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Influence of Ageless Liquid With or Without Piperine on Gentamicin-Induced Nephrotoxicity In Wistar Rats.

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ABSTRACT

To study possible protective role of ageless liquid on gentamicin-induced nephrotoxicity in in Sprague-Dawley rats. Nephrotoxicity was induced by intraperitoneal injection gentamicin 100 mg/kg/day i.p. to adult Sprague-Dawley rats weighing 1000–250 g. Ageless liquid in three different doses were given for 8 days. On 9th day, blood sample was taken and serum was obtained to assess the levels of urea and creatinine. Thereafter, all the rats were sacrificed by overdose of thiopentone sodium. Following immediate excision of left kidney, it was washed with ice-cold saline to remove as much blood as possible. Renal cortical tissues were separated into two parts for biochemical analysis and light microscopic examination. The gentamicin treated rats have shown a significant increase in MDA, creatinine, urea and a significant decrease in GSH, SOD, catalase and ATP level compared to control rats suggesting the renal damage. The different doses of ageless liquid with piperine and without piperine significantly reversed the renal damage done by gentamicin. The nephroprotective potential was found to be better for ageless liquid with piperine in comparison with ageless liquid without piperine.

Keywords: Ageless liquid, piperine, nephrotoxicity, gentamicin.

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INTRODUCTION

Ageless liquid consists of benfotiamine, cyanocobalamin, pyridoxamine and resveratrol. Resveratrol (3,5,4'-trihydroxy-trans-stilbene) is a natural compound found in red grape skin, Japanese knotweed (polygonum cuspidatum), peanuts, blueberries and some other berries. It is a powerful antioxidant produced by some plants to protect them against environmental stresses (1). Resveratrol has been promoted to have many health benefits such as protecting the heart and circulatory system, lowering cholesterol, and protecting against clots which can cause heart attacks and stroke(1). It has been reported that resveratrol attenuated the cisplatin-induced structural and functional renal changes by reducing free radicals and inhibiting inflammatory cell infiltrates(2) and has the potential to protect against kidney damage in populations exposed to arsenic(3).Further it is reported that these beneficial effects are at least partly due its antioxidant property(3). However, one of the drawback of resveratrol is its poor oral bioavailability. Piperine is an alkaloid obtained from black pepper and has been reported to be a bioavailability enhancer [4]. Further, Johnson et al has reported that piperine significantly improves the in vivo bioavailability of resveratrol [5]. In view of the piperine's effect on bioavailability we planned to see the effect of resveratrol with or without piperine on nephroprotection. Hence in the present study, we planned to investigate the nephroprotective potential of ageless liquid in gentamicin-induced nephrotoxic Wistar rats.

MATERIALS AND METHODS

Fifty four adult male Sprague-Dawley rats weighing 150–250 g were divided in to nine groups of six rats each. They were housed in polypropylene cages, maintained under standard conditions with temperature (22–240 C), 12- h light/12-h dark cycle and relative air humidity 40–60%. Rats had continuous access to standard rat pellet diet and to tap water. The animals were acclimatized to the laboratory conditions for one week before the start of the experiment. The experiment was conducted after approval from IAEC, Manipal and according to the ethical norms approved by Ministry of Social Justices and Empowerment, Government of India and Institutional Animal Ethics Committee Guidelines (Registration no. 94/1999/CPCSEA/ KMC).

All groups were treated over a period of 8 consecutive days as follows: Group I (Normal control) - Normal saline 1 ml/kg/day i.p. and 2% gum acacia 1 ml/kg/day; orally. Group II (Negative control) - Gentamicin 100 mg/kg/day i.p. and 2% gum acacia 1 ml/kg/day; orally. Group III (Positive control)- Gentamicin 100 mg/kg/day i.p. and vitamin C 45 mg/kg/day; orally. Group IV (Test 1A)- Gentamicin 100 mg/kg/day i.p. and ageless liquid with pieprine 4 mg/kg/day; orally. Group V (Test 1B)- Gentamicin 100 mg/kg/day i.p. and ageless liquid with pieprine 8 mg/kg/day; orally. Group VI (Test 1C) - Gentamicin 100 mg/kg/day i.p. and ageless liquid with pieprine 16 mg/kg/day; orally. Group VII (Test 2A) - Gentamicin 100 mg/kg/day i.p. and ageless liquid without pieprine 4 mg/kg/day; orally. Group VIII (Test 2B) - Gentamicin 100 mg/kg/day i.p. and ageless liquid without pieprine 8 mg/kg/day; orally. Group IX (Test1 2C) - Gentamicin 100 mg/kg/day i.p. and ageless liquid without pieprine 16 mg/kg/day; orally. Twenty-four hours after the administration of last doses of gentamicin and ageless liquid with or without piperine i.e. on 9th day, blood sample was taken from retro-orbital plexus following mild anaesthesia and serum was obtained to assess the levels of urea and creatinine. Thereafter, all the rats were sacrificed by overdose of thiopentone sodium. Following immediate excision of left kidney, it was washed with ice-cold saline to remove as much blood as possible. Renal cortical tissues were separated into two parts for biochemical analysis and light microscopic examination. Kidney homogenate (10% w/v) were prepared in cold 50 mM potassium phosphate buffer (pH 7.4) using a Remi homogenizer. The unbroken cells and cell debris were removed by centrifugation at 10000 rpm for 30 minutes using a cooling centrifuge (Hettich, Germany). The resulting supernatant was stored at -20˚C.

Biochemical analysis

Serum urea and creatinine levels: Serum urea and creatinine levels were determined according to the standard protocols given along with the commercially available kits.

Estimation of malondialdehyde (MDA): Lipid peroxidation as evidenced by the formation of TBARS and LH were measured by the method of Nichans and Samuelson [6]. About 0.1 ml of tissue homogenate was treated with 2 ml of 0.37% thiobarbituric acid (TBA) and 15% trichloroacetic acid (TCA) reagent and placed in a water bath for 15 min, cooled and centrifuged at room temperature for 10 min at 1000 rpm. The absorbance of the

clear supernatant was measured against a reference blank at 535 nm. The values are expressed as µmoles of malondialdehyde (MDA) formed /min/mg protein.

Estimation of reduced glutathione (GSH): The method was based on the reaction of reduced glutathione with 5, 5'-dithiobisnitrobenzoic acid (DTNB) to give a compound that absorbs at 412 nm. To the homogenate 0.1 ml of 10% TCA was added and centrifuged. About 0.1 ml of supernatant was treated with 0.5 ml of Ellman's reagent (19.8 mg of DTNB in 100 ml of 0.1% sodium nitrate) and 3.0 ml of 0.2 M phosphate buffer (pH 8.0) and the absorbance was read at 412 nm. Activity was expressed as µmoles/min/mg protein [7].

Estimation of adenosine triphosphate (ATP) content: ATP level was analyzed in accordance with the standard protocols given along with the commercially available colorimetric and ELISA kits respectively. The activity of catalase and superoxide dismutase was measured as per the standard protocols given along with the commercially available colorimetric assay kits.

Estimation of catalase (CAT): Catalase (CAT) activity was measured by the catalysis of H_2O_2 to H_2O in an incubation mixture adjusted to pH 7.0 was recorded at 254 nm. The reaction mixture contained 2.6 ml of 25 mM potassium phosphate buffer pH 7.0 and 0.1ml of tissue homogenate and was incubated at 37°C for 15 min and the reaction was started with the addition of 0.1 ml of 10 mM H_2O_2. The time required for the decrease in absorbance from 0.45 to 0.4 representing the linear portion of the curve was used for the calculation of enzyme activity. One unit of catalase activity was defined as the amount of enzyme causing the decomposition of µmol H_2O_2/mg protein/min [8]

Estimation of superoxide dismutase (SOD): SOD activity was determined by the inhibition of auto catalyzed adrenochrome formation in the presence of liver homogenate at 480 nm. The reaction mixture contained 150 µl of liver homogenate, 1.8 ml of carbonate buffer (30 mM, pH 10.2), and 0.7 ml of distilled water and 400 µl of epinephrine (45 mM). Auto oxidation of epinephrine to adrenochrome was performed in a control tube without the homogenate. Activity was expressed as µmoles/ min/mg protein [9].

Estimation of caspase-3 activity in kidney homogenate: Caspase-3 activity, catalase was measured as per the standard protocols given along with the commercially available kit.

Histological examinations

Assessment of renal tubular damage

For light microscopic examination, the right kidney were separated, rinsed in ice-cold saline and immediately fixed in 10% formalin for 24 h. Specimens were processed for paraffin embedding and 5 mm sections were prepared. Sections were stained with haematoxylin and eosin (H&E) and examined microscopically (magnification 100X). Images were captured and processed using Adobe Photoshop (version 8.0).

Data analysis

Using SPSS version 20.0, uniform data was expressed in terms of mean±standard deviation and analyzed by one way analysis of variance followed by post hoc Tukey test. P value less than 0.05 was considered as statistically significant.

RESULTS

7The gentamicin treated rats have shown a significant increase in MDA, creatinine, urea and a significant decrease in GSH, SOD, catalase and ATP level compared to control rats suggesting the renal damage (Table 1-9). The different doses of ageless liquid with piperine and without piperine significantly reversed the renal damage done by gentamicin (Table 1-9). Further, the reversal was better with ageless liquid with piperine compared to ageless liquid without piperine and it was dose related.

There was no significant change in body weight, isolated kidney weight and caspase 3 activities for the rats treated with both the formulations of Ageless liquid when compared to the gentamicin treated group (Table 1-9). Histopathological findings shows that there was significant loss of the proximal tubular brush

border, blebbing of apical membranes, tubular epithelial cell detachment from the basement membrane in gentamicin treated animals. The ageless liquid with and without piperine was able to reverse these changes. However the reversal was better with ageless liquid with piperine especially at the dose of 16 mg/kg in rats (Fig. 1-9).

Table 1: Effect on serum creatinine (mg/dl)

Groups	Dose	Mean±SD	P value	Significance
I	Normal saline 1 ml/kg/day + 2% gum acacia 1 ml/kg/day	0.48±0.23		
II	Gentamicin 100 mg/kg/day + 2% gum acacia 1 ml/kg/day	1.35±0.39	<0.001[a]	**S**
III	Gentamicin 100 mg/kg/day + Ascorbic acid 45 mg/kg/day	0.91±0.13	>0.05[b]	**NS**
IV	Gentamicin 100 mg/kg/day + Ageless liquid with piperine 4 mg/kg/day	0.84±0.15	0.030[b]	**S**
V	Gentamicin 100 mg/kg/day + Ageless liquid with piperine 8 mg/kg/day	0.6148±0.24	<0.001[b]	**S**
VI	Gentamicin 100 mg/kg/day + Ageless liquid with piperine 16 mg/kg/day	0.44±0.22	<0.001[b] 0.008[g] 0.019[h]	**S**
VII	Gentamicin 100 mg/kg/day + Ageless liquid without piperine 4 mg/kg/day	1.02±0.15	>0.05[b]	**NS**
VIII	Gentamicin 100 mg/kg/day + Ageless liquid without piperine 8 mg/kg/day	0.97±0.22	>0.05[b]	**NS**
IX	Gentamicin 100 mg/kg/day + Ageless liquid without piperine 16 mg/kg/day	0.91±0.36	>0.05[b]	**NS**

[a]compared to normal control, [b]compared to gentamicin intoxicated control, [c]compared to Ascorbic acid 45 mg/kg, [d,e,f]compared to Ageless liquid with piperine 4, 8, 16 mg/kg respectively, [g,h,i]compared to Ageless liquid without piperine 4, 8, 16 mg/kg respectively, S- Significant, NS- Not significant

Table 2: Effect on serum urea (mg/dl)

Groups	Dose	Mean±SD	P value	Significance
I	Normal saline 1 ml/kg/day + 2% gum acacia 1 ml/kg/day	40.88±4.30		
II	Gentamicin 100 mg/kg/day + 2% gum acacia 1 ml/kg/day	141.65±33.86	<0.001[a]	**S**
III	Gentamicin 100 mg/kg/day + Ascorbic acid 45 mg/kg/day	94.73±19.60	0.003[b]	**S**
IV	Gentamicin 100 mg/kg/day + Ageless liquid with piperine 4 mg/kg/day	106.33±19.90	>0.05[b]	**NS**
V	Gentamicin 100 mg/kg/day + Ageless liquid with piperine 8 mg/kg/day	77.50±19.75	<0.001[b]	**S**
VI	Gentamicin 100 mg/kg/day + Ageless liquid with piperine 16 mg/kg/day	50.50±10.33	<0.001[b] 0.007[c] <0.001[d]	**S**
VII	Gentamicin 100 mg/kg/day + Ageless liquid without piperine 4 mg/kg/day	91.87±16.17	0.002[b]	**S**
VIII	Gentamicin 100 mg/kg/day + Ageless liquid without piperine 8 mg/kg/day	82.16±13.68	<0.001[b]	**S**
IX	Gentamicin 100 mg/kg/day + Ageless liquid without piperine 16 mg/kg/day	70.58±21.14	<0.001[b]	**S**

[a]compared to normal control, [b]compared to gentamicin intoxicated control, [c]compared to Ascorbic acid 45 mg/kg, [d,e,f]compared to Ageless liquid with piperine 4, 8, 16 mg/kg respectively, [g,h,i]compared to Ageless liquid without piperine 4, 8, 16 mg/kg respectively, S- Significant, NS- Not significant

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Table 3: Effect on reduced glutathione in kidney homogenate (µmol/ml)

Groups	Dose	Mean±SD	P value	Significance
I	Normal saline 1 ml/kg/day + 2% gum acacia 1 ml/kg/day	21.37±8.27		
II	Gentamicin 100 mg/kg/day + 2% gum acacia 1 ml/kg/day	3.16±2.06	<0.001[a]	**S**
III	Gentamicin 100 mg/kg/day + Ascorbic acid 45 mg/kg/day	31.27±13.13	0.02[b]	**S**
IV	Gentamicin 100 mg/kg/day + Ageless liquid with piperine 4 mg/kg/day	57.77±15.28	<0.001[b]	**S**
V	Gentamicin 100 mg/kg/day + Ageless liquid with piperine 8 mg/kg/day	86.78±11.70	<0.001[b]	**S**
VI	Gentamicin 100 mg/kg/day + Ageless liquid with piperine 16 mg/kg/day	826.85±203.90	<0.001[b-i]	**S**
VII	Gentamicin 100 mg/kg/day + Ageless liquid without piperine 4 mg/kg/day	37.14±14.87	0.01[b]	**S**
VIII	Gentamicin 100 mg/kg/day + Ageless liquid without piperine 8 mg/kg/day	61.50±15.53	0.004[b]	**S**
IX	Gentamicin 100 mg/kg/day + Ageless liquid without piperine 16 mg/kg/day	433.92±118.77	<0.001[b-i]	**S**

[a]compared to normal control, [b]compared to gentamicin intoxicated control, [c]compared to Ascorbic acid 45 mg/kg, [d,e,f]compared to Ageless liquid with piperine 4, 8, 16 mg/kg respectively, [g,h,i]compared to Ageless liquid without piperine 4, 8, 16 mg/kg respectively, S- Significant, NS- Not significant

Table 4: Effect on malondialdehyde in kidney homogenate (nmol of MDA formed/ml)

Groups	Dose	Mean±SD	P value	Significance
I	Normal saline 1 ml/kg/day + 2% gum acacia 1 ml/kg/day	50.16±4.27		
II	Gentamicin 100 mg/kg/day + 2% gum acacia 1 l/kg/day	76.53±19.44	0.013[a]	**S**
III	Gentamicin 100 mg/kg/day + Ascorbic acid 45 mg/kg/day	60.57±11.04	>0.05[b]	**NS**
IV	Gentamicin 100 mg/kg/day + Ageless liquid with piperine 4 mg/kg/day	58.52±9.89	>0.05[b]	**NS**
V	Gentamicin 100 mg/kg/day + Ageless liquid with piperine 8 mg/kg/day	56.25±10.56	>0.05[b]	**NS**
VI	Gentamicin 100 mg/kg/day + Ageless liquid with piperine 16 mg/kg/day	34.88±15.68	<0.001[b] 0.018[c] 0.038[d] 0.007[h]	**S**
VII	Gentamicin 100 mg/kg/day + Ageless liquid without piperine 4 mg/kg/day	55.44±7.48	>0.05[b]	**NS**
VIII	Gentamicin 100 mg/kg/day + Ageless liquid without piperine 8 mg/kg/day	62.72±13.28	>0.05[b]	**NS**
IX	Gentamicin 100 mg/kg/day + Ageless liquid without piperine 16 mg/kg/day	41.50±11.38	<0.001[b]	**S**

[a]compared to normal control, [b]compared to gentamicin intoxicated control, [c]compared to Ascorbic acid 45 mg/kg, [d,e,f]compared to Ageless liquid with piperine 4, 8, 16 mg/kg respectively, [g,h,i]compared to Ageless liquid without piperine 4, 8, 16 mg/kg respectively, S- Significant, NS- Not significant

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Table 5: Effect on superoxide dismutase in kidney homogenate (U/ml)

Groups	Dose	Mean±SD	P value	Significance
I	Normal saline 1 ml/kg/day + 2% gum acacia 1 ml/kg/day	62.98±9.72		
II	Gentamicin 100 mg/kg/day + 2% gum acacia 1 ml/kg/day	20.96±17.10	0.04[a]	**S**
III	Gentamicin 100 mg/kg/day + Ascorbic acid 45 mg/kg/day	62.17±18.29	>0.05[b]	**NS**
IV	Gentamicin 100 mg/kg/day + Ageless liquid with piperine 4 mg/kg/day	81.29±13.54	0.01[b]	**S**
V	Gentamicin 100 mg/kg/day + Ageless liquid with piperine 8 mg/kg/day	114.03±26.10	<0.001[b]	**S**
VI	Gentamicin 100 mg/kg/day + Ageless liquid with piperine 16 mg/kg/day	157.25±64.50	<0.001[b,c,] 0.002[d]	**S**
VII	Gentamicin 100 mg/kg/day + Ageless liquid without piperine 4 mg/kg/day	57.66±23.80	0.02[b]	**S**
VIII	Gentamicin 100 mg/kg/day + Ageless liquid without piperine 8 mg/kg/day	70.88±30.85	0.06[b]	**S**
IX	Gentamicin 100 mg/kg/day + Ageless liquid without piperine 16 mg/kg/day	115.80±22.12	<0.001	**S**

[a]compared to normal control, [b]compared to gentamicin intoxicated control, [c]compared to Ascorbic acid 45 mg/kg, [d,e,f]compared to Ageless liquid with piperine 4, 8, 16 mg/kg respectively, [g,h,i]compared to Ageless liquid without piperine 4, 8, 16 mg/kg respectively, S- Significant, NS- Not significant

Table 6: Effect on catalase in kidney homogenate (nmol/min/ml)

Groups	Dose	Mean±SD	P value	Significance
I	Normal saline 1 ml/kg/day + 2% gum acacia 1 ml/kg/day	6733.39±1787.60		
II	Gentamicin 100 mg/kg/day + 2% gum acacia 1 ml/kg/day	3102.56±1195.76	0.02[a]	**S**
III	Gentamicin 100 mg/kg/day + Ascorbic acid 45 mg/kg/day	12300.46±1833.97	<0.001[b]	**S**
IV	Gentamicin 100 mg/kg/day + Ageless liquid with piperine 4 mg/kg/day	13206.80±1026.74	<0.001[b] 0.037[g]	**S**
V	Gentamicin 100 mg/kg/day + Ageless liquid with piperine 8 mg/kg/day	13163.30±728.81	<0.001[b] 0.034[g]	**S**
VI	Gentamicin 100 mg/kg/day + Ageless liquid with piperine 16 mg/kg/day	13075.59±1084.22	<0.001[b] 0.045[g]	**S**
VII	Gentamicin 100 mg/kg/day + Ageless liquid without piperine 4 mg/kg/day	9196.40±3796.31	<0.001[b]	**S**
VIII	Gentamicin 100 mg/kg/day + Ageless liquid without piperine 8 mg/kg/day	11892.58±713.97	<0.001[b]	**S**
IX	Gentamicin 100 mg/kg/day + Ageless liquid without piperine 16 mg/kg/day	11031.17±3406.19	<0.001[b]	**S**

[a]compared to normal control, [b]compared to gentamicin intoxicated control, [c]compared to Ascorbic acid 45 mg/kg, [d,e,f]compared to Ageless liquid with piperine 4, 8, 16 mg/kg respectively, [g,h,i]compared to Ageless liquid without piperine 4, 8, 16 mg/kg respectively, S- Significant, NS- Not significant

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Table 7: Effect on Caspase 3 in kidney homogenate (ng/ml)

Groups	Dose	Mean±SD	P value	Significance
I	Normal saline 1 ml/kg/day + 2% gum acacia 1 ml/kg/day	109.45±5.74		
II	Gentamicin 100 mg/kg/day + 2% gum acacia 1 ml/kg/day	156.67±15.42	>0.05[a]	**NS**
III	Gentamicin 100 mg/kg/day + Ascorbic acid 45 mg/kg/day	144.59±21.64	>0.05[b]	**NS**
IV	Gentamicin 100 mg/kg/day + Ageless liquid with piperine 4 mg/kg/day	120.18±34.78	>0.05[b]	**NS**
V	Gentamicin 100 mg/kg/day + Ageless liquid with piperine 8 mg/kg/day	121.87±23.89	>0.05[b]	**NS**
VI	Gentamicin 100 mg/kg/day + Ageless liquid with piperine 16 mg/kg/day	108.86±38.60	>0.05[b]	**NS**
VII	Gentamicin 100 mg/kg/day + Ageless liquid without piperine 4 mg/kg/day	131.25±24.07	>0.05[b]	**NS**
VIII	Gentamicin 100 mg/kg/day + Ageless liquid without piperine 8 mg/kg/day	114.86±25.76	>0.05[b]	**NS**
IX	Gentamicin 100 mg/kg/day + Ageless liquid without piperine 16 mg/kg/day	128.46±30.87	>0.05[b]	**NS**

[a]compared to normal control, [b]compared to gentamicin intoxicated control, [c]compared to Ascorbic acid 45 mg/kg, [d,e,f]compared to Ageless liquid with piperine 4, 8, 16 mg/kg respectively, [g,h,i]compared to Ageless liquid without piperine 4, 8, 16 mg/kg respectively, S- Significant, NS- Not significant

Table 8: Effect on Adenosine triphosphate kidney homogenate (mol/ml)

Groups	Dose	Mean±SD	P value	Significance
I	Normal saline 1 ml/kg/day + 2% gum acacia 1 ml/kg/day	0.64±0.02		
II	Gentamicin 100 mg/kg/day + 2% gum acacia 1 ml/kg/day	0.14±0.01	<0.001[a]	**S**
III	Gentamicin 100 mg/kg/day + Ascorbic acid 45 mg/kg/day	0.29±0.01	0.008[b]	**S**
IV	Gentamicin 100 mg/kg/day + Ageless liquid with piperine 4 mg/kg/day	0.42±0.05	0.002[b]	**S**
V	Gentamicin 100 mg/kg/day + Ageless liquid with piperine 8 mg/kg/day	0.64±0.04	<0.001[b]	**S**
VI	Gentamicin 100 mg/kg/day + Ageless liquid with piperine 16 mg/kg/day	1.12±0.04	<0.001[b]	**S**
VII	Gentamicin 100 mg/kg/day + Ageless liquid without piperine 4 mg/kg/day	0.39±0.08	0.004[b]	**S**
VIII	Gentamicin 100 mg/kg/day + Ageless liquid without piperine 8 mg/kg/day	0.52±0.01	<0.001[b]	**S**
IX	Gentamicin 100 mg/kg/day + Ageless liquid without piperine 16 mg/kg/day	0.98±0.02	<0.001[b]	**S**

[a]compared to normal control, [b]compared to gentamicin intoxicated control, [c]compared to Ascorbic acid 45 mg/kg, [d,e,f]compared to Ageless liquid with piperine 4, 8, 16 mg/kg respectively, [g,h,i]compared to Ageless liquid without piperine 4, 8, 16 mg/kg respectively, S- Significant, NS- Not significant

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Table 9: Effect on body weight (g)

Groups	Dose	Body weight (Mean±SD)		P value	Significance
		Basseline	Final		
I	Normal saline 1 ml/kg/day + 2% gum acacia 1 ml/kg/day	101.66±12.90	109.16±12.99		
II	Gentamicin 100 mg/kg/day + 2% gum acacia 1 ml/kg/day	189.50±4.88	153.33±8.98	>0.05[a]	**NS**
III	Gentamicin 100 mg/kg/day + Ascorbic acid 45 mg/kg/day	151.16±1.60	134.50±10.76	>0.05[b]	**NS**
IV	Gentamicin 100 mg/kg/day + Ageless liquid with piperine 4 mg/kg/day	136.00±1.67	127.50±4.18	>0.05[b]	**NS**
V	Gentamicin 100 mg/kg/day + Ageless liquid with piperine 8 mg/kg/day	145.16±9.98	136.16±4.91	>0.05[b]	**NS**
VI	Gentamicin 100 mg/kg/day + Ageless liquid with piperine 16 mg/kg/day	132.00±1.41	131.00±5.09	>0.05[b]	**NS**
VII	Gentamicin 100 mg/kg/day + Ageless liquid without piperine 4 mg/kg/day	140.50±1.51	126.50±4.72	>0.05[b]	**NS**
VIII	Gentamicin 100 mg/kg/day + Ageless liquid without piperine 8 mg/kg/day	119.50±10.19	117.66±16.78	>0.05[b]	**NS**
IX	Gentamicin 100 mg/kg/day + Ageless liquid without piperine 16 mg/kg/day	131.83±1.83	137.66±12.84	>0.05[b]	**NS**

[a]compared to normal control, [b]compared to gentamicin intoxicated control, [c]compared to Ascorbic acid 45 mg/kg, [d,e,f]compared to Ageless liquid with piperine 4, 8, 16 mg/kg respectively, [g,h,i]compared to Ageless liquid without piperine 4, 8, 16 mg/kg respectively, S- Significant, NS- Not significant

Figure 1: Section of kidney (H & E stain- 100X): Normal control rat

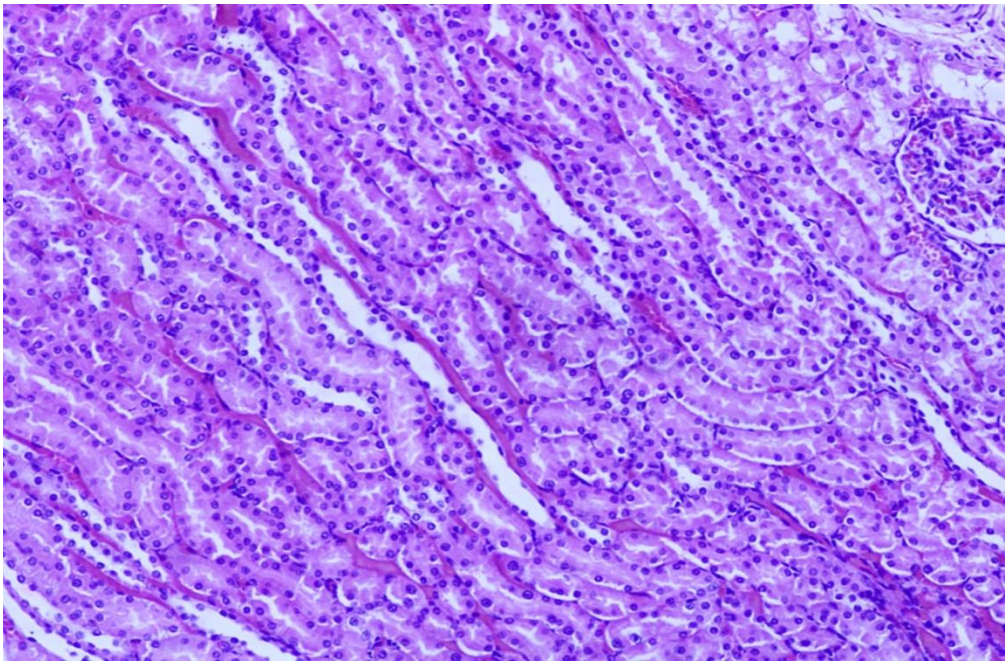

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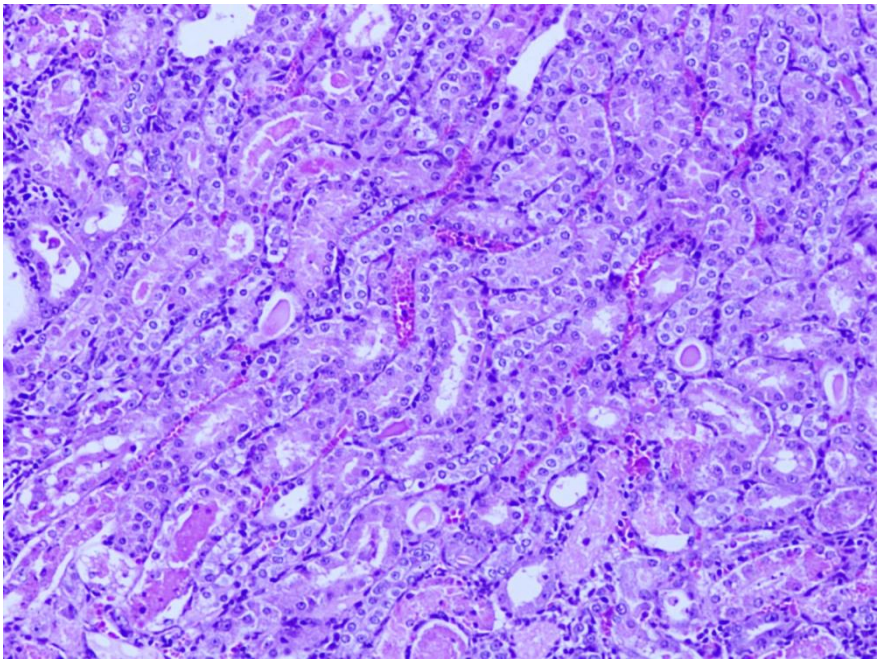
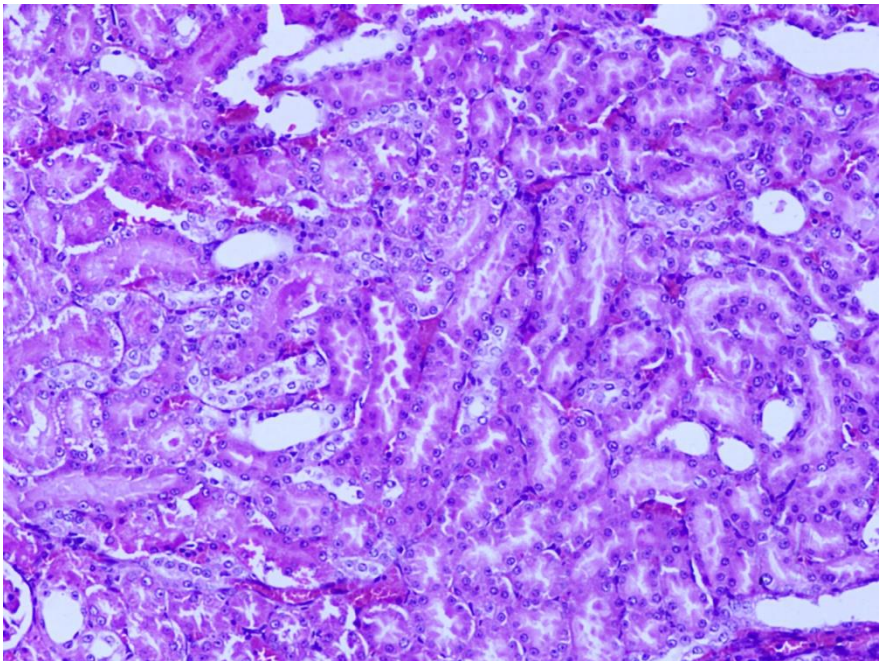
figure 2: Section of kidney (H & E stain- 100X): Gentamicin induced nephrotoxic control rat



Significant loss of the proximal tubular brush border, blebbing of apical membranes, tubular epithelial cell detachment from the basement membrane is seen.

Figure 3: Section of kidney (H & E stain- 100X): Nephrotoxic treated with Ascorbic acid 45 mg/kg/day



Loss of the proximal tubular brush border, blebbing of apical membranes, tubular epithelial cell detachment from the basement membrane by gentamycin was partly reversed by ascorbic acid.

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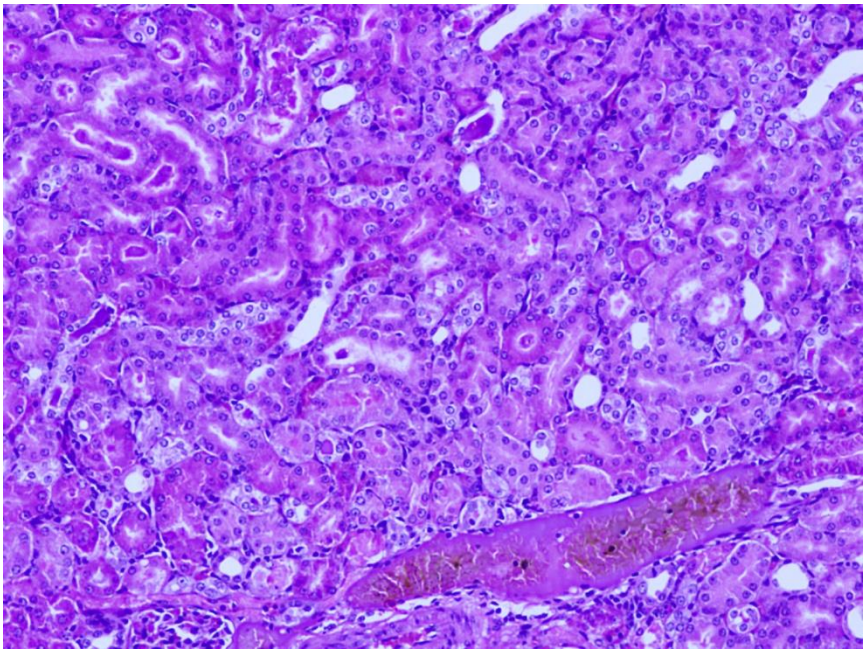
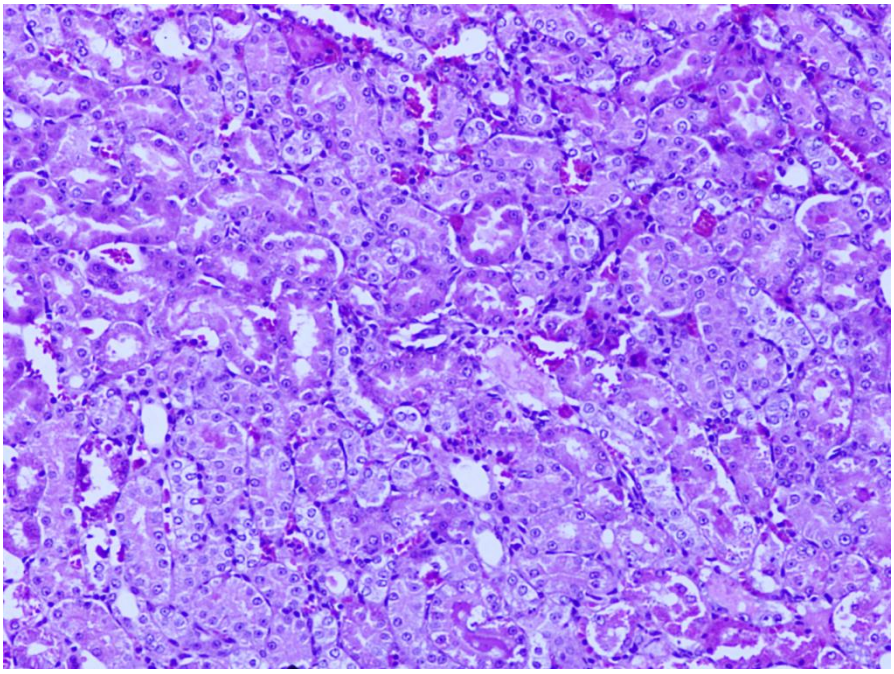
Figure 4: Section of kidney (H & E stain- 100X): Nephrotoxic treated with Ageless liquid with piperine 4 mg/kg/day



Loss of the proximal tubular brush border, blebbing of apical membranes, tubular epithelial cell detachment from the basement membrane by gentamycin was reversed by Ageless liquid with piperine. The reversal is better than ascorbic acid and Ageless liquid without piperine.

Figure 5: Section of kidney (H & E stain- 100X): Nephrotoxic treated with Ageless liquid with piperine 8 mg/kg/day



Loss of the proximal tubular brush border, blebbing of apical membranes, tubular epithelial cell detachment from the basement membrane by gentamycin was reversed by Ageless liquid with piperine. The reversal is better than ascorbic acid and Ageless liquid without piperine.

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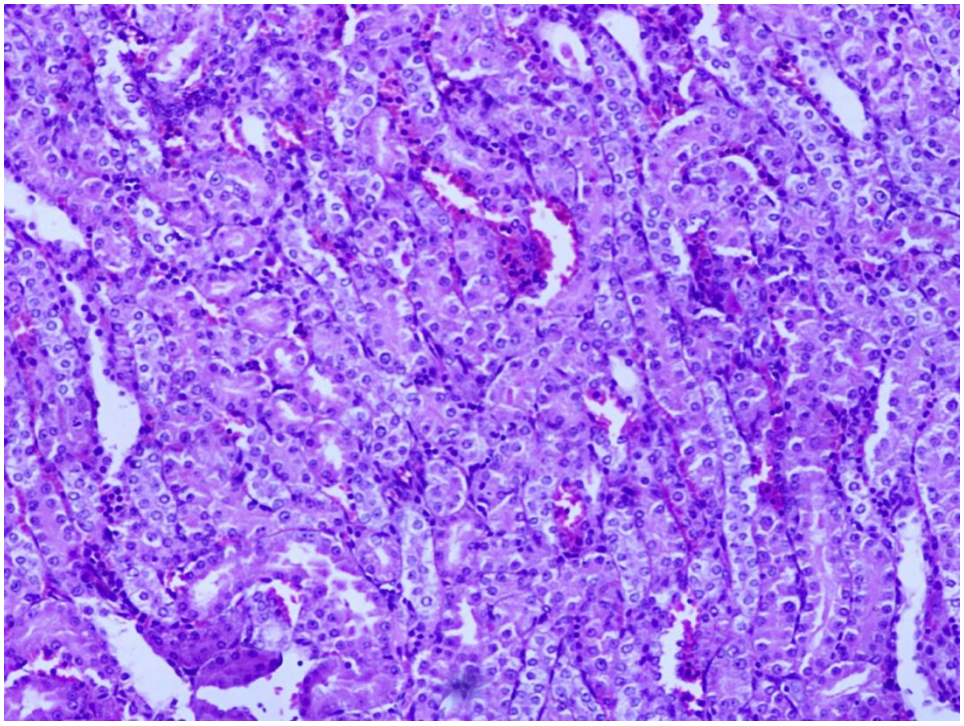
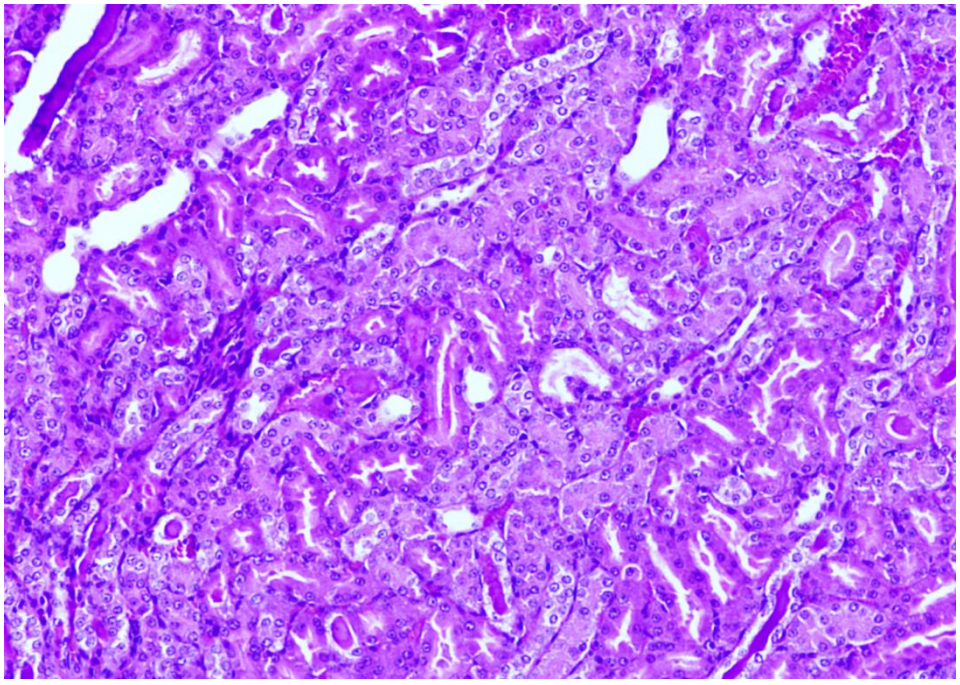
Figure 6: Section of kidney (H & E stain- 100X): Nephrotoxic treated with Ageless liquid with piperine 16 mg/kg/day



Loss of the proximal tubular brush border, blebbing of apical membranes, tubular epithelial cell detachment from the basement membrane by gentamycin was reversed by Ageless liquid with piperine. The reversal is better than ascorbic acid and Ageless liquid without piperine. At this dose level the reversal is almost close to control group.

Figure 7: Section of kidney (H & E stain- 100X): Nephrotoxic treated with Ageless liquid without piperine 4 mg/kg/day



Loss of the proximal tubular brush border, blebbing of apical membranes, tubular epithelial cell detachment from the basement membrane by gentamycin was partly reversed by Ageless liquid without piperine.

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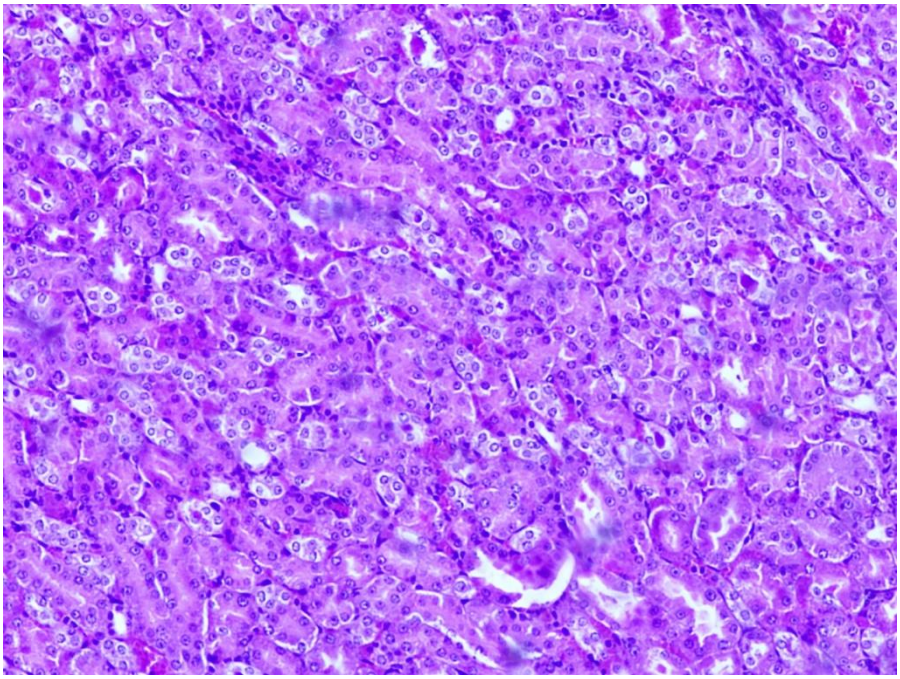
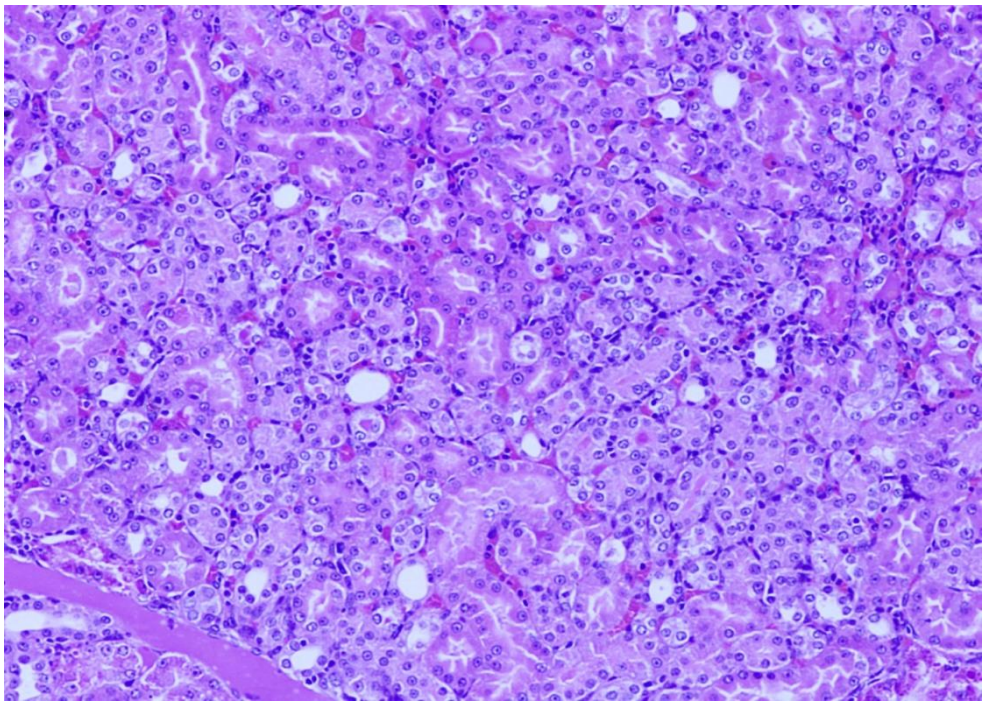
Figure 8: Section of kidney (H & E stain- 100X): Nephrotoxic treated with Ageless liquid without piperine 8 mg/kg/day



Loss of the proximal tubular brush border, blebbing of apical membranes, tubular epithelial cell detachment from the basement membrane by gentamycin was partly reversed by Ageless liquid without piperine.

Figure 9: Section of kidney (H & E stain- 100X): Nephrotoxic treated with Ageless liquid without piperine 16 mg/kg/day



Loss of the proximal tubular brush border, blebbing of apical membranes, tubular epithelial cell detachment from the basement membrane by gentamycin was partly reversed by Ageless liquid without piperine.

DISCUSSION

Data generated shows that gentamicin caused renal damage as evidenced by MDA, creatinine, urea and a significant decrease in GSH, SOD, catalase and ATP level compared to control rats which suggested involvement of free radicals in gentamicin-induced nephrotoxicity. Similar results are reported by earlier workers [Abdel]. It had been shown that gentamicin treatment causes enhanced generation of superoxide anion and hydrogen peroxide [10, 11] (Baliga et al., 1999; Walker et al., 1999) and accelerates lipid peroxidation in the renal cortex as reflected by increased MDA, an end product of lipid peroxidation, and by depression of poly unsaturated fatty acid (PUFA), which serve as substrates for free radical attack [12,13](Holub, 1987; Chance et al., 1979). Gentamicin has been shown to lead to release of iron from renal cortical mitochondria and to enhance generation of hydroxyl radical. These in vitro observations have been supported by in vivo studies in which scavengers of reactive oxygen metabolites and iron chelators have shown to be protective in gentamicin induced acute renal failure [14](Martínez-Salgado et al., 2002).

The different doses of ageless liquid with piperine and without piperine significantly reversed the renal damage done by gentamicin (Table 1-9). The nephroprotrective potential was found to be better for ageless liquid with piperine in comparison with ageless liquid without piperine and maximum therapeutic benefit was observed at 16 mg/kg of ageless liquid with piperine. This is due to the bioavailability enhancing property of piperine[4,5]. The mechanism by which piperine enhances the bioavailability is not fully known. But it has been found to inhibit human CYP3A4 and P-glycoprotein, enzymes important for the metabolism and transport of xenobiotics and metabolites[15,16]. In animal studies, piperine also inhibited other CYP 450 enzymes important for drug metabolism [17,18]. Though the observed effect on nephroprotection is due to all the component of ageless liquid, we believe that it is mainly due to resveratrol. Further, the antioxidant property of resveratrol might have protected the oxidative damage caused by gentamicin [1]. As there is species variation in response drugs the effect seen in animal studies cannot always be entirely extrapolated to humans. Hence, clinical evaluation should be performed to precisely define the role of ageless liquid with piperine in renal failure subjects.

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