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ORIGINAL PAPER

# The role of Protopine associated with Nuciferine in controlling adverse events during hyperthermic intravesical chemotherapy instillations. A nutraceutical approach to control adverse event during intravesical instillations

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***Summary*** *Objectives: The aim of this study was to analyse the role of two alkaloid, Protopine and Nuciferine, in the prevention and the treatment of the low and mild grade adverse events related to the use of HIVEC® (Hyperthermic IntraVEsical Chemotherapy) instillations.*
*Materials and methods: From September 2017 to September 2019, 100 patients were prospectively randomized into two groups: Group A = Protopine and Nuciferine syrup, 10 ml, once a day, for 8 weeks; Group B = placebo (flavoured coloured water), 10 ml, once a day, for 8 weeks. The primary endpoint was the evaluation of the efficacy of the therapy with Protopine and Nuciferine in controlling of the irritative symptoms. The secondary endpoint was the evaluation of the influences of the treatment on the uroflowmetric parameters.*
*Results: The patients of Group A showed a better International Prostatic Symptoms Score (IPSS) score, a better control of urgency symptoms (PPIUS) and tolerate well the pain (VAS score). The treatment doesn't modify Uroflow-$Q_{max}$ and seems to improve the Uroflow-Voided Volume (ml) without influencing the Uroflow-Post Void Residual volume (PVR).*
*Moreover, the treatment with Protopine and Nuciferine has been proven to be effective in the treatment of overactive bladder (OAB) symptoms. Patients' evaluation of the two different treatments assessed with Patient Global Impression of Improvement questionnaire (PGI-I), demonstrated improvements in the Group A, while the Group B showed a lower satisfaction.*
*Conclusions: Protopine and Nuciferine can be interesting nutraceutical compounds useful to control irritative and pain related symptoms of intravesical chemo/immunotherapy.*

*KEY WORDS: Complementary medicine; Bladder cancer; LUTS; Chemotherapy; Overactive bladder; Urodynamics.*

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## INTRODUCTION

In recent times, *Hyperthermic IntraVesical Chemotherapy* (HIVEC®) instillations has been added to the existing regimens as adjuvant and neoadjuvant treatment of bladder cancer (1). Most of the side effects were low grade and 97% of patients completed the HIVEC® protocol (2). In a population of 55 BCG (*Bacillus Calmette-Guérin*) unresponsive *non-muscle invasive bladder cancer* (NMIBC) patients, 5% of the patients did not complete at least 5 HIVEC® instillations because of facial swelling, urticaria and urinary tract pain Grade 3 according to *Common Terminology Criteria for Adverse Events* (CTCAE). Despite this, 7% of patients reported CTCAE Grade 1 bladder spams, 11% CTCAE Grade 1 urinary frequency/urgency, 24% CTCAE Grade 2 urinary frequency/urgency, 4% CTCAE Grade 1 urinary tract pain, 9% CTCAE Grade 2 urinary tract pain and 2% CTCAE Grade 3 urinary tract pain (3).
Despite the low number of adverse events, the patients treated with HIVEC® were significantly more likely to develop urinary frequency, haematuria and bladder spasm than passive mitomycin-C (4).
The aim of this study was to analyse the role of two alkaloid, Protopine and Nuciferinein the prevention and the treatment of the low and mild grade adverse events related to the use of HIVEC® instillations.

## MATERIALS AND METHODS

From September 2017 to September 2019, 100 patients with *Bacillus of Calmette Guerin* (BCG)-failure (*including BCG-refractory tumour, BCG-relapsing tumour and BCG unresponsive tumour*) NMIBC whose underwent HIVEC® Chemotherapy were prospectively enrolled in this study. The patients (pts) were randomized into two groups using block randomization in order to obtain two groups of equal sample size. In group A, we enrolled 50 patients who received *Protoves M1®* (*Protopine and Nuciferine*) syrup, 10 ml, once a day, for 8 weeks. In group B, we enrolled 50 patients who received placebo (flavoured coloured water), 10 ml, once a day, for 8 weeks.
They started the therapy two weeks before the beginning of HIVEC® treatment. Inclusion criteria covered all the patients with intermediate-and high-risk NMIBC patients who were planned to receive HIVEC® treatment. Patients with uncontrolled underlying diseases (ASA III or IV), post void residual urine ≥ 100 ml, bleeding tendency, drug abuse, chronic pelvic pain, urinary tract infection, neurological disease, bladder lithiasis, renal or liver failure, tachycardia and heart failure were

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excluded from the study. Male patients with *lower urinary tract symptoms* (LUTS) and *benign prostatic hyperplasia* (BPH) were not excluded from the study if they had normal values of uroflowmetry and they did not assume any medical treatment for BPH. At the baseline, data on demographic and anthropometric features (age, weight, height, BMI [body mass index]), lifestyle characteristics (smoke, alcohol), any comorbidities (hypertension, diabetes mellitus, etc.) were collected. All patients underwent a clinical evaluation (comprised general, genital and urologic examination). Before starting the treatment, the following measurements were collected: prostate volume by transrectal ultrasound, *prostate specific antigen* (PSA), uroflowmetry parameters (*Uroflow-$Q_{max}$, Uroflow-Voided Volume, Uroflow-PVR* [Post-Void Residual]), *International Prostatic Symptoms Score* (IPSS) questionnaire, *OverActive Bladder questionnaire-short form* (OABq-SF) 6 and 13, *patient perception of intensity of urgency scale* (PPIUS), and *visual analogue scale* (VAS). PSA and IPSS were investigated only in male pts. In addition, the patient impression of improvement was assessed.
Improvement was evaluated with the *Patient Global Impression of Improvement* questionnaire (PGI-I), a validated tool to estimate the improvement or the deterioration associated to the treatment. The primary endpoint was the evaluation of the efficacy of the therapy with Protopine and Nuciferine in controlling irritative symptoms, including nocturia, urinary frequency, bladder pain, urgency and urge incontinence related to the chemo-hypertermia treatment. The secondary endpoint we evaluated was the influence of the treatment on the uroflowmetric parameters. Evaluation was performed after 1 week and after 6 weeks of therapy. Statistical analyses were conducted using SAS version 9.3 software (*SAS Institute, Inc., NC*). Mean values with standard deviations (± SD) were computed and reported for all items. Statistical significance was achieved if p-value was ≤ 0.05 (two-sides).

## Results

Table 1 depicts patients' demographics and baseline characteristics. The two groups showed no difference in terms of patients' demographics as well as tumour characteristics in all variables.
Of all 100 patients enrolled, 2/50 (4%) of group B withdrew the study for adverse events of HIVEC treatment. In Table 2 and 3 we show a comparison of uroflowmetric parameters and questionnaires from baseline and one and six weeks follow up of treatment in the cases (group A) and in the controls (group B). The uroflow parameters (*Uroflow-$Q_{max}$, Uroflow-Voided Volume, Uroflow-PVR*) did not show statistical significant difference from baseline in the group A and one and six weeks follow-up. Otherwise IPSS total significantly increase at one week from baseline ($p < 0.001$), but this difference was not significant at six weeks when IPSS total was not significantly worse from baseline ($p = 0.09$). In group A the quality of life showed a significant worsening from baseline after one week and six weeks of treatment in all parameters and questionnaires analyzed (*OAB-q SF6, OAB-q SF13, PPIUS, VAS Scale*) but after six weeks of treatment a significant-

***Table 1.***
*Demographics and baseline characteristics of the 50 cases and 50 controls.*

| Variable | Value (cases) | Value (controls) | p |
|---|---|---|---|
| **N° (%)** | | | |
| Males | 40 (80%) | 39 (78%) | p = 0.8061 |
| Females | 10 (20%) | 11 (22%) | p = 0.8061 |
| Smokers | 12 (24%) | 11 (22%) | p = 0.8122 |
| Non-smokers | 38 (76%) | 39 (78%) | p = 0.8122 |
| Diabetes (Yes) | 5 (10%) | 7 (14%) | p = 0.5383 |
| Diabetes (No) | 45 (90%) | 43 (86%) | p = 0.5383 |
| C.I.S. (Yes) | 11 (22%) | 8 (16%) | p = 0.4444 |
| C.I.S. (No) | 39 (78%) | 42 (84%) | p = 0.4444 |
| **Mean ± SD** | | | |
| Age at surgery (years) | 58.52 ± 5.28 | 57.64 ± 5.38 | p = 0.4138 |
| BMI (kg/m$^2$) | 26.754 ± 1.96 | 26.916 ± 1.91 | p = 0.6764 |
| T.U.R.B. (N°) | 1.86 ± 1.18 | 2.08 ± 1.16 | p = 0.3495 |
| Tumors (last T.U.R.B.) (N°) | 3.5 ± 2.44 | 4.08 ± 2.25 | p = 0.2195 |
| PSA (ng/ml) | 2.33 ± 0.95 | 2.13 ± 0.95 | p = 0.2951 |
| Prostate volume (cc) | 42.43 ± 13.39 | 41.38 ± 13.56 | p = 0.6977 |
| Uroflow-$Q_{max}$ (ml/s) | 17.49 ± 2.39 | 17.39 ± 2.21 | p = 0.8285 |
| Uroflow-Voided Volume (ml) | 250.6 ± 39.8 | 242.2 ± 41.2 | p = 0.3023 |
| Uroflow-PVR (ml) | 15.4 ± 10.6 | 14.9 ± 10.1 | p = 0.8097 |
| IPSS total | 7.05 ± 3.88 | 7.79 ± 4.55 | p = 0.3837 |
| QOL score | 2.22 ± 1.23 | 2.34 ± 1.26 | p = 0.6310 |
| OAB-q SF 6 | 11.48 ± 5.18 | 11.92 ± 5.30 | p = 0,6755 |
| OAB-q SF 13 | 25.12 ± 11.50 | 26.02 ± 11.39 | p = 0.6950 |
| PPIUS | 0.94 ± 0.79 | 0.96 ± 0.78 | p = 0.8989 |
| VAS scale | 0.08 ± 0.27 | 0.18 ± 0.87 | p = 0.4395 |

*CIS: In Situ Carcinoma; BMI: Body Max Index; TURB: Trans Urethral Bladder Resection; PSA: Prostate Specific Antigen; PVR: post-void residual; IPSS: International Prostatic Symptoms Score; QOL: quality of life; OAB: overactive bladder; PPIUS: patient perception of intensity of urgency scale; VAS: visual analogue scale.*

***Table 2.***
*Comparison of uroflowmetry parameters and questionnaires from baseline and between one and six weeks of treatment (cases).*

| Variable | Value (baseline) | Value (one-week) | Value (six-weeks) |
|---|---|---|---|
| **Uroflow-$Q_{max}$ (ml/s)** | 17.49 ± 2.39 | 16.99 ± 2.55 | 16.62 ± 2.43 |
| p (from baseline) | | p = 0.3142 | p = 0.0742 |
| **Variable** | **Value (baseline)** | **Value (one-week)** | **Value (six-weeks)** |
| **Uroflow-Voided Volume (ml)** | 250.6 ± 39.8 | 239.7 ± 40.10 | 250.9 ± 39.22 |
| p (from baseline) | | p = 0.1756 | p = 0.9698 |
| p (from 1 and 6 weeks) | | | p = 0.1611 |
| **Uroflow-PVR (ml)** | 15.4 ± 10.6 | 18.18 ± 12.46 | 19.7 ± 12.01 |
| p (from baseline) | | p = 0.2324 | p = 0.0606 |
| p (from 1 and 6 weeks) | | | p = 0.5360 |
| **IPSS total** | 7.05 ± 3.88 | 11.18 ± 4.84 | 8.35 ± 3.81 |
| p (from baseline) | | p < 0.001 | p = 0.0941 |
| p (from 1 and 6 weeks) | | | p = 0.0016 |
| **OAB-q SF 6** | 11.48 ± 5.18 | 17.16 ± 4.82 | 14.46 ± 5.60 |
| p (from baseline) | | p < 0.001 | p = 0.0069 |
| p (from 1 and 6 weeks) | | | p = 0.0112 |
| **OAB-q SF 13** | 25.12 ± 11.50 | 36.42 ± 10.67 | 31.16 ± 11.57 |
| p (from baseline) | | p < 0.001 | p = 0.0102 |
| p (from 1 and 6 weeks) | | | p = 0.0201 |
| **PPIUS** | 0.94 ± 0.79 | 2.26 ± 0.88 | 1.66 ± 0.66 |
| p (from baseline) | | p < 0.001 | p < 0.001 |
| p (from 1 and 6 weeks) | | | p = 0.0002 |
| **PGI-I** | 4 ± 0 | 4.2 ± 0.60 | 2.46 ± 0.86 |
| p (from baseline) | | p = 0.1161 | p < 0.001 |
| p (from 1 and 6 weeks) | | | p < 0.001 |
| **VAS scale** | 0.08 ± 0.27 | 3.38 ± 1.35 | 2.34 ± 0.96 |
| p (from baseline) | | p < 0.001 | p < 0.001 |
| p (from 1 and 6 weeks) | | | p < 0.001 |

*PVR: post-void residual; IPSS:International Prostatic Symptoms Score; OAB: overactive bladder; PPIUS: patient perception of intensity of urgency scale; PGI-I: Patient Global Impression of Improvement questionnaire; VAS: visual analogue scale.*

***Table 3.***
*Comparison of uroflowmetry parameters and questionnaires from baseline and between one and six weeks of treatment (controls).*

| Variable | Value (baseline) | Value (one-week) | Value (six-weeks) |
|---|---|---|---|
| **Uroflow-$Q_{max}$ (ml/s)** | 17.39 ± 2.21 | 17.06 ± 2.25 | 17.02 ± 2.15 |
| p (from baseline) | | p = 0.4611 | p = 0.3982 |
| p (from 1 and 6 weeks) | | | p = 0.9278 |
| **Uroflow-Voided Volume (ml)** | 242.2 ± 41.22 | 220.4 ± 43.66 | 218.8 ± 43.21 |
| p (from baseline) | | p = 0.0118 | p = 0.0067 |
| p (from 1 and 6 weeks) | | | p = 0.8543 |
| **Uroflow-PVR (ml)** | 14.9 ± 10.13 | 13.8 ± 10.08 | 15.9 ± 9.98 |
| p (from baseline) | | p = 0.5875 | p = 0.6201 |
| p (from 1 and 6 weeks) | | | p = 0.2977 |
| **IPSS total** | 7.79 ± 4.55 | 13.64 ± 5.71 | 14.62 ± 5.92 |
| p (from baseline) | | p < 0.001 | p < 0.001 |
| p (from 1 and 6 weeks) | | | p = 0.4016 |
| **OAB-q SF 6** | 11.92 ± 5.3 | 20.58 ± 6.29 | 24.34 ± 6.3 |
| p (from baseline) | | p < 0.001 | p < 0.001 |
| p (from 1 and 6 weeks) | | | p = 0.0036 |
| **OAB-q SF 13** | 26.02 ± 11.39 | 43.78 ± 13.02 | 49.44 ± 12.65 |
| p (from baseline) | | p < 0.001 | p < 0.001 |
| p (from 1 and 6 weeks) | | | p = 0.0298 |
| **PPIUS** | 0.96 ± 0.78 | 2.12 ± 0.69 | 2.54 ± 0.64 |
| p (from baseline) | | p < 0.001 | p < 0.001 |
| p (from 1 and 6 weeks) | | | p = 0.0021 |
| **PGI-I** | 4 ± 0 | 4.82 ± 0.96 | 5.22 ± 1.02 |
| p (from baseline) | | p < 0.001 | p = 0.0462 |
| p (from 1 and 6 weeks) | | | p = 0.2719 |
| **VAS scale** | 0.08 ± 0.27 | 3.68 ± 1.88 | 3.92 ± 1.93 |
| p (from baseline) | | p < 0.001 | p < 0.001 |
| p (from 1 and 6 weeks) | | | p = 0.5302 |

*PVR: post-void residual; IPSS:International Prostatic Symptoms Score; OAB: overactive bladder; PPIUS: patient perception of intensity of urgency scale; PGI-I: Patient Global Impression of Improvement questionnaire; VAS: visual analogue scale.*

ly improvement of quality of life was demonstrated with respect of the values at one week. PGI-1 did not significantly show any change at one week but showed a significant decrease at six weeks follow-up with a greater satisfaction for the treatment. In the group B the uroflow parameters did not show a statistical significant difference from baseline at one and six weeks follow-up except for a significant reduction of Uroflow-Voided Volume (p = 0.0118) at one week. A significant worsening of IPSS score was seen at one and six weeks of follow-up (p < 0.001). In this group the quality of life showed a significant worsening after one week and six weeks of treatment from baseline in all parameters and questionnaires analyzed (OAB-q SF6, OAB-q SF13, PPIUS, VAS Scale). At six weeks follow-up a significantly worsening of quality of life was demonstrated from the values at one week. PGI-1 showed a significantly increase from baseline at one and six weeks of follow-up with a worse perception of the response to the therapy. VAS scale also showed a significantly increase from baseline at one and six weeks of follow-up. In both cases an increase of values was seen from the one and six weeks of follow-up without reaching a significant difference.

Table 4 shows a comparison of uroflowmetric parameters and results of questionnaires between cases and controls at one and six weeks. No differences were seen in the Uroflow-$Q_{max}$ and in the Uroflow-PVR at one (p = 0.8846) and six weeks (0.3951) while the Uroflow-Voided Volume was significantly reduced in the controls at one (p = 0.0234) and six (p = 0.0002) weeks follow-up. The IPSS questionnaire significantly get worse in the group B instead of group A at one (p = 0.0222) and six (p < 0.001) weeks of treatment. Group A showed a significantly better OAB-q SF 6 and OAB-q SF 13 at one and six weeks of follow-up, while the PPIUS and the VAS scale were significantly worse in the group B only at six weeks. PGI-1 showed a worst perception of the condition post-therapy in the group B both after one and six weeks of treatment. No adverse events were reported in both groups.

***Table 4.***
*Comparison of uroflowmetry parameters and results of questionnaires between cases and controls at one and six weeks.*

| Variable | Value (Group A 1-week) | Value (Group B 1-week) | p | Value (Group A 6-week) | Value (Group B 6-week) | p |
|---|---|---|---|---|---|---|
| **Uroflow-$Q_{max}$ (ml/s)** | 16.99 ± 2.55 | 17.06 ± 2.25 | 0.8846 | 16.62 ± 2.43 | 17.02 ± 2.15 | 0.3951 |
| **Uroflow-Voided Volume (ml)** | 239.7 ± 40.10 | 220.4 ± 43.66 | 0.0234 | 250.9 ± 39.22 | 218.8 ± 43.21 | 0.0002 |
| **Uroflow-PVR (ml)** | 18.18 ± 12.46 | 13.8 ± 10.08 | 0.0562 | 19.7 ± 12.01 | 15.9 ± 9.98 | 0.0611 |
| **IPSS total** | 11.18 ± 4.84 | 13.64 ± 5.71 | 0.0222 | 8.35 ± 3.81 | 14.62 ± 5.92 | < 0.001 |
| **OAB-q SF 6** | 17.16 ± 4.82 | 20.58 ± 6.29 | 0.0029 | 14.46 ± 5.60 | 24.34 ± 6.3 | < 0.001 |
| **OAB-q SF 13** | 36.42 ± 10.67 | 43.78 ± 13.02 | 0.0026 | 31.16 ± 11.57 | 49.44 ± 12.65 | < 0.001 |
| **PPIUS** | 2.26 ± 0.88 | 2.12 ± 0.69 | 0.3782 | 1.66 ± 0.66 | 2.54 ± 0.64 | < 0.001 |
| **PGI-I** | 4.2 ± 0.60 | 4.82 ± 0.96 | 0.0002 | 2.46 ± 0.86 | 5.22 ± 1.02 | < 0.001 |
| **VAS scale** | 3.38 ± 1.35 | 3.68 ± 1.88 | 0.3616 | 2.34 ± 0.96 | 3.92 ± 1.93 | < 0.001 |

*PVR: post-void residual; IPSS: International Prostatic Symptoms Score; OAB: overactive bladder; PPIUS: patient perception of intensity of urgency scale; PGI: Patient Global Impression of Improvement questionnaire; VAS: visual analogue scale.*

## Discussion

Several clinical trials have demonstrated a benefit for chemo-hyperthermia over intravesical chemotherapy alone for treating NMIBC (5-6). Neoadjuvant HIVEC consisted of intravesical chemotherapy with *Mitomycin-C* (MMC) combined with bladder hyperthermia, which was achieved in our cases with the *Bladder Recirculation System* (BRS) system from Combat Medical. The Combat BRS device is an external device that heats fluid (MMC in this case) in a sterile disposable bag and recirculates it to the urinary bladder at a constant and controllable temperature and flow rate through a three-way Foley catheter. Hyperthermia increases drug uptake into the cancer cells, affects drug metabolism, and impairs cellular DNA repair mechanisms that normally counteract drug effect (7-9).

This treatment might be a feasible option in BCG unresponsive NMIBC patients, potentially avoiding or postponing the need for radical surgery in a proportion of these patients (3, 10, 11).

Nevertheless, this treatment can increase the rate of local adverse events than passive mitomycin-C like urinary frequency, haematuria and bladder spasm (4).

Protopine has a demonstrated anti-

cholinergic- antimuscarinic (11, 12) and GABAergic (12-15) action and it is able to influence some neurological systems responsible of bladder functions. Moreover, its anti-acetylcholinesterase action give it an antiamnesic property that may hold significant therapeutic value in alleviating certain memory impairments observed in dementia (15, 16).

Protopine increase the p53-mediated transcriptional activity, resulting in stabilization of p53 protein. It exerts an antiproliferative activity and may have potential effect as a chemopreventive agent for human colon cancer (16, 17).

Nuciferin is a partial antagonist of D2-like receptor and has a demonstrated regulatory action on the dopaminergic system (responsible of urination onset (17, 18) and seems to reduce states of tension and anxiety on a psychological level (18-20). Nuciferine significantly inhibited the lipopolysaccharide (LPS)-induced inflammatory cytokine IL-6 and TNF-$\alpha$ production in RAW 264.7 cells having potential anti-inflammatory activities (20, 21).

Its use significantly decrease the expression of TLR4 in a dose-dependent manner and potently ameliorates LPS-induced mastitis by inhibition of the TLR4-NF-$\kappa$B signaling pathway (21, 22). In addiction nuciferine alleviated fructose-induced inflammation by inhibiting TLR4/PI3K/NF-$\kappa$B signaling and NLRP3 inflammasome activation in rat renal cortex and HK-2 cells, which may contribute to the improvement of renal injury (22, 23).

This molecule is an aporphine alkaloid of lotus leaf extract which can induce relaxation in contracted tracheal rings. It induce relaxation in tracheal rings mainly by inhibition of extracellular $Ca^{2+}$ influx through the blockade of voltage-dependent L-type $Ca^{2+}$ channels and/or nonselective cation channels, showing therapeutic effect on respiratory diseases associated with the aberrant contraction of airway smooth muscles and/or bronchospasm (23, 24).

In our experience the patients who underwent Protopine and Nuciferine syrup treatment, showed a better IPSS score, a better control of urgency symptoms (PPIUS) and tolerate well the pain related to the chemo-hyperthermia treatment (VAS score) compared to control group.

The treatment doesn't modify Uroflow-$Q_{max}$ at 1 and 6 weeks and seems to improve the Uroflow-Voided Volume (ml) withouth influence the Uroflow-PVR.

Moreover Protopine and Nuciferine syrup treatment has been proven to be effective in the treatment of OAB symptoms with a significant reduction of the symptoms assessed with *Overactive Bladder Symptoms Score questionnaire* (OAB-q SF6, OAB-q SF13) after six weeks of treatment but not after one week of treatment. This underline the need to start the treatment with Protopine and Nuciferine as soon as possible in order to enhance the effect of the treatment.

Patients' evaluation of the two different treatments (*Protopine and Nuciferine vs Placebo)* assessed with PGI-I, demonstrated improvements in the group of cases with a greater satisfaction expressed by patients at six weeks, while the control group showed a lower satisfaction at one and six weeks.

Moreover, the treatment with Protopine and Nuciferine syrup was well tolerated by all patients, none of them showing any side effect during the period study.

The study is a double-blind randomized study but is limited by the small number of patients and monocentric nature.

## Conclusions

Protopine and Nuciferine syrup can be an interesting alternative to antinflammatory and antimuscarinic agents to treat irritative and pain related symptoms of intravesical chemo/immunotherapy.

More studies should be carried out to clarify the precise role of the active ingredients of Protopine and Nuciferine syrup and their interactions.

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