International Journal of Medical Science and Research
www.medicaljournals.in
Online ISSN: 2664-8768, Print ISSN: 2664-875X
Received: 07-09-2025, Accepted: 08-10-2025, Published: 23-10-2025
Volume 7, Issue 2, 2025, Page No. 38-51

Investigation of nephroprotective, *In-vitro* antioxidant and cytotoxic potential of *Simarouba glauca DC*

Dr. A Krishnaveni[1*], Vaishnavi G[2], Sandhiya S[2], Manjula B[2], T Kavin[3]
[1] Assistant Professor, Department of Pharmacognosy, College of Pharmacy, Madurai Medical College, Madurai, Tamil Nadu, India
[2] Department of Pharmacognosy, College of Pharmacy, Madurai Medical College, Madurai, Tamil Nadu, India
[3] Veterinary Assistant Surgeon, Institute of Pharmacology, Madurai Medical College, Affiliated to The Tamil Nadu Dr. M.G.R. Medical University, Chennai, Tamil Nadu, India

Abstract
Objective: The present study aimed to evaluate the nephroprotective and antioxidant potential of aqueous extract of *Simarouba glauca* leaves in alloxan-induced diabetic rats through *in vivo*, in vitro, and biochemical assays.
Methods: Mature leaves of *Simarouba glauca* were collected, shade-dried, and powdered. Aqueous extract was prepared by boiling 10 g of the powder in 200 mL of water for 2 hours, followed by filtration and evaporation. *In-vitro* studies of Cellular assay such as MTT and malondialdehyde (MDA) were performed to evaluate cytotoxicity and lipid peroxidation. In addition, the antioxidant potential of the extract was assessed by Ferric Reducing Antioxidant Power, Phosphomolybdenum, DPPH radical scavenging, total antioxidant capacity, and nitric oxide assays to determine the IC_{50} values of *S. glauca*. For *in vivo* studies, diabetes was induced in Wistar rats by intraperitoneal injection of alloxan monohydrate (150 mg/kg body weight). The animals were divided into five groups: Group I (normal control), Group II (diabetic control), Group III (standard drug Ramipril, 10 mg/kg), Group IV (diabetic rats treated with *S. glauca* extract, 250 mg/kg), and Group V (diabetic rats treated with *S. glauca* extract, 500 mg/kg). Fasting blood glucose was measured 72 hours post-induction. Urine and blood samples were collected weekly for four weeks to estimate urine creatinine, urine urea, urine albumin, serum creatinine, serum urea, and blood urea nitrogen (BUN). Serum calcium levels were analyzed at the end of the study, followed by histopathological examination of kidney tissues.
Results: Treatment with *Simarouba glauca* extract significantly reduced fasting blood glucose, improved renal function parameters, and preserved kidney histoarchitecture in diabetic rats. Antioxidant assays demonstrated strong radical scavenging and reducing activity. In vitro results confirmed the cytoprotective and antioxidant potential of the extract.
Conclusion: The aqueous extract of *Simarouba glauca* exhibits significant nephroprotective and antioxidant properties, supporting its potential as a therapeutic agent in the management of diabetic nephropathy and oxidative stress-related complications.

Keywords: antioxidant, malondialdehyde, lipid peroxidation, paradise tree, ramipril

Introduction

Simarouba glauca, commonly known as 'Lakmitaru' or 'Paradise tree' belongs to Simaroubaceae. It is indigenous to Southern Florida, West Indies and Brazil [1], is considered exotic in India, Sri Lanka, Philippines and Myanmar [2]. It was first introduced by National Bureau of Plant Genetic Resources in the research station at Amravati in Maharashtra in 1966 [3, 4, 5] and later to the University of Agricultural Sciences, Bangalore in 1986.

Paniyas Tribes of Wayanad district, Kerala used the leaf decoction to treat cancer [6] some communities of Shimoha, Karnataka and for diabetes [7]. In Cuban folk medicine, the leaves are used for helmintic infection, dysentery and to exert hypotensive action [8]. Phytochemical survey reported the presence of alkaloid, flavonoid, terpenoid, anthraquinone, steroids, phenol, saponin, tannin, carbohydrate [9]. It is every effective in treating cancer and has predominant anti-cancerous property. Not only it is anti-cancerous but also antidiabetic [10], nephroprotective [11], antibacterial [12], antifungal [13], antioxidant [14], anticancer [15], anti-proliferation and pro-apoptotic avtivity [16], anti-inflammation [17], antimicrobial [18], antimalarial [19], antiamoebic [20], antiulcer [21], hepatoprotective [22], reducing patchy skin saturation [23], haemolytic [24], acaricidal [25], hypertensive [26], antidysenteric [27], analgesic [28] , anti-leukemic activitiy [29, 30]. It is crucial and timely to investigate the nephroprotective effect of *Simarouba glauca*, which has not been studied so far. Therefore, the present study aims to explore the nephroprotective potential of *Simarouba glauca* in the management of diabetic nephropathy.

Material and methods

Collection and authentication

Leaves ware collected from the village of Nazereth, Thoothukudi, Tamil Nadu in the month of February 2025. The leaves were identified and authenticated by Dr. S. Mutheeswaran, Scientist, Xavier Research Foundation, St. Xavier's College, Palayamkottai, Tamil Nadu. The herbarium of this specimen was kept in the department for further reference.

Preparation of Aqueous extract

The fresh matured leaves of *Simarouba glauca* were collected, thoroughly washed with water and shade-dried. The dried leaves were pulverized into a fine powder. About 10 g of the powdered material was boiled in 200 ml of water

for 2 hours. The extract was filtered and evaporated to dryness. As per the requirement, respective batches were prepared.

Effect of *Simarouba glauca* against Alloxan induced diabetes in rat

Experimental Animal

Male albino Wistar rats weighing between 120-225 g was acclimatized in separate cages for a week. Rats were housed at a temperature of 24 ± 1 °C and humidity of 65-70%, and were subjected to a 12 h light/dark cycle. All experimental procedures involving animals were conducted in accordance with Institutional guidelines and were approved by the Institutional Animal Ethics Committee (IAEC) of Madurai medical college, Madurai-20. Approval no: 14/2025 dated 05/06/2025.

Experimental design

Experimental rats were divided into five groups (n=6/ group). Diabetes was induced in overnight- fasted rats by a single I.P injection of freshly-prepared alloxan monohydrate (150 mg/kg/b. w), dissolved in a cold physiological saline (0.9% NaCl). Alloxan is a toxic glucose analogue that preferentially accumulate in pancreatic beta cells via the GLUT2 glucose transporter and is used for the experimental induction of diabetes mellitus in animals [31-33].

The rats were divided into 5 groups of 6 animals in each group;

Group	Treatment	Dose	Duration
Group I (Normal Control)	Saline	Normal saline	4 weeks
Group II (Disease control)	Alloxan monohydrate injected	150 mg/kg/ b. w	4 weeks
Group III (Standard)	Ramipril	10 mg/kg, oral	4 weeks
Group IV (Test 1)	*Simarouba glauca* low dose	250 mg/kg	4 weeks
Group V (Test 2)	*Simarouba glauca* high dose	500 mg/kg	4 weeks

After 72 h of alloxan injection, blood glucose levels were measured for confirming the onset of diabetes via tail vein at 0.2-0.3 ml of blood. Animals with blood glucose concentration equal or more than 200 mg/dL were considered diabetic rats.

Evaluation parameters

Metabolic parameters and tissue collection

- During the experimental period all the rats were provided with free access to rat chow and water. Rats were carefully monitored in order to assess variations in water and food intake, body weight, urine output and general behaviour monitored weekly Up to 4 weeks (7,14,21,28 days).
- Urinary creatinine, urea and albumin levels were determined Up to 4 weeks in urine sample using commercial kits in a semi-auto analyser [34].
- Serum creatinine, urea, BUN and were determined up to 4 weeks using 1.0 ml of blood collected via retrobulbar vein by enzymatic commercial kits using a semi- automated photometer [34].
- At the end of experimental period on 30th day (following overnight fasting after the 4-week treatment period), rats were euthanized using Ketamine (75 mg/kg, i.p) and Xylazine (10 mg/kg, i.p). blood was collected by cardiac puncture at 1.5-2mL, serum was separated for serum Calcium level analysis [35] and the kidneys were excised for histopathological examinations.

Renal Histology

Tissue processing begins by collecting the organ or tissue sample and fixing it in 10% formalin for 24 hours at room temperature to preserve its structure. After fixation, the tissue is rinsed under running tap water for 3 hours, then sequentially dehydrated using increasing concentrations of ethanol-50% for 1 hour, 70% overnight (which also serves as a storage medium), followed by 80%, 90%, and three rounds of 100% ethanol, each for 1 hour-to remove water. Clearing is then performed by immersing the tissue in xylene through three changes of 1 hour each, which replaces the ethanol and prepares the tissue for infiltration. Paraffinization follows, where the tissue is placed in melted paraffin wax at 58-60°C for three rounds of 1 hour each to allow wax infiltration. Finally, the tissue is embedded in fresh melted wax using a paper mould, left undisturbed overnight to solidify, and the resulting wax-embedded sample is called a block, ready for sectioning and microscopic analysis.

Sectioning

Cut thin slices (about 5 μm thick) from the wax block using a microtome. Warm a water bath to 45-50°C and float the sections in it to flatten them. Then, carefully transfer the sections onto clean microscope slides for further analysis.

Glass slides preparation

Separate fresh chicken egg whites from the yolks and discard the yolks. Beat the egg whites until smooth but still liquid. Spread the albumen on a slide and briefly pass it through a flame until it smokes-avoid overheating. Let the slide cool, then place the tissue section from the water bath onto it and bake as usual. Finally, let the slides dry for at least 24 hours.

Slide Processing

(Note: Tissue processing protocol has to be reversed for staining)

Start by placing the slide in xylene twice for 5 minutes each to remove paraffin (deparaffinization). Then, gradually rehydrate the tissue by transferring the slide through decreasing concentrations of ethanol: two rounds of 100% ethanol for 3 minutes each, followed by 95%, 70%, and 50% ethanol, each for 3 minutes. Finally, rinse the slide with distilled water to complete the process.

Staining

Stain the slide by placing it in haematoxylin for 5 minutes, then rinse under running tap water for another 5 minutes. Clean the slide with 70%, 80%, and 90% alcohol for 1 minute each. Dip the slide in eosin for 10 seconds, then transfer it to 90% ethanol for 2 minutes, followed by 100% ethanol for 2 minutes, and finally xylene for 2 minutes. Add DPX mount and cover with a cover slip. The slide is now ready for microscopic examination [36].

Statistical analysis
The data were expressed as mean ± standard deviation (SD). The Significance of differences among the group was assessed using one way analysis of variance (ANOVA) followed by Tukey's test P values less than 0.05 were considered as significance.

In-vitro* Diabetic nephropathy activity using *NRK-53E cell line

Cell culture
- Normal Rat Kidney Cells (NRK-52E) were cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10 % Fetal bovine serum (FBS), 1% L-glutamine and 1% Penicillin/Streptomycin.
- The cells were maintained at 37°C in a humidified incubator with 5 % CO_2 [37].

MTT Assay
The assay was carried out according to Mosmann (1983) [38] by using (3-(4,5-dimethyl thiazol-2yl)-2,5-diphenyl tetrazolium bromide (MTT). MTT is cleaved by mitochontrial succinate dehydrogenase and reductase of viable cells, yielding a measurable purple product formazan. This formazan production is directly proportional to the viable cell number and inversely proportional to the degree of cytotoxicity. Briefly, Trypsinised 10,000 cells were seeded in 96-well plate and incubated at 37°C for 48 hrs. After 48 h incubation, the NRK 52E cells were treated with *Simarouba glauca* aqueous extract in different concentrations for 24, 48 and 72 hours. After treatment, the media were removed and wells were added with MTT (5 mg/ml prepared in phosphate-buffered saline) and left for 4 hours at room temperature. Thus, the formazan crystals formed were dissolved in 100 µl DMSO and absorbance was read in a microplate reader at 570 nm. The *Simarouba glauca* extract 1 mg was mixed with sterile complete media and further diluted to produce the desired concentrations (10 µG, 50 µG, 100 µG) [38].

MDA Assay
NRK-52E cells were treated in triplicate with test extract of *S. glauca* at 100 µg/mL and incubated for 12 h. Cell culture supernatants were collected and centrifuged at 9000 × g for 10 min at 4 °C to remove debris. To 200 µL supernatant, 200 µL BHT solution was added and vortexed. Proteins were precipitated by adding 1.0 mL 10% (w/v) TCA, vortexed and centrifuged at 9000 × g for 10 min at 4 °C. 200 µL of the clarified supernatant was mixed with 600 µL TBA reagent (0.67% w/v TBA in 50 mM NaOH), heated in a boiling water bath at 95-100 °C for 20 min, cooled on ice for 5 min, centrifuged at 9000 × g for 10 min and the absorbance of the clear supernatant was measured at 532 nm. MDA standard curve (0-10 µM prepared from TEP) was run in parallel; results were expressed as nmol MDA/mg protein following normalization to cellular protein [39-41].

***In-vitro* antioxidant activity**
In vitro antioxidant potentials of successive extracts of leaf of *Simarouba glauca* (L.) were determined by various systems.

Determination of DPPH free radical scavenging assay
Various concentrations of the $AESG_L$ (20-100 µg/ml) were dissolved in water. To 1 ml of each extract solution, 3 ml of 0.1 mM DPPH solution (in methanol) was added and mixed well. For the control, 1 ml of methanol was used in place of the plant extract. The reaction mixtures were incubated in the dark at room temperature for 30 minutes. After incubation, the absorbance was measured at 517 nm using a UV-Visible spectrophotometer [42].

Determination of Hydrogen peroxide scavenging assay
To 1 ml of test solution of $AESG_L$ of different concentrations, 3.8 ml of 0.1 M Phosphate buffer solution (pH 7.4) and then 0.2 ml of hydrogen peroxide solution were added. The absorbance of the reaction mixture was measured at 230 nm after 10 min [43]. the reaction mixture without sample was used as blank. Ascorbic acid was used as standard.

Determination of nitric oxide scavenging assay
A 10 mM solution of sodium nitroprusside was prepared in Phosphate buffered saline (pH 7.4) and various concentrations of the $AESG_L$ were added to this solution. The resulting mixture were incubated at 37°C for 60 minutes under light to allow the generation of nitric oxide radicals. After incubation, half of each reaction mixture was taken and mixed with equal volume of freshly prepared Griess reagent reacts with nitrite, a stable end product of nitric oxide, to form a pink azo dye. The reaction mixture was then incubated at 25°C for 30 minutes in the dark to ensure complete color development. Following this, the absorbance of the resulting pink-colored solutions wad measured at 546 nm using a UV-Visible spectrophotometer against a suitable blank [44]. A decrease in absorbance compared to the control indicates the nitric oxide scavenging potential of the leaf extract.

Determination of ferric reducing antioxidant power (FRAP) activity
Different concentrations of the $MESG_L$ (10-50 µg/ml) were mixed with 2.5 ml of 0.2 M Phosphate buffer (pH 6.6) and 2.5 ml of 1 % Potassium ferricyanide. The mixture was vortexed and incubated at 50°C for 20 minutes. After incubation, 2.5 ml of 10% Trichloroacetic acid was added and centrifuged at 3000 rpm for 10 min. then, 2.5 ml of the resulting supernatant was mixed with 2.5 ml deionized water and 0.5 ml of 0.1% ferric chloride. The absorbance of the colored solution was measured at 700 nm against a blank using UV spectrophotometer [45]. Ascorbic acid was used as standard.

Determination of total antioxidant capacity content
$AESG_L$ in different concentration ranging from 100 µl to 500 µl were added to each test tube individually containing 3 ml of distilled water and 1 ml of molybdate reagent solution. These tubes were kept at 95°C for 90 min. after incubation, these tubes were normalized to room temperature for 20-30 min and the absorbance of the reaction mixture was measured at 695 nm [46]. Mean values from three independent samples were calculated for each extract. Ascorbic acid was used as positive reference standard.
All the antioxidant activities were evaluated and the percentage inhibition was calculated using the formula:
% Inhibition= [(Control- Test)/Control] x 100
The concentration of the sample required for 50 % reduction in absorbance (IC_{50}) was calculated using linear regression analysis.

Result and discussion

Effect of *Simarouba glauca* against Alloxan induced diabetes in rat

General behaviours

Table 1: Estimation of General behaviours of 4- week treatment of *Simarouba glauca* against Alloxan induced diabetic rat

S. No	Parameters	Mean ± SD (4th week)				
		Normal control	Disease control	standard	Test 1	Test 2
1	Body weight (g)	154.16 ±9.75	138.5 ±15.63	154.16 ±40.56	169.16 ± 10.17	169.16 ± 12.72
2	Food intake (g)	161.22±1.36	161.73 ±1.34	162.39 ±1.59	161.98 ± 1.14	162.10 ± 0.87
3	Water intake (ml)	17.25 ±4.95	50.08 ±4.44	114.02± 2.20	166.27 ± 6.35††††	134.50 ±7.95****††††
4	Urine output (ml)	8.42 ± 2.51	16.58 ±4.31	8.61 ± 2.53	13.79 ±3.68	9.37 ± 1.81

All parameters are calculated in One-way ANOVA followed by Tukey's test [Graph pas prism 10.6.0 (890)]
*P<0.05-significant with normal; **P<0.001-moderate significant with normal;
***P<0.0008-highly significant with normal; ****P<0.0001-very highly significant with normal
†P<0.05-significant with ramipril; ††P<0.001-moderate significant with ramipril;
†††P<0.0008-highly significant with ramipril; ††††P<0.0001-very highly significant with Ramipril.

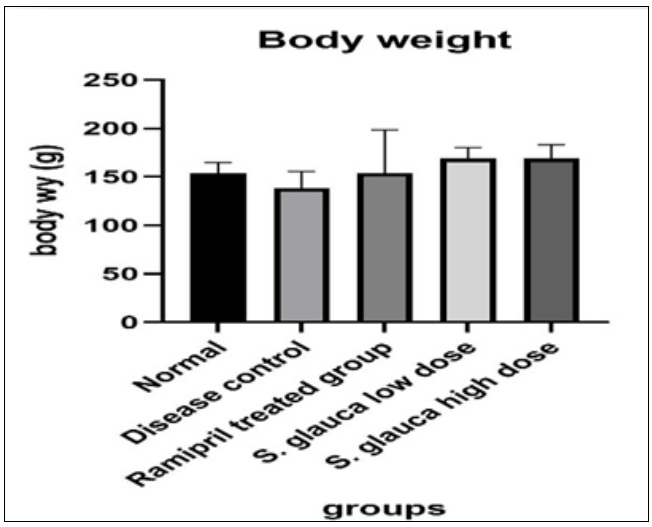


Fig 1a: body weight

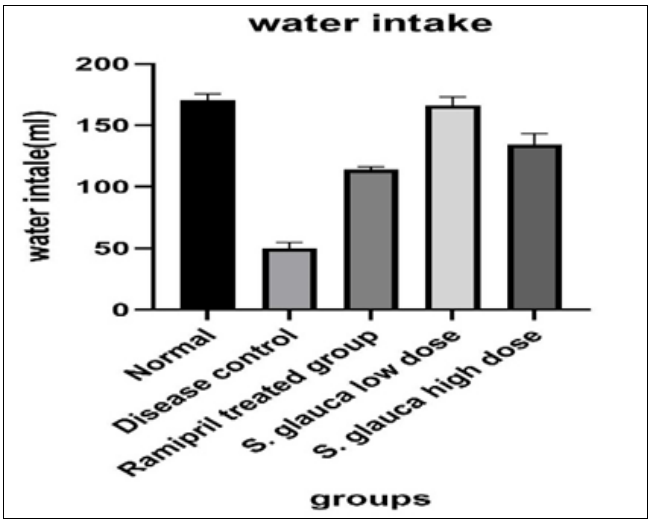


Fig 1b: water intake

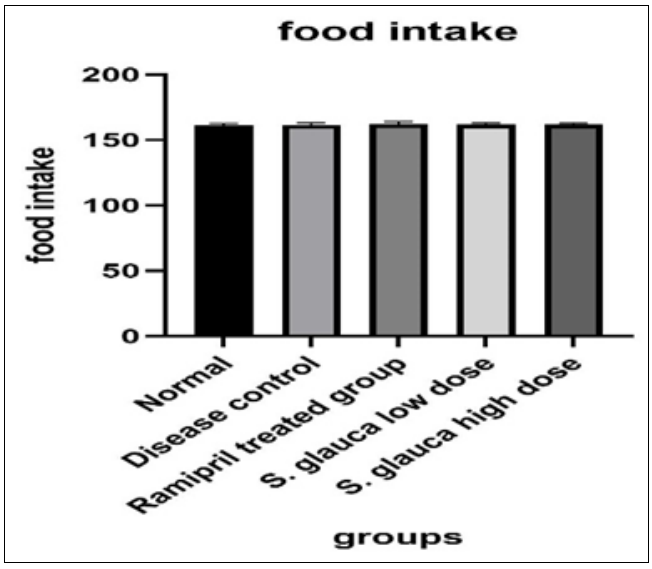


Fig 1c: Food intake

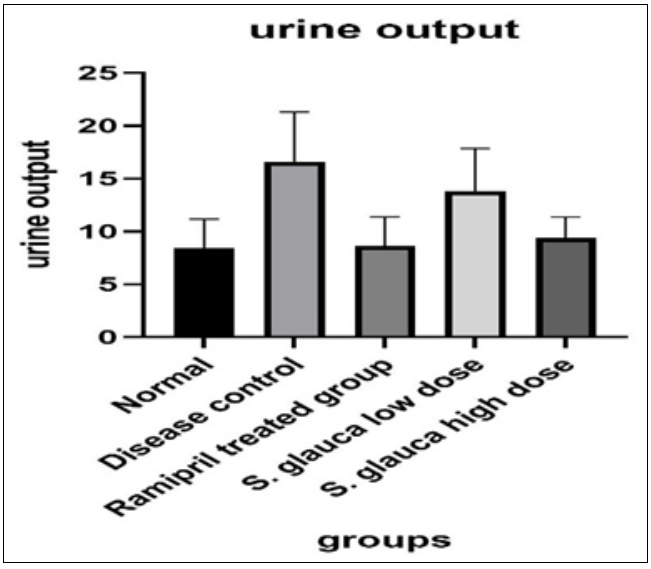


Fig 1d: Urine output

Urine output

In general behavioural parameters such as body weight, food intake and urine output did not show any significant difference when compared with the normal and Ramipril-treated groups. However, water intake was markedly elevated, treated with low and high doses of *Simarouba glauca* respectively, when compared with the Ramipril-treated group. Moreover, the water intake of the high-dose treated group (Group 5) also showed a highly significant elevation when compared with the normal control group.

Urinary profile

Table 2: Estimation of Urinary profile of 4- week treatment of *Simarouba glauca* against Alloxan induced diabetic rat

S. No	Parameters	Mean ± SD (4th week)				
		Normal control	Disease control	standard	Test 1	Test 2
1	Urine creatinine (mg/dL)	9.56 ±0.68	24.96 ±0.97	4.51 ± 1.68	6.84 ± 0.62**†††	7.46 ± 0.60*†††
2	Urine Urea (mg/dL)	35.03 ± 0.30	66.88 ± 0.38	54.08 ± 0.31	50.66 ± 0.29****††††	45.03 ± 0.51****††††
3	Urine Albumin (mg/dL)	2.96 ± 0.20	28.76 ± 0.45	15.9 ± 0.27	17.43 ± 0.30****††††	15.75 ± 0.33****

All parameters are calculated in One-way ANOVA followed by Tukey's test [Graph pas prism 10.6.0 (890)]
*P<0.05-significant with normal; **P<0.001-moderate significant with normal;
***P<0.0008-highly significant with normal; ****P<0.0001-very highly significant with normal
†P<0.05-significant with ramipril; ††P<0.001-moderate significant with ramipril;
†††P<0.0008-highly significant with ramipril; ††††P<0.0001-very highly significant with Ramipril.

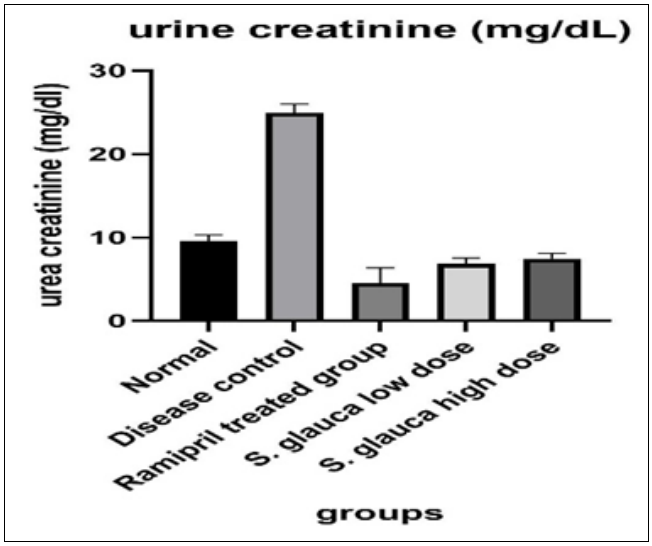


Fig 2a: Urine creatinine

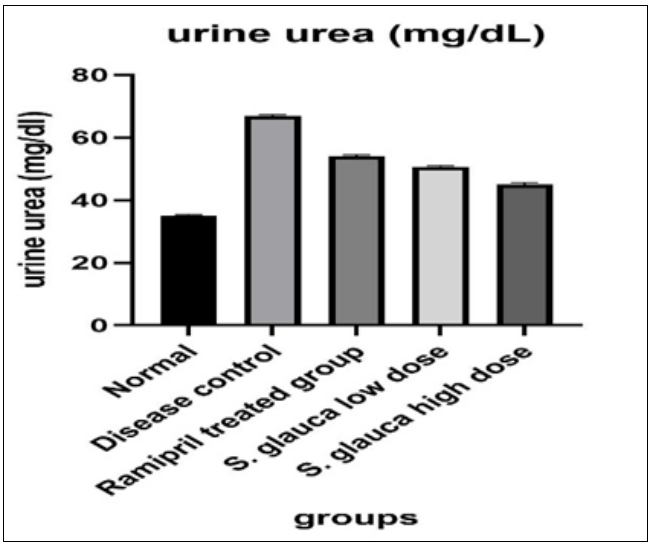


figure 2b: Urine urea

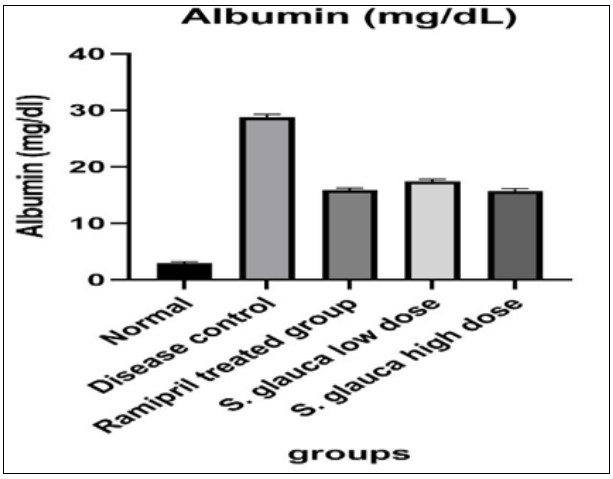


Fig 2c: Albumin

In the urinary profile, creatinine levels showed a moderate decrease in the low-dose *Simarouba glauca*-treated group when compared with the normal and Ramipril-treated groups. The high-dose group exhibited a significant decrease in creatinine compared with the normal control and a highly significant decrease when compared with the Ramipril-treated group. Urea levels were markedly decreased and found to be very highly significant in both low- and high-dose treated groups when compared with both the normal and Ramipril-treated groups. Albumin levels showed a very highly significant decrease in the low-dose group compared with both the normal and Ramipril-treated groups, whereas in the high-dose group the decrease in albumin levels was very highly significant only when compared with the disease control group.

Serum parameters

Table 3: Estimation of Serum parameters of 4- week treatment of *Simarouba glauca* against Alloxan induced diabetic rat

S. No	Parameters	Mean ± SD (4th week)				
		Normal control	Disease control	standard	Test 1	Test 2
1	Serum Creatinine (mg/dL)	0.45 ± 0.04	1.89 ± 0.01	0.43 ± 0.009	0.60 ± 0.006****††††	0.48± 0.009†
2	Serum Urea (mg/dL)	33.1 ± 0.23	78.95 ± 0.17	42.73 ± 0.19	46.08 ± 0.15****††††	41.75± 0.19****††††
3	BUN (mg/dL)	15.45 ± 0.09	36.86 ± 0.14	19.91 ± 0.13	21.45 ± 0.17****††††	19.51 ± 0.21****††
4	Serum Calcium (mg/dL)	6.9 ± 0.19	5.5 ± 0.18	6.4 ± 0.13	5.8 ± 0.18****††††	6.1 ± 0.14****†

All parameters are calculated in One-way ANOVA followed by Tukey's test [Graph pas prism 10.6.0 (890)]
*P<0.05-significant with normal; **P<0.001-moderate significant with normal;

***P<0.0008-highly significant with normal; ****P<0.0001-very highly significant with normal
†P<0.05-significant with ramipril; ††P<0.001-moderate significant with ramipril;
†††P<0.0008-highly significant with ramipril; ††††P<0.0001-very highly significant with Ramipril.

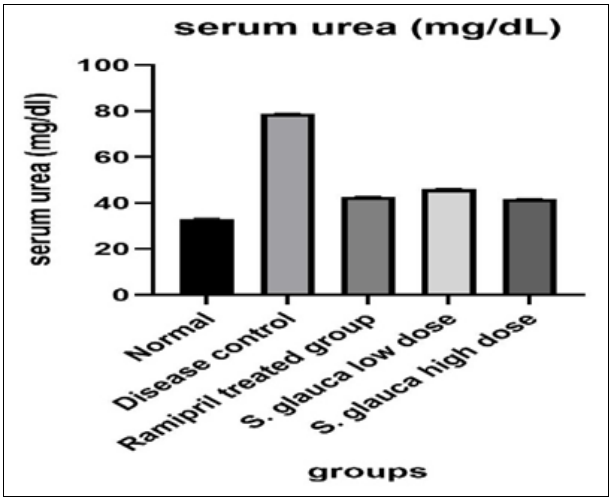


Fig 3a: Serum creatinine

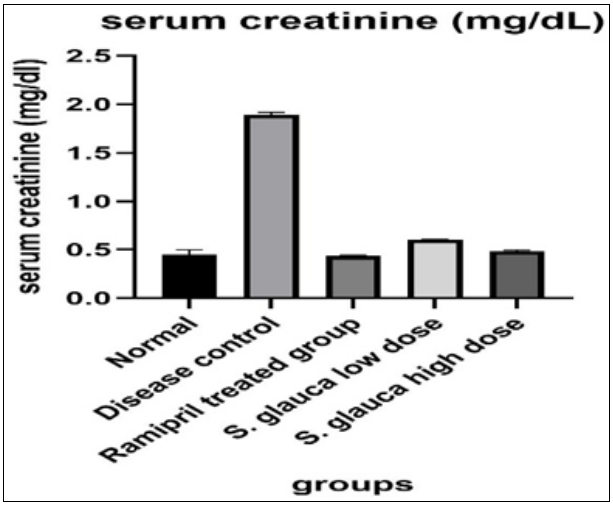


Fig 3b: Serum urea

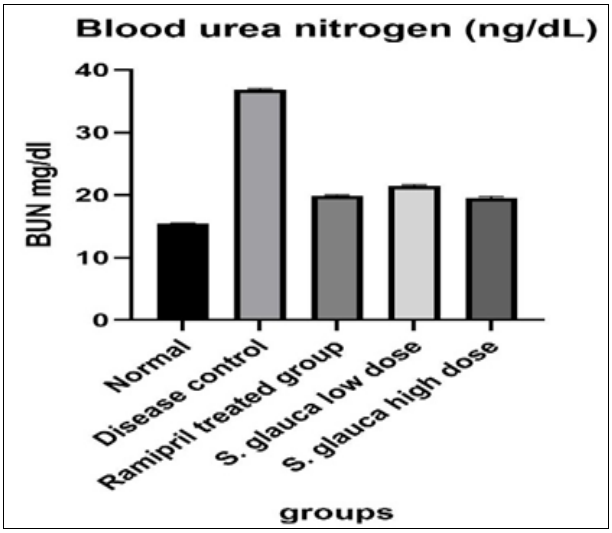


Fig 3c: BUN

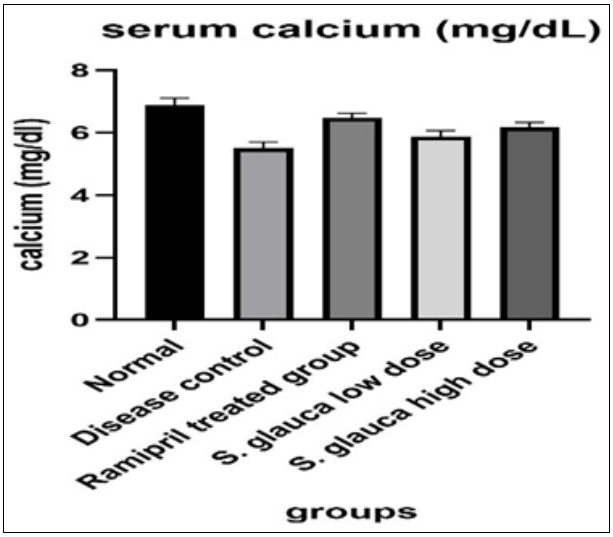


Fig 3d: Serum calcium

Fig 3: Estimation of Serum parameters of 4- week treatment of *Simarouba glauca* against Alloxan induced diabetic rat

In the serum profile, all measured parameters exhibited very highly significant decreases in the low-dose *Simarouba glauca*-treated group when compared with both the normal control and Ramipril-treated groups. In the high-dose group, serum creatinine showed a significant decrease compared with the Ramipril-treated group, whereas serum urea, BUN and calcium levels showed very highly significant decreases when compared with the normal control. Furthermore, when compared with the Ramipril-treated group, serum urea remained very highly significant, BUN showed moderate significance and calcium levels were significantly decreased.

The study showed that Ramipril provided strong nephroprotection significantly decreasing elevated renal parameters. The high dose of *Simarouba glauca* extract (500 mg/kg) offered partial nephroprotection also decreasing serum creatinine, urea, BUN and calcium more effectively than the low dose.

Renal Histology

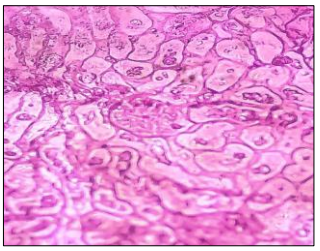


Fig 4a

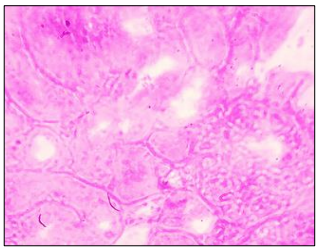


Fig 4b

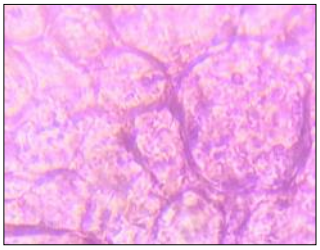


Fig 4c

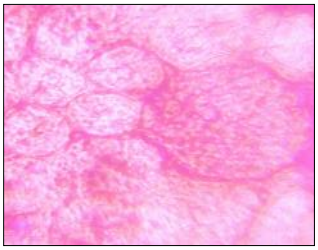


Fig 4d

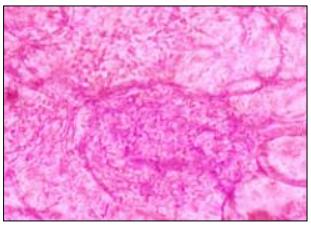


Fig 4e

a- Normal control; b- Disease control; c- Standard; d- *Simarouba glauca* (500mg/kg);
e- *Simarouba glauca* (250mg/kg)

Renal histopathological analysis across all experimental groups revealed distinct structural changes. The Normal Control (NG) group exhibited intact renal architecture with well-defined glomeruli and tubules, showing no pathological alterations. In contrast, the Diabetic Control (DM) group displayed severe damage, including glomerular basement membrane thickening, interstitial fibrosis, hyaline degeneration, epithelial vacuolar degeneration, hyaline cast formation, depicted the arteriolopathy-hallmarks of diabetic nephropathy. Treatment with Ramipril in the Diabetic control group led to partial amelioration of renal injury, with slight clumpy formations in glomeruli and tubules, indicating a modest protective effect. Similarly, S*imarouba glauca* High Dose treated group showed histological features comparable to the Ramipril-treated group, suggesting that high-dose of *Simarouba glauca* may exert equivalent nephroprotective effect. The *Simarouba glauca* Low Dose treated group demonstrated mild improvement, with partial preservation of glomerular and tubular structures, slight epithelial vacuolar degeneration, and minimal hyaline changes, reflecting a limited but noticeable protective response that was less pronounced than the high-dose or Ramipril treatments.

In-vitro* Diabetic nephropathy activity using *NRK-53E cell line

MTT assay

Table 4: Percentage Cell viability of *Simarouba glauca using NRK-53E cell line*

Percentage Cell Viability Of Simarouba Glauca			
Groups	24 hrs	48 hrs	72 hrs
Control	100	100	100
Osmotic Control	73.5	29.7	20.8
High Glucose	170.4	211.7	242.1
10 µG *(S. glauca)*	95.8	143.1	159.8
50 µG *(S. glauca)*	83.9	125.2	115.6
100 µG *(S. glauca)*	93.8	91.3	78

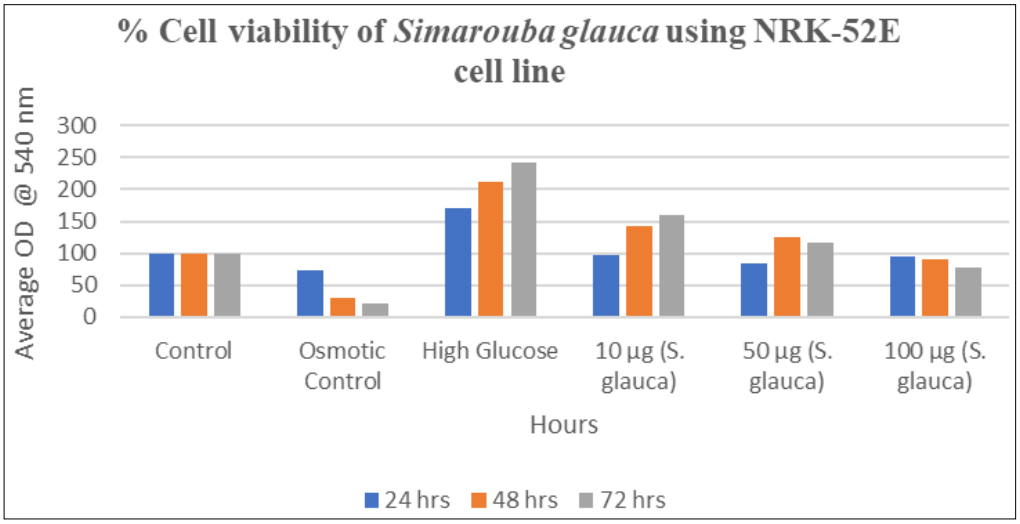
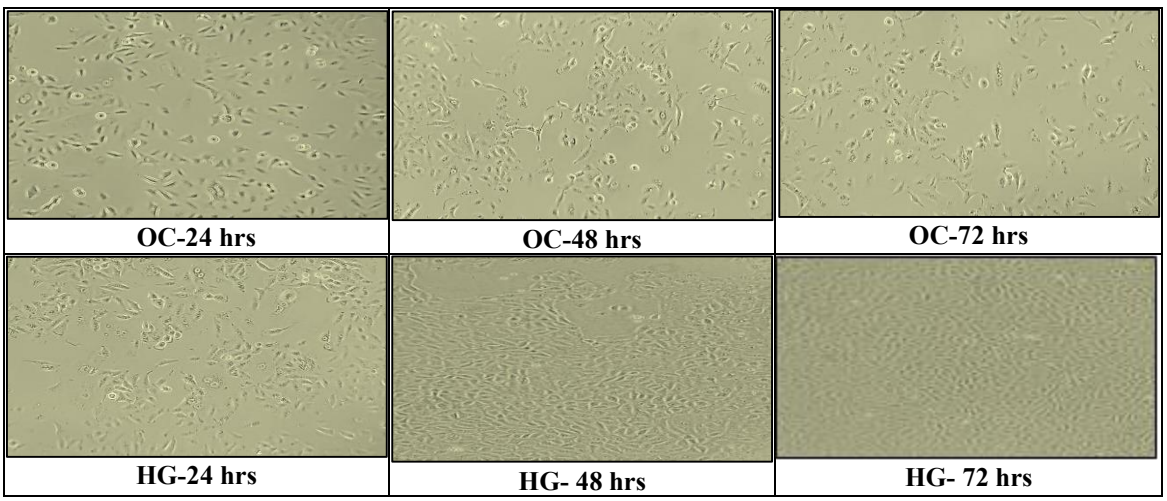


Fig 5: Percentage Cell viability of *Simarouba glauca using NRK-53E cell line*



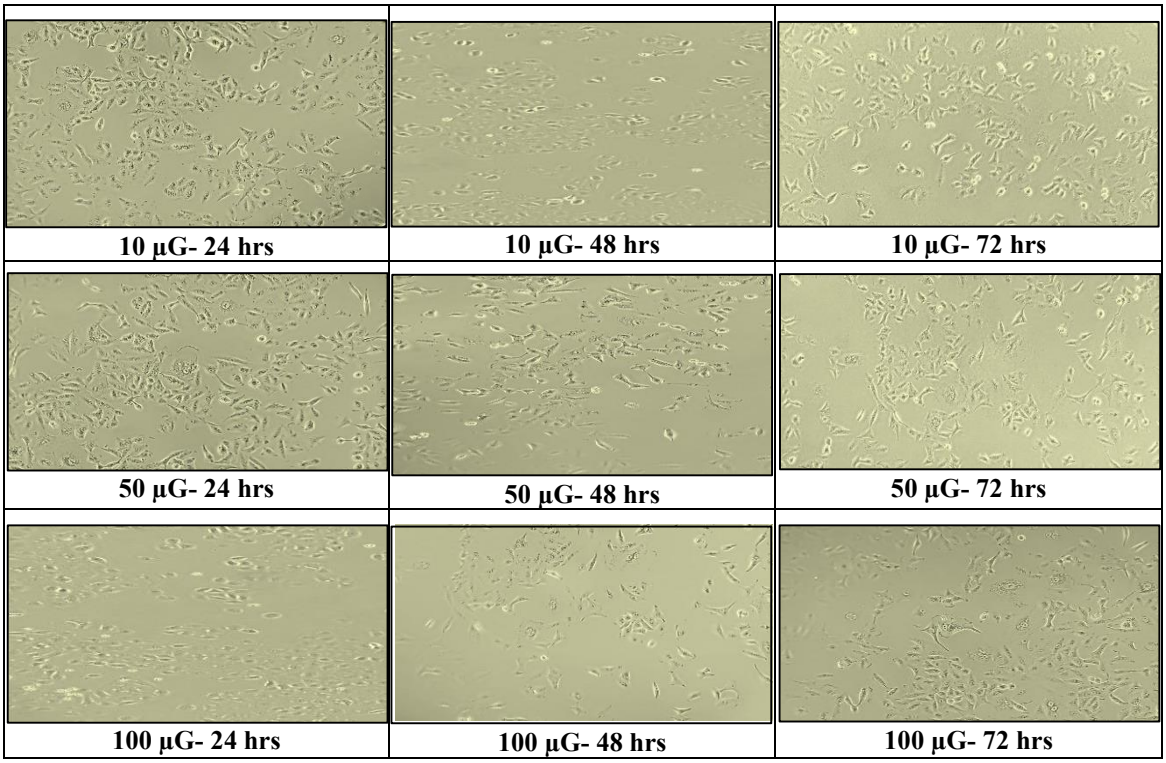


Fig 6: Percentage Cell viability of *Simarouba glauca* compared with control

The Cytotoxicity Study on Normal Rat Kidney cells (NRK-52E) showed that *Simarouba glauca* aqueous extract was safe at lower concentration, where 10 µg maintained and even improved cell viability under Hyperglycaemic stress, suggesting a protective effect. The 50µg dose showed moderate activity with partial decline at longer exposure, while 100 µg dose reduced cell survival indicating Cytotoxicity at higher concentration. Overall, the findings suggest that *Simarouba glauca* possesses dose-dependent effects with lower concentration supporting Kidney cell protection in Diabetic nephropathy, whereas higher concentrations may be harmful.

MDA assay

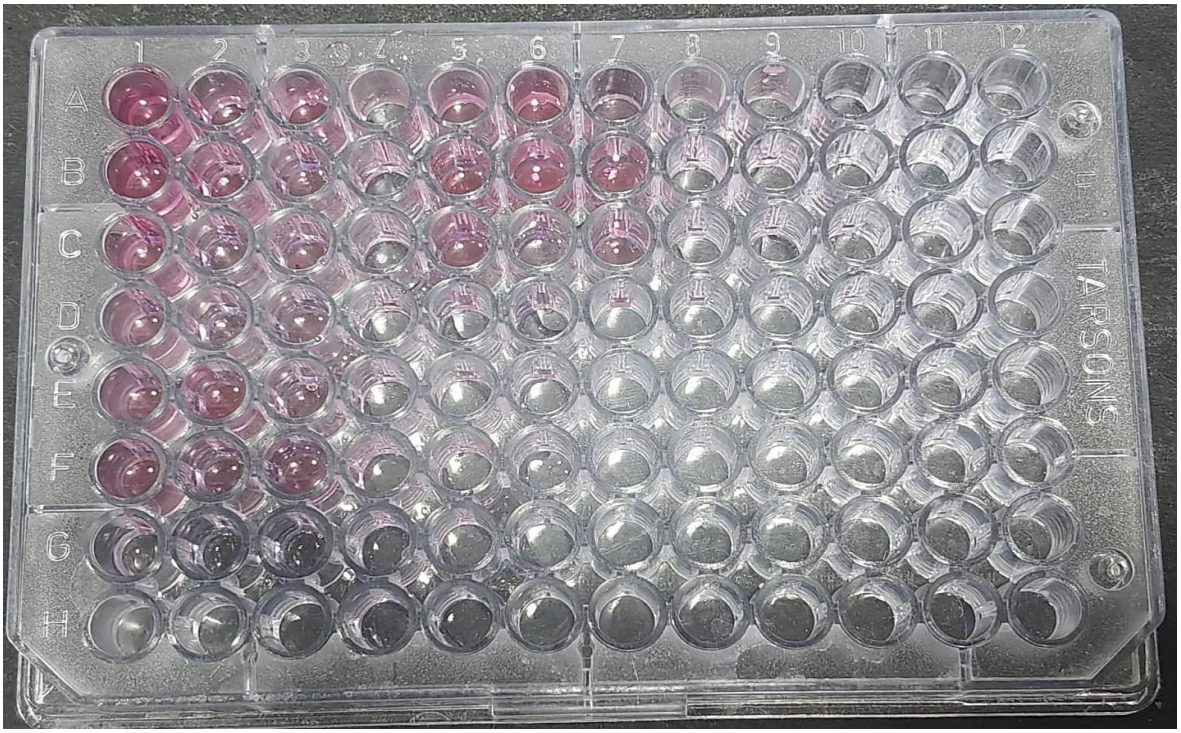


Fig 7: MDA assay 96-well plate

Labels: A1-A3-10 µM/mL; B1-B3-8 µM/mL; C1-C3-6 µM/mL; D-D3-4 µM/mL; E1-E3-2 µM/mL; F1-F3- 1µM/mL; G1-G3-0 µM/mL; A5-A7-High glucose; B5-B7-Normal glucose; C5-C7-Normal glucose+ 100 µg *S. glauca* extract.

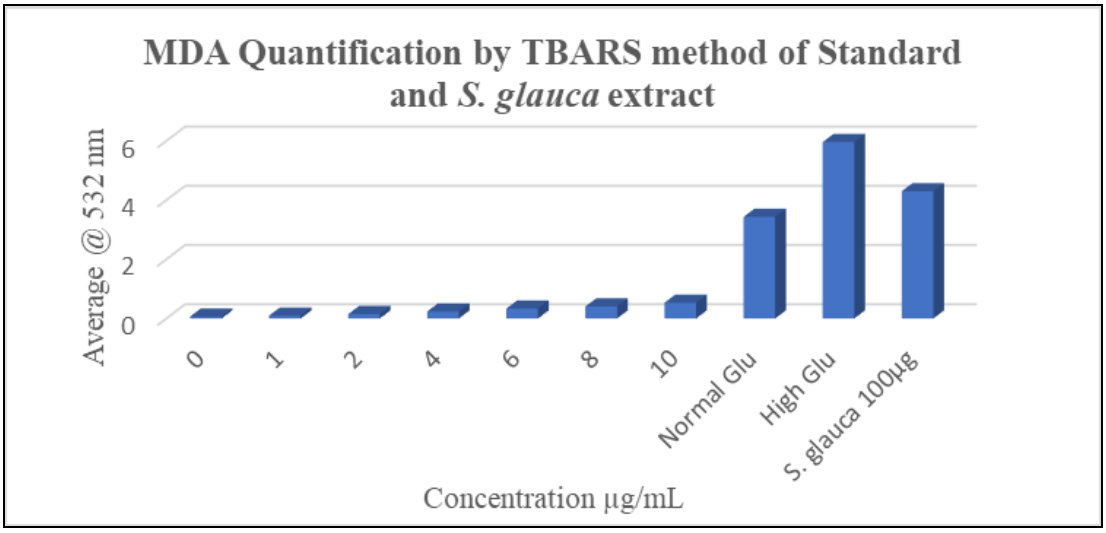


Figure 8: MDA Quantification by TBARS method of standard and *S. glauca* extract

Table 5: MDA Quantification by TBARS method of standard and *S. glauca* extract

S. No	MDA assay by TBARS Method	
	Concentration (µM/ml)	Average
1	Standard MDA (BHT)	
	1	0.0643
	2	0.098
	4	0.148
	6	0.2393
	8	0.3357
	10	0.4073
2	Normal glucose	0.5327
3	High glucose	3.434
4	*Simarouba glauca* 100 µg	5.95956

The *Simarouba glauca* extract 100 µg demonstrated a protective antioxidant effect against hyperglycemia-induced oxidative stress in NRK-52E cell lines. This was evidence by decrease in MDA concentration from 5.96 µM/mL (High glucose) to 4.30 µM/mL (*S. glauca* extract group) indicated a reduction in lipid peroxidation and cellular damage. Therefore, the S. glauca extract shows nephroprotective and antioxidant properties under hyperglycemic conditions.

In- Vitro* Antioxidant activity of *Simarouba glauca

The in-vitro antioxidant activity of *Simarouba glauca* were determined using five different methods and the results are presented below.

Determination of DPPH free radical scavenging assay of $AESG_L$

Table 6: Determination of DPPH free radical scavenging assay of $AESG_L$

S.no	Concentration (µg/ml)	Percentage inhibition of ascorbic acid (µg/ml)	Percentage inhibition of $AESG_L$(µg/ml)
1	0.5	29.92	17.75
2	1	34.15	21.79
3	1.5	43.83	41.61
4	2	50.00	49.55
5	2.5	55.28	51.59
	IC_{50}	7.9 µg/ml	8.7 µg/ml

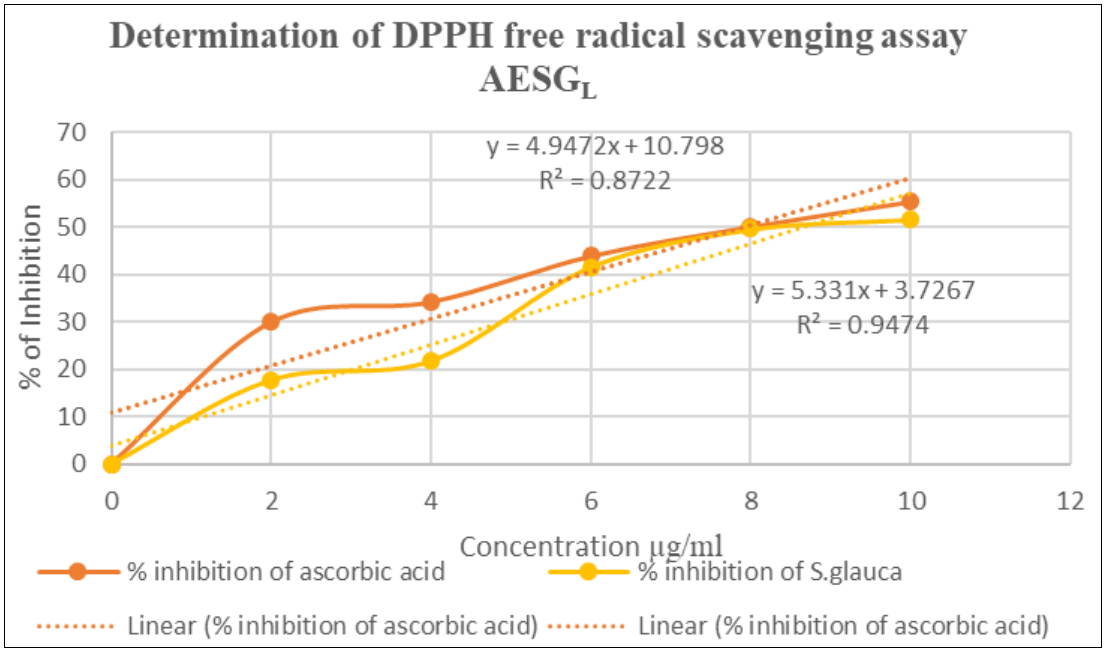


Fig 9: Graphical data of DPPH scavenging activity of $AESG_L$

The inhibitory concentration (IC_{50}) of *Simarouba glauca* leaf extract against the DPPH radical scavenging assay was found to be 8.7 µg/ml, when compared 7.9 µg/ml for ascorbic acid.

Determination of hydrogen peroxide scavenging activity of $AESG_L$

Table 7: Determination of hydrogen peroxide scavenging activity of $AESG_L$

S.no	Concentration (µg/ml)	Percentage inhibition of ascorbic acid (µg/ml)	Percentage inhibition of $AESG_L$(µg/ml)
1	2	29.92	13.95
2	4	32.03	31.31
3	6	47.23	44.52
4	8	50.08	52.3
5	10	63.26	54.4
	IC50	**7.3 µg/ml**	**8.01 µg/ml**

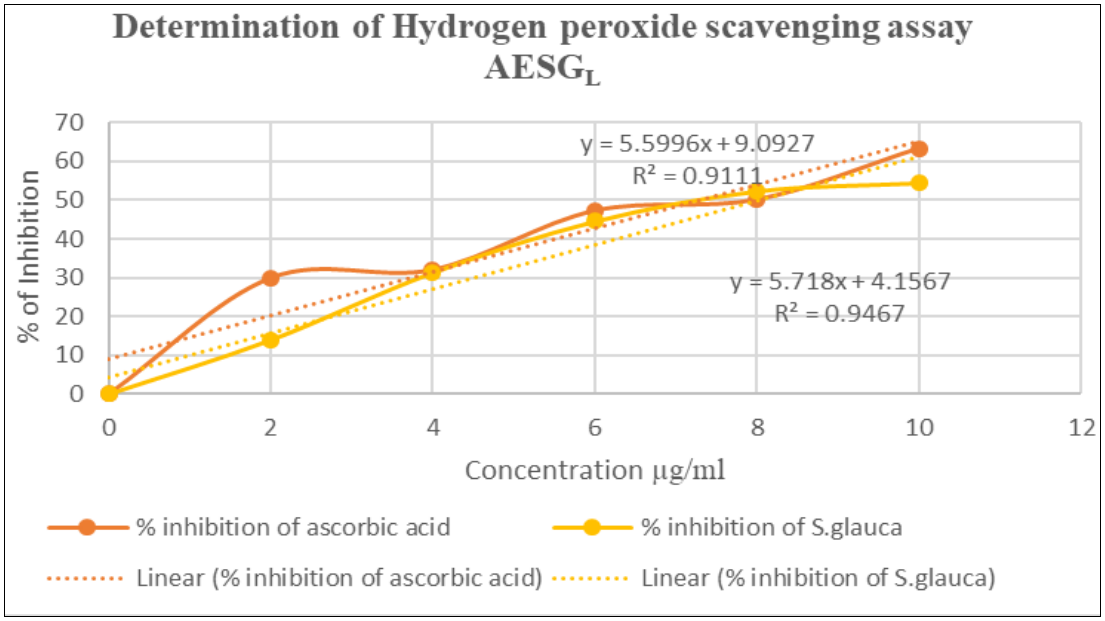


Fig 10: Graphical data of Hydrogen peroxide assay of $AESG_L$

The inhibitory concentration (IC_{50}) of *Simarouba glauca* leaf extract against the Hydrogen peroxide assay was found to be 8.01 µg/ml, when compared 7.3 µg/ml for ascorbic acid.

Determination of nitric oxide scavenging assay of $AESG_L$

Table 8: Determination of nitric oxide scavenging assay of $AESG_L$

S.no	Concentration (µg/ml)	Percentage inhibition of ascorbic acid (µg/ml)	percentage inhibition of $AESG_L$(µg/ml)
1	2	27.25669	9.9917
2	4	32.03546	22.8339
3	6	38.40715	34.1119
4	8	44.24787	34.256
5	10	50.08858	37.1887
	IC50	**9.09 µg/ml**	**12 µg/ml**

The inhibitory concentration (IC_{50}) of *Simarouba glauca* leaf extract against the Nitric oxide scavenging assay was found to be 12 µg/ml, when compared 9.09 µg/ml for ascorbic acid.

Determination of ferric reducing power of $AESG_L$

Table 9: Determination of ferric reducing power of $AESG_L$

S.no	Concentration (µg/ml)	Percentage inhibition of ascorbic acid (µg/ml)	Percentage inhibition of $AESG_L$(µg/ml)
1	2	31.1	34.36
2	4	37.29	37.8
3	6	43.32	42.06
4	8	47.23	44.1
5	10	53.42	44.9
	IC50	**8.2 µg/ml**	**9.4 µg/ml**

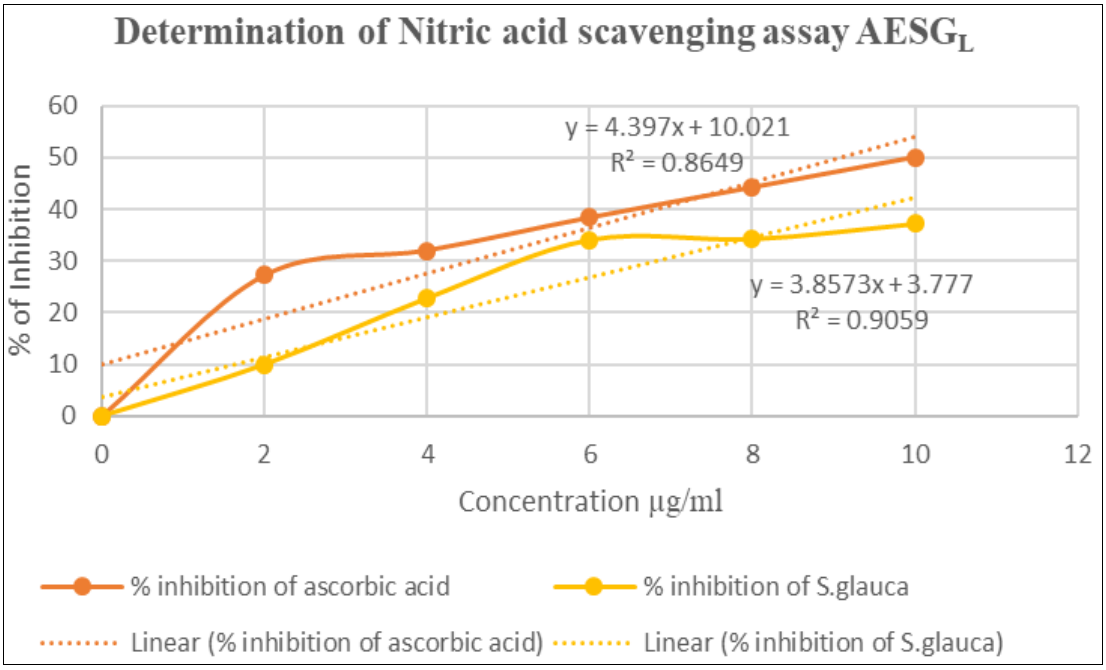


Fig 11: Graphical data of Nitric oxide Scavenging assay of $AESG_L$

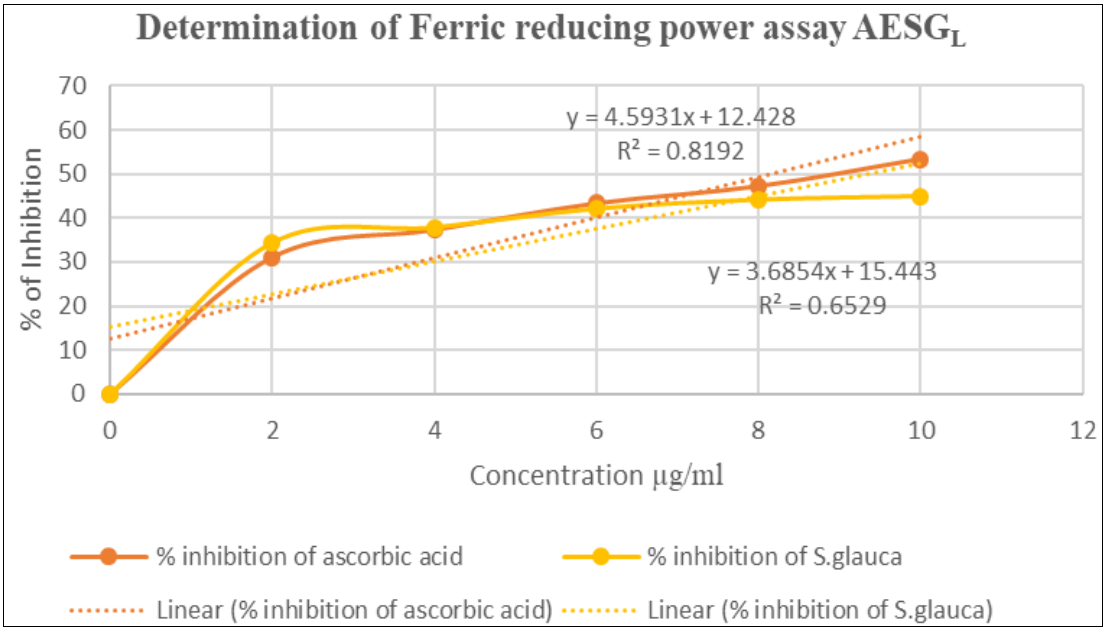


Fig 12: Graphical data of Ferric reducing power assay of $AESG_L$

The inhibitory concentration (IC_{50}) of *Simarouba glauca* leaf extract against the Ferric reducing power assay was found to be **9.4 µg/ml**, when compared **8.2 µg/ml** for ascorbic acid.

Determination of total antioxidant capacity of $AESG_L$

Table 10: Determination of total antioxidant capacity of $AESG_L$

S.no	Concentration (µg/ml)	Percentage inhibition of ascorbic acid (µg/ml)	Percentage inhibition of $AESG_L$(µg/ml)
1	2	28.23	29.23
2	4	36.47	37.47
3	6	53.23	54.23
4	8	73.67	64.67
5	10	91.17	71.17
	IC50	5.33 µg/ml	6.05 µg/ml

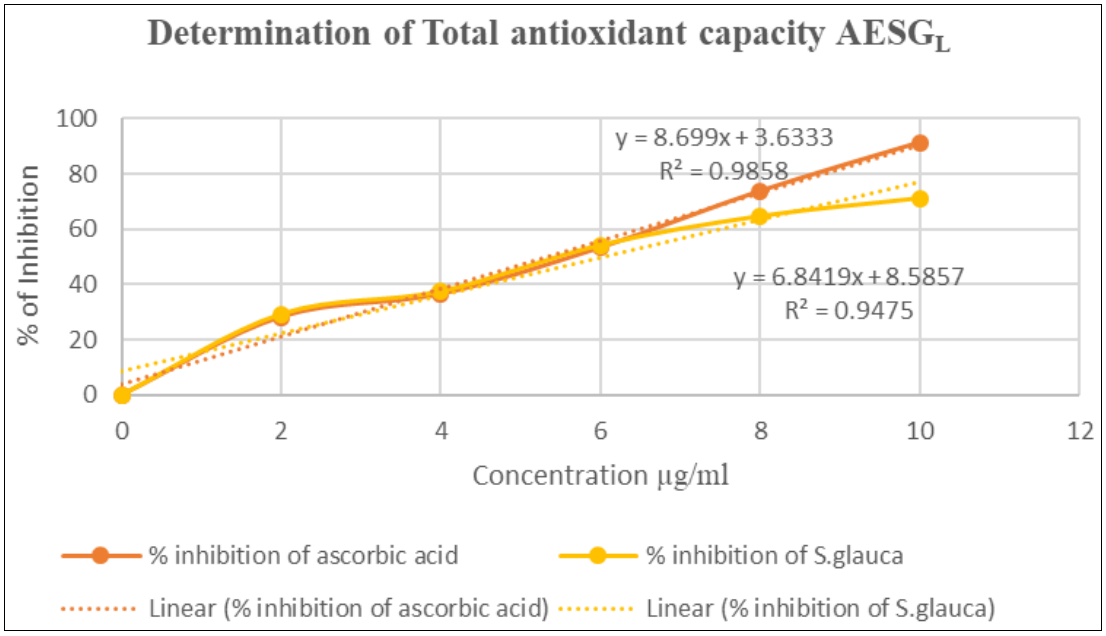


Fig 13: Graphical data of Total antioxidant capacity of AESG$_L$

The inhibitory concentration (IC_{50}) of *Simarouba glauca* leaf extract against the Total antioxidant capacity was found to be 6.05 µg/ml, when compared 5.33 µg/ml for ascorbic acid.

Conclusion

For the first time, the findings of this study demonstrate that the aqueous extract of *Simarouba glauca* leaves exhibits remarkable nephroprotective and antioxidant potential against alloxan-induced diabetic nephropathy. The extract showed strong free radical scavenging activity in vitro and effectively protected NRK-52E renal cells from oxidative damage. *In vivo* administration significantly improved renal function biomarkers, reduced hyperglycemia and preserved kidney histoarchitecture comparable to standard drug Ramipril. These results suggest that *Simarouba glauca* may serve as a promising natural therapeutic candidate for managing diabetic nephropathy and oxidative stress-related renal complications. Further studies are warranted to isolate and characterize the bioactive compounds responsible for these effects. This research scientifically validates its traditional use and establishes a foundation for future therapeutic applications in diabetic kidney disease.

Acknowledgement

The authors are greatly thankful to our beloved Dean Dr. L. Arul Sundaresh Kumar and respected Principal Dr. T. Venkata Rathina Kumar, Madurai medical college, Madurai-20 for providing necessary facilities to conduct the experiments.

Reference

1. Cronquist A. The old and the new in taxonomy. Bulletin of the Torrey Botanical Club,1944:71:226-246.
2. ICRAF Agroforestry Tree Database. http://ecocrop.fao.org/ecocrop/srv/en/cropView?id=9785, 2007.
3. Juyal SP, Singh RV, Lal UM, Singh JP. In strategies for making India self-reliant in vegetable oils (eds Rangarao V, Prasad MVR). Directorate of Oil Seed Research, Hyderabad,1991:335-377.
4. Bhagmal. In sustainability in oil seed (ed Prasad MVR). Indian Society of Oil Seed Research, Hyderabad, 1994, 83-90.
5. Rath T. Simarouba glauca an exotic edible oilseed. Indian Forester,1987:113(1):73-76.
6. Caceres A, Cano O, Samayou B, Aguilar L. Journal of Ethnopharmacology,1990:30(1):55-73.
7. Hingu B, Sake S, Gupta H, Vyas N, Thakur KS, Thakur M. Study on the phytochemical, antioxidant and in vitro anticancer activity on root extract of Simarouba glauca DC. Plant Science Today,2023:10(3):98-105. https://doi.org/10.14719/pst.2481
8. Sajeeda N, Kolgi RR, Shivakumara SL, Shivaraj Y, Karigar CS. Comparative phytochemical profile and antioxidant property of bark, flowers and leaves extracts of Simarouba glauca. Asian Journal of Pharmaceutical and Clinical Research,2019:12(9):56-63.
9. Nagaraj N, Hegde V, Gowda SK, Achur RN, Thippeswamy NB. Phytochemical analysis of Simarouba glauca DC and its antibacterial activity against MDR Salmonella Typhi. Journal of Pharmaceutical Sciences and Research,2021:13(6):351-356.
10. Paul A, Joseph N. Phytochemical analysis, molecular docking studies and anti-diabetic activity of Lakshmi taru. In: Sustainable Innovations in Functional Materials Developments and Applications, 2022, 29-36. ISBN: 978-81-957996-0-2.
11. Abdullah NT, Koneri R, Jha DK. Nephrotoxicity by using gentamicin in rats and screening of Simarouba glauca extract for its nephroprotective activity. International Journal of Pharmaceutical Sciences Review and Research,2018:51(11):182-186.
12. Mikawlrawng K, Kaushik S, Pushker A, Kumar S, Kameshwor M, Sharma G. Comparative in vitro antifungal activities of Simarouba glauca against

Fusarium oxysporum and Aspergillus parasiticus. Journal of Medicinal Plant Studies,2014:2:1-7.
13. Santhosh SK, Venugopal A, Radhakrishnan MC. Study on the phytochemical, antibacterial and antioxidant activities of Simarouba glauca. South Indian Journal of Biological Sciences,2016:2(1):119-124.
14. Shalal OS, Irayyif SM. Evaluation of cytotoxicity and apoptotic effects of Simarouba glauca on the prostate cancer cell lines PC3. Journal of Pakistan Medical Association, 2023, 73(9 Suppl 8):s113-s118. doi:10.47391/JPMA.IO024
15. Giridasappa A, Ismail SM, Rangappa D, Shanubhoganahalli Maheshwarappa G, Marilingaiah NR, Gollapalli SS, et al. Antioxidant, antiproliferative and antihemolytic properties of phytofabricated silver nanoparticles using Simarouba glauca and Celastrus paniculatus extracts. Applied Nanoscience,2021:11:2561-2576.
16. Jose SP, Sukumaran S, Mohanan R, Saji S, Asish A, George SM. Anti-inflammatory effect of squalene isolated from Simarouba glauca in experimental animal model. Pharmacognosy Research,2023:15(4):658-666.
17. Rudraswamy S, Hombarvali JS, Kenganora M, Doggalli N, Godhi BS, Srinivas S. Antimicrobial activity of Simarouba glauca leaf extracts against oral pathogens—An in vitro study. Journal of Orofacial Sciences,2023:15(1):8-15.
18. Franssen FF, Smeijsters LJ, Berger I, Medinilla Aldana BE. *In vivo* and in vitro anti-plasmodial activities of some plants traditionally used in Guatemala against malaria. Antimicrobial Agents and Chemotherapy,1997:41(7):1500-1513.
19. Wright CW, O'Neill MJ, Phillipson JD, Warhurst DC. Use of microdilution to assess in vitro antiamoebic activities of Brucea javanica fruits, Simarouba glauca stem, and a number of quassinoids. Antimicrobial Agents and Chemotherapy,1988:32(11):1725-1729.
20. Ridahunlang N. Evaluation of leaf extracts of Simarouba glauca on experimentally induced inflammatory bowel diseases in Wistar rats (Doctoral dissertation, KMCH College of Pharmacy, Coimbatore).
21. ohn PP, Jose N, Carla Sr B. Preliminary pharmacological screening of Simarouba glauca DC leaf extracts for hepatoprotective activity. World Journal of Pharmacy and Pharmaceutical Sciences,2016:5:714-724.
22. Hussain MS, Mohit, Khan MD. Pharmacological uses of Simarouba glauca: a review. Plant Archives,2021:21(1):648-655.
23. Lakshmi KS, Sangeetha D, Sivamani S, Tamilarasan M, Rajesh TP, Anandraj B. In vitro antibacterial, antioxidant, haemolytic, thrombolytic activities and phytochemical analysis of Simarouba glauca leaves extracts. International Journal of Pharmaceutical Sciences and Research,2014:5(2):432-437.
24. Santhana Bharathi N, Mahendran P. Exploration of acaricidal properties of certain seed oils against the Red Spider Mite (RSM), Oligonychus coffeae Nietner (Tetranychidae: Acarina) infesting tea. Journal of Entomology and Zoology Studies,2021:9(4):297-301.
25. Osagie-Eweka SD, Orhue NE, Amaechina FC, Omogbai EK, Moke EG. Preliminary investigative study on the blood pressure-lowering potential of aqueous leaf extract of Simarouba glauca (AESG) on normotensive adult Wistar rats. Biology, Medicine, & Natural Product Chemistry,2023:12(1):1-4.
26. Pawar KR, Janavale GB, Wagh SG, Panche AN, Daspute AA, Pohare MB, Harke SN. Phytochemical analysis of Simarouba glauca DC and comparison of its bioactivity. Asian Journal of Immunology, 2019, 1-11.
27. Antony J, Thomas A, Gnanasekaran D. Review study on pharmacological importance of Simarouba glauca. International Journal of New Technology and Research, 2016, 2(10).
28. Prajapati C, Reddy M, Bhatt M. Evaluation of anticancer activity using leaf extract of Simarouba glauca on leukemic cancer cell lines. Evaluation,2018:3(2):52-56.
29. Vikas B, Kunjiraman S, Rajam SS, Anil S. The apoptotic properties of leaf extracts of Simarouba glauca against human leukemic cancer cells. Asian Pacific Journal of Cancer Prevention, 2021, 22(4).
30. Hajjar D, Kremb S, Sioud S, Emwas A, Voolstra C, Ravasi T. Anti-cancer agents in Saudi Arabian herbals revealed by automated high-content imaging. PLoS One, 2017, 12(6):e0177316.
31. Carvalho VF, Barreto EO, Diaz BL, et al. Systemic anaphylaxis is prevented in alloxan-diabetic rats by a mechanism dependent on glucocorticoids. European Journal of Pharmacology,2003:472:221-227.
32. Ramprasath T, Kumar PH, Puhari SS, Murugan PS, Vasudevan V, Selvam GS. L-Arginine ameliorates cardiac left ventricular oxidative stress by upregulating eNOS and Nrf2 target genes in alloxan-induced hyperglycemic rats. Biochemical and Biophysical Research Communications,2012:428:389-394.
33. Sharma B, Siddiqui MS, Ram G, Yadav RK, Kumari A, Sharma G, Jasuja ND. Rejuvenating of kidney tissues on alloxan induced diabetic mice under the effect of Momordica charantia. Advances in Pharmacology, 2014, 439158.
34. Kumar V, Gill KD. Estimation of urea in serum and urine. In: Kumar V, Gill KD, editors. Basic Concepts in Clinical Biochemistry: A Practical Guide. Singapore: Springer Singapore, 2018, 67-70.
35. Eldjarn L, Nygaard O, Sveinsson SL. The determination of serum calcium: a comparison between the method of Clark and Collip and the titration with ethylenediamine tetra-acetate. Scandinavian Journal of Clinical and Laboratory Investigation,1955:7(1):92-94.
36. Aziz SJ, Zeman-Pocrnich C. Tissue processing, embedding, sectioning, and H&E staining. In: Methods in Molecular Biology: Histotechnology and Histopathology Protocols. New York: Springer, 2022, 47-63.
37. Ni W, Fang Y, Xie L, Liu X, Shan W, Zeng R, Liu X. Adipose-derived mesenchymal stem cells transplantation alleviates renal injury in streptozotocin-induced diabetic nephropathy. Journal of Histochemistry and Cytochemistry,2015:63(11):842-853.
38. Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. Journal of Immunological Methods,1983:65(1-2):55-63.

39. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Analytical Biochemistry,1979:95(2):351-358.
40. Buege JA, Aust SD. Microsomal lipid peroxidation. Methods in Enzymology,1978:52:302-310.
41. Draper HH, Hadley M. Malondialdehyde determination as index of lipid peroxidation. Methods in Enzymology,1990:186:421-431.
42. Shirwakar A, Shirwakar AR, Rajendran K, Punitha IRS. Biol. Pharm. Bull.,2006:29:1906. Prieto P, Pineda M, Aguilar M. Analytical Biochemistry,1999:269:337.
43. Rana MG, Katbamna RV, Padhya AA, Dudhrejiya AD, Jivani NP, Sheth NR. In vitro antioxidant and free-radical scavenging studies of alcoholic extract of Medicago sativa L. Romanian Journal of Biology - Plant Biology,1996:55(1):15-22.
44. McCune LM, Johns T. Antioxidant activity in medicinal plants associated with the symptoms of diabetic mellitus used by the indigenous peoples of the North American boreal forest. Journal of Ethnopharmacology,2002:82:197-205.
45. Vijayalakshmi M, Ruckmani K. Ferric reducing anti-oxidant power assay in plant extract. Bangladesh Journal of Pharmacology,2016:11(3):570-572.
46. Prieto P, Pineda M, Aguilar M. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. Methods in Enzymology,1999:299:379-385.