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ORIGINAL ARTICLE

Escin Attenuates Inflammation and Oxidative Stress and Preserves Renal Function in Hyperoxaluric Rats

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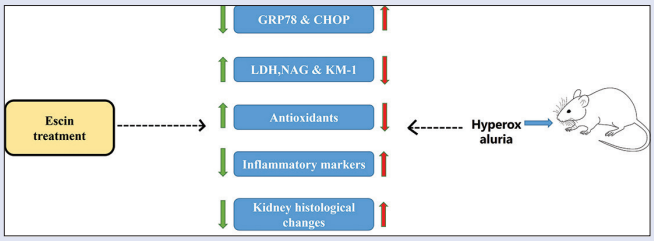
ABSTRACT

Background and Objectives: Oxalate is a toxic metabolite, which is predominantly excreted through the kidneys. Hyperoxaluria is a clinical condition characterized by the occurrence of excessive amounts of oxalate in the urine. Hyperoxaluria affects most of the patients with kidney stone. In this study, we aimed to reveal the therapeutic actions of escin against the hyperoxaluria-induced nephropathy in rats through the suppression of inflammation and oxidative stress. **Materials and Methods:** Wistar rats were induced with hyperoxaluria via administration of 0.4% ethylene glycol and 1% ammonium chloride through drinking water and treated with 50 mg/kg of escin for 28 days. The bodyweight, water intake, and urinary output was monitored and tabulated. The renal markers such as urinary kidney injury molecule-1 (KIM-1), N-acetyl-β-D-glucosaminidase (NAG), and lactate dehydrogenase (LDH) were examined using assay kits. The pro-inflammatory cytokines such as IL-1 β, IL-6, and monocyte chemoattractant protein-1 (MCP-1) were quantified using kits. The MDA and antioxidant enzymes such as superoxide/dismutase (SOD), glutathione peroxidase (GPx), and glutathione reductase (GR) were investigated using kits. The mRNA expression of CCAAT/enhancer-binding protein homologous protein and GRP78 was studied by reverse transcription–polymerase chain reaction analysis. **Results:** Escin remarkably improved the bodyweight and decreased the renal weight, water uptake, and urinary output in Wistar rats. The status of urinary oxalate, LDH, NAG, and KIM-1 was appreciably suppressed by the escin treatment. Escin also reduced the levels of interleukin (IL)-1 β, IL-6, MCP-1, and lipid peroxides and increased the activity of antioxidant enzymes such as SOD, GPx, and GR. It downregulated the expression of CHOP and GRP78. Furthermore, histological examination of escin-treated hyperoxaluric animals revealed improved kidney structures. **Conclusion:** The results of this study revealed that escin shows potent therapeutic actions against hyperoxaluria-induced nephropathy in rats.

Key words: Escin, hyperoxaluria, inflammation, oxidative stress, renal failure

SUMMARY

- Escin treatment appreciably increased the bodyweight and suppressed the renal weight, water uptake, and urinary output in hyperoxaluria-induced animals.
- Escin suppressed the status of inflammatory markers and improved the level of antioxidants in hyperoxaluria-induced animals.
- Escin appreciably downregulated the expression of CHOP and GRP78.



Abbreviations used: ROS: Reactive oxygen species; NAC: N-acetylcysteine; KIM-1: Kidney injury molecule-1; NAG: N-acetyl-β-D-glucosaminidase; LDH: Lactate dehydrogenase; LPO: Lipid peroxidation; MDA: Malondialdehyde; GPx: Glutathione peroxidase; GR: Glutathione reductase.

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INTRODUCTION

Hyperoxaluria is a clinical condition characterized by excessive amounts of oxalic acid (oxalate) in the urine.[1] It can be additionally categorized as primary and secondary hyperoxaluria. Primary hyperoxalurias are rare genetic disorders, which cause loss of enzyme activity of specific enzymes, thereby resulting in the accumulation of oxalate, whereas secondary hyperoxaluria is caused due to the overconsumption of oxalate-rich foods such as nuts and chocolate. Excessive amounts of calcium oxalate result in the formation of insoluble crystals that grow large in size, which could be passed via urinary tract thereby causing more pain, bloody urine, and kidney injuries.[2] Hyperoxaluria affects most patients with kidney stones.[3] It can lead to renal fibrosis, nephrocalcinosis, and ultimately kidney failure.[4] Till date, the cellular and molecular mechanisms of initiation and development of chronic oxalate nephropathy are unclear.[5]

The kidney is the most imperative organ needed by the body to accomplish various tasks such as detoxification, regulation of extracellular fluids, and excretion of toxic metabolites.[6] Toxic nephropathy is a reversible form of renal injury when it is diagnosed early.[7] It is caused due to the malfunction of kidney-specific excretion and detoxification process because of the renal injury.[8] The kidneys

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are highly susceptible to the oxalate crystals that could further result in nephrocalcinosis and even kidney failure.[9] Worldwide, the prevalence of hyperoxaluria is rapidly increasing.[10]

The elevated levels of oxalate result in oxidative stress in the kidneys that could provide an imperative role in the progression of kidney cell damage.[11] The renal failure is due to oxalate nephropathy which is an overwhelming difficulty of oxalate metabolism, overabsorption, and incorporation of a huge quantity of oxalate.[12-14] The elevated levels of oxalate in the renal tubular fluid can result in the formation of oxalate crystals. The crystals can damage the epithelial cells, which further leads to renal damage and reduced glomerular filtration rate.[15]

Oxidative stress is responsible for the pathological progression of nephrotoxicity. Reactive oxygen species (ROS) can be formed during hypoxia, which can cause oxidation of various proteins and damage the DNA, which can alter the structure of both plasma and mitochondrial membrane.[16] Inflammation is a highly regulated biological event that includes the formation and deliverance of pro-inflammatory regulators such as interleukin (IL)-1 β and IL-6.[17] A recent study has reported that IL-6 and IL-1 β play a critical role in various kidney diseases.[18] Ethylene glycol, also called ethane-1,2-diol, is a well-known nephrotoxic agent that can induce the formation of renal calcium oxalate stones, renal inflammation, and injury.[19] At present, the therapeutic options available for hyperoxaluria are primarily targeted toward the suppression of accumulation of oxalate even though these approaches are less effective and their success rate is marginal.[20]

Escin is a natural mixture of pentacyclic triterpenoid saponins isolated from *Aesculus indica* and *Aesculus hippocastanum*.[21] It is well known for its therapeutic activities such as anti-edematous,[22] anticancer,[23] and anti-inflammatory activities.[24] It has ameliorative effects against cardiac autonomic neuropathy[25] and demonstrates gastroprotective properties.[26,27] Therefore, in this study, we aimed to disclose the beneficial actions of escin against the hyperoxaluria provoked nephropathy in the rats via suppression of inflammation and oxidative stress.

MATERIALS AND METHODS

Chemicals

Escin, ethylene glycol, ammonium chloride, N-acetylcysteine (NAC), sodium azide, and other chemicals were obtained from Sigma-Aldrich (USA). The assay kits for biochemical analysis were obtained from Abcam (USA), MyBioSource (USA), and R&D Systems (Minneapolis, USA). The polymerase chain reaction (PCR) kits were obtained from the Biocompare (USA) and Biorad (USA).

Animal model

Male Wistar rats (120–150 g) were purchased from the Institutional Animal Facility. All rats were maintained under standard laboratory conditions, with 25 ± 3°C and 12 h light/dark cycle in a humidified cabin. Throughout this study, the animals were provided with regular pellet diet and acclimatized for a week prior to the initiation of work. Animals were divided into four groups: Group I (control animals were administered with normal saline via intraperitoneal (i.p.) route), Group II (hyperoxaluria induced in animals via administration of 0.4% ethylene glycol and 1% ammonium chloride via drinking water for 28 days), Group III (hyperoxaluria induced in animals supplemented with 50 mg/kg of escin via i.p. for 28 days), and Group IV (hyperoxaluria induced in animals administered with 50 mg/kg of NAC for 28 days as the standard drug). The bodyweight and water intake levels were monitored in a careful manner, and the data were tabulated. After the completion, the urine samples from all animals were gathered for additional investigations. Then, the animals were sacrificed via i.p. injections of sodium pentobarbital and the kidneys were harvested and analyzed further.

Collection of urine

In this study, on days 7, 14, and 28, animals were located on the metabolic confines and urine samples were gathered. Briefly, 0.02% of sodium azide was used as a preservative agent. The excretory volume and pH of the urine were examined and tabulated. Then, the urine samples were utilized for the examination of renal injury and inflammatory markers.

Quantification of renal markers

The oxalate levels in the urine samples of the control and treated animals were quantified using the assay kit and based on the manufacturer's instructions (Abcam, USA). The urinary kidney injury molecule-1 (KIM-1) was examined with the assay kit procured from MyBioSource (USA) according to the manufacturer's protocols. The activity of N-acetyl-β-D-glucosaminidase (NAG) in the urine sample was analyzed based on the instructions provided by the kit manufacturer (Abcam, USA). The activity of lactate dehydrogenase (LDH) was analyzed in the urine sample using the assay kit (Abcam, USA).

Analysis of pro-inflammatory cytokines

The pro-inflammatory cytokines such as IL-1 β, IL-6, and monocyte chemoattractant protein-1 (MCP-1) in the urine of the experimental animals were quantified using the respective assay kits as per the guidelines provided by the manufacturer (R&D Systems, Minneapolis, USA). Samples were examined in triplicates, and the outcomes were illustrated as data relative to the urinary creatinine detected concurrently (pg/mg creatinine).

Measurement of urea and creatinine clearance

The level of urea in the serum samples of control and treated animals was quantified using assay kits according to the protocols given by the manufacturer (Abcam, USA). Creatinine clearance was determined using standard clearance formula $C = U/S \times V$, where U is the creatinine amount in the urine, S – creatinine amount in the serum, and V – urine quantity (mL/min).

Measurement of oxidative stress and antioxidant markers

The level of lipid peroxidation (LPO) was estimated based on the previous protocol.[28] The technique involves measuring the level of malondialdehyde (MDA) formed, which is an LPO marker in the renal tissue homogenate. The data were presented as nanomole MDA formed per milligram protein (nmol MDA/mg protein). The activity of superoxide/dismutase (SOD) was examined as per the previous protocol.[29] The data of SOD activity are represented as units per milligram (U/mg) protein. Glutathione peroxidase (GPx) activity was measured as per the previous protocol.[30] The absorbance was read at 420 nm. The activity of glutathione reductase (GR) was performed based on the previous protocol.[31] The accumulation of ROS in renal cells was quantified using 2'-7'-Dichlorodihydrofluorescein diacetate (DCF-DA) staining.[32] The status of reduced and oxidized glutathione (GSH/GSSG) was detected with the aid of assay kits (MyBioSource, USA).

Reverse transcription–polymerase chain reaction analysis

PCR was conducted as per the protocol provided by the manufacturer (Trizol kit, Biocompare, USA). Briefly, the total RNA was isolated from the renal tissues of control and treated rats. The cDNA was constructed using the extracted RNA with the aid of kits (Biorad, USA). The PCR products were purified using 1.5% agarose gel and the values were standardized to GAPDH gene. The primers utilized are as follows: For CHOP, upstream 5′-TCCTGCGTCGGTGTATTC-3′; downstream 5′-CGTGAGTTGGTTCTTGGC-3′; GRP78 upstream 5′-GGAGCAGGAGAATGAGAG-3′; and downstream 5′-GACAGACAGGAGGTGATG-3′. Data were presented as the relative density of mRNA expression.

Histological study

The kidney tissues from the control and treated rats were excised and stabilized with 10% formalin and then embedded in paraffin. Then, 5 mm thick sections were prepared and stained with the hematoxylin–eosin (H and E) stain solution. Finally, the sections were observed under a microscope to detect the histological changes.

Statistical analysis

Data were analyzed using SPSS software (SPSS Inc., IL, USA). The results were analyzed via one-way analysis of variance and Student's *t*-test. $P < 0.05$ was regarded as statistically significant.

RESULTS

Effect of escin on the bodyweight, kidney weight, water intake, urine output, and pH levels in the hyperoxaluria-induced animals

The hyperoxaluria-induced animals demonstrated the major impairments in the bodyweight, kidney weight, and water intake of the animals. Hyperoxaluric condition diminished the bodyweight and elevated the kidney weight, water uptake, and the total urinary output. The pH of the urine was reduced slightly in the hyperoxaluric animals. These results show the hyperoxaluria-induced renal malfunctions. Escin notably modulated the hyperoxaluria-induced modifications in the rats. It improved the bodyweight and suppressed the kidney weight, water uptake, and the total urinary output in the hyperoxaluric animals. Escin treatment slightly improved the pH level of the urine in the hyperoxaluric rats [Table 1]. These results show that escin appreciably reverted the renal malfunctions in hyperoxaluria-induced rats.

Effect of escin on the urinary oxalate in the hyperoxaluria-provoked animals

Figure 1 shows the urinary level of oxalate in control and treated animals on days 7, 14, and 28 of the experiment. The hyperoxaluria-induced animals showed drastic elevation in the urinary status of oxalate. Interestingly, animals supplemented with escin exhibited a remarkable suppression in the urinary oxalate level. NAC, the standard drug, supplementation also decreased the urinary oxalate status in the hyperoxaluria-induced animals. These results demonstrate that the activity of escin is comparable to that of the standard drug NAC.

Effect of escin on the level of lactate dehydrogenase, N-acetyl-β-D-glucosaminidase, and kidney injury molecule-1 in the hyperoxaluria-induced animals

Hyperoxaluria-induced animals showed noticeable changes in the urinary status of LDH, NAG, and KIM-1. The level of LDH, NAG, and KIM-1 was examined on days 7, 14, and 28, and the results clearly demonstrate that these markers were found to be upregulated in the urine of hyperoxaluria-induced animals. Escin appreciably decreased the level of these markers in the urine of hyperoxaluria-induced animals. NAC also reduced the urinary status of LDH, NAG, and KIM-1. These results show that the activity of escin is similar to that of NAC [Figure 2].

Effect of escin on the lipid peroxides and the activity antioxidant enzymes in the hyperoxaluria-induced animals

The hyperoxaluria-induced animals demonstrated the drastic elevation in the level of LPO and suppressed the activity of antioxidant enzymes. The level of MDA was found to be elevated and the activities of SOD, GR, and GPx was significantly reduced in the renal tissues of hyperoxaluria-induced animals [Figure 3]. These changes were ameliorated by escin in the renal tissues of hyperoxaluria-induced animals. NAC also reduced the level of MDA and increased the activity of antioxidants SOD, GR, and GPx. These results show that escin and NAC provide comparable outcomes.

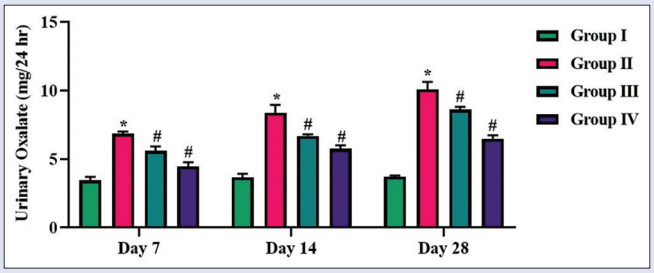

Figure 1: Effect of escin on the urinary oxalate in hyperoxaluric animals. The result was portrayed as mean ± standard deviation of triplicates. Values were scrutinized by one-way analysis of variance and Student's *t*-test. Note: "*" indicates that data significantly differ at $P < 0.05$ from control and "#" indicates significantly differ at $P < 0.05$ from hyperoxaluric group

Table 1: Effect of escin on the bodyweight, kidney weight, water intake, and urine output and pH levels in the hyperoxaluric animals

Groups	Bodyweight (g)		Kidney weight (g)	Water intake (ml/24 h)	Urinary output (ml/24 h)	Urine PH
	Initial	Final				
Group I	169.01±24.62	193.64±38.14	0.72±0.33	32.45±15.88	28.413±12.99	5.3±0.28
Group II	164.67±21.47	182.40±32.49*	0.96±0.49*	39.16±19.20*	34.88±17.61*	4.5±0.17*
Group III	174.70±28.39	191.51±37.88#	0.75±0.38#	37.49±17.88#	31.05±15.82#	5.16±0.26#
Group IV	161.096±19.92	190.79±36.09#	0.78±0.39*	35.86±16.60*	33.34±16.29*	4.76±0.18#

*Indicates that data significantly differ at $P<0.05$ from control, #Indicates significantly differ at $P<0.05$ from hyperoxaluric group. Result was portrayed as mean±SD of triplicates. Values were scrutinized by one-way ANOVA and Student's *t*-test. SD: Standard deviation; ANOVA: Analysis of variance

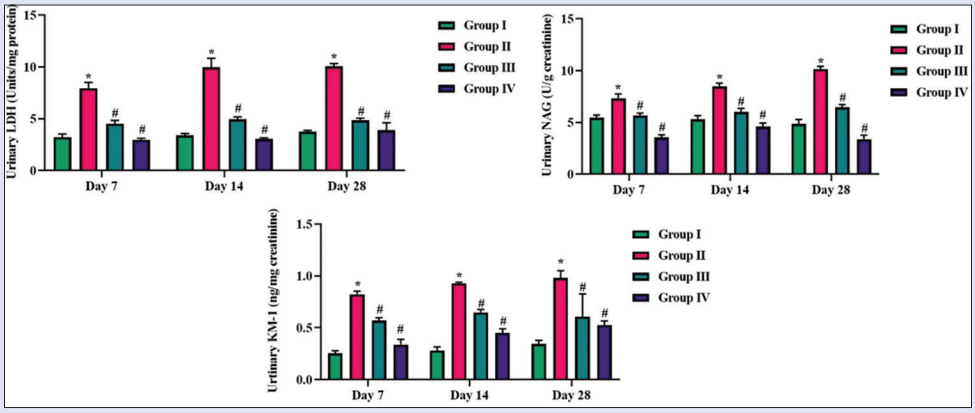

Figure 2: Effect of escin on the lactate dehydrogenase, N-acetyl-β- D-glucosaminidase, and Kidney injury molecule-1 status in the hyperoxaluric animals. The result was portrayed as mean ± standard deviation of triplicates. Values were scrutinized by one-way analysis of variance and Student's *t*-test. Note: "*" indicates that data significantly differ at $P < 0.05$ from control and "#" indicates significantly differ at $P < 0.05$ from hyperoxaluric group

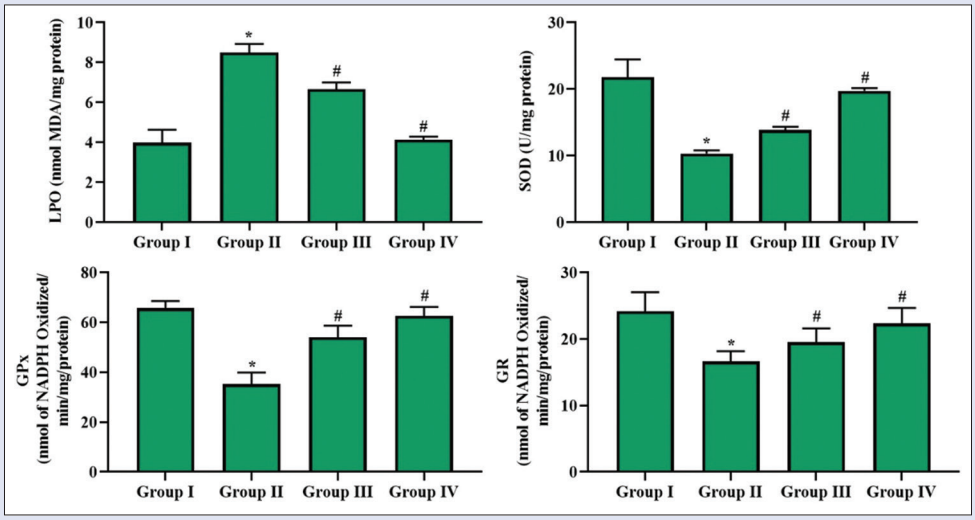

Figure 3: Effect of escin on the lipid peroxidation and antioxidant enzymes activity in the hyperoxaluric animals. The result was portrayed as mean ± standard deviation of triplicates. Values were scrutinized by one-way analysis of variance and Student's *t*-test. Note: "*" indicates that data significantly differ at $P < 0.05$ from control and "#" indicates significantly differ at $P < 0.05$ from hyperoxaluric group

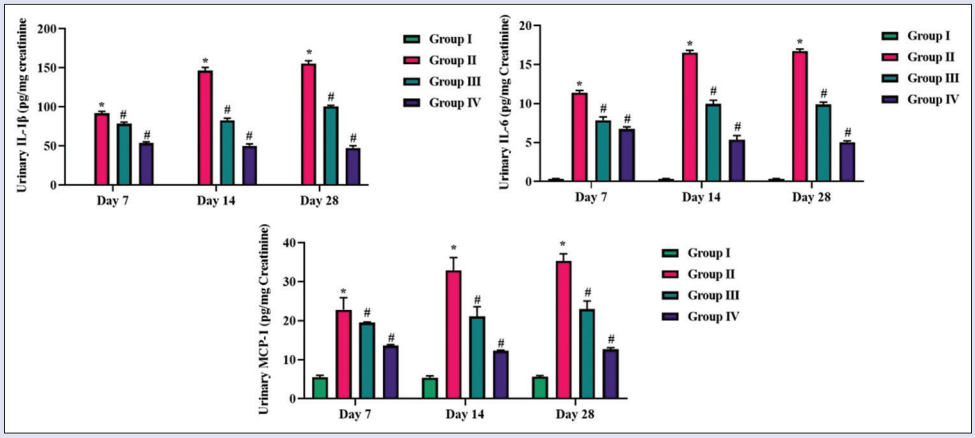

Figure 4: Effect of escin on the pro-inflammatory cytokines level of in the hyperoxaluric animals. The result was portrayed as mean ± standard deviation of triplicates. Values were scrutinized by one-way analysis of variance and Student's *t*-test. Note: "*" indicates that data significantly differ at $P < 0.05$ from control and "#" indicates significantly differ at $P < 0.05$ from hyperoxaluric group

Effect of escin on the level of pro-inflammatory cytokines in the hyperoxaluria-induced rats

Figure 4 shows the level of IL-6, IL-1 β, and MCP-1 on days 7, 14, and 28 of the experiment. The level of IL-6, IL-1 β, and MCP-1 was found to be increased in the urine of hyperoxaluria-induced animals. It indicates that hyperoxaluria triggered inflammatory condition. Escin reduced the inflammatory condition in the hyperoxaluria-induced animals by decreasing the level of pro-inflammatory markers. Escin reduced the level of IL-6, IL-1 β, and MCP-1 in the urine, which demonstrates its anti-inflammatory potential. Furthermore, NAC reduced the level of IL-6, IL-1 β, and MCP-1 in the urine of hyperoxaluria-induced animals [Figure 4]. These results show that escin and NAC show similar activity.

Effect of escin on the serum urea and creatinine clearance in the hyperoxaluria-induced animals

The level of serum urea was drastically elevated and the creatinine clearance was notably suppressed in the hyperoxaluria-induced animals. These results show that higher amounts of oxalic acid impair renal functions. Escin treatment remarkably modulated the creatinine clearance and serum urea in the hyperoxaluria-induced animals. Escin reduced the level of serum urea and noticeably improved creatinine clearance in the hyperoxaluria-induced animals [Figure 5]. Furthermore, NAC decreased the serum levels of urea and enhanced the creatinine clearance.

Effect of escin on the level of reactive oxygen species and GSH/GSSG in the renal tissues of hyperoxaluria-induced animals

Figure 6 shows the level of ROS and GSH/GSSG in the renal tissues of control and treated animals. In hyperoxaluria-induced animals, the level of ROS was severely increased and the activity of GSH/GSSG was remarkably diminished. Interestingly, escin suppressed the formation of ROS and improved the activity of GSH/GSSG status in the renal tissues of hyperoxaluria-induced animals. Furthermore, NAC reduced the formation of ROS and improved the activity of GSH/GSSG in the kidney tissues of hyperoxaluria-induced animals. The activity of escin and NAC were comparable to each other.

Effect of escin on the mRNA expressions of CHOP and GRP78 in the renal tissues of hyperoxaluria-induced animals

Figure 7 depicts the inhibitory effects of escin on the mRNA expressions of CHOP and GRP78 in the kidney tissues of hyperoxaluria-triggered animals. The mRNA expression level of CHOP and GRP78 was found to be upregulated in the renal tissues. Interestingly, escin remarkably downregulated the expression of CHOP and GRP78 in the kidney tissues of hyperoxaluria-induced animals. NAC also suppressed the expression of CHOP and GRP78 in the kidney tissues of hyperoxaluria-induced animals. Escin and NAC showed comparable results.

Effect of escin on the renal histopathology of hyperoxaluria-induced animals

Figure 8 shows the histological changes in the renal tissues of control and treated animals. The control animals have shown the higher amounts of typical glomerulus structures. The hyperoxaluria-induced animals demonstrated major alterations in the renal tissues. They also exhibited shrinkage of glomeruli and elevated urinary spaces. It also exhibited the occurrence of oxalate crystals in the lumen and increased penetration of leukocytes. In contrast, escin improved major renal abnormalities. Escin improved the tubular and glomerular structures. It also revealed improved renal histology. Furthermore, NAC treatment also improved the renal histology of the hyperoxaluric animals.

DISCUSSION

Oxalate is a toxic metabolite, which is predominantly excreted through the kidneys.[33] Hyperoxaluria has the potential to induce various processes that lead to significant amount of morbidity and mortality due to kidney damage and renal failure.[20,34] The renal exposure to oxalate is primarily via uptake of dietary oxalate, secretion and absorption in the gut, and endogenous accumulation through metabolism.[35] After the glomerular filtration, oxalate is reabsorbed and secreted along the nephron.[36] The net oxalate excretion through the urine is a measure of its uptake and endogenous accumulation. The elevated excretion of oxalate in the urine could be very toxic due to its tendency to crystallize and develop oxalate stones.[37] The oxalate exposure produces toxic reactions in renal epithelial cells and increase the inflammation and oxidative stress.[38,39] In this study, we observed that the hyperoxaluria-induced animals demonstrated the severe elevation in the level of urinary oxalate, which was reduced by the supplementation with escin.

The elevated levels of oxalate reduced the activity of antioxidant enzymes, increased the oxidative stress, as well as kidney damage.[40] The ROS levels were increased due to the increased oxidative stress.[41] The antioxidant systems maintain the antioxidant status in an organism.[42] Some of the antioxidants prevent the accumulation of free radicals, whereas the others neutralize the deleterious effects of existing free radicals. SOD and GPx protect the cells from the deleterious effects of free radicals by scavenging them.[43] In this study, we found that the level of MDA was elevated, and the activity of SOD, GR, and GPx was reduced in the renal tissues of hyperoxaluria-induced animals. Escin reduced the elevated

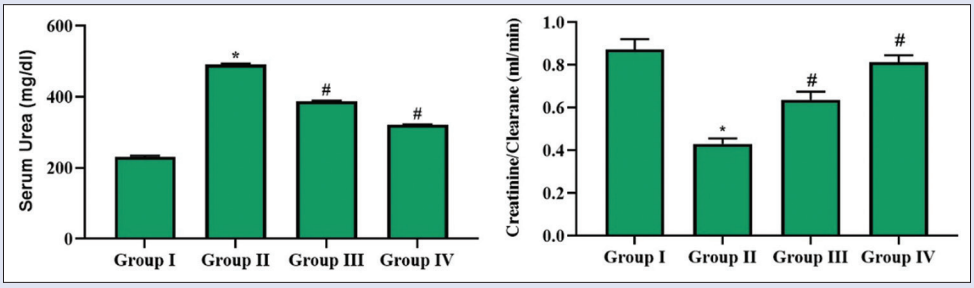

Figure 5: Effect of escin on the serum urea and creatinine clearance in the hyperoxaluric animals. The result was portrayed as mean ± standard deviation of triplicates. Values were scrutinized by one-way analysis of variance and Student's *t*-test. Note: "*" indicates that data significantly differ at $P < 0.05$ from control and "#" indicates significantly differ at $P < 0.05$ from hyperoxaluric group

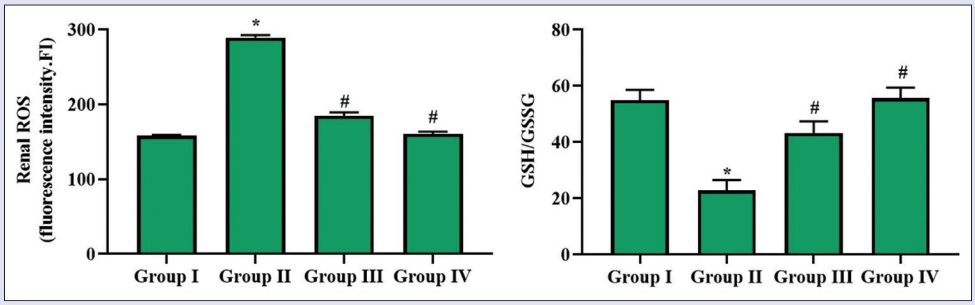

Figure 6: Effect of escin on the reactive oxygen species and GSH/GSSG status in the renal tissues of hyperoxaluric animals. The result was portrayed as mean ± standard deviation of triplicates. Values were scrutinized by one-way analysis of variance and Student's *t*-test. Note: "*" indicates that data significantly differ at $P < 0.05$ from control and "#" indicates significantly differ at $P < 0.05$ from hyperoxaluric group

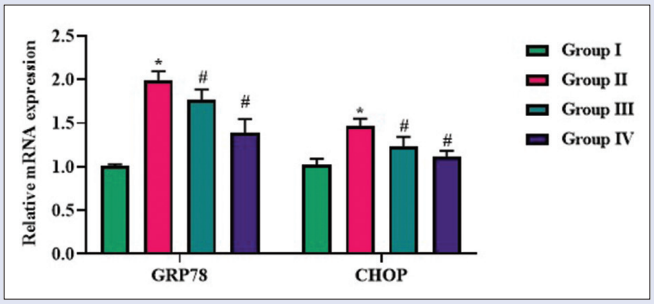

Figure 7: Effect of escin on the mRNA expressions of CHOP and GRP78 in the renal tissues of hyperoxaluric animals. The result was portrayed as mean ± standard deviation of triplicates. Values were scrutinized by one-way analysis of variance and Student's *t*-test. Note: "*" indicates that data significantly differ at $P < 0.05$ from control and "#" indicates significantly differ at $P < 0.05$ from hyperoxaluric group

levels of MDA and increased the activity of SOD, GR, and GPx in the renal tissues of hyperoxaluria-induced animals.

Inflammation is a highly regulated biochemical process. ROS accreted by immune cells which transferred into inflammatory processes which induces oxidative stress through suppressing the antioxidant potential. Excessive free radicals eventually interrupt the functions of the cell membrane as they bind with proteins and fatty acids present on the cell membrane.[44] ROS can activate tumor necrosis factor α via numerous cellular events. The level of IL-6 and IL-1 β has been reported to be augmented in the kidney tissues in various inflammatory conditions.[45,46] A previous study reported that renal parenchymal injury increases the level of IL-6.[47] Similarly, in this study, we found that the level of pro-inflammatory markers (i.e., IL-6, IL-1 β, and MCP-1) was enhanced in the urine of hyperoxaluria-induced animals. This shows that the inflammatory was severe in the hyperoxaluria-induced animals. However, supplementation with escin substantially reduced the formation of IL-6, IL-1 β, and MCP-1 in the urine of hyperoxaluria-induced animals, which shows the anti-inflammatory potential of escin.

It has been reported that in the case of nephrotoxic conditions, the level of creatinine, uric acid, and urea increases.[48-51] LDH is an important marker of kidney function, which is used to detect the pathological conditions of the kidneys.[52] A previous study has reported elevated levels of LDH in the serum using the renal injury model.[53] Furthermore, KIM-1 is an important marker to detect the damage caused to the kidneys, which is increased in hyperoxaluria-induced animals.[54] Estimating the level of KIM-1 in the urine is a sensitive and non-invasive technique to examine the renal damage. Previous studies have shown that the level of KIM-1 in the urine can get drastically elevated even prior to the increase in the level of serum urea and creatinine.[55] Furthermore, NAG is an important biomarker of renal damage, and it was said to be that NAG status was elevated in the hyperoxaluric animals models. Previous studies have revealed that in the case of nephrolithiasis, there is an elevation in the excretory status of NAG in urine. The elevation in the level of NAG is possible due to the renal tubular epithelial injury because of the increased oxalate.[56] In this study, we found that the hyperoxaluria-induced animals exhibited remarkable changes in the urinary levels of LDH, NAG, and KIM-1. These markers were found to be increased in the urine of hyperoxaluria-induced animals. Surprisingly, escin treatment exhibited a remarkable suppression in the level of urinary LDH, NAG, and KIM-1.

Shu *et al.*[57] found that the mRNA expressions of CHOP and GRP78 were upregulated in the hyperoxaluria-induced animals. Similarly, we also found that the mRNA expression of CHOP and GRP78 in the kidney tissues of hyperoxaluria-induced animals was upregulated, and the same was remarkably suppressed by the escin supplementation. Kidney injury could be examined through histological assessments and via detection of the status of kidney biomarkers such as urea and creatinine in the blood. In the case of renal toxicity, there are elevated levels of creatinine and urea and reduced glomerular filtration rate. A previous study has reported that these changes were due to the reduced glomerular filtration rate.[58] The serum level of urea was elevated and the creatinine clearance was diminished in the hyperoxaluria-induced animals. Escin decreased the level of urea in the serum and improved the creatinine clearance in hyperoxaluria-induced animals.

Hyperoxaluria can cause tissue damage, loss of membrane integrity, and renal inflammation by triggering the process of LPO and suppression of antioxidant enzymes.[59,60] The damaging of kidney epithelium could enhance the adherence of crystal and retaining as epithelial damage.[61] Similarly, hyperoxaluria-induced animals revealed shrinkage of glomeruli, occurrence of crystals, and increased leukocyte penetration. Surprisingly, escin improved the renal structures along with improvement in tubular and glomerular structures and less crystal formation in the hyperoxaluria-induced animals.

CONCLUSION

The findings of this study revealed that escin shows potent therapeutic actions against hyperoxaluric nephropathy in rats. Escin treatment appreciably increased the bodyweight and suppressed the renal weight, water uptake, and urinary output. The status of urinary oxalate and renal function markers were remarkably decreased by Escin treatment. Escin

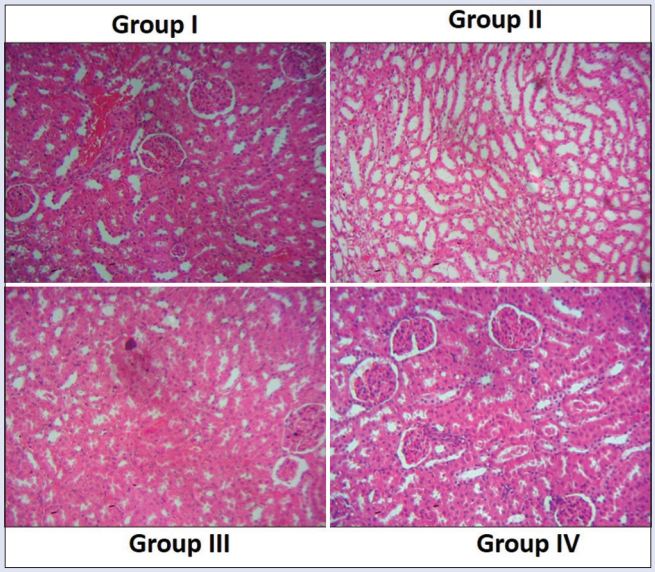

Figure 8: Effect of escin on the renal histopathology of hyperoxaluric animals. The control animals exhibited the normal renal histology (Group I). Hyperoxaluria-provoked animals displayed the glomeruli shrinkages, crystals formation, and increased leukocytes penetrations (Group II). Escin-supplemented hyperoxaluria animals displayed the major renal improvements in the hyperoxaluric animals (Group III). N-acetylcysteine treatment also improved the renal histology (Group VI)

suppressed the formation of inflammatory markers and improved the activity of antioxidant enzymes in hyperoxaluria-induced animals. It appreciably downregulated the expression of CHOP and GRP78. These findings demonstrate the therapeutic role of escin against hyperoxaluria in animals. In future, we recommend additional studies on this hypothesis, which could provide the key for the development of novel nephroprotective agent.

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Conflicts of interest

There are no conflicts of interest.

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