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Effect of *Morinda citrifolia* (Noni) Fruit Juice on Antioxidant, Hematological and Biochemical Parameters in N-Methyl-N-Nitrosourea (NMU) Induced Mammary Carcinogenesis in Sprague-Dawley Rats

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Abstract: N-Methyl-N-Nitrosourea (NMU) is a highly specific mammary gland carcinogen that act directly and does not require metabolic activation. The novel medicinal plant *Morinda citrifolia*, also called as Noni, has broad therapeutic effects such as antibacterial, antiviral, antifungal, anticancer, analgesic, anti-inflammatory, anti-oxidant and immune enhancing effects. The present study was conducted to assess the beneficial effects of *M. citrifolia* fruit juice on antioxidant, hematological and biochemical alterations caused by NMU induced mammary carcinogenesis in Sprague-Dawley rats. The rats were divided into five groups viz., vehicle control group-A (n = 8), *M. citrifolia* control group-B (n = 8), NMU control group C (n = 15), *M. citrifolia* prevention group-D (n = 15) and *M. citrifolia* treatment group-E (n = 15). By the end of the 28 weeks experimental period all the animals were euthanized, blood was collected by heart puncture. *M. citrifolia* treatment significantly (p<0.05) increased the anti-oxidant enzymes such as catalase, superoxide dismutase and significantly (p<0.05) decreased the lipid peroxidation activity when compared to NMU control group-C. *M. citrifolia* exhibited a preventive effect against anaemia, lymphocytosis and neutrophilia in group-D and group-E when compared to group-C. Biochemical analyses showed normal levels of enzymes of liver and kidney in *M. citrifolia* treated groups- B, D and E rats, whereas NMU control group-C showed significant (p<0.05) decrease in albumin and total protein levels. These findings indicate that *M. citrifolia* fruit juice did not show any hepatotoxic or nephrotoxic effects. It was concluded that the *M. citrifolia* fruit juice ameliorates the adverse effects of NMU carcinogenesis and could be useful to treat mammary tumours in humans and animals.

Key words: *Morinda citrifolia* fruit juice, N-Methyl-N-Nitrosourea induced mammary carcinogenesis, antioxidant, hematology, biochemical parameters, cancer, amelioration, treatment, rats

INTRODUCTION

Experimentally induced carcinogenesis models by using various chemicals in laboratory animals are widely used to study the biology of cancers and for developing evaluating the cancer prevention strategies. The most commonly used chemical carcinogenic agents are 3-methyl chloranthracene (MCA), 7, 12-dimethyl benzanthracene (DMBA), N-Methyl-N-Nitrosourea (NMU), diethylnitrosoamine (DEN) and azoxymethane (AOM) (Zarbl *et al.*, 1985; Russo *et al.*, 1990;

Thompson *et al.*, 1995; Mehta, 2000; Kubatka *et al.*, 2003; Roomi *et al.*, 2005). NMU is a directly acting carcinogen that does not require the metabolic activation steps in order to form DNA adducts and has a very short half-life. The molecular basis for NMU induced carcinogenesis is direct alkylation of DNA, thereby causing point mutations in codon 12 of the Ha-ras-1 gene (Zarbl *et al.*, 1985; Chan *et al.*, 2005; Perse *et al.*, 2009). NMU is a highly specific carcinogen for the mammary gland but it also causes tumours in other organs such as prostate, pancreas, liver, spleen, kidneys and lungs. NMU induced

mammary tumours are more estrogen dependent, aggressive, locally invasive and they are capable to metastasize (Gusterson and Williams, 1981; Russo *et al.*, 1990; Thompson *et al.*, 1995; Chan *et al.*, 2005). Most widely used standard dose for mammary tumour carcinogenesis is 50 mg NMU kg⁻¹ body weight administered intraperitoneally between 50-60 days of age. Moreover, NMU has been used to test whether animals are predisposed to neoplasia or susceptible to mutagens (Gusterson and Williams, 1981; Thompson *et al.*, 1995; Perse *et al.*, 2009).

India has a rich heritage of medicinal plants and herbs and a large number of plant extracts have been reported to be having high utility against several diseases and disorders including of cancers. In recent times, thousands of herbs and their preparations are being studied worldwide to identify their pharmacologically active components and scientific validation purposes which are playing crucial role in propagating and popularizing the use of wonderful drugs/medicines (Mahima *et al.*, 2012, 2013; Singh, 2012; Tiwari *et al.*, 2012; Dhama *et al.*, 2013; Saminathan *et al.*, 2013). The list of useful plants goes endless but most commonly used herbal medicinal plants include *Azadirachta indica* (neem), *Tinospora cardifolia* (giloy), *Astragalus membranaceus*, *Withania somnifera* (ashwagandha), *Embllica officinalis* (amla), *Ocimum sanctum* (tulsi), *Piper longum* (pipali), Aloe vera, *Allium sativum* (garlic), *Zingiber officinale*, (ginger), *Curcuma longa*, (turmeric) etc., (Mahima *et al.*, 2012; Tiwari *et al.*, 2012, 2014a, b; Dhama *et al.*, 2013).

An edible and tropical plant *Morinda citrifolia* L. has been widely used by polynesians in folk medicine for more than 2,000 years. It is commonly known as great morinda, Indian mulberry, nunaakai and Noni in India, Ba Jitianin in China, dog dumpling in Barbados, mengkudu in Indonesia and Malaysia, Nono in Tahiti; painkiller bush in the Caribbean; cheese fruit in Australia and beach mulberry or Noni in Hawaii (Morton, 1992; Wang *et al.*, 2002; Serafini *et al.*, 2011; Singh, 2012). *M. citrifolia* belongs to coffee family, *Rubiaceae*, made up of around 80 species. It is a short tropical evergreen plant and is found in open coastal and forest areas upto 1300 feet above sea level. It is native to the Pacific islands, Hawaii, Caribbean, Asia and Australia (Morton, 1992; Brown, 2012; Singh, 2012). In Southern India, *M. citrifolia* is found in the coastal regions of the Tamil Nadu and Kerala and also in the Mangalore area of Karnataka.

Different parts of the Noni plant have been traditionally used for treatment of various complaints for their therapeutic activities, including hypotensive action, analgesia, antibacterial effects, antituberculosis,

anti-inflammatory action and antioxidant effects. It is also used for curing osteoporosis and auditory improvement, wound healing, antiviral activity, anticataract, antigout, antifungal, neuronal protective, antidiabetes, anti-postoperative nausea and vomiting. Noni is also found useful in cancer chemoprevention and treatment of cancers due to its anti-angiogenesis effects and immune stimulation (Morton, 1992; Wang and Su, 2001; Wang *et al.*, 2002; Anitha and Mohandass, 2006; Dussosoy *et al.*, 2011; Serafini *et al.*, 2011; Brown, 2012; Singh, 2012; Saminathan *et al.*, 2013). It regulates cell function and regeneration of damaged cells. The Noni juice had been commercialized in the USA as "functional food" products in 1990s and is increasingly distributed all over the world (Brown, 2012; Singh, 2012). The pharmacologically active compounds derived from *M. citrifolia* fruits, leaves and roots are nowadays available as readymade capsules, teas and juices, the fruit juice being the most popular. Noni fruit contains alkaloids, scopoletin, damnacanthol and lots of other molecules, as a result the consumption of noni juice is currently high, not only in US but also in Japan, Europe and India (Su *et al.*, 2005; Liu *et al.*, 2007; Ikeda *et al.*, 2009; Singh, 2012).

The effects of *M. citrifolia* fruit juice on the antioxidant, hematological and biochemical alterations caused by N-Methyl-N-Nitrosourea (NMU) induced mammary carcinogenesis in Sprague-Dawley rats have not been recorded yet. Therefore, the present study was conducted to investigate the biological effects of *M. citrifolia* fruit juice in preventing and eliminating the adverse effects of NMU induced carcinogenesis by evaluating the *in vivo* antioxidant effects on blood and to investigate the safety of *M. citrifolia* by haematology and serum biochemical parameters.

MATERIALS AND METHODS

Animals: This experiment was approved by the Institute Animal Ethics Committee (IAEC) of Indian Veterinary Research Institute (IVRI), Izatnagar, India and Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) before its commencement. The guiding principles in the care and use of laboratory animals together with those described in the declaration of Helsinki and Indian standards were strictly adhered to in the conduct of all the experimental procedures. The out bred, female Sprague-Dawley rats, weighing 25-30 g were obtained from laboratory animal facility, Central Drug Research Institute (CDRI), Lucknow, India at 3 weeks of age. The rats were housed in polypropylene cages in the experimental animal house under environmentally

controlled conditions (temperature 25°C±2°C, relative humidity 30-70%) with a 12/12 h light/dark cycle. The rats were provided with standard rodent pelleted feed procured from Aashirwad Industries, Chandigarh (India) and water *ad libitum*. The animals were acclimatized for one week before the commencement of experiments.

Experimental induction mammary tumours: N-Methyl-N-Nitrosourea (NMU) (Sigma Aldrich, USA) was used as the chemical carcinogen. The NMU injection vials were wrapped with aluminium foil and kept in ice because NMU is sensitive to light and humidity. The NMU was dissolved immediately prior to its use in 4 mL of 0.9% NaCl solution and acidified to pH 4 with acetic acid in such a way that each mL was containing 5 mg of NMU and was administered at the dose rate of 50 mg kg⁻¹ body weight intra-peritoneal (i/p) following all necessary safety and sterile precautions. After dissolving, the NMU was used within 20 min and then the next vial of carcinogen was prepared. The injections were given along the ventral midline of the animal, half way between the third and fourth pair of mammary glands. Three doses were administered at 50, 80 and 110 days of age (Gusterson and Williams, 1981; Thompson *et al.*, 1995; Perse *et al.*, 2009).

Morinda citrifolia fruit juice: The fruit juice of *Morinda citrifolia* was purchased from World Noni Research Foundation, Chennai. *M. citrifolia* fruit juice was administered orally by gavage at a dose of 10% solution of 5 mL rat⁻¹ day⁻¹, in two divided doses.

Experimental design: A total of 61 rats were randomly divided into 5 groups (Group A, B, C, D and E). Group-A (n = 8) received only acidified saline (NMU vehicle), pH 4.0 by intra-peritoneal (i/p) route and served as vehicle control group. Group-B (n = 8) was administered with only *M. citrifolia* fruit juice at a dose of 10% solution of 5 mL rat⁻¹ day⁻¹ in two divided doses orally by gavage throughout the experiment. The animals were not administered with NMU for tumor induction and served as *M. citrifolia* control group. Group-C (n = 15) received only NMU at the dose rate of 50 mg kg⁻¹ body weight

intraperitoneally (i/p) three doses at 50, 80 and 110 days of age. The animals were not treated with *M. citrifolia* fruit juice and served as NMU control group. Group-D (n = 15) was administered with NMU as same protocol followed in group C and *M. citrifolia* was administered 15 days before NMU administration and continued for whole the study period and this group served as *M. citrifolia* prevention group. Group-E (n = 15) was administered with NMU as same protocol followed in group C and after appearance of palpable tumours, animals were treated with *M. citrifolia* fruit juice and this group served as *M. citrifolia* treatment group (Table 1). The whole experiment duration was of 28 weeks.

Anti-oxidant activity estimation: Anti-oxidant status was assessed in Red Blood Cells (RBC) hemolysate by estimation of catalase, superoxide dismutase and lipid peroxidation activity at the termination of experiment as per Aebi (1983), Marklund and Marklund (1974) and Shafiq-Ur-Rehman (1984), respectively.

Haematology: Red Blood Cells (RBC), haemoglobin (Hb), Packed Cell Volume (PCV), total White Blood Cells (WBC), Differential Leukocyte Count (DLC) and thrombocyte count was estimated using an automated blood analyzer (Cell Dyn® 3700, Abbott Diagnostic, USA).

Serum biochemistry

Liver Function Test (LFT): Blood was collected in sterile vial without anticoagulant for serum separation. Sera samples were analyzed for Liver Function Test (LFT), biochemical parameters viz. total protein, albumin, alanine aminotransferases (ALT/SGPT), aspartate aminotransferases (AST/SGOT) and alkaline phosphatase (ALP) using standard commercial kits (Span diagnostics, India).

Kidney function test: Blood was collected in sterile vial without anticoagulant for serum separation. Sera samples were analyzed for Blood Urea Nitrogen (BUN) and creatinine using standard commercial kits (Span diagnostics, India).

Table 1: Details of experimental design

Groups	No. of animals	NMU administration time*	Treatment with <i>M. citrifolia</i> fruit juice**	<i>M. citrifolia</i> treatment duration
A (Vehicle control)	8	-	-	-
B (<i>M. citrifolia</i> control)	8	-	Fruit juice	For whole study period
C (NMU control)	15	50, 80 and 110 days of age	-	-
D (Prevention)	15	50, 80 and 110 days of age	Fruit juice	15 days before MNU administration and will continue for whole study period
E (Treatment)	15	50, 80 and 110 days of age	Fruit juice	The day palpable tumour development noticed and continued till end of the experiment

NMU dose used was 50 mg kg⁻¹ b.wt, intra-peritoneal at 50, 80 and 110 days of age (*), *M. citrifolia* fruit juice dose used was 10% solution of 5 mL rat⁻¹ day⁻¹ in two divided doses, orally by gavage (**)

RESULTS

Oxidative stress related biochemical parameters

Effects on Lipid Peroxidation (LPO): The effects of *M. citrifolia* treatment on lipid peroxidation (LPO) in RBC haemolysate of rats in different groups are presented in Fig. 1. LPO levels were significantly ($p < 0.05$) increased (4.9 ± 0.09 nmol MDA mL^{-1}) in NMU control group-C. *M. citrifolia* treatment significantly ($p < 0.05$) decreased the LPO levels in group-E by 3.40 ± 0.09 nmol MDA mL^{-1} . *M. citrifolia* prevention group-D resulted in decreased LPO levels by 2.90 ± 0.03 nmol MDA mL^{-1} . The LPO values of *M. citrifolia* control group-B was statistically significant ($p < 0.05$) from vehicle control group-A, indicating *M. citrifolia* was reducing the LPO levels in normal body.

Effects on catalase (CAT): The effects of *M. citrifolia* treatment on catalase (CAT) in RBC hemolysate of rats in different groups are presented in Fig. 2. The activity of CAT was significantly depleted (10.41 ± 0.43 μmol of H_2O_2 min^{-1} mg^{-1} protein) in NMU control group-C. *M. citrifolia* treatment significantly increased the CAT activity in group-E and by 29.74 ± 0.59 μmol of H_2O_2 min^{-1} mg^{-1} protein. The CAT activity significantly increased in prevention group D by 45.70 ± 0.88 μmol of H_2O_2 min^{-1} mg^{-1} protein. *M. citrifolia* control group B was statistically significant ($p < 0.05$) from vehicle control group-A in terms of catalase activity, indicating *M. citrifolia* was increasing the CAT levels in normal body.

Effects on superoxide dismutase (SOD): The values of the superoxide dismutase (SOD) activity of RBC hemolysate in control and experimental rats are presented in the Fig. 3. SOD activity was significantly ($p < 0.05$) decreased by 14 ± 1.00 units g^{-1} of protein in rats exposed to NMU control group-C. *M. citrifolia* treatment significantly ($p < 0.05$) increased the SOD activity in group-E by 32 ± 1.1 units g^{-1} of protein. *M. citrifolia* used for prevention significantly ($p < 0.05$) increased the SOD activity by 41 ± 0.85 units g^{-1} of protein in group-D. The SOD levels in *M. citrifolia* control group B was statistically significant ($p < 0.05$) from vehicle control group-A, indicating *M. citrifolia* was increasing the SOD levels in normal body.

Haematology

Red Blood Cells (RBC), hemoglobin (Hb) and Packed Cell Volume (PCV) count: The NMU control group-C rats exhibited significant ($p < 0.05$) decrease in RBC, Hb and PCV levels indicating erythropania or anaemia when

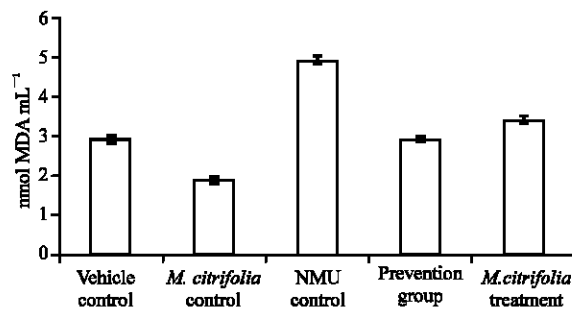


Fig. 1: Bar diagram showing effect of *M. citrifolia* fruit juice on Lipid Peroxidation (LPO) in different experimental groups

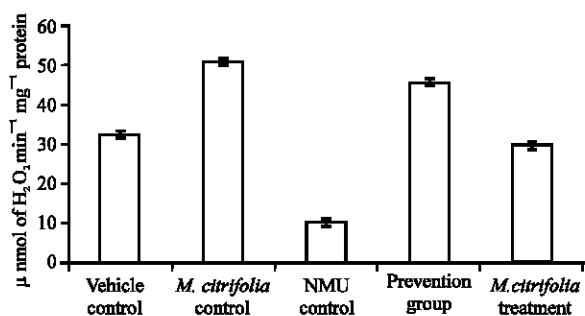


Fig. 2: Bar diagram showing effect of *M. citrifolia* fruit juice on catalase in different experimental groups

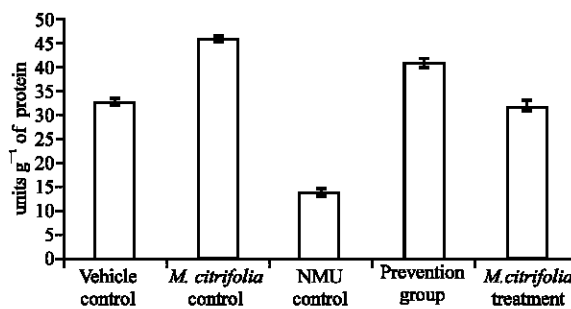


Fig. 3: Bar diagram showing effect of *M. citrifolia* fruit juice on superoxide dismutase (SOD) in different experimental groups

compared to group A, B, D and E rats. *M. citrifolia* treated prevention group-D and treatment group-E rats showed normal values of RBC, Hb and PCV (Table 2).

Thrombocyte count: Platelet count was significantly ($p < 0.05$) decreased in NMU control group-C rats when compared to group A, B, D and E rats. Whereas, *M. citrifolia* treated prevention group-D and treatment group-E rats showed normal levels of platelet count (Table 2).

Table 2: Effect of *M. citrifolia* fruit juice on hematological values in different experimental groups (Mean±SEM)

Parameters	Groups				
	A	B	C	D	E
RBC ($\times 10^6 \mu\text{L}^{-1}$)	7.4±0.2 ^a	6.9±0.06 ^a	5.6±0.24 ^b	7.6±0.12 ^a	7.3±0.15 ^a
WBC ($\times 10^3 \mu\text{L}^{-1}$)	7.1±0.09 ^a	7.3±0.13 ^a	16±0.68 ^b	8.6±0.19 ^a	10±0.60 ^a
Hb (g ⁻¹ dl)	14±0.22 ^a	15±0.28 ^a	11±0.48 ^b	14±0.39 ^a	13±0.41 ^a
PCV (%)	45±0.35 ^a	45±0.43 ^a	40±0.54 ^b	44±0.62 ^a	43±0.58 ^a
Lymphocyte (%)	84±0.71 ^a	83±0.6 ^a	145±5.3 ^b	95±1.4 ^a	112±2.0 ^a
Monocyte (%)	3.0±0.42 ^a	3.7±0.21 ^a	2.5±0.31 ^a	3.5±0.22 ^a	2.5±0.43 ^a
Neutrophil (%)	11±0.71 ^a	11±0.6 ^a	21±1.1 ^b	13±1.3 ^a	15±1.5 ^a
Eosinophil (%)	2.1±0.61 ^a	2.3±0.49 ^a	1.8±0.31 ^a	2.2±0.48 ^a	2.2±0.31 ^a
Basophil (%)	0±0.00 ^a	0±0.00 ^a	0.33±0.21 ^a	0±0.00 ^a	0±0.00 ^a
Platelet ($\times 10^3 \mu\text{L}^{-1}$)	707±4.3 ^a	708±3.2 ^a	610±5.10 ^b	706±3.6 ^a	654±9.4 ^a

Mean±SEM values with superscript 'b' indicates the significant differences ($p < 0.05$) between group-C and other groups, Group A: vehicle control, Group B: *M. citrifolia* control, Group C: NMU control, Group D: *M. citrifolia* Prevention and Group E: *M. citrifolia* treatment

Table 3: Effect of *M. citrifolia* fruit juice on liver enzyme levels in different experimental groups (Mean±SEM)

Parameters	Groups				
	A	B	C	D	E
ALT (U/L)	37±0.36 ^a	36±0.47 ^a	35±0.56 ^a	36±0.33 ^a	35±0.7 ^a
AST (U/L)	147±1.5 ^a	146±1.4 ^a	144±2.1 ^a	147±2.1 ^a	145±1.6 ^a
ALP (U/L)	68±1.3 ^a	69±0.98 ^a	69±1.9 ^a	68±1.3 ^a	67±2.7 ^a
Albumin (g dL ⁻¹)	3.9±0.09 ^a	4.1±0.1 ^a	3.0±0.15 ^b	4.0±0.16 ^a	3.8±0.18 ^a
Total Protein (g dL ⁻¹)	6.9±0.14 ^a	6.6±0.17 ^a	5.3±0.38 ^b	6.7±0.16 ^a	6.8±0.24 ^a

Mean±SEM values with superscript 'b' indicates the significant differences ($p < 0.05$) between group-C and other groups, Group A: vehicle control, Group B: *M. citrifolia* control, Group C: NMU control, Group D: *M. citrifolia* Prevention and Group E: *M. citrifolia* treatment

Table 4: Effect of *M. citrifolia* fruit juice on kidney function in different experimental groups (Mean±SEM)

Parameters	Groups				
	A	B	C	D	E
Creatinine (mg dL ⁻¹)	0.51±0.02 ^a	0.49±0.03 ^a	0.48±0.03 ^a	0.47±0.03 ^a	0.5±0.04 ^a
Urea (mg dL ⁻¹)	18±0.4 ^a	17±0.38 ^a	16±0.54 ^a	17±0.46 ^a	16±0.8 ^a

Mean±SEM values with superscript 'a' indicates no significant differences ($p < 0.05$) between the groups, Group A: vehicle control, Group B: *M. citrifolia* control, Group C: NMU control, Group D: *M. citrifolia* Prevention and Group E: *M. citrifolia* treatment

Total white blood cell (TLC) and Differential Leukocyte Count (DLC): The NMU control group-C rats showed significant ($p < 0.05$) increase in lymphocytes and neutrophils indicating lymphocytosis and neutrophilia, respectively, when compared to group B, D and E rats. *M. citrifolia* treated prevention group-D and treatment group-E rats showed significant decrease in lymphocytes and neutrophils and exhibited normal WBC levels (Table 2).

Serum biochemistry

Liver function test (LFT): The effects of *M. citrifolia* treatment on liver in terms of serum AST, ALT, ALP, total protein and albumin of rats in different groups are presented in Table 3. *M. citrifolia* treated groups-B, D and E rats showed no significant differences in the serum AST, ALT and ALP levels as compared to control groups-A and C. Group-C rats showed significant ($p < 0.05$) decrease in albumin and total protein levels indicating hypoproteinemia in comparison to groups-A, B, D and E rats, whereas *M. citrifolia* treated groups-D and E rats showed normal levels of albumin and total protein.

Kidney function test: The effects of *M. citrifolia* treatment on kidney in terms of serum BUN and creatinine levels of rats in different groups are presented in Table 4. No significant differences in serum BUN and creatinine levels were observed in rats exposed to *M. citrifolia* treatment as compared to control.

DISCUSSION

The N-Methyl-N-Nitrosourea (NMU) induced mammary tumours in rats mimics breast cancer in human because the histological resemblance of mammary gland tumours in rats, ovarian hormone dependant neoplasms, mammary ductal epithelial cells in origin, highly malignant rat tumours resembles intra-ductal and infiltrating ductal carcinomas in humans and altered expression of cyclin D1, erbB2 and TGFB (Gusterson and Williams, 1981; Russo *et al.*, 1990; Thompson *et al.*, 1995; Chan *et al.*, 2005). Therefore, the NMU induced mammary tumours model has been widely used to evaluate the chemopreventive and therapeutic agents against breast cancer in human (Mehta, 2000; Kubatka *et al.*, 2003; Roomi *et al.*, 2005).

Oxidative stress has been suggested to contribute to the pathogenesis of carcinogenicity and lipid peroxidation is one of the characteristic features of cancer (Halliwell, 2007; Klaunig *et al.*, 2010). The reactive intermediates, produced by oxidative stress, can alter the membrane bilayers and cause the lipid peroxidation of polyunsaturated fatty acids (PUFA) leading to the formation of lipoperoxyl radical (LOO[•]) which in turn, reacts with a lipid to yield a lipid radical and a lipid hydroperoxide (LOOH). LOOHs are unstable and they generate new peroxy and alkoxy radicals and decompose into secondary products (Halliwell and Chirico, 1993; Gago-Dominguez *et al.*, 2005). Malondialdehyde (MDA) is formed during oxidative degeneration as a product of free oxygen radicals which is accepted as an indicator of lipid peroxidation. MDA, the end product of lipid peroxidation, was reported to be higher in cancer tissues than in non-diseased organ (Nielsen *et al.*, 1997; Marnett, 1999).

In the present study, LPO levels were significantly ($p < 0.05$) increased in NMU control group-C rats. *M. citrifolia* treatment significantly ($p < 0.05$) decreased the LPO levels in prevention group-D and treatment group-E rats. Antioxidants, both enzymatic and non-enzymatic, are the first line of defence against free radical induced toxicity. A redox balance between pro-oxidants and antioxidants is essential for the normal cellular functioning (Nordberg and Amer, 2001; Rahal *et al.*, 2014). Impairment in the ratio of oxidants and antioxidants initiates the patho-physiological events that culminate in molecular and cellular damage to macromolecules and vital organs (Valko *et al.*, 2006). The antioxidant enzymes SOD and CAT play an important role in reducing cellular stress. SOD scavenges the superoxide radical by converting it to hydrogen peroxide and molecular oxygen (Robinson, 1998), while CAT brings about the reduction of hydrogen peroxides and protects higher tissues from the highly reactive hydroxyl radicals (Brioukhanov and Netrusov, 2004).

In the present study, the activity of CAT was significantly depleted in NMU control group-C rats. *M. citrifolia* treatment significantly increased the CAT activity in prevention group-D and treatment group-E rats when compared to NMU control. The SOD activity was significantly decreased in rats exposed to only NMU. *M. citrifolia* treatment significantly ($p < 0.05$) increased the SOD activity in prevention group-D and treatment group-E. Noni juice has excellent antioxidant activity which may guard individuals from oxygen free radicals and lipid peroxidation induced damage. These findings were corresponded with the observations of Wang and Su (2001) and Wang *et al.* (2002), who estimated the

in vitro Superoxide Anion Radicals (SAR) and quenched Lipid Peroxides (LPO) scavenging activity of Tahitian Noni Juice (TNJ) by Tetrazolium Nitroblue (TNB) assay and LMB assay, respectively. TNJ showed a concentration dependent inhibition of both LPO and SAR. The SAR scavenging activity of TNJ was 2.8 times that of vitamin C, 1.1 times that of grape seed powder and 1.4 times that of Pycnogenol. These results confirmed the antioxidant potential of TNJ by quenching the reactive oxygen free radicals. Wang and Su (2001) and Wang *et al.* (2002) also estimated the *in vivo* antioxidant activity of noni juice against carbon tetrachloride (CCl₄) induced liver injury model in female SD rats. CCl₄ is a hepatic carcinogen and potent inducer of lipid hydroperoxidation. Administration of 10% of TNJ in drinking water for a period of 12 days suppressed the levels of LPO and SAR in liver to 20% and 50%, respectively, 3 h after administration of CCl₄. Zin *et al.* (2002) and Su *et al.* (2005) reported that various parts of *M. citrifolia* (leaf, fruit and root) to have antioxidative activities. When compared to either leaf or fruit the polar and non-polar extracts of the root exhibited stronger antioxidative potential. Anitha and Mohandass (2006) reported that oral administration of 50 mg⁻¹ Kg⁻¹ day⁻¹ of crude methanol extract of *M. citrifolia* leaves for a period of 14 days significantly enhanced the anti-oxidant enzymes, such as glutathione peroxidase (GSHPx), catalase (CAT) and superoxide dismutase (SOD). Due to anti-oxidant activity there was reduction in lymphoma in mice. Liu *et al.* (2007) have reported that the antioxidative mechanism of Noni fruit juice was partially attributable to the group of phenolic compounds, such as isoscopoletin, quercetin and aesculetin in the EtOAc (ethanolic) extract. Ikeda *et al.* (2009) observed that both Noni and coumarin derivatives have scavenging activity on ROS such as superoxide (O₂⁻), singlet oxygen (¹O₂), hydroxyl radical (OH) and peroxynitrite (ONOO⁻) in a dose-dependent manner.

Cigarette smoke was reported to contain 227 possible carcinogens and each puff of cigarette smoke contains 1 × 10¹⁷ oxidant molecules (Chow, 1993). Wang *et al.* (2009a, 2009b) assessed the antioxidant activity of TNJ on plasma by estimating the SAR and LPO levels in current cigarette smokers. The smokers were provided daily with a dose of two ounces of TNJ twice a day for a period of 30 days. The LPO and SAR levels in the TNJ group showed 23% reduction and 27% reduction, respectively, when compared to placebo group. These results indicate that TNJ may guard individuals from tobacco smoke free radical induced damage. West *et al.* (2009a) also evaluated the antioxidant properties of roasted Noni leaf infusion. The infusion has 2, 2-diphenylpicrylhydrazyl

(DPPH) radical scavenging activity which was higher when compared to green tea infusion. Thani *et al.* (2010) recorded that the non-aqueous extracts from the leaves of Thai Noni/Yor showed antioxidant properties, giving IC50 values of 0.20-0.35 mg mL⁻¹. These results suggest that the leaves of *M. citrifolia* could be preferred as a food supplement for its antioxidative activities in epidermoid and cervical cancers over damnacanthal, rutin and scopoletin. Dussosoy *et al.* (2011) showed that Noni's anti-oxidant activities are possibly due to phenolic compounds, iridoids and ascorbic acid. Serafini *et al.* (2011) investigated the antioxidant activity of aqueous extract from *M. citrifolia* leaves against lipid peroxidation, hydroxyl and nitric oxide induced radicals. West *et al.* (2011) evaluated the antioxidant activity of *M. citrifolia* seed extract. The seed extract exhibited significant antioxidant potential against various types of free radical induced damage.

In the present study, hematological results showed significant ($p < 0.05$) decrease in RBC, Hb and PCV levels in NMU control group-C rats indicating a tendency to develop erythropania or anaemia. The group-C rats also showed significant ($p < 0.05$) increase in lymphocytes indicating lymphocytosis with neutrophilia. This indicates an inflammatory response in animals with large mammary tumours (Table 2). These findings were in agreement with that of Perse *et al.* (2009) and Hazilawati *et al.* (2010a), who observed anaemia and neutrophilia in NMU administered rats. Saffhill and Chaudhuri (1976) and Chang *et al.* (2012) observed leukaemia in NMU administered rats.

The *M. citrifolia* prevention group-D and treatment group-E showed normal levels of RBC, Hb and PCV. The *M. citrifolia* treated rats also showed normal levels of lymphocytes and neutrophils count. These findings were in agreement with Hazilawati *et al.* (2010a, 2010b), who observed daily supplementation of *M. citrifolia* at the dose of 3000 mg kg⁻¹ reduced the incidence of anaemia, neutrophilia and early stage of leukaemia in rats.

The soluble hepatic enzymes like aspartate transferase (AST), alanine transferase (ALT) and alkaline phosphatase (ALP) of blood-serum have been considered as indicators of the hepatic dysfunction and damage. The increase in the activities of these enzymes in serum is indicative of liver damage and thus causes alteration in liver function. The hepatic cell damage exhibits good correlation with these enzyme leakages. The possibility of *M. citrifolia* induced toxicity was assessed in rats consuming the equivalent of a recommended dose in women (<3 oz daily) for most of their adult life. Serum levels of the hepatic enzymes such as ALT, AST, ALP, albumin and total protein were examined in the

M. citrifolia treated rats, since these are considered markers of liver damage in humans. In addition, serum blood urea nitrogen (BUN) and creatinine a marker of renal function, was assessed. None of these markers in both liver and kidney were not elevated and the levels are similar to the control group. These results were supported histologically by the absence of toxin-induced damage to the tissue sections of liver and kidney from the *M. citrifolia* treated group. So long-term administration of *M. citrifolia* in the NMU induced mammary tumour cases did not have any detectable adverse effects on the liver and kidney tissues or markers assessed in this study.

These findings were almost similar to the observations of West *et al.* (2006, 2009b, 2009c) who performed with high doses of noni for shorter time periods, such as 90 mL kg⁻¹ daily for 3 months in rats and 750 mL⁻¹ day⁻¹ for 28 days in humans and showed lack of toxicity. Potterat and Hamburger (2007), Westendorf *et al.* (2007) and Hadijah *et al.* (2008) conducted the subchronic toxicity of an aqueous extract of *M. citrifolia* fruit on SD male rats. They found that a dosage of 20% juice (highest dose) was not safe for the rats as it indicated renal as well as liver injuries. However, lower dosages of the juice (5 and 10%) showed no significant changes, hence were considered safe. Rosly *et al.* (2011) conducted subchronic oral toxicity study to evaluate the safety of *M. citrifolia* in Sprague-Dawley (SD) rats. The dose levels of 2000 (low dose) and 5000 (high dose) mg kg⁻¹ b.w/day showed no toxicological significance. They concluded that the no-observed adverse-effect level (NOAEL) for *M. citrifolia* was 5000 mg kg⁻¹ body weight/day. However, few reports of hepatotoxicity do also exists. Yu *et al.* (2011) observed acute hepatotoxicity after ingestion of *M. citrifolia* juice in a 14-year-old boy. Millonig *et al.* (2005) and Stadlbauer *et al.* (2005, 2008) reported hepatotoxicity after consumption of *M. citrifolia*.

The possible mechanism of action responsible for anti-oxidant activity in NMU induced mammary carcinogenesis is that Noni fruits contain precursor of xeronine called proxeronine in significant quantity. Proxeronine is converted into xeronine in the body by proxeroninase. The most important function of xeronine is to regulate the rigidity and shape of specific proteins and is also a critical metabolic coregulator. Xeronine will act on abnormal protein and make it fold into its correct conformation that results in properly functioning protein (Heinicke, 1985). Noni has more than 160 phytochemical compounds. The major micronutrients are alkaloids, phenolic compounds, proteins, organic acids, minerals and vitamins. Among phenolic compounds, most important are anthraquinones damnacanthal, nordamnacanthal, morindone, rubiadin-1-methyl ether,

alizarin, rubiadin, aucubin, asperuloside and scopoletin (Wang and Su, 2001; Su *et al.*, 2005; Liu *et al.*, 2007; Potterat and Hamburger, 2007; Ikeda *et al.*, 2009; Singh, 2012). The organic acids mainly are caproic and caprylic acids, while the principal alkaloid is xeronine (Heinicke, 1985). The protein content of the fruit is surprisingly high and the main amino acids are glutamic acid, aspartic acid and isoleucine. Noni has six major substances namely anthraquinones, polysaccharides, epigallocatechin gallate (EGCg), coumarins, monoterpenes and terpenoid compounds which have been shown to fight cancer in different ways (Mathivanan *et al.*, 2005; Potterat and Hamburger, 2007).

CONCLUSION

The present study demonstrates for the first time the beneficial effects of *Morinda citrifolia* fruit juice on the antioxidant, haematological and biochemical alterations caused by N-Methyl-N-Nitrosourea (NMU) induced mammary carcinogenesis in Sprague-Dawley rats. It was found that *M. citrifolia* fruit juice ameliorates the effect of oxidative stress and brought the various anti-oxidant enzymes to a normal level, indicating that *M. citrifolia* fruit juice exhibits an anti-oxidant property in NMU induced mammary tumours in rats. The administration of *M. citrifolia* fruit juice did not produce any abnormalities in the Red Blood Cell (RBC), haemoglobin (Hb), Packed Cell Volume (PCV), White Cell (WBC), Differential Leukocyte Count (DLC) and platelet count. *M. citrifolia* fruit juice also exhibited a preventive effect against anaemia, lymphocytosis and neutrophilia. *M. citrifolia* fruit juice did not show any hepatotoxicity and nephrotoxicity at a dose of 10% solution of 5 mL rat⁻¹ day⁻¹ in two divided doses, orally by gavage. *M. citrifolia* fruit juice helped in maintaining the enzymes of liver and kidney within the normal levels. In conclusion, *M. citrifolia* fruit juice reduced the adverse effects caused by NMU induced mammary tumours and didn't show any toxicity on liver and kidney functions. However, further investigations are necessary in this therapeutic direction to provide clarifications regarding the molecular mechanisms, comprehensive phytochemical analysis, along with extensive study of the pharmacokinetics and pharmacotherapeutic potentials of Noni.

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