ISSN: 2454-9940

INTERNATIONAL JOURNAL OF APPLIED SCIENCE ENGINEERING AND MANAGEMENT

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www.ijasem.org

INTERNATIONAL JOURNAL OF APPLIED SCIENCE ENGINEERING AND MANAGEMENT

ISSN 2454-9940

www.ijasem.org

Vol 19, Issue 1, 2025

Pistia stratiotes shown renoprotective properties in ischaemia reperfusion injury models in rats with and without diabetes.

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Abstract

Renal reperfusion injury (RI/R) is characterised by oxidative and inflammatory bursts; Pistia stratiotes (PS) has a long history of use in avoiding these bursts. Some studies have shown that it may lower blood sugar and cholesterol levels simultaneously. Hence, the purpose of this study was to show that PS changed renal reperfusion damage in diabetic and non-diabetic rats.

DATA AND PROCEDURES:

The experiment allowed each rat 30 minutes of renal ischaemia (RI) before giving them an hour to recuperate. Oral administration of PS at a concentration of 100 mg/kg was given to the animals seven days before to the experiment. We then used the combination produced from the separated kidney tissues to evaluate the antioxidant, inflammatory, and histological effects. Findings demonstrated that aspartate aminotransferase, blood urea nitrogen, creatinine, myeloperoxidase, C-reactive protein, and tumour necrosis factor-alpha levels were lower in diabetic rats given PS as compared to RI/R rats.

Hyperglycaemic animals may have been protected against RI/R by PS, thus the conclusion. The anti-inflammatory, blood sugar-lowering, and free radical-fighting characteristics of PS may explain why it saved the test rats' kidneys.

Relevant Terms: Pistia stratiotes, renoprotective, injury, ischemia, reperfusion

Introduction:

When oxygen supply drops below oxygen demand, a pathological condition called schemia sets up. Reversal of ischaemia and subsequent reperfusion may cause cellular damage, which is called reperfusion injury, according to the literature.[1] Diabetic complications are a real possibility for people with Type 2 diabetes mellitus (T2DM). The risk of necrosis, or kidney damage, during surgical procedures is increased when ischaemia reperfusion (I/R) occurs. The development of nephropathy, a condition marked by reduced blood flow to the kidneys and other harmful effects, is thought by many to be heavily influenced by this. In this work, rats with type 2 diabetes were subjected to induced renal reperfusion injury (RI/R) in an effort to uncover the underlying

mechanisms of the disease. The study's stated goal was to provide light on the mechanisms by which heightened inflammatory response can cause irreversible damage to the I/R.

The inflammatory reaction caused by RI/R injury may harm several organs. Important actors in the pathophysiology of I/R damage include the possibility that it triggers an inflammatory response.A number of inflammatory cells, cell adhesion molecules, and cytokines have been identified as key players in the pathogenesis of I/R.the third The inflammatory cell type neutrophils are known to release copious quantities of reactive oxygen species in reaction to injury to the inner ear or respiratory tract. Myeloperoxidase (MPO) is an enzyme that neutrophils make, and it releases hypochlorous acid, an oxidative stressor that cells could be damaged by.People whose antioxidant defences are weak are more prone to experience oxidative stress and tissue damage caused by I/R [4].Potentially valuable properties of Pistia stratiotes (PS) include antimicrobial, antioxidant, and secondary metabolite activities. Essential minerals including calcium, carboxylic acids (like superoxide dismutase, or SOD), magnesium, zinc, the vitamin C in chlorophyll, DNA, RNA, and proteins are transported more easily by PS because to its high quantities of B-complex vitamins, particularly vitamin B12.According to [5], PS might therefore be a crucial keystone species in the field of medicine.

The Subjects and Procedures

Getting plant materials ready for useThe PS plant was purchased from the Botany Department of the Faculty of Science at Gujarat University, along with a taxonomist's certification. Due to drying out, the PS specimen was no longer viable. The dry powder was extracted at 50°C using 200 cc of 95% ethanol in a Soxhlet device. That being done, the mixture was allowed to sit until it reached 72°C. Under ideal temperature and humidity conditions, the dried extract was preserved in an airtight container.[6]

Analyzing plant compounds

Many compounds were identified throughout the analysis, including proteins, terpenoids, alkaloids, flavonoids, phenols, and saponins.

Contains phenolic compounds

Determine the total phenolic content using the FolinCiocalteu (FC) reagent. The FC reagent and plant extract were mixed and left to incubate at 22°C for 5 minutes. An equal volume of distilled water was used to dilute the FC reagent. Two milliliters of 20% Na2CO3 were then added. Following another 90 minutes of incubation at 22°C, the absorbance at 650 nm was measured. To measure the total phenolic content (mg/mL), acid was used as a reference.

Content of flavonoids

Total flavonoid concentration (mg/mL) was determined using the aluminium chloride (AlCl3) technique. Mixing 0.3 mL of water with 0.5 mL of plant extract and incubating for five minutes at 25°C made up the 50 sodium nitrite test combination. The reaction mixture was supplemented with 0.30 millilitres of 10% AlCl3 and 1 or 2 millilitres of 1 M NaOH. An important last step was to calculate the absorbance at 510 nm using quercetin as a reference.

ISSN 2454-9940
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Content of alkaloids

After vigorously mixing, 2 milliliters of hexane and half a milliliter of plant extract were filtered. Three milliliters of hydrochloric acid, which has a concentration of 2%, were then added to the plant extract. Extra heating and filtering were applied to the reaction mixture. A yellow precipitate was clearly formed when the filtrate was mixed with a little amount of acid, which indicated that alkaloids were present.

Contains terpenoids.

To get the deep reddish-brow color that indicates the presence of terpenoids, three milliliters of extract were mixed with one milliliter each of chloroform and powerful sulfuric acid.

Methodology and experimental setup for the operation

The study's experimental animal was a male Sprague-Dawley rat, which typically weighs between 250 and 300 grammes. The rat was acclimated in a 22°C controlled environment using a polypropylene cage. The lighting conditions went through a half-day cycle of light and dark. The Institutional Animal Ethics Committee gave its approval for this method (PIPH 16//18921//PO//ReBi//S/05/ CPCSEA), in compliance with CPCSEA regulations. Six rats without diabetes (Group 1) and six rats with diabetes (Group 2) underwent the same procedures without occluding the renal artery. Six rats were assigned to Group 3 to undergo renal ischaemia reperfusion injury (RI/R) following 30 minutes of obstruction and reperfusion; six diabetic rats were assigned to Group 4 to undergo diabetic ischaemia reperfusion injury (DRI/R) following the onset of diabetes; and six diabetic rats were assigned to Group 6 to receive PS (100 mg/kg p. o.) for one week before their renal arteries were narrowed for 30 minutes and reperfused for an hour on day 7. A diagnostic kit from Sigma Aldrich Pvt. Ltd was used to assess the degree of diabetes by measuring blood sugar levels, food and water consumption, weight increase, and weight loss.[7] After four weeks, diabetic and healthy rats were both put to sleep by injecting them with 60 mg/kg of ketamine and 5 mg/kg of diazepam intraperitoneally. This method was used to keep the blood temperature at 37.5% CI. The renal pedicles of both healthy and diabetic rats were exposed by a midline incision. To produce renal ischaemia (RI), bilateral renal pedicle clamping was applied for 30 minutes. The twenty-four-hour monitoring of the kidneys is an extension of the clamp. The rats were sedated and their kidneys were removed and preserved in nitrogen at a temperature of 70°C prior to doing further research on the antioxidant and oxidant parameters.[8] After seven days of intravenous streptozotocin development of diabetes at a dose of 65 mg/kg, the rats were administered 230 mg/kg of nicotinamide adenine dinucleotide (NAD) for a duration of fifteen minutes. In order to track diabetes, the standard diagnostic kits from Beacon Pvt. Limited were used to assess blood sugar levels, weight increase, food consumption, and water intake.[9]

Status of the kidneys

Span Diagnostics in the Indian state of Gujarat provided the industry-standard test kits that were used to analyze serum samples for blood urea nitrogen (BUN) (via Jaffe's colorimetric method), creatinine (through the DAM technique), and aspartate aminotransferase (AST).[10]

Diagnostic indicators of oxidative stress and inflammatory responses

This research assessed the levels of lipid peroxidation in renal tissue (NO), which was determined by measuring the content of malondialdehyde (MDA), and intrinsic antioxidant enzymes including reduced glutathione (GSH), superoxide dismutase (SOD), catalase (CAT), GSH peroxidase (GSHPx), and xanthine oxidase (XO).In order to assess the levels of tumor necrosis factor (TNF−) and C-reactive protein (CRP) in the blood, kits were obtained from Nicholas Pvt. Ltd. in India. Both the quantity of neutrophil infiltration in renal tissue and the activity of MPO[16] are being investigated in the present study.

Analysis of tissues by histology and DNA fragmentation

The paraffin-embedded kidneys were subjected to a histological examination. Thin 5 μm slices of tissue were cut using a microtome and then placed on glass slides coated with albumin. The hematoxylin-stained slices were then adhered to Canada balsam after a brief period of eosin staining and a series of escalating alcohol solutions. The photomicroscope used to capture the stained slices was an Olympus BX40. A seasoned pathologist or a blinded researcher evaluated the samples to look for histological changes. For each kidney slide, a minimum of 10 fields were analysed, and any changes seen were classified as either non-existent (−), slight (+), moderate (++), or severe (+++). The genomic DNA was extracted from the kidneys using a DNA extraction kit. A voltage of 80 volts was applied for 1-2 hours during the electrophoresis. Genomic ladders, which are structures created when DNA fragments in apoptotic tissues are examined under ultraviolet light, are documented here for reference purposes.18 and 17 Statistical analysis of data All of the data were represented as the standard error of the mean. Utilising a computer software (GraphPad, Software, 225, Franklin.Street.Fl.26Boston, MA02110) that executed the Bonferroni multiple comparisons test after one-way ANOVA, we ascertained the statistical significance of the two groups. P = 0.05 was used as the significance level.

Final Product

The impact of Pistia stratiotes on indicators of diabetes

Weight, caloric intake, insulin and blood sugar levels, RI and reperfusion in Diabetic Sham Operated (DSO) rats, Normal rats treated with Pistia stratiotes (NPS) and Diabetic rats treated with DPS, and RI/R in both normal and diabetic rats were among the many parameters recorded throughout the experiments. Table 1 shows the results of documenting this data using the DPS approach.

The impact of Pistia stratiotes on kidney function

Diabetic rats administered RI/R had elevated blood levels of AST, BUN, and C-Reactive Protein (CRP), suggesting glomerular dysfunction, in contrast to diabetic animals who had sham surgery. The RI/R and DRI/R groups also showed differences in the blood concentrations of AST, BUN, and CTN. Figure 1 shows that PS treatment reduced AST, BUN, and creatinine levels relative to the RI/R and DRI/R groups.

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ISSN 2454-9940
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Influence of Pistia stratiotes on indicators of oxidative stress

Quality factor or DRI/R-treated rats showed much greater MDA and XO levels than NSO or DSO-treated rats. The GSH, GSHPx, CAT, and SOD activity levels were lower in the RI/R and DR/IR rat groups as compared to the NSO and DSO groups. Table 2 reveals that compared to RI/R, PS therapy considerably reduced MDA and XO levels while enhancing GSH, GSHPx, CAT, and SOD activity.

Impact of Pistia stratiotes on inflammatory mediators and nitric oxide levels in kidney damage after reperfusionCompared to the NSO and DSO groups, the RI/R and DRI/R groups had much higher NO levels. On the other hand, the PS group had far lower NO levels than the RI/R and DRI/R groups. The MPO levels were shown to be considerably greater in the RI/R and DRI/R groups of rats compared to the NSO and DSO groups, but there was a noticeable drop in MPO levels in the rats who were treated with PS. Both the RI/R and DRI/R rats showed significantly

Groups	NSO	DSO	R/IR	DR/IR	NPS	DPS
Body weight (g)	279.81±20.07	172.7±20.1	221.46±17.30	174.41±21.37	196.34±12.25#	184.45±11.53@
Food intake (g/animal/day)	22.06±2.01	25.87±2.58	14.87±1.77	10.62±2.23@	21.75±2.18*,@	27.91±7.91
Water intake (mL/animal/day)	19.12±3.31	44.5±6.16	16.5±4.31	52.14±8.77	30.62±6.59*	39.71±16.1
Serum glucose (mg/dL)	101.75±29.43	378.62±22.45	114.37±6.96	323.41±31.32	122.37±8.56@	134.67±9.73
Serum insulin (µU/mL)	39.87±4.86	76.87±12.32*	56.35±4.59	84.5±11.21	46.12±6.78#	41.94±10.3

Values are mean ± SEM (n = 6), analyzed by one-way ANOVA followed by Bonfferoni's multiple comparison tests. *denote ($P < 0.05$) for chance differences vs NSO, #denote ($P < 0.05$) for chance differences vs DSO, @denote ($P < 0.05$) for chance differences vs RI/R in Body Weight, Food Intake, Water intake, Serum Glucose and serum insulin. Values are mean±SEM (n=6), analyzed by one-way ANOVA followed by Bonfferoni's multiple comparison tests. SEM=Standard error of mean, DPS=*Pistia stratiotes* - treated diabetic rats, R/IR=Renal reperfusion injury, NSO = Normal sham operated, DSO = Diabetic sham operated, NPS=Normal sham operated, DP=Diabetic rats Treated with Pistia stratiotes, DR/IR=Diabetic renal ischemia reperfusion injury

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ISSN 2454-9940
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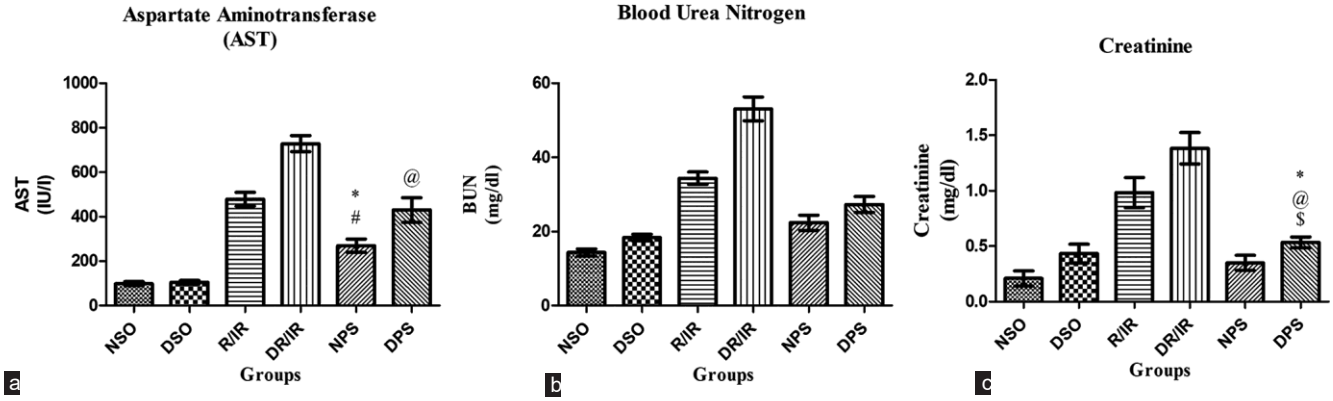

Figure 1: Effect of *Pistia stratiotes* on renal health as determined by (a) aspartate aminotransferase, (b) blood urea nitrogen, and (c) creatinine. The values are mean standard error of mean ($n = 6$), and they were evaluated using one-way ANOVA and Bonfferoni's multiple comparison tests. *($P < 0.05$) difference in chance versus NSO, #($P < 0.05$) difference in chance versus DSO, @($P < 0.05$) difference in chance versus RI/R, and $($P < 0.05$) difference in chance versus DR/IR rats. AST = Aspartate aminotransferase, BUN = Blood urea nitrogen, NSO = Normal sham operated, DSO = Diabetic sham operated, R/IR = Normal renal ischemia reperfusion injury, DR/IR = Diabetic renal ischemia reperfusion injury, NPS = Normal animals treated with *Pistia stratiotes*, DPS = Diabetic animals treated with *Pistia stratiotes*

more C-reactive protein than those who were treated with NSO or DSO. But compared to the PS-treated rats, the CRP levels in the control group were still much lower. When compared to the NSO and DSO groups, the TNF levels in the RI/R and DRI/R groups of rats were significantly higher. On the other hand, PS-treated rats showed much higher amounts. There was a statistically significant difference between the two results [Figure 3].

The impact of Pistia stratiotes on DNA fragmentation and renal histopathology

Lymphocytic proliferation and nephron destruction were shown in ten photos of eosin and hematoxylin-stained kidney tissues from the I/R groups compared to (A) and (B). As seen in (E) and (F), the administration of PS significantly ameliorated the harm in the rats used in the experiment. Histological damage in the cortical tubules varied from moderate (RI/R group) to severe (DRI/R + RI/R group) compared to the healthy (NSO and DSO categories) areas. These alterations were particularly pronounced in these areas. Table 2 summarises and rates the histopathological changes for the reader. The characteristics of cell death, genomic DNA laddering activity, was seen in the RI/R control, DRI/R, NPS, and DPS groups.

Subject under consideration

In order to treat RI/R, several of the presently available medicines are either used or studied for their possible nephroprotective benefits. Unfortunately, due to various patient and drug-related factors, most of these therapies failed to show substantial therapeutic effects. Researchers are working on new medications to block apoptosis in the kidneys, inflammation, and angiogenesis—all of which have been linked to the start of stroke.

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Within the framework of a conventional rat model of RI/R and DRI/R, the current investigation postulated that rats would be protected after receiving PS. According to reference, PS is used to treat nephrological diseases by reducing the effects of oxidative stress, inflammation, hyperglycemia, and tissue damage caused by ischemia.[12] The research aimed to establish the degree of oxidative damage and the preventive effects of PS by examining the amounts of free radical-neutralizing enzymes and NO in kidney tissue.

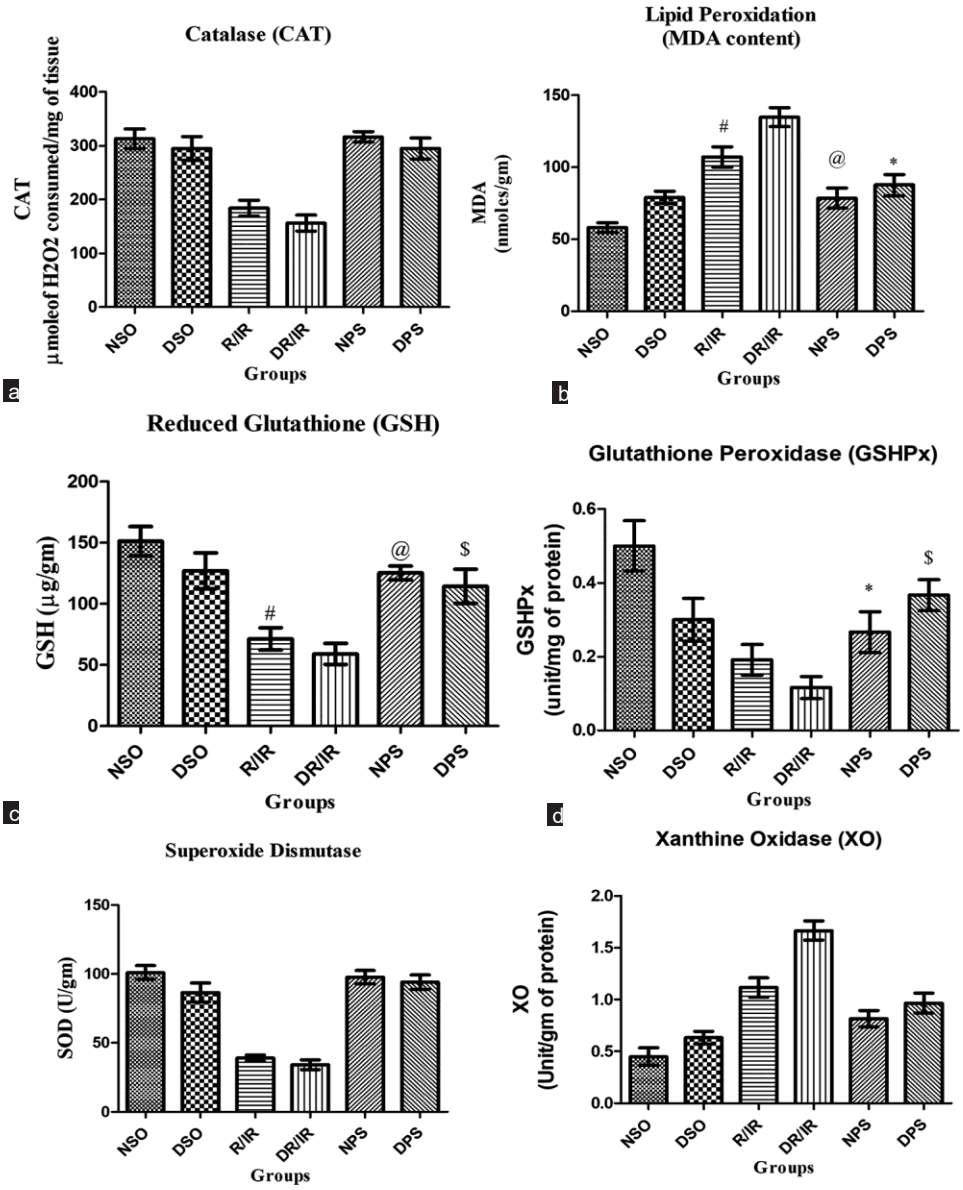

Figure 2: After renal reperfusion injury (RI/R) in NSO, DSO, and *Pistia stratiotes* -treated rats, the following changes were observed in the renal tissue: Catalase (a); MDA (b); glutathione (c); GSH peroxidase (d); super oxide dismutase (e); and xanthine oxidase (f). Values are mean standard error of mean (n = 6), and one-way ANOVA analysis is followed by multiple comparison tests by Bonfferoni. For chance variations compared to NSO, *(P < 0.05), $^{\#}$(P < 0.05), $^{@}$(P < 0.05), for chance variations compared to RI/R, and $^{\$}$($P$ < 0.05) for chance variations compared to DR/IR rats. CAT = Catalase, GSH = Glutathione, GSHPx = GSH peroxidase, XO = Xanthine oxidase,

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NSO = Normal Sham Operated, MDA = Malondialdehyde, DSO = Diabetic Sham Operated, R/IR = Normal renal ischemia reperfusion injury, DR/IR = Diabetic renal ischemia reperfusion injury, NPS = Normal animals treated with *Pistia stratiotes*, DPS = Diabetic animals treated with *Pistia stratiotes*

research found that animals given PS and then exposed to RI/reperfusion had much better kidney function.

The association between transient RI and nephrological problems is well-established. Arterial renal occlusion-induced RI/R injury may be protected against by PS, according to the results. It is from triphenyl that the red formazan pigment is made. After receiving PS, mice who underwent RI/reperfusion had significantly improved kidney function, according to the study.

It is well-established that temporary RI may cause kidney issues. The findings suggest that PS may provide protection against RI/R harm caused by arterial renal occlusion. The red formazan pigment is derived from triphenyl.

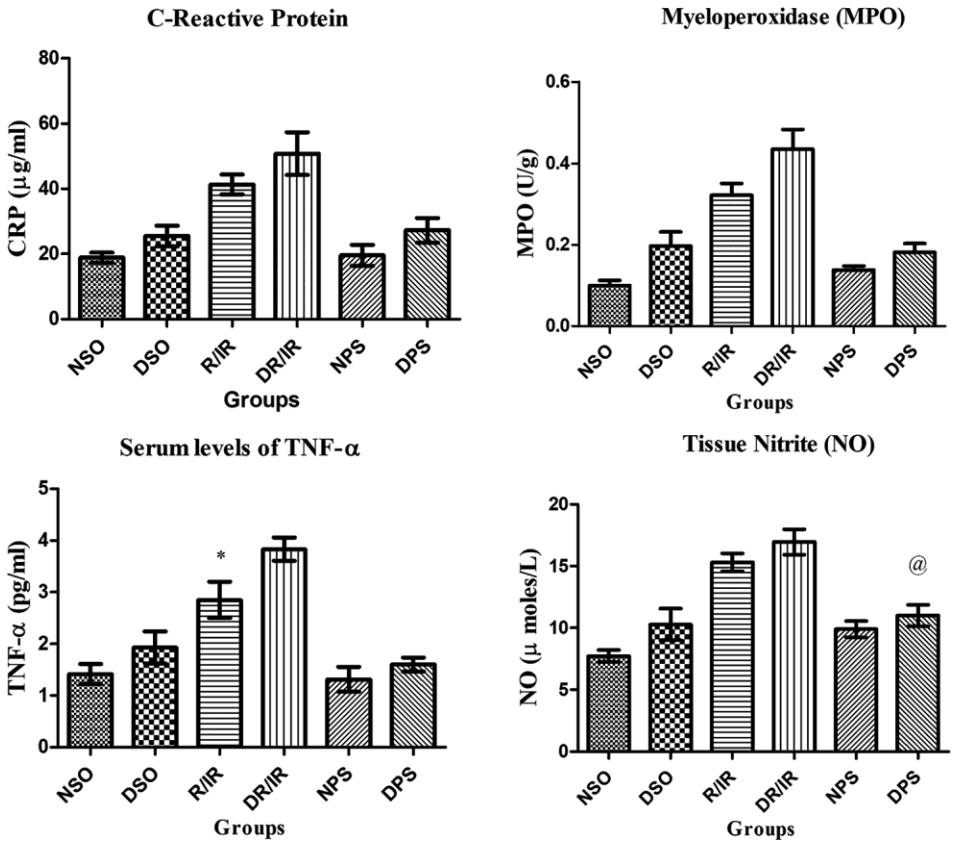

Figure 3: Following renal reperfusion injury (RI/R) in healthy, diabetic, and *Pistia stratiotes* -treated rats, renal tissue nitric oxide levels, C-reactive protein, myeloperoxidase, and serum tumor necrosis factor-□ levels were measured. Values are mean standard error of mean (n = 6), and one-way ANOVA analysis is followed by multiple comparison tests by Bonfferoni. For chance discrepancies vs. NSO, use * to signify ($P < 0.05$), and for chance discrepancies versus RI/R rats, use @ to signify ($P < 0.05$).

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MPO = Myeloperoxidase, NO = Nitric oxide, TNF = Tumor necrosis factor, NSO = Normal sham operated, DSO = Diabetic sham operated, R/IR = Normal renal ischemia reperfusion injury, DR/IR = Diabetic renal ischemia reperfusion injury, NPS = Normal animals treated with *Pistia stratiotes*, DPS = Diabetic animals treated with *Pistia stratiotes*

Table 2: Effect of *Pistia stratiotes* on renal histology (*n*=6)

Groups	Tubular cell swelling	Interstitial edema	Tubular dilatation	Necrosis of epithelium	Hyaline casts
NSO	—	—	—	—	—
DSO	—	—	—	—	—
RI/R	++	++	++	++	++
DRI/R	+++	+++	+++	+++	+++
NPS	+	+	—	—	—
DPS	+	+	—	—	—

Effect of EC on morphological changes of kidneys (*n*=6), as assessed by histopathological examination of the normal rats, and diabetic rats exposed to RI/R. DPS=*Pistia stratiotes* - treated diabetic rats, R/IR=Renal reperfusion injury, NSO = Normal sham operated, DSO = Diabetic sham operated, PS=Pistia stratiotes, NPS=Normal animals treated with *Pistia stratiotes*, DP=Diabetic rats Treated with Pistia stratiotes, DRI/R=Normal renal ischemia reperfusion injury

in the treatment of RI/R with PS. The histological analysis of the RI/R and DRI/R groups showed that the blood arteries were significantly constricted, neutrophils were infiltrated, and nephrons were necrosized. When compared to the RI/R and DRI/R groups, the PS-treated group showed a significant decrease in neutrophil infiltration.

Although NO may be harmful in certain situations, such oxidative stress, it performs an essential function as a modulator or messenger [20]. RI causes an increase in output

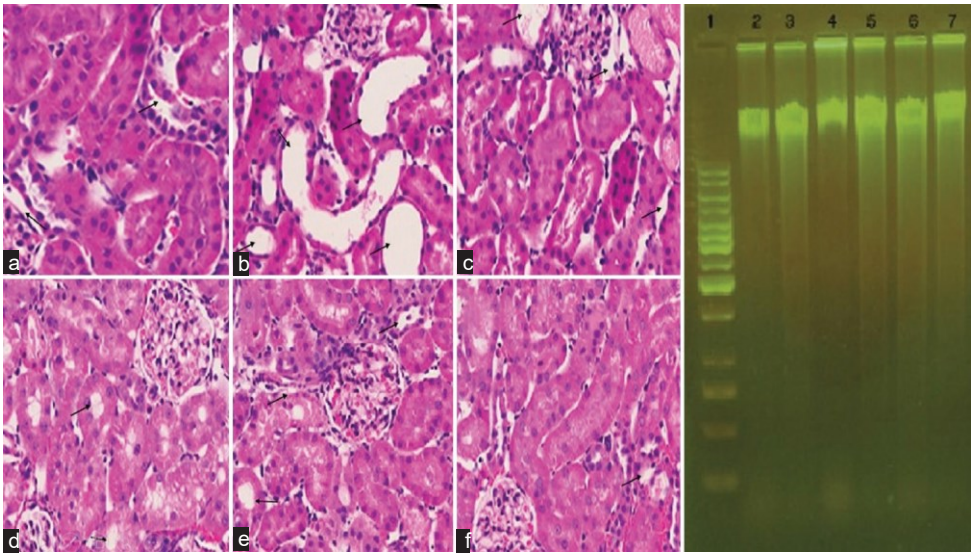

Figure 4: The fragmentation of DNA testing revealed typical DNA fragment laddering. (1) Standard Protein Sequence (2) NSO; (3) DSO; (4) RI/R; (5) DRI/R; (6) NPS subjected to RI/R; and (7) DPS The diabetic rats were treated with *Pistia stratiotes* (PS) and subjected to RI/R. Renal tissue slices from normal and diabetic rats that were treated to RI/R were examined under a BIOXL light

microscope to look for morphological alterations. Hematoxylin and eosin (×40) was used to capture images under light microscopy: are shown in (a-c and e), respectively. (d) are shown in (a e and f),respectively. (f) PS Took care of the diabetic rat's nephro ischemia reperfusion. NSO = Normal sham operated, DSO = Diabetic sham operated, R/IR = Normal renal ischemia reperfusion injury, DR/IR = Diabetic renal ischemia reperfusion injury, NPS = Normal animals treated with *Pistia stratiotes*, DPS = Diabetic animals treated with *Pistia stratiotes*

There is more evidence that oxidative stress harms kidneys since RI/R groups have higher quantities of enzymes that detoxify free radicals. Groups treated with PS had levels of CAT and SOD that were 19.67% and 22.14% higher, respectively. A 31.2% difference in GSH levels was seen between the PS-treated and RI/R-treated groups, according to the data. The levels of GSHPx rose by 18.37% in the groups who received PS treatment. This work contradicts previous research that indicated elevated levels of lipid peroxidation and NO and decreased levels of CAT, XO, GSHPx, and GSH in response to elevated blood oxygen species. This is thought to occur when detoxification mechanisms stay dormant and antioxidants degrade.According to prior research, molecular radicalisation, which begins with the MPO enzyme, triggers cell death and protein nitrotyrosination [22].[23] There was a statistically significant difference in the amounts of MPO between the ischaemia and control groups. The PS treatment group had much lower MPO levels than the RI/R and DRI/R groups, according to the findings of this study. In the groups that received PS, MPO levels decreased by 20.96%.
Sera levels of inflammatory cytokines may increase in both quantity and potency in reaction to elevated CRP levels. The study found that serum CRP levels increased significantly after RI. This finding is noteworthy because C-reactive protein (CRP) is a marker for evaluating the severity, prognosis, and recurrence of stroke.[24] The findings reveal that the PS group had significantly reduced CRP levels compared to the RI/R and DRI/R groups. In the groups who received PS, the levels of C-reactive protein were 22.13% lower.
Both intrinsic and acute RI-induced TNF production contribute significantly to the progression of renal injury. The potential consequences of RI include the production of inflammatory mediators, the activation and aggregation of multicore white blood cells, and the expression of tumour necrosis factor (TNF). Research done in the past has verified this.[25] There was a significant reduction of 24.48% in serum TNF levels in the PS group compared to the RI/R and DRI/R groups. The success of PS therapy may be measured by the return of normal blood flow and blood pressure, which might be caused by a decrease in renin release levels.
The creation of the apoptosome is triggered by the release of cytochrome from the cytosol, which in turn stimulates procaspases 3 and 9. The activation of caspase 3, which triggers caspase-triggered DNase, leads to DNA fragmentation [26,27]. It was also shown that renal tissue from RI/R animals dies off more quickly. In contrast, when comparing rats with DRI/R or NSO genotypes, this does not hold true. The findings showed that after PS treatment, DNA levels were lower.
apart from the other categories that received DPS and NPS. The results show that diabetic rats have reduced nuclear oxidative stress in renal tissue prior to RI/R.
The findings indicated that diabetic rats were protected against RI/R by PS. Renoprotective effects seen in the study participants may be due to PS's antioxidant, blood sugar-reducing, and

membrane-stabilizing properties. To make the most of PS's therapeutic potential in renovascular illnesses, more research is required in light of the novel ideas given by the findings.

In summary,

At long last, proof that PS might ward off RI/R in diabetic rats was shown. In addition, PS's antioxidant, blood sugar-lowering, and membrane-stabilizing characteristics may explain the kidney protection shown in animal investigations. More research is required to fully use Pistia stratiotes as a therapeutic tool for renovascular illnesses, although new potential are developing as a consequence of these findings.

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