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Hypolipidemic Effect of Aqueous Fruit Extract of Doum Palm (*Hyphaene Thebaica*) in Wistar Rat

Abdullahi A.N.[1*], Abdulmumin Y.[1], Abdulmumin T.M.[1], Sheshe S.M.[1], Ismail S.Y.[1], Murtala M.[1], Ibrahim A.M.[1], Hassan M.K.[1], Bichi S.A.[1], Sarki S.I[1]. and Abubakar S.[2]

[1]*Department of Biochemistry, Kano University of Science and Technology, Wudil along Maiduguri/Gaya Road, P.M.B. 3244, Kano state Nigeria.*

[2]*Department of Biochemistry and Molecular Biology, Federal University Birnin Kebbi, along Kalgo-Bunza Road, P.M.B. 1157, Birnin Kebbi, Kebbi State, Nigeria.*

[*]Correspondence:
Abdullahi Nazifi Abdullahi, Department of Biochemistry, Kano University of Science and Technology, Wudil, along Maiduguri/Gaya Road, P.M.B 3244, Kano state Nigeria, +2348024579597.

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ABSTRACT

Atherosclerotic cardiovascular diseases are characterized by strokes and coronary artery disease. Hyperlipidemia and free radicals generation in the body are among the main risk factors for cardiovascular diseases. Statin drugs such as lovastatin and atorvastatin are mostly prescribed for the treatment of hyperlipidemia. However, many patients show intolerance toward these drugs and develops mild symptoms, which include myalgia and cramps, to the most severe Rhabdomyolysis. In this study, we have qualitatively determined the phytochemicals in Doum (Hyphaene thebaica) fruit aqueous extract and the antioxidant activity using the DPPH assay. Furthermore, we have investigated the hypolipidemic potential of the extract in Wistar rats. Freshly prepared aqueous extract of the Doum fruit with concentration 200 - 400 mg/kg and 20mg/kg of atorvastatin were orally administered to the Wistar rats fed with high - fat diet for two weeks and were sacrificed for hyperlipidemic analysis. The qualitative determination of the phytochemicals indicates the presence of steroids, saponin, tannins, phlobatannins, terpenoids, alkaloid, glycoside, and flavonoids. The fruit extract shows antioxidant activity by scavenging the DPPH radicals with IC_{50} of 128 µm/mL. Both the fruit extract and atorvastatin decreased serum total glyceride cholesterol, total cholesterol, LDL, and increased HDL level significantly ($P < 0.05$). This result revealed the anti-hyperlipidemic, and antioxidant potential of Doum fruit. Therefore, it could be useful in managing hyperlipidemia, thereby reducing the risk factor of cardiovascular diseases in the end.

Keywords

Antioxidant, Doum fruits, Hyperlipidemia, Wistar rats.

Introduction

Hyperlipidemia is one of the major risk factors for atherosclerosis diseases such as stroke and coronary artery disease. Hyperlipidemia is defined as increase in plasma lipid concentrations (total cholesterol, low-density lipoprotein, and total triglycerides) [1]. However, oxidation of low-density lipoprotein by free radicals produced in the body accelerates the progression of atherosclerosis [2]. Lowering serum lipid concentrations and using antioxidants to scavenge the free radicals to be important steps in preventing cardiovascular disease. Statins (a group of hypolipidemic drugs) for example, have been shown to effectively lower serum lipid concentrations, through the inhibition of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase enzyme, which convert HMG-CoA to mevalonic acid during cholesterol synthesis in the liver [3]. However, the statins are not tolerated by some patients they show adverse effects from mild symptoms, which include myalgia (muscle pain), and cramps, to the most severe Rhabdomyolysis (muscle death) [4].

The search for an alternative to the usage of these statin drugs example using plant products cannot be overemphasized. Plants contain bioactive compounds otherwise known as phytochemicals that are produced as secondary metabolites to protect them from external threats [5]. These phytochemicals have therapeutic potential in humans; they are responsible for the antibacterial [6],

antioxidant [7], and antidiabetic activity [8] in humans. It is worthy to note that, the anti-hyperlipidemia effect of the plant extract has also been reported [9].

The *Hyphaene thebaica,* a Doum palm desert tree belongs to *Arecaceae* family. It is widely distributed in some parts of Africa and commonly alongside the Nile River in Egypt, and Sudan [10]. For the fact this plant survived a harsh desert condition, the plant system must have device a means of survival by producing enough antioxidants to scavenge the free radical generated due to oxidative stress and hence, this plant can be a good source of different bioactive compounds to suit our study. We have selected the fruit of this plant because it is the only edible part and consumed by the local populace. We prepared the aqueous extract and evaluated its anti-hyperlipidemic potential in Wistar rats. Moreover, we have investigated the antioxidant activity using a 2, 2-diphenyl-1-picrylhydrazyl (DPPH) assay.

Materials and Methods

Plant Material

Hyphaene thebaica fruits were purchased at Wudil market during a dry season in Wudil local government, Kano State, Nigeria. It was identified in the Biology Department at Kano University of Science and Technology.

Plant Extract

100 g of the powdered sample was soaked in 500 cm^3 of distill water for 24 hours. The mixture was filtered using Whatman filter paper No. 1. Volume of the extract administered to the Wistar rats was determined from a given relation:

$$Vol.\,cm^3 = \frac{weight\ of\ the\ rat(kg) \times dose(mg/kg)}{concentration\ of\ the\ extract(mg/cm^3)}$$

Phytochemical Screening

The phytochemicals were determined according to the previous studies; tannins, saponin, steroids, phlobatannins, terpenoids [11], alkaloid, glycoside [12], and flavonoids [13].

DPPH Radical Scavenging Assay

The scavenging Assay of the extract solution was conducted according to the previous study [13] with modifications. Briefly, 1 ml of the aqueous extract with concentrations ranging from 0.2 to 0.8 mg/ml was added to 1 ml of DPPH (1 ml, 0.135 mM) produced in methanol. The reaction mixture was vortexed to ensured complete mixing. The absorbance of the mixture was recorded at 517 nm using spectrophotometer and the scavenging ability was calculated using the given equation:

$$DPPH\ scavenging\ activity\ (\%) = \frac{Abs_{control} - Abs_{sample}}{Abs_{control}} \times 100$$

Where:

$Abs_{control}$ is the absorbance of DPPH + methanol and Abs_{sample} is the absorbance of DPPH radical + sample (sample or standard)

The IC_{50} was then calculated from the % inhibition.

Experimental Animals

Male Wistar rats with average weights 63g - 111g were purchased from the Biology department Bayero University Kano. They were housed in polycarbonate cages with dimension (48×33×19 cm) in the department of Biochemistry, Kano University of Science and Technology Wudil for the period of six weeks. They were provided with food, water, standard conditions, temperature (24 ± 1°C), relative humidity 55-70% with dark and light cycle (12h - 12h) and the environment be kept clean throughout the period. Ethical approval regarding the use of animals was obtained from the research and ethical committee of Kano University of Science and Technology, Wudil.

Formulation of High-Fat Diet

The high-fat diet was formulated by mixing standard vital feed diet (growers pelletized manufactured by grand cereals Ltd.) with egg yolk, and coconut oil in a percentage of 60%, 10%, and 30%, respectively.

Experimental Design

Forty-eight Wistar rats were divided into six groups A, B, $C_{1\text{-}3}$, and D consisting of eight rats each. Group A is the first control group and were given standard vital feed throughout the six weeks without administering the extract or the atorvastatin. After one week of acclimatization and adapting to the environment, Group B the second control was given the formulated diet throughout the five weeks without any treatment administration. Groups $C_{1\text{-}3}$ and D were fed the formulated diet for five weeks and this group was orally administered with fruit extract of *Hyphaene thebaica* at different concentrations of 200 mg/kg, 300 mg/kg, and 400 mg/kg and atorvastatin 20 mg/kg respectively for the last two weeks of the experiment.

Blood Serum Lipid Profile

After the animals were sacrificed, the serum was analyzed to measure the total cholesterol, triglyceride, low-density lipoproteins (LDL), and high-density lipoproteins (HDL) levels. All the analysis was ran using diagnostic kits made by micro lab (Merck Microlab Germany).

Statistical Analysis

All measurements were repeated in triplicate and analyses of variance (ANOVA) were carried out in Microsoft excel, differences in mean values $p < 0.05$ were considered significant. The graph is plotted using Veusz version 3.4 (a scientific plotting application copyright © 2003-2021).

Results and Discussion

Phytochemical Screening

A preliminary phytochemical screening of aqueous Doum fruit extract is presented in **Table 1.** The phytochemicals identified include tannins, saponins, steroids, phlobatannins, terpenoids, alkaloids, glycosides and flavonoids. The presence of these numerous phytochemicals is an advantage to the plant's ability to withstand and survive in the arid region and exert health benefits to humans against oxidative stress and other related diseases [15].

Table 1: Phytochemicals screened in Doum Fruit aqueous Extracts.

Phytochemicals	Status
Tannins	+
Saponins	+
Steroid	+
Phlobatannins	+
Terpenoids	+
Alkaloids	+
Glycoside	+
Flavonoids	+
The plus sign (+) indicates the presence of the compound	

DPPH Radical Scavenging Assay

The antioxidant activity of Doum fruit aqueous extract and the ascorbic acid (control standard antioxidant) was investigated using the DPPH assay and the result is presented in Figure 1 as mean values ± standard deviation. From the figure, it is indicated that both the tested sample and the control have antioxidant activity by scavenging DPPH* free radicals at all concentrations. The oxidized form of DPPH* is soluble and stable in methanol formed deep violet color solution and was reduced to DPPH-H in the presence of the antioxidant to form yellow solution [15]. Absorbance of the solution was measured at 517 nm and presented as percentage inhibitions of DPPH corresponding to the antioxidant activity.

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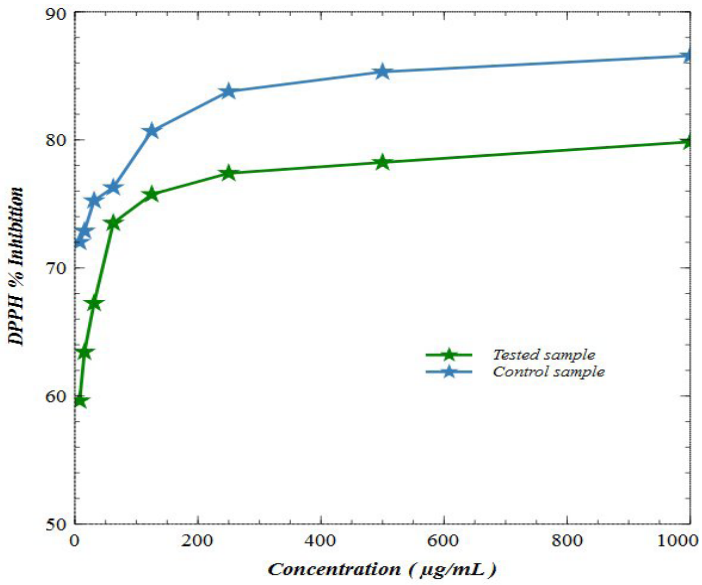

Figure 1: % inhibition of DPPH by different concentrations of the Tested sample (Doum fruit extract) and control (Ascorbic acid).

From our previous phytochemicals screening in this study, the aqueous fruit extract contained numerous phytochemicals, these phytochemicals are polyphenol compounds enriched with hydroxyl groups. Therefore, the antioxidant activity of the fruit extract could be emanated from different polyphenols and their abundance in the extract to reduce the oxidized DPPH* radical by donating their hydrogen atoms. This is evident from the strong antioxidant activity of the fruit extract even at low concentration with calculated IC_{50} % value of 128 µg/mL. The IC_{50} value of 128 µg/mL shows the tested sample have scavenged 50% of the DPPH* at 128 µg/mL. The lower IC_{50} value indicate a strong antioxidant activity [16].

Blood Serum Lipid Profile

The results of blood serum lipid profile of the Wistar rats after treatment with the fruit extract and the standard drug atorvastatin group C_{1-3} and D respectively are given in Table 2. Before the treatment, the Wistar rats were fed with a high-fat diet (40% lipid) for five weeks to induce hyperlipidemia, our approach is similar to the previous studies [17] whereby hyperlipidemia was induced after feeding rats with a high–fat diet (41% lipid) for two weeks. After two weeks and they were given the treatments. Both the treatment in the said groups decreased the serum total cholesterol, total glyceride LDL, and increased the HDL levels significantly ($p < 0.05$) compared to the two control groups A and B.

Table 2: Lipid profile of the Wistar rats.

Group n = 6	Total Cholesterol	Total Glyceride	HDL[a]	LDL[b]
A	4.33 ± 0.15	1.23 ± 0.35	1.8 ± 0.17	2.2 ± 0.31
B	6.55 ± 0.05	1.6 ± 0.50	2.45 ± 0.55	3.65 ± 0.55
C_1	3.20 ± 0.10	0.6 ± 0.10	2.35 ± 0.05	0.60 ± 0.10
C_2	3.25 ± 0.05	1.55 ± 1.15	2.10 ± 0.50	0.45 ± 0.05
C_3	2.90 ± 0.10	1.05 ± 0.05	1.85 ± 0.75	0.85 ± 0.05
D	3.65 ± 0.05	0.90 ± 0.10	2.55 ± 0.15	0.65 ± 0.05
The results are presented as mean ± SD. *P-value* compared with all groups $p < 0.05$ is considered significant.				

The mechanism of the effect exerted by atorvastatin, one of the statin groups of the hypolipidemic drug was explained in detail elsewhere [3]. The mechanism of hypolipidemic activity of the fruit extract can be explained from two viewpoints. The fruit extract may interfere with and reduced lipids absorption from the gastrointestinal tract. Usually, serum lipid absorbed from the gastrointestinal tract and or is synthesize by the *de novo* pathway (an endogenous synthesis pathway) [18]. The other possible reason could be attributed to the antioxidants present in the extract as we have shown the antioxidant activity of this extract in the previous section. These antioxidants prevent the LDL oxidation thereby lowering the serum cholesterol level [19].

Conclusion

The phytochemicals in the aqueous extract of the Doum fruit were qualitatively determined; it shows the presence of tannins, saponins, steroids, phlobatannins, terpenoids, alkaloids, glycoside, and flavonoids. These compounds in the aqueous extract shows a good antioxidant activity by scavenging DPPH free radicals with a low IC_{50} value of 128 µm/mL. We further investigated the hypolipidemic effect of this aqueous extract in Wistar rats that were fed with a formulated high-fat diet and found it to significantly reduced the serum total cholesterol, total glyceride, and LDL and increase the HDL level.

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