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(RESEARCH ARTICLE)

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Dabur Chyawankidz Gummies: A novel dosage form for immunomodulation

Baidyanath Mishra [1, *], Dharmendra Kumar [2], Ruchi Srivastava [3], Varun Kumar Singh [2], Yathapu Srinivasa Reddy [2] and Apratim Sai Rajesh [3]

[1] Head, Health Care Research, Dabur Research and Development Centre, Dabur India Limited, Ghaziabad, Uttar Pradesh – 201010, INDIA.
[2] Classical Ayurveda Health Care Research, Dabur Research and Development Centre, Dabur India Limited, Ghaziabad, Uttar Pradesh – 201010, INDIA.
[3] Medical Affairs and Clinical Research, Dabur Research and Development Centre, Dabur India Limited, Ghaziabad, Uttar Pradesh – 201010, INDIA.

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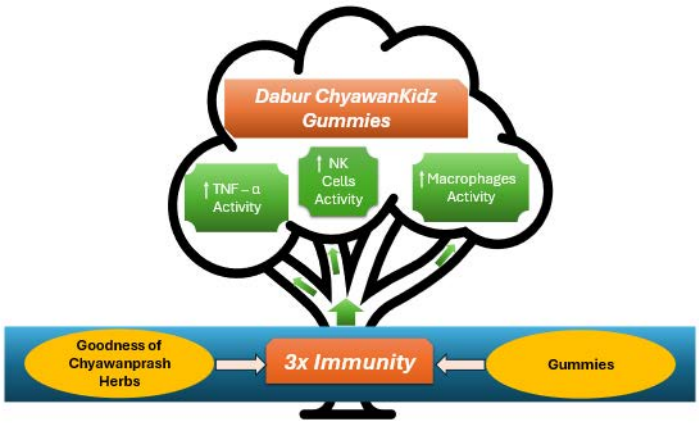
Abstract

Across age groups, a tasty eatable product is always cherished and so is immunity on the other hand, equally indispensable throughout lifetime. Optimal and sustained immunity is a contiguous requirement for growth and welfare of the body and goodness of herbs of Chyawanprash is a time-tested formula in Ayurveda for a healthy life. Combining the innovation of a chewable system of drug delivery with goodness of Ayurveda, Dabur India Limited has prepared the Dabur ChyawanKidz Gummies – a palatable, chewable and immune boosting formulation. To assess its efficacy, the active immune boosting formulation of the product were studied for its activity on NK Cell, Cytokines (TNF-α) and phagocytosis by Macrophages; wherein compared to the control groups, the test product showed up to 49% increased NK Cell activity, 66-fold increase in TNF-α (Cytokines) and 55% increased phagocytic activity by macrophages. The result indicates 3x (triple) immunity action of the Dabur ChyawanKidz Gummies, making it a unique choice of chewable immunomodulatory preparation, which appeals across ages, combining taste with immunity.

Keywords: Gummies; Immunity; NK Cells; Macrophages; Cytokines

Graphical Abstract



* Corresponding author: Baidyanath Mishra

1. Introduction

Immunity is a fundamental pillar of human life and existence, providing vital defense against external and internal pathogenic microbes and ensuring optimal physiology for a healthy body. Bulk of immune action is derived from the edible intake apart from physical activities and environment, underlining the importance of palatability of the immunity providing food items. The form of drug delivery plays an important role in bio-absorption and effectiveness of the dosage for enhancing therapeutic efficacy, thereby reducing toxicity for effective treatments [1]. Over the last couple of decades, drug delivery systems (DDSs) have undergone a huge remarkable transformation, transitioning from macroscale to nanoscale technologies and advancing towards intelligent, targeted delivery mechanisms [2]. Ayurveda – the proven ancient Indian system of medicine, is well studied and accepted for its immune boosting prescriptions and the goodness of Chyawanprash herbs with its immune protective and immune enhancing formulation has been well emphasized and established through numerous scientific studies [3].

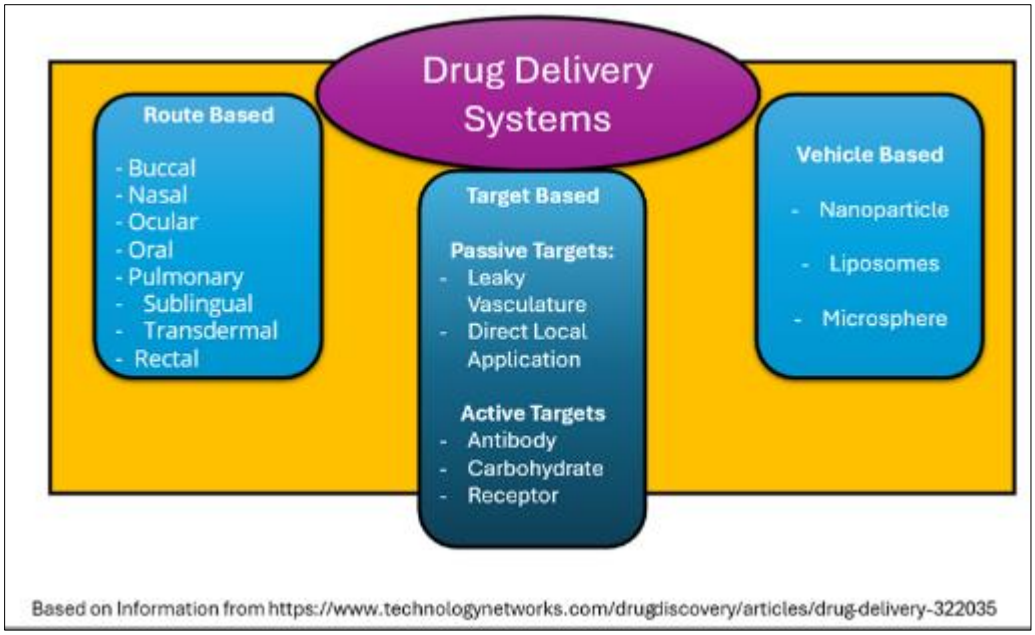


Figure 1 Concise Representation of Drug Delivery Systems

Multiple forms of drug delivery have evolved over time, summarized in Figure 1. Among these, although oral route is the most common, convenient and is easy to handle for drug delivery, chewable formulations such as chew tablets, gummies, gums are gaining attention due to their ease of administration, safety and form stability [4]. Chewing is a natural process of the digestive system with beneficial action on hypothalamic-pituitary-adrenal axis and autonomic nervous system [5]. Chewable form of drug delivery system has gained attention of late, and much research & development is underway to enhance the technique [6]. This is of advantage for the pediatric and geriatric populations, who may find it uneasy to consume direct medications complying with individual requirements.

Considering the importance of sustained immune boosting and benefits of Chyawanprash herbs and with an aim to innovate a novel process of palatable drug delivery system, the Dabur India Limited have formulated a new product – the Dabur ChyawanKidz Gummies (DCG). It includes the age-old advantage of Ayurvedic herbs, appealingly well coated and formulated into a 100% vegetarian gummy form. This study was aimed at evaluating the immunomodulatory potential of DCG.

2. Materials and Methods

2.1. Reagents and Culture media

Antibiotic solution, RPMI-1640, MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide), Fetal Bovine Serum (FBS), Triton-X, Lipopolysaccharide (LPS) (Sigma), Total Glutathione assay kit (Elabscience), Mouse TNF-α ELISA Kit (R&D Systems), CytoSelect™ 96-Well Phagocytosis Assay (Zymosan Substrate) (Cell Biolabs), DMSO (Rankem), and other regular chemicals were obtained used for the bioassays. The test substance – Dabur ChyawanKidz Gummies (DCG)

was prepared by the Formulation Division, Dabur Research and Development Centre, Dabur India Ltd., Ghaziabad, UP., India.

2.2. Animal & Single Cell Suspension

Male C57BL/6 (*Mus musculus*) mice were used for Splenocyte and Natural Killer (NK) cell activity assessment. The necessary ethical committee approvals (IAEC No. IAEC/90/1755 & 1756) were obtained for euthanization and collection of spleen to assess the *in vitro* TNF-α secretion and NK cell activity. The C57BL/6 mice were supplied by M/S Chromed Biosciences Pvt. Ltd. India, were active, no clinical signs of abnormality were noted. Spleen was aseptically excised and processed for single cell suspension as mentioned by Yathapu et al. [7]. Briefly, Cells were pelleted at 1200 rpm for 8 min. Erythrocytes in the splenocytes were lysed by treatment with lysis buffer (0.15 M NH_4Cl, 0.01 M $KHCO_3$, and 0.1 mM EDTA, pH 7.4). After lysis of RBCs, cells were washed twice in RPMI-1640 medium by centrifugation at 1200 rpm for 8 min and used for experiments.

2.3. Cytotoxicity assay

Non-cytotoxic concentrations of the test substances were determined before each experiment. The splenocytes were treated with test product (TP) in the concentration range of 1 µg/ml – 500 µg/ml (w/v) and cytotoxicity were studied by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay as per the methods of Denizot, 1986 [8]. The cell viability for immunity assessment was determined based on the cytotoxicity assay results.

Briefly, the trypsinized cell culture monolayer washed once with DPBS (*Dulbecco's Phosphate-Buffered Saline*) and test products in the range of (7.8 µg/ml – 1000 µg/ml) were added in the 96-well plates. The untreated cells were maintained as control groups for comparison. The plate was incubated at 37°C for 24hrs in 5% CO_2 atmosphere. The test solutions in the wells were discarded and 100µl of MTT diluted with DPBS was added to each well. The plate was gently shaken and incubated for 3hrs at 37°C in 5% CO_2 atmosphere. The supernatant was removed and 100µl of DMSO (Dimethylsulfoxide) was added and the plate was gently shaken to solubilize the formed formazan. The absorbance was measured using a micro plate reader (Bioteck, USA) at a wavelength of 570nm.

2.4. NK cell Activity

For estimation of NK cells activity, the action of splenocytes on Yac-1 cell line (murine lymphoma cell line) was studied as per reports of Shabsoug and Shang [9,10]. For the study purposes, non-treated cells were used as negative control, cells treated with 0.25% DMSO were included as Vehicle Control, cells treated with *Concanavalin – A* were used as positive control. To quantitatively determine the NK cells activity, they were suspended in 96 well culture plate and treated with test substance at concentrations ranging from 1µg/ml – 500µg/ml and incubated at 5% CO_2 incubator for 48 h. Subsequently, the treated NK cells were incubated together with 100 µl of CFSE stained YAC-1 cells for 24 hours. After which the NK cell mediated YAC-1 cell lysis was observed, using fluorescence concentration release method.

2.5. Cytokine (TNF-α) Activity

The immunomodulatory activity of the test item was assayed by investigating its action on secretion of Tumor Necrosis Factor alpha (TNF-α) in splenocytes ex vivo as per the methods Lee and Jung [11, 12]. For this purpose, spleen was aseptically isolated from C57BL/6 mice and single cell suspension was prepared. The action of the test product on spleen cells were evaluated against the positive control (lipopolysaccharide – LPS), while untreated cell served as negative control. After treatment the cells were incubated at 5% CO_2 for 24 hrs, post which the supernatant of cell was subjected to TNF-α levels estimation by ELISA methods, wherein the test cells were treated with TP at 1 µg/ml – 500 µg/ml concentration while the positive control cells were treated with 1 µg/ml - 25 µg/ml of LPS.

2.6. Macrophage Activity

For estimation of macrophage activity, RAW264.7 macrophage cell lines, widely reported by Ishida and Park, for immunomodulatory activity evaluation were used[13,14]. The non-treated cells were used as negative control, cells treated with 0.25% DMSO were included as Vehicle Control, cells treated with *Concanavalin – A* were used as positive control. The cells were plated at 50,000 cells/well 180 µL in 96-well plates and incubated for 24 h at 37°C, 5% CO_2 and 95% humidity. For quantifying the macrophage activity, the cells were treated with test items at concentrations ranging from 1µg/ml – 500µg/ml and incubated at 5% CO_2 for 24 h. The phagocytic activity was studied using CytoSelect™ 96-Well Phagocytosis Assay kit by Cell Biolabs Inc. USA, wherein measurement of engulfment of prelabeled zymosan particles by macrophages treated with Test Items was done in comparison to the control cells.

3. Results

3.1. NK Cell Activity

The cytotoxicity assessment of NK cells depicts cell viability percentage in the range of 94.4 – 197.19 (Figure 2), which is higher than 75%, suggesting the non-cytotoxic nature of test product in this case. The test product is seen to enhance the NK cells activity, as compared to the control group, with the percentage increase in the range of 16.2 % – 49.3%, wherein the minimum percentage increase in higher than the positive control at 15.6% (Figure 3).

Similarly, the percentage of YAC-1 cells eliminated by the NK cells upon treatment with the test product ranges from 43% to 76% for the concentration range of 1 µg/ml – 500 µg/ml, as compared to the action of positive control, which ranges from 42% - 55% in the concentration range of 1 µg/ml – 25 µg/ml.

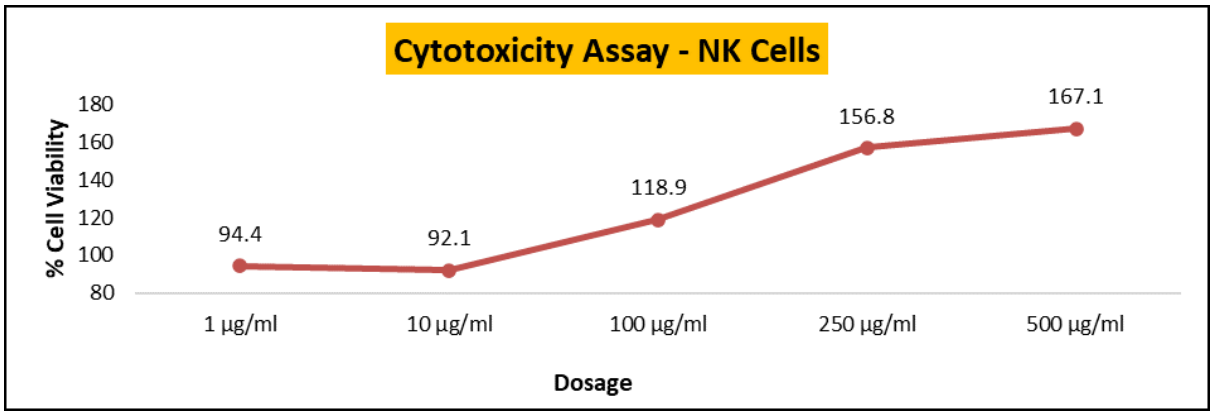


Figure 2 Cytotoxicity/Cell Viability of Splenocytes Cells with Test Product for NK Cells Assay

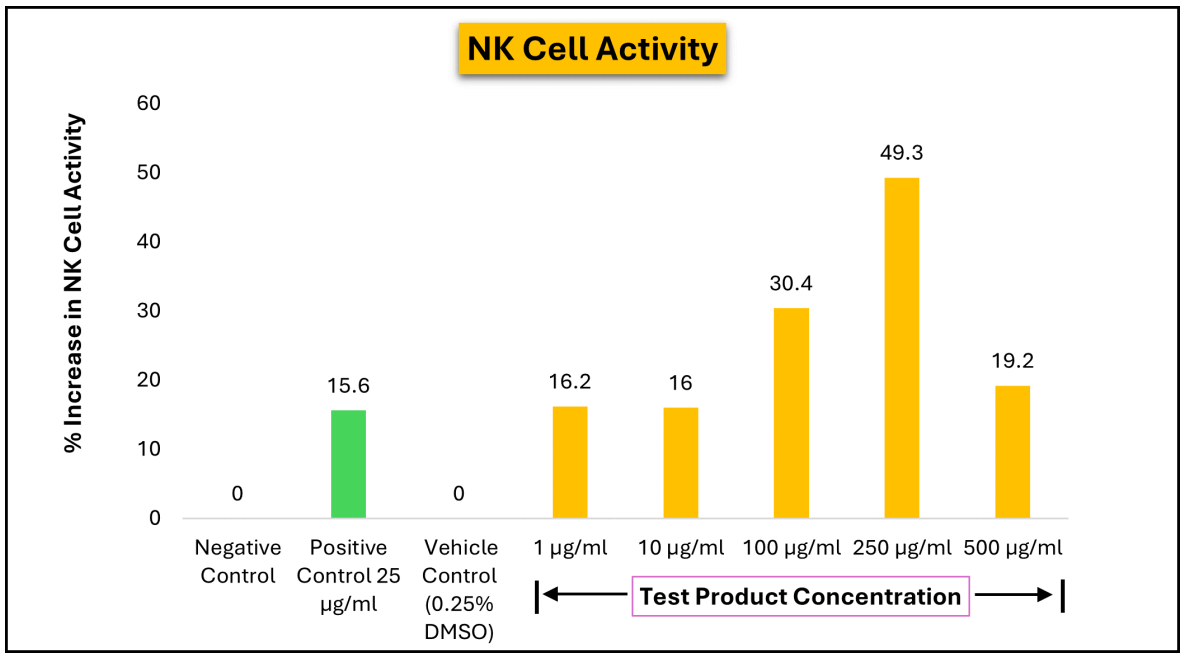


Figure 3 NK cells Activity of Test Product Compared to Control

Table 1 Action of NK cells on YAC – 1 Elimination

Samples	Concentration	% of Yac - 1 Cells Eliminated Compared to Control
Control (Untreated)	-	26.4
Positive Control (Concanavalin A)	1 µg/ml	42.0
	10 µg/ml	44.6
	25 µg/ml	55.1
Vehicle Control		26.8
DRDC/2024/019	1 µg/ml	43.0
	10 µg/ml	42.8
	100 µg/ml	57.2
	250 µg/ml	76.1
	500 µg/ml	46.0

3.2. Cytokine Activity (TNF – α)

In the cytokine activity study denoted by the action of TNF – α, the cytotoxicity assessment depicts cell viability percentage in the range of 93% - 141% (Figure 4), suggesting the non-cytotoxic nature of test products. There was a notable increase in TNF – α secretion, upon application of the test product in the concentration range of 1 µg/ml – 500 µg/ml, ranges from 1.6 – 66.7 fold in comparison to non-treated cells (Figure 5). The positive control in this case showed 92-fold increase of TNF – α levels. Similarly, the concentration of TNF-α in the splenocyte cells were also noted to increase from 4.9 pg/ml – 206.5 pg/ml, upon application of the test product from 1 µg/ml – 500 µg/ml, while the positive controls showed TNF-α concentrations of 316 – 383 pg/ml.

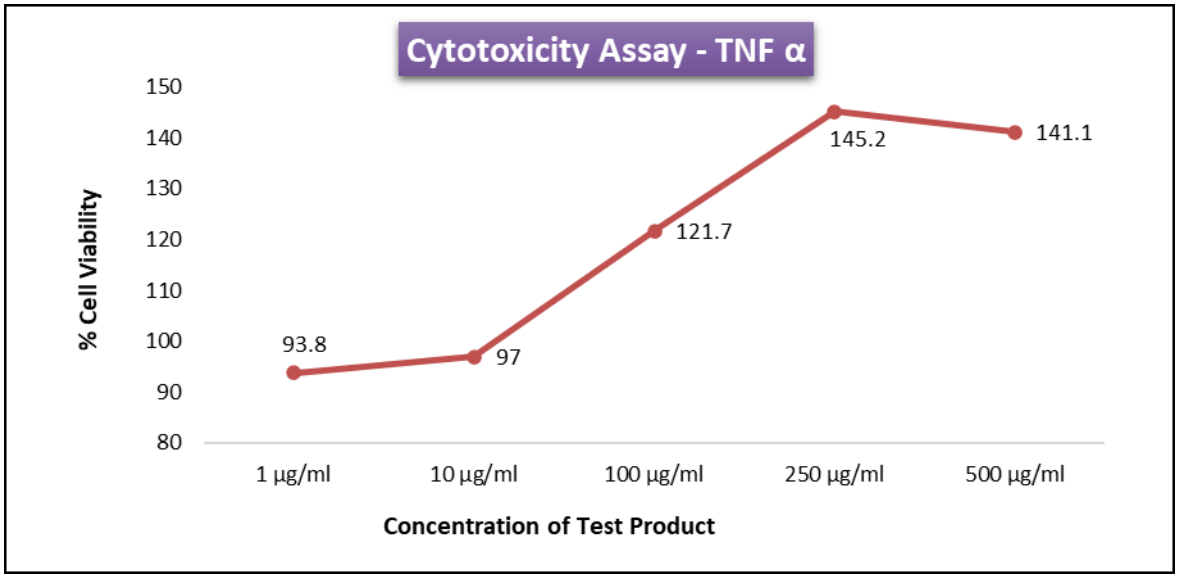


Figure 4 Cytotoxicity/Cell Viability of Splenocytes Cells with Test Product for TNF-α Assay

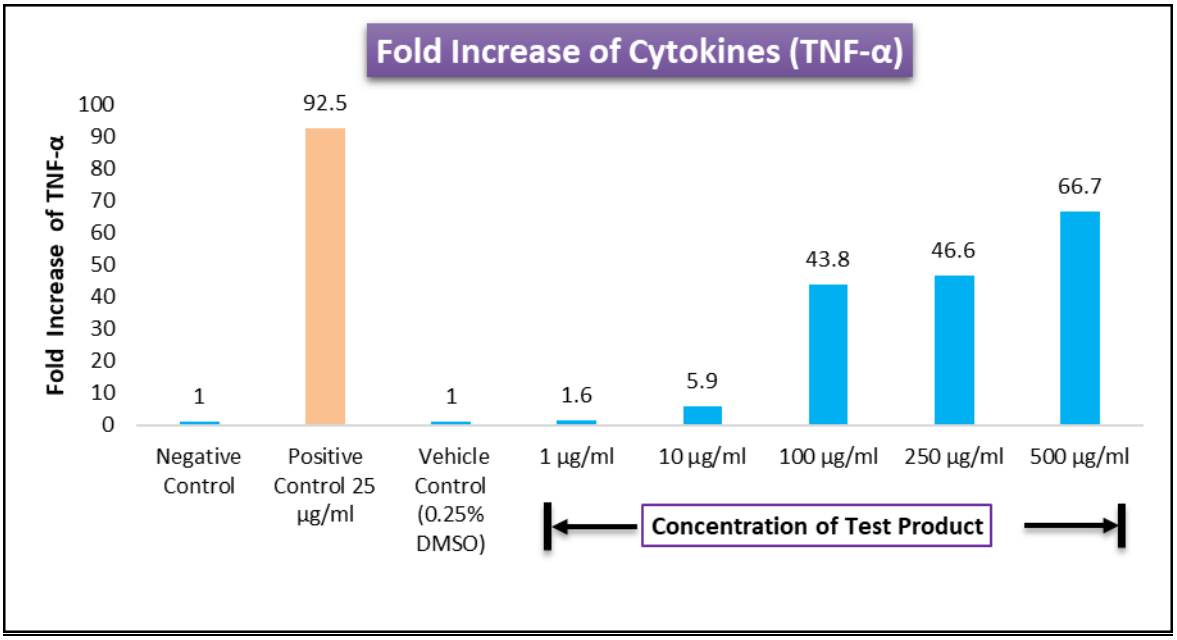


Figure 5 TNF-α Activity of Test Product Compared to Control

Table 2 Concentration of TNF-α secreted by Splenocytes

Samples	Concentration	Concentration of TNF-α (pg/ml)
Control (Untreated)	-	4.2
Positive Control (Concanavalin A)	1 µg/ml	316.5
	10 µg/ml	339.5
	25 µg/ml	383.8
Vehicle Control		3.1
DRDC/2024/019	1 µg/ml	4.9
	10 µg/ml	18.3
	100 µg/ml	135.7
	250 µg/ml	144.4
	500 µg/ml	206.5

3.3. Macrophages

For the study on phagocytic activity of the macrophages, the cytotoxicity assessment depicts cell viability percentage in the range of 90.4% - 100.9% (Figure 6), suggesting the non-cytotoxic nature of test products. The percentage increase in phagocytosis ranges from 27.2% – 55.4%, in concentration range of 1 µg/ml – 500 µg/ml (Figure 7), compared to non-treated cells. The positive control in this case showed 83.6% increase in phagocytosis, compared to the control cells.

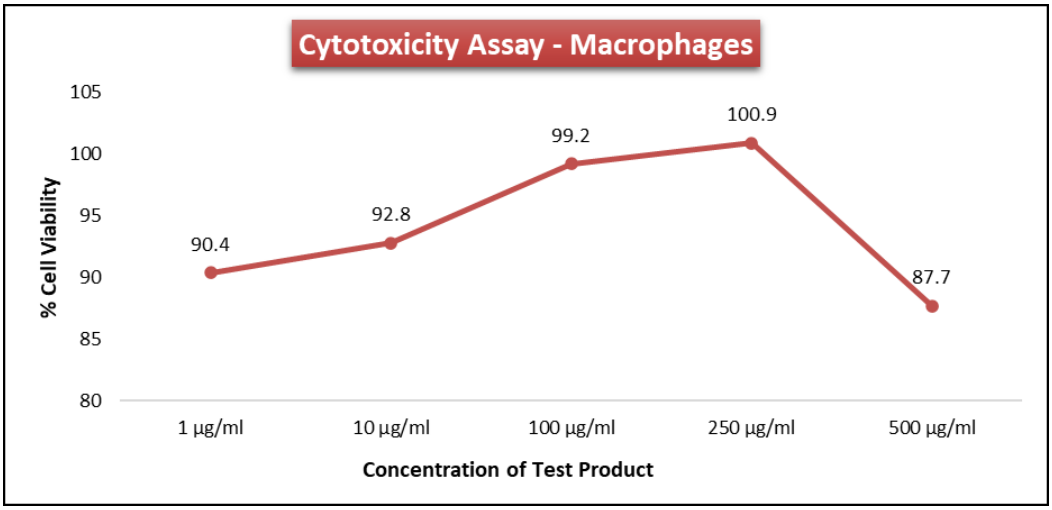


Figure 6 Cytotoxicity/Cell Viability of Macrophage Cells with Test Product for Macrophages Assay

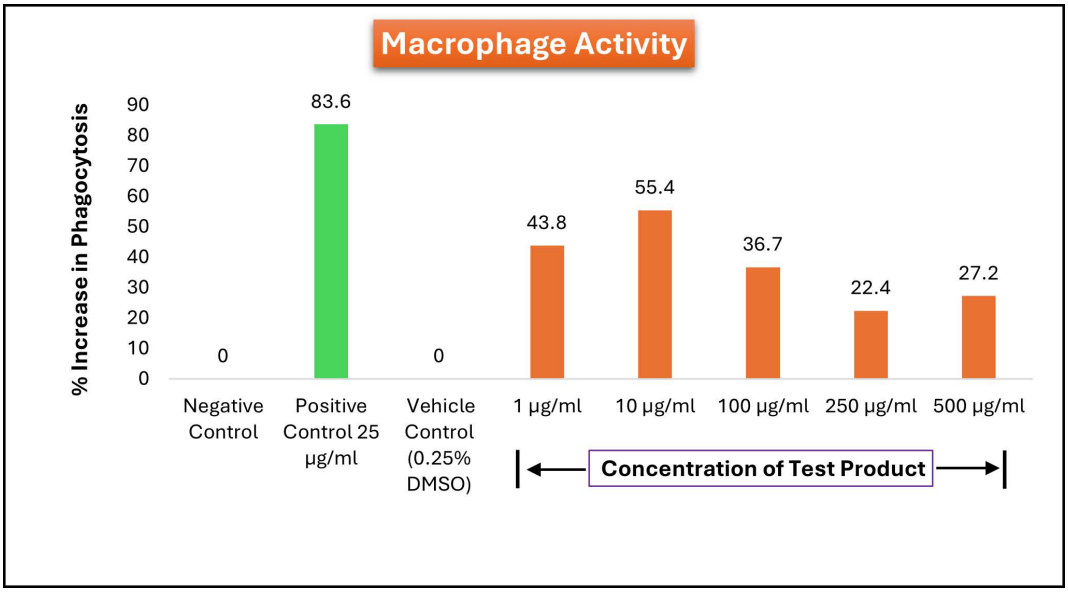


Figure 7 Macrophage Activity of Test Product Compared to Control

4. Discussions

4.1. NK cells

The current study reveals the potential of the test product to enhance the NK cells activity as demonstrated in Figure 3. As a critical component of the innate immune system, the NK cells primarily develop from hematopoietic stem cells in the bone marrows and have the capacity to recognize and neutralize a wide range of virally infected & malignant cells. They are recognized as the prime responders to offensive stimuli in various pathologies owing to multiple inhibitory or activating receptors [15]. With reports of memory-like responses [16] and low risk of toxicity [17] the NK cells are well recognized as innate immune regulatory cells, validated by many clinical studies [18].

4.2. Cytokines

Cytokines play a pivotal role in both innate and adaptive immunity [19] with therapeutic potential in tissue defense, growth and repair process [20]. Among them, TNF-α are small proteins with immunomodulatory action on chronic inflammation, intermediary metabolism and cardiovascular pathways [21]. TNF-alpha has a beneficiary effect against

ischemia and atherosclerosis [22], tuberculosis and antimicrobial functions [23]. It has also been reported to have tissue repair potential [24] and improved homeostasis in the Central Nervous System (CNS) [25]. As visible in Figure 5, the cell upon treatment with the test product, have the potential to increase the levels of cytokines thus underlining the immunomodulatory and other stated cytokine related function of the test product.

4.3. Macrophages

The action of macrophages is stimuli specific [26] and their role in maintaining human health is specific due to phagocytosis of cellular debris, tissue repair and maintenance in multiple organ systems including CNS [27]**.** They are well reported in promoting remyelination [28] and action in cellular injury [29], apart from phagocytosis. Moreover, the ability of macrophages to produce factors that stimulate angiogenesis, and fibroplasia has been firmly established [30]. In vitro studies suggest the dynamic capabilities of macrophages, from proinflammatory to anti-inflammatory [31, 32] and in wound healing [33]. The present study indicates the rise in macrophage activity (Figure 7), thus depicting the immunostimulatory and phagocytic potential of the test product.

The DCP gummies being prepared with a combination of multiple highly potent, time-tested Ayurvedic herbs such as Ashwagandha (*Withania somnifera*), Guduchi (*Tinospora cordifolia*), Shatavari (*Asparagus racemosus*), Gokshura (*Tribulus terrestris*), Draksha (*Vitis vinifera*), Punarnava (*Boerhavia diffusa*), Pippali (*Piper longum*), Sukshamaila (*Elettaria cardamomum*), Amalaki (*Emblica officinalis*) is an effective Ayurvedic combination. Such potent recipes integrated into a unique pectin-based gummy which is 100% vegetarian, palatable and suitable for all ages.

The immunological benefits of Dabur Chyawankidz Gummies are well elucidated by its NK cells, TNF-α and Macrophage activity enhancing potential, in the respective in vitro studies described before – thus underlining its 3x (triple) immunity action. This immune boosting potential coupled with benefits of chewing as discussed, make DCP Gummies a unique model of novel drug delivery for enhanced and sustained immunity, well suited across age groups and particularly beneficial for the growing ages.

5. Conclusion

The Dabur ChyawanKidz Gummies (DCG) is a unique preparation designed by Dabur India Limited, combining the significant portions of the potent Ayurvedic Chyawanprash herbs and delivered in form of fully vegetarian gummies, thereby establishing a novel system of immune boosting drug delivery. The result of the in vitro assays in this study suggests improved NK Cell, Cytokine and Macrophages activities.

Compliance with ethical standards

Disclosure of conflict of interest

The authors unanimously declare there are no conflict of interest in this research work.

Statement of ethical approval

The Institutional Animal Ethics Committee approval was obtained for the in vitro studies involving splenocytes for NK Cells and Cytokines (TNF-α) vide numbers IAEC/90/1755 and IAEC/90/1756, respectively, conducted on 14/MAY/2024.

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