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Original Article

Pharmacology Section

Nephroprotective Effect of Ethanolic Extract of Flax Seed

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ABSTRACT

Introduction: The treatment of Acute Kidney Injury (AKI) remains empirical and hence the quest for an effective agent for this condition still remains a challenge. The nephroprotective effect of flax seeds has been proven in pre-clinical models of kidney injury like lupus nephritis and in model of Type 2 diabetes mellitus where flax seeds meal ameliorated proteinuria.

Aim: To evaluate the nephroprotective effect of Ethanolic Extract of flax seed/*Linum Usitatissimum* (EELU) in Gentamycin induced nephrotoxicity in Wistar rats.

Materials and Methods: The study was initiated after Institutional Animal Ethics Committee approval. A total of 46 adult male Wistar rats were randomly divided into six groups with eight rats in each group except normal control (n=6) (Group I). The positive control (Group III) received alpha lipoic acid (25 mg/kg/day p.o). The three study groups received EELU in dose 0.7 (Group IV), 1.4 (Group V) and 2.8 (Group VI) mg/kg/day per oral (p.o.) respectively. All groups, except normal control group (Group I), received gentamycin 150 mg/kg/day intraperitoneal (i.p.) for 10 days. The nephroprotective effect of EELU was assessed using following variables body weight, Blood Urea Nitrogen (BUN), Serum creatinine, kidney Malondialdehyde (MDA), and Glutathione (GSH) levels and kidney injury on histopathology. One-way ANOVA was used to analyse parametric data while the non-parametric data was analysed using Kruskal Walis test.

Results: EELU in the dose of 2.8 mg/kg/day showed significant improvement in BUN (27.89±6.44 mg/dL), serum creatinine (1.3±0.29 mg/dL), MDA (196.88±17.88 mmol/gm), GSH (1.63±0.12 µgm/gm) and histopathology as compared to Disease control ($p>0.05$). These findings were statistically similar to positive control group ($p<0.05$). EELU in the dose of 1.4 mg/kg/day showed significant improvement in BUN (29.34±7.30 mg/dL), serum creatinine (1.89±0.26 mg/dL), MDA (235.71±22.42 nmmol/gm), GSH (1.37±0.22 µgm/gm) and histopathology as compared to Disease control ($p>0.05$). Improvement in BUN and histopathology was statistically similar to positive control group ($p<0.05$). EELU in the dose of 0.7 mg/kg/day showed significant improvement in BUN (34.24±3.10 mg/dL), serum creatinine (1.91±0.67 mg/dL), MDA (261.86±23.22 nmmol/gm) as compared to Disease control ($p>0.05$).

Conclusion: The present study demonstrated the nephroprotective effect of EELU in high dose (2.8 mg/kg/day) in the model of gentamycin-induced nephrotoxicity in Wistar rats and prevented the acute kidney injury most probably due to its antioxidative potential. Findings of the study suggest that EELU can be used as therapeutic agent in patients who are at risk of kidney injury and further clinical studies should be planned.

Keywords: α-lipoic acid, Acute kidney injury, Gentamycin, *Linum usitatissimum*, Serum creatinine

INTRODUCTION

Acute Kidney Injury which was previously known as acute renal failure is regarded as the sudden impairment of kidney function subsequently with the retention of nitrogenous and other waste products usually cleared by the kidneys and often accompanied by reduction in urine volume. Thus, AKI is defined by a rise in serum creatinine of at least 0.3 mg/dL or 50% higher than baseline within a 24-48-hours period or a reduction in urine output to 0.5 mL/kg per hour for longer than six hours [1]. Medications are one of the most common aetiological factors for development of AKI. Moreover, patients with acute illness, elderly patients with diabetes mellitus, patients with chronic kidney disease, patients undergoing general surgical procedures, patients receiving iodinated contrast etc., are at high risk for development of AKI [2]. Recent hospital studies have reported that 37.71% of patients who are admitted to the intensive care unit develop AKI. The mortality rate due to AKI is 51.9% [3].

The current standard of care for AKI is optimisation of systemic and renal hemodynamic, elimination of nephrotoxic agents e.g. Lithium, heparin, Amphotericin B, Tacrolimus and initiation of renal replacement therapy when indicated [4]. However, the treatment of AKI still remains empirical due to the heterogeneous nature of the disease which makes identification of a single therapy challenging. Moreover, the drugs like atrial natriuretic peptide, loop diuretics, a prostaglandin analog, and α-lipoic acid etc., are still being evaluated by researchers in human clinical trials or have filed to show effect in humans [5]. Thus, the quest for an effective agent for this condition still remains a challenge.

A study in patients with renal disease used compounds from traditional system of medicine like ginger, garlic juice, pomegranate seed oil, *Boerhavia Diffusa, Tribulus Terrestris, Echinacea pallida* etc [6]. In this light the present authors decided to study nephroprotective effect of flax seeds. Flax seed belongs to family '*Linaceae*' with more than 200 species. The Latin name of the flax seed is *Linum usitatissimum*, which means "very useful." Omega -3 fatty acid, alpha-linolenic acid, lignan, and fiber are active principles of *Linum usitatissimum*. It is a nutraceutical i.e., it provides health benefits in addition to its basic nutritional value [7]. The nephroprotective effect of flax seed has been proven in pre-clinical models of kidney injury like lupus nephritis [8]. In animal model of type 2 diabetes mellitus Flax seed meal ameliorated proteinuria [9].

Gentamycin is an aminoglycoside and is nephrotoxic. Experimental administration of gentamycin in the dose of 150 mg/kg i.p. results in production of situation similar to AKI [10]. The mechanism involved in the pathogenesis is believed to be oxidative stress. The probable mechanism of action of flax seed is due to its anti-oxidative potential. Thus, a study was planned to explore the nephroprotective potential

Flax seed in acute injury induced by Gentamycin in Wistar albino rats.

MATERIALS AND METHODS

A randomised, controlled, parallel arm preclinical study was done. The study was conducted in the central animal house facility of the institution. The study was carried out in 46 male Wistar rats each weighing between 200-250 grams, randomly bred in the central animal house of the institution. Prior to initiation of the study permission of the Institutional Animal Ethics Committee (60/PO/ReBi/S/99/CPCSEA) was obtained under reference number AEC/03/2016. The study procedures and maintenance of the study animals was done as per the guidelines by Committee for the purpose of control and supervision on experiments in animal (CPSCEA). Animals were housed in an air-conditioned area with 12-15 filtered fresh air changes, temperature 22±3°C, relative humidity 30-70%. Cages had a stainless steel top grill having facilities for food and drinking water in polypropylene bottles with stainless steel sipper tube. Standard rat feed was provided *ad libitum*. Aqua-guard pure drinking water was supplied to the animals *ad libitum*.

The inducing agent, Gentamycin was purchased as a vial of Gentimycin, containing 80 mg/ 2 mL of gentamycin sulfate, manufactured by Abbott Healthcare Pvt., Ltd. It was administered intraperitoneally in a dose of 150 mg/kg/day [10]. Alpha-lipoic acid (procured from Sigma-Aldrich; Batch no.D000011040) served as Positive control. It was administered in a dose of 25 mg/kg/day [11]. A 0.5% Carboxymethylcellulose (CMC) was used as a vehicle for all drugs except Gentamycin. Ethanolic Extract of *LinumUsitattissimum* (EELU) (procured from Konark Herbal Pvt., Ltd., India) was used as study drug with extractive value was 84.37%. The dose of EELU used in the current study was extrapolated from human dose [12] by using dose conversion chart. The dose obtained for rat was 1.4 mg/kg/day. In order to see the dose dependent effect of the EELU the present authors took a lower dose 0.7 mg/kg/day and a higher dose 2.8 mg/kg/day.

Study Procedure

On the day of commencement of study (day 0) the body weight of animal was measured; blood was collected by retro-orbital plexus and sent for estimation of BUN and SerumCreatinine levels. The rats with normal renal function were selected for the study. The animals were then randomly divided into six experimental groups as shown in [Table/Fig-1].

The Group I received 0.9% normal saline intraperitoneally (i.p.).

n=8 per group (Normal control=6)	Agent administered, intraperitoneally, per day	Treatment given, orally, per day
Group I (n=6)	Normal saline	-
Group II	Inj. Gentamycin sulphate (150 mg/kg/day)	-
Group III		Alpha-lipoic acid (25 mg/kg/day)
Group IV		EELU (0.7 mg/kg/day)
Group V		EELU (1.4 mg/kg/day)
Group VI		EELU (2.8 mg/kg/day)

[Table/Fig-1]: Study design.
Group I: Normal control, Group II: Disease control, Group III: Positive control, Group IV: EELU low dose, Group V: EELU Medium dose, Group VI: EELU High dose

Group II received Gentamycin 150 mg/kg/day intraperitoneally. The Group III received alpha lipoic acid 25 mg/kg/day in 0.5% Carboxymethylcellulose (CMC) and Gentamycin 150 mg/kg/day intraperitoneally. The study Groups IV, V and VI received Gentamycin 150 mg/kg/day intraperitoneally and in addition received 0.7 mg/kg,1.4 mg/kg and 2.8 mg/kg ethanolic extract of *Linum usitattissumum* (EELU) respectively, per orally (p.o.) for 10 days. On 11th day the animals were assessed for all the variables mentioned below. Two millilitres of blood was collected for estimation of parameters as mentioned below.

The rats were then sacrificed by administration of Ketamine 100 mg/kg by the intraperitoneal. route. Laparotomy was done, and both the kidneys were dissected carefully. The right kidney was cleaned gently using cold phosphate buffered saline. It was then dried, weighed using electronic mono pan balance. Later the kidney was divided into two parts. Two-third part was used for estimation of kidney Malondialdehyde (MDA) levels and one-third part used for the estimation of kidney reduced GSH levels. The left kidney was immediately immersed in 10% neutral buffered formalin and processed further for the preparation of histopathological sections. All dead and sacrificed animals were collected in plastic bags, stored in refrigerator and were disposed of according to Institutional biosafety committee (IBC).

Variables Assessed in the Study

The variables assessed on Day 0 and Day 11 (before administration of intraperitoneal injections of normal saline or gentamycin) included:

- Body weight (gm),
- Blood urea nitrogen (BUN) (mg/dL),
- Serum Creatinine (Sr. Creatinine) (mg/dL).

The other parameters assessed only on day 11 were:

- MDA and GSH levels in the kidney,
- Histopathology of the kidney.

Effect on body weight of the animal: It was measured using electronic weighing balance during the study at Day 0 and 11.

Estimation of blood urea nitrogen levels: Blood urea nitrogen was estimated on a semi-autoanalyser (ERBACHEM-5) using kits manufactured by Transasia Biomedicals.

Estimation of serum creatinine levels [13]: Serum creatinine was estimated using a semi-autoanalyser (ERBACHEM) using kits manufactured by Transasia Biomedicals. It was measured using modified Jaffe's reaction, initial rate method.

Estimation of kidney GSH (reduced glutathione) levels [14]: The kidney GSH levels, antioxidant biomarker, were estimated using a spectrophotometric procedure using 5-5′dithiobis-(2-nitrobenzoic acid) nDTNB reagent.

Estimation of kidney MDA (malondialdehyde) levels [15]: The kidney MDA levels, lipid peroxidation marker, were estimated using the method of Ohkawa et al. which is a spectrophotometric procedure using thiobarbituric acid reagent.

Histopathological examination: After dissection, the left kidney was removed and immediately immersed in 10% neutral buffered formalin. This was sent for histopathological examination where it was embedded in paraffin and stained with haematoxylin and eosin. The histopathological grading for renal damage was done by semi-quantitative analysis. The slides were observed under an image analyser microscope under 10X magnification attached to a camera for image capture, and the findings were captured using the image analyser software. The stained tissue sections were examined by a veterinary pathologist who remained blinded to the various experimental groups.

Each slide was assigned a particular grade. Histopathological scoring described by Houghton DC et al., was used for grading proximal tubule injury by light microscopy [16].

STATISTICAL ANALYSIS

Results were expressed as Mean±Standard Deviation (SD). The data were tested for normality (Kolmogorov-Smirnov test). The paired data in each group following normal distribution (parametric) was compared using a paired t-test. Between the groups, the data which were normally distributed was compared using one way

ANOVA test followed by Post Hoc Dunnett's test while, the data which were not normally distributed was compared using Kruskal-Wallis test. The histopathological scores between the groups were compared using Kruskal-Wallis test. If significance was detected; a post-hoc Dunn's the test was used. A p-value of <0.05 was considered to be as significant for all parameters. Analysis was done using GraphPadInStat, version 3.06.

RESULTS

Baseline Measurement

As shown in [Table/Fig-2], the baseline values for body weight BUN, Sr. Creatinine were comparable in all the groups (p>0.05)

Group n=8	Body weight (gm)	BUN (mg/dL)	Sr. Creatinine (mg/dL)
Group I (n=6)	238.8±8.84	16.4±4.46	0.5±0.2
Group II	236.13±13.68	18.06±3.78	0.83±0.31
Group III	235.88±13.75	19.44±5.48	0.70±0.28
Group IV	240.88±13.32	16.61±4.00	0.66±0.31
Group V	231.75±13.56	18.69±4.57	0.68±0.26
Group VI	235.00±13.62	15.84±3.51	0.63±0.25

[Table/Fig-2]: Baseline values of Body weight, BUN & Serum. Creatinine.
Values are expressed as Mean±SD. (n=8), Not significant using One-way ANOVA test (p>0.05).
BUN: Blood urea nitrogen
Group I: Normal control, Group II: Disease control, Group III: Positive control, Group IV: EELU low dose, Group V: EELU Medium dose, Group VI: EELU High dose

Effect on Various Parameters on Day 11

Effect on body weight: As seen in [Table/Fig-3], body weight of rats in all the study groups was comparable (p>0.05) on Day 11 and there was no statistical difference.

Effect on blood urea nitrogen: As seen in [Table/Fig-3] the BUN of animals in positive control (Group III) and all test groups was significantly lower than the disease control group (Group II) (p<0.05). EELU in the dose of 1.4 and 2.8 mg/kg/day showed comparable reduction in BUN as observed with the positive control (p>0.05).

Group n=8	Body weight (gm)	BUN (mg/dL)	Sr. Creatinine (mg/dL)
Group I (n=6)	248±10.13	15.88±3.17	0.47±1.4
Group II	255.62±13.55	41.26±4.14	3.43±0.48
Group III	253.25±16.31	23.03±3.79*	0.94±0.19*
Group IV	250.75±13.85	34.24±3.10*	1.91±0.67*
Group V	248.75±16.63	29.34±7.30*NS	1.89±0.26*
Group VI	250.12±12.68	27.89±6.44*NS	1.3±0.29*NS

[Table/Fig-3]: Values of Body weight, BUN & Serum. Creatinine at day 11.
Group I: Normal control, Group II: Disease control, Group III: Positive control, Group IV: EELU low dose, Group V: EELU Medium dose, Group VI: EELU High dose
Values are expressed as Mean±SD. (n=8). *p<0.05 vs. Group 2 Disease control, NS-Not significant vs. Group 3 positive control, One-way ANOVA with post-hoc Dunnett's test

Effect on serum creatinine: As seen in [Table/Fig-3] serum creatinine was significantly reduced in the positive control (Group III) and the EELU (all doses) as compared to disease control (Group II) (p<0.05). However, only the 2.8 mg/kg/day dose of EELU showed comparable results with the positive control group (p>0.05).

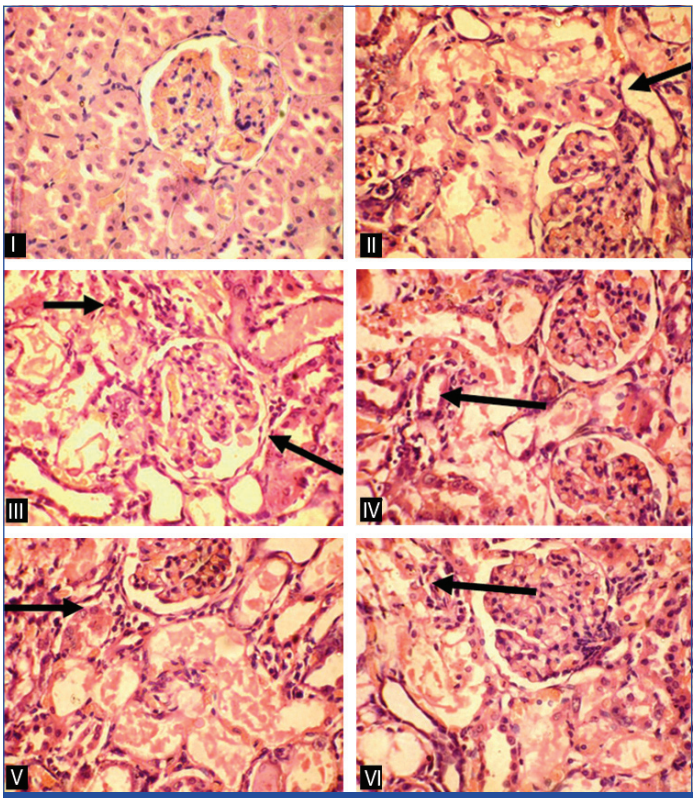
Effect on oxidative biomarkers (MDA, GSH): MDA levels were significantly lower in the positive control (Group III) and all the EELU groups when compared with disease control group (Group II) (p<0.05). The MDA levels in 2.8 mg/kg/day dose of EELU was comparable (p>0.05) with positive control as shown in [Table/Fig-4].

Kidney GSH was significantly higher in the positive control (Group III) when compared to disease control group (Group II) (p<0.05). The increase in GSH with 1.4 and 2.8 mg/kg/day doses was comparable (p>0.05) with positive control as shown in [Table/Fig-4].

Effect on renal histopathology: The low dose of EELU (0.7 mg/kg/day) failed to protect against gentamycin induced damage (p<0.05 versus disease control group). The medium and high dose (1.4 and 2.8 mg/kg/day) of EELU and the positive control group showed improvement in the histopathological scores as compared to the disease control (p<0.05). The improvement in histopathological scores seen in the above two groups was comparable with the positive control (p>0.05) Histopathological changes have been depicted in [Table/Fig-5].

Group (n=8)	MDA (nmmol/gm)	GSH (µgm/gm)	Histopathology (Median)
Group I (n=6)	212.32±10.49	1.61±0.17	0
Group II	276.62±17.04	1.05±0.16	3.5
Group III	178.29±18.44*	2.17±0.30*	1.5*
Group IV	261.86±23.22*	1.16±0.20*	3.5NS1
Group V	235.71±22.42*	1.37±0.22*NS	2.5*NS2
Group VI	196.88±17.88*NS	1.63±0.12*NS	1.5*NS2

[Table/Fig-4]: Values of MDA, GSH & Histopathology score at day 11.
Values are expressed as Mean±SD (n=8). *p<0.05 vs. Group 2 Disease control, NS-Not significant vs. Group 3 positive control, One way ANOVA with post-hoc Dunnett's test
For histopathology, p<0.05, using Kruskal- Wallis test, followed by post-hoc Dunn's multiple test, *p<0.05 vs. Group 2 Disease control NS 1-Not significant vs. Group 2 disease control, NS^2-Not significant vs. Group 3 positive control
Group I: Normal control, Group II: Disease control, Group III: Positive control, Group IV: EELU low dose, Group V: EELU Medium dose, Group VI: EELU High dose
MDA: Malondialdehyde, GSH: Reduced Glutathione



[Table/Fig-5]: Cross section of cortex of kidney (Haematoxylin- Eosin; 10X). I: Intact proximal renal tubule, II: Desquamation & necrosis, III: Desquamation but involve less than half of cortical tubule, IV: Desquamation but affect more than half of articles tubule, V: Proximal tubular necrosisbut involve less than half of cortical tubule, VI: Necrosed tubular epithelial cells in small foci.
Magnification: 10x. [1] Group I[2] Group II [3] Group III [4] Group IV [5] Group V [6] Group VI
Group I: Normal control, Group II: Disease control, Group III: Positive control, Group IV: EELU low dose, Group V: EELU Medium dose, Group VI: EELU High dose
EELU: Ethanolic extract of *Linum usitatissimum*

DISCUSSION

AKI is defined as a sudden (within hours) decline in kidney function, which incorporates both injury (structural damage) and impairment (loss of function) [17]. The prevalence of AKI is rising in developed countries [18]. At present there are no pharmacologic agents exists which can be used for prevention or treatment for AKI [19]. Therefore, investigators have tried using various herbs and nutraceutical agents like medicine like ginger, garlic juice, pomegranate seed oil, *Boerhavia diffusa, Tribulus terresteris, Echinacea pallida* etc [6].

One such natural plant product is flax seed which was found to be beneficial in the chronic pre-clinical models of kidney injury like lupus nephritis and diabetic nephropathy [8,9]. However, there are no studies evaluating the role of flax seeds in preventing AKI. Thus the present study was planned to evaluate the role of EELU in preventing the development of AKI and also establish its mechanism of action. AKI model of Gentamycin induced nephrotoxicity was chosen which causes kidney damage by oxidative mechanisms. The variables used to assess nephrotoxicity were kidney morphology, biochemical variables like serum creatinine, BUN and histopathological analysis of kidney. To assess the effect EELU on oxidative biomarkers MDA and GSH was used.

It was found that 2.8 mg/kg/day dose of EELU showed nephroprotective effect in Gentamycin-induced AKI by preventing rise in serum creatinine, BUN, showed better histopathological scores and higher GSH levels as compared to the disease control and were similar to that of positive control. All the doses of EELU showed significant decrease in MDA level while the increase in GSH levels shown by 2.8 mg/kg/day dose of EELU was similar to that of positive control.

The results of the present study are in coherence with Sayed HH et al., who observed a similar reduction in biochemical parameters like Uric acid, BUN, and serum Creatinine in the model of glycerol-induced nephropathy in male albino rats with flax seed. The authors attributed the effectiveness of flax seed in inhibiting oxidative stress to lignan which acts as an antioxidant in chronic kidney diseases [20]. Dietary flax seed meal had been shown to reduce proteinuria and ameliorate nephropathy in an animal model of Type II diabetes mellitus which was attributed to Secoisolariciresinol diglucoside (SDG) content (17 mg/gm) of flax seed [9]. Flax seed oil (1000 mg/kg) was found to produce nephroprotective effect even in lead acetate (20 mg/kg) induced acute nephrotoxicity in male rats. It was found to improve kidney histopathology, BUN, Sr. Creatinine and serum uric acid levels. The authors attributed these effects to the antioxidant effect of lignans of flax seed [21]. It is known that lignan contains SDG so it appears that the beneficial effects of flax seeds could be due to SDG content.

In the study of Hall AV et al., dietary flax seed showed an inhibitory effect in lupus nephritis in MRL/lpr mouse model. The pathology of Lupus involves immunologic mediation of inflammatory vascular injury and an accelerated rate of atherogenesis. Flax seed may affect this immune, inflammatory and atherosclerotic event to prevent renal failure in lupus nephritis [22].

A study by Ghule AE et al., showed that treatment with EELU (400 mg/kg) for four weeks after Renal ischemia-reperfusion injury (RIR) significantly restored the levels of BUN and Creatinine. They also showed attenuation of RIR induced oxidative damage, which was attributed to significant increase in antioxidant enzymes and membrane-bound enzymes like Na^+K^+-ATPase and Mg^{2+}-ATPase [23].

In Sodium Arsenite (NaAs) induced AKI model of rats, Flax seed oil had been shown to produce the nephroprotective effect when given for 14 days. Administration of NaAs led to a significant decline in the activities of Brush border membrane (BBM) enzymes {alkaline phosphatase (ALP), C-glutamyltranspeptidase (GGTase), Leucineaminopeptidase (LAP) and Acid Phosphatase (ACPase)} both in tissue homogenates and in the isolated membrane vesicles. Flax seed oil consumption in combination with NaAs treatment prevented NaAs-induced decrease in BBM enzyme activities in brush border membrane vesicles and homogenate. The inhibitory effect of Flax seed oil was attributed to its antioxidant and free radical scavenging properties associated with ω-3 Polyunsaturated fatty acids (PUFAs) and lignans [24].

Houghton DC et al., reported widespread proximal tubular desquamation and necrosis, with swollen epithelial cells and inflammatory infiltrate. Gentamycin accumulates in and affects the proximal tubular cells, but insignificantly affects the glomeruli [16]. In the present study, the present authors also found that gentamycin produced proximal tubular necrosis with desquamation involving more than half tubule in the disease control group while this damage was minimal in the Low dose 0.7 mg/kg/day flax seed treated group.

There was a significant improvement in histopathology (median grade: 1.5) with a dose of 1.4 and 2.8 mg/kg/day of EELU as compared to the disease control. The treatment showed a significant improvement in histopathology. Investigators noted that in the renal cortex, focal desquamation affected less than half of tubules. The proximal tubular cells appeared normal with decreased eosinophilia and inflammatory infiltrate. Velasquez MT et al., have shown that rats fed with flax seed meal, showed the lowest percentage of abnormal glomeruli, tubulointerstitial lesions indicating small focal injury of grade 2 when compared to rat fed with casein [9].

Ghule AE et al., reported the presence of mild (grade 2) vasocongestion, tubular and glomerular cell necrosis, in a high & medium dose of EELU in RIR in male Wistar rats, as compared to disease treated group showing >50% area of a kidney having vascular congestion, tubular cell necrosis, cytoplasmic vacuolization and nuclear pyknosis. The effects were attributed to restore myeloperoxidase activity and reduced TNF-α levels in addition to antioxidant properties exerted in terms of superoxide dismutase, glutathione peroxidase and reduced glutathione [23].

Literature has shown that flax seed is a rich source of α-linolenic acid (ALA), short chain Polyunsaturated fatty acids (PUFA), soluble and insoluble fibers, phytoestrogeniclignans (secoisolariciresinoldiglycoside-SDG) and proteins [25]. Lignan has established their anti-oxidant potential in chronic renal diseases by reducing renal inflammation and lipid peroxides in polycystic kidney disease as discussed and demonstrated by various investigators [26]. The present authors have also found similar effect on MDA and GSH which are oxidative markers further strengthening the concept. Beneficial effect of Flax seed observed in the present study could be attributed to the antioxidative potential of lignans. Flax seed can have therapeutic application in patients (e.g., chronic kidney disease, diabetes mellitus, heart failure, cancer, on medications like NSAID, amphotericin etc.,) who are at risk of acute kidney injury [27]. Thus, future studies can be planned to investigate effect of flax seed in population who are at risk of acute kidney injury.

LIMITATION

Proteinuria is one of significant markers of AKI but due to financial constraints and feasibility reasons could not be done in the current study. Findings of proteinuria, if done, can improve the outcomes observed in the current study.

CONCLUSION

The present study demonstrated the nephroprotective effect of extract of *Linum usitattissumum* (EELU) in the model of gentamycin-induced nephrotoxicity in Wistar rats with acute kidney injury. This nephroprotective effect of EELU is attributed to its antioxidant potential. The findings of this study should assist researchers in development of flax seed as nephroprotective agent in humans especially in patients who are at risk of acute kidney injury.

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