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Research Article

Study of Immunomodulatory Effect of Seeds of *Brassica nigra* and *Cuminum cyminum* in Albino Rats

T Nandini[1]*, D Ramya Sree[2], Shaik Janbee[3], K. Siva Sai kiran[4], G.Raveendra babu[5]

[1,2,3]*Arya College Of Pharmacy, Sangareddy (V), Medak (M), Telangana.*
[4] *D.C.R.M Pharmacy college, Inkollu, A.P, India*
[5]*A.K.R.G College Of Pharmacy, Nallajerla, A.P, India*

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ABSTRACT
The aim of the Present investigation is to assess the immunomodulatory effects of ethanolic extract of *Brassica nigra L.* in combination with *Cuminum cyminum* against pyrogallol induced immunosuppression in rats. Control group received a dose of pyrogallol 100mg/kg i.p., once daily from day 1 to 7, while the normal group received only vehicle. In another set of experiment the immunomodulatory activity of *Brassica nigra* 250 and 500 mg/kg p.o.,daily from day 1 to day 22 was screened in rats in whom immunosuppression was induced the pyrogallol. Besides the above treatments, the rats from all the groups received sheep red blood cells (SRBC) (0.5×10^9cells/100g, i.p.) on day 7 and 13, as the antigenic material to sensitize them for immunological studies. The immunological parameters that are assessed are humoral immune response, cellular immune response, carbon clearance test and white blood cell count. Invivo antioxidant studies were performed by assessing superoxide dismutase, catalase, reduced glutathione, lipid peroxidation and myeloperoxidase activity. Administration of high dose of *Brassica nigra* (500mg/kg p.o.) for 22 days significantly ($p<0.001$) prevented the influence of pyrogallol on primary and secondary humoral immune responses, increased the paw volume (index of cell-mediated immunity) when compared to control group. Administration of equal proportionate dose of *Brassica nigra* (250mg/kg, p.o.) and Cuminum cyminum (250mg/kg, p.o) for 22 days significantly ($p<0.01$) increased the paw volume, increased the phagocytic response, increased the WBC count when compared to control group. treatment with low and high dose of *Brassica nigra* significantly increased the superoxide dismutase, catalase, reduced glutathione, myeloperoxidase and significantly ($p<0.05$) decreased the lipid peroxidation respectively in a dose dependent manner when compared with control.

Key words: *Brassica nigra*, *Cuminum cyminum*, immunomodulatory and myeloperoxidase.

INTRODUCTION

Agents that suppress the immune system play an important role in the retention of organ or tissue grafts[1]. Agents that augment the immune response or selectively alter the balance of various components of the immune system are also becoming important in the management of certain diseases such as cancer, AIDS and autoimmune or inflammatory diseases. But the allopathic immunostimulants like levamisole and tetramisole side effects include skin toxicity and agranulocytosis. Allopathic immunosuppressants like cyclophosphamide, cyclosporine and azathioprine side effects include renal toxicity, hepatic toxicity and bone marrow depression. Most of the natural immunomodulatory products which are available in the market contain combination of crude drug powder and/or extracts of some herbs. When crude powders are used in the formulation there is a greater chance of variation in the active constituents present in it which in turn minimizes therapeutic benefit[2,3]. The chances of variation will be less with the use of fractions and extracts. Hence, for maximizing the potential of immunomodulatory herbal products, fractions or combination of fractions and extracts in the formulation has to be encouraged. *Brassica nigra and Cuminum cyminum* was claimed to have significant influence on the immune system. But no systemic pharmacological study was there to support the claim. So the objective of the present study was to study the immunomodulatory activity of ethanolic extract of *Brassica nigra L.in combination with Cuminum cyminum* against pyrogallol induced immunosuppression in rats.

MATERIALS AND METHODS

Brassica nigra seeds and *Cuminum cyminum* seeds were collected locally from supermarket at ECIL, hyderabad. Seeds were dried at room temperature and shade dried seeds were made into powder. The powder was extracted with ethanol: water (3:1) mixture on a reflex water bath for 3 hr. The cycle was repeated for three times. The extract was concentrated on rotary flash evaporator and air dried to get the semisolid extract.

Animals

Wistar rats of either sex 150-200 g body weight were housed under standard husbandry conditions, 25 ± 5^0C

**Author for Correspondence*

Table 1: Phytochemical screening of ethanolic extract of *Brassica nigra and Cuminum cyminum*

S. NO	Test	Ethanolic extract of *Brassica nigra*	Ethanolic extract of Cuminum cyminum
1.	Alkaloids	+	+
2.	Carbohydrates	_	_
3.	Proteins	_	_
4.	Flavonoids	_	+
5.	Tannins	+	+
6.	Glycosides	+	+

+ = Present - = Absent

temperature, 12 h light/dark cycle with standard rat feed with water *ad libitum*. The experimental protocol was approved by the Institutional Animal Ethical Committee (IAEC) of KGR Institute of Technology and Management (NO:1763/PO/Ere/s/14/CPCSEA).

Drugs and Chemicals

DTNB (5,5-di thio bis 2-nitro benzoic acid) was procured from Sigma-Aldrich Pvt. Ltd. India; Septilin syrup from Himalaya Ltd. India and all other chemicals and reagents used were of analytical grade, procured from SD fine chemicals Ltd. India.

Acute Toxicity Study

Acute oral toxicity study was performed as per OECD – 423 guidelines (acute toxic class method). Wistar albino rats of either sex were selected randomly and divided into six groups (n = 2). The animals were fasted overnight and extract in doses of 100, 250, 500, 1000 and 2000 mg/kg body weight, were administered orally to II – VI groups. Group I which received vehicle (water) served as control. The animals were observed continuously for 2 hr, and then intermittently for 6 hr and at the end of 24 hours, the number of deaths was noted to determine LD_{50} of the extract (Annie et al., 2004).

Preliminary phytochemical screening[4]

Preliminary phytochemical tests were performed for the ethanolic extract of *Brassica nigra and Cuminum cyminum* seeds to detect the presence of phytochemicals by following the standard methods described in the practical pharmacognosy of kokate and khandelwal. The results have been tabulated in table I.

TREATMENT SCHEDULE FOR ASSESING IMMUNOSTIMULANT POTENTIAL OF Brassica nigra:

Experimental rats were divided into five groups and each group consists of six animals (n=6). Control group received a dose of pyrogallol 100mg/kg i.p., once daily from day 1 to 7, while the normal group received only vehicle. In another set of experiment t he immunomodulatory activity of *Brassica nigra* 250 and 500 mg/kg p.o.,daily from day 1 to day 22 was screened in rats in whom immunosuppression was induced the pyrogallol. Besides the above treatments, the rats from all the groups received sheep red blood cells (SRBC) (0.5×10^9 cells/100g, i.p.) on day 7 and 13, as the antigenic material to sensitize them for immunological studies.

Immunologicalparameters

Humoral Immune Response

On day 13 and 20, blood was withdrawn from the retroorbital plexus of all antigenically challenged rats. 25µl of serum was serially diluted with 25µl of phosphate bufferd saline. SRBC (0.025×109 cells) were added to each of these dilutions and incubated at 37^oC for one hour[5]**.** The rank of minimum dilution that exhibited hemagglutination was considered as an antibody titer. The level of antibody titer on day 13 of the experiment was considered as the primary humoral immune response and the one on day 20 of the experiment was considered as the secondary humoral immune response (Joharapurkar et al., 2004).

Cellular Immune Response[6]

This was assayed by the footpad reaction method. The edema was induced in the right paw of rats by injecting SRBC (0.025×109 cells) in the subplantar region on day 20. The increase in the paw volume in 48h i.e., on day 22 was assessed on plethysmometer. The mean percentage increase in paw volume was considered as delayed type of hypersensitivity and as the index of cell mediated immunity. The volume of the left hind paw injected similarly with phosphate buffered saline served as a normal (Joharapurkar et al., 2004).

Carbon Clearance Test

Phagocytosis is a process by which certain body cells, collectively known as phagocytes, ingest and removes microorganisms, effector malignant cells, inorganic particles and tissue debris (Ghule *et al.*, 2006). The selected plant extracts were subjected to carbon clearance test by the following procedure (Dash *et al.*, 2006, Bizzo *et al.*, 1953). Different concentrations of the plant extracts i.e., 500, 1000mg//kg were administered orally for 7 days. At the end of seven days the rats were injected with 0.1 ml of carbon ink (Camel fountain pen ink) suspension (1.6 % v/v in 1% Gelatin, in saline) via the tail vein. Blood samples (about 50 µl) were drawn (in 0.15% w/v disodium EDTA solution, 50 µl) from the retro orbital vein, at intervals of 2 & 15 minutes after injection. A 25 µl sample was mixed with 0.1% sodium carbonate solution (2 ml) and the absorbance was measured at 660 nm taking 0.1% sodium carbonate solution as blank.

White Blood Cell (Wbc) Count

A drop of blood sample was withdrawn from the retro orbital venous plexus of rats. The collected blood was sucked up to 0.5 mark of WBC diluting pipette. The tip was cleaned and diluting fluids is sucked up to mark 11. The fluids were thoroughly mixed by rotating the pipette between the palms of two hands. A clean cover

Table 2: Effect of *Brassica nigra* & *Cuminum cyminum* on Humoral immune response

S.NO	GROUP	Hemagglutination (antibody titer) Primary	Secondary
1.	Normal	1± 0.0	1± 0.0
2.	Control	2.75± 0.25#	3.5± 0.28#
3.	Standard	1± 0.0*	1.25± 0.25*
4.	Test 1	2.05± 0.25	1.5± 0.28*
5.	Test 2	1± 0.0*	1.25± 0.25*
6.	Test 3	1.96± 0.0*	1.7± 0.35*

All values are shown as mean ± SEM and n=6. # indicate $p<0.001$ when compared to normal group.
* indicate $p<0.001$ when compared to control group.

Table 3: Effect of *Brassica nigra and Cuminum cyminum* on Cellular immune response

S. No.	Group	Treatment	Delayed type Hypersensitivity response (paw oedema) cm
1.	Normal	Distilled water	0.34 ± 0.02
2.	Control	Pyrogallol 100 mg/kg i.p./7days	0.22 ± 0.02##
3.	Standard	Pyrogallol 100 mg/kg i.p./7days + Septilin 1ml/100g/22days	0.34 ± 0.02**
4.	Test 1	Pyrogallol 100 mg/kg i.p./7days+ethanolic extract of *Brassica nigra* 250 mg/kg p.o/22days	0.32 ± 0.02*
5.	Test 2	Pyrogallol 100 mg/kg i.p./7days+ethanolic extract of *Brassica nigra* 500 mg/kg p.o/22days	0.34 ± 0.02**
6.	Test 3	Pyrogallol (100mg\kg – I.P/7days) +ethanolic extract of *Brassica nigra (25*0mg/kg) + *Cuminum cyminum (*250 mg/kg) p.o/22 days	0.30 ± 0.02*

All values are shown as mean ± SEM and n=6. ## indicate $p<0.01$ when compared to normal group.
* indicate $p<0.05$, ** indicate $p<0.01$ when compared to control group

Table 4: Effect of *Brassica nigra* on carbon clearance

S. No	Group	Treatment	Phagocytic Response
1.	Normal	Distilled water	0.015± 0.001
2.	Control	Pyrogallol 100mg/kg i.p./7days	0.002 ± 0.001#
3.	Standard	Pyrogallol 100mg/kg i.p./7days + Septilin 1ml/100g/22days	0.015 ± 0.001*
4.	Test 1	Pyrogallol 100mg/kg i.p./7days+ethanolic extract of *Brassica nigra* 250mg/kg p.o/22days	0.018 ± 0.003**
5.	Test 2	Pyrogallol 100mg/kg i.p./7days+ethanolic extract of *Brassica nigra* 500mg/kg p.o/22days	0.023 ± 0.003**
6.	Test 3	Pyrogallol (100mg\kg – I.P/7 days)+ ethanolic extract of *Brassica nigra (25*0mg/kg) + *Cuminum cyminum (*250 mg/kg) p.o/22 days	0.020 ± 0.001**

slip was placed over counting chambers. First few drops of diluting fluid present in capillary tube were discarded. A tiny drop was collected at the tip of pipette and was touched at junction of cover slip at the slide. Fluid was drawn in to chambers by capillary action. There should not be any air bubble. Examined under 10x. count the five fields[7,8]. Finally Calculate the WBCs/cmm by adding the cells in the 5 groups and multiplying by 40.

Statistical analysis

All the data was expressed as mean ± S.E.M. Statistical significance between more than two groups was tested using one way ANOVA followed by the Tukey test using computer based fitting program (Prism graph pad version 5.0).

RESULTS AND DISCUSSION

Acute toxicity studies

The alcoholic extract of *Brassica nigra was* found to be safe since no animal died even at the maximum single dose of 2000 mg/kg when administered orally, and the animals did not show any gross behavioral changes. Hence, 1/8 and 1/4 of maximum therapeutic dose (2000 mg/kg) was selected for the present study. Similarly the alcoholic extract of *cuminum cyminum* was found to be safe since no animal died even at the maximum single dose of 2000 mg/kg when administered orally, and the animals did not show any gross behavioral changes. Hence, 1/8 and 1/4 of maximum therapeutic dose (2000 mg/kg) was selected for the present study.

Table 5: Effect of *Brassica nigra* & *Cuminum cyminum* on WBC count

S. NO	GROUP	TREATMENT	WBC COUNT cu.mm
1.	Normal	Distilled water	9800 ± 142
2.	Control	Pyrogallol 100mg/kg i.p./7days	6431 ± 948#
3.	Standard	Pyrogallol 100mg/kg i.p./7days + Septilin 1ml/100g/22days	14600 ± 502***
4.	Test 1	Pyrogallol 100mg/kg i.p./7days+ethanolic extract of *Brassica nigra* 250mg/kg p.o/22days	9269 ± 735.5
5.	Test 2	Pyrogallol 100mg/kg i.p./7days+ethanolic extract of *Brassica nigra* 500mg/kg p.o/22days	11544 ± 439**
6.	Test 3	Pyrogallol (100mg\kg – I.P/7 days) +ethanolic extract of *Brassica nigra(25*0mg/kg) + *Cuminum cyminum (*250 mg/kg) p.o/22 days	1036 ± 225*

Table 6: Effect of *Brassica nigra and Cuminum cyminum* on Myeloperoxidase activity

S. NO	GROUP	TREATMENT	MPO ACTIVITY
1.	Normal	Distilled water	0.1030±0.03
2.	Control	Pyrogallol 100mg/kg i.p./7days	0.006±0.001#
3.	Standard	Pyrogallol 100mg/kg i.p./7days +Septilin 1ml/100g/22days	0.1028±0.001*
4.	Test 1	Pyrogallol 100mg/kg i.p./7days+ Brassica nigra 250mg/kg p.o/22days	0.1026±0.001*
5.	Test 2	Pyrogallol 100mg/kg i.p./7days+ Brassica nigra 500mg/kg p.o/22days	0.1032±0.001*
6.	Test 3	Pyrogallol (100mg\kg – I.P/7 days)+ ethanolic extract of *Brassicanigra (25*0mg/kg) + *Cuminum cyminum (*250 mg/kg) p.o/22 days	0.1015±0.005*

All values are shown as mean ± SEM and n=6. # indicate $p< 0.05$ when compared to normal group.
* indicate $p< 0.05$, ** indicate $p<0.01$, *** indicate $p<0.001$, when compared to control group.

Immunological Parameters

Effect of Brassica nigra and Cuminum cyminum on humoral, cellular immune response, carbon clearance and WBC count: Animals treated with pyrogallol (100mg/kg, i.p.) alone for seven days showed a significant (p<0.001) decrease in the primary and secondary humoral immune responses, phagocytic responses, decrease in the WBC count when compared to normal group. Treatment with pyrogallol (100mg/kg, i.p.) alone for seven days had not shown any increase in paw volume after the administration of SRBC (0.025×109 cells) when compared to normal group. Treatment with septilin syrup (1ml/100g, p.o.) for 22 days significantly (p<0.001) prevented the influence of pyrogallol on primary and secondary humoral immune responses, increased the paw volume (index of cell-mediated immunity), increased the phagocytic response, increased the WBC count when compared to control group. Animals receiving low dose of *Brassica nigra* (250mg/kg, p.o.) for 22 days had not shown any significant influence on the primary humoral immune response but significant (p<0.001) influence on the secondary humoral immune response was observed when compared to control group. Animals receiving low dose of *Brassica nigra* (250mg/kg, p.o.) for 22 days significantly (p<0.05) increased the paw volume, increased the phagocytic response, not shown significant influence on the WBC count when compared to control group. Administration of high dose of *Brassica nigra* (500mg/kg, p.o.) for 22 days significantly (p<0.001) prevented the influence of pyrogallol on primary and secondary humoral immune responses, increased the paw volume, increased the phagocytic response, increased the WBC count when compared to control group. Administration of equal proportionate dose of *Brassica nigra* (250mg/kg, p.o.) and Cuminum cyminum (250mg/kg, p.o.) for 22 days significantly (p<0.01) prevented the influence of pyrogallol on primary and secondary humoral immune responses, increased the paw volume, increased the phagocytic response and increased the WBC count when compared to control group and increase in paw volume is an index of cell-mediated immunity.

In vivo antioxidant parameters:

Effect of Brassica nigra on super oxide dismutase (SOD) Treatment with pyrogallol (100 mg/kg, i.p.) (G-II) showed a significant (p<0.01) decrease in SOD when compared to normal, treatment with septilin syrup (1ml/100g, p.o.) (G-III) significantly (p<0.01) increased the SOD and treatment with low and high dose of *Brassica*

nigra significantly increased the SOD respectively $p<0.05$ and $p<0.001$ in a dose dependent manner when compared with control.

Effect of Brassica nigra on catalase

Treatment with pyrogallol (100mg/kg, i.p.) (G-II) showed a significant ($p<0.001$) decrease in catalase when compared to normal, treatment with septilin syrup (1ml/100g, p.o.) (G-III) significantly ($p<0.001$) increased the catalase and treatment with low and high dose of *Brassica nigra* significantly increased the catalase respectively $p<0.001$ and $p<0.001$ in a dose dependent manner when compared with control .

Effect of Brassica nigra on reduced glutathione (RG)

Treatment with pyrogallol (100mg/kg, i.p.) (G-II) showed a significant ($p<0.001$) decrease in reduced glutathione when compared to normal, treatment with septilin syrup (1ml/100g, p.o.) (G-III) significantly ($p<0.001$) increased the reduced glutathione and treatment with low and high dose of *Brassica nigra* significantly increased the reduced glutathione respectively $p<0.001$ and $p<0.001$ in a dose dependent manner when compared with control.

Effect of Brassica nigra on lipid peroxidation (LPO)

Treatment with pyrogallol (100mg/kg, i.p.) (G-II) showed a significant ($p<0.05$) increase in lipid peroxidation when compared to normal, treatment with septilin syrup (1ml/100g, p.o.) (G-III) significantly ($p<0.05$) decreased the lipid peroxidation and treatment with high dose of *Brassica nigra* significantly ($p<0.05$) decreased the lipid peroxidation when compared with control but low dose of *Brassica nigra* had not shown any significant influence on the lipid peroxidation. The alcoholic extract of *Brassica nigra* not only attenuated the influence of pyrogallol on the immune system but was also found to prevent the changes in the oxidative stress parameters, which were induced by pyrogallol. The equal proportionate dose of *Brassica nigra and Cuminum cyminum* also significantly attenuated the influence of pyrogallol on the immune system. *Brassica nigra* was reported to possess isothiocyanate glycosides and fatty acids. Thus, the immunomodulatory activity of *Brassica nigra* could be due to its glycosidal and fatty acid components. These components may exert its immunomodulatory activity by altered membrane composition and changed cytokine biosynthesis.

CONCLUSIONS

Ethanolic extract of *Brassica nigra* prevented the influence of pyrogallol on immunological parameters and antioxidant parameters, which may due to the presence of fatty acids which will stimulate the immune system by altering the production of eicosanoids[9]. It can also be postulated that the presence of minerals which will increase the activity of antioxidants like SOD, catalase and the presence of isothiocyanate glycosides which chelate the metals that otherwise catalyse the oxidative chain reactions[10]**.** The equal proportionate dose of ethanolic estract of Brassica nigra and Cuminum cymium also showed a significant influence of pyrogallol on immunological parameters. Future studies are required for establishing *Brassica nigra and Cuminum cymium* as a therapeutic intervention for immunomodulatory activity by isolating its active principles.

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