# Betulinic acid attenuates renal fibrosis in rat chronic kidney disease model

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## ARTICLE INFO

## ABSTRACT

*Background:* Most chronic kidney diseases (CKDs), regardless of the nature of the initial injury, progress to end-stage renal disease (ESRD) characterized by fibrosis with irreversible loss of tissue and function. Thus, improved and more effective therapies are critical. Betulinic acid (BA), a pentacyclic triterpene is a compound in the pipeline of anti-cancer drug development. It has been shown to a possess variety of beneficial effects in many disease conditions. However, its efficacy against CKD is yet to be explored.
*Objective:* The present study was undertaken to investigate the effect of BA on renal fibrosis in the rat model of adenine-induced CKD.
*Results:* CKD rats gained significantly less weight during the experimental period when compared to control rats and BA treatment did not significantly increase the weight gain in CKD rats. CKD rats showed elevated levels of serum blood urea nitrogen (BUN), creatinine and uric acid along with increased levels of kidney injury markers such as cystatin C and neutrophil gelatinase-associated lipocalin (NGAL). Further, in comparison to control rats, kidney samples from CKD rats revealed increased profibrotic protein levels like transforming growth factor-beta (TGF-β), connective tissue growth factor (CTGF), fibronectin, collagen type I and hydroxyproline indicating a progressive fibrotic response. These data are further fortified by histological findings where kidney damage and fibrosis are clearly evident as dilatation of tubules, glomerular degeneration and vacuolation along with deposition of collagen fibers. However, the above-mentioned findings in CKD rats were significantly reversed by BA-treatment revealing its nephroprotective potential and anti-fibrotic activity.
*Conclusion:* The biochemical mechanism of the nephroprotective and anti-fibrotic effect of BA in the adenine-induced CKD rats might be mediated by inhibition of pro-fibrotic protein production thereby hindering the kidney tissue damage along with improvement in kidney function. Thus, BA could be an adjunct agent to retard fibrosis in CKD subjects.

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## 1. Introduction

Most chronic kidney diseases (CKDs), irrespective of etiology progress to renal fibrosis which leads to kidney failure and associated complications. Renal fibrosis involves an excess accumulation of extracellular matrix (ECM) and usually results in loss of function with normal tissue being replaced by scar tissue ultimately leading to end-stage renal failure requiring dialysis or kidney transplantation for the survival of the patient [1]. Although many different treatment modalities have been implemented for renal fibrosis, the results have not been promising and search for a better therapeutic agent will be a focused target in the area of renal fibrosis research.

Pathogenesis of kidney fibrosis is attributed to transforming growth factor-β1 (TGF-β1), a multifunctional cytokine that acts as a potent inducer of ECM synthesis. Activation of TGF-β1 results in the overproduction and deposition of type I collagen and other ECM proteins leading to the development of glomerulosclerosis and tubulointerstitial fibrosis [2]. Further, it is also found that blocking TGF-β signaling pathways attenuates fibrosis and

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targeting this pathway is apparently an attractive therapeutic strategy for the management of fibrotic conditions [3,4].

The adenine-induced CKD model in rats is considered as a standard method which reproduces clinical CKD in laboratory animals where adenine is given to rats in the feed at a concentration of 0.75%, w/w, for 4 weeks [5]. An excessive oral intake of adenine leads to the accumulation of 2,8-dihydroxyadenine (DHA) in renal tubules causing progressive renal dysfunction accompanied by interstitial fibrosis [6].

Betulinic acid {BA; 3β, hydroxy-lup-20(29)-en-28-oic acid} (Fig. 1) is a naturally occurring pentacyclic triterpenoid but can also be derived chemically from betulin, a substance found in abundance in the outer bark of white birch trees (Betula alba) [7]. BA is a minor bio-compound yet having a high efficacy in treating different diseases. BA and its derivatives have wide range of biological activities, including neuroprotective [8], anti-cancer [9], anti-inflammatory [10,11], anti-oxidant [12], anti-trichomonal [13] and anti-HIV properties [14]. Further, BA ameliorated diabetes-induced renal fibrosis and chemically-induced-hepatic fibrosis [15,16]. Research evidence suggest that BA has potential to retard experimentally-induced acute kidney injury [17,11]. However, its therapeutic potential against renal fibrosis, a final common pathway associated with all types of chronic kidney diseases is still unknown. In the view of the above findings, this study was designed to determine the effect of BA on renal fibrosis in the rat model of adenine-induced CKD.

## 2. Materials and methods

### 2.1. Experimental animals

Adult male albino Wistar rats (180–250 g) were procured from Laboratory Animal Resource (LAR) Section of the institute and housed in a temperature-controlled condition ($22 \pm 2\,°C$) with 12:12 light: dark cycle; maintained on standard rodent diet with water ad libitum. The animals were acclimatized to the laboratory conditions for 7 days prior to the start of the experiment. All animal experiments were duly approved by Institute Animal Ethics Committee.

### 2.2. Drugs and chemicals

BA from TCI, Tokyo, carboxymethyl cellulose (CMC) from Merck, USA, L-hydroxyproline, chloramine-T and p-dimethylaminobenzaldeyde (Ehrlich's reagent) from Sigma, St Louis, MO, USA were purchased. Adenine and other necessary chemicals were purchased from SRL, India. ELISA kits were purchased from Genetix Biotech Asia Pvt. Ltd.
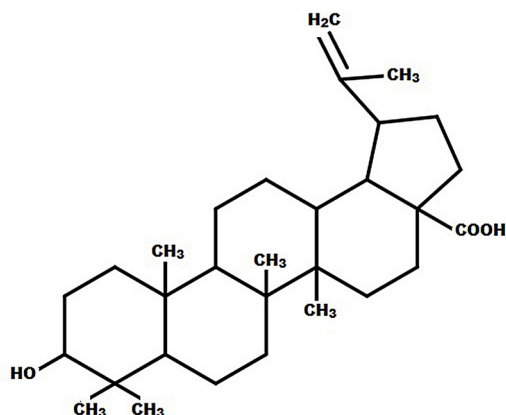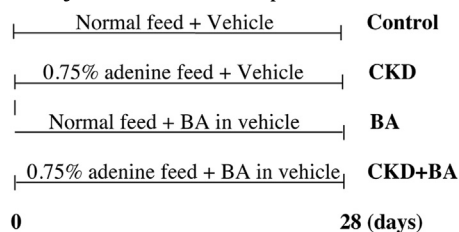


Fig. 1. Chemical structure of betulinic acid (BA).

### 2.3. CKD model

CKD in rats was induced by giving adenine mixed with the feed at a concentration of 0.75% w/w for 28 days.

### 2.4. Animal grouping and drug administration

The animals were initially divided into 4 groups containing 6 animals in each group. Control group rats received vehicle and normal feed for 28 days. CKD group rats received vehicle (0.5% CMC in distilled water) by oral route and adenine (0.75%) in feed for 28 days. BA group rats received BA in vehicle at the dose of 30 mg/kg body weight by oral route and normal feed for 28 days. CKD + BA group rats received BA in the vehicle at the dose of 30 mg/kg body weight by oral route and adenine (0.75%) in feed for 28 days. The schematic representation of the same is given below.

| Normal feed + Vehicle | **Control** |
| 0.75% adenine feed + Vehicle | **CKD** |
| Normal feed + BA in vehicle | **BA** |
| 0.75% adenine feed + BA in vehicle | **CKD+BA** |

**0**                              **28 (days)**

### 2.5. Body weight change and relative kidney weight

Body weight change (g) = [body weight of rat (g) at the end of experiment] – [body weight of the rat (g) at the start of experiment]; Relative kidney weight = kidney weight (g)/body weight (kg)

### 2.6. Collection of serum

Blood was collected from the rats by retro-orbital plexus puncture and allowed to clot in slanting position for 2 h. The clotted blood was centrifuged at 4000 rpm for 10 min. The clear serum was separated and stored at $-20\,°C$ until analyzed.

### 2.7. Preparation of kidney homogenates

After killing the animals by cervical dislocation, kidneys were removed, weighed and snap-frozen in liquid nitrogen. A small piece of tissue was excised and stored in the fixative (10% formalin or Bouin's fluid) for histology and the rest was then homogenized in lysis buffer solution with mammalian cocktail protease inhibitor (pH 7.4) at $4\,°C$ to make 5 or 10% tissue homogenate. The homogenate was centrifuged at 1500g for 10 min. The resulting supernatants were stored at $-20\,°C$ for biochemical parameter and protein analysis. Total protein content of the supernatant was estimated by a commercially available kit.

### 2.8. Estimation of blood urea nitrogen (BUN), creatinine, and uric acid

BUN, creatinine and uric acid were estimated by using commercially available kits from Span Diagnostics Ltd. India.

### 2.9. Estimation of cystatin C and neutrophil gelatinase-associated lipocalin (NGAL)

Serum cystatin C and NGAL were measured by ELISA kit as per the manufacturer's instructions to assess the kidney injury.