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Beneficial role of Galangin on Gentamicin-induced nephrotoxicity in Rat model: A Pharmaco-biochemical Study

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Abstract: *Background:* Gentamicin is among the golden standard antibiotics used in clinical medicine. But, it has a significant damaging effect on kidney owing to the production of oxidative insults. Galangin is a valuable polyphenol and plant derived-radical scavenger. This flavonoid is reported to possess antioxidant, anti-radical and anti-cancer potential. Even so, there is no research linked to nephroprotective effect of Galangin. The current study was focused at determining the nephroprotective effect of Galangin. *Material and Methods:* A dose 50, 100 and 200 µg/ kg was administered to the animals. Gentamicin (80 mg/ kg) was utilized to develop nephrotic damage. At the end of the protocol; body mass, kidney mass, levels of Urea, TNF-α, IL-1β, SOD, Catalase, GSH, GPx at renal homogenates and serum & urine Creatinine, BUN were performed along with histopathological findings. *Results:* Administration of Galangin resulted in restoration of kidney function parameters with markedly downregulation of inflammatory cytokines. Moreover, histological findings showed marginal tissue damage with localized inflammatory precipitates. *Conclusion:* Renal function tests was retrieved stepwise to normal values in response to Galangin administration. Histopathological findings also revealed improved renal anatomy with evidenced shrinkage of inflammatory area, suggesting possible renoprotective role against nephritic insult.
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Keywords: Galangin; nephritic damage; histopathology; renal biomarkers.

1. Introduction

Aminoglycosides play crucial role in antimicrobial chemotherapy. [1] These medications are often used in the treatment of multiple lethal infections. [2] Some of the benefits of aminoglycosides include decreased chance of resistance, concentration-dependent impact and economy are few of the benefits of aminoglycosides which render themselves a drug of choice. [3] But at the other side, these medications have a significant deleterious impact of nephrotoxicity. [4] Excretion of gentamicin is observed by glomerular filtration. Approximately 5 % of gentamicin is selectively absorbed into the body in proximal tubules that causes necrosis of S1-S2 region of the proximal tubule component. [5] There is an aggregation of 'undigested phospholipids' in lysosomes that hastens nephrotoxicity. [6] As gentamicin pervades the cell, it stimulates the apoptosis, affects the respiratory chain, lowers ATP synthesis and induces hydroxyl and superoxide production that contribute to oxidative stress. [7] There is also increment in plasma urea and creatinine levels [8] that results development stress in the biological system. Preventing and attenuating oxidative stress is a radical approach for managing oxidative stress.

Alpinia officinarum is an important drug in Asian traditional medicine. It is known for treatment of swelling, stomach-ache and colds. [9]. It is extracted from roots of Alpinia officinarum. In contemporary scientific world, Galangin is reported for numerous pharmacological effects. It is well studied for anti-inflammatory effects in lipopolysaccharides stimulated cellular system. [10] Galangin is found to improve spatial memory in memory deficit rats by increasing acetylcholine concentrations. [11] It is also reported to have protective and antioxidant effects of liver and kidney. [12,13] The free radical scavenging effects of Galangin are also well documented. [14,15] The objective of this work was to evaluate nephroprotective impact afforded by Galangin towards gentamicin-induced nephrotoxicity in experimental animals.

2. Material and Methods

Drugs and chemicals:

Galangin and Gentamicin were purchased Sigma Aldrich , USA. Other analytical grade reagents were used.

Animals:

Wistar albino rats (3-5 months; 100-150 g) were used in this study. The animals were kept in cages of polypropylene and kept under regular circumstances. They were supplemented by usual diet and ad libitum water.

Selection of dose:

The dose was selected based on previous studies reported by Sivakumar et al.,[12] Galangin at doses of 50, 100 and 200 µg/kg was used in this study.

Experimental protocol:

Rats in the study were categorized into five clusters with 6 animals in every group .

Group I : Distilled water (2ml/ kg)
Group II :Gentamicin (80mg/kg)
Group III : Gentamicin (80mg/kg) + Galangin (50 µg/kg)
Group IV : Gentamicin (80mg/kg) +Galangin (100 µg/kg)
Group V: Gentamicin (80mg/kg) +Galangin (200 µg/kg)

Upon dosing until day 8, each rat individually was kept in independent metabolic chamber for 24 hours to collect urine for assessment of creatinine. Our animal study protocols followed the guidelines of the Institutional Animal Care and Use Committee (IACUC) , Supervision of Experiments on Animals (CPCSEA) and ARRIVE (Animal Research: Reporting of In Vivo Experiments) and were duly approved by the Animal Care and Use Committee of Health Sciences College at Lieth, Umm Al- Qura University under the No. of 01–21-HSCL, Jan 2021.

Measured Parameters:

1.1 Body weight

The weight of animals participated in the study was measured on day 10.

1.2 Kidney function test

1.2.1 Blood urea nitrogen (BUN) levels

The estimation of BUN was performed according to Span Diagnostic Ltd's protocols.

1.2.2 Serum creatinine levels

Serum creatinine was determined using the method of Slot, using Jaffe's reaction. [16]

1.2.3 Urea levels

Urea levels was performed by reported method [17] on kidney homogenate.

1.2.4 Assay for TNF-α and IL-1β

The TNF-α and IL-1β level in tissue was estimated using an ELISA assay (eBioscience, Inc., San Diego., USA).

1.2.5 Assay of antioxidant enzymes

The assay of SOD was performed as according to Sun et al method. [18] The assay of catalase was performed by Goth et al method. [19] Reduced Glutathione (GSH) was estimated by Cohn method. [20] Glutathione peroxidase (GPx) was estimated as per the method reported by Folhe et al. [21]

1.3 Kidney Histology

Rats were sacrificed. The kidneys were removed from every animal. The removed kidneys ware fixed in 10% formalin. The staining was done by Hematoxylin and eosin. The slides were studied under a microscope.

1.4 Statistical Analysis

The results were expressed as mean ± SEM. Statistical analysis was carried out by using One way ANOVA followed by Dunnett's' test and $p<0.01$, $p<0.001$ was considered significant.

3. Results

Kidney and Body weight:

Gentamicin intake in experimental animals resulted in total body weight reduction. But, Galangin treatment (50, 100 and 200 µg/kg) retarded a notable decrement ($P < 0.001$) in loss of body weight (Figure 1). Concerning kidney mass, administration of Gentamicin increased percent kidney weight which was ameliorated due to the administration of Galangin (100 and 200 µg/ kg; Figure 2).

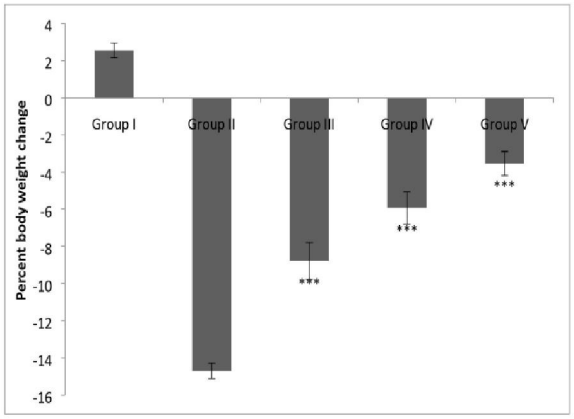

Figure 1. Effect of Galangin administration on body weight in gentamicin treated rats. Results are given as mean ± standard error of the mean (SEM) of six animals in each group. Control group compared with the extract-treated groups. Significance considered as *P < 0.05, **P < 0.01 and ***P < 0.001.

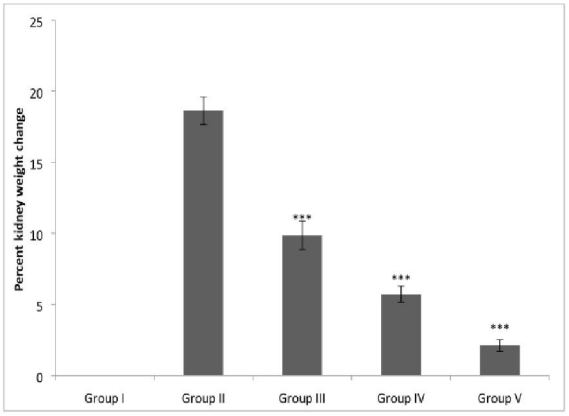

Figure 2. Effect of Galangin administration on kidney weight in gentamicin treated rats. Results are given as mean ± standard error of the mean (SEM) of six animals in each group. Control group compared with the extract-treated groups. Significance considered as *P < 0.05, **P < 0.01 and ***P < 0.001.

Serum and urine creatinine levels:

Serum creatinine levels were raised in animals treated with Gentamicin. While, The treatment with Galangin (Group III-V) (P< 0.01) showed apparent decrement in its levels (Figure 3). There was also restoration of urine creatinine levels due to Galangin treatment (50, 100, 200 µg/kg) (Figure 4).

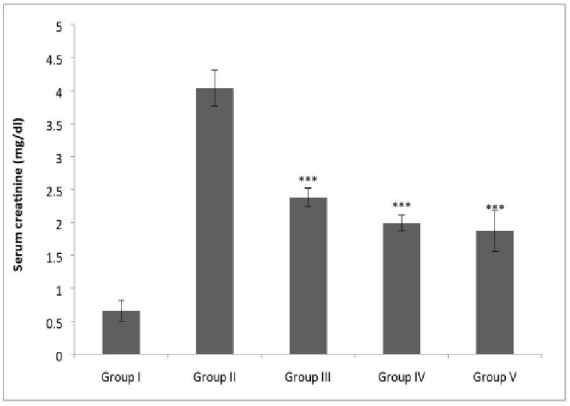

Figure 3. Effect of Galangin administration on serum creatinine levels in gentamicin treated rats. Results are given as mean ± standard error of the mean (SEM) of six animals in each group. Control group compared with the extract-treated groups. Significance considered as *P < 0.05, **P < 0.01 and ***P < 0.001

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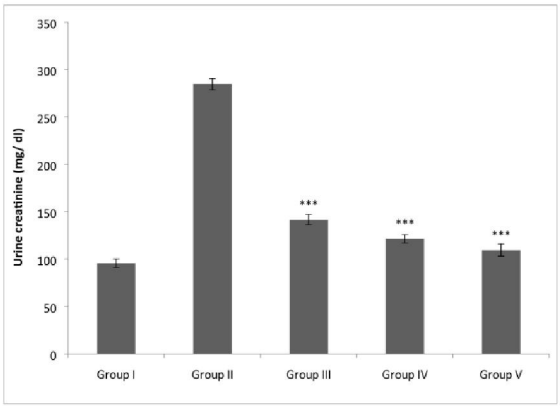

Figure 4. Effect of Galangin administration on urine creatinine levels in gentamicin treated rats. Results are given as mean ± standard error of the mean (SEM) of six animals in each group. Control group compared with the extract-treated groups. Significance considered as *P < 0.05, **P < 0.01 and ***P < 0.001.

Tissue Urea and BUN levels

BUN levels were elevated in the animals treated with Gentamicin (Group II). Treatment with Galangin (Group III-V) resulted in a notable reversal of toxicity (Figure 5). The treatment (Group III-V) with Galangin (50, 100 and 200 µg/kg) lead to notable decrement in urea (Figure 5) and BUN levels (Figure 6) in comparison with Group II.

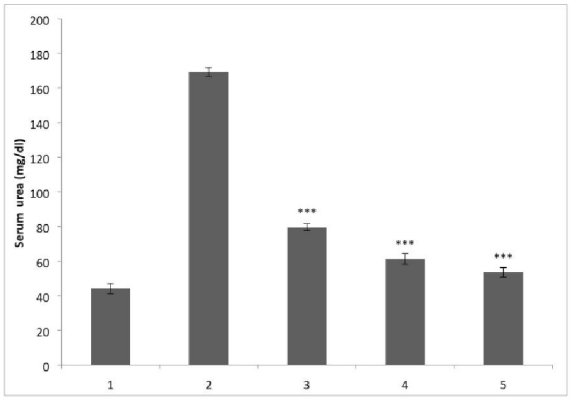

Figure 5. Effect of Galangin administration on tissue urea levels in gentamicin treated rats. Results are given as mean ± standard error of the mean (SEM) of six animals in each group. Control group compared with the extract-treated groups. Significance considered as *P < 0.05, **P < 0.01 and ***P < 0.001.

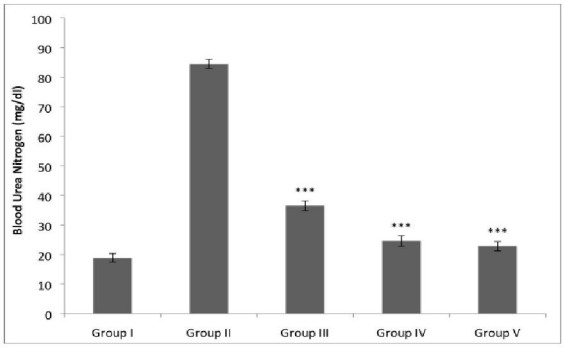

Figure 6. Effect of Galangin administration on BUN levels in gentamicin treated rats. Results are given as mean ± standard error of the mean (SEM) of six animals in each group. Control group compared with the extract-treated groups. Significance considered as *P < 0.05, **P < 0.01 and ***P < 0.001

Tissue TNF-α and IL-1β

Rats of the control group demonstrated normal levels of TNF-α and IL-1β. But, Rats treated with gentamicin resulted in increased levels of TNF-α. Administration of Galangin to gentamicin treated animals (Group III-V) showed notable drop in the TNF-α levels (Figure 7). IL-1β levels was elevated in Gentamicin treated animals (Group II). Administration of Galangin caused a notable change and tends to decrease the levels of IL-1β in animals (Group III-Group V) (Figure 8).

Table.

Treatment	SOD (U/mg protein)	Catalase (µmol/mg protein)	GSH (nmol/mg protein)	GPx (nmol/mg protein)
Group I	1.49±0.23	96.54±3.71	71.57±3.87	1657.63±449.35
Group II	0.74±0.17	59.21±2.27	35.84±2.54	1026.34±386.52
Group III	0.92±0.21***	67.62±3.51***	49.87±3.62***	1104.87±651.23***
Group IV	1.02±0.32***	74.36±2.98***	57.38±4.87***	129665±637.86***
Group V	1.31±0.29***	88.97±3.19***	66.25±3.23***	1586.21±520.34***

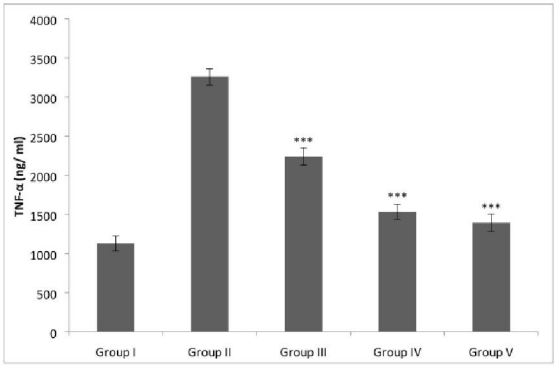

Figure 7. Effect of Galangin administration on tissue TNF-α levels in gentamicin treated rats. Results are given as mean ± standard error of the mean (SEM) of six animals in each group. Control group compared with the extract-treated groups. Significance considered as *P < 0.05, **P < 0.01 and ***P < 0.001.

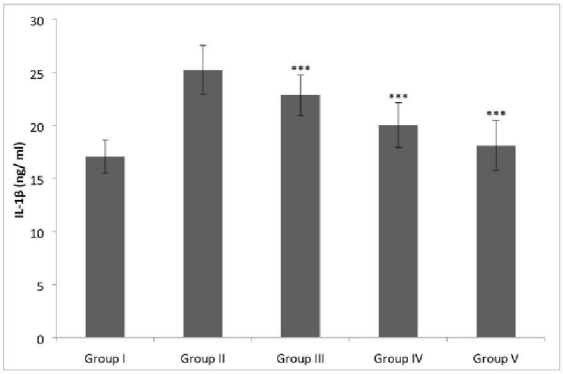

Figure 8. Effect of Galangin administration on tissue IL-1β levels in gentamicin treated rats. Results are given as mean ± standard error of the mean (SEM) of six animals in each group. Control group compared with the extract-treated groups. Significance considered as *P < 0.05, **P < 0.01 and ***P < 0.001.

Antioxidant enzymes

The levels of antioxidants enzymes are altered during any type of biological or oxidative stress. It is associated with oxidative insults that hamper their production. In the present study (Table 1) The levels of

SOD and catalase were decreased in group receiving Gentamicin. However, administration of Galangin in experimental dose (50, 100 and 200 µg/kg) resulted in their restoration. Glutathione is an endogenous antioxidant and plays a crucial role in prevention of inflammation. In the present study, administration of Galangin (50, 100 and 200 µg/kg) exhibited an increment reduced Glutathione (GSH) levels. Glutathione peroxidase (GPx) is imperative in catabolism of H2O2 into water, so that preventing cells from damage. The increase in the level of GPx due to treatment with Galangin demonstrates its antioxidant utility.

Table 1. Effect of Galangin administration on antioxidant enzymes in gentamicin treated rats.

Results are given as mean ± standard error of the mean (SEM) of six animals in each group. Control group compared with the extract-treated groups. Significance considered as $*P < 0.05$, $**P < 0.01$ and $***P < 0.001$

Histopathological inspection of kidney

Rats belonging to Group I demonstrated normal cellular structure of the kidney. The treatment with Gentamicin resulted in congestion of blood vessels along with cortical and peritubular damage. An interstitial inflammation was also observed. However, Administration of Gentamicin (Group III-V) resulted in restoration of kidney histoarchitecture damaged by Gentamicin (Figure 9).

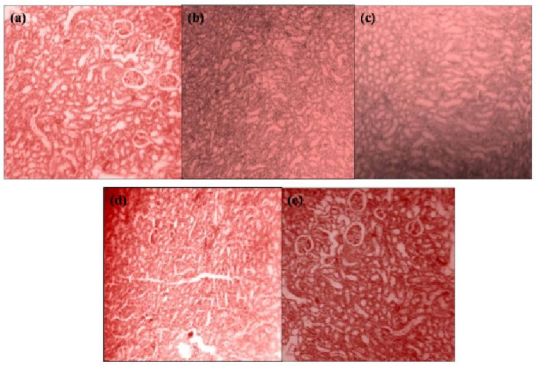

Figure 9. Evidence for the protective effect of Galangin in rats treated with gentamicin; (a) normal control, (b) toxicant, (c) Galangin 10 mg/kg p.o., and (d) Galangin 20 mg/kg p.o. (e) Galangin 40 mg/kg p.o.

4. Discussion

Gentamicin is among the major classes of antibiotics commonly utilized in the management of gram-negative bacterial infections. Nephrotic damage has become one of the main side-effects linked with it. The transfer and accumulation of Gentamicin at a greater percentage at renal proximal tubular cells' is seen during initial stage of nephritic damage. In subsequent stage, there is cellular damage associated with poly-cationic nature of drugs. [22] Transportation and build-up of this drug at higher amount by 'tubular cells' can be seen early stage of nephritic damage. In the second step, there is cellular damage which is due to this poly-cationic drugs. The subsequent oxidative stress appears to be the important determinants for such an etiology. [23]

The objective of this research was to assess the protective role of Galangin toward Gentamicin-associated nephrotoxicity. Gentamicin treatment in experimental animals resulted in 'acute renal failure,' which again was found due to an increase in serum creatinine. There was also an increase in urea levels. Administration of Galangin resulted in restoration of these markers. Body weight and kidney weight of rats of were determined to evaluate the detrimental consequences associated with Gentamicin administration in animals. It is generally observed that 'damaged kidneys' are more in weight which is observed due to the accumulation of necrotic and cellular infiltration. [24] together with a decrement in the total weight of animal body. Similar effects were observed in the present study. Galangin prevented a significant decrease in body weight and increased kidney weight.

TNF-α is a principal controller of inflammation. Excessive activity of this chemokine is associated with various inflammatory diseases like inflammatory bowel disease, renal dysfunction, rheumatoid arthritis and ankylosing spondylitis. [25] The restoration of the levels of TNF-α by administration of Galangin justifies its anti-inflammatory and antioxidant potential. Interleukin-1 is a pro - inflammatory cytokine which triggers either acute or chronic inflammation by inducing the expression of different genes. Interleukin-1 has two states, IL-1α and IL-1β. The two isoforms of IL are IL-1α and IL-1β. Thus, restricting expressed IL-1β can cause to prevent and treat several inflammatory disorders. [26] Administration of Galangin in experimental doses (Group III-V) resulted in significant decrement in IL-1β justifying nephroprotective effect .Renal disruption is associated with changes in the levels of uric acid, urea, and creatinine levels. The administration of Galangin in gentamicin-treated rats resulted in a substantial 'preservation' of these markers. Histological observations of normal control rat kidneys demonstrated normal anatomical characteristics.

Traumatic deposits and fibrotic breakdown were seen in histology of Gentamicin treated kidney of rats. Kidney of Galangin-treated rats showed marginal tissue damage and limited inflammatory precipitation with normal renal anatomy, which indicated the nephroprotective effect. Antioxidant enzymes play an important role in body by maintaining a steady state condition or homeostasis. The present study observed a change in the homeostasis as administration of Gentamicin (Group II animals) showed altered antioxidant enzymes. Superoxide dismutase catalyzes conversion of superoxide radical into molecular oxygen and H2O2. This enzyme levels are altered during diabetes, ischemia, hepatotoxicity and nephritic damage. [27] Galangin demonstrated protective effect on expression of superoxide dismutase by upregulating its levels. Catalase is another antioxidant enzyme that promotes conversion of H2O2 into water making biological system harmless and free from oxidative stress. [28] Treatment with Galangin restored the levels of catalase. Reduced Glutathione is a thiol containing peptide that exist in mammalian cell. Apart from various cellular functions.

Glutathione helps in maintaining redox state in the cell.[29] Galangin treatment afforded restoration of Glutathione levels. Glutathione peroxidase in an intracellular enzyme that is helpful in detoxifying and rendering cell free from oxidative damage. The restoration of this enzyme due to Galangin administration signifies its protective role. [30]

A previous study established nephroprotective effect of Galangin against cisplatin induced oxidative insults in mice kidney. The administration of Galangin resulted in attenuation of oxidative stress and inflammation. Such protective effect was observed as a result of inhibition of ERK and NF-kappa B signaling. [31] The present results are in association of previous findings whereby administration of Galangin in the present study resulted in decrement in oxidative stress in kidney that observed by biomarker analysis.

5. Conclusion

Galangin is well known as in vivo antioxidant. However, our recent study concluded that administration of Galangin to Gentamicin-treated rats indicated a beneficial nephroprotective effects evidenced by renal function analysis and histopathological findings. However, chronic studies are mandatory in order to affirm long-term impacts.

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