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EVALUATION OF THE HYPOLIPIDEMIC EFFECT OF *COCOS NUCIFERA* DRY FRUIT ENDOCARP (SHELL)

U. S. Jijith[1,*]

[1]College of Pharmaceutical Sciences, Govt. Medical College, Kozhikode-673008, Kerala, India.

***Corresponding Author: Dr. U. S. Jijith**
College of Pharmaceutical Sciences, Govt. Medical College, Kozhikode-673008, Kerala, India.

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ABSTRACT

Introduction: Plant principles are considered a good source for identification of new drug molecules **Objective:** We evaluated the hypolipidemic effect of Cocos nucifera dry fruit endocarp (shell) Moreover, this study may lead to new molecules in the drug discovery process. This study helps to justify the claims in folklore use of coconut endocarp as a hypolipidemic agent. **Materials and Methods:** The liver is the source of enzyme which is responsible for cholesterol biosynthesis in the body. Because of two reasons, we selected chicken liver instead of rat liver for this study First reason was to avoid the ethical issue and the second reason was its easy availability. Therefore, we preferred chicken liver homogenate for the present study. as a source of enzyme for in vitro biosynthesis of cholesterol and also include hot water extract of Cocos nucifera as test substance. Organic mixture n-hexane and isopropyl alcohol were used for lipid extraction from chicken liver. In order to make up the final volume of the test solution for estimation cholesterol content with Isopropyl alcohol was used. **Results**: These studies showed the dose-dependent inhibition of cholesterol biosynthesis when compared with standard drug as atorvastatin. **Conclusions**: From the result, we conclude hot extract of Cocos nucifera showed a significant hypolipidemic effect when compared with the standard drug. A further detailed study is required for the identification of the fraction responsible for this action produced by the Cocos nucifera.

KEYWORDS: n-hexane, HMG CoA, cholesterol, Isopropyl alcohol, dry fruit of endocarp cocos nucifera.

INTRODUCTION

Cardiovascular disease is the most common disease in the present world. Today lifestyle patterns are the major contributory factor for this. One of the major risk factors for this disease is a high level of cholesterol and triglycerides contents, which may be deposited on small blood vessels especially coronary arteries, the condition is called atherosclerosis. This results in the interference of blood flow towards the heart, which is the blood-pumping organ of the body. The absence of proper flow of blood to the heart may affect the improper function of the heart. This condition leads to heart difficulty to maintain the uniform circulation of blood throughout the body. Stains class of drugs are major drugs used to reduce or prevent hyperlipidemic conditions. But this class of drugs are not completely free from adverse effect and does not give complete cure. Hence the development of a new drug is worthy in the treatment strategies of high cholesterol content. Many plant parts were used to reduce the cholesterol content. But such remedies are not established because of a lack of scientific data to reveal their effectiveness. Plant-based studies are important in the drug discovery process. Once the effectiveness of active principles of the plant is established for a particular activity, then easy to make the drug synthetic or natural way. We have been blessed with medicinal plants unfortunately half of our medicinal plant's power to cure the disease is not established. Hence plant-based pharmacological screening is still worthy. Even many synthetic drugs are available for the treatment field, but we expect a single remedy for a particular disease or disorder or more active and side effects free drugs in the treatment strategies.

Plants have been used for healing purposes and in the treatment of various diseases from immemorial. The scientific name of the Coconut tree is *Cocos nucifera* and the family Arecaceae. The coconut fruit consists of an outer epicarp, a mesocarp, and an inner endocarp. The outer portion of fruit is called epicarp and next to the epicarp there is a heavy fibrous and tannins-containing part is called mesocarp. In dry conditions, this part can be used for many industrial and home purposes. The inner part of the fruit is the hardest part, which is the dark core The white albumen is present inside the endocarp. The thickness of the white part is varied according to the age of the fruit. There is liquid albumen is also present in the inner part of a fruit, which is sweet, slightly acidic, and called coconut water. The human lifestyle has been changed and human being is lived in their comfort zone. Hence many of us are affected the cardiovascular disease. Hyperlipidemia is a common

condition, which may lead to various cardiovascular diseases. Coconut endocarp is a cheap and widely available material. Hence the hypolipidemic effect of this material is of economic value.

MATERIALS AND METHODS

Materials

Isopropyl alcohol and n-Hexane were purchased from spectrum chemicals and supplies, India for lipid extraction from chicken liver. Hot Water extracts were obtained with bi-distilled H2O. The chemicals for cholesterol estimation were purchased from Sigma Chemicals All Other chemicals were used of analytical grade only.

METHODS

Extraction

The endocarp (shell) of coconut was collected. The collected endocarps were communited and prextracted with petroleum ether (60-80o C) in order to remove the waxy substance. The pretreated endocarp was transferred to the soxhlet apparatus. Hot water was used as the solvent for extraction. The extract was collected after 24 hrs of extraction and concentrate the volume 1/3 rd of its original volume. The different concentration of extracts was used for the evaluation of anti -hyperlipidemic activity of Cocos Nucifera

In vitro screening Chicken liver assay for anti-hyperlipidemic activity

Freshly isolated chicken liver was collected, chilled, and homogenized. The obtained liver homogenate was transferred into four iodine flasks with 20 g of the homogenate in each flask. Then 1 mL an aqueous solution of sodium acetate (100 mg/mL) was added to it and mixed well. In addition to this, Krebs buffer (5 mL) was added to the homogenate for facilitating proper mixing. The homogenate was then incubated by using a mechanical water bath kept at a temperature of 37ºC for 4 hours with or without atorvastatin added to it. The samples are described in detail in Table 1. After completion of the incubation period, the homogenate maintained in the iodine flask was treated with a mixture of n-hexane: isopropyl alcohol (50 mL) prepared in the ratio 3:2.for the lipid extraction. The extraction using this solvent mixture was carried out overnight in a shaker water bath. During incubation, the stopper of the iodine flask was kept secured to avoid the escape of the solvent by evaporation. After completion of the extraction, the mixture was filtered by using normal filter paper with a funnel. The filtrate was collected and the aqueous phase (less in quantity) was separated and discarded. The organic phase containing the extracted lipids was then evaporated to dryness at room temperature. The dried residue was dissolved in isopropyl alcohol under magnetic stirring at 600 rpm and finally made up to 10 mL using isopropyl alcohol. The obtained sample was estimated for cholesterol content using the Transasia ERBA® kit. The method involves incubation of 10 µL of the sample with 1 mL of the reagent for 10 min. The enzymatic degradation of cholesterol to a purple coloured product is measured in comparison to a standard provided in the kit.

Effect of Cocos nucifera dry fruit endocarp on acetate-mediated cholesterol biosynthesis in the chicken liver homogenate

Table 1: Details of sample used in the *in vitro* evaluation by chicken liver assay.

Sl. No.	Sample code	Liver homogenate (g)	Sodium acetate (mg)	Atorvastatin (mg) (=Dose X20g)	Test Extract
1.	A1	20	100	Nil	Nil
2.	A2	20	100	Nil	1 ml
3.	A3	20	100	Nil	2 ml
4..	A4	20	100	7.2	Nil
5.	A5	20	100	14.4	Nil

RESULT AND DISCUSSION

These in vitro studies showed a significant reduction of cholesterol content in lipid extract organic solvent. Here we compared the cholesterol inhibitory effect of the standard drug as atorvastatin with the effect of hot water extract of Cocos nucifera fruit endocarp. The liver is the major site for cholesterol synthesis in our body. Many enzymes are directly and indirectly involved in the cholesterol biosynthesis process. Hence the inhibitory effect of the test substance towards these enzymes may lead to the development of a new hypolipidemic agent. Enzyme inhibition is an important tool for pharmacological screening of anti-hyperlipidemic agents. The liver is a major source of the enzyme responsible for the cholesterol biosynthesis process. In order to avoid animal complexity in the experiment and ethical issues, we preferred chicken liver for this assay instead of rat liver. We have established a chicken liver assay for screening of anti-hyperlipidemic activity and already proved the effectiveness of this method for the evaluation of hypolipidemic activity with standards drug as atorvastatin. In vitro in vivo correlation was established in a previous study and good correlations were obtained by using in vitro and in vivo data. We found that some precursors are mandatory for the synthesis of cholesterol in chicken liver homogenate. Here we selected sodium acetate as precursors for cholesterol synthesis. Krebs solution was also used in this assay for providing energy and buffer action. In order to get the optimum temperature for the various enzymes involving cholesterol synthesis. We were maintained 37ºC throughout the assay. After

incubation lipid extraction from the liver homogenate was another task. But by using a mixture of organic solvent (n-Hexane and Isopropyl alcohol) lipid were extracted and evaporated. In order to get a good result, we removed the aqueous fraction by using a micropipette. The solid content is then diluted with isopropyl alcohol and stirred with a magnetic stirrer for avoiding any loss of cholesterol content. In each step, we took extra care and precaution for getting an accurate result. Moreover, the result of this assay expects more contribution towards the anti-hyperlipidemic treatments because Cocos nucifera dry fruit endocarp as test substances are easily and cheaply available. The high level of lipid content in the blood is a major reasonable cause factor of cardiovascular disease. Atorvastatin reduces cholesterol content by inhibiting the enzyme HMG CoA reductase, which is the important enzyme for the conversion of HMG CoA to mevalonic acid. This conversion is an important step involved in cholesterol biosynthesis. In the absence of mevalonic acid further steps of cholesterol synthesis are also blocked, finally, no cholesterol is formed. In the present study, the dose was calculated by the surface area ratio of rat basis to that of a human being. Here the human dose of atorvastatin is 10 mg, which is multiplied by factor 0.018. Hence the dose of 200 g rat is 0.18 mg. We took 20 g liver homogenate for this study. Therefore atorvastatin dose was multiplied with 20 g and obtained two doses as 7.2 mg and 14.4 mg of atorvastatin. In the present study, we selected two doses of atorvastatin to determine the hypolipidemic activity of Cocos nucifera dry fruit of endocarp. The treatment plan is given in Table-1. The in vitro assay result showed a reduction of cholesterol content that is somewhat similar to a standard drug-produced hypolipidemic effect Hence the mechanism of action of the test extract of Cocos nucifera dry fruit endocarp may be HMG CoA reductase inhibition. Further studies are required for the separation and isolation of chemical substances from extract responsible for the hypolipidemic action. Figure 1 and Table 2 showed cholesterol content in the control group is much higher than in test and standard groups. Hence the studies are significant. Does the dependable reduction of cholesterol content is also shown from the figure-1and table-2. The result obtained in the chicken liver assay clearly indicates the significant hypocholesterolemic effect of Cocos nucifera dry fruit endocarp.

Table 2: Data for in *vitro* evaluation by chicken liver assay.

Sl No	Sample Code	Cholesterol content in final solution (mg/dL)
1	A1	851.3310.40
2	A2	190.33±05.43
3	A3	165.33±14.84
4	A4	238.23±16.07
5	A5	166.66±05.11

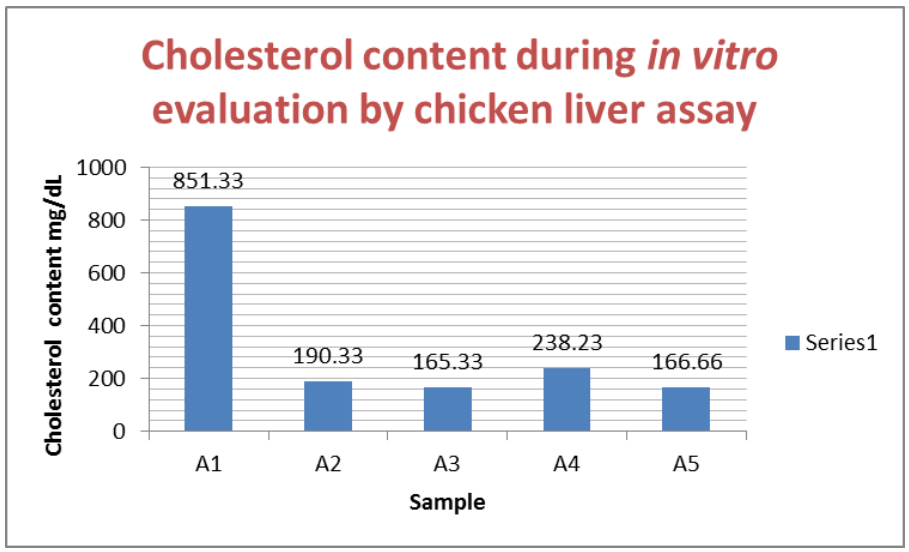

Figure 1: Cholesterol content during *in vitro* evaluation by chicken liver assay.

CONCLUSION

From the result of in vitro chicken liver assay for the evaluation of the hypolipidemic effect of Cocos nucifera dry fruit endocarp**,** we conclude hot extract of Cocos nucifera showed a significant cholesterol reduction effect when compared with standard drug. A further detailed study is required for the identification of the fraction responsible for this action produced by the Cocos nucifera dry fruit endocarp.

Conflict of interest: None

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