CURCUMA AMADA ROXB. RHIZOME EXTRACT MODULATES CELLULAR AND HUMORAL IMMUNE SYSTEM

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Summary

Immunomodulatory effect of *Curcuma amada*Roxb.was assessed using In-vitro Phagocytosis activity, delayed type hypersensitivity test and Hem agglutination test. Extraction of plant rhizome was performed using petroleum ether, chloroform, acetone and alcohol. Alcoholic extract showed maximum effect in in-vitro assay thus it was used for in-vivo investigation at 400 mg/kg, 200 mg kg and 100 mg/kg. Extract showed significant immunomodulatory effect at all selected dose.

Key words: *Curcuma amada* Roxb., Phagocytosis, Hemaggluination, immunomodulatory

Introduction

Any components which can alter immunity are considered as immunomodulators. They may either amplify or inhibit immunity. Medicinal plants and their active components have been shown to be an important source of immunomodulators. Effect of components from herbal origin on immunity is well documented [1]. Many herbs, e.g. *Hibiscus rosasinensis*linn. [2], *Cleome gynandra*linn[2], *Pouteriacambodiana*[3], *Clausenaexcavate*[4], *Acacia catechu* [5], *Moringaoleifera*[6], *Tinosporacordifolia*[7] *Terminaliabellerica*[8],*Alternantheratenella*colla[9] are reported to possess significant immunomodulatory activity.

Curcuma amada Roxb. is commonly known as mango ginger. It is a perennial, rhizomatous, aromatic herb belonging to the family Zingiberaceae. It is reported to possess therapeutic potential for its antioxidant activity [10], antibacterial activity[11], antifungal activity[12], anti-inflammatory activity[13], platelet aggregation inhibitory activity and cytotoxicity[14]. It consist of various bioactive chemical components like starch, phenolic acids, volatile oils, curcuminoids and terpenoids like difurocumenonol, amadannulen andamadaldehyde[15]. It is reported that *Curcuma amada* is used traditionally in Chandaka denudated forest patches of Bhubaneswar, Orissa, India, for treatment of various disease [16].

Materials and methods

Chemical and reagents

The minimum essential medium (MEM), bovine serum albumin and FicollHypaque were procured from Hi-Media Lab Pvt. Ltd., Mumbai. *Candida albicans* (ATCC-10231) was used for bioassay. All chemical and reagents were of analytical grade.

Sheep Red Blood Cells (SRBC)

SRBC was collected in Alsevier's solution, and were used for immunization in a concentration of 5 x 10^9 cells/ml.

Animals

Albino wistar rats weighing 200 ± 30 of either sex were selected at random from animal house of PBRI, Bhopal, India. Animals were further randomly divided into various treatment groups and kept in propylene cage with sterile husk as bedding. Animals were housed in relative humidity of 30.7 % at 22 ± 2 °C and 12:12 light and dark cycle. Animals were fed with standard pellets (Golden feeds, New Delhi, India) and water was available *ad libitum*. All animal experiments were approved by Instutional Animal Ethics Committee (IAEC) of PBRI, Bhopal (CPCSEA Reg No. - 1283/c/09/CPCSEA).

Polymorphonuclear leucocytes (PMN cells)

Healthy rats weighing 200 ± 30 were used for collection of PMN cells. Presence of any microbial contamination was strictly avoided.

Extract preparation

Rhizomes of *C.amada* was collected locally near Bhubaneswar region. Herbarium of plant was submitted, and authenticated by Dr. Ziaul Hasan, Botanist, Department of Botany, Safia Science College. Rhizome was washed with water and shade dried. Dried rhizomes were crushed and extracted with petroleum ether (CPEE), chloroform (CCE), acetone (CAE) and ethanol (CEE) successively in soxhlet apparatus. The extract was dried in rotary vacuum evaporator and collected in air tight container for further use.

Phagocytic assay (PMN function test)

FicollHypaque density gradient method was used for isolation of neutrophils from blood collected in heparinised tube from peripheral vein of healthy rats. Afterward dextral sedimentation was performed and supernatant with more than 90 % PMN cells were collected and used cell density was 1 x 10^6 cells/ml in MEM.*Candida albicans* in concentration of 1 x 10^6 cells/ml was used for present study.PMN cells and *C.albicans* were mixed and incubated in 5 % CO_2 at 37°C with different extract at different concentration. After incubation cytosmears were prepared and fixed in methanol and stained with Giemsa stain. Prepared slides were observed under 100X magnification for determination of phagocytic activity. Hundred neutrophil were analyzed and it was how many cells ingested microorganism. Percentage of PMN cells involved in phagocytosis (percentage phagocytosis) and ratio of number of microorganism engulfed to the total number of neutrophils (phagocytic index) was calculated for ascertaining *in-vitro* phagocytic activity.

Acute toxicity study
Acute oral toxicity study was performed as per OECD 423 guidleines. Extract (CEE) was given upto the maximum dose of 2000 mg/kg and animals were observed for mortality.

Delayed type hypersensitivity test
To measure cellular immunity delayed type hypersensitivity test was performed. SRBC was used as antigen in present study. In group I (six rats) vehicle was administered orally for five days. In group IV, III and IICEE was administered orally at the dose of 400 mg/kg, 200 mg/kg and 100 mg/kg respectively for five days. CEE and vehicle was administered on each two day before immunization, on the day of immunization and on each two day after immunization (i.e. -2,-1,0,+1+2). Rats were immunized by injecting 0.1 ml of SRBS subcutaneously into the right hind paw on day zero. Animals were challenged seven day later with same amount of SRBC into the left hind paw. Change in paw thickness was measured using digital caliper at 4^{th} and 24^{th}hour after challenge.

Hemagglutination reaction
To measure humoral immunity hemagglutination test was performed. In group I (six rats) vehicle was administered orally for five days. In group IV, III and II CEE was administered orally at the dose of 400 mg/kg, 200 mg/kg and 100 mg/kg respectively for five days. CEE and vehicle was administered on each two day before immunization, on the day of immunization and on each two day after immunization (i.e. -2,-1,0,+1+2). Rats were immunized by intraperitonealinjection of 0.5 ml SRBC. On the tenth day after immunization blood sample was collected by retro orbital puncture. Hemaggluination titer assay was performed for antibody level determination. Serial dilution of serum and 0.1% bovine serum albumin (100 µl: 100 µl) was prepared in sterile saline. One volume (100 µl) of 0.1 % SRBC in saline was added and mixed. They were allowed to settle at room temperature for 90 min till control tube showed a negative pattern (a small button formation). The value of highest serum dilution showing visible hemagglutination was considered as antibody titer.

Biostatical analysis
All data were analysed by One Way ANOVA followed by Dunnet's test. $P<0.05$ was considered as level of significance.

Results

Different extracts of *Curcuma amada* was investigated for its effect on PMN associated phagocytosis, observations are presented in Table 1. It was observed that chloroform extract of *Curcuma amada* (CCE) produced significant effect ($p<0.05$) on percentage phagocytosis at 2.0 and 3.0 mg/ml concentration, but at 1.0 mg/ml concentration effect was non significant ($p>0.05$) as compared to that of control group. Ethanolic extract of *Curcuma amada* (CEE) produced significant effect ($p<0.05$) on percentage phagocytosis at all selected concentration. When extracts were evaluated on the basis of Phagocytic index it was found that chloroform extract (CCE) at all selected concentration produced significant effect on Phagocytic index as compared to that of control group. Acetone extract (CAE) also produced significant effect at 3.0 mg/ml concentration ($p<0.05$) on phagocytic index. Ethanolic extract (CEE) also produced significant effect al all selected concentration of 1.0. 2.0 and 3.0 mg/ml on phagocytc index as compared to that of control group. Thus ethanolic extract showed best effect as compared to other exracts, so it was selected as test sample for further investigations.

Table 1. PMN function test for different extracts of *Curcuma amada*

Sample	Concentration (mg/ml)	Percentage phagocytosis	Phagocytic index
Control	--	28.66±3.66	1.6±0.09
CPEE	1.0	31.67±2.58	1.69±0.11
	2.0	32.5±3.15	1.76±0.12
	3.0	33.33±4.23	1.78±0.13
CCE	1.0	32.67±3.39	1.93±0.10 [a]
	2.0	36.17±1.94[a]	2.05±0.24 [a]
	3.0	38±4.56[a]	2.12±0.32 [a]
CAE	1.0	29.67±5.32	1.74±0.09
	2.0	29.83±3.97	1.85±0.10
	3.0	31±4	1.99±0.28 [a]
CEE	1.0	36.67±3.33 [a]	2.04±0.31 [a]
	2.0	37.5±5.79 [a]	2.12±0.21[a]
	3.0	52.83±8.57 [a]	2.19±0.40 [a]

*All data presented in mean ± SD
a-P<0.05 as compared to control group

In delayed type hypersensitivity (DTH) test it was observed that ethanolic extract (CEE) showed significant effect (p<0.05) at 100 mg/kg, 200 mg/kg and 400 mg/kg as compared to that of vehicle treated group. Results are mentioned in Table 2. Effect of extract was significant at 4th hour as well as on 24th hour.

Table 2. Effect of Ethanolic extract of Curcuma amada (CEE) in hypersensitivity reaction test

Group No	Treatment*	hypersensitivity reaction on[#]	
		4th hour	24th hour
01	Vehicle	0.38 ± 0.06	0.17 ± 0.08
02	CEE (100 mg/kg, p.o.)	0.70 ± 0.09 [a]	0.37 ± 0.05 [a]
03	CEE (200 mg/kg, p.o.)	0.79 ±0.07 [a]	0.46 ± 0.06 [a]
04	CEE (400 mg/kg, p.o.)	0.89 ±0.1 [a]	0.69 ± 0.12 [a]

* Each group consist of six animal
[#] Data presented in Mean ± SD
[a] P<0.05 as compared to vehicle treated group

Ethanolic extract of *Curcuma amada* (CEE) was evaluated for its immunomodulatory by using Hemagglutination antibody titer method. CEE produced significant effect (p<0/05) at 100 mg/kg, 200 mg/kg and 400 mg/kg as compared to that of vehicle treated group. Observation is mentioned in Table 3.

Table 3. Effect of ethanolic extract of *Curcuma amada* (CEE) on Hemagglutination

Group No	Treatment*	Hemagglutination antibody titer[#]
01	Vehicle	193.33 ± 44.96
02	CEE (100 mg/kg, p.o.)	248.5 ± 59.82
03	CEE (200 mg/kg, p.o.)	595.83 ± 252.87 [a]
04	CEE (400 mg/kg, p.o.)	966.33 ± 137.654 [a]

* Each group consist of six animal
[#] Data presented in Mean ± SD
[a] $P<0.05$ as compared to vehicle treated group

Discussion

In fighting against various diseases immune system plays an important role. Modulation of this immune response may help in treating and preventing many diseases. Agents that can modulate immunity in the presence of an impaired immune responsiveness can provide supportive therapy inmany treatment approaches[1].

Phagocytosis is an important part of immunity. It was observed that CEE elevated percentage phagocytosis at all selected dose thus for investigation on humoral immune responsehaemagglutination antibody titre (HAT) methodology was used. Augmentation of the humoral immune response to SRBCs by extract (CEE)evidenced by increase in the antibody titres in the blood of rats. B lymphocytes,plasma cells, IgG and IgM are the components involved in the complement activation, opsonization, and neutralization of toxins[17]. Thus they become an integral part of humoral immune system. Thus from HAT test it can be postulated that CEE showed its effect by modulating any of these factors involvedin humoral immune system.

SRBC-induced delayed type hypersensitivity was used to assess the effect of thefraction on cell-mediated immunity. Cell mediated immunity involves effector mechanisms carried out by T lymphocytes and lymphokines. Cell mediated immunity responses are critical to defence against infectiousorganisms, infection of foreign grafts, tumor immunity and delayed-type hypersensitivity reactions [17]. Thus stimulatory effect of CEE on delayed type hypersensitivity test revelaed that extract was having stimulatory effect on T lymphocytes.

Conclusion

Study concludes that ethanolic extract *Curcuma amada*Roxb. Possess significant immunomodulatory effect and this effect is mediated through stimulation of phagocytosis, B Cells and T cells.

References

1. Wagner H, Proksch A. eds. Immunostimulatory Drugs of Fungi and higher Plants. Economic and Medicinal Plant Research, ed. H. Wagner, H.Hikino, and N.R. Farnsworth. Academic Press: London1985: 113.

2. Gaur K, Kori ML,NemaRK.Comparative Screening of Immunomodulatory Activity of Hydro-alcoholic Extract of *Hibiscus rosasinensis*Linn. andEthanolic Extract of *Cleome gynandra*Linn. Glob J Pharmacol2009; 3 (2): 85-89.
3. Manosroia A, Saraphanchotiwitthay A, Manosroia J. Effects of *Pouteriacambodiana*Extracts on *in Vitro*Immunomodulatory Activity of Mouse Immune System.Fitoterapia 2006; (77): 189–193.
4. Manosroia A, Saraphanchotiwitthay A, Manosroia J. ImmunomodulatoryActivities of Fractions from Hot Aqueous Extract of Wood from *Clausenaexcavate.* Fitoterapia2004; (75): 302–308.
5. Syed I, Mohammed A. ImmunomodulatoryActivity of *Acacia catechu.* Indian J PhysiolPharmacol 2009; 53 (1): 25–33.
6. Sudha P, Syed MBA, Sunil S, Dhamingi and Chandrakala GK. ImmunomodulatoryActivity of Methanolic Leaf Extract of *Moringaoleifera*in Animals.Indian J PhysiolPharmacol 2010; 54 (2): 133–140.
7. Aher VD, Wahi AK. Pharmacological Study of *Tinosporacordifolia*as an Immunomodulator.J CurrPharmRes2010;4 (2): 52-54.
8. Aurasorn S, Pattana S, and Kornkanok I.Effects of *Terminaliabellerica*Roxb. MethanolicExtract on Mouse Immune Response *in vitro*.MjInt J Sci Tech2008;2(02), 400-407.
9. Biella AA, Salvador MJ, Dias DA, MarceloDB, Pereira LS. Evaluation of Immunomodulatory And Anti-Inflammatory Effects and Phytochemical Screening of *AlternantheraTenella*Colla(Amaranthaceae) Aqueous Extracts.MemInstOswaldo Cruz Rio de Janeiro 2008; 103(6): 569-577.
10. Prakash D, Suri S, Upadhyay G, Singh B. Total Phenol, Antioxidant and Free Radical Scavenging Activities of Some Medicinal Plants. Int J Food SciNutr2007;58: 18–28.
11. Chandarana H, Baluja S, Chanda SV. Comparison ofAntibacterial Activities of Selected Species of ZingiberaceaeFamily And Some Synthetic Compounds. TurJ Biol2005; 29: 83–97.
12. Singh G, Singh OP, Maurya S. Chemical and Biocidal Investigations on Essential Oils of Some Indian CurcumaSpecies. ProgCrystal Growth Charact2002; 45: 75–81.
13. Mujumdar AM, Naik DG, Dandge CN, Puntambekar HM. Anti-Inflammatory Activity of *Curcuma Amada*Roxb. in Albino Rats. Indian J Pharmacol2000; 32: 375–377.
14. Policegoudra RS, Aradhya SM. Structure and Biochemical Properties Of Starch From An Unconventional Source - A Mango Ginger (*Curcuma Amada*Roxb.) Rhizome. Food Hydrocoll2008;22:513–519.
15.Policegoudra RS, Aradhya SM, Singh L. Mango Ginger (*Curcuma amada*Roxb.)--A Promising Spice For Phytochemicals And Biological Activities.J Biosci 2001;36(4):739-48.
16. Behera KK, Sahoo S and Patra S. Floristic and Medicinal Uses of Some Plants of Chandaka Denudated Forest Patches of Bhubaneswar, Orissa, India. Ethnobotanical Leaflets 2008; 12: 1043-53.
17. Miller LE, Ludke HR, Peacock JE, Tomar RH. Manual of Laboratory Immunology.(Eds.), Lea and Febiger, London 1991: 1-18.