



ISSN : 2347-2251

**Indo-American Journal of
Pharma and Bio Sciences**



www.iajpb.com

iajpb.editor@gmail.com
editor@iajpb.com



In both healthy and diabetic rats, Pistia stratiotes showed renoprotective effects in ischemia reperfusion damage models.

Yadala Prapurna Chandra , S.Aliya afra, Sk.Phareedha ,P.Sailaja

Abstract

: Pistia stratiotes (PS) has a long history of usage in preventing oxidative and inflammatory bursts, which are significant components of renal reperfusion injury (RI/R). Reports indicate that it may reduce cholesterol levels as well as blood sugar levels. Therefore, this research set out to demonstrate how PS altered renal reperfusion injury in rats with diabetes and those without the disease.

DATA AND PROCEDURES:

Each rat suffered 30 minutes of renal ischemia (RI) and then had 1 hour to recover in the experiment. For seven days before to the experiment, the animals were administered PS at a dose of 100 mg/kg orally. We then assessed the antioxidant, inflammatory, and histological effects using the mixture derived from the kidney tissues that had been separated. The results showed that diabetic rats fed PS had reduced levels of certain urine enzymes compared to RI/R

rats, including aspartate aminotransferase, blood urea nitrogen, creatinine, myeloperoxidase, C-reactive protein, and tumor necrosis factor-alpha. Conclusion: PS may have protected hyperglycemic animals against RI/R. The fact that PS protected the test rats' kidneys might be because of its anti-inflammatory, blood sugar-lowering, and free radical-fighting properties.

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

Department of Pharmaceutics,

Ratnam Institute of Pharmacy, Pidathapolur (V), Muthukur (M), SPSR Nellore Dt.- 524346 A.P.,
India



Introduction :

A pathological condition known as ischemia is characterized by an increase in the body's oxygen demand and a decrease in oxygen supply. There is evidence in the literature that the reversal of ischemia and reperfusion may lead to cellular damage known as reperfusion injury.[1] One of the risks of developing kidney problems is having Type 2 diabetes mellitus (T2DM). Necrosis, or kidney destruction, may occur as a consequence of ischemia reperfusion (I/R) during surgical operations. Many people consider this to be a major component in develop nephropathy, which is characterized by a decrease in blood flow to the kidneys, and other detrimental consequences. The current study set out to identify the pathophysiological pathways of type 2 diabetes by studying the effects of induced renal reperfusion injury (RI/R) on rats with the condition. Specifically, the researchers hoped to learn how an increased inflammatory response may lead to the development of severe I/R damage.

Multiple organs may be damaged by the inflammatory response that is produced by RI/R injury. It is possible for an inflammatory response to be triggered by I/R damage, leading to major players in the pathophysiology of I/R damage. Research has shown that certain inflammatory cells, cell adhesion molecules, and cytokines play a major role in the pathophysiology of I/R.[3] In response to I/R damage, neutrophils, an inflammatory cell type, are known to produce large amounts of reactive

oxygen species. When neutrophils produce the enzyme myeloperoxidase (MPO), they are releasing hypochlorous acid, an oxidative stressor that may harm cells. I/R-induced oxidative stress and tissue damage are more likely to occur in individuals with inadequate antioxidant defenses [4]. Pistia stratiotes (PS) has antibacterial, natural antioxidant, and secondary metabolite capabilities that might make it useful. The high levels of B-complex vitamins, especially Vitamin B12, in PS facilitate the movement of essential minerals such as calcium, carboxylic acids (such as superoxide dismutase, or SOD), magnesium, zinc, the vitamin C found in chlorophyll, DNA, RNA, and proteins. PS may therefore be an important medicinal keystone species, as suggested by [5].

The Subjects and Procedures

Getting plant materials ready for use The 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

We used the aluminum chloride (AlCl₃) method to obtain the total flavonoid concentration (mg/mL). The 50 sodium nitrite test combination consisted of 0.3 milliliters of water, half a milliliter of plant extract, and five minutes of incubation at 25°C. To the reaction mixture, 0.30 milliliters of 10% AlCl₃ and either 1 or 2 milliliters of 1 M NaOH were added. Last but not least, quercetin was used as a reference to compute the absorbance at 510 nm.

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-brown 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

A male Sprague-Dawley rat weighing 250–300 grams was used as the experimental animal in this investigation. A polypropylene cage was used to acclimate the rat, and the temperature was maintained at 22°C. There was a half-day light and dark period in the lighting conditions. In accordance with CPCSEA guidelines, this technique was authorized by the Institutional Animal Ethics Committee (PIPH 16//18921//PO//ReBi//S/05/ CPCSEA). Group 1 (n = 1) comprised of six healthy rats that had all procedures performed without blocking the renal artery; Group 2 (n = 2) included six diabetic rats. Group 3 consisted of 6 rats that underwent renal ischemia reperfusion injury (RI/R) after 30 minutes of obstruction and subsequent reperfusion; Group 4 also consisted of 6 diabetic rats that underwent DRI/R after the onset of diabetes; Group 6 consisted of diabetic rats that received PS (100 mg/kg p. o.) for 1 week prior to having their renal arteries occluded for 30 minutes and then reperused for an hour on day 7. Sigma Aldrich Pvt. Ltd diagnostic kits were used to measure blood sugar levels, food and water intake, weight gain, and weight reduction in order to diagnose the severity of diabetes.[7] Following four weeks, rats with diabetes and healthy rats were both rendered unconscious by administering 60 mg/kg of ketamine intraperitoneally and 5 mg/kg of diazepam. Blood temperature was maintained at 37 0.5°C using this approach. Diabetic and



healthy rats' renal pedicles were exposed by a midline incision.

By applying bilateral renal pedicle clamping for 30 minutes, renal ischemia (RI) was induced. This is a continuation of the clamp, the kidneys were monitored for twenty-four hours. Before further investigation of the oxidant and antioxidant parameters could be carried out, the rats were put to sleep, and their kidneys were removed and stored in nitrogen at a temperature of 70°C.[8] Following a 7-day induction of diabetes by intravenous streptozotocin at 65 mg/kg, the rats were given 230 mg/kg of nicotinamide adenine dinucleotide (NAD) for 15 minutes. Blood sugar levels, weight gain, food consumption, and water intake were measured using the usual diagnostic kits from Beacon Pvt. Limited in order to monitor diabetes.[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

We performed a histological analysis on the kidneys that were embedded in paraffin. We used a microtome to slice the tissue into thin 5 μ m slices, which were then mounted on albumin-coated glass slides. The slices that had been stained with hematoxylin were then attached to Canada balsam after a short time of eosin staining and a course of increasing alcohol solutions. Using an Olympus BX40 photomicroscope, the stained slices were taken. To detect any histological changes, the samples were examined either by a seasoned pathologist or by a blinded research. At least ten fields were examined for every kidney slide, and any alterations seen were rated as either none (-), mild (+), moderate (++), or severe (+++). We used a DNA extraction kit to get the genomic DNA out of the kidneys. For the electrophoresis, a voltage of 80 volts was used for 1-2 hours. Genomic ladders formed by apoptotic tissue DNA fragmentation were studied under UV light, and their documentation is provided for future reference.[17 and 18] Data analysis using statistics

The standard error of the mean, which represents all of the data, was used. The statistical significance of the two groups was determined using a computer-based program (GraphPad, Software, 225, Franklin.Street.Fl.26Boston, MA02110) that conducted the Bonferroni multiple comparisons test after one-way ANOVA.



The significance threshold was set at $P = 0.05$.

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.

Influence of *Pistia stratiotes* on indicators of oxidative stress

Compared to rats given NSO or DSO, animals given quality factor or DRI/R exhibited significantly higher levels of MDA and XO. Compared to the NSO and DSO groups, the RI/R and DR/IR rat groups exhibited reduced levels of GSH, GSHPx, CAT, and SOD activity. Table 2 shows that in comparison to RI/R, PS treatment significantly decreased MDA and XO levels while increasing GSH, GSHPx, CAT, and SOD activity.

Impact of *Pistia stratiotes* on inflammatory mediators and nitric oxide levels in kidney damage after reperfusion Compared 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/R	DR/IR	NPS	DPS
Body weight (g)	279.81±20.0 7	172.7±20.1	221.46±17.3 0	174.41±21.3 7	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±29.4 3	378.62±22.4 5	114.37±6.96	323.41±31.3 2	122.37±8.56 @	134.67±9.7 3
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 Bonferroni'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 $\pm$ 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

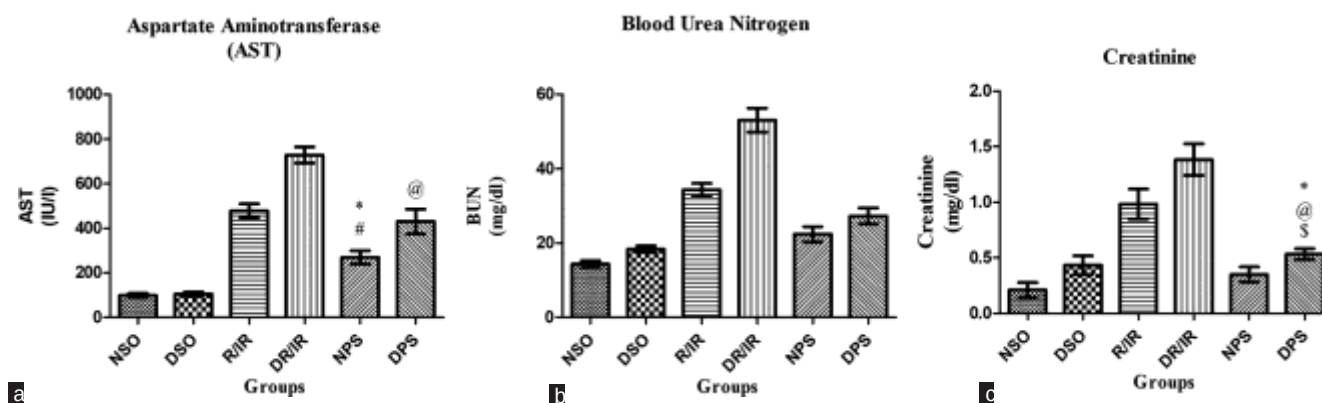


Figure 1: Effect of *Pistia stratiotes* on renal health as determined by (a) aspartate aminotransferase, (b) blood urea nitrogen, and (c) creatinine. The values are mean standard error of mean ($n = 6$), and they were evaluated using one-way ANOVA and Bonfferoni's multiple comparison tests. *($P < 0.05$) 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

Photos of kidney tissues stained with eosin and hematoxylin taken 10 times from the I/R groups and compared to (A) and (B) showed lymphocytic proliferation and nephron destruction. The damage in experimental rats was dramatically reversed after treatment with PS, as seen in (E) and (F). The most noticeable changes were in the cortical tubules, where histological damage ranged from mild (RI/R group) to severe (DRI/R + RI/R group) from healthy (NSO and DSO categories). The reader was provided with a summary and rating of the histopathological alterations [Table 2]. Genomic DNA laddering activity, a hallmark



of cell death, was seen in the RI/R control, DRI/R, NPS, and DPS groups. As compared to

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,

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. Right now,

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.

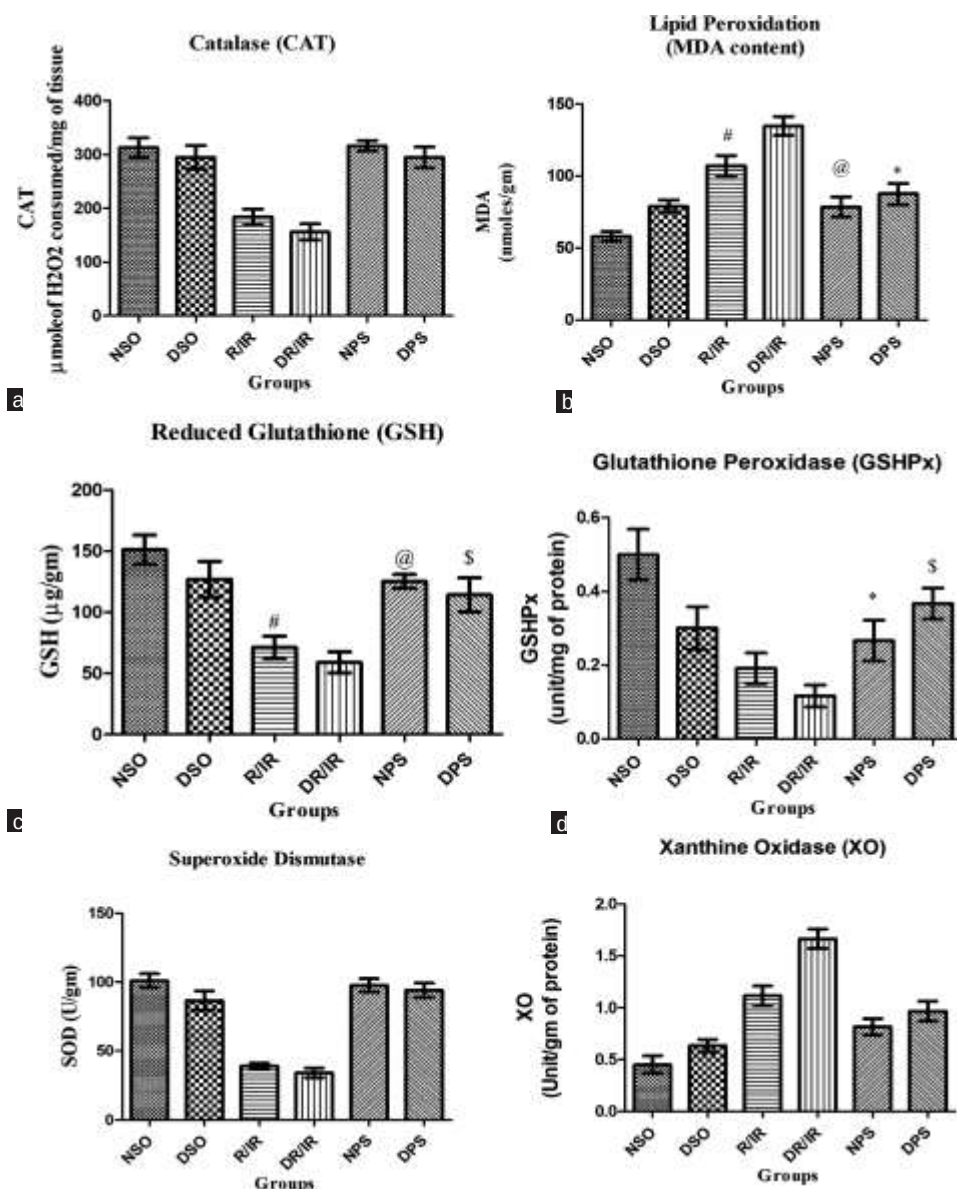


Figure 2: After renal reperfusion injury (RI/R) in NSO, DSO, and *Pistia stratiotes* -treated rats, the following changes were observed in the renal tissue: Catalase (a); MDA (b); glutathione (c); GSH peroxidase (d); super oxide dismutase (e); and xanthine oxidase (f). Values are mean standard error of mean ($n = 6$), and one-way ANOVA analysis is followed by multiple comparison tests by Bonferroni. 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, 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.

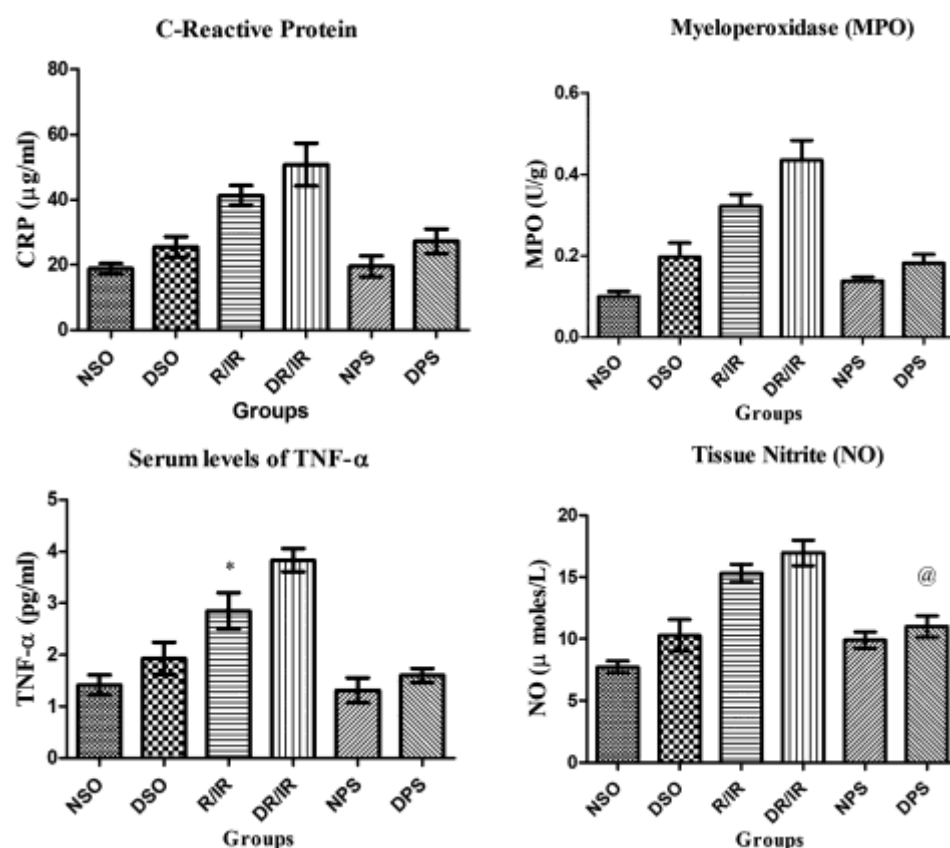


Figure 3: Following renal reperfusion injury (RI/R) in healthy, diabetic, and *Pistia stratiotes*-treated rats, renal tissue nitric oxide levels, C-reactive protein, myeloperoxidase, and serum tumor necrosis factor- α levels were measured. Values are mean standard error of mean ($n = 6$), and one-way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

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

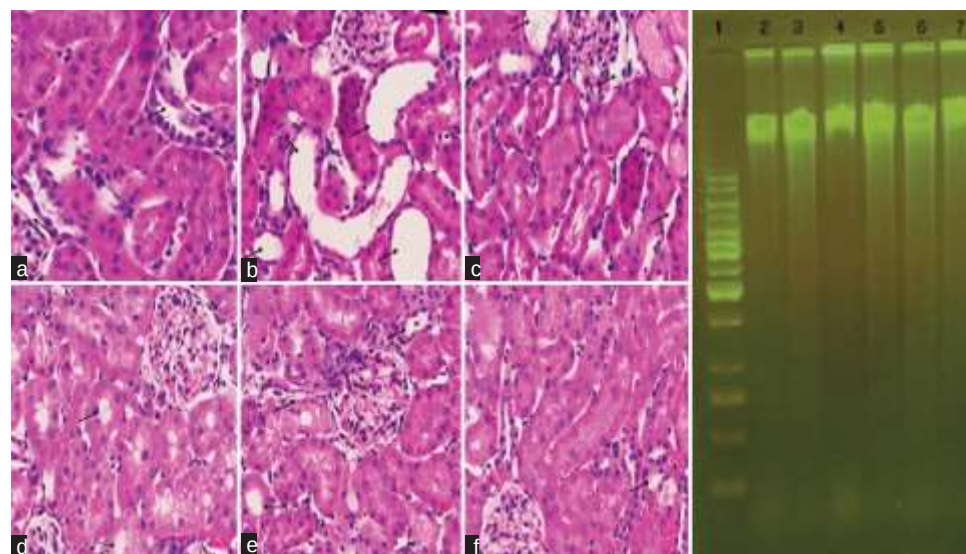


Figure 4: The fragmentation of DNA testing revealed typical DNA fragment laddering. (1) Standard Protein Sequence (2) NSO; (3) DSO; (4) RI/R; (5) DRI/R; (6) NPS subjected to RI/R; and (7) DPS The diabetic rats were treated with *Pistia stratiotes* (PS) and subjected to RI/R. Renal tissue slices from 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 ($\times 40$) 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*



RI/R groups' elevated levels of free radical detoxifying enzymes add credence to the idea that oxidative stress damages kidneys. A 19.67% increase in CAT levels and a 22.14% increase in SOD levels were seen in the groups that were treated with PS. The results show that GSH levels were significantly higher in the PS-treated groups compared to the RI/R-treated groups, with a difference of 31.2%. There was an 18.37% rise in GSHPx levels in the groups who were treated with PS. This study's results go counter to those of another that found that when there are too many oxygen species in the blood, lipid peroxidation and NO levels rise while CAT, XO, GSHPx, and GSH levels fall. It is believed that this happens when antioxidants break down and detoxifying systems become inactive.[22] is aMolecular radicalization, which is initiated by the MPO enzyme, activates apoptosis and protein nitrotyrosination, as previously shown in reference.[23] The groups experiencing ischemia and the control group showed significantly different levels of MPO. The results of this research demonstrate that the MPO levels were significantly lower in the PS treatment group compared to the RI/R and DRI/R groups. There was a 20.96% drop in MPO levels in the groups who were treated with PS.

The amount and strength of cytokines that promote inflammation serum levels may rise in response to increased concentrations of CRP. Serum CRP levels rose sharply after RI, according to the research. Because C-reactive protein (CRP) is a marker for assessing stroke severity, prognosis, and recurrence, this discovery is significant.[24] When comparing the PS treatment group to the RI/R and DRI/R groups, the results show that CRP levels are much lower in the PS group. The C-reactive protein levels were 22.13% lower in the PS-treated groups.

Renal damage progresses in large part due to TNF production, which may be either intrinsic or induced by acute RI. Inflammatory mediator generation, multicore white blood cell aggregation and activation, and TNF induction after RI are all possible outcomes. Previous study has confirmed this.[25] Serum TNF levels decreased by a substantial margin of 24.48% when comparing the PS group to the RI/R and DRI/R groups. Reestablishing normal blood flow and blood pressure, maybe due to a reduction in renin release levels, may be an indication of the efficacy of PS treatment.

The release of cytochrome from the cytosol triggers procaspases 3 and 9, which in turn lead to the formation of the apoptosome. Fragmentation of DNA occurs when caspase 3 is activated, which in turn activates caspase triggered DNase [26,27]. It was also determined that RI/R animals' renal tissue undergoes apoptosis at a higher rate. But this is not the case when comparing DSO rats to NSO rats or DRI/R rats. According to the study's results, lower levels of DNA were seen after PS treatment.

separation between the groups treated with NPS and DPS. This data reveals that pre-RI/R renal tissue nuclear oxidative stress was lower in diabetic rats.

According to the results, PS showed protection against RI/R in diabetic rats. The antioxidant, blood sugar-reducing, and membrane-stabilizing characteristics of PS might be responsible for the possible renoprotective benefits shown in the test subjects. Due to the new notions presented by the data, more study is needed to properly capitalize on PS's therapeutic potential in renovascular disorders.

**In summary,**

Finally, it was shown that diabetic rats were successfully protected against RI/R by PS. Furthermore, the protection of the kidneys shown in animal studies could be attributed to PS's antioxidant, blood sugar-lowering, and membrane-stabilizing properties. New opportunities are emerging as a result of these discoveries, and more study is needed to fully use *Pistia stratiotes* as a therapeutic tool for renovascular diseases.

Author contributions

Shantilal Padhiyar analyzed the data, wrote the first draft, and made all the necessary edits. The analysis and interpretation of information was helped along by Jitendra vaghasiya, Vashisth P. Bhavsar, and Ashish Patel. The design and modification of the work were done in part by Vashisth Bhavsar and Tejas B Patel. All authors have reviewed and approved the final version of the submitted manuscript.

Ethics clearance and permission to take part Acclimatization was conducted on male Sprague-Dawley rats weighing 250 to 300 g in a polypropylene cage under controlled circumstances with a temperature of $22^{\circ}\text{C} \pm 3^{\circ}\text{C}$ and a 12-hour cycle of light and darkness. This technique was carried out in compliance with CPCSEA rules and with the approval of the Institutional Animal Ethical Committee (No. PIPH 16//18921/PO/ ReBi//S/05/CPCSEA).

References

1. Dilek ON, Akbulut G, Yilmaz K, Serteser M, Koken T, Kahraman A. Kidney ischemia/reperfusion damage in mice: alterations in hepatic TNF- α levels, antioxidant state, and oxidation products. *Journal of the American Society for Surgery*, Volume 107, Issue 2, pages 234.
2. Ysebaert DK, et al. T lymphocytes have a mediating role in renal damage caused by ischemia and reperfusion. *The kidney journal* published the article in 2004 with the DOI 66:491–76.
3. Kilga S, Sahin YN, Akcay F, Yuldirim A, Gumus M. In rabbits, dehydroepiandrosterone enhances the antioxidant systems in the liver after renal ischemia-reperfusion damage. Publication date: 2003, *Annals of Clinical Laboratory Science*, volume 33, pages 459-464.
4. The authors of the study include Lara-Serrano JS, Rutiaga-Quiñones OM, López-Miranda J, Fileto-Pérez HA, Pedraza-Bucio FE, Rico-Cerda JL, and others. 1. The water hyacinth, scientifically known as *Eichhornia crassipes* (Mart.) Solms, was enumerated. The article is published in *BioResources* in 2016 and the DOI is 1172014-23.
5. Adegbola PI, Adedosu OT, EhigieL, Olaniyi AA, Agoola TO, and others made up the sixth group. Extract from the leaves of the water hyacinth plant (*Eichhornia crassipes*) and its chemical components, as well as its influence on certain enzymes found in *Periplaneta americana*. The article, published in 2020 in *BioTechnologia*, has the DOI number 10.5114/bta.2020.100424. Its pages number 321-336.



6. The authors of the paper include Goor Y, Peer G, Iaina A, Blum M, Wollman Y, Chernihovsky T, and others. Ischemic acute renal failure in streptozotocin- diabetic rats: nitric oxide's role. The article is published in *Diabetologia* in 1996 and can be found on pages 1036–1040.
7. Authors: Melin J, Hellberg O, Akyürek LM, Källskog O, Larsson E, and Fellström BC. The diabetic rat develops nephropathy quickly due to ischemia. The kidney journal published the article in 1997 with the DOI 52: 9,85–91.
8. The authors include Masiello, Broca, Gross, Roye, Manteghetti, Hillaire-Buys, and others. New model for experimental NIDDM using streptozotocin and nicotinamide in adult rats developed. The authors of the 1998 Diabetes article are Vargasiya J, Sheth N, Bhalodia Y, and Manek R. In diabetics, sitagliptin prevents kidney damage caused by ischemia-reperfusion. The article "Regul Pept 2011;166:48-54" states as much.
9. P. Slater and B. C. Sawyer. How carbon tetrachloride and other halogenoalkanes stimulate peroxidative processes in rat liver fractions in vitro for experimental purposes. General aspects of the systems employed. *Biochemical Journal* 1971;123:805–14.
10. The writings of Moron, Depierre, and Mannervik. The rat liver and lung glutathione levels and activities of glutathione reductase and glutathione S-transferase. Publication: 1979, *Biochim Biophys Acta*, 582: 67–78.
11. Thirteen. Misra HP, Fridovich I. The involvement of superoxide anion in the autoxidation of epinephrine and a simple test for superoxide dismutase. The citation for the 1972 article is *J Biol Chem* 247:3170–5.
12. Oxidoreductases that act on groups other than CHOH: Catalase (Aebi H.). Edited by Colowick SP, Kaplan NO, and Packer L. *Enzymology Procedures*. Chapters 121–125. London: Academic Press, 1984.
13. Prajda N, Weber G. Hypoxia-inducible oxidase activity in hepatomas: a malignant transformation-linked imbalance. Research published in *FEBS Lett* in 1975, volume 59, pages 245-249.
14. Contribution from Guevara I, Iwanejko J, Dembińska-Kieć A, Pankiewicz J, Wanat A, Anna P, and others. Analysis of human biological samples for nitrite and nitrate using the straightforward Griess reaction. *Clinical chemistry and related topics*, 1998, 274, 177–88.
15. Wu H and Frenkel K authored the 17th article. The connection between the in vivo promoting activity of phorbol ester-type tumor promoters and oxidative events and DNA oxidation in SENCAR mice. Published in *Carcinogenesis*, volume 14, pages 1195–201, in 1993.
17. *Eichhornia crassipes* protects normal and diabetic rats from brain ischemia reperfusion damage (Bhavsar V, Vaghasiya J, Suhagia BN, Thaker P.). The following is the reference for the article: "J Stroke Cerebrovasc Dis 2020;29:105385".



18. Ralph J.K. and Nancy J. Rothwell. A rat study found that blocking the interleukin-1 receptor prevented both ischemic and excitotoxic damage to neurons. The article "Brain Res Bull 1992;29:243–6" was published in 1992.
19. Chen L, Deng H, Cui H, Fang J, Zuo Z, Deng J, et al. Disorders in organs linked to inflammation and inflammatory reactions. An article published in Oncotarget in 2018 with the DOI number 9:7204-18.. Lively S, Schlichter LC. The reactions of microglia to pro-inflammatory factors

