Original Article

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Isosativan from Nigerian Propolis Modulates AMP-activated Protein Kinase, Glucose-6-Phosphatase and Lipoprotein Lipase levels in Rats with Insulin Resistance

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Abstract:

Objective: This study investigated the effects of isosativan, a bioactive compound isolated from Nigerian propolis, on the activities of key metabolic regulators, including AMP-activated protein kinase (AMPK), glucose-6-phosphatase (G6Pase), and lipoprotein lipase (LPL), in an animal model of insulin resistance.

Material and Methods: Male Wistar rats were fed a high-fat diet and fructose solution for 8 weeks to establish an animal model of insulin resistance. Eighteen of the animals were sorted into 3 groups: the insulin-resistant group, the isosativan group, and the metformin group, while a fourth group of 6 healthy rats served as the control group. The isosativan treatment group received daily oral administration of isosativan for 4 weeks, while the metformin group served as a positive control. Plasma glucose, insulin, and lipid profiles were measured, and the activities of AMPK, glucose-6-phosphatase in the liver, and lipoprotein lipase in the adipose tissue were assessed.

Results: Compared to the control group, the insulin-resistant group showed significantly increased fasting plasma glucose and homeostatic model assessment of insulin resistance (HOMA-IR) levels. The high-fat diet and fructose consumption also disturbed the lipid profile. Treatment with isosativan improved insulin resistance and modulated the altered activities of AMPK, glucose-6-phosphatase, and lipoprotein lipase in insulin-resistant rats, indicating its potential and probable mechanism of improving glucose and lipid homeostasis.

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Conclusion: Isosativan, a compound isolated from Nigerian propolis, demonstrated the ability to modulate key metabolic regulators involved in insulin resistance, suggesting its therapeutic potential for managing metabolic disorders.

Keywords: Insulin resistance, metabolic regulatory enzymes, Isosativan, Nigerian propolis

Introduction

Insulin resistance represents a significant public health concern, as it is a central factor contributing to the development of type 2 diabetes[1] and other metabolic disorders[2]. Examining the effects of natural compounds on key metabolic pathways involved in insulin resistance is a crucial area of research.

AMP-activated protein kinase (AMPK) is a master regulator of cellular energy homeostasis[3]. It plays a crucial role in governing glucose and lipid metabolism, and its impairment is strongly correlated with insulin resistance[4].

Glucose-6-phosphatase (G6Pase), a key enzyme in the gluconeogenic pathway[5], and lipoprotein lipase (LPL), a critical enzyme involved in lipid metabolism[6], have also been shown to exhibit altered activities in insulin-resistant states, contributing to the disturbance of glucose and lipid homeostasis[7,8].

Propolis, a gummy substance produced by honeybees from plant sources, has been the focus of growing scientific interest for its potential therapeutic applications. Notably, extensive research has demonstrated the antidiabetic potential of propolis derived from diverse geographical regions[9-12], including one of Nigerian origin[13], underscoring the efficacy of its crude form as a natural remedy for metabolic disorders such as insulin resistance.

However, isosativan is a relatively novel compound isolated from Nigerian propolis[14,15], and its ability to modulate key regulators of glucose and lipid metabolism in the context of insulin resistance warrants investigation.

This study aimed to investigate the effects of isosativan, a flavonoid isolated from Nigerian propolis, on the activities of AMP-activated protein kinase, glucose-6-phosphatase, and lipoprotein lipase in an animal model of insulin resistance.

Material and Methods

Experimental design

Twenty-four male Wistar rats were obtained from the Kwara State University animal facility and acclimatized for one week. During this time, they were kept under a 12-hour day/night cycle, at constant room temperature, and allowed unrestricted access to food. Subsequently, the animals were randomly divided into 4 groups: the control group, the insulin-resistant group, the isosativan group, and the metformin group. The study was approved by the Kwara State University Ethical Committee on Animal Studies.

Animal model

All groups, except the control group, underwent an 8-week dietary intervention to establish an animal model of insulin resistance. This involved consuming a high-fat diet composed of 45% fat, 35% carbohydrates, and 20% protein by mass, along with a 20% fructose solution as their drinking water. In contrast, the control group was maintained on a standard rat diet and plain drinking water throughout the study duration.

Isolation of isosativan

Isosativan used in this study was isolated from a Nigerian propolis extract using a combination of high-performance liquid chromatography and nuclear magnetic resonance techniques (Figure 1). The propolis extract was

initially fractionated using HPLC with a C18 reversed-phase column and a gradient elution system employing water and acetonitrile as the mobile phases. The fraction containing isosativan was then further purified and characterized through NMR spectroscopy to obtain the pure compound, which had a retention time of 16.4 minutes (Table 1).

Treatments

After confirming insulin resistance in the induction groups following the 8-week diet period, the insulin-resistant group continued to receive the high-fat diet and fructose solution without any treatment. Additionally, the isosativan group was administered a daily oral gavage of isosativan at a dose of 50 mg/kg body weight for 4 weeks, while the metformin group received metformin at a dose of 100 mg/kg body weight by oral gavage for the same duration.

Sample collection and Biochemical analyses

At the end of the 12-week study period, the animals were sacrificed, humanely, and blood, liver, and adipose tissue samples were collected. Plasma glucose, insulin, and lipid profiles were determined using standard assay kits purchased from Thermo Fisher Scientific (Waltham, Massachusetts, USA). The activities of AMPK and G6Pase in the liver, and LPL in the adipose tissue were measured using commercially available enzyme activity assay kits from Thermo Fisher Scientific, following the manufacturer's protocols.

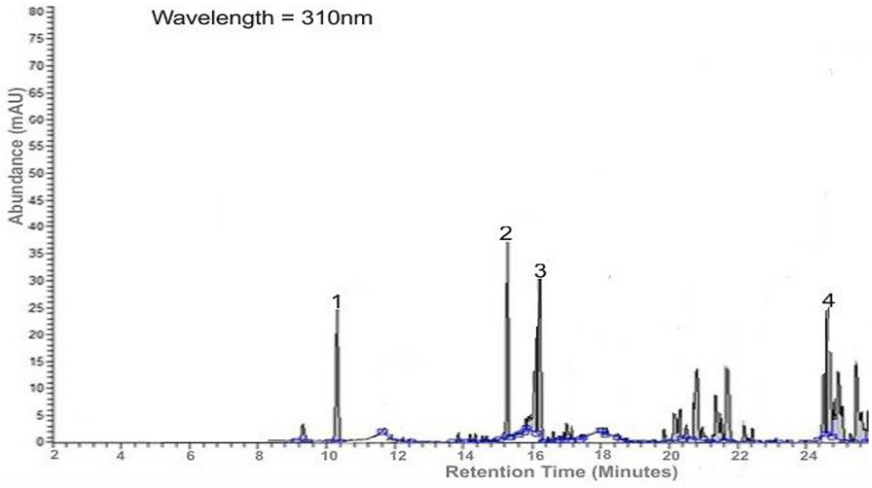


Figure 1 Isolation of isosativan using reversed-phase high-performance liquid chromatography (HPLC)

Table 1 Different compounds isolated from Nigerian propolis and their specific properties

	Peak	Retention Time (minutes)	Height (mAU)	Molecular formula	Area	Class
1	Epicatechin	10.2	26.12	$C_{15}H_{14}O_6$	33154	Flavonoid
2	Chrysin	15.3	37.94	$C_{15}H_{10}O_4$	24500	Flavonoid
3	Isosativan	16.4	32.08	$C_{17}H_{18}O_4$	60007	Flavonoid
4	Oleanolic acid	24.5	26.23	$C_{30}H_{48}O_3$	39012	Triterpene

mAU=milli-absorbance units

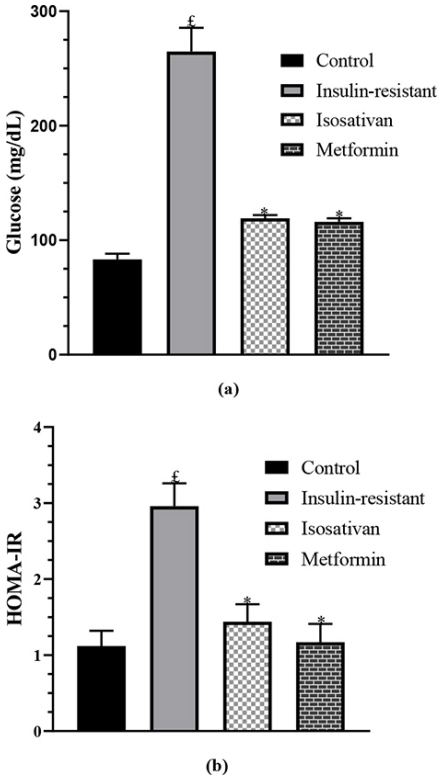
Statistical analysis

The data were analyzed using GraphPad Prism 8.0 software. All values are presented as mean±standard error of the mean. Comparisons between groups were performed using one-way analysis of variance followed by Tukey's post hoc test. Differences were considered statistically significant at p-value<0.05.

Results

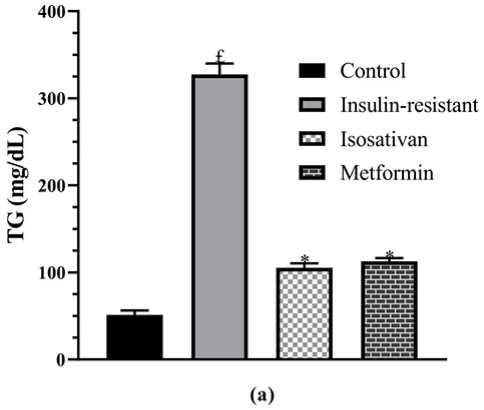
Plasma glucose, insulin, and lipid profile

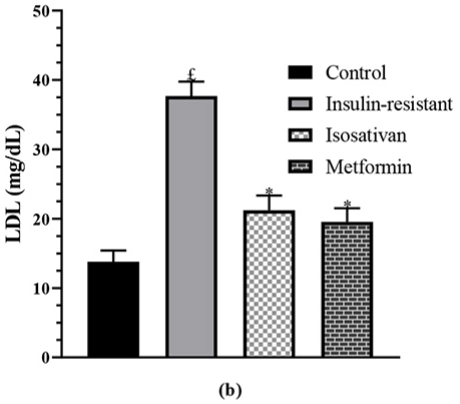
Consistent with the induction of insulin resistance, the insulin-resistant group exhibited a significant increase in fasting plasma glucose and homeostatic model assessment of insulin resistance (HOMA-IR) levels compared to the control group. Furthermore, the high-fat diet and fructose administration led to an adverse lipid profile, characterized by elevated triglycerides, low-density lipoprotein cholesterol, and reduced high-density lipoprotein cholesterol levels. Treatment with isosativan, however, demonstrated a significant enhancement in glucose homeostasis and insulin sensitivity, as evidenced by the reduction in fasting plasma glucose and HOMA-IR values relative to the insulin-resistant group (Figure 2(a) and Figure 2(b)). The isosativan group also exhibited a favorable modulation of the lipid profile, characterized by decreased triglycerides and low-density lipoprotein cholesterol, as well as increased high-density lipoprotein cholesterol levels (Figure 3(a), Figure 3(b), and Figure 3(c)).



All values are presented as mean±standard error of the mean. Comparisons between groups were performed using one-way analysis of variance followed by Tukey's post hoc test. Differences were considered statistically significant at p-value<0.05. (£) p-value<0.01 compared with the control group. (*) p-value<0.01 compared with the insulin-resistant group

Figure 2 Enhancement of glucose homeostasis and insulin sensitivity by isosativan





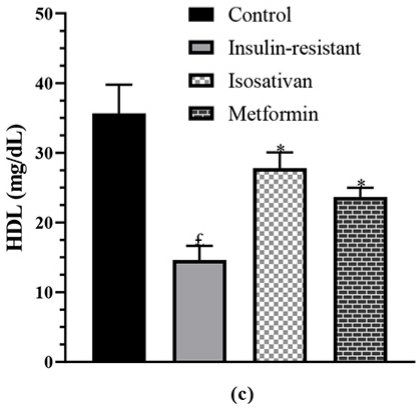


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Figure 3 Modulation of lipid profile by Iisosativan

AMP-activated Protein kinase

In Figure 4, the AMPK activity in the liver tissue was considerably diminished in the insulin-resistant rats compared to the control group. Notably, the administration of isosativan for 4 weeks significantly enhanced liver AMPK activity in the isosativan-treated group.

Glucose-6-Phosphatase

Similarly, in Figure 5, isosativan treatment was associated with a significant reduction in the activity of glucose-6-phosphatase, a key gluconeogenic enzyme, in the liver of insulin-resistant rats.

Lipoprotein lipase

Furthermore, the activity of lipoprotein lipase, a pivotal enzyme involved in triglyceride metabolism, was significantly enhanced in the adipose tissue of the isosativan-treated group compared to the insulin-resistant group. The improvements observed with isosativan treatment were comparable to those seen in the metformin-treated group, which served as a positive control (Figure 6).

Discussion

The findings from this study align with the growing body of evidence supporting the therapeutic potential of natural compounds, including those derived from propolis in managing metabolic disorders[16–18]. In this rat model, we have demonstrated that isosativan, a constituent of Nigerian propolis, can ameliorate insulin resistance and the associated dysregulation of glucose and lipid metabolism.

The observed improvements in glucose homeostasis and insulin sensitivity with isosativan treatment may be attributed to its ability to modulate the activity of the AMPK pathway in the liver. AMPK is a critical intracellular energy sensor that coordinates a broad array of metabolic pathways, including glucose and lipid metabolism[19], thereby playing a pivotal role in maintaining cellular energy homeostasis[20]. Activating the AMPK signaling pathway by isosativan may underlie its capacity to improve insulin sensitivity. This could involve stimulating glucose absorption, facilitating fatty acid oxidation, and suppressing gluconeogenesis – metabolic mechanisms that collectively contribute to ameliorating insulin resistance.

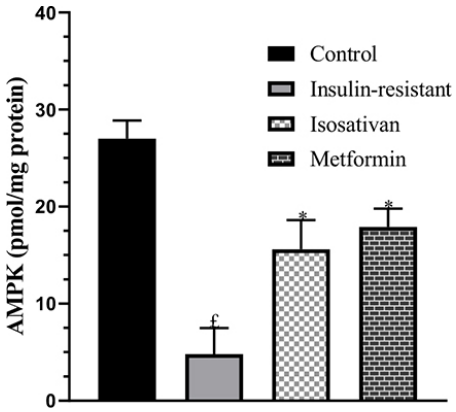

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Figure 4 Isosativan enhances liver AMP-activated protein kinase (AMPK) activity

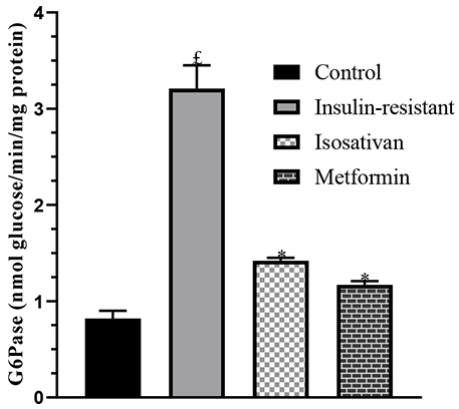

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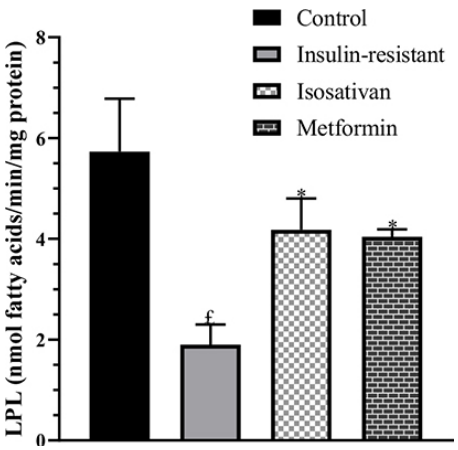
Figure 5 Reduction of glucose-6-phosphatase (G6Pase) activity by isosativan



All values are presented as mean±standard error of the mean. Comparisons between groups were performed using one-way analysis of variance followed by Tukey's post hoc test. Differences were considered statistically significant at p-value<0.05. (£) p-value<0.01 compared with the control group. (*) p-value<0.05 compared with the insulin-resistant group

Figure 6 Isosativan enhances adipose tissue lipoprotein lipase (LPL) activity.

Furthermore, the reduced G6Pase activity in the liver of isosativan-treated animals indicates that the compound may inhibit gluconeogenesis, thereby enhancing glucose regulation. Previous studies have reported that propolis extract can suppress the expression of G6Pase and/or its activity[21,22], a crucial enzyme involved in the gluconeogenic process[23,7].

Additionally, the enhanced LPL activity in the adipose tissue of the isosativan-treated group may have contributed to the favourable modulation of the lipid profile observed in this investigation. Lipoprotein lipase is a key enzyme that removes triglyceride-rich lipoproteins from the bloodstream[24]. The increased activity of this enzyme following isosativan treatment may have helped improve lipid balance and reduce the dyslipidaemia associated with insulin resistance.

The study found that isosativan can modulate the activities of important metabolic enzymes, such as AMP-activated protein kinase, glucose-6-phosphatase, and lipoprotein lipase, in an animal model of insulin resistance. These metabolic changes were associated with improvements in glucose regulation and lipid profile, suggesting isosativan may have potential as a treatment for insulin resistance and related metabolic disorders.

Conclusion

The findings of this study indicate that treatment with isosativan, a flavonoid derived from Nigerian propolis, improved glucose homeostasis, lipid profile, and the regulation of key metabolic enzymes, including AMP-activated protein kinase, glucose-6-phosphatase, and lipoprotein lipase. These results suggest that isosativan may have potential as a natural therapeutic option for the management of insulin resistance and related metabolic disorders, warranting further investigation in order to assess its efficacy and safety in clinical settings.

Conflict of interest

No conflict of interest declared

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