How to Cite:
Gomez, S., Prabha, S., Kanjoormana Aryan, M., Raghavamenon, A., Kuruvila, B., & Anjali Chandran. (2024). Indian gooseberry (Emblica officinalis Gaertn) drink spiked with spices-Immunomodulatory properties. *International Journal of Health Sciences*, *8*(S1), 866–886.
https://doi.org/10.53730/ijhs.v8nS1.14920

Indian gooseberry (Emblica officinalis Gaertn) drink spiked with spices Immunomodulatory properties

Saji Gomez
Department of Postharvest Management, College of Agriculture, Kerala Agricultural University, Vellanikkara, Thrissur, Kerala, India
Corresponding author email: saji.gomez@kau.in

Silpa Prabha
Assisted in immunomodulatory studies, Amala Cancer Research Centre, Thrissur, Kerala, India
Email: silpaprabha@gmail.com

Manu Kanjoormana Aryan
Investigation of Immunomodulatory Properties of Herbal Drink, Amala Cancer Research Centre, Thrissur, Kerala, India
Email: manu.aryan@amalaims.org

Achuthan Raghavamenon
Investigation of Immunity Boosting Properties of the Drink, Amala Cancer Research Centre, Thrissur, Kerala, India
Email: ragav@amalaims.org

Bintu Kuruvila
Assisted in Phytochemical Analysis of the Product, Department of Postharvest Management, College of Agriculture, Kerala Agricultural University, Vellanikkara, Thrissur, Kerala, India
Email: mariyamkuruvilaolickal@gmail.com

Anjali Chandran
Assisted in Phytochemical Analysis of the Product, Department of Postharvest Management, College of Agriculture, Kerala Agricultural University, Vellanikkara, Thrissur, Kerala, India
Email: canjali377@gmail.com

***Abstract*---**Indian gooseberry juice (20% (v/v) was reconstituted with 5% ginger juice (v/v) extracted from freshly harvested rhizomes, 2% each of turmeric rhizome powder and black pepper powder (w/v), followed by raising the total soluble solids to 13^{0}Brix by adding sugar

syrup. Finally, 5% acid lime juice of 2.45% of titratable citric acid was added to the entire mixture in order to impart a fruity flavour to the product. The entire mixture was homogenized at 175 Bar (2500 psi) with a speed of 235 rpm and was subsequently pasteurized at 100^0C for 10 minutes. LC-MS/MS analysis detected phenolic compounds (2-galloyl glucose, 2,6-galloyl glucose), capsaicinoids, curcuminoids, gingerol and shogaol. Though cyclophosphamide significantly inhibited the relative weight of thymus from 0.193 ± 0.048 in normal mice to 0.077 ± 0.034, treatment with the herbal drink significantly increased the relative weight of thymus, showing increased T cell differentiation. Mice treated with the drink showed marginal increase in the WBC count, indicating recovery of immunity. Recovery of bone marrow cellularity in mice fed with the herbal drink was directly proportional to the concentration of the drink. Increase in α-esterase activity was also directly proportional to the concentration of herbal beverage.

***Keywords*---**Indian gooseberry, 2-galloyl glucose, capsaicinoids, WBC, bone marrow cellularity, curcuminoids.

1. Introduction

Awareness on nutrition and health has created increased demand for foods that are natural, plant-derived and even supplements, especially after the incidence of corona virus disease. It was reported that people with low levels of immunity are the ones worst affected due to infection by corona virus. This realization prompted even people who did not have any apparent health problems to turn to natural remedies which could either maintain or boost their immunity levels. Many phytochemicals have definite role in the prevention or delay of life-style related diseases. Antioxidants like ascorbic acid, carotenoids, polyphenols and certain minerals have proven health benefits like neutralizing the ill-effects of oxidative stress, besides retarding the progress of degenerative diseases like cancer, arthritis, premature ageing and cardiovascular problems. Substances having nutraceutical properties may range from nutrients in food items, food supplements, dietary ingredients, fruit and vegetable beverages, and also, herbal products (Kesrvani et al., 2010). Indian gooseberry, popularly known as *aonla* or amla belongs to the family Euphorbiaceae and is believed to have originated in the central and southern parts of India. The country ranks first in the area and production of this fruit (Priya & Khatkar, 2013). It is a very rich source of vitamin C and phenolic compounds, two potent antioxidants. The vitamin C content of this fruit ranges from 450 to 600 mg $100g^{-1}$, which is almost 10 times higher than in citrus fruits, a content which is rarely seen in fresh fruits. The hydrolysable tannin content of Indian gooseberry fruits, a major phenolic compound in combination with the high vitamin C are thought to provide the antioxidant and anticancerous properties. The major phenolic compounds identified in the fruit are tannins, gallic acid, ellagic acid, pyrogallol, chebulagic acid and quercetin. Among the tannins, ellagic acid has been shown to have antiproliferative properties against colon, prostate cell lines and breast cancer. Corilagin, another tannin found in the fruit has been found effective against

ovarian cancer cells, liver cancer cells and hepatocarcinoma xenografts. Pyrogallol has been effective against lung cancer cells, gastric cancer cells, and lung adenocarcinoma xenografts. Chebulagic acid could retard retinoblastoma colon cancer, breast cancer, prostate cancer, and leukemia. Gallic acid could slow down the progress of breast and lung cancer. Quercetin, a flavonoid found in abundance in Indian gooseberry fruits, could retard numerous cancer lines from multiple tissue types, transgenic murine model of breast cancer, and leukemia xenograft (Zhao et al., 2015). The leucoanthocyanins in the fruit along with the high amounts of phenolics are considered to provide stability to the ascorbic acid content. Indian gooseberry is widely used in traditional forms of medicines like Ayurveda and Unani to treat diseases like diabetes, jaundice, dyspepsia, diarrhea, scurvy, bronchitis, skin diseases and various types of cancers. The bioactivity of Indian gooseberry fruit extract in its anticancer and antitumour functions are thought to be imparted by the high levels of phenolics and flavonoids. Another important crop which is known for its immunity boosting properties is the spice, turmeric, wherein the rhizome of the plant is used in culinary preparations. Besides the bright yellow colour imparted by the rhizome, it is also consumed widely due to its flavouring properties. Curcumin in turmeric has been shown to possess antioxidant, anti-inflammatory and anti-proliferative properties. Curcumin is chemically 1, 7-bis (4-hydroxy-3-methoxyphenyl)-1, 6-heptadiene-3, 5-dione (Hewlings and Kalman, 2017). In many food preparations, turmeric is added in conjunction with black pepper, a spice crop wherein the berries formed in long spikes are the economic parts. Black pepper is an indispensable ingredient in many cuisines due to the hot, pungent flavour imparted by the alkaloid, piperine which is chemically 1-peperoyl piperidine. Antioxidant activity of *Piper nigrum*, P. *guineense* and P. *umbellatum* on hamsters fed with atherogenic diet at the rate of 1g/kg and 0.25g/ kg for 12 weeks resulted in protective activity against oxidative stress occurring in cardiac, hepatic and renal tissues (Agbor et al., 2012). Swollen rhizomes of the spice crop ginger, is invariably used in various dishes across the world for its unique flavouring and health-protective properties. Ginger is known for its pungency attributed by the ketone, gingerol, the primary ingredient responsible for its unique flavour. Gingerol is chemically 1-(4′-hydroxy-3′- methoxyphenyl)-5-hydroxy-3-decanone (Bode and Dong, 2011). A very high level of total antioxidants (3.85 mmols/100g) was reported in ginger rhizomes which was next only to pomegranate and some berry fruits (Halyorsen, 2002). Acid lime, a member of the citrus group of fruits, grown widely in tropical and sub-tropical regions, is hugely popular for its fruity flavour, besides being a good source of vitamin C and flavonoids.

As the demand for natural food items which are rich in health-protective phytochemicals is surging, particularly after the outbreak of Covid-19 pandemic, an attempt was made at Kerala Agricultural University during 2020-21 to develop an herbal nutraceutical drink incorporating plant parts which are widely grown, easily available and, also known for their health-protective properties, particularly those having immunity enhancing functions. Thus, the herbal drink from Indian gooseberry fruit juice incorporated with powder from turmeric and black pepper, in combination with juice extracts of ginger rhizome and acid lime fruits, was developed with the main objective of testing its efficacy in boosting immunity in experimental animals. Apart from being completely natural and devoid of synthetic food additives like flavouring agents and preservatives, the product was

developed in a ready to drink form with the view of exploring its potential as an alternative to synthetic drugs, which are known to have negative side effects.

2. Methods

2.1. Preparation of functional herbal drink and assessment of phytochemical contents/ LC-MS/ MS analysis

Juice from Indian gooseberry fruits was extracted using fruit crusher, followed by expelling the juice in a basket press. Being highly acidic and astringent, 20% (v/v) of juice was reconstituted with 5% ginger juice (v/v) extracted from freshly harvested rhizomes, 2% each of turmeric rhizome powder and black pepper powder (w/v) and the total soluble solids was raised to 13 ^{0}Brix by adding sugar syrup. Finally, 5% acid lime juice of 2.45% of titratable citric acid was added to the entire mixture in order to impart a fruity flavour to the product. The drink prepared thus was subjected to homogenization in a two-stage stainless steel homogenizer with an operating pressure of 175 Bar (2500 psi) with a speed of 235 rpm. The homogenized herbal drink was filled into 500 ml capacity glass bottles, followed by screw capping and was subsequently pasteurized at 100 ^{0}C for 10 minutes. Ascorbic acid was estimated by dissolving the product in 3% metaphosphoric acid, followed by titration with 2, 6-dichlorophenol indophenol dye. Ascorbic acid content was expressed in mg100g^{-1} (AOAC, 1998).

Liquid chromatography-mass spectrophotometry (LC-MS/MS)

The analysis was performed on a Q-TOF mass spectrometer (Agilent Technologies, USA). The compounds were detected and identified with the help of data acquisition software, Agilent MAss Hunter and the data processing software used was Agilent MAss Hunter Qualitative Analysis B.06. The chromatography was performed on a Hypersil GOLD C18 100x2.1mm-3 MICRON column. The mobile phase (Solvent A) was 0.1 % formic acid in Milli-Q water while solvent B was acetonitrile. The entire procedure was carried out at SAIF-IIT, Bombay.

2.2 Sensory analysis

A group of 50 semi-trained panelists in the age group of 20 -50 were asked to rate the product in a 9-point Hedonic scale (1-dislike extremely to 9-like extremely) and the average hedonic score for each attribute was computed which indicated the consumer acceptability of the product (Chakraborty et al., 2020). The individual attributes evaluated were as follows; appearance- denotes the colour shade of the drink, aroma- indicates the olfactory perception, flavour-represents a combined perception of taste and aroma, taste-signifies the desired sweet-sour effects, after taste-is an assessment of the lingering astringency imparted by Indian gooseberry fruits, consistency-is a sign of the flow characteristics of the drink assessed visually. Mouth feel is a combination of both physical (thin or thick and chalky or particulate) and chemical (acidic or astringent) sensations. All these attributes were taken into consideration to arrive at the overall acceptability or impression of the product.

2.3 Immunomodulatory studies

Balb/c mice consisting of 6 groups (6 animals/ group) forming a total of 36 animals (female; 4–6 weeks old; 20–25g body weight) were divided into 5 groups. Group 1 was treated as normal control. Group 2 was treated with cyclophosphamide (50mg/kg) and Groups 3 to 5 were treated with cyclophosphamide along with different doses of herbal drink for 7 consecutive days. Blood was collected from caudal vein and parameters such as relative thymus weight, total WBC count (haemocytometer) were recorded before administration of the drug. Body weight of the animals was recorded before sacrifice, 24 h after the last dose of the drug and weight of the lymphoid organ, thymus was recorded and expressed as relative organ weight. The femurs from experimental animals were taken, followed by collection of bone marrow cells and was finally made into single cell suspension. The cell number was determined using haemocytometer (Sredni et al, 1992). Bone marrow cells prepared thus, was smeared on clean glass slides and stained with Harri's hematoxylin to determine the non-specific α-esterase activity by the azodye coupling method (Bancroft & Cook, 1992). The experimental design consisted of the following treatments.

Treatment 1: Normal (control) (6 mice/ group), Treatment 2: cyclophosphamide 50 mg kg^{-1} body weight (6 mice/ group), Treatment 3: Herbal drink 2.5 ml kg^{-1} body weight+ cyclophosphamide (6 mice/group), Treatment 4: Herbal drink 5.0 ml kg^{-1} body weight+ cyclophosphamide (6 mice/group, Treatment 5: Herbal drink 10.0 ml kg^{-1} body weight+ cyclophosphamide (6 mice/group).

2.4 Statistical analysis

Analysis of data was done by single-factor one-way analysis of variance (ANOVA) to determine significant difference using Microsoft Excel 2007 software. Results of the study were presented as mean ±standard deviation (SD). *P* values less than 0.5 were considered significant.

3. Results and Discussion

The phytochemical constitution of the nutraceutical herbal drink, and also the impact of feeding the experimental animals (mice) with the herbal drink on relative thymus weight, total WBC, bone marrow cellularity and α-esterase activity are presented under the following heads.

3.1 Assessment of phytochemical contents and LC-MS/MS analysis

Ascorbic acid, popularly known as vitamin C, is one of the potent antioxidants. Fresh Indian gooseberry is one of the richest sources of ascorbic acid among fruits. The herbal drink had an ascorbic acid content of 61.0 $mg100g^{-1}$. The phytochemical constitution and antioxidant activity of this herbal drink was reported by Gomez et al (2023). LC-MS/MS analysis revealed the presence of 2-galloyl glucose, 2-6 digalloyl glucose, capsaicinoids like capsaicin, dihydrocapsaicin and, also dipeperamide, gingerol, shogaol, curcumin, demethoxycurcumin and bisdemethoxycurcumin. The compounds were identified by retention time, mass, score and height. Target compounds

having a score above 50 out of 100 were considered as the compounds detected in the herbal drink.The compounds detected in LC-MS/MS analysis and their physiological/ pharmacological functions are presented in Tables 1a and 1b.

3.2. Sensory analysis

Several sensory attributes contribute to the overall acceptability of beverages, especially the sugar: acid blend, flavour, mouth feel etc. Fruits having naturally high bitter/astringent/ acidic flavour can be improved by blending with sweeter ones in order to mask the unpleasant aspects. Alternatively, spices can be incorporated into the juice so as to have a pleasantly altered palatability. In this study, spices like black pepper, ginger and turmeric were incorporated into the drink, besides raising its TSS to 13^0Brix. The overall acceptability score of the herbal drink was 7.75 (Fig.1) on a maximum score of 9.0, with the predominant attributes being body/consistency (7.9), followed by taste and appearance scoring 7.8 each. The lowest score (7.5) was for the attribute, after-taste. Addition of spices and increasing the TSS content might have resulted in masking the astringent and acidic taste of the drink from Indian gooseberry fruits by imparting a slightly pungency and sweetness to the product. The lowest score of 7.5 for the sensory parameter, after-taste could be due to the lingering astringency on the tongue as it normally persists for a few seconds after consuming beverages made from Indian gooseberry fruits. Chakraborty et al (2020) reported that blending of Indian gooseberry juice with that of pomegranate and muskmelon helped in reduction of bitterness and improved the overall quality attributes of the beverage. They also reported that panelists appreciated the formulation with higher proportion of pomegranate juice as it contributed to sweet taste and attractive colour. Mishra and Sangma (2017) also opined that the bitter-sour taste of amla (Indian gooseberry) is the dominant suppressor for its limited usage in mixed fruit beverages from their study on functional ready to serve beverage made from amla blended with aloe, sweet lime and ginger.

3.3 Relative thymus weight

Thymus is a lymphoid organ where the T-lymphocytes mature. A well-functioning immune system will continuously produce T cells in the thymus, which in turn will ward off pathogens detrimental to human health. The capacity and size of thymus is negatively correlated with age, the reason why older people are more sensitive to immunity related diseases. The relative weight of thymus can be correlated with T- cell differentiation process. Treatment with cyclophosphamide significantly inhibited the relative weight of thymus from 0.193 ± 0.048 in normal mice to 0.077 ± 0.034 in the animals treated with the drug, showing reduced T-cell differentiation activity. While treatment with the beverage significantly increased the relative weight of thymus, showing increased T cell differentiation in the beverage treated cells (Table 2). The increase in thymus weight may be due to the combined effect of the ingredients like Indian gooseberry, turmeric, ginger, black pepper etc. Indian gooseberry fruits are rich sources of bioactive compounds that can impart immunomodulatory, hepatoprotective, anti-tumour, and antioxidant properties (Maheswary et al., 2022). Similar properties have been

reported in the other ingredients used in the preparation of the drink. Moreover, inclusion of vitamin C in diet in adequate amounts will benefit in warding off many diseases related to metabolism and immunity and also, will improve the overall well-being. Ascorbic acid is highly bio-available and is also soluble in water. The protective effect of amla (Indian gooseberry) against oxidative damage, apoptosis of lymphocytes and decline in cell viability was proved by Singh et al., (2013) in mice exposed to arsenic toxicity. Inhibition of humoral immunity in cyclophosphamide treated mice was found to have a decreasing effect upon feeding the animals with amla. The amount of antioxidative enzymes in normal mice returned to their original levels even in animals induced with cyclophosphamide toxication, after supplementation of diet with amla extract (Haque et al., 2001). Balcells et al., (2022) also reported that balanced diet and nourishment have remarkable effects on immunity and thymus weight. They observed that mice fed with a probiotic supplemented diet showed a sustained and constant thymus/ body weight relationship over time. According to Jiang et al., (2021) bioactive compounds from plants can boost immunity through multiple mechanisms such as influencing the immune organs, cellular immunity, humoral immunity, non-specific immunity, and immune-related signal transduction pathways.

3.3 Total WBC

White blood cells (WBCs) are also known as leucocytes. They are further classified as phagocytes and lymphocytes. There are three types of phagocytes, namely, granulocytes (basophils, eosinophils and neutrophils), monocytes and macrophages. There are three categories of lymphocytes as well. They are T-lymphocytes, B-lymphocytes and natural killer cells. T-lymphocytes are further classified into helper T cells, consisting of CD4 molecules, and cytotoxic T cells which contain CD8 molecules (Calder & Kew, 2002). When a pathogen enters our body, the first response would be from the innate immune system activated by phagocytes that produce toxic chemicals such as superoxide and hydrogen peroxide radicals. T-lymphocytes play an important role in the acquired immune system by identifying the pathogen and subsequently producing the antibody required to neutralize the antigen. Total WBC count in mice was significantly inhibited after the treatment with cyclophosphamide (Figure 2). Treatment with the beverage increased the WBC count marginally in the cyclophosphamide treated animals, showing recovery of immunity. Immunomodulatory, antiviral, antioxidant and anti-inflammatory properties of vitamin C and polyphenols in Indian gooseberry, curcumin in turmeric, the alkaloid piperine in black pepper and gingerol in ginger might have contributed to the recovery of immunity in mice even after being treated with cyclophosphamide. Consumption of Indian gooseberry enhanced spleen weight, a secondary lymphoid organ consisting of many phagocytes as well as T and B lymphocytes (Haque et al., 2001). Various polyphenols found in Indian gooseberry such as ellagic acid, corilagin, pyrogallol, chebulagic acid, gallic acid and quercetin imparted anticancer properties in irradiated mice (Rekha et al., 2000). Delecroix et al., (2017) reported that consumption of a diet supplemented with 2 g of curcumin and 20 g of piperine was helpful in alleviating muscle soreness in rugby players after grueling practice sessions. Numerous health

benefits have been reported for curcumin and these benefits are primarily due to the anti-inflammatory and anti-oxidant properties (Hewlings & Kalman, 2017). A study conducted in Nigeria among HIV infected patients who were given fruits and vegetables highly rich in antioxidants such as vitamins C, E, A and other carotenoid derivatives in addition to the antiretroviral drugs, a significant increase in CD4 cells (252.1±40.902 cells/μL) was noticed compared to the control group (201±35.29 cells/μL) who were given only antiretroviral drugs (Nweze et al., 2015). Zak & Pokora (2017) investigated the effect of long-term green tea extract supplementation on peripheral blood leukocytes in cross-fit trained and untrained men. The results did not reveal any significant difference in the total white blood cell count of the study participants. However, the percentage of peripheral blood eosinophils increased while monocyte and neutrophil counts declined. They noticed that the changes in white blood cells were less pronounced in cross-fit trained compared to the untrained men.

3.4 Bone marrow cellularity

Bone marrow is the seat of hematopoietic cells. Cellularity varies with age and it diminishes with advancement of age with a relative increase in adipocytes. Treatment with cyclophosphamide significantly inhibited the bone marrow cellularity in mice which brought down the cellularity by almost 50 % (Figure 3). Treatment with herbal drink recovered the bone marrow cellularity which was directly proportional to the concentration of the drink. There was significant increase in the 5mL and 10 $mLkg^{-1}$ treated groups. The strong antioxidant and anti-inflammatory properties of the ingredients used in the preparation of herbal drink might have resulted in the restoration of bone marrow cellularity in cyclophosphamide treated mice. The finding is an indication that the beverage might have helped in increasing the stem cell pool of blood cells. Sai Ram et al., (2002) opined that Indian gooseberry is abundant in vitamin C which is responsible for the curative, restorative and prophylactic activity. Rekha et al., (2000) reported that the bone marrow cellularity of irradiated mice declined (3.8x 10^{6} cells/ femur) significantly compared to that of normal mice (12.7x 10^{6} cells/femur) whereas *Brahma Rasayana* (BR), a herbal preparation of sixty plants, increased bone marrow cellularity significantly which was on par with normal animals or even higher on different days of administration. Moreover, curcumin, piperine and gingerol also might have contributed to increased bone marrow cellularity. Piperine is known to enhance bioavailability of many nutrients and drugs by interfering in metabolic pathways. This property of piperine is being utilized in the preparation of several drugs aimed at alleviating or even curing many metabolic disorders. Curcumin has established antioxidant activity as evident from its efficacy on oxidative stress indicators such as plasma activities of superoxide dismutase, catalase and also, in serum concentrations of glutathione peroxidase as well as lipid peroxides (Sahebkar et al., 2015). Three curcuminoids viz. curcumin, demethoxycurcumin and bisdemethoxycurcumin in clinical trials have been found to have good tolerability, of doses up to 12000 mg/ day (Lao et al., 2006). Ginger is well known for its antioxidant properties and has been found to retard oxidative stress related to ageing process. Halyorsen et al., (2002) reported a very high level of total antioxidants (3.85 mmols/100g) in ginger rhizomes. A clinical trial involving humans were given ginger at doses ranging from 100 mg to 2.0 g as a single oral dose and the findings revealed that free

forms of gingerol and shogaol could not be detected; instead the glucuronide of these compounds were detected in the conjugated form (Zick et al., 2008). Tadokoro & Hirao (2022) are of the opinion that dietary interventions can prevent the aging of hematopoietic stem cells which could promote their preservation in a healthy state. Nutraceuticals and functional foods containing plant metabolites such as quercetin, curcumin, epigallocatechin gallate, berberin, thymoquinone, polyphenols etc. have broad potential for preventing the mechanisms of viral infection and modulating immune responses (Haslberger et al., 2020). Investigations on bone marrow functioning revealed synergism between malnutrition and inflammation in mediating changes in bone marrow composition and bone health. After inflammatory challenge, the bone marrow of malnourished mice had increased inflammatory cytokine expression and caspase 1-dependent IL-1β production, which led to decrease in bone formation and promote bone resorption, ultimately resulting in bone fragility (Osorio et al., 2022).

3.5 α-esterase activity

Alpha esterase activity show differentiation of stem cells. Bone marrow cells are the stem cell pool of blood cells. Bone marrow cells can differentiate into different blood cells involved in both innate and adaptive immunity. Treatment with cyclophosphamide significantly inhibited alpha esterase activity of bone marrow cells in mice. But treatment with the herbal drink significantly increased the alpha esterase activity of the bone marrow cells (Figure 4). Increase in α-esterase activity was directly proportional to the concentration of herbal beverage. Herbal drink at all the three concentrations helped in the recovery of the enzyme activity which was significantly higher than the cyclophosphamide treated animals. Recovery of α-esterase activity might be due to the protective and curative properties of active principles / bioactive compounds like vitamin C, polyphenols, gingerol, piperine etc. Addition of spices and condiments like turmeric, black pepper and ginger might have compounded the antioxidant capacity of the product as curcumin in turmeric, gingerol in ginger and piperine in black pepper are compounds with proven antioxidant and anti-inflammatory properties. Rekha et al., (2000) also reported recovery of α-esterase activity in irradiated mice after treatment with *Brahma Rasayana* (BR), an herbal formulation of sixty medicinal plants. Das K, (2022) too listed Indian gooseberry (amla) fruits as having immunomodulatory properties with therapeutic activity which could impart protection against Covid-19 disease. The bioactivity of Indian gooseberry fruit extract in its anticancer and antitumour functions are thought to be imparted by the high levels of phenolics and flavonoids. Polyphenols present in tea are powerful antioxidants that induce phase 2 detoxification enzymes, which in turn reduce the risk of cancer by reducing damage to DNA in the cell which would otherwise lead to malignancy (Hayat et al., 2015). The role of 3, 4 dihydroxycinnamic acid in stimulating the immune system in male Swiss albino mice was studied by Ishwarya & Narendhirakannan, (2017). 3, 4 dihydroxycinnamic acid, popularly known as caffeic acid, is the main hydroxycinnamic acid present in fruits such as berries, cherries, apples, kiwi fruit and plums. In spite of treatment with cyclophosphamide, the animals that received dihydroxycinnamic acid (DCA) @ 50 mg/kg and 100 mg/kg body weight showed significant increase in the number of α-esterase positive cells compared to the normal mice. The animals that received DCA @ 100 mg/kg body weight had

almost double the number of α-esterase positive cells than that of the normal mice. The increase in the α-esterase positive bone marrow cells could be taken as an indication that DCA could also boost up the productivity of the stem cell.

4. Conclusions

The findings of this study revealed that apart from vitamin C, the compounds detected in LC-MS/MS analysis such as 2-galloyl glucose, 2-6 digalloyl glucose, capsaicin, dihydrocapsaicin, dipeperamide, gingerol, shogaol, curcumin, demethoxycurcumin and bisdemethoxycurcumin etc. having strong immunity boosting properties. Food items in various forms including beverages, containing these ingredients could be consumed in order to sustain or even enhance the immunity levels, as revealed in experimental animals. Though the physiological functions in mice were adversely affected after treatment with cyclophosphamide, feeding with the herbal drink resulted in recovery of immunity as revealed by significant increase in relative thymus weight and marginal increase in WBC counts. Results also revealed that the herbal drink was effective in improving bone marrow cellularity and α-esterase activity. The study establishes the combined effects of plant metabolites such as ascorbic acid, phenolics, carotenoids, piperine and gingerol, when formulated into an herbal drink and its subsequent administration in mouse could recover the hampered immunity. The findings also offer scope for product diversification wherein fruit juices can be incorporated with herbs or plant parts containing metabolites known for their health protective properties. Consumption of plants or their parts having these metabolites could have long term health protective properties, particularly in sustaining immunity levels in human beings as well. Though preliminary findings in mice revealed recovery of immunity after being fed with the drink, clinical studies involving human subjects might be required to understand how these metabolites function in protecting health of individuals.

Conflict of Interest Statement
The authors do not have any conflict to declare

Acknowledgements
The authors are grateful to Department of Science and Technology and SAIF/CRNTS Office, Indian Institute of Technology (IIT), Mumbai for providing HRLCMS analytical facility for the research work.

References

Agbor, G.A.L., Akinfiresoye, J., Sortino, J.R., Johnson, R., & Vinson, J.A. (2012).
AOAC. (1998). *Official method of analysis of AOAC International* (16 Ed.). Association of Official Agricultural Chemists, Washington DC, p899
Balcells, F., Martínez Monteros, M.J., Gómez, A.L., Cazorla, S.I., Perdigón, G., & Maldonado-Galdeano, C. (2022). Probiotic Consumption Boosts Thymus in Obesity and Senescence Mouse Models. *Nutrients*, 14, 616.https://doi.org/10.3390/nu14030616
Bancroft, J.D., & Cook, H.F. (1992). *Manual of Histologic Techniques*. London: Churchill Livingstone, pp. 171–174

Bischoff-Kont, I.and Furst, R. (2021). Benefits of ginger and its constituent 6-shogaol in inhibiting inflammatory processes.*Pharmaceuticals*.14 (6):1-19.

Bodagh, M.N., Maleki, I. And Hekmatdoost, A.(2019). Ginger in gastrointestinal disorders: a systematic review of clinical trials. *Food Science and Nutrition*. 7(1): 96-108.

Bode, A.M., & Dong, Z. (2011).The Amazing and Mighty Ginger. *Herbal Medicine: Biomolecular and Clinical Aspects*. 2nd edition. Boca Raton (FL): CRC Press/Taylor & Francis *British Journal of Nutrition*, 88(S2), S165-S176. https://doi.org/10. 1079/bjn2002682

Calder, P.C., & Kew, S. (2002). The immune system: A target for functional foods? *British Journal of Nutrition*. 88 2:S165-77. doi: 10.1079/BJN2002682. PMID: 12495459.

Chakraborty, S., Bhalerao, P.P., Mahale, S.A., & Dhar, R. (2020). Optimizing the formulation for a pomegranate-amla-muskmelon based mixed fruit beverage using sensory analysis and evaluating its thermal stability. *LWT- Food Science and Technology*, 133, 109907. https://doi.org/10.1016/j.lwt.2020.109907

Crocker, P.R., Paulson, J.C. and Varki, A. (2007). Siglecs and their roles in the immune system. *Nature Reviews Immunology*, 7(4): 255-266.

Das, K. (2022). Herbal plants as immunity modulators against COVID-19: A primary preventive measure during home quarantine. *Journal of Herbal Medicine*, Mar; 32:100501. doi: 10.1016/j.hermed.2021.100501

Delecroix, B., Abaïdia, A.E., Leduc, C., Dawson, B., Dupont, G., Nelson, K.M., Dahlin, J.L., Bisson, J., Graham, J., Pauli, G.F., & Walters, M.A. (2017). The Essential Medicinal Chemistry of Curcumin: Miniperspective. *Journal of Medicinal Chemistry*, 60: 1620–1637

Gomez, S., Anjali, C., Kuruvila, B., Maneesha, P.K., & Joseph, M. (2023).Phytochemical constitution and antioxidant activity of functional herbal drink from Indian gooseberry (*Emblica officinalis* Gaertn.) fruits containing spices and condiments. *Food Prod Process and Nutr* **5**, 12 (2023). https://doi.org/10.1186/s43014-022-00127-8

Gul, M., Liu, Z.W., Rabail, R., Faheem, F., Walayat, N., Nawaz, A., Shabbir, M.A., Munekata, P.E., Lorenzo, J.M. and Aadil, R.M.(2022). Functional and nutraceutical significance of Amla (*Phyllanthus emblica* L.): a review. *Antioxidants*, 11(5): 1-15.

Halyorsen, B.L., Holte, K., Myhrstad, M.C., Barikmo, I., Hvattum, E., Remberg, S.F., Wold, A.B., Haffner, K., Baugerød, H., & Andersen Moskaug, L.F. (2002). A systematic screening of total antioxidants in dietary plants. *Journal of Nutrition* 132(3), 461-471. https://doi.org/10.1093/jn/132.3.461

Haque, R., Bin-Hafeez, B., Ahmad, I., Parvez, S., Pandey, S., & Raisuddin, S. (2001). Protective effects of Emblica officinalis Gaertn. in cyclophosphamide-treated mice. *Human & Experimental Toxicology*, 20(12), 643-650. https://doi.org/10.1191/096032701718890568

Haslberger, A.G., Jakob, U., Hippe, B., & Karlic, H. (2020). Mechanisms of selected functional foods against viral infections with a view on COVID-19; Mini review. *Functional Foods in Health and Disease*, 10(5); 195-209. https://doi.org/10.31989/ffhd.v10i5.707

Hayat, K., Iqbal, H., Malik, U., Bilal, U., & Mushtaq, S. (2015). Tea and its consumption: benefits and risks. *Critical Reviews in Food Science and Nutrition*, 55, 939–954. https://doi.org/10.1080/10408398.2012.678949

He, C., Miyazawa, T., Abe,C., Ueno, T., Suzuki, M., Mizukami, M., Kurihara, K. and Toda, M.(2023). *International Journal of Molecular Sciences.* 24(11):1-14

Hewlings, J.S., & Kalman, D.S. (2017). Curcumin: A review on its effects on human health. *Foods*, (2017) 6, 92; doi: 10.3390/foods6100092

Ishwarya, M., & Narendhirakannan, R.T. (2017). 3, 4 Dihydroxycinnamic acid stimulates immune system function by modifying the humoral antibody response – An in vivo study. *Cellular Immunology*, 314:10-17. ttp://dx.doi.org/10.1016/j.cellimm.2017.01.006

Izui,S., Sekine, S., Maeda, K., Kuboniwa, M., Takada, A., Amano, A. and Nagata, H.(2016). Antibacterial activity of curcumin against periodontopathic bacteria. *Journal of Periodontology* 87(1): 83-90.

Jiang, L., Zhang, G., Li, Y., Shi, G & Li, M. (2021). Potential Application of Plant-Based Functional Foods in the Development of Immune Boosters. *Frontiers in Pharmacology*, 12:637782.doi: 10.3389/fphar.2021.637782

Keservani, R.K., Kesharwani, R.K., Vyas, N., Jain, S., Raghuvanshi, R., & Sharma, A.K. (2010). "Nutraceutical and Functional Food as Future Food: A Review", *Der Pharmacia Lettre*, 2 (1): 106-116

Lao, C.D., Ruffin, M.T., Normolle, D., Heath, D.D., Murray, S., Bailey, J.M., Boggs, M.E., Crowell, J., Rock, C.L., & Brenner, D.E. (2006). Dose escalation of a curcuminoid formulation. *BMC Complementary and Alternative Medicine*, 1, 6, 10

Luo, X.J. Peng, J. and Li, Y.J.(2011). Recent advances in the study on capsaicinoids and capsinoids. *European Journal of Pharmacology*. 650(1):1-7.

Maheshwari, S., Kumar, V., Bhadauria, G., & Mishra, A. (2022). Immunomodulatory potential of phytochemicals and other bioactive compounds of fruits: A review. *Food Frontiers*, 3, 221–238. https://doi.org/10.1002/fft2.129

Mishra, L. K., & Sangma, D. (2017). Quality attributes, phytochemical profile and storage stability studies of functional ready to serve (RTS) drink made from blend of Aloe vera, sweet lime, amla and ginger. *Journal of Food Science & Technology,* 54(3), 761–769. https://doi.org/10.1007/s13197-017-2516-9.

Nweze, C.C., Yako, A.B., Uzoukwu, A.E., & Rasaq, N.O. (2015). Effect of antioxidant nutraceuticals and functional foods on blood serum and immune activation of HIV infected patients in Abuja, Nigeria. *International Journal of Innovative Science, Engineering & Technology*, 2 (2), 148-155

Okwute, S.K. and Egharevba, H.O.(2013). Piperine-type amides: review of the chemical and biological characteristics. *International Journal of Chemistry*, *5*(3):99-122.

Osorio, E.Y., Gugala, Z., Patterson, G.T., Palacios, G., Cordova, E., Uscanga-Palomeque, A., Travi, B.L., & Melby, P.C. (2022). Inflammatory stimuli alter bone marrow composition and compromise bone health in the malnourished host. *Frontiers in Immunology*, 13:846246. doi: 10.3389/fimmu.2022.846246

Piper species protect cardiac, hepatic and renal antioxidant status of atherogenic diet fed hamsters. *Food Chemistry*, 134: 1354-1359

Priya, M.D., & Khatkar, B.S. (2013). Effect of processing method on keeping quality of aonla preserve. *International Food Research Journal*, 20(2):617- 622

Rekha, P.S., Kuttan, G., & Kuttan, R. (2000). Effect of herbal preparation, brahma rasayana, in amelioration of radiation induced damage. *Indian Journal of Experimental Biology*, 38: 999-1002

Sahebkar, A., Serbanc, M.C., Ursoniuc, S., & Banach, M. (2015). Effect of curcuminoids on oxidative stress: A systematic review and meta-analysis of randomized controlled trials. *Journal of Functional Foods*, 18, 898–909

Sai Ram, M.., Neetu, D., Yogesh, B., Anju, B., Dipti, P., Pauline, T., Sharma, S.K., Sarada, S.K.S., Ilavazhagan, G., Kumar, D., & Selvamurthy, W. (2002). Cyto-protective and immunomodulating properties of amla (*Emblica officinalis*) on lymphocytes: An in-vitro study. *Journal of Ethnopharmacology*, 81(1), 5-10. https://doi.org/10.1016/s0378-8741(01)00421-4

Shahrajabian, M.H., Sun, W. and Cheng, Q. (2019). Clinical aspects and health benefits of ginger (*Zingiber officinale*) in both traditional Chinese medicine and modern industry. *Acta agriculturae scandinavica, section b—Soil & Plant Science*, 69(6): 546-556.

Singh, M. K., Yadav, S.S., Gupta, V., & Khattri, S. (2013). Immunomodulatory role of Emblica officinalis in arsenic induced oxidative damage and apoptosis in thymocytes of mice. *BMC Complementary and Alternative Medicine*, 13, 193. https://doi.org/10.1186/1472-6882-13-193

Sredni, B.M., Albeck, G., Kazimmirsky, F., & Shalet. (1992).The immunomodulator administered orally as a radio protective agent. *International Journal of lmmunopharmacology*, 14, 619

Tadokoro, Y., & Hirao, A. (2022). The Role of Nutrients in Maintaining Hematopoietic Stem Cells and Healthy Hematopoiesis for Life. *International Journal of Molecular Sciences*, 23, 1574. https://doi.org/10.3390/ijms23031574

Thongin, S., Den-Udom, T., Uppakara, K., Sriwantana, T., Sibmooh, N., Laolob, T., Boonthip, C., Wichai, U., Muta, K. and Ketsawatsomkron, P. (2022). Beneficial effects of capsaicin and dihydrocapsaicin on endothelial inflammation, nitric oxide production and antioxidant activity. *Biomedicine & Pharmacotherapy*. 153:1-10.

Vo, T.S., Vo, T.T.B.C., Vo, T.T.T.N. and Lai, T.N.H. (2021). Turmeric (Curcuma longa L.): Chemical components and their effective clinical applications. *Journal of the Turkish Chemical Society Section A: Chemistry*, *8*(3):883-898.

Walker, B.C. and Mittal, S. (2020). Antitumor activity of curcumin in glioblastoma. *International Journal of Molecular Sciences*. 21(24):1-11.

Yu, L., Hu, X., Xu, R., Ba, Y., Chen, X., Wang, X., Cao, B., & Wu, X. (2022). Amide alkaloids characterization and neuroprotective properties of *Piper nigrum* L.: a comparative study with fruits, pericarp, stalks and leaves. *Food Chemistry*. 368:130832. https://doi.org/10.1016/j.foodchem.2021.130832

Zak, A & Pokora, I. (2017). Effect of long-term green tea extract supplementation on peripheral blood leukocytes in cross-fit trained and untrained men. *Central European Journal of Sport Sciences and Medicine*, 19 (3), 67-76. DOI: 10.18276/cej.2017.3-06

Zhao, T., Sun, Q., Marques, M., & Witcher, M. (2015). Anticancer properties of Phyllanthus emblica (Indian Gooseberry). *Oxidative Medicine and Cellular Longevity*, 1-7. https://doi.org/10.1155/2015/950890

Zick , S.M., Djuric , Z., Ruffin , M.T., Litzinger , A.J., Normolle , D.P., Alrawi, S., Feng , M.R., & Brenner, D.E. (2008). Pharmacokinetics of 6-gingerol, 8-gingerol, 10-gingerol, and 6-shogaol, and conjugate metabolites in healthy human subjects. *Cancer Epidemiology, Biomarkers and Prevention*, 17 (8): 1930-6. doi: 10.1158/1055-9965.EPI-07-2934

Table 1a: Compounds identified by LC-MS/MS based on retention time, mass, score and height obtained during the representative run in the positive mode

Name	Retention time	Mass	Mass DB	DB diff (ppm)	Score	Height	Pharmacological/ physiological functions
Curcumin	10.331	368.1227	368.126	9.01	74.98	560357	Antibacterial (Izui *et.al.*, 2016), hypolipidemic (He *et.al.*, 2023) antitumor (Walker and Mittal, 2020), anti-diabetic, anti-inflammatory, against anorexia, hepatic disorders (Vo *et.al.*, 2021)
Demethoxycurcumin	3.394	338.1172	338.1154	-5.29	60.78	2849	
Bisdemethoxycurcumin	1.717	308.1062	308.1049	-4.44	71.16	3824	
Gingerol	13.993	294.184	294.1831	-2.87	73.27	2624	Antiulcer,Anti-inflammatory,Anti-oxidant (Bodagh, *et.al.,* 2019)
Dipiperamide E	12.722	570.2687	570.273	7.48	55.11	158958	
Nordihydrocapsaicin	14.465	293.1987	293.1991	1.2	98.84	35895	Analgesic,Anti-cancer, Anti-inflammatory, Anti-oxidant (Luo *et.al.*, 2011; Thongin *et.al.*, 2022)
Capsaicin	14.816	305.1978	305.1991	4.9	94.76	16874	
Dihydrocapsaicin	15.229	307.2137	307.2147	3.44	96.02	51846	
Homocapsaicin	15.803	319.2135	319.2147	3.75	90.75	18048	
Homodihydrocapsaicin	16.328	321.2292	321.2304	3.84	94.95	33821	
Pipercide	16.458	355.2108	355.2147	11.15	68.42	31667	Neuroprotective properties (Yu *et. al.*, 2022)
Pipercyclobutanamide B	17.291	596.2827	596.2886	10.02	63.04	31667	
Glutamyl-lysine	12.175	275.15	275.1481	-6.96	63.43	29448	
Triterpenoid	12.474	552.3162	552.3121	-7.46	72.24	21027	
Monoglyceride citrate	1.428	266.0631	266.0638	2.43	97.88	32273	
Neuraminic acid	1.526	267.0922	267.0954	12.22	43.7	17955	Adaptive immune systems through glycan recognition (Crocker et al, 2007)
10-Hydroxydihydrosanguinarine	1.648	349.0968	349.095	-5.08	77.79	15883	
Benzoyl meso-tartaric acid	5.174	254.0418	254.0427	3.27	96.3	23167	
cis-Sinapic acid	10.503	224.0676	224.0685	3.7	96.86	20923	
4,4-Disubstituted cyclohexenone	8.901	347.1692	347.1733	11.82	67.36	17985	
Dihydroferuperine	14.96	289.1668	289.1678	3.27	82.72	20778	
(E,E)-2,4-Decadienoic isobutylamide	14.762	223.191	223.1936	11.53	64.4	26885	
Humilixanthin	10.645	326.1119	326.1114	-1.45	96.62	28216	
(S)-Edulinine	8.652	291.1458	291.1471	4.32	93.93	13406	
(E,E,E)-Sylvatine	18.012	383.2426	383.246	9	75.21	32254	Antifungal, antimicrobial and anti-tumour (Okwute and Egharevba, 2013)

Table 1b: Compounds identified by LC-MS/MS based on retention time, mass, score and height obtained during the representative run in the negative mode

Name	Retention time	Mass	Mass DB	DB diff (ppm)	Score	Height	Health promoting activities
[6]-Shogaol	15.032	276.171	276.1725	5.61	95.24	3364	Anti-inflammatory, anti-allergic (Bischoff-Kont and Furst, 2021)
6-Paradol	14.69	278.1872	278.1882	3.6	86.34	5322	Anti- emetic,anti-pyretic (Shahrajabian *et al.*, 2019).
Sanguiin H11	6.072	952.0826	952.0818	-0.9	63.17	264239	
Heterophylliin A	5.766	786.0925	786.0916	-1.15	96.48	154108	
Isoterchebin	5.186	954.0995	954.0974	-2.2	95.04	342479	
Punicacortein B	5.184	634.0817	634.0806	-1.66	97.44	1045063	
2,6-Digalloylglucose	4.507	484.086	484.0853	-1.45	97.73	765247	Anti-oxidant (Gul *et al.*, 2022). Superpathway of hydrolysable tannin biosynthesis
2-Galloylglucose	1.771	332.0744	332.0743	-0.09	98.88	200090	

Table 2. Effect of herbal drink on relative thymus weight of mice
CTX – Cyclophosphamide; HD- Herbal drink; b.wt-body weight

Treatments	Relative thymus weight (Mean + SE)
Normal	0.193 ± 0.048[a]
CTX 50 mg/kg b.wt.	0.077 ± 0.034[e]
HD 2.5 mL/kg b.wt. + CTX	0.128 ± 0.034[d]
HD 5 mL/kg b.wt. + CTX	0.134 ± 0.016[c]
HD 10 mL/ kg b. wt. + CTX	0.164 ± 0.042[b]
CV (%)	**0.718**
CD	**0.002**

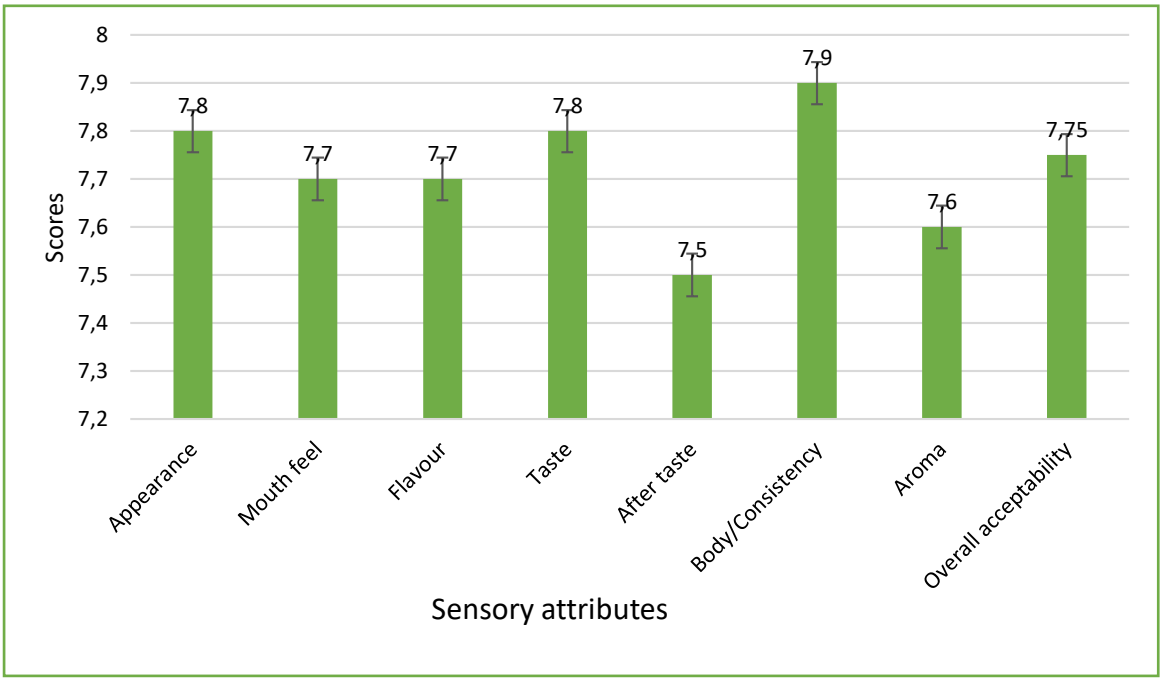
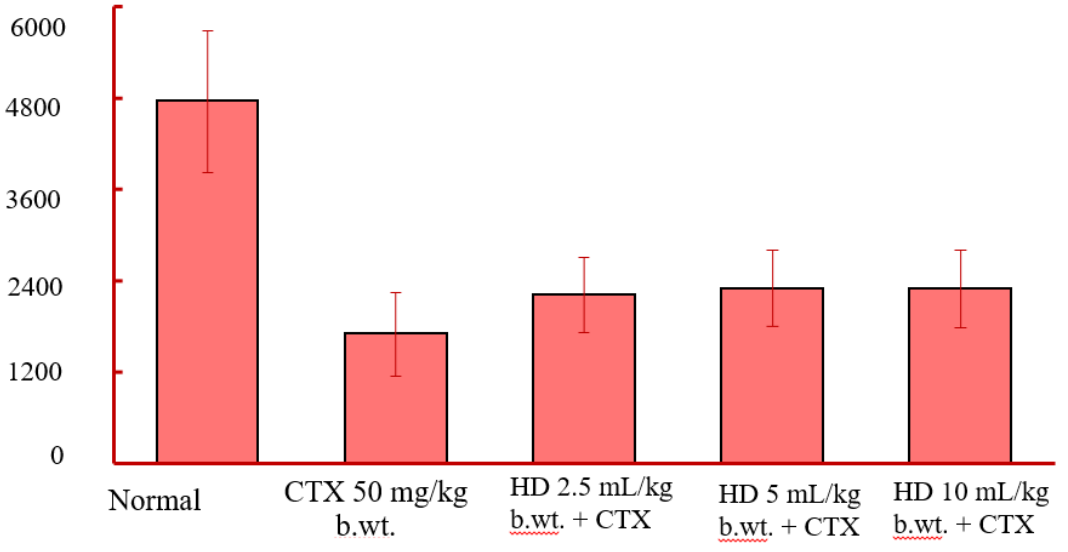
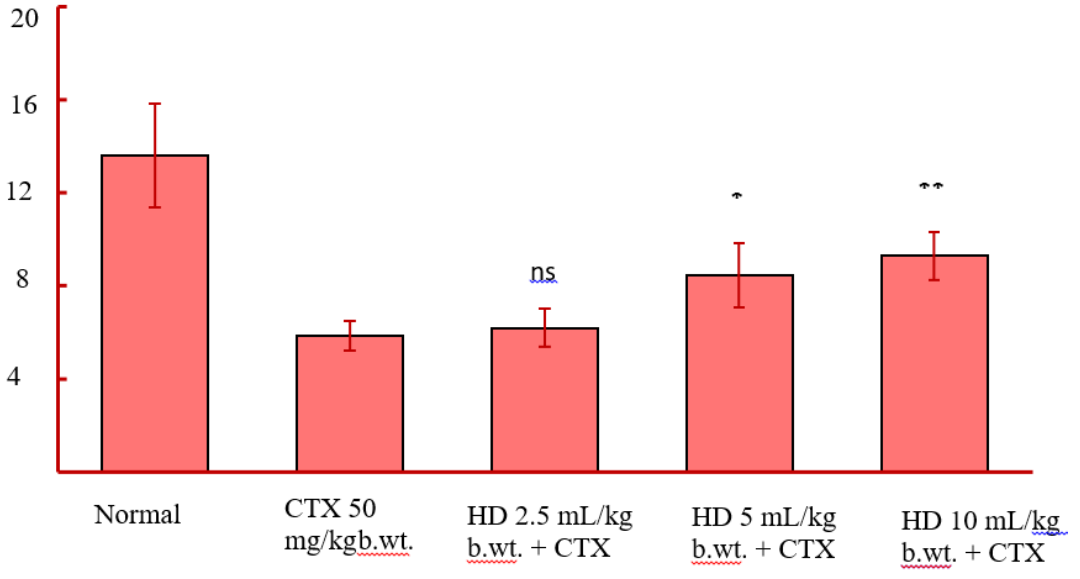


Figure 1. Sensory quality of herbal drink from Indian gooseberry fruits



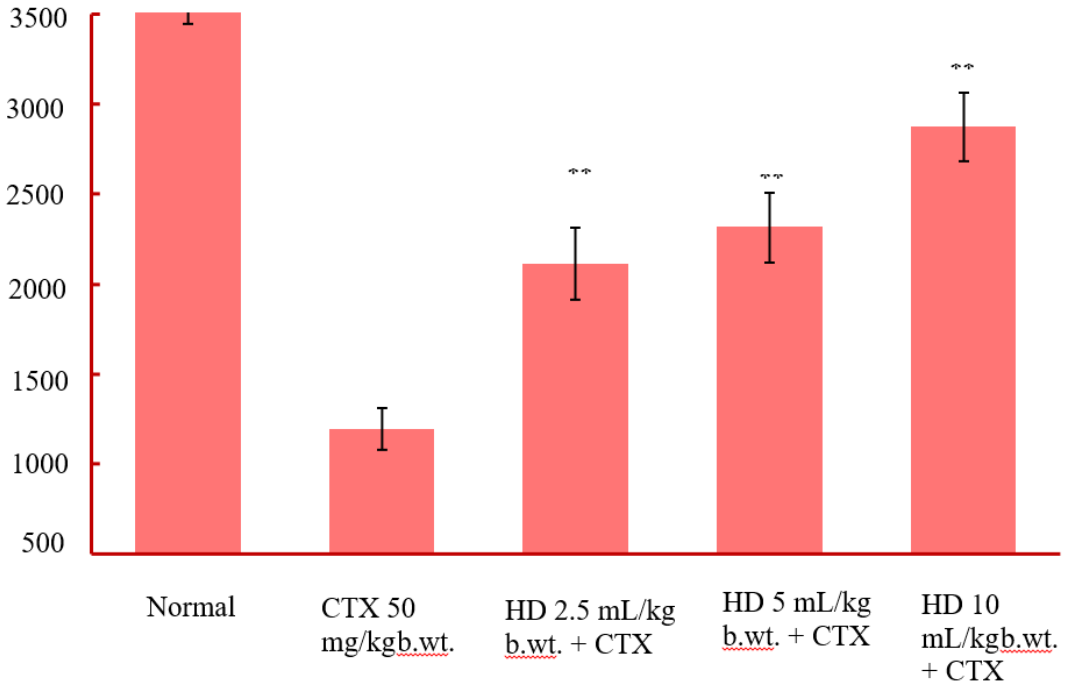
CTX – Cyclophosphamide; HD- Herbal drink; b.wt- body weight

Figure 2. Effect of herbal drink on Total WBC (cells/cc mm) of mice



CTX – Cyclophosphamide; HD- Herbal drink; b.wt- body weight

Figure 3. Effect of herbal drink on bone marrow cellularity ('n' x cells/femur/mL x 10^6) of mice



CTX – Cyclophosphamide; HD- Herbal drink; b.wt- body weight

Figure 4. Effect of herbal drink on α-esterase activity (no. of positive cells/4000 BMC) of mi

Nutraceutical herbal drink