RESEARCH ARTICLE

Nephroprotective Effects of Polyherbal Formulation on Gentamicin-induced Toxicity in Wistar Rats

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Abstract

Effect of a polyherbal formulation (polyherbal alcoholic extract; PAE) prepared from Withania somnifera (root), Aegle marmelos (leaves), Tribulus terrestris (fruit) was studied on gentamicin-induced nephrotoxicity. Thirty Wistar rats were equally divided into five groups; group I (control), group II (gentamicin alone), group III (prophylactic), group IV (curative) and group V (formulation alone). Gentamicin caused signs of depression, dullness, polyurea, restlessness, reduced body weight, and reduced water and feed intake. These toxicity signs were recovered in the PAE pre-treated prophylactic group; however, in the PAE-treated therapeutic group, some mild toxicity signs were noticed and subsided gradually. Gentamicin increased relative kidney weight but in groups III and IV animals, no significant effect was observed. The Hb, PCV, TEC, TLC and MCV of group II animals revealed a significant decrease, whereas; BUN, creatinine, AST, ALT, ALP, total protein, albumin and globulin were significantly increased. The PAE treatment caused significant recovery in these hematobiochemical parameters in groups III and IV. Gentamicin caused histopathological alterations in kidney represented by focal renal degenerative and necrotic changes, with intratubular hyaline cast. In PAE-treated groups III and IV, minimal histopathological alterations were seen. In conclusion, PAE showed significant protective and moderate therapeutic potential against gentamicin-induced nephrotoxicity in Wistar rats.

Keywords: Gentamicin, Nephrotoxicity, PAE, Wistar rats, Withania.

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Introduction

Gentamicin, a bactericidal antibiotic of the aminoglycoside class, is effective against Gram-negative bacterial infections (Balakumar *et al.*, 2010). The drug is known to cause nephrotoxicity in longer course of use. The nephrotoxicity caused by aminoglycoside class characterised by a slow rise in serum creatinine, blood urea nitrogen, and severe proximal renal tubular necrosis that end by renal failure, and hypo-osmolar urinary output. (Al-Majed *et al.*, 2002). In a study, more than 30% of patients treated with gentamicin up to 7 days showed signs of nephrotoxicity, edema and inflammatory alterations in proximal tubular epithelial cells, resulting in an increase in kidney weight (Jeyanthi and Subramanian, 2009). The drug generates reactive oxygen species (ROS) that change in the number and size of lysosomes, and mitochondrial vacuolization, followed by functional alterations such as DNA damage, and apoptosis, acute tubular necrosis, resulting in renal impairment (Abdel-Raheem *et al.*, 2009; Yadav *et al.*, 2017).

Nearly 80% of the world's population depends on herbal remedies for their healthcare needs and plants account for more than 30% of pharmaceutical formulations (Shinwari and Khan, 1998). *Withania somnifera*, also known as Ashwagandha, Indian Ginseng or Winter Cherry, has been used in Indian ayurvedic and traditional medicine from antiquity. *W. somnifera* has multiferous properties viz. antioxidant, anti-inflammatory, mind-boosting, rejuvenating,

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anti-stress, anti-tumor, and immune-enhancing (Umadevi *et al*., 2012). *Aegle marmelos*, Bael (Hindi) a member of the Rutaceae family, is widely distributed in the plains and hills of the Indian subcontinent and Southeast Asian countries. The various parts of Bael are used to treat anemia, diabetes, asthma, fever, snake venom antidote, jaundice, chronic diarrhea, dysentery and heart disorders (Bhalerao and Ghadigaonkar, 2018). The leaves have analgesic, cardiotonic, antioxidant, antifungal and nephroprotective properties (Kore *et al*., 2011). *Tribulus terrestris* (Zygophyllaceae), known as Gokharu, is a well known valuable herb for its use in Indian traditional medicine. It has numerous pharmacological properties like analgesic, cardiotonic, astringent, lithotriptic, aphrodisiac, anti-urolithic, hypolipidemic, stomachic, diuretic, hepatoprotective, anti-diabetic, absorption enhancing, hypolipidemic, antihypertensive apoptosis, anti-oxidant, vasodilator properties and urinary anti-infective (Chhatre *et al.,* 2014; Shalaby and Hammouda, 2014; El-Shaibany *et al*., 2015). The fruit T. terrestris removes debris from the urine and stones from the bladder (Kavitha and Jagadeesan, 2006).

W. somnifera, A. marmelos and *T. terrestris* have been used in Indian traditional medicine for different kidney problems. It has been observed that polyherbal formulations are more often used than individual herbs for their better results, possibly due to synergistic actions. There is scarce information available on use of polyherbal formulation containing above three mentioned herbs for their potential nephroprotective effect against renal toxicities; therefore, the present study was conducted to evaluate a polyherbal formulation of these herbs against gentamicin-induced nephrotoxicity in Wistar rats.

Materials and Methods

Preparation of Polyherbal Alcoholic Extract

The roots of W. somnifera, leaves of A. marmelos were collected from the campus of the PGIVAS, Akola, and fruits of *T. terrestris* were obtained from local suppliers and authenticated by an expert taxonomist before use. The roots, leaves and fruits were washed with clean water and dried in the shade for some days. The powders of *W. somnifera* root, *A. marmelos* leaves and *T. terrestris* fruit were taken in equal proportion (1:1:1) to prepare polyherbal formulation. The freshly prepared powder was soaked in 70% ethanolic solution in cotton plugged flask and kept on orbital shaker at 150 rpm at room temperature for 24 h. The flask's content was initially filtered through a muslin cloth and then through Whatman No. 1 filter paper. The final filtrate was transferred to a large petri dish for complete evaporation of solvent at room temperature. The final remaining extract obtained was kept in a desiccator until used.

Table 1: Effect of PAE on relative kidney weight in control and gentamicin treated rats

Group	Treatment	Kidney Weight (g)	Relative Kidney Weight
I	Control	1.6017 ± 0.11a	0.734 ± 0.020a
II	Gentamicin	2.1467 ± 0.09b	1.394 ± 0.098b
III	PAE + Gentamicin (Protective group)	1.6550 ± 0.06a	0.775 ± 0.032a
IV	Gentamicin + PAE (Curative group)	1.7867 ± 0.04a	0.861 ± 0.017a
V	PAE	1.6217 ± 0.10a	0.740 ± 0.027a

Values indicate mean ± S.E.
Means bearing different superscript in the same column differ significantly (P≤0.05)

Experimental Design

The experiment was carried out in 30 Wistar rats after approval from Institutional Animal Ethics Committee of the Post Graduate Institute of Veterinary and Animal Sciences, Akola (IAEC approval No. 312/01/2000/21).

Thirty Wistar rats of either sex were divided randomly into five groups, each group containing six rats of either sex. Group I rats were control, and group II received gentamicin @ 80 mg/kg b.wt. i.p. for 8 days, group III (prophylactic) rats received PAE @ 500 mg/kg b.wt. orally for 21 days along with gentamicin @ 80 mg/kg b.wt. from day 14 to day 21, group IV (curative) rats received gentamicin @ 80 mg/kg b.wt. for 8 days along with PAE @ 500 mg/kg b.wt. from day 9 to day 21 and group V rats received only PAE @ 500 mg/kg b.wt. for 21 days.

General Clinical Observations

Rats of each group were observed four to five times a day for the appearance of any clinical signs and symptoms or mortality during the entire experimental period.

Relative Kidney Weight

Rats fasted overnight before sacrifice and the live body weight (g) was recorded at the end of the experiment before being euthanized. The kidneys were isolated and weighed to calculate the relative kidney weight of each animal.

All the rats were sacrificed on 22nd day by cervical dislocation and blood and renal tissue were collected for the estimation of Hb, PCV, TEC, TLC, MCV, MCH, BUN, creatinine, AST, ALT, ALP, total protein, albumin, globulin and A/G ratio and histopathological study following routine method in practice.

Statistical Analysis

The statistical data were expressed as Mean ± S.E. and analyzed by one-way analysis of variance followed by Tukey's post hoc tests in IBM SPSS Statistics, Version 22 Application/ Software (2013). P-value of less than 0.05 was considered significant.

Results and Discussion

Animal in group II treated with gentamicin exhibited toxicity signs such as depression, dullness, polyurea, restlessness, and

reduced water and feed intake. Khalid *et al.* (2003) observed similar clinical signs in animals treated with gentamicin. Similar mild symptoms also seen in group IV. However, these symptoms subsided gradually towards the end of the experiment. The group III animals that received PAE along with gentamicin showed no toxicity symptoms during the experiment.

The average and relative kidney weight of gentamicin alone treated group was found to be significantly ($p<0.05$) increased in the control group. Pre-treatment with PAE in group III rats effectively ($p<0.05$) protected the increase in mean kidney weight and relative kidney weight. The PAE treatment in group IV rats also showed a significant ($p<0.05$) curative effect on the reduction in relative kidney weight. Similar to the present findings Govindappa *et al.* (2019) and Kilany *et al.* (2020) reported a significant ($P<0.05$) increase in relative kidney weight and average kidney weight in gentamicin toxicity.

The mean values of Hb, PCV, TEC, TLC and MCV of gentamicin alone treated rats decreased significantly ($P<0.05$). The PAE treated group III and IV shows improvement in Hb, PCV, TEC, TLC and MCV values as compared to the gentamicin-alone treated group (Group II). The decrease in hemoglobin and TEC levels was primarily due to anemia caused by a lack of erythropoietin hormone following gentamicin-induced kidney injury (Naeshiro *et al.,* 1997).

A significant ($p<0.05$) increase in BUN and creatinine level was observed in gentamicin-treated rats compared to the control group. The values of BUN and creatinine in PAE treatment groups III and IV were significantly ($p<0.05$) recovered from the gentamicin-treated group. Pre-treatment with PAE in group III prevented the alteration in BUN and

Table 2: Effect of PAE on hematological values in control and gentamicin treated rats

Group	Treatment	Hb	PCV	TEC	TLC	MCV	MCH
I	Control	13.10 ± 0.28b	41.86 ± 0.64b	7.60 ± 0.23b	9.80 ± 0.33c	55.23 ± 1.50b	17.27 ± 0.41
II	Gentamicin	10.90 ± 0.37a	28.88 ± 1.75a	6.39 ± 0.24a	6.82 ± 0.22a	44.61 ± 1.29a	17.15 ± 0.71
III	PAE + Gentamicin (Protective group)	12.37 ± 0.41b	39.88 ± 1.28b	7.07 ± 0.26ab	7.83 ± 0.40ab	56.79 ± 2.78b	17.64 ± 0.97
IV	Gentamicin + PAE (Curative group)	12.13 ± 0.25ab	37.66 ± 1.20b	6.83 ± 0.30ab	7.66 ± 0.52a	55.70 ± 2.96b	17.98 ± 1.03
V	PAE	13.23 ± 0.35b	40.61 ± 1.84b	7.57 ± 0.26b	9.48 ± 0.53bc	53.89 ± 2.80ab	17.62 ± 0.88

Values indicate mean ± S.E.
Means bearing different superscript in the same column differ significantly (P≤0.05)

Table 3: Effect of PAE on biochemical parameters in control and gentamicin treated rats

Group	Treatment	BUN	Creatinine	AST	ALT	ALP
I	Control	18.77 ± 0.75a	0.62 ± 0.03a	50.68 ± 1.49a	23.06 ± 1.55a	74.70 ± 1.59a
II	Gentamicin	66.15 ± 3.10b	2.12 ± 0.13b	109.33 ± 2.47d	39.62 ± 1.89b	141.77 ± 1.49c
III	PAE + Gentamicin (Protective group)	19.74 ± 0.96a	0.72 ± 0.04a	71.92 ± 4.30b	24.88 ± 1.87a	80.48 ± 2.12ab
IV	Gentamicin + PAE (Curative group)	21.00 ± 0.46a	0.87 ± 0.13a	85.95 ± 2.14c	29.98 ± 0.83a	85.58 ± 2.92b
V	PAE	19.31 ± 0.55a	0.68 ± 0.05a	55.65 ± 4.90a	24.50 ± 2.15a	75.92 ± 1.80a

Values indicate mean ± S.E.
Means bearing different superscript in the same column differ significantly (P≤0.05)

Table 4: Effect of PAE on serum total protein, albumin, globulin, and A/G ratio in control and gentamicin-treated rats

Group	Treatment	Total protein (g/dL)	Albumin	Globulin (g/dL)	A/G ratio (g/dL)
I	Control	7.04 ± 0.21a	4.06 ± 0.09a	2.98 ± 0.18a	1.39 ± 0.08
II	Gentamicin	9.09 ± 0.24b	4.71 ± 0.13b	4.45 ± 0.29b	1.09 ± 0.10
III	PAE + Gentamicin (Protective group)	7.40 ± 0.29a	4.17 ± 0.12ab	3.23 ± 0.25a	1.34 ± 0.13
IV	Gentamicin + PAE (Curative group)	7.55 ± 0.16a	4.23 ± 0.19ab	3.32 ± 0.15a	1.29 ± 0.10
V	PAE	7.11 ± 0.28a	4.13 ± 0.12a	2.98 ± 0.27a	1.45 ± 0.16

Values indicate mean ± S.E., Significance P≤0.05

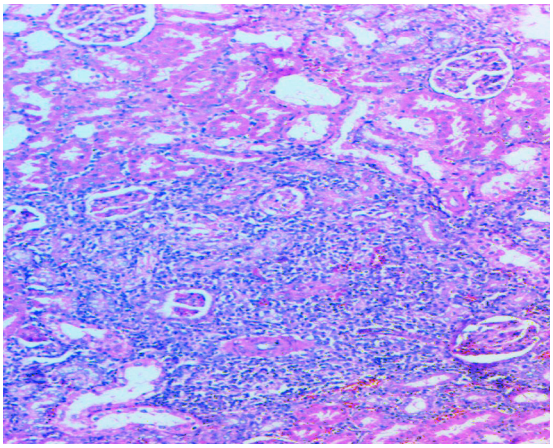

Fig. 1 Diffuse interstitial mononuclear cells infiltrations with hydropic changes in the renal tubular cells

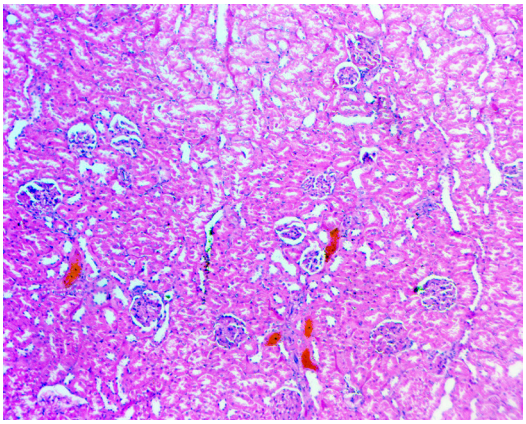

Fig. 2 The cells infiltration is intratubular with degeneration of some others tubules

creatinine in gentamicin-treated rats. Similarly, the curative treatment of PAE in group IV showed retrieval in BUN and creatinine compared to the gentamicin-treated group. In a related study, Phillips *et al.* (2006) demonstrated that *T. terrestris* extract improved BUN and creatinine levels by inhibiting the activity of the angiotensin-converting enzyme, stimulating the release of nitric oxide from vascular endothelium and hyperpolarizing vascular smooth muscles resulting in vasodilation. Govindappa *et al*. (2019) reported that W. somnifera significantly increased glutathione and superoxide dismutase antioxidant activities in Wistar rats to protect renal tissue damage from gentamicin. Bhalerao and Ghadigaonkar, (2018) reported that A. marmelos leaf extract effectively improved BUN and creatinine levels in gentamicin-induced nephrotoxicity.

The mean AST, ALT and ALP values of group II gentamicin intoxicated rats were significantly ($p<0.05$) increased as compared to the control group. The serum AST, ALT and ALP values in group III and group IV indicated a significant recovery with the PAE treatment and in protective group there was no significant increase in ALT and ALP, although, AST activity was quite low as compared to control but still significant increase in the activity was noted. In the present study, liver damage observed in gentamicin-treated rats is correlated with their increased levels of serum AST, ALT and ALP. Significantly reduced serum AST and ALT values, which returns to almost normal levels in the PAE pre-treated gentamicin-intoxicated rats, appreciate PAE's ameliorative effects.

The mean total protein, albumin, and globulin values of gentamicin-treated animals were significantly ($p<0.05$) higher than the control and different treatment groups. Mean values of total protein, albumin and globulin of group III and IV were in a normal range and comparable to the control group.

In the present study, gentamicin @ 80 mg/kg b.wt. significantly elevated the mean values of BUN, creatinine, AST, ALT, ALP, total protein, albumin and globulin. Similar to the results of the present finding the alterations in biochemical parameters in gentamicin toxicity were reported by various investigators (Lopez-Giacoman and Madero, 2015; Abuelezz *et al.,* 2016;; Teo and Endre, 2017; Govindappa *et al.,* 2019). Increased levels of ALT and AST in gentamicin-induced liver injury indicate cellular leakage and loss of membrane integrity of liver cells. The animal treated with PAE reversed the increased levels of ALT and AST, which might be caused by plasma membrane stabilization and the repair of gentamicin-induced hepatic cell damage.

The histological sections of the kidney of the gentamicin-treated group showed mild to moderate degree of focal tubular necrosis and presence of hyaline cast in the tubules, peritubular mononuclear cell infiltration, tubular degenerative changes, venous congestion and intra- and inter-tubular hemorrhages (Fig 1). From the PAE treated rats of group III and group IV kidney sections showed mild histopathological alterations (Fig 2). In agreement with the present study, Kalita *et al.* (2017), and Rashid *et al.* (2021) found similar histopathological lesions in gentamicin intoxicated rats.

CONCLUSION

In conclusion, polyherbal formulation prepared from alcoholic extracts of *W. somnifera* (root), *A. marmelos* (leaves), *T. terrestris* fruit and shows significant ameliorative as well as moderate curative effects on gentamicin-induced nephrotoxicity toxicity in Wistar rats.

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