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RESEARCH ARTICLE

Hepatoprotective and body weight lowering effects of the aqueous leaf extract of *Phyllanthus pentandrus* Schumach. and Thonn (*Phyllanthaceae*) in nonalcoholic fatty liver disease induced by a high-fat diet in Wistar rats

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ABSTRACT

Background: Some members of the genus *Phyllanthus* have been widely used in global traditional medicine to manage a variety of liver diseases and experimentally documented to have hepatoprotective activity. However, it is not known whether *Phyllanthus pentandrus* (PP) (a member of the genus endogenous to Northwestern Nigeria) possesses hepatoprotective properties. **Aims and Objective:** The objective was to investigate the hepatoprotective and body weight lowering effects of the aqueous leaf extract of PP using a high-fat diet (HFD)-induced rat model of nonalcoholic fatty liver disease (NAFLD). **Materials and Methods:** After obtaining ethical permission from a departmental committee, Wistar rats were randomly assigned to one of the following treatments for 7 weeks: vehicle + normal diet, vehicle + HFD, orlistat + HFD (at 400 mg/1000 g of HFD), or the PP extract +HFD (500 mg/100 g of HFD). Serum levels of liver enzymes (alkaline phosphatase [ALP], aspartate transaminase [AST], and alanine transaminase), conjugated and total bilirubin were determined. Determination of final body weight gain, adipose tissue weight, liver weight, as well as liver histology, was also performed on the animals. **Results:** Compared with normal controls, HFD-fed rats exhibited a significant elevation in ALP, severe liver steatosis, body weight gain, and increased adipose tissue mass. Treatment with the PP extract prevented the increase in the level of ALP, reduced the serum level of AST and ameliorated the hepatic steatosis, and adipose tissue gain. **Conclusion:** These findings suggest that PP may be beneficial in the management of NAFLD commonly associated with obesity.

KEY WORDS: *Phyllanthus pentandrus*; Fatty Liver; High-Fat Diet; Rats

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD), a common comorbid condition associated with obesity, is one of the most common liver disorders worldwide.[1] It progresses from benign and reversible fatty infiltration to subsequent more serious liver fibrosis. It is categorized into two subtypes: (1) Simple steatosis or steatosis with nonspecific abnormal features and (2) non-alcoholic steatohepatitis, a subtype associated with a higher risk of progression to hepatic fibrosis or cirrhosis. It seems that the mobilization of visceral fat, resulting in the release of free fatty acids into the portal circulation is the primary factor responsible for the liver dysfunction.[2] This effect primarily driven by insulin resistance represents the first hit according to the two-hit theory for the pathogenesis of NAFLD, with the second hit being the peroxidation of the fatty acids due to various factors producing oxidative stress.[3,4]

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Ethnicity, metabolic diseases (type 2 diabetes mellitus and polycystic ovarian syndrome), obesity, chronic infections (HIV, hepatitis C virus), primary aldosteronism, and myotonic dystrophy are some of the risk factors associated with NAFLD.[5]

Phyllanthus pentandrus (PP) (family: *Phyllanthaceae* and *Genus*: *Phyllanthus*) is an annual or perennial erect or decumbent plant, which is widely distributed in African countries. It is known as "geron tsuntsaye" in Hausa language. Members of the genus *Phyllanthus* are traditionally employed in the management of disease conditions related to NAFLD. For example, *Phyllanthus amarus* is used in the management of obesity[6] and liver disorders.[7] Previous studies have reported the hepatoprotective and body weight-lowering effects of extracts from some members of the genus *Phyllanthus*.[8-11] However, to the best of our knowledge, no previous study has investigated the hepatoprotective or weight-reducing properties of PP.

This study is aimed at evaluating aqueous leaf extract of PP for potential hepatoprotective activity in a high-fat diet (HFD)-fed rat model of NAFLD and investigating the weight-reducing properties of the extract.

MATERIALS AND METHODS

Plant Identification

The plant selected was identified by Prof A.A. Aliero of Botany unit, Department of Biological Sciences, Faculty of Science, Usmanu Danfodiyo University, Sokoto. The voucher specimen (V/N/PCG/UDUS/EUP/0010) was kept at the herbarium of the Faculty of Pharmaceutical Sciences, Usmanu Danfodiyo University, Sokoto.

Test Drugs and Chemicals

Orlistat (Xenical, Roche Ltd., Switzerland), was purchased from reputable pharmaceutical stores. Kits for alkaline phosphatase (ALP), aspartate transaminase (AST), alanine transaminase (ALT), and total and conjugated bilirubin were purchased from Randox Laboratories (UK). Other chemicals used were of analytical grade.

Animals

Wistar rats

Thirty male Wistar rats weighing 180–230 g were used for the study. They were acclimatized for 1 week on a HFD before starting the experiment and were given free access to water. Before the start of the experiment, ethical permission was obtained from a departmental committee, Department of Pharmacology and Therapeutics, College of Health Sciences, Usmanu Danfodiyo University, Sokoto.

Formulation of HFD

The HFD was formulated according to the method of Yang *et al*. (2007).[12] Forty grams of sheep tallow (animal fat around the kidneys and loins), 20 g of whole milk, 4 g of sugar, and 36 g of standard formula feed were mixed, giving 100 g of HFD. Based on the baseline average daily feed intake of the animals (100 g of feed/kg bwt of animals), 500 mg of the aqueous leaf extract of PP was incorporated into the HFD. The feeds were stored in a deep freezer to avoid putrefaction until time for use.

Plant Preparation and Extraction

The leaves of the plant were air-dried to constant weight, ground into a fine powder before subjecting them to aqueous extraction (40 g of the dried leaves in 200 ml of water) using plain bottled water. Frequent stirring for 30 min and then rapid filtration through a clean cloth followed this. The marc was squeezed to obtain a combined filtrate.

Ten milliliters aliquot of the combined filtrate was evaporated to estimate the stock concentration, which was stored in a freezer and can be further diluted to required concentration when needed.

Qualitative phytochemical analysis[13] was conducted on water extract of the plant for alkaloids, saponins, tannins, flavonoids, cardiac glycosides, anthraquinones, and phytosterols.

Design of the Study

Twenty four rats were randomized into 4 groups (n=6 for each group):

Group 1: Negative control on standard low-fat chow
Group 2: Negative control on the HFD
Group 3: Positive control treated with orlistat incorporated into a HFD (at 0.04 g%)
Groups 4: Treated with the extract incorporated into HFD (at 0.5 g%).

The parameters evaluated include baseline and final body weight (after 7 weeks of treatment). Others are weights (in g/100 body weight) of the liver, white (perirenal and epididymal) adipose tissue weight, serum levels of ALP, ALT, AST, and total and conjugated bilirubin, which were determined at the end of the study (after 7 weeks treatment). Microscopic histology of liver samples with hematoxylin and eosin staining (magnification: ×100 and ×200) was also performed.

Statistical Analysis

Data were analyzed as mean ± SD using GraphPad Prism version 6. ANOVA with Tukey-Kramer post-test was used to compare the means of different groups. $P < 0.05$ was considered significant.

RESULTS

The qualitative phytochemical analysis revealed the presence of saponins, flavonoids, tannins, phytosterols, and cardiac glycosides, but absence of alkaloids and anthraquinones [Table 1].

Effect of the Aqueous Leaf Extract of PP on Serum Levels of Liver Enzymes, Total and Conjugated Bilirubin, and Relative Liver Weights

Reduction in serum levels of ALP and AST was significant in the PP extract-treated group [Table 2]. No significant difference in the serum levels of alanine transaminase (ALT), and total and conjugated bilirubin [Table 2].

Effect of the Aqueous Leaf Extract of PP on Weight Gain, White Adipose Tissue Mass, and Liver weight

The HFD-fed control group of animals exhibited a significant increase in weight gain and white adipose tissue mass compared to the negative control group on normal diet [Table 3]. On the other hand, compared to the HFD-fed group, a significant reduction in white adipose tissue mass, but not in weight gain, was observed in the group treated with the conventional drug Orlistat [Table 3]. Treatment with the PP extract (at 500 mg/kg) resulted in a significant reduction in the body weight gain and the adipose tissue mass when compared with the HFD-treated control group. However, no significant difference in the relative liver weights at autopsy was observed between the six groups [Table 3].

Table 1: Phytochemical constituents of the aqueous leaf extract of *Phyllanthus pentandrus*

Phytochemical	Result of qualitative phytochemical analysis
Alkaloids	Negative
Cardiac glycosides	Positive
Saponins	Positive
Phytosterols	Positive
Anthraquinones	Negative
Flavonoids	Positive
Tannins	Positive

Histological Examination of the Liver

HFD-fed group showed severe liver steatosis, which was ameliorated in the PP-treated group. The liver appeared normal in normal control and ORL-treated groups [Figures 1a-d].

DISCUSSION

The use of HFD is currently the most common strategy of inducing NAFLD in rats.[14] The standard chow used to induce NAFLD in this study contains 7% fat and 15% protein. By adding moderate amount of sheep fat (40 g/100 g diet and whole milk powder (20 g/100 g diet), the resultant HFD contains about 11% protein (since the whole milk contains 27% protein), a level, though low that will not lead to deleterious effects.[15,16] The added sheep tallow (derived from perirenal fat by low-temperature cooking) consists, predominantly, of saturated fat, which is more likely to induce lipid accumulation in the liver, weight gain, and insulin resistance in rodents than unsaturated fat.[17] The animals fed with a HFD developed fatty liver disease (evidenced by macrovesicular and microvesicular liver steatosis) as well as a significant increase in weight gain and white adipose tissue mass. The elevation in the level of

Table 2: Effect of the aqueous leaf extract of *Phyllanthus pentandrus* on liver enzymes (ALP, AST, and ALT) and bilirubin (total and conjugated)

Treatment groups	ALP (U/l)	AST (U/l)	ALT (U/l)	Total bilirubin (mg/dl)	Conjugated bilirubin (mg/dl)
Normal control	41.91±12.93^{b}	101.22±32.20^{a}	25.06±4.34^{a}	0.31±0.18^{a}	0.13±0.06^{a}
HFD control	102.54±18.42^{a}	107.54±14.91^{a}	30.22±11.75^{a}	0.26±0.15^{a}	0.10±0.07^{a}
ORL+HFD	99.32±54.41^{a}	115.52±14.23^{a}	25.43±4.50^{a}	0.33±0.26^{a}	0.14±0.08^{a}
PP+HFD	46.12±2.90^{b}	47.40±14.71^{b}	27.01±4.22^{a}	0.43±0.07^{a}	0.21±0.06^{a}

Values are the mean±standard deviation (n=6). Means with different lower case superscripts in the same column are significantly different at P<0.05.
HFD: High-fat diet, ORL: Orlistat, PP: *Phyllanthus pentandrus*

Table 3: Effect of the aqueous leaf extract of *Phyllanthus pentandrus* on weight gain, adipose tissue weight, and liver weight

Treatment groups	Weight gain at week 7	White adipose tissue weight (g/100 g body weight)	Liver weight (g/100g body weight)
Normal control	30.86±8.64^{b}	1.11±0.30^{c}	3.02±0.24^{a}
HFD control	70.50±13.12^{a}	5.04±1.22^{a}	3.73±1.01^{a}
ORL+HFD	53.50±9.83^{a}	2.12±0.61^{bc}	2.72±0.44^{a}
PP+HFD	17.88±10.40^{b}	3.10±0.83^{b}	3.31±0.80^{a}

Values are the mean±standard deviation (n=6). Means with different lower case superscripts in the same column are significantly different at P<0.05.
HFD: High-fat diet, ORL: Orlistat, PP: *Phyllanthus pentandrus*

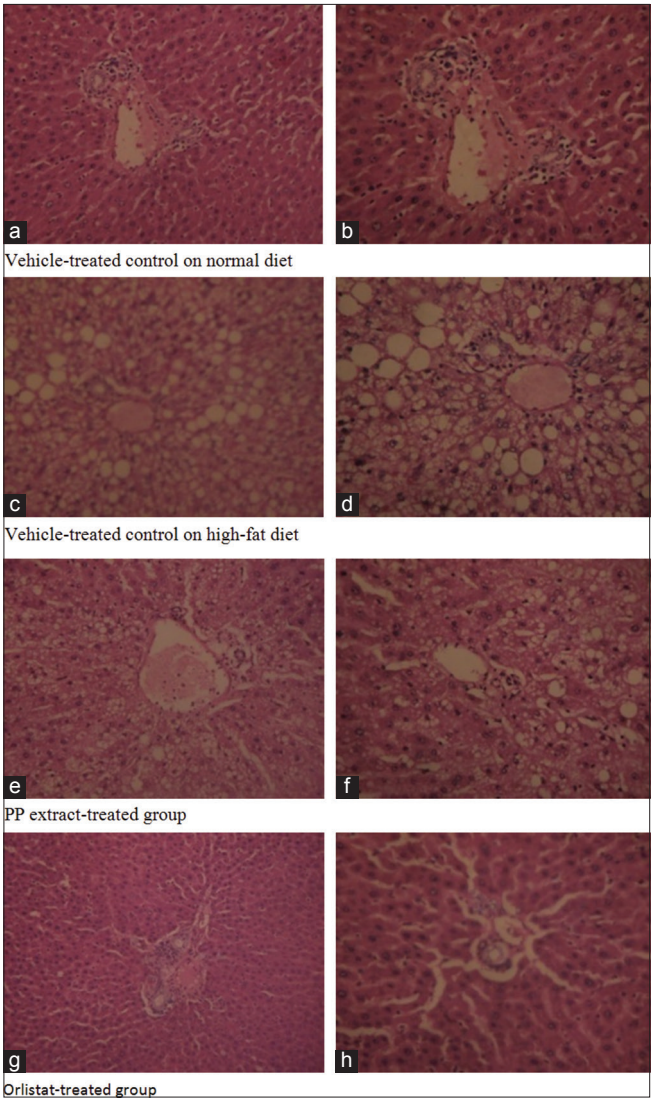

Figure 1: Effect of aqueous leaf extract of *Phyllanthus pentandrus* and orlistat on high-fat diet **(**HFD**)**-induced liver steatosis after 7 weeks of treatment. Hematoxylin and eosin staining for microscopic histopathology of liver (magnification: ×100 and ×200) showing portal triad composed of portal vein, hepatic artery, and biliary duct surrounded by normal hepatocytes for normal rats administered with vehicle (a and b) and rats administered with HFD + orlistat (g and h) Rats administered with HFD + vehicle for 7 weeks showed severe steatosis (c and d), which was ameliorated by treatment with the PP extract (e and f)

the enzyme ALP (present in bile canaliculi) is indicative of obstructive liver disease. However, this study did not find any significant elevation in serum levels of other liver enzymes commonly tested for evaluation of liver, namely AST and ALT. Both orlistat and the PP extract ameliorated the observed fatty infiltration in the liver and the increase in adipose tissue mass. The PP extract, but not orlistat, reduced the observed body weight gain and brought back to normal the elevated ALP enzyme. Furthermore, contrary to orlistat, administration of the PP extract was beneficial in reducing the level of AST enzyme. Elevation in the serum level of this enzyme (found in hepatocellular cytosol) suggests liver disease; hence, reducing its level is a desirable pharmacological effect. The current study revealed that the aqueous leaf extract of PP possesses some phytochemical constituents such as tannins, cardiac glycosides, saponins, flavonoids, and phytosterols, but lacks alkaloids and anthraquinones.

The normal levels of ALT and AST in the HFD-fed rats as observed in this study agree with previous findings using a similar rat model of non-alcoholic steatohepatitis.[18] This is not surprising, since even in humans, up to 79% of patients with non-alcoholic steatohepatitis have normal serum levels of aminotransferase enzymes[19,20] and some patients with hepatic fibrosis as a sequela of steatohepatitis may present with a normal level of ALT.[21] Furthermore, an isolated elevation of ALP has been documented in some patients with NAFLD.[22] The observed potential beneficial effect of PP extract agrees with the findings that some members of the genus *Phyllanthus* ameliorated the features of NAFLD.[9,10] In addition, the body weight lowering effect of the extract is consistent with findings of previous studies which reported antiobesity activity of some species in the genus.[8] These properties may be, at least partly, attributed to the documented antioxidant and anti-inflammatory activities of some members of the genus *Phyllanthus*.[23-25] The observed beneficial effect of orlistat in reducing hepatic steatosis has been documented.[26] The drug is an inhibitor of pancreatic and gastric lipases, leading to reduction in the absorption of dietary fats.[27] The lack of beneficial effect of the standard drug orlistat in reducing the levels of liver enzymes, as found in this study, has previously been documented.[28] This indicates the superiority of PP extract over the standard drug in this respect. Some of the phytochemicals identified in the extract have been documented to possess hepatoprotective[29-32] and antiobesity properties [33-35] and any of them, alone or in combination, could, therefore, be contributory to such activities observed in the extract.

Strength and Limitations of the Study

The current study is the first to report the potential beneficial effect of PP in reducing hepatic steatosis and causing weight loss in an animal model of NAFLD. The study has several limitations. Firstly, the constituents of the extract have not been precisely characterized using high-performance liquid chromatography and mass spectrometry. However, we have qualitatively identified certain phytoconstituents that have been found to exhibit hepatoprotective and antiobesity properties. In addition, we only investigated the effect of the PP extract at a single dose of 500 mg/kg/day (chosen based on a preliminary study showing it to be well tolerated by the rats).

CONCLUSION

Aqueous extract of PP possesses hepatoprotective as well as body weight lowering effects; thus, it may be potentially useful in managing NAFLD.

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