Prevention of nephrotoxicity with nutrient *morinda citrifolia* in acetaminophen induced rats

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Abstract **- Objective: To investigate the nephroprotective activity of ethanolic extract of *Morinda citrifolia* leaves against acetaminophen induced nephrotoxicity in male Wistar rats.**

Materials & Methods: The method of nephrotoxicity study in male wistar rats were divided into four groups (n=6). Group-I normal and Group-II served as toxic controls respectively, while Group-III and Group-IV were the treatment groups which were pretretment with *Morinda citrifolia* ethanolic extract 250, 500 mg/kg/day, respectively for about 28 days, simultaneously each dose of acetaminophen (750 mg/kg/day) for 14 days from 15th to 28th days, urine and blood samples were collected and estimated for biochemical parameters, kidneys for histopathological studies were obtained under the inhaled ether anaesthesia.

Results: Acetaminophen treatment caused nephrotoxicity was evidenced by marked elevation in blood urea, serum creatinine, serum total protein, urinary creatinine and urinary total protein and also reduced creatinine clearance. Pretreatment and Co-administration of *Morinda citrifolia* extract reduced the blood urea, serum creatinine, serum total protein, urinary creatinine and urinary total proteins. Apart from these histopathological changes also shown the protective nature of *Morinda citrifolia* extract against acetaminophen induced necrotic damage of renal tissues.

Conclusion: It was observed that the ethanolic extract of *Morinda citrifolia* leaves conferred

Nephroprotective in acetaminophen induced nephrotoxic rats.

Index Terms **- acetaminophen, nephrotoxicity, *Morinda citrifolia*, nephroprotective**

INTRODUCTION

Nephrotoxicity is a poisonous effect of some substances, both toxic chemicals and medication on the kidneys. There are various forms of toxicity, Nephrotoxicity should not be confused with the fact that some medications have a predominantly renal excretion and need their dose adjusted for the decreased renal function. Mainly nephrotoxicity can be produced by the excess use of NSAID (Non steroidal anti-inflammatory drugs) like Acetaminophen, it induced liver necrosis and renal insufficiency occurs in approximately 1–2% of patients with acetaminophen overdose (Ahmed et al., 2008). Acetaminophen metabolised by the microsomal P-450 enzyme system, a highly reactive intermediate, N-acetylpbenzoquinone imine (NAPQI) is produced. NAPQI directly reacts with glutathione (GSH) and at overdoses of acetaminophen, the depletion of cellular GSH occurs, leads to nephrotoxicity (Ghosh et al., 2010).

Most of the drugs that can cause acute tubular necrosis are excreted by the kidney; these include aminoglycoside antibiotics, amphotericin B, cisplatin, radiocontrast agents, pentamidine, cocaine, and intravenous immunoglobulins (Cayco et al., 1997). Mechanisms of injury are multiple but may overlap, including direct tubular toxicity, deranged cellular energy production, free radical injury, heme tubular toxicity, abnormal phospholipid metabolism, and intracellular calcium toxicity (Zager, 1997). The Acetaminophen (APAP) is a widely used analgesic and antipyretic drug and is safe at therapeutic doses but accidental or intentional overdose causes acute

liver and kidney failure (Boutis and Shannon, 2001; Larson et al., 2005).

The mechanisms leading to hepatic injury have been extensively studied (Latchoumycandane et al., 2007; Ghosh and Sil, 2009), but the molecular mechanisms regarding APAP-induced nephro-toxicity are poorly defined. Although nephro-toxicity is less common than hepatotoxicity in APAP overdose, renal tubular damage and acute renal failure can occur even in the absence of liver injury (Jones and Vale, 1993; Eguia and Materson, 1997). This allows NAPQI to bind to cellular proteins and initiate lipid peroxidation, leading to renal injury (Hart et al.,1994).when the patient is taking NSAIDs and RAAS inhibitors (Ashley, et al., 2009)

Noni is the Hawaiian name for the fruit of Morinda citrifolia L. (Rubiaceae). Its various vernacular names are: ''Indian mulberry'', ''nuna'', or ''ach'' on the Indian subcontinent, ''mengkudu'' in Malaysia, ''nhau'' in Southeast Asia, ''painkiller bush'' in the Caribbean, or ''cheese fruit'' in Australia (Morton, 1992; Neilson, 2001; Cardon, 2003). Noni is native from Southeast Asia to Australia and is cultivated in Polynesia, India, the Caribbean, Central and northern South America (Dixon et al., 1999). The Polynesians have been using the noni plant for food and medicinal purposes for more than 2000 years. In traditional pharmacopoeia, the fruit is claimed to prevent and cure several diseases. It is primarily used to stimulate the immune system and thus to fight bacterial, viral, parasitic and fungal infections; it is also used to prevent the formation and proliferation of tumors, including malignant ones. Noni juice is also claimed to relieve inflammation. Most noni is consumed as juice, although leaves, flowers, bark and roots can also be used (Dixon et al., 1999 ;).

Morinda citrifolia, has been reported to possess antithrombotic, antioxidant, analgesic and anti-inflammatory and xanthine oxidase inhibitory (Palu et al., 2009) activities. There are also preliminary studies reporting its blood pressure lowering (Dang-Van-Ho et al., 1955)] and vasodilatory (Runnie et al., 2009) 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. A protective effect against carbon tetrachloride-induced liver injury in female Sprague Dawley rats has also been described.

MATERIALS AND METHODS

Collection of plant material

Fresh leaves of Morinda citrifolia L. were procured from local market and its authentication was done by prof. Dr.K.Madhava chetty, Department of Botany, Sri Venkateshwara University, Thirupathi(Dist.), Andra Pradesh(state), India.

Chemicals: Ethanol,

Preparation of plant extract:

The fresh leaves were dried in shade and ground to fine powder. This powder (150-180g) was extracted with soxhalet apparatus for 24 hours using 1.5 liters of ethanol. The alcoholic extract was filtered with filter paper; filtrate was collected and evaporated under reduced pressure using vacuum evaporator. The concentrated material obtained was reduced to thick mass at room temperature and water was removed by placing it in desiccators. The extract was dissolved in water and used for the studies.

Animals: Healthy, adult male albino waster rats between 2 and 3 months of age and weighing between 150-200g were used for the study. The animals were procured from Sainath agencies, Hyderabad. The animals were kept in polypropylene cages (6 in each cage) and animals were acclimatized to our lab environment for about a week prior to the study, so that they could adapt to the new environment. Animal house were maintained under standard hygienic conditions, at 25 ± 2^0C, humidity (60 ± 10 %) with 12 hrs day and night cycle, with food and water ad libitum. The experiments were carried out prior approval from Institutional Animal Ethical Committee (IAEC).

Induction of nephrotoxicity and treatment procedure:

Nephrotoxicity was induced by Acetamiphen (750 mg/kg p.o) for 14 days in toxic groups and Ethonolic extracts of morinda citrifolia (250mg/kg, 500mg/kg) were treated in preventive groups and at the end of experimental period, from all the animals urine was collected with help of metabolic cages for 24hrs on 15^{th} day after acetaminophen administration and the urine samples were subjected for estimation of urinary functional parameters such as urinary creatinine (Ucr), urinary total protein (UTP), and All the animals were sacrifice under diethyl ether anesthesia. Blood samples were collected by puncturing retro-orbital plexus,

allowed to clot. Serum was separated by centrifusing at 3000 rpm for 10min and used for the measurement of serum creatinine (Scr), blood urea nitrogen (BUN), and serum total protein (STP) using a commercial kits and results were expressed as mg/dl.

In all the groups, the abdomen was opened and kidney was dissected out and the left kidneys were processed for histopathological examination under light microscopic observation, according to standard procedures. For histopathological examination, the kidneys were fixe in phosphate buffered formalin (10%formaldehye in normal saline) and then embedded in paraffin wax, sectioned (4-5µm) and stained with hematoxylin and eosin. In all the groups, the right kidneys were removed and maintained at -80^0c. On the way of analysis, the kidney tissues were homogenized in cold KCL solution (1.5%). To give a 10% homogenate suspension an usefor biochemical assays.

Experimental design:

Male Wister rats were divided into 4 groups (n=6)

Group I: Normal control (drinking water per oral (p.o)

Group II: Acetaminophen (AP) (750mg/kg/day i.p, 1-28 days)

Group III: MCEE (250mg/kg *p.o* 1-28 days) + AP (750mg/kg/day *i.p*, 15-28 days)

Group IV: MCEE (500mg/kg *p.o* 1-28 days) + AP (+750mg/kg/day *i.p*, 15-28 days)

Biochemical parameters

The biochemical parameters were estimated as per the standard procedure prescribed by the manufacturer's instruction manual provided in the standard kits. Estimated in Serum and urine Estimation of serum markers: as determination of Blood Urea Nitrogen (BUN), Serum Creatinine (Scr), Serum total proteins (STP) and Estimation of urinary functional parameters: determination of Urinary Total Proteins (UTP), Urinary Creatinine (Ucr) and Creatinine Clearance (Clcr).

RESULTS

In the present study results obtained shows that acute dose of acetaminophen nephrotoxicity were reliably established with 750mg/kg/day single oral dose of acetaminophen as evidence by significant ($p<0.05$, $p<0.01$, $p<0.001$) elevation in the serum urea, uric acid and creatinine in acetaminophen treated control (group II) rats when compared to untreated controls (group I) rats . However, oral pre-treatment dose of extract of MCEE significantly attenuated elevated serum concentration of these parameters, in dose related pattern.

The blood urea nitrogen (BUN), serum creatinine and serum total protein values were significantly raised in acetaminophen (750mg/kg) treated groups. Where as in the preventive group treated with MCEE, the levels were found to be decreased compared to inducing group. Pretreated rats showed significant ($P<0.001$) decrease in the levels of serum markers. The results were represented in figures 1, 2 and 3 respectively.

The urinary parameters like urinary creatinine, urinary total protein and urinary clearance were found to be elevated in acetaminophen (750mg/kg) treated groups. Where as in the preventive group treated with MCEE, the levels were found to be decreased compared to inducing group. The results were represented in figures 4, 5 and 6 respectively.

The normal group kidney section revealed that glomeruli and tubules had normal morphology and no evidence of interstitial lymphocytic infiltration. In toxic control group there was a dilated congested vessels and areas of interstitial haemorrhages were seen. In Morinda citrifolia L. prevention groups the sections were showed less hemorrhagic regions.

In histopathological study of control group showing normal histological structure of the glomeruli and renal tubules in the cortex and normal tubules in the medulla. Acetaminophen treated group (group II) showing diffuse glomerular congestion, tubular casts, peritubular congestion, epithelial desquamations, blood vessel congestion, interstitial edema, mononuclear inflammatory cells and cellular necrosis, while treatment group (i.e. group III), shows only some of the blood vessels are dilated and congested within the interstitium. Also few scattered mononuclear inflammatory infiltration is seen within the interstitium and treatment group (i.e. group IV) shows no sign of histological changes or cellular necrosis and showed normal glomerular and tubular histology. From histopatological results we can conclude that Morinda citrifolia extract at dose of 250mg/kg have partial protective effect while 500mg/kg have more protective effect on acetaminophen induced nephrotoxicity.

DISCUSSION

In the present study, we investigated the effect of noni fruit juice on Acetaminophen-induced nephrotoxicity in a murine model. Results of this study confirmed that Acetaminophen at a dose of 750 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 (Agharazii et al., 1999; Burry et al., 1997; Schwarz, 1987). Pretreatment with noni fruit provided marked functional and histological protection against acute renal damage in rats treated with Acetaminophen. This study revealed that orally administered noni fruit juice has a significant and dose dependent protective effect in Acetaminophen-induced nephrotoxicity in rats as evident by the significant decrease in serum urea, uric acid, creatinine and blood urea nitrogen levels. A relationship between oxidative stress and nephrotoxicity has been well demonstrated in many experimental animal models (Curry et al., 1982; Keaton, 1988).

In Acetaminophen treated rats, a significant increase in lipid peroxidation products (MDA) suggesting the involvement of oxidative stress has been reported. A role of lipid peroxidation in Acetaminophen-induced acute renal failure has also been described in previous studies (Mugford et al., 1997). Moreover, pretreatment of rats with hydroxyl radical scavengers has shown protection against Acetaminophen induced acute renal failure. It has been demonstrated that Noni juice contains some antioxidative ingredients both in vivo and in vitro (Wang et al., 2004). In fact, the ingredients contained in Noni juice which demonstrate an antioxidative effect have been identified (Mohd-Zin et al., 2002). The previous reported that intake of 10% of Tahitian Noni juice for 12 days inhibited the lipid hyperoxidation in the liver (Wang et al. 2008).

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 (Su et al., 2005). This is also the mechanism by which noni fruit juice provided protection against carbon-tetrachloride induced liver damage in Sprague-Dawley rats (Palu et al., 2008). Other research revealed anti-oxidative activity that scavenges reactive oxygen species (ROS) and quenches lipid peroxides (LPO) in smokers (Wang et al., 2009). 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 (Heinicke, 1985). Therefore, it is not unreasonable to assume that the nephroprotection shown by noni fruit juice extract in Acetaminophen 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 Acetaminophen-induced lipid peroxidation. However other mechanisms of protection (Beauchamp et al., 1997).

Additional studies are warranted in order to test these assumptions, such as the measurement of Acetaminophen urinary excretion, the examination of Acetaminophen and noni fruit juice interactions with brush border membranes, and the effect of treatment on intracellular Ca2+. Further studies exploring and linking the antioxidant activities to the nephroprotective effect and evaluation of glomerular filteration 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 Acetaminophen-induced nephrotoxicity by noni fruit.

CONCLUSION

The result of this study illustrated that ethanolic extract of the plant produced significant protection against acetaminophen induced alterations in serum and urinary marker enzymes and cellular damage. Combined effect of active principles present in the ethanolic extract of Morinda citrifolia L. might offer protection against renal damage rendered by acetaminophen in rats. Thus, ethanolic extract of leaves of Morinda citrifolia L. exhibited significant nephroprotective activity in rats.

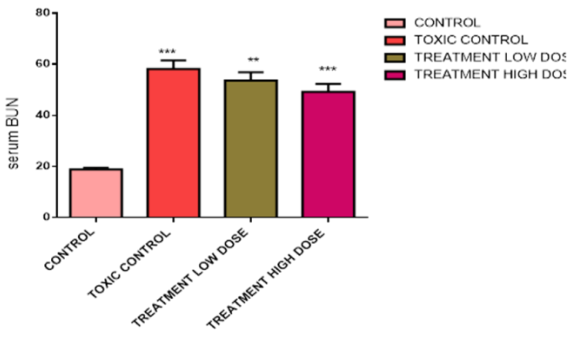

Fig 1: Estimation of BUN in serum (Data were expressed in Mean ± SD (n=6) p- values were (where, ** p <0.01 and***P<0.001, compared with control, toxic control, treatment groups)

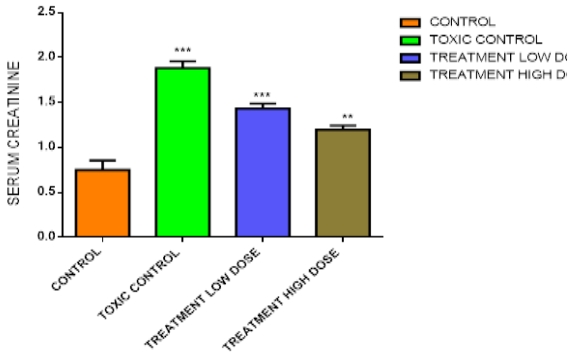

Fig 2: Estimation of Serum Creatinine in blood (Data were expressed in Mean ± SD (n=6) p- values were (where, ** p <0.01 and***P<0.001, compared with control, toxic control, treatment groups)

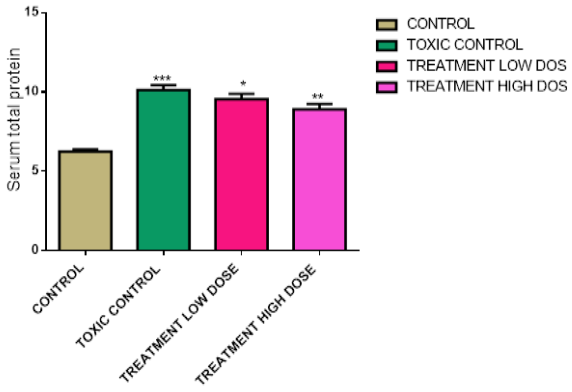

Fig 3. Estimation of total proteins in serum (Data were expressed in Mean ± SD (n=6) p- values were (where, ** p <0.01 and***P<0.001, compared with control, toxic control, treatment groups)

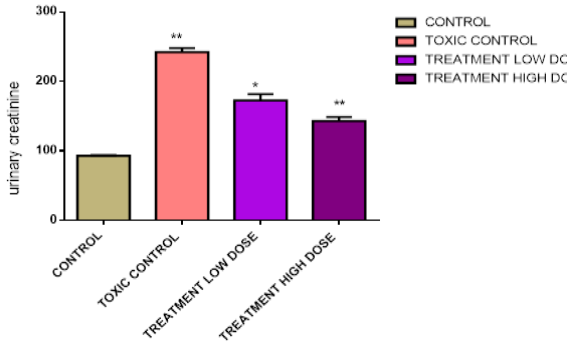

Fig 4: Estimation of Urinary creatinine in urine (Data were expressed in Mean ± SD (n=6) p- values were (where, ** p <0.01 and***P<0.001, compared with control, toxic control, treatment groups)

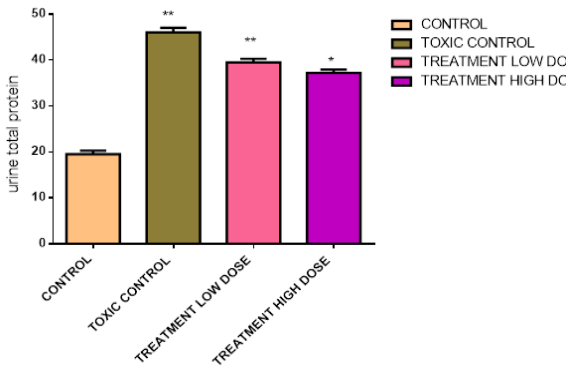

Fig 5: Estimation of urinary protein in urine (Data were expressed in Mean ± SD (n=6) p- values were (where, ** p <0.01 and***P<0.001, compared with control, toxic control, treatment groups).

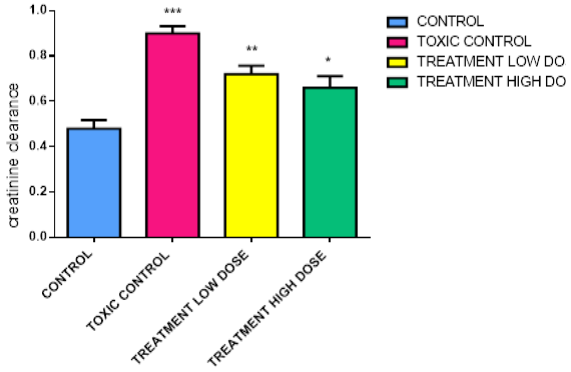

Fig 6: Estimation of creatinine clearance in urine (Data were expressed in Mean ± SD (n=6) p- values were (where, ** p <0.01 and***P<0.001, compared with control, toxic control, treatment groups)

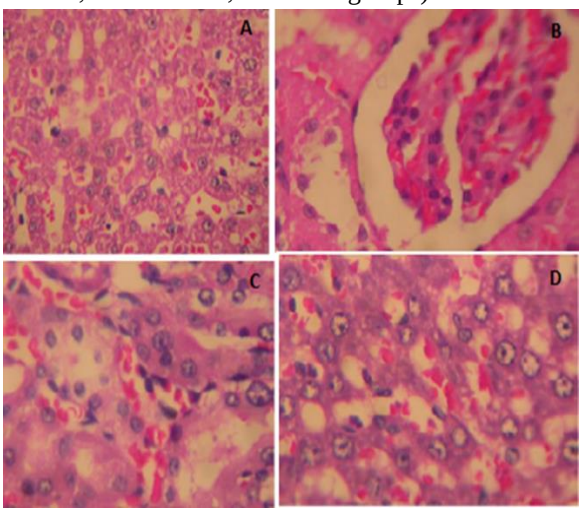

Fig 7: A) Control B) toxic control C) Treatment with low dose (250mg/kg) D) Treatment high dose (500mg/kg).

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Conflict of interest: No Conflict of interest

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