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NEPHROPROTECTIVE ROLE OF NEERI-KFT (A POLYHERBAL FORMULATION) AGAINST GENTAMICIN INDUCED NEPHROTOXICITY IN EXPERIMENTAL RAT MODEL: A PRE-CLINICAL STUDY

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ABSTRACT

The present manuscript is mainly focused to experimentally elucidate the nephroprotective potential of Neeri-KFT (a polyherbal formulation for renal disorders hereinafter mentioned as NR-KFT) against aminoglycoside antibiotic Gentamicin (80mg/kg, i.p.) induced nephrotoxicity in albino Wistar rats. The nephroprotective potential of NR-KFT was also compared with ethanolic extract of *Boerhaavia diffusa* (Family: Nyctaginaceae), which is a vital constituent of NR-KFT and also individually used in renal disorders, to elucidate the synergism or additive effect of multiple herbal drugs present in NR-KFT. Since, many researchers mainly focused on antioxidants for nephroprotective potential therefore to elucidate that NR-KFT is not merely antioxidant, but exhibit nephroprotective potential, it was also compared with antioxidant Vitamin E. The experimental findings proved that NR-KFT possessed significant nephroprotective potential along with antioxidant activity; and the different herbal constituents present also potentiated the nephroprotective effect to produce wholesome better effect of correcting altered renal architecture and improving renal physiology. Conclusively, the NR-KFT showed significant nephroprotective potential in these pre-clinical studies.

KEYWORDS: Neeri-KFT, Nephrotoxicity, Gentamicin, Nephroprotective, *Boerhaavia diffusa*, Vitamin E.

INTRODUCTION

The kidneys perform the most of major excretory processes in the human body, hence become the prime target organ for various circulating toxins that may cause nephrotoxicity. Nephrotoxicity is a major side effect of many drugs like some NSAIDs, aminoglycoside antibiotics, anti-cancer drugs, etc.[1] Gentamicin is a potent broad spectrum aminoglycoside antibiotic which mostly used against Gram-negative microbes and causes nephrotoxicity in 10-30% cases.[2-4] It also causes mitochondrial dysfunction and generation of reactive oxygen species that leads to oxidative stress, inflammation and apoptosis in the proximal convoluted tubular cells of the kidney.[4, 5]

Neeri-KFT (NR-KFT), a polyherbal sugar free syrup manufactured by Aimil Pharmaceuticals India Ltd., is especially designed for restoring biochemical parameters and correcting the impaired renal physiology. It is mainly prescribed for improving the poor kidney functions that may be due to many conditions like diabetes, glomerulonephritis, hypertension, generalized odema, nephrotoxicity etc. The NR-KFT syrup contains many herbal drugs like *Boerhaavia diffusa, Tinospora cordifolia, Nelumbo nucifera, Butea monosperma, Tribulus terrestris, Moringa oleifera, Veteveria zizanioides, Crataeva nurvala, Amaranthus spinosus* etc along with some classical ayurvedic formulations like Panchtrinmool. The main aim of present manuscript is to elucidate the nephroprotective role of NR-KFT against Gentamicin induced nephrotoxicity in experimental model. In this manuscript, the authors have compared the nephroprotective potential of NR-KFT with Vitamin E (antioxidant) and the extracts of *Boerhaavia diffusa* - one of the vital constituents of NR-KFT and other formulations used in various renal disorders.[1, 6, 7] It is to elucidate that NR-KFT is not merely antioxidant that are usually protective in various conditions, but also having nephro-corrective actions that might be due to the changes in renal architecture; and it is a multiherbal composition to produce optimal effects via multiple actions and/or synergism.

MATERIALS AND METHODS

Experimental Animals

Thirty five healthy adult Wistar rats of either sex, age between 4-5 months, weighing about 150-250g were procured from Panacea Biotec Ltd, Lalru (Punjab), India and housed in polypropylene cages. They were kept under standard laboratory conditions; fed with standard pellet diet and water *ad libitum*. Prior to experimental work, all the animals were acclimatized to experimental laboratory conditions for at least seven days. The study protocol was duly approved by the Institutional Animal Ethics Committee (IAEC) [Protocol No. IAEC-CTIPS/2014/IV/0021(PCL-M)] and all the experiments were performed as per the guideline of the Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA), Ministry of Environment & Forests, New Delhi.

Chemicals and Reagents

Gentamicin Sulphate was purchased from Ranbaxy Fine Chemical Limited, (India), Vitamin E from Hi Media Laboratories Pvt. Ltd. (India), Thiobarbituric acid (TBA) from Avarice Laboratories Pvt. Ltd. (India) and Trichloroacetic acid (TCA) from Thermo Fisher Scientific Pvt. Ltd. (India). Assay kits for estimation of biochemical parameters like creatinine, urea, albumin, total protein and glucose were purchased from ERBA Diagnostic Mannheim GmbH, (Germany). All other chemicals used were of analytical grade.

NR-KFT and Plant Materials

The polyherbal formulation NR-KFT and authenticated whole plant of *Boerhaavia diffusa* (Family: Nyctaginaceae) was obtained as a gift sample from Aimil Pharmaceutical India Limited, New Delhi.

Extraction

The dried whole plant of *B. diffusa* was extracted with 60% ethanol by hot and continuous extraction using a Soxhlet apparatus. The excessive solvent from the extract was recovered with rotary vacuum evaporator and then the concentrated extract was dried to constant weight in a hot air oven at 40ºC. The percentage yield of the extract was found to be approximately 8.0%w/w.

Preliminary Phytochemical Screening

The preliminary phytochemical screening of both NR-KFT formulation and *B. diffusa* extract for the presence of various phytoconstituents like alkaloids, carbohydrates, flavonoids, glycosides, proteins, tannins, terpenes etc. was performed as previously carried out in our laboratory using the standard procedures.[8]

Induction of Nephrotoxicity

For inducing nephrotoxicity in albino rats, Gentamicin sulphate (80mg/Kg/day, i.p.) for 8 consecutive days was used.

Experimental Protocol

The 35 albino rats were divided into seven groups, each having five animals.

- Group I (Normal Control): Administered with normal saline only, for 28 days.
- Group II (Negative Control): Treated with Gentamicin (80mg/Kg/day, i.p.) for initial 8 days and then with normal saline for a remaining period of study.
- Group III (Positive Control): Co-administered with Gentamicin (80mg/Kg/day, i.p.) for 8 days and Vitamin E (100mg/Kg/day, p.o.) from day 1to 28.
- Group IV (NR-KFT I): Co-administration of Gentamicin (80mg/Kg/day, i.p.) for 8 days and NR-KFT I (667.50 mg/Kg, p.o.) from 1st to 28th days.
- Group V (NR-KFT II): Co-administration of Gentamicin (80mg/Kg/day, i.p.) for 8 days and NR-KFT II (1112.50 mg/Kg, p.o.) from 1st to 28th days.
- Group VI (EtBd I): Co-administration of Gentamicin (80mg/Kg/day, i.p.) for 8 days and EtBd I (667.50 mg/Kg, p.o.) from 1st to 28th days.
- Group VII (EtBd II): Co-administration of Gentamicin (80mg/Kg/day, i.p.) for 8 days and EtBd I (1112.50mg/Kg, p.o.) from 1st to 28th days.

Estimation of Biochemical Parameters for Assessing Nephroprotective Potential

On the 29th day, each animal was individually placed in metabolic cages for 24hrs and the urine sample of each was collected. The blood samples were obtained and the serum was separated by centrifugation at 3000 rpm for 15minutes in a micro-centrifuge. The urine was then quantitatively analyzed for creatinine, glucose, protein and; the serum for total proteins, albumin, urea and creatinine by using autoanalyzer. The lipid peroxidation level (i.e. a marker of oxidative stress) was determined by the method described by Stocks and Dormandy[9] as previously carried out in our laboratory.[8]

Histopathological study of kidney

For the histopathological study, one rat from each group was sacrificed by cervical dislocation at the end of the experiment. The kidneys were removed, washed with cold saline and preserved in 10% formalin in buffered form. After embedding in paraffin, the sections were cut, stained with hematoxylin and eosin. Now the slides were then examined for renal architecture at the magnification of 450X using a laboratory light microscope attached with camera.[10]

Statistical Analysis

All the data were expressed as mean±SEM (standard error of mean) and analyzed by one-way Analysis of Variance (One-way ANOVA) followed by "Tukey's multiple comparison test" by using GraphPad Prism-5. The $P<0.05$ was considered to be significant.

RESULTS AND DISCUSSION

Gentamicin-induced nephrotoxicity is one of the important and validated models used to elucidate the

nephroprotective potential.[11, 12] The different mechanisms involved in Gentamicin nephrotoxicity consist of oxidative stress, apoptosis, necrosis, up regulation of transforming growth factors, elevation of endothelin level, an increase in the infiltration of monocyte/macrophages, etc.[13, 14] Gentamicin nephrotoxicity is usually characterized functionally by increased levels of serum creatinine, blood urea nitrogen, and decreased glomerular filtration rate.[15] It is morphologically characterized by proximal tubule epithelial desquamation, tubular necrosis, tubular fibrosis, epithelial edema and glomerular hypertrophy.[16]

In the present study, Gentamicin raised the serum level of creatinine and urea when compared with normal group as shown in the Table 1 and indicated the nephrotoxicity. It also caused proteinuria as shown in table 2. The NR-KFT I & II both significantly decreased the serum creatinine level by increasing the creatinine clearance as shown in the Table 1, 2 and Figure 1, 2. The results also showed that EtBd I, II and Vitamin E also significantly decreased the serum creatinine levels in comparison with that of nephrotoxicated rats. The elevation of serum creatinine in treated groups was lower than that of negative group and also in comparison to positive group. In treated groups, the urinary creatinine level was found to be comparatively increased. It indicated that both the experimental drug and formulation exerted the preventive effect on degeneration. The wholesome effect of NR-KFT was found to be better than that of the single drug extract of EtBd.

The data revealed that the herbal drugs may possibly have both protective effects against degeneration by virtue of antioxidant property or may have influence on metabolic alterations in the production of creatinine or do help in augmenting the creatinine clearance.

Table 1: Effect of NR-KFT and EtBd on various serum biochemical parameters

Groups	Creatinine (mg/dl)	Albumin (g/dl)	Total Protein (g/dl)	Urea (mg/dl)
Normal Control	0.52±0.03	5.01±0.08	64.38±0.73	12.88±1.19
Negative Control	1.17±0.07***	2.77±0.12***	37.24±1.49***	35.54±1.36***
Positive Control	0.72±0.02***	4.08±0.08***	49.40±1.07***	24.78±1.82***
NR-KFT I	0.64±0.08***	3.72±0.05***	54.30±2.19***	22.83±0.92***
NR-KFT II	0.51±0.03***	4.65±0.09***	62.56±0.78***	17.89±1.91***
EtBd I	0.66±0.12***	3.32±0.07*	54.18±1.94***	24.30±1.21***
EtBd II	0.58±0.04***	3.73±0.03***	58.22±1.13***	21.10±0.87***

All the values are expressed as mean±SEM. (n=5) and analyzed by One-way ANOVA followed by Tukey's multiple comparison test. The negative control group was compared with normal group while all other groups were compared with negative control group i.e. nephrotoxic group. The *indicated significance at P<0.05, ** at P<0.01 and *** at P<0.001.

Table 2: Effect of NR-KFT and EtBd on various urinary biochemical parameters

Groups	Creatinine (mg/dl)	Glucose (mg/dl)	Protein (g/dl)
Normal Control	10.39±0.51	0.32±0.11	2.20±0.35
Negative Control	13.34±1.07*	3.51±0.14***	10.46±0.46***
Positive Control	9.92±0.44**	1.91±0.09***	7.42±0.35***
NR-KFT I	10.34±0.25*	1.28±0.11***	5.20±0.25***
NR-KFT II	10.24±0.34*	0.95±0.18***	3.74±0.20***
EtBd I	11.83±0.54	2.03±0.28***	5.54±1.10***
EtBd II	11.23±0.65	1.23±0.25***	4.70±0.21***

All the values are expressed as mean±SEM. (n=5) and analyzed by One-way ANOVA followed by Tukey's multiple comparison test. The negative control group was compared with normal group while all other groups were compared with negative control group i.e. nephrotoxic group. The *indicated significance at P<0.05, ** at P<0.01 and *** at P<0.001.

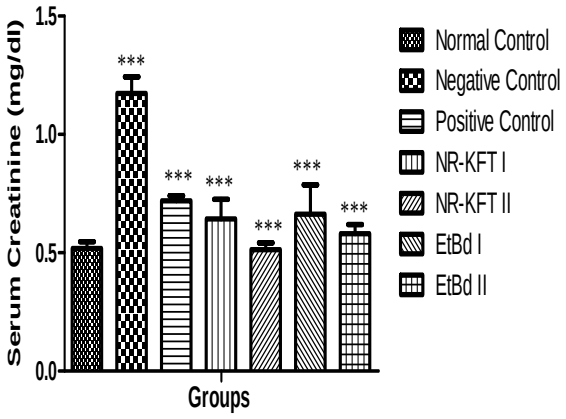

Fig. 1: Effect on serum creatinine level. All the values are expressed as mean±SEM. (n=5) and analyzed by One-way ANOVA followed by Tukey's multiple comparison test. The negative control group was compared with normal group while all other groups were compared with negative control group i.e. nephrotoxic group. The *indicated significance at P<0.05, ** at P<0.01 and * at P<0.001.**

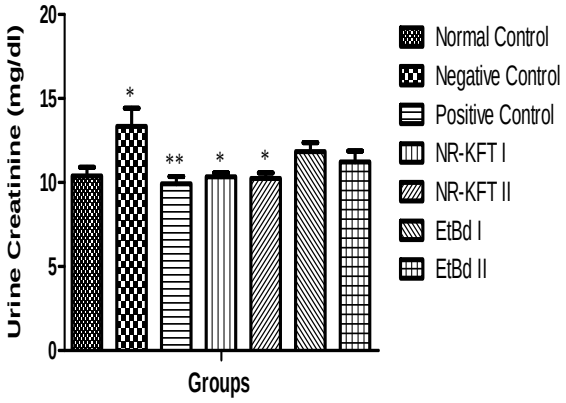

Fig. 2: Effect on urinary creatinine level. All the values are expressed as mean±SEM. (n=5) and analyzed by One-way ANOVA followed by Tukey's multiple comparison test. The negative control group was compared with normal group while all other groups were compared with negative control group i.e. nephrotoxic group. The *indicated significance at P<0.05, ** at P<0.01 and * at P<0.001.**

The serum and urinary data of estimated proteins represented in table 1, 2 and figure 3, 4 respectively, clearly indicated that both the NR-KFT and EtBd significantly prevented proteinuria and elevated the serum protein level. The effect of NR-KFT was found to be comparatively better than that of the single drug EtBd, which may be due to the synergistic effect of multiple herbs included in NR-KFT. Both the formulation and extract also raised the serum albumin as shown in table 1 and figure 5. These may also reduce the albuminuria and again the effects of NR-KFT were observed better.

The data expressed in table 1 and figure 6 showed that both NR-KFT and EtBd significantly prevented the elevation of the urea level in serum when compared with nephrotoxic group. It indicated the improvement in the impaired renal functions that might be due to the prevention of renal degeneration. Further the values for urinary glucose level expressed in table 2 and figure 7 clearly indicated that both NR-KFT and EtBd reduced the glucosuria and the effect of NR-KFT was observed comparatively better. It was observed from the above said data expressed in table 1 and 2 that Vitamin E (positive control group) also produced nephroprotective potential to some extent but the wholesome effects of NR-KFT were better. It elucidated that NR-KFT also exhibited the nephroprotective effect in along with antioxidant potential.

Further the data expressed in table 3 and figure 8, 9 showed that both the NR-KFT and EtBd increased the water intake as well as urine output when compared with nephrotoxic i.e. negative control group and restored these parameters towards the normal range. It elucidated the improvement in renal physiological status.

Table 3: Effect of NR-KFT and EtBd on water intake and urine output

Groups	Water Intake (ml)	Urine Output (ml)
Normal Control	24.80±1.28*	22.00±1.30***
Negative Control	14.80±0.97	10.20±0.37
Positive Control	17.20±2.67	14.60±2.79
NR-KFT I	19.40±1.03	17.00±0.95
NR-KFT II	22.00±2.07	19.40±2.06*
EtBd I	18.60±1.57	15.80±1.32
EtBd II	20.40±3.20	18.00±3.16

All the values are expressed as mean±SEM. (n=5) and analyzed by One-way ANOVA followed by Tukey's multiple comparison test. The negative control group was compared with normal group while all other groups were compared with negative control group i.e. nephrotoxic group. The *indicated significance at P<0.05, ** at P<0.01 and *** at P<0.001.

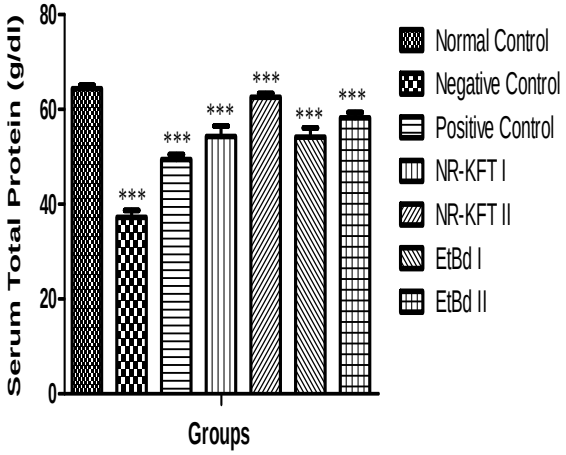

Fig. 3: Effect on serum total protein level. All the values are expressed as mean±SEM. (n=5) and analyzed by One-way ANOVA followed by Tukey's multiple comparison test. The negative control group was compared with normal group while all other groups were compared with negative control group i.e. nephrotoxic group. The *indicated significance at P<0.05, ** at P<0.01 and * at P<0.001.**

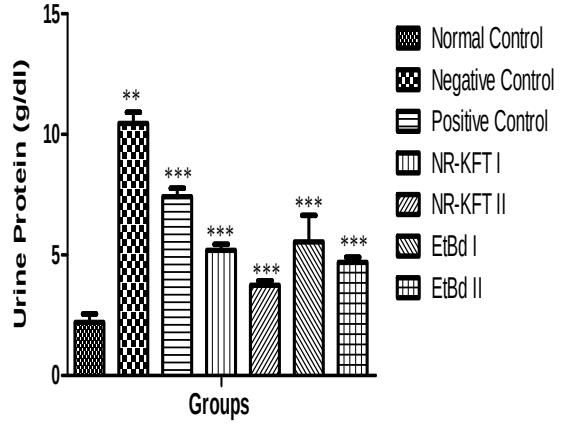

Fig. 4: Effect on urinary protein level. All the values are expressed as mean±SEM. (n=5) and analyzed by One-way ANOVA followed by Tukey's multiple comparison test. The negative control group was compared with normal group while all other groups were compared with negative control group i.e. nephrotoxic group. The *indicated significance at P<0.05, ** at P<0.01 and * at P<0.001.**

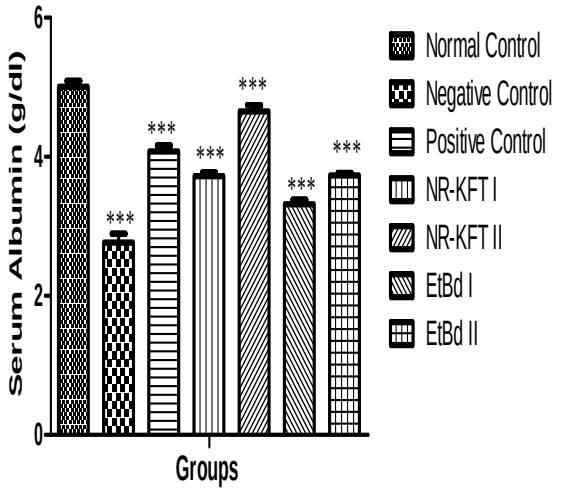

Fig. 5: Effect on serum albumin level. All the values are expressed as mean±SEM. (n=5) and analyzed by One-way ANOVA followed by Tukey's multiple comparison test. The negative control group was compared with normal group while all other groups were compared with negative control group i.e. nephrotoxic group. The *indicated significance at P<0.05, ** at P<0.01 and * at P<0.001.**

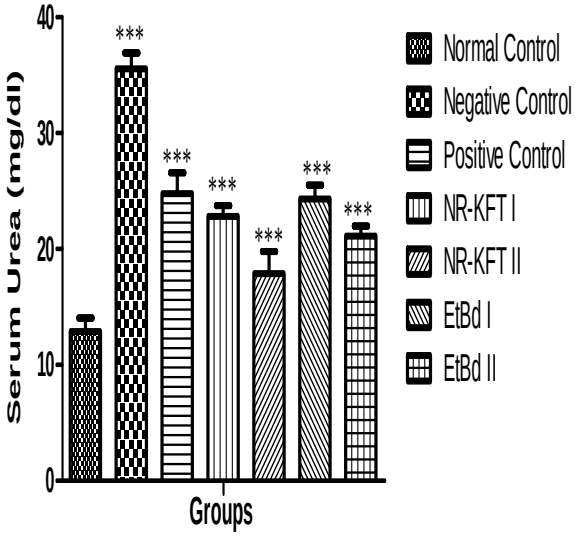

Fig. 6: Effect on serum urea level. All the values are expressed as mean±SEM. (n=5) and analyzed by One-way ANOVA followed by Tukey's multiple comparison test. The negative control group was compared with normal group while all other groups were compared with negative control group i.e. nephrotoxic group. The *indicated significance at P<0.05, ** at P<0.01 and * at P<0.001.**

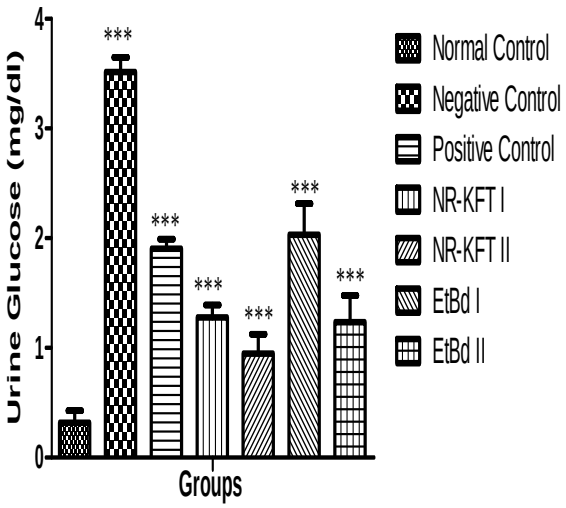

Fig. 7: Effect on urinary glucose level. All the values are expressed as mean±SEM. (n=5) and analyzed by One-way ANOVA followed by Tukey's multiple comparison test. The negative control group was compared with normal group while all other groups were compared with negative control group i.e. nephrotoxic group. The *indicated significance at P<0.05, ** at P<0.01 and * at P<0.001.**

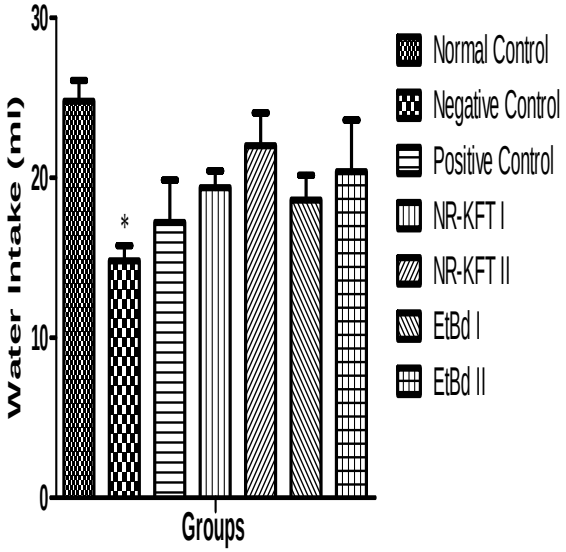

Fig. 8: Effect on water intake. All the values are expressed as mean±SEM. (n=5) and analyzed by One-way ANOVA followed by Tukey's multiple comparison test. The negative control group was compared with normal group while all other groups were compared with negative control group i.e. nephrotoxic group. The *indicated significance at P<0.05, ** at P<0.01 and * at P<0.001.**

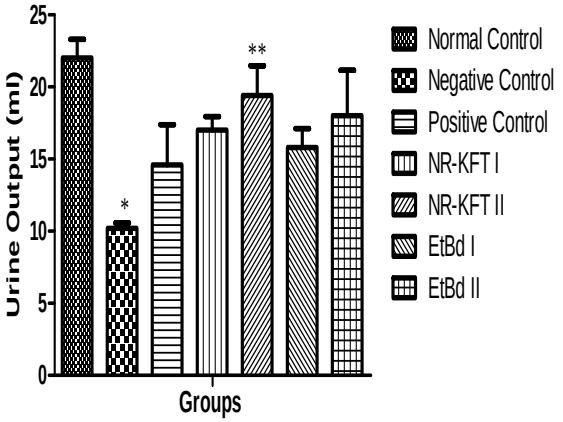

Fig. 9: Effect on urine output. All the values are expressed as mean±SEM. (n=5) and analyzed by One-way ANOVA followed by Tukey's multiple comparison test. The negative control group was compared with normal group while all other groups were compared with negative control group i.e. nephrotoxic group. The *indicated significance at P<0.05, ** at P<0.01 and * at P<0.001.**

The result expressed in figure 10 showed that the antioxidant level was significantly decreased and TBARS contents increased by Gentamicin in the negative control group indicating increased oxidative stress that lead to cell damage. The NR-KFT, EtBd and Vitamin E all showed a significant protective effect as compared to the negative control group. It showed the free radical scavenging ability that prevented the membrane lipid per-oxidation.

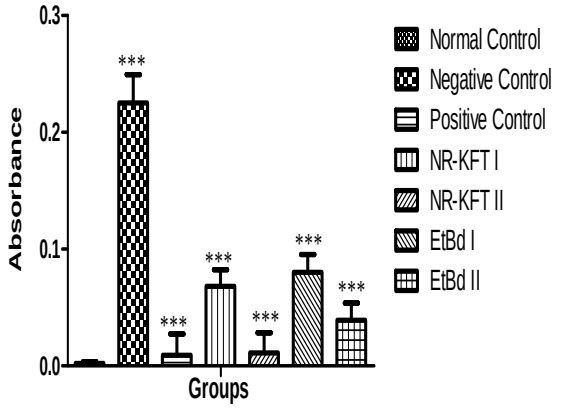

Fig. 10: Effect on TBARS. All the values are expressed as mean±SEM. (n=5) and analyzed by One-way ANOVA followed by Tukey's multiple comparison test. The negative control group was compared with normal group while all other groups were compared with negative control group i.e. nephrotoxic group. The *indicated significance at P<0.05, ** at P<0.01 and * at P<0.001.**

Most researchers against Gentamicin nephrotoxicity focused on the use of various antioxidants as these inhibited or attenuated Gentamicin-induced nephrotoxicity in rats.[17] Usage of antioxidants improved histological injuries such as tubular necrosis, tubular cell edema and apoptosis in Gentamicin-injected rats.[18] The data also showed that NR-KFT is not merely antioxidant, but having nephroprotective prospective also. The nephroprotective potential of NR-KFT might be due to the presence of multiple active constituents like glycosides, steroids, alkaloids, tannins, proteins, flavonoids, terenoids and carbohydrates as qualitatively estimated by preliminary phytochemical screening. The EtBd was also found to contain steroids, glycosides, tannins, flavonoids, terpenes etc. NR-KFT also showed a significant nephroprotective potential against heavy metal, i.e. Lead (Pb) induced nephrotoxicity in rats.[8]

The nephroprotective potential of NR-KFT against Gentamicin-induced toxicity was also supported by histopathological findings as shown in the figure 11. Gentamicin caused deranged and necrosed renal tubular cells in the negative control group in comparison to normal tubular brush border, intact glomerulus and Bowman's capsule of normal group. The NR-KFT, EtBd and Vitamin E showed significant protective effects compared to negative group, but the effects of the NR-KFT II was found to be comparatively more better indicating that NR-KFT also exhibit significant nephroprotective potential in addition to antioxidant and other activities.

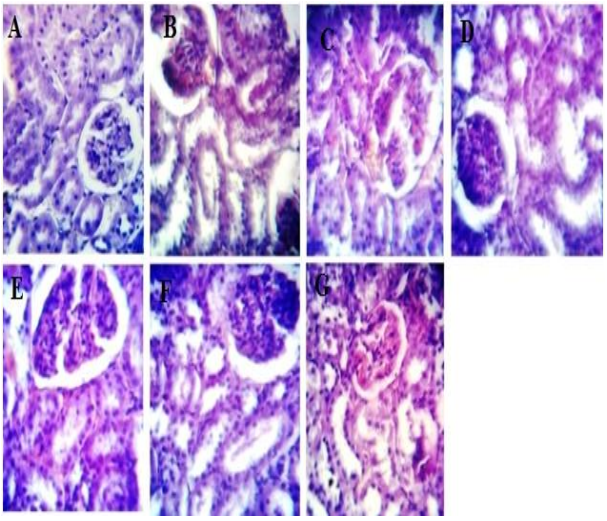

Fig. 11: Histopathological Findings (45X): (A) Normal group: Normal glomerulus and tubular structure. (B) Negative Control: Glomerular necrosis and deranged tubular structure. (C) Positive Control: Dilated glomerulus and tubular cells. (D) EtBd I: The dilated tubules and glomerular membrane lining. (E) EtBd II: Restoration of the tubular and glomerular structures toward normalization. (F) NR-KFT I: The dilated tubules and glomerular membrane lining. (G) NR-KFT II: Restoration of the tubular and glomerular structures toward normalization.

CONCLUSION

In the present experimental findings, the polyherbal formulation NR-KFT, single drug EtBd and antioxidant Vitamin E all showed beneficial effects in Gentamicin-induced nephrotoxicity in rats. The comparatively better nephroprotective potential of NR-KFT concluded that NR-KFT is not merely antioxidant, but nephroprotective also and it showed synergism for improving renal functions probably because of its multiherbal composition.

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