

## ANTICANCER EFFICACY OF NANOPARTICLES SYNTHESISED FROM *MORINDA CITRIFOLIA* LINN (FRUIT SAMPLES) ON EHRlich ASCITES CARCINOMA IN ALBINO RATS

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### Abstract

*The aim of the stud was to investigate the anticancer efficacy of nanoparticles synthesised from Morinda citrifolia linn (fruit samples) on Ehrlich ascites carcinoma in albino rats. The nanoparticles synthesized from extract of Morinda citrifolia Linn was effective in inhibiting the tumor growth in ascitic and solid tumor models. The biochemical studies supported its antioxidant and anticancer properties.. The plant merits further investigation in an ascitic model at low doses and to elucidate its mechanism of action and isolation of its active constituents. However, the components responsible for the antioxidative activity are currently unclear. Therefore, further investigations need to be carried out to isolate and identify the antioxidant compounds present in the plant extract.*

**Keywords:** *Morinda citrifolia*, Nanoparticles, Ehrlich ascites, Cancer

### INTRODUCTION

Cancer is a group of diseases caused by loss of cell cycle control. Cancer is associated with abnormal uncontrolled cell growth. Cancer is caused by both external factors (tobacco, chemicals, radiation and infectious organisms) and internal factors (inherited mutations, hormones, immune conditions, and mutations that occur from metabolism). (Balachandran and Govindarajan 2005). Cancer is a significant worldwide health problem generally due to the lack of widespread and comprehensive early detection methods, the associated poor prognosis of patients diagnosed in later stages of the disease and its increasing incidence on a global scale. Indeed, the struggle to combat cancer is one of the greatest challenges of mankind.(Parinitha et.al, 2005)

Noni (*Morinda citrifolia* L. family Rubiaceae) is one of the traditional folk medicinal plant. The plant has been shown to have potent antioxidant activity both *in vitro* and *in vivo* studies. (Krishnamurthi 2007). Noni fruit is rich in phenolic compounds like ellagic acid, gallic acid, quercetin, rutin, rosmarinic acid, caffeic and chlorogenic acid which are highly potent antioxidant. EGCg is a polyphenolic flavonoid antioxidant found in abundance in noni fruit having antioxidant properties which inhibit the quinol oxidase (NOX). NOX enzymes are found in various types of cells and tissues where they react with oxygen to generate ROS, the free radical forms of oxygen that damage the DNA of normal cell (Divisi etal 2006). Antioxidants maintain free radicals into balance thus lowering the risk of oxidative stress. It is well documented that many herbal antioxidants reduced the toxicity due to oxidative stress, elicited by several medications and prevent the damage to normal cells (Palu et.al, 2008). There is additionally sensible evidence that antioxidants reversed nephrotoxicity caused by oxidative

stress due to one or the other reasons. Reported that antioxidants protected the kidney cells from the toxicity elicited by CIS ( Gupta and Singh 2013).

*Morinda citrifolia* L (Noni) (Figure 1), belongs to Rubiaceae family, a small evergreen tree 3-10 m in height; bright green and elliptical leaves, white tubular flowers and ovoid 'grenade like' yellowish white fleshy fruit 5-10cm long has a lumpy surface covered by polygonal shaped section, triangular and reddish brown seeds and fruit has a foul taste and odour.(Mathivanan, 2005).



**Figure 1; Shows the Morphology of *Morinda citrifolia* plant with flower and fruit samples**

*Morinda citrifolia* L. is also known as Indian mulberry and has been used in folk remedies by Polynesians for over 2000 years. The plant is mentioned in *Ayurved* as *Achuka*, which means longevity, and was used as a balancing agent and *Charak* described it as *Akshiki phala* or *Ashyuka*. This is known as other vernacular name in Indian such as Aal in Hindi, *Achuk*, *Akshi Phal* and *Ranjandru* in Sanskrit, *Bartondi* and *Surangi* in Marathi, *Sarogi* in Gujarati and *Numaakai* in Tamil language.(Singh et.al, 1984).

Noni is used for various traditional treatments for malaria, general febrifuge, and analgesic, laxative, jaundice, hypertension, ulcers, rheumatism, and sore throat. Fruit is believed to be as an appetite and brain stimulant. It has been reported to have a broad range of health benefits for cancer, infection, arthritis, diabetes, asthma.(Charak et.al, 1986). Ayurveda adds that noni is a *Kapha* stabilizer and helps to remove excess *Pitta* (fire element) from the body. There is an impressive list of body systems which "have all been effectively influenced by noni circulatory, digestive, respiratory, integumentary (skin), endocrine, immune, nervous, skeletal system. In Indian system of medicine, leaves and roots are used as astringent, deobsterent, emmengogue and to relieve pain in the gout.. It is tonic, antipyretic, regularize menses and useful in dysentery. Root is purgative, Leaves are used for infantile diarrhea, dysentery, to heal the wounds, ulcers and the pain of gout. Poultice of leaves is used in wound healing, charred unripe fruits mixed with salt relieve diseased gums.(Bhandari et.al 1998)

Chemotherapy is an effective treatment against various types of cancer either singly or in combination with surgery and/or radiotherapy. However, chemotherapeutic effects of most of the drugs showed limited efficacies due to the development of various side effects. This fostered our attempts to evaluate some plant products against cancer as they are less likely to cause serious side effects (Raju et.al, 2010).The rich and diverse plant sources of India are likely to provide effective anticancer agents. One of the best approaches in the search for anticancer agents from plant resources is the selection of plants based on ethnomedical leads (Kintzios SE. , 2006).

The effect of morinda citrifolia on hepatotoxicity, renotoxicity, microbes have been already studied. However, the antitumour efficacy of Morinda Citrifolia on EAC is very limited. Thus, the present study is focused on anticancer effect of Morinda Citrifolia on EAC induced carcinoma in rats.

## MATERIALS AND METHODS

### Animals

The Male Swiss albino rats weighing 150-200g were used in the present study. They were acclimatized to the laboratory conditions prior to the study. The animals were kept at  $25 \pm 2$  °C and a relative humidity of 40–45% with alternative day and night cycles of 12 h each. They were fed with pelleted rat chow and water ad libitum. Anesthetic procedures and handling with animals were approved by and complied with the ethical guidelines of CPCSEA.

### Synthesis of Silver nanoparticles

45 ml of 1 mM aqueous AgNO<sub>3</sub> solution added to 5 ml of fruit extract to the conical flask. The flask was then cubated in the dark at 4 hrs (to minimize the photo activation of silver nitrate), at room temperature. A control setup was also maintained without plant extract. The Ag nanoparticle solution thus obtained was purified by repeated centrifugation at 10,000 rpm for 15 min followed by re-dispersion of the pellet in de-ionized water. (Arunachalam *et al.*, 2012). Then the Ag nanoparticles air dried in desiccator for 6 hrs. Dried nanoparticle was used for biological activities.

### Transplantation of tumour:

Ehrlich ascites carcinoma (EAC) cells were obtained. The EAC cells were maintained in vivo in Swiss albino rats by intraperitoneal inoculation of  $2 \times 10^6$  cells per rat. From the peritoneal cavity of the mice, the EAC cells were aspirated, washed with saline and were given intraperitoneally to develop ascitic tumor. Ehrlich Ascites Carcinoma (EAC) cells maintained in the peritoneal cavity of Swiss albino rats (male) were collected from an animal having 8–10 days old ascitic tumor by aspirating the ascitic fluid in sterile isotonic saline. The viable EAC cells were counted (trypan blue indicator) under microscope. Rats were injected with  $1 \times 10^7$  EAC cells intraperitoneally on day 0. A day of incubation was allowed for multiplication of the cells.

#### Animals were divided into four groups.

**Group-I (Normal Control)** was served as normal control treated with saline control (5 ml/kg i.p.)

**Group-II – (Cancer Induced)** was served as EAC tumor control group

**Group-III (Cancer +Standard drug)** was served as EAC tumour control administered with 5 – Flouro uracil (5-FU) (20 mg/kg, i.p.) for 14 days.

**Group-IV- (Cancer + Plant sample)** was served as EAC treated tumour control treated with nanoparticle samples from Morinda Citrifolia fruit samples (2 mg/kg/bwt) for 14 days.

The standard 5-FU and the plant extract were administered after 72 hrs of tumour inoculation. All the drugs were administered for 15 days continuously and the biochemical parameters were evaluated.

The body weight of rats in each group was measured in gram (g) just before tumor inoculation and at the end of the experiment. The rat bearing EAC were dissected and the

ascitic fluid was collected from the peritoneal cavity. The volume of the collected ascitic fluid in ml was measured by taking it in a graduated centrifuge tube.

#### **Collection of samples:**

At the end of the experiment and after 48 h, blood samples from each rat were collected from the eyes by sino-orbital puncture of mice using micro-capillary tubes. Blood samples were withdrawn in clean and dry test tubes containing ethylene diamine tetra acetic acid (EDTA) and were then centrifuged at 3000 rpm for 15 minutes. The estimation red blood cell (RBC) count and white blood cell (WBC) count was measured using standard procedures.

The supernatant obtained were used superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and LPO.

#### **Assay of Antioxidant enzymes**

Renal enzymatic antioxidants of Superoxide dismutase (SOD) was assayed by the method of Kakkar et al., (1984) , Catalase (CAT) activity was assayed by the method of Sinha AK (1972) Glutathione peroxidase (GPx) activity was assayed by the method of Rotruck et al., (1973) . Cycloperoxides are formed as a result of peroxidation reaction, which give MDA by cleavage. MDA forms a pink colored complex with thiobarbituric acid whose absorbance can be read at 532 nm. The results were expressed as nmoles MDA/mg protein using molar extinction coefficient of MDA-thiobarbituric acid chromophore. Malondialdehyde was estimated as per the method of Beuge and Aust (1978)

#### **Statistical Analysis**

The Results were expressed as the mean value  $\pm$  SD. Group comparisons were performed by using one-way analysis of variance (ANOVA) test. Significant difference between normal control and experimental groups were assessed by student's t-test. A probability level of less than 5% ( $P < 0.05$ ) was considered as significant

### **RESULTS AND DISCUSSION:**

The effect of *M. Citrifolia* on body weight, tumour volume, RBC and WBC in different experimental Groups were given in Table. 1. There was a significant ( $P < 0.05$ ) increase in body weight, tumor volume in cancer induced rats (Group II) when compared to normal Control (Group I). The level of these parameters were found to be reversed in rats treated with standard drug 5-Flurouracil (Group III) and *Morinda citrifolia* treated groups (Group IV).

The level of RBC was found to be decreased and increased significantly ( $P < 0.05$ ) in group II as compared to group I (Normal control). The results of the present study were supported by Hariom Singh et.al, 2013 and Mohammad alia (2016).

**Table 1 : Effect of Nanoparticles synthesis from *Morinda Citrifolia* Linn on Body weight, Tumor volume, RBC and WBC in Different Experimental Groups of albino rats**

Groups	Body Weight (g)	Tumor Volume (ml)	RBC (cells/ml x 10 <sup>6</sup> )	WBC (cells/ml x 10 <sup>3</sup> )
I	20.00±0.01	00.00±0.00	05.12±0.02	07.40±0.04
II	27.5±0.04 <sup>*a</sup>	07.50±0.10 <sup>*a</sup>	03.65±0.04 <sup>*a</sup>	16.10±0.08 <sup>*a</sup>
III	20.5±0.03 <sup>*b</sup>	01.20±0.02 <sup>*b</sup>	04.95±0.01 <sup>*b</sup>	09.50±0.06 <sup>*b</sup>
IV	22.5±0.01 <sup>*b</sup>	02.00±0.01 <sup>*b</sup>	04.20±0.02 <sup>*b</sup>	10.40±0.03 <sup>*b</sup>

All values were expressed as mean±SD (n=6). Statistically significant of <sup>\*a</sup>p < 0.05 compared to Normal control group (I), <sup>\*b</sup>p < 0.05 compared to EIC treated group (II). Same superscript letters were not significantly (p<0.05) different from each other

It was found that Swiss albino mice having EAC-cell lines showed rapid and regular increase in scites fluid. Ascitic fluid is the nutritional requirement for the growing tumor cells and a sharp increase in tumor fluid with tumor growth would be necessary to meet the nutritional demand of the developing tumor. (Prasad and Giri 1994).

Cancer chemotherapy leads to the major problems such as myelosuppression and anemia. Anaemia in EAC cell bearing mice is just because of the decrease in Hb content and RBC count and this occur because of iron deficiency or because of myelopathic condition. Moreover, administration of freeze dried Noni restores the haemoglobin level, RBC, and WBC count more or less to normal levels and this confers that the freeze dried Noni possess protective Solid tumor implantation by EAC cells in swiss albino mice cells lead to morphological and metabolic changes as structural basis, reduced number of mitochondria, nucleotides loss such as purines and pyrimidines, nucleosides and bases, decreased DNA and RNA synthesis, fall in turnover and pool of ATP, protein synthesis, glutathione concentration also decreases and triglycerides, cholesterol esters and free fatty acids gets increased. It was found that Noni reduces tumor weight, tumor volume and retards the tumor growth when compared to EAC control. (Price and Greenfield 1958) (Hogland HC 1982)

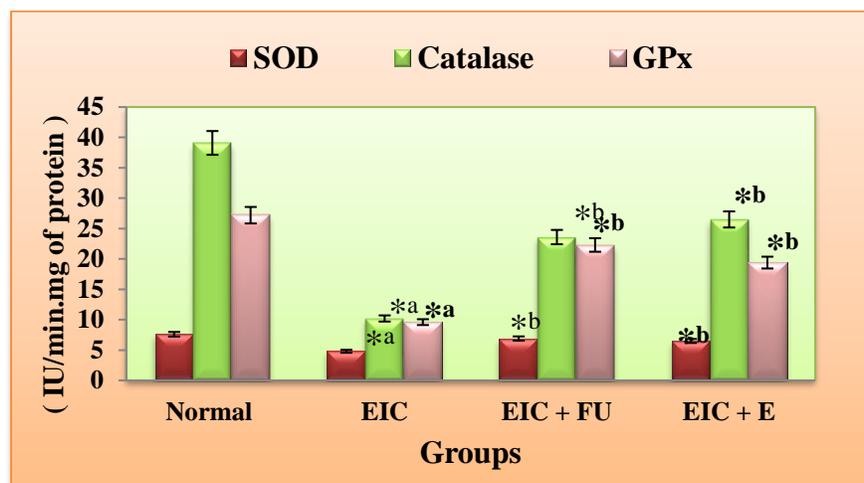
The effect of *Morinda Citrifolia* on antioxidant enzymes (SOD, CAT and GPx) on EAC induced carcinoma in rats is shown in Table 2, Fig 1 and Fig 2. The level of these enzymes was found to be significantly decreased in EAC induced cancer rats (Group II) as compared to normal Control (Group I) rats. The level of LPO was found to be increased in EAC induced rats (Group II) when compared to normal control rats (Group I).

**Table 2 : Effect of Nanoparticles synthesized from *Morinda Citrifolia* Linn on SOD, Catalase, GPx and LPO in Different Experimental Groups of albino rats**

Groups	SOD (IU/min/mg protein)	Catalase (IU/min/mg protein)	GPx (IU/min/mg protein)	LPO (μM/ g tissue)
I	07.60±0.01	39.10±0.00	27.20±0.02	162.60±0.04
II	04.80±0.04 <sup>*a</sup>	10.20±0.10 <sup>*a</sup>	09.60±0.04 <sup>*a</sup>	403.10±0.08 <sup>*a</sup>
III	06.90±0.03 <sup>*b</sup>	23.60±0.02 <sup>*b</sup>	22.30±0.01 <sup>*b</sup>	190.40±0.06 <sup>*b</sup>
IV	06.50±0.01 <sup>*b</sup>	26.50±0.01 <sup>*b</sup>	19.40±0.02 <sup>*b</sup>	205.10±0.03 <sup>*b</sup>

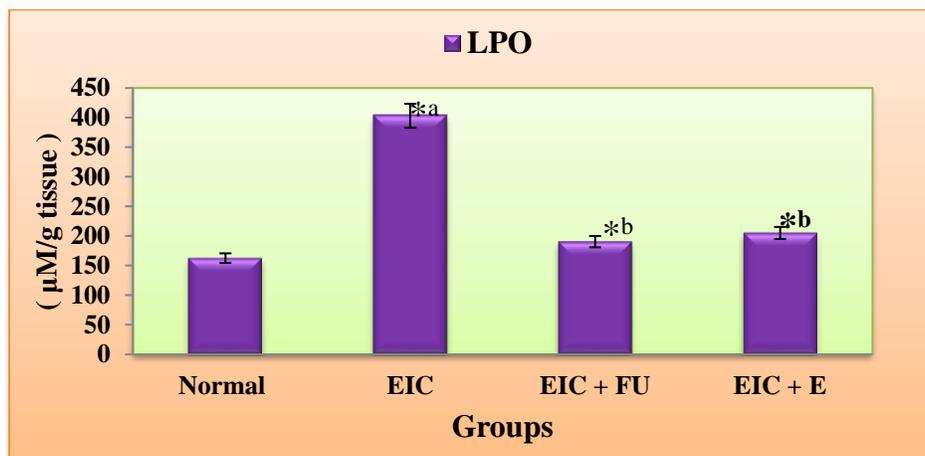
All values were expressed as mean±SD (n=6). Statistically significant of <sup>\*a</sup>p < 0.05 compared to Normal control group (I), <sup>\*b</sup>p < 0.05 compared to EIC treated group (II). Same superscript letters were not significantly (p<0.05) different from each other

**Fig.3 : Effect of Nanoparticles synthesized from *Morinda Citrifolia* on SOD, Catalase, GPx and LPO in Different Experimental Groups of albino rats**



All values were expressed as mean±SD (n=6). Statistically significant of <sup>\*a</sup>p < 0.05 compared to Normal control group (I), <sup>\*b</sup>p < 0.05 compared to EIC treated group (II). Same superscript letters were not significantly (p<0.05) different from each other.

**Fig.4: Effect of Nanoparticles synthesized from *Morinda Citrifolia* on LPO in Different Experimental Groups of albino rats**



All values were expressed as mean±SD (n=6). Statistically significant of  $*ap < 0.05$  compared to Normal control group (I),  $*bp < 0.05$  compared to EIC treated group (II). Same superscript letters were not significantly ( $p < 0.05$ ) different from each other

*In vivo* research has demonstrated that noni juice increases superoxide dismutase (SOD) and glutathione peroxidase (GPx) enzyme activities. The superoxide anion radical (SAR) is a major cellular reactive oxygen species and may be generated via enzymatic and nonenzymatic process or may come from exogenous sources, including cigarette smoke [13]. SOD catalyzes the dismutation of SAR to hydrogen peroxide and oxygen [14]. GPx is capable of reducing free hydrogen peroxide to water (Zin et.al 2002). GPx also reduces lipid hydroperoxides, as well as prevents free radical attack on polyunsaturated fatty acids in cellular membranes. As such, the effect of noni juice on these two enzymes may be at least two of the major antioxidant mechanisms of action through which it protects lymphocyte DNA and lowers plasma concentration of tobacco smoke-induced free radicals and peroxides. (Mickoy et.al, 2002)

Noni juice also showed a preventive effect against anemia, lymphocytosis and neutrophilia when compared to NMU control group. They concluded that Noni juice have liver and kidney protective effect in NMU induce carcinogenesis and could be useful to treat mammary gland in humans and animals. (G.C.Mills, 1957). The antioxidant properties of ethanol and ethyl acetate extracts of root, fruit and leaf of *M. citrifolia* assessed by ferric thiocyanate method (FTC) and thiobarbituric acid methods (TBA) which indicated that the root extract showed higher activity than the fruit or leaf extracts. (McCay, 1976)

### CONCLUSION

In conclusion, the nanoparticles synthesized from extract of *Morinda citrifolia* Linn was effective in inhibiting the tumor growth in ascitic and solid tumor models. The biochemical studies supported its antioxidant and anticancer properties.. The plant merits further investigation in an ascitic model at low doses and to elucidate its mechanism of action and isolation of its active constituents. However, the components responsible for the antioxidative activity are currently unclear. Therefore, further investigations need to be carried out to isolate and identify the antioxidant compounds present in the plant extract

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