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Original Article

IMMUNOSTIMULATORY EFFECTS OF ETIOLATED WHEAT GRASS, *TRITICUM AESTIVUM* L. ON DEXAMETHASONE INDUCED IMMUNOSUPPRESSED ALBINO RATS

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ABSTRACT

Objective: Present work explore the immune stimulatory effects of fresh etiolated wheat grass juice (FEGJ) in dexamethasone induced immunosuppressed albino rat through study of neutrophil adhesion percentage and phagocytic efficiency via carbon clearance test.

Methods: Albino rats of both sexes were divided into four group (n=6). Group I (NC) was kept as normal control. Group II (ISC) animals were injected intra peritoneally with dexamethasone phosphate (5mg/kg) twice a day for 5 days and kept as immune suppressed control. Group III (FEGJ 20ml/kg) and Group IV (FEGJ 40 ml/kg) both were injected intraperitoneally with dexamethasone phosphate (5mg/kg) twice a day for 5 days. After this, from 5th to 15th day, animals were orally administrated with FEGJ at the dose of 20ml/kg and 40 ml/kg respectively.

Results: In dexamethasone induced immune suppressed, FEGJ treated groups (FEGJ 20ml/kg and FEGJ 40ml/kg) significant increase in neutrophil adhesion percentage and significant decrease in mean carbon clearance time were observed.

Conclusion: Fresh etiolated wheat grass juice show promising immune stimulatory activity and reversed the dexamethasone induce immune suppression.

Keywords: Etiolated wheat grass, Dexamethasone, Phagocytic efficiency, Neutrophil adhesion, Immune suppression.

INTRODUCTION

The mammalian immune system is comprises of two intercommunicating branches: innate and acquired immunity. The innate immune system is the first line of host defense against potentially invading pathogens and is mediated by phagocytes including macrophages and dendritic cells (DCs)[1]. Appropriate recognition of threats and induction of the inflammatory cascade are essential steps in the removal of these organisms from the system. Failure of the innate system to identify pathogens delay the induction of the immune response and may worsen outcomes of infection[2]. Primary immunodeficiency (PID) is the condition of impairment of innate as well as acquired immune system due to defects in immune system development. PID includes about 130 disorders that result from genetic as well as acquired impairment of immune system[3]. Disorders such as PID, HIV infection and regular consumption of glucocorticoides result into suppression of immune function than normal.

Dexamethasone is the synthetic glucocorticoid and it acts as a potent immunosuppressive agent[4]. Glucocorticoids are the most commonly used drugs, and are widely used for the management of inflammatory diseases. These drugs inhibit various immune functions by affecting gene transcription events[5-6]. Immunosuppressive effect of glucocorticoids exerted by various combined mechanisms, including pre- and post-transcriptional means and cytosolic glucocorticoid receptor mediated modification of gene regulation in target cells. *Triticum aestivum* L. belongs to family Poaceae, commonly known as bread wheat, native to Southwest Asia and the Mediterranean region. Besides serving as a major food source globally, *T. aestivum* has number of health beneficial properties. Few pharmacological research, in past, stated the anti cancerous activity[7], anti-thalassemic activity[8], hypoglycemic activity[9], anti oxidant activity[10] and hepatoprotective activity[11]. In present study, we have investigated the unexplored immunostimulatory effects of etiolated wheat grass through neutrophil index and phagocytic activity.

MATERIALS AND METHODS

Plant material

In order to get etiolated yellow grass, *T. aestivum* grains were grown in 24 hrs dark on bamboo stick pad (60cm diameter). Pad was layered with three inch thick 3:1 soil compost mixture. Overnight soak grains were sown evenly and sprinkled with sufficient water every day for proper growth. On 14th day, yellow grass was harvested just 2 cm above the surface. Fresh and concentrated juice was prepared in laboratory with mortar and pastel at each time of dosing. To fulfill the requirement of plant material for experiment, other ten pads also processed at one day interval with same procedure.

Experimental animal

Albino rats of both sexes, weighing 180-240 gm were used for the experiment. Animals were cared for and used in accordance with the Institutional Animal Ethics Committee (IAEC), P.G.T. Department of Zoology, RTM Nagpur University, Nagpur (Registration no.-478/01/a/CPCSEA).

Phytochemical screening

Fresh wheat grass juice was screened phytochemically using standardized protocols to check the presence of major bioactive components[12].

Immunosupressant

Dexamethasone phosphate injection (Pemadex®, China), was used to induce immunosuppression in experimental animals. Each vial contained 4 mg of dexamethasone per ml of Pemadex.

Neutrophil adhesion test

For this test, animals were divided into four groups (n=6): Group I (NC) was kept as normal control.Group II (ISC) animals were injected intraperitoneally with dexamethasone phosphate (5mg/kg) twice a day for 5 days and kept as immunosuppressed control.

Group III (FEGJ 20ml/kg) was injected intraperitoneally with dexamethasone phosphate (5mg/kg) twice a day for 5 days. Then, from 5th to 30th day, animals were orally administrated with FEGJ at the dose of 20ml/kg.

Group IV (FEGJ 40 ml/kg) was injected intraperitoneally with dexamethasone phosphate (5mg/kg) twice a day for 5 days. From 5th to 30th day, animals were orally administrated with FEGJ at the dose of 40mg/kg. On the 30th day, blood samples were collected into vials coated with anticoagulant substance, by retro-orbital puncture from rats of every group. Analysis of initial counts of total leukocytes and differential leukocytes were done by fixing the blood smears and staining with Leishman's stain. After this, blood samples from all groups were incubated with nylon fibers (NF, 80 mg/ml of blood sample) at 37^0 C for 15 min. Analysis of initial counts of total leukocytes and differential leukocytes were repeated for incubated blood. Neutrophil index was calculated as the product of total leukocytes and percent neutrophil. For the calculation of percent neutrophil adhesion following formula was used.

$$\text{Percent neutrophil adhesion} = \frac{\text{NIu} - \text{NIt}}{\text{NIu}} \text{ x } 100$$

NIu represents neutrophil index before incubation while NIt is the neutrophil index after incubation with the nylon fiber[13].

Phagocytic activity

Estimation of phagocytic activity was performed with the help of carbon clearance test. In this test, rats were divided into four groups (n=6).

Group I (NC) was kept as normal control.

Group II (ISC) animals were injected intraperitoneally with dexamethasone phosphate (5mg/kg) twice a day for 5 days and kept as immunosuppressed control.

Group III (FEGJ 20ml/kg) and Group IV (FEGJ 40 ml/kg) both were injected intraperitoneally with dexamethasone phosphate (5mg/kg) twice a day for 5 days. Then, from 5th to 15th day, rats were orally administrated with FEGJ at the dose of 20 ml/kg and 40 ml/kg respectively.

The carbon ink suspension was made with black carbon ink (3ml), 3% gelatin solution (4ml), and saline (4 ml). Each animal of every group was injected with carbon ink suspension (10ml/kg) intravenously, 48 hrs after completion of 15 days dose. Blood samples were withdrawn from retro-orbital puncture within 15 min after injection of carbon suspension. In order to lyses the RBCs, 14 drops of this blood were mixed with 0.1% w/v sodium carbonate (4ml). Absorbance was measured at 650 nm using UV spectrophotometer in order to measure the half life ($t_{1/2}$ in sec.) of carbon in the blood. Phagocytic activity was expresses as phagocytic index (K) which was calculated using following formula[13].

$$K = \frac{(lnOD1 - lnOD2)}{(t2 - t1)};\ t1/2 = \frac{0.693}{K}$$

In above equation OD1 and OD2 represent optical densities at t1 (initial time) and t2 (final time) respectively.

Statistical analysis

The results were expressed as Mean ± SEM, comparison between groups was done with t-test using GraphPad Prism version 6. Significance level was set at P<0.05 and P<0.01.

RESULTS AND DISCUSSION

Preliminary screening of extract

Major bioactive component found in FEGJ is presented in table-1.

Table1: Phytochemical screening of FEGJ.

Test	Inference
Reducing sugar	+
Alkaloids	+
Phenols	-
Steroids	-
Flavenoids	-
Saponin	+
Tanin	-
Protein	+

Effect of FEGJ on neutrophil adhesion

Table-2 represents the dose dependent effects of FEGJ on neutrophil adhesion percentage. When compare with the ISC, significant dose dependent increase in the neutrophil adhesion was observed. FEGJ dose of 20 ml/kg causes significant (P<0.05) increase in neutrophil adhesion by 13.07 % when compared with ISC while the higher dose of FEGJ (40 mg/kg) causes significant (P<0.01) increase in neutrophil adhesion by 18.26% compared to ISC. LFA -1 and Mac-1 are two most abundant CD 11/ CD18 integrins found on neutrophil[14]. LFA-1 mediates adhesion of neutrophil and Mac-1 play important role in its extravasations[14]. Dose dependent increase of neutophil adhesion percentage indicates that FEGJ may upregulate the expression of these two CD11/CD18 integrins and provoke the dexamethasone induced inactive inflammatory barrier of the innate immune system.

Table 2 Effect of FEGJ on percent neutrophil adhesion.

Groups	Neutrophil index		Neutrophil adhesion (%)
	NF untreated Blood	NF treated Blood	
NC	218.53±11.27	172.72±9.55	20.94±1.65
ISC	151.66±6.29	130.94±3.89	13.41±1.48**
FEGJ(20ml/kg)	338.23±16.43	245.96±13.42	26.48±4.80*
FEGJ(40ml/kg)	370.49±5.09	248.47±7.15	31.67±2.59**

Results are expressed as Mean ± SEM, (n=6), **P<0.05 statistically significant when FEGJ 20ml/kg was compared with ISC and ,*P<0.01 statistically significant when FEGJ 40ml/kg compared with ISC.

Effect of FEGJ on phagocytic activity

Effect of FEGJ on phagocytic activity expressed in terms of phagocytic index and mean carbon clearance time is shown in table-3. Both doses, 20ml/kg and 40ml/kg of FEGJ very significantly (P<0.01) increases the phagocytic activity when compared to the NC and ISC. Carbon clearance time represents the half life of carbon in

the blood which has inverse relation with the phagocytic index i.e. more the activity of phagocytic cell, less will be the $t_{1/2}$. Macrophages are capable of engulfing and killing microbes, but perhaps their most important functions are supervisory. Through the elaboration of chemotactic cytokines, they recruit other myeloid cells, particularly polymorphonuclear phagocytes, to the site of infection. Macrophages and even more so dendritic cells also initiate the adaptive immune response to most pathogens by presenting antigen to CD^{4+} T cells via class II MHC antigen[15]. Thus, by stimulating the activity of phagocytic cells, FEGJ also play vital role in better interaction of innate and acquired immune system.

Values are expressed as Mean ± SEM, (n=6), P<0.01 statistically significant when groups FEGJ 20 ml/kg and FEGJ 40ml/kg were compared with ISC.

Table3: Effects of FEGJ on phagocytic index and $t_{1/2}$ of carbon in blood.

Groups	Mean Phagocytic Index (K)
NC	0.13 ± 0.005
ISC	0.047 ± 0.005
FEGJ 20 (ml/kg)	0.38 ± 0.03
FEGJ 40 (ml/kg)	0.67 ± 0.043

Values are expressed as Mean ± SEM, (n=6), P<0.01 statistically significant when groups FEGJ 20 ml/kg and FEGJ 40ml/kg were compared with ISC.

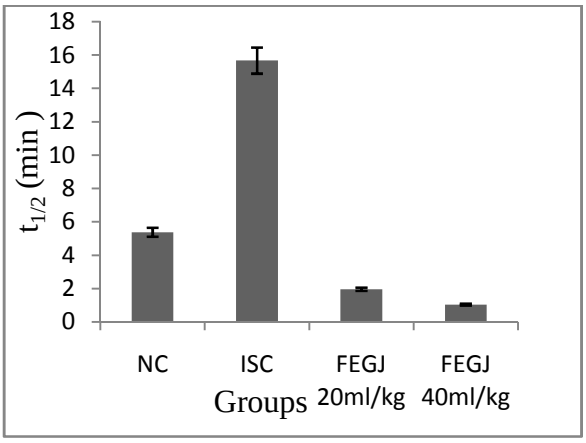

Fig 1: Effect of dexamethasone and FEGJ on mean carbon clearance time, $t_{1/2}$.

CONCLUSION

FEGJ show stimulatory effects on reticuloendothelial system by increasing the phagocytic activity, which plays a vital role in eliminating pathogens and other foreign antigens thereby, contributes to improve the first line of defense. Since etiolated wheat grass contains negligible chlorophyll, reversal of dexamethasone induced immunosuppression and immunostimulatory effects observed in present work indicate that these are chlorophyll independent properties of FEGJ. On the basis of result obtained, we encourage the use of FEGJ in immunodeficiency disorders.

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