Original Article

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Piperine, Isolated from Nigerian Propolis, Modulates GLUT-4 Gene Expression and NF-ΚB in a Rat Model of Chronic Fructose and Glucose Intake

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Abstract:

Objective: This study investigated the effects of piperine from Nigerian propolis on glucose metabolism and inflammatory pathways in a rat model of chronic fructose and glucose intake.

Material and Methods: Twenty male Wistar rats were randomly divided into 4 groups: control group, fructose-glucose (unsupplemented) group, fructose-glucose plus piperine group, and fructose-glucose plus metformin group. The fructose-glucose (unsupplemented) group received continuous oral infusion of a 50% fructose and 50% glucose solution (at a concentration of 10% (w/v) each) without any supplements, while the fructose-glucose plus piperine and fructose-glucose plus metformin groups received daily oral administration of piperine and metformin as supplements, respectively, in addition to fructose-glucose infusion. Insulin sensitivity was evaluated by computing the homeostatic model assessment of insulin resistance index (HOMA-IR). Real-time Polymerase Chain Reaction (PCR) was performed to quantify the mRNA expression of the Glucose Transporter-4 (GLUT-4) gene. The protein expression levels of pro-inflammatory transcription factor Nuclear Factor Kappa B (NF-ΚB) and p-Akt (phosphorylated protein kinase B) were analysed using western blotting techniques.

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Results: After 8 weeks, biochemical analyses showed that the piperine from Nigerian propolis increased GLUT-4 expression and decreased NF-NF-ΚB expression compared to the fructose-glucose (unsupplemented) group, where insulin insensitivity persisted. Piperine also significantly lowered blood glucose levels and significantly improved insulin sensitivity by lowering the HOMA-IR in the supplemented animals compared to the unsupplemented infusion group.
Conclusion: The results of the study suggest that piperine, an alkaloid from Nigerian propolis, may have the therapeutic potential to mitigate the detrimental effects of chronic fructose and glucose intake on glucose metabolism and inflammatory pathways.

Keywords: fructose, glucose, GLUT-4, NF-NF-ΚB, Nigerian propolis, piperine

Introduction

Dietary intake of fructose and glucose, which are usually present in high concentrations in highly processed "fast foods", has been associated with the development of metabolic disorders, including insulin resistance and type 2 diabetes[1-3]. Elevated levels of these sugars in the blood can contribute to increased oxidative stress, inflammation, and impaired glucose homeostasis[4,5]. Recent studies have suggested that natural compounds found in propolis, a resinous substance collected by honeybees, may have therapeutic potential for metabolic diseases[6].

One such compound is piperine, an alkaloid isolated from Nigerian propolis[7]. The ethanolic extract of Nigerian propolis (a type of propolis rich in piperine) has been shown in previous research to exhibit hepatoprotective and pancreatoprotective properties in animal models, indicating its potential to mitigate metabolic dysfunction[6]. According to a study by[8], piperine demonstrated anti-diabetic and anti-inflammatory properties. However, the specific mechanisms by which piperine may modulate glucose metabolism and inflammation in the context of chronic fructose and glucose intake have not been elucidated.

The present study aimed to evaluate the antihyperglycemic effect of piperine isolated from Nigerian propolis in a rat model of chronic fructose and glucose infusion. This was accomplished by assessing the expression of the Glucose Transporter-4 (GLUT-4) and the inflammatory mediator Nuclear Factor Kappa B (NF-NF-ΚB).

Material and Methods

Animals and study design

The study utilized 20 male Wistar rats as the experimental subjects. The rats were obtained from the Kwara State University Animal Facility; they were well acclimatized, housed in a controlled environment with a 12-hour light/dark cycle, and had unrestricted access to food and water. All experimental procedures were reviewed and approved by the Kwara State University Ethical Committee on Animal Studies (11670) and conducted in accordance with relevant international guidelines and regulations. The rats were randomly divided into 4 experimental groups: a control group, a fructose-glucose (unsupplemented) group, a fructose-glucose (fructose and glucose) plus piperine supplementation group, and a fructose-glucose plus metformin group. The control group received a continuous administration of normal saline solution throughout the experiment. The fructose-glucose group received a continuous oral infusion of a 50% fructose and 50% glucose solution, while the fructose-glucose plus piperine group received the same infusion in addition to the daily oral administration of 100 mg/kg piperine. The 100 mg/kg dose was chosen for efficacy studies, following the experimental determination of the LD50 to be 430 mg/kg and the TD50

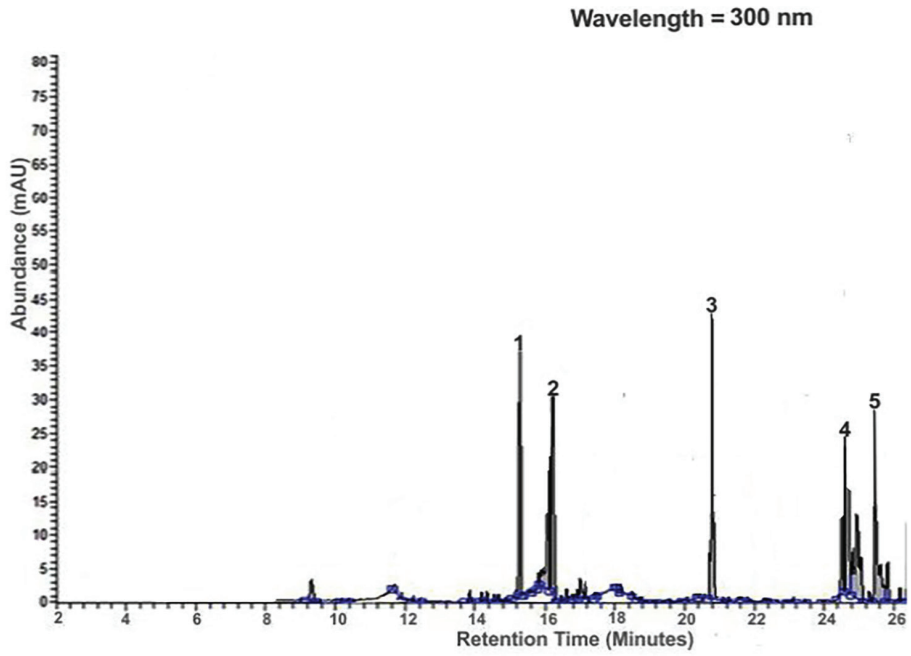
to be 250 mg/kg. The chosen dose falls well below both the toxic and lethal thresholds, providing a favourable safety margin. The fructose-glucose plus metformin group was administered the 50% fructose and 50% glucose infusion together with the daily oral administration of 200 mg/kg metformin[9]. The vehicle for metformin/piperine was distilled water.

Oral infusion with glucose and fructose

The rats were subjected to a chronic oral infusion of a glucose and fructose solution to induce a diabetic state. The infusion was administered daily for 8 weeks. The glucose and fructose solution was prepared by dissolving the respective sugars in the drinking water at a concentration of 10% (w/v) each. The rats had free access to the glucose-fructose solution throughout the study period. The animals' plasma glucose levels were closely monitored, and their drinking water containing the solutes was constantly replenished in order to keep track of the development of the hyperglycaemic state.

High-performance liquid chromatography (HPLC) isolation of piperine from nigerian propolis

Propolis samples were obtained from the South-Western region of Nigeria. The piperine used in this study was isolated from ethanolic extracts of Nigerian propolis using high-performance liquid chromatography. The structure and identity of the isolated compounds were confirmed by NMR spectroscopy and compared to the reference standards, as highlighted in Figure 1 and Table 1. In the HPLC chromatogram, a peak corresponding to piperine was observed at a retention time of 20.8 minutes. The isolation process ensured the experimental administration of a purified piperine for the supplementation studies.



mAU=milli-absorbance unit

Figure 1 Chromatogram (HPLC) of Nigerian propolis used for this study. Piperine is marked "3". Properties of marked compounds are shown in Table 1.

Table 1 Properties of some compounds separated from Nigerian propolis

	Peak name	Retention time	Area	Height (mAU)	Class	Molecular formula
1	Chrysin	15.3	24050	38.24	Flavonoid	$C_{15}H_{10}O_4$
2	Pinocembrin	16.2	53462	31.80	Flavonoid	$C_{15}H_{12}O_4$
3	Piperine	20.8	31546	46.01	Alkaloid	$C_{17}H_{19}NO_3$
4	Oleanolic acid	24.5	47800	25.37	Triterpene	$C_{30}H_{48}O_3$
5	Glycyrrhizin	25.4	72701	28.61	Saponin	$C_{42}H_{62}O_{16}$

mAU=milli-absorbance unit

Biochemical analyses

At the end of the 8-week study period, blood samples were obtained from the rats, centrifuged at 6000 rpm to separate the serum. The serum glucose concentration was measured after a 9-hour fast, using the On Call Plus Glucometer by ACON Laboratories, Inc., while insulin levels were quantified with the Insulin ELISA Kit by Sigma Aldrich after the same fasting period. Additionally, gastrocnemius muscle samples were harvested, homogenized in an appropriate buffer, and stored at -80 degrees Celsius for further biochemical analyses. The expression of the GLUT-4 gene was quantified using the GLUT-4 Assay Kit by Gold Standard Diagnostics, California, USA. Furthermore, the expression of Nuclear Factor Kappa B (NF-ΚB) and phosphorylated Protein Kinase B (p-Akt) was measured using proteins from Western Blot Kits provided by Hunan ZR-HuXin Biomed Co., Ltd., Changsha, China.

Glucose and insulin analyses

Glucose and insulin concentrations in the serum were determined using commercially available assay kits as mentioned in the previous section. Insulin sensitivity was evaluated by computing the homeostatic model assessment of insulin resistance index (HOMA-IR)[10].

HOMA-IR=(Fasting Plasma Glucose (mg/dL)× Fasting Insulin (μU/mL))/405

GLUT-4 gene expression analyses

Real-time Polymerase Chain Reaction (PCR) was performed to quantify the mRNA expression of the GLUT4 gene. Total RNA was extracted from the muscle tissue homogenate and reverse transcribed into cDNA (complementary DNA). The cDNA samples were then subjected to real-time PCR amplification using GLUT4-specific primers (forward primer: 5'-ATGGAGACAAGACTCAAGCG-3', reverse primer: 5'-CTCAATGTAGCCCTCATAGC-3') and fluorescent probes. The mRNA levels of GLUT4 were normalized to glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and quantified using the comparative Ct method.

NF-ΚB and pAkt analyses

The protein expression levels of pro-inflammatory transcription factor NF-ΚB and phosphorylated protein kinase B p-Akt were analysed using western blotting techniques. Muscle tissue homogenates were subjected to SDS-PAGE, and the resolved protein bands were transferred to a membrane. The membrane was then probed with a specific antibody against the NF-ΚB p65 subunit and p-Akt, and the relative expression levels were quantified using densitometry.

Statistical analysis

Statistical analyses were conducted using version 9.0 of GraphPad Prism software. All data were expressed as mean±standard error of the mean. Differences between groups were analysed using one-way ANOVA followed by Tukey-Kramer post-hoc tests. A p-value less than 0.05 was accepted as statistically significant.

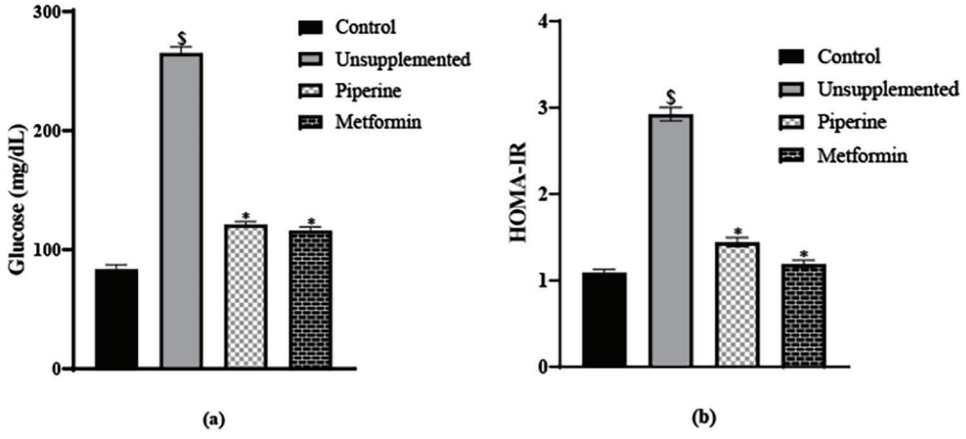
Results

Hyperglycaemia, glucose, and insulin

Rats in the glucose/fructose infusion (Unsupplemented) group developed features characteristic of the hyperglycaemic state. These animals exhibited significantly elevated fasting blood glucose levels compared to the control group, indicating the successful induction of hyperglycaemia through the chronic carbohydrate overload. The elevated blood glucose levels were accompanied by impaired insulin sensitivity, as demonstrated by the increased homeostatic model assessment of insulin resistance index (HOMA-IR) in the infusion group. These metabolic disturbances were effectively prevented by the administration of piperine, which was able to significantly lower blood glucose levels and significantly improve insulin sensitivity in the supplemented animals compared to the unsupplemented infusion group (Figure 2 a and b).

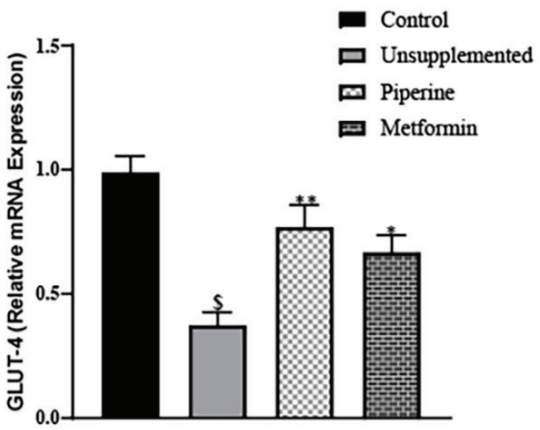
GLUT-4 gene expression

The expression of the GLUT-4 gene, a key regulator of glucose uptake, was significantly decreased in the gastrocnemius muscle of the fructose-glucose group compared to the control group (Figure 3). However, piperine supplementation significantly increased GLUT-4 expression compared to the fructose-glucose (unsupplemented) group, indicating that this natural compound may enhance glucose utilization in skeletal muscle. Similarly, metformin, a widely used antidiabetic drug, also showed a comparable effect in improving GLUT-4 expression.



($) p-value<0.05 compared to the control group. (*) p-value<0.05 compared to the unsupplemented group.
HOMA-IR=homeostatic model assessment of insulin resistance

Figure 2 Prevention of hyperglycaemia and insulin-insensitivity by piperine. Data are expressed as mean±standard error of the mean. Differences between groups were analysed using one-way ANOVA followed by Tukey-Kramer post-hoc tests. A p-value less than 0.05 was accepted as statistically significant.

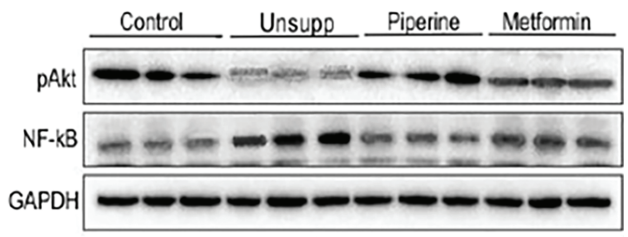


($) p-value<0.05 compared to the control group. (*) p-value<0.05 compared to the unsupplemented group. (**) p-value<0.01 compared to the unsupplemented group.

Figure 3 Piperine supplementation increases GLUT-4 expression. Data are expressed as mean±standard error of the mean. Differences between groups were analysed using one-way ANOVA followed by Tukey-Kramer post-hoc tests. A p-value less than 0.05 was accepted as statistically significant.

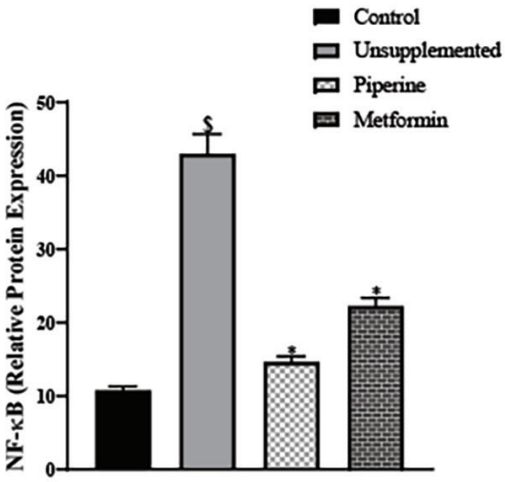
NF-ΚB and pAkt analyses

In Figures 4 and 5, expression of Nuclear Factor Kappa B (NF-ΚB) was significantly elevated in the fructose-glucose (unsupplemented) group compared to the control group. Piperine supplementation significantly decreased the NF-ΚB levels compared to the fructose-glucose (unsupplemented) group. In contrast, as seen in Figure 4 and 6, the expression of phosphorylated Protein Kinase B (p-Akt) was significantly decreased in the fructose-glucose group, and piperine supplementation significantly restored the p-Akt level.



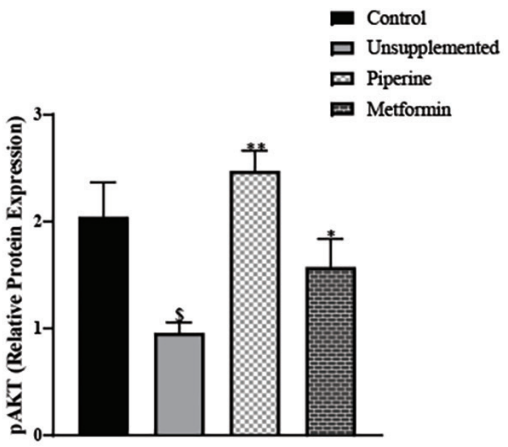
Unsupp=unsupplemented

Figure 4 Western blot analyses of pAkt and NF-ΚB.



($) p-value<0.05 compared to the control group. (*) p-value<0.01 compared to the unsupplemented group.

Figure 5 Piperine supplementation decreases NF-ΚB levels. Data are expressed as mean±standard error of the mean. Differences between groups were analysed using one-way ANOVA followed by Tukey-Kramer post-hoc tests. A p-value less than 0.05 was accepted as statistically significant.



($) p-value<0.05 compared to the control group. (*) p-value<0.05 compared to the unsupplemented group. (**) p-value<0.01 compared to the unsupplemented group.

Figure 6 Piperine supplementation increases pAkt protein expression. Data are expressed as mean±standard error of the mean. Differences between groups were analysed using one-way ANOVA followed by Tukey-Kramer post-hoc tests. A p-value less than 0.05 was accepted as statistically significant.

Discussion

The findings from this study suggest that piperine, a natural alkaloid compound isolated from Nigerian propolis, has the potential to mitigate the detrimental effects of chronic fructose and glucose intake on glucose metabolism and inflammatory pathways. The model of chronic oral carbohydrate overload used in this study has been well-established to reliably induce features of diabetes in rodents[11,12], simulating the metabolic disturbances observed in human populations with diets high in refined carbohydrates[13-16]. Piperine supplementation was able to effectively mitigate the adverse metabolic changes induced by the chronic exposure to high levels of glucose and fructose, including elevated blood glucose, impaired insulin sensitivity, altered expression of key proteins involved in glucose homeostasis, and inflammation.

Previous research has demonstrated possible anti-diabetic and anti-inflammatory properties of piperine[17]. Similar to these reports, the current study shows that piperine supplementation was able to effectively normalize blood glucose levels and improve insulin sensitivity in the fructose-glucose-infused rats. There was a marked reduction of fasting blood glucose in the piperine-supplemented group compared to the unsupplemented glucose/fructose infusion group, where blood glucose levels remained significantly elevated. The homeostatic model assessment of insulin resistance index (HOMA-IR) has been reliably used to assess insulin sensitivity in experimental rat models of diabetes[18]. Administration of fructose and glucose significantly impaired insulin sensitivity in the infusion group, as indicated by the increased HOMA-IR values. This insulin resistance was effectively prevented by piperine supplementation, which normalized the HOMA-IR index in the treated animals. These metabolic improvements were accompanied by an increase in the expression of the GLUT-4 gene, a key regulator of glucose uptake in skeletal muscle[19,20].

The upregulation of GLUT-4 expression in skeletal muscle by piperine suggests a potential mechanism by which this natural compound enhances glucose utilization and improves insulin sensitivity. GLUT-4 is the primary glucose transporter responsible for insulin-stimulated glucose uptake in peripheral tissues[21], and its decreased expression has been linked to the development of insulin resistance and type 2 diabetes[22]. By increasing GLUT-4 levels, piperine may facilitate greater glucose disposal and alleviate hyperglycaemia.

Additionally, piperine was able to suppress the activation of the pro-inflammatory transcription factor, Nuclear Factor Kappa B (NF-ΚB). NF-ΚB is known to play a central role in the pathogenesis of insulin resistance and

diabetes-related complications[23,24]. Chronic inflammation is a hallmark of insulin resistance[25,26], and the inhibition of NF-ΚB signalling by piperine may represent another mechanism by which this compound protects against metabolic disturbances.

The observed high increase in the phosphorylation of Protein Kinase B (Akt), a key mediator of insulin signalling[27], further supports the insulin-sensitizing effects of piperine. Akt activation is crucial for the translocation of GLUT-4 to the cell membrane and the subsequent uptake of glucose[28]. The ability of piperine to enhance Akt phosphorylation suggests that this compound can potentiate insulin-stimulated glucose disposal, thereby substantially improving glucose homeostasis.

The interplay between improved glucose handling and reduced inflammation may be a key mechanism underlying the therapeutic potential of piperine. As the accumulation of visceral fat and chronic inflammation are closely linked to the development of insulin resistance and type 2 diabetes, the ability of piperine to modulate both of these pathways could be particularly advantageous.

Additionally, some of these activities of piperine isolated from Nigerian propolis might partly be due to its source (Nigerian propolis), as it is suggested that the source and method of extraction of piperine can influence its biological activity[29].

Conclusion

The findings from this study suggest that piperine, an alkaloid isolated from Nigerian propolis, has the potential to mitigate the detrimental effects of chronic fructose and glucose intake on glucose metabolism and inflammatory pathways. The observed increase in GLUT-4 expression and decrease in NF-ΚB expression in the piperine-treated group indicate that this natural compound may enhance glucose uptake and utilization while also suppressing inflammatory processes.

These results provide valuable insights into the therapeutic potential of piperine in the context of chronic fructose and glucose consumption, and warrant further investigation to elucidate the probable additional molecular mechanisms with which piperine exerts its effects on glucose metabolism and inflammation.

Conflict of interest

No conflict of interest declared.

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