A Comparative Evaluation of the Anticaries Efficacy of Herbal Extracts (Tulsi and Black Myrobalans) and Sodium Fluoride as Mouthrinses in Children: A Randomized Controlled Trial

Abstract

Background: Dental caries is a multifactorial disease in which microorganisms play an important role. Recently, herbs have been tried as mouthrinses to combat the side effects of chemical mouthrinses. The anticaries efficacy of Sodium fluoride, Tulsi leaf, and Black myrobalans fruit extracts on Streptococcus mutans (S. mutans) have been reported in the literature, but no comparative study has been done yet. Aim: This study aims to observe the change in the pH of saliva and to assess the efficacy of the herbal rinses-Tulsi and Black myrobalans on S. mutans count while comparing it with Sodium fluoride mouthrinse. Methods: Herbal ethanolic extracts of Tulsi (4%) and Black myrobalans (2.5%) were prepared as mouthrinses and compared with sodium fluoride mouthrinse (0.05%). Sixty high caries risk patients were selected and allocated randomly into three groups [n = 20], categorized as Group A-Sodium fluoride mouthrinse, Group B-Tulsi mouthrinse, and Group C-Black myrobalans mouthrinse. They were instructed to rinse their mouth with their assigned mouthrinses for 7 days. Salivary samples were collected and sent to the laboratory at baseline, 1 h postrinsing and after 7th day of rinsing for determining the salivary pH and S. mutans count. The increase in pH and reduction of S. mutans were determined. The values obtained were tabulated and statistically analyzed. Results: There was a significant increase in the salivary pH and reduction in S. mutans count after rinsing in all the three groups. Increase in salivary pH was more in the Sodium fluoride mouthrinse when compared to the experimental herbal groups (Group B and Group C). While S. mutans counts reduced more with Tulsi mouthrinse at 1 h postrinsing and after the 7th day of rinsing more reduction was seen in Black myrobalans mouthrinse. Conclusion: The results of the study suggest that herbal mouthrinses could be tried as an adjunctive anticaries agent against dental caries causing microorganisms.

Keywords: Antimicrobial agents, child, dental caries, herbal, mouthrinses, Streptococcus mutans

Introduction

Dental caries is a disease of complex etiology, in which microorganisms play an important role.[1] Endogenous oral bacterial species such as Streptococcus mutans (S. mutans), Lactobacillus, Streptococcus sobrinus, and their metabolites have a complex role in the initiation and progression of dental caries.[2] The effective prevention of dental caries can be achieved by mechanical removal of dental plaque through proper brushing and flossing. However, the majority of the population, particularly children, may not perform mechanical plaque removal efficiently and thus antimicrobial mouthrinses such as triclosan, sodium fluoride, and chlorhexidine have been used to limit plaque related oral infections, especially in high caries risk children.[3] Sodium fluoride mouthrinses are effective in reducing caries by inhibiting the oral microorganisms from utilizing carbohydrates by blocking the enzymes which are involved in the bacterial glycolytic pathway.[4] Consequently, the side effects caused by the chemical mouthrinses have prompted to look for herbal alternatives. From this perspective, the use of herbal rinses has been considered as a useful adjunct in children.[2]

Tulsi (Ocimum sanctum) the holy basil is a traditional plant which is considered sacred by the Hindus. It is also known as the incomparable one, elixir of life, queen of herbs, or the mother medicine of nature.[5] It has been found to be effective against infections, especially in high caries risk children.[6] Sodium fluoride mouthrinses are effective in reducing caries by inhibiting the oral microorganisms from utilizing carbohydrates by blocking the enzymes which are involved in the bacterial glycolytic pathway.[4] Consequently, the side effects caused by the chemical mouthrinses have prompted to look for herbal alternatives. From this perspective, the use of herbal rinses has been considered as a useful adjunct in children.[2]

How to cite this article: Megalaad N, Thirumurugan K, Kayalvizhi G, Sajeve R, Kayalvizhi EB, Ramesh V, et al. A comparative evaluation of the anticaries efficacy of herbal extracts (Tulsi and Black myrobalans) and sodium fluoride as mouthrinses in children: A randomized controlled trial. Indian J Dent Res 2018;29:760-7.
both Gram-positive and Gram-negative bacteria while these properties were comparable with the effectiveness of clove oil. Black myrobalans (Terminalia chebula) is called the “King of Medicine” in Tibet because of its exceptional power of healing. The aqueous extract of Black myrobalans actively inhibited the growth, glucan-induced aggregation, and sucrose-induced adherence of S. mutans. In dentistry, few studies have evaluated the anticaries efficacy of Tulsi leaf and Black myrobalans extracts on S. mutans, but no comparative study has been done. Hence, this clinical study was planned to observe the change in the pH of saliva and to assess the efficacy of the herbal rinses-Tulsi and Black myrobalans on S. mutans count and comparing it with the commercially available Sodium fluoride mouthrinse.

Materials and Methods

The study was conducted among school children of St. Johns Residential School in Villupuram district. Permission to conduct the study was obtained from the school Principal. This study was approved by the Institutional Ethical Committee, Internal Review Board, IGIDS (IRB REFERENCE NO: IGIDSIRB 2014PEDO02PGNMDP). Consent from the parents and child’s assent was obtained before enrolling the child for the study. A total of 105 children were screened and recorded in the case sheet. The case sheet consisted of vital statistics with oral hygiene index, plaque index, decayed filled teeth/decayed filled surfaces and decayed, missing, and filled teeth/decayed, missing, and filled surfaces index. The individuals were selected for the study based on the following inclusion and exclusion criteria.

Children between 6 and 12 years of age and children with high caries risk according to the Caries-risk Assessment Tool for >6 years old- (AAPD guidelines 2011) were included whereas children with any systemic disease, physically and mentally challenged children, children with a history of taking antibiotics upto one week before the study, children undergoing orthodontic treatment or wearing any intraoral appliances and those with any intraoral soft tissue pathology were excluded.

From the 105 children screened, 60 children were randomly selected based on sample size calculation. The selected children were randomly categorized as Group A - Sodium fluoride rinse 0.05% (Kidodent Mouthrinse-Warren company) (Control group), Group B - Tulsi mouthrinse 4% (Experimental group), and Group C – Black myrobalans 2.5% (Experimental group) by lottery method. After baseline saliva collection, each group was given their assigned mouthrinse.

Preparation of mouthrinses

**Tulsi leaf extract oral rinse (Group B)**

Tulsi leaves were washed with purified water. This was done to lessen the microbial load on the plant material. They were allowed to air dry and ground to a powdered form. The leaf powder was extracted with 100% ethanol for 30 h. Three hundred grams of fine powdered Tulsi was mixed with 1 L of ethanol; then, it was centrifuged at 12000 rpm for 10 mins. The supernatant was collected, and the solvent was evaporated in hot air oven at 50°C. From 300 g of Tulsi powder dissolved in 1 L of ethanol, 18 g of residue (extract) was obtained. The extract obtained after processing was then desiccated and stored in an air tight container. Final mouthrinse was formulated by dissolving 0.4 g of extract in 10 ml of deionized water.

**Black myrobalans fruit extract oral rinse (Group C)**

The dried ripe fruit of Black myrobalans was screened, and impurities were removed. They were allowed to air dry and ground to a powdered form. The dried fruit powder was extracted with 100% ethanol for 30 h. Then, it was centrifuged at 12,000 rpm for 10 minutes. The supernatant was then collected and the solvent was evaporated in a hot air oven at 50°C. The fruit powder and ethanol were added in 1:6 ratio. The extract thus obtained was then desiccated and stored in an air tight container. Final mouthrinse was formulated by adding 2.5 g of the extract to deionized water.

**Saliva collection**

Baseline saliva samples were collected. Participant’s unstimulated saliva (1.5 ml) was collected using spitting method. Individuals were then asked to spit the collected saliva into a vial containing thioglycollate transport medium. Salivary pH analysis was performed each time immediately after the collection of saliva, using a pH meter. The collected samples were sent to the laboratory for microbial analysis. After baseline saliva sample collection, individuals were instructed to rinse approximately 5 ml of their assigned mouthrinse for 1 min. Salivary samples were collected again 60 min post rinsing. Then, the individuals were instructed to rinse 5 ml of their assigned mouthrinse twice per day for 7 days, and saliva samples were collected.

Salivary pH values and microbial count data were collected and entered in the data collection pro forma. Descriptive statistics is shown by calculating mean and standard deviation. Wilcoxon Signed rank test and Kruskal–Wallis’ ANOVA test was used for comparison between the three groups. The significance level was set at 0.05.

**Results**

A total of sixty children included in the study were randomly divided into three groups as Tulsi, Black myrobalans and Sodium fluoride. After baseline saliva collection, each group was given their assigned mouthrinse. The mean age for the Sodium fluoride group was found to be $8.25 \pm 1.410$, Tulsi and Black myrobalans group was $9.45 \pm 1.504$ and $9.70 \pm 1.658$. The study participants included 45% males and 55% females in the Sodium fluoride group.
flouride group, 60% males and 40% females in Tulsi group and equal distribution in Black myrobalans group.

On comparing Salivary S. mutans count for all the three groups (sodium fluoride, Tulsi, and T. chebula) using Wilcoxon Signed rank test, during the time intervals such as baseline, at 1 h postrinse and 8th day postrinse, the results were found to be statistically significant [Table 1-3].

While comparing salivary pH of Sodium fluoride group between baseline and at 1 h postrinse, baseline and 8th day postrinsing, statistically significant result was found (P ≤ 0.001). However, no significant difference (P = 0.134) was found in the salivary pH between 1 h postrinse and 8th day postrinse (7.025 ± 0.107). Whereas both the experimental groups (Tulsi group and Black myrobalans group), was found to be statistically significant (P ≤ 0.001) during baseline, at 1 h postrinse and 8th day postrinse [Tables 4-6].

Among 3 groups, no significant differences were found between the baseline salivary pH in the sodium fluoride control group (6.085 ± 0.275) and the experimental groups (tulsi and black myrobalans) (6.390 ± 0.441) (6.170 ± 0.499) with Kruskal–Wallis’ ANOVA test [Table 7]. Besides, same results were observed with S. mutans count in all the three groups [Table 8].

While comparing the salivary pH after 1 h between the different mouthrinses, it was found to be statistically significant (P < 0.001*) [Table 9]. It was found that in all the three groups, there was an increase in the salivary pH, while more increase was seen in the control group when compared to the experimental groups. Among the experimental groups, less increase was seen in the Black myrobalans group [Graph 1].

The comparison of S. mutans levels between the three mouthrinses at 1 h postrinsing was done using Kruskall–Wallis’ ANOVA test and statistically significant results were found (P ≤ 0.001*) [Table 10]. When compared with the baseline values, it was found that there was a reduction in the S. mutans count in all the three groups. Tulsi leaf extract mouthrinse showed more reduction in the S. mutans count; Sodium fluoride showed minimal reduction when compared to the Tulsi and Black myrobalans mouthrinses. Thus, these results reveal that Tulsi mouthrinse has the potential to reduce S. mutans immediately [Graph 2].

From the results obtained on comparing salivary pH at 8th day, it was found that both the experimental groups and the control group were statistically significant (P ≤ 0.023*). Raise in salivary pH was found to be high in the control group (Sodium fluoride), followed by Tulsi leaf extract and Black myrobalans mouthrinse was found to be the least [Table 11]. On comparing the S. mutans count among these groups, they were found to be statistically significant (P ≤ 0.001*). However, Black myrobalans mouthrinse showed increased reduction in S. mutans count, thus, it is known to have a prolonged effect on S. mutans [Table 12].

**Discussion**

The present clinical study was conducted in a residential school; to avoid any bias in the outcome as all the individuals had the same dietary and oral hygiene practices. In addition, daily rinsing could be monitored by the teachers

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**Table 1: Comparison of Salivary Streptococcus mutans at different time intervals in Sodium fluoride (Kidodent) group using Wilcoxon Signed rank test**

| S mutans count in CFU × 10^3 | Mean | n  | Std. deviation | Mean difference | P     |
|-----------------------------|------|----|----------------|----------------|-------|
| Pair 1                      |      |    |                |                |       |
| pH Baseline                 | 4.885| 20 | 1.893          | 1.695          | 0.005*|
| At 1 hour pH                | 3.185| 20 | 1.162          |                |       |
| Pair 2                      |      |    |                |                |       |
| pH Baseline                 | 4.885| 20 | 1.162          | 0.895          | 0.002*|
| After 7 days pH             | 2.293| 20 | 1.072          |                |       |
| Pair 3                      |      |    |                |                |       |
| At 1 hour pH                | 4.885| 20 | 1.893          | 2.591          | <0.001*|
| After 7 days pH             | 2.293| 20 | 1.072          |                |       |

*Denotes statistically significant (P<0.05*)

**Table 2: Comparison of Salivary Streptococcus mutans at different time intervals in Tulsi group using Wilcoxon Signed rank test**

| S mutans count in CFU × 10^3 | Mean | n  | Std. deviation | Mean difference | P     |
|-----------------------------|------|----|----------------|----------------|-------|
| Pair 1                      |      |    |                |                |       |
| pH Baseline                 | 4.785| 20 | 4.334          | 3.429          | <0.001*|
| At 1 hour pH                | 1.356| 20 | 1.250          |                |       |
| Pair 2                      |      |    |                |                |       |
| pH Baseline                 | 4.785| 20 | 4.334          | 1.067          | <0.001*|
| After 7 days pH             | 0.288| 20 | 0.252          |                |       |
| Pair 3                      |      |    |                |                |       |
| At 1 hour pH                | 1.356| 20 | 1.250          | 4.496          | <0.001*|
| After 7 days pH             | 0.288| 20 | 0.252          |                |       |

*Denotes statistically significant (P<0.05*)

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and the researchers. We had selected children randomly in the age group of 6–12 years of age since the caries prevalence was considered to be more in this age group.\textsuperscript{14}

Fluoride plays a significant role in the prevention of caries, and thus improves oral and dental health. Sodium fluoride (0.2\%) mouthrinses are effective in reducing caries and inhibit carbohydrate utilization of oral microorganisms by blocking enzymes involved in the bacterial glycolytic pathway studies.\textsuperscript{5,15-19} have shown sodium fluoride mouthrinse to be effective in reducing \textit{S. mutans} counts. Thus sodium fluoride was selected as a control in our study.

There has been a change in the global awareness. With a growing tendency toward the natural products and due to
Table 8: Intergroup comparison - Baseline Streptococcus mutans count in CFU × 10³

| Intergroups                                      | Groups                        | Mean  | Std. deviation | Mean difference | P   |
|-------------------------------------------------|-------------------------------|-------|----------------|-----------------|-----|
| Sodium fluoride vs. Tulsi                        | Sodium fluoride (Kidodent)    | 4.885 | 1.8930         | 0.1000          | 1.00|
| vs. Black myrobalans                            | Tulsi                         | 4.785 | 4.3340         |                 |     |
| Tulsi vs. Black myrobalans                       | Tulsi                         | 4.785 | 4.3340         | 0.1700          | 0.998|
| Black myrobalans                                | Black myrobalans              | 4.615 | 2.5903         |                 |     |

Table 9: Intergroup comparison: One hour after rinsing - Salivary pH

| Intergroups                                      | Groups                        | Mean  | Std. deviation | Mean difference | P   |
|-------------------------------------------------|-------------------------------|-------|----------------|-----------------|-----|
| Salivary pH after one hour rinsing               | Sodium fluoride (Kidodent)    | 6.985 | 0.1785         | 0.2600*         | 0.027*|
| vs. Tulsi                                       | Tulsi                         | 6.725 | 0.3226         |                 |     |
| Tulsi vs. Black myrobalans                       | Tulsi                         | 6.725 | 0.3226         | 0.2600*         | 0.027*|
| Black myrobalans                                | Black myrobalans              | 6.465 | 0.3746         |                 |     |
| Sodium fluoride (Kidodent) vs. Black myrobalans  | Sodium fluoride (Kidodent)    | 6.985 | 0.1785         | 0.5200*         | 0.000*|
| vs. Black myrobalans                            | Black myrobalans              | 6.465 | 0.3746         |                 |     |

*Denotes statistically significant (P<0.05*)

the side effects of conventional mouthwashes; we had selected herbal leaves and fruits in our study. Phytochemical constituents of tulsi leaves are responsible for its antimicrobial activity. A few in vitro[10,20] and clinical studies[21] have found tulsi to be effective against S. Mutans. Black myrobalans/T. chebula chemical constituents also exhibit antibacterial activity.[10,21] Studies have reported that its tannic acid content is bactericidal and bacteriostatic against few Gram-positive and Gram-negative pathogens.[6] Besides, studies have found it to reduce cariogenic microorganisms clinically.[10,11,22-24] The extracts were prepared using ethanol because in the previous studies it was stated that ethanolic extracts of Tulsi and Black myrobalans were found to be more effective.[10,13,24,25] Ethanol was used only as a solvent because its phytochemical constituents are easily soluble and display enhanced activity when compared to water alone.[22] It has stronger extraction capacity and produces a greater number of active constituents responsible for antibacterial activity.[26] The final extract, however, did not contain ethanol as it was removed from the extract[22] using a rotary evaporator.

In the present study, saliva samples were collected from the individuals similar to the previous study[11,16,21,22,27-30] than plaque. Recently, it has been shown that dental caries is associated with characteristic bacterial profiles of saliva.[31] We collected unstimulated saliva samples collection in the morning in between 11.00 and 11.30 am, to match with the normal circadian rhythm as it represents the basal salivary flow rate. Previous studies[10,21-23,27] have
Megalaa, et al.: Herbal mouthrinse versus sodium fluoride mouthrinse

The salivary pH of the two experimental groups (Tulsi and Black myrobalans) and control group showed almost same amount of raise in pH. Sodium fluoride mouthrinse showed maximum increase followed by Tulsi group on 1 h postrinsing, whereas after 7 days, it was more in Black myrobalans group. Among the groups, reduction in S. mutans count was more in Black myrobalans group, followed by Tulsi group and then by very effective in increasing the salivary pH up to 90 min and the peak increase was at 30 min postrinsing. Similar studies also found an increase in salivary pH at 10 min and 5 h after rinsing.[28] This shows that both the Tulsi and Black myrobalans extract mouthrinses possess substantivity and thus they could be tried as an effective mouthrinse.

In this study, there was a significant reduction in the S. mutans count on using Sodium fluoride mouthrinse at 1 h and 8th day postrinsing. Similar results were observed from other studies on sodium fluoride.[5,16] In the current study, we have made an attempt to assess the antibacterial effect of 4% Tulsi leaf extract mouthrinse against S. mutans count and found a greater reduction on 8th day postrinsing. These results were similar to Agarwal et al. study where they investigated different concentrations of Tulsi extract against Smutans and found its 4% concentration to display the maximum zone of inhibition.[1]

We found a significant reduction in the S. mutans count on using 2.5% of the Black myrobalans mouthrinse, with more reduction on the 8th day when compared to 1 h postrinse. Our results are in comparison with Nayak et al. and Aneja and Joshi studies.[24] Similar studies Jagtap and Karkera also gave same results up to 3 h postrinsing but used 10% aqueous extract of T. chebula,[28] while Carounanidy et al. also used 10% aqueous extract of Black myrobalans and found a 65% decrease in the S. mutans count.[11] Pratap Gowd MJS et al. studied various concentrations (5%, 10%, 25%, and 50%) of T. chebula extract and found its 10%, 25%, and 50% exhibited greater antimicrobial efficacy against S. Mutans count.[36]

In the present study, no significant differences was observed on comparing the baseline salivary pH and S. mutans between the experimental and control groups. On comparing the S. mutans count after 1 h of rinsing between the three groups, significant results were found. Reduction of S. mutans count was more in Tulsi group, followed by Black myrobalans and Sodium fluoride mouthrinse. From this, it could be assessed that Tulsi mouthrinse has an immediate effect on S. mutans count. The antibacterial effect of Tulsi may be attributed to the presence of methyl ester of salicylic acid which accelerates granulation and regeneration of tissues.[27] In addition, ursolic acid and carvacrol might be responsible for its antimicrobial activity.[37] Some authors have found its fixed oil to exhibit good antibacterial activity due to its higher linolenic acid content.[38]

The salivary pH of the two experimental groups (Tulsi and Black myrobalans) and control group showed almost same amount of raise in pH. Sodium fluoride mouthrinse showed maximum increase followed by Tulsi group on 1 h postrinsing, whereas after 7 days, it was more in Black myrobalans group. Among the groups, reduction in S. mutans count was more in Black myrobalans group, followed by Tulsi group and then by

Table 12: Intergroup comparison: 7 days after rinsing - Streptococcus mutans count in CFU \times 10^3

| Intergroups | Groups | Mean | Std. deviation | Mean | P value | difference |
|------------|-------|------|---------------|------|---------|------------|
| Streptococcus mutans count in CFU × 10^3 after 7th day rinsing | Sodium fluoride (Kidodent) vs. Tulsi | 2.29315 | 1.072528 | 2.004200 | <0.001* |
| | Tulsi vs. black myrobalans | 0.28895 | 0.252001 | 0.28450 | 0.959 |
| | Sodium fluoride (Kidodent) vs. Black myrobalans | 2.29315 | 1.072528 | 2.032650 | <0.001* |
| | Black myrobalans | 0.26050 | 0.134535 | | |

*Denotes statistically significant (P<0.05*)

also used unstimulated saliva for assessing the salivary pH and S. mutans count. Saliva has been recognized as having the ability to reduce the incidence of dental caries. It also serves as the host defense mechanism by repairing the demineralization that occurs when the plaque pH is below 5.5–6.0 and can remineralize enamel.[12] Thus, evaluating the causative factors such as pH and S. mutans count in saliva of individuals at risk to dental caries could pave the way to make recommendations that will cater specifically to individual’s needs.[13]

Samples were collected at baseline to compare the effectiveness of the mouthrines after subjecting them to intervention. In previous studies, saliva samples have been collected 60 min[11,22,23] and 7 days after rinsing.[23] Hence, samples were collected at three times (baseline, 60 min after rinsing and 7 days after rinsing) to evaluate the efficacy of the three mouthrines over a period.

It was found that as the duration of using the mouthrinse increased, there was an increase in the salivary pH. These results are in correlation with previous studies using sodium fluoride,[16-18] while contradictory to Fadaei and Amir et al. study as they found no improvement in reducing caries with its use for almost 8 years.[34] With Tulsi mouthrinse, there was increase in the pH in an orderly manner, i.e., from baseline to 1 h postrinse and after 7th day postrinse in the present study. The results of this study cannot be compared with other studies because there is no previous study, which assessed the salivary pH on using Tulsi mouthrinse. With Black myrobalans extract mouthrinse, it was found that there was gradual increase in the salivary pH, which is in correlation with Nayak et al.[10,22] study, who observed an increase in salivary pH till 1 h and Carounanidy et al.[11] found T. chebula mouthrinse to be
the Sodium fluoride group. The chief constituents of Black myrobalans are hydrolysable tannins. Tannic acid, gallic acid, and chebulic acid are the major constituents of the ripe fruit of *T. chebula*. This long-term effectiveness of Black myrobalans could be attributed to the presence of tannins, Tannic acid is known to be well adsorbed to the hydroxyapatite of the tooth or to the salivary mucins, alternatively, it can bound to the anionic groups on the surface of the bacterial cells, which could result in protein denaturation and ultimately bacterial cell death. No sweeteners were added because it might alter the result. Both Tulsi and Black myrobalans mouthrinses were acceptable by the patients in our study.

Thus, from the present study, it was found that Black myrobalans were found to be effective on long term use, whereas Tulsi showed effectiveness immediately. Here, both the herbal mouthrinses (Tulsi and Black myrobalans) were found to be as effective as the Sodium fluoride mouthrinse. Thus, these herbal mouthrinses could be tried as a safe and effective alternative in children with further research.

Further studies should be undertaken with a larger sample size. Long-term studies need to be conducted to determine the long-term effect of mouthrinses on oral health. Besides, the long-term patient acceptability and compliance need to be assessed.

**Conclusion**

Tulsi and Black myrobalans mouthrinses possessed anticaries efficacy by effectively increasing the salivary pH and reducing the *S. mutans* counts. All the three mouthrinses effectively increased salivary pH and reduced the *S. mutans* counts. Tulsi seems to show its efficacy immediately, whereas, Black myrobalans mouthrinse displayed a prolonged effect on *S. mutans*. The use of medicinal plants against *S. mutans* can be a viable alternative to other antimicrobial agents as these herbal mouthrinses offer a cheap and effective module in controlling the bacteria responsible for causing caries.

**Financial support and sponsorship**

Nil.

**Conflicts of interest**

There are no conflicts of interest.

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