



DURATIONAL ENHANCED ANTIULCEROGENIC POTENTIALS OF ALOE VERA WITH OMEPRAZOLE IN GASTRIC ULCER

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ABSTRACT

A comparative study of curative potentials of Aloe Vera juice with proton pump inhibitor; omeprazole was carried on 30 adult albino wister rats for the period of 28 days. Rats administered with 122.47mg/kg of Aloe Vera juice had decrease ($P < 0.05$) in ulceration i.e high rate of ulcer healing but there was a significant increase ($P < 0.01$) in gastric acid secretion compared to omeprazole and control

groups. However, there was a significant decrease ($P < 0.01$) in gastric output but increase in ulceration, ($P < 0.05$) in omeprazole treated group than in Aloe Vera and the control groups. Clinically gastric ulceration predisposes to morbidity and mortality rates. It is concluded that Aloe Vera juice could be used as combined therapy with omeprazole in the treatment of gastric ulcer.

KEYWORDS: Aloe Vera, omeprazole, gastric acidity, ulcer scores.

INTRODUCTION

Plants have been proven as very important source of medication and the World Health Organization (WHO) estimates that about 80% of people derive treatment of ailments through plant and its extract and many drugs used today are derivatives of plants, (Jansen 2011). This has made natural products to be the emerging source of contemporary alternative therapies in most cases of challenging and upsurgent diseases. Aloe Vera is a member of Liliacea (Lily family). The genus Aloe contains 250 species and Aloe Vera is the best known of all species (Nutraceutical, 1996). Vera means true, coined by Linnaeus. The plant is characterized by thick taped spiny leaves that grow in rosette pattern from a short stalk at the base. It is used as herbal remedy in the treatment of various ailments; ulcerative colitis,

constipation, osteoarthritis, diabetes, asthma, epilepsy, amenorrhea, depression, glaucoma, multiple sclerosis, hemorrhoids, burns, wounds, Sunburn, (Fragoso, 2009). However, some of these ailments cure-claims are not scientifically documented. It is used as daily supplement and nutraceutical purposes (Vaidya, 2007) due to its antioxidant properties. The phytochemical properties of Aloe Vera e.g. anthroquinone, barbaloin acemmanan, beta, sitosterol enhance some of its therapeutic functions. For e.g. its barbaloin contents prevents opening of chloride channel in the colonic mucosa resulting in increase in peristaltic activity in the large intestine and also soften stool (Boudraau, 2006), hence its use in the treatment of constipation. The acemmanan content of Aloe Vera contains a beta 1, 4 glycosidic bonds claimed as responsible for the immunomodulating and antiviral and anti tumor properties of Aloe Vera (Sun 2010). The beta-sitosterol content in Aloe Vera is a sterol found in its sap and has anti-inflammatory and angiogenic properties (Langmeal, 2004), Lee, 1998). The healing efficacy of Aloe Vera has not been thoroughly substantiated but the period has been put for about 9 days, its efficacy on healing has not yet been fully established due to it various contents, (Maethaisong, 2007) (Boundreau, 2006). The uncertainty of this wound healing effect and the duration prompted our 28 days period in our investigation. Gastric ulcer is a gastrointestinal disease characterized by an excoriated area of the mucosa caused by digestive action of gastric juice which is acidic, (Guyton, 2006). The ulcer is often situated along the lesser curvative of the antrial end of the stomach an area susceptible for the ulceration. Gastric ulcer can be caused by diet e.g. tea and coffee beverages, (Jimmy, 2014) high acidic gastric content, irritation, poor blood supply (Guyton, 2006) infection as in helicobacter pylori, (Kikkawa, 2005), by nonsteroidal anti-inflammatory drugs NSAIDS, (Hawkey, 2003), by smoking, (Shin, 2002).

Omeprazole is one of the most potent antiulcerogenic drugs for the treatment of peptic ulcer. It is a proton pump inhibitor (PPI) as it blocks the hydrogen potassium Atpase ($H^+ - K^+ - ATpase$) and inhibit gastric acid secretion (Shin 2009) (Uda, 2008). It is also used in the treatment of gastro esophageal reflux disease (GERD), dyspepsia, Laryngopharyngeal reflux (LPR) and Zollinger-Ellison syndrome.(Guyton 2006). It is metabolized by hepatic CYP 450 (Bertram, 2004). Omeprazole has been found to inhibit iodine uptake resulting in enlargement of thyroid gland and atrophy of thyroid gland. Proton pump inhibitors like omeprazole is given in an inactive form which is neutrally charged i.e. lipophillic and readily crosses cell membrane into intracellular compartments e.g. parietal cell canalicus with acidic environment. In this environment, the inactive drug is protonated and rearranged into its

active form and will covalently and irreversibly binds to the gastric proton pump and hence causing deactivation of the pump, (Betram, 2004). It is in the forms of tablets and capsules with various concentration, 20mg, 40mg, 60mg, 80mg. The dosage in the treatment of gastric ulcer is 40mg daily orally i.e. once a day for four to eight weeks, (Peura, 2004). However, for other ailments e.g. Zollinger-Ellison syndrome the starting dose is 60mg daily, (Metz, 1993). Omeprazole competes with other drugs through their enzymes, e.g. CYP2C19 which drugs like diazepam, phenytoin and warfarin depends for their activities hence concomitant administration would delay their activities, (Stedman, 2000). It also affects the absorption of other drugs that are acid-environment dependent, e.g. ketoconazole or atazanavir, (Paul Magnus, 2001). The absorption of omeprazole takes place in the small intestine within 3-6 hours and its bioavailability is 60% but could be impaired by food hence should be taken without food and the period of waiting for meal after taking the drug is not specified, (Jansen, 2011). It is metabolized by cytochrome p450 system in the liver, (Anderson, 1993). Metabolites: sulfone, sulfide, hydroxyl-omeprazole are excreted in urine and feces (Lind 1989). Omeprazole is superior to H₂ antagonists e.g. ranitidine and cimetidine as it inhibits both fasting and meal stimulated acid secretion as it blocks the final common pathway of acid secretion i.e. the proton pump. The adverse effects of omeprazole include headache, diarrhea, abdominal pain, nausea, dizziness, sleep deprivation (Kenneth, 2009). Iron and vitamin deficiency this is controversial, (Madanick, 2001). But this drug is associated with osteoporosis related fractures (Targowink, 2008), (Yang 2006), it is also associated with clostridium associated diarrhea, (Madanick, 2010). Use of omeprazole as antacid preparations to suppress acid mediated breakdown proteins is said to lead to food and drug allergies due to sensitization of undigested proteins in the gastrointestinal system. However, the effects of such preparation on long term or short term basis are not explained (Pali-Scholl, 2011). But the use of antacid preparations of omeprazole is said to be associated with pneumonia, (Herzig, 2009). Other adverse effects include acute tubulointerstitial nephritis, an inflammation of the kidneys (Ray, 2010), (Thompson 2010) and fundic gland polyposis. However, despite the adverse effects of this drug which are normal in all drugs, omeprazole still stands out as the most effective proton pump inhibitor (PPI) in the treatment of peptic ulcer hence the interest of this drug in comparison with herbal extract for effective fight against peptic ulcer scourge.

MATERIALS AND METHODS

Collection and identification of the plants, Aloe Vera

The plants, Aloe Vera were collected in its fresh state from the University of Uyo Pharmacy Farm. It was identified and authenticated by a taxonomist in the Department of Botany, Faculty of Science, University of Uyo, Akwa Ibom State, Nigeria.

Preparation of Extract of Aloe Vera

The leaves were obtained from the plant stem washed and pulverized into pieces and 2000g of it extracted in 100ml of water as aqueous extract for 72 hours. The liquid filtrates were concentrated and evaporated to dryness at 40⁰C using rotary evaporator. The method of Trease and Evans 1996 was used for the extraction. The pure extract was stored at 40⁰C for use.

Phytochemical Screening of the Aloe Versa Extract

The screening for alkaloids, saponin, tannins, terpenes, flavonoids and cardiac glycosides were carried out using methods of (Adeniyi, 2006), (Trease and Evans, 2002) (Sofowara, 1995) and (Clark, 1975).

Acute Toxicity Study (LD50)

The acute toxicity expressed in Lethal dose is that dose of the compound that will cause death of 50% of the experimental animal which the compound was administered. This is used as a measure of the toxicity of drugs; (Rang, 2002). The LD50 of the Aloe Vera extract was determined using Lorke; method (1983). A total of 30 mice of average weight 18g – 25g were used for the study. The animals were divided into three phases of studies. In the first phase, 9 mice were divided to 3 groups of 3 mice each and were given dosage of Aloe Vera extract intraperitoneally as follows, 5000mg/kg, 3000mg/kg 1000mg/kg, and were observed for 24 hours for signs of toxicity. In the second phase, 9 mice were used and divided into three groups of 3 mice each with the following extract dosage, 2500, 2000, 1500mg/kg. And the third phase, 12 mice were divided into 4 groups of 3 mice each in a group with the following dosage of the extract, 1000, 800, 600, 400mg/kg and allowed for toxicity observation for 24 hours. Groups that received 1500 to 500mg/kg had 100% mortality while those with doses of 400 to 100mg/kg had zero mortality. Signs of toxicity were convulsions, staggering, decreased respiratory rate. The LD50 of the Aloe Vera extract was calculated as follows.

$$LD50 = \sqrt{AB}$$

Where A = Maximum dose that produced 0% mortality

B = Minimum dose that produced 100% mortality

A = 1000mg/kg

B = 1500mg/kg

$$\text{Therefore } LD50 = \sqrt{1000 \times 1500} = 1224.74\text{mg/kg}$$

The dosage effective for pharmacological activities but not toxic to the animals were chosen as 10 and, 20% and 30% of the LD50.

Inducement of gastric ulcer ulcerscores and treatment with extract

A total of 30 male and female albino rats weighing 100-150g were starved for 24 hours and induced with gastric ulcer using 2.5ml/kg ethanol administered orally using canula by-passing esophagus and delivered into the stomach, (Nwafor, 2005). The rats were anaesthetized with chloroform, stomach removed and fixed in formaldehyde. The ulcerations were observed by opening along the greater curvature of the stomach. The ulceration were observed using hand lens and each ulcer point scored according to the method of (Vogel et al 1997) was used. The ulcers were scored as follows, 5 as multiple ulcers along the entire length of the gastric fold. 4 as ulcers which followed appropriately 80% of the fold. 3 as ulcers 1-4mm length of 80% of the fold, 2 as at least 2 ulcers of approximately 2mm in length and 1 as the presence of ulcer and generalized erythema, 0 as ulcer not visible.

Grouping and Treatment of Ulcerated rats with Aloe Vera Extract

The animals were divided into 3 groups of 10 per each group. Groups 1 served as control and were given distilled water, and the group with animals without ulcer. Group 2 had ulcer and treated with Aloe Vera extract of dosage of 122.47mg/kg body weight for 4 weeks while group 3 also with ulcer were treated with 20mg/kg of omeprazole for 4 weeks.

Preparation of administered extract and drugs

2g of extract was dissolved in 20ml of distilled water to obtain a stock dosage (Concentration of 200mg/ml). From this stock, a dose for each animal was obtained and administered orally according to body weight using canula by-passing esophagus and delivered into the stomach, Bertram 2004, Robert 1979.

Preparation of Omeprazole

A capsule of omeprazole which contains 20mg was dissolved in 20ml of distilled water. The drugs were prepared fresh on each treatment i.e. for 4 weeks.

Collection of Sample (Gastric acid)

Samples; gastric acid were collected from the isolated stomach of the rats after anesthesia with chloroform. Incisions were made into linea alba with sterile scissors to expose the stomach which was opened to remove the gastric contents

Titration acidity: This was determined according to the methods of (Silverton, 1985). The gastric contents were emptied into a beaker and 5ml of distilled was added to it. It was filtered and then titrated with 0.2m sodium hydroxide using phenolphthalein as indicator. The volume of the initial gastric volume before addition of 5ml distilled water was recorded and the titration volume also recorded to the volume of the acid as the titration acidity i.e. acid output was then calculated.

$$= \frac{X \cdot 20 \text{ml}}{Y}$$

Where X = Initial volume of secretion
Y = Final volume of secretion

Statistical Analysis: Student t-test was used to analyze the data obtained.

RESULTS

The results of acid output and ulcer scores in all the groups studied in Aloe Vera and omeprazole is expressed as \pm SEM and are presented in the graphs below:

Fig. 1 showed significant ($P < 0.05$) decrease ulcer scores in group 2 treated with Aloe Vera compared to group 1 and 3 and a significant ($P < 0.05$) decrease in acid output in group 3. Fig. 2 explains the weekly acid output and ulcer scores for group 1, fig. 2, fig. 3 explained the mean acid output and ulcer scores for group 2, a = $P < .001$, vs day 1, b = $P < 0.01$ vs day 1. The fig. 3 further detailed the increase in acid output significantly on day 14 and 28 compared to day 7. And also a significant ($P < 0.001$) decrease in day 21, and a significant ($P < 0.01$) decrease in ulcer scores in day 14, 21 and 28 compared to day 7 fig. 4 explained the comparison of mean acid output and ulcer scores on weekly basis for group 3 treated with

omeprazole. The results showed a significant ($P < 0.00$) decrease in acid output but increase in ulcer scores.

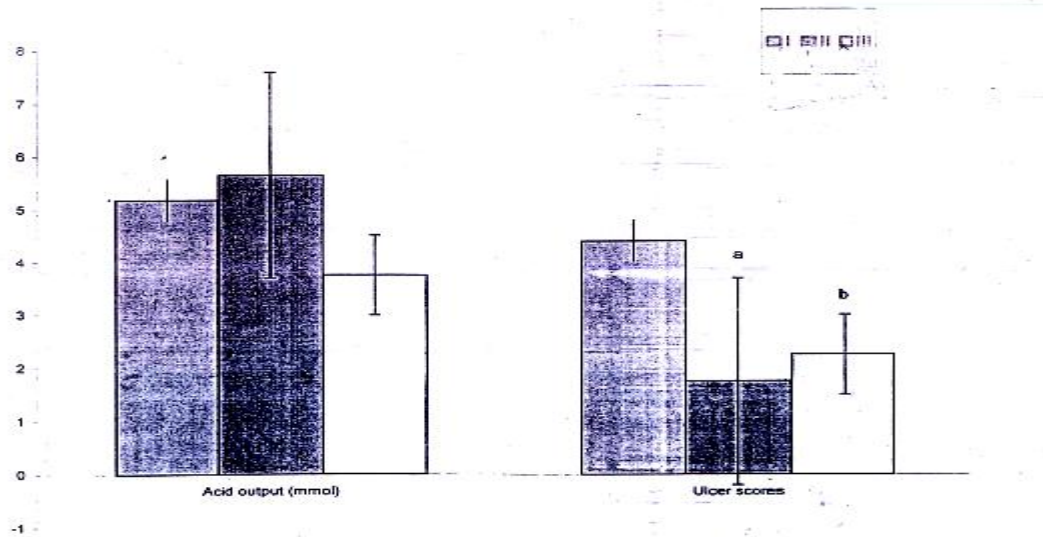


Fig. 1: Comparison of acid outputs and ulcer scores in various experimental groups following aloe vera and omeprazole administration a = ($p < 0.05$) b = ($p < 0.001$)

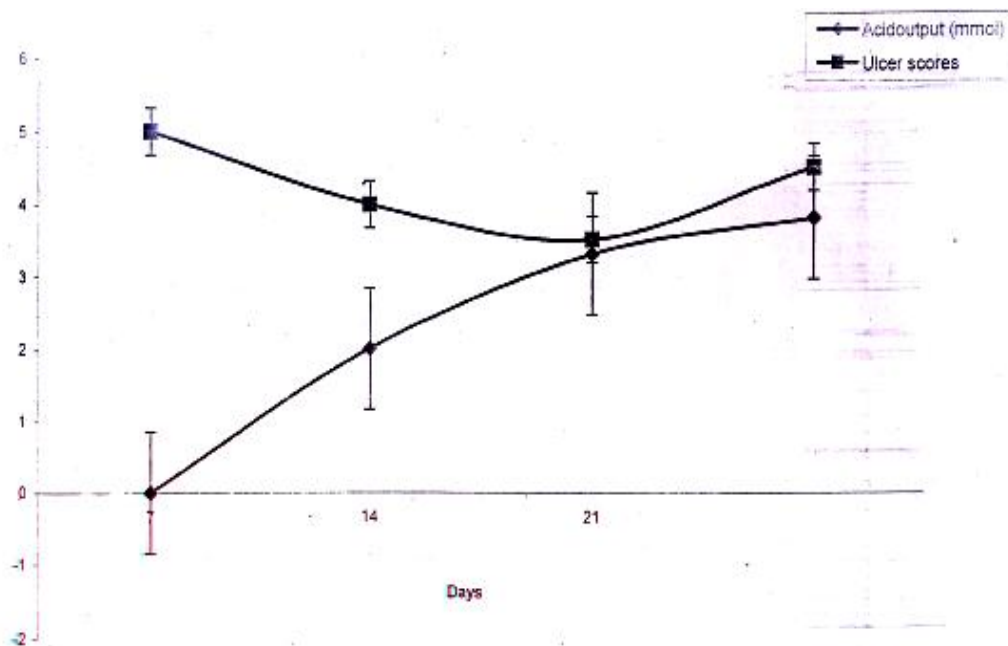


Fig. 2: The weekly acid output and ulcer scores for group I

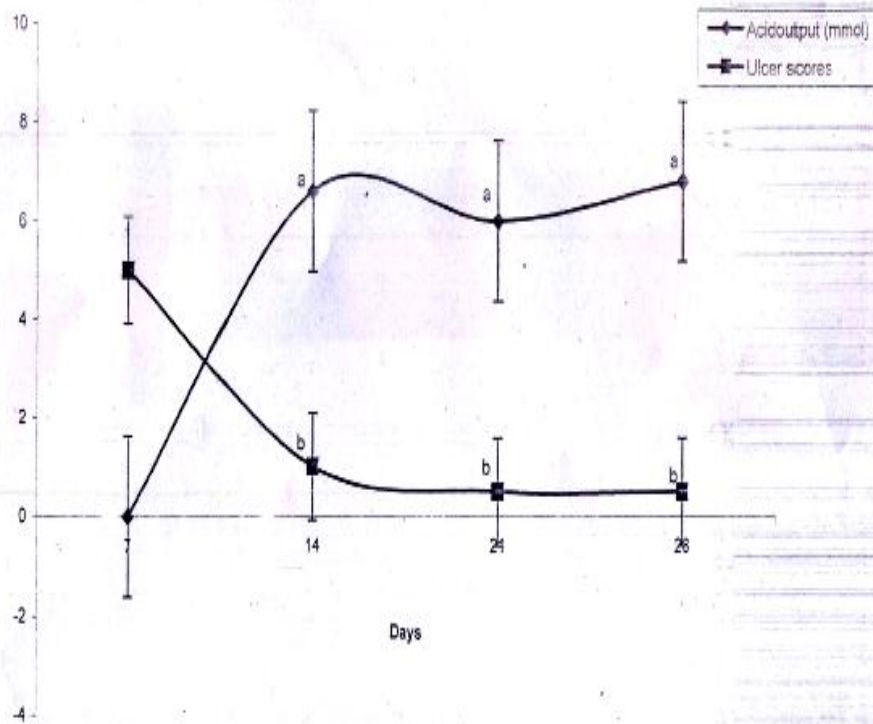


Fig. 3: The mean acid output and ulcer scores for group II. a = $p < 0.001$ vs Day 1, b = $p < 0.01$ vs Day 1.

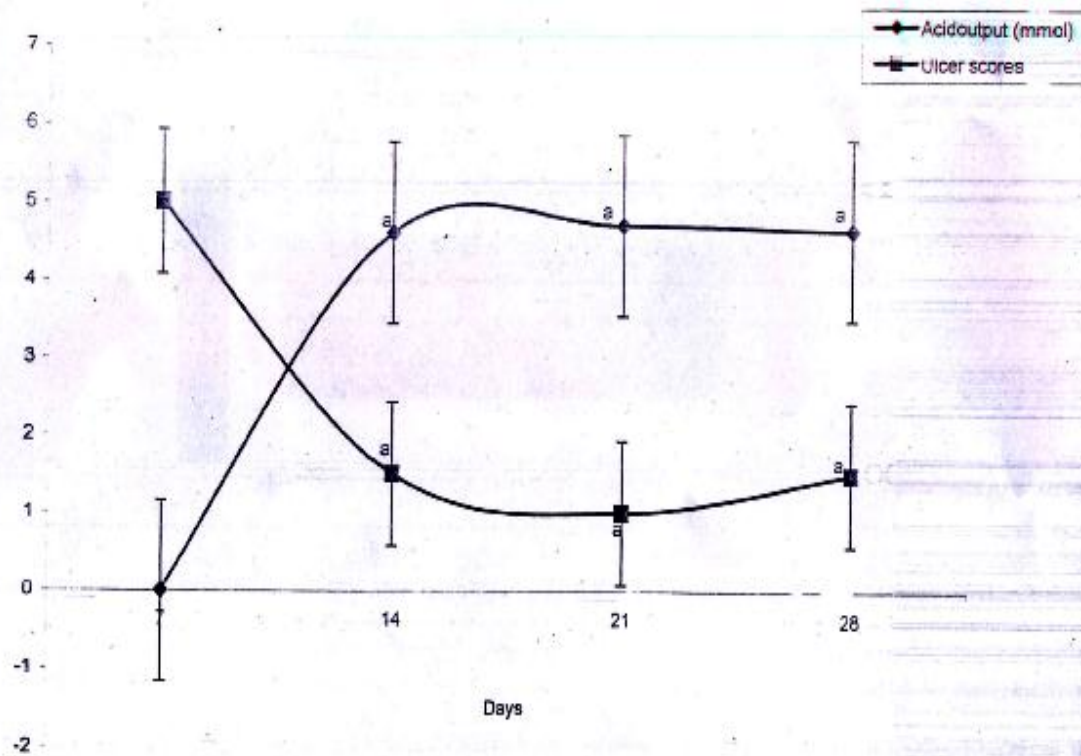


Fig. 4: The mean acidoutput and ulcer scores for group III. a = $p < 0.001$ vs Day 1.

DISCUSSION

The results of the study have shown the antiulcerogenic potentials of Aloe Vera juice compared with a known drug for ulcer treatment, the proton pump inhibitor; omeprazole. Interestingly the effects of Aloe Vera juice is on the direct healing of the ulcer rather than the acid output. Clinically, mortality and morbidity of peptic ulcer depend on the ulceration (Noquiera, 2003). The therapeutic action of Aloe Vera observed specifically on the ulcer may be a function of its multi-phytochemical contents, anthraquinone, flavonoids, tannins, saponins, beta, 1, 4-glycosides Terpenes (Alok,2011), (Fragoso, 2009) (Langmead, 2004) (Ramamorthy, 1996), (Wombe, 1988). However, the specific action of these contents are not very certain due to their mixed contents, (Macthaisong, 2007) (Boundreau, 2006). But alkaloids are found to stimulate gastric acid secretion (Maeda, 1982). Perhaps this is why there was an increase in acid output in the group administered with Aloe Vera. The confirmation of alkaloid acidity stimulation as evident in the group of Aloe Vera is seen in group 2 with only omeprazole treatment. Increase in acid output is expected to also stimulate gastrin negative feed back release which would reversely reduce its output. But ideally gastrin release leads to acid secretion (Guyton 2006). The reduction in ulcer scores despite increase in acid output in Aloe Vera treated group indicates the likely neutrality or alkalinity properties of Aloe Vera. This is because increase acidity often leads to ulceration as such affects the mucosa directly based on membrane gastric barrier susceptibility to high acidity. Aloe Vera may therefore also serve as antacid or H₂ antagonist, its immunological properties could also be an additional factor in decreasing the ulcer score or healing (Ramamorthy, 1996); particularly the antibactericidal properties which potentially enhance healing of wound infected by micro-organisms.

Our study is very particular about the durational effect of the Aloe Vera juice on the gastric ulceration. It is observed in the study that there was a steady decrease in ulceration on days 7, 14, 21 and 28. This means that Aloe Vera given in long duration will enhance more healing. The study did not combine Aloe Vera with omeprazole. But the activity of omeprazole in decreasing acid output on same period, days 7, 14, 21 and 28 means that Aloe Vera and omeprazole are potent combined therapy in the treatment of gastric ulcer. There was increase in ulcer scores as the period of observation increased that is 28 days, for the untreated group, this confirmed that Aloe Vera and omeprazole are ideally potent antiulcerogenic drugs indicating also that increase in ulceration is also a function of increase acid output.

However, caution need be taken in taking Aloe Vera due to its alkaloid associated gastric acid stimulation.

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