

Content available at: <https://www.ipinnovative.com/open-access-journals>

Indian Journal of Pharmacy and Pharmacology

Journal homepage: <https://www.ijpp.org.in/>

## Original Research Article

Nephroprotective effects of *Oroxylum Indicum* fruit extract in diabetes induced nephropathy in ratsVarun V Joshi<sup>1</sup>, Manoj S Mahajan<sup>1</sup>, Aman B Upaganlawar<sup>1</sup>, Chandrashekhar D Upasani<sup>1</sup><sup>1</sup>Dept. of Pharmacology, SNJB's Shriman Sureshdada Jain College of Pharmacy, Chandwad, Maharashtra, India

## ARTICLE INFO

## Article history:

Received 09-03-2021

Accepted 10-03-2021

Available online 19-04-2021

## Keywords:

Oroxylum indicum  
Diabetic nephropathy  
Streptozotocin  
Hyperglycemia  
Oxidative stress

## ABSTRACT

**Background:** Nephropathy is one of the important microvascular complications of diabetes. In the present study, *Oroxylum indicum* fruit extract was screened for its probable nephroprotective effects in streptozotocin-induced diabetic rats.

**Materials and Methods:** Male albino rats of Wistar strain (200-250gm) were used in the study. Rats were divided into various groups each containing six rats. Nephropathy was induced by the injection of freshly prepared streptozotocin (60 mg/kg, i.p.). After confirmation of diabetic nephropathy, rats were treated with *Oroxylum indicum* fruit extract at three different doses i.e. 100, 200, 400 mg/kg/orally for four weeks. Nephropathy was assessed by evaluating biochemical parameters such as blood glucose, total protein, albumin, urea, uric acid, creatinine, and total bilirubin from serum and urine. The markers of oxidative stress such as lipid peroxidation, reduced glutathione, and tissue nitrite level were estimated from kidney homogenates. Histopathology of the kidney sample was also carried out.

**Result and Conclusion:** Nephropathy-induced rats showed a significant alteration in biochemical and markers of oxidative stress. Treatment with *Oroxylum indicum* fruit extract significantly attenuated the altered parameters towards normal. The fruit extract showed dose-dependent effects, where 400 mg/kg showed good nephroprotective effects compared to 100 and 200 mg/kg. This dose-dependent activity of the fruit might be due to the presence of polar flavonoid and tannin which has been reported as strong free radical scavengers.

**Key Messages:** *Oroxylum indicum* fruit has nutritional value and it is used for various medicinal purposes. This study gives an idea about the use of *Oroxylum indicum* fruit in diabetic nephropathy.

© This is an open access article distributed under the terms of the Creative Commons Attribution License (<https://creativecommons.org/licenses/by/4.0/>) which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

## 1. Introduction

Diabetes mellitus (DM) is a metabolic disorder which is characterized by chronic hyperglycemia which may occur due to insulin resistance or reduced secretion of insulin from pancreatic beta-cell.<sup>1</sup> Persistent hyperglycemia may lead to the development of secondary complications such as neuropathy, nephropathy, and retinopathy.<sup>2</sup> It has been reported that about 30-40% of diabetic patients reported nephropathy. Diabetic nephropathy (DN) is characterized by glomerular hypertrophy, proteinuria, reduced glomerular function, and subsequent alteration in renal function.<sup>3</sup> It

has been reported that persistence hyperglycemia starts the generation of free radicals and oxidative stress played an important role in the genesis of DN.<sup>4-6</sup> *Oroxylum indicum* commonly known as the "Indian Trumpet tree" belongs to the Family Bignoniaceae. The *Oroxylum indicum* plant has been used as traditional medicine. In Asia as traditional medicinal systems of medicine, this plant has been used as an analgesic, anti-inflammatory, antimicrobial, hepatoprotective, nephroprotective, antidiabetic, and antioxidant for the prevention & treatment of diseases.<sup>7-10</sup> The plant is reported to contain flavonoids that are the major constituents of all parts of the plant. The flavonoids include chrysin, oroxylin A, baicalein, and oroxylin B.<sup>11</sup>

E-mail address: [amanrx@gmail.com](mailto:amanrx@gmail.com) (A. B. Upaganlawar).

The aqueous and ethanolic extracts of stem, bark, root, and fruit were found to be safe.<sup>12</sup> The literature reviewed showed that the fruit of *O. indicum* possesses strong antioxidant properties.<sup>13,14</sup> Considering the reported study and available literature the present study is designed to assess the nephroprotective effects of aqueous extract of *O. indicum* fruit in diabetes-induced nephropathy in albino rats.

## 2. Materials and Methods

### 2.1. Chemicals and reagents

Streptozotocin (STZ) was purchased from Sigma Aldrich USA Ltd. Ethanol use as solvent for extraction, was purchased from Loba Chemical. The diagnostic kits used for biochemical analysis were purchased from the Coral Clinical Systems. All other chemicals and reagents used were of AR grade.

### 2.2. Collection and preparation of plant extract

The powder of fruit part of *O. indicum* was collected from Kurnol District, Andhra Pradesh, India. The powder was passed through a 120# mesh sieve to remove fine and coarse particles, and then the powder was extracted using the Soxhlet extraction method using ethanol as a solvent. After completion of the extraction, the solvent was distilled off and the concentrated extract was air-dried.<sup>15</sup> The extract was kept at vacuum desiccators till further use. The percentage yield of the extract was found to be 4.3%.<sup>16</sup>

## 3. Experimental animals

Adult male albino rats of Wistar strain (200-250 gm) were used in the study. Rats were divided into five different groups and each group contains six rats (6 rats). The animals were procured from Wockhardt Ltd. Aurangabad, Maharashtra, India. Rats were placed separately in polypropylene cages with paddy husk as bedding. The animals were maintained under standard laboratory conditions at a temperature of  $23 \pm 2$  °C, relative humidity of  $55\% \pm 10\%$ , and 12-h light and 12-h dark cycle throughout all the experiments. Animals had free access to water and standard laboratory feed (Nutrivet Lab, Pune, India). The experimental procedures and protocol were reviewed and approved by the Institutional Animal Ethics Committee (IAEC) of SSDJ College of pharmacy, Neminagar, Chandwad (647/02/c/CPCSEA dated: 19/07/2002).

### 3.1. Induction diabetic nephropathy

Nephropathy in rats was induced by a single injection of streptozotocin (60 mg/kg i.p.) prepared freshly in ice-chilled 0.1M citrate buffer (pH 4.5).<sup>17</sup> The control rats received an equal volume of vehicle. Diabetes was confirmed after

72 hours of STZ injection, the Blood glucose level was checked by Glucometer (Dr. Morepen BG-03 Gluco One Glucometer). The rats having a blood glucose level of more than 250 mg/dl were considered as diabetic and further checked for the development of nephropathy. Body weight, food consumed, and fluid intake was measured before and after 24 hours of STZ administration.<sup>18</sup>

## 4. Experimental design

Rats were randomly divided into five groups each consisting of six animals.

1. Group, I served as normal control and received an equal volume of citrate buffer (CN).
2. Group II served as STZ induced diabetic nephropathy (STZ-DN).
3. Group III, DN rats received *O. indicum* fruit extract (100 mg/kg, p.o.).
4. Group IV, DN rats received *O. indicum* fruit extract (200 mg/kg, p.o.).
5. Group V, DN rats received *O. indicum* fruit extract (400 mg/kg, p.o.).

STZ induced diabetic rats were tested weekly for urine protein level. After 4 weeks the urine protein level was found to be significantly increased and that period was considered as development for nephropathy and the treatment continued for 4 more weeks. The total protocol for the model was 8 weeks (4 weeks for nephropathy development and 4 weeks for treatment).

### 4.1. Assessment of diabetic nephropathy

#### 4.2. General parameters

The animals were monitored for changes in body weight (BW), feed intake (FI), water intake (WI) and, 24 hours urine volume. At the end of the treatment period, kidney weight was recorded and the hypertrophy index was calculated.<sup>19</sup>

#### 4.3. Biochemical parameters

Biochemical parameters were estimated from serum and urine samples. At the end of the 8<sup>th</sup> week, an individual animal from each group was placed in the metabolic cage and 24 hours urine was collected. Blood was withdrawn from the retro-orbital plexus using light ether anesthesia. The serum and urine sample was used for the estimation of glucose level, total protein, albumin, uric acid, urea, creatinine, and total bilirubin, and these parameters were measured using a diagnostic kit under Biochemical autoanalyzer.<sup>20,21</sup> The urinary albumin excretion rate (UAER), as an indication of albuminuria, was calculated by using the formula.<sup>22</sup>

$$\text{UAER } (\mu\text{g}/\text{min}) =$$

$$\frac{\text{Albumin (mg/dl)} \times \text{Volume of urine in timed collection} \times 1000}{\text{Time of urine collection (min)}}$$

#### 4.4. Urinary ions

The 24 hr. urine sample was used for the estimation of urinary ions ( $\text{Na}^+$  and  $\text{K}^+$ ) using a flame photometer. The normal levels of  $\text{Na}^+$  ion in urine are 135-153 mEq/L and  $\text{K}^+$  ion in urine 3.5-5.0 mEq/L.<sup>23</sup>

#### 4.5. Tissue antioxidant parameters

The animals were euthanasiously sacrificed and the kidney was isolated, it was homogenized and centrifuged using a high-speed cooling centrifuge. The clear supernatant was for the estimation of the following parameters.<sup>24</sup> Lipid peroxidation (LPO) was determined by using the method of Slater and Sawyer<sup>25</sup> and the value was expressed as nM of MDA/mg of tissue. Reduced Glutathione (GSH) was determined by using the method of Moron et al.<sup>26</sup> and the value was expressed as  $\mu\text{g}$  of GSH/mg protein. Tissue Nitrite level (NO) was determined by using the method of Guevara<sup>27</sup> and the value was expressed as nmol/gm of tissue.<sup>24</sup>

#### 4.6. Histopathology

The kidney was rapidly dissected out and washed with saline and fixed in 10% buffered formalin. Small sections of tissue were cut and stained with Hematoxylin and Eosin (H&E).<sup>28</sup> The sections were examined under a light microscope for general morphological evaluation and photomicrographs were taken.

#### 4.7. Statistical analysis

Data were expressed as Mean  $\pm$  SEM and analyzed using a one-way analysis of variance followed by Dunnett's multiple comparison test as appropriate to identify the difference between various groups understand. Statistical Significant was defined as  $p < 0.05$ . All statistical analysis was performed using statistical software (Graph Pad Prism, version 5.0).

### 5. Results

#### 5.1. Effect of *O. indicum* fruit extract on body weight, feed intake, and water intake

At the end of the 8<sup>th</sup>-week body weight, feed intake, and water intake from all the groups were monitored. The body weight of STZ-DN rats was found to be significantly ( $p < 0.001$ ) reduced as compared to CN rats. While rats treated with 200 and 400 mg/kg showed a significantly ( $p < 0.01$ ) increased in body weight as compare to STZ-DN, 100 mg/kg. 200 and 400 mg/kg rats also exhibited significantly ( $p < 0.05$ ) improved food intake compared to STZ-DN and

100 mg/kg rats. On the other hand, STZ-DN rats show a significant ( $p < 0.001$ ) increased in water intake as compared to CN. *O. indicum* at a dose of 400 mg/kg showed better effects in the prevention of altered body weight, feed intake, and water intake (Table 1).

#### 5.2. Effect of *O. indicum* fruit extract on 24 hr. urine volume, kidney weight, and hypertrophy index

At the end of the 8<sup>th</sup> week, 24 hr. urine volume, kidney weight, and hypertrophy index of STZ-DN rats were found to be significantly ( $p < 0.001$ ) increased as compared to CN rats. The treatment with 100, 200, and 400 mg/kg rats showed a significant ( $p < 0.05$ ) decreased in 24 hr. urine volume compared to STZ-DN rats. The treatment with 200 and 400 mg/kg rats showed significantly ( $p < 0.05$ ) increased kidney weight and hypertrophy index compared to 100 mg/kg rats (Table 2).

#### 5.3. Effect of *O. indicum* fruit extract on serum parameters

A significant increase in blood glucose levels was observed in all groups at week 0 after 72 h of STZ injection as compared to control animals. This hyperglycemia was persistent in diabetic rats until the end of the 8<sup>th</sup> week compared to control rats. The blood glucose level in the STZ-DN group was found to be significantly ( $p < 0.001$ ) increased as compared to CN animals. STZ-diabetic rats treated with 100, 200, and 400 mg/kg dose for 4 weeks show a significant ( $p < 0.01$ ) reduction in blood glucose level compared to DN (Table 3). At 4<sup>th</sup> -week STZ-DN rats showed a significant ( $p < 0.001$ ) increase in the levels of total protein, albumin, uric acid, urea, creatinine, and total bilirubin compared to CN. Diabetic rats receive treatment with *O. indicum* fruit extract contains 100 mg/kg, 200 mg/kg, and 400 mg/kg shows a significant ( $p < 0.05$ ) decrease in levels of serum parameters compared to STZ-DN (Table 3). The group 400 mg/kg dose showed more beneficial effects on serum parameters as compared to diabetic rats treated with 100 and 200 mg/kg dose.

#### 5.4. Effect of *O. indicum* fruit extract on urine parameters

At 4<sup>th</sup> -week STZ-DN rats showed a significant ( $p < 0.001$ ) increase in the levels of urine sugar, total protein, albumin, uric acid, urea, creatinine, and total bilirubin compared to CN. Diabetic rats receive treatment with *O. indicum* fruit extract contains 100 mg/kg, 200 mg/kg, and 400 mg/kg shows a significant ( $p < 0.05$ ) decrease in levels of urine parameters compared to STZ-DN (Table 4). Urinary albumin excretion rate (UAER) was calculated by using the formula as mentioned. STZ-DN rats showed a significant

**Table 1:** Effect of *O. indicum* fruit extract on body weight, feed intake, and water intake at the 8<sup>th</sup> week

Groups	Body weight (gm)	Feed intake (gm)	Water intake (ml)
CN	310.45 ± 7.31	25.57 ± 0.45	60.19 ± 5.18
STZ-DN	215.99 ± 0.80***	47.83 ± 2.79***	115.00 ± 7.56***
STZ + 100 mg/kg	210.57 ± 9.47 <sup>#</sup>	40.61 ± 3.78 <sup>#</sup>	113.50 ± 4.92 <sup>#</sup>
STZ + 200 mg/kg	237.66 ± 4.10 <sup>#</sup>	40.00 ± 2.47 <sup>#</sup>	99.52 ± 8.30 <sup>#</sup>
STZ + 400 mg/kg	250.61 ± 10.33 <sup>##</sup>	42.00 ± 1.69 <sup>#</sup>	98.23 ± 12.97 <sup>#</sup>

Values are expressed as Mean ± SEM; n = 6; \*p < 0.05, \*\*p < 0.01, \*\*\*p < 0.001, compared with diabetic nephropathy control; <sup>#</sup>p < 0.05, <sup>##</sup>p < 0.01, <sup>###</sup>p < 0.001 compared with treatment group.

**Table 2:** Effect of *O. indicum* fruit extract on 24 hr. urine volume, kidney weight, and hypertrophy index

Groups	24 hr. Urine volume (ml/Day)	Kidney weight (gm)	Hypertrophy index (%)
CN	06.16 ± 0.57	1.19 ± 0.016	0.42 ± 0.006
STZ-DN	70.07 ± 2.91***	1.35 ± 0.055***	0.63 ± 0.026***
STZ + 100 mg/kg	32.87 ± 3.78 <sup>#</sup>	1.28 ± 0.012	0.54 ± 0.006 <sup>#</sup>
STZ + 200 mg/kg	30.45 ± 3.47 <sup>##</sup>	1.25 ± 0.048 <sup>#</sup>	0.54 ± 0.021 <sup>#</sup>
STZ + 400 mg/kg	31.70 ± 3.71 <sup>##</sup>	1.21 ± 0.014 <sup>##</sup>	0.50 ± 0.005 <sup>##</sup>

Values are expressed as Mean ± SEM; n = 6; \*p < 0.05, \*\*p < 0.01, \*\*\*p < 0.001, compared with diabetic nephropathy control; <sup>#</sup>p < 0.05, <sup>##</sup>p < 0.01, <sup>###</sup>p < 0.001 compared with treatment group.

**Table 3:** Effect of *O. indicum* fruit extract on serum parameters.

Groups	BGL (mg/dl)	Total Protein (gm/dl)	Albumin (gm/dl)	Uric acid (mg/dl)	Urea (mg/dl)	Creatinine (mg/dl)	Total Bilirubin (mg/dl)
CN	126.66 ± 03.37	3.84 ± 0.42	2.46 ± 0.65	3.50 ± 0.16	14.85 ± 4.15	1.18 ± 0.07	0.22 ± 0.06
STZ-DN	458.50 ± 27.61***	6.03 ± 0.16***	3.92 ± 0.14***	7.15 ± 1.05***	33.51 ± 25.89***	2.16 ± 1.67***	0.98 ± 0.08***
STZ + 100 mg/kg	345.42 ± 32.82 <sup>#</sup>	5.60 ± 0.23 <sup>#</sup>	3.53 ± 2.72 <sup>#</sup>	5.91 ± 1.13 <sup>#</sup>	26.64 ± 3.34 <sup>#</sup>	1.80 ± 0.06 <sup>#</sup>	0.86 ± 0.12 <sup>#</sup>
STZ + 200 mg/kg	338.42 ± 23.0 <sup>#</sup>	5.30 ± 0.18 <sup>#</sup>	3.33 ± 0.25 <sup>#</sup>	5.50 ± 0.83 <sup>#</sup>	25.01 ± 2.48 <sup>#</sup>	1.64 ± 0.12 <sup>#</sup>	0.60 ± 0.14 <sup>#</sup>
STZ + 400 mg/kg	304.85 ± 17.43 <sup>##</sup>	5.20 ± 0.16 <sup>#</sup>	3.05 ± 0.65 <sup>#</sup>	5.32 ± 0.48 <sup>#</sup>	21.45 ± 0.63 <sup>#</sup>	1.45 ± 0.08 <sup>#</sup>	0.46 ± 0.12 <sup>#</sup>

Values are expressed as Mean ± SEM; n = 6; \*p < 0.05, \*\*p < 0.01, \*\*\*p < 0.001, compared with diabetic nephropathy control; <sup>#</sup>p < 0.05, <sup>##</sup>p < 0.01, <sup>###</sup>p < 0.001 compared with treatment group.

(p < 0.001) increase in UAER due to renal impairment compared to CN. Whereas diabetic rats treated with 100, 200 and 400 mg/kg dose shows a significant (p < 0.001) decrease in UAER compared to STZ-DN (Table 5).

### 5.5. Effect of *O. indicum* fruit extract on urinary ions (Na<sup>+</sup> and K<sup>+</sup>)

The level of urinary ions (Na<sup>+</sup> & K<sup>+</sup>) in urine was significantly (p < 0.001) increased in the STZ-DN group as compared to the normal control group. The treatment with *O. indicum* fruit extract contains 100 mg/kg, 200 mg/kg, and 400 mg/kg groups show significantly (p < 0.001) decreased level of urinary ions (Na<sup>+</sup> & K<sup>+</sup>) in urine as compared to the STZ-DN group (Table 6).

### 5.6. Effects of *O. indicum* fruit extract on Tissue antioxidant status

Lipid peroxidation was significantly (p < 0.001) increased in diabetic rats. Treatment with *O. indicum* fruit showed a significantly decreased in the levels of LPO compared to the STZ-DN group (Figure 1 A). There was a significant (p < 0.001) reduction in the levels of reduced glutathione (GSH) in STZ-DN as compared to the normal control group. Treatment with *O. indicum* fruit extract contains dose-dependently showed a significant (p < 0.001) increased in the levels of GSH than the STZ-DN group (Figure 1 B). Tissue Nitrite level was significantly increased in the kidney tissue of the STZ-DN group compared to CN. Administration of *O. indicum* fruit extract showed a significant reduction in renal nitrite levels as compared to the STZ-DN group (Figure 1C). Overall *O. indicum* 400 mg/kg orally was found to displayed good activity compared to 100 and 200

**Table 4:** Effect of *O. indicum* fruit extract on urine parameters.

Groups	Glucose Level (mg/dl)	Total Protein (gm/dl)	Albumin (gm/dl)	Uric acid (mg/dl)	Urea (mg/dl)	Creatinine (mg/dl)	Total Bilirubin (mg/dl)
CN	99.53 ± 40.63	3.01 ± 0.44	2.27 ± 0.27	2.24 ± 0.35	14.05 ± 1.56	1.08 ± 0.85	0.23 ± 0.01
STZ-DN	436.56 ± 29.48***	6.16 ± 0.20***	4.44 ± 0.36***	7.58 ± 1.11***	34.67 ± 1.33***	2.73 ± 0.30***	1.01 ± 0.08***
STZ + 100 mg/kg	376.99 ± 29.30 <sup>#</sup>	5.65 ± 0.22 <sup>#</sup>	3.63 ± 0.07 <sup>#</sup>	6.91 ± 0.71 <sup>#</sup>	28.65 ± 2.02 <sup>#</sup>	1.97 ± 0.20 <sup>#</sup>	0.86 ± 0.12 <sup>#</sup>
STZ + 200 mg/kg	346.55 ± 12.49 <sup>#</sup>	5.30 ± 0.18 <sup>#</sup>	3.33 ± 0.25 <sup>#</sup>	5.85 ± 0.74 <sup>#</sup>	25.00 ± 2.48 <sup>##</sup>	1.64 ± 0.12 <sup>##</sup>	0.60 ± 0.14 <sup>#</sup>
STZ + 400 mg/kg	323.23 ± 5.61 <sup>###</sup>	3.95 ± 0.22 <sup>###</sup>	2.78 ± 1.03 <sup>#</sup>	5.48 ± 0.54 <sup>#</sup>	17.41 ± 0.66***	1.21 ± 0.21 <sup>###</sup>	0.44 ± 0.29 <sup>#</sup>

Values are expressed as Mean ± SEM; n = 6; \* p< 0.05, \*\* p< 0.01, \*\*\* p< 0.001, compared with diabetic nephropathy control; <sup>#</sup>p< 0.05, <sup>##</sup>p< 0.01, <sup>###</sup>p< 0.001 compared with treatment group.

**Table 5:** Effect of *O. indicum* fruit extract on urinary albumin excretion rate (UAER)

Groups	UAER (µg/min)
CN	1.14 ± 6.56
STZ-DN	9.3 ± 26.40***
STZ + 100 mg/kg	1.99 ± 46.01 <sup>#</sup>
STZ + 200 mg/kg	1.28 ± 61.78 <sup>#</sup>
STZ + 400 mg/kg	1.16 ± 22.27 <sup>#</sup>

Values are expressed as Mean ± SEM; n = 6; \* p< 0.05, \*\* p< 0.01, \*\*\* p< 0.001, compared with diabetic nephropathy control; <sup>#</sup>p< 0.05, <sup>##</sup>p< 0.01, <sup>###</sup>p< 0.001 compared with treatment group.

**Table 6:** Effect of *O. indicum* fruit extract on urinary ions (Na<sup>+</sup> and K<sup>+</sup>)

Groups	Na <sup>+</sup> ion (mEq/L)	K <sup>+</sup> ion (mEq/L)
CN	41.43 ± 0.45	1.28 ± 0.012
STZ-DN	161.3 ± 0.47***	3.16 ± 0.030***
STZ + 100 mg/kg	111.4 ± 0.52 <sup>###</sup>	2.52 ± 0.043 <sup>###</sup>
STZ + 200 mg/kg	97.54 ± 0.57 <sup>###</sup>	2.12 ± 0.061 <sup>###</sup>
STZ + 400 mg/kg	55.07 ± 0.26 <sup>###</sup>	1.53 ± 0.043 <sup>###</sup>

Values are expressed as Mean ± SEM; n = 6; \* p< 0.05, \*\* p< 0.01, \*\*\* p< 0.001, compared with diabetic nephropathy control; <sup>#</sup>p< 0.05, <sup>##</sup>p< 0.01, <sup>###</sup>p< 0.001 compared with treatment group.

mg/kg dose.

### 5.7. Histopathology

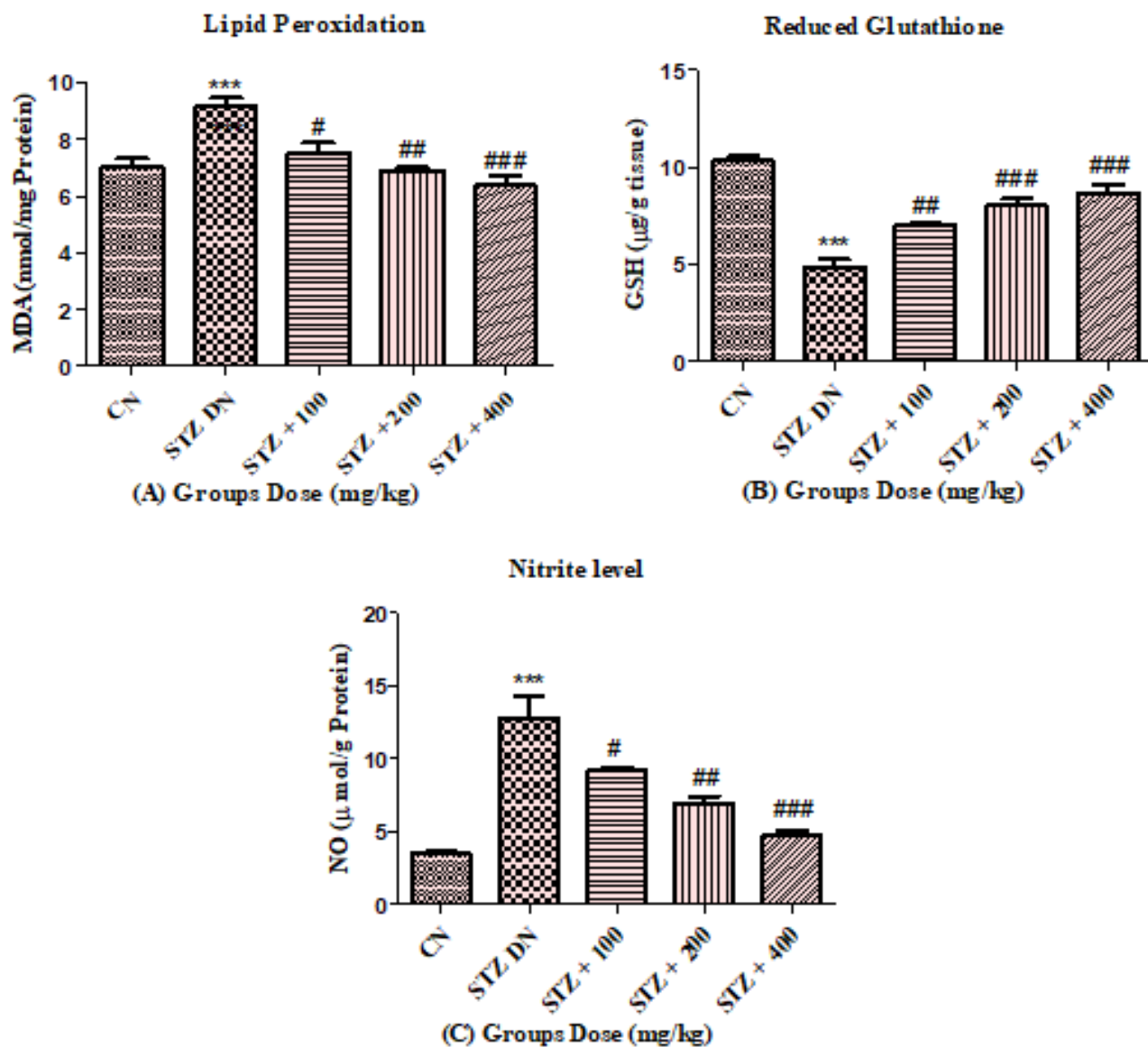
Evaluation of photomicrographs of kidney tissue confirmed by Motic microscope (10X) (DMWB1 – 223ASC series, PAL system, China). In the control group, the normal architecture of kidney tissue was observed. In the diabetic group necrosis of the glomerulus, tubular dilatation, and tubular architectural impairment was noted. Treatment with 100 mg/kg dose shows significant pathology alteration. Rats treated with 200 and 400 mg/kg dose shows improvement in kidney structure as compared to the diabetic group (Figure 2).

## 6. Discussion

The present study was aimed to explore the antioxidant properties of *O.indicum* fruit extract in diabetes-induced

nephropathy in rats. The involvement of reactive oxygen species and hyperglycemia is reported to be one of the important factors involved in the formation of secondary complications such as diabetic nephropathy.<sup>29</sup> Diabetic nephropathy is one of the major complications of DM, in which the kidney is the most affected organ. At the chronic stage, it leads to end-stage renal disease (ESRD).

STZ is a chemical agent used for the induction of diabetes mellitus in animals. STZ can develop destruction in pancreatic beta cells which results in dysfunction of insulin secretion and thereupon hyperglycemia due to the production of free radicals.<sup>30</sup> In the present study administration of STZ leads to a significant increase in blood glucose after 72 hr. which was significantly reduced on treatment with *O. indicum* fruit extract at 4th-week treatment. In an initial treatment period, there is no significant difference was observed in the blood glucose level of the *O. indicum* fruit extract-treated group as



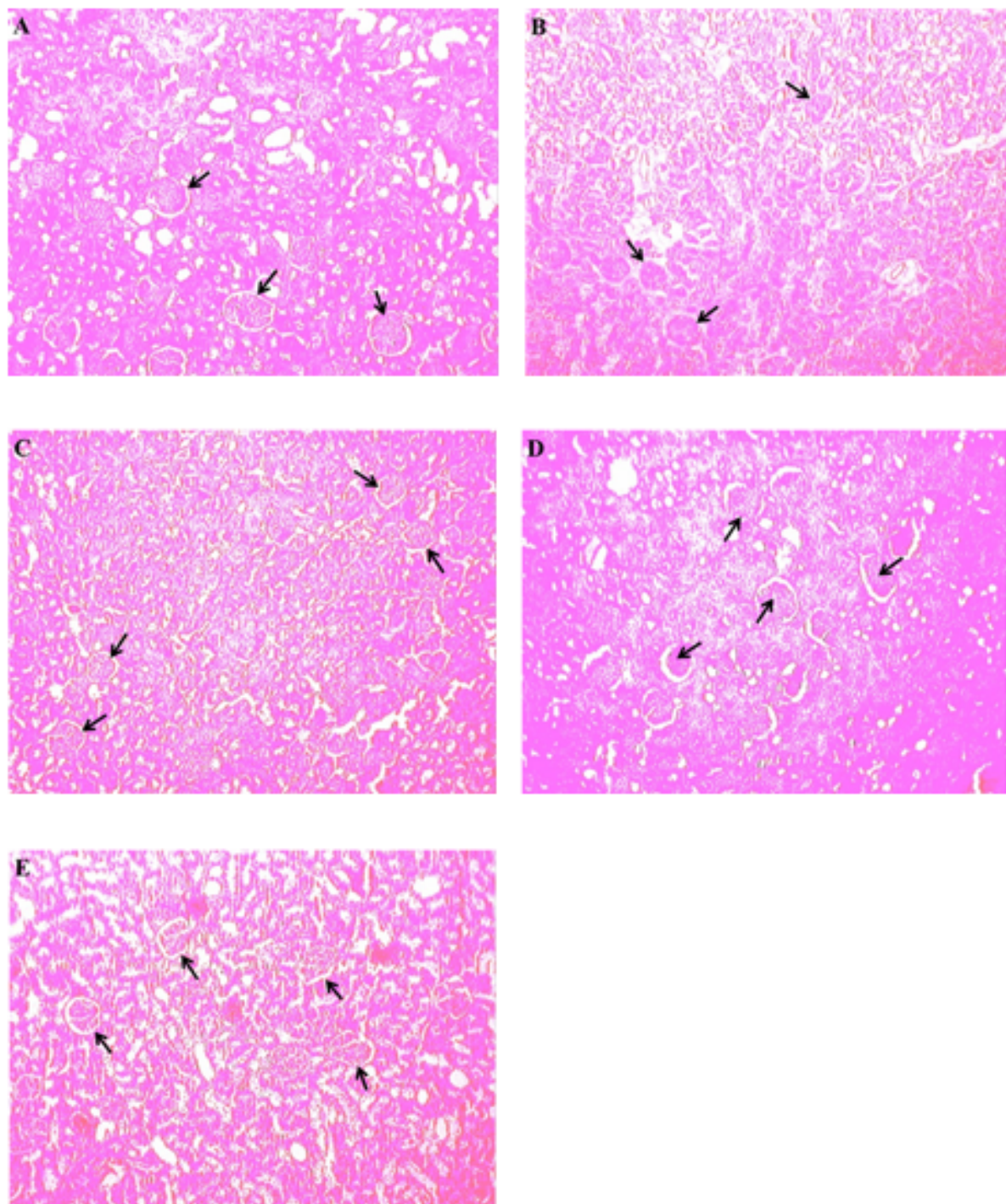
**Fig. 1:** Effect of *O. indicum* fruit extract on tissue antioxidant parameters; A) LPO, B) GSH and C) NO levels in kidney homogenate. (Values are expressed as Mean  $\pm$  SEM; n = 6; \*p < 0.05, \*\*p < 0.01, \*\*\*p < 0.001, compared with diabetic nephropathy control; #p < 0.05, ##p < 0.01, ###p < 0.001 compared with treatment group).

compared to the diabetic group. This might be due to the presence of active constituents in the extract. At 4th-week STZ diabetic rats shows a significant reduction in blood glucose level compared to DN.

Diabetic nephropathy in rats is associated with significant changes in body weight, feed intake, and water intake which might be due to persistent hyperglycemia, less insulin secretion, loss of tissue proteins, and increase muscle degradation.<sup>13</sup> Treatment with *O. indicum* extract showed a significant improvement in general parameters. These changes might be due to the nutrition value and antioxidant property of *O. indicum* fruit extract which

improves altered body weight, feed intake, and water intake. The present results are in line with previously presented literature.<sup>20,31</sup>

An increase in 24 hr. urine volume and in the weight of the kidney (hypertrophy index) in proportion to body weight was observed in STZ-induced diabetes in rats. The nephron contains proximal convoluted tubule cells and glomerular mesangial cells; In DN due to persistence hyperglycemia causes alterations in the kidney structure and the formation of development of renal hypertrophy.<sup>19</sup> The formation of protein synthesis and a decrease in the levels of extracellular components in the kidney leads to the formation of renal



**Fig. 2:** Photomicrographs of kidney (Hematoxylin-Eosin staining under a light microscope at 10x magnification), **A:** Normal Control group; **B:** STZ DN (Diabetic group); **C:** 100 mg/kg *O. indicum* fruit extract-treated group; **D:** 200 mg/kg *O. indicum* fruit extract-treated group; and **E:** 400 mg/kg *O. indicum* fruit extract-treated group.

hypertrophy.<sup>32</sup> Treatment with *O. indicum* fruit extract showed a significant decrease in 24h urine volume and hypertrophy index.

STZ-DN rats showed a significant increase in serum and urine markers. In DM, chronic hyperglycemia starts the development of pathological conditions like the presence of protein in the urine.<sup>33</sup> The presence of protein in the urine shows the formation of renal failure. STZ-induced diabetic rats significantly increase the levels of total protein and albumin. Also, the nephropathy-induced rats showed abnormal levels of uric acid, urea, creatinine, and total bilirubin in serum and urine samples. A previous study shows that STZ induced DM shows an increase in levels of serum parameters and a decrease in levels of urine parameters. This can be due to deteriorated excretory and regulatory renal function to maintain constant homeostasis of these parameters.<sup>28,31</sup> As treatment with *O. indicum* fruit extract significantly normalizes the levels of altered serum and urine parameters. Nephropathy rats show the presence of glucose in the urine. This might be due to the presence of protein in the urine sample. The protein accumulates in the vessel, leads to the altered metabolism of carbohydrates and fats. According to this glucose will not be metabolized and will be excreted in the urine.<sup>3,34</sup> As treatment with *O. indicum* fruit extract shows a decrease in the glucose level in urine.

Albuminuria is the main factor related to deteriorating kidney function. Albuminuria primarily refers to increased urinary excretion of albumin. STZ-induced diabetic rats show an increased albuminuria level. A previous study shows that STZ induced DM corresponds to a rise in UAER levels. As urinary albumin level is a predictor of glomerular injury and the rise in Albumin Excretion Rate (AER) indicates progressive nephropathy. This results in a loss of renal function. Several studies have suggested that the reduction of microalbuminuria shows a stronger prognosis in diabetic nephropathy.<sup>19,35</sup> As treatment with *O. indicum* fruit extract significantly reduces the level of albuminuria and it shows a beneficial role against albuminuria.

The body contains a wide range of ions or electrolytes that perform many functions. Six electrolytes are most essential to maintaining body function: sodium, potassium, chloride, bicarbonate, calcium, and phosphate. Sodium ( $\text{Na}^+$ ) is the major extracellular cation and plays a part in the distribution of body fluids. Increase or decrease levels of sodium ion are referred to as hypernatremia and hyponatremia, respectively.<sup>36</sup> Potassium ( $\text{K}^+$ ) is the major cation present in cells. Proper potassium levels are important for normal cell function. An abnormal increase or decrease in potassium affects the nervous system and the heart and can be fatal when severe.<sup>37</sup>  $\text{Na}^+$  &  $\text{K}^+$  are important ions for maintaining sodium-potassium pump ( $\text{Na}^+$ - $\text{K}^+$  Pump) in the body. STZ-induced diabetic rats show an increased urinary ions ( $\text{Na}^+$  &  $\text{K}^+$ ) level. This might

be due to persistent hyperglycemia, less insulin secretion, loss of tissue proteins, and increase muscle degradation.<sup>28</sup> A previous study shows that STZ induced DM shows to increase in levels of  $\text{Na}^+$  &  $\text{K}^+$  in albino Wistar rats which affects the sodium-potassium pump. This is due to the obstruction present in the urinary bladder.<sup>34</sup> In the present study treatment with *O. indicum* fruit extract normalized levels of urinary ions ( $\text{Na}^+$  &  $\text{K}^+$ ) in urine as compared to STZ-induced diabetic rats.<sup>23</sup>

In STZ-induced diabetic rats, blood glucose level persistently increases which leads to the formation of oxidative stress. Oxidative stress in STZ-induced diabetic rats shows an increase in the production of reactive oxygen species and a sharp reduction in antioxidant defenses. Lipid peroxidation seems to be a key component in the development of diabetic nephropathy. Glutathione is a major intracellular non-protein sulfhydryl agent; it plays an important role in the generation of cellular redox state and is thus the imbalance in reduced GSH to oxidized glutathione ratio is a prospective predictor of cellular oxidative stress.<sup>38</sup>

Oxidative stress is involved in various disorders, DN induced by STZ in rats produces free radicals and thereby an imbalance between oxidants and antioxidants inside the body. In the present study, the markers of oxidative stress (LPO, GSH, and NO) were tested. An STZ-induced diabetic rat shows significantly increases the LPO and NO levels and treatment with *O. indicum* fruit extract significantly decrease levels of LPO and NO. The decreased level of GSH in STZ-induced diabetic rats shows an increased level of GSH after treatment with *O. indicum* fruit extract.

Histopathology study of STZ-induced diabetic rats showed necrosis of glomerulus, tubular dilatation, and tubular architectural impairment. Treatment with *O. indicum* fruit extracts significantly alterations as above shows improvement in a tubular structure, thus the presence of a protective role in kidney damage.

## 7. Conclusion

In the present study, *Oroxylum indicum* fruit extract was screened at three different doses in diabetic nephropathy. The fruit extract showed dose-dependent protective effects, whereas 400 mg/kg showed good effects compared to 100 and 200 mg/kg. This dose-dependent activity of *Oroxylum indicum* fruit extract might be due to the presence of polar flavonoid and tannin which showed strong free radical scavenging activity.

## 8. Source of Funding

None.

## 9. Conflicts of Interest

The authors declare that there is no conflict of interest.



## 10. Acknowledgement

The authors are grateful to the Management of SNJB's Shriman Sureshdada Jain College of Pharmacy for providing the necessary facility for smooth conductance and completion of this research.

## References

- Dabla PK. Renal function in diabetic nephropathy. *World J Diabetes*. 2010;1(2):48–56. doi:10.4239/wjcd.v1.i2.48.
- Foggensteiner L, Mulroy S, Firth J. Management of Diabetic Nephropathy. *J R Soc Med*. 2001;94(5):210–7. doi:10.1177/014107680109400504.
- Kiran G, Nandini CD, Ramesh HP, Salimath PV. Progression of early phase diabetic nephropathy in streptozotocin-induced diabetic rats: Evaluation of various kidney-related parameters. *Indian J Exp Biol*. 2012;50:133–40.
- Zhang H, Fang B, Zhang Q, Ji X, Chen L. Renoprotective effect of lidocaine on streptozotocin-induced diabetic nephropathy. *Int J Clin Exp Med*. 2016;9(7):14254–9.
- Qian X, Li X, Ma F, Luo S, Ge R, Zhu Y. Novel hydrogen sulfide-releasing compound, S-propargyl-cysteine, prevents STZ-induced diabetic nephropathy. *Biochem Biophys Res Commun*. 2016;473(4):931–8.
- Wang G, Lu X, Li W, Zhao X, Zhang C. Protective effects of Luteolin on diabetic nephropathy in STZ-induced diabetic rats. *Evid Based Complement Alternat Med*. 2011;2011:323171. doi:10.1155/2011/323171.
- Upaganlawar AB, Tenpe CR. In vitro antioxidant activity of Oroxylin indicum Vent. Leaves extracts. *Int J Biomed Res*. 2007;2(3):300–4.
- Chopade VV, Upaganlawar AB, Yeole PG. Antimicrobial activity of Oroxylin indicum root extract. *Antiseptic J Med Surg*. 2008;105(3):146–7.
- Upaganlawar AB, Tenpe CR, Yeole PG. Analgesic activity of Oroxylin indicum leaves extract in rats. *Indian J Nat Product*. 2007;23(2):30–2.
- Upaganlawar AB, Tenpe CR, Yeole PG. Anti-inflammatory activity of aqueous extract of Oroxylin indicum vent. leaves extract-preliminary study. *Pharmacologyonline*. 2009;1:22–6.
- Dinda B, SilSarma I, Dinda M, Rudrapaul P. Oroxylin indicum (L.) Kurz, an important Asian traditional medicine: From traditional uses to scientific data for its commercial exploitation. *J Ethnopharmacol*. 2015;161:255–78. doi:10.1016/j.jep.2014.12.027.
- Ahad A, Ganai AA, Sareer O, Najim MZ, Kausar MA, Mohd M. Therapeutic Potential of Oroxylin Indicum: a Review. *J Pharm Res Opin*. 2012;2(10):163–72.
- Singh J, Kakkar P. Modulation of liver function, antioxidant responses, insulin resistance and glucose transport by Oroxylin indicum stem bark in STZ induced diabetic rats. *Food Chem Toxicol*. 2013;62:722–31. doi:10.1016/j.fct.2013.09.035.
- Sannigrahi S, Mishra SL, Sinhamahapatra PK, Nayak A, Das R. In vitro Antioxidant Potential of Different Parts of Oroxylin indicum: A Comparative Study. *Indian J Pharm Sci*. 2010;72(2):267–9. doi:10.4103/0250-474x.65013.
- Azwanida AA. A Review on the Extraction Methods Use in Medicinal Plants, Principle, Strength and Limitation. *Med Aromatic Plants*. 2015;04(03):1–6.
- Gahlot M, Bhatt P, Joshi J. Study on Yield of Plant Extracts Using Different Solvents and Methods. *The Bulletin of Environment. Pharmacol Life Sci*. 2018;7(6):65–7.
- Tesch GH, Allen TJ. Methods in Renal Research: Rodent models of streptozotocin-induced diabetic nephropathy. *The Asian Pacific Society of Nephrology*. 2007;12(3):261–6.
- Singh AK, Singh J. Evaluation of anti-diabetic potential of leaves and stem of Flacourtia jangomas in streptozotocin-induced diabetic rats. *Indian J Pharmacol*. 2010;42(5):301–5.
- Somani R, Singhai AK, Shivgunde P, Jain D. Asparagus racemosus Wild (Liliaceae) ameliorates early diabetic nephropathy in STZ induced diabetic rats. *Indian J Exp Biol*. 2012;50(7):469–75.
- Mestry SN, Gawali NB, Pai SB, Gursahani MS, Dhodi JB, Munshi R. Punica granatum improves renal function in gentamicin-induced nephropathy in rats via attenuation of oxidative stress. *J Ayurveda Integr Med*. 2017;11(1):1–9.
- Kishore L, Singh R. Ameliorative effect of Cephalaria indica homeopathic preparation in STZ induced diabetic nephropathy rats. *J Ayurveda Integr Med*. 2017;10(4):255–61.
- Chavan VU, Sayyed AK, Durgawale PP, Sontakke AV, Nilakhe SD. Practical Aspects of Calculation, Expression and Interpretation of Urine Albumin Measurement. *Natl J Integr Res Med*. 2011;2(1):29–34.
- Pauline MH. The Flame Photometer for the Measurement of Sodium and Potassium in Biological Materials. *J Biol Chem*. 1997;167:499–510.
- Alan N, Bristi NJ, Rafiqzaman. Review on in vivo and in vitro methods evaluation of antioxidant activity. *Saudi Pharm J*. 2013;21(2):143–52. doi:10.1016/j.jsps.2012.05.002.
- Slater TF, Sawyer BC. The stimulatory effects of carbon tetrachloride and other halogenoalkanes on peroxidative reactions in rat liver fractions in vitro. General features of the systems used. *Biochem J*. 1971;123(5):805–14. doi:10.1042/bj1230805.
- Moron M, Depierre J, Mannervik B. Levels of glutathione, glutathione reductase and glutathione S-transferase activities in rat lung and liver. *Biochim Biophys Acta*. 1979;582(1):67–78. doi:10.1016/0304-4165(79)90289-7.
- Guevara I, Iwanejko J, Dembińska-Kieć A, Pankiewicz J, Wanat A, Anna P, et al. Determination of nitrite/nitrate in human biological material by the simple Griess reaction. *Clin Chim Acta*. 1998;274(2):177–88. doi:10.1016/s0009-8981(98)00060-6.
- Mestry SN, Dhodi JB, Kumbhar SB, Juvekar AR. Attenuation of diabetic nephropathy in streptozotocin-induced diabetic rats by Punica granatum Linn. leaves extract. *J Tradit Complement Med*. 2017;7(3):273–80. doi:10.1016/j.jtcm.2016.06.008.
- Minaz N, Razdan R. Therapeutic insight into molsidomine, a nitric oxide donor in streptozotocin-induced diabetic nephropathy in rats. *Indian J Pharmacol*. 2016;48(5):544–9. doi:10.4103/0253-7613.190744.
- Rajendiran D, Packirisamy S, Gunasekaran K. A Review on Role of Antioxidants in Diabetes. *Asian J Pharm Clin Res*. 2018;11(2):48–53. doi:10.22159/ajpcr.2018.v11i2.23241.
- Manojkumar MS, Chandrashekhar UD, Aman UB, Vishal GS. Renoprotective Effect of Co-Enzyme Q10 and N-Acetylcysteine on Streptozotocin-Induced Diabetic Nephropathy in Rats. *Int J Diabetes Clin Res*. 2020;7(2):1–12. doi:10.23937/2377-3634/1410123.
- Zafar M, Naqvi SN. Effects of STZ-Induced Diabetes on the Relative Weights of Kidney, Liver and Pancreas in Albino Rats: A Comparative Study. *Int J Morphol*. 2010;28(1):135–42. doi:10.4067/s0717-95022010000100019.
- Julian BA, Suzuki H, Suzuki Y, Tomino Y, Spasovski G, Novak J. Sources of urinary proteins and their analysis by urinary proteomics for the detection of biomarkers of disease. *Proteomic Clin Appl*. 2009;3(9):1029–43. doi:10.1002/prca.200800243.
- Sangar VC, Singh J. Effects of Streptozotocin-Induced Type I Diabetes Mellitus on cation contents in urinary bladder tissues of the rat. *Int J Pharm Sci Res*. 2016;7(2):789–97.
- Jain PG, Nayse PG, Patil DJ, Shinde SD, Surana SJ. The possible antioxidant capabilities of formononetin in guarding against streptozotocin-induced diabetic nephropathy in rats. *Future J Pharm Sci*. 2020;6(1):1–9. doi:10.1186/s43094-020-00071-9.
- Pirahanchi Y, Jessu R, Aeddula NR. Physiology, Sodium Potassium Pump (Na+ K+ Pump). StatPearls Publishing House; 2020.
- Balci AK, Koksak O, Kose A, Armagan E, Ozdemir F, Inal T, et al. General characteristics of patients with electrolyte imbalance admitted to emergency department. *World J Emergency Med*. 2013;4(2):113–6. doi:10.5847/wjem.j.issn.1920-8642.2013.02.005.
- Phaniendra A, Jestadi DB, Periyasamy L. Free Radicals: Properties, Sources, Targets, and Their Implication in Various Diseases. *Indian J*

*Clin Biochem.* 2015;30(1):11–26. doi:10.1007/s12291-014-0446-0.

**Chandrashekhar D Upasani**, Professor and Principal

### Author biography

**Varun V Joshi**, Student  <https://orcid.org/0000-0003-4035-2564>

**Manoj S Mahajan**, Assistant Professor

**Aman B Upaganlawar**, Associate Professor

**Cite this article:** Joshi VV, Mahajan MS, Upaganlawar AB, Upasani CD. Nephroprotective effects of *Oroxylum Indicum* fruit extract in diabetes induced nephropathy in rats. *Indian J Pharm Pharmacol* 2021;8(1):78-87.