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Protective effect of *Vitex negundo* L. in diclofenac induced nephrotoxicity

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Major morbidity and mortality are related to kidney damage, a complex clinical disease. About 19–33% of hospitalized patients' AKI episodes are caused by drug-induced nephrotoxicity.Non-steroidal anti-inflammatory medications, like diclofenac, are thought to be safe, but in recent years, they have drawn particular attention because of the possibility of kidney damage.*Vitex negundo*, or nigundi, has anti-inflammatory qualities. In this study, we looked into how well Nirgundi (*Vitex negundo*) worked to prevent Wistar rats from developing nephrotoxicity from diclofenac. After a 7 day intramuscular course of diclofenac 10 mg per kg b/w, we found that it increased in urea, creatinine, electrolytes (potassium, sodium, and chloride), and decreased urine output.Furthermore, diclofenac caused morphological alterations that were consistent with renal injury and elevated oxidative stress in renal tissue (raising MDA levels and decreasing SOD levels). However, *Vitex negundo* decreased creatinine, blood urea, and electrolytes brought on by diclofenac while also boosting urine production. *Vitex negundo*, prevented the kidneys from producing cytokines, oxidative stress, morphological alterations, and apoptosis when exposed to diclofenac. *Vitex negundo*, is effective in preventing nephrotoxicity caused by diclofenac sodium.

Keywords: Antioxidants, Kaphavatahar, Nephrotoxicity, Oxidative stress, Vishaghna, *Vitex negundo* L.

Nephrotoxicity (NT), a disorder unique to the kidneys, is defined by the rapid degradation of kidney function brought on by the acute effects of nephrotoxic substances. Nephrotoxic substances account for about 20% of NT, while comorbidities and poly-medications can increase this number to 60% in the elderly[1]. Normally, the kidney regulates intra-glomerular pressure, which is primarily dependent on renal prostaglandin, to maintain a steady glomerular filtration rate (GFR)[2]. Therefore, by altering intra-glomerular pressure, medications that influence prostaglandins (PG), such as non-steroidal anti-inflammatory drugs (NSAIDs), cause glomerular dysfunction[3]. Both selective and non-selective COX inhibitors have the potential to cause acute or chronic renal insufficiencies because the renal tissues are the primary site of expression for both isoforms of the PG metabolising enzymes (COX-1 and COX-2)[4]. Since diclofenac (DCF) inhibits PG generally, it has adverse effects *via* inhibiting COX-1, which protects against acute renal and other organ damage[5]. By blocking PG production, DCF has been shown to decrease GFR and other renal functions in a dose-dependent way[6]. Furthermore, DCF can damage renal proximal tubules by increasing intracellular osmolality and triggering autolysis, which results in kidney tubular dysfunction and lowers GFR[6]. *Vitex negundo* L. is a member of the Verbenaceae family[7]. It consists of over 2500 species and 75 genera in a broad family of trees, shrubs, and herbs[8]. It's Nirgundi in Hindi and Sindhuvara in Sanskrit are two examples of common names.Trifoliate or pentafoliate leaves are typically found on quadrangular branches, which produce bluish-purple flowers in branching tomentose[9]. Flowers bloom all year long[10]. Ayurveda, Unani, and Siddha are among the ancient medical systems that currently use *Vitex negundo* L. in clinical settings to treat a variety of conditions, such as headache, inflammation, leucoderma, spleen enlargement, rheumatoid arthritis, gonerrhea, bronchitis, fever, cold, and cough[11].Numerous chemicals, including lignin, volatile oils, flavonoids, iridoids, terpenes, and steroids, have been found in *Vitex negundo* L. The root of *Vitex negundo L* contains sitosterol, 3-formyl-4.5-dimethyl-8-oxo-5H-6, 7-dihydronaphtho (2, 3-b) furan, vitexin, isovitexin, negundin-A, negundin-B,

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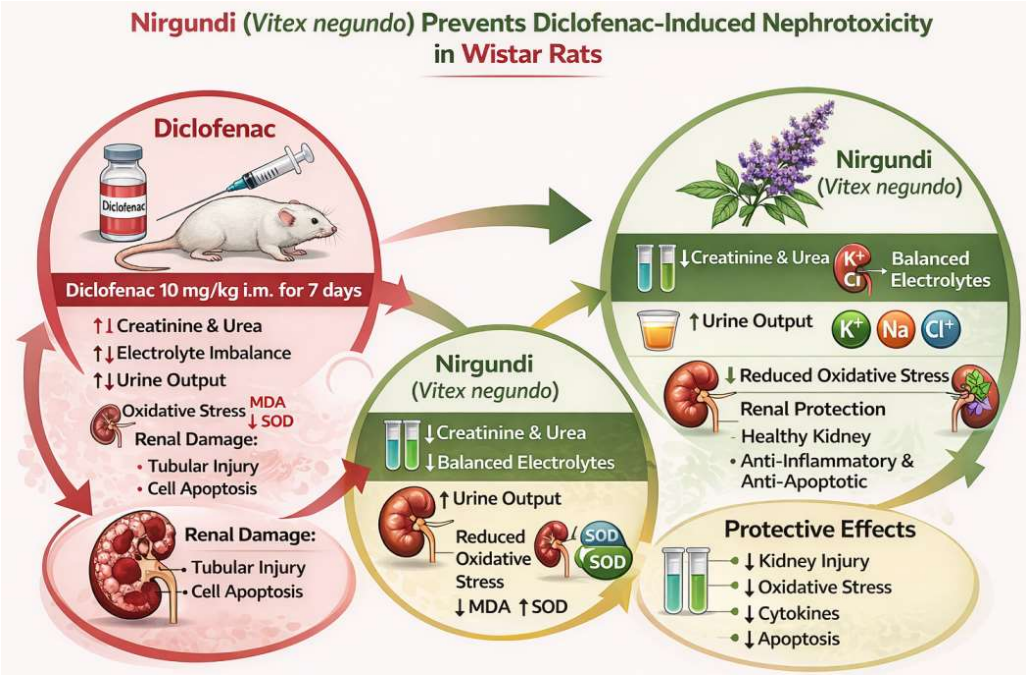
Suppl. data available on respective page of NOPR



Graphical abstract

(+)-diasyringaresinol, (+)-lyoniresinol, vitrofolal-E and vitrofolal-F, acetyl oleanolic acid, and 2β, 3α-diaceto xyoleana-5, 12 dien-28-oic acid[12]. *Vitex negundo* L. has anti- antioxidant[13] and inflammatory[14] qualities. This study aims to assess the efficacy of *Vitex negundo* in diclofenac sodium-induced nephrotoxicity in Wistar rats.

Materials and Methods

Plant material

The root of *Vitex negundo* were collected from pharmacy garden, Dr. D.Y. Patil Ayurved college, Pimpri Pune Maharashtra. India.

The botanical identity of the plant material was authenticated by Botanical Survey of India, Pune, Maharashtra, India.

The standardization of root powder done at Authorized standard laboratory.

Drugs and Chemicals: Inj. Diclofenac sodium was used to induce nephrotoxicity

Preparation of root powder

Traditional samhita procedure of making churna (Powder) was followed. (According to ayurdveda powder dose is 5 g for adult. mean 5000 mg for 60 kg human.83.33 mg per kg body weight for human. Animal factor bt surface area is 6.17. Animal dose mg/kg b.w.=Human dose mg/kg b.w. x surface factor. 83.33 x 6.17=514.16 mg/kg b.w.)

Experimental animals

Wistar Rats 6 (3male 3 female) in each group of age 6 weeks, weight 200 ±20 g. They were housed in clean polypropylene cages under standard conditions of humidity (50±5%), temperature (22±3°C), and light (12 h light/12 h dark cycle), and fed with standard diet and water *ad libitum*. This study was approved by the Institutional Animal Ethics Committee (IAEC approved number SC/IAEC/2223/003).

Experimental design

After one week of acclimatization period, Wistar rats were divided into three groups of six animals each.

Group I: Vehicle control(VC)-Normal control rats were treated with oral dose of distilled water for 21 days.

Group II: Disease control(DC)-Rats were treated with I.M. dose of diclofenac sodium (10 mg/kg of body weight) for 7 days.

Group III: Test drug(TD)-Rats were treated with I.M. dose of diclofenac sodium (10mg/kg of body weight) for 7 days, then rats were treated with oral dose of *Vitex negundo* (514 mg/ kg of body weight/day) from 8th day to 21st day for 14 days. After the experimental period, collected blood samples were used for the studies of biochemical and hematological studies in all groups.

Parameters assessed for renal function

Urine Output and Specific Gravity

Urine collected by placing animals in metabolic cages and sample collected in special tubes.Urine

output was measured for all animals on Day 0, 7, 14 and 21.

Biochemical analysis

Sodium, Potassium, Chloride, Urea, Creatinine levels were analysed on day 0, 7, 14 and 21 using commerce- ally available kits (Pathozyme) and as per kit manufa cturers protocol using Biochemistry Analyzer Chem Xpress.

Tissue parameters

MDA and SOD were performed on day 21st from Kidney tissue as per previously reported protocols (Anand et al., 2021; Beauchamp & Fridovich, 1971; Buege & Aust, 1978) with the help of Multiskan SkyHigh Plate Reader.

Histopathology

Kidney Tissues were stored in 10% NBF for fixation. After fixation these tissues were trimmed and processed routinely. Tissue processing was done to dehydrate in ascending grades of alcohol, clearing in xylene and embedded in paraffin wax. Paraffin wax embedded tissue blocks were sections at 3-5 µM thickness with the Rotary Microtome. Slides of colons were stained with Haematoxylin & Eosin (H & amp; E) stain.

Statistical analysis

The Results were expressed as the mean value ± SD. Within group comparisons were performed by the analysis of variance using paired *t*-test.Significant difference between the groups were assessed by ANOVA-test. A probability level of less than 5% ($P< 0.05$) was considered as significant.

Observations

Detailed observations were made, documented, and statistically examined. They were arranged in tables and charts. On the 0th, 7th, 14th, and 21st days of treatment, changes in the parameters were noted.Utilising statistical methods, changes in the parameters on days 0, 7, 14, and 21 of treatment were compared. The information was utilised to produce a thorough conclusion. A report on the histopathology of renal tissue has also been mentioned.

Urine output

Urine output values are changing on each day. First 7 days of study, DC group and TC group has given Diclofenac sodium. DC group shows urine output decreased significantly (p value 0.001) as compare to VC group. TD group shows urine output decreased significantly (p value 0.009) as compare to VC group. TD group has given *Vitex negundo* L. root powder for next 14 days. On day 7 to day 14 the urine output of Test drug group goes increased significantly ($P< 0.05$) as compare to DC group. On day 14th -day -21st the changes in urine output seen more significant urine output increased in TD group significantly ($P< 0.05$). TD shows significant ($P< 0.000$) increased urine output between day 7 - day 21 as compare to DC group.

Urine Specific Gravity

Specific gravity of urine sample tested it shows significant changes.On day 0 normal parameters recorded.

On day 7 specific gravity of urine sample raised in DC and TD group, On day 7 –day 14 specific gravity of TD group decreased ($P< 0.05$) as to DC group. On day 21 Specific gravity significantly ($P< 0.000$) normalized in TD group as compare to DC group

Microscopic observations of Urine (Epithelial Cells)

On day 0 urine sample shows absence of epithelial cells inVC, DC and TD group. On day 7th sample epithelial cells are present in DC and TD group.After giving *Vitex negundo* L.root powder they were absent in TD group on 21st day sample.

Sr. Urea

On day 0 sr.urea level recorded after giving Diclofenac sodium on day 7th sr. urea level raised in disease control DC and TD group significantly ($P< 0.001$). On day 7th-14th in TD group sr.urea value decreased significantly ($P< 0.0001$) as compare to DC.On day 7th-21st sr.urea value of TD group significantly decreased ($P< 0.0001$) as compare to DC.

Sr. Creatinine

On day 0 normal parameter of sr. creatinine recorded, after giving Diclofenac sodium on day 7th sr.creatinine level raised in DC ($P<0.000$) and TD group ($P<0.05$) significantly.On day 7th-14th in TD group sr. creatinine value decreased significantly ($P< 0.05$) as compare to DC. On day 7th - 21st sr. creatinine value of TD group decreased significantly ($P<0.01$) as compare to DC.

Electrolyte

a) Sr.Sodium

On day 0 normal parameter of sr.sodium recorded, after giving Diclofenac sodium on day 7th sr. sodium

level raised in DC ($P< 0.001$) and Test drug group ($P< 0.000$) significantly.On day 7-14th in TD group sr. sodium value decreased significantly ($P< 0.000$) as compare to DC.On day 7th - 21st sr. sodium value in TD group decreased significantly ($P< 0.000$) as compare to DC.

b) Sr. Potassium

On day 0 normal parameter of sr.Potassium recorded, after giving Diclofenac sodium on day 7th sr. Potassium level raised in DC ($P< 0.001$) and TD group ($P< 0.01$) significantly. On day 7th-14th in TD group sr. Potassium value mild decreased as compare to DC. On day 7th - 21st sr. Potassium value of TD group decreased mild significantly as compare to DC.

c)Sr. Chloride

On day 0 normal parameter of sr. chloride recorded, after giving Diclofenac sodium on day 7th sr. chloride level raised in DC($P< 0.000$) and TD group ($P< 0.000$) significantly. On day 7th-14th in TD group sr. chloride value mild significantly ($P< 0.05$) decreased as compare to DC. On day 7th - 21st sr. chloride value of TD group decreased significantly ($P< 0.01$) as compare to DC

Tissue parameters

a) MDA

On day 21 MDA value is seen increased significantly ($P< 0.01$) in DC group as compare VC group. TD group shows normalize MDA value ($P< 0.001$) as compare to DC group

b) SOD

On day 21 SOD value is seen decreased significantly ($P< 0.001$) in DC group as compare VC group.TD group shows normalize SOD value ($P< 0.001$) as compare to DC group

Histopathology

Histopathological studies

Results

Effect on urine output

Tables 1 & 2 and Figures 1 & 2 showed the effect of Diclofenac sodium and *Vitex negundo* on urine out and specific gravity of different groups over the duration of the study.The urine output of the animals (group II and group III) which received Diclofenac sodium significantly decreased when compared to the VC.When *Vitex negundo* root powder was given to group lll (TD), the urine output significantly increased

Table 1 — Urine output (mL/24 h)

Group	Animal ID	Urine output (ml/24 hr)			
		Day 0	Day 7	Day 14	Day 21
Vehicle Control	1	1.5	1.6	1.6	1.5
	2	1.7	1.5	1.6	1.7
	3	1.6	1.6	1.7	1.8
	4	1.5	1.6	1.5	1.8
	5	1.6	1.7	1.6	1.5
	6	1.6	1.6	1.9	1.7
	Mean	1.58	1.60	1.65	1.67
	SD	0.08	0.06	0.14	0.14
Disease Control	1	1.9	1	0.8	1
	2	1.5	1.2	0.8	0.9
	3	1.7	1.1	1	0.8
	4	1.7	1.1	0.9	0.8
	5	1.8	0.9	0.8	0.8
	6	1.5	1	0.9	1.1
	Mean	1.68	1.05	0.87	0.90
	SD	0.16	0.10	0.08	0.13
Test Group	1	1.5	1.2	1.1	1.5
	2	1.5	1.1	1.1	1.4
	3	1.7	0.8	1.2	1.3
	4	1.6	0.9	1.1	1.5
	5	1.8	0.8	1.2	1.5
	6	1.5	1.2	1.2	1.6
	Mean	1.60	1.00	1.15	1.47
	SD	0.13	0.19	0.05	0.10

Table 2 — Urine Specific Gravity

Group	Animal ID	URINE Specific Gravity			
		Day 0	Day 7	Day 14	Day 21
Vehicle Control	1	1.03	1.031	1.029	1.029
	2	1.02	1.021	1.025	1.026
	3	1.021	1.025	1.025	1.025
	4	1.028	1.026	1.029	1.026
	5	1.025	1.025	1.025	1.026
	6	1.026	1.029	1.021	1.029
	Mean	1.03	1.03	1.03	1.03
	SD	0.00	0.00	0.00	0.00
Disease Control	1	1.036	1.061	1.06	1.056
	2	1.025	1.065	1.05	1.055
	3	1.03	1.059	1.065	1.062
	4	1.056	1.06	1.065	1.056
	5	1.058	1.055	1.055	1.055
	6	1.06	1.056	1.059	1.056
	Mean	1.04	1.06	1.06	1.06
	SD	0.02	0.00	0.01	0.00
Test drug(Nirgundi)	1	1.031	1.056	1.045	1.035
	2	1.025	1.065	1.046	1.036
	3	1.026	1.059	1.048	1.035
	4	1.03	1.059	1.042	1.035
	5	1.031	1.056	1.05	1.036
	6	1.025	1.052	1.045	1.035
	Mean	1.03	1.06	1.05	1.04
	SD	0.00	0.00	0.00	0.00

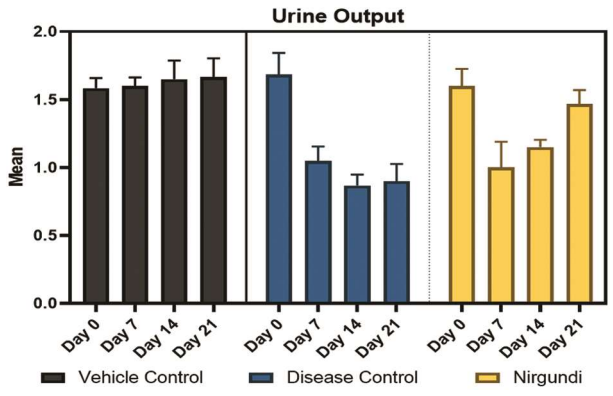


Fig. 1 — Urine output

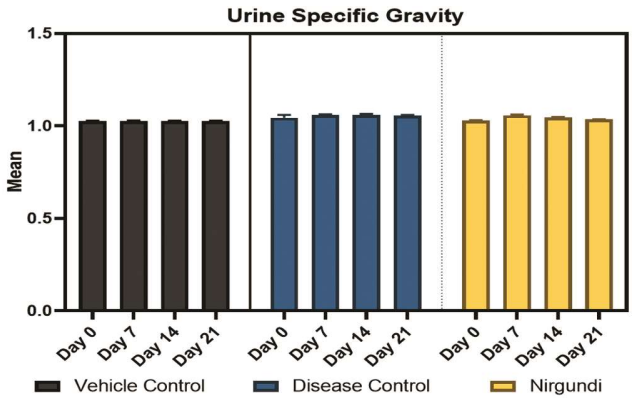


Fig. 2 — Urine Specific Gravity

when compared to Diclofenac sodium treated group (DC). The specific gravity of group TD changes significantly as compare to VC group.

Effect on Epithelial cells

On day 0 urine sample showed absence of epithelial cells in VC, DC, and TC group. After received Diclofenac sodium, epithelial cells were present in DC and TD group. After giving *Vitex negundo* root powder it seen absent in TD group on 21st day sample. It shows anti inflammatory action of *Vitex negundo* helps to reduce inflammation.

Effect on Biochemical parameters

Tables 3 to11 and Figures 3 to11 showed the effect of *Vitex negundo* root powder on kiadney markers in serum in all groups of animals over the duration of the study. Blood urea, creatinine, sodium potassium and chloride significantly increased in DC group when compared with VC. However, after oral administration of *Vitex negundo* in TD group significantly ($P< 0.05$) increased Blood urea, creatinine, sodium potassium and chloride when compared with Diclofenac sodium treated group (DC).

Table 3 — Microscopic observations of Urine (Epithelial Cells)

Group	Animal ID	Male				Animal ID	Female			
		Day 0	Day 7	Day 14	Day 21		Day 0	Day 7	Day 14	Day 21
Vehicle Control	1	-	-	-	-	4	-	-	-	-
	2	-	-	-	-	5	-	-	-	-
	3	-	-	-	-	6	-	-	-	-
Disease Control	1	-	+	+	+	4	-	+	+	+
	2	-	+	+	+	5	-	+	+	+
	3	-	+	+	+	6	-	+	+	+
Test drug	1	-	+	-	-	4	-	+	-	-
	2	-	+	-	-	5	-	+	-	-
	3	-	+	+	-	6	-	+	+	-

Table 4 —(Sr. Urea)

		Sr. UREA			
Group	Animal ID	Day 0	Day 7	Day 14	Day 21
Vehicle Control	1	62.03	69.05	68.03	45.79
	2	68.83	63.74	63.09	58.15
	3	68.05	64.96	69.05	59.88
	4	54.69	89.2	53.93	63.03
	5	62	52.62	53.45	53.15
	6	70.53	53.82	73.84	47.83
	Mean	64.36	65.57	63.57	54.64
	SD	5.94	13.27	8.38	6.89
Disease Control	1	65.42	159.01	149.06	135.8
	2	77.54	171.72	163.54	153.93
	3	76.09	124.98	119.03	150.65
	4	57.25	160.36	165.08	174.21
	5	70.73	167.22	176.5	178.21
	6	72.99	158.87	186.41	160.87
	Mean	70.00	157.03	159.94	158.95
	SD	7.58	16.52	23.69	15.73
Test drug	1	64.7	152.32	105.07	64.4
	2	86.46	160.56	106.72	55.99
	3	72.79	160.44	112.8	52.42
	4	68.14	142.92	104.21	50.19
	5	80.31	162.81	108.31	49.7
	6	76.59	168.91	106.87	50.43
	Mean	74.83	157.99	107.33	53.86
	SD	8.00	9.10	3.04	5.66

Table 5 — Serum Biochemistry: Creatinine (mg/dL)

		Serum Biochemistry: Creatinine (mg/dL)			
Group	Animal ID	Day 0	Day 7	Day 14	Day 21
Vehicle Control	1	0.63	0.79	0.75	0.66
	2	0.61	0.82	0.78	0.69
	3	0.59	0.71	0.68	0.58
	4	0.61	0.68	0.65	0.71
	5	0.63	0.67	0.64	0.6
	6	0.63	0.64	0.61	0.65
	Mean	0.62	0.72	0.69	0.65
	SD	0.02	0.07	0.07	0.05

(*Contd.*)

Table 5 — Serum Biochemistry: Creatinine (mg/dL) (*Contd.*)

Serum Biochemistry: Creatinine (mg/dl)					
Group	Animal ID	Day 0	Day 7	Day 14	Day 21
	2	0.57	1.07	1.02	1.06
	3	0.66	1.08	1.03	1.03
	4	0.64	0.91	0.97	0.97
	5	0.54	0.93	0.98	0.97
	6	0.6	0.93	0.98	0.89
	Mean	0.61	1.00	1.00	1.02
	SD	0.04	0.08	0.03	0.11
Test drug	1	0.55	0.99	0.79	0.81
	2	0.56	1.07	0.72	0.74
	3	0.58	1.06	0.82	0.86
	4	0.63	0.84	0.8	0.76
	5	0.65	0.98	0.84	0.75
	6	0.7	0.84	0.8	0.74
	Mean	0.61	0.96	0.80	0.78
	SD	0.06	0.10	0.04	0.05

Table 6 — Serum Biochemistry: Sodium (mmol/L)

Serum Biochemistry: Sodium (mmol/L)					
Group	Animal ID	Day 0	Day 7	Day 14	Day 21
Vehicle Control	1	145.51	146.23	145.17	147.44
	2	147.85	147.26	145.25	147.17
	3	136.49	146.21	146.15	148.65
	4	142.97	140.02	143.83	147.12
	5	146.03	141.91	146.86	145.76
	6	149.08	145.38	147.98	149.93
	Mean	144.66	144.50	145.87	147.68
	SD	4.51	2.87	1.45	1.44
Disease Control	1	145.46	163.93	168.5	178.8
	2	146.09	161.81	171.01	176.21
	3	149.29	168.91	172.98	179.11
	4	147.29	158.57	163.46	167.35
	5	149.02	160.28	166.59	168.58
	6	146.84	164.75	167.21	165.08
	Mean	147.33	163.04	168.29	172.52
	SD	1.55	3.67	3.37	6.23
Test drug	1	145.19	164.78	159.31	149.14
	2	142.94	165.11	154.67	149.3
	3	143.56	162.53	153.17	148.88
	4	140.87	164.88	149.22	148.01
	5	148.25	163.84	156.2	148.45
	6	144.88	164.84	157.2	148.31
	Mean	144.28	164.33	154.96	148.68
	SD	2.49	0.99	3.51	0.50

Table 7 — Serum Biochemistry: Potassium (mmol/L)

Serum Biochemistry: Potassium (mmol/L)					
Group	Animal ID	Day 0	Day 7	Day 14	Day 21
Vehicle Control	1	5	5.08	5.67	4.79
	2	5.63	5.07	4.74	5.62
	3	4.76	5.65	4.27	5.38

(*Contd.*)

Table 7 — Serum Biochemistry: Potassium (mmol/L) (*Contd.*)

Serum Biochemistry: Potassium (mmol/L)					
Group	Animal ID	Day 0	Day 7	Day 14	Day 21
	4	5.34	5.62	5.2	5.9
	5	5.65	5.11	5.77	4.89
	6	6.33	5.53	6.12	5
	Mean	5.45	5.34	5.30	5.26
	SD	0.55	0.28	0.69	0.44
Disease Control	1	5.11	8.14	7.75	8.9
	2	5.78	7.43	7.08	9.23
	3	5.66	7.13	6.79	7.01
	4	6.08	9.2	8.76	7.47
	5	5.26	8.07	7.69	9.08
	6	5.66	7.36	7.01	8.71
	Mean	5.59	7.89	7.51	8.40
	SD	0.35	0.76	0.72	0.93
Test drug	1	5.43	8	7.62	5.08
	2	5.59	6.45	6.14	7.26
	3	5.32	8.84	8.42	6.8
	4	5.63	9.87	9.4	6.42
	5	5.21	9.18	8.74	6.04
	6	6.08	7.83	7.46	7.74
	Mean	5.54	8.36	7.96	6.56
	SD	0.31	1.20	1.15	0.94

Table 8 —Serum Biochemistry: Chloride (mmol/L)

Serum Biochemistry: Chloride (mmol/L)					
Group	Animal ID	Day 0	Day 7	Day 14	Day 21
Vehicle Control	1	101	101.38	100.17	101.42
	2	103.31	105.55	104.29	109.29
	3	102.24	104.08	106.74	105.14
	4	101.53	104.58	107.22	109.33
	5	103.23	107.93	106.6	109.98
	6	101.3	105.24	103.09	104.78
	Mean	102.10	104.79	104.69	106.66
	SD	0.99	2.14	2.74	3.42
Disease Control	1	105.35	142.86	146.06	134..75
	2	103.35	159.33	151.74	137.25
	3	103.22	153.04	145.75	145.15
	4	105.12	151.28	144.08	132.5
	5	103.25	154.68	147.31	141.06
	6	103.09	160.68	153.03	138.31
	Mean	103.90	153.65	148.00	138.85
	SD	1.04	6.41	3.58	4.68
Test drug	1	98.56	155.77	129.3	106.38
	2	103.99	140.74	134.04	114.15
	3	100.81	143.31	136.49	110.09
	4	126.33	160.49	136.92	111.73
	5	103.58	153.59	127.13	103.79
	6	102.17	158.5	126.83	105.99
	Mean	105.91	152.07	131.79	108.69
	SD	10.20	8.17	4.60	3.94

Table 9 — MDA (nM/mg of Tissue)

MDA (nM/mg of Tissue)	1	2	3	4	5	6	Mean	SD
Vehicle Control	17.9	18.21	20.21	22.05	19.79	19.56	19.62	1.50
Disease Control	50.59	48.59	52.44	35.31	31.31	51.03	44.88	9.13
Test drug	14.82	14.97	15.33	16.97	14.54	20.67	16.22	2.35

Table 10 — SOD (U/min/gm of Tissue)

SOD (U/min/gm of Tissue)	1	2	3	4	5	6	Mean	SD
Vehicle Control	6.22	9.76	8.78	11.14	13.26	10.4	9.93	2.36
Disease Control	2.54	2.1	1.92	2.18	5.02	4.18	2.99	1.29
Test drug	5.98	6.38	5.1	7.44	6.08	7.52	6.42	0.93

Table 11 — Histopathological observations

Group	Histopathological Microscopic observations of Kidney tissue	Pathological Grade
Vehicle Control-1	Normal renal parenchyma histomorphological characteristics include intact renal tubule and glomerulus walls and nucleus. Glomeruli showed normal cellular details of Bowman`s capsule and glomerular tuft. Absence of pathogenic or inflammatory alterations in the renal parenchyma.	NAD
Vehicle Control-2	Normal histomorphological features of renal parenchyma with intact nucleus and cell borders of renal tubules and glomeruli. Glomeruli showed normal cellular details of Bowman`s capsule and glomerular tuft. Absence of inflammatory or pathological changes in renal parenchyma.	NAD
Vehicle Control-3	Normal histomorphological features of renal parenchyma with intact nucleus and cell borders of renal tubules and glomeruli. Glomeruli showed normal cellular details of Bowman`s capsule and glomerular tuft. Absence of inflammatory or pathological changes in renal parenchyma.	NAD
Disease Control-1	Mild degenerative changes of renal tubules and glomerular tissue were noted. The renal tubules showed cellular swelling of tubular epithelium with focal loss of tubular epithelium and granular cytoplasm of degenerated tubules. Focal areas with atrophic changes of glomeruli were noted. Mild areas with presence of eosinophilic deposits in the lumen of tubules were noted. Mild congested vascular tissue in renal parenchyma and focal interstitial hemorrhages in renal tissue.	Mild (+2) to Moderate (+3)
Disease Control-2	Mild degenerative changes of renal tubules and glomerular tissue were noted. The renal tubules showed cellular swelling of tubular epithelium with focal loss of tubular epithelium and granular cytoplasm of degenerated tubules. Focal areas with atrophic changes of glomeruli were noted. Focal areas with presence of eosinophilic deposits in the lumen of tubules were noted. Mild congested vascular tissue in renal parenchyma and focal interstitial hemorrhages in renal tissue.	Mild (+2)
Disease Control-3	Mild degenerative changes of renal tubules and glomerular tissue were noted. The renal tubules showed cellular swelling of tubular epithelium with focal loss of tubular epithelium and granular cytoplasm of degenerated tubules. Focal areas with atrophic changes of glomeruli were noted. Mild areas with presence of eosinophilic deposits in the lumen of tubules were noted. Mild congested vascular tissue in renal parenchyma and focal interstitial hemorrhages in renal tissue.	Mild (+2) to Moderate (+3)
Test drug -1	Focal vascular tissue congestion in the parenchyma of the kidney. Normal renal parenchyma histomorphological characteristics include intact renal tubule and glomerulus cell boundaries and nucleus. Glomeruli showed normal cellular details of Bowman`s capsule and glomerular tuft.	NAD
Test drug -2	Focal vascular tissue congestion in the parenchyma of the kidney. Normal renal parenchyma histomorphological characteristics include intact renal tubule and glomerulus walls and nucleus. Glomeruli showed normal cellular details of Bowman`s capsule and glomerular tuft. Focal and minimal degenerative changes of renal tubules were observed occasionally.	Minimal (+1)
Test drug -3	Focal vascular tissue congestion in the parenchyma of the kidney. Normal histomorphological characteristics of the renal parenchyma include the cell borders of the renal tubules and glomeruli and an intact nucleus.. Glomeruli showed normal cellular details of Bowman`s capsule and glomerular tuft.	NAD

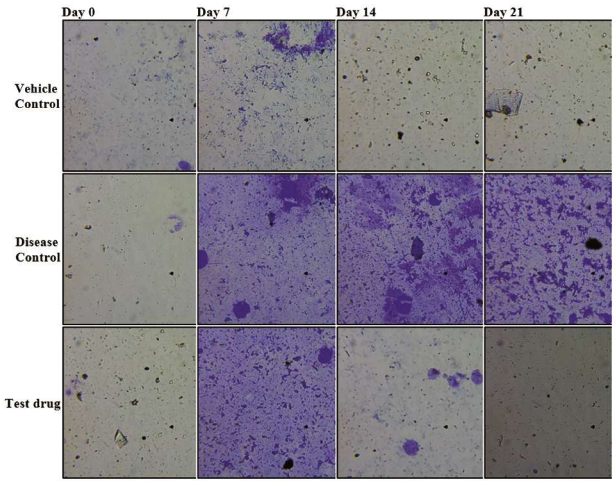


Fig. 3 — Microscopic Observations of Urine

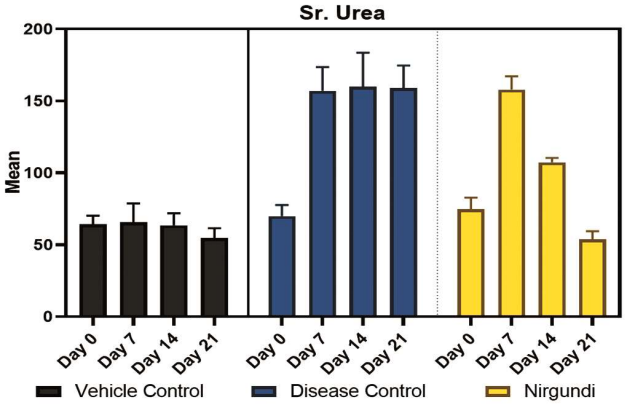


Fig. 4 — Sr. Urea

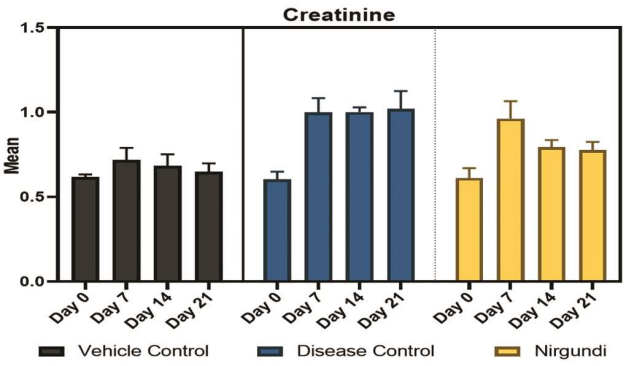


Fig. 5 — Creatinine (mg/dl)

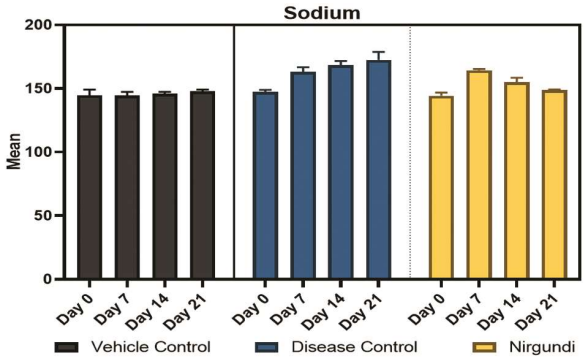


Fig. 6 — Sodium (mmol/L)

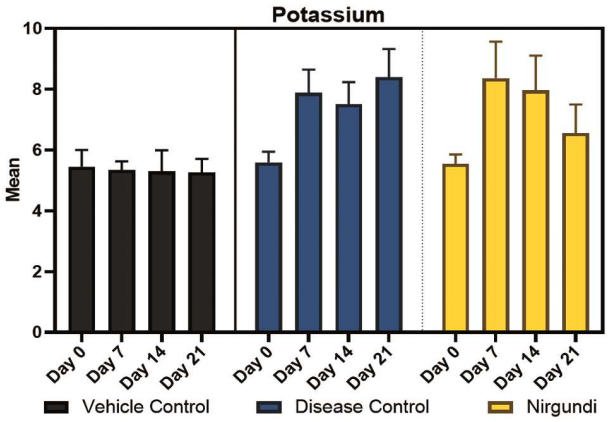


Fig. 7 — Potassium (mmol/L)

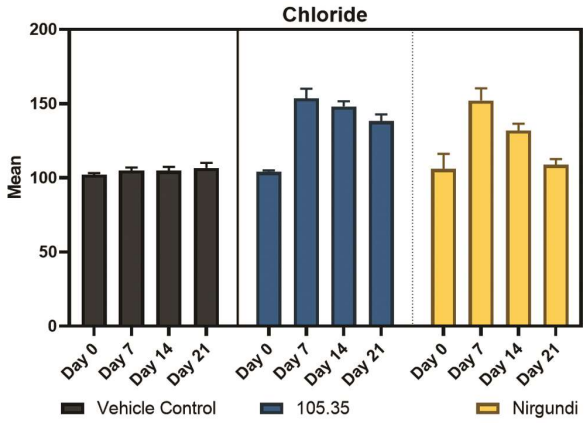


Fig. 8 — Chloride (mmol/L)

Effect on Tissue parameters

MDA value is seen increased significantly ($P< 0.01$) in DC group as compare VC group. TD shows normalize MDA value ($P< 0.001$) as compare to DC group.

SOD value is seen decreased significantly ($P< 0.001$) in DC group as compare VC group. TD group shows normalize SOD value ($P< 0.001$) as compare to DC group.

Histopathology

Normal renal tissue histopathology observed in VC group whereas DC group showed Mild degenerative changes of renal tubules and glomerular tissue, cellular swelling, atrophic changes of glomeruli, Mild congested vascular tissue in renal parenchyma and focal interstitial hemorrhages in renal tissue.TD group showed normal to mild renal parenchyma histomorphological characteristics

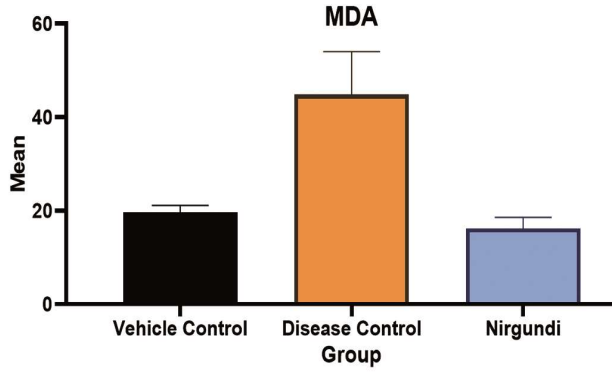


Fig. 9 — MDA (nM/mg of Tissue)

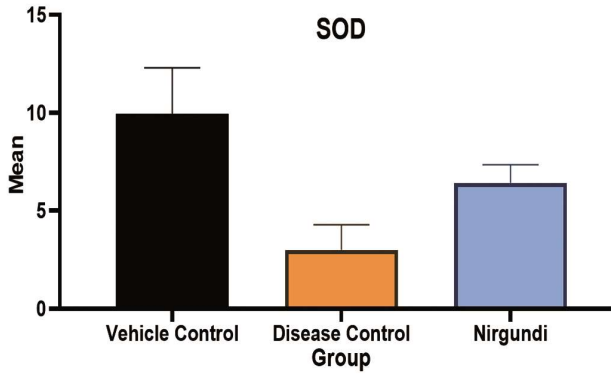


Fig. 10 — SOD (U/min/gm of Tissue)

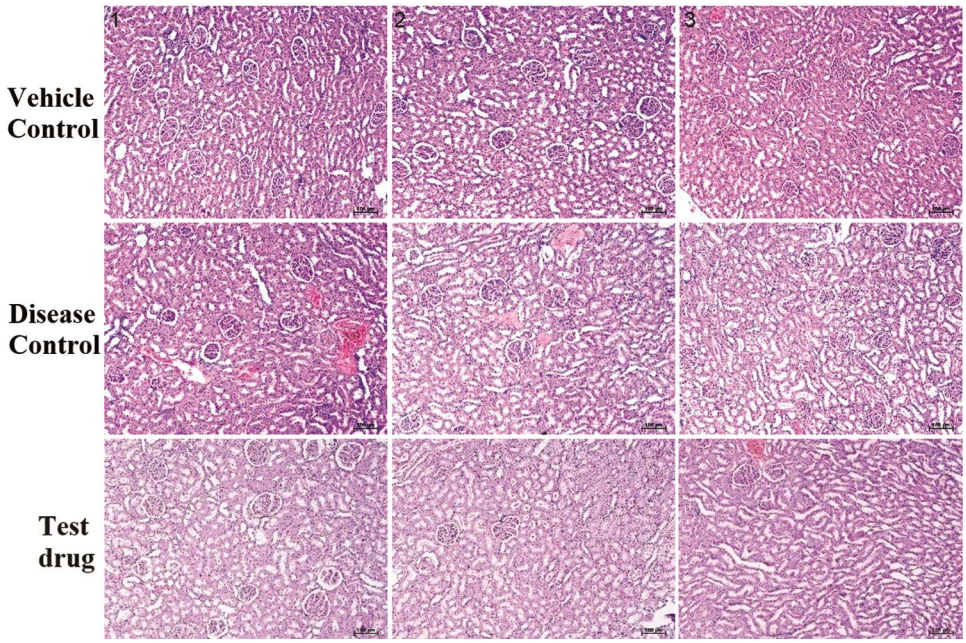


Fig. 11 — Histopathological Images

include intact renal tubule and glomerulus cell boundaries and nucleus. Focal and minimal degenerative changes of renal tubules were observed occasionally.

Discussion

The most commonly reported symptom of many common illnesses, including osteoarthritis, rheumatoid arthritis, and ankylosing spondylitis, is pain. It is the most common complaint from which patients suffer.

In order to get relief from pain, people usually tend to take various analgesics. NSAIDs are the most common type of analgesics that might be used. With a market share of around 30% on average among the 15 nations, Diclofenac was the most widely used NSAID. Diclofenac sodium is frequently used NSAID. The main mechanisms of Diclofenac-induced nephrotoxicity are oxidative stress, free radicalgeneration and lipid peroxidation.

Discussion regarding efficacy of test drug

Nirgundi (Vitex negundo) is easily available and cost effective herble remedies. Ayurvedic texts have described various qualities of *Vitex negundo* L. *as* Antioxidantsderived from plants are not only effective but also comparatively safer than those derived from synthetic materials[13]. *Vitex negundo* L. holds a significant place in Ayurvedic literature, as it is part of the *Vishaghna mahakashaya* in Charak Samhita[14]and is known as a *vishanashaka,* or antitoxic, in various Ayurvedic texts. It possesses *shothanashaka* (anti inflammatory) properties, which means it is effective at reducing inflammation[15]. It's *tikta rasa* (bitter test) is beneficial for rakta (blood) which helps in purifying blood[16]. It's kaphavatahara property helps in eradicating the accumulated kaphavata which causes avarodha (obstruction) of

tubules.Its diuretic action helps in increasing urine output and to reduce nephrotoxicity.

The *Vitex negundo* root included many compounds, including vitexin, isovitexin, negundin A and B, vitrofolal E and F, sitosterol, oleanolic acid, *etc*[17]. Many phytoconstituents, such as terpinoids, flavones, and steroidal entities, have been shown to possess strong cytoprotective and anti-oxidant qualities[18]. Phytochemical study of the ethanolic extract has revealed the presence of alkaloids, carbohydrates, glycosides, phenolic compounds, saponins, and sterols. The potent anti-inflammatory effect might be attributed to these phytoconstituents[19, 20].

Discussion on observation and result

In both the DC and TD groups, the induction of toxicity by Diclofenac sodium resulted in a significant decrease in urine output from day 0 to day 7, as well as a significant increase in serum creatinine, serum urea, serum sodium, serum potassium, and serum chloride on day 7.

Urine Output: Measuring urine output is a crucial part of the current diagnostic and staging criteria for acute renal injury since it is a useful indicator of kidney function. In the present investigation, we found a significant variation in the urine production of the DC group on day 0 and day 21. This observation implies that the toxicity seems irreversible and that there is no chance of recovery to their normal function in the absence of intervention or treatment. The TD, on the other hand, showed no discernible change in urine production from the first to the 21st day. This result suggests that by the end of the treatment period, the treatment according to this observation, the treatment resulted in a complete reversal to toxicity by the conclusion of the treatment period with regard to urine production.

Urine specific gravity

From day 0 to day 7, there was increase in urine specific gravity in the DC group and TD group. Day 7 to day 21, there was a persistent increase in urine specific gravity in the DC group, suggesting that the body's natural recovery processes were not able to offset the toxic effects. On the other hand, by the 21st day, the Test drug group showed a noticeable decrease in urine specific gravity. This finding confirms that the experimental medications were successful to maintain urine specific gravity.

Serum urea

Deamination is a process through amino acids connected to proteins are broken down to produce ammonia. The next step involves the conversion of ammonia to urea by liver enzymes. Because the body depends on the renal system to reduce urea excretion, urea is useful in the analysis of renal function. Serum urea may increase in response to a decrease in renal excretion.

Serum urea levels in the DC group showed a significant difference from the first day to the twenty-first day. This disparity strongly implies that the body cannot spontaneously reverse the harmful effects on kidney function in the absence of intervention or treatment.

On the other hand, there was no discernible change in serum urea levels between the first and the 21-day in the Test drug group. This observation suggests that the treatment was successful in facilitating a whole reversal of toxicity by the end of the treatment period with respect to serum urea levels.

Serum creatinine

Creatinine concentrations in plasma and urine samples are measured to show the glomerulus's capacity to filter substances, also referred to as the glomerular filtration rate (GFR). When renal disease results in a decreased GFR, the kidneys' capacity to eliminate creatinine is compromised. After then, the decreased GFR will cause the plasma creatinine concentration to start rising. Serum creatinine is therefore regarded as a crucial measure to evaluate renal function. The current investigation found a significant difference in the DC group's serum creatinine levels from day zero to day twenty-one. This result provides unambiguous proof that renal damage caused by poisoning cannot be fully reversed naturally without the use of therapy. On the other hand, there was a noticeable change in the TD group's serum creatinine levels from day 0 to day 21. This finding supports the test drug's ability to lower serum creatinine levels by the end of the research.It suggests that kidney function has improved. therapy. On the other hand, there was a noticeable change in the TD group's serum creatinine levels from day 0 to day 21. This finding supports the test drug's ability to lower serum creatinine levels by the end of the research.It suggests that kidney function has improved.

Serum electrolytes

The kidneys play a critical role in maintaining the body's normal levels of fluid and electrolytes by filtering blood and excreting certain chemicals to maintain homeostasis. Electrolytes are charged

particles that help nerve and muscle impulses travel throughout the body. Renal disorders can lead to an imbalance of certain electrolytes because they impair the kidney's capacity to maintain fluid and electrolyte balance. Because of this, the serum electrolyte level may be used as a measure to evaluate renal function.

Serum sodium

Between the first day (day zero) and the 21st day, there was a substantial variation in the serum sodium levels in the DC group. This disparity shows unequivocally that when toxicity is present, treatment must be administered in order for the body to fully return to normal sodium levels. Conversely, between the first and the twenty-first day, there was no discernible change in the serum sodium levels in the Test drug group. This result emphasises that the treatment successfully allowed for a whole reversal of toxicity by the end of the treatment period with respect to serum sodium levels.

Serum potassium

There was a noticeable variation in the serum potassium levels on day zero and day 21 of the current investigation. This remarkable disparity emphasises that when poisoning is prevalent, the body cannot naturally and fully restore its normal potassium levels without medical assistance.On the other hand, there was change in serum potassium levels which was mild significant between day zero and day 21 in the Test drug group. This result emphasises that by the end of the treatment period, the treatment had successfully facilitated a complete reversal of toxicity with regard to serum potassium levels.

Tissue Parameters

MDA

On day 21, the MDA (malondialdehyde) values for the TD group were noted. The results indicated that the test substance, *Vitex negundo* L. root powder, considerably reduced the MDA value in comparison to DC.A drop in MDA levels indicated that the nephrotoxicity had reversed.

SOD

On day 21, the TD group's SOD (superoxide dismutase) readings were noted. It revealed that as compared to DC, the test medication, *Vitex negundo* L. root powder, dramatically raised the SOD value. A rise in SOD levels indicated that the nephrotoxicity had reversed.

After analyzing all these observations result shows *Nirgundi* (*Vitex negundo*) root powder capable to treat nephrotoxicity induced by Diclofenac sodium in Wistar rats.

Conclusion

According to the study's findings, powdered *Vitex negundo* root reduces kidney damage in rats after they ingest diclofenac sodium. This may be done by reducing inflammation and preserving or enhancing the biochemical and haematopoietic potentials. These results imply that the root powder of *Vitex negundo* is likely effective as a new nephroprotective drug, which may be because it contains flavonoids and phenolic chemicals. Therefore, Nephroprotective effect of *Vitex negundo* may benefit for patients with renal insufficiency.

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