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Original article

Nephroprotective effect of ethanolic extract of *Azima tetraacantha* root in glycerol induced acute renal failure in Wistar albino rats



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ABSTRACT

The gravity of the impact of renal failure on human health is well known and as there is no specific pharmacotherapy for renal failure, the current study was undertaken to evaluate the effect of root extract of *Azima tetraacantha*, an ancient medicinal plant used in Siddha and Ayurvedic systems of medicine.

The experiment was done in glycerol-induced acute renal failure in Wistar albino rats. Thirty rats were divided into five groups. Group 1 was given normal saline (10 ml/kg) per oral, group 2 with single dose of hypertonic glycerol (8 ml/kg) by intramuscular injection into the hind limbs, group 3 with glycerol and ethanolic extract of *A. tetraacantha* root (ATR) 250 mg/kg, group 4, glycerol and ATR 500 mg/kg and group 5, 500 mg/kg ATR. Extract was given orally 60 min prior to glycerol injection. 24 h urine output, serum creatinine, blood urea nitrogen, total proteins and albumin were measured for all the groups. Kidneys were examined for histopathological changes.

The antioxidant activity of the extract was tested *in vitro* and *in vivo*. Rats treated with ATR showed significant improvement in biochemical parameters and histopathological changes compared to glycerol treated group. The protective effect was highly significant at 500 mg/kg. Both *in vitro* and *in vivo* assays showed significant antioxidant activity. The *in vitro* activity was comparable to vitamin-C.

The ethanolic extract of ATR has nephroprotective effect in glycerol-induced acute renal failure and the mechanism of action could be the antioxidant effect.

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1. Introduction

The term acute renal failure (ARF) is currently substituted by acute kidney injury (AKI). AKI is a reversible condition in which there is a sudden decline in renal function, manifested by hourly/daily/weekly elevation in serum creatinine and blood urea nitrogen (BUN).¹

Different organizations such as Acute Dialysis Quality Initiative (ADQI), Acute Kidney Injury Network (AKIN) and Kidney Disease International Global Outcome (KDIGO) have provided different definitions for acute kidney injury. Among these definitions of AKI, the most acceptable one is that of KDIGO²:

AKI is any of the following³

- “Increase in serum creatinine (SCr) by ≥ 0.3 mg/dl (≥ 26.5 $\mu\text{mol/l}$) within 48 h” or
- “Increase in SCr to ≥ 1.5 times baseline, which is known or presumed to have occurred within the prior 7 days “ or
- “Urine volume < 0.5 ml/kg/h for 6 h”.

The incidence of AKI in the community is 2147 and 4085 per million populations per year (pmp) in developing and developed

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nations.^{4,5} Recent reports in the developed world indicate that AKI is seen in 3.2–9.6% of hospital admissions with overall mortality of 20%–50% in ICU patients.^{6,7} AKI demanding renal replacement therapy is 5–6% with a high in-hospital mortality rate of 60%. It is estimated that nearly 2 million people die of AKI every year globally.^{8,9} Those who survive AKI are at a greater risk for later development of chronic kidney disease (CKD).

The causes of AKI could be pre-renal, renal and post-renal ones. Pre-renal failure, the commonest form of acute renal failure, is due to decrease in renal blood flow primarily as a result of hypovolemia. It is reversible if the cause of the decreased renal blood flow can be identified and rectified before kidney damage occurs. Pre-renal causes account for 40–70% of all AKI cases.¹⁰ Intrinsic renal causes of AKI include diseases that affect the renal parenchyma which can be divided based on the compartment of the kidney that is affected like tubular injury, tubulointerstitial diseases, diseases of the renal microcirculation and glomeruli and diseases of larger renal vessels.¹¹ Post-renal causes of AKI are diseases associated with urinary tract obstruction which account for 5% of renal failures.¹¹

1.1. Management of ARF

The management of ARF is mainly supportive and in refractory patients renal replacement therapy (RRT) may be the best option.¹² RRT refers to life supporting measures which include hemodialysis, peritoneal dialysis, hemofiltration and renal transplantation. In spite of RRT, mortality in patients with AKI remains high and this may be due to the severity of the disease or the adverse effects of RRT.¹³

As there is no effective pharmacotherapy for AKI, seeking alternative treatments becomes a necessity. Use of medicinal plants in renal failure goes back to ancient days. More than 221 plants have been screened for nephroprotective activity both in acute and chronic renal failure models.¹⁴

Some of the medicinal plants such as *Ocimum sanctum*, *Aerva lanata*, *Aegle marmelos*, *Pongamia pinnata*, *Salviae radix*, *Ginkgo biloba*, *Allium sativum*, *Cassia auriculata*, *Nigella sativa*, *Drynaria fortune*, *Tribulus terrestris* and others are reported to have nephroprotective activity.¹⁵

Azima tetraacantha (AT) has been traditionally used for many diseases including renal disease.¹⁶ It belongs to the family, Salvadoraceae and known as mulchangu in Siddha and kundali in Ayurvedha.¹⁷ Its common names are Needle brush (English), Kantagurkamai (Hindi) and Cankakiranam (Tamil).¹⁸

AT has been reported to have antimicrobial, antifungal, anti-inflammatory, analgesic, antioxidant, antipyretic, antiulcer, anticancer, anti-snake venom, diuretic and hepato-protective activities.^{19–23}

Though AT is traditionally used for nephroprotective effect, it has not been evaluated by either preclinical or clinical studies. Hence this study was undertaken to evaluate its action in animal models of renal failure.

Root and stem: flavonoids – myricetin, quercetin, rutin, isorhamnetin, rhamnetin and rhamnazin.

Leaf and stem: fatty acids.

Leaf: terpenoid – friedelin.

2. Objectives

The objectives were to evaluate the nephroprotective activity of ethanolic extract of *A. tetraacantha* root in glycerol-induced acute renal failure in Wistar albino rats and to find out the probable mechanism.

3. Materials and methods

The study was initiated after getting IAEC (Institutional Animal Ethics Committee) approval.

3.1. Chemicals and reagents

Chemicals for *in vitro* and *in vivo* antioxidants assays were purchased from Sigma Aldrich, New Delhi, India.

3.2. Preparation of *A. tetraacantha* root (ATR) extract

The plant was collected from Tindivanam, Villupuram district, Tamil Nadu, India and it was identified and authenticated by Dr. Narasimhan, Associate Professor of Botany, Madras Christian College, Chennai, Tamil Nadu. One kg of roots was thoroughly washed with water and air dried under shade at room temperature for 30 days and milled into a coarse powder. The dried powder obtained was subjected to continuous extraction with 95% v/v ethanol in Soxhlet apparatus for 15 cycles. The extract was then dried in a flash evaporator. 55.3 g of green colored, thick and sticky residue was obtained. It was reconstituted in 1% gum acacia before administering to animals. The phytochemical analysis was carried out by HPTLC studies.

3.3. Animals

Thirty healthy male Wistar albino rats weighing between 150 and 250 g were obtained from the central animal house of Chettinad Hospital and Research Institute.

3.4. Study of *in vitro* antioxidant activity

The antioxidant activity of ATR ethanolic extract was evaluated by 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay, nitric oxide (NO) assay, hydrogen peroxide (H₂O₂) and ferric reducing antioxidant power (FRAP) assay. Vitamin C was used as a positive control. All assays were performed using spectrophotometer and the values were calculated by the following formula.

$$\text{Percentage inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of test} \times 100}{\text{Absorbance of control}}$$

1.2. Phytochemical components in different parts of *A. tetraacantha*^{24,25}

Whole plant: alkaloids – azimine, azacarpaine and carpaine.

Root and fruit: glycosides – N-methoxy-3-indolylmethyl-glucosinolate, iodole glucosinolate and N-methoxy indole 3- corbinol.

The ATR extract was prepared in increasing concentrations from 25 to 1600 µg/ml for all the antioxidant assays.

3.4.1. DPPH scavenging assay

One ml of ATR extract solution was mixed with 1 ml of ethanolic solution of DPPH (200 µM). Another 1 ml DPPH solution was mixed

with 1 ml of ethanolic solution of vitamin- C (200 µg/ml). A mixture of 1 ml of ethanolic solution of DPPH (200 µM) and 1 ml of ethanol served as control. After mixing, all the solutions were incubated in dark for 2 min and absorbance was measured at 517 nm.²⁶ The experiments were carried out in triplicate and the average percentage of inhibition was calculated by the above formula.

3.4.2. NO scavenging assay

Three ml of 10 mM sodium nitroprusside in phosphate buffer is added to 2 ml of ATR extract and the reference compound. The resulting solutions were then incubated at 25 °C for 60 min. 5 ml of the incubated sample and 5 ml of Griess reagent (1% sulphanilamide, 0.1% naphthyl ethylenediamine dihydrochloride in 2% phosphorous acid) were added and absorbance of the chromophore formed was measured at 540 nm.²⁷

3.4.3. H₂O₂ scavenging assay

Two ml ATR extract was mixed with 0.6 ml of 4 mM hydrogen peroxide solution prepared in phosphate buffer (0.1 M pH 7.4) and incubated for 10 min. The absorbance of the solution was taken at 230 nm.²⁸

3.4.4. FRAP scavenging assay

The assay mixture contained 2.5 ml of 300 mM acetate buffer at pH 3.6, 0.25 ml of 10 mM 2, 4,6-tripyridyl-s-triazine solution in 40 mM HCl, 0.25 ml of 20 mM ferric chloride and ATR extract at various concentrations in 0.1 ml of methanol. The absorbance was measured after 30 min incubation at 593 nm. FRAP values were measured by comparing the absorption change in the test mixture with that obtained from increasing concentrations of Fe³⁺.²⁹

3.5. Acute toxicity study

Acute oral toxicity study was conducted as per OECD-423 guidelines. Female rats (150–200 g), nulliparous and non-pregnant were randomly distributed to four groups (1–4) of 3 each. The rats were fasted overnight and given orally 5, 50, 300 and 2000 mg/kg of ATR extract. They were observed continuously for the first 2 h and hourly for the next 6 h and at 24, 48, and 72 h for general behavior, convulsions and mortality.

No mortality or toxicity was observed up to the dose of 2000 mg/kg. Hence, the drug was found to be safe. Based on the acute toxicity studies 250 and 500 mg/kg of the ethanolic extract were used for the current study.

3.6. In vivo evaluation

Induction of ARF: ARF was induced by intramuscular injection of single dose of 50% hypertonic glycerol (8 ml/kg) into both the hind limbs in equal volume as per the standard methods prescribed.^{30,31}

3.7. Experimental design

Rats were divided into 5 groups of 6 animals in each. Group 1 was treated with normal saline (10 ml/kg) per oral, group 2 with single dose of hypertonic glycerol (8 ml/kg) by intramuscular injection into both the hind limbs, group 3 with glycerol and ATR extract 250 mg/kg, group 4 with glycerol and ATR extract 500 mg/kg and group 5 was treated with ATR extract 500 mg/kg alone. The ATR extract was given by oral route 60 min prior to glycerol injection. The animals were observed for general behavior and activity. Urine output was measured for all the animals for 24 h.

After 24 h, the blood samples were collected by retro orbital sinus puncture and tested for serum creatinine, blood urea nitrogen, total proteins and albumin. All the levels were measured using

diagnostic kits.³² The animals were then sacrificed by exposure to halothane. The kidneys were isolated immediately and sent for histopathological examination.

3.8. Estimation of antioxidant activity

The homogenized kidney was used for estimation of antioxidants. The antioxidants, superoxide dismutase (SOD),³³ reduced glutathione (GSH),³⁴ glutathione reductase (GR),³⁵ glutathione peroxidase (GPx),³⁶ and catalase (CAT)³⁷ were determined using standard methods prescribed.

3.9. Histopathology

Kidneys were rinsed with normal saline and then fixed in 10% neutral buffered formalin solution, embedded in paraffin and used for histopathological examination. The sections were 2 µm thick, deparaffinized, hydrated and stained with haematoxylin and eosin. The renal sections were examined for the extent of damage to glomeruli, tubules and interstitium as well as for capillary congestion and hemorrhage.

3.10. Statistical analysis

The data were expressed as mean ± S.E.M. One way analysis of variance (ANOVA) traced by Tukey's multiple range test was applied to calculate the statistical significance among different groups. P < 0.05 was considered to be statistically significant.

4. Results

4.1. HPTLC profile

The results from HPTLC finger print scanned at wavelength 254 nm for ethanolic extract of ATR showed 15 polyvalent phyto-constituents (Graph) and corresponding retarding fraction (Rf), height, area and lambda max (Table 1).

4.2. In vitro antioxidant activity

Ethanolic extract of *A. tetraantha* root in graded concentrations was tested for antioxidant activity in four different *in vitro* methods and compared with the standard antioxidant, vitamin C.

4.2.1. DPPH assay

Percentage of inhibition of DPPH radicals by different concentrations of ATR extract was 46% at 25 µg/ml and 89% at 1600 µg/ml. Standard drug vitamin- C showed 50 % inhibition at 25 µg/ml and 95% at 1600 µg/ml (Table 2).

4.2.2. NO assay

The percentage inhibition of NO by ATR extract was 24% at 25 µg/ml and 91% at 1600 µg/ml. Vitamin-C showed 28 % and 95% inhibition at 25 and 1600 µg/ml respectively (Table 3).

4.2.3. H₂O₂ assay

ATR extract showed 23% and 88% at 25 and 1600 µg/ml and vitamin C at the same concentration showed 28 and 99% of inhibition (Table 4).

4.2.4. FRAP assay

ATR extract had shown 22% inhibition at 25 µg/ml and 87% inhibition at 1600 µg/ml. Vitamin-C showed 28 % and 98% inhibition at 25 and 1600 µg/ml respectively (Table 5).

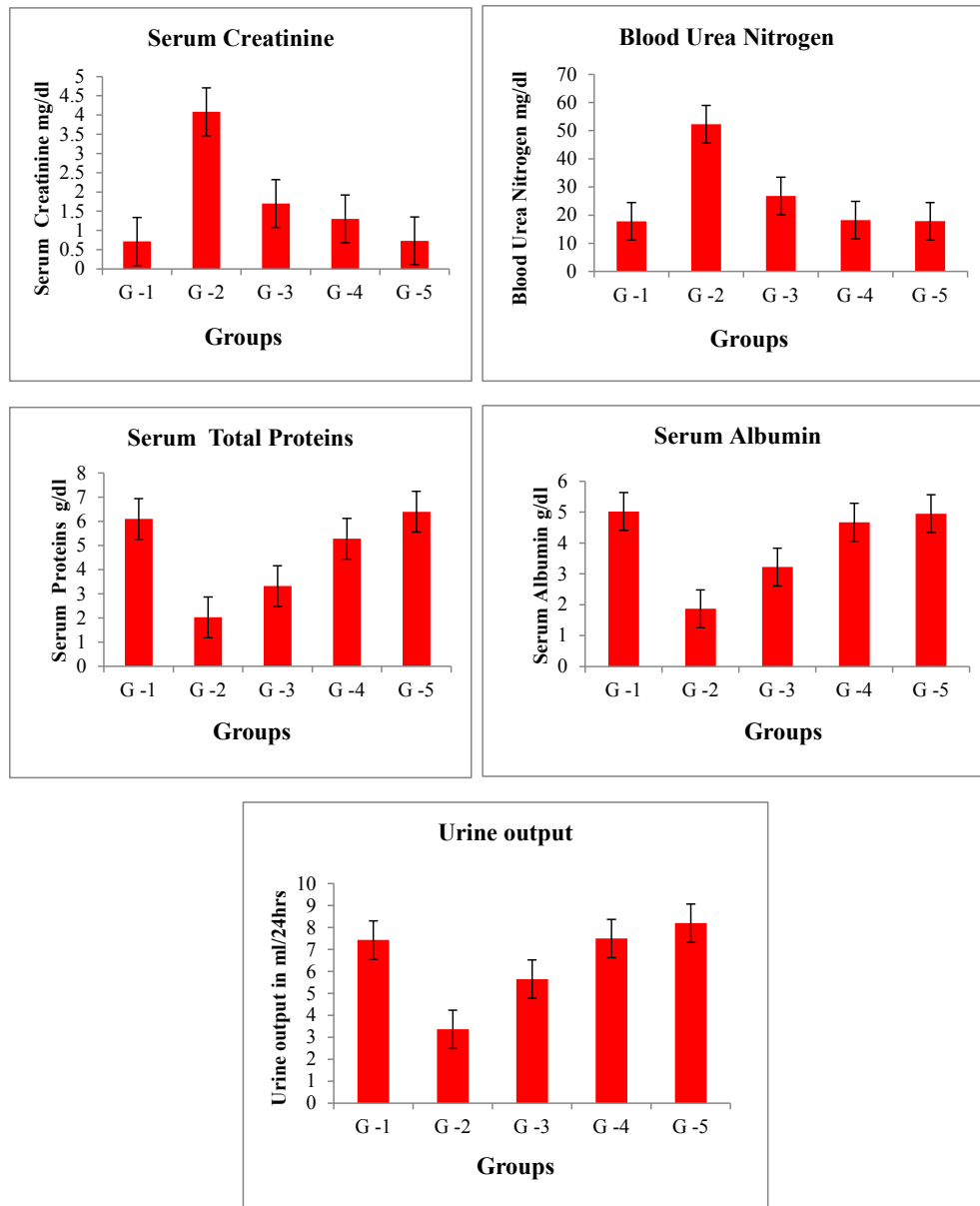


Fig. 1. Biochemical parameters.

4.3. Nephroprotective activity

Nephroprotective activity was assessed by the levels of serum creatinine, blood urea nitrogen, total proteins, albumin and urine output as well as histopathological changes (Table 6).

Increased levels of serum creatinine, BUN and decreased total proteins, albumin and urine output in glycerol treated animals were seen (group 2) compared with normal saline treated animals (group 1) confirming renal failure. In ATR extract treated groups there was a significant decrease ($p < 0.05$) in serum creatinine, blood urea nitrogen and increase in total proteins, albumin and urine output at both the doses of 250 and 500 mg/kg. The group treated with ATR extract alone (group 5) did not show any change in the above parameters. (Fig. 1, biochemical parameters).

4.4. Enzymatic antioxidants

SOD, GSH, GR, GPx and CAT levels were significantly ($p < 0.05$) increased in group 3 and 4 compared with group 2. ATR extract at

500 mg/kg had shown higher antioxidant levels than 250 mg/kg (Fig. 2, *in vivo* antioxidant activity).

4.5. Histopathology

Rat kidney in the control group had shown normal glomeruli with intact Bowman's capsule, normal proximal convoluted and distal convoluted tubules and there was no capillary congestion or hemorrhage (group 1). Severe distortion of glomeruli, capillary congestion, hemorrhages and apical blebbing were seen in glycerol treated group indicating renal damage. In group 3 and 4, treated with 250 and 500 mg/kg of ATR extract showed markedly reduced capillary congestion, tubular damage and glomerular distortion compared to glycerol treated group. The protective effect was found to be better with 500 mg/kg than 250 mg/kg. Group 5 animals were not sacrificed as they did not show abnormalities in biochemical parameters. The histopathological changes were found to be in correlation with biochemical changes. (Fig. 3, histopathology).

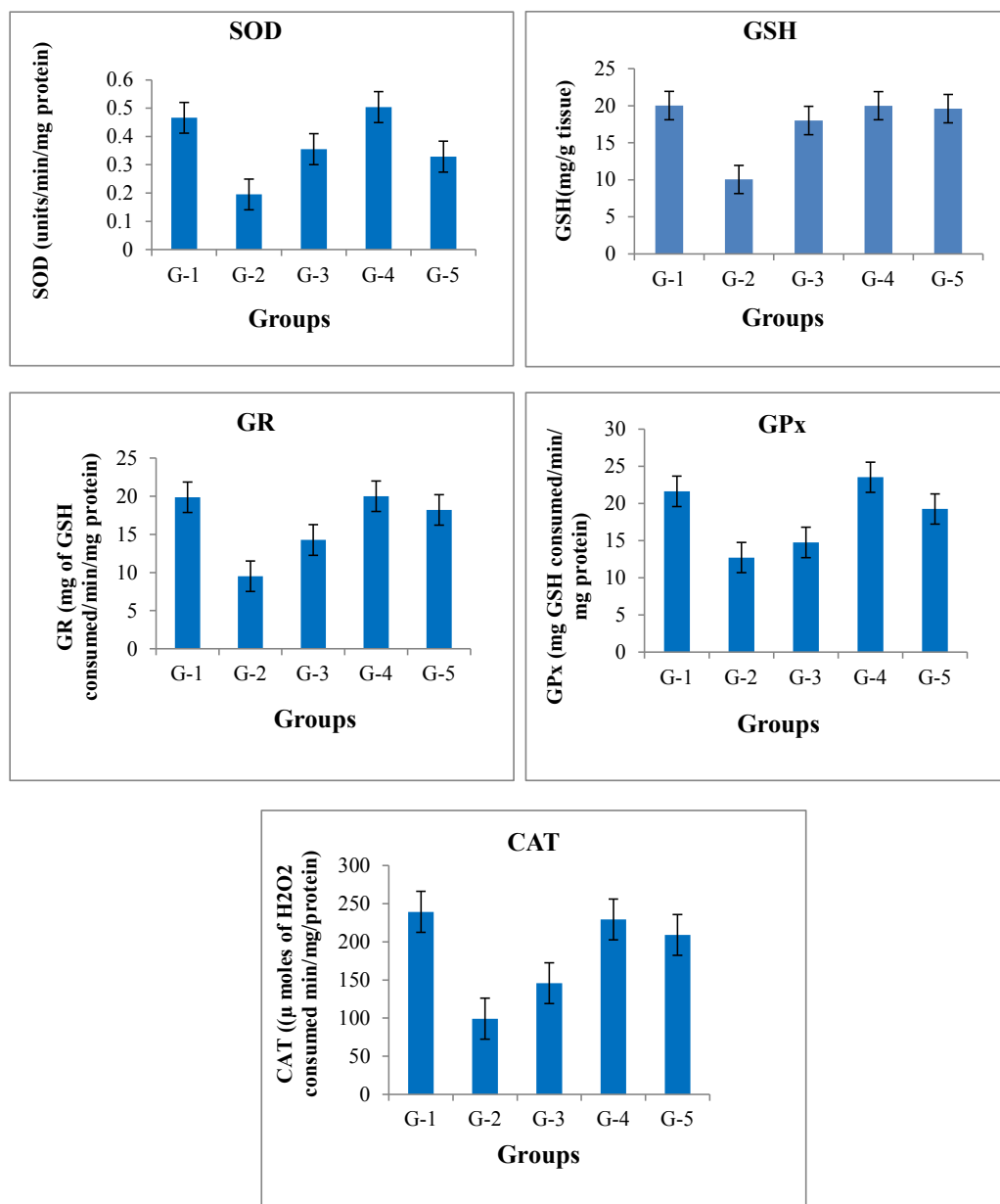


Fig. 2. *In vivo* antioxidant activity.

5. Discussion

The present study has evaluated the effect of *A. tetraacantha* root extract in glycerol-induced acute renal failure and its antioxidant potential both *in vitro* and *in vivo*.

Glycerol-induced acute renal failure is one of the commonest animal models used for evaluation of nephroprotective activity.^{30,31} The mechanisms involved in glycerol-induced renal failure include oxidative stress, inflammation and apoptosis^{38,39} which lead to rhabdomyolysis resulting in myoglobinemia, myoglobinuria and cast formation.⁴⁰ Cast formation causes tubular obstruction and reduced GFR. Rhabdomyolysis causes cytokine activation and release of inflammatory mediators such as IL-1, ICAM-1 and TNF α .⁴¹ These pro-inflammatory cytokines mediate the inflammatory reaction causing afferent and efferent renal arteriolar vasoconstriction⁴² eventually leading to renal failure. In general, about 10–40% of patients with rhabdomyolysis develop ARF.⁴³

As oxidative stress and inflammatory damage have been mainly attributed to the development of acute kidney injury, any compound or drug having antioxidant & anti-inflammatory activity will have a protective action against renal damage.

In our study ATR extract has shown a protective effect evidenced by its inhibition of alteration of renal biochemical parameters and reversal of histopathological changes. Prior administration of ATR extract has caused reduction in serum creatinine, blood urea nitrogen and increase in total proteins and albumin. In addition the urine output increased in ATR treated animals. The histopathological examination has shown significant reduction in cellular infiltration, capillary congestion, glomerular damage, tubular necrosis and cast formation induced by glycerol.

The possible mechanisms for the nephroprotective effect of ATR extract could be due to the antioxidant and anti-inflammatory actions.

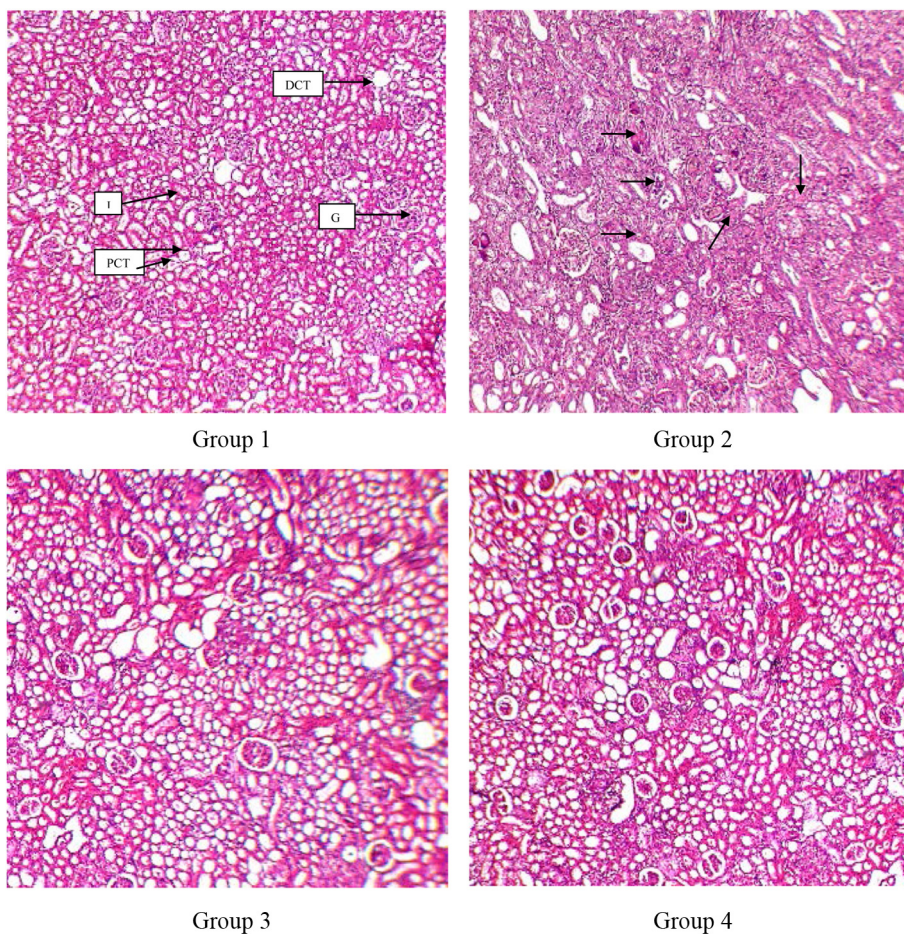


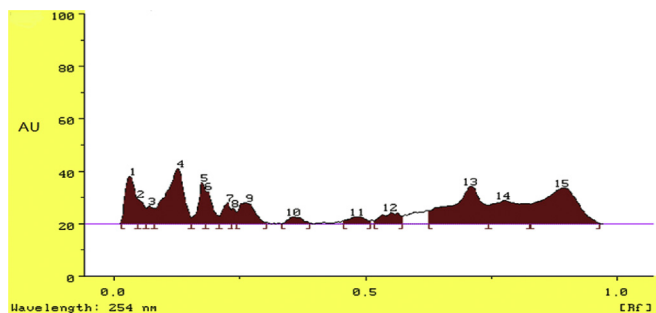
Fig. 3. Histopathology. The kidney sections are shown in 10× magnification stained with haematoxylin and eosin. Group 1, Normal kidney: Normal kidney with glomerulus (G), intact Bowman’s capsule, proximal convoluted tubules (PCT) and distal convoluted tubules (DCT). No capillary congestion, hemorrhage and interstitial damage (I). Group 2, Glycerol treated kidney: The arrows show several ruptured glomeruli, undefined proximal, distal convoluted tubules, interstitial damage, capillary congestion and hemorrhages. Group 3, Glycerol + ATR extract 250 mg/kg: Picture shows intact glomeruli with Bowman’s capsules, defined proximal, distal convoluted tubules with less interstitial damage, capillary congestion and hemorrhages. Group 4, Glycerol + ATR extract 500 mg/kg: Many intact glomeruli with well defined tubules and resolving interstitial damages show marked protection in this group.

The root extract has shown antioxidant activity both *in vitro* and *in vivo* assays. The *in vivo* antioxidant effect of ethanolic extract ATR on the levels of SOD, GSH, GPx, GR and CAT is shown in Fig. 2. Glycerol treatment has reduced the levels of these antioxidants, which get utilized in scavenging the free radicals generated during glycerol-induced oxidative damage of renal parenchyma. ATR

Table 1
HPTLC profile of ethanolic extract of ATR.

S. No	Rf	Height	Area	Lambda max
1	0.03	18.0	249.6	297
2	0.04	9.4	75.6	297
3	0.07	6.8	69.1	287
4	0.12	21.3	535.1	205
5	0.17	15.8	152.9	266
6	0.18	12.5	116.7	267
7	0.22	8.0	88.3	207
8	0.23	6.0	37.2	210
9	0.26	8.0	181.7	294
10	0.36	2.8	54.9	213
11	0.48	2.8	67.2	213
12	0.55	4.3	109.1	215
13	0.71	14.4	622.4	299
14	0.78	9.0	398.7	219
15	0.89	13.7	706.0	214

In vitro antioxidant activity.



Graph. HPTLC profile of ethanolic extract of ATR.

Table 2
DPPH scavenging activity.

Percentage inhibition of ATR extract and Vitamin-C		
Concentration $\mu\text{g/ml}$	ATR extract	Vitamin-C
25	46.33 \pm 1.45	50.99 \pm 0.57
50	67.32 \pm 1.15	70.00 \pm 0.57
100	73.23 \pm 0.88	77.33 \pm 1.20
200	78.32 \pm 0.33	81.99 \pm 0.57
400	85.31 \pm 0.57	88.00 \pm 0.59
800	87.36 \pm 0.66	93.66 \pm 0.33
1600	89.33 \pm 0.33	95.37 \pm 0.57

Values were expressed as Mean \pm SEM, n = 3.

Table 3
NO scavenging activity.

Percentage inhibition of ATR extract and Vitamin-C		
Concentration $\mu\text{g/ml}$	ATR extract	Vitamin-C
25	24.33 \pm 0.88	28.66 \pm 0.33
50	51.33 \pm 2.60	56.33 \pm 0.57
100	77.33 \pm 1.20	79.33 \pm 0.33
200	87.00 \pm 0.57	86.33 \pm 1.20
400	89.66 \pm 0.66	90.66 \pm 0.33
800	90.66 \pm 0.33	91.33 \pm 0.33
1600	91.66 \pm 0.33	94.95 \pm 0.88

Values were expressed as Mean \pm SEM, n = 3.

Table 4
 H_2O_2 scavenging activity.

Percentage inhibition of ATR extract and Vitamin-C		
Concentration $\mu\text{g/ml}$	ATR extract	Vitamin-C
25	23.33 \pm 0.88	29.66 \pm 2.33
50	36.66 \pm 1.20	43.66 \pm 0.66
100	72.33 \pm 0.33	84.33 \pm 1.20
200	79.66 \pm 0.88	88.00 \pm 0.57
400	83.66 \pm 0.68	88.33 \pm 1.20
800	85.66 \pm 0.66	90.66 \pm 1.20
1600	88.33 \pm 0.62	98.12 \pm 0.26

Values were expressed as Mean \pm SEM, n = 3.

Table 5
FRAP scavenging activity.

Percentage inhibition of ATR extract and Vitamin-C		
Concentration $\mu\text{g/ml}$	ATR extract	Vitamin-C
25	22.33 \pm 0.83	28.33 \pm 1.33
50	35.99 \pm 1.20	44.66 \pm 0.66
100	70.33 \pm 0.22	83.33 \pm 1.22
200	75.66 \pm 0.66	87.22 \pm 0.57
400	80.66 \pm 0.99	89.33 \pm 1.20
800	84.66 \pm 0.66	91.66 \pm 0.20
1600	87.33 \pm 0.62	98.12 \pm 0.26

Values were expressed as Mean \pm SEM, n = 3.

Table 6
Effect of ATR extract on biochemical parameters & urine output.

Values are mean \pm SEM						
Parameters	Groups					
	Control	Glycerol (G)	G+ATR (250 mg/kg)	G+ATR (500 mg/kg)	ATR (500 mg/kg)	P -value
Creatinine (mg/dl)	0.71 \pm 0.02	4.18 \pm 0.07	1.73 \pm 0.13	0.71 \pm 0.08	0.72 \pm 0.01	0.0001
BUN (mg/dl)	17.8 \pm 0.19	52.2 \pm 0.34	26.8 \pm 0.23	18.2 \pm 0.19	17.8 \pm 0.34	0.0001
Total proteins (g/dl)	6.10 \pm 0.10	2.03 \pm 0.18	3.31 \pm 0.21	5.28 \pm 0.16	6.40 \pm 0.09	0.001
Albumins (g/dl)	5.01 \pm 0.19	1.86 \pm 0.19	3.21 \pm 0.09	4.66 \pm 0.20	4.95 \pm 0.19	0.001
Urine output (ml)	7.43 \pm 0.17	3.36 \pm 0.13	5.65 \pm 0.11	7.45 \pm 0.07	8.16 \pm 0.16	0.001

extract has reduced the oxidative damage as evidenced by the dose dependent increase in the level of the antioxidants. The importance of protecting the antioxidants pool in preventing renal damage has been emphasized by Singh et al.⁴⁴ The *in vitro* antioxidant effect exhibited in different assays has further supported the antioxidant property of ATR extract.

The phytochemicals found to be present in the root extract are the flavonoids, terpenoids, alkaloids, tannins, saponins & glucosinolates.^{45,46} Among them tannins, triterpenoids, flavonoids and saponins could be responsible for antioxidant property as these phytoconstituents are already reported to have antioxidant activity.

The anti-inflammatory activity could be due to the presence of flavonoids which are well known to have anti-inflammatory action. The root extract also contains tannins. Tannins have vasodilatory activity.⁴⁷ Renal vasodilatation can improve the GFR and urine output. This could be a reason for using ATR extract as a diuretic agent in traditional systems. Hence terpenoids, tannins and flavonoids present in the root extract may have contributed to the protection from renal damage induced by glycerol by their anti-inflammatory, antioxidant and vasodilatory as well as diuretic actions.

The extract of AT leaves has been found to have anti-inflammatory activity.²⁰ The leaves contain freidelin which is a terpenoid. Freidelin has been established to have antioxidant, free radical scavenging, hepato-protective⁴⁸ anti-inflammatory, analgesic and antipyretic activities.⁴⁹ Beta sitosterol, also a terpenoid has been reported to have antipyretic and anti-inflammatory activity.⁵⁰ The terpenoids in the roots can similarly produce these actions.

The glucosinolate and N-hydroxy-3-indolylmethyl-glucosinolate were detected in ATR and not in the other parts of the plant.²⁴ Glucosinolates have vasodilatory effect like tannins and flavonoids.^{51,52} Renal vasodilatation by these components can improve renal blood flow, reduce the ischemic changes and also increase urine output.

Thus the *in vitro* and *in vivo* findings suggest that protection from oxidative damage, anti-inflammatory and vasodilatory actions of the various phytochemicals present in ATR extract could have contributed to the nephroprotective actions of *A. tetraantha* root extract in acute renal failure. Between the 2 doses administered, 250 and 500 mg/kg, 500 mg/kg is found to have significant nephroprotective action.

6. Conclusion

The ethanolic extract of ATR is found to have nephroprotective effect in glycerol-induced acute renal failure in Wistar albino rats. ATR extract is found to have significant action at 500 mg/kg compared to 250 mg/kg. The possible mechanisms could be the antioxidant, anti-inflammatory, vasodilatory and diuretic activity of its phytochemical constituents such as flavonoids, terpenoids, tannins, glucosinolate and saponins.

Conflicts of interest

None declared.

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