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Research Article

In vitro Evaluation of Antioxidant and Immunomodulatory Activity of *Khajurprash*: A Traditional Health Supplement Preparation

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Abstract

The traditional preparations were developed over a period with combination of strong theoretical knowledge of natural ingredients and unique preparation processes. The Indian Ayurvedic text has plethora of such preparations as therapeutics, health and food supplement to support the 'quality of life' of human being. *Khajurprash*, an extention of Chyawanprash under proprietary category for achieving Rasayana(rejuvenation) has been meticulously prepared, possessing the Date palm fruit (*Phoenix dactylifera L.*), Amalaki (*Emblica officinalis*) and a combination of nearly 40 variety of herbs/spices. *Dabur Khajurprash* (D-KP) has been evaluated for its antioxidant, immunomodulatory and innate immune response induction property using the *in vitro* and *ex vivo* system. The antioxidant potential of D-KP was assessed in HepG2 cell for GSH levels, immunomodulatory activity by splenocyte TNF-α secretion, innate immunity response by NK cell function and phagocytosis by RAW 264.7 cultured cells. The results suggest that D-KP has antioxidant activity, assessed by reduced form of GSH levels, and was at *par* with Quercetin. A concentration dependent increase of TNF-α secretion by splenocytes up to 100 µg/mL pf D-KP and reduction of TNF-α levels with further higher concentrations of D-KP suggest the immunomodulatory potential. The D-KP has significantly increased the NK cell mediated YAC-1 cell lysis and phagocytosis assessed using the RAW 264.7 cells. Results of current study conclude that D-KP has antioxidant, immunomodulatory and innate immune response stimulation potential.

Keywords: *Khajurprash; Chyawanprash; Antioxidant; Immunomodulatory; Innate Immune Response*

Introduction

According to Ayurveda, the Indian traditional system of medicine, *Rasayana* deals with rejuvenation and revitalization of energy, and addressing the issues with progression of age (Geriatric). Use of plant/herbal based materials such as root, shoot, bark, fruits, leaf etc. as extracts is the key process of Ayurveda, being practiced since ancient period. Several countries have their own traditional curative or remedial forms which are firmly rooted in their culture and history. According to World Health Organization (WHO), traditional medicine is 'the sum total of the knowledge, skill, and practices based on the theories, beliefs, and experiences indigenous to different cultures, whether explicable or not, used in the maintenance of health as well as in the prevention, diagnosis, improvement or treatment of physical and mental illness' [1]. In the ancient system of Ayurveda, preparation of *Rasayana* in different storage forms like, *Churna*, *Awaleha, Vati* etc. are well defined and have been practiced for prevention and cure of diseases, along with wellbeing of individuals.

Khajurprash is one such type of *Rasayana Awaleha*, comprising mainly of date palm fruit i.e. *Khajur* (*Phoenix dactylifera L.*), *Amalaki (Emblica officinalis)* and a combination of nearly 40 various herbal ingredients. Date palm fruit has attributed for various properties such as tonic, antimicrobial, rejuvenation of energy, etc. *Khajur* comprises of 70% carbohydrates (majority are simple sugars), high amounts of dietary fiber, B-complex vitamins and minerals, viz. iron, potassium, and calcium [2]. The pharmacological activities such as antioxidants, anti-cancer, immunomodulatory etc. of date fruit were attributed to the active phytochemical constituents such as anthocyanins, phenolics, sterols, carotenoids, and flavonoids, which vary between variety and stage of maturity and ripening of fruit [3]. Continuous consumption of Dates is known to enhance bone marrow function and blood hemoglobin levels as it contains Iron and Calcium.

The fruits of Amla (*Emblica officinalis*) have beneficial role in diabetes, gastric ulcers, digestion related issues and various other diseases. *Amalaki* has antioxidant, immunomodulatory, antipyretic, analgesic, cytoprotective, antitussive and gastroprotective actions [4]. The phytochemical constituents such as gallic acid and ellagic acid are high in Amalaki along with Vitamin C and are known for their biological properties mentioned above. Similarly other herbal ingredients of *Khajurprash* such as *Draksha (Vitis vinifera), Shatavari (Asparagus racemosus), Ashvagandha (Withania somnifera),* etc., have proven beneficial effects in rejuvenation of energy, immunomodulatory and antioxidant activities. Over last 2 – 3 decades substantiation of claims attributed to the 'traditional preparations' utilizing the scientific advancement of *in vitro*, *ex vivo*, *in vivo* etc. test systems, has gained importance and has become the essentiality for public acceptance [5]. In the current study *Khajurprash* (D-KP) developed by Dabur India Ltd., was evaluated for its bioactivities using a battery of *in vitro* and *ex vivo* systems.

Materials and Methods

Reagents and culture media

Antibiotic solution, RPMI-1640, H_2O_2 (Hi-Media), 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 i.e. Khajurprash was obtained from M/S Dabur India Ltd., Ghaziabad, UP., India. Composition of *Dabur Khajurprash* (D-KP) is given in Table – 1.

S. No.	Name of the Ingredient	Quantity (g/100g)
1	Kharjura (*Phoenix dactylifera*) extract	52.000
2	Kharjura (*Phoenix dactylifera*) pulp	26.000
3	Amalaki (*Emblica officinalis*)	27.000
4	Draksha(*Vitis vinifera*)	7.000
5	Tila tail (*Sesamum indicum*)	0.657
6	Pippali (*Piper longum*)	0.630
7	Sukshmaila (*Elettaria cardamomum*)	0.460
8	Vamsa (*Bambusa bambos*)	0.350

9	Vidari (*Pueraria tuberosa*)	0.324
10	Varahi (*Dioscoria bulbifera*), Satavari (*Asparagus racemosus*), Ashvagandha (*Withania somnifera*)*	0.648
11	Bala (*Sida cordifolia*)	0.205
12	Tvak (*Cinnamomum zeylanicum*)	0.195
13	Lavanga (*Syzygium aromaticum*)	0.122
14	Bilva (*Aegle marmelos*), Agnimantha (*Clerodendrum phlomidis*), Shyonaka (*Oroxylum indicum*), Patala (*Stereospermum suaveolens*), Gambhari (*Gmelina arborea*), Shalaparni (*Desmodium gangeticum*), Prishniparni (*Uraria picta*), Brihati (*Solanum indicum*), Kantkari (*Solanum surattense*), Gokshura (*Tribulus terrestris*), Mudgaparni (*Phaseolus trilobus*), Mashaparni (*Teramnus labialis*), Karkatshringi (*Pistacia integerrima*), Tamalaki (*Phyllanthus niruri*), Draksha (*Vitis vinifera*), Pushkara (*Inula racemosa*), Haritaki (*Terminalia chebula*), Guduchi (*Tinospora cordifolia*), Karchura (*Curcuma zedoaria*), Musta (*Cyperus rotundus*), Punarnava (*Boerhavia diffusa*), Utpala (*Nymphaea stellata*), Vasa (*Adhatoda vasica*), Yashti (*Glycyrrhiza glabra*), Kakanasika (*Martynia annua*)**	2.700
15	Abhrak Bhasma	0.100
16	Akarkarabha (*Anacyclus pyrethrum*)	0.070
17	Tvakpatra(Cinnamomum tamala), Nagkesara(Mesua ferrea)***	0.130
18	Jivanti (*Leptadenia reticulata*)	0.011
19	Sucralose	0.013
20	Sodium Benzoate	0.2

Table 1: True List of Composition Active Ingredients in - D-KP.

*Each ingredient - 0.216 g.; ** Each ingredient - 0.108 g.; *** Each ingredient - 0.065g.

Animal and 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 were obtained for euthanization and collection of spleen to assess the *in vitro* TNF-α secretion and NK cell activity. Spleen was aseptically excised and processed for single cell suspension as mentioned by Yathapu., *et al.* [6]. 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.

Antioxidant activity

The antioxidant activity of *Khajurprash* (D-KP) was evaluated using HepG2 (human Hepatocarcinoma) cell line, obtained from National Centre for Cell Science (NCCS), Pune, India. D-KP dissolved in DMEM containing 2% inactivated FBS was used for cytotoxicity evaluation. Trypsinized HepG2 cells were adjusted to 1,00,000 cells/mL using MEM media with 10% FBS (inactivated) and antibiotic solution (Penicillin-100 IU/mL, Streptomycin-100 µg/mL and Amphotericin B-5 µg/mL). The cytotoxicity of D-KP was determined (1000 – 7.8 µg/mL) as per the standard protocol of MTT assay. Then, the non-cytotoxic concentrations of D-KP i.e. 500 µg/mL and 250 µg/mL were used for antioxidant activity evaluation through GSH assay. HepG2 cells were exposed to H_2O_2 (20 µg/mL), the exogenous free radical generating agent; then Quercetin (250 µg/mL) was used as reference standard and D-KP (500 µg/mL and 250 µg/mL) as test substance. After 24h incubation, cells were washed and subjected for lysis through freeze – thawing process, and clear cell lysate was used for antioxidant activity (ability of GSH level modulation) evaluation. The GSH levels present in test system were quantified by DNB (5,5-Dithiobis (2-nitrobenzoic acid)), which form the yellow product TNB (5′-thio -2-nitrobenzoic acid) being read at 412 nm.

TNF-α secretion by splenocytes

Immunomodulatory potency of D-KP was evaluated using *ex vivo* cultured splenocyte TNF-α secretion. Single cell suspension of Splenocytes were prepared as mentioned above and used for cytotoxicity assessment of D-KP (1 µg/mL – 500 µg/mL) following standard protocol of MTT assay. The TNF-α secretion by splenocytes in presence of D-KP was evaluated, with reference to LPS (1 µg/mL – 25 µg/mL) as positive control in 24 well culture plate. After 24h incubation, the supernatants were collected for TNF-α quantification using the 'Mouse TNF-α ELISA test kit (R&D Systems)', following the manufacturer's instructions.

Natural Killer cell activity

Splenocytes comprised of B cells, T cells, Natural killer cells (NK cells) and macrophages. Hence, immunostimulatory ability of D-KP was assessed considering the splenocytes as effector cells and YAC–1 (murine lymphoma) cells as target cells, Concanavalin A (Con. A), the mitogen, was used as positive control. The NK cells (splenocytes) treated with either D-KP, or Con A and untreated controls were incubated at 37oC for 48 h. Meanwhile, YAC-1 cells were stained with fluorescent compound i.e. Carboxyfluorescein diacetate succinimidyl ester (CFSE 25 µM per 1.5x106 cells/mL by incubating at 37°C for 1 h) and unbound fluorescein was washed with serum free medium. The CFSE stained YAC-1 cells were co-incubated with NK cells at a density of 10000 cells/well. After 24 h of co-incubation at 37°C and 5% CO_2, NK cell mediated YAC-1 cell lysis/killing was determined by removing the supernatant and addition of 100 µL of 1% Triton X-100 in 0.5 M borate buffer (pH 9.0)/well. Fluorescence was measured at excitation wavelength of 485/20 nm and emission wavelength of 528/20 nm.

RAW 264.7 cell Phagocytosis assay

The RAW 264.7 (Macrophage cell line) cells cultured in DMEM were plated at a density of 50,000 cells/well in 96-well plates and incubated for 24 h at 37°C, 5% CO_2. Cells were treated with either D-KP or Con. A, and untreated cells as control were incubated for another 24h. The phagocytotic ability of treated and untreated RAW 264.7 cells were measured by engulfment of prelabelled zymosan particles using CytoSelect™ 96 well Phagocytosis Assay kit (Zymosan, Colorimetric Format) as per manufacturer's instructions (Cell Biolabs Inc., USA).

Results

Antioxidant activity

The information of cytotoxic effect of D-KP against HepG2 is given in Figure 1. More than 75% of HepG2 cell viability was noted, even at as high as 1000 µg/mL of D-KP. The data suggests that CTC_{50} of D-KP is higher than 1000 µg/mL for HepG2 cells (Figure 1). Antioxidant activity of D-KP was carried out with non-cytotoxic concentrations i.e. 250 µg/mL and 500 µg/mL, keeping in view of more than 85% of cells are viable at these concentrations.

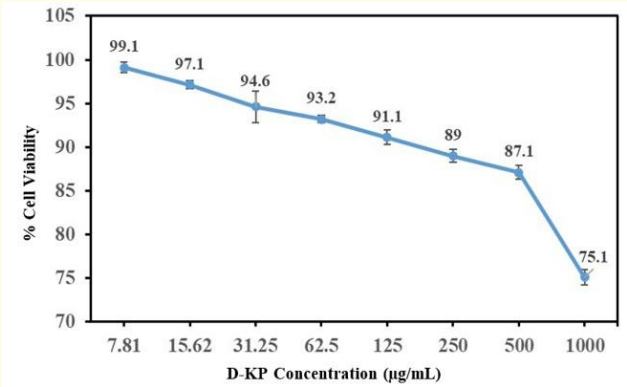

Figure 1: Effect of *Dabur Khajurprash* on HepG2 Cell Viability.

The HepG2 cells were cultured in MEM containing antibiotic solution and 10% FBS and exposed to 7.8 to 1000 µg/mL of D-KP followed by incubation at 37°C and CO_2 5% , in humidified atmosphere/24 h. The standard protocol of MTT assay was followed to determine cell viability.

Antioxidant potential (assessed by GSH level modulation) of D-KP in HepG2 cells treated with exogenous H2O2 is depicted in Figure 2. A significant ($p < 0.05$) decrease in GSH levels were noted among the H_2O_2 treated HepG2cells in comparison to control. Whereas the HepG2 cells treated with either Quercetin (standard reference) or D-KP have shown improved GSH levels (Figure 2). Quercetin has significantly ($p < 0.05$) increased the GSH levels in comparison to controls, H_2O_2 alone and D-KP at a concentration of 250 µg/mL. Similarly, the 500 µg/mL of D-KP have shown significant ($p < 0.05$) increase in GSH levels among the HepG2 cells (control), exposed to H_2O_2 alone and D-KP (250 µg/mL) (Figure 2).

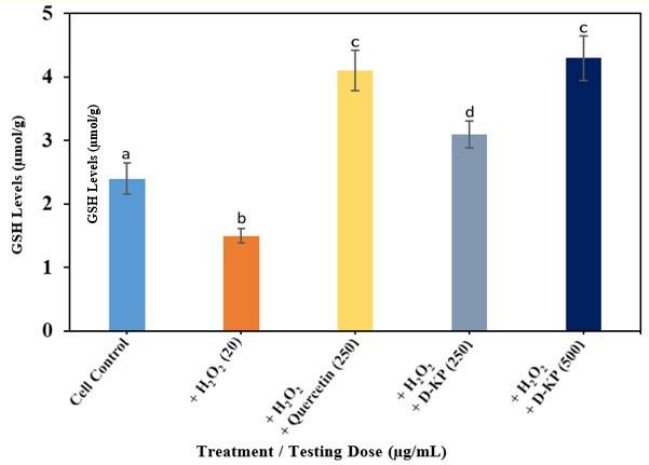

Figure 2: Antioxidant activity (GSH levels) of *Dabur Khajurprash.*

The HepG2 cells were exposed to exogenous H_2O_2 followed by addition of either 250 and 500 µg/mL of D-KP or Quercetin, as reference molecule. After48 h of incubation (37°C and 5% CO_2), cell lysates were prepared and GSH levels were measured. Bars represent mean ± SD of GSH levels. Different superscripts are significantly different at $p < 0.05$.

TNF-α secretion by splenocytes

The *ex vivo* cultured splenocytes upon incubation with D-KP at a concentration range of 1 µg/mL to 500 µg/mL have shown proliferation instead of cytotoxicity (Figure 3). A steady increase in cell proliferation was noted from as low as 10 µg/mL to 500 µg/mL. The TNF-α levels of splenocytes treated with LPS (positive control) and D-KP were given in Figure 4. A significant ($p < 0.05$) increase in TNF-α levels was noted in splenocytes exposed to as low as 1.0 µg/mL LPS. Further higher concentrations of LPS (10.0 and 25.0 µg/mL) have induced the TNF-α secretion (Figure 4). Similarly, the splenocytes incubated with D-KP at the concentrations of 1.0 and 10.0 µg/mL have minimal TNF-α secretion. On the other hand, 100.0 µg/mL D-KP have shown significant ($p < 0.05$) elevated TNF-α levels in comparison with control (Figure 4). Whereas further higher concentrations, i.e. 250 and 500 µg/mL of D-KP resulted in comparatively decreased levels of TNF-α, but still significantly ($p < 0.05$) higher than untreated control. The overall fold increase of TNF-α levels of splenocytes treated either with LPS or D-KP are given in Table – 2. LPS exposure has shown 'dose-dependent increase of TNF-α levels' from 76.3 to 92.5 folds, whereas increasing dose of D-KP has umbrella shaped response for TNF-α secretion (Table 2 and Figure 4).

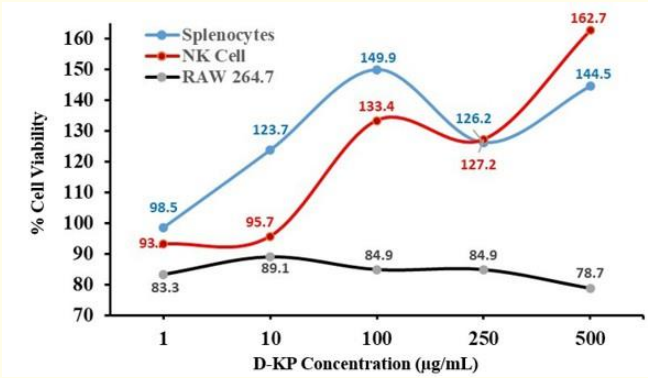

Figure 3: Effect of *Dabur Khajurprash* on Splenocytes, NK cells and RAW 264.7 cell Viability.

The splenocytes and NK cells were cultured in RPMI – 1640 medium, whereas DMEM was used for RAW 264.7 cells. The cell viability/proliferation against the D-KP (1 – 500 µg/mL) was determined by standard protocol of MTT assay.

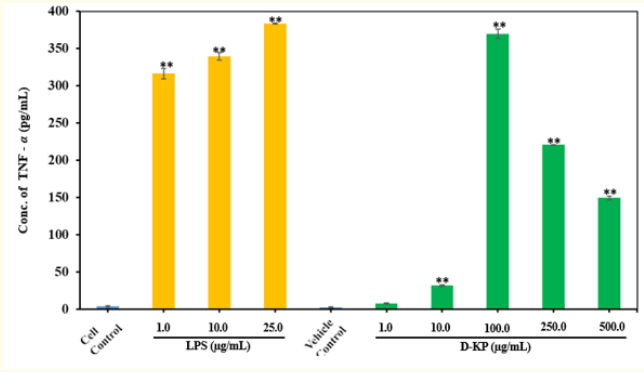

Figure 4: Immunomodulatory Activity (TNF – α Secretion).

The splenocytes were exposed to either D-KP (1 – 500 µg/mL) or LPS for a period of 24h. Then the supernatants were collected and quantified the TNF-α levels followed by ELISA kit method. Bars represent TNF-α levels (pg/mL).

S. No.	Test Substance and Concentration	Fold Increase of TNF-α over Control
1	LPS - 1.0 µg/mL	76.3
2	LPS - 10.0 µg/mL	81.8
3	LPS - 10.0 µg/mL	92.5
4	D-KP - 1.0 µg/mL	2.6
5	D-KP - 10.0 µg/mL	10.3
6	D-KP - 100.0 µg/mL	119.4
7	D-KP - 250.0 µg/mL	71.5
8	D-KP - 500.0 µg/mL	48.3

Table 2: TNF-α Secretion by Splenocytes.

LPS: Lipopolysaccharide (Positive control); D-KP: *Dabur Khajurprash*.

Natural Killer cell activity

The NK cell (splenocytes) viability in presence of D-KP is depicted in Figure 3. No cytotoxicity was noticed even at higher concentration of 500 µg/mL of D-KP. Instead decrease in cell viability, NK cell proliferation was observed as dose-response of D-KP from 10 to 500 µg/mL (Figure 3). YAC-1 cells lysis/killing ability of NK Cell upon co-incubation in presence of Con A and D-KP is given in Figure 5. Untreated control NK cells have shown ~26% YAC-1 cell lysis, whereas both mitogen (Con A) and D-KP have increased 1.6 to 2.8-fold and ~ 1.6-fold, respectively. A dose dependent increase of YAC-1 cells lysis by NK Cells was observed in presence of Con A, whereas moderate range of cell lysis was noted by NK cells treated with various doses of D-KP (Figure 5).

RAW 264.7 cell phagocytosis assay

The RAW 264.7 macrophage cell viability upon treatment with various doses of D-KP are given in Figure 3. More than 78% of cells are viable even at highest concentration i.e. 500 µg/mL of D-KP tested, suggesting the CTC_{50} was higher than 500 µg/mL. The phagocytotic ability of RAW 264.7 cells treated with Con-A has increased significantly ($p < 0.05$) in comparison to control (Figure 6). Ten µg/mL of Con-A has triggered 80% phagocytosis by RAW 264.7 cells, whereas dose-dependent response was not observed with further higher concentration (25 µg/mL) of Con-A. Similarly, the RAW 264.7 cells treated with various doses of D-KP have shown 33 – 62% higher phagocytosis with reference to control (Figure 6).

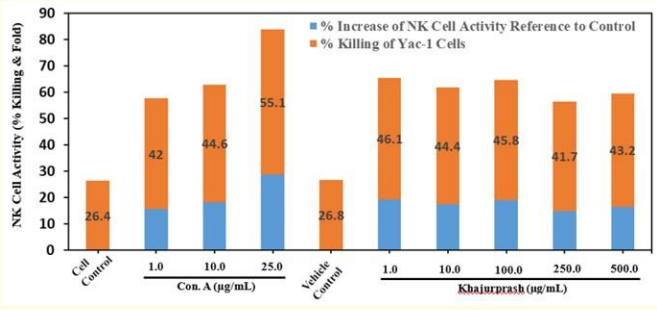

Figure 5: Natural Killer Cell Activity.

The NK cells (splenocytes) were exposed to either D-KP (1 – 500 µg/mL) or Con-A for a period of 48h. Then, co-incubated with CFSE stained YAC-1 cells/24 h. The supernatants were collected and quantified the TNF-α levels followed by ELISA kit method. Bars represent TNF-α levels (pg/mL). NK cell mediated YAC-1 cell lysis/killing was determined by addition of Triton X-100 in 0.5 M borate buffer (pH 9.0)/well to supernatant remove cells. Fluorescence was measured at excitation wavelength of 485/20 nm and emission wavelength of 528/20 nm. The bars represent % increase of NK cell activity with reference to controls and % killing of YAC – 1 cells.

Interestingly, RAW 264.7 cells incubated with 10.0 µg/mL of D-KP has shown significantly higher percentage of phagocytosis than the other concentrations of D-KP.

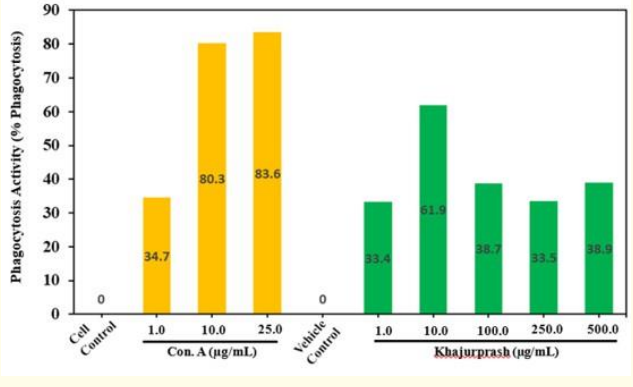

Figure 6: Phagocytosis Activity of RAW 264.7 Cells.

The RAW 264,7 cells were treated with either D-KP or Con-A for 24h. Then phagocytotic activity of RAW 264,7 cells were assessed by engulfment of prelabelled zymosan particles using CytoSelectTM 96 well Phagocytosis Assay kit (Zymosan, Colorimetric Format; Cell Biolabs Inc., USA). Bars represent % phagocytotic activity of RAW 264,7 cells.

Discussion

Use of medicinal plants, herbs and spices for prevention and healing of diseases/infections have been brought by human civilization through ancient period. Single ingredient and/or combination of more than one botanical, rich in natural minerals and vitamins preparations have proven for their efficacy and is gaining the momentum over the few decades [7]. The Indian Ayurvedic text has well documented the 'method of preparation of natural ingredient-based therapeutics, health and food supplements', along with their mode of action. *Khajurprash*, an extension of Chyawanprash, possess the Date palm fruit (*Phoenix dactylifera L*) and Amalaki as key ingredients. Immunomodulatory activity of *Khajurprash* has attributed for their phytoconstituents. Ability of *Dabur Khajurprash* (D-KP) to induce TNF-α secretion by *ex vivo* splenocytes suggests the pro-inflammatory activity up to certain dose range (100 µg/mL) and reduction of TNF-α secretion at further higher concentrations (≥250 µg/mL) suggests it's immunomodulatory activity (Figure 4). This observation corroborated with Ghonime et al., [8] findings of significant proliferation of splenocytes (BLAB/c mice and C57/BL6) cultivated in presence of aqueous extract of black cumin, along with anti-inflammatory and immunomodulatory effects in a concentration-dependent manner. In the current study also, splenocyte (C57/BL6) proliferation was noted upon incubation with D-KP in dose dependent manner (Figure 3), instead of cytotoxicity. In contrary, the dendritic cells (DC) treated with Chyawanprash has shown the 'immunostimulatory potential' by secreting the TNF-α in response to higher doses [9].

The D-KP comprised of ~27% of Amalaki, rich source of Vitamin C, could be a reason for suppression of TNF-α at a higher dose of D-KP. Similar findings of inhibition of ROS, DNA damage, TNF-α, IL-6, and p38 expression was noted in LPS induced cultured macrophages in presence of Vitamin C [10]. The anti-inflammatory activity of D-KP was attributed to the vast phytoconstituents such as anthocyanins, phenolics, sterols, carotenoids, flavonoids, gallic acid and ellagic acid, Vitamin C etc. present in the key ingredients [3,4]. These phytoconstituents have free radical scavenging activity, hence H_2O_2 induced HepG2 cells upon treatment with D-KP have shown significant elevated levels of reduced Glutathione, considered as an essential thiol-based antioxidant molecule (Figure 2). Antioxidant potential of D-KP (500 µg/mL) is on par with pure form of Quercetin (250 µg/mL), hence it's regular consumption may provide the intended health benefits. A study carried out among middle aged women consuming Date fruit have reduced expression of IL-1, TGF-, COX-1 and COX-2 expression [11].

Another part of innate immune response is elimination of pathogen/foreign agent by lysis/killing by creating pores in pathogen cells or engulfment of entire pathogen, and processing for antigen presentation to mount the T cell and B cell mediate immune response. In the current study, D-KP has shown significant increase in proliferation of NK cells and induced the NK cell mediated lysis of YAC-1 cells. Such NK cell mediated lysis activity induction of D-KP was on par with Con-A, the mitogen (Figure 5). These findings were substantiated by Chyawanprash treated NK cells which also exerted similar lysis activity against the YAC-1 cells [9]. The *in vitro* phagocytotic activity evaluated by Zymosan particle engulfment by RAW 264.7 cells treated with 100 µg/mL of D-KP have exerted maximum activity (Figure 6). This could be due to the cell viability of RAW 264.7 cells was higher at 100 µg/mL of D-KP and cytotoxicity was noted at higher concentrations of D-KP (Figure 3). A study with Chyawanprash effect on RAW 264.7 cell viability and it's phagocytotic activity has shown linear increase with dose increment [9].

The cell viability/cytotoxicity and proliferation data of current study could draw an interesting finding, when HepG2 cells, RAW 264.7 cells and primary cell suspension i.e. splenocytes and NK cells were exposed to different doses of D-KP have responded uniquely (Figure 1 and Figure 3). The HepG2 and RAW 264.7 cell lines are the carcinoma originated cells, whereas splenocytes and NK cells are derived from spleen of C57/BL6. The D-KP has exerted cytotoxicity against the HepG2 and RAW 264.7 cells in a dose response manner, surprisingly both Splenocytes and NK cells were proliferated in presence of D-KP even at higher concentrations also (Figure 1 and Figure 3). These findings substantiate the anti-tumour property of natural ingredients with their plethora of phytoconstituents [3,4,11,12].

Conclusion

The result of present study concludes that *Dabur Khajurprash* has antioxidant activity, immunomodulatory potential in terms of pro-inflammatory cytokine secretion and anti-inflammatory property as dose-dependent response. In addition, the D-KP could induce the innate immune response through activation of NK cell mediated lysis and Phagocytosis processes.

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