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Evaluation of the Aqueous Extract of *Emblica Officinalis* (Storg C) for Bioavailability, Antioxidant, and Immune-Boosting Properties Compared to Synthetic Vitamin

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Abstract The aim of this study was to evaluate the *in vitro* antioxidant, *in-vivo* immunomodulatory, and bioavailability profiles of *Emblica officinalis* (Storg C) in comparison to synthetic vitamin C using SD rats. The antioxidant activity of Storg C and synthetic vitamin C was assessed *in vitro* using the DPPH assay. Immunosuppression was induced by the administration of cyclophosphamide subcutaneously in all treated groups, and treatment with the test drug was continued for 14 days. For bioavailability analysis, a single oral dose of 200 mg/kg of Storg C and synthetic vitamin C was given to the rats, and pharmacokinetic parameters were measured at various time intervals using the LC-MS/MS method. Storg C exhibited significant free radical scavenging activity with an IC_{50} value of 44.24 µg/mL, outperforming the standard BHT (IC_{50} 60.52 µg/mL) but slightly less effective than synthetic vitamin C (IC_{50} 33.15 µg/mL). In immunomodulatory tests, Storg C at doses of 50 and 100 mg/kg enhanced both primary and secondary immune responses in a dose-dependent manner, showing better performance in blood indicators and immune response compared to the control, negative groups, and synthetic vitamin C. Additionally, Storg C demonstrated superior bioavailability, achieving a maximum plasma concentration of 135.68 ng/mL and synthetic vitamin C (126 ng/mL) at a peak time of 1 hour. These findings suggest that Storg C has notable antioxidant and immunomodulatory properties, along with enhanced bioavailability, making it a promising alternative to synthetic vitamin C.

Keywords: *Antioxidant, Bioavailability, Vitamin C, Immune response, Emblica officinalis*

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1. Introduction

Mother Nature has provided mankind with an abundance of medicinal plants to create a life that is disease-free and healthy. The medicinal plants are presented in the Indian traditional systems of medicine (like Ayurveda, Unani, and Siddha); the most used one amongst them is Indian gooseberry, or Amla, also known as *Phyllanthus emblica* Linn. (Syn. *Emblica officinalis* Gaertn.) belongs to the family Euphorbiaceae [1]. This fruit grows profusely throughout India's tropical and subtropical regions. It is referred to as Nellikai in Tamil, Avala in Marathi, Nelli in Kannada, and Amalki in Sanskrit. It is a popular medicinal herb in India that has several health advantages. It contains several chemical constituents like tannins, alkaloids, and phenols. Among all hydrolysable tannins, emblicanin A and B, gallic acid, and ellagic acid are reported to possess biological activity [2]. Amla fruits are an important source of lipids, proteins, minerals (iron, calcium, and phosphorus), fiber, carbohydrates, and ascorbic acid [3]. It can support longevity, improve digestion, ease constipation, reduce fever, purify the blood, lessen coughing, ease asthma, strengthen the heart, benefit the eyes, encourage hair development, and improve intelligence [4].

Plants, being a valuable source of a wide range of secondary metabolites, are used as pharmaceuticals, agrochemicals, flavors, fragrances, colors, biopesticides, and food additives. The number of factors, such as climate, altitude, rainfall, and other conditions, may affect the growth of plants, which in turn affects the quality of herbal ingredients [5]. It is necessary to consider natural isotopic fractionation that occurs during photosynthesis [6]. Seasonal variations in carbon isotope deposition, specifically $\delta^{13}C$, in medicinal plants depend on environmental factors such as temperature, humidity, and photosynthetic activity. Studies suggest that $\delta^{13}C$ values can reflect changes in water-use efficiency and stomatal

conductance across seasons. During periods of lower water availability or higher temperatures, $\delta^{13}C$ values tend to be enriched, indicating reduced stomatal opening and increased water-use efficiency. Conversely, during cooler and wetter seasons, $\delta^{13}C$ may decrease due to more open stomata and less selective carbon assimilation [7]. Leaves are often collected during cooler and wetter seasons because lower carbon deposition in plant extracts during these periods results in reduced $\delta^{13}C$ levels. This occurs due to increased stomatal conductance and greater discrimination against carbon-13 during photosynthesis, making these leaves ideal for applications requiring lower isotope concentrations.

Amla has a broad range of phenolic compounds, such as anthocyanins, flavonols, ellagic acid, and its derivatives, that offer protection against the deleterious effects of reactive oxygen species (ROS) and manifest a multiplicity of biological actions, such as antitumor, anti-inflammatory, antibacterial, and hepatoprotective properties [8]. Amla is highly nutritious and is one of the richest sources of vitamin C, amino acids, and minerals [9]. Several studies revealed that vitamin C possesses antimicrobial properties, thus reducing the risk of infections, and has immunomodulatory functions, particularly in high concentrations [2]. A high vitamin C content aids in the body's production of the neurotransmitter norepinephrine, which helps dementia patients' brains work better. The enzyme dopamine β-hydroxylase, which transforms dopamine into norepinephrine, requires vitamin C as a cofactor. Proper norepinephrine synthesis requires adequate vitamin C levels, and a deficit may affect the balance of neurotransmitters in the brain, which may lead to cognitive loss associated with dementia [10,11]. Also, essential for wound healing, protecting the integrity of bones and cartilage throughout the body, and preserving healthy connective tissues [12]. Additionally, it works well for anti-aging and is utilized to make skin care products [13]. It aids in the production of collagen protein, which protects skin from damaging UV rays and promotes youthful, flexible skin. The fruits of *E. officinalis* are more widely employed for the treatment of many infectious and non-infectious diseases, either by themselves or in conjunction with other traditional herbs, even though all parts of the plant are utilized medicinally [14]. Researchers have proved that vitamin C and several other phytochemicals present in amla are effective antioxidants, and they render it immunomodulatory properties too [15]. It has been postulated long ago that amla is abundant in vitamin C, and the curative, restorative, and prophylactic activity of amla is attributed to this factor [16]. Vitamin C is an excellent antioxidant because it neutralizes the harmful ROS generated in the cells connected with the immune system [17].

Recent *in vitro* studies have also demonstrated that fruit extract of amla is able to relieve the immunosuppressive effects of chromium in rat lymphocytes [18]. Several studies revealed that experimentally induced vitamin C deficiency reduces cellular [19,20] and humoral immune responses [21]. Vitamin C also acts as a cofactor for many enzymes, including those involved in the production of amino acid-derived macromolecules, neuropeptide hormones, neurotransmitters, and bile salts [22]. In addition, this vitamin reduces inflammation by suppressing the expression of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) and cyclooxygenase (COX)-2 and also prevents immune cells from entering the microcirculation by inhibiting the expression of intracellular adhesion molecules [23,24]. Furthermore, vitamin C improves blood circulation, boosts several components of the immune system, and inhibits apoptosis [25,26]. Interestingly, vitamin C is one of the most critical micronutrients that are used as an immunostimulant agent [27]. The current work demonstrated the antioxidant, immunomodulatory, and bioavailability properties of vitamin C derived from the aqueous extract of *Emblica officinalis* (Storg C). The study was effectively carried out by combining *in vitro* and *in vivo* methods.

2. Materials and Methods

2.1. Drug

Storg C is manufactured, patented (Mother patent No. **437203**, IND Appln No: 202043054120, US Appln No: 17024731, EU Appln No: EP21193681.0, PCT Appln No: PCT/IB2020/062000) and registered by Star Hi Herbs Pvt. Ltd, Jigani, Bangalore, Karnataka, India. Storg C is an organic *Emblica officinalis* extract standardized for 50% w/w.

2.2. Biofortification Method of Storg C (Enrichment process)

The raw material is amla fruit juice. A membrane filtration is carried out on the raw material amla fruit juice, and a filtrate and a supernatant are collected separately. The filtrate is passed through a column packed with an ion exchange resin. The column is eluted with hot water (temperature 75°C) in the presence of 5% organic lemon juice. The eluate is collected, and the eluate is concentrated at 70°C to 80°C to obtain a concentrated solution having a TDS of 30 to 40%. The membrane purification was carried out until the desired concentration was achieved. The concentrated solution is spray-dried to obtain a dried powder.

2.3. Antioxidant Activity

2.3.1. 2, 2-diphenyl-1-picryl-hydrazyl-hydrate Free Radical-scavenging Activity (DPPH assay)

The Storg C ability to scavenge free radicals was evaluated using the DPPH radical scavenging assay, as described by the Blois method [28]. The ability of the Storg C (*Emblica officinalis*) to donate hydrogen atoms was determined by decolorizing a methanol solution with 2, 2-diphenyl-1-picrylhydrazyl (DPPH). In methanol solutions, DPPH generates a violet or purple colour that fades to shades of yellow when antioxidants are present. A 0.1 mM DPPH (Sigma-Aldrich, USA) solution was made, and 1.6 mL of Storg C at varying doses (20, 40, 60, 80, and 100µg/ml) was combined with 2.4 mL of this solution. Following a thorough vortex, the material was exposed to room temperature for thirty minutes in the dark. At 517

nm, the sample's absorbance was determined using spectrophotometry. Various concentrations of butylated hydroxyl toluene (BHT) (Sigma-Aldrich, USA) (20, 40, 60, 80, and 100 µg/ml) and synthetic vitamin C (Sigma-Aldrich, USA) (10, 20, 30, 40 and 50 µg/ml) were utilized as a reference. The following formula was used to determine the percentage of DPPH radical scavenging activity:

$$\%\ of\ DPPH\ radical\ scavenging\ activity = (A_0 - A_1)/A_0 \times 100$$

Where A_0 represents the absorbance of the control and A_1 is the absorbance of the Storg C. The IC_{50} was determined by graphing the percentage of inhibition against concentration. At each concentration, the experiment was repeated three times over.

2.4. Immunomodulatory Evaluation in Rats

2.4.1. Animals

Sprague-Dawley (SD) rats weighing 150-200 g were used in this study. They were supplied by the animal house, Invivo Biosciences, Bangalore, Karnataka, India. The studies were conducted in accordance with ethical guidelines, and clearance was obtained from the Institutional Animal Ethics Committee (IAEC) (Invivo/017/2023) prior to the experiment. The rats were housed in polypropylene cages with provision for water bottle holder and feed hopper with corn cobs as bedding material and kept under standard air-conditioned laboratory conditions (temperature 24±1°C and relative humidity 55±7%) with a 12/12 hr dark/light cycle. Before the trial, all of the animals were acclimatized to laboratory conditions for a week.

2.4.2. Grouping of Animals

Group I animals served as the control group. Group II animals were treated as negative control group i.e.100 mg/kg cyclophosphamide (Sigma-Aldrich, USA). Group III and IV animals served as treatment groups i.e.50 mg/kg (low dose) and 100 mg/kg (high dose) of Storg C respectively. Group V animals were treated with 100 mg/kg synthetic vitamin C (SVc).

2.4.3. Antigen Preparation

Freshly made Alsever's solution was diluted 1:1 with sheep blood obtained from the external jugular vein. Collected blood is centrifuged at 2500 rpm for 10 min to separate SRBCs and then washed with pyrogen-free normal saline (0.9% w/v). The concentration was adjusted to 0.1 ml of 20% SRBC [29].

2.4.4. Hemagglutination Antibody Titer

The test substance administration was done from day 1 to day 21. On days 7 and 14 all the groups except the control were administered 0.1 mL of 20% SRBC. On the 9th and 16th days, all the groups except control were administered cyclophosphamide to suppress immunity. This is also called the immunization of animals. Blood was withdrawn on the 14th and 21st days from the retro-orbital plexus under mild ether anesthesia from all the antigenically sensitized and challenged rats, and serum was separated for antibody titer checks. Each well of the microtiter plate (96-well plate) was filled initially with 20 µL of saline, and 20 µL of serum was mixed in the first well of the microtiter plate. Subsequently the 20µL diluted serum was removed from first well and added to the next well to get twofold solution of the antibodies present in the serum. Further serial dilutions take place till the last well of the second row. 20 µL SRBC (0.1% of SRBCs) was added to each of these dilutions, and the plates were incubated at 37°C for one hour and then observed for hemagglutination and antibody reactions.

2.5. Hematological Analysis

The blood was collected from each group by retro-orbital plexus and placed into heparinized collecting tubes. To estimate hematological parameters, 0.08 ml blood was mixed with 0.02 ml of ethylene diamine tetraacetic acid (33.33 mg/ml) (Himedia, India) and fed to the autoanalyzer (Beckman Coulter, Inc., Fullerton, CA, USA). The parameters such as WBC, RBC, and total platelet count were determined [30].

2.6. Bioavailability Study

2.6.1. Experimental Design

SD rats (weighing about 150-200 g) were selected as the animal model. The age of the rats was 8−12 weeks. Prior to the study, the rats that were chosen for it weren't administered any medicine for two weeks. Twelve hours before drug administration, food was taken away from the rats until 24 hr post-dosing, while water was available for rats throughout the study. The Storg C dose of 200 mg/kg p.o. and the SVc dose of 200 mg/kg p.o. based on the animal body weight were administered to rats using a gavage needle. Blood samples (0.6 mL) were withdrawn from the retro-orbital plexus at time intervals of 0, 0.5, 1, 2, 4, 8, and 24 hours after administration. EDTA disodium salt was used as an anticoagulant. Plasma was separated by centrifugation at 6000 rpm for 10 min, and the resulting plasma sample from each blood sample was divided into two aliquots and stored in suitably labeled heparin tubes at –20°C until used. Estimation of pharmacokinetic parameters of Storg C and SVc was done by using LCMS/MS. The study was approved by the Institutional Animal Ethical Committee (Invivo/017/2023).

2.7. Chemicals

Every standard was bought from Sigma-Aldrich. The standards were stored in the original packages at -18°C prior to use.

Methanol (JT baker for LC-MS), formic acid (Optima from Fisher Chemicals, LC-MS grade), Milli-Q-water from Sartorius arium mini. The analysis specification of Storg C is shown in Table 1.

Table 1. The analysis specification of Storg C

TEST	SPECIFICATION	PROTOCOL
PHYSICAL		
Description	Cream to Creamish yellow powder with characteristic taste	Organoleptic
Identification	To comply by TLC	TLC
Solubility	Soluble in water Not less than 90% w/w	USP
Loss on drying	Not more than 6.00% w/w (dried at 105^0 C)	USP<731>
Sieve test (passes through)-40#	Not less than 98% w/w	USP<786>
CHEMICAL ANALYSIS		
Natural Vitamin C	Not less than 50.0% w/w	HPLC
HEAVY METALS		
Lead	Not more than 3.0 ppm	ICP-MS
Arsenic	Not more than 1.0 ppm	ICP-MS
Cadmium	Not more than 1.0 ppm	ICP-MS
Mercury	Not more than 0.1 ppm	ICP-MS
PESTICIDES RESIDUE	Should be Absent	USP
MICROBIOLOGICAL PROFILE		
Total Plate count	Not more than 10000 cfu/g	USP<2021>
Total Yeast and Mould count	Not more than 1000 cfu/g	USP<2021>
Salmonella	Negative/10g	USP<2022>
E. Coli	Negative/10g	USP<2022>
Staph. Aureus	Negative/10g	USP<2022>
P. aeruginosa	Negative/10g	USP<62>
Coli forms	Negative/10g	USP<62>
ADDITIONAL INFORMATION		
Sanitizing treatment	Non-irradiated and Not-treated with ETO	
Certification Status	Kosher and Halal certified	
Genetic Modification status	GMO free	
BSE/TSE status	BSE/TSE free	
Country of Origin	India	
Cultivated or wild crafted	Cultivated	
Manufactured by	Star Hi Herbs Pvt Ltd, Plot No 50,3rd Road,1St phase ,KIADB Ind Area, Jigani, Bangalore 560105	
Shelf Life	3 years	

2.7.1. LC-MS/MS Analysis

Liquid chromatography and mass spectrometry were performed by a Shimadzu LCMS-8050, a high-end model of its UFMS (Ultra-Fast Mass Spectrometry) with the Nexera X3 UHPLC series. A binary analytical system was used, which consisted of a Solvent Delivery Unit LC-40 Series with Mobile Phase Monitor MPM-40, an autosampler SIL-40, and a Column Oven CTO-40 Series. Heated ESI source that uses a heated gas in addition to the nebulizer gas to increase ionization efficiency and improve desolvation. The UF sweeper® III collision cell optimizes the pressure of the collision cell and a PC using Lab Solution and Insight software to increase CID efficiency.

A chromatographic column Shim-pack GIS C18-120 (100 x 4.6 mm, 3 μm) kept at 40°C was fitted with the LC-MS/MS system. To make mobile phase A, one milliliter of formic acid (0.1% formic acid in water) was added to one thousand milliliters of water. The mobile phase B used was 100% methanol. Prior to analysis, every mobile phase was freshly prepared, filtered using a 0.2 μm nylon filter, and degassed.

A flow rate of 0.5 mL/min was utilized to accomplish chromatographic separation using the isocratic condition of mobile phases A and B (60:40). The injection volume was 10 μL.

Multiple reactions monitoring (MRM) in ESI positive mode was used to finish the mass spectrometry analysis using the following optimized parameters:

Nebulizing gas flow (Nitrogen) =3 mL/min, Heating gas flow (Zero Air) = 5 mL/min, interface temperature =300°C, DL temperature =180°C, heat block temperature =350°C, drying gas flow (Nitrogen) =10°C, interface voltage =4 kV, CID gas (Argon) =270 kPa.

2.8. Standard Preparation

An adequate quantity of Standard (Vitamin C) was dissolved in water containing 8% acetic acid to create standard stock solutions. A 20000 ng/mL concentration of vitamin C was prepared and stored at -18°C. The standard solution of 20000 ng/mL was prepared freshly in an amber-colored glass volumetric flask (5 mL), and the calculated amount of each standard stock solution was diluted with acidified water.

Fresh calibration solutions were prepared by diluting the 20,000 ng/mL standard solution in 1.8 mL brown glass HPLC vials. Seven different concentrations of stock standard solution were prepared from 3 ng/mL to 300 ng/mL. The prepared stock standard solution had a total volume of 1 mL for each calibration solution.

2.9. Sample Preparation

For the sample analysis, 40 μL of serum sample was taken in a 2 mL Eppendorf tube, followed by 160 μL of methanol in the tube. The mixture was vortexed for 3mins and then centrifuged for 10 min at 6000 rpm at 4°C. After centrifugation, transfer 100 μL of clarified supernatant and 200 μL of water to the brown glass HPLC vial (1.8 mL) for further analysis. Vortex for 2 mins and inject

For the spiked sample analysis, 40 μL of serum sample was taken in a 2 mL Eppendorf tube, followed by 10 μL of an appropriate standard solution and 160 μL of methanol in the tube. The mixture was vortexed for 3 mins and then centrifuged for 10 min at 6000 rpm at 4°C. After centrifugation, transfer 100 μL of clarified supernatant and 200 μL of water to the brown glass HPLC crimp vial (1.8 mL) for further analysis. Vortex for 2 mins and inject.

2.10. Statistical Analysis

The average of all data was compiled, and SEM was calculated. All the data were compiled using one-way ANOVA followed by Dunnett's multiple comparison tests. *P* values < 0.05 were considered statistically significant (Graph pad Prism version. 10.0.3(273)).

3. Results

3.1. *In-vitro* Antioxidant Activity Study

3.1.1. DPPH Assay

The antioxidant activity of the Storg C is shown in Figure 1. A test compound's ability to reduce lipid peroxidation was found to be closely correlated with its DPPH radical scavenging activity [31]. The Storg C antioxidant activity was compared with that of standard BHT and SVc. The free radical antioxidant scavenging activity of standard BHT, SVc, and Storg C was found to have IC_{50} values of 60.52, 33.15, and 44.24 µg/mL, respectively. A substance is more effective at scavenging DPPH if its IC_{50} value is lower, which suggests that it has a higher level of antioxidant activity. That is, the stronger a substance's antioxidant power, the lower its concentration is required to inhibit 50% of free radicals [32]. According to the current study's findings, Storg C has strong free radical scavenging properties compared to the standard drug BHT and also shows notable antioxidant activity in addition to SVc.

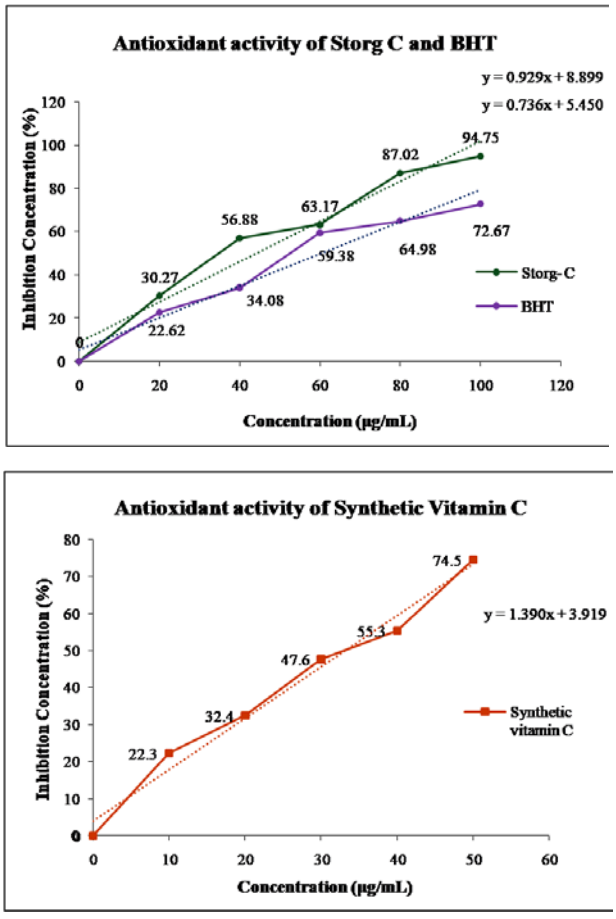
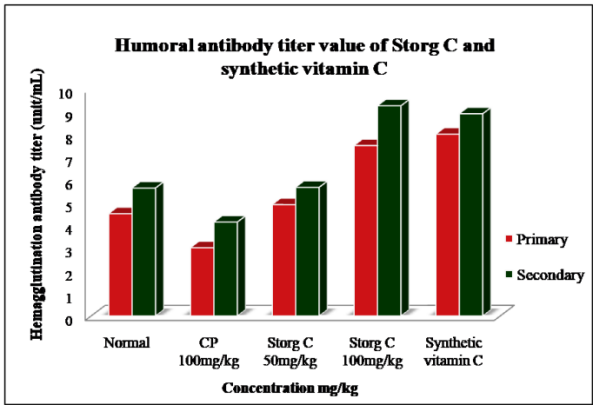


Figure 1. DPPH free radical scavenging activity and IC_{50} of Storg C, Synthetic vitamin C and Standard BHT

3.2. *In-vivo* Immunomodulatory Activity Study

3.2.1. Hemagglutination Antibody Titer

The humoral immune response was evaluated using the HA titer. Cyclophosphamide has shown inhibition of antibody titer response as it suppresses the B lymphocyte proliferation. Administration of 50 mg/kg and 100 mg/kg of Storg C shows a significant rise in HA titer value when compared with the negative control group. When comparing with SVc, 100 mg/kg of Storg C (shows a notably higher response in immunity. The test substance stimulates the immune response by counteracting the suppression of both primary and secondary humoral responses induced by cyclophosphamide. Augmentation of humoral response indicates the enhanced activity of T and B lymphocytes (Figure 2).



Values are mean ± SEM (n=8). *p <0.05, Test drug treated groups were compared with cyclophosphamide group (Statistically analyzed by one-way ANOVA followed by Dunnett's multiple comparison tests).

Figure 2. Effect of Storg C on primary and secondary antibody response on HA titer

3.3. Hematological Parameters

Analysis of hematological parameters was carried out by autoanalyzer. The count of RBC, WBC, platelets, and DLC was reduced in the cyclophosphamide group due to myelosuppressive activity. All of the cyclophosphamide-induced alterations in hematological parameters were significantly reduced by treatment with Storg C and showed the immunostimulant action. It also has a significant effect when compared with the SVc treatment group (Table 2).

Table 2. Effect of Storg C on Hematological parameters

Group	Treatment	RBC 10^6cells/mm^3	Platelet 10^3/mm^3	WBC 10^3/mm^3
I	Normal	5.3±0.13	15.9±1.2	9.5±0.10
II	Cyclophosphamide	4.1±0.43	9.8±1.3	8.6±0.3
III	Storg C 50mg/kg	5.42±0.10	16.0±1.12	9.5±0.17
IV	Storg C 100mg/kg	5.98±0.99	19.2±1.01	10.5±0.12
V	SVc 100mg/kg	5.52±0.88	17.2±0.18	10.2±0.23

Values are mean ± SEM (n=8). * p <0.05, Test drug treated groups were compared with cyclophosphamide group (Statistically analyzed by one-way ANOVA followed by Dunnett's multiple comparison tests).

3.4. Bioavailability Study

The LCMS/MS technique reveals the higher influence of Storg C bioavailability in the current investigation. Storg C shows a maximum concentration (C_{max}) of 135.68 ng/mL, and SVc possesses a C_{max} of 126 ng/mL at the T_{max} of 1 hour (Table 3 and Figure 3). SVc and Storg C have an effective bioavailability, which has an elimination half-life of 3 and 5 hours, respectively. The metabolism regulates or controls activities that keep ascorbic acid levels in tissues constant. As a result, Storg C has a

considerable level in the systemic circulation and a higher gastrointestinal absorption.

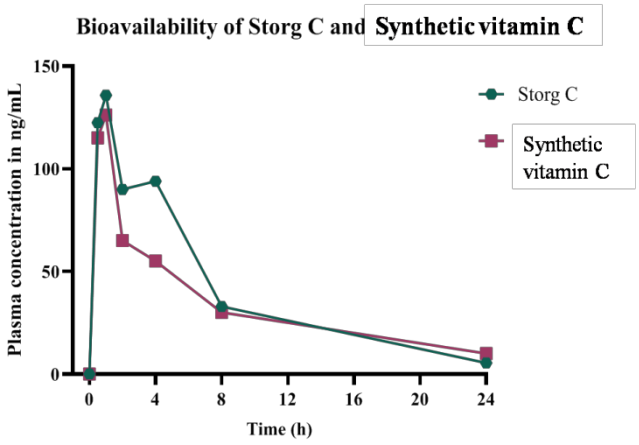


Figure 3. The plasma concentration–time curves of Storg C and Synthetic vitamin C in SD rats at a single dose of 200mg/kg body weight (mean ± SEM, n =8)

Table 3. Pharmacokinetic parameters of Storg C in rats at a dose of 200mg/kg

Parameters	C_{max} (ng/mL)	T_{max} (hour)	AUC (ngh/mL)	$t_{1/2}$
Storg C (200mg/kg/b.w.)	135.68	1 hour	951.23	5 hours
SVc (200mg/kg/b.w.)	126	1 hour	794.5	3 hours

Data are expressed as mean ± SEM (n = 6)

4. Discussion

Ascorbic acid, often known as vitamin C, is a vital component that is necessary for both nourishment and maintaining human health. In addition to being essential for the immune system's proper operation, vitamin C has a significant impact on cellular proliferation and differentiation. Acute and insufficient vitamin C shortage results in reduced collagenous structures, slowed wound healing, and a compromised immune system that affects resistance to infections [20].

Vitamin C has a potent antioxidant ability that helps shield cells from the harmful effects of ROS and endogenous reactive oxygen radicals. Amla has been found to have protective properties against free radical damage and to have positive impacts on the biological system. Amla's antioxidant capacity could be attributed to its abundance of phytoconstituents, which maximize conjugation with free radical species to lower the amount of accessible free radicals and the degree of cellular damage [33,34].

Amla is recognized as an immunological enhancer due to its pharmacological activities and immunomodulation. At the time of stress and infection, the count of vitamin C declines rapidly in the blood plasma and initiates the immunological response. Vitamin C is considered a stimulant agent for leukocytes, especially monocytes and neutrophils [35]. The leukocytes that actively acquire vitamin C against the concentration gradient. Neutrophils accumulate vitamin C via the sodium-dependent vitamin C transporter (SVCT2) pathway and can increase intracellular concentrations of vitamin C through oxidative burst stimulation. The accumulation of such elevated vitamin C concentrations appears to indicate significant roles within these cells [36]. Vitamin C can inhibit the activation of the nuclear factor kappa-B (NF-κB) pathway and other pro-inflammatory mediators such as chemokines, inflammatory mediators, adhesion molecules, etc. Along with that, it can also inhibit TNF-α and IL-6 in a dose-dependent manner [37,38]. Studies suggest that vitamin C hinders the overproduction of cytokines that cause positive responses in other immune cells by regulating the activation and proliferation of B cells, T cells, and natural killer cells (NK cells) [39,40]. A minimum concentration of vitamin C is required to exert a humoral and cellular immune response, and it also exhibits NK cell activity, accelerated phagocytosis, prevention of necrosis, and chemotaxis [41].

Our study results showed the antioxidant, immunomodulatory, and bioavailability activity of Storg C. The DPPH assay is a commonly used method for antioxidant activity. Inhibitory concentration (IC_{50}) is defined as the amount of an antioxidant molecule needed to scavenge half (50%) of the initial DPPH radicals. The molecule at scavenging DPPH is more potent at a lower IC_{50} value, implying a higher level of antioxidant activity [32]. *E. officinalis* has been shown to possess potent antioxidant properties, which may be attributed in part to the presence of flavonoids and a number of derivatives of gallic acid, such as epigallocatechin gallate [42]. Storg C scavenges the free radicals either by inhibiting the formation of ROS or eliminating them before they endanger the cell's essential components. Absorbance of the sample (517 nm) showed more % inhibition in the DPPH assay. Hence, the findings showed that Storg C has significant antioxidant activity.

In an immunomodulatory study, a hemagglutination assay helps to determine the presence and concentration of antibodies in a sample. Humoral immune response is mediated by antibodies secreted from B cells. When the antigen binds to the B cell receptors, the signal from the antigen-bound B cell and T helper cells produces antibodies [43]. In this study, cyclophosphamide is used as an immunosuppressant agent that is capable of inhibiting both primary and secondary humoral responses and cell-mediated immune responses. The negative group treated only with cyclophosphamide showed a decrease in antibody titers after the first exposure to the antigen. The Storg C-treated group of animals showed a significant rise in antibody titers in the primary response. During the subsequent exposure to the antigen, the negative control group remains decreased in antibody titer, and the Storg C-treated group shows an increase in antibody response. It was also found that, after the administration of Storg C, the leukocyte count increased in cyclophosphamide-induced treated groups. By comparing with the normal control, there was significant proliferation in total leukocyte and lymphocyte counts in the Storg C treated groups. Lymphocytes improve the immune response against host cells. The level of neutrophils also increased in the Storg C-treated groups, which elevates the pathogenic action. These results show the enhanced immune response and immunostimulant action. SVc has shown effective results in the immune response by acting

against cyclophosphamide-induced effects. The stimulating impact of vitamin C on humoral immune responses may account for the increase in circulating HA antibody titre in mice treated with it [44]. It has also shown significant results in hematological parameters such as RBC, WBC, and platelets, as well as modulating effects on lymphocytes and neutrophils. By comparing both Storg C and SVc, Storg C results in a notably higher response towards immunity.

Vitamin C is an incredible supplement used for repairing damaged tissue and in the production of vital neurotransmitters, which play a prominent role in the proper functioning of the immune system. In humans, vitamin C distribution, metabolism, and absorption are all highly intricate processes. The suppression of multiple variables that disrupt the absorption activity of vitamin C either fully or partially affects its absorption [45]. The LCMS/MS technique has been applied to quantify the amount of Storg C in blood. The body exhibits its greatest plasma concentration (C_{max}) at a time known as the peak time (T_{max}, h). The concentration at T_{max} was used to calculate the maximum concentration [46]. Drug absorption that is erratic or delayed will result in lower peak concentrations that may happen later. The variation in absorption rate could potentially impact the $t_{½}$. A longer half-life may increase the duration of the drug effect [47]. The mechanism of vitamin C absorption and excretion in the small intestine is highly dose-dependent and includes active transport via SVCT and diffusion [48]. Studies have demonstrated that when vitamin C is consumed at levels of up to 200 mg at a time, 100% absorption efficiency is achieved [49]. Storg C possessed enhanced systemic absorption, which attains its maximum concentration at 1 hour, than SVc. The prolonged half-life of Storg C ($t_{1/2}$ -5 hours) lengthens the duration of the drug's effect on systemic circulation. SVc attains maximum concentration and remains unaltered with a half-life of 3 hours. Hence, Storg C exhibits better absorption and bioavailability. When comparing Storg C to SVc (synthetic vitamin C), the LCMS/MS estimation of vitamin C in this investigation showed that Storg C has the highest bioavailability.

5. Conclusion

The present investigation suggests that Storg C exhibits significant antioxidant and immunomodulatory activity. At doses of 50 and 100 mg/kg, it exerts an immune response in a dose-dependent manner and promotes lymphocyte proliferation. Moreover, results indicate that Storg C has greater systemic absorption and ideal bioavailability. Our research suggests that Storg C can be used as an alternative to synthetic vitamin C and is a promising antioxidant and immunopotentiator. However, additional clinical research on Storg C's immunomodulatory and antioxidant properties is required for a better understanding of its impact on human health.

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Statement of Competing Interests

The authors have no competing interests.

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List of Abbreviations

BHT -butylated hydroxyl toluene, DLC- differential lymphocyte count, DPPH- 2, 2-diphenyl-1-picryl-hydrazyl-hydrate, EDTA- ethylene diamine tetra acetic acid, LCMS-Liquid chromatography and Mass Spectrometry, NK cells- natural killer cells, SD- Sprague-Dawley, SRBC- sheep red blood cells, SVc-synthetic vitamin C, SVCT- sodium-dependent vitamin C transporter

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