

Ulcer protective action is improved by washing fresh *Aloe vera* gel: a comparative study with unwashed gel in indomethacin and ethanol induced ulcers in rats

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ABSTRACT

Introduction: The *Aloe vera* and its components has been widely suggested for its ulcer protective action. Traditional Indian Siddha medical literature claims that washing the fresh *Aloe vera* gel (FAVG) has improved ulcer protective action due to the removal of bitter yellow latex. In this study, we compared the ulcer protective effect of unwashed as well as washed fresh *Aloe vera* gel in rats. **Methods:** We prepared washed and unwashed FAVG. Albino rats were fed with normal saline, ranitidine suspension, washed and unwashed FAVG for 8 days. Drugs have been tested in two ulcer models, in which indomethacin (20mg/kg) and ethanol (80%) were used for the induction of ulcer after animals were fasted for 24 hours. The animals were sacrificed and stomach was cut open to calculate mean ulcer index and a section was sent for histopathological examination. One Way ANOVA was done for analysis. **Results:** In indomethacin induced ulcer model, the mean ulcer index in control, ranitidine, washed and unwashed FAVG treatment groups were 7.67 ± 1.51 , 1.92 ± 1.12 , 2.17 ± 1.16 and 4.0 ± 1.09 respectively. The percentage of ulcer protection in washed FAVG treatment (71.71%) is comparable with ranitidine treatment (74.97%), whereas the unwashed FAVG showed only 47.84% ulcer protection. In ethanol induced ulcer model, the mean ulcer index in control, ranitidine, washed and unwashed FAVG treatment groups were 7.83 ± 1.51 , 1.83 ± 0.82 , 2.33 ± 1.37 and 4.17 ± 1.47 respectively. The percentage of ulcer protection in washed FAVG treatment (70.24%) is comparable with ranitidine treatment (76.63%), whereas the unwashed FAVG showed only 46.74% ulcer protection. In both the above ulcer models, washing the FAVG showed statistically significant ($p < 0.05$) reduction in ulcer index and improved ulcer protection. **Conclusions:** Washed fresh *Aloe vera* gel has shown higher ulcer protective activity compared to unwashed gel.

Keywords: *Aloe vera* gel, Aloe latex, Gastric Ulcer, Indomethacin, Ethanol, Siddha, Ayurveda

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INTRODUCTION

Peptic ulcer is a remitting, relapsing lesion that is most often diagnosed in middle-aged to older adults which impairs the quality of life; it is one of the leading causes of gastrointestinal surgery with high morbidity and mortality rates.^[1]The pathogenesis of ulcers includes mainly aggressive factors (acid, pepsin, bile and *Helicobacter pylori* infection) which is complemented by factors such as stressful lifestyle, alcohol consumption, smoking, use of steroidal and nonsteroidal anti-inflammatory drugs (NSAIDs) and

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lower socioeconomic status.^[2-5]

In addition to allopathy drugs, herbal plants are also sought after in anti-ulcer drug market due to their safer potential, effectiveness and convenience. *Aloe vera* has been used by eastern herbalists for its amazing healing powers to wounds and ulcer of the skin.^[6,7] In past quarter of a century, western medicine has started to analyse the medicinal properties of *Aloe vera*.^[8] clinical benefit was recorded in clinical cases as early as early 1960s.^[9] It contains more than two hundred ingredient including essential amino acids, enzymes, glucose and more. Anti-ulcer activities is may due to its anti-oxidant property, anti-inflammatory, mucus secreting, cytoprotective or healing activities.^[8] It can be used to treat peptic ulcer with lesser side effects and is accessible for people with lower economical standard.

In studies measuring gastric acid secretion by Keshavarziet. al.^[10], *Aloe vera* group showed significant decrease in gastric acid levels compared to gastric ulcer group. Formation of acute gastric lesions has been shown to be prevented after pre-treatment with oral administration of powdered fresh gel of *Aloe vera* gel (150mg kg⁻¹).^[11] Borraet. al. found that *Aloe vera* powder mixed with gum acacia showed statistically significant anti-ulcer activity comparable to standard drug omeprazole.^[12] Emalamnet. al. showed that the gastric ulcer healing property could be due to reduction of leukocyte adherence and TNF- α level and elevation of IL -10 level.^[13]

In Nepal, people use fresh whole *Aloe vera* leaf gel, just peeling the outer covering of plant. In the fresh leaf fresh gel, contain two primary components gel and latex or exudates. *Aloe vera* latex is yellow brownish colour with bitter taste (often referred to as Aloe sap), present between the plants outer skin and the pulp.^[14,15] The latex has long been recognized for its purgative action and has diarrhoea, abdominal cramps and other gastro intestinal side effects.^[15] These side effects of latex may possibly antagonize the anti ulcerogenic activity of *Aloe vera* gel. Traditional Siddha Medical literature claim that washing of the leaf with water for 7 times can reduce concentration of bitter latex from the gel which might be beneficial in healing of gastric ulcer.^[16] The influence of washing of *Aloe vera* fresh gel on its protective effect against gastric ulcer remains to be assessed.

Thus, the purpose of this study was to investigate the anti-ulcer effects of washed and unwashed whole leaf fresh gel of *Aloe vera* in preventing indomethacin and ethanol induced gastric ulcer.

METHODS

Preparation of Aloe vera gel

Whole leaves of *Aloe vera* were collected from local area of Dhulikhel, Nepal. Before categorising group, whole leaf fresh gel of *Aloe vera* gel was obtained from the plant by peeling off outer covering and collecting gel with help of spatula. Fresh *Aloe vera* gel was collected. For washed group, Fresh *Aloe vera* gel was washed seven times in order to remove yellowish mucilage, *Aloe vera* latex which tastes bitter and has laxative and irritant effect. For unwashed group, the fresh whole leaf *Aloe vera* gel was not washed. The gel was liquefied with distilled water by motor grinder and fed to rats. This fresh gel was prepared on the day of each administration.

Experimental animals

The experimental protocol was approved by Institutional Review Committee (KUSMS/IRC). The protocol approval number is 67/12. The study was performed in the experimental laboratory in department of Pharmacology, Kathmandu University School of Medical Sciences (KUSMS). Healthy albino Wistar rats, 5-6 months old, of either sex weighing 150-200 gm were used. The rats were maintained under standard environmental condition in an animal house and were provided with standard diet and water *ad libitum* as approved by the committee. The animals were kept for two weeks prior to experiment to acclimatize. They were randomly allocated to different experimental groups and placed in cages.

Acute Toxicity Study

Acute toxicity study was conducted in rats of either sex weighing 150-200 gm. Rats were fasted overnight and divided into six groups of two rats each. Whole leaf fresh gel of washed and unwashed *Aloe vera* gel was administered orally with the help of a feeding tube to each group in ascending and widely spaced doses viz. 1, 30, 100, 300, 1000, 3000 mg/kg. The rats were observed continuously for two hours and then occasionally for further four hours and finally overnight mortality was recorded. Mortality and behavioural changes were not observed even at maximum dose of 3000 mg/kg of both washed and unwashed gel of *Aloe vera*. Therefore, 300 mg/kg was selected for the study.^[17]

Study on anti-ulcer activity

Study plan: Seventy two rats of either sex were grouped.

Half of the mouse were kept in indomethacin model (group 1-6) and other half in ethanol model (group 7-12). The detail is shown in Table 1

Indomethacin induced ulcer

The control groups (group 1 and group 4) received normal saline (2ml/kg), positive control groups (group 2 and 5) received ranitidine (20 mg/kg) and test group (group 3 and 6) received fresh washed and unwashed gel of *Aloe vera* (300 mg/kg) respectively, orally for eight days. After eight days, animals were fasted for 24 hours. Ulcer in this method was produced by oral administration of aqueous suspension of indomethacin (20mg/kg) and the animals were sacrificed four hours later. The stomach were cut open to observe presence of ulcers, ulcer index were calculated and sent to the Pathology Laboratory of Dhulikhel Hospital for histopathological examination.

Ethanol induced Ulcer

The control groups (group 7 and group 10) received normal saline (2ml/kg), positive control groups (group 8 and 11) received ranitidine (20 mg/kg) and test group (group 9 and 12) received fresh washed and unwashed gel of *Aloe vera* (300 mg/kg) respectively, orally for eight days. After eight days, animals were fasted for 24 hours. In this method ulcer was induced by administration of 80% ethanol (2 ml) and the animals were sacrificed after one hour. The stomach were cut open to observe presence of ulcers, ulcer index were calculated and sent to the Pathology Laboratory of Dhulikhel Hospital for histopathological examination.

Ulcer Index

The dissected stomachs were cut open along the less curvature and inner surface was examined for ulceration. The open stomachs were studied by individuals who were blinded for test drugs and control animals.

Ulcer indexing was done according to the modified scoring system of Adami et al. as follows: 0 = no lesions, 1 = hemorrhagic suffusions, 2 = 1 to 5 small ulcers up to 3 mm size, 3 = 5 small ulcers or 1 ulcer of ≥ 3 mm, 4 = many ulcers of more than 3 mm, 5 = perforated ulcers. The mean scores for each group were then calculated and the results were analysed.

The percentage of ulcer protection was determined as follows:

$$\% \text{ protective} = \frac{CMUI - TMUI}{CMUI} \times 100\%$$

CMUI = Control Mean Ulcer Index

TMUI = Test Mean Ulcer Index

Histological studies

The stomachs were isolated and fixed in 5% buffered neutral formalin solution. After fixation, tissues were embedded in paraffin; solid sections were cut at 5 μ m and stained with hematoxylin and eosin. The sections were examined with the help of pathologist under light microscopy.

Statistical Analysis

The values for ulcer index were expressed in terms of mean \pm SD for six rats in each group. The measured indexes were tabulated in MS excel and analysed using SPSS (statistical Package for Social Sciences Version 7.5)

Hypothesis testing method included One Way Analysis of Variance (ANOVA) followed by post hoc followed with Least Significance Difference (LSD) test. P values less than 0.05 were considered to be statistically significant.

RESULTS

The gastric ulcer of control group (washed and unwashed) presented with features of ulceration. On gross examination, serosal surface of stomach showed disruption, thickening of walls, dilated blood vessels and multiple areas of haemorrhagic sites. Mucosal surface showed multiple acute ulcers of variable sizes ranging from pin point to 0.5 cm with clot formation at the centre. Sites of perforation of mucosa were also discerned. Remaining of mucosa presented with hyperaemic, congestion and edema (Figure 2a & Figure 3a). The ulcer index (UI \pm SD) was 7.67 \pm 1.51 (Table 2).

Microscope examination showed breach in the lining mucosa extending beyond muscularis mucosa with dense, acute inflammatory cells. There was absence of fibrous reaction in the ulcer bed.

The one way of ANOVA of mean ulcer index is shown in table 2.

A normal mucosal surface of stomach of rat is shown in figure 2. The photograph of mucosal surface of

Table 1: Different groups of rats with different treatment

	Oral pre-treatment	Ulcer induction 20mg/kg indomethacin	Ulcer induction 80% Ethanol (2ml)
Washed	2 ml Saline	Group 1	Group 7
	20 mg/kg Ranitidine	Group 2	Group 8
	300 mg/kg <i>Aloe vera</i> gel (washed)	Group 3	Group 9
Unwashed	2 ml Saline	Group 4	Group 10
	20 mg/kg Ranitidine	Group 5	Group 11
	300 mg/kg <i>Aloe vera</i> gel (unwashed)	Group 6	Group 12

Table 2: One way analysis of Mean Ulcer Index(mean ± SD)and percentage protection against indomethacin(20mg/kg) and Ethanol(80%) induced ulcer by Ranitidine, washed fresh gel of Aloe vera and unwashed fresh gel of Aloe vera

Groups	Dose	Indomethacin Induced Ulcer (20 mg/kg)		Ethanol Induced Ulcer (2ml, 80% Ethanol)	
		Mean Ulcer Index ± SD	Percentage Protection (%)	Mean Ulcer Index ± SD	Percentage Protection (%)
Control (saline)	2 ml/kg	7.67± 1.51		7.83(1.51)	
Ranitidine	20 mg/kg	1.92 ±1.12* ⁺	74.97	1.83 ±0.82* ⁺	76.63
<i>Aloe vera</i> Washed	300 mg/kg	2.17± 1.16* ⁺	71.71	2.33 ±1.37* ⁺	70.24
<i>Aloe vera</i> unwashed	300 mg/kg	4.0 ±1.09*	47.84	4.17 ±1.47*	46.74

n=6 animals in each group

*statistically significant with respect to control (P<0.05) at 95% Confidence Interval

+statistically significant with respect to *Aloe Vera* unwashed (P<0.05) at 95% Confidence Interval



Figure 1: Photograph showing normal rat stomach

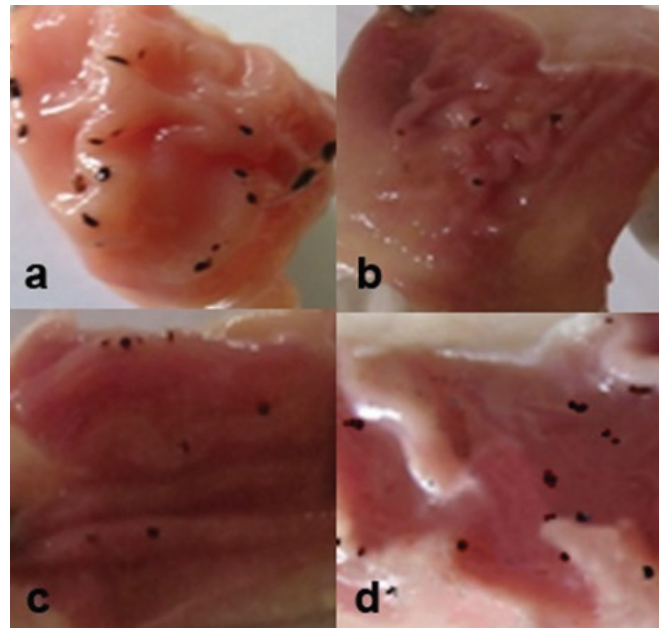


Figure 2: Photograph showing indomethacin induced ulcers in rat stomach. a. control b. ranitidine pretreated c. washed *Aloe vera* gel d. unwashed *Aloe vera* gel

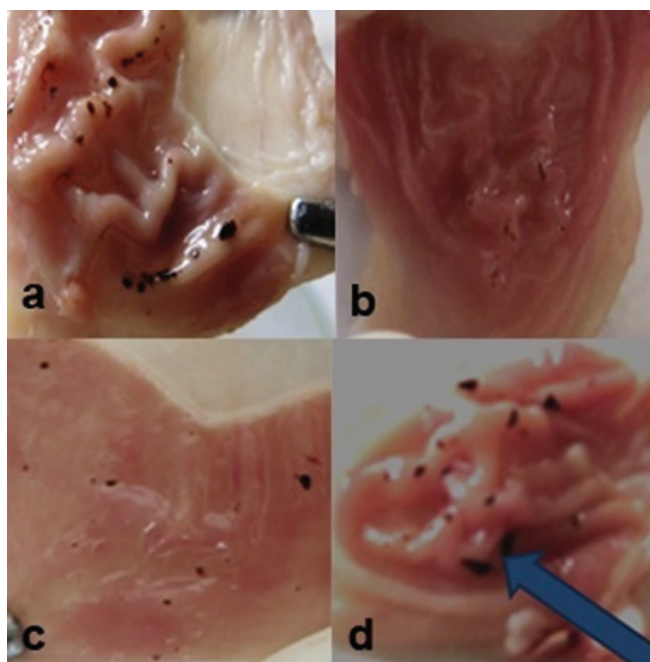


Figure 3: Photograph showing ethanol induced ulcers in rat stomach. a. control b. ranitidine pretreated c. washed *Aloe vera* gel d. unwashed *Aloe vera* gel

indomethacin induced ulcer and ethanol induced ulcer is shown in figure 3 and 4.

Indomethacin induced ulcer

As shown in Table 2, mean ulcer index for control was 7.67 ± 1.51 . The mean ulcer index in pretreated groups with Ranitidine, Washed *Aloe vera* gel and unwashed *Aloe vera* gel was 1.92 ± 1.12 , 2.17 ± 1.16 and 4.0 ± 1.09 respectively. The result is statistically significant with respect to control at P VALUE. Similarly, mean ulcer index of *Aloe vera* gel washed and Ranitidine was found to be significantly different from ulcer index of *Aloe vera* gel unwashed ($p=0.018$ & $p=0.000$ respectively).

Ethanol induced ulcer

The mean ulcer for control was 7.83 ± 1.51 and for Ranitidine pre-treated, Washed *Aloe vera* gel pre-treated group and unwashed *Aloe vera* gel pre-treated group was 1.83 ± 0.82 , 2.33 ± 1.37 and 4.17 ± 1.47 which were found to be statistically significant with respect to control. Similarly, mean ulcer index of *Aloe vera* washed ($p=0.026$) and Ranitidine ($p=0.006$) was found to be significantly different from ulcer index of *Aloe vera* gel unwashed as well.

The mean ulcer index in Indomethacin Induced Ulcer and Ethanol ulcer did not vary statistically between each other showing that drug involved in induction of ulcer did not influence the measure of ulcer index.

DISCUSSION

In the present study, the anti-ulcer effect of washed and unwashed whole leaf fresh gel of *Aloe vera* gel in Non-Steroidal Anti-Inflammatory Drug (indomethacin) and ethanol induced peptic ulcer was observed. The mean ulcer index of washed and unwashed whole leaf fresh gel of *Aloe vera* gel is 2.17 ± 1.16 and 4.0 ± 1.09 respectively in indomethacin induced ulcer and 2.33 ± 1.37 and 4.17 ± 1.47 respectively in ethanol induced ulcer. In this study we have tried to investigate whether or not the presence of the latex alters the antiulcer effect, so we compared with the washed and unwashed whole leaf fresh gel of *Aloe vera* gel. Unwashed *Aloe vera* gel is observed to have less protective effect against gastric ulcer (47.84% and 46.74%) compared to washed *Aloe vera* gel (71.71% and 70.24%).

In most of the cases, non-steroidal anti-inflammatory drug like indomethacin is known to induce numerous puncti form and fili form gastric ulcers during the course of anti-inflammatory therapy. Although, the mechanism underlying the ulcerogenicity of indomethacin are not completely known, but suppression of mucosal prostaglandins synthesis and a direct irritative, topical effect may be important^[18]. Furthermore, ethanol damage to the gastrointestinal mucosa starts with micro-vascular injury, namely disruption of the vascular endothelium resulting in increased vascular permeability, edema formation and epithelial lifting. Ethanol produces necrotic lesions in the gastric mucosa by its direct toxic effect, reducing the secretion of bicarbonates and production of mucus.^[19]

The *Aloe vera* whole leaf fresh gel, contain two primary components (the gel and the latex or exudates) of the leaf, which have been used for various reasons in traditional medicine. Generally, the gel is used topically to soothe wounds, burns, skin irritations and the latex is recognized to possess cathartic effects.^[14] The leaf of *Aloe vera* is protected by a thick, green epidermis layer (skin or rind), which surrounds the mesophyll. Immediately beneath he rind are located the vascular bundles, which are composed of three types of tubular structures: the xylem, the phloem and the large pericyclic tubules, which stores and transports bitter yellow latex (often referred to as Aloe sap) along the margin of the leaf along the margin of the leaf.^[14] The pericyclic portion of the vascular bundle is adherent to the rind.^[20] The inner leaf pulp, the parenchyma makes up the majority of the plant by volume which is the major part of the leaf by volume, contains a clear mucilaginous gel (known as *Aloe vera* gel).^[21,22]

Aloe vera latex, present between the plant's outer skin

and pulp. These species are harvested for their bitter leaf exudate or ‘latex’, important in trade as the source of drug aloes or ‘aloin’, used for its purgative effects. The C- Glycosides, barbaloin and isobarbaloin present in *Aloe vera* latex have been shown to be a chief constituent exerting purgative effect on gastrointestinal tract. Similarly, Aloin was reported to have laxative effect greater than placebo which was stronger than stimulant laxative phenolphthalein in a double blind, randomized control trial performed by Chapman and Pitelli in 1974. The use of *Aloe vera* latex as laxative has also been associated with several gastrointestinal adverse effects like vomiting, diarrhoea and electrolyte imbalance. All these factors might counteract with the protective effect of unwashed *Aloe vera* gel.

The anti-ulcer activity of *Aloe vera* gel is due to its anti-inflammatory^[23], cytoprotective^[24], healing^[25] and mucus stimulatory effects.^[26] The anti-inflammatory activity of *Aloe vera* gel has been revealed by a number of *in vitro* and *in vivo* studies. *In vivo* studies have demonstrated that *Aloe vera* gel promotes wound healing by directly stimulating the activity of macrophages and fibroblasts.^[27] Fibroblast activation by *Aloe vera* gel has been reported to increase both collagen and proteoglycan synthesis, thereby promoting tissue repair.^[27] The active monosaccharides, mannose 6-phosphate of *Aloe vera* gel, can bind to the growth factor receptors on the surface of the fibroblasts and thereby enhance their wound healing properties.^[27,28] Furthermore, acemannan, a complex carbohydrate isolated from *Aloe vera* gel is a potent macrophage-activating agent and therefore may stimulate the release of fibrogenic cytokines. The growth factors may directly bind to acemannan, promoting their stability and prolonging their stimulation of granulation tissue thereby accelerate wound healing and reduce radiation induced skin reactions.^[29,30] The therapeutic effects of *Aloe vera* gel also include prevention of progressive dermal ischemia caused by burns, frostbite, electrical injury and intra-arterial drug abuse. *In vivo* analysis of these injuries demonstrates that *Aloe vera* gel acts as an inhibitor of thromboxane A₂, a mediator of progressive tissue damage.^[27,31]

Fresh *Aloe vera* gel significantly reduced acute inflammation in rats (carrageenin-induced paw oedema), although no effect on chronic inflammation was observed^[29]. *Aloe vera* gel appears to exert its anti-inflammatory activity through bradykinase activity and thromboxane B₂ and prostaglandin F₂ inhibition.^[32-34] Furthermore, three plant sterols in *Aloe vera* gel (lupeol, campesterol, and β -sitosterol) reduced inflammation by up to 37% in croton oil-induced oedema in mice.^[28] Lupeol, one of the sterol compounds found in *Aloe vera*, was the most active and reduced inflammation

in a dose dependent manner.^[28]

The species *Aloe buettneri* increased gastric mucus production.^[35] Iranian journal of pharmaceutical research: IJPR, page: 69-74, volume: 10, issue: 1, source: PubMed, abstract: Aloe buettneri A. Berger is commonly used in traditional Togolese medicine to treat inflammatory and gastric ulcers. The present study examined the gastro-protection effect of the hydro-alcoholic extract of *A. buettneri* on mucus production and gastric pH. A gastric ulcer is induced by ethanol 95° alone (1 mL/kg body weight). Gastric mucus is a viscous, elastic, adherent and transparent gel formed by water and glycoproteins covering the entire gastrointestinal mucosa. Therefore, *Aloe vera* induces mucus which protects the gastric mucosa against irritants, such as ethanol, HCL and acetyl acid.^[36] Journal of Ethnopharmacology, page: 215-224, volume: 104, issue: 1-2, source: PubMed, abstract: The hydroethanolic extract of the leaves (HEL). Hence administration of *Aloe vera* gel decreases the ulcer index and ulcerated surface. The cyto-protective action of *Aloe vera* gel may be due to its active ingredients like tannins, saponins and flavonoids.^[37]

A large number of biological activities have been ascribed to *Aloe vera* to explain its purported health benefits, including antimicrobial, anti-inflammatory, lipid and glucose lowering, antiproliferative, immunostimulatory, and antioxidant functions.

A number of potentially active ingredients in the latex and gel of *Aloe vera* have been identified; however, much has yet to be determined about their mechanisms of action. Further studies are also required to determine the active properties of *Aloe vera* constituents and to explore the competitive of synergistic actions of particular combinations of ingredients.

CONCLUSIONS

Our study shows activity ulcer protective activity of both washed and unwashed whole leaf fresh gel of *Aloe vera* against indomethacin and ethanol induced gastric ulcer. Washed *Aloe vera* gel has shown more protective effect compared to unwashed *Aloe vera* gel. The mean ulcer indices were significantly reduced, suggestive of anti-ulcerogenic activity of *Aloe vera* gel. Thus, our study supports the rational for the claim mentioned in Siddha Medicine literature of washing *Aloe vera* gel for 7 times, while using for peptic ulcer. However, the exact cellular mechanism

for these actions remain to be established.

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