PRECLINICAL EVALUATION OF IMMUNOMODULATORY POTENTIAL OF NUTRACEUTICAL FORMULATION NC30 IN SPRAGUE DAWLEY RATS

Avula Manikanta Reddy[1], Prof. Chakka Gopinath[1*], N.Yamini[2], O.Sreenivasa Krishna[3]

[1,1*,3] *Department of Pharmacology, JNTUA-Oil Technological and Pharmaceutical Research Institute, Jawaharlal Nehru technological university Anantapur, Andhra Pradesh-515001*

[2,3] *Assistant professor Department of Pharmacology, JNTUA-Oil Technological and Pharmaceutical Research Institute, Jawaharlal Nehru technological university Anantapur, Andhra Pradesh-515001*

[1*] *Professor, Department of Pharmacognosy, JNTUA-Oil Technological and Pharmaceutical Research Institute, Jawaharlal Nehru technological university Anantapur, Andhra Pradesh-515001*

ABSTRACT

The immunomodulatory potential of NUTRACEUTICAL FORMULATION NC30 which included the different plant products { i.e., jower, foxtailmillet, littlemillet, fingermillet, pearlmillet, saggubiyyam, barley, barnyardmillet, porsomillet, greengram, soyabean, blackeyedbean, bengalgram, horsegram, redgram, blackgram, toordal, maize, sunflowerseeds, flaxseed, pumpkinseeds, almond, groundnut, cashewnut, drydates, elachi, pepper} are collected,dryed and powered is used as test drug. The Acute toxic studies andVarious pharmacological screening models like delayed hypersensitivity,Humoral antibody (HA) titer,Total leukocyte count ,Determination of total serum protein, were employed in Sprague dawley rats for hypersensitivity and hemagglutination reactions, using sheep red blood cells (SRBC) as the antigen. Distilled water served as a control in all the tests.

The NUTRACEUTICAL FORMULATION NC30 exhibited a significant increase in the percentage phagocytosis versus the control. In the *in vivo* studies, was found to exhibit a dose related increase in the hypersensitivity reaction, to the sheep red blood cells (SRBC) antigen, at concentrations of 200 and 400 mg/kg. It also resulted in a significant increase in the antibody titer value, to SRBC, at doses of 200 and 400 mg/kg in animal studies and found to stimulate cell mediated and antibody mediated immune responses in rats.

KEY WORDS: Hypersensitivity,phagocytosis,Hemagglution,Sheep red blood cell.

Introduction

The immune system is one of our most complex biological systems in the body. The basic role of the immune system is to distinguish self from non-self1 . This non-self could be an infectious organism, a transplanted organ or an endogenous cell that can be mistaken as a foreign. The immune responses of the human body against any non-self are of two types: (a) innate (or natural or non-specific) and (b) adaptive (or acquired or specific). Immune system disorders results in autoimmune diseases, inflammatory diseases, cancer and immunodeficiency.

Immunomodulators are the biological or synthetic substances, which can stimulate, suppress or modulate any of the immune system including both adaptive (humoral and cell mediated) and innate arms of the immune response. Immuomodulators with enhanced

immune reaction is called as an immunostimulative drug which primarily implies stimulation of non-specific system i.e., granulocytes,macrophages, complement, certain T-lymphocytes and different effector substances. Immunosuppressant implies mostly to reduce resistance against infections, stress and may occur on account of environmental or chemotherapeutic factors. Many proteins, amino acids, and natural compounds have shown a significant ability to regulate immune responses, including interferon-γ (IFN-γ), steroids. A number of Indian medicinal plants have been claimed to possess immunomodulatory activity and use of plant derived products as immunomodulators is still in a developing stage.

A variety of plant-derived compounds such as polysaccharides, lectins, peptides, flavonoids, tannins, sterols and sterolins have been reported to modulate the immune system. Since ancient times, several diseases have been treated by administration of plant extracts based on traditional medicine. The natural immunomodulators act to strengthen weak immune systems, about three quarters of the world population relies on the plants and plant extracts for healthcare. India has an extensive forest cover, enriched with plant diversity.

Nutraceutical Formulation NC30 which included the different plant products { i.e., jower, foxtailmillet, littlemillet, fingermillet, pearlmillet, saggubiyyam, barley, barnyardmillet, porsomillet, greengram, soyabean, blackeyedbean, bengalgram, horsegram, redgram, blackgram, toordal, maize, sunflowerseeds, pumpkinseeds, flaxseed,almond, groundnut, cashewnut, drydates, elachi, pepper} are collected, dryed and powered is used as test drug

Mentioned plants were used in dysentery, diarrhea, diabetes leucorrhoea, menorrhagia, antioxident, nervous disorders, tonic and astringent

2) MATERIALS & METHODS

2.1.Collection of Materials, Chemicals & Drugs:

- The Sheep Red Blood Cells (SRBCs) were procured from local market Hyderabad.
- The Levamisole (Cipla Limited- India) was purchased from local pharmacy, Hyderabad.
- The Humoral antibody tests were performed in Teena laboratories. All chemicals were procured from Teena laboratories,Hyderabad.

2.2.Collection of Nutraceutical formulation NC30:

¬ Date of collection: 15- 01- 2023.
¬ Place of collection: JNTUA-OTPRI.
¬ Time of collection: Early morning hours (10-11 am).

Powder of few seeds which were collected from fields. The plant material was given by**Prof. Chakka Gopinath-M.Pharm., Ph.D., M.Sc.(Psychology)., B.A., PGDEM, (PGDCE)**, **JNTUA-OTPRI**. The drug material was dried under shade for about 14 days, powdered & stored in an air tight container.

2.3.Experimental Animals

The experiment was carried out by using Sprague Dawley Rats, which were procured from central animal house of the Teena labs, bachupally, hyderabad. The experimental protocol has been approved by institutional animal ethical committee, hyderabad. Rats of Sprague Dawley strain weighing between 150 to 200 gm were maintained under standard laboratory conditions. They were provided with a standard diet supplied by Pranav agro industries Ltd India.

2.4.Acutetoxicitystudy

Acute toxicity for*Nutraceutical formulation NC30*will be done according to theoffice of pollution prevention and toxics (OPPT) The overnight fasted rat is weighedand selected. The extracts will be dosed in a stepwise procedure, by using up anddownorstaircasemethod.Thetwoanimalsselectedwithadoseof50mg/kg.Orally and examined for 24h for mortality. Subsequent dose are then increased toattainmaximumnonlethalandminimumlethaldose.TheNC30 wasfoundtobesafeatthedoseof5g/kgperoral.Maximumsafedose(5g/kg)correspondingto500mg/kgand 100 mg/kgwereselected as high andlow doses respectively.

***2.5.In vivo* immunomodulatory activities**

2.5.1)Delayed Type Hypersensitivity (DTH) Response

For the evaluation of delayed type of hypersensitivity (DTH) test animals were divided in to four groups, having six animals in each. Group I, the control, was given 2ml of 5% normal saline and to group II.III was administered of 200 mg and 400 mg/kg body weight of *Nutraceutical formulation NC30* orally for ten days. On 10th day 0.1ml of SRBC solution was injected subcutaneously in to the right footpad. After 24,48,72,96 hrs, thickness of footpad was measured by plethisometer. Difference in the footpad thickness in control and treated group has been taken as the measure of the DTH reaction **(Dikshit *et al.,* 2000)**

2.5.2) Humoral Antibody Titre

The animals were immunized by injecting 0.1 ml of SRBCs suspension, containing 1X 10 8 cells, intraperitonially, on day 0. Blood samples were collected in micro centrifuge tubes from individual animals of all the groups by retro orbital vein puncture on day 10. The blood samples were centrifuged and the serum separated. Antibody levels were determined by the hemagglutination technique.

Method for Serial dilution

This was performed by using 96 wells (12x8) U bottomed titre plate. The wells were marked from I to XII. In the first (I) and last well (XII) 25 microliter of serum collected from treated animals was added and inactivated at 56 degree Celsius for 30 minutes.

Afterwards to all the wells except well number XII, 25 microliter of PBS was added.25 microliter was taken from first well and added to 2nd well again 25 microliter from second well was taken and added to third well and continued the same procedure up to well number XI. After this 25 microliter of sample from well number XI was discarded. Finally 25 microliter of 1% SRBC was added to all the wells and was kept at room temperature for two hours **(Vinod S Pawar *et al.,* 2012)**

2.5.3)Total Leukocyte Count

W.B.C diluting pipette: It has got three graduations. Two graduations 0.5 and 1 are present on the stem of the pipette and the third mark 11 is placed just above the bulb. Blood is drawn up to mark 0.5 and the rest of the bulb is filled by sucking up diluting solution up to the mark 11, the bulb of the pipette is so constructed that it holds exactly 20 times the volume of fluid contained in the stem of the pipette up to mark 1.Although fluid is drawn up to 11, the dilution of the blood will be 20 because the last part of the fluid remains locked up in the stem and is not available for dilution

The Counting Chamber

The ruling area consists of 9 square millimeters. The central of the smallest squares are separated by triple lines in which RBC will be counted. The side of each square for counting WBC is ¼ mm.

Diluting Fluid for WBC (Turks fluid)

Commonly the fluid is made up as follows

1. Glacial acetic acid : 1.5ml
2. 1%solution of gentian violet in water : 1ml
3. Distilled water : 98ml

The glacial acetic acid haemolysis the red cells, while the gentian violet stains the nucleus of leukocytes

Method of Counting W.B.C

The white cells are counted in four corners of 1 square millimeter ruled area on both sides. The white cells are recognized by the retractile appearance and by the slight color given to them by the stain contained in the diluting fluid. The cells touching the left side and upper side of boundary line are not counted.

Calculations

Theareaof thesmallest	=	1/16mm3square
Volume ofsmallest square	=	1/160 mm3
Totalnumberof squarecounted	=	16×4=64
Totalnumber ofcells counted	=	X
64/160 mm3ofdiluted bloodcontains	=	Xcells
So,1mm3 ofdilutedblood contains	=	160/64 × Xcells
1mm3ofundiluted bloodcontains	=	160/64 × 20 ×Xcells.

2.5.4 Determination of Total Serum Protein

Total Protein (Biuret Method):

Total Protein-To exactly 4 cc. of 10 per cent sodium hydroxide in a 10 ml standard flask and add 0.1 cc. of fresh serum with a Folin micropipette. Rinse out the pipette three times with sodium hydroxide solution.

Mix by rotating and add 0.5 cc. of 1% copper sulphate. Shake vigorously five to six times. Allow to stand for 25 minutes and absorbance read in a U.V Spectrophotometer at 540 nm.

STATISTICAL ANALYSIS:

The resulting experimental data were statistically analysed using Graph Pad prism 3 software and MS excel was used for statistical analysis of data. All the results were expressed as mean±standard error of mean (SEM), analyzed for ANOVA and Dunnet's t-test (Multiple). Differences between groups were considered significant at $p<0.05$, $p<0.01$ levels.

3.Results and discussion:

3.1.Acutetoxicitystudy

For toxicity studies, Nutraceutical formulation NC30 was administered orally to thethree groups having two rats in each with graded doses (50mg/kg-500mg/kg bodyweight).Mortalityrateswereobservedafter7days**(Choudhary *et al.*, 1997)**

Table3.1: Determination of acute toxicity ofNutraceutical formulation NC30

Sl.No	Dose(mg/kg.Bodyweight)	Percentmortality
1	50	0
2	100	0
3	150	0
4	200	0
5	250	0
6	300	0
7	350	0
8	400	0
9	450	0
10	500	0

3.2.Delayed Type Hypersensitivity (DTH) Response

The effect of test drug&standard drugs on the DTH response in Sprague Dawley Rats using SRBCs as antigen, administration of Nutraceutical formulation NC30 at the dose of 200mg/Kg & 400mg/Kg & Levamisole 50mg/Kg treatments which were given orally. After 24, 48, 72, 96 hrs showed significant increase in paw edema compared to control group.

Table3.2 Effect of Nutraceutical formulation NC30 on delayed type of hypersensitivity response

Sl. No	Groups	Pawvolume (mm)			
		24 Hrs	48 Hrs	72 Hrs	96 Hrs
I	Control	1.43±0.027	0.72±0.019	0.41±0.013	0.16±0.012
II	Nutraceutical formulation NC30 (200 mg/kg body weight)	1.50±0.011	0.88±0.018	0.51±0.015	0.20±0.021*
III	Nutraceutical formulation NC30 (400 mg/kg body weight)	1.54±0.016	0.93±0.019	0.57±0.012	0.23±0.014**
IV	Standard-Levamisole (50 mg/kg body weight)	1.58±0.010	1.02±0.029	0.65±0.021	0.32±0.016***

n= 6. Tabulation values represents mean ± SD (*P<0.05, **P<0.025, ***P <0.001)

3.3 Humoral Antibody Titre

Administration of Nutraceutical formulation NC30 at the dose of (200 & 400 mg/kg) & Levamisole 50mg/Kg treatments which were given orally for 14 days showed highly significant increase in antibody titre values compared to control group. The results are shown in below table 3.3.

Table3.3Effect of Nutraceutical formulation NC30 on Humoral Antibody titre

Sl. No	Group	Humoral antibody titre
I	Control	11±1.0210
II	Nutraceutical formulation NC30 (200 mg/kg body weight)	337.47±2.0401*
III	Nutraceutical formulation NC30 (400 mg/kg body weight)	412±1.5010**
IV	Levamisole (50 mg/kg)	461± 2.6861***

n=6, humoral antibody titre value mean ± SEM(*P<0.05,**P<0.025,***P <0.001)

3.4 Total Leukocyte Count

The effect of test drug&standard drugs on Total Leukocytes in Sprague Dawley Rats , administration of Nutraceutical formulation NC30 at the dose of (200,400 mg/kg) & Levamisole 50mg/Kg treatments which were given orally for 14 days. The low dose (200 mg/kg) show effect on TLC count compared to control group, whereas the 400mg/Kg &standard drug Levamisole 50mg/Kg showed significant increase in total leukocytes count values compared to control group. The results are shown in below table 3.4.

Table3 .4 Effect of Nutraceutical formulation NC30 on Humoral Antibody titre.

Sl. No	Group	Mean Leukocyte count
I	Control	5.01×103 cu.mm± 0.2640
II	Nutraceutical formulation NC30 200 mg/kg	6.93×103 cu.mm± 0.2461*
III	Nutraceutical formulation NC30 400 mg/kg	9.56×103 cu.mm± 0.3101**
IV	Levamisole 50 mg/kg	15.01×103 cu.mm± 0.1381***

n= 6, total leukocyte count means ±SEM (*P<0.05,**P<0.025,***P <0.001)

3.5 Determination of Total Serum Protein

The effect of test drug&standard drugs on Total serum protein in Sprague Dawley Rats , administration of Nutraceutical formulation NC30 at the dose of (200,400 mg/kg) & Levamisole 50mg/Kg treatments which were given orally for 14 days. The low dose (200 mg/kg) & large dose (400 mg/kg),standard drug Levamisole 50mg/Kg showed significant increase in total serum values compared to control group. The results are shown in below table 3.5.

Table 3.5. Effect of Nutraceutical formulation NC30 on total serum protein

Sl. No	Group	Total serum protein (g/200 ml)
I	Control	7±0.1201
II	Nutraceutical formulation NC30 200 mg/kg	8.5±0.952
III	Nutraceutical formulation NC30 400 mg/kg	10.6±0.1012
IV	Levamisole 50 mg/kg	14.1±0.010

n= 6, total serum value means ± S,D

4.CONCLUSION

Many of the above mentioned plants have been reported for immunomodulatory activity. However,combination of 30 types of plant nutritional dried material is not been reported for the same.Physicochemical evaluation studies showed the properties of dried Nutraceutical formulation properties. Acute toxicity study was done and no mortality reported at doses between 50mg/kg to 500mg/kg. Determined Delayed type hypersensitivity response show significance paw volume of lower and higher concentration as compared to control. Evaluation of Humoral antibody titre value showed highly significant increase in antibody titre values compared to control group.Determined the low dose of test drug(200 mg/kg) show effect on TLC count compared to control group .Estimation of total serum protein show significant increase of total serum value compared to control. **Nutraceutical formulation NC30 showed moderate immunomodulatory activity compared with standard drug.**

5.Reference

1.Nutraceuticals in Immune School of Dentistry and Medical Sciences, Charles Sturt University, Bathurst, NSW 2795, Australia; sooi@csu.edu.au* Correspondence:

2.Immune boosting functional foods and their mechanisms A critical evaluation of probiotics and prebiotics journal homepage: www.elsevier.com/locate/biopha

3. Therapeutic Potential of Nutraceuticals and Dietary Supplements in the Prevention of Viral Diseases Front. Nutr., 17 September 2021

4. Nutraceuticals: A Review on current status Namdeo Shinde1, Bhaskar Bangar1,Sunil Deshmukh1, Pratik Kumbhar2.1Department of Pharmaceutics, Satara College of Pharmacy, Satara- 415004, (MS) India. 2Department of Pharmaceutics, Yashoda Technical Campus, Satara- 415011, (MS) India. *Corresponding Author E-mail: pr.shindenamdeo@gmail.

5. New Concepts in Nutraceuticals as Alternative for PharmaceuticalsHamid Nasri,Azar Baradaran1, Hedayatollah Shirzad2, Mahmoud Rafieian-Kopaei

6. A REVIEW ON NUTRACEUTICALS: CLASSIFICATION AND ITS ROLE IN VARIOUS DISEASES Garima Verma* and Manoj Kumar Mishra Shambhunath Institute of Pharmacy, Jhalwa, Allahabad, Uttar Pradesh-211012, India.

7.Essential Oils in Food Preservation, Flavor and Safety 2016, Pages 295-301 Chapter 33 - Cardamom (Elettaria cardamomum Maton) Oils Author links open overlay panelFarooqAnwar12Anwar-ul-HassanGilani56
https://doi.org/10.1016/B978-0- 12416641-7.00033

8.https://www.researchgate.net/publication/282907291_Nutritional_composition_of_raw_fresh_cashew_Anacardium_occidentale_L_kernels_from_different_origin

9. Medicinal properties of cardamom Elettaria cardamomum VS Korikanthimathm, D Prasath and Govardhana Rao Indian Institute of Spices Research, Cardamom Research Center, Appangala Madikeri, Kodagu, Karnataka, India.

10. Ahmad Ateeq et al / Int. J. Res. Ayurveda Pharm. 4(3), May – Jun 2013

11. Zarqa et.al Medicinal Uses of Cashew (Anacardium occidentale)

12. Nutraceutical snack pr aceutical snack prepared from sprouted r outed rough rice and gr ough rice and green gram and its physicochemical and its physicochemical properties and in vitro glycemic index

13. Horse gram- an underutilized nutraceutical pulse crop: a review Saroj Kumar Prasad & Manoj Kumar Singh

14. PEARL MILLET: A FUNDAMENTAL REVIEW ON UNDERUTILIZED SOURCE OF NUTRITION Monika Satankar 1* , Amit Kumar Patil 2 , Sheshrao Kautkar 3 and Utkarsh Kumar4 1 PhD Scholar, Division of Agricultural Engineering,ICAR- IARI, (New Delhi)

15. Ali Esmail Al-Snafi Chemical Constituents and Pharmacological Activities of Arachis hypogaea.

16. Medicinal and biological potential of pumpkin doi:10.1017/S0954422410000107

17. Preventive and Therapeutic Role of Functional Ingredients of Barley Grass for Chronic Diseases in Human Beings Yawen Zeng, Xiaoying Pu, [...], and Tao Yang