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Inhibition of Innate Immune Responses of Polymorphonuclear Leucocytes and Monocytes by Rhinacanthin-C

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ABSTRACT

Phagocytosis is a pivotal microbicidal function of innate immune cells by which phagocytes would engulf and eliminate invading pathogens. The functioning and efficacy of immune system is altered by many pathogens *e.g.* bacteria and fungi and the nonspecific kind of innate immunity is responsible for providing protection against these invading pathogens. The aim of this study is to assess the inhibitory effect of rhinacanthin-C on phagocytic activities to obtain important insights into its ability to suppress phagocytes. Phagocytosis activity of innate immune cell was monitored using a flowcytometer and myeloperoxidase activity assay was conducted using a myeloperoxidase activity colorimetric assay. Rhinacanthin-C at 100.0 and 6.25 µg/ml significantly showed strong inhibition of phagocytosis with percentage of 26.40% and 30.59% respectively, in comparison with the positive control ($p< 0.001$). A dose-dependent (50.0, 25.0, 12.5, 6.25 and 3.13 µg/ml) inhibition of MPO activity of rhinacanthin-C was observed and show high activity compared to control cells ($p< 0.001$). This finding indicated that rhinacanthin-C was able to suppress human phagocyte response and emphasizing their potency as immunomodulatory agents. Nevertheless, further investigations are needed to elucidate other immunomodulatory responses.

Keywords: macrophage, monocyte, myeloperoxidase, phagocytosis, rhinacanthin-C

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INTRODUCTION

Immune system is responsible for maintaining homeostasis within the body of host. An agent, synthetic or natural that has the potency to suppress or improve both specific and non-specific immunological responses, is termed as immunomodulator. The functioning and efficacy of immune system is altered by many pathogens *e.g.* bacteria and fungi and the nonspecific kind of innate immunity is responsible for providing protection against these invading pathogens[1]. Components of innate immune system *viz* monocytes, macrophages and polymorphonuclear leucocytes (PMNs), performance essential role to eradicate the pathogens[2]. Upon infection, these professional phagocytic cells accumulate at the site of infection and engulf the invaders causing their destruction. Through the activation of myeloperoxidase (MPO) and nicotinamide adenine dinucleotide phosphate-oxidase (NADPH) stimulated phagocytes release reactive oxygen species (ROS)[3]. Under normal situation, ROS are known to participate in destruction of pathogens, which have been considered as the most efficient microbicide mechanisms. Beside their defensive purpose during infections, overproduction of ROS can promote the inflammatory process. It also contributes in the development of various diseases including rheumatoid disorders[4]. Therefore, it needs to discover the inhibitors of reactive oxidants production for the treatment of inflammation. Recently, there is a great interest in investigating medicinal plants having potency in modulating the immune system. Many bioactive compounds derived from medicinal plants such as curcumin, codifolioside A, syringin, phyllantin and hypophyllantin exhibited immunomodulatory activity[5,6].

Rhinacanthus nasutus, a medicinal herb native to Thailand and Southeast Asia, has traditionally been used in the treatment of various disorders including diabetes mellitus[7]. In China and Taiwan it has been consumed as an herbal drink[8]. Rhinacanthin-C, a major phytochemical of *Rhinacanthus nasutus* leaf has been recently reported for some pharmacological activities like antibacterial, antifungal, antiproliferative, anti-inflammatory, antidiabetic and hyperlipidemic and pancreatic protection effects in diabetic rats[9]. However, no information is available about the activity of rhinacanthin-C in modulating phagocytosis of innate

immune response. The aim of this study is to assess the inhibitory effect of rhinacanthin-C on phagocytic activities to obtain important insights into its ability to suppress phagocytes.

MATERIALS AND METHODS

Chemicals and solutions

Rhinacanthin-C (purity ≥98%) was isolated in our laboratory from green leaves of *Rhinacanthus nasutus* as per the method described by Bhushal *et al.* (2014)[10]. Indomethacin (purity >99%) was purchased from Sigma Aldrich (Sigma-Aldrich Chemicals Pvt. Ltd. Bengaluru, India). All other drugs were dissolved in distilled water. The doses for all freshly prepared drug solutions were expressed in terms of their free bases.

Isolation of polymorphonuclear leucocytes and monocytes

The PMNs isolation was conducted as described previously by Septama *et al.* (2018)[11]. Fresh goat blood was obtained from the authorized slaughterhouse. Briefly, the blood was mixed with the mixture of PBS and dextran (1:1) and left for sedimentation at 25 °C for 45 min. PMNs were isolated by centrifugation on Ficoll density gradient and washed with distilled water. PMNs were then collected from the base of the tubes. A modified method was used to isolate monocytes[12]. The blood was mixed with physiological solution in an equal volume. The mixture was then carefully added with lymphoprep and centrifuged (400 × g, 45 min). Monocytes were retained at the medium interface, removed and washed with PBS (250 × g for 10 min) and re-suspended in PBS. The cells suspensions were then counted using heamocytometer and light microscope, then diluted with PBS to adjust approximately 1×10^6 cells/ml.

Phagocytosis assay

Phagocytosis activity of innate immune cell was monitored using a flowcytometer. Briefly, 20 µl samples of rhinacanthin-C (6.25 and 100.0 µg/ml) and 100 µl whole blood were incubated with 20 µl FITC-labelled opsonized *Escherichia coli* for 30 min in shaking water bath (60 rpm/min). Positive control was cells without samples. While cell remained on ice was used as a negative control. After incubation, tubes were immediately kept on ice to stop phagocytosis and added with quenching solution. The cells were then mixed with 2 ml of lysing solution and incubated at room temperature for 20 min. After that, the cells were washed with PBS and then re-suspended in 200 µl DNA staining solution. Phagocytosis was observed using flowcytometer. Percentage of phagocytosis was considered as phagocytic activity[11].

Myeloperoxidase activity assay

The assay was conducted using a myeloperoxidase activity colorimetric assay kit (OxiSelect) Briefly, PMNs suspensions (1×10^6 cells/ml) were incubated with samples at final concentrations of 50.0, 25.0, 12.5, 6.25 and 3.13 µg/ml, at 37 °C for 10 min. The cells were then activated with PMA (8×10^{-7} M) and incubated at 37 °C, 30 min. After that, the cells were centrifuged (450 × g, 10 min). The supernatants were collected to determine MPO activity. Indometachin was used as a positive control. Supernatant obtained from PMA stimulated neutrophil without treatment was considered as 100% of MPO release. Fifty µl supernatant of samples, positive control and normal control were added into 96-wells plate, then 50 µl of reaction mix (40 µl MPO buffer assay + 10 µl MPO substrate) was added to each well. While, 50 µl of the sample background control (40 µl MPO buffer assay + 10 µl dH_2O) was added to background control (PBS). The mixture was then incubated at 25 °C for 30 min. Two microlitre stop mix were added to all samples, positive control, normal control and background control and incubated for 10 min to stop reaction. Fifty µl TNB standard was added 50 µl to all wells. Then, the reading was taken using microplate reader at 412 nm.

Statistical analysis

Results were expressed as mean ± S.E.M. The data were analyzed by one way analysis of variance (ANOVA) tracked by Tukey's multiple comparison tests. Probability values less than 0.05 were considered statistically significant in all the cases.

RESULTS AND DISCUSSION

Phagocytosis is a pivotal microbicidal function of innate immune cells by which phagocytes would engulf and eliminate invading pathogens. Many receptors are competent to initiate engulfment of opsonized bacteria. Fcc receptors, such as FccRII and FccRIII will bind to immunoglobulincoated particles, while complement-opsonized bacteria are recognized by CR1 (CD35) and CR3 (CD11b)[13]. The effect of rhinacanthin-C on phagocytosis was analyzed by flowcytometer. Rhinacanthin-C at 100.0 and 6.25 µg/ml significantly showed strong inhibition with percentage of phagocytosis of 26.40% and 30.59% respectively, in comparison with the positive control ($p< 0.001$). Positive control was the untreated cells at 37 °C in which condition phagocytes enabled to engulf opsonized bacteria. Normal condition at 0 °C was used a negative control. Phagocytosis is essential for effective control of infection. Killing invading bacteria by neutrophil may be critical point of the outcome of inflammation. The result indicates that flavonoids were able to inhibit phagocytosis. This result was supported by a previous work which reported the inhibitory effect of phytoconstituents like artocarpin and artocarpanone on phagocytosis activity against invading bacteria[11] (Table 1).

Myeloperoxidase is a haemic enzyme expressed in PMNs. Upon stimulation by phorbol myristate acetate (PMA), PMNs release MPO, which catalyze the production of hypochlorous acid (HClO), as an important microbicide agent. However, in some inflammation conditions, MPO released induce severe damage to cell membrane and host tissue[14]. Therefore, reducing the activity of MPO might be beneficial to the treatment of inflammation. A dose-dependent inhibition of MPO activity of rhinacanthin-C was observed and show high activity compared to control cells ($p< 0.001$) (Figure 1). The result exhibited the potency of rhinacanthin-C to reduce the deleterious effect of MPO and is supported by several studies. According to previous works, epigallocatechin gallate, eriodictyol, artocarpanone and artocarpin inhibited MPO activity in human neutrophil[11], hence the presented research is supported by previous works which reported the inhibitory effect of phytoconstituents on MPO.

Table 1: Percentage phagocytic activity of rhinacanthin-C on phagocytes.

Sample	Percent phagocytic activity	
	100.0 µg/ml	6.25 µg/ml
Positive control	72.86 ± 0.93	-
Rhinacanthin-C	26.40 ± 0.17*	30.59 ± 0.14*
Negative control	20.95 ± 0.12	-

Results are expressed as Mean ± S.E.M. (n = 3). Data was analyzed by one way repeat measure ANOVA followed by Tukey's multiple comparison test. Significance: *p< 0.001 when compared with positive control group. Positive control was normal cells incubated at 37 °C and negative control was normal cells incubated on ice (0 °C).

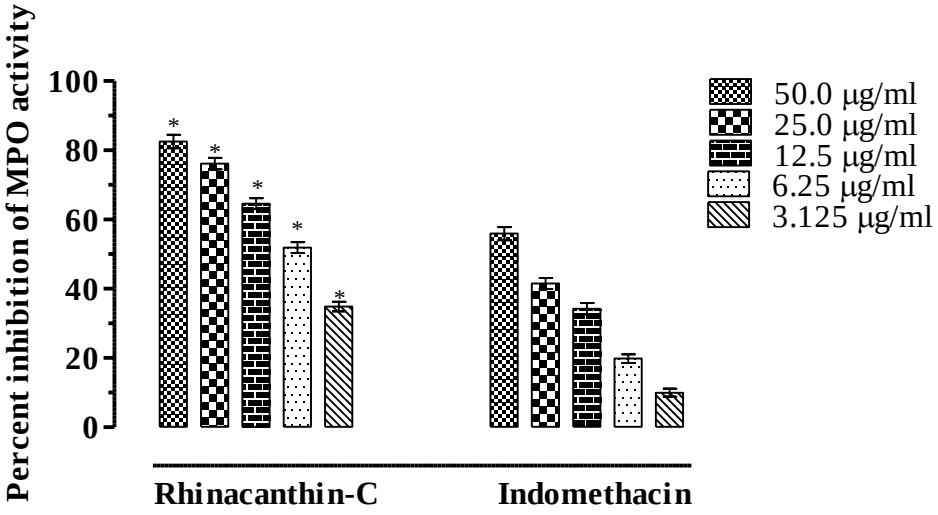

Figure 1: Percentage inhibition of myeloperoxidase activity by rhinacanthin-C in polymorphonuclear leucocytes.

Results are expressed as Mean ± S.E.M. (n = 3). Data was analyzed by one way repeat measure ANOVA followed by Tukey's multiple comparison test. Significance: *p< 0.001 when compared with respective control.

CONCLUSIONS

In conclusion, rhinacanthin-C exhibited strong inhibitory effects at different steps of phagocytosis. This finding indicated that rhinacanthin-C was able to suppress human phagocyte response and emphasizing their potency as immunomodulatory agents. Nevertheless, further investigations are needed to elucidate other immunomodulatory responses.

CONFLICT OF INTEREST

The author has no conflict of interest to declare.

REFERENCES

1. Turvey SE, Broide DH, Innate immunity, J Allergy Clin Immunol, 2010; 125:S346.
2. Underhil DM, Goodridge HS, Information processing during phagocytosis, Nat Rev Immunol 2012; 12:492-502.
3. Vejrazka M, Micek R, Stipek S, Apocynin inhibits NADPH oxidase in phagocytes but stimulates ROS production in nonphagocytic cells, Biochem Biophys Acta, 2005; 1722:143–147.
4. Afonso V, Champy R, Mitrovic D, Collin P, Lomri A, Reactive oxygen species and superoxide dismutases: role in joint diseases, Joint Bone Spine, 2007; 74:324–329.
5. Sharma U, Bala M, Kumar N, Singh B, Munshi RK, Bhalerao S, Immunomodulatory active compounds from *Tinospora cardifolia,* J Enthnopharmacol, 2012; 141:918–926.
6. Yuandani, Ilangkovan M, Jantan I, Mohamad HF, Husain K, Abdul Razak AF, Inhibitory effects of *Phyllanthus amarus* and *Phyllanthus urinaria* and their marker compounds on phagocytic activity of human neutrophils, Evid Based Complement Alternat Med, 2013; 2013:603-634.
7. Brimson JM, Tencomnao T, Medicinal herbs and antioxidants: Potential of *Rhinacanthus nasutus* for disease treatment? Phytochem Rev, 2014; 13:643-51.
8. Huang RT, Lu YF, Inbaraj BS, Chen BH, Determination of phenolic acids and flavonoids in *Rhinacanthus nasutus* (L.) Kurz by high-performance-liquid-chromatography with photodiode-array detection and tandem mass spectrometry, J Funct Foods, 2015; 12: 498-508.
9. Adam SH, Giribabu N, Rao PV, Sayem AS, Arya A, Panichayupakaranant P, Rhinacanthin C ameliorates hyperglycaemia, hyperlipidemia and pancreatic destruction in streptozotocin-nicotinamide induced adult male diabetic rats, Eur J Pharmacol, 2016; 771:173-90.
10. Bhusal N, Panichayupakaranant P, Reanmongkol W, *In vivo* analgesic and anti-inflammatory activities of a standardized *Rhinacanthus nasutus* leaf extract in comparison with its major active constituent rhinacanthin-C, Songklanakarin J Sci Technol, 2014: 36:325-331.
11. Septama AW, Jantan I, Panichayupakaranant P, Flavonoids of *Artocarpus heterophyllus* Lam. heartwood inhibit the innate immune responses of human phagocytes, J Pharm Pharmacol, 2018; 70:1242-1252.
12. Ahmad W, Jantan I, Kumolosasi E, Bukhari SN, Immunostimulatory effects of the standardized extract of *Tinospora crispa* on innate immune responses in Wistar Kyoto rats, Drug Des Devel Ther, 2015; 9:2961–2973.
13. Lee WL, Harrison RE, Grinstein S, Phagocytosis by neutrophils, Microbes Infect, 2003; 5:1299–1306.
14. Tabart J, Frank T, Kevers C, Oincemail J, Serteyn D, Defragne JO, Dommes J, Antioxidant and anti-inflammatory activities of *Ribes nigrum* extracts, Food Chem, 2012; 131:1116–1122.