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# THE METHANOLIC EXTRACT OF *ALBIZIA ODORATISSIMA* BARK ATTENUATED THE DEVELOPMENT OF DIABETIC NEPHROPATHY IN RATS

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**ABSTRACT: Purpose:** The present study has been designed to investigate the protective effect of methanolic extract of *Albizia odoratissima* (AO) bark in prevention of streptozotocin (STZ) induced-diabetic nephropathy (DN) in Wistar rats. **Methods:** Healthy Wistar rats of either sex weighing 180-200g were employed in the present study. Experimental diabetes was induced in rats by injecting STZ at a dose of 45 mg/kg (i.p.). Assessment of DN was done by estimating glucose level in blood and urine samples. Moreover, different antioxidant parameters like catalase, superoxide dismutase (SOD) and thiobarbituric acid reactive substances (TBARS) in kidney tissue samples were assessed. Additionally, inflammatory cytokines like interleukin-1 (IL-1), transforming growth factor beta (TGF-β), and tumour necrosis factor-alpha (TNF-α) were assessed in renal tissue. **Results:** Methanolic extract of AO bark (AOB) has shown significant prevention against diabetes-associated nephropathy. The bark extract decreased glucose levels both in urine and blood sample. The AO extract, either alone or in combination with a standard drug (glibenclamide) showed a significant reduction in oxidative stress in renal tissue, as demonstrated by increased catalase and SOD levels or decreased TBARS levels compared to diabetic rats. Additionally, methanolic extract of AOB alone or in combination with glibenclamide significantly reduced inflammatory cytokines like IL-1, TGF-β, and TNF-α in diabetic rats. **Conclusion:** Our studies suggest that methanolic extract of AOB might be beneficial for the treatment of DN. The ability of AO to attenuate DN may be mediated by the inhibition of oxidative stress and inflammatory cytokines by AOB extract.

**INTRODUCTION:** This is well accepted that the incidence of Diabetes mellitus (DM) is increasing worldwide [1, 2]. DM is a chronic metabolic disorder characterized by hyperglycemia, glycosuria, polyuria, polydipsia, insulin resistance, and relative insulin deficiency [3, 4].

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Moreover, this is documented that the number of people affected with DM is expected to double in the next decade, ultimately increasing the burden on healthcare providers.

According to the world health organization (WHO), about 422 million people have diabetes, accounting for about 1.5 million deaths each year [5]. DM mortality results from the complications coupled with it, which include nephropathy, neuropathy, retinopathy and cardiomyopathy [2, 6]. Surprisingly, diabetic nephropathy (DN) affects approximately one-third of people with DM and is

considered the most lethal complication of DM. It has been demonstrated that oxidative stress accounts for the pathophysiological feature in DN, which can be caused by hyperglycemia [7]. The enhanced oxidative stress involves the increased production of reactive oxygen species (ROS) [7, 8]. In addition, it has been reported that oxidative stress has been associated with increased pro-inflammatory cytokines, such as interleukin-1 (IL-1), tumor necrosis factor-alpha (TNF-α), and transforming growth factor beta (TGF-β), which ultimately lead to the development and progression of DN [9, 10].

The herbal plants and plant products have been used to cure many fatal diseases for ages, as they lack the side effects associated with chemical medicines [11]. The potential of herbal medicine to treat various metabolic disorders is attributed to the different types of the photochemical present in them, such as flavonoids, tannins, alkaloids, polysaccharides, and hormones [12, 13]. *Albizia odoratissima* (AO) (family: Mimosaceae), commonly known as Black Siris in Hindi, is known by different names in different languages like Bhusirisah in Sanskrit, Karmaru in Punjabi, Cinduga in Telgu, Karuvagai in Tamil and Ceylon rose-wood in English [14, 15]. The plant is characterized by a large erect tree 15-25 m tall, which is well distributed in sub-Himalayan tracts, slopes, and valleys. The plant generally exists in peninsular India, Assam, West Bengal, and the western ghats of South India [15].

AO is a deciduous tree having dark grey bark, bipinnate leaves with 4-15 pairs of leaflets. The flowers are pale yellowish white, fragrant in terminal heads and redish brown at maturity [14, 15]. AO bark showed potential effects in traditional Indian medicine for the treatment of leprosy, ulcers, cough, skin diseases, rheumatism, bronchitis, diabetes, and burning sensation [14, 15]. The phytochemical screening of AOB's methanolic extract has shown the presence of flavonoids, glycosides, tannins and phenolic compounds, phytosteroids and saponins [15, 16]. These organic compounds show a budding resource for the sighting and development of new antidiabetic molecules. Although studies have shown the potential of AO bark in different diseases, its impact on the mitigation of DN has not been investigated. Hence, the present study was designed to investigate the potential effect of methanolic extract of AOB bark in the prevention of streptozotocin (STZ) induced-DN in Wistar rats.

## MATERIALS AND METHODS:

**Procurement and Identification of Plant Material:** The bark was collected from Haryana, India. The plant's bark was identified as *Albizia odoratissima* (family: Mimosaceae) by Dr. N. K. Yadav, Chairperson Department of Botany, Indira Gandhi University, Rewari, Haryana and also by the National Institute of Science Communication and Information Resources, New Delhi with Ref. No. NISCAIR/RHMD/ Consult/2020/3737-38 dated 31/12/2020.

**Preparation of Plant Extract:** The bark of *Albizia odoratissima* was dried in the shade for two weeks. The dried bark was coarsely powdered and sieved (#40). Afterward, the powdered and sieved bark was stored in an air-tight container at room temperature. The dried powder was subsequently extracted with methanol, which was preserved in the refrigerator at 4 °C[15].

**Preliminary Phytochemical Screening:** The preliminary phytochemical screening of Methanolic AOB extract was carried out following the standard procedure.

**Animals:** Healthy Wistar rats weighing 180-200 gm were obtained from BMRL, Rajasthan, India. The animals were maintained at 12-hour light and dark cycle in the registered animal house at BMRL, Rajasthan. The rats were fed with a standard food pellets diet. Water was provided to rats as *ad libitum*.

Rats were left for one week for acclimatization before starting the study. Animal experiments were carried out according to the standard guidelines of the Committee for the purpose of control and supervision of experiments on animals (CPCSEA), India. The experimental protocol was approved by the Institutional Ethical Committee (IAEC) of BMRL, Rajasthan (Reg.No.-2005/PO/RcBT/S/18/ CPCSEA).

**Induction and Assessment of Experimental DN:** Experimental DN was induced by injecting STZ at a dose of 45 mg/kg (i.p.), which was freshly

prepared in 0.1 citrate buffer at pH 4.5 in rats [17]. Seven days after the induction of diabetes, the blood glucose level was measured from the retro orbital plexus.

The fasting blood glucose levels were determined in blood samples using a strip-operated glucometer (Accu-Check, Roche Diagnostics Pvt. Ltd., New Delhi, India). In addition, urine glucose levels were assessed in the urine samples collected under a layer of toluene by 3, 5-dinitrosalicylic acid method.

The animals with blood glucose levels exceeding 250 mg/dl were regarded as diabetic and used for further study. The blood and urine glucose levels were measured before treatment and on the first, second, third, and fourth weeks respectively.

After the completion of four weeks, the experimental animals' blood was withdrawn via retro orbital plexus.

**Experimental Design:** All experimental animals are divided into Seven groups, each group having six animals, and treated once a day for 28 days as follows:

Group I: Normal control: given only vehicle.

Group II: Diabetic control (STZ 45 mg/kg,i.p).

Group III: AO bark extract per se Group (500 mg/k.g, p.o).

Group IV: Glibenclamide (2.5 mg/kg body weight) treated group.

Group V: AO bark extract (250 mg/kg, p.o) treated group.

Group VI: AO bark extract (500 mg/kg, p.o) treated group.

Group VII: AO bark extract (500 mg/kg, p.o) + Standard drug (Glibenclamide) treated group.

Blood Samples and urine samples were collected on 48 hrs and after every week and centrifuged. The estimation of various biochemical parameters was conducted in blood serum.

**Kidney Antioxidant Parameters:** A 10%, w/v kidney homogenate was prepared with 0.1 M PBS. Subsequently, it was centrifuged at 12000 g for 10 minutes. The commercially available kits were used to determine catalase [19], superoxide dismutase [20] and TBARS levels [21] in the supernatant.

**Measurement of Inflammatory Cytokines:** For the measurement of inflammatory cytokines in renal tissue, a total of 20 micrograms of renal tissue were rapidly frozen in liquid nitrogen. Subsequently, in order to collect the supernatant, the tissue was thawed, homogenized, and centrifuged at 13,400×g at 4°C for 15 min.

Further, the IL-1, TGF-β, and TNF-α levels in the renal tissue were measured using a rat IL-1 ELISA kit, rat TGF-β ELISA kit, and rat TNF-α ELISA kit, respectively.

**Statistical Analysis:** Dennett's test was used for statistical comparison, and p values ($P<0.05$) were considered significant for control. The data were further analyzed by a one-way ANOVA test followed by Tukey multiple comparison tests. The values are expressed as mean ± SD. The p values ($P<0.05$) were considered statistically significant.

**RESULTS:**

**Preliminary Phytochemical Screening:** The phytochemical screening of methanolic AOB extract revealed the presence of numerous phytoconstituents like alkaloids, flavonoids, phenolic compounds, terpenoids, tannins, and saponins.

**Effect of Methanolic AOB Extract on Blood and Urine Glucose Levels:** STZ administration produced significantly raised fasting blood glucose levels in the diabetic group compared to the normal control.

Also, the urine glucose levels were significantly increased in STZ-treated rats. Treatment with methanolic extract of AOB at different doses of 250 mg/Kg and 500 mg/kg alone or in combination with glibenclamide showed a gradual and significant reduction in the fasting blood glucose and urine glucose levels when compared to diabetic control **Tables 1-2**.

The results indicate the potential of methanolic extract of AOB in the gradual reduction of blood and urine glucose levels.

**TABLE 1: EFFECT OF METHANOLIC AOB EXTRACT ALONE AND IN COMBINATION WITH GLIBENCLAMIDE ON URINE GLUCOSE LEVEL**

| | Normal Control | Diabetic Control | AO *per se* | Glibenclamide 2.5 mg/kg | AO 250 mg/kg | AO 500 mg/kg | AO 500 mg/kg + Glibenclamide |
|---|---|---|---|---|---|---|---|
| 0 Week | 1.72 ± 0.33 | 1.95 ± 0.35 | 1.87± 0.23 | 2.22 ± 0.19 | 1.655 ± 0.16 | 1.82 ± 0.35 | 2.26 ± 0.25 |
| Week 1 | 1.89± 0.26 | 52.21 ± 1.42* | 1.12 ± 0.42 | 49.54 ± 1.25** | 58.27 ± 2.74** | 54.14 ± 0.89** | 44.65 ± 3.53** |
| Week 2 | 2.02 ± 0.39 | 69.51 ± 1.41* | 1.52 ± 0.63 | 46.51 ± 0.51** | 51.18 ± 0.85** | 46.21 ± 0.45** | 38.12 ± 0.45** |
| Week 3 | 2.15 ± 0.35 | 72.45 ± 1.23* | 2.21 ± 0.45 | 42.21 ± 0.41** | 47.56 ± 0.32** | 40.12 ± 0.31** | 31.92 ± 0.39** |
| Week 4 | 1.86 ± 0.40 | 78.54 ± 1.21* | 2.32 ± 0.35 | 35.21 ± 0.45** | 43.15 ± 0.19** | 33.82 ± 0.28** | 25.68 ± 0.29** |

All the values are expressed as mean ±SD. *P<0.05 compared with normal control, **P<0.05 compared with diabetic control

**TABLE 2: EFFECT OF METHANOLIC AOB EXTRACT ALONE AND IN COMBINATION WITH GLIBENCLAMIDE ON BLOOD GLUCOSE LEVEL**

| | Normal Control | Diabetic Control | AO *per se* | Glibenclamide 2.5 mg/kg | AO 250 mg/kg | AO 500 mg/kg | AO 500 mg/kg + Glibenclamide |
|---|---|---|---|---|---|---|---|
| 0 Week | 93.6 ± 2.81 | 95.6 ± 3.22 | 95.6 ± 2.81 | 97.1 ± 2.12 | 98.1 ± 2.52 | 95.3 ± 3.62 | 94.1 ± 2.83 |
| 48 Hours | 95.2 ± 2.63 | 522.6 ± 3.51* | 97.2 ± 2.63 | 552.2 ± 2.82** | 554.6 ± 2.62** | 557.9 ± 4.22** | 533.6 ± 10.63** |
| Week 1 | 96.7 ± 1.72 | 540.9 ± 2.56* | 97.7 ± 1.72 | 503.1 ± 1.82** | 525.5 ± 1.82** | 499.5 ± 4.72** | 479.1 ± 8.26** |
| Week 2 | 95.2 ± 2.22 | 549.3 ± 3.12* | 96.2 ± 2.22 | 458.2 ± 2.53** | 492.3 ± 3.72** | 482.3 ± 3.74** | 413.8 ± 4.82** |
| Week 3 | 94.2 ± 1.35 | 552.8 ± 3.13* | 96.2 ± 1.35 | 413.4 ± 1.92** | 476.8 ± 3.82** | 432.8 ± 3.92** | 353.7 ± 7.53** |
| Week 4 | 92.9 ± 1.16 | 562.2 ± 4.72* | 95.9 ± 1.16 | 363.7 ± 1.55** | 420.6 ± 1.72** | 381.1 ± 4.25** | 289.5 ± 4.53** |

All the values are expressed as mean ±SD. *P<0.05 compared with normal control, **P<0.05 compared with diabetic control

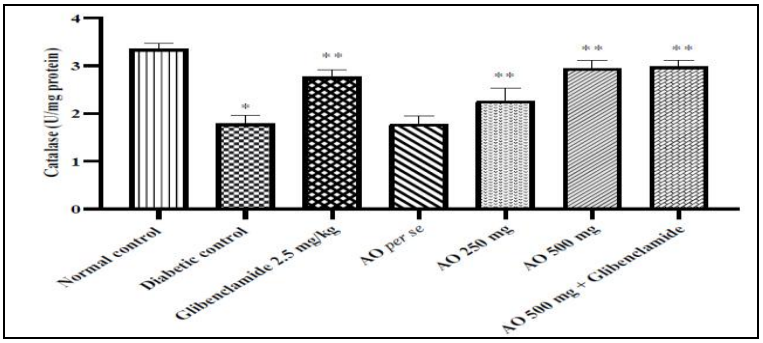


**FIG. 1: EFFECT OF METHANOLIC AOB EXTRACT ALONE AND IN COMBINATION WITH GLIBENCLAMIDE ON CATALASE LEVELS.** All the values are expressed as mean ±SD. *P<0.05 compared with normal control, **P<0.05 compared with diabetic control.

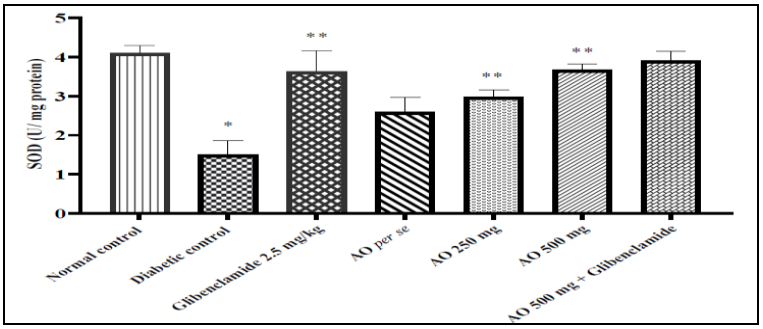


**FIG. 2: EFFECT OF METHANOLIC AOB EXTRACT ALONE AND IN COMBINATION WITH GLIBENCLAMIDE ON SOD LEVELS.** All the values are expressed as mean ±SD. *P<0.05 compared with normal control, **P<0.05 compared with diabetic control.

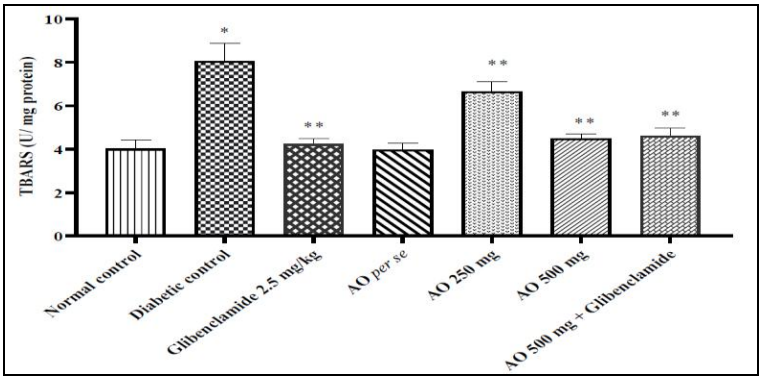


**FIG. 3: EFFECT OF METHANOLIC AOB EXTRACT ALONE AND IN COMBINATION WITH GLIBENCLAMIDE ON TBARS LEVELS.** All the values are expressed as mean ±SD. *P<0.05 compared with normal control, **P<0.05 compared with diabetic control.

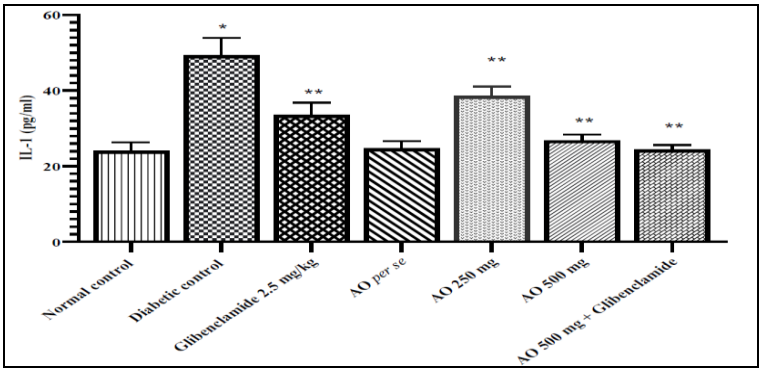


**FIG. 4: EFFECT OF METHANOLIC AOB EXTRACT ALONE AND IN COMBINATION WITH GLIBENCLAMIDE ON IL-1 LEVELS.** All the values are expressed as mean ±SD. *P<0.05 compared with normal control, **P<0.05 compared with diabetic control.

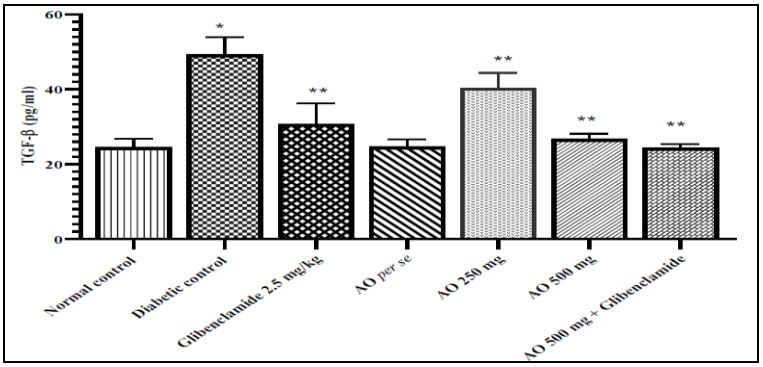


**FIG. 5: EFFECT OF METHANOLIC AOB EXTRACT ALONE AND IN COMBINATION WITH GLIBENCLAMIDE ON TGF-B LEVELS.** All the values are expressed as mean ±SD. *P<0.05 compared with normal control, **P<0.05 compared with diabetic control.

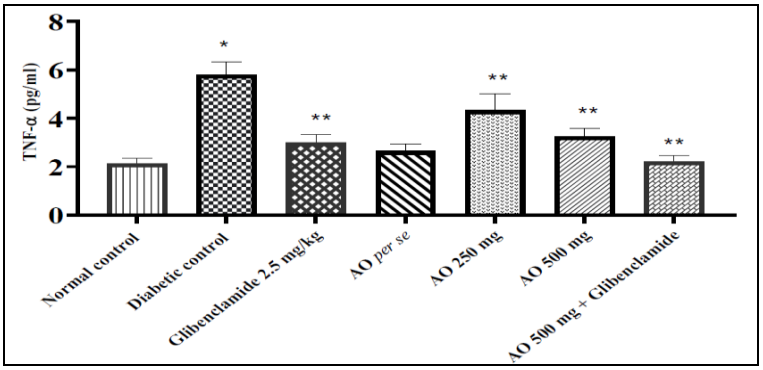


**FIG. 6: EFFECT OF METHANOLIC AOB EXTRACT ALONE AND IN COMBINATION WITH GLIBENCLAMIDE ON TNF- A LEVELS.** All the values are expressed as mean ±SD. *P<0.05 compared with normal control, **P<0.05 compared with diabetic control.

**Effect of Methanolic Extract of AOB on Renal Oxidative Stress Parameters:** Renal oxidative stress parameters like catalase and SOD were significantly decreased, and TBARS levels were augmented in the diabetic group when compared to the normal group. Administration of methanolic extract of AOB at different doses of 250 mg/Kg and 500 mg/kg alone or in combination with glibenclamide showed a gradual and significant decline in oxidative stress in renal tissue, as demonstrated by increased catalase and SOD levels and decreased TBARS levels when compared to diabetic control (**Fig. 1-3**). The results indicate the reduction of oxidative stress parameters by treatment with methanolic AOB extract.

**Effect of Methanolic Extract of AOB on Inflammatory Cytokines Parameters:** The inflammatory cytokines like IL-1, TGF-β, and TNF-α were significantly increased in diabetic rats compared to the normal group. Treatment with methanolic extract of AOB at different doses of 250 mg/Kg and 500 mg/kg alone or in combination with glibenclamide significantly reduced inflammatory cytokines like IL-1, TGF-β, and TNF-α when compared to diabetic control rats (**Fig. 4-6**). These results indicate that methanolic extract of AOB significantly ameliorates renal inflammatory cytokines in diabetic rats gradually.

**DISCUSSION:** DN represents a serious complication of DM, which is widely accepted to be the widespread cause of end-stage renal failure [7, 8]. This has been reported that nearly one-fourth percent of type 1 and one-third percent of patients with type-II diabetes experience DN [2]. Although numerous therapeutic agents are available in treating diabetes and its complications, widespread interest has been developed during the last few decades about the use of herbal drugs in treating patients presented with DN [22, 23]. Hence the present study investigated the potential of methanolic AOB extract in experimental DN. Nephropathy has been well demonstrated to result from damage due to high blood and urine glucose in patients presented with diabetes [7]. The high blood and urine glucose level have been found to affect the filtration of blood from kidney arteries, thereby resulting in the development and progression of DN [8, 24]. In the present study, STZ was used for the induction of experimental diabetes in rats. Administration of methanolic extract of AOB lowered the STZ-induced increase in blood and urine glucose levels, which confirms the antihyperglycemic activity of the extract as reported by earlier reported studies. Prolonged hyperglycemia has been reported to be an important factor for the progression and development of DN. Hyperglycemia has been well reported to attenuate the antioxidative mechanism and lead to high oxidative stress [25]. Furthermore, DN-induced enhanced oxidative stress leads to dysfunction in the defense system against free radicals. This results in the generation of more ROS/TBARS and inactivation of SOD and CAT, which ultimately leads to the deleterious effects on the kidney as a result of superoxide anion and hydrogen peroxide accumulation and damage [25, 26].

In the present study, enhanced oxidative stress in diabetic kidneys was observed by the elevation of renal concentration of TBARS and decreased levels of SOD and CAT in diabetic animals. The administration of methanolic extract of AOB showed significant improvement in renal function by decreasing TBARS levels and increasing the SOD and CAT levels. This suggests the inhibitory effect of AOB extract on oxidative stress parameters and the protective effect against DN-induced kidney damage.

This has been demonstrated that chronic hyperglycemia-mediated DN induces oxidative damage and inflammation. In addition, the generation of ROS results in the elevation of IL-1, TGF-β, TNF- α, and other inflammatory mediators [27]. Hence, this can be concluded that hyperglycemia contributes greatly to kidney injury in diabetes. In addition, a strong association has been found between oxidative stress and inflammation metabolically [28]. This has been well reported that oxidative stress-induced pro-inflammatory cytokine generation mediates the production of ROS. This further enhances oxidative stress and ultimately leads to DN [25, 28]. The diabetic rats showed increased pro-inflammatory cytokines like IL-1, TGF-β, and TNF-α in both serum and renal tissue. Treatment of diabetic rats with methanolic AOB extract significantly suppressed the inflammation, as confirmed by the decreased expression of renal pro-inflammatory cytokines in the renal tissue. The above findings suggest the potent antioxidant and anti-inflammatory effects of

methanolic AOB extract in experimental DN. However, few studies have investigated the antidiabetic and renoprotective effects of AOB extract. The present study presents the evidence that methanolic extract of AOB ameliorated hyperglycemia, oxidative stress, inflammation, and renal injury in STZ-induced diabetic rats, which may pave the way to further studies.

**CONCLUSION:** In the present study, the enhanced blood and urine glucose levels were significantly decreased by administering methanolic extract of *Albizia odoratissima* bark in STZ-induced DN. Moreover, treatment with methanolic AOB extract significantly showed renal protection in DN-induced enhanced oxidative stress. In addition, the inflammatory cytokines were significantly reduced by the administration of methanolic extract of AOB that showed potential nephroprotection against STZ-induced DN. Further studies are needed in this regard to assess the potential of the plant extract in experimental DN.

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**CONFLICTS OF INTEREST:** The authors have no conflicts of interest regarding this investigation.

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