

## Studies of Phytochemical Screening, Acute Toxicity and Anti-Diarrhoeal Effect of Aqueous Extract of Kenyan *Tithonia diversifolia* Leaves in Rats

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**Abstract:** *Tithonia diversifolia* (family-Asteraceae), a wildy growing plant has been reported to possess a number of medicinal properties being used traditionally in tropics especially Kenya and Nigeria. This study evaluated the phytochemicals, acute toxicity (100-10,000 mg/kg) and anti-diarrhoeal effect of *Tithonia diversifolia* on group of Wistar rats. The anti-diarrhoeal effect of aqueous extract of *Tithonia diversifolia* leaves (200, 400 and 800 mg/kg doses) was studied using castor-oil-induced-diarrhoea model (dropping test), castor-oil-induced enteropooling (secretory test) and gastrointestinal transit test (charcoal transit) in rats. In castor-oil-induced diarrhoea test, the rats' droppings were observed and noted as wet or dry with wet signifying characteristic diarrhoea. The castor-oil-induced enteropooling was done to determine the volume of intestinal content induced by castor oil while in gastrointestinal transit, the speed and percentage distance travelled by charcoal meal were noted to determine the anti-motility properties of the extract. The results showed that *Tithonia diversifolia* leaves' aqueous extract reduced wet faecal output in castor-oil-induced diarrhoea but with slightly greater frequencies in comparison with loperamide treated animals and had less volume of intestinal contents as compared with the negative control (distilled water treated animals). It also had a significant ( $p < 0.05$ ) non-dose dependent reduction in speed and distance travelled by charcoal in gastrointestinal tract but slightly higher speed and longer distance than the atropine treated rats. Therefore, *Tithonia diversifolia* leaves' aqueous extract has a remarkable anti-diarrhoeal effect in castor-oil-induced diarrhoea, enteropooling and gastrointestinal motility models attesting to its utility in a wide range of diarrhoeal states traditionally.

**Keywords:** Acute toxicity, anti-diarrhoea, leaves, phytochemicals, rats, *Tithonia diversifolia*

### INTRODUCTION

*Tithonia diversifolia* (family-Asteraceae) is a wildy growing composite shrub common on field boundaries in eastern Africa especially in Western and Central Provinces, coastal regions and parts of the Rift Valley of Kenya and is locally called 'Aketch'. It is moderately resistant to drought. It is a native shrub to Colombia, Guatemala, Honduras, Mexico, Nicaragua, Panama, US, Zanzibar and exotic in India, Kenya, Philippines (Orwa *et al.*, 2009). This shrub is an important medicinal plant in tropical Africa whose leaf

is the major organ used alone or in combination with other plants for the treatment of a wide variety of ailments such as stomach pains, indigestion, sore throat, liver diseases and pain. This is because the leaf is considered to have most of the active constituents (Orwa *et al.*, 2009). It has been reported to possess antiplasmodial activity (Ajaiyeoba *et al.*, 2006; Goffin *et al.*, 2002) testifying to the presence of sesquiterpene lactones as well as an artemisinin acid analog from *T. diversifolia* (Kuo and Chen, 1998; Bordoloi *et al.*, 1996); anti-inflammatory and analgesic activities (Owoyele *et al.*, 2004); bile, kidney, urinary and

venereal diseases, testicular inflammation, frigidity, sterility, heavy menstruation, rheumatism and arthritis, upper respiratory tract infections, ranging from cough to tuberculosis, intestinal worms and schistosomiasis, cancer chemopreventive activity (Jian-Qiao *et al.*, 2002); cytotoxic properties (Wu *et al.*, 2001; Coyle *et al.*, 1994) and antimicrobial activity (Ogundare, 2007; Singleton, 1999). Aerial parts of *Tithonia diversifolia* collected in São Paulo State (Brazil) afforded two new heliangolides in addition to the heliangolides tagitinin F and 1, 2-epoxytagitinin C, one known guaianolide and the flavone hispidulin whose structures were established by spectroscopic studies (Paulo *et al.*, 1997). A 70% ethanol extract of the aerial parts of *Tithonia diversifolia*, at a concentration used to treat malaria (*Plasmodium*), displayed kidney and liver toxicity at the lowest dose tested. Therefore the use of this plant extract against malaria raises concerns over its safety (Elufioye *et al.*, 2009).

Diarrhoea is the passage of three or more loose or liquid stools per day (or more frequent passage than is normal for the individual). It has been recognized as one of the most important health problems in the developing countries (Snyder and Merson, 1982) and is still one of the major health threats to population in tropical and subtropical countries (Heinrich *et al.*, 2005). The WHO has estimates that 4-5 billion cases occur each year with 1 billion in children below the age of 5 and 5 million deaths result from diarrhoea annually with 50% in children below the age of 5 (Abdullahi *et al.*, 2001). Diarrhoea and the associated faecal urgency and incontinence result from an imbalance between the absorptive and secretory mechanisms in the intestinal tract accompanied by hypermotility. This results in excess loss of fluid and electrolytes in faeces (Chitme *et al.*, 2004). To combat the problems of diarrhoea, the World Health Organization (WHO) has constituted a Diarrhoeal Diseases Control Programme (CDD) which includes the study of traditional medical practices, together with the evaluation of health education and prevention approaches (Lutterodt, 1989; Snyder and Merson, 1982; WHO Expert Committees, 1964). Due to the limited availability of affordable conventional pharmaceutical medicines in many tropical countries, about 80% of the rural population in Africa still depends on traditional herbal remedies to treat a variety of diseases including diarrhoea (WHO, 2002). It thus becomes important to identify and evaluate commonly available natural drugs as alternative to currently used anti-diarrhoeal drugs, which are not completely free from adverse effects. Several studies have evaluated the effectiveness of some traditional medicines in treating diarrhoea, in all different continents (Offiah and Chikwendu, 1999; Rani *et al.*, 1999; Zavata *et al.*, 1998; Onwukaeme and Udoh, 1998; Hutchinson and

Dalziel, 1957). Despite the widespread use of this plant in the traditional treatment of diarrhoea in Kenya and Uganda, there was no available literature on its use as an anti-diarrhoeal agent. This study was to identify the phytochemical constituents, acute toxic effects and justify the use of *Tithonia diversifolia* leaves' aqueous extract as an anti-diarrhoeal agent traditionally.

## MATERIALS AND METHODS

**Preparation of plant extract:** The *Tithonia diversifolia* plant was taxonomically identified by a botanist from Kampala International University-Western Campus (KIUWC) Ishaka, Bushenyi, Uganda. Fresh leaves of *Tithonia diversifolia* were then collected on 20<sup>th</sup> of April 2010 from Kisumu-Kenya in the morning hours during rainy season. A voucher specimen of the plant was deposited in the Herbarium of the Pharmacognosy Unit of School of Pharmacy KIUWC, Ishaka, Bushenyi, Uganda. The leaves were washed off sand and particles, separated and allowed to dry under a shade. The dry leaves were grounded into fine powder and sieved. Two hundred grams of the fine powder was dissolved in 1 L of distilled water, then loaded on a shaker for 24 h after which it was filtered and placed on a water bath at 40°C and left to evaporate till a concentrated solution was obtained and then weighed as *Tithonia diversifolia* leaves' aqueous extract (TDE).

**Preliminary phytochemical screening:** Qualitative phytochemical tests were conducted on the aqueous extract of *T. diversifolia* using standard methods (Trease and Evans, 2002).

**Laboratory animal acquisition and maintenance:** Male and female Wistar rats (weighing not less than 100 g) were used for this study. These animals were bred and housed in the Animal Facility Centre of the School of Pharmacy, KIUWC. The animals were separated and kept for 1 week to allow for acclimatization in a cage lined with wood shavings, at room temperature with adequate ventilation, under a naturally illuminated environment with 12 h of light and 12 h of darkness. They were fed on standard diet (Nuvita® Animal Feed Ltd, Jinja Uganda) and had access to clean drinking water *Ad libitum*. Animal care protocols were used according to the National Institute of Health Guide for the care and use of laboratory animals (NIH, 1996) and ethical considerations during investigation were adhered to.

**Determination of acute toxicity:** The Lorke (1983) method was modified (use of rats instead of mice) and used. This was conducted in two phases. In phase one, 3 groups of three rats per group ( $n = 3$ ), each received single oral dose of aqueous extract of *T. diversifolia* (group 1-100, group 2-500 and group 3-1000 mg/kg body weight, respectively) and the rats observed for six hours post administration for signs of toxicity and after 24 h, they were scored for mortality and general behaviour. In phase two, 3 groups of one rat each ( $n = 1$ ) were given geometrical doses of *T. diversifolia* leaves' aqueous extract based on findings in phase 1 which recorded no death after 24 h; (group 1-2,500, group 2-5,000 and group 3-10,000 mg/kg body weight, respectively) and then observed as in phase 1 above. The metrical mean of the smallest dose that killed a rat and the highest dose that did not kill a rat was taken as the mean lethal dose ( $LD_{50}$ ) of the extract.

**Castor oil-induced diarrhoea in rats (dropping test):** The method of Awouters *et al.* (1978) was adopted with slight modifications. The rats were fasted 12 hours prior to the commencement of the experiment. They were then divided into five groups of six rats each ( $n = 6$ ). Rats in the first group received loperamide 3 mg/kg (p.o), the second, third and the fourth groups received 200, 400 and 800 mg/kg body weight of *T. diversifolia* leaves' aqueous extract respectively, while the fifth group received 10 mL/kg (p.o) distilled water. After 30 min of administration of extract, 2 mL of castor oil (p.o) was administered. The animals were then placed in individual cages lined with adsorbent clean white paper. The animals were observed for 6 h for the presence of characteristic diarrhoea droppings and recorded using a predetermined scoring index (Di Carlo *et al.*, 1994) as follows: (+ +) for copious, (+) for mild and 0 for lack of diarrhoea. The total score of each group were taken, mean total number of droppings determined and activity expressed as % inhibition of diarrhoea.

**Castor oil-induced enteropooling (fluid accumulation method):** This was done according to Robert *et al.* (1976) modified by Di Carlo *et al.* (1994). In this method, the rats were deprived of food and water for 18 h prior to the experiment. Five groups of six animals each ( $n = 6$ ) were used; rats in the first group received loperamide 3 mg/kg (p.o) as a standard drug for comparison, the second, third and the fourth groups received 200, 400 and 800 mg/kg body weight of *T. diversifolia* leaves' aqueous extract respectively, while the fifth group received 10 mL/kg (p.o) of distilled

water. After 30 min of administration of extract, 2 mL of castor oil (p.o) was administered. All the rats were sacrificed with cervical dislocation after 30 min and the entire length of the intestine from the pylorus (the distal aperture of the stomach opening into the duodenum) to the caecum was dissected out and its contents collected into a clean crucible and measured using a 5 mL syringe. The volume obtained from the control (group 5) was used to compare the rest. Values less than the negative control (group 5) were recorded as a protection from diarrhoea and the percentage protection calculated.

**Gastrointestinal motility test (charcoal transit method):** In this method, rats were fasted for 18 h and placed in five groups of six ( $n = 6$ ) in each. Each animal was given 1 mL of charcoal meal (marker) (3% deactivated charcoal in distilled water). The first group received atropine (0.2 mg/kg body weight (i.p)) as a standard drug for comparison; the second, third and the fourth groups received 200, 400 and 800 mg/kg body weight of *T. diversifolia* leaves' aqueous extract respectively, while the fifth (control) group received 10 mL/kg (p.o) distilled water. The rats were sacrificed with cervical dislocation after 1 h and the distance travelled by charcoal meal from the pylorus towards the caecum was measured and expressed as percentage of the total length of the intestine from the pylorus to caecum (Mascolo *et al.*, 1994). The speed of transit of charcoal meal was also calculated.

**Statistical analysis of data:** The results for all the experiments were expressed as mean values  $\pm$  Standard Error of Mean (SEM) and group data comparisons were evaluated by an independent sample t-test with significance at  $p < 0.05$  using SPSS version 17.0.

## RESULTS

**Phytochemical analysis:** Phytochemical analysis of *T. diversifolia* leaves' aqueous extract gave positive reactions to alkaloids, flavonoids, phlobatannins, tannins, terpenoids, saponins and reducing sugar.

**Acute toxicity test:** Oral administration of *T. diversifolia* leaves' aqueous extract to rats up to 10,000 mg/kg caused no death in the two phases of the test after 24 h. Thus, the  $LD_{50}$  of *T. diversifolia* extract in rats was estimated to be greater than 10,000 mg/kg.

**Castor oil-induced diarrhoea (dropping) test:** Sixty minutes after administration of castor oil, the diarrhoea

Table 1: Effect of *T. diversifolia* extract on castor oil-induced diarrhoea in rats (dropping test)

Treatment	Diarrhoea score			Total score	Mean diarrhoea score	Inhibition of wet defaecation (%)
	++	+	0			
Loperamide 3 mg/kg	0	0	30	0	0.00±0.00	100.0
TDE 200 mg/kg	2	5	23	9	1.80±1.11*	60.9
TDE 400 mg/kg	3	7	20	13	2.60±1.21	43.5
TDE 800 mg/kg	1	6	23	8	1.60±0.93*	65.2
Distilled water 10 mL/kg	10	3	13	23	4.60±1.86	0.0

n: 6; values presented as mean±standard error of mean (S.E.M); \*: p<0.05 was considered significant when compared with distilled water-treated group (control) (independent sample t-test); Inhibition of defaecation was calculated relative to the control

Table 2: Effect of *T. diversifolia* extract on castor oil-induced enteropooling in rats (fluid accumulation test)

Treatment	Volume of intestinal contents (mls)	Inhibition of intestinal content (%)
Loperamide 3 mg/kg	0.68±0.07*	34.00
TDE 200 mg/kg	0.98±0.12	4.90
TDE 400 mg/kg	0.82±0.05	20.40
TDE 800 mg/kg	0.82±0.06*	20.40
Distilled water 10 mL/kg	1.03±0.16	0.00

n: 6; values presented as mean±standard error of mean (S.E.M); \*: p<0.05 was considered significant when compared with control (independent sample t-test)

was clinically apparent in most animals of control group (distilled water treated animals) which continued for the next 4 h. Diarrhoea was completely inhibited (100%) by 3 mg/kg of loperamide administered per oral throughout the period of the experiment (Table 1). A marked non dose dependent reduction in the number of defaecations over four hours was achieved with TDE in doses of 200, 400 and 800 mg/kg (p.o) with 200 (60.9%) and 800 mg/kg (65.2%) doses recording statistically significant proportions (p<0.05) and higher inhibition than 400 mg/kg (43.5%) when compared with the control. Oral dose of 400 mg/kg TDE delayed the onset of diarrhoea (wet faeces) and only 28% of animals on treatment showed diarrhoea during the first hour. The 200 and 800 mg/kg, p.o. doses of the extract had minimal effect on severity and onset of diarrhoea.

**Castor oil-induced enteropooling:** Castor oil caused accumulation of water and electrolytes in intestinal loop. Castor oil-induced enteropooling was significantly (p<0.05) inhibited by loperamide in rats (3 mg/kg, p.o) at 34% when compared with the control. Each dose of the extract produced a non dose dependent reduction in intestinal content volume. Two hundred mg/kg, (p.o) dose of extract produced 4.9% inhibition of volume of intestinal content. However, 400 and 800 mg/kg (p.o) doses each produced 20.4% inhibition of volume of intestinal content with 800 mg/kg producing significant reduction of volume of intestinal content at p<0.05 (Table 2).

**Gastro intestinal transit test (charcoal transit):** The percentage of the intestinal charcoal transit was

increased in distilled water treated rats (40.07±5.13%) but much more markedly reduced by atropine (19.75±7.26%) and recorded non dose dependent reduction among the three doses of extract (200, 400 and 800 mg/kg (p.o), with 200 and 400 mg/kg doses producing statistically significant inhibition (29.06±2.12)% and (35.92±8.46%) of intestinal transit respectively; whereas 800 mg/kg dose produced a non-statistically significant 39.27±1.84% inhibition of castor oil induced charcoal meal transit (Table 3). The speed of intestinal transit followed the same pattern as in the intestinal transit as follows: it increased with distilled water (0.73±0.0004 cm/min), but much more markedly reduced by 0.2 mg/kg (i.p) atropine (0.36±0.001 cm/min). Also the transit speed was reduced in each of the three doses of extract (p.o), with 200 and 400 mg/kg producing 0.49±0.0004 cm/min and 0.61±0.03 cm/min intestinal transit speed respectively; whereas 800 mg/kg dose produced 0.39±0.002 cm/min charcoal meal transit speed (Table 3).

## DISCUSSION

The phytochemical analysis of the extract showed the presence of alkaloids, reducing sugars, flavonoids, tannins, terpenoids, phlobatannins and saponins; either one or combination of which may be responsible for the observed anti-diarrhoeal effect of *T. diversifolia*.

Acute toxicity test on the TDE in rats established a high LD<sub>50</sub> which suggests that the aqueous extract of the leaves of *T. diversifolia* may be generally regarded as safe with a remote risk of acute intoxication and sedation at high doses of between 5,000 mg/kg and 10,000 mg/kg of body weight. The high degree of safety is also consistent with its popular use locally. However, caution should be exercised with its rampant use because (Elufioye *et al.*, 2009) reported that a 70% ethanol extract of the aerial parts of *Tithonia diversifolia* at a concentration used to treat malaria (*Plasmodium*) exhibited kidney and liver toxicity at the lowest dose tested thereby calling for sub-chronic and chronic safety evaluation of this widely used plant.

Diarrhoea results from an imbalance between the absorptive and secretory mechanisms in the intestinal

Table 3: Effect of *T. diversifolia* extract on gastro intestinal transit of charcoal in rats (git motility test)

Treatment	Total mean length travelled (cm)	Mean distance travelled by maker (cm)	Intestinal transit (%)	Speed of transit (cm/min)
Atropine 0.2 mg/kg	110.37±4.12	21.73±8.70*	19.75±7.26	0.36±0.0010
TDE 200 mg/kg	101.60±2.49	29.58±2.73*	29.06±2.12	0.49±0.0004
TDE 400 mg/kg	101.18±1.82	36.27±12.13*	35.92±8.46	0.61±0.0300
TDE 800 mg/kg	100.17±2.72	23.52±1.66	23.27±1.84	0.39±0.0020
Distilled water 10 mL/kg	110.03±8.13	44.00±3.96	40.07±5.13	0.73±0.0004

n: 6; values presented as mean±standard error of mean (S.E.M); \*: p<0.05 was considered significant when compared with control (independent sample t-test); GIT: Gastro Intestinal Tract

tract; accompanied by hypermotility, resulting in an excess loss of fluid in the faeces. In some diarrhoea, the secretory component predominates, while other diarrhoeas are characterized by hypermotility. The use of castor oil induced diarrhoea model in our study is logical because the autocoids and prostaglandins have been implicated in the causation of diarrhoea in man (Horton *et al.*, 1968; Greenbargena *et al.*, 1978). The liberation of ricinoleic acid from castor oil results in irritation and inflammation of the intestinal mucosa, leading to release of prostaglandins, which stimulate motility and secretion (Pierce *et al.*, 1971). The results of the present study showed that the extract of *T. diversifolia* (TDE) produced a statistically significant reduction in the severity and frequency of diarrhoea induced by castor oil. It was also noted that the extract significantly inhibited castor oil induced intestinal fluid accumulation exhibiting a non-dose dependent reduction in volume of intestinal content. There was also a significant non-dose dependent reduction in the intestinal transit of charcoal meal. Atropine drastically reduced intestinal transit time and this might possibly be due to its anti-cholinergic (antimuscarinic) effect (Brown and Taylor, 1996).

Castor oil induced diarrhoea increases the volume of intestinal content by prevention of the reabsorption of water. Liberation of ricinoleic acid results in irritation and inflammation of the intestinal mucosa thus leading to release of prostaglandins which results in stimulation of secretion (Pierce *et al.*, 1971) thereby prevents the reabsorption of NaCl and H<sub>2</sub>O (Galvez *et al.*, 1993). These observations reasonably suggest that the extract inhibits gastrointestinal hyper-secretion and enteropooling by enhancing electrolytes (reabsorption of NaCl), solutes and water absorption from the intestinal lumen by decreasing intestinal motility as observed by the decrease in intestinal transit of charcoal meal. The anti-diarrhoeal activity of the extract may also be due to the presence of denatured proteins forming protein tannates (a salt of tannin); protein tannates make the intestinal mucosa more resistant and reduce secretion (Tripathi, 1994). The secretory diarrhoea is associated with an activation of Cl<sup>-</sup> channels, causing Cl<sup>-</sup> efflux from the cell, the efflux

of Cl<sup>-</sup> results in massive secretion of water into the intestinal lumen and profuse watery diarrhoea (Ammon and Soergel, 1985). The extract may inhibit the secretion of water into the lumen by reverting this mechanism.

Castor oil is a suitable model of diarrhoea in rats, since it allows the observation of measurable changes in the number of stools and intestinal content volume. *T. diversifolia* leaves' aqueous extract resulted in a marked reduction in the number of diarrhoea (wet stools) and the reduction in the volume of the intestinal contents signifying the usefulness of this model and the clinical effect of the TDE.

The remarkable anti-diarrhoeal effect of TDE in castor oil-induced diarrhoea model attests to its wide range of utility in secretory and functional diarrhoeas. This study did not go further to demonstrate whether the extract specifically altered the activity of Na<sup>+</sup>K<sup>+</sup>ATPase or activation of chloride channels. Whatever may be the mechanism of action, TDE may be useful in a wide range of diarrhoeal states, due to both disorders of transit e.g., functional and radiation diarrhoeas or due to abnormal secretory mechanisms like in cholera or *E. coli* enterotoxin induced diarrhoea.

Hypermotility characterizes forms of diarrhoea where the secretory component is not the causative factor (Chitme *et al.*, 2004). Pre-treatment with the TDE suppressed the propulsive movement or transit of charcoal meal through the gastrointestinal tract which clearly indicates that the *T. diversifolia* leaves' aqueous extract may be capable of reducing the frequency of stooling in diarrhoeal conditions. Delay in gastric motility causes further absorption of water from faeces and may additionally contribute to reducing its watery texture. Although not directly supported by our experimental data, it is likely that the extract inhibits gastrointestinal hypermotility in diarrhoea through anticholinergic effect. Anticholinergic agents are known to inhibit gastrointestinal hypermotility. Gastrointestinal hypermotility has been suggested to be indirectly mediated by the cholinergic system since it is inhibited by atropine, a known anticholinergic agent (Brown and Taylor, 1996).

Anti-dysenteric and antidiarrhoeal properties of medicinal plants can be attributed to their

phytochemical constituents. Research testifies of anti diarrhoeal properties due to tannins, alkaloids, saponins, flavonoids, sterols and/or triterpenes and reducing sugars (Longanga-Otshudi *et al.*, 2000). The sesquiterpene lactones, a large group of compounds with anti-inflammatory properties have the ability to relax smooth muscles and thereby relieve gastrointestinal distress (Heinrich *et al.*, 2005). It should be recalled that the phytochemical analysis of the extract revealed the presence of alkaloids, reducing sugars, flavonoids, tannins, terpenoids, phlobatannins and saponins. Sesquiterpenoids, in addition, have also been reported to have been isolated from *T. diversifolia* by Jian-Qiao *et al.* (2002). These constituents may mediate the antidiarrhoeal property of the *T. diversifolia* leaves' aqueous extract. Although the antidiarrhoeal properties of the reported active terpenoids are well established, aspects of their mechanism of action remain poorly understood. Sesquiterpenes, diterpenes, terpenes, flavonoids and terpenoid derivatives are known for inhibiting release of autocoids and prostaglandins, thereby inhibit the motility and secretion induced by castor oil (Veiga *et al.*, 2001; Nikiema *et al.*, 2001; Vimala *et al.*, 1997; Milanova *et al.*, 1995). Flavonoids have been shown to possess anti-diarrhoeal activity attributable to their ability to inhibit intestinal motility and hydro-electrolytic secretion (Rao *et al.*, 1997; Di Carlo *et al.*, 1993). Whatever mechanisms and constituents are involved, the results of this study showed that the extract reduced gastric contents and watery texture of diarrhoeal stools as well as gastrointestinal motility thus leading to the much desired reduction in frequency of stooling in diarrhoeal disease.

However, further studies are needed to completely understand the actual mechanisms and the specific constituent (s) responsible for the anti-diarrhoeal action of *Tithonia diversifolia* leaves' aqueous extract.

### CONCLUSION

The aqueous extract of *Tithonia diversifolia* leaves has a considerable anti-diarrhoeal effect in castor-oil-induced diarrhoea, enteropooling and gastrointestinal motility models confirming the reason for its wide use in traditional treatment of various diarrhoeal conditions.

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