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Modulatory Effect of a Unani Formulation *(Jawarish amla sada)* on Cyclophosphamide-induced Toxicity in Tumour Bearing Mice

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Authors' contributions

FA wrote the protocol, performed the statistical analysis, and wrote the first draft of the manuscript. HR, MK and SA managed the literature searches. KB, HR and RAA managed the analyses of the study. SR designed the study, wrote the protocol and edited the first and final drafts of the manuscript. All authors read and approved the final manuscript.

Research Article

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ABSTRACT

Aims: Our aim was to study the modulatory effect of a Unani herbal formulation *Jawarish amla sada* against cyclophosphamide-induced toxicity in tumour bearing mice.
Study Design: Non randomized control study.
Place and Duration of Study: The study was conducted at the Department of Medical Elementology and Toxicology, Jamia Hamdard, New Delhi during 2008-10.
Methodology: Study was conducted in Swiss albino mice divided in five groups (*n*=6). Animals were challenged with Ehrlich's ascites tumour cells ($1x10^6$ cells). Cyclophosphamide (50 mg/kg body weight), an alkylating anticancer drug that especially affects humoral immune functions, was injected intraperitoneally in a single dose. The protective effect of Unani drug *Jawarish amla sada* (250 mg/kg body weight) was studied in tumour bearing animals treated with cyclophosphamide. Immune function assessment

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test such as plaque forming cell assay (PFC) and biochemical parameters such as activities of antioxidant enzymes and reduced glutathione were measured in mice.
Results: *Jawarish amla sada* significantly modulated the immunosuppressive effect of cyclophosphamide as compared to the group treated with cyclophosphamide. *Jawarish amla sada* also protected activities of antioxidant enzymes such as catalase, glutathione peroxidase, glutathione reductase and glutathione *S*-transferase and significantly restored level of reduced glutathione in liver and kidney of tumour bearing mice exposed to cyclophosphamide. Similar protective effect of *Jawarish amla sada* was observed against elevated lipid peroxidation in these tissues.
Conclusion: *Jawarish amla sada* showed potential to provide protection against toxic effects of cyclophosphamide in tumour bearing mice. The mechanism of action of the drug may be attributed to various antioxidants fortified in this herbal Unani formulation, which is used in the traditional system of medicine in Indian subcontinent against several liver ailments.

Keywords: Cyclophosphamide; Ehrlich's ascites tumour; immunosuppression; Unani herbal formulation; protection; antioxidants.

1. INTRODUCTION

Cyclophosphamide (CP) is an oxazaphospharine class of alkylating agent widely used in cancer treatment (Schmidt and Koelbl, 2012). All major anticancer drugs such as CP, doxorubicin, cisplatin, bleomycin etc. are associated with toxic side effects of varying degree affecting various vital systems (Hassan et al., 2011; Christie et al., 2012; Pabla and Dong, 2012). CP frequently causes hematopoetic depression, nausea, vomiting, alopecia, hemorrhagic cystitis, water retention, cardiac damage, gonadotrophy and carcinogenicity (Bhatia et al., 2008; Panahi et al., 2012). CP also induces mutagenicity in mice (Sharma et al., 2001). The suppressive effects of CP on lymphoid organs, WBC counts and other immune functions are well documented (Zaidi et al., 1990; Bin-Hafeez et al., 2001; Jena et al., 2003; Ramadan et al., 2011; Shreder et al., 2012).

The immunosuppressive effect of CP on humoral immunity is of major concern in the cancer patients (Sazliyana et al., 2011). CP also induces oxidative stress in various tissues resulting from the production of reactive oxygen species (ROS) (Sulkowska et al., 1998; Il'yasova et al., 2011). Production of ROS leads to decrease in the antioxidant enzyme level in the cells (Sun, 1990; Abraham et al., 2011). Moreover, decrease of the tissue and serum levels of reduced glutathione (GSH) and activities of glutathione reductase (GR), superoxide dismutase (SOD) and catalase (CAT) of rats and mice has also been reported in case of CP treatment (Kaya et al., 1999; Rekha et al., 2001).

In a tumour bearing mice excessive production of free radicals results in oxidative stress, which leads to damage of macromolecules such as lipids leading to lipid peroxidation (LPO) *in vivo* (Yagi, 1991; Bhattacharya et al., 2011). Increased LPO would cause degeneration of tissues. Lipid peroxides formed in the primary site would be transferred through the circulation and provoke damage by propagating the process of LPO (Sinclair et al., 1990). LPO was reported to be higher in carcinomatous tissue than in non-diseased organs (Yagi, 1991; Chaiswing et al., 2011). A large number of single natural products and herbal extracts

containing these products have shown protective effects against CP-induced toxicity (Kaneko et al., 1999; Sharma et al., 2001; Haque et al., 2003; Bhatia et al., 2006, 2008). However, there is limited knowledge about the efficacy of such natural products and extracts in the animals exposed to CP and also concurrently challenged with tumour cells. Such effects of protection therapy may be desirable as it will not only enhance the therapeutic index but also be a rational approach to minimize the toxic manifestations of anticancer drugs in already immunosuppressed/immunocompromised host. Earlier, it has been shown that aqueous gooseberry (*Emblica officinalis*) extract protects mice from CP toxicity (Sharma et al., 2000). *E. officinalis* is widely used as an antioxidant constituent of a large number of traditional and folklore herbal formulations (Chopra et al., 1956). One such herbal formulation, *Jawarish amla sada* has *E. officinalis* as major constituent (~ 7- 8%) (Hamdard Pharmacopeia of Eastern Medicine, 1969). We wanted to study whether *Jawarish amla sada* which is widely prescribed in Unani system of medicine against several liver ailments and has *E. officinalis* as its major constituent has ability to modulate the toxic effects of CP in tumour bearing animals. Therefore, it would be interesting to observe protective effects of *Jawarish amla sada* under these conditions.

2. MATERIALS AND METHODS

2.1 Chemicals

Bovine serum albumin (BSA), butylated hydroxy toluene (BHT), ethylenediamine tetra acetic acid (EDTA) disodium salt, Folin's reagent, nitroblue tetrazolium (NBT) and sulfosalicylic acid were procured from SRL (Mumbai, India). CP, dithiobis-2-nitrobenzoic acid (DTNB), 1-chloro-2,4-dinitrobenzene (CDNB), GSH and nicotinamide adenine dinucleotide phosphate reduced (NADPH) were purchased from Sigma–Aldrich Co. (St. Louis, MO, USA). Ortho phosphoric acid (OPA) was procured from CDH Chemicals (Mumbai, India). RPMI-1640 medium, dimethylsulfoxide (DMSO), phosphate buffer saline (PBS) and 2-thiobarbituric acid (TBA) were procured from Hi-Media Labs (Mumbai, India).

2.2 *Jawarish amla sada* (JAS)

Jawarish amla sada was procured in ready to use form from the market. In the traditional system of medicine it used as a stomachic and liver tonic (Hamdard Pharmacopeia of Eastern Medicine, 1969). Its chief ingredients are so incorporated as to be neither too coarse nor large or small, they are all ground to medium sized in the *sufuf* (powder) so that the action of Jawarish is prolonged. The composition of *Jawarish amla sada* is as follows (Source: Hamdard Pharmacopeia of Eastern Medicine, 1969):

Table 1. Composition of *Jawarish amla sada*

Ingredients	Percentage composition
Dried *Emblica officinalis* (*amla khusk*)	7.94%
Valeriana officinalis (*balchar*)	0.70%
Sawdust of *Santalum album* (*burada sandal safaid*)	1.58%
Rosa damscena (*gul-i-surkh*)	0.70%
White sugar syrup (*qiwam shaker safaid*)	88.26%
Sodium benzoate (*nitrum-i-bunjavi*) as preservative	0.08%

2.3 Animals and Ehrlich's Ascites Tumour (EAT)

The study was conducted in Swiss albino male mice (body wt. 25±2 g) provided by the Central Animal House Facility of Jamia Hamdard. The study protocol was approved by the Institutional Animal Ethics Committee (IAEC approval Project No: 318). Animals were bred and maintained under the standard conditions (temperature 25± 2ºC; photoperiod of 12 h). Commercial pellet diet and water were given *ad libitum*. The EAT cells were obtained from the Institute of Nuclear Medicine and Allied Sciences (INMAS), Delhi, India. The ascitic fluid was removed by opening the belly and carefully collecting all the fluid with the help of a sterile 5 ml syringe. EAT cell counts were done in a Neubauer hemocytometer. The cells were found to be more than 99% viable by the Trypan blue dye exclusion method. The EAT cells were maintained by weekly *intraperitoneal (i.p.)* inoculation of $1x10^6$ cells/mouse for further use in outbred mice. A batch of 24 animals was inoculated with $1x10^6$ EAT cells *(i.p.)* three days (day -3 of experimentation) before the commencement of the JAS dosing as shown in Table 2. Treatment of JAS was commenced after three days of tumour inoculation so that EAT tumours reach exponential growth phase as described by Luksiene et al. (2003) and also to bring the animals in the state where a partially grown tumour is treated with the drug. Animals were randomized and divided into four groups (*n*=6).

2.4 Doses and Treatment Schedule

CP and JAS were suspended in normal saline. The control received 1 ml of normal saline orally each day. The same volume was administered to JAS treated animals also. The dose volume administered *i.p.* for each treatment was 0.2 ml in normal saline. Dosing was done in such a manner that all the animals could be sacrificed on the same day. The doses were administered as indicated in Table 2.

Table 2. Description of treatment groups

Group	Treatment	Route of dosing	Dose schedule
I	Normal control*	Oral	Normal saline for 14 days (day 1 to 14).
II	EAT cell challenge control*	*i.p.*	Single inoculums of tumour cells on (–) 3 day.
III	EAT cell challenge + CP (50 mg/kg body wt)	*i.p.*	Single inoculums of tumour cells on (–) 3 day and single dose of CP on day 12.
IV	EAT cell challenge + JAS (250 mg/kg body wt) + CP (50 mg/kg body wt)	*i.p.* (CP)+oral (JAS)	Single inoculums of tumour cells on (–) 3 day, JAS treatment for 14 days from day 1 to 14 and single dose of CP on day 12.
V	EAT cell challenge + JAS (250 mg/kg body wt)	Oral (JAS)	Single inoculums of tumour cells on (–) 3 day and JAS treatment for 14 days from day 1 to 14.

CP: cyclophosphamide; JAS: Jawarish amla sada, EAT: Ehrlich's ascites tumour

The dose of CP was taken based on previously published report (Haque et al., 2003) and dose of JAS was taken on the basis of dose deciding study undertaken separately (data not shown). For the study of immunological parameters all the animals were injected with sheep red blood corpuscles five day before the sacrifice.

2.5 Preparation of Tissue Homogenate and Post-mitochondrial Supernatant (PMS)

The liver and kidney were weighed and tissue was homogenized in chilled phosphate buffer (0.1 M, pH 7.4), using a Potter Elvehjem homogenizer to prepare a 10% homogenate (w/v). The supernatant was centrifuged at 10,500 *g* for 30 min at 4°C to obtain the PMS.

2.6 Lipid Peroxidation (LPO)

LPO was measured as the major indicator of oxidative stress using the procedure of Uchiyama and Mihara (1978). The reaction mixture consisted of 10 mM BHT, 0.67% TBA, 1% chilled OPA and tissue homogenate (10% w/v). The mixture was incubated at 90 °C for 45 min. The absorbance of supernatant was measured at 535 nm. The rate of LPO was determined as nmol of TBA reactive substances (TBARS) formed/h/g of tissue using a molar extinction coefficient of 1.56×10^5 $M^{-1}cm^{-1}$.

2.7 Measurement of Antioxidants

2.7.1 Glutathione reduced (GSH)

GSH content was measured in the PMS by the method of Haque et al. (2003). PMS (1 ml) was precipitated with 1 ml of 4% sulfosalicylic acid (Hi-Media Labs, Mumbai, India). The samples were incubated at 4ºC for 1 h and then centrifuged at 1,200 *g* for 15 min at 4ºC. The assay mixture consisted of 0.2 ml of filtered aliquot, 2.6 ml of sodium phosphate buffer (0.1 M, pH 7.4) and 0.2 ml of 100 mM DTNB in a total volume of 3 ml. The absorbance of reaction product was measured at 412 nm and results expressed as nmol GSH/gm tissue.

2.7.2 Glutathione *S*-transferase (GST)

GST activity was measured by the method of Haque et al. (2003). The reaction mixture consisted of 1.675 ml phosphate buffer, 0.2 ml of 1 mM GSH, 0.025 ml of 1 mM CDNB and 0.1 ml of PMS (10%) in a total volume of 2 ml. The change in absorbance was recorded at 340 nm and the enzyme activity calculated as nmol CDNB conjugates formed/min/mg protein using a molar extinction coefficient of 9.6×10^3 $M^{-1}cm^{-1}$.

2.7.3 Glutathione peroxidase (GPx)

Method described by Haque et al. (2003) was followed for estimating the activity of GPx in the samples. The assay mixture consisted of 1.44 ml sodium phosphate buffer, 0.1 ml of 1 mM EDTA, 0.1 ml of 1 mM sodium azide, 0.05 ml of 1 IU/ml GR, 0.1 ml of 1 mM GSH, 0.1 ml of 0.02 mM NADPH, 0.01 ml of 0.25 mM H_2O_2 (0.25 mM, Sigma), and 0.1 ml PMS (10%) in a total volume of 2 ml. Oxidation of NADPH was recorded spectrophotometrically at 340 nm. The enzyme activity was calculated as nmoles of NADPH oxidized/min/mg of protein using molar extinction coefficient of 6.22×10^3 $M^{-1}cm^{-1}$.

2.7.4 Glutathione reductase (GR)

The GR activity was estimated by the method described by Mohandas et al. (1984). The assay mixture consisted of 1.65 ml phosphate buffer, 0.1 ml of 0.5 mM EDTA, 0.05 ml of 1mM GSSG, 0.1 ml of 0.1 mM NADPH and 0.1 ml of PMS (10%) in a total volume of 2.0 ml. The enzyme activity was measured as disappearance of NADPH at 340 nm and was calculated as nmoles of NADPH oxidized/min/mg protein using a molar extinction coefficient of 6.22×10^3 $M^{-1}cm^{-1}$.

2.7.5 Catalase (CAT)

Activity of CAT was assayed by the method described by Claiborne (1985). The assay mixture consisted of 1.95 ml phosphate buffer, 1 ml of 0.9 M H_2O_2 and 0.05 ml of 10% PMS in final volume of 3 ml. Change in absorbance was recorded kinetically at 240 nm. CAT activity was calculated as nmoles H_2O_2 consumed/min/mg protein.

2.8 Protein Content Measurement

Protein contents in various samples were estimated by the method of Lowry et al. (1951) using Folin's reagent and BSA as standard. The assay mixture consists of 20 µl of PMS, diluted to 1 ml with distill water followed by addition of alkaline copper reagent (consisting of NaOH, Na_2CO_3, $CuSO_4$, Na-K-tartarate) followed by *Folin Ciocalteu* reagent after 15 minutes incubation which was followed by 30 min incubation time. The OD of resultant product solution was measured at 660 nm on spectrophotometer (Shimadzu, UV-1201).

2.9 Immunological Studies

2.9.1 Relative weights of lymphoid organs

The relative weight of lymphoid organs (spleen and thymus) was calculated as gm/100 gm body weight.

2.9.2 Plaque forming cell assay (PFC)

PFC assay was performed by the method of Raisuddin et al. (1991). Animals were immunized with 0.2 ml of sheep red blood corpuscles (SRBC) on fifth day before sacrifice of animals. On fifth day of the immunization with SRBC the mice were killed by cervical dislocation, the spleen were removed, cleaned free of extraneous tissues and a single cell suspension of 10^6 cells/ml was prepared in RPMI-1640 medium. For PFC assay the SRBC were prepared at a cell density of $5x10^8$ cells/ml in PBS. One ml of SRBC in medium along with 0.5 ml of diluted guinea pig complement (1:10 diluted with normal saline) was added to 1 ml of spleen cell suspension. Cunningham chambers were prepared using glass slides, cover slips and double-sided tape (Scotch Brand, St. Paul MN, USA). The chambers were loaded with a known volume of assay mixture, sealed with petroleum jelly and incubated at 37ºC for 1 h. The plaques were counted under a light microscope and expressed as PFC per 10^6 spleen cells.

2.10 Data Presentation and Statistical Analysis

The values are expressed as means ± S.E. One-way analysis of variance (ANOVA) was applied to determine significant differences in results of various groups over respective controls. *P*-values < 0.05 were considered significant. The statistical analysis of data was performed using Graph Prism Software.

3. RESULTS

3.1 Effect of *Jawarish amla sada* on LPO

Table 3 shows LPO data of liver and kidney of mice from different treatment groups. It was observed that tumour challenge in animals caused enhancement of LPO in liver as well as in kidney. However, the effect was significant (*P*<0.01) only in kidney. CP treatment in tumour bearing animals (group III) further increased level of LPO both in liver and kidney, but the effect was not significant. JAS treatment in tumour bearing animals with treatment of CP caused reduction in LPO in a significant manner in both the organs. However, JAS treatment in tumour bearing animals without CP treatment reduced the LPO values significantly only in kidney.

Table 3. LPO in liver and kidney of mice

Parameter	Group I	Group II	Group III	Group IV	Group V
Liver	174.9±5.4	190.0±4.6	212.3±6.1	176.8±7.1§§	172.2±3.1
Kidney	155.5±4.1	183.8±6.0**	196.9±5.8	157.2±3.9§§§	144.3±6.1^{c}

*A detailed description of groups is given in Table 2. LPO: Lipid peroxidation (nmol of TBARS formed/h/g of tissue) ** P<0.01 compared with group I , §§ P<0.01, §§§ P<0.001 compared with group III ; c P<0.001 compared with group II. Values are expressed as means ± S.E.*

3.2 Effect of *Jawarish amla sada* on Antioxidants

EAT challenge in animals caused a significant decrease in activities of all the four antioxidant enzymes viz. CAT, GPx, GR, GST in liver (Table 4). Liver of group III animals which were first challenged with tumour cells and then administered with CP showed further decrease in activities of these enzymes which was significant in case of CAT and GR when compared with group II (EAT cell challenge only). GSH values also showed a similar pattern. Group IV and V animals which were given JAS treatment showed protective effect of JAS against the reduction in activities of antioxidant enzymes resulting from either tumour challenge or tumour challenge + CP treatment in CAT activity. However, effect of JAS on the activities of other antioxidant enzymes was not statistically significant. JAS also afforded protection in case of GSH in liver. It was observed that JAS did not provide complete protection when the values of antioxidants in group IV and V were compared with group I i.e. normal control.

The antioxidant profile of kidney in different groups of animals showed a pattern similar to the liver in group II with a significant reduction in the activities of antioxidant enzymes (Table 5), while CP treatment in tumour bearing mice significantly reduced CAT activity when compared with Group II. Although reduction in GSH values of both group II and III were observed, the change was not significant. In case of kidney also JAS was effective in reducing the CP toxicity to antioxidants, except for GPx, GST and GSH values which

showed no statistically significant protection afforded by JAS, although an improvement was observed.

Table 4. Antioxidants in liver of mice in different treatment groups

Parameter	Group I	Group II	Group III	Group IV	Group V
CAT (nmoles H_2O_2 consumed/min/mg protein)	173.2±4.7	112.4±4.4***	79.88±3.7^{b}	113.51±5.5§§§	170.8±6.3^{c}
GPx (nmoles of NADPH oxidized/min/mg of protein)	299.1±9.3	221.8±5.9***	194.2±5.5	224.8±15.6	281.1±15.6^{a}
GR (nmoles of NADPH oxidized/min/mg protein)	238.4±13.6	184.8±7.8**	135.1±7.2^{a}	169.7±7.9	210.9±9.6
GST (nmol CDNB conjugates formed/min/mg protein)	291.8±5.8	195.6±5.6***	166.4±4.6	183.3±8.1	217.1±13.7
GSH (nmol GSH/gm tissue)	4.76±0.21	3.29±0.19***	2.44±0.12^{a}	2.78±0.17	3.78±0.23

*A detailed description of groups is given in Table 2. CAT: Catalase; GPx: Glutathione peroxidase; GR: Glutathione reductase; GST: Glutathione S-transferase; GSH: Glutathione reduced; **P<0.01, ***P<0.001 compared with group I; §§§ P<0.001 compared with group III; ^{a}P<0.05, ^{b}P<0.01, ^{c}P<0.001 compared with group II. Values are expressed as means ± S.E.*

Table 5. Antioxidants in kidney of mice in different treatment groups

Parameter	Group I	Group II	Group III	Group IV	Group V
CAT (nmoles H_2O_2 consumed/min/mg protein)	162.3±2.6	88.36±4.4***	66.92±3.6^{a}	91.68±6.0§	149.8±5.9^{c}
GPx (nmoles of NADPH oxidized/min/mg of protein)	259.2±4.8	194.0±5.4***	169.5±7.1	179.2±6.7	217.1±9.7
GR (nmoles of NADPH oxidized/min/mg protein)	263.1±18.4	181.2±5.1**	167.0±6.2	181.1±5.9	255.1±18.6^{b}
GST (nmol CDNB conjugates formed/min/mg protein)	256.8±5.8	200.8±4.6***	164.8±5.2^{a}	182.2±7.6	208.6±9.9
GSH (nmol GSH/gm tissue)	3.28±0.24	2.50±0.15	2.01±0.07	2.59±0.20	2.82±0.24

*A detailed description of groups is given in Table 2. CAT: Catalase; GPx: Glutathione peroxidase; GR: Glutathione reductase; GST:Glutathione S-transferase; GSH : Glutathione reduced; ** P < 0.01, *** P < 0.001 compared with group I , § P < 0.05 compared with group III , ^{a} P < 0.05, ^{b} P < 0.01, ^{c} P < 0.001 compared with group II. Values are expressed as means ± S.E.*

3.3 Effect on Immunological Parameters

Tumour cell challenge in normal mice resulted in decrease relative weights in thymus and spleen (Figs. 1 and 2). This reduction was significant when compared with normal control animals ($P < 0.001$). Here also CP treatment caused further reduction in weights of these lymphoid organs in group III animals (EAT cell challenge followed by CP treatment) but the reduction was not significant. JAS treatment restored weights of spleen in tumour bearing animals exposed to CP (group IV).

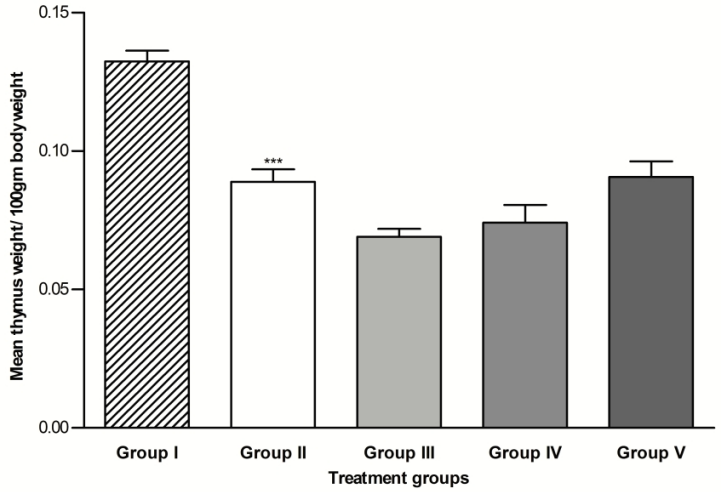

Fig. 1. Effect of different treatments on thymus weight in mice
*A detailed description of groups is given in Table 2.,*** P<0.001 compared with group I. Values are expressed as means ± S.E.*

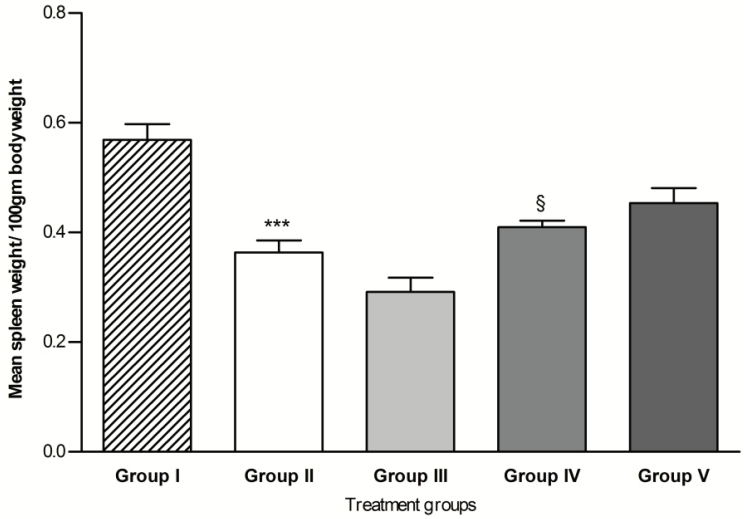

Fig. 2. Effect of different treatments on spleen weight in mice
*A detailed description of groups is given in Table 2. ***P<0.001 compared with group I; § P<0.05 compared with group III, Values are expressed as means ± S.E.*

PFC assay which was performed to measure humoral immune response revealed that EAT significantly affected primary antibody response which was demonstrated by a significant decrease in PFC response in group II animals (P<0.01, Fig. 3). As expected, CP treatment caused a severe decrease in PFC response in mice challenged with EAT cells (P<0.01 when compared with group II). *Jawarish amla sada* provided protection to the animals in case of EAT challenge + CP treatment.

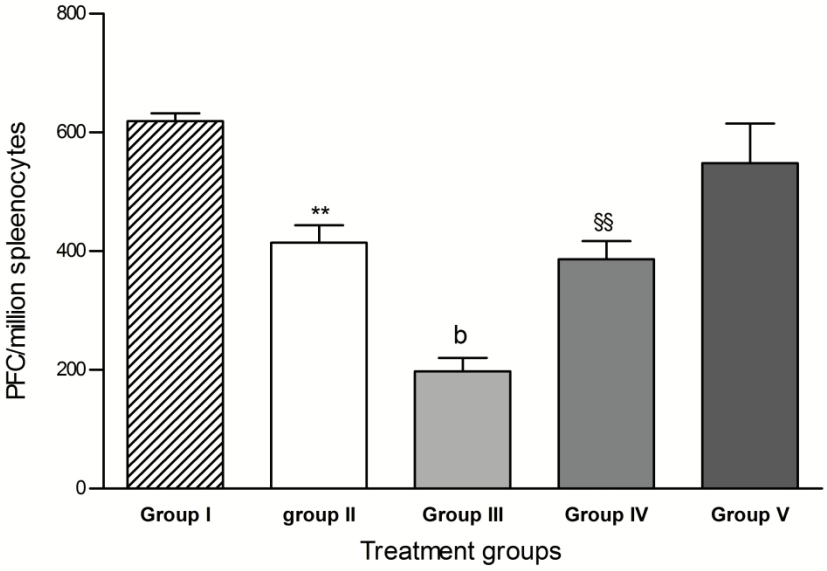

Fig. 3. Effect on different treatments on plaque forming cell assay
*A detailed description of groups is given in Table 2.; **P<0.01, [b]P<0.01 compared with group II; [§§] P<0.01 compared with group III. Values are expressed as means ± S.E.*

4. DISCUSSION

One of the major causes of concern with the use of chemotherapeutic drug is their toxicity which overweighs in certain cases against the beneficial effects of chemotherapeutic drugs (Wiesburger et al., 1975; Seiber, 1977). CP is no exception which is otherwise an efficient chemotherapeutic drug against several forms of cancers such as breast cancer, Hodking's lymphoma, lymphoma etc (Pico et al., 1995; Debaere et al., 2011). Immunotoxicity of CP is also well recognized (Bin-Hafeez et al., 2001). It mainly affects the humoral immune functions (Bin-Hafeez et al., 2001) PFC assay is a robust parameter of humoral immune response measurement which reflects the IgM secreting capacity of B-cells (Biella et al., 2008). It was observed that the PFC response of animals was significantly decreased in tumour bearing animals which were treated with CP. This demonstrates that in case of cancer, if there is a chemotheurapeutic treatment, the immunosuppressive effects may aggravate leading to immunocompromised situation. In this study tumour bearing animals treated with CP showed suppressed humoral immune response measured by PFC assay. All the groups of EAT bearing animals have shown a certain degree of decrease in PFC when compared with the normal control animals. This is an indication that the tumour load has a suppressive effect on the host immune system. It has been demonstrated that EAT growth leads to suppression of immune responses (Mandal and Poddar, 2008; Torello et al., 2012). Animals challenged with EAT cells also showed reduction in weights of lymphoid organs. CP

treatment in such animals showed magnified response. Both tumour challenge as well CP treatment are known to cause reduction in weight of spleen and thymus. Chen et al. (2007) reported that CP treatment in mice caused a drastic reduction in weights of these organs.

Tumour challenge if not handled by immune surveillance of host may cause immunosuppression (Wheatley and Easty, 1965; Pal et al., 2005). In the immunosuppressed host, the chemotherapeutic treatment has its implications, which may limit the efficiency of the treatment. It was observed that JAS not only provided protection in tumour bearing animals but also in tumour bearing animals treated with CP. The EAT carcinoma is a cancer with very fast potential to establish itself in the peritoneal cavity of the animals (Karthikeyan et al., 2008). During the growth of EAT it has been observed that both non specific and specific immune responses are severely suppressed in the later stages of tumourogenesis. Although we did not analyze the macrophage function in the present study, earlier it has been shown that macrophage function, which is a parameter of non specific immune response in severely compromised in EAT bearing mice (Raisuddin et al., 1991). JAS with *E. officinalis* as a major constituents demonstrated its protective effects by ameliorating the humoral immune response and also by mitigating the toxic effects of CP on antioxidants in tumour bearing animals. EAT caused disruption of antioxidants including depletion of reduced glutathione. It has been reported that during tumorogenesis there is a severe crunch of antioxidant (Gupta et al., 2004). Therefore, antioxidants are invariably associated with anticancer as well as tumour burden reducing activities (Bonmassar et al., 1968; Sannigrahi et al., 2010). Glutathione supplementation in particular has shown to elicit response which leads to increased apoptosis of tumour cells (Navarro et al., 1999). A majority of herbal extracts which have shown protection against cytotoxicty induced by chemotherapeutic drug acts by modulating the antioxidant status of blood and tissues (Bhattacharyya et al., 2003; Li et al., 2008; Patra et al., 2012). We also observed that JAS protected animals against the decrease of activities of antioxidant enzymes such as CAT, GPx, GST and GR. The decrease in activities of these enzymes was significantly higher in the tumour bearing animals treated with CP compared with only tumour bearing animals. It reflects that herbal extracts with potent antioxidant constituents may be effective in mitigating these toxic effects. JAS which has over 7% of *E. officinalis* extract, efficiently mitigated the toxic effects of CP in tumour bearing animals on antioxidants. Such protective effect of JAS was also observed in case of lipid peroxidation. *E. officinalis* extracts have demonstrated abrogating effects on CP toxicity and other chemotherapeutic drugs (Sharma et al., 2000). It was observed that the treatment of animals with JAS showed an overall improvement in the level of antioxidants. It may be helpful in restoring the overall well being of the tumour bearing host. JAS treatment has also improved the immune system of the animals which were affected by EAT and CP treatment.

The traditional and folklore medicine systems work on holistic approach whereby it is envisaged that the whole extracts of plants or combinations of different extracts with medicinal efficacy have higher therapeutic efficacy compared to the purified compounds (Dhir et al., 1991; Schoenherr and Jewell, 1997). Therefore, we herewith conclude that the JAS which has total extract of *E. officinalis* along with other total extracts of three more herbs (Table 1) may be a good complimentary treatment for reducing the CP induced toxic manifestations.

5. CONCLUSION

In spite of several toxic side effects recognized decades earlier, cyclophosphamide and its various isoforms are still widely used in chemotherapy. A number of herbal extracts have

shown varying degrees of efficacy in mitigating toxic side effects of cyclophosphamide. However, limited data are available on the efficacy of herbal extracts or formulations containing total herbal extracts against the toxic effects of cyclophsphamide in tumour bearing animals. A tumour bearing host has to cope with immunosuppression which is amplified in case of chemotherapy with cyclophosphamide and other cytotoxic chemotherapeutic drugs. In this study, we demonstrated that a traditional herbal formulation used in Unani system of medicine, which has high content of antioxidant *Emblica officinalis*, modulated the toxic side effects of cyclophosphamide in tumour bearing animals. However, further studies may strengthen the evidence of modulatory effect of *Jawarish amla sada* on the deleterious effect associated with treatment of cyclophosphamide, an anticancer drug, which also causes suppression of the immune functions.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

ETHICAL APPROVAL

The study was performed as per guidelines of the Institutional Animal Ethics Committee (IAEC approval for Project No. 318). No human subjects were involved in this study.

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