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Research Article

AMLEORATIVE ROLE OF ESCULETIN-MEDIATED RENOPROTECTION AGAINST GENTAMICIN-INDUCED NEPHROTOXICITY AND POSSIBLE INVOLVEMENT OF N-METHYL-D-ASPARTATE RECEPTORS

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ABSTRACT

Objective: In this investigation, the amleorative role of esculetin (Esc) was investigated in gentamicin (Genta) nephrotoxicity in rats and the possible role of N-methyl-D-aspartate receptors (NMDAR) in genta-induced nephrotoxicity.

Methods: Genta (100 mg/kg/day, i.p. for 7 days) was administered to rats for the induction of nephrotoxicity, and subsequently, the extent of renal damage was measured by estimating creatinine clearance (CrCl), blood urea nitrogen (BUN), uric acid, microprotienuria and fractional excretion of sodium, and potassium. In addition, renal superoxide anion generation (SAG), Thiobarbituric acid reactive substance (TBARS), and reduced glutathione (GSH) level were used to evaluate renal oxidative parameters. Renal myeloperoxidase (MPO) activity was used to measure renal inflammation. D-serine, NMDA agonist was used in this study to evaluate the role of NMDA antagonist in genta-induced nephrotoxicity. Histopathological examination was performed using hematoxylin and eosin staining method.

Results: Genta-treated rats exhibited remarkable changes in renal parameters like increase in BUN, uric acid, microprotein fractional excretion of sodium and potassium with decrease in CrCl and similarly biochemical parameters like increase in SAG, thiobarbituric acid reactive species (TBARS), MPO activity with decrease in GSH level. Treatment with Esc (5 and 10 mg/kg/day, i.p for 7 days), NMDAR antagonist attenuated the genta-induced nephrotoxicity but did not shown significant effect on combined use of genta and D-serine treated group. Histopathological examination of genta-treated rats. The coadministration of Esc + genta-protected kidney tissue from nephrotoxic effect of genta as illustrated by normalization of tubules but not with the combined use of Esc + genta + D-serine treated rats.

Conclusion: Esc displayed protective effect in genta-induced nephrotoxicity but combined effect of Esc + genta + D-serine abolished the protective effect of Esc thus confirming that NMDAR may be involved in genta-induced nephrotoxicity.

Keywords: Nephrotoxicity, Gentamicin, D-serine, Esculetin, N-methyl-D-aspartate receptor.

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INTRODUCTION

Nephrotoxicity occurs when kidney-specific detoxification and excretion do not work properly due to the damage or destruction of kidney function by exogenous or endogenous toxicants [1]. Gentamicin (Genta) is one of the most effective aminoglycoside antibiotics used against Gram-negative infections. However, nephrotoxicity is the major untoward outcome of genta treatment. Recent reports suggest that 30% of the patients treated with genta for more than 7 days develop nonoligouric renal dysfunction and results in apoptosis as well as necrosis of tubular epithelial cells [2,3]. The occurrence of nephrotoxicity with genta is up to 60% in case of patients admitted in intensive care units [4].

N-methyl-D-aspartate receptors (NMDARs) belong to a class of ionotropic glutamate receptors that also include the α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid receptors and kainate receptors [5]. NMDAR is glutamate-gated cation channel with high calcium permeability and critical for the development of the central nervous system [6]. NMDARs are also expressed across a wide spectrum of non-neuronal cells, including central and peripheral glial cells, endothelium, kidney, bone, pancreas, and others [5]. NMDAR forms a heterotetramer composed of NR1 and NR2 (A-D) subunits and more rarely, NR3 subunits [7]. The NR1 subunit is the main subunit of the NMDAR essential for channel activity, whereas the NR2 subunits, although not essential for function, can confer modulatory properties [8]. NR1 is present in total rat kidney, cortex, and medulla and out of the NR2 subunits, only the NR2C subunit protein is present in the kidney [9].

Esculetin (Esc) is a coumarin derivative contained in many plants, such as *Artemisia capillaris* (Compositae), the leaves of *Citrus limonia* (Rutaceae), and *Ceratostigma willmottianum* [10,11] that are used as folk medicines. Various studies have reported Esc to possess antioxidant, antitumor, neuroprotective, and anti-inflammation activities [12]. Esc has been reported to inhibit NMDA neurotoxicity by modulating the expression of NMDA [13]. The renoprotective role of NMDA antagonist has been suggested in ischemia-reperfusion-induced renal dysfunction [14] and drug-induced renal dysfunction [8]. Recently, the renoprotective role of Esc has been evinced in streptozotocin induced diabetic rats [15,16]. Therefore, Esc was selected to evaluate its efficacy in genta-induced nephrotoxicity and its effect as NMDAR antagonist.

D-serine was used as a NMDA agonist in this study. It is relevant endogenous NMDAR ligand which act as coagonist at "glycine site" of NR1 subunit [17]. Therefore, this study has been designed to evaluate the role Esc mediated renoprotection against genta-induced nephrotoxicity and possible involvement of NMDAR.

METHODS

Animals

Sprague Dawley rats weighing (200-250 g) of age 3-5 months (National institute of Pharmaceutical Education and Research, Mohali, Punjab,

India) were employed in this study. Animals were provided with standard laboratory feed (Ashirwad Industries, Tirpari, Kharar District, Mohali, Punjab, India) and clean drinking water *ad libitum* and were exposed to natural cycle of light and dark. The experimental protocol was approved by the Institutional Animal Ethics Committee and care of the animals were taken as per the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment and Forest Government of India (1201/9/08/CPCSEA/013).

Drugs and chemicals
Esc and D-serine were purchased from Sigma-Aldrich and Himedia Laboratories Pvt. Ltd., India. Genta was obtained from Nitin Life sciences Ltd., India. Eosin and haematoxylin were procured from S.D. Fine Chemicals Ltd., India. Dithiobis nitro benzoic acid, reduced glutathione (GSH), and nitro blue tetrazolium were obtained from Loba Chemie, India. All other reagents used in the study were of analytical grade.

Induction of nephrotoxicity
The nephrotoxicity in rats was induced by administering genta at a dose of 100 mg/kg; i.p. for 7 days [18,19]. Animals were placed in metabolic cages for urine collection. On 8th day, animals were anesthetized using diethyl ether. The blood samples were collected using retro-orbital puncture, and rats were sacrificed by cervical dislocation. The plasma isolated from blood was used for estimation of creatinine, blood urea nitrogen (BUN), uric acid, sodium and potassium level. Moreover, the creatinine, sodium, potassium, and protein content in urine were estimated. The kidneys were removed and washed with 1.17% potassium chloride (KCl) solution. A part of renal tissue was preserved in neutral buffered formalin for histological studies. A small portion was used for estimation of superoxide anion generation (SAG), and the rest of tissue was minced and homogenized (10% w/v) in 1.17% KCl solution using teflon homogenizer. The contents were centrifuged at 800 g for 20 minutes. The pellet obtained was used for estimation of myeloperoxidase (MPO) activity, whereas the clear supernatant was used to estimate lipid peroxides and reduced GSH levels.

Estimation of creatnine clearance
The estimation of creatinine in plasma and urine samples was done by using commercially available kit by ERBA Diagnostics Pvt. Ltd., India.

Estimation of BUN level
The BUN level was estimated in plasma by using commercially available kit by Meril Diagnostics Pvt. Ltd., India.

Estimation of plasma uric acid level
The uric acid was estimated in the plasma sample using commercially available kit by ADI Diagnostics Pvt. Ltd., India.

Estimation of fractional excretion of sodium
The sodium level was estimated in the plasma and urine samples by using commercially available kit by Reckon Diagnostics Pvt. Ltd., India.

Estimation of fractional excretion of potassium
The potassium level was estimated in the plasma and urine samples by using commercially available kit by Reckon Diagnostics Pvt. Ltd., India.

Estimation of microproteinuria
The microproteins were estimated in urine samples by using commercially available kit by ERBA Diagnostics Pvt. Ltd., India.

Estimation of MPO activity
The MPO activity which is measured as an index of neutrophil accumulation was measured using method of Bradley *et al*. [20].

Estimation of thiobarbituric acid reactive substance
The quantitative measurement of thiobarbituric acid reactive substances (TBARS), an index of lipid peroxidation in renal tissue was performed according to method of Ohkawa *et al*. [21].

Estimation of total protein content
The total protein content was determined using Lowry's method [22] using bovine serum albumin as a standard.

Estimation of SAG
The SAG in the renal tissue has been evaluated using method described by Wang *et al*. [23].

Hematoxylin and eosin staining
The renal tissues preserved in 10% formalin were dehydrated in graded concentrations of ethanol, immersed in xylene and then embedded in paraffin. The sections of 4 μm thickness were cut and placed on slide using commercial Baker's mounting fluid. Paraffin wax was removed by warming the slide gently, until the wax melted and then was washed with xylene. This was followed by washings with absolute alcohol and water to hydrate the sections and stained with haematoxylin and eosin described by Clayden [24]. The hydrated sections were stained with hematoxylin for 15 minutes. The stained sections were washed with water and treated with 1% acid alcohol mixture for 20 seconds. The acid alcohol mixture was washed off with water, and sections were counterstained with 1% aqueous solution of eosin for 2 minutes. After washing with water to remove excess of eosin, the sections were dehydrated using absolute alcohol and then mounted using Canada balsam as mounting agent. The slides were observed for gross histopathological changes and neutrophil accumulation.

Experimental protocol
About 6 groups were employed in the present study, each comprising 6 rats.

Group I (control)
Animals were exposed to normal conditions for 7 days.

Group II (Esc per se)
Animals in this group were administered Esc (10 mg/kg; i.p.) for 7 days.

Group III (genta)
Animals in this group were treated with genta (100 mg/kg; i.p.) for 7 days.

Group IV (genta + Esc 5 mg/kg)
Animals were treated with Esc (5 mg/kg; i.p.) 1 hr before the administration of genta (100 mg/kg; i.p.) for 7 days.

Group V (genta + Esc 10 mg/kg)
Animals were treated with Esc (10 mg/kg; i.p.) 1 hr before the genta (100 mg/kg; i.p.) administration for 7 days.

Group VI (D-serine + genta + Esc 10 mg/kg)
D-serine (80 mg/kg; i.p.) was administered to animals for 7 days followed by the similar treatment as mentioned in Group V.

Statistical analysis
Results were expressed as mean ± standard error of mean. The data obtained from various groups was statistically analysed using one-way ANOVA followed by Tukey's multiple range test. The $p<0.05$ was considered to be statistically significant.

RESULTS

Effect of various pharmacological interventions on creatinine clearance (CrCl)
The administration of genta (100 mg/kg; i.p. for 7 days) produces significant decrease in CrCl, when compared to control rats. The administration of Esc per se (10 mg/kg; i.p. for 7 days) did not show any significant effect on CrCl. The administration of Esc (5 mg/kg; i.p.; 10 mg/kg; i.p.) for 7 days to genta-treated rats; attenuated genta

decreased level of CrCl. The administration of D-serine (80 mg/kg i.p.) + Esc (10 mg/kg; i.p.) + genta (100 mg/kg i.p.) for 7 days did not produce significant effect on CrCl (Fig. 2).

Effect of various pharmacological interventions on BUN level

The administration of genta (100 mg/kg; i.p. for 7 days) produces significant increase in BUN level, when compared to control rats. The administration of Esc per se (10 mg/kg; i.p. for 7 days) did not show any significant effect on BUN level. The administration of Esc (5 mg/kg; i.p.; 10 mg/kg; i.p.) for 7 days to genta-treated rats; attenuated genta increased level of BUN. The administration of D-serine (80 mg/kg; i.p.) + Esc (10 mg/kg; i.p.) + genta (100 mg/kg; i.p.) for 7 days did not produce significant effect on BUN level (Fig. 3).

Effect of various pharmacological interventions on plasma uric acid level

The administration of genta (100 mg/kg; i.p. for 7 days) produces significant increase in plasma uric acid level, when compared to control rats. The administration of Esc per se (10 mg/kg; i.p. for 7 days) did not show any significant effect on plasma uric acid level. The administration of Esc (5 mg/kg; i.p.; 10 mg/kg; i.p.) for 7 days to genta-treated rats; attenuated genta increased level of plasma uric acid. The administration of D-serine (80 mg/kg i.p.) + Esc (10 mg/kg; i.p.) + genta (100 mg/kg i.p.) for 7 days did not produce significant effect on plasma uric acid level (Fig. 4).

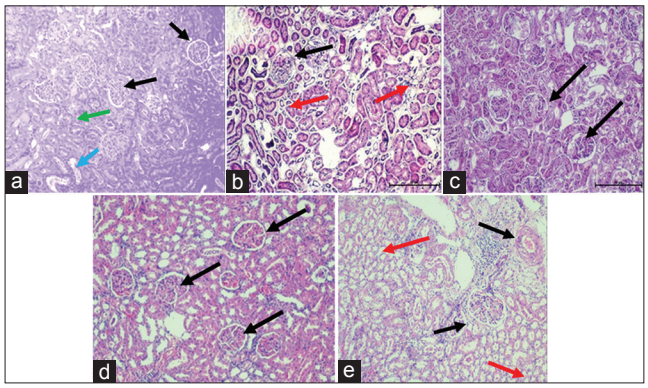

Fig. 1: Histopathological investigation, (a) normal control: Glomerular capillaries (black arrow) with both PCT (green arrow) and DCT (blue arrow) appears normal, (b) gentamicin treated group. Intestitial oedema seen by separation of tubules as well as congestion of capillaries. Tubules show epithelial degeneration, severe necrosis (red) and glomerular capillaries are widened (black), (c) esculetin (5 mg/kg) treated group: The histological features are improved as compared to gentamicin treated group. Glomerular capillaries retain their normal appearance and tubular epithelium is diminished, (d) esculetin (10 mg/kg/day) treated group: The histological features greatly improved and come to their normal structure of glomerular capillaries and tubular epithelium, (e) D-serine (80 mg/kg) + gentamicin (100 mg/kg) + esculetin (10 mg/kg) treated group: The histopathological feature witnessed tubular necrosis (black arrow), interstitial edema and cluster of inflammatory cells (neutrophil accumulation) (red arrow)

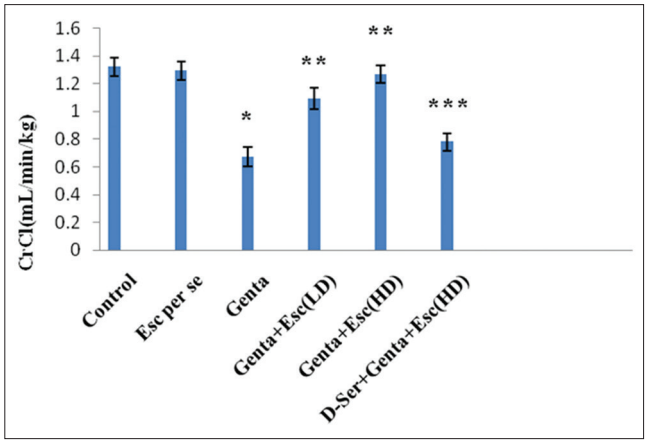

Fig. 2: Effect of various pharmacological interventions on creatinine clearance. Esculin (LD): Esculetin low dose (5 mg/kg, i.p.), Esculin (HD): Esculetin high dose (10 mg/kg, i.p.), values are expressed as mean ± standard error of mean (STDEV), n=6, one-Way ANOVA followed by Tukey's multiple range test. *p<0.005 as compared to control. **p<0.005 as compared to Gentamicin-treated group. ***p<0.005 as compared to esculetin high-dose treated group

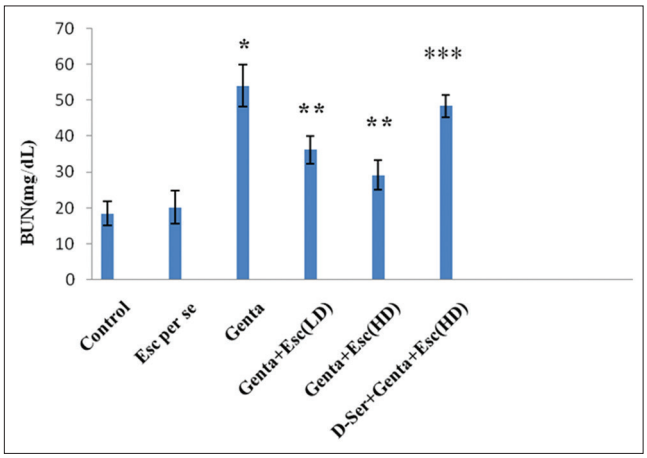

Fig. 3: Effect of various pharmacological interventions on blood urea nitrogen level. Esculin (LD): Esculetin low dose (5 mg/kg, i.p.), Esculin (HD): Esculetin high dose (10 mg/kg, i.p.), values are expressed as mean ± standard error of mean (STDEV), n=6, one-Way ANOVA followed by Tukey's multiple range test. *p<0.005 as compared to control. **p<0.005 as compared to Gentamicin-treated group. ***p<0.005 as compared to esculetin high-dose treated group

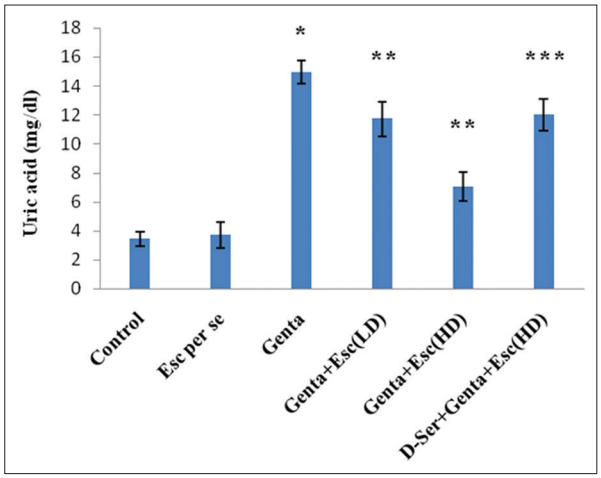

Fig. 4: Effect of various pharmacological interventions on plasma uric acid level. Esculin (LD): Esculetin low dose (5 mg/kg, i.p.), Esculin (HD): Esculetin high dose (10 mg/kg, i.p.), values are expressed as mean ± standard error of mean (STDEV), n=6, one-Way ANOVA followed by Tukey's multiple range test. *p<0.005 as compared to control. **p<0.005 as compared to Gentamicin-treated group. ***p<0.005 as compared to esculetin high-dose treated group

Effect of various pharmacological interventions on microproteinuria

The administration of genta (100 mg/kg; i.p. for 7 days) produces significant increase in microproteinuria level, when compared to control rats. The administration of Esc per se (10 mg/kg; i.p. for 7 days) did not show any significant effect on microproteinuria level. The administration of Esc (5 mg/kg; i.p.; 10 mg/kg; i.p.) for 7 days to genta-treated rats; attenuated genta increased level of microproteinuria. The administration of D-serine (80 mg/kg i.p.) + Esc (10 mg/kg; i.p.) + genta (100 mg/kg i.p.) for 7 days did not produce significant effect on microproteinuria level (Fig. 5).

Effect of various pharmacological interventions on Fe_K

The administration of genta (100 mg/kg; i.p. for 7 days) produces significant increase in Fe_K level, when compared to control rats. The administration of Esc per se (10 mg/kg; i.p. for 7 days) did not show any significant effect on Fe_K level. The administration of Esc (5 mg/kg; i.p.; 10 mg/kg; i.p.) for 7 days to genta-treated rats; attenuated genta increased level of Fe_K. The administration of D-serine (80 mg/kg i.p.) + Esc (10 mg/kg; i.p.) + genta (100 mg/kg i.p.) for 7 days did not produce significant effect on Fe_K level (Fig. 6).

Effect of various pharmacological interventions on Fe_{Na}

The administration of genta (100 mg/kg; i.p. for 7 days) produces significant increase in Fe_{Na} level, when compared to control rats. The administration of Esc per se (10 mg/kg; i.p. for 7 days) did not show any significant effect on Fe_{Na} level. The administration of Esc (5 mg/kg; i.p.; 10 mg/kg; i.p.) for 7 days to genta-treated rats; attenuated genta increased level of Fe_{Na}. The administration of D-serine (80 mg/kg i.p.) + Esc (10 mg/kg; i.p.) + genta (100 mg/kg i.p.) for 7 days did not produce significant effect on Fe_{Na} level (Fig. 7).

Effect of various pharmacological interventions on TBARS

The administration of genta (100 mg/kg; i.p. for 7 days) produces significant increase in TBARS level, when compared to control rats. The administration of Esc per se (10 mg/kg; i.p. for 7 days) did not show any significant effect on TBARS level. The administration of Esc (5 mg/kg; i.p.; 10 mg/kg; i.p.) for 7 days to genta-treated rats; attenuated genta-increased level of TBARS. The administration of D-serine (80 mg/kg i.p.) + Esc (10 mg/kg; i.p.) + genta (100 mg/kg i.p.) for 7 days did not produce significant effect on TBARS level (Fig. 8).

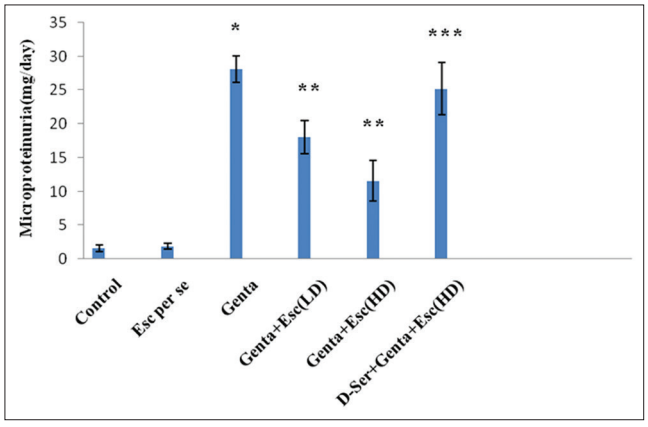

Fig. 5: Effect of various pharmacological interventions on microproteinuria. Esculin (LD): Esculetin low dose (5 mg/kg, i.p.), Esculin (HD): Esculetin high dose (10 mg/kg, i.p.), values are expressed as mean ± standard error of mean (STDEV), n=6, one-Way ANOVA followed by Tukey's multiple range test. *p<0.005 as compared to control. **p<0.005 as compared to Gentamicin-treated group. ***p<0.005 as compared to esculetin high-dose treated group

Effect of various pharmacological interventions on GSH level

The administration of genta (100 mg/kg; i.p. for 7 days) produces significant decrease in GSH level, when compared to control rats. The administration of Esc per se (10 mg/kg; i.p. for 7 days) did not show any significant effect on GSH level. The administration of Esc (5 mg/kg; i.p.; 10 mg/kg; i.p.) for 7 days to genta-treated rats; attenuated genta decreased level of GSH level. The administration of D-serine (80 mg/kg i.p.) + Esc (10 mg/kg; i.p.) + genta (100 mg/kg i.p.) for 7 days did not produce significant effect on GSH level (Fig. 9).

Effect of various pharmacological interventions on MPO activity

The administration of genta (100 mg/kg; i.p. for 7 days) and D-serine (80 mg/kg; i.p. for 7 days) produces significant increase in MPO

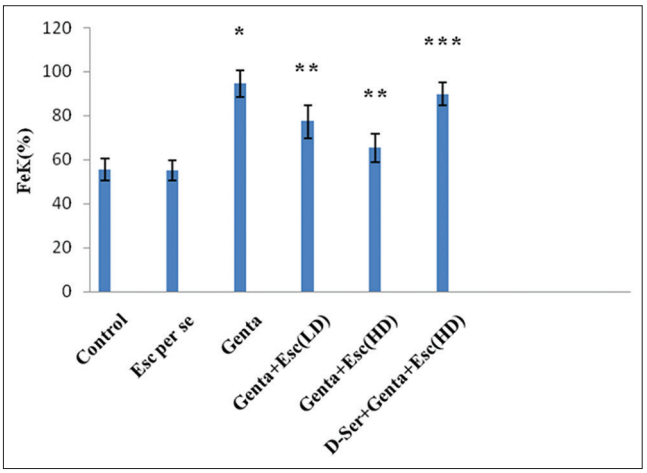

Fig. 6: Effect of various pharmacological interventions fractional excretion of potassium (Fe_K). Esculin (LD): Esculetin low dose (5 mg/kg, i.p.), Esculin (HD): Esculetin high dose (10 mg/kg, i.p.), values are expressed as mean ± standard error of mean (STDEV), n=6, one-Way ANOVA followed by Tukey's multiple range test. *p<0.005 as compared to control. **p<0.005 as compared to Gentamicin-treated group. ***p<0.005 as compared to esculetin high-dose treated group

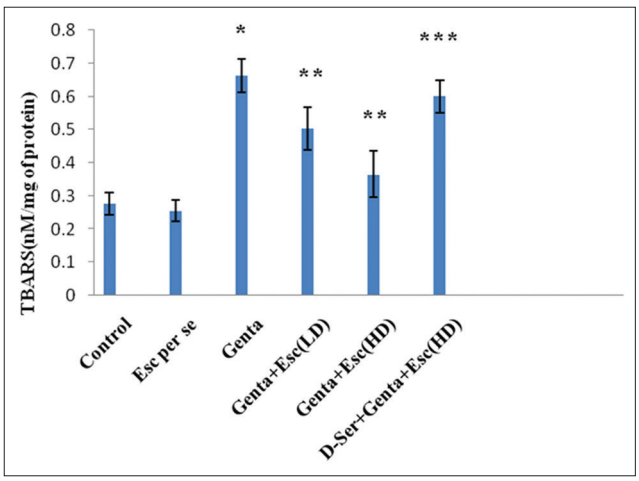

Fig. 7: Effect of various pharmacological interventions on fractional excretion of sodium (Fe_{Na}). Esculin (LD): Esculetin low dose (5 mg/kg, i.p.), Esculin (HD): Esculetin high dose (10 mg/kg, i.p.), values are expressed as mean ± standard error of mean (STDEV), n=6, one-Way ANOVA followed by Tukey's multiple range test. *p<0.005 as compared to control. **p<0.005 as compared to Gentamicin-treated group. ***p<0.005 as compared to esculetin high-dose treated group

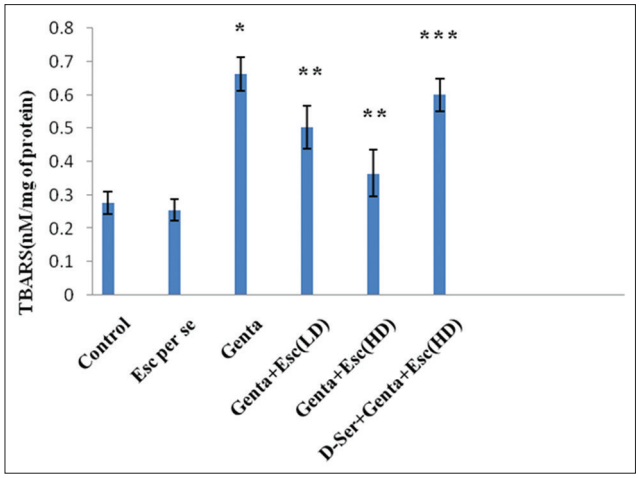

Fig. 8: Effect of various pharmacological interventions on thiobarbituric acid reactive oxygen substances. Esculin (LD): Esculetin low dose (5 mg/kg, i.p.), Esculin (HD): Esculetin high dose (10 mg/kg, i.p.), values are expressed as mean ± standard error of mean (STDEV), n=6, one-Way ANOVA followed by Tukey's multiple range test. *p<0.005 as compared to control. **p<0.005 as compared to Gentamicin-treated group. ***p<0.005 as compared to esculetin high-dose treated group

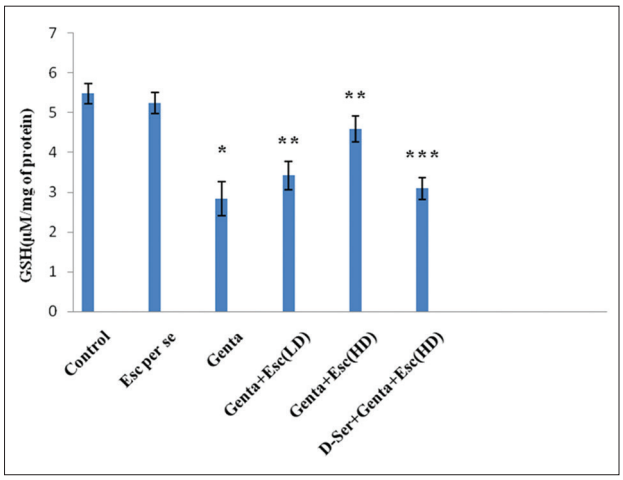

Fig. 9: Effect of varoius pharmacological interventions on (reduced glutathione). Esculin (LD): Esculetin low dose (5 mg/kg, i.p.), Esculin (HD): Esculetin high dose (10 mg/kg, i.p.), values are expressed as mean ± standard error of mean (STDEV), n=6, one-Way ANOVA followed by Tukey's multiple range test. *p<0.005 as compared to control. **p<0.005 as compared to Gentamicin-treated group. ***p<0.005 as compared to esculetin high-dose treated group

level, when compared to control rats. The administration of D-serine (80 mg/kg; i.p.) + genta (100 mg/kg; i.p.) for 7 days also produces significant increase in MPO level when compared to control rats. The administration of Esc per se (10 mg/kg; i.p. for 7 days) did not show any significant effect on MPO level. The administration of Esc (5 mg/kg; i.p.; 10 mg/kg; i.p.) for 7 days to genta-treated rats; attenuated genta-increased level of MPO. The administration of D-serine (80 mg/kg i.p.) + Esc (10 mg/kg; i.p.) + genta (100 mg/kg i.p.) for 7 days did not produce significant effect on MPO level (Fig. 10).

Effect of various pharmacological interventions on SAG level

The administration of genta (100 mg/kg; i.p. for 7 days) produces significant increase in SAG level, when compared to control rats. The

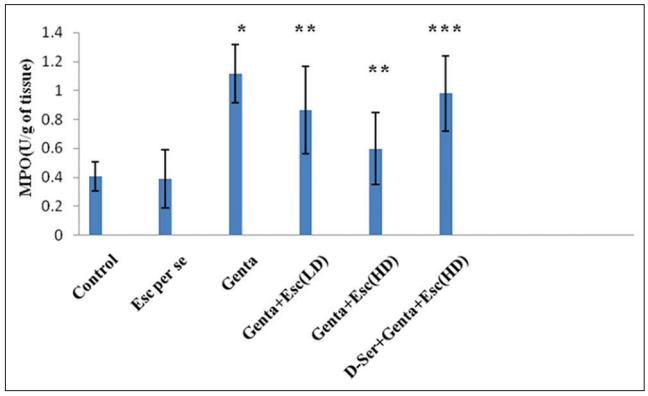

Fig. 10: Effect of various pharmacological interventions on myeloperoxidase activity. Esculin (LD): Esculetin low dose (5 mg/kg, i.p.), Esculin (HD): Esculetin high dose (10 mg/kg, i.p.), values are expressed as mean ± standard error of mean (STDEV), n=6, one-Way ANOVA followed by Tukey's multiple range test. *p<0.005 as compared to control. **p<0.005 as compared to Gentamicin-treated group. ***p<0.005 as compared to esculetin high-dose treated group

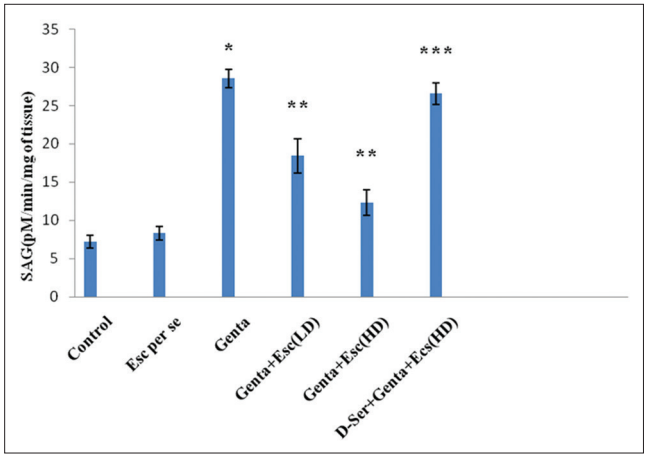

Fig. 11: Effect of various pharmacological interventions on superoxide anion generation. Genta - Gentamicin (100 mg/kg, i.p.), Esculin (LD): Esculetin low dose (5 mg/kg, i.p.), Esculin (HD): Esculetin high dose (10 mg/kg, i.p.), values are expressed as mean ± standard error of mean (STDEV), n=6, one-Way ANOVA followed by Tukey's multiple range test. *p<0.005 as compared to control. **p<0.005 as compared to Gentamicin-treated group. ***p<0.005 as compared to esculetin high-dose treated group

administration of Esc per se (10 mg/kg; i.p. for 7 days) did not show any significant effect on SAG level. The administration of Esc (5 mg/kg; i.p.; 10 mg/kg; i.p.) for 7 days to genta-treated rats; attenuated genta increased level of SAG. The administration of D-serine (80 mg/kg i.p.) + Esc (10 mg/kg; i.p.) + genta (100 mg/kg; i.p.) for 7 days did not produce significant effect on SAG level (Fig. 11).

DISCUSSION

This study is based on ameliorative role of Esc-mediated renoprotection against genta-induced nephrotoxicity and possible involvement of NMDAR. genta is an antibiotic that exhibits a broad spectrum of activity and is particularly valuable in severe sepsis. Its use is, however, restricted because of the development of ototoxicity and nephrotoxicity [25,26]. Nephrotoxicity has been related to a selective accumulation of genta in the renal cortex and resulting morphologic lesions of proximal tubules [26,27] genta is associated with an induction of tubular necrosis, epithelial oedema of proximal tubules, cellular desquamation,

tubular fibrosis, glomerular congestion, perivascular edema and inflammation, which ultimately show the way to renal dysfunction [28], genta induces reactive oxygen free radicals mediated apoptosis [27] and endothelin-1, TXA_2 and PAF mediated mesangial cells contraction that results in decreased GFR [29]. The exact mechanism underlying nephrotoxic behavior of genta is still not known.

In the present study, genta (100 mg/kg; i.p) was administred to Wistar rats for 7 days. After 7 days of administration of genta resulted in considerably renal damage. Histopathological examination of genta-treated rats witnessed marked morphological changes including proximal and distal tubular damage, interstitial edema seen by sepration of tubular as well as congestion of capillaries, tubular show epithelial degeneration and severe necrosis as compared to control group (Fig. 1). Renal functions are important biomarkers to access nephrotoxicity. Genta-treated group showed significant changes in renal functions such as there was decreased level of CrCl, elevates BUN level, uric acid level, microproteinurea, fractional excretion of sodium, and potassium. The coadministration of Esc (5 mg/kg; 10 mg/kg) and genta showed increased level of CrCl and decreased elevated blood urera nitrogen and uric acid level and changes in fractional excretion of sodium and potassium levels where as combined effect of D-serine + genta + Escultin did not showed any significant changes in histopathological (Fig. 1) and renal parameters.

Genta-treated group also showed elevation in TBARS, SAG, MPO and reducing GSH levels suggesting oxidative stress and inflammation in genta-induced injury. The combined administration of Esc and genta significantly increased the activity of GSH and reduced elevated level of TBARS, SAG and MPO activity indicating that Esc has antioxidant activity by decreasing oxidative stress and has anti-inflammatory activity. However, combined effect of D-serine + genta + escultin abolished the above effects.

Esc (6,7-dihydroxycoumarin) has been reported to possesses NMDAR antagonist activity. NMDAR activation leads to opening of an ion channel that is selective for cations, resulting in the influx of Na^+ and Ca^{2+} ions and efflux of K^+ ions [30]. Excessive Ca^{2+} influx through NMDAR - coupled channels have been linked to cell death [31,32] and D-serine (D-Ser) used as NMDAR agonist which act at "glycine site" of NR1 subunit to mediate ion influx [17]. NMDAR subunit expression is increased in short-term genta animals and thus mediates renal damage [8]. Therefore, D-serine + genta + escultin did not show any significant results as D - serine abolished the effect of Esc.

Esc also reported to possesses analgesic, anti-inflammatory activity [33] by blocking NF-kB activation, by inhibiting the generation of ROS, by inhibiting LPS-induced nitric oxide and prostaglandin E2 production. It also significantly suppresses the production of inflammatory cytokines, including tumor necrosis factor-α and interleukin-1β [34] and in cocultured macrophages and adipocytes through up-regulation of heme oxygenase-1 (HO-1) [35]. Esc efficiently attenuated the oxidative stress induced cell damage via its antioxidant properties such as DPPH radical scavenging, hydroxyl radical scavenging, and intracellular ROS scavenging activities, also resulted in the protection of cells from lipid peroxidation, protein carbonyl, and DNA damage induced by H_2O_2 [12,36]. The renoprotective role of Esc against streptozotocin-induced diabetic rats [15] and cisplatin-induced nephrotoxicity [37] has been attributed to its antioxidant activity and modulation of p53/Akt/phosphatase and tensin homolog expression, respectively. Therefore with support from literature and data in hand, it may be suggested that Esc has shown amleorative effect in genta-induced nephrotoxicity.

The role of Esc in genta-induced nephrotoxicity and involvement of NMDAR was explored for the first time in this study. The administration of genta (100 mg/kg) for 7 days resulted in significant renal damage as indicated by decreased CrCl and increased levels of BUN, uric acid, Fe_{Na}, Fe_K, microproteinuria and significant rise in oxidative stress parameters TBARS, SAG and MPO along with depletion of GSH, an established indicator of antioxidant defense of the body. The treatment with Esc (5 mg/kg; 10 mg/kg) significantly attenuated genta-induced renal damage.

Hence, on the basis of above discussion, it is concluded that the treatment with Esc protects kidneys from genta-induced oxidative stress and dysfunction. Moreover, the administration of D-serine abolished the effect of Esc and thus indicating that NMDAR may be involved in the genta-induced nephrotoxicity.

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