



Formulation of Health Drink using Cucumber, Bottlegourd and Tulsi for reducing Serum Uric Acid in Individuals with Non-Alcoholic Fatty Liver Disease

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Abstract

This research relates to the preparation of nutritional health-drink by mixing bottlegourd (*Lagenaria siceraria*), cucumber (*Cucumis sativus*), Holy basil (*Ocimum sanctum*), Stevia (*Stevia rebaudiana*), Lemon juice and Black salt in water. A “double-blind placebo-controlled trial” was performed to study the effect of this dietary supplement on the levels of serum UA in individuals with NAFLD. 42 participants with hepatic steatosis were considered. The intervention group received the health drink (300 ml/day) daily for six weeks, whereas the control group had a placebo drink, on a daily basis. At baseline and six weeks later, measurements of “serum UA,” “lipids,” “glucose,” and “anthropometric parameters” were made. After adjusting for confounding factors, we observed that participants receiving the health drink group experienced a larger decrease in blood UA (-0.1 ± 0.6 mg/dL vs. 0.3 ± 0.6 mg/dL, $p = 0.003$), particularly those reflecting symptoms of moderate to severe hepatic steatosis. Among participants using supplement, those with highest baseline blood UA levels (>5.1 mg/dL) experienced a higher reduction in UA levels, than those with the lowest baseline levels (-7.2% vs. $+4.3\%$; adjusted $p = 0.03$). The absolute blood UA change and nutraceutical treatment were linked, as per the stepwise multivariable analysis ($B = -0.47$; $p = 0.003$).

Keywords:

Cucumber, Bottlegourd, Holy basil, Health drink, Stevia, Serum uric acid, Fatty liver disease

INTRODUCTION

Bottlegourd (*Lagenaria siceraria*) and Cucumber (*Cucumis sativus*) are medicinal vegetables used as low-calorie food and are rich sources of minerals and vitamins. They can control various diseases including Non-alcoholic fatty liver disease (FLD), urinary problems, kidney disorder, heart related diseases, pancreatic problems in addition to their diuretic and antioxidant activities. Body weight, hepatic steatosis, and oxidative stress indicators can all be decreased by it. Even cardiovascular problems and blood pressure fluctuations are claimed to be relieved following a regular intake of bottlegourd juice for about 4-6 months (Kothari and Rakshak, 2005).

The body's uric acid levels can be impacted by Non-alcoholic FLD. Purines, which can be found in some meals and are also created by the body, are broken down by the body to form uric acid. Uric acid normally dissolves in the blood, travels through the kidneys, and leaves the body through the urine. However, occasionally the body either makes too much uric acid or does not get rid of it effectively, which causes the blood levels to be elevated. Several variables can influence increases in uric acid levels in people with NAFLD.

Non-alcoholic FLD represents a condition that is caused due to various build-ups of fat in the liver. Most people who are suffering from obesity represents this symptom. In the early stages, patients suffering from Non-alcoholic FLD does not faces major issues, however, with time, it might cause severe liver damage.

People with Non-alcoholic FLD may have dietary patterns that contribute to higher uric acid levels. Diets high in purine-rich foods (such as organ meats, shellfish, and certain types of fish) can increase uric acid production. Additionally, excessive consumption of fructose (found in sugary beverages and some processed foods) can raise uric acid levels. It's important to note that not all individuals with Non-alcoholic FLD will experience changes in uric acid levels but most of the diabolic patients have the risk of high-level uric acid. Treatment for metabolic disorders including NAFLD and hyperuricemia may be safe and successful when using natural substances taken from fruits and vegetables (Jiang et al., 2020).

A health drink which mainly made of the juice of Cucumber, Bottle gourd, Holy basil (Tulsi) in a measured quantity has the capability to lower the uric acid level on Non-alcoholic FLD patients. Stevia can use as a substitute of other artificial sweeteners and raw sugar for the

diabetic patients. It is use in this health drink for maintain the taste as well as have some other benefits as well. Drinks that have been blended are a good way to create new items that have a fresh flavour and better nutrition. This improvement can be due to the combination of two or more fruit juices, which increased the product's vitamin, mineral, and nutritional value. (Jain and Khurdiya, 2004).

Bottlegourd, also known as Lauki, is a vegetable used in Indian cuisine. It offers a range of nutritional advantages. It has a high percentage of fibre, vitamins, minerals, and various proteins. Bottle gourd plays a significant role as a diuretic and anti-bilious when prepared as a vegetable. It is crucial in the management of urinary diseases.

Bottlegourd has potential uses in Non-alcoholic FLD remedies like it has a low glycemic index, its effect on blood sugar levels is negligible. In those with Non-alcoholic FLD, eating foods low in glycemic index can help control blood sugar levels. It contains a lot of dietary fibre, which can help slow down the blood sugar absorption. This may assist maintain better blood sugar regulation and lessen post-meal glucose surges. It is a good source of vitamins C, B, and K as well as minerals like Ca, Mg, and K. These nutrients are crucial for general health and can help Non-alcoholic FLD patients to maintain a balanced diet. Bottlegourd have the potential to maintain the uric acid level in Non-alcoholic FLD patents. The high-water content of bottle gourds can aid with hydration. For the kidneys to effectively remove waste materials, including uric acid, staying hydrated is crucial. Purines are naturally occurring compounds present in some foods that decompose into uric acid. Bottlegourd is low in purines. For people with high uric acid levels and disorders like gout, limiting purine-rich meals can be helpful. Bottlegourd has antioxidant qualities that may aid to lessen inflammation in the body (Lopez et al., 2005; Ghule et al., 2006). Higher uric acid levels have been linked to increased inflammation. By include foods high in antioxidants, such as bottlegourd, in your diet, you may be able to support general health, which may include uric acid management.

Another important component of this health drink is Cucumber. Manganese, potassium, silicon, chlorophyll, and vitamin A are all present in cucumbers in good amounts. As a fruit, cucumbers are in the same family as pumpkins and are free of fat, cholesterol, and salt. Cucumbers are beneficial in treating liver, pancreas, renal, and urinary bladder diseases because of their nutritional qualities (Mukherjee et al., 2013).

Cucumbers are low in calories and carbohydrates, making them a healthy option for diabetics who need to manage their blood sugar levels and maintain a healthy weight. Cucumbers can add bulk and fullness to meals without dramatically influencing blood sugar levels. Cucumbers' high water content aids in maintaining proper hydration. For optimal blood sugar control and general health, it's crucial to stay hydrated. The skin of cucumbers, in particular, is a good source of dietary fiber which assists in digestion, reduces the rate at which sugar is absorbed into the bloodstream, and regulates blood sugar levels.

Cucumbers' high water content aids in maintaining proper hydration. It's critical to maintain appropriate hydration to help the kidneys operate correctly and dilute uric acid in the body, which helps with its excretion. Cucumbers are low in purines, which are organic substances that decompose into uric acid. Purine-rich food restriction is a good way to control uric acid levels, especially for people with illnesses like gout. The antioxidant content of cucumbers gives them their anti-inflammatory qualities. Inflammation and pain in the joints can result from high uric acid levels. Cucumbers and other anti-inflammatory foods can help lessen inflammation brought on by high uric acid levels. Because they are low in calories, cucumbers can be included in a healthy diet to help you lose weight. For those with NAFLD and high uric acid levels, maintaining a healthy weight is crucial since obesity raises the risk of developing both illnesses. If necessary, losing weight can aid in lowering uric acid levels. Although cucumbers may help manage uric acid levels, it's important to have a comprehensive strategy to managing Non-alcoholic FLD and excessive uric acid.

The plant Tulsi, commonly referred to as holy basil is another main ingredient of this health drink which highly prized in conventional medicine, notably in Ayurveda. The overall impact of Tulsi on the uric acid levels in Non-alcoholic FLDN people have received attention by many clinical trials (Satapathy et al., 2016).

Because of Holy basil's well-known anti-inflammatory qualities, the body's inflammation may be lessened. Increased uric acid production may be caused by increased inflammation. It may indirectly aid in uric acid regulation by lowering inflammation. Antioxidants in Holy basil, like flavonoids and phenolic substances, aid in the fight against oxidative stress. Higher uric acid levels have been linked to oxidative stress. Its antioxidant properties might lessen oxidative stress and promote uric acid management. It is thought that Holy basil has nephroprotective qualities, which could support and protect kidney function. Filtering and

excreting uric acid from the body is a critical function of the kidneys. Tulasi may help to keep ideal uric acid levels in check by encouraging healthy renal function.

The *Stevia rebaudiana* plant yields stevia, a natural sweetener. It is a popular option for those with Non-alcoholic FLD because of its well-known extreme sweetness without adding calories or changing blood sugar levels. In this health drink stevia is used as sweetener which balance the taste of the juice.

Stevia, a non-nutritive sweetener, does not cause an increase in blood sugar when ingested. Steviol glycosides are the substances that give it its sweet flavor but are not metabolised by the body. As a result, stevia has a negligible effect on blood glucose levels and can be substituted for sugar by those who have Non-alcoholic FLD. Stevia has been demonstrated to have little to no impact on insulin levels. For people with Non-alcoholic FLD, it's crucial to keep their insulin levels consistent because insulin is the hormone that controls blood sugar levels (Park et al., 2022). Stevia does not promote the release of insulin, which is good for controlling Non-alcoholic FLD.

Lemon is also an important ingredient of this health drink which adds a flavor and also, it's a fruit that may be advantageous for those with Non-alcoholic FLD and high uric acid levels. Citric acid, which is present in lemons, has been said to help lower uric acid levels in the body (Wang et al., 2017). In order to facilitate the dissolution and excretion of uric acid, citric acid may assist raise the pH of urine. It's crucial to remember that each person may respond differently to the citric acid in lemons in terms of how it affects uric acid levels. Lemons are a strong source of vitamin C, an antioxidant that may protect against problems caused by uric acid. In several studies, vitamin C has been linked to reduced uric acid levels.

Lemon water is a low-calorie beverage option that may help with weight loss. For those with Non-alcoholic FLD and high uric acid levels, maintaining a healthy weight is crucial since obesity raises the risk of developing both illnesses. If necessary, losing weight can aid in lowering uric acid levels.

Black salt is a form of rock salt which is used in this health drink. It is mainly used as a flavoring ingredient here. The iron compounds, sulphates, and sulphides are present in small levels in black salt, sodium chloride makes up the majority of the substance. These Sulphur compounds are thought to be responsible for the distinctively sulfuric flavor and scent of

black salt and this Sulphur compounds may have some health advantages, such as possible antibacterial and digestive qualities.

In order to investigate the effects of the health drink containing totally natural ingredients on SUA levels in Non-alcoholic FLD participants, we conducted a secondary analysis of a clinical trial.

MATERIALS AND METHODS

A. Formulation of the Health-Drinks:

Raw materials viz. bottle gourd, cucumber, Holy basil, lemon, stevia and black salt were purchased from the local market in Kolkata, India. The purchased vegetables and herbs were sorted and cleaned with water. Then, the cucumber and bottle gourd were peeled separately and cut into small pieces. Then, blanching of the minced vegetables was done by heating them separately in water at 80 °C for 2 mins followed by decantation. Juices of the vegetables were prepared with a Juicer grinder (Inalsa, India) by taking the cucumber and bottle gourd individually.

Lemon Juice was extracted from lemon by wooden hand pressure equipment (Reliance Enterprise, India).

For holy basil and stevia leaves extraction, firstly leaves were separately ground in a mixture at 10% (w/v) in water by using a mixer-grinder (Inalsa, India) followed by screening to obtain the desired holy basil and stevia extract.

A known quantity of the vegetable juices and herb extracts were mixed in water according to formulations cited in Table-1. Holy basil extract of 5% (v/v), lemon juice 3% (v/v), stevia 2% (v/v) and black salt 1% (v/v) were used according to the formulations.

Sensory tests performed for identifying the best percentage quantities of the health drink.

After preparing the final health drink is kept in refrigerator for cooling at 4 °C for 1 hour, then it was ready to serve. Fig.1 provides complete health drink preparation method.

B. Analysis:

Analysis of the final product was done in three replications.

I. Physico-Chemical Analysis:

Physico-chemical analysis comprises following procedures:

1. pH:

A pH meter was used to measure the sample's pH.(Elico, India).

2. Total soluble solids: (⁰Brix)

Total soluble solids (⁰Brix) of the final health-drink samples were determined by using a refractometer (Trans Instruments, Singapore).

3. Total acidity:

Natural fruit juices have are slightly acidic due to the presence of different organic acids in them. In our product, citric acid was incorporated in the form of lemon juice. Fruit juice's acidity was measured using a straightforward “direct titration” with 0.1M NaOH and phenolphthalein as an indicator. (AOAC, 1985).

4. Sugar content:

“Reducing,”“non-reducing” and “total sugar contents” were assessed through the use of Lane and Eynon method (Raganna. 2000).

II. Sensory Analysis:

A panel of 30 participants with some training assessed the sample juices for colour, flavour, and general acceptability. Samples are served individually in succession and each is taken and rated before the next sample is served. The sample juice was provided with a glass of water and was instructed to ‘take a drink’ during the 40-60 seconds rest period between the samples. Typically, samples are identified by a number or letter code. The responses are recorded in 9-point hedonic scale. In the scale 9 phrases are arranged along a line, designed (Table-2) to suggest only a single continuum which is emphasized by the successive degrees of effect of the verbal description. In the measurement, 9 is scored for extreme like and 1 is for extreme dislike.

C. Study Design:

The research included the use of analysis technique for “the randomized, double blinded control trial,” that was performed by the Department of Biotechnology of Bengal Institute of Technology, Kolkata, West Bengal, India. The period of the research was between 10th March to 30th April, 2023. The design of this study was further approved by the Central Licensing Authority hereby registers and permits Institutional Ethics Committee, Ruby Hospital KasbaGolpark, E.M. Bypass Kolkata, West-Bengal to perform duties of ethics committee as specified in the New Drugs and Clinical Trials Rules, 2019.

D. Population and Randomization

We enrolled participants for the clinical test who had been invited by social media advertisements and mutual contacts to have transient elastography (TE) performed to check for hepatic steatosis. In humans, uric acid (UA) is an endogenous substance and the result of purine metabolism. (Maiuolo et al., 2016). It is produced and eliminated through the kidneys and intestines (De Oliveira et al., 2012). Due to gender-related characteristics, males often have greater serum uric acid levels than females (SUA) as they age (Dehlin et al., 2020). Numerous studies have found a high association between SUA and NAFLD (Yu et al., 2021) demonstrating that SUA is a risk factor for the onset of fatty liver disease and its progression to non-alcoholic steatohepatitis (NASH) (Fernández Rodríguez et al., 2019). When the controlled attenuation parameter (CAP) value was >216 dB/m as measured by TE as previously reported, we made the diagnosis of Non-alcoholic FLD. We included individuals with hepatic steatosis aged between, 30 and 65 years for the study, including all genders. We did not include patients with Non-alcoholic FLD brought on by other conditions. Additionally, we disqualified those who were using dietary supplements, functional foods, or nutraceuticals to treat hepatic steatosis, as well as people who had allergies to maize or any of their ingredients, had diabetes, or had serum triglyceride levels that were higher than 250 mg/dL.

The health drink was given to 42 adults who were randomly assigned, with Non-alcoholic FLD in the ratio of 1:1, over a 6-week period along with a placebo. Every day, 300ml of a typical store-bought health drink was given to one group of patients and placebo given another group of people. The health drink given to the control group as placebo having 1% (w/v) black salt and 3% (w/v) saccharin in water.

The clinical test of 45 days (or 6 weeks) was successfully completed by the patients in this secondary analysis. 42 people in total participated in the analysis process.

E. Outcomes

The change in SUA seen in patients with Non-alcoholic FLD following a 6-week clinical test intervention served as the study's endpoint. The changes in SUA based on gender after the intervention lasted 6 weeks was the outcome of the secondary analysis. The main report (Ferro et al., 2020) included an explanation of the primary and secondary endpoints of clinical test. In order to continue their Mediterranean diets without cutting back on their overall energy intake, the study participants received both written and spoken recommendations. However, patients who were overweight were instructed to keep their caloric intake between 400 and 500. It was ensured that none of the participants knew who drank the "placebo" and who drank the "actual" health beverage.

G. Anthropometrical assessment and CVD Risk Evaluation

After a 12-hour overnight fast and without having eaten breakfast, the randomly chosen person's body weight was measured using "calibrated digital scale," which has an overall accuracy of 0.1 kg. The weight of the light aprons these people were wearing was deducted from the outcome. The stadiometer was utilized to assess the individual's body height. The Body mass index or (BMI) was calculated as follows: weight/height^2 (kg/m²). These people were found to be obese if their BMI was greater than or equal to 30 kg/m². Over the light apron the patients were wearing, the hip and waist circumferences were also measured using a "non-stretchable tape."

The measurements were taken between the iliac crest and the costal border, respectively, at the level of the buttocks' widest diameter and the naked abdomen's narrowest point.

Over the light apron the patients were wearing, the hip and waist circumferences were also measured using a "non-stretchable tape." The measurements were taken between the iliac crest and the costal border, respectively, at the level of the buttocks' widest diameter and the naked abdomen's narrowest point. The ratio between the waist and hips was also assessed.

In addition to being evaluated based on their clinical histories, the individuals chosen also had their potential risk factors for cardiovascular accidents, such as smoking-related events, hypertension, and hyperlipidemia, estimated (Ferro et al., 2020). Moreover, during the sixth

week of the intervention, the blood pressure level was also detected. The assessment portion of the study took into account variables such “fasting blood glucose, above or equivalent to 126 mg/dL” and anti-diabetic medications.

H. Blood Measurement

The venous blood was drawn from the vacutainer tubes after an overnight fast, and it was then centrifuged for four hours. After carefully following the usage guidelines and instructions, chemicals and components such as “insulin, serum, glucose, creatinine, cholesterol, triglyceride, lipoprotein cholesterol, ALT, AST, GT, total bilirubin, and UA” were analysed using the “chemiluminescent immunoassay on COBAS 8000.” Additionally assessed were “Low density lipoprotein cholesterol (LDL-C) level and homeostatic model assessment index.”

I. Statistical Analysis:

On Formulation: -

The gathered data underwent statistical analysis to determine the mean and standard deviation.

On Clinical study

The data has been reported as mean \pm standard deviation or percentage, through the application of the “Kolmogorov Smirnov normality test,” which was employed for analysis of the continuous variable’s distribution. Furthermore, the “Chi-Square test” was also performed to analyses the pervasiveness b/w the treatment groups and the SUA tertiles. Additionally, the “independent unpaired samples T test” was performed to determine the difference between the mean value obtained after 6 weeks, as per the severity of the Non-alcoholic FLD assessment at the baseline. Moreover, “an ANOVA test” was executed to assess the mean between the “SUA Tertiles” along with the “Fisher’s LSD test” in form of a “post-hoc analysis.”

A "multi-variable linear regression analysis" was performed on the full population to determine the link between the absolute SUA change and the confounding variables that were different during the independent unpaired samples t-test and "the 2 test." The clinical study specifically evaluated how the SUA alters and the treatment (Health drink or Placebo), along

with factors such as age, gender, antiplatelet agents, urate-lowering drugs, calcium channels, diuretics, and so on in alignment with the alteration of weight after 6 weeks of consumption.

At $p < 0.05$ (two-tailed) significant differences were assumed. SPSS 22.0 for Windows (PIBM Corporation, New York, NY, USA) was utilized to perform all these comparisons.

RESULTS AND DISCUSSION

After performing the sensory test of all the formulated health drink sample Formulation-2 sample of Table -1 provided the best possible result. The sensory test analysis result has been mentioned on Table -3.

The mean overall acceptability value just after preparation, i.e., 8.0 is comparable to that found in RSM optimised formulation of sugarcane-cucumber juice (Kauret al., 2018).

Of the 42 participants who finished the initial phase of the study after 6 weeks of medication with Non-alcoholic FLD were evaluated on this test.

Table 4 displays the clinical traits of the subjects by treatment. Age, BMI, CAP score, and SUA were comparable between the groups at the outset. The health drink group had a greater smoking prevalence than the placebo group (45 Health drink vs. 30% placebo group, $p = 0.07$) (Table 4). In contrast to the two people in the placebo group, no participants in the health drink group used urate-lowering medications ($p = 0.49$). Throughout the six weeks of treatment, none of the subjects altered their regular medicine regimen.

After 6 weeks of treatment, our secondary examination of a clinical test showed for the first time that nutraceuticals comprising bioactive compounds from cucumber, bottle gourd, holy basil can significantly lower SUA levels in individuals with Non-alcoholic FLD.

In comparison to the placebo group, the health drink group significantly reduced SUA by 1% after 6 weeks (Table 5). After correcting for confounding factors, we discovered that participants suffering from moderate to severe fatty-liver steatosis who took the health drink had a higher drop in SUA level as compared to the placebo control groups.

These findings are incredibly intriguing because hyperuricaemia is linked to a more advanced state of histological liver injury because Non-alcoholic FLD patients have higher SUA levels than controls. In fact, experimental investigations have demonstrated that hyperuricemia can

cause organ damage and cardio-metabolic disorders by activating “oxidative stress,” “inflammation,” and the “fibrosis pathway.”

While the impact on clinical outcomes is yet uncertain, anti-hyperuricemia medications may improve surrogate endpoints of CVD like blood pressure, endothelial function, carotid intima media thickness, and proteinuria. The results of our investigation are still highly intriguing since they support the creation of novel combinations of natural compounds with beneficial nutraceutical properties for the treatment of metabolic disorders.

Uric Acid is the end product of “purine nucleotide catabolism,” which can be influenced by a variety of factors, such as food and consumption of animal proteins, which significantly contribute to the purine pool. Xanthine and hypoxanthine are examples of the intermediate products of this metabolism. The last two phases in the metabolic chain that produces UA are catalysed by the XO enzyme: hypoxanthine is transformed to xanthine, which is then turned to UA. At the glomerulus level, urate is freely filtered, albeit up to 90% of the filtered urate is reabsorbed. The two primary transporters involved in tubular reabsorption are GLUT9 and URAT1/SLC22A12. This clinical analysis intended to assess how this nutraceutical affected the accumulation of fatty liver and/or the derived indicators of liver injury. Additionally, data obtained only revealed a modest decrease in SUA levels compared to what was accomplished with dietary adjustments alone or medication. Although only one serving was taken each day to achieve this reduction, it is possible to reduce SUA levels even more by doubling the daily serving of this health drink. Due to this, the results must be interpreted carefully and can only serve as the basis of new research hypotheses.

CONCLUSION

The report has conceptualised regarding the process of developing health drink using Bottlegourd and cucumber juice and further using it treat conditions such as Non-alcoholic Fatty liver disease. The various ingredients used for the preparation of the health drink like Cucumber, Bottle gourd, Holy basil, Lemon, Stevia. The key benefits of these ingredients of the health drink reduces the uric acid level significantly of Non-Alcoholic Fatty Liver Disease individuals. The methodology to made this health drink is quite easy and can be made in home as well. The methods involved to perform the clinical trial are also illustrated in this report. A statistical analysis of the data obtained is made in this report and a suitable discussion regarding the overall impact of consuming this health drink among the selected participants with Non-Alcoholic FLD are discussed.

REFERENCES

- AOAC. (1985) Official Methods of Analysis of Association of Official Analysis Chemist. 13th ed., Association of Official Analytical Chemists, Washington, DC., USA.
- Dehlin M., Jacobsson L., Roddy E. Global epidemiology of gout: Prevalence, incidence, treatment patterns and risk factors. *Nat. Rev. Rheumatol.* 2020;16:380–390.
- De Oliveira E.P., Burini R.C. High plasma uric acid concentration: Causes and consequences. *Diabetol. Metab. Syndr.* 2012;4:12.
- Fernández Rodríguez C.M., Aller R., Gutiérrez García M.L., Ampuero J., Gómez-Camarero J., Martín-Mateos R., Burgos-Santamaría D., Rosales J.M., Aspichueta P., Buque X., et al. Higher levels of serum uric acid influences hepatic damage in patients with non-alcoholic fatty liver disease (NAFLD) *Rev. Esp. Enferm. Dig. Organo Of. Soc. Esp. Patol. Dig.* 2019;111:264–269
- Ferro, Y., Montalcini, T., Mazza, E., Foti, D., Angotti, E., Gliozzi, M., Nucera, S., Paone, S., Bombardelli, E., Aversa, I. and Musolino, V., (2020). Randomized clinical trial: bergamot citrus and wild cardoon reduce liver steatosis and body weight in non-diabetic individuals aged over 50 years. *Frontiers in Endocrinology*, 11, p.494.
- Ghule, B. V., Ghant, M. H., Saoji, A. N. and Yeole, P. G. (2006) Hypolipidemic and antihyperlipidemic effects of *Lagenaria siceraria* (Mol) fruit extracts. *Indian J. Expt. Biol.* 44, 905.
- Heena, K, V., Kaur, J., Gat, Y., Chandel, A., Suri, S. and Panghal, A. (2017) Optimisation of the different variables for the development of a cucumber-based blended herbal beverage. *Beverages*. 3(50).
- Jain, S. K. and Khurdiya, D. S. (2004) Vitamin-C enrichment of fruit juice based ready-to-serve beverages through blending of Indian gooseberry juice. *Plant Food Hum Nutr.* 59, 63-66.
- Jiang L.L., Gong X., Ji M.Y., Wang C.C., Wang J.H., Li M.H. (2020) Bioactive Compounds from Plant-Based Functional Foods: A Promising Choice for the Prevention and Management of Hyperuricemia. *Foods*. 9: 973.

- Kaur, G., Kumar, V., Goyal, A., Tanwar, B. and Kaur, J. (2018) Optimization of nutritional beverage developed from radish, sugarcane and herbal extract using response surface methodology. *Nutr. Food Sci.*
- Kothari, M. and Rakshak, H. (2005) Laukiras, Hridaya Mitra Mandal, Nagpur, India
- Lopez, P., Sanchez, C., Batlle, R. and Nerin, C. (2005) Solid- and vapor-phase antimicrobial activities of six essential oils: Susceptibility of selected food borne bacterial and fungal strains. *J. Agric. Food Chem.* 53, 6939-6946.
- Maiuolo J., Oppedisano F., Gratteri S., Muscoli C., Mollace V. Regulation of uric acid metabolism and excretion. *Int. J. Cardiol.* 2016;213:8-14.
- Mukherjee PK, Neema NK, Maity N and Sarkar BK. (2013) Phytochemical and therapeutic potential of cucumber. *Fitoterapia.* 24, 227-236.
- Ranganna, S. (2000) Handbook of Analysis and Quality Control for Fruits and Vegetable Products. 2nd ed., Tata McGraw-Hill Publishing Company Ltd., New Delhi, India.
- Satpathy S., Das, N., Bandopadhyay D., Mahapatra S. C., Sahu D. S., and Meda M. (2016) Effect of Tulsi (*Ocimum sanctum* Linn.) supplementation on metabolic parameters and liver enzymes in young overweight and obese subjects. *Indian journal of Clinical Biochemistry.* 32 (3): 357-363.
- Wang H., Cheng L., Lin D., Ma Z., Deng X. (2017) Lemon fruits lower the blood uric acid levels in humans and mice. *Scientia Horticulturae.* 220: 4-10
- Wei F., Li J., Chen C., Zhang K., Cao L., Wang X., Ma J., Feng S., Li W.-D. Higher serum uric acid level predicts non-alcoholic fatty liver disease: A 4-year prospective cohort study. *Front. Endocrinol.* 2020;11:179.
- Yu H., Zhao L., Liu L., Li Y., Sun J., Liu Y. Relationship between serum uric acid level and nonalcoholic fatty liver disease in type 2 diabetes patients. *Medicine.* 2021;100
- Zheng X., Gong L., Luo R., Chen H., Peng B., Ren W., Wang Y. Serum uric acid and non-alcoholic fatty liver disease in non-obesity Chinese adults. *Lipids Health Dis.* 2017;16:202.

Table – 1: Formulations of Health-drink

Ingredients	Formulation-1 (%)	Formulation-2 (%)	Formulation-3 (%)
Bottle gourd	20	25	30
Cucumber	20	25	30
Holy basil	5	5	5
Lemon	3	3	3
Stevia	2	22	2
Black Salt	1	1	1

Table -2: Sensory Analysis Scores

Score/Rating	Std. Hedonic Scale
9	I like extremely
8	I like very much
7	I like moderately
6	I like slightly
5	I neither like nor dislike
4	I dislike slightly
3	I dislike moderately
2	I dislike very much
1	I dislike extremely

Table – 3: Sensory Score of different formulated Health Drink samples

Sensory-Attributes	Sensory Score		
	Formulation-1	Formulation-2	Formulation-3
Color	6	8	7
Flavor	7	9	7
Taste	7	9	5
Overall Acceptability	6	8	6

Table-4: Baseline demographic and clinical traits of individuals with NAFLD

Variables	Placebo (<i>n</i> = 21)	Health drink (<i>n</i> = 21)	<i>p</i> -Value
Age (years)	42 ± 10	43 ± 11	0.32
Weight (Kg)	80.0 ± 10	79.9 ± 13	0.95
BMI (Kg/m ²)	27.9 ± 3	27.1 ± 2	0.76
WHR	0.93 ± 0.1	1.2 ± 1.4	0.19
SBP (mmHg)	115 ± 17	114 ± 15	0.92
DBP (mmHg)	67 ± 12	69 ± 9	0.53
CAP score (dB/m)	276 ± 38	284 ± 37	0.29
IQR	10 ± 5	11 ± 4	0.93
Glucose (mg/dL)	92 ± 7	90 ± 7	0.73
HOMA-IR	2.18 ± 1.0	2.22 ± 1.1	0.81
TC (mg/dL)	183 ± 35	178 ± 32	0.41
TG (mg/dL)	113 ± 50	101 ± 46	0.14
HDL-C (mg/dL)	47 ± 12	49 ± 12	0.27
LDL-C (mg/dL)	118 ± 33	112 ± 31	0.43
AST (IU/L)	22 ± 15	19 ± 6	0.19
ALT (IU/L)	31 ± 28	22 ± 13	0.04
γGT (UI/L)	25 ± 20	24 ± 18	0.78
SUA (mg/dL)	5.3 ± 1.3	4.9 ± 1.2	0.85

Variables	Placebo (n = 21)	Health drink (n = 21)	p-Value
Creatinine (mg/dL)	0.84 ± 0.1	0.79 ± 0.1	0.70
Total bilirubin (mg/dL)	0.6 ± 0.3	0.59 ± 0.3	0.93
Prevalence			
Gender (Male, %)	75	70	0.8
Physical activity (%)	45	44	1
Smokers (%)	30	45	0.07
Obesity (%)	60	52	0.89
Hypertension (%)	32	34	1
Hyperlipidemia (%)	49	42	0.61
Antihypertensive drugs (%)	22	28	0.79
Beta-blockers (%)	7	5	1
Diuretics (%)	5	9	0.67
Lipid-lowering agents (%)	7	14	0.3
Antiplatelet agents (%)	5	10	0.41
Urate-lowering drugs (%)	5	0	0.41

Note: Body mass index (BMI), waist to hip ratio (WHR), systolic blood pressure (SBP), diastolic blood pressure (DBP), controlled attenuation parameter (CAP), interquartile range (IQR), and homeostatic model assessment of insulin resistance (HOMA-IR) are abbreviations for measurements of body mass. AST, ALT, GT, and SUA all stand for aspartate aminotransferase, alanine aminotransferase, low density lipoprotein cholesterol, triglycerides, high density lipoprotein cholesterol, and gamma glutamyl transferase.

Table – 5: Alterations in clinical indicators during the 6-week follow-up

Variables	Placebo (<i>n</i> = 21)	Health drink (<i>n</i> = 21)	<i>p</i> -Value
Weight (Kg)	-2 ± 1.7	-3 ± 2.2	0.03
BMI (Kg/m ²)	-0.6 ± 0.4	-1 ± 0.6	0.029
WHR	-0.001 ± 0.03	-0.17 ± 1.1	0.26
Glucose (mg/dL)	-0.3 ± 6.1	-0.65 ± 6.6	0.81
HOMA-IR	-0.2 ± 0.7	-0.4 ± 1	0.46
TG (mg/dL)	-12.7 ± 42	0.5 ± 57	0.17
HDL-C (mg/dL)	-0.3 ± 7	-2.8 ± 6	0.39
LDL-C (mg/dL)	-2.7 ± 30	-8.4 ± 16	0.28
AST (IU/L)	-2.2 ± 8	-0.9 ± 8	0.32
γGT (UI/L)	-3.5 ± 7	-5.5 ± 7	0.29
SUA (mg/dL)	0.29 ± 0.5	-0.08 ± 0.5	0.003
aSUA (mg/dL) *	0.27 ± 0.3	-0.16 ± 0.3	0.004
SUA (%)	6.2 ± 15	-1.3 ± 13	0.007
aSUA (%) *	6.4 ± 3	-1.1 ± 3	0.017
Creatinine (mg/dL)	-0.005 ± 0.08	-0.007 ± 0.07	0.96

SUA adjusted for age, gender, smokers, sartans, calcium channel, β blockers, diuretics, urate-lowering drugs, antiplatelet agents, and weight change. Note. BMI = body mass index, WHR = waist to hip ratio, HOMA-IR = homeostatic model assessment of insulin resistance, TG = triglycerides, HDL-C = high density lipoprotein cholesterol, LDL-C = low density lipoprotein cholesterol, AST = aspartate aminotransferase, ALT = alanine aminotransferase, γGT = gamma glutamyl transferase, SUA = serum uric acid. * Adjusted for age, gender, smoking habit, drugs, and weight change.

Table – 6:

Individuals with Non-Alcoholic Fatty Liver Disease (NAFLD): Factors linked with absolute SUA change in multivariable linear regression analysis.

Dependent Variable	<i>p</i> -Value	C.I. 95%	
		LL	UL
SUA Change			
Treatment	0.003	-0.7 1	-0.1 4

Note. Age, gender, smokers, and weight change were excluded from the analysis. Confidence intervals are denoted by the letters CI, LL, and UL.

Fig -1: Formulation method of the health drink

