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Piperine, an Alkaloid Component of Nigerian Propolis, Improves the Glomerular Filtration Rate (GFR) and other Markers of Renal Health in Nephropathic Diabetic Rats

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Abstract:

Objective: This study investigated the effects of piperine isolated from Nigerian propolis on renal function in nephropathic diabetic rats and evaluated its efficacy when combined with the antidiabetic drug metformin.

Materials and Methods: Male Wistar rats were induced with diabetes using streptozotocin and subsequently developed nephropathy. The rats were divided into 5 groups: a healthy control group, a diabetic (untreated) control group, a piperine-treated group, a metformin-treated group, and a group receiving a combination of piperine and metformin. Each treatment group received its respective interventions for 6 weeks. Renal function was assessed by measuring glomerular filtration rate (GFR) using inulin clearance tests. Biochemical markers of kidney injury and inflammation were also analyzed.

Results: The results indicate that the combination of piperine and metformin was more effective at improving renal function in nephropathic diabetic rats compared to either treatment alone. The rats receiving the combined therapy exhibited significantly higher GFR values and reduced markers of kidney injury and inflammation. In contrast, the individual treatments with piperine or metformin alone produced only moderate improvements. The untreated diabetic control group had substantially impaired renal function compared to all the treatment groups, while the healthy control group maintained normal GFR levels.

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Conclusion: The synergistic effects of piperine from Nigerian propolis and metformin significantly enhanced GFR in nephropathic diabetic rats compared to either treatment alone. The combination therapy appears to offer a more effective approach for improving renal function in diabetic nephropathy.

Keywords: diabetic nephropathy, glomerular filtration rate, metformin, Nigerian propolis, Piperine

Introduction

Diabetes is a prevalent chronic metabolic disorder[1,2] that can lead to a range of complications, including nephropathy[3,4]. Diabetic nephropathy (DN) is a major cause of end-stage renal disease[5,6] and is characterized by a progressive decline in glomerular filtration rate[7,8]. Identifying effective treatments to preserve and enhance kidney function in diabetic patients is a critical public health priority.

Propolis, a natural resin produced by honeybees[9,10], possesses various pharmacological properties[11,12], including anti-inflammatory[13], antioxidant[14,15], antimicrobial[16], and antidiabetic[17,18] effects. Propolis constituents are highly varied based on the geographic origin and plant sources[19,20], hence the need for standardization[21,22] and isolation of specific bioactive compounds[23,24].

Piperine, an alkaloid component found in *Piper nigrum* (black pepper), has been attracting attention for its potential therapeutic applications[25,26]. Studies have suggested that piperine may have beneficial effects on various disease states[27-29], including diabetes and its complications[30,31]. However, its impact on kidney function in the context of diabetic nephropathy remains understudied. This study aimed to examine the impact of piperine, alone and in combination with the antidiabetic drug metformin, on the glomerular filtration rate in a rat model of diabetic kidney disease. By shedding light on the potential renoprotective properties of piperine, this research may help in the development of new therapeutic approaches in order to maintain kidney health in individuals with DN.

Material and Methods

Induction of nephropathic diabetes in rats

Male Wistar rats were rendered diabetic by administering streptozotocin intraperitoneally at a dose of 60 mg/kg body weight. Diabetes was confirmed by measuring the blood glucose levels, and only animals with blood glucose levels exceeding 300 mg/dL were included in the diabetic model. The development of nephropathy, characterized by progressive renal dysfunction and reduced glomerular filtration rate, was subsequently confirmed by evaluating various markers of kidney function before the initiation of treatment.

Experimental design and treatments

The study utilized a randomized, controlled experimental design to investigate the effects of piperine, both alone and in combination with metformin, on the glomerular filtration rate in a rat model of nephropathic diabetes. The nephropathic diabetic rats were randomly divided into 5 groups: a healthy control group, a diabetic control (untreated) group, a piperine-treated group, a metformin-treated group, and a group receiving a combination of piperine and metformin. The piperine-treated group received 50 mg/kg body weight of piperine per day, the metformin-treated group received 150 mg/kg body weight of metformin per day, and the combination group received both piperine and metformin at the same doses. All treatments were administered orally for 6 weeks. This experimental design allowed for comparison between the effects of the individual treatments and the combination

therapy on the glomerular filtration rate, and other markers of kidney function in the nephropathic diabetic rats.

Determination of glomerular filtration rate

The glomerular filtration rate was determined using the inulin clearance method, which is considered the gold standard for assessing kidney function. Rats were placed in metabolic cages and allowed to acclimatise for a 24-hour period. During this acclimation period, the animals had unrestricted access to water while fasting to ensure accurate collection of urine samples. After the acclimation period, an intravenous infusion of inulin was initiated. Blood and urine samples were collected at regular intervals, and the concentration of inulin in these samples was measured using established analytical techniques. The glomerular filtration rate (GFR) was then calculated using the standard formula: GFR=(U inulin × V)/ P inulin, where U inulin is the urine inulin concentration, V is the urine flow rate, and P inulin is the plasma inulin concentration.

The study was approved by Ahmadu Bello University Ethical Committee on the use of animals for research.

Isolation and characterization of piperine

Propolis samples were collected from the Ibadan region of Southern Nigeria. The piperine compound was isolated from the propolis using a multistep extraction and purification process. The chemical structure and purity of the isolated piperine were confirmed using a combination of mass spectrometry (MS) and high-performance liquid chromatography (HPLC) (Figure 1 and Table 1). Further analyses were conducted to evaluate the bioavailability and pharmacokinetic properties of the purified piperine compound, ensuring its suitability for the in vivo experiments.

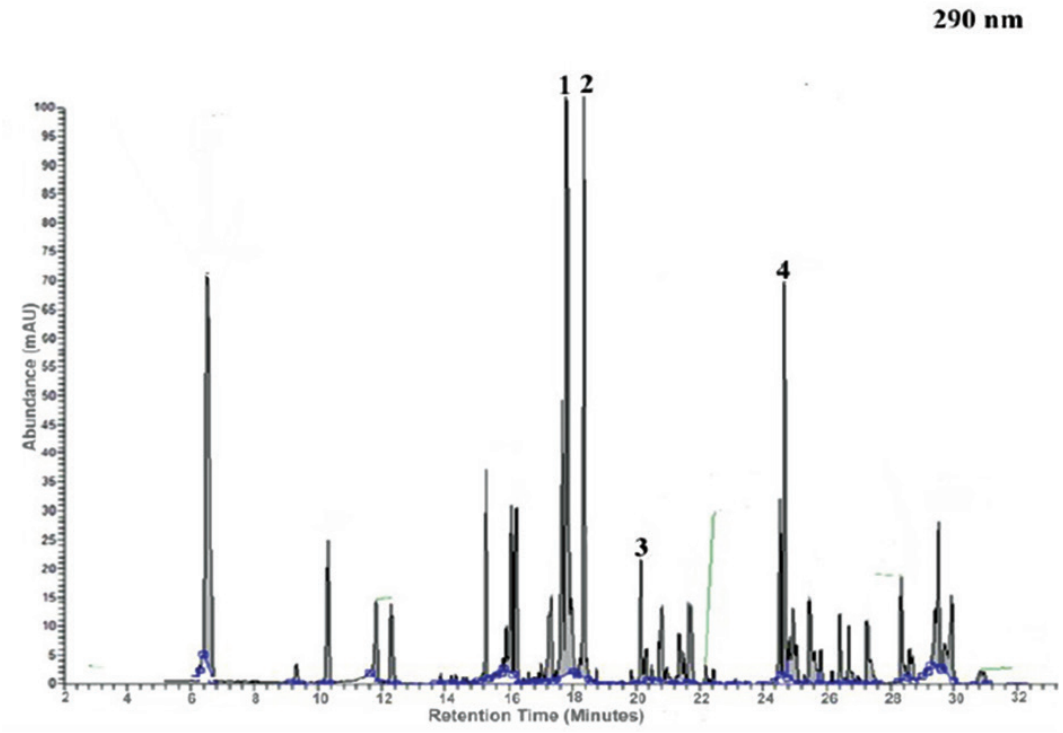


Figure 1 High-performance liquid chromatography chromatogram of the isolation of piperine from Nigerian propolis. The peak labelled "3" is piperine.

Table 1 Properties of some of the characterized constituents of Nigerian propolis

	Height (mAU)	Area	Retention time (minutes)	Class	Molecular formula	Peak name
1	99.96	109036	17.8	Flavonoid	$C_{15}H_{12}O_4$	Pinocembrin
2	99.94	86500	18.5	Flavonoid	$C_{15}H_{10}O_4$	Chrysin
3	23.08	30007	20.3	Alkaloid	$C_{17}H_{19}NO_3$	Piperine
4	73.23	39012	24.6	Saponin	$C_{42}H_{62}O_{16}$	Glycyrrhizin

Biochemical analyses

Biochemical analyses were conducted to evaluate the effects of the treatment interventions on various markers of kidney function. Serum and urine samples were collected at regular intervals throughout the study period and analyzed for key parameters, including blood urea nitrogen (BUN) (using the BUN Assay Kit from Sigma-Aldrich), serum creatinine (using the Creatinine Assay Kit from Abcam), and albumin-to-creatinine ratio (ACR) (using the Albumin-to-Creatinine Ratio Assay Kit from Cayman Chemical). These biomarkers provided insights into the overall renal health of the animals and the potential protective effects of piperine, alone and in combination with metformin, against the progression of DN.

Statistical analysis

Statistical analyses were performed using one-way analysis of variance, followed by Tukey's post-hoc test, to compare the effects of the different treatment groups. The threshold for statistical significance was set at $p\text{-value} < 0.05$, and all data are presented as mean±standard error of mean (SEM).

Results

Glomerular filtration rate

The results of the study showed that treatment with piperine, either alone or in combination with metformin, significantly increased the glomerular filtration rate in the nephropathic diabetic rats compared to treatment with metformin alone. The combination of piperine and metformin appeared to be particularly effective in improving glomerular filtration when compared to the diabetic control group (Figure 2).

Blood urea nitrogen and creatinine

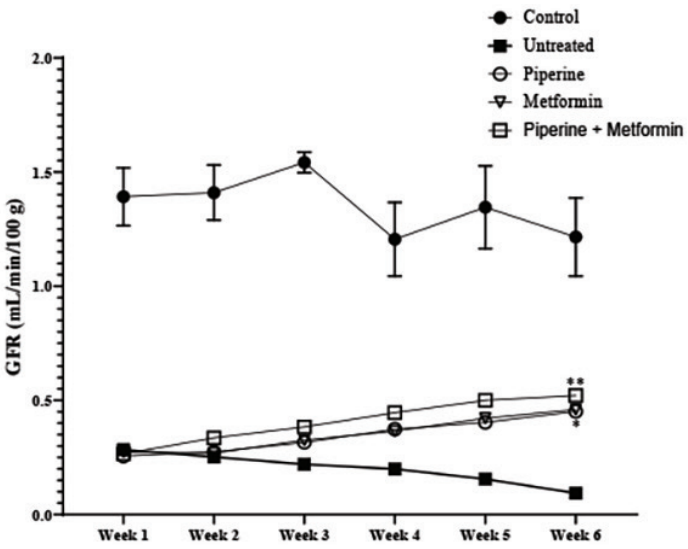
Biochemical analyses revealed that piperine treatment, both alone and in combination with metformin, significantly reduced blood urea nitrogen (BUN) and serum creatinine levels in the nephropathic diabetic rats compared to the diabetic control group (Figure 3 and Figure 4). The combination therapy was more effective in improving these markers of kidney function than either piperine or metformin alone.

Albumin-to-creatinine ratio

The ACR, a marker of kidney damage, was substantially lower in the piperine-treated groups compared to the diabetic control group. The combination of piperine and metformin was the most effective in reducing the ACR (Figure 5).

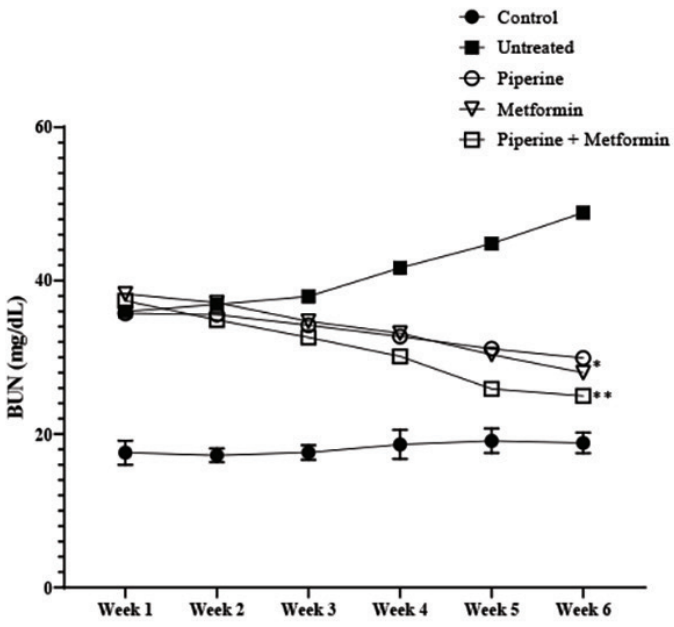
Discussion

The results of this study provide important insights into the potential therapeutic applications of piperine, an alkaloid component of Nigerian propolis, for the management of diabetic nephropathy. The finding that piperine, either alone or in combination with metformin, significantly improved the glomerular filtration rate and biomarkers of renal health in nephropathic diabetic rats suggests that this natural compound has beneficial effects on kidney function in the context of this debilitating diabetes complication.



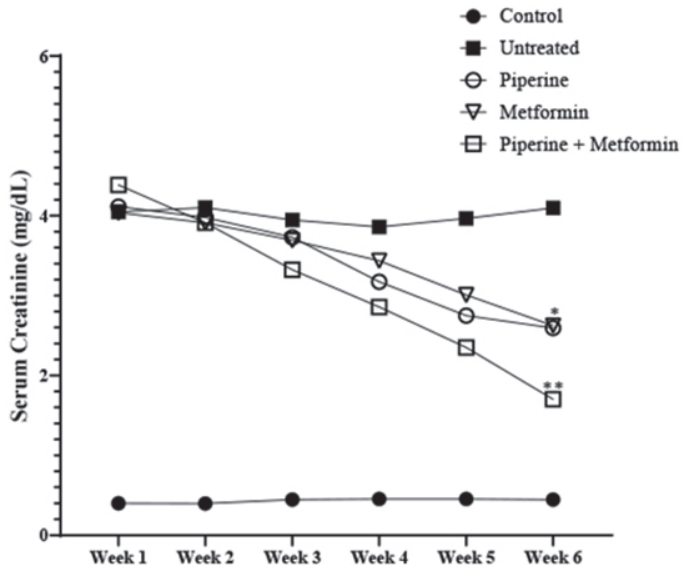
A p-value<0.05 was accepted as statistically significant. (*) signifies p-value<0.01 compared with the untreated while (**) signifies p-value<0.001 compared with the untreated.

Figure 2 Glomerular filtration rate improved in the treatment groups. Results are presented as mean±SEM.



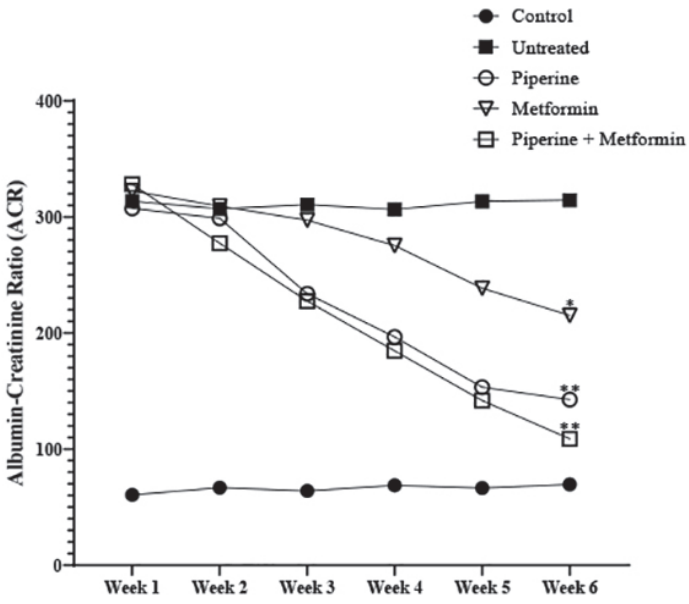
Data are presented as mean±SEM with p-value<0.05 accepted as statistically significant. (*) p-value<0.01 compared with the untreated group. (**) p-value<0.001 compared with the untreated group.

Figure 3 Piperine treatment reduced blood urea nitrogen over the course of the experiment



Data are presented as mean±SEM with p-value<0.05 accepted as statistically significant. (*) p-value<0.05 compared with the untreated group. (**) p-value<0.01 compared with the untreated group.

Figure 4 Piperine treatment reduced serum creatinine over the course of the experiment



A p-value<0.05 was accepted as statistically significant. (*) p-value<0.01 compared with the untreated group. (**) p-value<0.001 compared with the untreated group.

Figure 5 Piperine treatment (alone, or with metformin) significantly improved the albumin-creatinine ratio over the course of experiment. Data are presented as mean±SEM.

The observed improvements in glomerular filtration rate with piperine treatment are particularly noteworthy, as preserving and improving kidney function is a critical goal in the management of DN. The progressive decline in glomerular filtration rate is a hallmark of this condition[32,33], eventually leading to end-stage renal disease if left unchecked[34]. Therefore, the ability of piperine to raise the glomerular filtration rate in this animal model indicates that it may have valuable renoprotective properties that could be leveraged to benefit diabetic patients at risk of or already experiencing nephropathy.

Moreover, the synergistic effects observed with the combination of piperine and metformin are particularly promising. The enhanced improvements in glomerular filtration rate suggest that piperine may be able to potentiate the benefits of standard antidiabetic therapies, potentially offering a novel adjunctive treatment approach for diabetic nephropathy. This could be an important finding, as many diabetic patients may require a multi-pronged treatment strategy to effectively manage their kidney complications[35]. Furthermore, BUN and creatinine levels are known to be elevated in diabetic nephropathy[36], and the observed reductions in these biomarkers with piperine treatment indicate that this compound may have a protective effect on overall kidney health. Also, compromise of the glomerular filtration membrane in DN leads to proteinuria[37]. The appearance of albumin in the urine[7,38] causes an increase in the ACR[39]. The lower ACR found in the piperine-treated groups further supports the idea that piperine may help preserve the glomerular filtration barrier, preventing the excessive leakage of albumin into the urine that is characteristic of this disease.

While the current study was limited to an animal model, the results lay the groundwork for further investigations into the mechanisms by which piperine exerts its renoprotective effects and its potential translation to human clinical applications. Elucidating the underlying pathways and evaluating the efficacy and safety of piperine in diabetic patients with nephropathy are the next crucial steps necessary to fully harness the therapeutic potential of this natural compound. Additionally, exploring the optimal dosing and timing of piperine administration, both alone and in combination with standard treatments, will be important for optimizing its clinical utility.

Conclusion

This study has demonstrated that treatment with piperine, an alkaloid component of Nigerian propolis, can significantly raise the glomerular filtration rate in nephropathic diabetic rats undergoing treatment with metformin. The synergistic effects observed with the combination of piperine and metformin suggest that piperine may be a promising adjunctive therapy for the management of diabetic nephropathy.

Conflict of interest

No conflict of interest declared.

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