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Galangin attenuates adenine-induced chronic renal failure by inhibiting transforming growth factor beta (TGF-β) expression

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ABSTRACT

Introduction: Galangin (3,5,7 tri-hydroxy flavone), naturally active flavonoid is derived from the rhizomes of Alpinia officinarum is proven to be effective antioxidant, anti-inflammatory, anti-cancer. However, protective effects of galangin in chronic renal failure (CRF) is not explored.
Objectives: This study is aimed to investigate the nephroprotective activity of galangin in adenine-induced CRF rats.
Materials and Methods: Adenine induced rats were administered galangin 20 mg/kg and 40 mg/kg body weight (BW) serum renal and hepatic parameters, and renal antioxidant-lipid peroxidation parameters, histological studies were carried out. The mechanism of action was investigated by flow cytometry.
Results: Galangin has normalized serum renal and hepatic parameters, reduced oxidative stress and lipid peroxidation. Histopathology confirms nephroprotection. The percentage number of cells expressing transforming growth factor beta (TGF-β) was reduced with galangin treatment.
Conclusion: Galangin exerts nephroprotection in adenine induced CRF by inhibiting TGF-β expression.

Implication for health policy/practice/research/medical education:
The data suggests that galangin administration in rats has reduced the progression of chronic renal failure in adenine induced CRF. Thus, it can be a useful alternate or adjunct in CRF.

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Original

Introduction

Chronic renal failure (CRF) is a condition where the glomerular filtration rate is less than 60ml/min for more than three months. The condition is characterized by increased serum creatinine and urea nitrogen. Further kidney biopsy shows glomerular sclerosis, tubular atrophy, and interstitial fibrosis (1). Family history, drug misuse, diabetes, hypertension, environmental pollution, infections, and obesity contribute to CRF (2). CRF has connections with fibrosis, a regenerative mechanism that contributes to short-term repair. Persistent repair and regeneration lead to scarring of tissue. These events progress to end-stage renal failure (3). Adenine causes oxidative stress, lipid peroxidation, inflammation, apoptosis, and fibrosis leading to CRF (4). Natural small molecules have been effective in reversing fibrosis progression with low toxicity, which indicates efforts to explore these natural molecules in CRF are important (5). Galangin (3,5,7tri-hydroxy flavone), naturally active flavonoid is derived from the rhizomes of *Alpinia officinarum*. It possesses osteoinduction (5), antimicrobial (6) antioxidant & anti-aging (7), anti-inflammatory (8), anti-cancer & antiangiogenic (9). It has also been proven nephroprotective against cisplatin (10, 11) and diabetic nephropathy (12).

Objectives

The current study aims to explore the nephroprotective activity of galangin against adenine-induced CRF.

Materials and Methods

The present study is an experimental study conducted as per protocol.

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Chemicals

Adenine and losartan were procured from Sisco Laboratories, Hyderabad and galangin (>97.0%) was purchased from Sigma Aldrich. Male Sprague Dawley rats were procured from Jeeva Life Sciences, Hyderabad, India.

Experiment design

Adult male Sprague Dawley rats weighing around 200 grams acclimatized for a 12 hours light/dark cycle at around 22°C temperature with 60% relative humidity, provided pellet diet and tap water *ad libitum* for one week. After a week, rats were weighed and randomly divided into five groups (n = 6). Adenine 200 mg/kg solution was prepared in 0.5% sodium carboxymethyl cellulose, and administered by oral gavage to all groups except the normal control group. The remaining four groups were administered standard losartan 20 mg/kg, galangin 20 mg/kg, and 40 mg/kg, along with adenine by oral gavage for 4 weeks. Body weight and feed intake were recorded every week. Rats were overnight fasted at the end of 28 days, and on the next day, rats were anesthetized ketamine-xylazine mixture, and blood was collected from cardiac puncture. Kidneys were isolated, cleaned, and weighed, and left kidneys were stored in 10% formalin and sent for histopathology studies. Right kidneys were placed in an ice-cold buffer and used for further analysis. Blood was centrifuged for 15 minutes at 3000 rpm; serum was separated, and stored at -20°C, used for biochemical estimations. Kidneys were homogenized in an ice-cold buffer and the homogenate was estimated for antioxidant and lipid peroxidation markers. Renal cell suspension was prepared as per the literature (13). The cells were labeled with 20 µL of anti-human LAP (TGF-β). Five hundred microliters of DPBS was mixed thoroughly, and the cells were analyzed by FACS on FL1 channel. The percentage of cells expressing TGF-β was analyzed by flow cytometry (14).

Statistical analysis

All data were shown as mean ± SD and were analyzed by GraphPad Prism 9.5.1 using one-way ANOVA followed by Dunnett's multiple comparison tests. A P value < 0.05 is considered statistically significant.

Results

Adenine administration had reduced the body weights is rats that can be correlated to the feed consumption changes in rats. An increase in kidney weights may be due to hypertrophy caused by adenine. Galangin has improved body weights, feed consumption and kidney weights in adenine induced rats (Table 1).

Creatinine, urea nitrogen and uric acid are often considered as important biochemical parameters in renal failure. A significant increase in the serum biomarker levels was observed in experimentally induced CRF rats. Galangin at both doses had reduced these renal biochemical parameters. Electrolyte alterations are often associated with renal failure that subsequently causes metabolic changes. Serum sodium and potassium levels were decreased and increased, respectively in adenine treated rats. These levels were normalized in galangin treated rat groups (Table 2).

Table 1. Effect on body weight, feed intake, and kidney weight data at the end of the 28th day

Groups	Body weight (g)	Feed intake (g/24 h/rat)	Kidney weight (mg)
Normal	189±0.707	10.3±1.241	764±0.535
Adenine 200 mg/kg	109±1.414	6.8±0.707	1181.6±2.522
Adenine + losartan 20 mg/kg	165±2.212	9.0±1.122	836.80±1.875
Adenine + galangin 20 mg/kg	173±3.252	8.2±0.421	903.4±0.705
Adenine + galangin 40 mg/kg	172±4271	9.0±1.125	862.2±1.5

Data are shown as mean ± SEM (n=6).

Table 2. Effects of galangin on renal parameters in serum

Groups	Creatinine (mg/dL)	Urea nitrogen (mg/dL)	Uric acid (mg/dL)	Sodium (mEq/L)	Potassium (mEq/L)
Normal	0.645±0.077	18.02±0.167	4.70±0.045	148±2.135	4.15±0.233
Adenine 200 mg/kg	4.893±0.470	47.48±0.707	8.75±0.331	134.5±4.207	6.58±2.527
Adenine + losartan 20 mg/kg	2.5±0.357	28.89±2.221	5.75±0.211	144.5±2.657	5.75±2.223
Adenine + galangin 20 mg/kg	3.058±0.520	29.16±0.707	6.91±0.045	138.5±5.242	5.85±2.355
Adenine + galangin 40 mg/kg	2.203±0.357	25.83±0.707	6.003±0.332	141.75±1.414	5.35±2.334

Data are shown as mean ± SEM (n=6).

Alterations in liver enzymes depicts metabolic changes induced by adenine. Adenine has almost doubled the alanine aminotransferase (ALT) levels and also raised aspartate aminotransferase (AST) levels that describe adenine causing metabolic alterations. Galangin has decreased these changes bringing the aminotransferases to normal ranges (Table 3).

Adenine increase blood uric acid and produces reactive oxygen species. This can be shown through increased peroxidation of lipids, decreased endogenous antioxidants and antioxidant effect producing enzymes in kidney. The effects are reversed by galangin treated rats (Table 4).

Histopathology studies reveal the changes produced by adenine compared to normal group. Severe nephritis was observed in adenine treated kidneys. Galangin dosing has transformed the severe form of nephritis to mild nephritis in kidney sections. The effects of galangin 40 mg/kg were similar to losartan histopathology studies (Figure 1).

Transforming growth factor is the fibrotic biomarker, usually expresses in response to repeated damage and repair of tissue, finally transforms normal tissue into scar tissue, leading to loss of functional properties. Adenine produces repetitive oxidative stress that induces expression of TGF-β by the kidney cells causing loss of kidney functions. Flow cytometry analysis of renal tissue homogenate have shown that adenine has increased significantly the number of cells expressing fibrotic marker TGF-β, that are relatively halved in galangin treated rat kidneys (Table 5).

Table 3. Effects of galangin on ALT, AST

Groups	ALT (IU/dL)	AST (IU/dL)
Normal	26.66±0.707	35±2.325
Adenine 200 mg/kg	57.33±2.121	56.5±2.123
Adenine + losartan 20 mg/kg	38.33±2.121	36±1.414
Adenine + galangin 20 mg/kg	44±2.121	42.5±0.166
Adenine + galangin 40 mg/kg	37±0.707	37.5±0.707

Data are shown as mean ± SEM (n=6).
ALT, Alanine aminotransferase; AST, Aspartate aminotransferase.

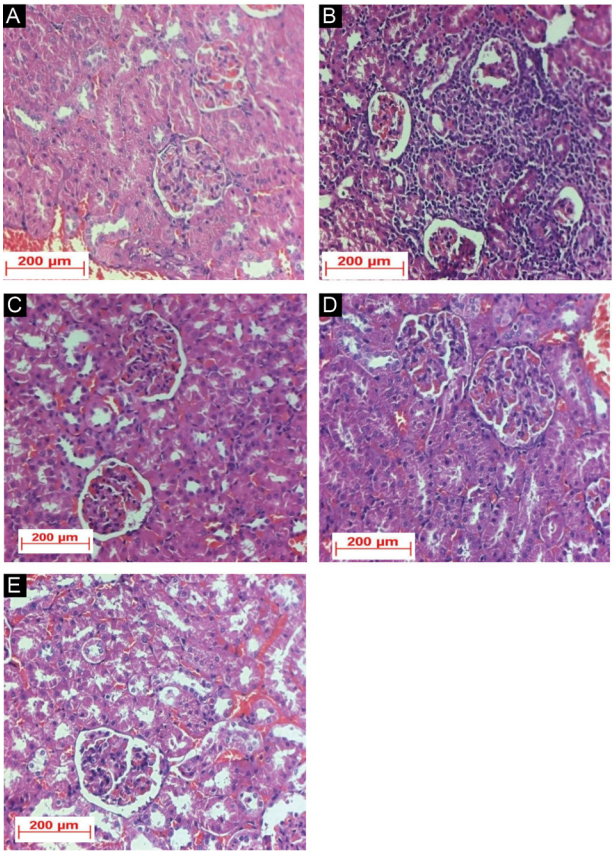

Figure 1. Hematoxylin & Eosin staining under 200×. (A) Normal, (B) Adenine 200 mg/kg, (C) Adenine+ Losartan 20 mg/kg, (D) Adenine + Galangin 20 mg/kg, and (E) Adenine+ Galangin 40 mg/kg.

Discussion

Galangin, a flavonoid isolated from *Propolis* and *Alpinia officinarum* (13), exhibits various pharmacological activities because of its antioxidant and anti-inflammatory effects. The current study has been carried-out to explore the protectiveness of galangin in adenine-induced CRF. Adenine is hypothesized to produce renal failure through oxidative stress and apoptotic mechanisms. The model is selected based on existing literature that claims the

Table 4. Effects of galangin on renal glutathione, superoxide dismutase, and MDA levels

Groups	GSH (µg/g wet tissue)	SOD (U/mg tissue)	MDA (nmol/g wet tissue)
Normal	35.5±2.121	72±2.82	26.5±0.707
Adenine 200 mg/kg	17.5±0.707	37.5±0.707	58±1.414
Adenine + losartan 20 mg/kg	32±1.414	67.3±0.707	23.5±0.707
Adenine + galangin 20 mg/kg	22.5±0.707	45.5±0.707	43.5±2.121
Adenine + galangin 40 mg/kg	31.25±0.353	55±1.414	31.5±2.121

Data are shown as mean ± SEM (n=6).
GSH, glutathione; SOD, superoxide dismutase; MDA, malondialdehyde.

Table 5. Effect of galangin on renal expression of fibrotic marker (TGF-β) expressed as % of cells expressing the markers

Groups	TGF-β expression
Normal	1.09±2.121%
Adenine 200 mg/kg	55±4.256%
Adenine + losartan 20 mg/kg	21.6±1.359%
Adenine + galangin 40 mg/kg	23.73±3.423%

TGF β, transforming growth factor beta.

adenine model of CRF is similar to features of CRF produced in human beings (14). In the present study, adenine has significantly reduced body weight and feed intake, whereas galangin at both doses has increased the body weight and feed intake. Galangin 40 mg/kg have effects similar to losartan 20 mg/kg. Kidney weights increased from 761mg in the normal group to 856 mg in the adenine group. Kidney weights were significantly decreased in losartan and galangin 40 mg/kg groups. Serum creatinine, urea nitrogen, and uric acid significantly increased in adenine-treated rats and were reduced by galangin. Serum electrolytes-sodium and potassium levels were not significantly affected in adenine and other treated groups. Liver enzymes (ALT and AST) were significantly involved in adenine-treated rats that showed metabolic alterations with adenine. The levels of ALT and AST have been brought down by galangin same as with losartan 20 mg/kg. Galangin has shown a dose-dependent effect in this aspect. Oxidative stress and lipid peroxidation are characteristic features of kidney damage. The current study assessed glutathione (GSH), superoxide dismutase (SOD), and malondialdehyde (MDA) levels. The levels of these parameters were almost halved in adenine 200 mg/kg treated rats, which clearly show oxidative stress induced by adenine. GSH and SOD levels were significantly raised with galangin treatment. Free radicals produce lipid peroxidation in kidney tissue that further aggravates damage. The lipid peroxidation marker, MDA, has been increased in the adenine group significantly, and galangin 40 mg/kg dose has shown an effect similar to standard losartan. H&E Staining reveals severe nephritis and glomerular, tubular damage along with WBC migration into the cortex under 200× in adenine-treated groups. The features were reduced from severe to mild and normal in galangin-treated groups. On analyzing single-cell kidney homogenate through flow cytometry, it was observed that the normal control group kidney has the least percentage of cells expressing TGF-β (1.09%), whereas adenine-treated groups have almost increased to 55%. Galangin treated rat kidneys have lowered the cells % expressing TGF-β to 23%.

Conclusion

The present study concludes that galangin is effective against adenine-induced CRF. Serological, histological, and advanced biological techniques validate the results. From the present study it can be concluded that the protective effects of galangin in adenine-induced CRF rats are mediated through TGF-β inhibition.

Limitations of the study

The present study did not use any immuno-histochemical staining protocols for determining molecular mechanisms. Further studies can be conducted by maximizing sample size of rats.

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Authors' contribution

Conceptualization: Deepthi Rayilla.
Data curation: Deepthi Rayilla.
Formal analysis: Deepthi Rayilla.
Investigation: Deepthi Rayilla.
Methodology: Deepthi Rayilla.
Project administration: Ganta Suhasin.
Resources: Deepthi Rayilla.
Supervision: Ganta Suhasin.
Validation: Deepthi Rayilla.
Visualization: Ganta Suhasin.
Writing–original draft: Deepthi Rayilla.
Writing–review & editing: Ganta Suhasin.

Conflicts of interest

The authors declare that they have no competing interests.

Ethical issues

The protocol of this study was in accordance with the guidelines of animal studies and approved by Animal Ethics Committee of Care College of Pharmacy and Jeeva Life Sciences Pvt. Ltd. Accordingly, we tried to conduct the guidelines related to animal experiments, approved by the United States National Institutes of Health (NIH, 1978). Ethical issues (including plagiarism, data fabrication, double publication) have been completely observed by the authors.

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