POTENTIAL OF *MORINDA CITRIFOLIA* IN KIDNEY AN *IN VIVO* STUDY

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ABSTRACT

The aim of the present study was to assess the activity of *Morinda citrifolia* extract in tubular enzymes and in urinary albumin of Gentamicin (GM) induced renotoxic rats. The study was conducted for fourteen days in twenty-four rats in four groups and the study was designed as Group I: Control group received 0.5% CMC; Group II: GM 100mg/kg/day i.p. Group III: *Morinda citrifolia* extract (200mg/kg/day p.o.) + GM (100mg/kg/day i.p.); Group IV: *Morinda citrifolia* extract (400mg/kg/day p. o) + GM (100mg/kg/day i.p.). After the last injection of GM, 24-hour urine samples were collected from all the groups to analyze the levels of tubular marker enzymes N acetyl glusosaminidase (NAG), Gama Glutamyl transpeptidase (GGT), Alkaline phosphatase (ALP) and Lactate dehydrogenase (LDH) and albumin in urine to determine the extent of renal tubular damage by measuring renal enzymuria and albuminuria in toxic rats induced by Gentamicin. Levels of tubular marker enzymes and albumin were increased in Group-II (0.90 ± 0.07) and in treatment with *Morinda citrifolia* extract with different doses brought back to normal levels in tubular marker enzymes as well as albumin in urine for Group- III (0.49 ± 0.12) and Group- IV (0.35 ± 0.08). The present study revealed that *Morinda citrifolia* extract possess potent protective effect in both doses due to its high antioxidant property against renal enzymuria and albuminuria.

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INTRODUCTION

Plants synthesize potent biochemicals which constitute the components of phytomedicine since times immemorial (Farombi *et al.,* 2006). Medicinal plants typically contain several different pharmacologically active compounds that may act individually, additively or in synergy to improve health. Recently there has been an upsurge of interest in the therapeutic potentials of medicinal plants as antioxidants in reducing free radical induced tissue damage. Since ancient times, many herbs have been potentially used as an alternative remedies for treatment of many infections, diseases, and also as food preservatives suggesting the presence of antimicrobial and antioxidant constituents (Tao *et al.,* 2000). Antioxidants play an important role in inhibiting and scavenging free radicals, thus providing protection to human against infections and degenerative diseases. Current research is now focused towards natural antioxidants originated from plants due to safe therapeutics. Natural antioxidants can protect the human body from free radicals by retarding the progress of many chronic diseases as well as retard lipid oxidative rancidity in foods. Many oxidative stress related diseases are as a result of accumulation of free radicals in the body (Whiting *et al.,* 1996). Free radicals are implicated for more than 80 diseases including Diabetes Mellitus, Arthritis, Cancer, and Aging etc. In the treatment of these diseases, antioxidant therapy has gained an utmost importance. Plants are endowed with free radicals scavenging molecules such as vitamins, terpenoids, phenolic acids, lignins, tannins, flavonoids, quinones, coumarins, alkaloids, amines, betalains and other metabolites which are rich in antioxidant activity. The many number of medicinal plants are used in the treatment of cellular and metabolic disease. There are some speculations that the generation of free radicals inside the body in some physiological conditions is resulted in the cellular changes and development of cancer etc and this would be neutralized by the antioxidants from different medicinal plants. *Morinda citrifolia* belonging to the family Rubiaceae, commonly known as noni and it is a most popular drug in ayurvedic medicine. It is tropical and subtropical plant grown in the pacific islands and has been used to treat a broad range of diseases for over 2000 diseases. Commercial noni juice and encapsulated noni powder have become popular in Asia, North America and Europe. Various unsubstantiated claims are currently made from noni products such as immune system stimulant, anticancer agent, menstrual cycle regulator and blood cleanser. Various biological compounds such as glycosides, polysaccharides, alkaloids, lignans, fattyacid esters, anthroquinones, scopoletin, morindin, vitamins and minerals have been isolated from noni fruits, roots and leaves (Dadsena *et al.*, 2013).

The kidney is a common target for toxic xenobiotics, due to its specific capacity in clearing the toxic substances. A number of drugs, chemicals, heavy metals play a role to alter its structure and function but acute and chronic intoxication have been demonstrated to cause nephropathy with various levels of severity ranging from tubular dysfunctions to acute renal failure (Barbier *et al.,* 2005). Gentamicin (GM), an aminoglycoside antibiotic, very effective in treating against life threatening gram-negative bacterial infections (Ho and Barza, 1987). The use of GM was limited due to its ototoxicity and reno toxicity (Well wood *et al.,* 1976). GM was essentially

eliminated by glomerular filtration and partially reabsorbed by proximal tubular cells (Black *et al.,* 1963) and this was simplified as, an increased kidney uptake of antibiotic, mainly in the proximal tubular cells (Beauchamp *et al.,* 1985). Earlier detection of renal impairment might provide an opportunity to minimize the risk of severe or persistent of the renal injury caused by the nephrotoxic drugs. Clinical manifestations of impairment, such as elevations in blood urea nitrogen (BUN) or serum creatinine, not become evident until 50-75% of the nephrons have been damaged (Lilis and Landrigan, 1988). Hence, there has become an ongoing interest in developing innovative and early biomarkers of renal damage (Sara Taylor *et al.,* 1997). Measurements of the activities of urinary enzymes were considered as a useful non-invasive test in detecting the deterioration of renal function in the initial stage. The renal enzyme such as N-acetyl-glusosaminidase (NAG) and the brush border enzyme Gama glutamyl transpeptidase (GGT) have also been used to assess renal toxicity (Periyasamy *et al.,* 2009). The damage in the tubules leads to the excretion of NAG and GGT into the lumen of the tubules that were detected in the urine. However, limiting factors in the use of these enzymes, particularly NAG is the considerable intra/inters individual variation in urinary enzyme activity (Naidu and Lee, 1994). Interest in the antioxidants related to food and herbal medicines was popularized owing to their multifaceted activities like natural origin, cost effectiveness, and it is less repercussion (Kullu *et al.,* 2013). These provide enormous scope in correcting the disease conditions, serving humans as food components, seasonings, beverages, cosmetics as well as medicine. Several organic products were utilized to protect the toxicities induced by drugs (Kumar *et al.,* 2013). Herbs were usually considered safe and proved effective against various human ailments and their medicinal uses have been gradually increasing in developed countries (Kaur and Kapoor, 2002).

The researchers in the same plant did common parameters for GM induced renotoxicity. However, the activities of tubular marker enzymes were not yet studied in this plant extract. Therefore, our present study aims to evaluate the efficacy of *Morinda citrifolia* with two different doses on the activity of tubular marker enzymes of GM induced renotoxic rats.

MATERIALS AND METHODS

Collection of plant material
Morinda citrifolia (MC) fruits were purchased from the local areas around Chennai, Tamil Nadu, India. The fruits were washed thoroughly with water to eliminate the earthy matters, freed from the debris, shade dried under room temperature for a few weeks, and coarsely powdered in a food processor. The collected fruits was identified and authenticated by Botanical survey of India (BSI), Tamil Nadu Agriculture University, Coimbatore, Tamil Nadu, India. A voucher specimen (No.BSI/SRC/93558) has been deposited at the herbarium of the Botany Department.

Extract preparation
Extraction was performed by hot continuous percolation method using Soxhlet apparatus. About 500gms of coarsely powdered *Morinda citrifolia* was extracted in 70% ethanol by the continuous hot extraction method at 50ºC. The extract with 70% ethanol was decanted from the Soxhlet apparatus and the filtrate was evaporated for the total elimination of alcohol using a Rota flash Vacuum evaporator. The concentrated liquid extracts obtained were transferred to a China dish and kept in a water bath for 50ºC for dryness. The residual extracts were transferred to an airtight container free from contamination for further experimental works.

Drug and chemicals
Main drug Gentamicin (GM) procured from Aptus Therapeutics Pvt. Ltd (Hyderabad, India). The Standards such as p-nitrophenol, p-nitroaniline, pyruvate and crystalline phenol for the assay were obtained from Sisco Research Laboratories Pvt. Ltd., (Mumbai, India). The Substrates p-nitrophenol N-acetyl-glucosaminide from Himedia Laboratories (Mumbai, India). The other substrates such as L-ā-glutamyl-p-nitroanilide, NAD^+ and Disodium phenyl phosphate were procured from Sisco Research Laboratories Pvt. Ltd., (Mumbai, India). The other chemicals such as sodium citrate, sodium hydroxide, glycine, tris powder, hydrochloric acid, glycyl glycine, Acetic acid, lithium lactate, DNPH, sodium carbonate, sodium bicarbonate, citric acid, Folin's phenol reagent were obtained from Sisco Research Laboratories Pvt. Ltd. (Mumbai, India).

Animals
Male Sprague Dawley rats weighing 200-250g were obtained from the Amala institute of medical sciences, Thrissur, Kerala, India was used for the study. Animals were housed in individual polypropylene cages in a ventilated room (air cycles: 15/min; 70:30 exchange ratio) under an ambient temperature of 22 ± 2ºC and 40-65% relative humidity, with a 12-h light/dark artificial photoperiod. They were provided with food (M/s: Hi-tech Animal Feed Pvt Ltd, Madurai, Tamil Nadu, India) and purified water ad libitum. All the animals were acclimatized at least for 7 days in the laboratory conditions prior to experimentation. CPSCEA guidelines were followed throughout the study. The Institutional Animal Ethical Committee of Kovai medical centre research and educational trust, Coimbatore, India (IAEC/ KMCRET/ AICTE/01/2014-2015), approved the experimental study.

Experimental study design
After acclimation, the rats were grouped into four of six animals each. The group I serve as Normal control (0.5% CMC); Group II serves as a Toxic control (100mg/kg i.p GM, +0.5% CMC); Group III serves as Test control with low dosage (200 mg/kg p. o MC +100mg/kg i.p GM); Group IV serves as MC +GM group (400 mg/kg p.o MC +100mg/kg i.p GM).GM 100 mgs/kg/d was injected intra peritoneal for 14 days, except for Group I and MC extract was administered orally every day with two different doses, one hour before Gentamicin injection.

Collection of urine sample
After the last injection of Gentamicin (on day 14), all the animals were kept in individual metabolic cages to collect 24-hour urine samples, Sodium azide were added to the urine samples, Sodium azide were added to the urine collection vessels as a preservative and a small portion of the urine was used to assay the albumin content. The remaining part of the Urine samples was centrifuged for 10 minutes at 1000g in a refrigerated centrifuge and separated from the sediment. The

supernatant considered as crude urine. This crude urine sample was used for dialysis (Berscheid *et al.*, 1983) using the standard RC Dialysis tubing, Part. No 132655 (Spectrum laboratories, Rancho Dominguez, California) at room temperature for 90 minutes against flowing tap water and later was used for the determination of various enzyme levels in urine.

Analysis of albumin and tubular enzyme levels in urine

The activity of N-acetyl-glusosaminidase (Moore and Morris, 1982), Gama glutamyl transpeptidase (Rosalki and Rau, 1972), alkaline phosphatase (King, 1965b) and Lactate dehydrogenase (King, 1965c) were determined using the above- cited references. Albumin levels in urine were assayed using commercially available kits from Accurex biomedical Pvt. Ltd. (Mumbai, India). All the assays were measured using Semi Automatic Biochemical Analyzer and assays were done in replicate to determine the concordant values.

Statistical analysis

The results were given as Mean $\pm$ Standard error of Mean (SEM). Data were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison tests using the Graph Pad Prism 5.03 (Graph Pad Software, San Diego, CA, USA). The statistical significance of the difference was taken as $P < 0.05$. $P < 0.01$ and $P < 0.001$.

RESULTS AND DISCUSSION

The present study was attempted to test the protective activity of hydroethanolic extract of *Morinda citrifolia* in the albumin and tubular marker enzyme levels in experimental animals were shown in Table 1.

The albumin level in urine was found to be significantly increased ($P < 0.01$) in the GM induced group and it was reverted towards normal with significance ($P < 0.01$ and $P < 0.001$) in both the doses of MC (200mg and 400mg/kg/day). Well-marked responses have been observed in the high dose (400mg/kg/day) of MC as showed in Table-1. The levels of ã - GT, LDH and ALP were considered to be increased with significance ($P < 0.01$) in the urine of GM induced tubular toxic rats. On the other hand, the activities of GGT, LDH, and ALP was reverted towards normal with significant ($P < 0.05$ and $P < 0.01$) in low and high dose of MC (200mg and 400mg/kg/day). NAG activity was significantly ($P < 0.001$) increased in the urine of GM induced toxic rats and in turn the activity was reverted towards normal with significant ($P < 0.01$ and $P < 0.001$) in low and high doses of MC.

The following studies report (Ali *et al.*, 2005) that oxidative Stress is the major contributor in GM- induced nephrotoxicity. The increased excretion of albumin in urine shows, the extent of renal tubular injury caused by GM in the toxic group, which comes to a normal level in the present study by the dose dependent administration of MC with GM and the present report, was agreement with the earlier report (Kakalij *et al.*, 2014). GM induces conspicuous and characteristic changes in lysosomes of proximal tubular cells consistent with the accumulation of polar lipids (myeloid bodies). These changes are preceded and accompanied by signs of tubular dysfunctions or alterations leads to release of brush border and lysosomal enzymes; decreased re-absorption of filtered proteins. Under normal conditions, only few enzymatic activities of the serum enter the urine through glomerular filtrations. NAG is discovered predominantly in lysosomes of proximal tubule (Ali *et al.*, 2008) of kidney and excreted in increased concentration in urine during GM induced renal tubular toxicity (Li *et al.*, 2009) and it is the indication of proximal tubular injury of the kidney (Waring and Moonie, 2011).

In the present study, the NAG excretion in urine is increased in the GM induced group because of proximal tubule lesions (Emeigh Hart, 2005) but the condition is reversed by oral graded dosage of MC even in the presence of GM proving the protectiveness of the MC on toxic kidney which was supported by the previous report (Kadkhodaee *et al.*, 2005). GGT, LDH and ALP the enzymes of the brush border located in the proximal renal tubule are usually present in small amount in urine because of tubular cell shedding. An elevated level of these enzymes in urine is the major indication of proximal tubular Injury (Racusen *et al.*, 1991) which was said to be the earlier biomarkers of renal damage (Sarah Taylor *et al.*,1997).

A GM administration for 14 sequential days in the present study indicates, a well-developed tubular injury caused by GM induction by the excretion of tubular enzymes LDH (Kadkhodaee *et al.*, 2005), GGT (Mohamed *et al.*, 2013) and ALP (Kadkhodaee *et al.*, 2005) in urine. Oral administration of graded doses of MC even in the presence of GM in rats reverts to the normal level of these tubular marker enzymes LDH (Kadkhodaee *et al.*, 2005), GGT (Mohamed *et al.*, 2013), ALP (Kadkhodaee *et al.*, 2005) and albuminuria (Table-1) implies restoring the normal function of proximal tubular cells from toxic injury caused by GM. Most of the Medicinal plants are rich in bioactive constituents tannins, phenols, flavonoids, alkaloids, saponins, glycosides, carbohydrates which possess antioxidant property (Annie *et al.*, 2005)

Table 1: Effect of hydro ethanol extract of *Morinda citrifolia* and GM induced renotoxicity in rats.

Experiments	Groups Control	GM	MC + GM	MC + GM
Albumin(mg/dl)	0.45 ± 0.009	0.90 ± 0.07$^{\$\$}$	0.49 ± 0.12	0.35 ± 0.08***
NAG	12.16 ± 2.48	28.67 ± 2.52$^{\$\$}$	11.38 ± 0.70	11.04 ± 2.82***
GGT	2.19 ± 0.03	3.48 ± 0.05$^{\$\$}$	2.15 ± 0.99	2.12 ± 0.32***
LDH	1.32 ± 0.44	2.05 ± 0.77$^{\$\$}$	1.29 ± 0.59	1.20 ± 0.1***
ALP	0.15 ± 0.11	0.69 ± 0.04$^{\$\$}$	0.14 ± 0.01	0.10 ± 0.02***

GM:Gentamicin; MC: *Morinda citrifolia* extract; NAG: N acetyl glusosaminidase; γ-GT: Gama glutamyl transpeptidase; LDH: Lactate dehydrogenase; ALP:Alkaline phosphatase; Units: NAG: μmoles of phenol liberated/ mg ptn./min; g- GT-(IU/l);LDH-μmoles of pyruvate liberated/mg ptn. / min;ALP -μmol ofp-nitrophenol liberated/ mg ptn./ min; Data's were expressed as mean ± SEM (n=6) ; $^{\$\$}$P < 0.01 and $^{\$\$\$}$P < 0.001 when compared to control; *P < 0.05, **P < 0.01 and ***P < 0.001 when compared to Toxic Group.

The present study concludes from the discussion part, that the *Morinda citrifolia* are rich in phyto constituents proving its antioxidant property from the researchers report, may contributes to its renoprotective activity against tubular enzymuria and albuminuria of GM induced renotoxic rats. However, further studies are required to identify the exact mechanism and active phyto-constituents involved behind this reno protective effect.

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