

Immunostimulant Effects of Binweed (*Convolvulus arvensis*) Extract in Rabbits

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Abstract: Subcutaneous administration of 10 mg kg⁻¹ aqueous extract of binweed (*Convolvulus arvensis*) to rabbits significantly ($p < 0.05$) increased total leukocyte count, lymphocyte (%) and lysosomal activity compared to saline treated animals. The extract enhanced the phagocytic function and phagocytic index and blocked the immunosuppressive effect produced by dexamethasone. The alcoholic extract was without effect. These results suggest that aqueous extract of binweed has immunostimulant effects.

Key words: Binweed, rabbits, immune cells, date palm, alcoholic, Saudi Arabia

INTRODUCTION

Binweed (*Convolvulus arvensis*) is a creeping weed widely distributed in the Middle East (Yeruham and Shcosbery, 2004). It became the dominant plant growing under the date palm trees in Eastern region of Saudi Arabia (Mandaville, 1990) and due to overgrazing with periodic drought, animals may be forced to consume varying amounts of this plant. The root and also a resin made from the root is cholagogue, diuretic, laxative and strongly purgative (Chopra *et al.*, 1986). Tea made from the flowers is laxative and is also used in the treatment of fevers and wounds (Foster and Duke, 1990). A cold tea made from the leaves is laxative and is also used as a wash for spider bites or taken internally to reduce excessive menstrual flow (Foster and Duke, 1990). All parts of the plant contain tropane alkaloids with atropine-like action (Todd *et al.*, 1995). Binweed has rarely been associated with poisoning in animals (Todd *et al.*, 1995). The plant may be of therapeutic value to animals. One possible effect is immunostimulation.

Plants or plant products that stimulate immune cells in ways beneficial to the body include garlic, nigella sitiva (Black seed), mushroom proteoglycans and various Chinese herbs (Ali and Erwa, 1993; Bocci, 1993; Sinclair 1998; Fatani *et al.*, 1999; Kidd, 2000). However, immunosuppressive effects of plants such as sinomenine (Kidd, 2000) have also been reported. This study was carried out to investigate the immunopharmacological effects of binweed in rabbits.

MATERIALS AND METHODS

Collection and preparation of plant material: Fresh leaves (aerial portions) of binweed locally known as Fidagh was

collected from date palm farms in Al-Ahsa oasis, Saudi Arabia during the rainy season from December-February 2009. Botanical identification of the plant was made by College of Science, King Faisal University, Saudi Arabia.

The aqueous extract of binweed was prepared according to the method of Meng *et al.* (2002). The fresh raw material was mixed in distilled water at a concentration of 0.16 g mL⁻¹ using a commercial blender. The mixture was boiled for 30 min allowed to cool and filtered with a 100 micron sieve. The filtrate was centrifuged at 11,300 rpm for 15 min at 4°C. The supernatant was filtered at 1.5 µm fiberglass and 1.2 µm nylon filters and then concentrated using a pressurized stir cell apparatus (Model CH2, Millipore, Bedford, MA) with a 30 Kda YM-30 (Millipore) membrane. This concentrate was lyophilized (Freeze Zone 6 Freeze Drying Apparatus, Labconco Inc., Kansas City, MO) to produce the extract powder.

Alcoholic extract of binweed was prepared according to the method of Todd *et al.* (1995). The aerial parts (100 g wet wt.) were extracted at room temperature for 24 h with MeOH. The mixture was filtered and the MeOH evaporated to leave about 25 mL of gummy solution which was taken up in 250 mL of 0.5 M HCl. The solution was extracted with ethanol (Et₂O; 6 × 75 mL) and then with chloroform (CHCl₃; 6 × 50 mL) to remove nonbasic material. The solution was made basic to pH 11 with K₂CO₃ and then extracted with CHCl₃ (6 × 50 mL). CHCl₃ solution was evaporated to yield 17 mg of crude alkaloid residue. The residue was dissolved in 1 mL of MeOH, streaked on a silica gel plate and the plate double developed (CHCl₃-MeOH, 4:1). The edge of the plate was sprayed with iodoplatinate to reveal the alkaloid-containing bands.

Under these conditions, alkaloids revealed were hygrine, tropinone, tropine, pseudo-tropine, cuscohygrine stereoisomer and cuscohygrine (Todd *et al.*, 1995).

Animals and treatment: About 36 adult rabbits (*Oryctolagus cuniculus*) with a mean body weight of 3.1 ± 0.33 kg were recruited for the study. The animals were maintained in a regulated schedule of 12 h light, 12 h darkness with food and water available *ad libitum*. Animals were randomly divided into 6 equal groups.

Group 1: Animals were treated with saline and kept as controls.

Group 2: Animals treated with dexamethasone (Sigma, UK) at a dose of 2 mg kg^{-1} SC for 7 days.

Group 3: Animals were treated with aqueous extract at a dose of 10 mg kg^{-1} body weight for 3 days.

Group 4: Animals were treated with alcoholic extract at a dose of 10 mg kg^{-1} body weight for 3 days.

Group 5: Animals were treated with dexamethasone for 7 days followed by aqueous extract.

Group 6: Animals were treated with dexamethasone followed by alcoholic extract.

Collection of blood samples: Blood samples were collected by jugular vein puncture into heparinized syringes for hematology or plain tubes for serum preparation. Serum was stored at -30°C until analysis.

Effects of binweed extracts on phagocytic function of macrophages

Reticuloendothelial System phagocytic (RES) function:

On the 4th day after aqueous, alcohol or saline treatment the reticuloendothelial system function was determined in rabbits by measuring the intravascular clearance of carbon colloid as described by Al-Ankari and Homeida (1996).

The colloid carbon (Gunther, Wagner, Hanover) was administered intravenously at a dose of 0.08 mg kg^{-1} body weight into the left Jugular vein. About $50 \mu\text{L}$ of blood was collected from cannulated Jugular vein at various times after carbon injection into heparinized tubes.

Blood was then hemolyzed with 4 mL of 0.1% sodium carbonate. The samples were then centrifuged at 500 g for 5 min. The relative amount of carbon remaining in the supernatant of the samples was estimated spectro-

photometrically at 675 nm by using the samples collected before carbon injection as the zero value. The carbon concentrations were plotted as percentage of the injected dose semi-logarithmically against time in minutes and thus intravascular half life ($t_{1/2}$) in minutes was calculated.

Restoration of phagocytic function of reticulo system by binweed extracts in dexamethasone-treated rabbits: In group 1, 2, 5 and 6 carbon colloid was administered and carbon concentrations determined. The graph of absorbance against time was plotted and phagocytic index calculated (Pallabe *et al.*, 1998) using the formula:

$$\text{Phagocytic index} = \frac{R_{ct}}{R_{cc}}$$

Where:

R_{ct} = Regression coefficient (treatment)

R_{cc} = Regression coefficient (control)

Serum lysozyme activity: Serum lysozyme concentrations were measured using *Micrococcus lysodieticus* as a substrate (lysozyme reagent kit, Worthington Biochemical, Co. Freehold, NJ) according to the manufacture's recommendations (Al-Ankari and Homeida, 1996).

The percentage changes in transmission (510 nm) per min were immediately recorded using a spectrophotometer (Hitachi, Japan). The values were compared to a standard curve simultaneously prepared using a known concentration of egg white lysozyme.

Statistical analysis: Results are expressed as mean \pm SD and presence of significant differences among means of the groups was determined using one way ANOVA with a Tukey-Kramer post-test for significance. Values were considered significant when $p < 0.05$.

RESULTS AND DISCUSSION

The effects of binweed extracts on carbon clearance by reticular endothelial system are shown in Fig. 1. Aqueous extract but not alcoholic extract significantly ($p < 0.05$) increased the intravascular carbon clearance and accelerated the vascular half-life at about twofold compared to controls, the clearance rate values were 9.4 ± 1.3 , 4.7 ± 0.30 and 8.6 ± 1.2 min for control, aqueous and alcoholic extract treated rabbits, respectively.

The phagocytic and leukocyte are shown in Table 1. Dexamethasone group and showed a value of index of 0.49. These values were significantly lower than in control

animals. Likewise the leukocyte and lymphocytes number were decreased ($p<0.05$). Aqueous extract of binweed produced significantly ($p<0.05$) higher index in normal rats (1.53) and even more higher ($p<0.001$) in dexamethasone treated rabbits (1.73).

Aqueous extract significantly ($p<0.01$) increased total leukocyte count and percentage lymphocytes by 30 and 27%, respectively. The extract also inhibited the effect of dexamethasone on leukocyte and lymphocyte. No effect of aqueous extract on neutrophil was observed. Alcoholic extract was without effect on the variables studied.

Administration of aqueous extract to rabbits increased total leukocyte count and lymphocytes. Similar studies of aqueous binweed extract on human lymphocyte growth in the laboratory have shown between 35 and 46% increase in the number of lymphocyte (Kidd, 2000).

Serum lysozyme activity was significantly increased in rabbits treated with aqueous extract. Serum lysozyme activity is considered to be an index of macrophage function (Currie, 1976). Similar studies showed that suppression of macrophage activity with methyl palmitate was associated with reduction of lysozyme enzyme release in serum, while activation of macrophages with glucan was associated with a marked release of the

enzyme in serum (Koskoshis and di Luzio, 1979). The data obtained revealed a significant induction of reticuloendothelial system phagocyte function in rabbits treated with aqueous extract of binweed. The extract increased the phagocytosis efficiency to remove foreign particles such as carbon colloid which could be attributed to induction of reticuloendothelial system function which was reflected by increase of intravascular carbon clearance and acceleration of vascular half-life at about two fold compared to controls.

It is known that phagocytosis is a complex process that involves opsonisation followed by adsorption onto the macrophage surface (Wools and di Luzio, 1962), endocytosis and eventually digestion (phagosome-lysosome fusion) (Dedon and Tannenbaum, 2004).

The phagocytic indices of the different groups are shown in results section. Dexamethasone group showed a value of 0.49 which implies that the phagocytic function of the reticulo endothelial system has decreased. Aqueous extract group were active in normal (1.53) and more active in immunosuppressed animals (1.73) thus restoring the suppressed phagocytic function.

When proper colloidal suspension of particle sizes which do not cross the capillary barriers were injected intravenously, the particles were phagocytosed by the kupffer cells of the liver and the reticular cells of the spleen (Biozzi *et al.*, 1958).

The suppressive effect on immune system by dexamethasone is indicated by its less activity in carbon clearance in part functionally impairing cells of mononuclear phagocytic system (Jain, 1986).

Similarly, increased rate of carbon clearance from the blood by levamisole was reported by Sarker *et al.* (2000). Mushroom proteoglycans have been shown to have an increase immune cell counts and to enhance infiltration of T-lymphocytes and dendritic cells into tumors (Kidd, 2000). Moreover, they have been shown in clinical trials to enhance survival times of patients with a variety of cancers and to ameliorate sides effects of chemotherapy (Kidd, 2000).

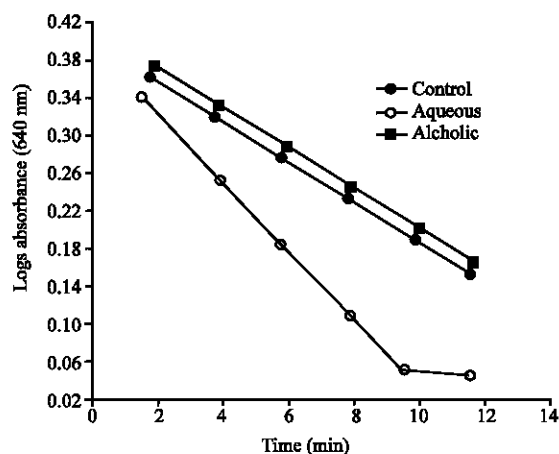


Fig. 1: Effect of binweed extracts on carbon clearance by reticular endothelial system in rabbits

Table 1: Effect of binweed extracts on phagocytic function and leukocyte indices in dexamethasone treated rabbits

Groups	Phagocytic index	Total leukocytes 1000 cells mL ⁻¹	Lymphocytes (Percentage of leukocytes)	Neutrophils (Percentage of leukocytes)
I (Control)	1.00	20.4±0.2	55.0±3.1	22.0±0.8
II (dexamethasone)	0.49*	14.1±0.1	36.1±2.2	23.0±0.6
III (aqueous extract)	1.53*	26.6±0.3	70.1±3.2*	21.2±0.8
IV (alcoholic extract)	0.90	21.1±0.2	44.3±4.1	22.3±0.6
V (dexamethasone+aqueous extract)	1.73*	26.1±0.2	68.2±2.5*	21.6±0.7
VI (dexamethasone+alcoholic extract)	0.53*	13.2±0.1	38.1±2.1	22.4±0.6*

$p<0.05$, significantly different from controls

CONCLUSION

From the findings, it is that the mechanism of action for this extract may be related to immune cell function enhancement. In this way, the aqueous *Convolvulus arvensis* extract is similar to plant proteoglycans and glycoproteins described in the literature (Meng *et al.*, 2002). These molecules are of interest in cancer research for a variety of reasons. They are an important part of the extracellular matrix and they serve in some fashion to prevent tumor cell migration.

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