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Cross River State Grown *Andrographis paniculata* Ethanol Leaf Extract Attenuates Insulin Resistance and TNF Alpha in Obese Rats

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Abstract

This study investigated the ethanol leaf extract of Andrographis paniculata and its potential role in attenuating insulin resistance and decreasing turmor necrosis factor alpha levels, raised in obese condition. Twenty five (25) rats were grouped into five (5); Group I: served as normal control and received normal saline and normal rats pellet, Group II: served as obesity control and was fed a high-fat diet but not treated, Group III: served as positive control, fed with a high-fat diet and 30 mg/kg body weight Orlistat, Group IV: fed with a high-fat diet and treated with 500 mg/kg body weight A. paniculata, and Group V: fed with a high-fat diet and treated with 1000 mg/kg body weight A. paniculata. The results showed significant ($P<0.05$) increases in both TNF-α and HOMAR-IR index in obese rats but decreased in obese rats treated with 30 mg/kg bw Orlistat, 500 and 1000 mg/kg bodyweight A. paniculata. It can be concluded, that A. paniculata has properties that could play a role in attenuating insulin resistance and inflammation induced by obesity.

Keywords: Obesity, TNF-α, insulin resistance, inflammation, HOMA-Index, A. paniculata

INTRODUCTION

Obesity has been described as excessive abnormal accumulation of lipids in the adipose tissue, which poses health risks (Purnell, 2023). Lifestyle, eating habits, and diseases influence obesity. The availability of food coupled with a sedentary lifestyle has been implicated in the induction of obesity. Obesity causes derailment of metabolic processes and cell and tissue dysfunction and alters the levels of related biomarkers (Purnell, 2023). Complications are not limited to retinopathy, apnea, hypertrophy, and cardiovascular diseases; they also include insulin resistance and increased levels of tumor necrosis factor alpha (TNF-α), an inflammatory biomarker (Wondmkun, 2020).

According to Ana *et al.* (2014), insulin resistance is characterized by impairment of insulin action, and the state of insulin resistance is defined by impairment of glucose uptake in muscle and the increment of endogenous glucose production by the liver resulting in hyperglycemia, simultaneously in fasting and postprandial states. Generally, insulin resistant involves the impairment of insulin action on lipid metabolism by way of increasing breakdown of adipose tissue lipids or impairment of protein synthesis in muscle, predisposing to sarcopenia. Moreover, the functions of other organs such as vessels, brain, pancreas and bone are affected by insulin resistance (Kawai *et al.* 2021; Jo *et al.* 2009). Ana *et al.* (2014) described the mechanisms involved in the etiopathogenesis of insulin resistance related to obesity, encompassing pre-receptor, receptor and postreceptor defect. For instance, decreased access of insulin to muscle secondary to free fatty acids excess is classified as "pre-receptor". Insulin receptor downregulation secondary to hyperinsulinemia is classified as "receptor" and inhibition of intracellular cascades by several adiposity-related factors (*e.g.* increased FFA, impaired adipokines and/or cytokines secretion) is classified as "post receptor" (Stears *et al.,* 2021).

TNF-α is an adipocytokine whose blood level increases in obese condition. In addition, increased levels of serum TNF-α in diabetic conditions has also been implicated in insulin resistance (HOMA-IR), type 2 diabetes mellitus and complications associated with energy metabolism (Tzanavari *et al.,* 2010). Occasionally, these complications are sometimes managed by the use of synthetic drugs. However, these drugs have side effects that may outweigh the symptoms of the diseases. Moreover, their cost and bottleneck processes is a major problem for the management of these metabolic diseases in health care system.

Consequently, products from natural sources, such as medicinal plants have received increased investigations into their bioactivity, with a view to harnessing their product for the treatment and management of metabolic diseases. *Andrographis paniculata* (*A. paniculata)*, a shrub also called "King of bitters" is largely grown and cultivated in Asian countries, including India, Srilinka, and Pakistan (Muhammad and Ali, 2022). Of recent interest, *A. paniculata* has been imported, identified and grown by the National Institute for Pharmaceutical Research and Development (NIPRD), Abuja and subsequently spread to other parts of Nigeria, including Cross River State (Ameh *et al.,* 2010). *A. paniculata* is commonly known as "Kazu'kwel" by the people of Obudu and Bekwarra, Cross River State, and called "vinegar" by other Cross Riverians due to its extremely bitter taste. *A. paniculata* has been used traditionally as for typhoid fever, stomach ache and upset, inflammation and to reduce the sugar content of local wine (palm wine). *A. paniculata* has been reported with antidiabetic potency in diabetic models and found to normalize dyslipidaemia. There is a need to investigate its bioactivity in assuaging insulin resistance and normalizing levels of serum TNF-α in high fat diet-induced obese Wistar rats.

MATERIALS AND METHODS

Collection of Plant Samples

Fresh leaves of *Andrographis paniculata* were harvested from local households across the three (3) senatorial zones (North, Central and South) in Cross River State, Nigeria. The plant was identified and authenticated in the department of plant science, University of Cross River State, Calabar, with the voucher number: CRUTECH/PSB/0031 and deposited in the herbarium.

Whole Leaf Extract

Fresh leaves of *A. paniculata* were harvested, rinsed to remove debris, and air dried at room temperature. The dried leaves were pulverized using a Loonier Grander Mill (Japan). 1.3kg of the pulverized plant material was dissolved in absolute ethanol in a ratio of 1:4 and allowed for 24 hours. The sample was filtered using cheese cloth and White Man No 1 filter paper. The filtrate was oven-dried at 40°C to obtain a solvent-free extract and then stored in a refrigerator till when needed for use.

Animals

Sixty (60) male Wistar rats were obtained from the animal house of the department of Medical Biochemistry, University of Cross River State, Okuku Campus. The animals were allowed to acclimatize for a period of one (1) week, in a well-ventilated room with 12 h natural light- dark cycle. They were fed normal animal feed (pellet). Good hygiene was maintained by daily cleaning and removal of faeces and spills from the animals' cages, and strictly adhered to animal handling precautions and standards.

Induction of Obesity

After a week of acclimatisation, 60 Wistar rats were assigned into two (2) groups, labeled 1 and 2. Group 1 served as the normal control and was served normal diet (rats pellet) and saline while group 2 was fed with high fat diet (HFD) for five (5) weeks to induce obesity. Obesity was induced as described by Megalli *et al.* (2005) with slight modifications. Rats with above 20% weight increase compared with average weight gain of the normal control was considered obese (lee, 1929), and used in the study.

Standard Drug Preparation

Orlistat was purchased in tablet form at strength of 40mg/tab from Benz pharmacy, Calabar. Tablets were crushed into powder, dissolved in normal saline, and administered.

Design of the Experiment and Treatment Plan

The experimental design and treatment schedule are shown in Table 1.

Table 1: Experimental Design and Treatment Schedule

Groups	Title	Treatment
1	Normal group	Normal diet for 11 weeks
2	Control I	High-fat diet for 11 weeks
3	Control II	High-fat diet for 11 weeks + 30 mg/kg bw Orlistat from week 6-11 (6 weeks)
4	Treatment I	High-fat diet for 11 weeks + 500 mg/kg *A. paniculata* extract from week 6-11 (6 weeks)
5	Treatment II	High-fat diet for 11 weeks + 1000 mg/kg *A. paniculata* extract from week 6-11 (6 weeks)

N = 5

Treatment

The reference anti-obesity drug- Orlistat was prepared in normal saline solution and administered at a dose of 30 mg/kg body weight. Normal and obese controls received saline. All treatments (extract, standard drug and saline) were by oral gavage for six (6) weeks.

Termination of the Experiment and Sample Collection

At the end of the 6-week treatment, Wistar rats were euthanized by brief exposure to a mixture of ether and chloroform in an air-tight desiccator, and whole blood collected by cardiac puncture into plain tubes. The collected blood was allowed to clot for about an hour, and centrifuged at 2000 revolution per minute (rpm) for 10 minutes. The serum was carefully withdrawn and used for biochemical analysis.

Determination of the Serum Tumor Necrosis Factor Alpha

Serum tumor necrosis factor-alpha (TNF-α) was determined with the aid of enzyme-linked immunosorbent assay (ELISA) kits.

Insulin Resistance Determination

Insulin resistance was determined using the Homeostasis Model Assessment-estimated Insulin Resistance (HOMA- IR)

In the model, HOMA-IR = Insulin x Glucose/250

Statistical Analysis

Data obtained was analysed using One Way Analysis of Variance (ANOVA) with post hoc test at $P< 0.05$. The Statistical Package for Social Science (SPSS) Software version 25.0 was used for the analysis followed by Microsoft Excel for graphical representation.

RESULTS AND DISCUSSION

The results showed higher ($P<0.05$) insulin resistance index in obese control rats compared with normal controls (Figure 1). Rats fed the HFD were treated with 30mg/kg bwt. Orlistat, 500 and 1000 mg/kg bwt. extracts of *A. paniculata* showed higher insulin resistance index but was non-significant in rats treated with higher dose of the plant extract, when compared with the normal control. Rats treated with 30 mg/kg bwt. Orlistat, 500 and

1000mg/kg bwt. extracts of *A. paniculata* were significantly lower in HOMA-IR when compared with the index in obese control.

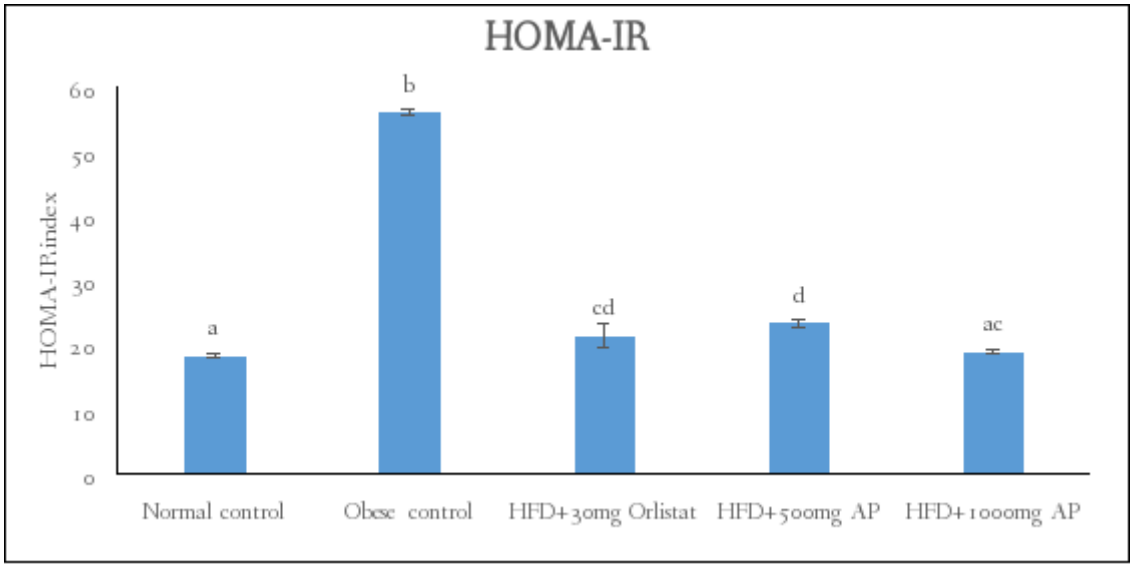

Figure 1: Effect of the ethanol leaf extract of *A. paniculata* on HOMA-IR in high fat diet-induced obese Wistar rats. Values are presented as the mean ±SEM (n=5). Values with different letters (a, b, c, d) among bars are statistically significant ($P<0.05$). **Legend**: Normal control = fed with normal rat pellets and tap water, Obese control = Fed with high fat diet and orally administered tween 80, HFD+30mg Orlistat = Obese rats administered 30mg/kg bwt. Orlistat orally, HFD+500mg AP = Obese rats orally administered 500 mg/kg bwt. ethanol leaf extract of *A. paniculata* and HFD+1000mg AP = Obese rats orally administered 1000mg/kg bwt. ethanol leaf extract of *A. paniculata.*

Results presented in Figure 2 revealed that there was a significant higher level ($P<0.05$) of TNF-α in obese control rats when compared with normal control. Obese rats treated with 30 mg/kg bwt. Orlistat, 500 mg/kg bwt. extract of *A. paniculata* and 1000mg/kg bwt. extract of *A. paniculata* showed higher levels of TNF-α in regard to normal control but significantly lowered when compared with levels in obese control. There were no significant ($P>0.05$) difference between groups of rats treated with Orlistat and 500mg/kg bwt. ethanol leaf extracts of *A. pamiculata.* Rats treated with a higher dose of the extract (1000mg/kg bwt.) showed significantly lowered level of TNF-α when compared with rats

treated with 30mg/kg bwt. orlistat and 500mg/kg bwt. *A. paniculta*; but was non-significant in respect to the normal control rats.

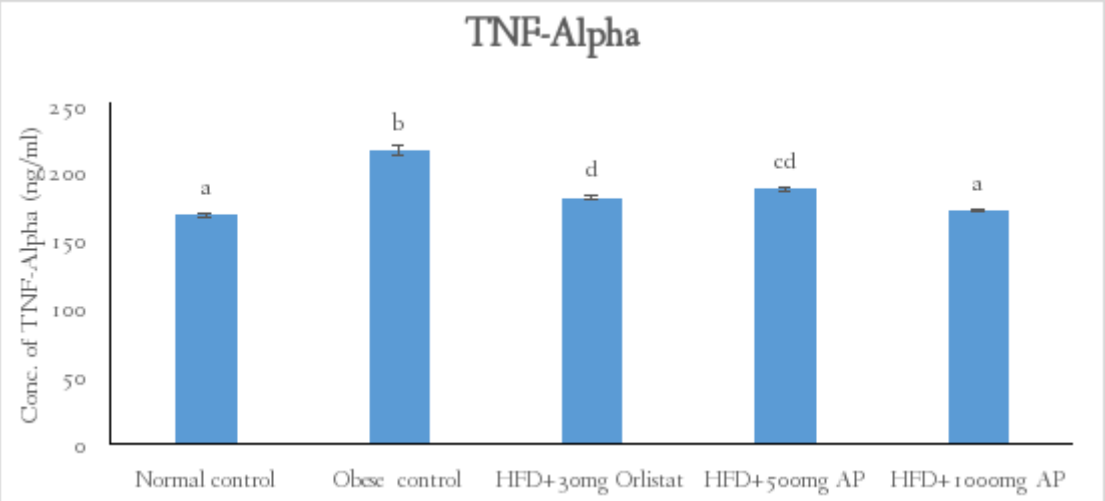

Figure 2: Effect of the ethanol leaf extract of *A. paniculata* on serum Tumor Necrosis Factor- alpha in high fat diet-induced obese Wistar rats. Values are presented as the mean ±SEM (n=5). Values with different letters (a, b, c, d) among bars are statistically significant (P<0.05). **Legend**: Normal control = fed with normal rat pellets and tap water, Obese control = Fed with high fat diet and orally administered tween 80, HFD+30mg Orlistat = Obese rats administered 30mg/kg bwt. Orlistat orally, HFD+500mg AP = Obese rats orally administered 500 mg/kg bwt. ethanol leaf extract of *A. paniculata* and HFD+1000mg AP = Obese rats orally administered 1000mg/kg bwt. ethanol leaf extract of *A. paniculata.*

The findings revealed higher TNF-α and insulin resistance index in obese rats. Insulin resistance in obese individuals has been reported by different researchers including, Arneth (2024), Yue *et al.* (2022); Steppan and Lazar (2002). According to Steppan and Lazar (2002), the accumulation of free fatty acids in abnormal amounts in insulin-sensitive nonadipose tissues causes ectopic deposition of lipid, leading to lipotoxicity. This lipotoxicity is therefore, an important cause of insulin resistance. Furthermore, Ana *et al.* (2014) identified adipocytes and adipose tissue as key players in the pathogenesis of insulin resistance associated with obesity. According to the authors, abnormal enlargement and dysfunction of adipocytes in visceral adipose tissue and upper body subcutaneous adipose tissue result in increased release of free fatty acids (FFAs) and altered amounts of leptin, resistin,

adiponectin and other adipokines in circulation. Consequently, Ana *et al.* (2014) described insulin resistance in obesity with the portal and spillover hypothesis, following elevated level of free fatty acids. In the portal theory, an increase in central abdominal fat tissue results in an elevation of the delivery of FFA to the liver through its portal vein drainage; consequently, hepatic insulin resistance and glucose rise occur. Based on the spillover hypothesis, a limited or reduced ability of adipose tissue to expand in the face of a positive energy balance, would lead to a spillover of FFA to the visceral fat compartment and to non-adipose tissues like the liver, muscle, pancreas, kidney and bone (Virtue and Vidal-Puig, 2010). The limited ability of non-adipose tissues to oxidize and/or store FFA results in the ectopic accumulation of FFA and/or its metabolic active derivatives leading to insulin resistance. In addition, tissues with more capability to oxidize FFAs would experience less occurrence of obesity and insulin resistance. This approach could serve as a therapeutic target for the management of insulin resistance and obesity (Sakuma and Yamaguchi, 2013).

Insulin resistance in obesity may also arise from disorientations of insulin receptors as a result of enlargement of the adipocytes. Adipocytes accumulate triglyceride as stored energy. However, excessive abnormal accumulation leads to expansion of the cells, which causes displacement of the position of receptors on the cell surface – making insulin not to fit into its receptor, a process of lock and key model as in ligand-receptor binding. In such conditions, even with sufficient insulin, there is no alignment of receptor to bind with insulin, and can lead to insulin resistance.

Insulin resistance with correspondent rise in TNF-α observed in this study is common. Reports have shown that different biomarkers of chronic and systemic inflammation, such as tumor necrosis factor-α (TNFα), interleukin 6 (IL-6), and C-reactive protein (CRP), are significantly associated with the emergence of insulin resistance (Swaroop *et al.,* 2012). Kitade *et al.* (2012) included TNFα concentrations to be high among obese individuals who developed resistance to insulin. Jais and Bruning, (2017) buttressed that obesity and chronic inflammation affect insulin sensitivity in all metabolic organs including the nonadipose organs and white adipose tissue (WAT). The enlargement of white adipose tissue in obese individuals has been implicated in inducing both mechanical and endoplasmic reticulum stress in adipocytes. This stress resulted in the release of FFA and inflammatory cytokines, and the recruitment of immune cells to obese WAT subsequently enhance local and systemic inflammation.

In WAT with obesity, proinflammatory macrophages are recruited and form crown-like structures around dead adipocytes and thereby contribute to the obesity-induced, low-grade inflammation (Revelo *et al.,* 2014). The elevated number of macrophages in WAT from obese individuals was reported by Weisberg *et al.* (2003) to be the main source of TNF-α increase in obesity.

Upon treatment, orlistat and *A. paniculata* extracts exhibited a lower HOMA IR index, indicating that the plant extracts could possess anti-glycemic potency similar to the conventional drug, orlistat. It might have activated insulin and enhanced its activities in reducing excess glucose in the blood (Subramanian *et al.,* 2008). Basically, a target on alpha pancreatic amylase is a good rationale for reducing excess glucose molecules in the body. The plant extract might have served as an inhibitor of the enzyme (amylase) - leading to reduction of glucose level. Moreover, FFAs that mediate insulin resistance, can be regulated via cholesterol synthesis. Orlistat is a statin drug whose mechanisms of ameliorating dyslipidemia are associated with the inhibition of HMG-CoA reductase, a key regulatory molecule in cholesterol biosynthesis. Ding *et al.* (2014) reported the preventive effects of andrographolide on high fat diet induced obesity. The authors explained that andrographolide, which is the major active component of *A. paniculata*, downregulates sterol regulatory element-binding proteins (SREBPs) by downregulation. SREBPs are said to be the major transcription factors regulating the expression of genes involved in biosynthesis of cholesterol, fatty acids and triglycerides (Ding *et al.,* 2014). The observed decreased insulin resistance in this study could be attributable to the active ingredient andrographolide contained in the plant extract which might have played regulatory role in the expression of HMG-CoA reductase, thereby, reducing the production of FFAs implicated in insulin resistance.

The decrease in TNF-α level by orlistat and extracts of *A. paniculata* could be tied to the explanation that orlistat acts as a reversible inhibitor of gastric and pancreatic lipases which prevents the hydrolysis of triglycerides; thus free fatty acids are not absorbed. This could also explain the action of *A. paniculata* in reducing TNF-alpha as reported in the previous study by Patmi *et al.* (2018). This anti-inflammatory property of *A. paniculata* has been reported (Nishanthini *et al.,* 2014; Aparana *et al.,* 2012), and it is due to the presence of its phytochemicals including, andrographolide, 14-octadecenoic acid, 1-(cyclopropyl-nitro-methyl)-cyclopentanol, and hexadecanoic acid (palmitic acid) (Ati *et al.,* 2023).

CONCLUSION

The current study evaluated the impact of locally grown *Andrographis paniculata* leaf extracts on insulin resistance and TNF-α in obese rats. In this study, *A. paniculata* showed a good competitive effect with Orlistat. This study demonstrated that *A. paniculata* has properties that could possibly play a role in attenuating insulin resistance and inflammation induced by obesity.

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Conflicts of interest: None

Ethics statement: Ethics approval for the study was obtained from the Faculty of Basic Medical Sciences Animal Research Committee of the Cross River University of Technology, Calabar, Nigeria.

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