International Journal of Pharmacy and Integrated Life Sciences
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RESEARCH ARTICLE ISSN: 2320 - 0782 V2-(I10) PG(73-81)

Cell Culture Study On The Effects Of "Cureit"- A Novel Bio Available Curcumin On Boosting Phagocyte Mediated Immunity

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ABSTRACT

Turmeric, is commonly used as a spice in curries, food additive and also, as a dietary supplement. It has been used to treat various illnesses in the Indian subcontinent from the ancient times. Turmeric finds its use in one form or the other in the textile and pharmaceutical industries. Turmeric has been used as a nontoxic drug in Ayurveda for centuries to treat a wide variety of disorders including rheumatism, bodyache, skin diseases, intestinal worms, diarrhea, intermittent fevers, hepatic disorders, as immunity enhancer etc. Curcumin is known to affect the immune response by interacting uniquely with various cells of the immune system. The major drawback of curcumin is its poor bio availability. The novel formulation to enhance the bio availability was successful and it was branded as "cureit". The phagocytic activity of "cureit" was studied in this article.

KEYWORDS : Curcumin, Immunity, phagocytosis, bio availability

Article received on: 25/08/2014 Article accepted on: 05/09/2014

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INTRODUCTION

Turmeric is a spice derived from the rhizomes of *Curcuma longa*, which is a member of the ginger family (*Zingiberaceae*). Rhizomes are horizontal underground stems that send out shoots as well as roots. The bright yellow color of turmeric comes mainly from fat-soluble, polyphenolic pigments known as curcuminoids. Curcumin, the principal curcuminoid found in turmeric, is generally considered its most active constituent . Other curcuminoids found in turmeric include demethoxycurcumin and bisdemethoxycurcumin. In addition to its use as a spice and pigment, turmeric has been used in India for medicinal purposes for centuries. More recently, evidence that curcumin may have anti-inflammatory and anticancer activities has renewed scientific interest in its potential to prevent and treat disease. Traditionally, the turmeric powder was extensively used to enhance the immunity. There are many literatures explains the immunity enhancement potential of curcumin as an active ingredient. Clinical trials in humans indicate that the systemic bioavailability of orally administered curcumin is relatively low and that mostly metabolites of curcumin, instead of curcumin itself, are detected in plasma or serum following oral consumption. In the intestine and liver, curcumin is readily conjugated to form curcumin glucuronides and curcumin sulfates or, alternately, reduced to hexahydrocurcumin. Curcumin metabolites may not have the same biological activity as the parent compound. The bio availability issue was addressed by Aurea Bio labs (A Plantlipids company) and made a highly bio available curcumin formulation known as "cureit". The present article deals with the phagocytic activity of "cureit"

PHAGOCYTE MEDIATED IMMUNITY.

In 1883, Elie Metchnikoff was the first person to demonstrate that cells contributed to the immune state of an animal. He observed that certain white blood cells, which he termed as **phagocytes,** were able to ingest (phagocytose) microorganisms and other foreign material. Noting that these phagocytic cells were more active in animals that had been immunized, Metchnikoff hypothesized that cells, rather than serum components, were major effectors of immunity. The active phagocytic cells identified by Metchinikoff were blood monocytes and neutrophils.

MONONUCLEAR PHAGOCYTES.

The Mononuclear phagocytic system consists of **monocytes** circulating in the blood and **macrophages** in the tissues. During hematopoieses in the bone marrow, granulocyte-monocyte progenitor cells differentiate into promonocytes, which leave the bone marrow and enter the blood, where they further differentiate into mature monocytes. Monocytes circulate in the bloodstream for about 8 hours, during which they enlarge, then they migrate into the tissues and differentiate into specific tissue macrophages or into dendritic cells.

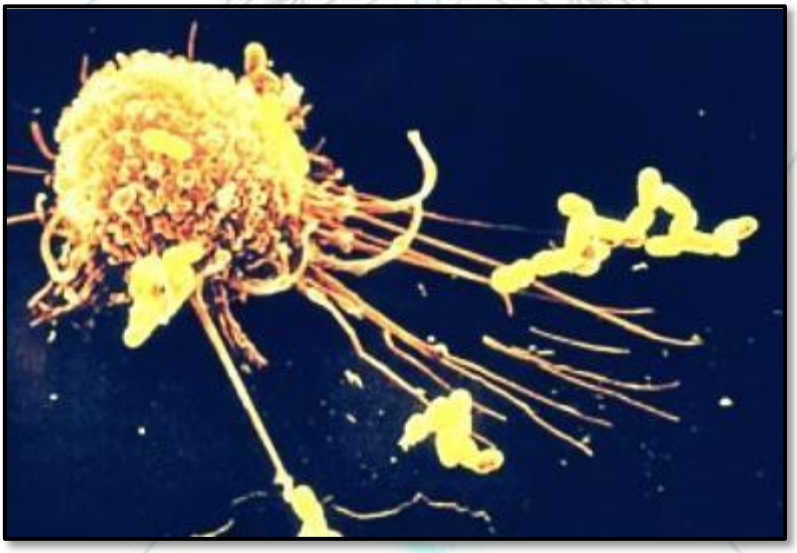

Figure 1: Photomicrograph of a phagocyte engulfing bacteria (x3000)

Macrophage like cells serve different functions in different tissues and are named according to their tissue location:

- **Alveolar Macrophages** in the lungs
- **Histiocytes** in connective tissues.
- **Kupffer cells** in the liver.
- **Mesangial cells** in the kidney
- **Microglial cells** in the brain
- **Osteoclasts** in bone

Although normally in a resting state, macrophages are activated by a variety of stimuli in the course of an immune response. Phagocytosis of particulate antigen serves as an initial activating stimulus. However, macrophage activity can be further enhanced by cytokines secreted by activated T_H cells by mediators of the inflammatory response and by components of bacterial cell walls. Activated macrophages are more effective than resting ones in eliminating potential pathogens, because they exhibit greater phagocytic activitiy, an increased ability to kill ingested microbes, increased secretion of inflammatory mediators and increased ability to activate T cells. In addition, the activated macrophages but not resting ones, secrete various cytotoxic proteins that help them eliminate a broad range of pathogens including virus infected cells, tumor cells and intracellular bacteria. Activated macrophages also express higher levels of class II MHC (Major HistoCompatability) molecules, allowing them to function more effectively as antigen-presenting cells.

PHAGOCYTOSIS.

Macrophages are capable of ingesting and digesting exogenous antigens such as a whole microorganism and insoluble particles and endogenous matter such as injured or dead cells, cellular debris and activated clotting factors. In the first step of phagocytosis, macrophages are attracted by and move toward a variety of substances generated in an immune response; process is called **chemotaxis.** The next step is adherence of the antigen to the macrophage cell membrane. Complex antigens such as whole bacterial or viral particles also tend to adhere well and are readily phagocytocised. Adherence includes membrane protrusions called **psueopodia,** to extend around the attached material. Fusion of the pseudopodia encloses the material within a membrane-bounded structure called a **phagosome,** which then enters the endocytic processing pathway. In this pathway, aphagosome moves towards the cell interior, where it fuses with a **lysosome** to form a **phagolysosome.** Lysosomes contain mysozyme and a variety of other hydrolytic enzymes that digest the ingested material. The digested contents of the phagolysosome are then eliminated in a process called **exocytosis.** [13]

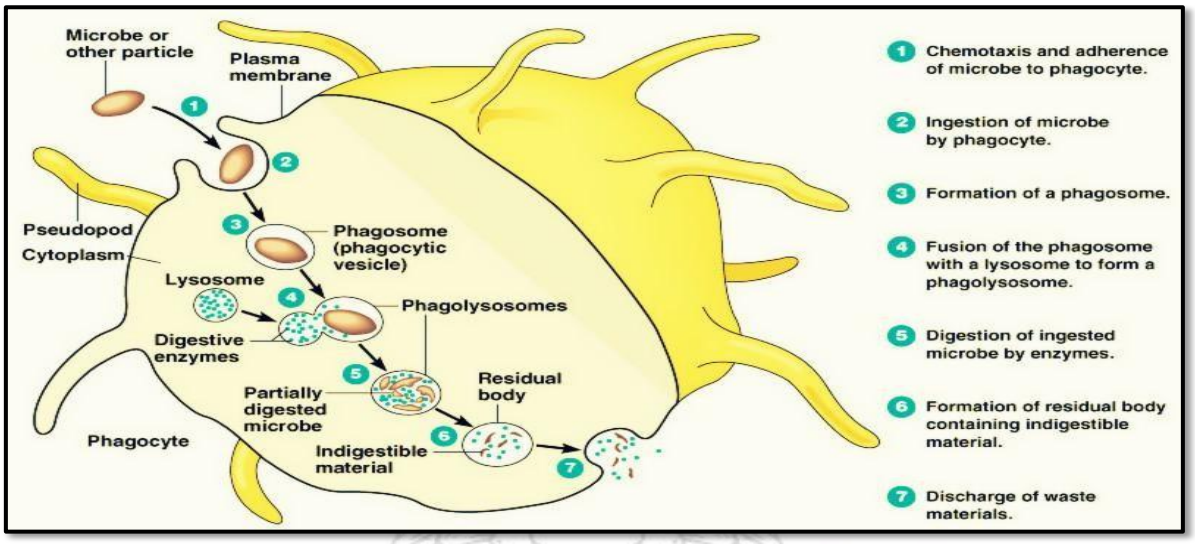

Figure 2: Phases of Phagocytosis.

TRYPAN BLUE DYE EXCLUSION TEST – CELL VIABILITY ASSAY FOR PHAGOCYTOSIS INDEX

The dye exclusion test has been used as a simple standard to differentiate and count the viable cells from the non-viable cells in a suspension. Though simple but efficiently accurate with its measurements of viable cells, the assay cannot differentiate between necrotic and apoptotic cells. This assay has been used in microscopy to assess cell viability in cultures of cells and tissues.

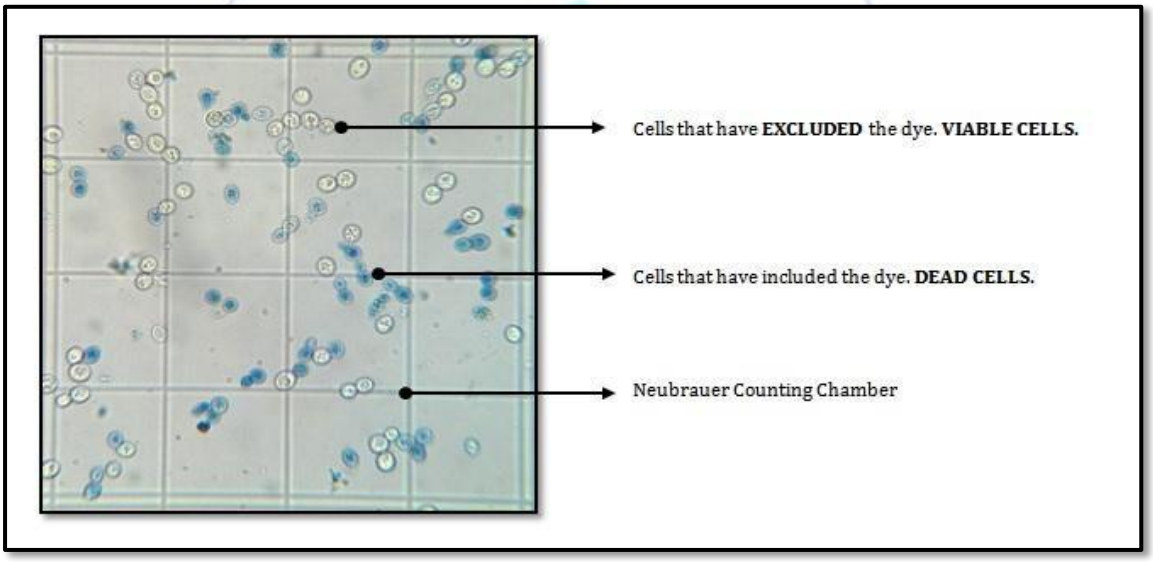

Figure 3: Trypan Blue Dye Exclusion Assay.

Thus, a cell suspension mixed with dye colors the dead cells, which allows effective counting of the number of viable cells using a neubrauer counting chamber (hemocytometer) (14)

REAGENTS AND MATERIALS USED.

- Test Compound - Curcumin
- Cell Culture - Murine Peritoneal Macrophages
- RPMI 1640 (Rosewell Park Memorial Institute Medium)
- ELISA plate
- Antigen 1 - Opsonized Cells of Candida albicans
- Antigen 2 - Spores of Aspergillus fumigates
- Trypan Blue Dye – 0.4% solution.
- Phosphate Buffer Solution (pH 7.4)
- Neubrauer Chamber (hemocytometer)

Methodology

Cell culture of murine peritoneal marcophages were used for the study. The cells were cultured in RPMI 1640 (Rosewell Park Memorial Institute Medium) in a flat bottomed ELISA plate. The growth supplements for macrophages and antimicrobial agents to limit the microbialgrowth were used. Oponised cells of Candida albicans and spores of Aspergillus fumigates were used as antigents for the culture. Cells of antigens were adjusted to the ratio of 1:16 per culture well of phagocytes. The test compound – "cureit" – bio available curcumin was dissolved in normal saline. Three concentrations of test compounds – 10, 20 and 30 µg/ml was prepared. Three individual sets of cultures phagocytes were treated with each concentration of the test compound – "cureit". After one hour of treatment, the cells were washed with RPMI 1640 medium and the cells were re-suspended in the same medium.

The pre-treated phagocytes (cultured phagocytes treated with test compound – "cureit") were infected with antigens. The pre-treated and infected cultures of phagocytes were then incubated for 3 hours. After 3 hours, microscopy was used to determine the viability of cells.

6. RESULTS AND DISCUSSION.

The following is the result of the assay performed.

Antigens	**Treated cells in triplicates/ratio obtained from average**			**Untreated cells - CONTROL**	**Treated/ Uninfected**	**Untreated/ Uninfected**
	10 µg/ml	20 µg/ml	30 µg/ml			
C. albicans	1:8	1:11	1:16	1:5	-	-
A. fumigatus	1:11	1:14	1:16	1:7	-	-

CONCLUSION

The results shows that the test compound – "cureit" has increased phagocytic ability by 2x-3x times. The test compound has also increased the mortality rate of the phagocytes as the cell viability had not affected in the case of the test whereas the cell death of the phagocytes was high in the case of control. By potentiating the viability of phagocytes, the test compound- "cureit" is proved to have immune boosting potential.

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