



RESEARCH ARTICLE

Effects of New *Bouldia laevis* Leaf Extract and Homeopathic Remedies on Kidney Markers of Streptozotocin-Induced Diabetic Rats

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ABSTRACT

Diabetic nephropathy is a serious complication of diabetes mellitus and remains one of the leading causes of kidney dysfunction, often resulting from persistent hyperglycemia, oxidative stress, and metabolic imbalance. This research was designed to determine the effects of *Newbouldia laevis* leaf derived extract and homeopathic formulations on kidney function markers in streptozotocin-induced diabetic rats. The extract was prepared in ethanol to produce a mother tincture, which was subsequently diluted through a 10-fold serial dilution and succussion to obtain different homeopathic potencies (1X, 2X, 3X, 6X, and 30C). Experimental Diabetes was induced through a single intraperitoneal injection of Streptozotocin (STZ) (50 mg/kg), after which the rats were divided into eleven groups, including three crude extract doses (200, 400, and 600 mg/kg), five homeopathic potencies, normal and diabetic controls, and a standard drug (glibenclamide) group. Treatments were administered orally for 21 days, and kidney markers were assessed using standard biochemical methods. Results showed that diabetes induction significantly elevated ($p < 0.05$) urea levels, indicating impaired renal function, while *N. laevis* leaf extract and most homeopathic formulations helped stabilize urea in comparison with the untreated diabetic controls. The extract produced a dose-independent effect, with no meaningful variations among the three doses, although the 1X and 30C formulations were less effective. Similarly, creatinine levels were considerably elevated in diabetic groups, but intervention with *N. laevis* extract and lower potencies (1X–3X) reduced creatinine in a dose-responsive manner, with the 3X formulation and higher extract doses showing effects comparable to glibenclamide. These findings suggest that the renoprotective potential of *N. laevis* is strongest at lower dilutions and higher extract doses, while higher dilutions appear less effective.

Key words: Diabetic nephropathy, *Newbouldia laevis*, Homeopathy, Renoprotective effects, Streptozotocin-induced diabetes.

INTRODUCTION

Diabetes mellitus is a group of metabolic disorders defined by elevated blood glucose levels resulting from deficiencies in either the effectiveness or production of insulin, or both (American Diabetes Association, 2009). Hyperglycemia, a key feature of diabetes mellitus, is linked to disruptions in carbohydrate, fat, and protein metabolism,

often leading to complications like cardiovascular disease and neuropathy if not properly managed (American Diabetes Association, 2020). Additionally, sustained hyperglycemia can lead to the glycation of body proteins and damage to the kidneys, arteries, eyes, and nerves (Owolabi et al., 2011). In many developed countries, including the US, diabetic nephropathy is the primary cause of end stage renal disease (Rabkin, 2003).

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Controlling blood sugar levels is essential for preventing these complications, and traditional medicine has gained recognition for its possible role in diabetes management. Among these remedies, *Newbouldia laevis*, a plant native to West Africa, has been used for various therapeutic purposes (Etuk *et al.*, 2010) and in ethnomedicine to treat diabetes (Mbagwu *et al.*, 2020).

N. laevis is a native flowering plant of tropical West Africa (Eluu *et al.*, 2023). It belongs to the Bignoniaceae family and is commonly known for its leaves, which have been traditionally used in treating numerous ailments and have been evaluated for their analgesic, anti-inflammatory, and anticonvulsant properties (Ukwubile *et al.*, 2023). This may be due to the phytochemical bioactive compounds present in *N. laevis* leaves, such as alkaloids, flavonoids, tannins, and phenolic acids (Ayoola *et al.*, 2016; Obum-Nnadi *et al.*, 2020). These compounds may play a protective role against diabetes-induced oxidative stress and organ damage, including nephropathy.

Around the world, homeopathy is a popular complementary therapy, and the growing number of clinical research papers and meta-analyses reflects increasing scientific interest in its therapeutic effectiveness (Tournier *et al.*, 2019). Homeopathy, a complementary and alternative system of medicine, employs highly diluted remedies prepared through serial dilution and succussion (Bell and Koithan, 2012). Homeopathic preparations may exert biological effects, possibly through modulation of oxidative stress, inflammation, and immune response.

This research, therefore, designed to investigate the impacts of *Newbouldia laevis* leaf extract and its homeopathic potencies on kidney markers in STZ-induced diabetic rats. The findings are expected to provide scientific evidence on the potential use of *N. laevis* in managing diabetic nephropathy and to contribute to the broader understanding of integrating herbal and homeopathic therapies in diabetes care.

MATERIALS AND METHODS

Animals

The study involved fifty-five male Wistar rats (80–120 g) obtained from the Department of Zoology and Environmental Biology, University of Nigeria, Nsukka. All procedures complied with IACUC guidelines for laboratory animal care (ICMR, 2001).

Plant material

N. laevis leaves were collected from Agulu, Anambra State, and the authentication carried out by Dr. C.F. Iroka, a taxonomist from the Botany Department, Nnamdi Azikiwe University, Awka, Nigeria.

Methods

Plant extraction

The extraction process involved washing *N. laevis* leaves, shade-drying them for seven days, and grinding them

into a coarse powder. This powder was cold-macerated in ethanol (1:1) for 48 h. The filtrate obtained was then concentrated at 40 °C using a rotary evaporator and subsequently stored under refrigeration until needed.

Preparation of potentized homeopathic remedies

The potentized homeopathic remedies were prepared as described by Rawat (2016).

Preparation of the homeopathic mother tincture

The *N. laevis* extract homeopathic mother tincture was prepared by dissolving one part by weight of the *N. laevis* extract (4mg) in 9 parts by weight (36ml) of Absolute Ethanol (99.5% w/w) (Rawat, 2016).

Preparation of dilute ethanol

87% ethanol was prepared by mixing one part of distilled water to seven parts of absolute ethanol.

Dilute ethanol (61%) was prepared by incorporating three parts of distilled water to 7 parts of the 87% ethanol (Rawat, 2016).

Preparation of the homeopathic X potencies

The 1X potency was prepared by mixing one part of the homeopathic mother tincture with nine parts of dilute ethanol and succussing the mixture 100 times. Subsequent potencies (2X to 6X) were made by serially diluting one part of the previous potency with nine parts of dilute ethanol and subsequent succussion. This process of serial dilution and succussion continued progressively up to the 6X potency.

Preparation of the homeopathic C potencies

The 1C potency was prepared by diluting one part of the homeopathic mother tincture with 99 parts of dilute ethanol, followed by 100 succussions. Each subsequent potency (2C to 30C) was prepared by serially diluting one part of the previous potency with 99 parts of ethanol and succussing the mixture. This process was repeated up to the 30C potency, with dilutions beyond 12C likely containing little to no molecules of the original substance due to exceeding Avogadro's limit.

Induction of diabetes

The rats were induced with diabetes using a single dose of streptozotocin (50 mg/kg body weight) diluted in cold citrate buffer (pH 4.5), intraperitoneally. The diabetic rats were randomly allocated into 10 groups, each consisting of five rats. An additional control group of five non-diabetic, untreated rats was also included. After forty-eight hours, blood samples were collected, and blood glucose levels were measured to confirm the development of diabetes (Tella *et al.*, 2022).

Experimental design

A total of fifty-five male albino rats were divided into eleven groups, each containing five rats. Group 1 was served as the normal control, whereas Group 2 served as the diabetic control. Group 3 administered

glibenclamide as a standard treatment. Groups 4 to 6 were treated with 200, 400, and 600 mg/kg of the ethanolic crude extract, respectively. Groups 7 to 11 received homeopathic dilutions of the extract at potencies 1X (4.5×10^{-3} mg/dose), 2X (4.5×10^{-4} mg/dose), 3X (4.5×10^{-5} mg/dose), 6X (4.5×10^{-8} mg/dose), and 30C (4.5×10^{-62} mg/dose), administered as three oral drops per dose every eight hours, and the study lasted 21 days.

Kidney function assessment

The concentrations of serum urea and creatinine were analyzed following the method of Tietz (1994) as outlined in the Randox kit, UK.

Statistical analysis

Values were expressed as mean \pm standard deviation. One-way analysis of variance (ANOVA) was carried out using the Statistical Package for the Social Sciences (SPSS) to compare the means across the different groups.

RESULTS AND DISCUSSION

Effect of *N. laevis* leaf extract and formulations on creatinine levels in diabetic rats over time

The impact of *N. laevis* leaf extract on creatinine levels among diabetic rats over time is presented in Fig. 2. Creatinine is a vital biomarker for assessing renal function, and elevated levels are commonly associated with an impaired glomerular filtration rate (GFR), especially in diabetic nephropathy. At baseline, there were no statistically significant differences ($p > 0.05$) in creatinine levels between the groups, confirming a consistent renal status before treatment (Fig. 1). However, following diabetes induction, all diabetic induced groups exhibited significantly elevated ($p < 0.05$) creatinine levels relative to the uninduced non-treated group. This corroborates with previous reports that hyperglycemia contributes to progressive kidney damage through mechanisms involving oxidative stress, advanced glycation end-products, and mesangial expansion (Forbes & Cooper, 2013; Wu et al., 2023; Na & Chun, 2024).

By day 11 post-treatment, the uninduced untreated group continued to exhibit notably lower ($p < 0.05$) creatinine levels than all other groups. Among the treated groups, there were no significant differences ($p > 0.05$) were observed in creatinine levels were observed between those exposed to 200 mg/kg of *N. laevis*, 600 mg/kg of *N. laevis*, 1X, and 2X. However, the other treatment groups showed significantly lower creatinine concentrations of *N. laevis*. There were no statistically significant differences ($p > 0.05$) among the standard drug group and the other treatment groups, except for the 200 mg/kg of *N. laevis* and 2X groups, which showed significantly different ($p < 0.05$) creatinine levels. Additionally, no notable significant differences were found among the groups subjected to 200 mg/kg of *N. laevis*, 400 mg/kg of *N. laevis*, 600 mg/kg of *N. laevis*, 1X, and 2X.

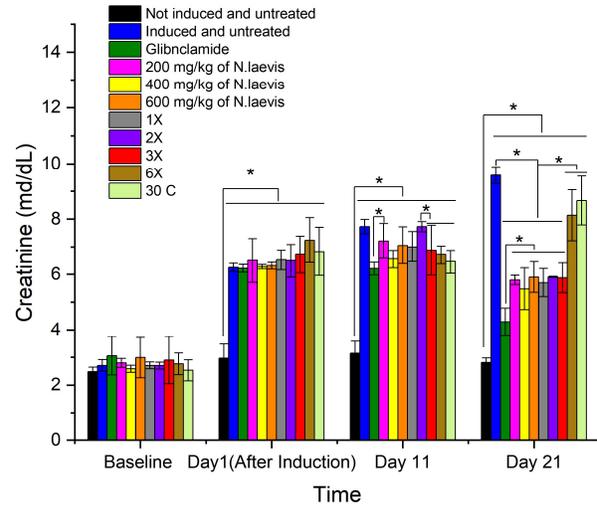


Fig. 1: Effect of *N. laevis* extract on creatinine concentration in induced and treated groups over time. Asterisk (*) shows that the mean difference between groups is significant at $p < 0.05$.

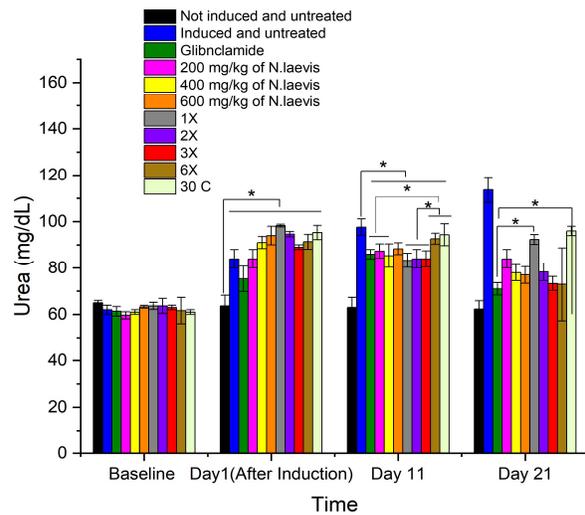


Fig. 2: Effect of *N. laevis* extract on urea level in induced and treated groups over time. Asterisk (*) indicates that the mean difference between groups is significant at $P < 0.05$.

The 3X group exhibited significantly lower ($p < 0.05$) creatinine concentrations contrasted with the 2X group, but the 200 mg/kg of *N. laevis*, 400 mg/kg of *N. laevis*, 600 mg/kg of *N. laevis*, and 1X groups had no significant differences ($p > 0.05$) relative to the group treated with 3X. The 6X and 30C groups showed no significant differences ($p > 0.05$) from most treatment groups but had considerably higher ($p < 0.05$) creatinine levels compared to the 2X group. The result demonstrated that *N. laevis* possesses a dose-responsive renoprotective capacity in diabetic rats, capable of ameliorating creatinine elevation. Particularly, the 3X dilution and higher extract doses emerge as viable alternatives to standard antidiabetic therapy in preserving kidney function under diabetic stress. The histopathological evidence further supports this biochemical improvement, with kidneys from the 3X

and high-dose extract groups demonstrating less vacuolar degeneration and tubular necrosis compared to untreated and poorly performing groups (6X and 30C), thereby validating the renoprotective actions at the tissue level.

At the conclusion of the treatment period, the induced untreated group continued to exhibit the highest creatinine concentrations, indicating unchecked progression of diabetic nephropathy. The 30C group also failed to show significant renal protection, as the induced untreated group had notably higher ($p > 0.05$) creatinine levels relative to all other groups, apart from the 30C group, which demonstrated no significant difference ($p > 0.05$). Furthermore, the groups subjected to 200 mg/kg, 400 mg/kg, and 600 mg/kg of *N. laevis*, along with the 1X, 2X, and 3X groups, did not significantly vary ($p > 0.05$) from one another. However, the 6X and 30C groups exhibited substantially higher ($p < 0.05$) creatinine levels than all other groups, although there were no appreciable difference ($p > 0.05$) among these two. This indicates that homeopathic preparations below 3X may retain some bioactivity, while higher dilutions (6X and 30C) appear ineffective, casting doubt on the physiological significance of highly diluted remedies (Shang *et al.*, 2005).

Effect of *N. laevis* leaf extract and formulations on urea levels in diabetic rats over time

The impact of *N. laevis* leaf-based extract and homeopathic formulations on urea levels in diabetic rats over time is shown in Fig. 2. Urea is a critical marker of renal function, protein metabolism, and nitrogen excretion. In diabetes mellitus, hyperglycemia and oxidative stress can impair kidney function, leading to altered urea levels (Dabla, 2010; Gyurászová *et al.*, 2020). At baseline, there were no notable differences ($p > 0.05$) in urea concentrations among the experimental groups, confirming similar renal function before diabetes induction. However, following the induction of the condition, the diabetic groups exhibited significantly higher ($p < 0.05$) urea levels as opposed to the untreated non-diabetic groups. Diabetes is often associated with increased urea levels due to protein catabolism and renal dysfunction (Wang *et al.*, 2025). By day 11, the uninduced untreated group had considerably lower ($p < 0.05$) urea concentrations than the other groups (Fig. 2). There were no notable differences ($p > 0.05$) in urea concentrations between the induced untreated group and those treated with 6X and 30C, suggesting that these formulations may not have had a strong effect on renal function. The lack of significant differences ($p < 0.05$) among the 200 mg/kg, 400 mg/kg, and 600 mg/kg extract-treated groups, as well as the 1X, 2X, and 3X formulations, indicates a potential dose-independent stabilizing effect of *N. laevis* on urea levels. This aligns with reports on the nephroprotective properties of *N. laevis*, which can be attributed to its bioactive compounds, such as flavonoids and alkaloids (Ayoola *et al.*, 2016; Innih *et al.*, 2023). Additionally, the groups

exposed to 600 mg/kg of *N. laevis*, 6X, and 30C showed no meaningful differences ($p > 0.05$) in urea concentrations. After treatment, the urea concentration in the uninduced untreated group did not deviate significantly ($p > 0.05$) from that of the treatment groups, except for the 1X and 30C groups, which exhibited significantly higher ($p < 0.05$) urea concentrations (Fig. 2). This suggests that while most *N. laevis* formulations contributed to maintaining normal renal function, the 1X and 30C formulations may have had less pronounced effects. Furthermore, the induced untreated group exhibited notably higher ($p < 0.05$) urea concentrations than all treatment groups, except for the 1X and 30C groups, which did not differ significantly ($p > 0.05$). Overall, there were no statistically significant differences ($p > 0.05$) in urea concentrations among the various treatment groups when compared to each other. The findings indicate that *N. laevis* extract helps regulate urea levels in diabetic rats, supporting its potential role in mitigating diabetes-related renal dysfunction.

Conclusion

The study demonstrates that *N. laevis* leaf extract and low-potency homeopathic formulations (1X–3X) provide significant renoprotective influence in streptozotocin-induced diabetic rats by stabilizing kidney function markers and reducing renal damage.

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