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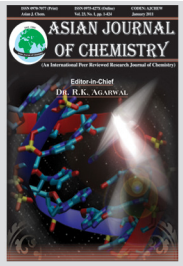

Inhibition Effect of Ethyl Acetate Soluble Fraction of *Citrullus colocynthis* on *In Vitro* Cholesterol Crystallization

N. SAMPATH KUMAR[1,*] and G. MADHURAMBAL[2]

[1]Department of Chemistry, Chettinad College of Engineering & Technology, Karur-639 114, India
[2]Department of Chemistry, ADM College for Women, Nagapattinam-611 011, India

*Corresponding author: Tel: +91 4324250930; E-mail: arunaiguru@gmail.com

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Cholesterol is one of the important compounds in the body for the proper function of several systems. When the presence of cholesterol exceeds its maximum level it leads to cardiovascular disease (CVD) which is due to the deposition (crystallization) of cholesterol on the interior sides of the blood vessels. The crystallization depends on factors like solvents, concentration, pH and temperature. The crystallization process can be affected by the presence of phytoactive compounds like flavanoids, terepenoids, triterepenoids *etc*. Phytoactive compounds present in the ethyl acetate fraction of the fruits of *Citrullus colocynthis* are capable of altering the rate of crystallization and the morphology of the crystals when they are introduced to the *in vitro* crystallization process.

Key Words: Cardiovascular disease, *Citrullus colocynthis*, Crystallization, Morphology, Ethyl acetate soluble fraction.

INTRODUCTION

Cholesterol ($C_{27}H_{46}O$) is the most important steroid which is found in most of the parts of the body. The deposition of cholesterol crystals may produce cardiovascular diseases. Plant extracts with phytoactive compounds are used to control cholesterol deposition by Ayurvedic medicines. The morphology of cholesterol crystals in blood vessels is reported to be plate like shape[1]. The *in vitro* cholesterol crystals also have the same morphology as like in blood vessels[2]. Cholesterol was crystallized from different solvents under various conditions and the effect of the solvents on cholesterol crystals were reported[3]. Gel is an ideal medium to grow cholesterol crystal because its structure is similar to the mucus in the living organisms and the effect of ethyl acetate soluble fraction of *Citrullus colocynthis* on the growth of cholesterol crystals in sodium meta silicate medium is discussed. *Citrullus colocynthis* is also known as bitter apple, bitter cucumber. It is an ancient medicine used to cure some diseases like bronchial asthema, constipation, rheumatic diseases and tumour diseases[4]. Plant sterols which have similar structure to cholesterol and they can influence the nucleation of cholesterol. The influence of sterols on cholesterol is characterized by X-ray analysis.

EXPERIMENTAL

The single test-tube diffusion method was employed for growing cholesterol crystals in the gel medium[5]. The stock solution of specific gravity 1.03 g/cc was prepared by dissolving sodium meta silicate (SMS) powder in double distilled water at room temperature. The solution was filtered and kept in a flask. This solution was mixed with acetic acid to adjust the pH of the solution to 5.3. The gels were set in 5 days and the acetone solution of cholesterol and the acetone solutions of ethyl acetate soluble fractions were prepared as supernatant solutions.

The control was prepared by mixing 5 mL of acetone with 1 % (w/v) cholesterol in acetone solution and kept with out any disturbance. Treatment was kept by adding the solutions of ethyl acetate soluble fraction in varying concentrations *viz*. 10, 20 and 30 % to the top solution. All tubes were observed and noted that there is change in the nucleation of cholesterol crystal in the presence of ethyl acetate soluble fraction. The optimum conditions set for the growth of crystals were tabulated in Table-1.

RESULTS AND DISCUSSION

Inhibition effect: It was observed that the time taken to start the nucleation and the growth decreased with the increase in the concentration of ethyl acetate soluble fraction of *Citrullus colocynthis* (Table-2).

The variations in the morphology of the crystals were also observed in the crystals grown in the presence of ethyl acetate soluble fraction of *Citrullus colocynthis* (Table-3).

TABLE-1
OPTIMUM CONDITIONS SET FOR THE GROWTH OF CHOLESTEROL CRYSTAL

Density of sodium meta silicate solution	1.03
Acid used to adjust pH of gel	Acetic acid
pH value of set gel	5.3
Temperature	32 ºC
Concentration of cholesterol solution	1 % (W/V)
Concentration of ethyl acetate soluble fraction	0.001 g/L
Solvent used	Acetone
Gel setting period	4 days
Observed period of growth	70 days

TABLE-2
CONCENTRATION OF INHIBITOR *VERSUS* NUMBER OF CRYSTALS

Percentage of ethyl acetate soluble fraction	Number of crystals formed in 24 h (after the addition of cholesterol and inhibitor)
0	15
10	13
20	11
30	8
40	5
50	2

TABLE-3
VARIATION OF MORPHOLOGY IN THE PRESENCE OF INHIBITOR

Nature of the solution	Solvent medium	Shape of the crystals
Control	Acetone	Plate
Ethyl acetate soluble fraction of *Citrullus colocynthis*	Acetone	Needle

Characterization of cholesterol crystals: The X-ray diffractogram of the grown crystals of pure cholesterol and the crystals grown in the presence of ethyl acetate soluble fraction of *Citrullus colocynthis* were recorded in Rigaku, Ultima III computer controlled diffrractometer using Cu K_α radiation. The intensity values for the corresponding 2θ values decreased enormously from 4520-2567 cps (Fig. 1). This supports that the inhibitor prevent the crystal formation of cholesterol. The X-ray powder diffraction analysis was done and the cell parameters of cholesterol grown under inhibition are found[6] to be a = 12.00; b = 32.2; c = 8.5 Å. There are 8 independent molecules of cholesterol per unit cell z = 8 and it has the triclinic symmetry. The effect of ethyl acetate soluble fraction of *Citrullus colocynthis* on the growth specimen has obviously an inhibitory nature which not only reduced the growth rate and also changed the nature of the material[7].

The IR spectrum was recorded and the observed values were seen to be in good agreement with those reported for the free crystals (Fig. 2). Generally aromatic compounds give peaks in the range of 3100-2700 cm^{-1} region due to C-H stretching[8]. Both CH_2 and CH_3 groups give rise to bands near 1400 cm^{-1} due to hydrogen bonding vibrations. The bands involving in-plane bending vibration of the C-H are observed in the 1300-1000 cm^{-1} region. The C-H out of plane bending vibration depends on the type of substitutions and absorbs between 1090-800 cm^{-1} [9,10].

The bands observed around 3400 cm^{-1} indicate the presence of water group along with the OH group which is

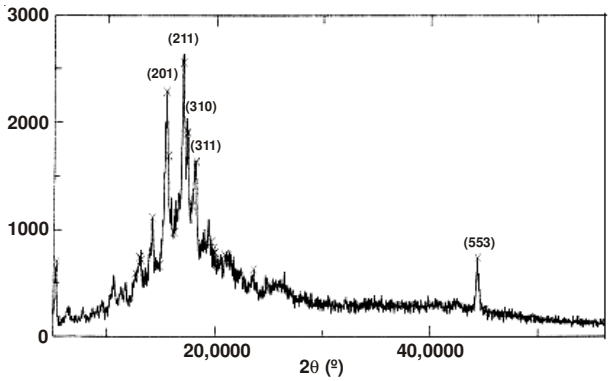

Fig. 1. XRD spectrum of cholesterol crystal grown in the presence of inhibitor

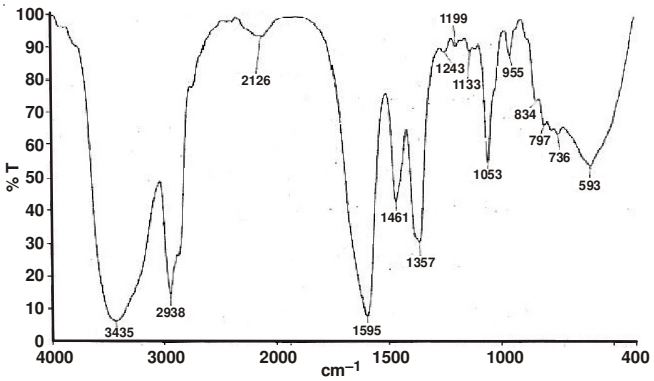

Fig. 2. FTIR spectrum of cholesterol crystal grown in the presence of inhibitor

attached to the cholesterol ring. H_2O bending vibrations are expected around 1700 cm^{-1}. The C-OH out of plane bending occurs near 590 cm^{-1}. The absence of the band at 1722 cm^{-1} of IR spectrum of cholesterol under ethyl acetate soluble fraction of *Citrullus colocynthis* treatment indicates the absence of H_2O bending vibration. This suggests dehydration of the material in the presence of the additive which is further supported by the change in morphology of the crystals[7]. The band around 1700 cm^{-1} not observed in the IR spectrum of the cholesterol in the treatment shows that it is anhydrous form which is indicative of the growth reduction seen. Change in the finger print region indicates the change in morphology of the crystals grown.

Conclusion

Growth experiment conducted using the ethyl acetate soluble fraction of *Citrullus colocynthis* added to the super annuated solution of cholesterol show that it has got an inhibitory effect on the crystallization. The addition of the inhibitor showed not only a delay in nucleation but also a change in morphology. The change in morphology is a clear indication of inhibition of cholesterol crystal growth which may be attributed to ethyl acetate soluble fraction of *Citrullus colocynthis*. This fact is supported by IR studies in which hydroxyl bonds seen in pure cholesterol were absent in the crystals grown in the presence of ethyl acetate soluble fraction of *Citrullus colocynthis*. In oxidation of secondary alcohol to ketone, one learns to expect the disappearance of hydroxyl (O-H) stretch and appearance of carbonyl (C=O). X-Ray studies proved it to be triclinic system. An addition of ethyl

acetate soluble fraction of *Citrullus colocynthis* in the growth stage can reduce the growth of cholesterol crystal *i.e*., additional crystallization of cholesterol can be avoided. Therefore ethyl acetate soluble fraction of *Citrullus colocynthis* is a suitable agent for the control of cholesterol crystallization.

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