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Original Article

# Protective Effect of Arjunakwatha and Arjunasheeta in Paracetamol-induced Kidney Injury in Rat Model

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## ABSTRACT

**Background:** Chronic kidney disease is now a global burden with an increased grade of morbidity and mortality due to the unavailability of particular medicines except for high-cost treatments like dialysis or kidney transplantation. Tribal people use their own indigenous preparation of medicinal plants for the prevention and treatment of kidney ailments. The present study was aimed at preparing Arjunakwaatha and Arjunasheeta, an indigenous preparation of the bark of Terminalia arjuna (TA) and its supplementation in the kidney injury rat model. **Materials and Methods:** In this study, rats were induced to kidney injury (KI) by intraperitoneal injection of paracetamol 15 mg/kg b.w. for 14 days and supplementation of Arjunakwaatha and Arjunasheeta with various doses continued the experimentation for 28 days. **Results:** Results showed that urea, creatinine, C-reactive protein (CRP), glutamine oxalic transaminase (GOT), glutamate pyruvate transaminase (GPT) in plasma, and malondialdehyde (MDA) in kidney tissue, urinary protein, and KIM-1 were significantly ($p<0.05$) increased in kidney injury rats when compared to normal control rats. Supplementation of Arjunakwaatha and Arjunasheeta with kidney injury rats significantly ($p<0.05$) decreased urea, creatinine, CRP, GOT, GPT, MDA, SOD, CAT, and GSH levels as compared to kidney injury rats. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis showed new low molecular weight urinary protein bands in kidney injury rats. The protective effects of Arjunakwaatha and Arjunasheeta present no band at this molecular level in normal rats. **Conclusion:** It has been concluded that Arjunakwaatha is the best indigenous preparation for kidney protection.

**Keywords:** Arjunakwaatha, Arjunasheeta, Kidney injury, KIM-1, CKD.

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## INTRODUCTION

According to World Kidney Day (WKD), globally 850 million people are currently affected by kidney disease reflected a picture of 1 in 10 adults.[1] Chronic kidney disease (CKD) is a common comorbid disease of diabetes, hypertension, and heart disease which is estimated as 30% diabetes,[2] 50% heart disease,[3] and 70% hypertension[4] patients were affected by CKD. However, there was no proper treatment strategy for CKD patients except kidney transplant and dialysis. So, early diagnosis and prevention of CKD are very much important for the survival of kidney disease patients. The plant *Terminalia arjuna* L. (TA), commonly known as Arjuna or Arjun tree belongs to the Combretaceae family and grows abundantly throughout the Indian subcontinent, Sri Lanka, Burma, and Mauritius.[5] The various parts of the TA plant like bark, root, leaves, and fruits are used to treat many ailments due to the presence of so many bioactive compounds like luteolin, arjungenin, terminic acid, arjunoside I, arjunoside II, arjunolic acid, etc.[6] Moreover, previous literatures have demonstrated the protective effects of TA extract against hepatotoxicity,[7] inflammation, oxidative stress and apoptosis,[8] cardiac hypertrophy,[9] and nephrotoxicity.[10] In our laboratory, the bark extract of TA had experimentally proved antioxidative, [11] antimicrobial,[12] anti-uremic,[10] and nephroprotective[14] in the CKD rats model. Tribal people from West Bengal's Jangalmahal area are said to use indigenous preparation of bark of TA for their kidney ailments. The present study aimed at the effect of indigenous preparation (from the source of the tribal population) of the bark of TA on urinary protein, kidney injury markers, and oxidative stress markers of the paracetamol-induced CKD model in rats.

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## MATERIALS AND METHODS

### Chemicals and reagents

Major biochemical parameters were measured using diagnostic kits like urea, creatinine, total protein, C-reactive protein (CRP),

glutamine oxalic transaminase (GOT), and glutamate pyruvate transaminase (GPT), purchased from Agape Hills, Ernakulam, Kerala, India. We used Enzyme-Linked Immunosorbent Assay (ELISA), kits like kidney injury molecule-1 (KIM-1) purchased from Caltech life science, Bhubaneswar, Odisha, India. Paracetamol (neomol®) was purchased from a local market, Medinipur. All other chemicals like sodium dodecyl sulfate (SDS), glycerol, bromophenol blue, pyrogallol, tris buffer, trichloroacetic acid (TCA), thiobarbituric acid (TBA), and hydrochloric acid (HCL), sucrose, hydrogen peroxide ($H_2O_2$), ethanol, hematoxylin, eosin, xylene, potassium dihydrogen phosphate ($KH_2PO_4$), sodium hydrogen phosphate ($Na_2HPO_4$), sulfosalicylic acid, sodium chloride (NaCl), Tween 20, bovine serum albumin (BSA), nickel ammonium sulfate, cobaltous chloride were purchased from SRL, India, and MERCK, India, HiMedia Laboratories Pvt. Ltd., Mumbai, India, and Crest Biosystems Goa, India.

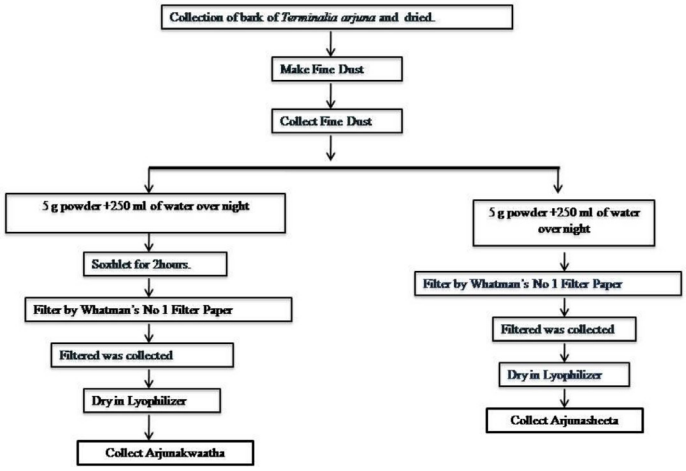


**Figure 1:** Preparation of Arjunakwaatha and Arjunasheeta from powder bark of TA.

## Instruments

Biochemical parameters like urea, creatinine, total protein, C-reactive protein (CRP), Glutamic oxalic transaminase (GOT), and Glutamate pyruvate transaminase (GPT), were measured by Semiautoanalyser (AGAPPE), oxidative stress markers malondialdehyde (MDA), antioxidant enzyme parameters like superoxide dismutase (SOD), catalase (CAT), glutathione (GSH) were measured by absorbance UV-VIS double beam Spectrophotometer (systronics, India), KIM-1 was measured by ELISA plate reader (Thermofisher Scientific), SDS-PAGE was done by a full set up of gel apparatus (Tarsons, India), Western blot analysis was done by a full set up of trans-blot turbo apparatus (Bio-Rad, India), extract bark of *Terminalia arjuna* (TA) was performed using Electrical Blender (Philips, India), soxhlet apparatus (Yoma, India), Microcentrifuge (Remi, India), BOD incubator, Incubator with shaker (Indian Instruments Ltd.). All materials weighted by Digital weight balance (Accuracy-0.1mg) Adhair Dutta and Sons.

## Collection of *Terminalia arjuna* (TA) bark

The bark of TA was collected from Raja Narendralal Khan Women's College campus of Midnapore, Paschim Medinipur district of West Bengal. The material was identified by the taxonomist of the Botany Department at our college, Midnapore. The voucher specimen was kept in the Botany Department. At first, TA bark was dried at 40 ± 1°C in the incubator and the dried parts were crushed using an electric grinder the powder was then separated.

## Extract preparation

Some tribal population of Paschim Medinipur district, West Bengal in India uses traditional medicine to prevent and or cure various ailments due to the high cost of drugs. Their traditional medical practices are the consumption of various medical plants or herbs by following common two methods-Some people soak one or more parts of plants in water overnight and consume filtrate the next morning on empty stomach and another some people boil one or more parts of medicinal plants in water and consume the filtrate. Followed by those practices, we collected some information regarding traditional medical practices from tribal by directly interacting with them. A tribal people said " I take bark dust of TA about 1 glass of water overnight then boiled for 2 hr and make ¼ part of the solution. Then cool and filter and consume the filtrate in the next morning in empty stomach to keep healthy kidney". Another people told " I take 1 teaspoon bark dust of TA and dissolved in 1 glass of water for overnight to soak then filter and consume the filtrate in the next early morning in empty stomach for a healthy kidney. We followed their traditional practices and we prepared two extracts of bark of TA called Arjunakwaatha and Arjunasheeta (Figure 1). Arjunakwaatha and Arjunasheeta were separately stored in a refrigerator at 4°C and used for our experiments. The percentage yield of arjunakwaatha extract was 1.2g% and arjunasheeta extract was 1g%.

## Induction of kidney injury

Kidney injury was prompt through intraperitoneal injection of freshly prepared paracetamol (neomol®) (15 mg/kg body weight) in 0.2 ml of normal saline for 14 days. At the time of induction, vehicle control rats were injected with 0.2 ml normal saline alone.

## Selection of animals and care

Adult male albino outbred Wistar rats weighing 150-170 g were obtained from the Centre for Translational Animal Research, Bose Institute, Kolkata. The animals were acclimatized to laboratory conditions for 2 weeks prior to experimentation. Animals were housed in three rats/cages in a temperature-controlled room (22 ± 20°C) with 12–12 h dark-light cycles (8.00–20.00 hr light, 20.00– 8.00 hr dark) at a humidity of 50 ±10%. All experimental procedures on animals were in accordance with the guidelines

on the regulation of scientific experiments on animals stated by the Committee for the Purpose of Control and Supervision of Experiments of Animals (CPCSEA), 2018 and our Institutional Animal Ethics Committee (IAEC) (Regd. No. 1905/PO/Re/S/2016/CPCSEA).[14]

## Experimental animals

In this experiment, 45 rats were divided into 9 groups comprising 5 animals each. Control (C) rats received a normal diet and water for 28 days. Vehicle control (VC) rats have injected with a single dose of 0.2 ml saline water peritoneally and fed normal diet for 14 days. Kidney injury (KI) rats were administration Paracetamol (intraperitoneally) at the dose of 15 mg/ kg body for first 14 days. Another 6 groups of rats were subjected to KI for 14 days and supplemented arjuna bark preparation for 28 days as follows- KI+Arjunakwaatha 5, KI+Arjunakwaatha 10, KI+Arjunakwaatha 15 fed Arjunakwaatha at the dose of 5mg/ kg b.w., 10mg/ kg b.w., 15mg/ kg b.w./day for 28 days. KI+ Arjunasheeta 4, KI+ Arjunasheeta 8, KI+ Arjunasheeta 12 fed Arjunasheeta at the dose of 4mg/ kg b.w., 8mg/ kg b.w., 12mg/ kg b.w./day for 28 days.

## Animal sacrificed and plasma and organ collection

This experimental design was continued for 28 days. On the 29$^{th}$ day, the animals were sacrificed to collect blood samples from the aorta for analysis of plasma renal markers, hepatic functional markers, oxidative stress markers, and anti-oxidant enzyme markers. After blood collection, the specific organs like kidneys were dissected and weighed. Samples of organs were used for different biochemical analyses. The collected blood was centrifuged at 3000 rpm/min and plasma fractions were separated for biochemical parameter assessment.

## Estimation of plasma renal markers
## Plasma urea, creatinine, CRP and total protein level

The Plasma urea (mg/dL), creatinine (mg/dL), CRP (mg/dL), and total protein (g/dL) level was measured according to our earlier laboratory established method by the semi-auto analyzer (AGAPPE, India) using standard AGAPPEE kit.[11,13]

## Estimation of kidney injury markers in urine
## Urine urea, creatinine, and total protein level

The urine urea (mg/dL), creatinine (mg/dL), and total protein (g/dL) level was measured according to our earlier laboratory established method by the semi-auto analyzer (AGAPPE, India) using a standard AGAPPEE kit.[11,13]

## Levels of KIM-1

KIM-1 (pg/dL) of urine was measured by using a commercially available standard kit (Thermofisher Scientific, Invitrogen Bioservices India Pvt. Ltd) with standard protocol by ELISA (Thermofisher Scientific) plate reader in our laboratory.[14] In brief samples and standard was pipette in 96 well plates that have been pre-coated with specific antibodies for KIM-1. In the next step, wells were washed and a biotinylated antibody specific for KIM-1 was added and incubate for 1 hour. After that, the wells were washed and streptavidin- HBP solution was added and followed by washing and the solution was read at 450 nm using an ELISA plate reader.

## Biochemical estimation of GOT, GPT level in plasma

For the assessment of kidney function in plasma, we measured GOT, and GPT levels by our earlier established protocol by the semi-auto analyzer (AGAPPE, India) using the kit manual protocol.[11,14]

## Biochemical assay of SOD activity in kidney tissue

The kidney was homogenized in ice-cold 100 mM Tris-cacodylate buffer to give a tissue concentration of 50 mg/mL and centrifuged at 10,000 rpm for 20 mins at 4°C. The SOD activity of the supernatant was estimated by measuring the percentage of inhibition of the pyrogallol auto-oxidation by SOD according to our established method. One unit of SOD was defined as the enzyme activity that inhibited the auto-oxidation of pyrogallol by 50%.[11,13]

## Biochemical assay of CAT activity in kidney tissue

CAT was measured biochemically in tissue supernatant by our laboratory established method and reading of absorbance was noted using a spectrophotometer at 240 nm. These homogenates were centrifuged separately at 10,000 rpm at 4°C for 10 min.[11,13] For the evaluation of CAT in the kidney were homogenized separately in 0.05 M Tris Hydrochloric acid (HCl) buffer solution (pH-7.0) at a tissue concentration of 50 mg/mL.

## Biochemical assay of GSH activity in kidney tissue

GSH was measured biochemically in tissue supernatant by our laboratory established method and reading of absorbance was noted using a spectrophotometer at 240 nm. These homogenates were centrifuged separately at 10,000 rpm at 4°C for 10 min.[15] For the evaluation of GSH in the kidney were homogenized separately in 0.05 M Tris Hydrochloric acid (HCl) buffer solution (pH-7.0) at a tissue concentration of 50 mg/mL.

## Urinary peptidomics Assay
## Collection and preparation of urine sample

Urine samples were collected before scarification of all animals and lyophilized to concentrate urine. Then, the concentrated urine will store at −20°C to be used for further analysis.

## Polyacrylamide gel electrophoresis (PAGE)

Denaturing sodium dodecyl sulfate (SDS)-PAGE was made after over 28 day period of either Paracetamol administration or coadministration of different prepared doses of Arjunakwaatha and Arjunasheeta in rats with normal feeding rats. A urine sample

was concentrated on a lyophilizer. Pellet was mixed with an equal volume of ×2 electrophoresis sample buffer (0.125 m Tris-Hydrochloride, 4% SDS, 20% v/v glycerol, 0.2 M Dithiothreitol, and 0.02% bromophenol blue, pH 6.8), 5 µg protease cocktail and heated at 95°C for 5 min, was analyzed by SDS-PAGE according to the method of Laemmli using 15% acrylamide resolving gels and 10% stacking gel. Electrophoresis was performed on the Tarson electrophoresis apparatus. After washing in water for 5 min, the gels were stained with Coomassie Brilliant Blue R250 (0.1% in 25% methanol, and 10% acetic acid). The distaining step was done with Coomassie Brilliant Blue by incubation in 30% acetic acid distinguished with blue stain for protein bands.[13]

### Analysis of Western Blot for identifies KIM-1

Urine and conditioned media were prepared approximately 1 h before analysis; thereafter, it was vortexed and centrifuged at 3000×g to filter out impurities. Protein concentrations were determined according to the manufacturer's instructions using a protein assay kit (Bio-Rad, Hercules, CA, USA). Equal amounts of protein were loaded onto 6–15% SDS-PAGE gel. After electrophoresis, the PAGE was transferred to a polyvinylidene difluoride (PVDF) membrane (Bio- Rad Laboratories, USA). After transfer, the membrane was blocked at 25°C for 1 h using 4% bovine serum albumin (BSA). Then, the membrane was incubated overnight with primary antibodies at 4°C with a gentle shake and after incubation washed three times (5 minutes each time) with Tris buffer saline-Tween 20 (TBST). Subsequently, membranes were incubated with anti-goat IgG (1:10,000) or anti-rabbit IgG (1:10,000) horseradish peroxidase (HRP)-conjugate with 2% BSA for 4 hours at room temperature with gently shake. After that, the membrane was washed three times (5 minutes each time) with TBST. Finally, the blots were developed by using a DAB solution with 0.2 (M) phosphate buffer saline (PBS).[16,17]

### Statistical analysis

Statical analysis was performed using one-way analysis of variance (ANOVA). All results were expressed as mean ± standard error (SE) and 5 % level of significant ($P \leq 0.05$).[11]

## RESULTS

### Body weight (b.w.) and somatic index of kidney

The study expressed that the mean body weight and somatic index of kidney tissue significantly ($p<0.05$) increases at the end of the experiment in normal control, vehicle control, arjunakwatha at different doses of 5, 10, and 15 mg/kg of b. w./day dissolved in water as well as when treated with arjunasheeta at the different doses of 4, 8, and 12 mg/kg b. w./ day than kidney injury group in rats. Only kidney injury rats progressively lost body weight compared to healthy control and vehicle control rats (Table 1).

### Effects of Arjunakwaatha and Arjunasheeta on the levels of plasma urea,creatinine, and CRP

Plasma urea level, creatinine level, and CRP level were significantly ($p<0.05$) increased in paracetamol treated kidney injury rats as compared to control and vehicle control rats. The administration of Arjunakwatha at different doses 5mg/kg b.w., 10mg/kg b.w., 15mg/kg b.w., and 4mg/kg b.w., 8mg/kg b.w.,12mg/kg b.w., orally for 28 days were significantly ($p<0.05$) decreased in plasma urea, plasma creatinine and CRP levels as compared with kidney injury rats. When compared with kidney injury rats the plasma urea, creatinine, and CRP levels were significantly ($p<0.05$) decreased in Arjunasheeta at the dose of 4mg/kg b.w., 8mg/kg b.w.,12mg/kg b.w., orally for 28 days. Surprisingly treated with Arjunakwaatha at the dose of 10 mg/kg b.w./day and Arjunasheeta at the dose of 8 mg/kg b.w./day not only decreases the level of urea, creatinine, and CRP levels in plasma but also go back to the normal control value (Table 2).

**Table 1: Effects of different doses of arjunakwaatha and arjunasheeta on body weight of Paracetamol–induced kidney injury oxidative stress and uremia condition in rats. Data are expressed as mean ± standard error, $n=5$, $p \leq 0.05$.**

| Groups | Initial body weight (g) | Final body weight (g) | Elevation/diminution in body growth (g%) | Hepatosomatic index | Renosomatic index |
|---|---|---|---|---|---|
| Control | 190.8±3.7 | 224±4.3 | 33.2 | 2.21±0.04 | 0.49±0.02 |
| Vehicle Control | 192.4±3.8 | 224.2±4.02 | 31.8 | 2.23±0.04 | 0.49±0.01 |
| Kidney Injury | 199.8±3.4 | 166.2±2.4$^{*}$ | 33.6 | 3.94±0.1$^{*}$ | 1.35±0.11$^{*}$ |
| Arjunakwaata 5 | 195.6±1.8 | 207.4±2.4$^{*\#}$ | 11.8 | 3.01±0.1$^{*\#}$ | 1.08±0.09$^{*\#}$ |
| Arjunakwaata 10 | 197.6±1.9 | 216±2.6$^{\#}$ | 18.4 | 2.23±0.06$^{\#}$ | 0.5±0.09$^{\#}$ |
| Arjunakwaata 15 | 195.4±3.7 | 217.6±4.1$^{\#}$ | 22.2 | 2.78±0.06$^{\#}$ | 0.86±0.07$^{\#}$ |
| Arjunasheeta 4 | 194±1.6 | 206.8±2.1$^{*\#}$ | 12.8 | 3 ±0.06$^{*\#}$ | 1.07±0.08$^{*\#}$ |
| Arjunasheeta 8 | 194.4±2.08 | 212.2±2.4$^{\#}$ | 17.8 | 2.35±0.1$^{\#}$ | 0.53±0.1$^{\#}$ |
| Arjunasheeta 12 | 194.2±3.4 | 215±4.05$^{\#}$ | 20.8 | 2.76±0.1$^{\#}$ | 0.88±0.14$^{\#}$ |

(*): $p \leq 0.05$; significantly compared with KI group. (#): $p \leq 0.05$; significantly compared with control group, (↑): Increase, (↓): Decrease.

## Effects of Arjunakwaatha and Arjunasheeta on urine urea, creatinine, total protein and urinary KIM-1

Urine urea and creatinine level were significantly ($p$<0.05) decreased whereas urine total protein and urinary KIM-1 levels were significantly ($p$<0.05) increased in kidney injury rats as compared to control and vehicle control rats. The administration of Arjunakwaatha at different doses of 5mg/kg b.w., 10mg/kg b.w., 15mg/kg b.w., and 4mg/kg b.w., 8mg/kg b.w.,12mg/kg b.w., a dose of Arjunasheeta orally for 28 days significantly ($p$<0.05) increase in urine urea and creatinine whereas urine total protein and urinary KIM-1 level were significantly($p$<0.05) decreased as compared with kidney injury in rats. Surprisingly, Arjunakwaatha at the dose of 10 mg/kg b.w./day and Arjunasheeta at the dose of 8 mg/kg b.w./day not only increases the level of urea, but creatinine in urine also go back to the normal control value (Table 3).

## Effects of Arjunakwaatha and Arjunasheeta on plasma GOT, GPT and plasma total protein

Plasma GOT level and GPT level were significantly ($p$<0.05) increased whereas total protein levels were significantly($p$<0.05) decreased in kidney injury rats as compared to control and vehicle control in rats. The administration of Arjunakwaatha at different doses of 5mg/kg b.w., 10mg/kg b.w., 15mg/kg b.w., and 4mg/kg b.w., 8mg/kg b.w.,12mg/kg b.w., a dose of Arjunasheeta orally for 28 days was significantly ($p$<0.05) decreased GOT and GPT levels in plasma whereas total protein was significantly($p$<0.05) increased as compared with kidney injury in rats. However, the total protein level of plasma not only increases but also goes back to the normal level at the dose of Arjunakwaatha 10 and Arjunasheeta 8 groups in rats (Table 4).

## Effects of Arjunakwaatha and Arjunasheeta on SOD, CAT, GSH and MDA activity of kidney tissue

Here, the present study showed that the oxidative stress parameters like CAT, SOD, and GSH levels were significantly ($p$<0.05) decreased in kidney injury rats when compared with control as well as vehicle control rats. After 28 days of oral administration of Arjunakwaatha and Arjunasheeta in all treatment groups, the levels of CAT, SOD, and GSH were gradually increased compared with kidney injury rats (Figure 2, Figure 3 and Figure 4).

In kidney injury rats, the MDA activity increased significantly ($p$<0.05) when compared to control as well as vehicle control rats. After 28 days of oral administration of Arjunakwaatha and

**Table 2: Effects of different doses of arjunakwaatha and arjunasheeta on plasma urea, creatinine, and CRP level of Paracetamol-induced kidney injury in rats. Data are expressed as mean ± standard error, $n$=5, $p$<0.05.**

| Groups | Plasma Urea (mg/dL of plasma) | Plasma Creatinine (mg/dL of plasma) | CRP (mg/dL of plasma) |
|---|---|---|---|
| Control | 26.59±0.15 | 0.31±0.01 | 2.44±0.14 |
| Vehicle Control | 26.91±0.1 | 0.34±0.01 | 2.52±0.12 |
| Kidney Injury | 76.33±0.3* | 1.11±0.02* | 7.04±0.12* |
| Arjunakwaatha 5 | 57.93±0.28*# | 0.82±0.01*# | 4.06±0.08*# |
| Arjunakwaatha 10 | 27.9±1.7# | 0.32±0.01# | 2.82±0.07# |
| Arjunakwaatha 15 | 29.15±0.03# | 0.43±0.01# | 2.94±0.08# |
| Arjunasheeta 4 | 58.59±0.22*# | 0.84±0.01*# | 4.16±0.14*# |
| Arjunasheeta 8 | 28.36±2.1# | 0.33±0.01# | 2.88±0.1# |
| Arjunasheeta 12 | 30.66±0.03# | 0.44±0.01# | 2.97±0.11# |

(*): $p \leq 0.05$; significantly compared with KI group.( #): $p \leq 0.05$; significantly compared with control group.

**Table 3: Effects of different doses of arjunakwaatha and arjunasheeta on urine urea, creatinine, urinary KIM-1, and urine total protein of Paracetamol-induced kidney injury in rats. Data are expressed as mean ± standard error $n$=5, $p \leq 0.05$.**

| Groups | Urine urea (mg/dL) | Urine creatinine (mg/dL) | Urinary total protein (g/dL) | Urinary KIM-1 (pg/dL) |
|---|---|---|---|---|
| Control | 89.1±0.08 | 4.44±0.2 | 0.22±0.22 | 169±0.1 |
| Vehicle Control | 89.2±0.13 | 4.34±0.2 | 0.22±0.3 | 169.1±0.06 |
| Kidney Injury | 40.2±0.11* | 1.68±0.2* | 0.69±0.5* | 398.1±0.05* |
| Arjunakwaatha 5 | 56.8±0.08*# | 2.43±0.2*# | 0.34±0.3*# | 298.1±0.02*# |
| Arjunakwaatha 10 | 89.1±0.18# | 4.14±0.2# | 0.29±0.2# | 196.2±0.01# |
| Arjunakwaatha 15 | 89.01±0.35# | 3.96±0.1*# | 0.31±0.3*# | 198.1±0.01*# |
| Arjunasheeta 4 | 57.04±0.09*# | 2.18±0.1*# | 0.35±0.3*# | 299.3±0.01*# |
| Arjunasheeta 8 | 89.2±0.06# | 4.04±0.1# | 0.3±0.4# | 197.1±0.01*# |
| Arjunasheeta 12 | 89.04±0.53# | 3.58±0.1*# | 0.32±0.3*# | 199.2±0.01*# |

(*): $p \leq 0.05$; significantly compared with KI group.( #): $p \leq 0.05$; significantly compared with control group.

**Table 4: Effects of different doses of aqueous extract of arjunakwaatha and arjunasheeta on plasma toxicity level and plasma total protein on Paracetamol-induced kidney injury in male rats. Data are expressed as mean ± standard error, *n*=5, *p*≤ 0.05.**

| Groups | GOT (U/L of plasma) | GPT (U/L of plasma) | Plasma total protein (g/dL of plasma) |
|---|---|---|---|
| Control | 18.1±0.32 | 26.2±0.34 | 6.06±0.09 |
| Vehicle Control | 18.7±0.51 | 26.1±0.33 | 6.12±0.13 |
| Kidney Injury | 73.5±0.23* | 71.4±0.21* | 4.6±0.18* |
| Arjunakwaatha 5 | 42.4±0.21*# | 60.2±0.27*# | 4.86±0.23*# |
| Arjunakwaatha 10 | 27.4±0.22# | 33.1±0.39# | 6.04±0.2# |
| Arjunakwaatha 15 | 29.1±0.37# | 34±0.27* | 5.9±0.33# |
| Arjunasheeta 4 | 43.3±0.3*# | 61.3±0.18*# | 4.98±0.29# |
| Arjunasheeta 8 | 28.5±0.23# | 33.5±0.22# | 5.94±0.39# |
| Arjunasheeta 12 | 30±0.27# | 34±0.28# | 5.86±0.34# |

(*): *p*≤ 0.05; significantly compared with KI group.( #): *p*≤ 0.05; significantly compared with control group.

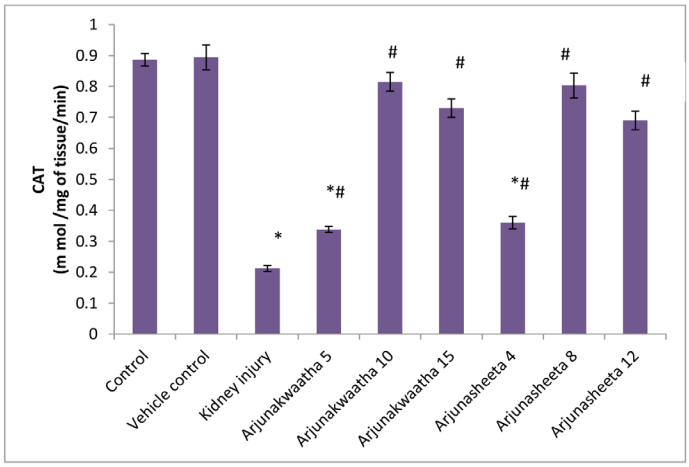


**Figure 2:** Effects of different doses of Arjunakwaatha and Arjunasheeta on CAT activity of kidney tissue on Paracetamol-induced kidney injury in male rats. Data are expressed as mean ± standard error (*n*=5), (*): *P*≤ 0.05; significantly compared with KI group.( #): *P*≤ 0.05; significantly compared with control group.

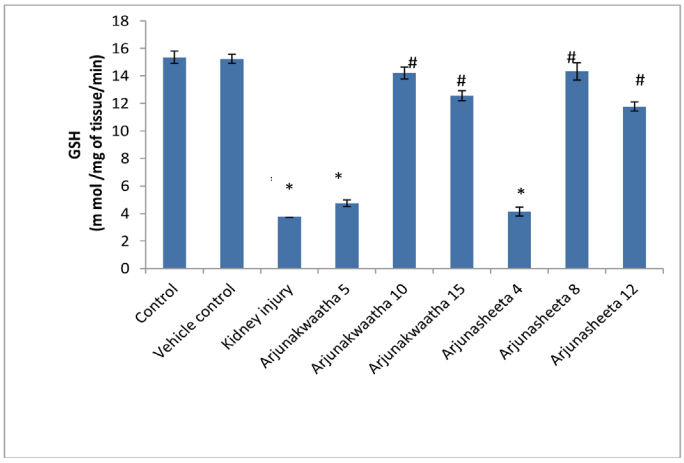


**Figure 4:** Effects of different doses of Arjunakwaatha and Arjunasheeta on GSH activity of kidney tissue on Paracetamol-induced kidney injury in male rats. Data are expressed as mean ± standard error (*n*=5), (*): *P*≤ 0.05; significantly compared with KI group.( #): *p*≤ 0.05; significantly compared with control group.

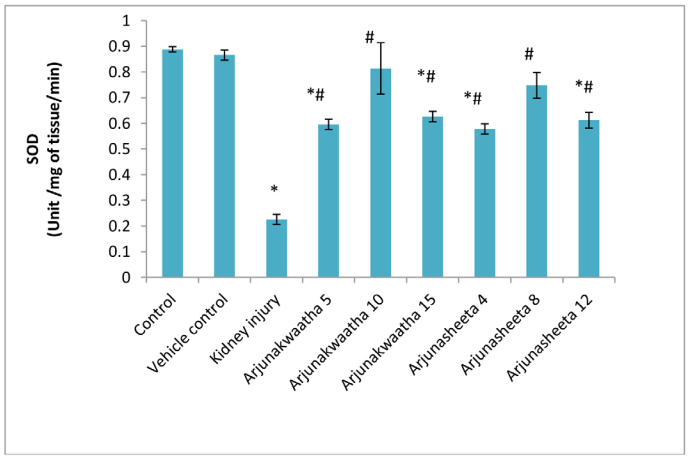


**Figure 3:** Effects of different doses of Arjunakwaatha and Arjunasheeta on SOD activity of kidney tissue on Paracetamol-induced kidney injury in male rats. Data are expressed as mean ± standard error (*n*=5), (*): *P*≤ 0.05; significantly compared with KI group.( #): *P*≤ 0.05; significantly compared with control group.

Arjunasheeta extract in all treatment groups significantly ($p<0.05$) reduced the MDA activity compared to the kidney injury group. However, compared with the kidney injury group Arjunakwaatha 10 and Arjunasheeta 8 shows surprisingly the preferred effect in reducing the MDA activity (Figure 5).

## SDS-PAGE and Western blot analysis of urinary protein

SDS-PAGE showed different protein aggregated bands (Figure 6) corresponding to the molecular weight marker (MWM). MWM represented ten separate protein bands showing 70 kDa to 10 kDa in descending orders (Laned). Lane 2 and lane 3 represented the urinary protein bands at between 18 kDa and 14 kDa of control and vehicle control group rats and there was no other band. Urinary protein bands in lane 4 observed new bands at 35,18 15, and 10 kDa of kidney injury group rats when compared to lane 2 and lane 3 and also observed that band intensity was higher than lane 2 and lane 3. The co-administration of Arjunasheeta

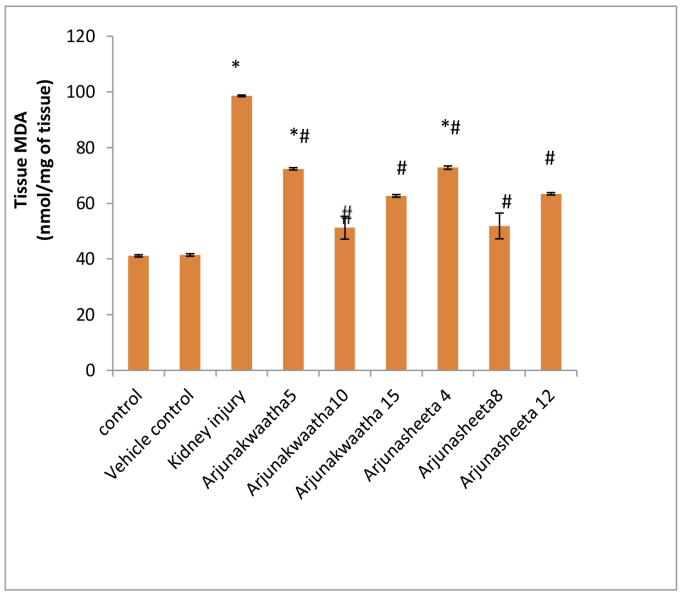


**Figure 5:** Effects of different doses of Arjunakwaatha and Arjunasheeta on MDA activity of kidney tissue on Paracetamol-induced kidney injury in male rats. Data are expressed as mean ± standard error ($n$=5), (*): $P \leq 0.05$; significantly compared with KI group.( #): $P \leq 0.05$; significantly compared with control group.

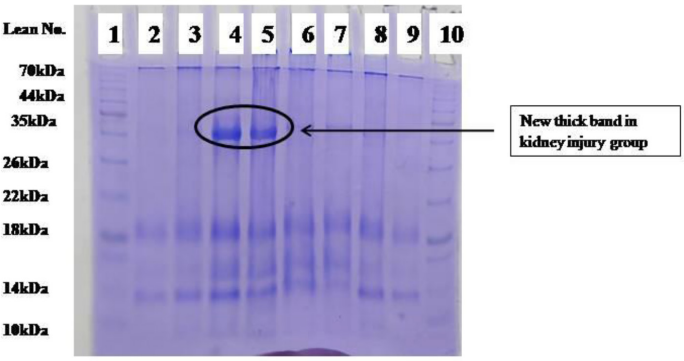


**Figure 6:** SDS-PAGE analysis from urine samples of Control, Vehicle Control, Kidney injury (KI), KI+ Arjunakwaatha 10, KI + Arjunasheeta 8 performed with standard molecular weight markers (MWM). Lane 1 and 10, represent the MWM with 9 separate protein bands showing 70 kDa to 10 kDa in descending orders, Lane 2 control and Lane 3 Vehicle control, Lane 4 and 5 kidney injury, Lane 6 and 7 Arjunasheeta 8, Lane 8 and 9 Arjunakwaatha 10.

and Arjunakwaatha in different doses represented the light or no urinary protein bands in lane 5, lane 6, lane 7 lane 8 lane 9, and lane 10 between 35 kDa to 14 kDa. However, Arjunakwaatha 10 mg/kg b.w. shows no bands in 35 kDa to 14 kDa and Arjunasheeta 8 mg/kg b.w. observed that band intensity was less than Lane 4.

Western blot results represented (Figure 7) high levels of urinary KIM-1in kidney injury rats whereas control and vehicle control rats did not show KIM-1. The quantification of KIM-1 in the urine by ELISA corresponded well with KIM-1 Western blot analysis.

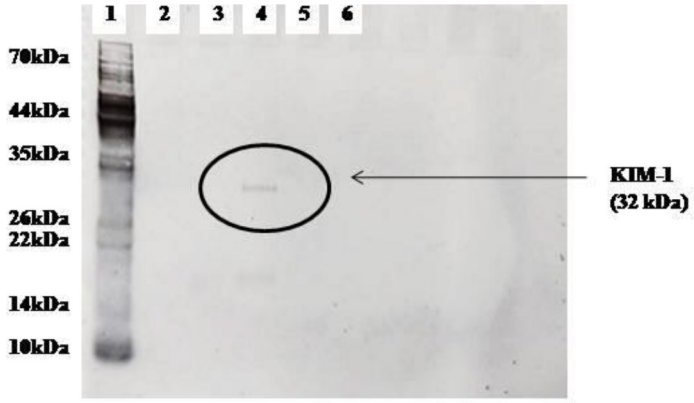


**Figure 7:** Western blot analysis from urine samples of Control, Vehicle Control, Kidney injury (KI), KI+ Arjunakwaatha10, KI + Arjunasheeta 8 performed with standard molecular weight markers (MWM). Lane 1, represent the MWM, Lane 2 control, Lane 3Vehicle control, Lane 4 kidney injury, Lane 5 Arjunasheeta 8, and Lane 6 Arjunakwaatha 10.

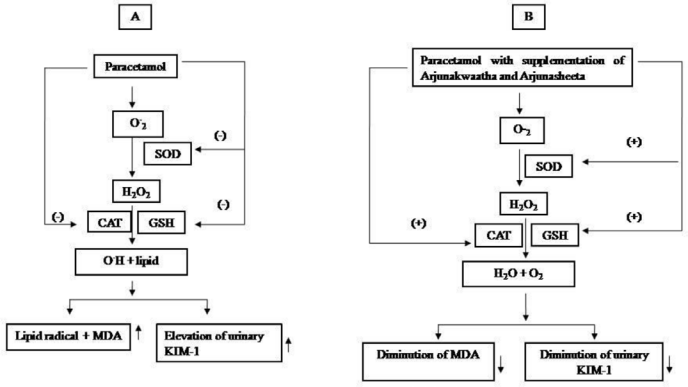


**Figure 8:** A. Paracetamol reduce SOD, CAT, GSH activity and increase oxidative stress and lipid peroxidation. B. Supplementation of Arjunakwaatha and Arjunasheeta expand SOD, CAT, GSH and decrease oxidative stress and lipid peroxidation.

'+': Induction, '-': Inhibition".

'↑': Increase , '↓': Decrease

## DISCUSSION

Some tribal populations of Paschim Medinipur district, West Bengal, India use Traditional Medicine to prevent and or cure various ailments due to the high cost of drugs. Their traditional medical practises are the consumption of various medical plants or herbs by soaking one or more parts of plants in water overnight and consuming the filtrate the next morning on an empty stomach. Other people boil one or more parts of medicinal plants in water and consume the filtrate. The present study followed those practices, and we prepared the Arjunakwaatha and Arjunasheeta from the bark of *Terminalia arjuna* (TA). Arjunakwaatha and Arjunasheeta were found as potential nephroprotectives in a paracetamol-induced kidney injury rat model.

In this study, paracetamol overdose in rats caused a significant increase in plasma levels of urea, creatinine, CRP, GOT, and, GPT

indicating kidney injury in the rats. Further, kidney injury by excess paracetamol injection was proved by significant urinary elevation of KIM-1 and significant diminution of urinary urea and creatinine levels. It has also been revealed that the kidney injury has been detected by detecting urinary KIM-1 expression with mouse monoclonal antibodies in western blot analysis and a new urinary protein band in SDS-PAGE analysis. Other kidney injuries were also established by other workers using excess paracetamol injections.[18-20]

Excess paracetamol causes the overproduction of reactive oxygen species (ROS) in kidney tissue by altering the oxidant-antioxidant balance with an increase in lipid peroxidation.[22] In the study, we observed that paracetamol treated rats were associated with increased MDA levels and a significant decrease in SOD, CAT, and GSH activity. The SOD-CAT-GSH renal antioxidant enzyme axis maintains the oxidant-antioxidant balance in the normal kidney where disbalance of that axis indicates kidney injury[20] (Figure 8). SOD reduces the super oxide radical into hydrogen peroxide. The catalase enzyme converts hydrogen peroxide into water and oxygen with the help of GSH. The elevated level of MDA in the kidney focuses the increase in lipid peroxidation, indicating the damaged tissue and failure of the activity of the renal antigen enzyme axis.

KIM-1 is a type I transmembrane protein found in urine due to proximal tubular injury and a high urinary level of KIM-1 indicates serious tubular damage.[20] CRP is an acute-phase protein synthesised by macrophages of inflamed renal tissue. A high CRP level in the plasma indicates renal and hepatic injury.

Elevations of urea and creatinine levels were dramatically reduced in the Arjunakwaatha and Arjunasheeta receiving groups. Arjunakwaatha and Arjunasheetha contain various antioxidant components due to their being prepared from bark of *Terminalia arjuna* (TA).[22] Giri *et al*. found that an aqueous extract of TA lowered urea and creatinine levels in rats with paracetamol-induced CKD,[13] which matched the results of the current investigation. Arjunakwaatha and Arjunasheeta improved kidney tissue antioxidant capacity and reduced the concomitant oxidative stress, as indicated by elevations in GSH levels and SOD activity, an increase in CAT activity, and the decreases in the tissue levels of MDA compared to those of kidney injury rats (Figure 8). A similar study has been carried out by Das *et al*., in which they found the beneficial effect of TA on reducing oxidative stress and improving antioxidant capacity in kidney tissue.[11] Arjunakwaatha and Arjunasheeta markedly reduced KIM-1 urinary levels. This reduction is most likely due to the more powerful direct antinecrotic ability of Arjunakwaatha and Arjunasheeta, leading to a low urinary level of KIM-1. However, these remedies may also counteract the kidney injury indirectly by blocking urinary KIM-1 release through scavenging oxidative stressors.

## CONCLUSION

Arjunakwaatha and Arjunasheeta has a significant potential against kidney injury rats by reducing high level of urea, creatinine, KIM-1, oxidative stress and increasing anti-oxidant enzyme activity. However, more studies are needed to investigate the precise mechanism of Arjunakwaatha and Arjunasheeta function at the molecular level in future.

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## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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