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Quantification of Immunity Status of Dabur Chyawanprash - A Review Part-1 (Experimental Studies)

KEYWORDS: Dabur Chyawanprash, Immunity, Pre-clinical Safety and efficacy, NK cells and Dendritic Cells

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ABSTRACT *Ayurveda, the oldest Indian system of medicine, recommends the administration of a specified group of formulations known as Rasayana for preventing occurrence of diseases. Ancient scholars like Caraka (500 BC) have mentioned that, the use of Rasayana would impart a long, healthy and a disease free life while promoting intelligence, memory and lustre. Chyawanprash is a well-known Ayurvedic Rasayana formulation. In the present review, the preclinical studies conducted by Dabur Research and Development Centre in quantifying the immunity benefits of Dabur Chyawanprash (DCP) are reviewed. Details of studies to establish the safety and efficacy, the anabolic effect as also the anti-allergic and Immunomodulatory activities of NK and Dendritic Cells are elaborated.*

1. INTRODUCTION:

Chyawanprash (CP) is a well-known Ayurvedic recipe, which is being used since ancient times for rejuvenation and immune boosting effects on the human body. It is claimed to be useful in reducing the effects of ageing on human body and to provide resistance against disease[1]. There are[3] about 16 different CP formulations mentioned in the 57 books mentioned under First Schedule of Drugs & Cosmetics Act, 1940 (India). CP is made up of a number of herbs where Amla (Emblica officinalis) is the main ingredient. The other important constituents are different active principles of the herbal ingredients[2]. Dabur Chyawanprash (DCP) is one of the time-tested immunity booster, in India containing about forty five constituents. There are several preclinical as well as clinical observations documented over the last 50 years (mostly the immunity benefits of CP). Investigations suggest that, CP has an anabolic effect. It has been observed to promote positive nitrogen balance, enhance serum protein level and contribute to accelerated weight gain, as well as keep a balanced steroidal content. It is also known to possess adaptogenic properties and maintain a well - balanced mental status[3-5]. There exists a battery of evidence suggesting its multi faceted biological activity in favour of positive health.

Dabur Chyawanprash (DCP) is being manufactured following the traditional methods for more than 60 years. Dabur Research and Development Centre (DRDC) is involved in several pre-clinical and clinical studies on DCP. A review is being made here covering all those studies which were instrumental in quantifying the immunity status of subjects who consume DCP. In the present study (Part 1) the preclinical studies, both in-vivo as well as in-vitro studies conducted by DRDC in quantifying the immunity benefits of DCP are reviewed. Clinical studies DRDC in quantifying the immunity benefits will be reviewed in subsequent parts (2).

2. AIMS AND OBJECTIVES

In this review, it is intended to rationally analyze the quantification of immunomodulatory benefits of DCP on immune status through pre-clinical evaluation methods. Both the safety as well as efficacy studies are reviewed critically.

3. PRE-CLINICAL STUDIES

I. Safety Studies

a) Acute Oral Toxicity[6]

Acute Dose Toxicity Study (LD_{50}) of DCP in Rodents by oral route was conducted at Althea Life Sciences, New Delhi, India in November 2008 on rodents. Both the sexes of Wistar rats and Swiss albino mice were treated orally with single oral dose DCP at 2000 mg/kg bw. The test formulation was prepared by dissolving 8 g of test substance in 16 ml of 5 % dextrose to obtain a final concentration of 500 mg/ml. Control animals were administered 5 % dextrose. Animals were observed for 14 days after the treatment for product related toxic signs and symptoms, body weight and mortality.

DCP did not cause any signs of toxicity and body weight loss after the dosing and all animals survived throughout the study period of 14 days. The LD_{50} value of DCP in both sexes of rats and mice was found to be greater than 2000 mg/kg of the body weight.

b) Chronic Oral Toxicity

a. 28 days repeated dose oral toxicity study of DCP in Wistar Rats by oral route[7]

A 28 day repeated dose oral toxicity study was conducted at Althea Life Sciences in August 2010 to determine the toxicity profile of DCP when administered orally for 28 consecutive days to Wistar rats of either sex at different dose levels (250 mg/Kg, 500 mg/Kg and 1000 mg/Kg bw) with 5 animals per dose group per sex. High dose reversal group (1000 mg/Kg) comprised of 5 animals per sex. Control animals were administered deionised water corresponding to the highest dose volume of DCP. The test formulation was administered using sterile disposable syringes tipped with an oral gavage needle.

All the animals were observed at least once daily for 28 days, for DCP related toxicities and morality. After the 28 day treatment period, animals of the reversal groups were further observed for 14 days without treatment to detect delayed occurrence of toxicity or recovery from toxic effects if any.

No clinical signs of toxicities were observed in all the treated groups and control group animals.

Male and female animals from all the treated groups including the reversal group exhibited comparable body weight gain with respect to the control animals throughout the dosing period of 28 days and the 14 day post dosing recovery period.

All the animals from the control and the treated dose groups survived throughout the dosing period of 28 days and the 14 days recovery period.

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Feed consumption was recorded weekly till the end of the study. Feed consumption of control animals and DCP treated animals were found comparable throughout the study period of 28 days and the 14 days recovery period.

Haematological investigation and blood biochemistry parameters were examined at the end of test period from all the animals except reversal group animals.

At the termination of study, all the animals were subjected to detailed necropsy and examination of the external surface of the body.

Gross pathological examination did not reveal any abnormality and the relative organ weights were comparable to vehicle control. Histopathological examination was carried out on 3 representative animals of both the sexes in vehicle control and high dose animals treated with test item and did not reveal any test item related changes.

The no observed adverse effect level (NOAEL) of DCP when administered orally once every day for a period of 28 days in both the sexes of Wistar rats upon oral administration was found to be 1000 mg/Kg bw.

b. 90 day repeated dose oral toxicity study of DCP in Rodents[8]
The 90 day repeated dose oral toxicity study was conducted at Althea Life Sciences in July 2009 to determine the toxicity profile of DCP when administered daily for 90 days in Wistar rats of either sex, 6-8 weeks of age and weighing 130-300 g. 15 animals per sex per group were selected. The reversal group comprised of 10 animals per sex. The animals were acclimatized for 5 days. DCP was administered at dose levels of 250, 500 and 1000 mg/Kg BW. An additional group (reversal group) was dosed at 1000 mg/Kg bw to evaluate the reversibility or delayed occurrence of symptoms, if any. Control animals were administered 5 % dextrose. The test substance was administered equivalent volume of 5 % dextrose (corresponding to the highest dose). Observations comprised of mortality, clinical signs, body weight and food consumption, clinical laboratory investigations, organ weights and macro-microscopic pathology.

All the animals from the control and the treated groups survived throughout the dosing period of 90days and the recovery period. There were no treatment related mortalities in any of the groups. Few instances of mortality recorded were incidental, but these animals did not show any characteristic clinical signs and on necropsy, revealed no abnormality in vital organs. Male and Female animals from all the treated animals including the reversal group exhibited comparable body weight gain with respect to the controls throughout the dosing period of 90 days and the recovery period.

Food consumption of control and treated animals were found comparable throughout the dosing period of 90 days and the recovery period. Haematology parameters analysed at the end of dosing period in week 13 and at the end of post dosing recovery period on day 105 and on day 119 were within the normal range for Wistar rats.

Blood biochemistry carried out at the end of dosing period in week 13 and at the end of post dosing recovery period on day 105 and on day 119 revealed no abnormalities. Urine parameters studied for all animals on day 86 and 87 and reversal group animals on day 104 and on day 118 were within normal limits.

On necropsy organ size shape and weight did not reveal any significant differences between groups when compared to control. Gross pathological examination also did not reveal any abnormality.

Histopathological examination was carried out on 5 representative animals of both the sexes in control and high dose animals. The organs (adrenal glands, brain, heart, kidney, liver, lung, spleen, testis/ovary, epididymis, aorta, eye, lymph node, pancreas, pituitary, skin, thigh muscle, trachea, thyroid, thymus, urinary bladder, stomach, duodenum, jejunum, colon and sternum for bone marrow) were collected and the haematoxylin & eosin stained sections were prepared. No test substance related histological change was seen in any of the animals.

The no observed adverse effect level (NOAEL) of DCP when administered orally once every day for a period of 90 days in both the sexes of Wistar rats upon oral administration was found to be 1000 mg/Kg.

II. Efficacy Studies
The following pharmacological evaluation is done both at *in-vivo* and *in-vitro* level[9-14]

- Evaluation of anti allergic potential of DCP by estimation of levels of Immunoglobulin E (IgE) in serum.
- Evaluation of anti allergic potential of DCP by estimation of histamine in plasma.
- Evaluation of Immunomodulatory potential of DCP by estimation by phagocytosis assay using mouse macrophages.
- Evaluation of Immunomodulatory activity of DCP by Natural Killer Cells.
- Evaluation of Immunomodulatory activity of DCP against Dendritic Cells.

1.) *In-vivo* studies
a. Anti allergic potential of DCP by estimation of IgE in mouse[9]
Allergic disorders include hay fever, asthma, atopic dermatitis and food allergies allergic rhinitis and sinusitis. The symptoms are associated with high levels of serum Immunoglobulin E.

The present study evaluated the anti allergic potential of DCP against ovalbumin induced allergy where mice were treated orally with the DCP at the dose of 1 g/kg extracts. After 1h of administration of DCP, the test animals were challenged with ovalbumin for the induction of allergy on day 0. Animals were challenged with booster dose on day 7 and day 14 respectively. IgE release assay was then performed with the serum of mice collected on day 7, 14 and 21. Cetrizine (10 mg/kg), a widely used anti allergic drug was used as positive control.

It was observed that serum IgE levels rose significantly 26 and 39 fold at 14 and 21 day, respectively with respect to vehicle control. However, pre treatment with DCP led to a significant decrease of 12 and 41 fold at 14 and 21 day and respectively of Ovalbumin induced serum IgE release. Pre treatment with DCP and Cetrizine in the respective groups kept serum IgE levels almost to that of the vehicle treated group when mice were challenged with ovalbumin after 1h of their administration. This effect is indicative of potent anti-allergic activity of DCP and Cetrizine.

DCP possess potent anti allergy activity and oral intake of the same as a dietary supplement may contribute to the prevention or treatment of mast cell mediated allergic diseases.

b. Evaluation of anti allergic potential of DCP[10]: In humans, mast cells are important mediators of inflammatory responses such as allergy and anaphylaxis (immediate type of allergy). Allergic and anaphylactic reactions are induced by the sudden systemic release of inflammatory mediators, such as histamine, heparin and various cytokines from mast cells, which are constituents of virtually all organs and tissues.
Since the measurement of histamine levels in plasma can provide unique information about degree and mechanism of allergic reactions, the inhibitory effect of the DCP on plasma histamine release induced by an allergen provides a major index for predicting its anti-allergy potential.

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In the present study, the anti-allergic potential of DCP against compound induced allergy was evaluated. Rats were treated with DCP at the dose of 1 g/Kg followed by a challenge with the compound for induction of allergy. The blood samples were then collected and plasma histamine levels were determined.

It was observed that plasma histamine levels rose significantly up to 3 fold above the baseline levels immediately within 3 min of administration of the allergen compound. Pre treatment with DCP and Control in respective groups kept plasma histamine levels almost to baseline and led to a significant suppression plasma histamine release at all time points when rats were challenged with allergen. This effect was indicative of potent anti allergic activity of DCP.

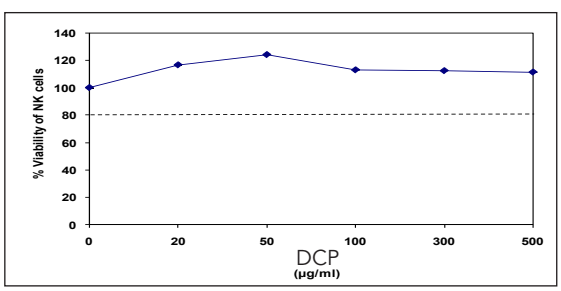
DCP possess potent anti allergy activity and oral intake of the same as a dietary supplement may contribute to the prevention or treatment of mast cell – mediated allergic diseases.

2.) In-vitro studies

a. Evaluation of Immunomodulatory activity of DCP by Natural Killer Cells[11 and 13,14]

Natural Killer cells are essential elements in the immune defense against pathogens and tumor cells by modulating both innate and adaptive immune responses. Activated NK cells are potent anti-tumor effector cells and play a key role in generation of a protective immune response. The immunomodulatory potential of a compound is evaluated by assessing its effect on NK-cell mediated lyses of target tumor cell population (Figure 1).

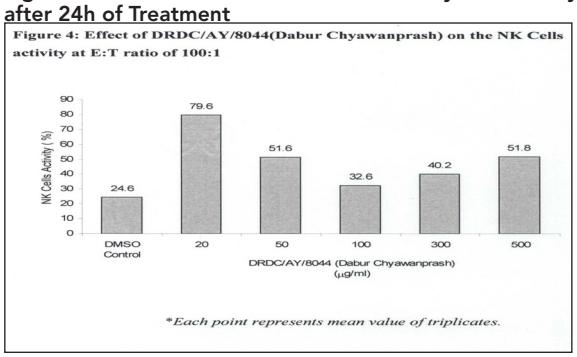
Figure 1: Effects of DCP on NK cells viability and activity after 24h of Treatment



The present study was conducted to evaluate the immunomodulatory activity of DCP using Natural Killer (NK) cells. NK cells were treated with DCP at concentrations ranging from 20 µg /ml to 500 µg /ml for 24 h. Any modulation in NK cells mediated killing of co-cultured target tumor cells (YAC-1) was determined as compared to control cells.

DCP treatment for 24 h resulted in marked enhancement of NK cells activity mediated by specific lyses of target murine lymphoma, YAC-1 cells. At both concentrations, DCP treatment led to an increase in NK cells activity as compared to DMSO treated controls

Figure 2: Effects of DCP on NK cells viability and activity after 24h of Treatment



NK cells activity was found to be increased by 1.8 fold to 10.3 and 1.3 to 3.2 fold in different concentration ratios of NK cells and target cells upon treatment with DCP 20 µg/ml – 500 µg/ml (Figure 2).

Treatment with DCP resulted in increased NK cells activity by enhanced target cell killing, when compared to control cells, which strongly suggest potential immunostimulatory activity.

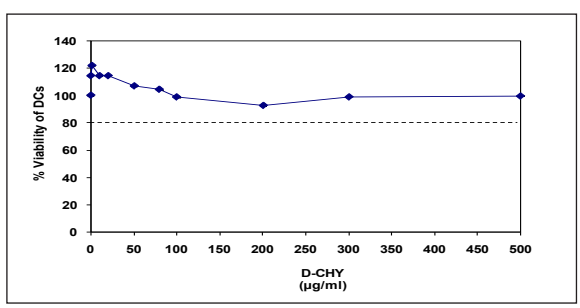
Marked elevation in Natural Killer cells activity strongly suggests that DCP possesses considerable immunostimulatory activity.

b. Evaluation of Immunomodulatory activity of DCP against Dendritic Cells[12, 13]

Dendritic cells are professional antigen-presenting cells that play pivotal role in the development of protective immune responses against a variety of pathogens. DCs represent an ideal target cell population to evaluate the immunomodulatory action of a test agent.

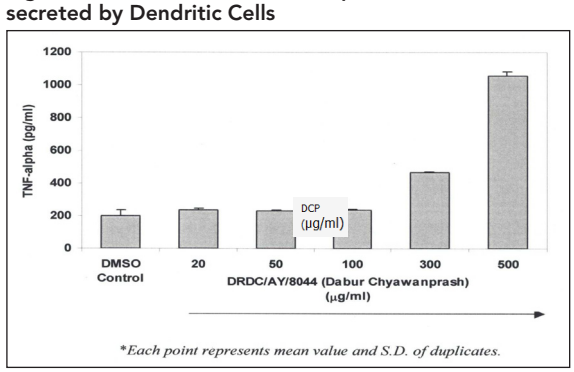
In the present study, morphological observation of DCs demonstrated a dose dependant maturation of DCs upon treatment with DCP (20 µg/ml –500 µg/ml). Increased population of adherent and branched cells was observed after 24 hrs of treatment of DCP as compared to control DCs treated with DMSO. This finding is an indicative of immunomodulatory activity.

Figure 3: Effects of DCP on viability and morphology of Dendritic Cells



DCP treatment also resulted in increased levels of TNF-α, IL-1-β and MIP-1- α secreted by DCs over a concentration range of 20 µg/ml –500 µg/ml, as compared to control DCs treated with DMSO after 24 h of treatment (Figure 3)

Figure 4: Effect of DCP on the expression levels of TNF-α secreted by Dendritic Cells



TNF-α is a key pro-inflammatory cytokine secreted by activated DCs, which is an important marker for immunostimulation. Treatment of DCs with DCP resulted in 1.2 fold – 5.2 fold increased secretion of TNF-α, as compared to control DCs treated with DMSO. IL-1—β is also a pro inflammatory cytokine, which play a central role in mediating immune & inflammatory responses to injury and infection. Stimulation in IL-1-β levels by 1.2 fold – 2.2 fold was observed upon treatment with DCP MIP-1- α is another proinflammtory chemokine that is involved in a va-

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riety of immune and inflammatory responses. It induces chemo attractant and pro adhesive effects on T-cells, NK cells, cytotoxic T cells, B cells, basophils & eosinophils. Treatment with DCP resulted in elevation of MIP-1- α secretion by 1.4 fold – 3.3 fold as compared to DMSO treated control cells (Figure 4).

Figure 5: Effect of DCP on the expression levels of IL-1-β secreted by DCs

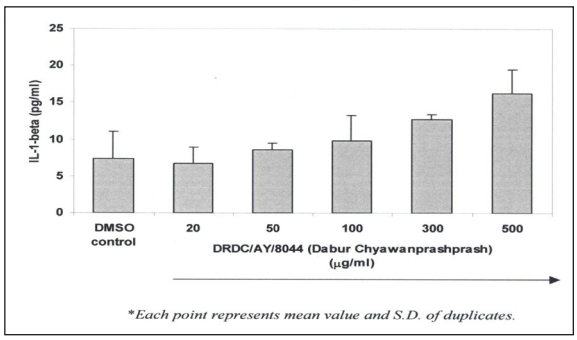
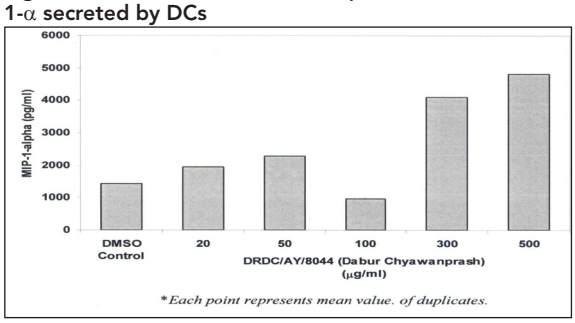


Figure 6: Effect of DCP on the expression levels of MIP-1-α secreted by DCs

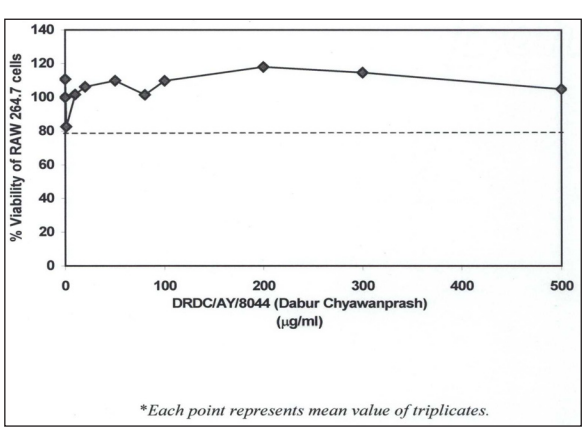


The considerable elevation in levels of the immunity markers after treatment with DCP strongly suggests that DCP possesses considerable immunostimulatory activity (Figure 5, 6).

c. Immunomodulatory activity of DCP by phagocytosis assay using mouse macrophages[14]:

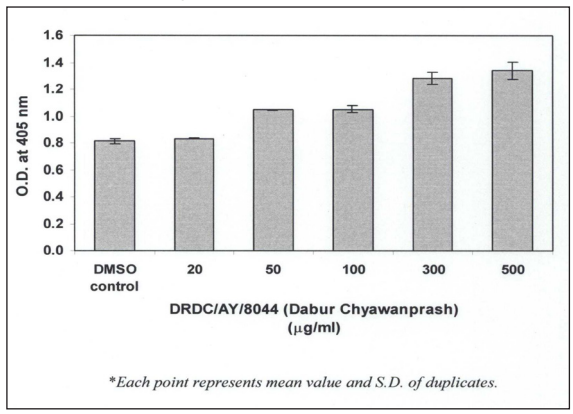
Macrophages play a significant role in the immune system as part of the host's defence mechanism. Macrophages are important regulatory cells, which recognize invading foreign bodies and are central to cell-mediated and humoral immunity. Phagocytosis is the process of internalization, which is followed by pathogen killing and destruction. This process is an essential element of the innate immune system and the first step of defence against infections.

Figure 7: Effect of DCP on viability of Mouse Macrophages after 24 h of treatment



The present study was conducted to evaluate the immunomodulatory activity of DCP using phagocytosis assays employing macrophages. Mouse macrophages were treated with DCP at non-cytotoxic concentration and phagocytosis was assessed. Any modulation in extent of phagocytosis by DCP treated macrophages was determined as compared to control cells (Figure 7 & 8).

Figure 8: Effect of DCP on the phagocytic activity of mouse macrophages



DCP treatment for 24 hours resulted in marked enhancement of phagocytic activity as compared to the controls. Phagocytic activity was found to be increased by 28.9% -65.2% upon treatment with DCP in the concentration of 20 µg/ml - 500 µg/ml.

Elevation in phagocytic activity strongly suggests that DCP possesses considerable immunostimulatory activity.

4. DISCUSSION

Chyawanprash is an age old formulation described in classical Ayurvedic texts. It is having multiple Ayurvedic ingredients which possess rejuvenating, strength and bulk promoting, nourishing and antioxidant properties. The beneficial effects of Chyawanprash are being mentioned in various Ayurvedic literatures and formularies (that are mentioned in first schedule of Drug & Cosmetic Act, 1940) like Charak Samhita, Bhaisajya ratnavali, Ayurvedic Formulary of India etc. As per Charaka Samhita and Ayurvedic Formulary of India, Chyawanprash is useful in various conditions like respiratory conditions such as cough, weakness and debility, improving the quality of life and acts as rejuvenator etc.

Chyawanprash has been studied for its medicinal properties extensively in many studies. DCP is properly standardized and follows the quality norms of Govt. of India to maintain the product quality. DCP was found to be safe in the recommended dosage for the defined period of time in the toxicity studies. In all the toxicity studies i.e. acute dose oral toxicity, 28 day repeated dose oral toxicity study and in the 90 day repeated dose oral toxicity studies, DCP did not cause any signs of toxicity and body weight loss and all animals survived throughout the study periods. In acute toxicity, the LD50 value of DCP in both sexes of rats and mice was found to be greater than 2000 mg/kg of the body weight. The no observed adverse effect level (NOAEL) of DCP was found to be 1000 mg/Kg bw in both 28 days and 90 days toxicity studies.

DCP is scientifically established to possess anti-allergic and immunomodulatory benefits. Pre-treatment with DCP and Control in respective groups kept plasma histamine levels almost to baseline and led to a significant suppression of plasma histamine release at all time points when rats were challenged with allergen. When DCP was studied against Ovalbumin induced allergy it was observed that pre treatment with DCP led to a significant decrease of 12 and 41 fold at 14 and 21 day and respectively of Ovalbumin induced serum IgE release. This effect establishes the anti-allergic ac-

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tivity of DCP along with its probable mechanism of action for the same.

NK cells activity was found to be increased by 3.2 to 10.3 times in comparison to DMSO control in different concentration ratios of NK cells and target cells upon treatment with DCP. When Dendritic cells were treated with DCP, it resulted in increased secretion of TNF-α, Stimulation in IL-1-β levels and elevation of MIP-1- α secretion in comparison to DMSO treated control cells. Phagocytic activity was also found to be increased.

Therefore, decrease/suppression of plasma histamine release and IgE Levels, suggests anti-allergic potential of DCP; and the increased levels of the immunity markers (TNF-α, IL-1—β and MIP-1- α), along with enhancement of NK cells and phagocytic activity suggests that it possesses potential immunomodulatory properties.

The above mentioned observations are basis preclinical studies. However, DCP has been studied in various clinical studies; few of them are specifically conducted to evaluate the immunomodulatory properties of the same. The review of the Clinical studies will be published as part II review of this series for the Quantification of Immunity Status of DCP.

5. CONCLUSION:

CP is claimed to best Rasayana i.e., best immunity booster according to Ayurvedic texts. the above studies provide the evidence for different path ways through which the immunomodulatory activity is assessed and quantified. Also, the mechanism of action for the three times more immunity for DCP is established. On the basis of N K Cell activity and other relevant studies the quantification of the immunity benefits of DCP (Dabur Chyawanprash) is made possible. DCP has been found safe in the given doses in various pre clinical repeated dose in vivo toxicity studies.

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