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Evaluation of Renoprotective Effects of Ethanolic Extract of *Morinda citrifolia* L. in a Murine Model of Gentamicin-induced Nephrotoxicity

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Abstract - AIM: The present study was undertaken to evaluate noni fruit juice for its protective effects on gentamicin-induced nephrotoxicity in rats.

METHODS: Wistar albino rats of either sex, weighing 150-200g were divided into 4 groups; normal saline, gentamicin 80 mg/kg, i.p., for 8 days, noni fruit juice 5 and 10 mg/kg, p.o., for 8 days, noni fruit juice 3 days prior and concurrently with gentamicin for 5 days. Blood urea, serum creatinine, serum uric acid and blood urea nitrogen analyses and microscopic examination of kidney were performed after the treatment.

RESULTS: Gentamicin treatment caused nephrotoxicity as evidenced by marked elevation in blood urea and serum creatinine. Serum urea, serum uric acid, serum creatinine and blood urea nitrogen were increased with gentamicin compared to saline-treated animals (162.33 ± 9.92 mg/dl, 3.13 ± 0.12 mg/dl, 6.85 ± 0.35 mg/dl and 75.86 ± 4.64 mg/dl respectively). Co-administration of noni fruit juice with gentamicin decreased the rise in these parameters in a dose dependent manner. Study of renal morphology by light microscope showed epithelial loss with intense granular degeneration involving >50% renal cortex in gentamicin treated rats, whereas in noni fruit juice plus gentamicin treated rat revealed insignificant changes in tubular epithelium.

CONCLUSION: To conclude, our data suggest that supplementation of noni fruit juice may be useful in reducing gentamicin nephrotoxicity in rats.

Keywords: *Morinda citrifolia*, nephroprotective, gentamicin, noni fruit

I. INTRODUCTION

Herbal and natural products of folk medicine have been used for centuries in every culture throughout the world. Scientists and medical professionals have shown increased interest in this field as they recognize the true health benefits of these remedies. "Let food be your medicine and let medicine be your food" was advised by the father of medicine, Hippocrates, over two millennia ago. Among the medicinal plants discovered by the ancestors of Polynesians, *Morinda citrifolia* L (Noni) is one of the traditional folk medicinal plants that have been used for over 2000 years in Polynesia [1]. Noni is a native plant from Southeast Asia to Australia and is cultivated in Polynesia, India, the Caribbean, Central and northern South America [2, 3]. It has been reported to have a broad range of therapeutic and nutritional value [4].

Morinda citrifolia Linn (Rubiaceae), also known as Noni or Indian mulberry, Ba Ji Tian, Nono or Nonu, Cheese Fruit, and Nhau in various cultures throughout the world, is a small evergreen tree. It is identifiable by its straight trunk, large, bright green and elliptical

leaves, white tubular flowers, and its distinctive, ovoid, "grenade-like" yellow fruit. The fruit can grow in size up to 12 cm or more and has a lumpy surface covered by polygonal-shaped sections. The seeds, which are triangular shaped and reddish brown, have an air sac attached at one end, which makes the seeds buoyant. The mature Noni fruit has a foul taste and odor [5]. It has been reported to have a broad range of health benefits for cancer, infection, arthritis, diabetes, asthma, hypertension, and pain [6]. Several animal studies suggest noni may have anti-cancer [7, 8], immune enhancing [9] and pain-relieving properties [10]. Most recently Takashima et al. demonstrated the medicinal uses of new constituents isolated from noni leaves [11]. Furthermore, it has been demonstrated that Noni juice contains some antioxidative [12] and anti-inflammatory ingredients [13].

Morinda citrifolia, has been reported to possess antithrombotic [14], antioxidant [15], analgesic and anti-inflammatory [16] and xanthine oxidase inhibitory [17] activities. There are also preliminary studies reporting its blood pressure lowering [18] and vasodilatory [19]

properties. On the downside, reports of serious hyperkalemia due to its high content of potassium (56.3 meq/L), which is similar to orange and tomato juices have been published [20]. A protective effect against carbon tetrachloride-induced liver injury in female Sprague Dawley rats has also been described [21].

Gentamicin induced renal damage is a popular model to study the effects of potential renoprotective drugs [22]. A therapeutic approach to protect or reverse gentamicin-induced kidney injury would have significant clinical value in acute renal failure as well as drug induced renal damage. Nephrotoxicity induced by gentamicin is a complex phenomenon characterized by an increase in plasma creatinine and urea levels and severe proximal renal tubular necrosis, followed by deterioration and renal failure [22, 23]. The toxicity of gentamicin is believed to be related to the generation of reactive oxygen species (ROS) in the kidney [23, 24]. The cellular antioxidant status determines the susceptibility to oxidative damage and usually alters in response to oxidative stress [25]. Various studies have claimed antioxidant property of drugs for their nephroprotective effects in gentamicin induced renal damage [26, 27, 28, 29].

Since noni fruit has proven antioxidant and hepatoprotective activity (attributed to its antioxidant activity), we decided to explore the nephroprotective effects of *Morinda citrifolia* L (Noni) fruit juice in a murine model of gentamicin induced renal damage.

II. MATERIALS AND METHODS

Animals: Wistar albino rats of either sex weighing 150-200 gram inbred in our own central animal house were used for the study. Rodents were housed in clean polypropylene cages, with dust free rice husk as a bedding material; three rats per cage; under controlled laboratory conditions (Temperature: $25^{\circ} \pm 2^{\circ}\text{C}$, humidity ($60\% \pm 10\%$) and 12 h light/dark cycle as per CPCSEA guidelines) and fed with standard rodent diet and water *ad libitum*. The rodents were allowed to acclimatize to these conditions for one week prior to the commencement of the study. The study was approved by the institutional animal ethics committee.

Drugs: Gentamicin sulfate (*Piramel Health Care Ltd*) was used to induce renal damage.

Test drug extract:

Ripe noni fruits, washed and air dried, were weighed and placed in a food grade plastic container for 4-5 days. During this time noni fruit juice dripping from the pulp was collected in the container, decanted (separating the juice from other sediments), filtered and preserved using 10% sodium methyl paraben IP and 5%

sodium propyl paraben. This juice was then bottled and stored in a cool dry place.

Evaluation of nephroprotective activity:

Experiment protocol:

After acclimatization, the animals were divided randomly into four groups (n=6), and placed in metabolic cages separately for collecting 24-hour urine samples. After collecting the first urine samples, the animals were grouped into the normal control group (fed with normal saline 1mg/k i.p.), the disease control group (fed with gentamicin 80mg/kg/day i.p.) [26] and test drug groups (fed with Noni fruit juice-5mg/kg/day and 10mg/kg/day, started 3 days prior orally + Gentamicin i.p.). The drugs were administered for a total duration of 8 days. The test drug was started 3 days prior to the commencement of the study. Injections of gentamicin were made daily at 10:00 hours to minimize the circadian variation in nephrotoxicity [30].

Sample collection and biochemical assays:

Twenty-four hours after the last injection, urine samples were collected and the animals were euthanized under ether anesthesia. Blood samples were collected by cardiac puncture for measuring urea and creatinine as an indicator of kidney damage, using urease (a nickel-metallo enzyme that catalyzes the degradation of urea to ammonia and carbon dioxide), and Jaffé (the combined use of creatinine, amidohydrolase, and alkaline sodium picrate) methods, respectively [31]. Serum uric acid and blood urea nitrogen were also estimated.

Histopathological examination:

Kidneys from all the four groups were weighed and processed for histopathological evaluation. The kidneys fixed in 10% neutral buffered formalin were processed and embedded in paraffin wax and sections were taken using a microtome. Sections (5 microns) were then stained with haematoxylin and eosin and examined under light microscope. They were evaluated and assigned scores as follows [26]:

Score 0=normal

Score 1=areas of focal granulovacuolar epithelial cell degeneration and granular debris in tubular lumens with or without evidence of tubular epithelial cell desquamation of small foci (< 1% of total tubule population)

Score 2= tubular epithelial necrosis and desquamation easily seen but involving less than half of cortical tubules

Score 3= more than half of proximal tubules showing desquamation of necrosis but involved tubules easily found

Score 4=complete or almost complete tubular necrosis.

Statistical analysis:

Data were expressed as mean ± standard error of mean (SEM). Statistical evaluation was done using SPSS (version 17.0). The differences among treated groups were analyzed by one-way ANOVA followed by Tukey’s test. P < 0.05 was considered statistically significant.

III. RESULTS

In the present study, gentamicin (80 mg/kg) when injected for 8 consecutive days caused marked nephrotoxicity as is evident from Table 1, showing significant (P < 0.001) increase in serum urea (162.33 ± 9.92 mg/dl) serum uric acid (3.13 ± 0.123 mg/dl), serum creatinine (6.85 ± 0.348 mg/dl) and blood urea nitrogen (75.86 ± 4.636 mg/dl) as compared to normal control animals.

The test drug, noni fruit juice extract showed a significant nephroprotective effect as evidenced by a decrease in the renal parameters i.e. Serum urea, uric acid, creatinine and blood urea nitrogen levels when compared to the Gentamicin treated group. Moreover, the results as depicted in table1 also suggest an interesting dose dependent renoprotective effect”.

Table: 1 Effect of Gentamicin and noni fruit extract on serum urea, uric acid, creatinine and blood urea nitrogen levels

Parameters	Control group	Gentamicin group	Nonifruit juice 5mg/kg/day	Noni fruit juice 10mg/kg/day
Serum urea (mg/dl)	41.17 ± 0.703	162.33 ± 9.92*	137.83 ± 4.64**	99 ± 2.65**
Serum uric acid (mg/dl)	1.62 ± 0.31	3.13 ± 0.12*	2.42 ± 0.11**	1.57 ± 0.13**
Serum Creatinine (mg/dl)	0.58 ± 0.075	6.85 ± 0.35*	5.63 ± 0.39**	2.42 ± 0.3**
Serum Blood urea nitrogen (mg/dl)	19.24 ± 0.329	75.86 ± 4.64*	64.41 ± 2.17**	46.26 ± 1.24**

Values are mean ± SEM
 P< 0.001 when compared to the normal control
 P< 0.001 when compared to gentamicin induced non treated disease control

sections that the kidneys from the saline treated appeared histologically normal (Score 0) whereas the gentamicin treated group showed tubular epithelia loss

with intense granular degeneration involving > 50% renal cortex. In addition to the tubular epithelial loss, some of the tubular epithelium contains tubular casts (Score 4). The histomorphology of rats treated with noni fruit extract showed moderate tubular epithelial degeneration with desquamation in patchy areas of the renal cortex (Score2-3).

Table 2: Histopathological features of kidneys of rats of different treatment groups

Glomerular congestion	-	++++	++	+
Blood vessel congestion	-	+++	++	+
Interstitial edema	-	+++	+	+
Inflammatory cells	-	++	+	++
Necrosis & tubular casts	-	++++	++	+
Average score	0	4	3	2

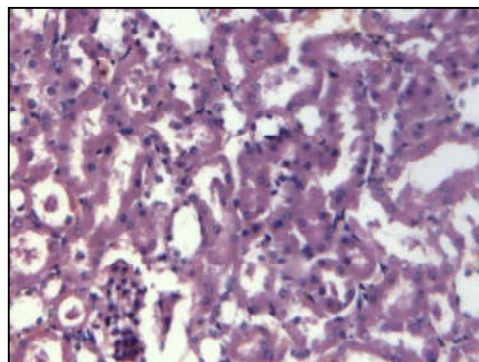


Fig 1: Photomicrograph of kidney sections showing normal kidney (40X, H & E)

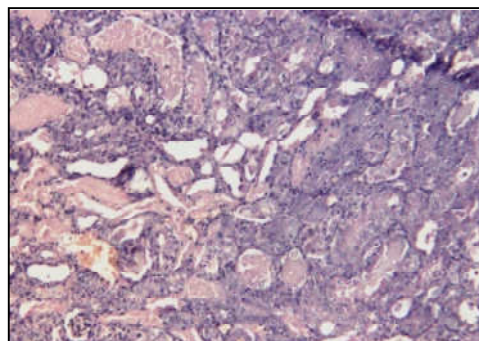


Fig 2: Photomicrograph of kidney sections Gentamicin treated group: showing tubular epithelial damage with intense granular degeneration and intense widespread necrosis of PCT- score4 (10X, H & E)

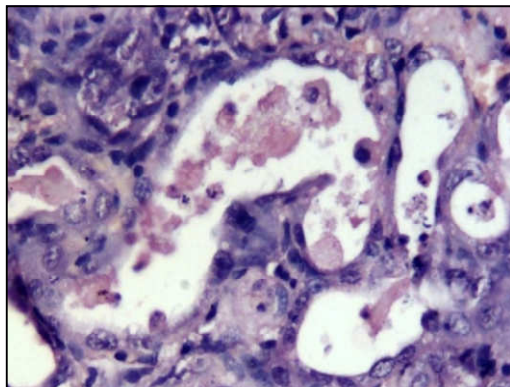


Fig 3: Photomicrograph of kidney sections Noni fruit juice treated group: showing dilated PCT with epithelial necrosis and desquamation with granular debris in lumen- score2 (10X, H & E)

IV. DISCUSSION

In the present study, we investigated the effect of noni fruit juice on gentamicin-induced nephrotoxicity in a murine model. Results of this study confirmed that gentamicin at a dose of 80 mg/kg produces significant renotoxicity as evidenced by increase in blood urea nitrogen, serum creatinine, urea and uric acid and renal tubular necrosis which corroborated with previous reports [23, 26, 27, 28, 29, 32]. Pretreatment with noni fruit provided marked functional and histological protection against acute renal damage in rats treated with gentamicin (fig 3). This study revealed that orally administered noni fruit juice has a significant and dose dependent protective effect in gentamicin-induced nephrotoxicity in rats as evident by the significant decrease in serum urea, uric acid, creatinine and blood urea nitrogen levels (Table 1).

A relationship between oxidative stress and nephrotoxicity has been well demonstrated in many experimental animal models [26, 27, 28, 29]. In gentamicin treated rats, a significant increase in lipid peroxidation products (MDA) suggesting the involvement of oxidative stress has been reported [23, 24]. A role of lipid peroxidation in gentamicin-induced acute renal failure has also been described in previous studies [22]. Moreover, pretreatment of rats with hydroxyl radical scavengers has shown protection against gentamicin induced acute renal failure [26].

It has been demonstrated that Noni juice contains some antioxidative ingredients both in vivo and in vitro [33]. In fact, the ingredients contained in Noni juice which demonstrate an antioxidative effect have been identified [34]. Wang *et al.* reported that intake of 10% of Tahitian Noni juice for 12 days inhibited the lipid hyperoxidation in the liver [21]. The European Union-

approved form of noni fruit juice from Tahiti (TNJ) has been found to exert an antioxidant effect in human athletes, thereby increasing exercise endurance [35]. This is also the mechanism by which noni fruit juice provided protection against carbon-tetrachloride-induced liver damage in Sprague-Dawley rats [36]. Other research revealed anti-oxidative activity that scavenges reactive oxygen species (ROS) and quenches lipid peroxides (LPO) in smokers [37]. Noni is rich in proxeronine, which combines with enzymes in the body to form an essential substance known as xeronine. It activates the immune system at cellular level thereby repairing and protecting kidney from damage [38].

Therefore, it is not unreasonable to assume that the nephroprotection shown by noni fruit juice extract in Gentamicin induced nephrotoxicity is mediated through its potent antioxidant effects that help to preserve intracellular GSH levels. The antioxidant activity of noni fruit might have contributed to its nephroprotective effect by inhibiting gentamicin-induced lipid peroxidation.

However other mechanisms of protection [39] like inactivation of the aminoglycoside by electrostatic complex formation or preventing its binding to the brush border membrane or by forming complexes at acidic pH and preventing phospholipid overloading in lysosomes cannot be negated also. Additional studies are warranted in order to test these assumptions, such as the measurement of gentamicin urinary excretion, the examination of gentamicin and noni fruit juice interactions with brush border membranes, and the effect of treatment on intracellular Ca^{2+} . Further studies exploring and linking the antioxidant activities to the nephroprotective effect and evaluation of glomerular filtration rate in noni fruit juice treated rats might shed more light on the mechanism of renoprotective action of noni fruit. Therefore, further investigations should be conducted in order to better characterize the attenuation of gentamicin-induced nephrotoxicity by noni fruit.

V. CONCLUSION:

To conclude, this study provides scientific evidence of the nephroprotective effects of orally administered noni fruit juice in toxicant that directly induces renal damage. It further proposes that observed protective effects of noni fruit juice in gentamicin nephrotoxicity could be attributed to its well-known antioxidant potential.

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