The National Ribat University

Faculty of Graduate Studies and Scientific Research

Invivo Hypocholesterolemic Effect of *Adansonia digitata* Ethanolc Fruits Extract on Rats

A THESIS SUBMITTED IN THE FULFILLMENT OF THE REQUIREMENTS OF M.Sc. DEGREE IN HUMAN NUTRITION AND DIETETICS PROGRAM

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قال الله تعالى:

(والأَرض مَدَّنَاهَا وَأَلْقَيْنَا فِيهَا رُوَاسِيَ وَأَنْبِتْنَا فِيهَا مِن كُلِّ شَيْءٍ مَّوْزُونٍ)

صدق الله العظيم [الحجر: 19]
Dedication

I dedicate this thesis to my family for their matchless affection and dedicated partnership for success.

Ryan Mustafa El Siddig
Acknowledgement

I would like to express my appreciation to Dr. Reem Hassan Ahmed for her guidance during this study, without her a valuable assistance this work would not have been completed.

Also I indebted to National Ribat University, Faculty of Medicine and postgraduate Faculty.

Finally, a very special thanks to my husband Dr. Khadir Ibrahim Khadir for his support and encouragement.
Abstract

The present study was aimed to investigate the potential hypocholesterolemic effect of ethanolic extract of *Adansonia digitata* in two different doses (200, 400 mg/kg) in induced hypercholesterolemic rats for a period of 14 days.

Twenty five Wistar Albino rats were divided into five groups five animals each. Animals' weights were recorded (60-92 g). Serum levels of (total cholesterol, triglycerides, LDL low-density lipoproteins and HDL high-density lipoproteins - cholesterol) were determined at the beginning and after completion of the experiments.

The first group was fed with normal diet and act as control group (control 1). Groups from two to five (2-5) were fed with high cholesterol diet (HCD) for 2 weeks. Then rats' weights and serum readings were evaluated. Group (2) was administered orally with 20 mg/day of the standard drug atorvastatin daily for two weeks. 200, 400 mg/kg of *Adansonia digitata* were administered orally as a single dose per day to induced hypercholesterolemic rats (groups 3, 4) for a period of 14 days. Group (5) was left as un-treated and considered as (control 2) group.

At the end of the four weeks period of experimentation, results showed that 400 mg/kg of *Adansonia digitata* extract caused a reduction in the serum total cholesterol level and in the low density lipoprotein levels after 28 days. While 200mg/kg of the extract showed a decreasing effect on the triglyceride levels.

Thus, these findings shows that fruits of *A. digitata* extract has a potential hypocholesterolemic effect on hypercholesterolemia induced by feeding of high cholesterol diet and a beneficial lowering effect on Low density lipoprotein levels.
المستخلص

هدفت هذه الدراسة للتحقق من التأثير المحتمل الخافض للكوليسترول للمستخلص الابثنولي للتبلدي لجرعتين مختلفتين (200, 400 ملجم/كلجم) على الفئران التي سبق رفع الكوليسترول في الدم لديها لمدة 14 يوم.

قسمت 25 من فئران التجربة لخمس مجموعات بواقع خمس فئران في كل مجموعة. سجل الوزن ومستويات دهون الدم (الكوليسترول الكلي، الجليسيريدات الثلاثية، والليبيبروتينات مرتفعة ومنخفضة الكثافة) في بداية التجربة وبعد انتهاء مدتها.

المجموعة الأولى غذت على وجبة عادية ومثلت مجموعة كنترول (كنترول 1) للمجموعات من الثانية إلى الخامسة (2-5) غذت على وجبة عالية الدهون والكوليسترول لمدة أسبوعين، ثم قدرت مستويات دم ونسبة الوزن.

المجموعة الثانية أعطيت بالفم 20 ملجم/اليوم من الأتورفاستاتين يوميأ لمدة أسبوعين، ثم أجريت قراءة مستويات دم ونسبة الوزن.

المجموعة الثالثة أعطيت بالفم 200، 400 ملجم/كلجم من مستخلص التبلدي أعطيت بالفم كجرعة واحدة في اليوم للفئران مرتفعة كوليسترول الدم (المجموعة 3.4) لمدة 14 يوم.

المجموعة الخامسة تُرِكت من دون معالجة واعتبرت (كنترول 2).

بمجرد الانتهاء من 4 أسابيع (مدة التجربة) أظهرت النتائج أن 400ملجم/كلجم من المستخلص سبب نقصاً ملحوظاً في مستوي الكوليسترول الكلي والليبيبروتينات منخفضة الكثافة، بينما 200ملجم/كلجم من المستخلص أظهرت تأثير خافض لمستوي الجليسيريدات الثلاثية.

لذلك تظهر هذه المعطيات أن مستخلص ثمرة التبلدي تأثير خافض للكوليسترول في الفئران التي لديها ارتفاع كوليسترول الدم (ولا يوجد بها جدال عالية المحتوى من الكوليسترول) ومفيد في تخفيض مستوي الليبيبروتينات منخفضة الكثافة.
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Chapter one

Introduction

Hypercholesterolemia as a disease means elevated blood cholesterol level. High serum cholesterol level and specifically a high LDL cholesterol is one of the key causes of CHD (coronary heart disease), stroke, and mortality. (Mahan and Escott, 2008).

Medicinal and aromatic plants and their derivatives represent an integral part of life in Sudan. Indigenous remedies are the only form of therapy available to the majority of poor people. It has been estimated that only 11% of the population has access to formal health care.

Examples of some medicinal plants from different vegetative regions of Sudan and their medicinal uses are: Boscia senegalensis (Kursan; Mukheit used locally as Anti-rheumatic, against gonorrhea, for urinary tract inflammations, as Anthelmintic, for eye wash and against tuberculosis. Senna alexandrina Mill. (Senna Mekka) is used as Laxative and against gastro-intestinal disorders “GID”. Acacia Senegal (L.) Willd. (Hashab) is used against stomach ulcers and as anti-diarrheal (Hassan et al., 2012).

The medicinal value of these plants lies in some chemical substances that produce a definite physiological action on the human body. The most important of these bioactive constituents of plants are alkaloids, tannins, flavonoids, and phenolic compounds. Many of these indigenous medicinal plants are used as spices and food plants. They are also sometimes added to foods meant for pregnant and nursing mothers for medicinal purposes. (Edeoga et al., 2005).
Several studies has been directed towards the evaluation of several naturally occurring botanicals and herbs, potentially useful as nutraceutical ingredients. Ethnobotanical research was recently focused on *Adansonia digitata* L. (*Bombacaceae*), an African plant known as baobab tree. Leaves, bark and fruits of this tree are traditionally employed in several African regions as foodstuffs and for medicinal purposes, and for that reason baobab is also named “the small pharmacy” or “chemist tree” (*Vertuani et al.*, 2002).

Thus, the current study is directed to evaluate the potential capability of *A. digitata* L. fruits in the treatment of experimentally induced hypercholesterolemia.

**Objectives**

1. To verify the traditional use of *A. digitata* in the treatment of hypercholesterolemia.

2. To evaluate the potential hypocholesterolemic effect of *A. digitata*.

3. To determine its efficiency level as a hypocholesterolemic agent.

**Justification:** Major phytochemical constituents of *A. digitata* were proved to have anti–oxidant, antimicrobial, antiviral and anti- inflammatory activities. And dietary phytosterols content which can inhibit the absorption of both dietary and biliary cholesterol.

**Chapter two**

**Literature review**

2.1 Hyperlipidemia
Hyperlipidemia is defined as an elevated level of triglycerides and cholesterol. It is one of the most common complications in increasing the risk of premature atherosclerosis, coronary and myocardial infarction, which in turn are major causes of cardiovascular (CV) morbidity and mortality. Hyperlipidemia and reduced high-density lipoproteins (HDL-C) occur as a consequence of several interrelated factors which may be lifestyle, genetic, metabolic or other conditions that influence plasma lipoprotein metabolism. (Saxena and Saxena, 2009).

Cardiovascular diseases constitute one of the leading causes of death. Elevation of serum cholesterol, more particularly low density lipoprotein cholesterol (LDL.c) is a primary risk factor of cardiovascular diseases and reduction of (LDL.c) levels and increase of high density lipoprotein (HDL c) reduces the risk of cardiovascular diseases (James et al., 2005).

The current anti-hyperlipidemic therapy includes principally Statins and Fibrates medicines. The Statins act by inhibiting the biosynthesis of cholesterol while Fibrates act by enhancing the clearance of triglyceride rich lipoproteins.

It is thought that investigation of lipid lowering activity of natural products will be a useful strategy in the discovery of new lead molecules eliciting improved activity with fewer side effects. Medicinal plants extract maintaining the lipid metabolism can be used in treating hyperlipidemia of varied etiology factors (Saxena and Saxena, 2009).

Hence, reduced levels of dietary cholesterol are desirable. This can be achieved by reduction of animal fats in the diet and / or by removal of cholesterol from animal fats by supercritical carbon dioxide extraction or molecular distillation (Srinivasan et al., 2008).
2.2 Hypercholesterolemia

It is thought to be a contributing factor in heart disease because high serum cholesterol, is common in clients with atherosclerosis. Atherosclerosis is a cardiovascular disease in which plaque (fatty deposits containing cholesterol and other substances) forms on the inside of artery walls, reducing the space for blood flow. When the blood cannot flow through an artery near the heart, a heart attack occurs. When this is the case near the brain, a stroke occurs. It is considered advisable that blood cholesterol levels not to exceed 200 mg/dl (200 milligrams of cholesterol per 1 deciliter of blood). A reduction in the amount of total fat, saturated fats, and cholesterol level and an increase in the amounts of monounsaturated fats in the diet, weight loss, and exercise all help to lower serum cholesterol levels. Soluble dietary fiber also is considered helpful in lowering blood cholesterol because the cholesterol binds to the fiber and is eliminated via the feces, thus preventing it from being absorbed in the small intestine. In some cases, medication may be prescribed if diet, weight loss, and exercise do not sufficiently lower serum cholesterol. Because the development of plaque is cumulative, the preferred means of avoiding or at least limiting its development is to limit cholesterol and fat intake throughout life. If children are not fed high-cholesterol foods on a regular basis, their chances of over consuming them as adults are reduced. Thus, their risk of heart attack and stroke is also reduced (Ruth, 2011).

2.3 Hypercholesterolemia prevalence

Hypercholesterolemia prevalence is estimated as 17.6% among children and adolescents, with strong association of family history for cardiovascular disease risk factors. (Santiago et al., 2002). In a
prospective cardiovascular trial, male participants, ages 40 to 65 years, who had been free of myocardial infarction or stroke at the time of entry and had been followed up for 4 years, longitudinal data analysis shows that hypertension, diabetes mellitus, and hyperlipidemia are independent risk factors for CHD. The concomitant occurrence of these factors leads to a cumulative increase in CHD risk. Hyperlipidemia is a more significant risk factor for CHD than hypertension or diabetes mellitus. (Assmann and Schulte, 1988). A previous study conducted among 4043 men and 1333 women, ages 50 to 65 years, showed that more than 50% of all diabetics are hypertensive and cholesterol is slightly increased in male hypertensives and diabetics of either sex, whereas low-density lipoprotein cholesterol is slightly raised in male hypertensives and female diabetics only. The serum triglyceride concentrations are higher for hypertensives and markedly higher for diabetics of both sexes. High-density lipoprotein cholesterol concentrations are decreased in hypertensives, especially in hypertensive women, and even more so in diabetics. The European Consensus Conference for primary prevention of CHD has classified hyperlipidemia into five groups (A to E). For hypertensives, the proportion of patients in group D (cholesterol between 200 and 300 mg/dl and triglyceride levels between 200 and 500 mg/dl) is 20.4% for men and 6.2% for women, about twice as high as those in the control groups. The occurrence of combined (group D) or massive hyperlipidemia (group E: cholesterol >300 mg/dl and/or triglycerides >500 mg/dl) is prevalent in more than 30% of all diabetics: two to three times more frequently than in nondiabetic patients. When concomitant hypertension is included, this prevalence increases to more than 40% for diabetic men. Among those patients endangered by three risk factors, approximately 40% of all men and 60% of all women have the particularly atherogenic combination that includes lowered high-density lipoprotein cholesterol (Assmann and Schulte, 1988).
Hypertension and hypercholesterolemia are important modifiable risk factors for cardiovascular disease (CVD), thus, increased efforts to improve treatment of these conditions are needed (Wong et al., 2006).

2.4 Cholesterol

It is not a true fat but a fat-like substance that exists in animal foods and body cells. (Ruth, 2011). Is the major sterol found in animal lipids and can be found in plant lipids as a minor sterol component. The hydroxyl group at carbon 3 of sterols makes these compounds surface active. Cholesterol therefore can orient itself into cell membranes where it is important in stabilizing membrane structure. It is also important because it is the precursor of the synthesis of bile acids and 7 dehydrocholesterol is the precursor for the production of vitamin D in the skin by ultraviolet irradiation.

Cholesterol is essential for the synthesis of sex hormones, cortisone, and is needed by every cell in the body. The body manufactures 800 to 1,000 mg of cholesterol a day in the liver. Cholesterol is a common constituent (part) of one’s daily diet because it is found so abundantly in egg yolk, fatty meats, shellfish, butter, cream, cheese, whole milk, and organ meats (liver, kidneys, brains, sweetbreads). (Srinivasan et al., 2008).

2.4.1 Trans Fatty Acids

Trans fatty acids have recently gained attention in their unique role in heart disease through their ability to both increase LDL-cholesterol and decrease high-density lipoprotein (HDL) cholesterol. This behavior is partially due to the geometric configuration of Trans fatty acids that is more similar to saturated fatty acids than unsaturated fatty acids. Originally, trans fatty acids are included in the unsaturated fatty acid. While a large
amount of research has been devoted to the negative aspects of dietary lipids on health, evidence is growing that some dietary lipids can reduce the risk of several diseases. These bioactive lipids include ω-3 fatty acids, phytosterol, carotenoids.

2.4.2 Phytosterols

The major phytosterols in food are sitosterol, campesterol, and stigmasterol. Dietary phytosterols are practically nonabsorbed in gastrointestinal tract. Their bioactivity lies in the fact that they can inhibit the absorption of both dietary and biliary (produced by intestinal cells) cholesterol. The intake of 1.5-2g per day of phytosterols can reduce LDL-cholesterol by 8-15%. Since phytosterols primarily inhibit cholesterol absorption, they are most effective when consumed with a cholesterol containing meal. (Srinivasan et al., 2008)

2.5 Hypocholesterolemic medicinal plants:

The leaves of Moringa oleifera Lam (Moringaceae) are used by the Indians in their herbal medicine as a hypocholesterolemic agent in obese patients. (Ghasi et al., 2000).

Lipid lowering effect of T. foenum-graecum (fenugreek seed) extract was evaluated in high fat diet induced hyperlipidemic models of albino rats. Aqueous seed extract of fenugreek (120 mg/kg, p.o.) inhibited the elevation in plasma cholesterol in high fat diet administrated rats (Saxena and Saxena, 2009).

In a rabbit model of atherosclerosis, a protein isolate from L. albus reduced cholesterolaemia and exerted a remarkable protective activity against atherosclerosis progression. (Marchesi et al, 2008)
2.6 *Adansonia digitata*

Family: (Bombacaceae) is a majestic tree revered in Africa for its medicinal and nutritional value (*Kamatou et al., 2011*).

2.6.1 Morphological description and ethnobotanical uses

The genus *Adansonia*:

The genus comprises deciduous trees, some massive and up to 30m tall, others such as A. gibbosa less than 10m, and two species, A. rubrostipa and A. madagascarensis 5-20m. Crowns are usually compact and trunks taper from top to bottom or are large and cylindrical or bottle-shaped. Diameter of trunk can vary 2-10m. Bark is colored red to grey and the inner bark possesses longitudinal fibers. Wood is soft and arranged in sheets with mucilaginous gum produced when damaged. (*Sidibe and Williams, 2002*).

Baobab is a very long-lived tree with multipurpose uses. The different plant parts are widely used as foods, medicines and the bark fibers are also used. The tree provides food, shelter, clothing and medicine as well as material for hunting and fishing. Every part of the baobab tree is reported to be useful. (*Caluwe, 2010*). Bark of baobab is used for the treatment of fever in Nigeria. Drinking of the aqueous extract of bark of *A. digitata* is used in Nigeria traditional medicine as treatment of sickle cell anemia.

Leaves are used medicinally as a diaphoretic and as astringent. They have hypo-sensitive and antihistamine properties, which are used to treat kidney and bladder diseases, asthma, general fatigue, diarrhoea, guinea worm. The baobab fruit pulp is therapeutically employed as febrifuge, analgesic, anti-dysentery and for the treatment of small pox and measles. (*Ganiyat et al., 2010*). The leaves of the baobab tree are a staple for many populations in Africa, especially the central region of the continent. Young
leaves are widely used, cooked as spinach, and frequently dried, often powdered and used for sauces over porridges, thick gruels of grains, or boiled rice. The baobab fruit pulp is probably the most important foodstuff. It can be dissolved in water or milk. The liquid is then used as a drink, a sauce for food, a fermenting agent in local brewing, or as a substitute for cream of tartar in baking (Caluwe, 2010). Leaves and fruits are eaten, commonly used as medicine. Phytochemical investigation revealed the presence of flavonoids, phytosterols, amino acids, fatty acids, vitamins and minerals. It is used in scurvy related diseases, laxative purpose, antidiabetic, anti-diarrhoeal, anti-trypanosomal activities (Sundarambal et al., 2015). The dry baobab fruit pulp have particularly high values for carbohydrates, energy, calcium, potassium (very high), thiamine, nicotinic acid and vitamin C (very high). When eaten raw, the pulp is a rich source of calcium and vitamins B and C. It contains sugars but no starch, and is rich in pectins. Baobab seeds can be eaten fresh, or they may be dried and ground into a flour which can either be added to soups and stews as a thickener, or roasted and ground into a paste, or boiled for a long time, fermented and then dried for use (Caluwe, 2010).

2.6.2 Chemical Composition of Adansonia digitata:

Phytochemical investigation revealed the presence of flavonoids, phytosterols, amino acids, fatty acids, vitamins and minerals. The seeds are a source of significant quantities of lysine, thiamine, calcium and iron (Kamatou et al., 2011). The pulp is acidic, due to the presence of the organic acids citric, tartaric, malic, succinic and ascorbic, with pH 3.3 (Caluwe, 2010).

2.6.3 Nutritional value of Adansonia digitata

Chadare et al; (2009) estimated the contribution of baobab pulp to the recommended daily intake (RDI) for energy, carbohydrates and protein for
children and pregnant women. The reported lowest and highest carbohydrate content of the pulp allows coverage of 21.5% and 40.6% of the RDI when 60 g is consumed by a child. A consumption of 100 g pulp will cover 26 to 50% of the carbohydrate RDI for pregnant women. On the other hand, the energy content of the pulp is rather low when compared with the RDI for children and pregnant women.

2.6.4 Biological activities of Adansonia digitata:

It is known that free radicals play a fundamental role in several diseases. The biochemical damages caused by free radicals to cells and tissues, lead to the development of diseases such as artherio-sclerosis, high blood pressure, cancer, inflammation, renal failure and liver disease (Latifou et al., 2012).

The major interest on baobab product relies in its ascorbic acid (AA, Vitamin C) and dietary fibers content. In particular baobab fruit pulp represents the most important natural sources of AA, while the leaves are characterized by the content on provitamin A. In this regards, the baobab fruit pulp can be considered a highly valuable source containing levels of vitamin C ranging from 2.8-3 g/kg (Vertuani et al., 2002).

Also it was found that Adansonia digitata leaves, fruit-pulp and seeds were acquired and extracted with three different solvents showed anti-viral activity against influenza virus, herpes simplex virus and respiratory syncytial virus (Vimalanathan and Hudson, 2009). Methanolic and hydroalcoholic extract of adansonia digitata inhibited the growth of S. aureus; S. aureus methicillin resistant (SARM) and S. epidermidis. (Latifou et al., 2012)
Chapter three

Materials and Methods

3.1 Materials:

3.1.1 Plant Material

*Adansonia digitata* L. (Bombacaceae) fruit specimen was purchased from a local market in Khartoum State- Sudan (March 2016). It was authenticated by taxonomists in Medicinal and Aromatic Plants Research Institute- National Centre for Research- Sudan.
3.1.2 Animals

Twenty five Male Albino Wister rats with a body weight of (60-92 g) were obtained from National Laboratory Animal House- National Centre for Research. They were kept within the premises of Medicinal and Aromatic Plants Research Institute in a standard conditions.

3.2 Methods:

3.2.1 Plant extraction:

One Kg of *Adansonia digitata* fruit sample, was macerated in one Leter of 80% ethanol for about seventy two hours with daily filtration and evaporation. Solvent was evaporated under reduced pressure to dryness using rotary evaporator until the extract was dried. *(Ganiyat et al., 2010).*

Yield percentage was calculated as followed:

\[
\text{Crude plant: 1000g} \\
\text{Weight of extract: 90.5 g} \\
\text{Yield = (Weight of extract / weight of crude plant)*100} \\
\text{= 90.5/1000× 100 = 9.05%}
\]

3.2.2 Dosage calculation:

Doses are calculated as follows:

Every 200 and/or 400 mg of the extract are used per 1 kilogram of rat’s weight (200 and or 400 mg/kg body weigh).

Concentration of the extract:

40 mg of extract is freshly dissolved in 1 ml of distilled water (40 mg/ml).
3.2.3 Blood samples:

Blood samples were obtained from the retro-orbital plexus of rats. Blood was centrifuged at 5000 r.p.m. and plasma was collected for the evaluation of biochemical parameters determined by Enzymatic Endpoint Method with a spectrophotometer. (Marti and Okid 2009)

3.2.3 Experimental animals Model:

Twenty five Albino Wister rats were divided into five groups five animals each. Animals' weights were recorded. Serum levels of total cholesterol, triglycerides, LDL-cholesterol, HDL-cholesterol) were determined at the beginning of the experiments (zero readings).

The first group one was fed with normal diet and act as the (control1) group.

Groups' two to five (2-5) were fed high cholesterol diet (HCD) for 2 weeks. Water was provided ad libitum to rats.

Then rats weights and serum readings (total cholesterol, triglycerides, LDL-cholesterol, HDL-cholesterol) were evaluated.

Group (5) was left un-treated and considered as (HCC) (control2) group.

Group (2) was administered orally with 20 mg/day of atorvastatin daily for two weeks. (Marti and Okid, 2009).

Groups (3) and (4) were administered daily with 200 and 400 mg/kg body weight of Adansonia digitata extract for two weeks respectively.

At the end of the four weeks period of experiment, serum total cholesterol, triglycerides, LDL-cholesterol, HDL-cholesterol were re-evaluated (Saxena and Saxena, 2009).

3.2.4 Preparation of High fat diet:

The composition of the high fat diet was adapted from Yugarani et al. (1992). The entire ingredients were individually weighed; wheat flour,
minced meat and egg they were mixed well. Water was added to the above mixture. High fat diet was prepared manually (Saxena and Saxena, 2009).

3.2.5 Statistical analysis:

Data generated were subjected to SAS. Two factor CRD was assessed; where factor A = days and factor B = treatment. Percent changes of all parameters were calculated, and then means were separated according to DMRT. (Montgomery, 2001).
Chapter Four

Results

4.1 Effect of *Adansonia digitata* ethanolic extract on body weight (gm) of rats:

Effect of administration of *A. digitata* ethanolic extract on the means of the initial body weights (gm) of experimental rats, 14 and 28 days after experimentation is shown in Table [1], Figure (1).

In the group that was fed with the high cholesterol diet and treated with ( 20mg) Atorvastatin (HCA), there was an increase of ( 8.1 %) and (13.2%) in the means of body weight of rats after 14 and 28 days of treatment respectively, however, these increments were not significant at $( P \leq 0.05)$.

The group of rats that were fed with the high cholesterol diet and treated with 200 mg/kg body weight of *A. digitata* extract, there was an increase of (21.2% ) and ( 34.6 % ) on days 14 and 28 of experiment respectively, however it was also not significant.

The group fed the high cholesterol diet and treated with 400 mg/kg of extract showed an obvious weight gain which was found to be (19.5%) after 14 days of treatment.

In the group fed the high cholesterol diet with no treatment and acted as control group, (HCC), there was an increase of (18.9%) and a significant increase $(P \leq 0.05)$ of (40.8 %) on days 14 and 28 of experiment respectively.

Table (1): Effect of *Adansonia digitata* extract on body weight (gm) of experimental rats
<table>
<thead>
<tr>
<th>Treatment</th>
<th>Initial</th>
<th>14 days</th>
<th>28 days</th>
<th>Overall treatments</th>
</tr>
</thead>
<tbody>
<tr>
<td>A(NDG)</td>
<td>80.40&lt;sup&gt;def&lt;/sup&gt; ±12.22</td>
<td>78.80&lt;sup&gt;def&lt;/sup&gt; ±8.41</td>
<td>87.20&lt;sup&gt;bcddef&lt;/sup&gt; ±14.06</td>
<td>82.13&lt;sup&gt;BC&lt;/sup&gt;</td>
</tr>
<tr>
<td>B(HCA)</td>
<td>74.20&lt;sup&gt;ef&lt;/sup&gt; ±9.78</td>
<td>80.20&lt;sup&gt;def&lt;/sup&gt; ±16.18</td>
<td>84.00&lt;sup&gt;cdef&lt;/sup&gt; ±23.23</td>
<td>79.46&lt;sup&gt;C&lt;/sup&gt;</td>
</tr>
<tr>
<td>C(HCE200)</td>
<td>76.40&lt;sup&gt;def&lt;/sup&gt; ±14.12</td>
<td>92.60&lt;sup&gt;abcde&lt;/sup&gt; ±14.54</td>
<td>102.80&lt;sup&gt;abcd&lt;/sup&gt; ±17.61</td>
<td>90.60&lt;sup&gt;AB&lt;/sup&gt;</td>
</tr>
<tr>
<td>D(HCE400)</td>
<td>79.80&lt;sup&gt;def&lt;/sup&gt; ±8.98</td>
<td>95.40&lt;sup&gt;abcd&lt;/sup&gt; ±10.19</td>
<td>108.25&lt;sup&gt;a&lt;/sup&gt; ±6.72</td>
<td>94.48&lt;sup&gt;A&lt;/sup&gt;</td>
</tr>
<tr>
<td>E(HCC)</td>
<td>71.00&lt;sup&gt;f&lt;/sup&gt; ±5.96</td>
<td>84.40&lt;sup&gt;bcdef&lt;/sup&gt; ±10.29</td>
<td>100.00&lt;sup&gt;abc&lt;/sup&gt; ±9.38</td>
<td>85.13&lt;sup&gt;ABC&lt;/sup&gt;</td>
</tr>
<tr>
<td>Overall days</td>
<td>76.36&lt;sup&gt;C&lt;/sup&gt;</td>
<td>86.28&lt;sup&gt;B&lt;/sup&gt;</td>
<td>96.45&lt;sup&gt;A&lt;/sup&gt;</td>
<td></td>
</tr>
</tbody>
</table>

P-value | 0.0358<sup>*</sup>

Lsd<sub>0.05</sub> | 16.31

SE± | 5.767

Mean±SD values having different superscripts (small letters for interaction and capital for overalls) are significantly different (P≤0.05).

17
**Key:**

A ≡ Normal diet (control-1)

B ≡ High cholesterol diet (20 mg of Atrovastation)

C ≡ High cholesterol diet (extraction 200 mg)

D ≡ High cholesterol diet (extraction 400 mg)

E ≡ High cholesterol diet (control-2)

---

Fig. (1): Effect of *Adansonia digitata* ethanolic extract on body weight of experimental rats
4.2 Effect of *A. digitata* extract on serum cholesterol level (mg/g):

The effect of administration of *A. digitata* ethanolic extract on serum cholesterol level of experimental rats is presented on Table [2] and figure (2).

Animals fed with high cholesterol diet and the standard drug Atorvastatin (HCA) has showed a significant decrease (P≥0.05) after 28 days of treatment (39.8 %) compared to the initial level before treatment.

After 28 days of treatment, the serum cholesterol level of the group of rats fed with high cholesterol diet and 200 mg/kg of *A. digitata* extract, the serum cholesterol level maintained the normal range.

In the group that was fed with high cholesterol diet and 400 mg/kg of extract (HCE 400), the serum cholesterol level showed a decrease of (20 %) after 28 days after treatment compared to its initial level however it was not proven statistically.

Control rats given high cholesterol diet without treatment (HCC) cholesterol level showed a significant increase (P≥0.05) on day 14 (27.1 %) however it come down to normal range on day 28.
Table (2): Effect of *Adansonia digitata* ethanolic extract on serum cholesterol level (mg/g):

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Initial</th>
<th>14 days</th>
<th>28 days</th>
<th>Overall treatments</th>
</tr>
</thead>
<tbody>
<tr>
<td>A(NDG)</td>
<td>76.26&lt;sup&gt;abc&lt;/sup&gt; ±12.54</td>
<td>84.00&lt;sup&gt;ab&lt;/sup&gt; ±5.14</td>
<td>46.43&lt;sup&gt;ef&lt;/sup&gt; ±12.67</td>
<td>68.90&lt;sup&gt;AB&lt;/sup&gt;</td>
</tr>
<tr>
<td>B(HCA)</td>
<td>63.42&lt;sup&gt;cd&lt;/sup&gt; ±8.67</td>
<td>86.20&lt;sup&gt;a&lt;/sup&gt; ±11.03</td>
<td>38.20&lt;sup&gt;f&lt;/sup&gt; ±5.10</td>
<td>62.61&lt;sup&gt;B&lt;/sup&gt;</td>
</tr>
<tr>
<td>C(HCE200)</td>
<td>60.08&lt;sup&gt;de&lt;/sup&gt; ±4.88</td>
<td>86.53&lt;sup&gt;a&lt;/sup&gt; ±19.41</td>
<td>69.32&lt;sup&gt;bcd&lt;/sup&gt; ±14.47</td>
<td>71.98&lt;sup&gt;A&lt;/sup&gt;</td>
</tr>
<tr>
<td>D(HCE400)</td>
<td>55.57&lt;sup&gt;de&lt;/sup&gt; ±8.43</td>
<td>81.64&lt;sup&gt;ab&lt;/sup&gt; ±11.40</td>
<td>44.99&lt;sup&gt;ef&lt;/sup&gt; ±5.55</td>
<td>60.73&lt;sup&gt;B&lt;/sup&gt;</td>
</tr>
<tr>
<td>E(HCC)</td>
<td>59.35&lt;sup&gt;de&lt;/sup&gt; ±9.35</td>
<td>75.46&lt;sup&gt;abc&lt;/sup&gt; ±11.25</td>
<td>56.74&lt;sup&gt;de&lt;/sup&gt; ±13.75</td>
<td>64.00&lt;sup&gt;AB&lt;/sup&gt;</td>
</tr>
<tr>
<td>Overall days</td>
<td>63.03&lt;sup&gt;B&lt;/sup&gt;</td>
<td>82.77&lt;sup&gt;A&lt;/sup&gt;</td>
<td>51.14&lt;sup&gt;C&lt;/sup&gt;</td>
<td></td>
</tr>
</tbody>
</table>

P-value 0.0027**

Lsd<sub>0.05</sub> 13.90

SE± 4.915

Mean±SD values having different superscripts (small letters for interaction and capital for overalls) are significantly different (P≤0.05).
Fig. (2): Effect of *Adansonia digitata* ethanolic extract on cholesterol level of experimental rats

**Key:**

A(NDG) ≡ Normal diet group (control-1)
B(HCA) ≡ High cholesterol diet (20 mg of Atrovastation)
C(HCE200) ≡ High cholesterol diet and 200 mg/kg extract
D(HCE400) ≡ High cholesterol diet and 400 mg/kg extract
E(HCC) ≡ High cholesterol diet without treatment (control-2)
4.3 Effect of *A. digitata* extract on serum triglycerides level (mg%):

The effect of *A. digitata* extract on serum triglycerides level is shown in Table [3] and figure (3).

Results showed that rats given high cholesterol diet and Atorvastatin (HCA), showed a significant decrease (P≥0.05) in the triglyceride level on day 28 of treatment estimated by (56.6 %) when compared with the initial level.

While rats given high cholesterol diet and 200 mg/kg of *A. digitata* extract (HCE200) showed a decrease in the triglyceride level after 28 days of treatment estimated by (29 %) compared to its initial level but was not proven statistically.

The other group of rats that were given high cholesterol diet and 400 mg/kg of *A. digitata* extract in the triglyceride content kept the same level after 28 days of treatment compared to the initial level.

The triglyceride level of control un- treated group that was given high cholesterol diet (HCC) was kept in the same level after 28 days of experimental time.
Table (3): Effect of *Adansonia digitata* ethanolic extract on T.G level (mg%) of experimental rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Initial</th>
<th>14 days</th>
<th>28 days</th>
<th>Overall treatments</th>
</tr>
</thead>
<tbody>
<tr>
<td>A(NDG)</td>
<td>48.74&lt;sup&gt;cd&lt;/sup&gt; ±9.37</td>
<td>56.36&lt;sup&gt;bcd&lt;/sup&gt; ±18.07</td>
<td>14.60&lt;sup&gt;f&lt;/sup&gt; ±2.32</td>
<td>39.90&lt;sup&gt;C&lt;/sup&gt;</td>
</tr>
<tr>
<td>B(HCA)</td>
<td>66.51&lt;sup&gt;abc&lt;/sup&gt; ±13.09</td>
<td>78.02&lt;sup&gt;a&lt;/sup&gt; ±12.83</td>
<td>28.86&lt;sup&gt;ef&lt;/sup&gt; ±14.54</td>
<td>57.80&lt;sup&gt;AB&lt;/sup&gt;</td>
</tr>
<tr>
<td>C(HCE200)</td>
<td>61.18&lt;sup&gt;abcd&lt;/sup&gt; ±10.48</td>
<td>74.84&lt;sup&gt;ab&lt;/sup&gt; ±26.91</td>
<td>43.41&lt;sup&gt;de&lt;/sup&gt; ±14.09</td>
<td>59.81&lt;sup&gt;A&lt;/sup&gt;</td>
</tr>
<tr>
<td>D(HCE400)</td>
<td>46.66&lt;sup&gt;cde&lt;/sup&gt; ±6.11</td>
<td>57.93&lt;sup&gt;bcd&lt;/sup&gt; ±8.81</td>
<td>42.24&lt;sup&gt;de&lt;/sup&gt; ±8.56</td>
<td>48.95&lt;sup&gt;BC&lt;/sup&gt;</td>
</tr>
<tr>
<td>E(HCC)</td>
<td>56.44&lt;sup&gt;bcd&lt;/sup&gt; ±13.61</td>
<td>64.89&lt;sup&gt;abc&lt;/sup&gt; ±12.46</td>
<td>48.00&lt;sup&gt;cd&lt;/sup&gt; ±14.68</td>
<td>56.44&lt;sup&gt;AB&lt;/sup&gt;</td>
</tr>
<tr>
<td>Overall days</td>
<td>55.91&lt;sup&gt;B&lt;/sup&gt;</td>
<td>66.41&lt;sup&gt;A&lt;/sup&gt;</td>
<td>35.42&lt;sup&gt;C&lt;/sup&gt;</td>
<td></td>
</tr>
</tbody>
</table>

P-value 0.0261<sup>*</sup>
Lsd<sub>0.05</sub> 17.11
SE± 6.049

Mean±SD values having different superscripts (small letters for interaction and capital for overalls) are significantly different (P≤0.05).
Fig. (3): Effect of *Adansonia digitata* ethanolic extract on T.G level of experimental rats

**Key:**
A(NDG) ≡ Normal diet group (control-1)
B(HCA) ≡ High cholesterol diet (20 mg of Atrovastation)
C(HCE200) ≡ High cholesterol diet and 200 mg/kg of extract
D(HCE200) ≡ High cholesterol diet and 400 mg/kg of extract
E(HCC) ≡ High cholesterol diet (control-2)
4.4 Effect of *Adansonia digitata* extract on HDL level (mg%):  

The effect of *Adansonia digitata* extract on levels of high-density lipoproteins is shown in Table [4] Figure (4)  

Levels of high-density lipoproteins in the group of rats that was given high cholesterol diet and Atorvastatin (HCA) has showed a significant decrease (P≥0.05) by (46.8%) after 28 days experimental.

In group that given high cholesterol diet and 200 mg of extract showed no change in the HDL level after 28 days of experiment and it remained unaffected. While the group of rats fed with high cholesterol diet and 400 mg/kg of *A. digitata* extract were subjected to a significant decrease (P≥0.05) by (23.2%) after 28 days of treatment.

Animals fed with high cholesterol diet and act as control (HCC), has showed a significant decrease (P≥0.05) in high-density lipoproteins level after 28 days of treatment (20.1%) compared to the initial level before treatment.
Table (4): Effect of *Adansonia digitata* extract on HDL level (mg%) of experimental rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Initial</th>
<th>14 days</th>
<th>28 days</th>
<th>Overall treatments</th>
</tr>
</thead>
<tbody>
<tr>
<td>A(NDG)</td>
<td>72.09&lt;sup&gt;abc&lt;/sup&gt; ±15.41</td>
<td>85.89&lt;sup&gt;a&lt;/sup&gt; ±16.95</td>
<td>48.15&lt;sup&gt;e&lt;/sup&gt; ±8.32</td>
<td>68.71&lt;sup&gt;A&lt;/sup&gt;</td>
</tr>
<tr>
<td>B(HCA)</td>
<td>83.10&lt;sup&gt;ab&lt;/sup&gt; ±8.67</td>
<td>45.09&lt;sup&gt;e&lt;/sup&gt; ±7.74</td>
<td>44.19&lt;sup&gt;e&lt;/sup&gt; ±7.27</td>
<td>57.46&lt;sup&gt;B&lt;/sup&gt;</td>
</tr>
<tr>
<td>C(HCE200)</td>
<td>76.63&lt;sup&gt;abc&lt;/sup&gt; ±9.23</td>
<td>68.73&lt;sup&gt;bcd&lt;/sup&gt; ±13.76</td>
<td>72.69&lt;sup&gt;abc&lt;/sup&gt; ±7.77</td>
<td>72.68&lt;sup&gt;A&lt;/sup&gt;</td>
</tr>
<tr>
<td>D(HCE400)</td>
<td>74.31&lt;sup&gt;abc&lt;/sup&gt; ±7.53</td>
<td>74.86&lt;sup&gt;abc&lt;/sup&gt; ±9.05</td>
<td>57.07&lt;sup&gt;de&lt;/sup&gt; ±3.47</td>
<td>68.75&lt;sup&gt;A&lt;/sup&gt;</td>
</tr>
<tr>
<td>E(HCC)</td>
<td>85.31&lt;sup&gt;a&lt;/sup&gt; ±7.63</td>
<td>68.14&lt;sup&gt;bcd&lt;/sup&gt; ±13.57</td>
<td>63.90&lt;sup&gt;cd&lt;/sup&gt; ±3.80</td>
<td>72.45&lt;sup&gt;A&lt;/sup&gt;</td>
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<tr>
<td>Overall days</td>
<td>78.29&lt;sup&gt;A&lt;/sup&gt;</td>
<td>68.54&lt;sup&gt;B&lt;/sup&gt;</td>
<td>57.20&lt;sup&gt;C&lt;/sup&gt;</td>
<td></td>
</tr>
</tbody>
</table>

P-value 0.00**

Lsd<sub>0.05</sub> 13.17

SE± 4.656

Mean±SD values having different superscripts (small letters for interaction and capital for overalls) are significantly different (P≤0.05).
**Fig. (4): Effect of *Adansonia digitata* ethanolic extract on HDL level f**

**experimental ra**

**Key:**

A ≡ Normal diet (control-1)
B ≡ High cholesterol diet (20 mg of Atrovastation )
C ≡ High cholesterol diet (extraction 200 mg)
D ≡ High cholesterol diet (extraction 400 mg)
E ≡ High cholesterol diet (control-2)
4.5 Effect of *Adansonia digitata* extract on low-density lipoproteins LDL (mg%):

The effect of administration of *A. digitata* ethanolic extract on low-density lipoproteins level of experimental rats is presented on Table [5] and figure (5).

Low-density lipoproteins level (mg/g) of experimental rats fed with normal diet (NDG) showed a significant increase (P≥0.05) on day 14 (26.5%) and retained it’s normal level on day 28.

Animals fed with high cholesterol diet and the standard drug Atorvastatin (HCA) showed a significant decrease (P≥0.05) after 28 days of treatment (36.9%) compared to the initial level before treatment.

After 14 days of treatment, the low-density lipoproteins level of the group of rats fed with high cholesterol diet and 200 mg/kg of *A. digitata* extract, the level of low-density lipoproteins decrease of (16.8 %) and a slight increase on day 28.

In the group that fed with high cholesterol diet and 400 mg/kg of extract (HCE 400), the low-density lipoproteins level showed a decrease of (19.4%) after 14 days and a slight decrease after 28 days of treatment compared to its initial level.

Control rats given high cholesterol diet without treatment (HCC) showed a slight decrease on low-density lipoproteins day 28 (7.6%).
Table (5): Effect of *Adansonia digitata* ethanolic extract on LDL level (mg%) of experimental rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Initial</th>
<th>14 days</th>
<th>28 days</th>
<th>Overall treatments</th>
</tr>
</thead>
<tbody>
<tr>
<td>A(NDG)</td>
<td>$57.34^{gh}$ ±6.10</td>
<td>$72.53^{bcde}$ ±15.22</td>
<td>$40.80^{i}$ ±8.21</td>
<td>$56.89^{C}$</td>
</tr>
<tr>
<td>B(HCA)</td>
<td>$84.90^{ab}$ ±4.38</td>
<td>$59.33^{fgb}$ ±6.27</td>
<td>$53.60^{hi}$ ±6.53</td>
<td>$65.94^{B}$</td>
</tr>
<tr>
<td>C(HCE200)</td>
<td>$83.19^{abc}$ ±6.66</td>
<td>$69.20^{cdefg}$ ±11.08</td>
<td>$92.05^{a}$ ±11.28</td>
<td>$81.48^{A}$</td>
</tr>
<tr>
<td>D(HCE400)</td>
<td>$76.29^{bcde}$ ±12.16</td>
<td>$61.46^{efgh}$ ±11.70</td>
<td>$74.71^{bcde}$ ±8.77</td>
<td>$70.82^{B}$</td>
</tr>
<tr>
<td>E(HCC)</td>
<td>$81.03^{abcd}$ ±10.22</td>
<td>$66.25^{defgh}$ ±4.31</td>
<td>$74.85^{bcde}$ ±21.77</td>
<td>$74.04^{AB}$</td>
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<tr>
<td>Overall days</td>
<td>$76.55^{A}$</td>
<td>$65.75^{B}$</td>
<td>$67.20^{B}$</td>
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</tr>
<tr>
<td>P-value</td>
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<td></td>
<td>0.00**</td>
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<tr>
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<td>13.43</td>
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<td>SE±</td>
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<td>4.748</td>
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</table>

Mean±SD values having different superscripts (small letters for interaction and capital for overalls) are significantly different (P≤0.05).
**Key:**

A ≡ Normal diet (control-1)
B ≡ High cholesterol diet (20 mg of Atrovastation)
C ≡ High cholesterol diet (extraction 200 mg)
D ≡ High cholesterol diet (extraction 400 mg)
E ≡ High cholesterol diet (control-2)

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**Fig. (5):** Effect of *Adansonia digitata* ethanolic extract on LDL level of experimental rats

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Chapter Five

Discussion

The current study is conducted to evaluate the potential hypocholesterolemic activity of two different doses of the fruits of Adansonia digitata (Baobab tree) ethanolic extract in induced hypercholesterolemic rats by feeding a high fat cholesterol diet. This plant was known to have many proven biological activities with very valuable nutritional capabilities.

The present study came to the facts that Adansonia digitata fruit extract affect the gain weight obtained by feeding rats with high cholesterol diet, The increase in weights of those treated rats was lower that detected in the (control un-treated rats) specially on day 28 of experimentation.

The effect of administration of two different doses of Adansonia digitata extract revealed that the higher dose of extract (400 mg/kg) has culminated in a reduction of cholesterol level in treated rats on day 28 of experiment compared to that administrated the standard drug (Atorvastatin). Similar results were observed by Osman et.al (2011), who stated that hypercholesterolemia-induced diet has developed hypercholesterolemia in rats which was marked by a significant (P≥0.05) increase in plasma total cholesterol level. Supplementation with bitter and sweet Lupin seeds showed a significant (P≥0.05) falls in total cholesterol levels compared with the hypercholesterolemic un-treated group.

Controversal results were shown by the methanol extract of Costus igneus rhizome as stated by Pazhanichamy and Eevera (2011). This study it was found that the lipid profile of hypercholesterolemic rats showed a significant (p<0.05) increase of total serum cholesterol after 30 days of treatment.
On the other hand, administration of 200 mg/kg of *Adansonia digitata* extract caused a marked decrease in triglyceride levels of animals fed with high cholesterol diet. These results similar to the result obtained by Osman *et al.* (2011) who stated that the levels of triglycerides was decreased significantly (P≥0.05) upon supplementation of induced hypercholesterolemic animals with bitter and sweet lupin seeds compared with the control hypercholesterolemic group. Our results were not in line to those results carried out by Pazhanichamy and Eevera (2011) who reported that the lipid profile of induced hypercholesterolemic animals showed a significant (p<0.05) increase in triglyceride levels, compared to normal control for 30 days of treatment with the methanolic extract of *Costus igneus* rhizome.

High-density lipoproteins carry cholesterol from the cells to the liver for eventual excretion. The level at which low HDL becomes a major risk factor for heart disease has been set at 40 mg/dl. Research indicates that an HDL level of 60 mg/dl or more is considered protective against heart disease. High-density lipoproteins are sometimes called *good cholesterol.* (Ruth, 2011). Thus, HDL protect against atherosclerosis. In addition, some factors that increase coronary heart disease (CHD risk) are obesity, smoking, inactivity, and male gender, these factors also cause a reduction in HDL. It is not known whether raising HDL will help to reduce CHD risk, but weight loss, physical activity, and smoking cessation can all independently help to lower risk (Sharon *et al.*, 2009).

In the current study, it was found that rats given high cholesterol diet and 200 mg of extract caused the HDL levels to remain in the normal range after 28 days of experiment compared to the control group which showed a decrease in HDL levels. This effect of the extract although it was not the targeted increase but may be considered as potential as it did not cause a
decreasing effect. These results may be compared to previous study where there was a significant (p<0.05) decrease in HDL serum levels in animals administered with the methanol extract of *Costus igneus* (Pazhanichamy and Eevera.,2011).

On the other hand, Osman *et.al* (2011) observed that rats fed the hypercholesterolemia-induced diet and bitter and sweet lupin seeds has shown an increase in plasma high density lipoprotein cholesterol (HDL-C) compared with the control hypercholesterolemic group.

Studying the effect of *Adansonia digitata* extract on LDL level (mg%) of experimental rats revealed that, 400 mg/kg of the extract has caused a decrease in the levels of LDL-C after 14 days and a slight decrease on day 28. Elevated blood levels greater than 130 mg/dl of LDL are thought to be contributing factors in atherosclerosis. Low-density lipoprotein is sometimes termed *bad cholesterol*. *(Ruth 2011).* Thus, these results highlight the beneficial role of *Adansonia digitata* in reducing the risks of coronary disease.

**Conclusion**

The high costs for therapeutic treatment has compelled physicians and researchers to look for alternative and less cost medicines for the treatment of hypercholesterolemia and minimize its complications. Therefore, medicinal plants and their products can be the best substitutes for the treatment of hypercholesterolemia due to their availability, low cost, minimum side effects and greater acceptance amongst users.
The present study result’s shows that the higher hypocholestremic effect of \textit{Adansonia digitata} extract is reported by a dose of 400 mg/kg for a period of 28 days of treatment in addition to hypotriglyceridemic effect and a mild reduction of LDL levels.

Thus, \textit{Adansonia digitata L. (Bombacaceae)} may contribute to decreasing the level of cholesterol and this indicates that it may have beneficial effects as a hypocholesterolemic agent.

\textbf{Recommendations:}

- Further studies with different types of extracts of the plant must be conducted to isolate and characterize the active constituent responsible for the treatment of hypercholesterolemia and its lipids associated with complications.
- \textit{Adansonia digitata} extract can be performed in different therapeutic doses.

6. References


Conference on Bioscience, Biochemistry and Bioinformatics, Periyar Maniammai University. vol. 5.


