

## Evaluation of noni extract on mouse macrophages for its immune-enhancer property

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### Abstract

*Morinda citrifolia* L. has been used in a traditional Polynesian medicine for over 2000 years. The fruit are consumed by various communities all over the world. Due to its nutritional value, it has received an increasing importance. The present study analyzed the phagocytic property of *Morinda citrifolia* L (Noni drug). The BD dose (0.266 mg / 25 gm mice) of Noni drug was used to test the immunoenhancer property on Swiss albino mice. Animals were divided in to 4 groups of both either sex. Test group animals in each molecular weight were treated with OD and BD dose of noni orally, control group animal were treated with plain drinking water and standard control animal were treated with Cyclosporine A according mice weight. The BD treated animal exhibited 77.89% phagocytic activity when compared with control which exhibited 27.23%, OD group animal 47.77% and standard group animal 18.75%.

**Keywords:** OD - Omnie die, BD – Bis die, DDW – Double distilled water.

### Introduction

The immune system is a biological structure and processes within an organism that protect against disease. Antibody can combine specially with the antigen that stimulate their production and hasten its destruction. Second type of immune response has to be employed. The infected cells have to be destroyed special killer cells called lymphocytes. This process is called cell-mediated immunity [1].

Macrophages are large phagocyte cells found through the body produced by the differentiation of monocytes in tissue. Their role is to phagocytose, or engulf and then digest, cellular debris and pathogens, either as stationary or as mobile cells. Macrophages are of major importance in immunity since they are actively phagocytic. The function is to trap foreign materials and then process it so that it can be recognized by the cell of immune system. Macrophages contain a Golgi apparatus and rough endoplasmic reticulum and can synthesize and secrete proteins such as the enzyme lysosome and the complement component C2, C3, C4 and C5, are secreted continuously other proteins are released only during phagocytosis. Phagocytosis is an important feature of cellular innate immunity performed by cells called phagocytes that engulf, or eat, pathogens or particles. Phagocytes generally patrol the body searching for pathogens, but can be called to specific locations by cytokines. Once a pathogen has been engulfed by a phagocyte, it becomes trapped in an intracellular vesicle called phagosomes, which subsequently fuses with another vesicle called a lysosome to form a phagolysosomes. The pathogen is killed by the activity of digestive enzymes or following a respiratory burst that releases free radicals into the phagolysosomes.

When a macrophage ingests a pathogen, the pathogen becomes trapped in phagosomes, which then fuse with a lysosome, within the phagolysosome, enzyme and toxic peroxide digest the pathogen. Activated macrophages, NK cells and cytotoxic T Lymphocytes are generally involved with its anti-tumor activity macrophage may play a role in antitumor in a part due to the production of effector molecules such as NO, TNF- and IL-1 $\beta$ . Thus macrophage derived mediators have been recognized for their cytostasis properties against tumor cell [2]. Macrophages are classified in to two main groups designated M1 and M2, M1 macrophages are activated by LPS and IFN-gamma and secrete high level of IL-12 and low level of IL-10. M2 macrophages are activated by IL-4 and produce high levels of IL-10 and low level of IL-12. Tumor associated macrophages are thought to be M2 macrophages [3]. M1 macrophages are activated by LPS and IFN-gamma and secrete high level of IL-12 and low level of IL-10. M2 macrophages are activated by IL-4 and produce high levels of IL-10 and low level of IL-12. Tumor associated macrophages are thought to be M2 macrophages. [4]

Immunoenhancers are substances (drug and nutrients) that enhance the immune system by inducing activation or increasing activity of any of its components. Noni is one of the most important traditional Polynesian medicinal plants with tremendous medicinal properties [5]. Noni is the Hawaiian name for the fruit of *Morinda citrifolia* L. (Rubiaceae) [6]. Its various vernacular names are: "Indian mulberry", "nuna", "ash", on the Indian subcontinent, "mengkudu" in Malaysia, "nhau" in South East Asia, "painkiller bush" in the Caribbean, or "cheese fruit" in Australia [7-9]. Noni is native from Southeast Asia to Australia and is cultivated in Polynesia [10,11]. It is primarily used to stimulate the immune system and thus



to fight bacterial, viral, fungal, and parasitic infection. Hence, the present study was undertaken to analyze the phagocytic property of *Morinda citrifolia* L. (Noni drug).

## Material and Methods

### Experimental animals and research approval

Swiss albino mice (20-30 gm) of either sex were provided from Jawaharlal Nehru cancer hospital and research center, Bhopal (M.P). Animals were maintained at a temperature of 25±1°C and relative humidity 45-50% under 12-h light: 12-h dark cycle. The animals had free accesses to standard food pellets & water was

available. The experimental protocol was approved by the institutional animal ethics committee (IAEC) constituted in accordance with the rules and guidelines of the Committee for the purpose of control and supervision on experimental animals (CPCSEA), India (CPCSEA Registration number : CPCSEA/a/500/2001)

### Groups and Treatment

Animal were divided in four groups. Each group included three animals.

**Table 1 - Drug dosing in experimental groups**

S.No.	Group	Dosing	Mice quantity	Dosing Days	Drug Conc.
1	Group 1	Control	3	5	Only DDW
2	Group 2	Std control	3	5	0.113 mg / 25 gm mice
3	Group 3	OD	3	5	0.113 mg/25 gm mice
4	Group 4	BD	3	5	0.226 mg/25 gm mice

### Phagocytic index

Noni drug were administered to the experimental animals for 5 days regularly. Chilled PBS and yeast injected to mice Trans Abdominally. Abdominal was gently massaged after 5 min. Peritoneal fluid aspirated and centrifuged at 1000 rpm for 15 min. The supernatant was discarded and the pellet collected then fixed with cornoy's fixative. Slide prepared, air dried and stained with giemsa for 15 min and observed the under 40X magnification in light microscope. Percentage of inhibition of yeast digestion and macrophage index was calculated by using the formulae.

$$\% \text{ of inhibition of yeast} = \frac{\text{No. of active macrophage}}{\text{Total no. of macrophage}}$$

## Result

### Statistical analysis

The data were expressed as mean ± SD. The statistical significance between means was analyzed using one way analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparison test. D \*\*\* P<0.001 was considered as statically significant. Data was analyzed by ANOVA test.

Our present study results have shown that Noni Drug has maximum 47.77 % and 77.89 % (OD and BD Dose, respectively) yeast digestion as compared to control 25.75% and Standard group 18.75% (Table 2).

**Table 2 – Phagocytosis index**

S.No.	Group	Total No. of A.M	Mean ± SEM	Y.E.M %
1	Normal Control	160	53 ± 7.311	27.23
2	Std Control	33	11 ± 1.155	18.75
3	OD Dose	168	56 ± 16.093	47.77
	BD Dose	694	231.33 ± 88.380	77.89

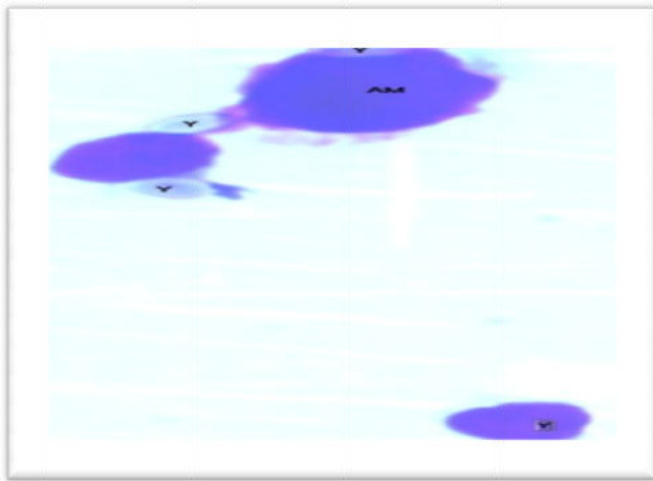
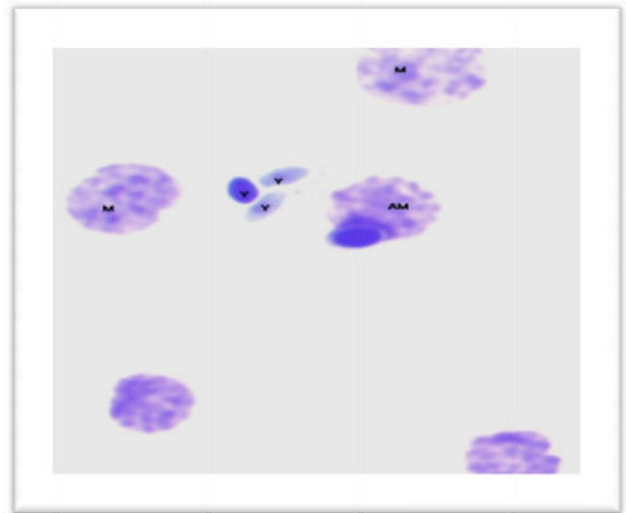
SEM - Standard error mean AM - Activated Macrophages  
 OD - Omnie die BD - Bis die  
 YEM - Yeast Engulfing Macrophage

Activated Yeast engulfing Macrophages was analyzed using Tukey-Kramer Multiple Comparisons Test, revealed P value as 0.0354, considered significant. Variation among column means is significantly greater than expected by chance.

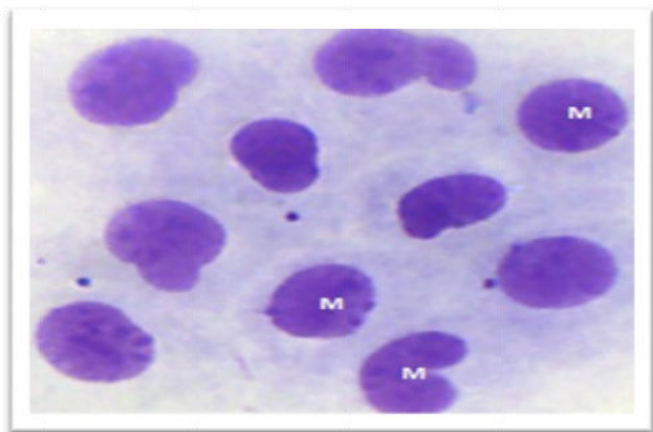
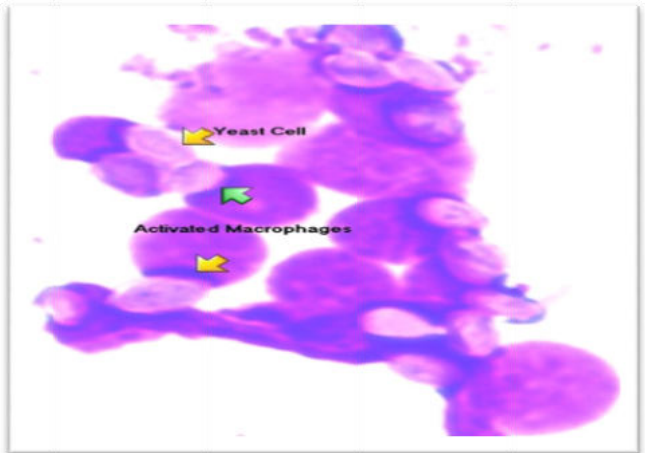
**Table 3 - Activated Yeast engulfing Macrophages (One-way ANOVA)**

S.No.	Comparison	Difference	Q Value	P value
1	Normal Control Vs Std Control	42.333	0.9393	ns P>0.05
2	Control Vs OD	-2.667	0.05917	ns P>0.05
3	N Control Vs BD	-178.00	3.949	ns P>0.05
4	Std Control Vs OD	-45.000	0.9985	ns P>0.05
5	Std Control Vs. BD	-220.33	4.889	* P<0.05
6	OD dose Vs BD	-175.33	3.890	ns P>0.05

None significant

**Figure: 1** Test Group 1 (Control Group)**Figure: 3** Test Group 3 (OD)

Significant

**Figure-2** Test Group 2 (Standard Control Group)**Figure: 4** Test Group 3 (BD)

M - Macrophage, AM - Activated Macrophage, Y - Yeast

## Discussion

Immune potentiality defines when the immune response or cellular defense mechanism against antigen. The *Morinda citrifolia* plant, and especially its fruit, has been used for centuries in folk medicine. The present study was an attempt to test immunoenhancer property of Noni extracts. Our study supports the previous findings showed that there was T cell activation by Noni extract [12].

A series of cytokine assay and NO determination corroborated the speculation that Noni ppt can induce macrophage activation. Noni ppt effectively enhanced the production of NO, TNF- IL-1 $\beta$  and IL-12 p70 from thioglycollate-elicited adherent PEC, which are mainly composed of macrophage. These macrophage-derived produce

are considered important mediator of tumor cytostasis and cytotoxicity. NO may produce cellular toxicity through the inhibition of DNA synthesis and mitochondrial respiration [13] TNF- may be able to directly cause haemorrhagic necrosis of tumor [14].

## Conclusion

Therefore present study revealed that noni extract is a better option to be subjected as an adjuvant to cancer treatment. It enhances not only the immunity but also give signature to protect genetic imbalance. The futuristic scopes of the present study will be a boon for chemopreventive, genetic stability and Immunoenhancer therapy. This will also act as poor man friendly a cost effective therapy.

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