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IMMUNOSTIMULATORY POTENTIAL OF AN ALCOHOLIC EXTRACT (ACP) FROM *CARICA PAPAYA* AGAINST SRBC IN BALB/C MICE

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ABSTRACT

Administration of an alcoholic extract from the powdered leaves of the plant *Carica papaya* (ACP) was found to stimulate immunological activity in BALB/c mice against T dependent antigen (SRBC). The data obtained in the present study showed oral administration of ACP (50– 200 mg/kg) stimulated the IgM and IgG titre expressed in the form of haemagglutination antibody (HA) titre. It also elicited a dose related increase in the delayed type hypersensitivity reaction (DTH) after 24 and 48 h compared with the control. Besides augmenting the humoral and cell-mediated immune response, it induced macrophage phagocytosis which resulted in a high degree of in vivo carbon clearance. The results in these studies demonstrated the immunostimulatory effect of ACP in a dose-dependent manner with respect to the macrophage activation possibly expressing the phagocytosis.

KEYWORDS: *Carica papaya,* Haemagglutination antibody titre, Delayed type hypersensitivity reaction, Carbon clearance, Phagocytosis.

INTRODUCTION

The survival of an organism in this environment, teeming with a multitude of potentially harmful microorganisms, depends upon its ability to resist a wide variety of infections (Abram, *et al.,* 2007). Indian medicinal plants are a rich source of substances which are claimed to induce paraimmunity, the non-specific immunomodulation of essentially granulocytes, macrophages, natural killer cells and complement functions (Sainis, *et al.,* 1997). The papaya or pawpaw is the fruit of the plant *Carica papaya*, the sole species in the genus *Carica* of the plant family Caricaceae. It is native to the tropics of the Americas, and was first cultivated in Mexico several centuries before the emergence of the Mesoamerican classical civilizations. Papaya fruit is a rich source of nutrients such as provitamin A carotenoids, vitamin C, B vitamins, dietary minerals and dietary fibre. Papaya skin, pulp and seeds also contain a variety of phytochemicals, including natural phenols. Danielone is a phytoalexin found in the papaya fruit. This compound showed high antifungal activity against *Colletotrichum gloesporioides*, a pathogenic fungus of papaya (Echeverri, *et al.*, 1997). Preliminary medical research in animals has confirmed the potential contraceptive and abortifacient capability of papaya, and also found that papaya seeds have contraceptive effects in adult male langur monkeys, and possibly in adult male humans (Lohiya, *et al.*, 2002). Unripe papaya is especially effective in large amounts or high doses. Ripe papaya is not teratogenic and will not cause miscarriage in small amounts. Phytochemicals in papaya may suppress the effects of progesterone (Oderinde, *et al.*, 2002). Convincing phytochemical

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research studies shows that *Carica papaya* a good source of both flavonoids and glycosides that are a rich source of powerful antioxidants. The immune-stimulatory potential of *C. papaya* on immune system has not yet been explored. Therefore, the objective of the present study was evaluation of immunomodulatory activity of an alcoholic extract (ACP) from *C. papaya* against SRBC in BALB/c mice.

MATERIALS AND METHODS

Collection and authentication of plant material

The leaves of plant *Carica papaya* was collected from the field of Department of Silviculture, Nauni University, Solan. The botanical identity was confirmed by Dr. R. Raina, qualified taxonomist from the Department of Forest Products, Dr. Y.S. Parmar University of Horticulture and Forestry, Nauni, Solan (H.P.).

Extraction

The extraction is done through soxhlet apparatus (Soxhlet, 1879). The sample (powder of Carica papaya 50 gm.) was weighed and placed in the thimble made from thick filter paper, which was then loaded into the main chamber of the Soxhlet extractor (Yadav *et al.*, 2008). The extractor was then placed onto a flask containing the extraction solvent (50% methanol 500 ml).The Soxhlet was then equipped with a condenser. The solvent was heated to reflux. The chamber containing the solid material was slowly filled with warm solvent to dissolve some of the desired compound. When the Soxhlet chamber was almost full, the chamber was automatically emptied by a siphon side arm, with the solvent running back down to the distillation flask. This cycle was allowed to repeat many times, over 36 hrs. During each cycle, a portion of the non-volatile compound dissolved in the solvent. The extract was passed through a filter paper. The filtrates were concentrated with a vacuum pump at 40°C, giving a yield of 8.93%, which was stored in universal bottles and refrigerated at 4°C prior to use.

Animals

The study was conducted on male Balb/c mice (18–22 g) procured from CPCSEA approved animal breeder and maintained under standard laboratory conditions: temperature (25±2 °C) and photoperiod of 12 h. Commercial pellet diet and water were given ad libitum. Experiments on animals were performed by the following guidance of Institutional Animal Ethical committee (IAEC).

Phytochemical investigation

Qualitative tests for the presence of plant secondary metabolites such as carbohydrates, alkaloids, tannins, flavonoids, proteins, saponins and glycosides were carried out on extract using standard procedures (Sofowora, 1984).

Immunization schedule

Sheep red blood cells (SRBC) were used as a source of T-dependent antigen. For this purpose, the blood was withdrawn from a healthy sheep in Alsever's solution (Alsever and Ainslie, 1941). SRBC used for immunization were prepared in pyrogen-free normal saline. Mice were divided into eightgroups, each consisted of six animals. ACP at 50, 100 and 200 mg/kg (in 200 µL of 1% gum acacia) was administered orally by gavage for 15 days, daily. The dose volume was 0.2 mL. Control group received 1% gum acacia. Levamisole, a known immune-stimulator reported to augment the antibody response (Tempero, *et al.*, 1995), was given orally as positive control, at a dose of 2.5 mg/kg body weight. All groups were immunized with 0.2 mL of SRBC (5×10^9) per mouse intraperitoneally (i.p.) on day 0 of drug treatment (Sidiq, 2011). Additional three immunized groups, challenged on day 7 with SRBC, were used for DTH and different immunoglobulin and phagocytic assays.

Treatment

Animals were divided into five groups of six animals each: (Group I) normal control, received 1% gum acacia; (Group II)positive control, received levamisole (2.5 mg/kg body weight); (Group III) alcoholic extract of *Carica papaya* (ACP) (50 mg/kg body weight); (Group IV) received ACP (100 mg/kg body weight) and (Group V) received ACP (200 mg/kg body weight).

Positive control, received levamisole Normal and vehicle control mice received 1% gum acacia administered per oral (p.o.). The alcoholic extract (ACP) was dissolved in 1% gum acacia and was administered per oral for 14 days. The dose volume was 0.2 mL.

Haemagglutination antibody (HA) titre

The animals were immunized by injecting 0.2 mL of 10% of fresh SRBC suspension intraperitonially on day 0. Blood samples were collected in micro centrifuge tubes from individual animals by retro-orbital plexus on day 7 for primary antibody titre and day 15 for secondary antibody titre. Serum was separated and antibody levels were determined by the haemagglutination technique (Gupta, *et al.*, 2006). Briefly, equal volumes of individual serum samples of each group were pooled. Two fold dilutions of pooled serum samples were made in 25 µL volumes of normal saline in a microtitration plate to which were added 25 µL of 1% suspension of SRBC in saline. After mixing, the plates were incubated at room temperature for 1 h and examined for haemagglutination under the microscope. The reciprocal of the highest

dilution of the test serum giving agglutination was taken as the antibody titre.

DTH reaction

ACP was administered 2 h after SRBC injection and once daily on consecutive days. Six days later, the thickness of the left hind footpad was measured with a sphero-micrometer (pitch, 0.01 mm) and was considered as a control. The mice were then challenged by injecting 20 µL of 5×10^9 SRBC/mL intradermally into the left hind footpad. The foot thickness was measured again after 24 h (Khajuria *et al.*, 2008).

In vivo carbon clearance by reticulo-endothelial system

Phagocytic function of the RES (reticulo-endothelial system) was assayed in groups of 5 mice each by injecting i.v. 160 mg/kg b.wt. of1.6% suspension of gelatin stabilized carbon particles of 20–25 µm size. Blood samples were collected from the retro-orbital plexus immediately before and at various intervals between 0 and 60 min after carbon injection. An aliquot (10 µL) of blood samples was lysed with 2 mL of0.1% acetic acid and transparency determined spectrophotometrically at a wavelength of 675 nm (Uvikon 810, spectrophotometer, KontronLtd., Switzerland) until transparency equivalent to standard (original pre-injection blood sample) was obtained (Sidiq, *et al.*, 2011).

Statistical analysis

Data are expressed as Mean ± S.E.M. and statistical analysis was carried out using one-way ANOVA (Bonferroni correction multiple comparison test). Dunnett's test was used to analyze the different variables in the same subject and P values less than 0.05 were being taken as statistically significant.

RESULTS

Preliminary phytochemical screening

The phytochemical screening revealed that alcoholic extract contained saponin, alkaloids, cardiac glycosides and carbohydrates.

Effect of ACP on serum antibody (anti-SRBC) titre

Anti-SRBC antibody (IgM and IgG) titers were measured in mice serum of different groups, collected retro-orbitally on 7 and 14 days after immunization and treatment. Anti-SRBC titers increased in mice treated with four doses of ACP 50, 100 and 200 mg/kg after 7 days when compared with control. A similar profile was obtained after 14 days, with IgG predominating over IgM (Tab. 1.)

Effect of ACP on DTH reaction to SRBC

DTH reaction to SRBC is given in Tab. 2, in which data are expressed in terms of the swelling of the footpad. After administration of the ACP (50–200 mg/kg, p.o.), a significant dose-related increase in footpad thickness was found at 24 and 48 h as compared with the control group.

Effect of ACP on carbon clearance

The effect of ACP on carbon clearance is shown in Tab. 3. The effect was observed in peritoneal macrophages of ACP treated mice and the effect was found that carbon get cleared with time.

Table 1. Effect of ACP on HA titre

Treatment	Dose (mg/kg)	Antibody response log-2 titre	
		7^{th} day	14^{th} day
Control	-	6.34 ± 0.21	6.27 ± 0.20
Levamisole	2.5	10.3 ± 0.20**	9.8 ± 0.23**
ACP	50	8.8 ± 0.22*	8.4 ± 0.22*
	100	9.9 ± 0.3**	9.4 ± 0.22**
	200	8.3 ± 0.22*	7.7 ± 0.26*

Values are expressed as Mean ± SEM, n=6,*****p < 0.05, ******p<0.01 compared to control (group 1)

Table 2. Effect of ACP on DTH reaction

Treatment	Dose (mg/kg)	DTH response Paw edema (mm)	
		24 h	48 h
Control	-	0.97 ± 0.03	0.84 ± 0.019
Levamisole	2.5	1.61 ± 0.02**	1.21 ± 0.02**
ACP	50	1.41 ± 0.03*	1.11 ± 0.03*
	100	1.49 ± 0.04**	1.09 ± 0.03*
	200	1.31 ± 0.02*	1.01 ± 0.02*

Values are expressed as Mean ± SEM, n=6,*****p < 0.05, ******p<0.01 compared to control (group 1)

Table 3. Effect of ACP on carbon clearance

Treatment	Dose (mg/kg)	Carbon clearance				
		0 min	15 min	30 min	45 min	60 min
Control	-	0.236 ± 0.02	0.389 ± 0.015	0.362 ± 0.012	0.349 ± 0.017	0.322 ± 0.015
Levamisole	2.5	0.23 ± 0.012	0.382 ± 0.01	0.303 ± 0.014	0.258 ± 0.02*	0.233 ± 0.014**
ACP	100	0.241 ± 0.015	0.392 ± 0.012	0.312 ± 0.015	0.273 ± 0.019*	0.249 ± 0.013**

Values are expressed as Mean ± SEM, n=6,*****p < 0.05, ******p<0.01 compared to control (group 1)

DISCUSSION

In the present study, immunomodulatory potential of ACP was explored extensively on the modulation of both T and B cells in relation to serum immunoglobulins IgM and IgG to T dependent antigen SRBC. Primarily, the antibody response to SRBC was observed by the hemagglutination titre. The augmentation of humoral antibody response to T-dependent antigen (SRBC) reveals the increased responsiveness of macrophages since the antibody production is closely associated with the co-operation of macrophages, T and B lymphocyte responsiveness (Benacerraf, 1978). The T cells in turn participate in the expression of cell mediated immunity contributing to DTH. A DTH reaction is an expression of cell-mediated immunity and plays a role in many inflammatory disorders (Gongora *et al.,* 2000). Treatment with ACP enhanced the DTH reaction, as reflected by the increased footpad thickness compared to the control group, suggesting heightened infiltration of macrophages to the inflammatory site. The present results showed that the ingestion of carbon particles by the peripheral blood leucocytes, killing activity of neutrophils and monocytes increased in ACP-treated mice, showing its potential of enhancing the non-specific immune response.

CONCLUSION

It is clear from this study that ACP played an important role in the modulation of the immune response and thus may have applications in combating various life-threatening infections. Therefore, it could be a drug of choice, effective in treating the diseases where the underlying defect is a T-cell and B-cell deficiency. Further detailed study for immunomodulation is currently under way to isolate the characterized the active principle of the plant extract.

REFERENCES

Abram M, Vučković D, Bubonja M, Dorić M. Immunohistopathological study of *Listeria monocytogenes* infection in pregnant mice. Acta *Microbiologica et Immunologica Hungarica*, 52, 2005, 1-184.

Alsever JB, Ainslie RB. A new method for the preparation of dilute blood plasma and the operation of a complete transfusion service. *New York State journal of medicine*, 4, 1941, 126-131.

Benacerraf B. A hypothesis to relate the specificity of T lymphocytes and the activity of I region-specific Ir genes in macrophages and B lymphocytes. *Journal of Immunology*, 120,1978, 1809-1812.

Echeverri F, Torres F, Quinones W, Cardona G, Archbold R, Roldan J, Brito I, Luis JG, Lahlou UEH. Danielone, a phytoalexin from papaya fruit. *Phytochemistry*, 44, 1997, 255-256.

Gongora L, Manez S, Rosa M, Giner RM, Recio MC, Rios JL. On the activity of trifluoperazine and palmitoyl carnitine in mice: delayed hypersensitivity models. *Life Science*, 66, 2000, 183-188.

Gupta A, Khajuria A, Singh J, Bedi KL, Satti NK, Dutt P, et al. Immunomodulatory activity of biopolymeric fraction RLJ-NE-205 from Picrorhiza kurroa. *International Immunopharmacology*, 6, 2006, 1543-1549.

Khajuria A, Gupta A, Suden P, Singh S, Malik F, Singh J, *et al.* Immunomodulatory activity of biopolymeric fraction BOS 2000 from *Boswellia serrata*. *Phytotherapy Research*, 22, 2008, 340-348.

Lohiya NK, Manivannan B, Mishra PK, Pathak N, Sriram S, Bhande SS, Panneerdoss. Chloroform extract of *Carica papaya* seeds induces long-term reversible azoospermia in langur monkey. *Asian Journal of Andrology*, 4, 2002, 17-26.

Oderinde O, Noronha C, Oremosu A, Kusemiju T, Okanlawon OA. Abortifacient properties of Carica papaya (Linn) seeds in female Sprague-Dawley rats. *Nigerian Postgraduate Medical Journal*, 9, 2002, 95-98.

Sainis KB, Sumariwalla PF, Goel A, Chintalwar GJ, Sipahimalani AT, Banerji A. Immunomodulatory Properties of Stem Extracts of *Tinospora cordifolia*: Cell Targets and Active Principles. (Eds.) Narosa Publishing House, New Delhi, India, 1997, 95.

Sidiq T, Khajuria A, Suden P, Sharma R, Singh S, Suri KA, *et al.* Possible role of macrophages induced by an irridoid glycoside (RLJ-NE-299A) in host defense mechanism. *International Immunology*, 11, 2011, 128-135.

Sofowora A. Medicinal plants and traditional medicine in Africa. *John Wiley, Chichester*, 1984 142-145.

Soxhlet, F. Die gewichtsanalytische Bestimmung des Milchfettes. *Polytechnisches Journal*, 1879, 232-461.

Yadav AV, Kawale LA, Nade VS. Effect of *Morus alba* L. (Mulrerry) Leaves on Anxiety in mice. *Indian Journal of Pharmacology*, 40, 2008, 32-36.