



**RESEARCH ARTICLE**

**THE HYPOLIPIDEMIC ACTIVITY OF T-COLOSTROL, A SIDDHA PREPARATION IN  
HYPERCHOLESTEROLEMIA INDUCED ALBINO WISTAR RATS**

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**ABSTRACT**

Siddha medicine is one of the oldest medical systems known to mankind. Contemporary Tamil literature holds that the system of Siddha medicine originated in Southern India, in the state of Tamil Nadu. In this present study, a Siddha preparation called T-colostron, known local hypolipidemic drug, containing a mixture of *Musa paradisiaca* stem juice, Black salt and Ferrous oxide was evaluated for its hypolipidemic activity in albino Wistar rats. Cholesterol powder, tween 80 and distilled water was given for induction. The present study proved that the hypolipidemic activity of *T-Colostron* was effective to rats which showed decreased cholesterol level compared to induced control. Statin drugs, commonly used as lipid reducer in allopathic system was used as a positive control and the results of T-colostron is comparable to some extent with such drug. This study strongly suggests that the Siddha preparation could effectively be used as a hypolipidemic therapeutic drug

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**INTRODUCTION**

Siddha Medicine is one of the oldest medical systems known to mankind. Contemporary Tamil literature holds that the system of Siddha medicine originated in Southern India, in the state of Tamil Nadu, as part of the trio Indian medicines - ayurveda, siddha and unani. Reported to have surfaced more than 2500 years ago, the Siddha system of medicine is considered one of the most ancient traditional medical systems. "Siddhargal" or Siddhars were the premier scientists of ancient days.

Generally the basic concepts of the Siddha medicine are almost similar to ayurveda. The only difference appears to be that the siddha medicine recognizes predominance of vata, pitta and kapha in childhood, adulthood and old age, respectively, whereas in ayurveda, it is totally reversed: kapam is dominant in childhood, vata in old age and pitham in adults. According to the Siddha medicine, various psychological and physiological functions of the body are attributed to the combination of seven elements: first is saaram (plasma) responsible for growth, development and nourishment; second is chenneer (blood) responsible for nourishing muscles, imparting colour and improving intellect; the third is ooun (muscle) responsible for shape of the body; fourth is kozhuppu (fatty tissue) responsible for oil balance and lubricating joints; fifth is elumbu (bone) responsible for body structure and posture and movement; sixth is moolai (brain) responsible for nerve strength; and the last is sukila (semen) responsible for reproduction.

Like in Ayurveda, in Siddha medicine also, the physiological components of the human beings are classified as vata (air), pitta (fire) and kapha (earth and water). It is assumed that when the normal equilibrium of the three humors (vata, pitha and kapha) is disturbed, disease is caused. The factors, which assumed to affect this equilibrium are environment, climatic conditions, diet, physical activities, and stress. Under normal conditions, the ratio between these three humors (vata, pitha and kapha) i.e.: (Vadham, Pittham, Kabam in Tamil) is 4:2:1, respectively.

According to the siddha medicine system, diet and lifestyle play a major role, not only in health but also in curing diseases. This concept of the siddha medicine is termed as pathyam and apathya, which is essentially a list of "do's and dont's". This indigenous knowledge, passed down from generation to generation in various parts of the world, has significantly contributed to the development of different traditional systems of medicine (Jachak and Saklani, 2007) as well as helped in exploration of different medicinal plants to find the scientific basis of their traditional uses. Banana is a familiar tropical fruit. From its native Southwestern Pacific home, the banana plant spread to India by about 600 BC and later on it spread all over the tropical world. It is possibly the world's oldest cultivated crop. It even spread into the Islands of the Pacific and to the West Coast of Africa as early as 200-300 BC. *Musa paradisiaca* (Family: Musaceae) is a perennial tree like herb. It is commonly known as banana or kela in Hindi and is widely found in northern India. The potential use of banana fruit pulp to treat cholesterol has been explored by a number of investigators (Singh *et al.*, 2007). *M. Paradisiaca* (Banana) has thick stem composed of convolute leaf sheaths,

Leaves are very large and oblong. It is distributed throughout India and Malaysia. And belong to the musaceae family. Roots of *M. paradisiaca* are antihelmintic (Kirtikar *et al.*, 1991). Flowers are astringent (Joshi *et al.*, 2000). Banana fruit is mild laxative. It aids in combating diarrhea and dysentery and promotes healing of intestinal lesions in ulcerative colitis (Joshi *et al.*, 2000). It is useful in celiac disease, constipation and peptic ulcer. Banana powder is effective in treatment of celiac disease (Joshi *et al.*, 2000).

This study was aimed at finding hypolipidemic activity of T-colostrum extract of *Musa paradisiaca* drugs from Siddha & Ayurveda. The most of the drugs belonging to this system are said to be free from side effects. So, if a medicine is found out for hyperlipidemic problems, from this system, it would be better to use the same for treatment after following clinical studies.

## MATERIALS AND METHODS

### Siddha Preparations

(T- Colostrum)

<i>Musa paradisiaca</i>	-	50.02%
Ferrous oxide	-	16.66%
Extract of Black Salt	-	33.2%

Dosage: 5-10 drops mixed with 10ml water for 14 days

### Animals

Animals are divided into three groups, each group containing 6 animals. Adult male *albino* rat of Wistar strain weighing around 250 to 300gms were procured from Tamil Nadu Veterinary and Animal Sciences University, Chennai. The animals were kept in polypropylene cages (four in each cage) at an ambient temperature of 25±2°C and 55-65% relative humidity. A 12±1hr light and dark schedule was maintained in the animal house till the animals were acclimatized to the laboratory conditions, and were fed with commercially available rat chow (Hindustan Lever Ltd., Bangalore, India) and had free access to water. The experiments were designed and conducted in accordance with the institutional animal ethics committee.

### Animal Handling

The rats were kept in well-ventilated house conditions (temperature: 28-31° C; photoperiod; 12hr natural light and 12hr dark; humidity; 50-55%). The animals were housed in a barriered building and identified by an individual unique tattoo on the tail. The three animals used for this study were randomly divided into 2 experimental groups (A&B). The body weights of the rat was approximately 120 to 160 gm on the day the experiment began. The rats were kept in individual cages. The cages, cage trays, food hoppers and water bottles were sanitized at regular intervals. All animals were given free access to both food and tap water. There was no indication that any (non-nutrient) substance was present in the diet or in the drinking water that influenced the effects of the test compound.

### Cholesterol powder

The Cholesterol powder was diluted in physiological saline (NaCl) with a pH of 7.0. The concentration of the test formulations were 0.5 mg / ml.

### Dosage Given

**Group 1-** No induction and treatment- Routine food.

**Group 2-** Induction with 0.5 mg of Cholesterol was diluted in 1 ml of saline given orally to the rat for 14 days.

**Group 3;** 1 mg of Fe oxide + 0.5 mg of Cholesterol in 1ml of saline given orally for 14 days.

**Group 4;** 1 mg of black salt solution in water + 0.5 mg of Cholesterol in 1ml of saline given orally for 14 days

**Group 5;** 1 drop of *Musa* stems extract in water + 0.5 mg of Cholesterol in 1ml of saline given orally for 14 days.

**Group 6;** 1 drop of T-colostrum + 0.5 mg of Cholesterol in 1ml of saline given orally for 14 days.

**Group 7:** 1 ml 50 mg/kg of Statin drug and 0.5 mg of Cholesterol in 1ml of saline given orally for 14 days

### Soxhlet Procedure

The sample was placed into a soxhlet extractor covered with a filter paper. A solvent-Acetone was placed in the flask at the bottom of the apparatus. The flask, Soxhlet extractor glass ware, and water-cooled condenser were assembled. The electric hot plate boiled the solvent, and the vapours were directed through the extractor to the top-mounted condenser. The condensed vapours dripped down into the sample, filling the extraction cup with the solvent. The soluble chemicals of the sample diffuse from the sample into the solvent, which siphons back into the lower flask when the cup was filled. The clean solvent vapours continued to condense, refilled the Soxhlet cup, and flushed into the flask. This cycling action extracted the acetone soluble chemical from the sample and collected them in the flask (Dhanapal *et al.*, 2005).

### Sample Collection and Preparation

The rats were sacrificed at the end of the experimental period and the venous blood was collected into clean sample bottles. This was allowed to clot and then centrifuged at 3000rpm for 5 mins after which the serum was separated and stored frozen until needed for analysis.

## QUALITATIVE PHYTOCHEMICAL SCREENING OF T-COLOSTROL

### Detection of Alkaloids

#### Preparation of filtrate

Solvent free extract (50 mg) is stirred with few ml of dilute hydrochloric acid and filtered.

#### a) Mayer's test

To a few mL of filtrate, a drop or two of Mayer's reagent are added by the side of the test tube. A white precipitate indicates the test as positive.

#### Mayer's reagent preparation

Mercuric chloride (1.358g) is dissolved in 60 mL of water and potassium iodide (5.0g) is dissolved in 10 mL of water. The two solutions are mixed and made up 100 mL with water.

#### b) Wagner's test:

To a few mL of filtrate, few drops of Wagner's reagent are added by the side of the test tube. A reddish brown precipitate confirms the test as positive

### **Wagner's reagent preparation**

Iodine (1.27 gram) and potassium iodide (2 gram) is dissolved in 5ml of distilled water and made up to 100 ml distilled water

### **Detection of Carbohydrate**

#### **Preparation of filtrate**

The extract (100 mg) is dissolved in 5ml of water and filtered

#### **Molish's Test**

To 2 ml of filtrate 2 drops of alcoholic solution of alpha naphthol were added, the mixture is shaken well and 1 ml of concentrated sulphuric acid was added slowly along the side of the test tube and allowed to stand. A violet ring indicates the presence of carbohydrate

#### **Fehling's Test**

One ml of filtrate is boiled on water bath with one ml each of Fehling's solution A and B. A red precipitate indicates the presence of sugar Fehling's solution A: copper sulphate (34.66g) is dissolved in distilled water and made up to 800 ml of distilled water

Fehling's solution B: Potassium sodium tartarate (173g) and sodium hydroxide (50g) is dissolved in water and made up to 500ml.

#### **Benedict's test**

To 0.5 ml of the filtrate and 0.5 ml of Benedict's reagent was added. The mixture is heated on a boiling water bath for two minutes. A characteristic red coloured precipitate indicates the presence of sugars.

#### **Benedict's reagent Preparation**

Sodium citrate (173g) and sodium carbonate (100g) are dissolved in 800 mL of distilled water and boiled to make it clear. Copper sulphate (17.3g) dissolved in 100 mL distilled water is added to it.

#### **Barford's Test**

To 1ml of filtrate, 1ml of Barford's reagent was added and heated on a boiling water bath for 2 minutes. Red precipitate indicates presence of sugar.

#### **Barford's Reagent Preparation**

Copper acetate, 30.5g is dissolved in 1.3 ml of glacial acetic acid.

#### **Detection of Saponins**

Extract (50 mg) was diluted with distilled water and made up to 20ml. the suspension is shaken in a graduated cylinder for 15 min. A two cm layer of foam indicates the presence of saponins.

#### **Detection for Proteins and Amino acids Preparation of filtrate**

The extract (100 mg) was dissolved in 10 mL of distilled water and filtered through Whatmann no.1 filter paper and the filtrate is subjected to tests for proteins and amino acids.

#### **Millon's test**

To 2ml of filtrate, few drops of Millon's reagent were added. A white precipitate indicates the presence of proteins.

### **Millon's reagent preparation**

Mercury (1 g) was dissolved in 9 mL of fuming nitric acid. When the reaction was completed, equal volume of distilled water was added.

#### **Biuret Test**

An aliquot of 2 mL of filtrate is treated with 1 drop of 2% copper sulphate solution. To this, 1 mL of ethanol (95%) was added, followed by excess of potassium hydroxide pellets. Pink color in the ethanolic layer indicates the presence of proteins.

#### **Ninhydrin test**

Two drops of ninhydrin solution (10 mg of ninhydrin in 200 mL of acetone) was added to two mL aqueous filtrate. A characteristic purple colour indicates the presence of amino acids.

### **Detection of Phytosterols**

#### **Libermann- Burchard's Test**

The extract (50 mg) was dissolved in 2 mL of acetic anhydride. To this one or two drop of concentrated sulphuric acid was added slowly along the sides of the tube. An array of colour shows the presence of phytosterols.

### **Detection of Phenolic Compounds and Tannins**

#### a) **Ferric chloride test:**

The extract (50 mg) was dissolved in 5 mL of distilled water. To this few drops of neutral ferric chloride solution was added. A dark green colour indicates the presence of phenolic compounds (Mace Gorbach, 1963).

#### b) **Lead acetate test**

The extract (50 mg) was dissolved in distilled water and to this 3 mL of 10% lead acetate solution is added. A bulky white precipitate indicates the phenolic compounds.

#### c) **Alkaline reagent test**

An aqueous solution of the extract is treated with 10% ammonium hydroxide solution. Yellow fluorescence indicates the presence of flavonoids.

### **Detection of Tannins**

About 0.5 g of the dried powdered samples was boiled in 20 mL of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or a blue black colouration.

### **Detection of Terpenoids**

#### **Salkowski Test**

Five ml of each extract was mixed in 2 ml of chloroform and concentrated sulphuric acid was added to form a layer. A reddish brown colouration of the interface was formed to show positive results for presence of terpenoids.

### **Detection of Steroids**

Two ml of acetic anhydride was added to 0.5 g ethanol extract of each sample with 2 ml of sulphuric acid. The colour changed from violet to blue green in some samples indicates the presence of steroids.

### **Determination of Serum Lipid Parameters**

Estimation of LDH, Estimation of Cholesterol, Estimation of HDL (By Wybenga & Pillegi Method) and Estimation of

Triglycerides were carried out for the blood (serum) samples from animals

**Test for Low Density Lipoprotein (LDL);**

The cholesterol, HDL and triglyceride values were found out. From this LDL was calculated.

$$LDL = \frac{Cholesterol - \frac{TG}{5}}{HDL}$$

TG – Values of Triglycerides

HDL- values of HDL

**Test for Very Low Density Lipoprotein (VLDL)**

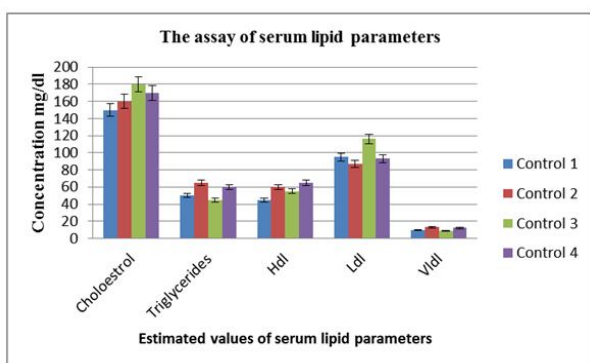
The triglyceride values were found out. From this VLDL was calculated.

$$VLDL = \frac{Triglycerides}{5}$$

**RESULTS**

**Table 1** Estimation of Cholesterol, Triglycerides HDL, LDL and VLDL in Normal rats

S.No.	Normal group	Cholesterol	Triglycerides	HDL	LDL	VLDL
1	Control I	150	50	45	95	10
2	Control II	160	65	60	87	13
3	Control III	180	45	55	116	9
4	Control IV	170	60	65	93	12

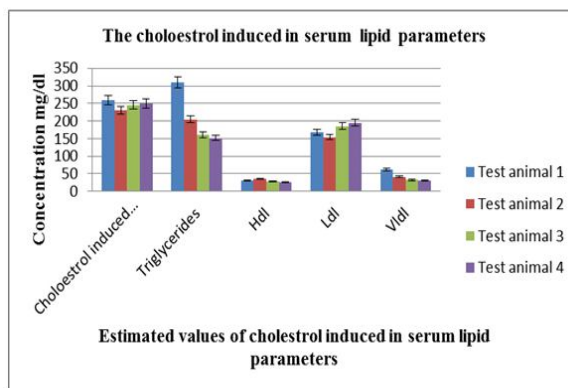


**Interpretation**

Normal level of Cholesterol, Triglycerides, HDL, LDL and VLDL was seen.

**Table 2** Estimation of Cholesterol, Triglycerides, HDL, LDL, and VLDL in Cholesterol induced rats.

S.No.	Cholesterol induced animals	Cholesterol	Triglycerides	HDL	LDL	VLDL
1	Test animal I	260	310	30	168	62
2	Test animal II	230	205	35	154	41
3	Test animal III	245	160	28	185	32
4	Test animal IV	250	152	25	194.6	30.4

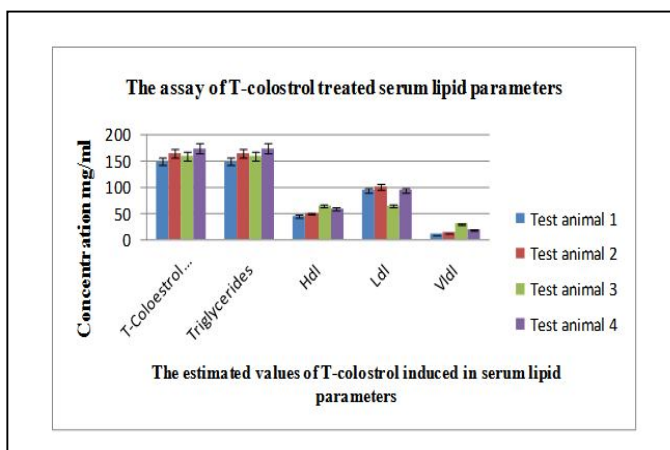


**Interpretation**

Due to Cholesterol feeding, the Cholesterol level was increased and it denotes the coronary heart diseases, Arteriosclerosis

**Table 3** Estimation of Cholesterol, Triglycerides, HDL, LDL, and VLDL in T-Colostrol treated rats

S.No.	T- Colostrol treated animals	Cholesterol	Triglycerides	HDL	LDL	VLDL
1	Test animal I	150	150	45	95	10
2	Test animal II	165	165	50	102	13
3	Test animal III	160	160	65	65	30
4	Test animal IV	175	175	60	95	20

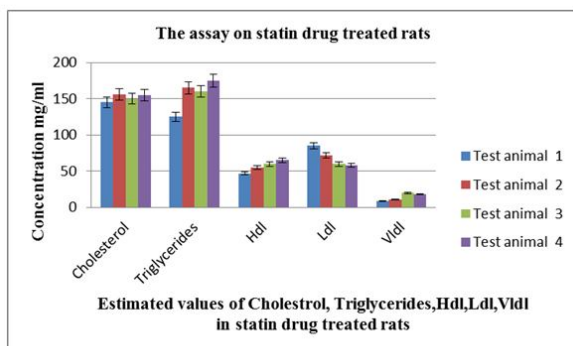


**Interpretation**

The T-Colostrol significantly decreased the Heart disease conditions and HDL Cholesterol level was comes to normal on Cholesterol induced rats.

**Table 4** Estimation of Cholesterol, Triglycerides, HDL, LDL, and VLDL in Statin drug treated rats

S.No.	T- Colostrol treated animals	Cholesterol	Triglycerides	HDL	LDL	VLDL
1	Test animal I	145	125	47	85	9
2	Test animal II	156	165	55	72	11
3	Test animal III	150	160	60	60	20
4	Test animal IV	155	175	65	58	18



### Interpretation:

The Statin drug has significantly decreased the Heart disease conditions and HDL Cholesterol level was comes to normal on Cholesterol induced rats.

### DISCUSSION

Lipid disorders are common problems, nowadays, because of the modern lifestyle and heredity. To overcome such problems, apart from lifestyle change, a treatment option is also mandatory for the normal process. Hyperlipidemia is a common problem, which is the main cause for many heart ailments such as hypertension and arteriosclerosis affecting majority of the population, which is increasing year by year. Allopathy way of treatment is encouraging, but the side effects are innumerable than the main effects. In our country, from time immemorial many herbals are being used to treat such ailments.

This study was aimed at finding the effectiveness of some known traditional herbal medicines such as *T-Colostrol*. In this study Cholesterol were given orally. After 7 days, the analysis of serum levels of cholesterol, LDL, VLDL, LDH, Triglycerides was made and the results were interpreted and a significant increased level are seen. The affected rats were given herbal preparations for about 7 days and afterwards the serum levels were checked for any reduction.

During the treatment time they were not allowed to take any other medicines meant for hypercholesterolemia and heart diseases. This study has clearly indicated that this preparation could be given to treat hyperlipidemic individuals. The result of this study suggests that *T-Colostrol* would be the drug of choice for better control over hyperlipidemia associated diseases. The drugs were given to human being directly because of its traditional use in the society. Though, the toxicity should be studied in animals and human to a molecular level.

The results of this study corroborates with the following study to some extent. Barks extracts of four different trees (*Azadirachta indica*, *Acacia nilotica*, and *Eugenia jambolana* Lam.) in three different solvents 80% methanol, 80% ethanol, and 80% acetone (solvent: water, 80:20 v/v) were evaluated for their antioxidant activity, total phenolic (TP), and total flavonoids (TF) contents. Antioxidant activity (AA) was determined by measuring reducing power, inhibition of peroxidation using linoleic acid system and 2,2'-diphenyl-1-picrylhydrazyl radical (DPPH) scavenging activity. Significant ( $P < 0.05$ ) differences were observed in the TP, TF, inhibition

of linoleic acid oxidation and DPPH scavenging activity of different bark extracts. Nevertheless, minute variation was observed in reducing power. All the bark extracts exhibited wide range of total phenolic, 7.8–16.5 gallic acid equivalents and total flavonoid contents, 1.59–4.93 catechin equivalents. Reducing power at 10 mg/mL extract concentration ranged from 1.34 to 1.87. Different bark extracts inhibited oxidation of linoleic acid by 44–90% while DPPH radical scavenging activity ranged from 49% to 87%. Extraction efficacy of components with antioxidative properties was lowering in the following order: ethanol > methanol > acetone. Good correlation was observed between TP and DPPH scavenging activity among the extracts. *A. nilotica* bark had the highest amounts of TP, ranging from 9.2 to 16.5 g/100 g, while the highest AA as measurement by inhibition of linoleic acid oxidation is offered by bark from *E. jambolana* Lam. The same tree showed the highest DPPH scavenging activity and reducing power. The correlation among the results of different antioxidant assays although revealed a strong relationship between some of the assays, however, a number of different methods may be necessary to adequately assess the in vitro antioxidant activity of a specific plant material. (Bilbis *et al.*, 2002)

A rapid, sensitive and reproducible reversed phase high performance liquid chromatographic method with photo diode array detection is described for the simultaneous quantification of major oleane derivatives: arjunic acid (1) in *T-Colostrol* extract, arjungenin (2), and arjunetin (3), arjunolic acid (4). The method involves the use of a Waters Spherisorb S10 ODS2 column (250×4.6 mm, I.D., 10 μm) and binary gradient mobile phase profile. The various other aspects of analysis viz. Extraction efficiency, peak purity and similarity were validated using a photo diode array detector. (Shin *et al.*, 2007)

Cholesterol (1, 3, 7 trimethylxanthine) affects the cardiovascular system, with potential toxic effects ranging from a moderate increase in heart rate to more severe cardiac arrhythmias. Telemetry transmitters were implanted in Wistar rats in the peritoneal cavity with a pressure catheter in the aorta and electrodes for electrocardiogram (ECG) recording subcutaneously. After a single oral administration of saline, each rat was administered single oral doses of 5, 15 and 45 mg/kg b.w. of Cholesterol. Cholesterol was found to induce, to various degrees, a dose-dependent early increase in spontaneous physical activity, heart rate, dp/dt and systolic–diastolic blood pressure. No arrhythmias or visual changes were observed in the ECG complex. High doses induced more strong responses and of longer duration. The increase in systolic blood pressure at the median dose remained in the rats until 20 h after administration. However, the highest dose of Cholesterol (45 mg/kg b.w.) induced a biphasic response, with an early and pronounced increase in body temperature, spontaneous physical activity, systolic and diastolic blood pressure that later decreased, except for the systolic blood pressure. The results show that the dose level for long-lasting signs of intoxication to develop in the rat, in terms of effects on spontaneous physical activity, body temperature and cardiovascular function, was reached after a single oral dose of Cholesterol at 45 mg/kg b.w. (Newairy *et al.*, 2002).



*T-Colostrol* like medicines has been marked as a potential cardioprotective agent since Vedic period. The present study was aimed to investigate the effects of butanolic fraction of *T-Colostrol* bark (TA-05) on Doxorubicin (Dox)-induced cardio toxicity. Male wistar rats were used as in vivo model for the study. TA-05 was administered orally to Wistar rats at different doses (0.42 mg/kg, 0.85 mg/kg, 1.7 mg/kg, 3.4 mg/kg and 6.8 mg/kg) for 6 days/week for 4 weeks. Thereafter, all the animals except saline and TA-05-treated controls were administered 20 mg/kg Dox intraperitoneally. There was a significant decrease in myocardial superoxide dismutase (38.94%) and reduced glutathione (23.84%) in animals treated with Dox. Concurrently marked increase in serum creatine kinase-MB (CKMB) activity (48.11%) as well as increase in extent of lipid peroxidation (2.55-fold) was reported. Co-treatment of TA-05 and Dox resulted in an increase in the cardiac antioxidant enzymes, decrease in serum CKMB levels and reduction in lipid peroxidation as compared to Dox-treated animals. Electron microscopic studies in Dox-treated animals' revealed mitochondrial swelling, Z-band disarray, focal dilatation of smooth endoplasmic reticulum (SER) and lipid inclusions, whereas the concurrent administration of TA-05 led to a lesser degree of Dox-induced histological alterations. These findings suggest that butanolic fraction of *T-Colostrol* bark has protective effects against Dox-induced cardio toxicity and may have potential as a cardioprotective agent (Sharma *et al.*, 2005).

The results indicated that the plant can effectively be used in the treatment of heart ailments particularly atherosclerosis and lipid related disorders.

## CONCLUSION

Lipid disorders are common problems, nowadays, because of the modern lifestyle and heredity. To overcome such problems, apart from lifestyle change, a treatment option is also mandatory for the normal process. Hyperlipidemia is a common problem, which is the main cause for many heart ailments such as hypertension and arteriosclerosis affection majority of the population, which is increasing year by year. Allopathy way of treatment is encouraging, but the side effects are innumerable than the main effects. In our country, from time immemorial many herbals are being used to treat such ailments. This study was aimed at finding the effectiveness of some known traditional herbal medicines such as *T-Colostrol*.

In this study Cholesterol were given orally. After 7 days, the analysis of serum levels of cholesterol, LDL, VLDL, LDH, Triglycerides was made and the results were interpreted. Significant increased levels were seen. The affected rats were given herbal preparations for about 7 days and afterwards the serum levels were checked for any reduction. During the treatment time they were not allowed to take any other medicines meant for hypercholesterolemia and heart diseases. This study has clearly indicated that this preparation could be given to treat hyperlipidemic individuals.

The result of this study suggests that *T-Colostrol* would be the drug of choice for better control over hyperlipidemia and its associated diseases.

The drugs were given to human being directly because of its traditional use in the society. Though, the toxicity should be studied in animals and human to a molecular level. The results indicated that the plant can effectively be used in the treatment of heart ailments particularly atherosclerosis

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