Lipid-Lowering Effect of *Parkia Biglobosa* Leaf Saponins in Triton-X 1339-Induced Hyperlipidemic Rats

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ABSTRACT

*Parkia biglobosa* has popular ethnomedicinal use in the treatment of cardiovascular-related disorders. Hyperlipidemia has been described as the greatest risk factor contributing to the prevalence and severity of coronary heart diseases. Here, we investigated the effect of saponins from *Parkia biglobosa* leaf on triton-induced hyperlipidemic rats. Hyperlipidemia was induced in wistar rats by single intraperitoneal (i.p) injections of Triton X-1339 at a dose of 350 mg/kg b.w following oral pretreatment with *P. biglobosa* saponin mixture (PBS) (30- or 60mg/kg/day) or nicotinic acid (500 mg/kg/day) for five days. Another group of rats received PBS (40 mg/kg/day) alone. Animals were euthanized by decapitation 20 h after triton induction to harvest serum used in biochemical analysis. PBS prevented triton-induced increase in serum cholesterol, triglyceride, low density lipoprotein (LDL) and decrease in high density lipoprotein (HDL) in a statistically significant manner (P<0.05) and reversed the elevated serum LDL/HDL ratio comparably to nicotinic acid. PBS alone also caused significantly reduced serum lipid profile in rats. The results demonstrated the mitigation of triton-induced hyperlipidemia in rats by PBS. Consequently, we can hypothesize that part of the *P. biglobosa*-mediated therapeutic effects is associated with its hypolipidemic components.

Keywords: Hyperlipidemia; saponin; Parkia biglobosa; Triton –X-1339

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INTRODUCTION

*Parkia biglobosa* (Jacq.) Benth., commonly called the African locust bean is a perennial deciduous tree that has dark green and alternate bipinnate leaves [1]. It is known to occur in a diversity of agro ecological zones ranging from tropical rain forests to arid zones and therefore found scattered around the many countries of West Africa where it is a popular food plant. The Hausa people of Northern Nigeria call it Dorowa while amongst the Yoruba it is known as Igba or Irugba. The seeds of *P. biglobosa* are fermented to make a strong smelling tasty food rich in protein popularly called Iru- by the Yoruba people of South-western Nigeria or Dawadawa – by the Hausa people of Northern Nigeria. *Parkia biglobosa* (Jacq.) Benth., tree was largely prescribed in traditional medicine for its multiple medicinal virtues in South western Nigeria. The bark, seeds and leaves were prescribed for the treatment of arterial hypertension, piles, amoebiasis, bronchitis, cough, burn, zoster, and abscess [1].

The presence of phytochemicals like saponins, tannins, flavonoids and cardiac glycosides in *Parkia biglobosa* has been reported [2,3]. The medicinal values of plants lie in their component phytochemicals, which produce definite physiological actions on the human body [4]. Several biological effects have been ascribed to saponins. Saponins from different sources have been shown to lower serum cholesterol levels in a variety of animals including human subjects as well as causing selective reduction of the more harmful LDL-cholesterol in the serum of rats and human subjects (5,6).

Hyperlipidemia, ranked as one of the greatest risk factors contributing to the prevalence and severity of coronary heart diseases, is characterized by elevated serum total cholesterol, low-density lipoprotein and decreased high-density lipoprotein levels [7]. Hyperlipidemia associated lipid disorders are considered to cause atherosclerotic cardiovascular disease. Treatment of patients with hyperlipidemia is usually aimed at reducing the risk of developing ischemic heart disease or the occurrence of further cardiovascular or cerebrovascular disease [8].

Triton X-1339 belong to the same class of drugs with Triton WR 1339 (Tyloxapol: oxyethylated tertiary octyl phenol formaldehyde polymer) and Triton-X-100 (polyoxyethylene octyl phenyl ether or octyl phenol ethoxylate). They are non-ionic surfactant which interferes with the uptake of plasma lipids [9], and have been widely used to block clearance of triglyceride-rich lipoproteins to induce acute hyperlipidemia in several animals [10].

Finding sustainable hypolipidemic agents from natural sources that could replace some of the currently available synthetic ones is now receiving much attention. This is crucial owing to a number of side effects associated with the use of synthetic hypolipidemic drugs [11].

The paucity of information on the hypolipidemic effect of *Parkia biglobosa* saponins has therefore necessitated the current research aimed at providing scientific information on the effects of saponins from study plant on triton-induced hyperlipidemia in rats.
MATERIALS AND METHODS

Chemicals

Triton -X-1339 (A non-ionic detergent, Isooctyl poly oxyethylene phenol) and solvents used were obtained from Qualikems Chemicals Co, India. Water used was glass distilled.

Collection of Plant Materials

Fresh leaves of Parkia biglobosa were collected in Isua-Akoko, Ondo State, Nigeria. Botanical Identification and authentication were carried out at the herbarium of the Forestry Research Institute (FRIN) Ibadan, Oyo state, Nigeria where a voucher specimen (no 109603) was deposited.

Isolation of Saponins from Parkia biglobosa leaf

200g of ground sample was extracted with 1L of petroleum ether (40-60°C) in a soxhlet extractor for 12 hours. The air-dried, defatted sample was extracted with methanol (500ml) for 12 hours. The methanolic extract was partitioned between mixture of n-butanol and water (1:1.v/v). After a thorough shaking and allowing to stay overnight, the n -butanol layer was separated. The aqueous layer was washed five times with aliquots of n -butanol until it became colourless. The pooled butanolic layer was evaporated in vacuo to give a residue which was dissolved in 100ml methanol and precipitated by adding a large amount of diethyl ether to obtain a solid crystalline compound [12].

Qualitative tests for P. biglobosa saponins

Confirmatory tests for saponins were carried out as described by El-Olemy et al. [13].

Frothing test: 0.5ml of extracted saponin-rich aliquot was diluted in equal volume of water and shaken strongly. Presence of relatively persistent froth was observed.

Emulsifying properties: A portion of aliquot was diluted in two portions of distilled water. Equal volume of castor oil was added followed by violent shaking for several minutes and observed for the presence of stable thick white emulsion.

Hemolytic effect on red blood cell (RBC): 5ml of 5% suspension of RBC in normal saline solution was put into test tubes A and B. 5ml of normal saline solution was added to A while B contained saponin fraction in which 0.045g NaCl had previously been added to render it isotonic with normal saline. The two tubes were shaken gently. B was observed for the formation of a clear red liquid indicative that the red blood cells have been hemolysed by the surfactant effect of saponins on the RBCs. A was observed for opacity due to the intactness of the red blood corpuscles.
Animals

Adult male albino rats (wistar strain) weighing between 250-280g, purchased from the Central Animal house of University of Ibadan were used for the study. They were housed in the primate colony of the Department of Biochemistry, Federal University of Technology, Akure, Nigeria. The animals were kept in wire mesh cages under controlled light cycle (12h light/12h dark), fed with commercial rat chow (Vital Feeds Nigeria Limited) ad libitum, and liberally supplied with water. The protocol of study was approved by the appropriate review board of the ethic committee of the Institution and conforms to the guidelines of National Institute of Health (NIH publication 85-23, 1985) for laboratory animal care and use.

Experimental design

Animals were divided into six groups (I-V) of six animals per group.

**Group I:** Animals in this group received distilled water only throughout the duration of the experiment and served as the control.

**Group II:** Animals received single intraperitoneal (i.p) dose of Triton X-1339 (350 mg/kg) only on day 5 of the experiment.

**Group III:** Animals were administered Nicotinic acid (500 mg/kg b.w) by gavage once daily for 5 consecutive days and later administered Triton X-1339 (350 mg/kg) on the 5th day.

**Group IV:** Animals were administered *Parkia biglobosa* saponins (30 mg/kg) by gavage once daily for 5 consecutive days and later administered Triton X-1339 (350 mg/kg) on the 5th day.

**Group V:** Animals were administered *Parkia biglobosa* saponins (60 mg/kg) by gavage once daily for 5 consecutive days and later administered Triton X-1339 (350 mg/kg) on the 5th day.

**Group VI:** Animals were administered *Parkia biglobosa* saponins (40 mg/kg) by gavage once daily for 5 consecutive days only.

Animals were euthanized by decapitation 20h after triton induction and blood collected through cardiac puncture to prepare the serum for biochemical analyses.

Biochemical Parameters

The serum levels of total cholesterol, triglycerides, low density lipoprotein and high density lipoprotein were measured in experimental animals using assay kits from Randox Laboratories Ltd., UK according to the instructions of the manufacturer.

Statistical analysis

The data were analysed using one-way analysis of variance followed by Newman-Keuls Multiple Comparison Test. The significance level was set at $p < 0.05$. Statistical Analysis and graphing were performed using Graph Pad Prism (ver.5.0a).

RESULTS
Qualitative tests conducted on the isolate from *Parkia biglobosa* showed positive results for saponins (Table 1). Triton-X 1339 (350 mg/kg i.p) caused dramatic and statistically significant increase (P<0.001) in serum cholesterol and triglyceride levels of rats when compared with the normal control rats (Fig 1 and 2). This effect was inhibited significantly in the groups pretreated with *P. biglobosa* saponins (PBS). Pretreatment with *P. biglobosa* saponins (30- and 60 mg/kg b.w) and nicotinic acid (500 mg/kg) prevented triton-induced hypercholesterolemia and hypertriglyceridemia in rats. *P. biglobosa* saponins (40 mg/kg) alone decreased serum triglyceride and cholesterol levels. The hypotriglyceridemic effect of PBS was significant (P<0.01).

**Table 1:** Qualitative screening for saponins isolated from *P. biglobosa* leaf

<table>
<thead>
<tr>
<th>Tests</th>
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<tr>
<td>Frothing test</td>
<td>Persistent froth</td>
<td>Positive for saponins</td>
</tr>
<tr>
<td>Red blood cell lytic test</td>
<td>Hemolysis of red blood cells</td>
<td>Positive for saponins</td>
</tr>
<tr>
<td>Emulsifying test</td>
<td>Formation of stable thick white emulsion</td>
<td>Positive for saponins</td>
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Statistically significant decrease in serum HDL and increase in serum LDL level was observed in the rats treated with the ionic surfactant alone (Fig 3 and 4). Triton effects on serum levels of lipoprotein were inhibited in PBS- and nicotinic acid pretreated animals. A dose-dependent inhibition of triton-induced increase in serum LDL was observed in PBS (30- and 60 mg/kg) pretreated rats. Nicotinic acid and PBS (30 mg/kg) pretreatment resulted in serum HDL levels that were significantly the same as that of the normal control. PBS (40 mg/kg) alone however, caused significant reduction in both serum HDL and LDL levels of rats.

![Graph showing effect of saponins from *Parkia biglobosa* leaf on serum cholesterol levels of Triton-X-1339 intoxicated rats.](image)

**Fig 1:** Effect of saponins from *Parkia biglobosa* leaf on serum cholesterol levels of Triton-X-1339 intoxicated rats.
TRT 350: Triton treated group; NIC 500 + TRT 350: Nicotinic acid pretreated group; PBS 30 + TRT 350 & PBS 60 + TRT 350: groups pretreated with Parkia biglobosa saponins 30 & 60mg/kg; PBS 40: group treated with Parkia biglobosa saponins 40 mg/kg alone. Values are expressed as mean±SD (n=6). ***P<0.001 vs control; #P<0.05 vs TRT 350.

Fig 2: Effect of saponins from Parkia biglobosa leaf on serum triglyceride level of Triton-X-1339 intoxicated rats.

Serum LDL: HDL ratio was significantly increased (P<0.001) in serum of rats treated with triton alone when compared with the normal control rats. *P. biglobosa* saponins (30- and 60 mg/kg) and nicotinic acid pretreated groups showed significantly reduced serum LDL: HDL ratio when compared with triton-induced group. Rats pretreated with 30 mg/kg PBS and those placed on 40 mg/kg PBS alone showed serum LDL: HDL ratio that was not statistically different from the control values (Fig 5).

Fig 3: Effect of saponins from *Parkia biglobosa* leaf on serum LDL level of Triton-X-1339 intoxicated rats.
TRT 350: Triton treated group; NIC 500 + TRT 350: Nicotinic acid pretreated group; PBS 30 + TRT 350 & PBS 60 + TRT 350: groups pretreated with Parkia biglobosa saponins 30 & 60mg/kg; PBS 40: group treated with Parkia biglobosa saponins 40 mg/kg alone. Values are expressed as mean±SD (n=6). *P<0.05, **P<0.01, ***P<0.001 vs control; #P<0.05 vs TRT 350.

Fig 4*: Effect of saponins from Parkia biglobosa leaf on serum HDL level of Triton-X-1339 intoxicated rats.

Fig 5*: Effect of saponins from Parkia biglobosa leaf on serum LDL: HDL ratio of Triton-X-1339 intoxicated rats.

TRT 350: Triton treated group; NIC 500 + TRT 350: Nicotinic acid pretreated group; PBS 30 + TRT 350 & PBS 60 + TRT 350: groups pretreated with Parkia biglobosa saponins 30 & 60mg/kg; PBS 40: group treated with Parkia biglobosa saponins 40 mg/kg alone. Values are expressed as mean±SD (n=6). **P<0.01, ***P<0.001 vs control; #P<0.05 vs TRT 350.
DISCUSSION

The use of triton-induced hyperlipidemia has been described as an important approach to screen the action of natural or chemical hypolipidemic drugs [14].

Triton is widely used to block clearance of triglyceride-rich lipoproteins to induce acute hyperlipidemia in several animals [10] and induction of hyperlipidemia by a parenteral administration of a dose of Triton to adult rats have been demonstrated [14]. In the present study, the hyperlipidemic effect of triton-X 1339 in rats was manifested by the significant increase in serum levels of cholesterol, triglyceride and low density lipoprotein. Hyperlipidemia associated lipid disorders are considered to cause atherosclerotic cardiovascular disease [15], with hypercholesterolemia and hypertriglyceridemia being closely related to ischemic heart disease [16]. In the same vein, increased level of serum LDL-cholesterol results in increased risk for the development of atherosclerosis [17].

The reduction of total cholesterol in triton-treated rats by *P. biglobosa* saponins was associated with a decrease of its LDL fraction which is the target of several hypolipidemic drugs. The hypotriglyceridemic effect of PBS was also evident and compared well with the standard nicotinic acid. LDL-cholesterol possesses a risk of cardiovascular disease when it invades endothelium and becomes oxidized rendering it more easily retained by the proteoglycan. Increase in this fraction of cholesterol is thus associated with atherosclerosis, heart-attack, stroke and peripheral vascular disease [18].

This activity of *P. biglobosa* saponins might result from the rapid catabolism of LDL-cholesterol through its hepatic receptors for final elimination in the form of bile acids [19]. Specifically, saponin is known to elicit serum cholesterol lowering activity by causing resin-like action, thereby reducing the enterohepatic circulation of bile acids [20]. In the process, the conversion of cholesterol to bile acid is enhanced in the liver resulting in concomitant hypocholesterolemia [21]. Saponins are also reported to increase the lipoprotein lipase activity (LPL) which is considered as helpful in faster removal of free fatty acids from circulation that causes in turn a decrease in cholesterol [22]. They are known to reduce the uptake of certain nutrition especially cholesterol at the gut through intraluminal physiochemical interactions. Saponins have strong affinity for the aglycone moiety of membrane sterols, particularly cholesterol, with which they form insoluble complexes [23] and this is believed to be the basis of both their hypocholesterolic [24] and hemolytic effects [23].

HDL-cholesterol can remove cholesterol from antheroma within arteries and transport it back to the liver for its excretion or reutilization, thus high level of HDL-cholesterol protect against cardiovascular disease [25]. *P. biglobosa* saponins at the study doses might have an HDL-cholesterol boosting effect since a significant inhibition of triton-induced reduction in serum HDL-cholesterol was observed. Increased cholesterol excretion and decreased cholesterol absorption through gastrointestinal tract upon PBS pretreatment might be responsible for the observed increase in level of HDL-cholesterol and the corresponding decrease in total cholesterol and LDL-cholesterol levels. On the overall, the hypolipidemic effect
of P. biglobosa saponins was demonstrated by the observed decrease in serum lipid profile of rats placed on PBS alone.

The LDL/HDL-cholesterol ratio has been described as a valuable tool to evaluate coronary heart disease risk and is increasingly being recognized as a stronger risk predictor of cardiovascular diseases than each lipid parameter [26,27]. Changes in ratios have been shown to be better indicators of successful CHD risk reduction than changes in absolute levels of lipids or lipoproteins [28,29]. It is also an excellent monitor for the effectiveness of lipid-lowering therapies [28,30]. That PBS was able to reduce triton-induced elevation in LDL/HDL-cholesterol ratio may be lending pharmacological support to its lipid-lowering efficacy and the possibility of cardioprotection by P. biglobosa as often described in folkloric medicines.

CONCLUSION

This study has been able to demonstrate the hypolipidemic effect and the improvement in serum lipid profile of triton-induced hyperlipidemic rats by Parkia biglobosa saponins. This probably suggests that PBS could recover the disorders in lipid metabolism noted in hyperlipidemic state and possibly serve as a useful hypolipidemic agent. Consequently, we can hypothesize that part of the P. biglobosa-mediated therapeutic effects may be associated with its hypolipidemic components.

REFERENCES