



ISSN Print: 2394-7500
ISSN Online: 2394-5869
Impact Factor: 5.2
IJAR 2017; 3(1): 169-173
www.allresearchjournal.com
Received: 22-11-2016
Accepted: 23-12-2016

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Protective effect of flavonoid rich fraction of *Helicteres isora* (L.) fruit on gentamicin induced nephrotoxicity in wistar rats

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Abstract

Objective: Herbal and polyherbal formulations are at boom owing to lesser side effects and higher efficacy and are considered for management of renal complications. The present study evaluates the renoprotective effect of flavonoid rich fraction of *Helicteres isora* (L.) fruit in gentamicin induced nephrotoxicity wistar rats.

Methods: Nephrotoxicity were induced in wistar rats of either sex by intraperitoneal administration of gentamicin 80 mg/kg body weight for ten days. The extract of flavonoid rich fraction of *Helicteres isora* (L.) fruit (100, 200, 400 mg/kg) was suspended in 2% w/v gum acacia and administered orally to gentamicin treated rats for next ten days. Rats were subjected to evaluate biochemical and oxidative parameters and histopathology of renal tissue were performed at the end of ten days treatment.

Results: The elevated serum creatinine, uric acid, urea, blood urea nitrogen levels, oxidative stress of kidney and reduced levels of urine creatinine, glomerular filtration rate in gentamicin treated rats were reverted back approximately near to normal after the treatment of flavonoid rich fraction of *Helicteres isora* (L.) fruit at a doses of 100, 200 and 400 mg/kg.

Conclusion: Flavonoid rich fraction of *Helicteres isora* (L.) fruit possesses protective action against gentamicin induced nephrotoxicity in wistar rats which may be attributed by its antioxidant effect.

Keywords: Gentamicin, nephrotoxicity, flavonoid rich fraction of *Helicteres isora* (L.) fruit (FRFHI)

1. Introduction

As kidney is an essential organ required by the body to perform several important functions including the maintenance of homeostasis, regulation of the extracellular environment such as detoxification and excretion of drugs and toxic metabolites it is major target organ for exogenous toxicants. Nephrotoxicity is a kidney specific feature in which excretion does not go smoothly owing to toxic chemicals or drugs. Approximately 20% of nephrotoxicity is induced by drugs, but medication of the elderly increases the incidence of nephrotoxicity up to 66% as the average life span increases. Nephrotoxicity is an adverse side effect of chemotherapy or anticancer medicines [1]. Many drugs, natural products, industrial chemicals and environmental pollutants are mainly responsible for nephrotoxicity. e.g. therapeutic drugs like aminoglycoside antibiotics, Non-steroidal anti-inflammatory drugs (NSAID's), chemotherapeutic agents chemical reagents like ethylene glycol, carbon tetrachloride, sodium oxalate and heavy metals such as lead, mercury, cadmium and arsenic produces acute renal failure [2]. Most drugs found to cause nephrotoxicity exert their effects by one or more common pathogenic mechanisms. These include altered intraglomerular hemodynamics, tubular cell toxicity, inflammation, crystal nephropathy, rhabdomyolysis, and thrombotic microangiopathy [3]. Aminoglycosides preferentially affect the proximal tubular cells. These agents are freely filtered by the glomeruli and quickly taken up by the proximal tubular epithelial cells, where they are incorporated into lysosomes after first interacting with phospholipids on the brush border membranes. They exert their main toxic effect within the tubular cell by altering phospholipid metabolism. In addition to their direct effect on cells, aminoglycosides also cause renal vasoconstriction [4]. Thus the present study was undertaken to investigate the Protective Effect of Flavonoid Rich Fraction of *Helicteres isora* (L.) (FRFHI) fruit on Gentamicin Induced Nephrotoxicity in Wistar Rats.

2. Material and Methods

2.1 Drugs and chemicals

The dried fruits of *Helicteres isora* (L.) were collected from Western Ghats of Konkan region, Maharashtra, India. The plant was authenticated by Dr. Miss. A. S. Upadhye, Agharkar Research Institute, Pune, India. Gentamicin (Wemycin) was procured from local market of Pune. The diagnostic kits were obtained from Biolab (Mumbai, India) and Robonik (Navi Mumbai, India). All required chemicals were of laboratory grade obtained from local suppliers of Pune.

2.2 Preparation of plant Extract (Methanolic extract)

Dried fruits were coarsely powdered and extracted with methanol: water (9:1) by maceration process. The extract was filtered and concentrated in vacuum and kept in a vacuum desiccator for complete removal of solvent [5]. Methanolic extract of fruits of *Helicteres isora* was obtained in the yield of 0.3% w/w.

2.3 Experimental animals

Thirty Wistar rats of either sex, weighing 200-250 g were procured from National Institute of Bioscience, Pune. The rats were housed in polypropylene cages with paddy husk as bedding. The animals were maintained under standard laboratory conditions at temperature 23 ± 2 °C with relative humidity $55 \pm 10\%$ under 12 h light and 12 h dark cycle throughout the experiment in the animal house of Sinhgad college of Pharmacy, Pune. The animals were fed with standard pellet diet (Nutrivet Life Sciences, Pune, India) and water *ad libitum*. All the experimental procedures and protocols used in this study were reviewed and approved (SCOP/IAEC/2015-16/01/204) by the Institutional Animal Ethics Committee (IAEC) of Sinhgad College of Pharmacy, Pune.

2.4 Experimental procedure

Thirty wistar rats of either sex were randomly divided into five groups (n=6). Group I was control group and group II was gentamicin control group. Group III, IV and V were gentamicin treated groups. Group III received 80 mg/kg/day gentamicin i.p. and 100 mg/kg/day of FRFHI fruit p.o. for eight days. While group III and group IV were received 80 mg/kg/day gentamicin i.p. as well as 200 and 400 mg/kg/day of FRFHI fruit p.o. for eight days respectively.

One day before sacrifice each rat was individually placed in a metabolic cage for 24 h for urine collection. Urine was centrifuged at 1000 rpm for 10 min. to remove cells and debris. Blood was collected from retro orbital plexus under light anaesthesia and the serum was separated by centrifugation at 3000 rpm for 15 min. At the end of the experiment rats were killed by cervical dislocation under ether anaesthesia. The abdominal cavity was immediately opened and both kidneys were removed and processed for antioxidant as well as histopathological examinations.

2.5 Body weight, kidney weight and relative kidney weight

The body weight of all animals before and after the experiment was measured by gravimetric method using electronic weighing balance and their difference was expressed as body weight change. After sacrificing the experimental animals one of the kidneys was rinsed in

chilled saline, decapsulated blotted on filter paper and quickly weighed. For standardization, total kidney weight was normalized as kidney/body-weight ratio.

Relative kidney weight (%) = $\frac{\text{Absolute kidney weight}}{\text{Body weight at sacrifice}} \times 100$

2.6 Biochemical estimations in serum and urine

At the end of the treatment, serum and urinary levels of creatinine, serum uric acid, serum urea and Blood urea nitrogen (BUN) were estimated as per the instruction of commercial diagnostic kits. Whereas creatinine clearance was calculated as per the following formula;

$$\text{Ccr (mL/min/kg)} = \frac{\text{urinary Cr (mg/dL)} \times \text{urinary volume (mL)}}{\text{serum Cr (mg/dL)}} \times \frac{1000}{\text{body weight (g)}} \times \frac{1}{1440 \text{ (min)}}$$

2.7 Evaluation of oxidative stress in rat kidney

At the end of experiment, rats were sacrificed by decapitation and kidneys were excised quickly, washed immediately with ice cold physiological saline (0.9% NaCl), dried and weighed. Right kidney was taken for histopathological examination while left kidney was homogenized in chilled 50mM phosphate buffer saline (pH 7.4). The homogenates were centrifuged at 10000 rpm for 15 min at 4°C. The supernatant was used to determine the levels of malondialdehyde (MDA) [6], nitric oxide (NO) [7], reduced glutathione (GSH) [8] and superoxide dismutase (SOD) activity [9].

2.8 Histopathological examination of kidney

The isolated renal tissue was fixed in 10% formalin solution, embedded in paraffin and cut into semi-thin sections (2 µm). The tissue sections were stained with hematoxylin and eosin (H &E) and observed under light microscopy (100 X).

2.9 Statistical analysis

All the data were expressed as the mean \pm S.E.M (n=6). Data were subjected to one-way analysis of variance (ANOVA) followed by the Tukey's multiple comparison test. $P < 0.05$ was considered as minimum level of significance. Data was analysed using Graph Pad Prism version 7.0.

3. Results

3.1 Body weight, kidney weight and relative kidney weight

Significantly decreased body weight and increased kidney weight as well as relative kidney weight was noted in non-treated gentamicin control rats in comparison with control rats. ($P < 0.001$, $P < 0.01$) Treatment of FRFHI at doses 100, 200 and 400 mg/kg significantly restored the body weight. Significant increase in body weight was observed in the rats treated with FRFHI fruit 200 and 400 mg/kg dose as compared to rats treated with 100 mg/kg dose ($p < 0.01$ and $p < 0.05$ respectively). Rats treated with FRFHI fruit at a dose of 100, 200 and 400 mg/kg produced significant decrease in kidney weight as well as relative kidney weight ($p < 0.01$, $p < 0.01$ and $p < 0.001$; respectively) in comparison with gentamicin control rats. However significant decrease in kidney weight was observed in the rats treated with FRFHI fruit at 400 mg/kg dose in comparison with FRFHI fruit 200 and 100 mg/kg dose ($p < 0.001$).

Table 1: Effect of FRFHI fruits on body weight, kidney weight and relative kidney weight on normal and diabetic rats.

Group	Body weight (g)	Kidney weight (g)	Relative kidney weight (%)
NC	234.5± 5.69	0.77±0.034	0.323±0.019
GC(80)	222.5± 5.83 ^c	1.37±0.027 ^c	0.613±0.020 ^c
GC+FRFHI (100)	220.3 ± 5.04	1.15±0.016 [#]	0.516±0.016 [@]
GC+FRFHI (200)	224.7± 4.74 ^{\$}	1.07±0.014 [#]	0.475±0.012 [@]
GC+FRFHI (400)	231.7± 5.43 ^{@&}	0.9±0.010 ^{#+%}	0.383±0.009 ^{#+%}

^c*P*<0.001 Vs NC ; [#]*P*<0.001, [@]*P*<0.01 Vs GC ; [%]*P*<0.001, [&]*P*<0.01, ^{\$}*P*<0.05 Vs FRFHI (100) ; ^{+P}<0.001 Vs FRFHI (200)

3.2 Biochemical estimations in serum and urine

In present study gentamicin produced prominent kidney damage as evidenced by significantly higher levels of serum creatinine, blood urea nitrogen and decreased urine creatinine as well as marked reduction in creatinine clearance. However, the rats treated with FRFHI fruit at a dose of 100, 200 and 400 mg/kg significantly decreased the elevated serum creatinine and blood urea nitrogen levels as well as increased urine creatinine and creatinine clearance as compared to gentamicin treated rats (*p*<0.001). Gentamicin

induced nephrotoxicity produced significant increase in the 24 h total urine volume and Serum urea levels as compared to normal control rats (*p*<0.001) which was significantly decreased with the treatment of FRFHI fruit at a dose of 100, 200 and 400 mg/kg. Nephrotoxic rats treated with FRFHI fruit at a dose of 100, 200 and 400 mg/kg significantly decreased the elevated serum uric acid levels when compared to control rats (*p*<0.01, *p*<0.001, *p*<0.001 respectively).

Table 2: Effect of FRFHI fruits on various biochemical parameters.

PARAMETERS	NC	GC(80)	GC+FRFHI (100)	GC+FRFHI (200)	GC+FRFHI (400)
Serum Creatinine (mg/dl)	0.57±0.029	4.39±0.377 ^C	1.73±0.255 [#]	1.12±0.263 [#]	0.68±0.117 ^{\$}
Urine Creatinine (mg/dl)	73.09±3.814	48.83±4.661 ^C	63.81±2.31 [*]	71.68±2.702 [#]	79.3±2.59 ^{\$}
Urine Volume (ml)	12.08±0.611	20.08±0.663 ^C	17.62±0.465 [*]	16.05±0.641 [@]	10.22±0.873 ^{@\$^}
Creatinine Clearance (ml/min/kg)	1.077±0.087	0.166±0.021 ^C	0.513±0.093	0.85±0.134 [#]	0.923±0.135 [#]
Serum Urea Level (mg/dl)	41.98±0.712	134.7±2.888 ^C	110.5±3.16 [#]	92.97±2.482 ^{#%}	71.48±2.058 ^{#+%}
Serum Uric Acid Level (mg/dl)	0.481±0.013	1.587±0.137 ^C	1.147±0.071 [@]	0.871±0.052 [#]	0.551±0.043 ^{#%^}
Blood Urea Nitrogen (mg/dl)	16.87±0.764	50.17±0.869 ^C	43.94±2.004 [*]	27.89±1.65 ^{#%}	21.14±0.873 ^{#%^}

^c*P*<0.001 Vs NC ; [#]*P*<0.001, [@]*P*<0.01, ^{*}*P*<0.05 Vs GC; ^{\$}*P*<0.05, [%]*P*<0.001 Vs FRFHI (100); ^{^P}<0.05, ^{+P}<0.001 Vs FRFHI (200)

3.3 Antioxidant parameters

Gentamicin has been found to increase the generation of reactive oxygen species in the renal cortex that lead to renal damage and necrosis via several complex mechanisms. In the present investigation we have observed the significant increase in the levels of renal MDA (*P*<0.001) and NO (*P*<0.001) concentration, which was significantly decreased with FRFHI fruit treatment at a dose of 100, 200 and 400 mg/kg (*p*<0.05 *P*<0.001 and *p*<0.001 respectively).

Intraperitoneal administration of gentamicin also decreased renal GSH (*p*<0.001) as well as renal SOD (*p*<0.001) activity compared to control rats which was significantly increased with FRFHI fruit treatment at a dose of 200 and 400 mg/kg (*P*<0.001 and *p*<0.001 respectively). Significant improvement was observed in SOD and GSH activity at a dose level of 400 mg/kg body weight compared to 100 mg/kg body weight dose (*p*<0.05 and *p*<0.001 respectively).

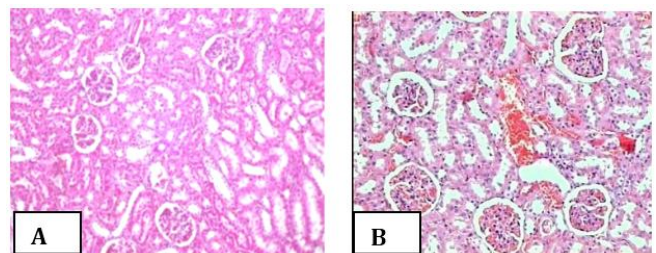
Table 3: Effect of FRFHI on renal oxidative stress.

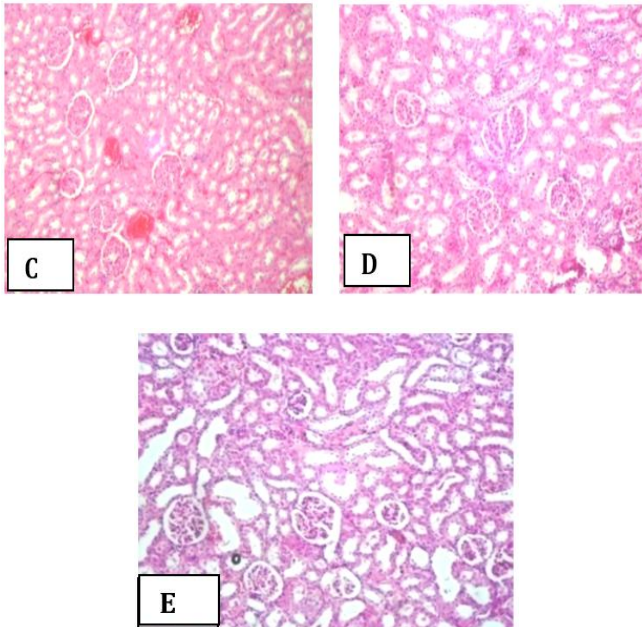
Treatment (mg/kg)	MDA (nmol/mg of protein)	SOD (U/ mg of protein)	GSH (ng/ mg of protein)	NO (nmol/mg of protein)
NC	7.33±0.71	34.47±0.58	54.17±1.16	134±1.065
GC (80)	14.25±0.49 ^C	23.05±0.77 ^C	40.67±1.59 ^C	234.1±1.17 ^C
GC+FRFHI (100)	11.75±0.36 [*]	30.75±1.03 [#]	36.08±2.37	213.4±1.48 [#]
GC + FRFHI (200)	8.96±0.44 ^{#&}	32.97±0.55 [#]	45.97±0.57 [%]	189±1.71 ^{#%}
GC + FRFHI (400)	6.76±0.37 ^{#%^}	34.33±0.99 ^{\$}	54.75±0.69 ^{#%?}	148.8±1.86 ^{#%+}

^c*P*<0.001 Vs NC; [#]*P*<0.001, ^{*}*P*<0.05 Vs GC; ^{\$}*P*<0.05, [%]*P*<0.001, ^{?P}<0.01 Vs FRFHI (100); ^{^P}<0.05, ^{?P}<0.001, ^{+P}<0.001 Vs FRFHI(200)

3.4 Histopathological study of the kidney

Light microscopic examination of gentamicin treated rat kidney showed more extensive and marked tubular necrosis, blood vessel congestion, tubular cellular swelling. However no abnormalities were observed in control rats. Rats treated with FRFHI fruit (100 mg/kg) showing mild effect on necrosis. However, rats treated with FRFHI fruit (200 and 400 mg/kg) showing normal renal structure with mild congestion. Histopathological investigation suggests a high dose of FRFHI fruit has a protective effect against gentamicin induced structural damage on kidney.





4. Discussion

Disorders of civilization are slow progressing, long lasting, largely preventable illnesses that result from numerous common modifiable risk factors [10]. Drug-induced Nephrotoxicity is an extremely common condition and is responsible for a variety of pathological effects on the kidneys. It is resulted due to availability of over-the-counter medications especially non-steroidal anti-inflammatory drugs (NSAIDs), antibiotics, etc. Drug-induced acute renal failure (ARF) accounted for 20% of all ARF cases in India [11]. The kidney is a common target for toxic xenobiotics, due to its capacity to extract and concentrate toxic substances [12]. Most commonly used aminoglycoside antibiotics such as gentamicin and amikacin induce a dose-dependent nephrotoxicity in 10–25% of therapeutic courses [13–15]. The human population is witnessing the overall limelight being gained by the herbal and polyherbal formulations owing to their effectiveness, reduced side-effects and cost effectiveness of the therapy. As reported in literature, *Helicteres isora* (L.) possesses wide range of pharmacological activities including antioxidant property [16] and various pharmacological actions like antibacterial [17], hepatoprotective [6], anticancer [18], cardioprotective [19], hypoglycemic [20], antihyperlipidaemic [20], and thus is considered as a potent moiety for maintaining good health. Intraperitoneal administration of gentamicin (80 mg/kg) produces significant reduction in body weight [21]. Also edema caused by drug induced acute tubular necrosis increases kidney weight [22]. Oral treatment with FRFHI fruit (200 and 400 mg/kg) significantly increased the body weight in comparison to non-treated gentamicin control rats. Significant decrease in kidney weight and relative kidney weight was observed with FFHI fruit treatment at dose of 200mg/kg and 400mg/kg. Elevated levels of serum creatinine, serum urea, serum uric acid and BUN are a potent indicator in the first phases of kidney disease [23]. In present study administration of FRFHI fruit (400 and 200 mg/kg) in gentamicin treated rats significantly reduced the elevated levels of serum creatinine, serum urea, serum uric acid and BUN compared to at a dose of 100 mg/kg. Administration of FRFHI fruit (400, 200 and 100 mg/kg) dose in gentamicin treated rats significantly increased the

urinary creatinine levels and also creatinine clearance was also significantly increased.

Increased oxidative stress is the major contributor towards the development of renal damage and necrosis via several complex mechanisms [24]. Non-treated gentamicin rats showed a significantly increased oxidative stress as elevated renal MDA and NO levels while decreased renal GSH and SOD levels. Significant effect on the restoration of antioxidant defence such as decreased MDA and NO as well as increased GSH and SOD concentration was observed in the rats treated with FRFHI fruit treatment (100, 200 and 400 mg/kg), suggesting strong antioxidant property possessed by FRFHI fruit.

Histopathological examination reveals the tissue toxicity and effect associated with the administered drug [25]. In present study, marked tubular necrosis, blood vessel congestion was observed in gentamicin control rats, whereas control rats showed no abnormalities. Rats treated with FRFHI fruit at doses of 200 and 400 mg/kg attenuated these changes.

5. Conclusion

Helicteres isora (L.) being a potent antioxidant, antihyperglycaemic, antihyperlipidaemic and cardioprotective moiety, plays an important role in health management. In the present investigation it successfully maintained the body weight, kidney weight and relative kidney weight. Renoprotective action of the same was evident owing to its ability to restore the serum and urinary levels of levels of creatinine, urea, uric acid, BUN and creatinine clearance. It significantly restored the antioxidant enzyme system and on histopathological examinations, marked restoration of altered renal architecture was observed. Thus, it can be concluded that flavonoid rich fraction of *Helicteres isora* (L.) fruit possesses protective action against Gentamicin Induced Nephrotoxicity in Wistar Rats.

6. Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper

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