.27*	28.17±1.72*	12.50±1.05	13.83±0.98	181.17±17.51*
200 mg/Kg	6.38±1.37*	8.00±1.79*	28.50±8.46*	14.50±1.05*	30.67±7.69*	197.00±35.18*
400 mg/Kg	6.97±1.12*	7.50±2.07*	30.00±1.41*	14.17±1.72*	42.33±16.60*	181.33±59.89*
800 mg/kg	6.68±1.83*	8.00±1.79*	33.17±6.65*	13.17±1.33	31.33±4.50*	182.00±24.45*
Berenil	7.70±0.25*	10.17±1.47*	39.33±3.39*	22.33±4.32*	16.73±0.38	206.00±13.31*
Normal Cont.	9.05±0.46*	8.63±0.68*	48.50±5.13*	18.83±1.47*	16.97±0.65	258.67±31.51*
Neg. Control	3.53±0.51	4.50±0.49	20.50±3.08	11.33±0.82	11.17±0.75	110.00±22.80
Extract Contr	8.47±0.55*	8.92±0.48*	48.67±6.56*	17.17±2.14*	16.17±0.75	245.83±32.93*
Prophylactic	7.63±0.46*	8.50±3.89*	29.83±0.75*	15.67±0.82*	26.33±12.34	214.67±37.54*

Table 8. The Haematological parameters of rat infected with *T. brucei* and treated with *Carica papaya* seed methanol extract

White blood cells indices of rats			
Treatment groups	**Wbc (×1000)**	**Lymphocytes (%)**	**Neutrophils (%)**
100 mg/Kg	1.83±0.75	83.67±4.84	18.00±6.54
200 mg/Kg	4.33±2.50*	78.00±4.60	22.00±4.60
400 mg/Kg	3.68±1.32*	84.33±6.06	15.67±6.06
800 mg/kg	5.49±3.17*	83.17±3.52	16.83±8.61
Berenil	9.50±1.64*	70.00±7.29*	30.00±2.48*
Normal Cont.	12.67±1.21*	66.00±6.48*	34.00±2.98*
Neg. Control	1.00±0.00	83.17±9.54	16.83±9.54
Extract Contr	12.67±1.21*	71.50±7.01*	28.50±7.01*
Prophylactic	6.50±1.87*	76.50±7.77*	25.17±6.56*

**Significantly different from negative control down the group at P<0.05. Values are Mean ± SEM for determinations n=7*

which have anti-inflammatory and antioxidant effect. Tauheed et al. [34] also reported that the leaf extract of *L. inermis*has shown to protect the erythrocyte membrane against trypanosome-induced osmotic fragility and therefore, may ameliorate anaemia associated with trypanosome infection in animals.

4. CONCLUSION

This study has demonstrated that the methanol extract, hexane, ethyl acetate and butanol fractions of C. papaya seed has significant anti-trypanosoma activity, it was able to eliminate T. brucei *in vitro* within few minutes at higher concentration, which means that it is dosage dependent. Although it was not able to eliminate the parasite in vivo, but it was able to suppressed replication of the parasite and extended the life span of the treated rat more than the negative control. However, this is a promising potential drug to use associated with other commonly used anti-T. brucei drugs, such as benznidazol, berenil (Diminazene aceturate) for improving therapeutic effectiveness.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. WHO. Author. Medicinal plants - guidelines to promote patient safety and plant conservation for a US$ 60 billion industry; 2004.
Available:http://www.who.int/mediacentre/notes/2004/np3/en on 20th November 2018.
2. Victor AM, Beatty VM, Patricia IK. *In vitro* effect of aqueous extract and fraction iv portion of ximenia americana stem bark on trypanosoma congolense DNA. Journal of Parasitology. 2014;904318(5)1155-904318.
3. Getachew A. Trypanosomosis in Ethiopia. Journal of Biol Sci. 2005;4:75-121.
4. Peter B, Adekunle OS, Wethyton AO, Adetayo FF, Nnaemeka CI. Current analysis of Chemotherapy S trategies for the Treatment of Human Africa Trypanosomiasis, Pathog Globa Health. 2013;107(5):242-252.
5. Bezie M, Girma M, Dagnachew S, Tadesse D, Tadesse G. African trypanosomes: Virulence factors, pathogenicity and host responses. Journal of Veterinary Advance. 2014;4(11):732-745.
6. Biu AA, Buratai LB, Ahma AA, Hambali IU, Ngulde SI, Zakariah M, Lawa JR. Photochemistry, toxicity and efficacy of crude aqueous extract of *C. papaya* leaf against *Trypanosomal brucei*. Bangladesh. Journal of Veterinary Medicine. 2016; 14(1):99-102.
7. Stijlemans B, Vankrunkelsven A, Brys L, Magez S, Baetselier P. Role of iron homeostasis in trypanosomiasis–associated anaemia. Immunobiology. 2008;213:823-835.
8. Ngozi JN, Akachukwu I, Fidele N-K, Michael UA, Chika JM. Anti trypanosomal

activity of nigerian plants and their constituents molecules. Molecule Review. 2015;1420-3049.
9. Marín PA, Soto-Ospina A. Redox mechanism of Trypanosoma cruzi resistance to nitro prodrugs Benznidazole and Nifurtimox. International Journal of Bioinformatics and Computational Biology. 2020;5(1):1-7.
10. Legros D, Ollivier G, Gastellu-Etchegorry M, Paquet C, Burri C, Jannin J, Buscher P. Treatment of human African trypanosomiasis-Present situation and needs for research and development. The Lancet, Infectious Diseases. 2002;2:437–440.
11. Mehta K, Patel BN, Jain BK. Phytochemical analysis of leaf extract of phyllanthusfraternus. Research Journal of Recent Sciences. 2013;2:129–140.
12. Krstin S, Silva H, Peixoto, Wink M. Combinations of alkaloids affecting different molecular targets with the saponin digitonin can synergistically enhance trypanocidal activity against *Trypanosoma brucei brucei*. Antimicrobial agents and Chemotherapy. American Society for Microbiology; 2016.
13. Merschiohann K, Sporer F, Steverding D, Wink M. *In vitro* Effect of alkaloids on bloodstream forms of *Trypanosoma brucei* and *T. congolense*. Planta Med. 2001;67: 623–627.
14. Hoet S, Pieters L, Muccioli GG, Habib-Jiwan JL, Opperdoes FR, Quetin-Leclercq J. Antitrypanosomal activity of triterpenoids and sterols from the leaves of strychnosspinosa and related compounds. J Nat Prod. 2007;70(8):1360–1363.
15. Shekins OO, Iliemene U, Timothy T, Liman B, Dayo O. Proximate analysis, phytochemical screening and antitrypanocidal potentials of *Bucholzia coriacea* in *Trypanosoma brucei brucei*-infected mice. Journal of Pharmacy and Biological Sciences. 2014;9(4):69-77.
16. Adelanwa MA, Haruna HB. Survey of some plants found in Gurara Local Government area of Niger State IJBR. 2013;5(1):45-53.
17. Anitha B, Raghu N, Gopenath TS, Karthikeyan M, Gnanasekaran A, Chandrashekrappa GK, Basalingappa KM. Medicinal uses of *Carica papaya*. Journal of Natural & Ayurvedic Medicine. 2018; 2(6):000144.
18. Nugroho A, Heryani H, Choi J, Park J. Identification and quantification of flavonoids in *Carica papaya* leaf and peroxynitrite-scavenging activity Asian Pacific Journal of Tropical Biomedicine. 2017;7(3):208-213.
19. Das K, Tiwan RKS, Shivasteva DK. Techniques for evaluation of medicinal plant products as anti-microbial agent: Current methods and future trends. Journal of Medicinal Plants Research. 2010;4:104 –111.
20. Kokate CK, Khandelwal KR, Pawar AP, Gokhale SB. Practical pharmacognosy, (45th Ed.), NiraliPrakashan. 2010;2.5-2.7.
21. Herbert WJ, Lumsden WHR. *Trypanosoma brucei*: A rapid "matching" method for estimating the host's parasitemia. Experimental Parasitology. 1976;40:427-431.
22. Kanadi MA, Alhassan AJ, Ngulari AL, Yaradua AI, Nasir A, Wudil AM. Acute toxity studies and phytochemicals constituents of different solvents extract of *Carica papaya* seeds. Asian Journal of Resaerch in Botany. 2019;2(3):1-9.
23. Ogbole E, DashakD. Ab, Nvau JB, Daben MR, Abongaby G.1, Obaloto OB, Oladipo OO, Igweh AC. Phytochemical screening and *in vitro* evaluation of the antitrypanosomal action of the methanolic leaf extract of *Corym biatorelliana* Internationa Journal of Ethnomedicine and Pharmacognosy. 2016;3(1):20-29.
24. Eze JI, Anosa GN, Ozota CA. *In vitro* and *in vivo* trypanocidal activity of Combretum racemosum leaves. African. Journal. Biotechnology. 2012;11:10611-10616.
25. Ermias M, Getachew T, Tilahum T, Workined S. Phytochemical screening and *in vitro* antitrypanosomal activity of aqueous and methanol leave extract of Clutiaabyssinica against T. congolense. Journal of Pharmacologia. 2015;6(3):79-82.
26. Wink M, Schmeller T, Latz B. Mode of action of allele chemical; alkaloid intereactions with neuroreceptors, DNA and other molecular target. Journal of Chemical Ecology. 1998;24:1881-1987.
27. Bezie M, Girma M, Dagnachew S, Tadesse D, Tadesse G. African Trypanosomes: Virulence Factors, Pathogenicity and Host Responses. Journal of Veterinary Advance. 2014;4(11):732-745.

28. Amisigo CM, Antwi CA, Adjimani JP, Gwira TM. *In vitro* anti-trypanosomal effects of selected phenolic acids on Trypanosoma brucei. Plos One. 2019; 14(5):21-68.
29. Albadrani BA. Clinical and hematological study of *Trypanosoma brucei* and Trypanosoma congolense in cattle in Mosul City, Iraq Res OpinAnim Vet Sci. 2012;2:92-97.
30. Kennedy PG. Clinical features, diagnosis, and treatment of human African trypanosomiasis (sleeping sickness) Lancet Neurol. 2013;12:186–194.
31. Allam L, Ogwu D, Agbede RIS, Sackey AKB. Hematological and serum biochemical changes in gilts ematological and serum biochemical changes in gilts experimentally infected with experimentally infected with *Trypanosoma brucei*. Vet. Arhiv. 2011;81:597-609.
32. Ikede BO, Lule Margaret, Terry RJ. Anaemia in trypanosomiasis: Mechanisms oferythrocyte destruction in mice infected with "Trypanosoma congolense" or "T. brucei". Acta Tropica. 2018;34:53-60.
33. Chamond N, Cosson A, Blom-Potar MC, Jouvion G, D'Archivio S, Medina M. Trypanosoma vivax Infections: Pushing Ahead with Mouse Models for the Study of Nagana. I. Parasitological, Hematological and Pathological Parameters. PLoS Negl Trop Dis. 2010; 4(8):792.
34. Tauheed AM, Shittu SH, Suleiman MM, Habibu B, Kawu MU, Kobo PI, Yusuf PO. *In vivo* ameliorative effects of methanol leaf extract of Lawsoniainermis Linn on experimental Trypanosomacongolense infection in Wistar rats, International Journal of Veterinary Science and Medicine. 2016;4(2):33-40.

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