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Doi: doi.org/10.4314/jeca.v21i1.12

Original Article

# Histological Evaluation of Wound Healing Potential of Aqueous Extracts of *Tamarindus indica* Seed Extract in Wistar Rats

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## Abstract

**BACKGROUND AND AIM:** *Tamarindus indica* has been used as a traditional medicine for various purposes. Wound healing is a complex process that involves a series of immunological and biological events. This study aimed to evaluate the wound healing potential of *Tamarindus indica* seed extract in Wistar rats.
**METHODOLOGY:** The study involved creating a 1.5cm full thickness wound on the dorsal region of 16 healthy Wistar rats. After the excision of the wound, the animals were grouped into four (4) groups (A, B, C and D) of four animals per group. Untreated, Silver Sulphadiazine, 1% *Tamarindus indica* seed extract and 2% *Tamarindus indica* seed extract were respectively applied to group (A, B, C and D) for the period of 13 days. And the histological analysis of the wound area was performed on the 14th day.
**RESULTS:** The results showed that 2% of aqueous *Tamarindus indica* seed extract significantly improved wound healing rates and was better in term of efficacy when compared to the standard drug (Silver Sulphadiazine). It also enhanced the cellular proliferation, blood vessel formation, epidermal regeneration, granulation tissue formation, and collagen formation.
**CONCLUSION:** The study revealed that *Tamarindus indica* seed extract had wound healing potential, and that the effect was dose dependent.

**Keywords:**
Histological, Wound, Healing, *Tamarindus indica*, Wistar, Rats.

Submitted: 6th April, 2024
Revised: 8th June, 2024
Accepted: 11th June, 2024
Published: 30th June, 2024

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## INTRODUCTION

Wound is a break in the integrity of the skin or tissues often which may be associated with disruption of the structure and function (Enoch and Price, 2004; Alam *et al.*, 2011; Mohammad *et al.,* 2022). (Robson, *et al.,* 2001; Al-Ghanayem, *et al.,* 2022a). This can range from a simple break in the epithelial integrity of the skin or it can be deeper, involving subcutaneous tissue with damage to other structures such as tendons, muscles, nerves, parenchyma organs and even bone. Wound is caused by process that begins externally or internally that involved organ (Bischoff, *et al.,* 1999; Al-Ghanayem, *et al.,* 2022a). Wound can be classified according to the level of contamination as; clean wound (wound caused by sterile object) and contaminated wound (wound caused by accident characterized by presence of pathogens and foreign bodies, example infected wound and colorized wound). Wound can also be grouped into two types. Open wound are classified according to the object that caused the wound. Some examples include incision wound, irregular tear-like wound and abrasion wound. Closed wound has fewer categories. Some examples include hematomas, crush, and gunshot wound (Robson, *et al.,* 2001; Enoch and Price, 2004). The body undertakes a series of actions collectively known as the wound healing process. Wound healing is a cascade of immunologic and biologic events resulting in a closed wound and every wound is unique, with a unique set of physiological and social circumstances preventing or retarding wound healing. The normal wound healing process consists of three phases that occur in a



**How to cite this article**: Mohammed, M.O., Angbagh, O., Muhammad, A.T., Mohammed, I., Abubakar, U., Salihu, A., Umar, A., Bello, M., Iyiola, A.O. and Isah Y.A. Histological Evaluation of Wound Healing Potential of Aqueous Extracts of *Tamarindus indica* Seed Extract in Wistar Rats. J Exp Clin Anat 2024; 21(1): 78-83. https://dx.doi.org/10.4314/jeca.v21i1.12

sequence; inflammation, proliferation and lastly remodelling (Enoch Price, 2004; Mohammad, *et al.,* 2022). The universal role of plants extracts such as T*amarindus indica* and many other plants in the treatment of wound from generation to generation cannot be over emphasized. *Tamarindus indica* is commonly identified and known as Tamarind (common name), Tamiya (local name) in traditional system of medicine. Its fruits, tender leaves and flowers are used extensively for treating various ailments including wound healing (Evan, 2009). The plant has been reported to possess several medicinal properties such as anti-oxidant, anti-inflammatory, analgesic, anti-arthritic activities (for seed); anti-oxidant, anti-microbial activities (for fruit), anti-bacterial, hepatoprotective effect (for flowers), anti-microbial activities (for leaves), and anti-microbial activities (for bark) (De Caluwé, *et al.,* 2010 ; Suralkar *et al.,* 2012). The use of standard wound healing drug such as silver sulphadiazine is associated with many risks such as high cost, toxicity, storage condition and even death. It becomes imperative to seek potential alternatives to the synthetic drugs that are easily available, less expensive and less toxic. The aim of the study was to histologically evaluate the wound healing potential of aqueous seed extract of *Tamarindus indica* in Wistar rats.

## MATERIALS AND METHODS

### Purchased and Identification of *Tamarindus indica* Seed

The pods of *Tamarindus indica* were purchased from Sokoto South Local government, Sokoto State. The *Tamarindus indica* Seeds were identified at the Department of Pharmacognosy and Ethnomedicine, Faculty of Pharmaceutical Sciences, College of Health Science, Usmanu Danfodiyo University, Sokoto State with a specimen voucher number PCG/UDUS/FABA/0007 allocated.

### Preparation of Plant Extract

After identification, large quantity of pods were collected and dried under shade for 7 days. The dried samples were milled into fine powder by pounding manually in a chemically clean mortar with a pestle. The powder were collected into an air tight water proof polythene bag, labeled for easy identification and kept in a cool dry place for further use. Sixty grams (60g) of the pods was weighed and extracted with 450ml of distilled water using maceration method. The maceration involves the soaking of powdered plant material in a stopper container (Beaker) with the distilled water and allowed to stand at room temperature for a period of 3 days with frequent agitation, after which the mixture was sieved with a clean cloth and filtered using Whatman number one filter paper to get a clean supernatant. The distilled water was evaporated in an electric blast oven set at 50$^{o}$c to a dry powder. The powder was stored with desiccators in a moisture free place. Then, 1% and 2% of the extract were prepared by dissolving in sterile injection water for wound healing effect assessment.

### Experimental Animals

A total of sixteen (16) males and females healthy Wistar rats of 10 – 12 weeks of age, weighing between 200g and 250g were used for this study. The rats were kept in a clean stainless steel cages at temperature of 15±5 and fed with standard rat/mouse pellet in the Department of Pharmacology and Toxicology Animals House, College of Health Science, Usmanu Danfodiyo University Sokoto. The study was performed in accordance with the approval of Ethical Committee of animal care and use of the Department of Pharmacology and Toxicology, College of Health Science, Usmanu Danfodiyo University Sokoto with Ethical Approval number, AET NO: PTAC/MO/(Ee)/Ta/52-23.

The animals were randomly assigned into four (4) groups of four rats per group. The rats were grouped as follows:

- Group A (Untreated (UNTRD), allowed to heal naturally)
- Group B (Treated with 1% Silver Sulphadiazine (SSDZ)
- Group C (Treated with 1% *Tamrindus indica* (TI1) seed extract)
- Group D (Treated with 2% *Tamarindus indica* (TI2) seed extract)

### Surgical Procedures and Topical Application of Intervention Substances

The back of each of the rat was disinfected with 70% ethanol, shaved to expose the dorsal skin. The animals were anesthetized via chloroform inhalation. About 1.5cm length of full thickness wound was made on the shaved dorsum area of each rat using a surgical blade and the extract was applied topically daily on the wound. The treatments continued for thirteen days and the animals were sacrificed on the 14th day.

### Biophysical Wound Healing Assessment Method

Various biophysical parameters of wound healing were assessed by measuring wound length on days 4, 7, 10 and 13 in cm unit. The percentage of wound healing was calculated using the formula;

$$\text{Percentage of wound healing} = \frac{\text{wound surface area on day one} - \text{wound surface area on day} \times}{\text{Wound surface area on day one}} x100$$

Where × is the day when wound length was measured.

### Animal Sacrifice and Sample Collection

After 13days of topical application, the rats were sacrificed on day 14 upon chloroform anesthesia and about 2cm by 2cm square of the wounded areas were surgically excised using surgical blade and dissecting forceps. The excised samples were washed with normal saline and were fixed immediately in 10% buffered formal saline for 24hours.

### Tissue Processing

About 5mm portion of wounded skin samples were dehydrated in an ascending grades of alcohol, cleared in three changes of xylene and impregnated in paraffin wax using an automatic tissue processor. The tissues were then embedded using tissue tek mould and embedding centre machine. Finally, the embedded tissue block were trimmed and sectioned at 10 µm and 3 µm respectively using rotary microtome machine. The microscopic cut sections were stained with Haematoxylin and Eosin staining and Weigert Van Gieson is staining methods. For Haematoxylin and Eosin staining, the sections were dewaxed and with three changes of xylene for ten minutes with each batch, hydrated with descending grades (absolute alcohol, 90%, 70% and 50%) of alcohols for two minutes with each grade and then rinsed with water. They were then stained with Harris haematoxylin solution for ten minutes and rinsed in water briefly. The sections were differentiated with 1% acid alcohol briefly and rinsed in water. They were blued with Scott's tap water for five minutes and rinsed with water. The sections were counterstained with 1% eosin for three minutes, rinsed with water, dehydrated with ascending grades (50%, 70%, 90% and absolute alcohol) of alcohol for two minutes with each grade and cleared with three changes of xylene for two minutes with batch and mounted with dibutylphtalate xylene (DPX). And for the Weigert Van Gieson is staining method, The sections were dewaxed with three changes of xylene for ten minutes with each batch, hydrated with descending grades (absolute alcohol, 90%, 70% and 50%) of alcohol for two minutes with each grade and rinsed with water. They were stained with freshly mixed equal volume of Weigert haematoxylin solution A and B for ten minutes, differentiated with 1% acid alcohol briefly and rinsed with water thoroughly with water for five minutes. The section were then counterstained with Van Gieson stain for three minutes and blotted with whatman number one filter paper. The sections were dehydrated with ascending grades (50%, 70%, 90% and absolute alcohol) of alcohols for two minutes with each grade, cleared with three changes of xylene for two minutes with each batch and mounted with dibutylphtalate xylene (DPX).

The wound healing parameters such as new blood vessels, epidermal regeneration, epithelisation, proliferating fibrous tissue and inflammation were assessed with haematoxylin and eosin stained slides and scored as poor-0, scanty-1, moderate-2 and adequate-3. While Van Gieson stained slides were used to assess the collagen fibers and scored poor-0, scanty-1, moderate-2 and adequate-3.

### STATISTICAL ANALYSIS

The data analysis was performed using IBM Statistical Package for Social Science (SPSS) software version 25.0. The values were expressed as mean±standard error of the mean (SEM), and analyzed using analysis of variance (ANOVA) and *Post Hoc* Test.

## RESULTS

The effects of the interventions on wound length is shown in table 1. The results showed that on days 4 and 7, there was no significant reduction in wound length in silver sulphadiazine-treated group compared to untreated group but there were significant reductions in wound length of *T. indica*-treated groups compared to the untreated group. On days 10 and 13, wound lengths were significantly reduced in all the treated groups compared to the untreated group. However, wound length was significantly reduced in the group treated with 2% of *T. inidica* compared to silver sulphadiazine-treated group. The effects of the interventions on percentage wound recovery is shown in table 2. The results showed that on days 4 and 7, there was no significant increase in percentage wound recovery in silver sulphadiazine-treated group compared to untreated group but there were significant increase in percentage wound recovery of *T. indica*-treated groups compared to the untreated group. On days 10 and 13, percentage wound recovery were significantly increased in all the treated groups compared to the untreated group. However, percentage wound recovery was significantly increased in the group treated with 2% of *T. inidica* compared to silver sulphadiazine-treated group.There were improvements in epidermal regeneration, epidermal layer, fibroblast, hair follicle, granulation tissue, tissue thickness, and angiogenesis in all other groups when compared with untreated group (plate 1). Also, there were improvements in collagen fibers deposit in all groups when compared with untreated group (plate 2).

## DISCUSSION

From the current study, the extract of *Tamarindus indica* seeds has been established as one the important plants used for wound healing. This is in agreement with the previous report by Mohammed *et al.* (2022) who found that *Tamarindus indica* seeds had a wound healing potential. The current study revealed that the treated groups showed significant differences in wound length reduction and increased percentage of wound healing compared to the untreated group on the 4th, 7th, 10th, and 13th days. This finding is consistent with a previous report by Naik *et al.* (2017), which showed that *Tamarindus indica* significantly increased the percentage of wound contraction compared to the control group on the 9th, 12th, 18th, and 21st days after wounding. Similarly, Susanti *et al.* (2017) also establish a similar pattern where it was established that, *Tamarindus indica* seed extracts, had significant differences in wound length reduction and increased percentage of wound recovery improvement on the 4th, 7th, 10th, and 13th days. Also in an agreement with our finding is result reported by Naik *et al*. (2017) where it was found that tamarind turmeric juice had a significant effect on the duration of perineal wound healing in postpartum mothers. The wound healing effect may not be unconnected with the presence antioxidant (curcumin), which had been effective in wound healing process and may reduce inflammation and speed up the wound healing process. The

current study also established that the H and E stained sections revealed increased in some histological structures such as epidermal regeneration, epidermal layer, fibroblast, hair follicle, granulation tissue, tissue thickness, and angiogenesis in test groups when compared with control group. This finding aligns with the previous report by Al-Ghanayem *et al.* (2022b), which demonstrated that *Tamarindus indica* seed extract enhance wound contraction, reduce the time required for epithelialization, and increase antioxidant and enzyme activities, proliferation activities, epithelialization, capillaries density, and collagen formation.

It was established that the wound healing properties of the extracts are dose-dependent and this is in line with previous report by Tofiq *et al.* (2021), which demonstrated potential wound healing property of *Tamarindus indica at* various concentrations as a dose-dependent agent.

In conclusion, the research revealed that the aqueous seed extracts of *Tamarindus indica seed* had wound healing potential and that the effect was established to be dose dependent

Further research should be conducted to evaluate the major active phytoconstituents of the extract and its mode of action through which the extracts exhibits its wound healing effects.

**Table 1. Descriptive statistics of wound length (cm) measurements of all study groups across various days.**

| Day | Interventions (Extracts) | Minimum | Maximum | Mean±SEM |
|---|---|---|---|---|
| 4 | UNTRD | 0.90 | 1.10 | 1.00±0.04$^{a}$ |
| | SSDZ | 0.80 | 0.90 | 0.88±0.03$^{a,b}$ |
| | TI1 | 0.70 | 1.00 | 0.80±0.07$^{b}$ |
| | TI2 | 0.70 | 0.70 | 0.70±0.00$^{b}$ |
| **7** | UNTRD | 0.90 | 1.00 | 0.95±0.03$^{a}$ |
| | SSDZ | 0.70 | 0.90 | 0.80±0.04$^{a,b}$ |
| | TI1 | 0.60 | 0.90 | 0.70±0.07$^{b}$ |
| | TI2 | 0.60 | 0.70 | 0.63±0.03$^{b}$ |
| **10** | UNTRD | 0.70 | 0.90 | 0.80±0.04$^{a}$ |
| | SSDZ | 0.60 | 0.70 | 0.65±0.03$^{b}$ |
| | TI1 | 0.50 | 0.60 | 0.53±0.03$^{b,c}$ |
| | TI2 | 0.30 | 0.50 | 0.40±0.04$^{c}$ |
| **13** | UNTRD | 0.40 | 0.50 | 0.45±0.03$^{a}$ |
| | SSDZ | 0.20 | 0.50 | 0.28±0.08$^{a,b}$ |
| | TI1 | 0.10 | 0.20 | 0.15±0.03$^{b,c}$ |
| | TI2 | 0.05 | 0.10 | 0.07±0.01$^{c}$ |

**Table 2. Descriptive statistics of percentage wound recovery (%) of all study groups across various days.**

| Day | Interventions (Extracts) | Minimum | Maximum | Mean± SEM |
|---|---|---|---|---|
| 4 | UNTRD | 26.70 | 40.00 | 33.33±2.71$^{a}$ |
| | SSDZ | 40.00 | 46.70 | 41.68±1.68$^{a,b}$ |
| | TI1 | 33.30 | 53.30 | 46.65±4.71$^{b}$ |
| | TI2 | 53.30 | 53.30 | 53.30± 0.00$^{b}$ |
| **7** | UNTRD | 33.30 | 40.00 | 36.65±1.93$^{a}$ |
| | SSDZ | 40.00 | 53.30 | 46.68±2.72$^{a,b}$ |
| | TI1 | 40.00 | 60.00 | 53.33±4.71$^{b}$ |
| | TI2 | 53.30 | 60.00 | 58.33±1.68$^{b}$ |
| **10** | UNTRD | 40.00 | 53.30 | 46.68±2.72$^{a}$ |
| | SSDZ | 53.30 | 60.00 | 56.65±1.93$^{b}$ |
| | TI1 | 60.00 | 66.70 | 65.03±1.68$^{b,c}$ |
| | TI2 | 66.70 | 80.00 | 73.53±2.72$^{c}$ |
| **13** | UNTRD | 66.70 | 73.30 | 68.35± 1.65$^{a}$ |
| | SSDZ | 66.70 | 86.70 | 81.70± 5.00$^{b}$ |
| | TI1 | 86.70 | 93.30 | 90.00±1.91$^{b,c}$ |
| | TI2 | 93.30 | 96.70 | 95.68±0.81$^{c}$ |

**Key:** like superscripts mean no significant difference between group ($p$>0.05) while unlike superscripts mean significant difference between group ($p$<0.05). UNTRD (untreated/Group A), SSDZ (silver sulphadiazine/Group B), TI1 (1% *Tamarindus indica*/Group C), TI2 (2% Tamarindus indica/Group D) and SME (standard error of mean)

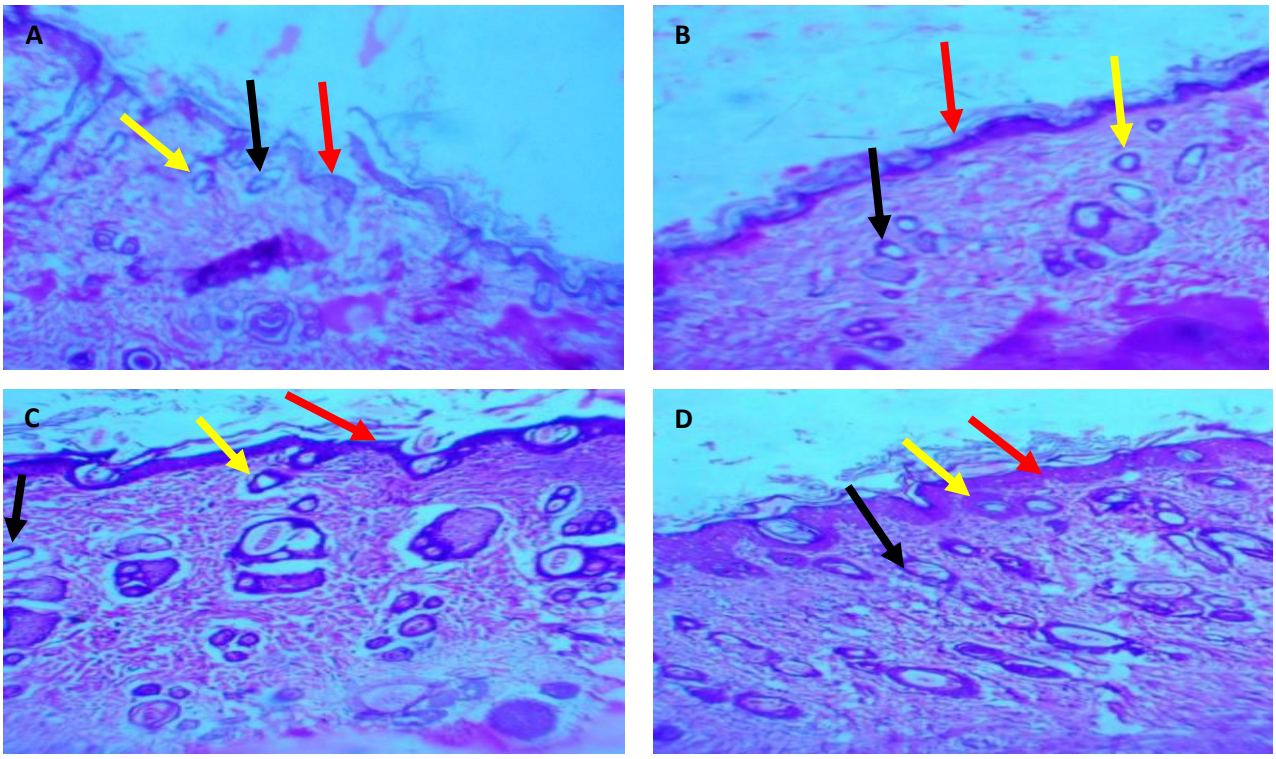


**Plate 1:** Photomicrograph showing scored of histological structures across various groups. **Key:** Group A (untreated), Group B (treated with 1% silver sulphadiazine), Group C (treated with 1% *Tamarindus indica*) and Group D (treated with 2% *Tamarindus indica*). **H&E(X400). Yellow arrow**: Artery, **Black arrow**: Vein, **Red arrow**: Epidermal re-generation

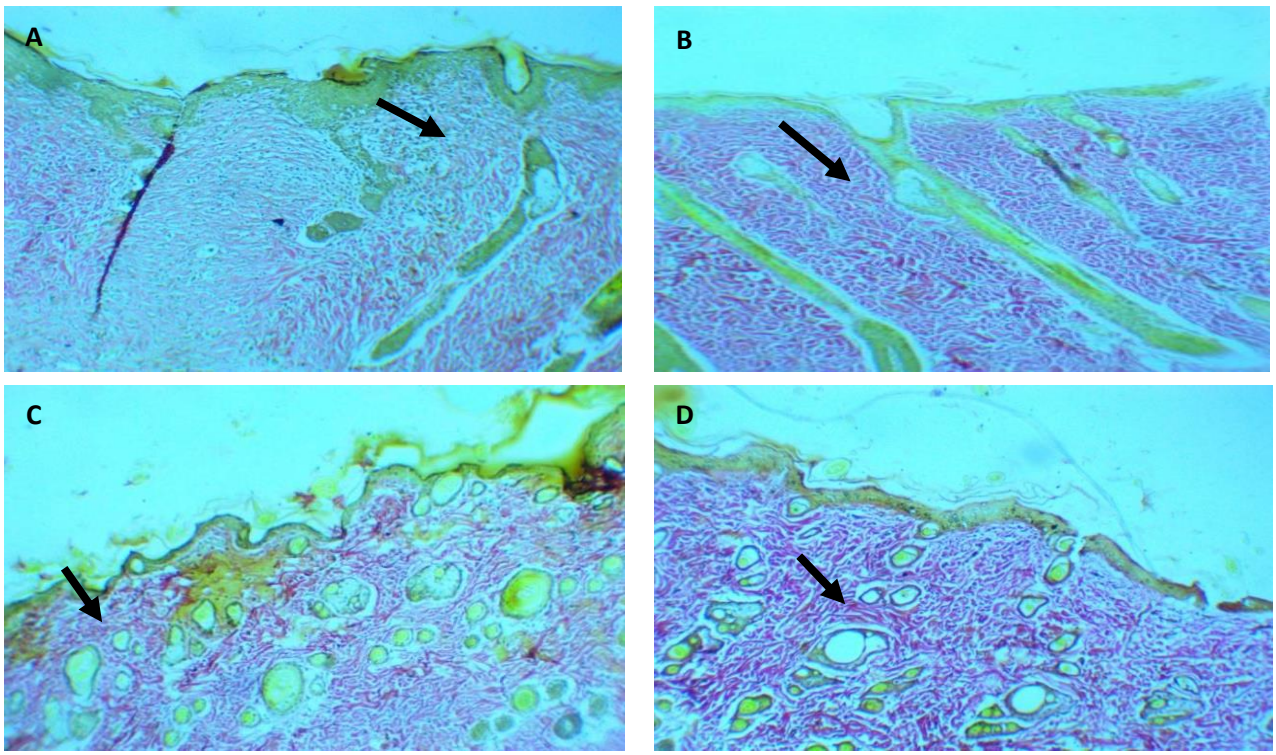


**Plate 2:** Photomicrograph showing scored collagen across various groups. **Key:** Group A (untreated), Group B (treated with 1% silver sulphadiazine), Group C (treated with 1% *Tamarindus indica*) and Group D (treated with 2% *Tamarindus indica*). **W.V.G (X400)**. **Black arrow:** collagen fibers.

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