RESEARCH PAPER | Biochemistry | Volume : 5 | Issue : 8 | August 2015 | ISSN - 2249-555X

Anti-Hyperlipidemic Activity of the Bark Extract of Terminalia Arjuna in Caffeine Induced Mice

KEYWORDS: Cholesterol, Terminalia Arjuna, Caffeine, Coronary heart diseases

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ABSTRACT *The present study was aimed to investigate the effect of bark extract Terminalia Arjuna (2.4g/kg of body weight) on caffeine (150 mg/kg body weight) induced coronary heart disease. Male Swiss albino mice weighing about 20 and 22g were used as the experimental animal for the study. Caffeine dissolved in physiological saline (NaCl) with a pH of 7.0 administered orally to Swiss albino mice continuously for about 14 days. Thereafter, all the animals sacrificed and the blood sample is collected for all groups. The samples were estimated quantitative examinations of Low density lipoprotein cholesterol, Very low density lipoprotein cholesterol, High density lipoprotein cholesterol, triglyceride and Total cholesterol. That the values are compared against with Terminalia Arjuna treated animals. All the animals at the end of experiment showed significant elevation in the level of cholesterol, LDL-cholesterol, VLDL-cholesterol and triglycerides and also a decrease level of HDL-cholesterol ($p<0.001$). These finding suggest that the bark extract of Terminalia Arjuna has protective effects against caffeine induced coronary heart disease and may have potential as a cardio protective agent.*

2. INTRODUCTION

The word cholesterol is derived from Greek word, *chole=bile; steros=solid; ol=alcohol*. Cholesterol widely distributed in the body. In a 70 kg man a total of about 140 g of cholesterol is available; which is distributed as about 30 g in brain and nerves, 30 g in muscles, 30 g in adipose tissues, 20 g in skin, 10 g in liver and spleen, 5 g in bone marrow, 3 g in alimentary tract and 2 g in adrenal gland. Cholesterol is a constituent of all cell membranes. It's necessary for synthesis of all steroid hormones, bile salt and vitamin D, Abnormality of cholesterol metabolism my lead to cardiovascular accident and heart attacks. Cholesterol consists of three major components that they are very low density lipoprotein cholesterol (VLDL), Low density lipoprotein cholesterol (LDL) and High density lipoprotein cholesterol (HDL). The lipids are insoluble in water; they need the help of carriers in plasma. Therefore, they are complex with protein to form lipoprotein.

A word may be devoted here to the general subject of hypercholesterolemia. The blood lipids, cholesterol in particular, may be elevated unrelated condition. Primary hypercholesterolemia is a rare familial condition with a genetic basis. Secondary hypercholesterolemia in which a other lipids may be involved, occurs in a variety of diseases. Of these atherosclerosis is the most important. It may also be marked in diabetic mellitus, hypothyroidism, lipoid nephrosis, and xanthomatous biliary cirrhosis. Soft yellow nodules known as xanthomas may be formed, more particular in the cyelids, but also in wrinkles or over pressure point. These consist of collections of macrophages filled with lipid in the subcutaneous tissue. Hyperlipidemias are of utmost clinical significance. The elevation of lipid in plasma leads to the deposition of cholesterol on the arterial walls, leading some cardiac problem.

Dr. D.S. Fredrickson is considered by many to be the founding father of lipidology. An exceptional scientist, Fredrickson discovered Tangier disease and cholesteryl ester storage disease, two genetic conditions caused by aberrant lipid metabolism. His identification of various apolipoprotein components contributed to our current understanding of lipid transport and physiology. His classification of lipoprotein abnormalities, established on the basis of electrophoretic plasma lipoprotein patterns, was accepted by the World Health Organization as a standard of clinical practice and provoked global interest in dyslipidemic disorders [1].

Table 1: The most accepted Frederickson's classification.

Phenotype	Lipoprotein(s) Elevated	Serum Cholesterol Concentrations	Serum Triglyceride Concentrations	Relative Frequency%
I	Chylomicrons	Normal to ↑	↑↑↑↑	<1
IIa	LDL	↑↑	Normal	10
IIb	LDL and VLDL	↑↑	↑↑	40
III	IDL	↑↑	↑↑↑	<1
IV	VLDL	Normal to ↑	↑↑	45
V	VLDL AND Chylomicrons	↑and ↑↑	↑↑↑↑	5

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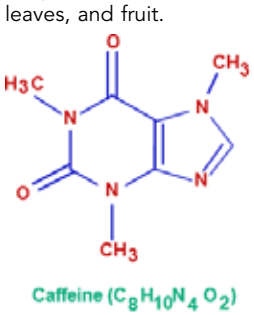
Heart is a myogenic muscular organ found in all animals with a circulatory system, which pumps blood throughout the blood vessels by repeated, rhythmic contraction. The term cardiac (as a cardiology) means "related to the heart" and come from the Greek word. The human heart has a mass of between 250 and 350 grams and is about the size of a fist. It is located anterior to the vertebral column and posterior to the sternum. It is enclosed in a double-walled sac called the pericardium. The superficial part of this sac is called the fibrous pericardium. This sac protects the heart, anchors it is surrounding structures, and prevents overfilling of the heart with blood.

The outer of the human heart is composed of three layers. The outer layer is called the epicardium, or visceral pericardium since it is also the inner wall of the pericardium. The middle layer is called the myocardium and is composed of cardiac muscle which contracts. The inner layer is called the endocardium and is in contact with the blood that heart pumps. Also it merges with inner lining (endothelium) of blood vessels and heart valves.

The following diagnostic test will help for early detection of cardiac problem. The enzymes are helpful identification cardiac problems so that the particular enzymes are noted as "cardiac bio markers" that enzymes are troponin test, creatine kinase, Lactate dehydrogenase, aspartate transaminase, and myoglobulin.

2. CAFFEINE

Caffeine was isolated from coffee in 1820 by the Fredlieb Ferdinand Range, German chemist. Caffeine is a bitter, white, crystalline Xanthine alkaloid that acts as a stimulant drug. Caffeine is found in varying quantities in the seeds, leaves, and fruit.



Caffeine ($C_8H_{10}N_4O_2$)

Structure of caffeine

IUPAC name : 1, 3, 7-Trimethylpurine-2,6dione
Chemical Name : Methyl therobromine
Molecular Mass : 194.19 g/mol
Appearance : Odourless, white powder,
Density : 1.23 g /cms
Melting Point : 227 – 228 Boiling
Point : 178°C

3. PLANT DESCRIPITION:

Kingdom	**Plant**
Division	Magnoliphyta
Class	Mangnolipsida
Oder	Myrtales
Family	Combretaceae
Genus	Terminalia
Species	T.arjuna
Binomial name	Terminalia Arjuna

Table 2: Plant descriptions

4. COLLECTION OF PLANT:

The Healthy root of *Terminalia* has to use in this experiment. The Bark of plant has collected from the agriculture office Thiruvannamali, Tamilnadu, India. The barks of Terminalia selected and then clean dried. These dray bark are grained mechanical to make powder form.

5. METHOD AND MATERIAL:

5.1 Animals

Healthy male swiss albino mice (8 – 14 week age) 25 – 30 g weight animal purchase from Tamilnadu veterinary and animal science university (TANUVS), Madhavaram, Chennai and warehouse under statured condition of temperature (26 H) and illumination (12 h light day cycle) water and standard rodent food IAC, chayyar. Ethical number is IAC/IAE/21/12. Animal are were categorized in to five group each have six animals. First group animals treats as (normal or) control animals, second group consist of hyperlipidimic induced animals, third group animals treated with plant extract, fourth group animals are hyperlipidimic induced with treatment of plant extract, well know group of drug traded animals are categorized as group five.

5.2 Plant Extract

The collected plant extract powder was mixed with 70% ethanol at room temperature for 3days after the supernatant was collected in to china disk. The china disk over boiling 45°C, semi solid extract was obtained after complete elimination alcohol. The extract was made up to a know volume in distilled water just before oral administration.

5.3 Estimation Biochemical Parameter

Serum Glucose, Uric acid, Triglyceride, Cholesterol, High density lipoprotein cholesterol (HDL), Low density lipoprotein cholesterol (LDL), Lactate dehydrogenises (LDH), Aspartate Aminotranferase (AST) and Alanine Aminotransferase were estimated according to the method of Reitman and Frankel, [10].Creatinine kinase was estimated by Disk and subharow's method. Urea was determined by the diacetylmonoxime (DAM) method [13]. Serum creatinine was estimated according to Jaffe's Reaction [14]. Serum total protein was determined by Lowry *et al.*, [8]. The biochemical parameters compared with the control animals. The experimental of all group values are showed in table 3 and their values showed in graphically in image 1.

SNO	EXPERIMENTAL MODEL	Glucose (mg/dl)	Creatinine (mg/dl)	Uric acid (mg/dl)	Urea (mg/dl)	SGOT (U/L)	SGPT (U/L)	Cholestrol (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	CK (U/L)	LDH (U/L)	Total Protein (g/dl)	Triglycerides (mg/dl)
1	Group I	98	1.1	5.5	28.6	32.1	30.8	152.6	31	86.5	137.3	176.5	6.3	125.6
2	Group II	101.3	1.2	5.8	30.5	33	32	162.1	33.5	91.8	139.5	180	6.4	128.3
3	Group III	183.5	2.8	8.2	60	67.6	50	291	63.8	157.6	211.8	311	9.7	218
4	Group IV	105	1.2	6.0	32.6	34	32	169	34.1	94.2	148	189	6.7	131.1

Table 3: Estimation of Biochemical parameters.

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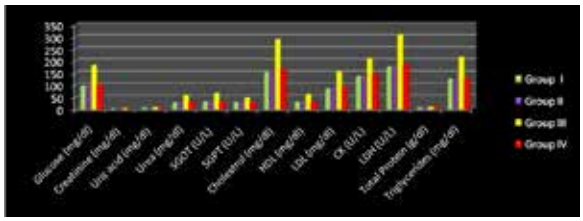


Fig 1 Estimation of Biochemical parameters

5.4 Comparison Biochemical Parameters

The leave of Serum Glucose, Cholesterol, LDH and CK in (normal or) Control animals, Test control animals, Induced animals and *Terminalia Arjuna* treated with caffeine induced animals. The values are expressed as MEAN+SD for six animals in each group. The values are showed in Table 2 with graphical image 1. The leave of Serum SGOT, SGPT and urea in (normal or) Control animals, Test control animals, Induced animals and *Terminalia Arjuna* treated with caffeine induced animals. The values are expressed as MEAN+SD for six animals in each group. The values are showed in Table 3 with graphical image 2. The leave of Serum creatinine and total protein in (normal or) Control animals, Test control animals, Induced animals and *Terminalia Arjuna* treated with caffeine induced animals. The values are expressed as MEAN+SD for six animals in each group. The biochemical parameters compared with the control animals. The values are showed in Table 4 with graphical image 2.

Table 4: Levels of serum Glucose, Urea, Creatinine and Uric Acid in normal, test control, induced animals and caffeine induced with *Terminalia Arjuna* treated animals. The values are expressed as MEAN+SD for all groups of animals.

PARAMETER	GLUCOSE	UREA	CREATININE	URIC ACID
NORMAL	98 ±0.56	28.6±0.25	1.1±0.15	5.5±0.32
TEST CONTROL	101.3±0.68	30.5±0.31	1.2±0.17	5.8±0.38
INDUCED	183.5 ±1.56	60±0.42	2.8±0.19	8.26±0.56
TREATED	105±0.56	32.6±0.54	1.2 ±0.17	6±0.41

Values are expressed as MEAN+SD for all groups of animals.

Fig 2: Levels of serum Glucose, Urea, Creatinine and Uric Acid in experimental mice

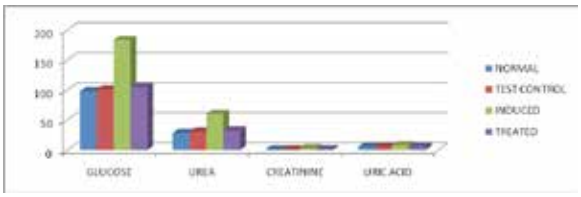
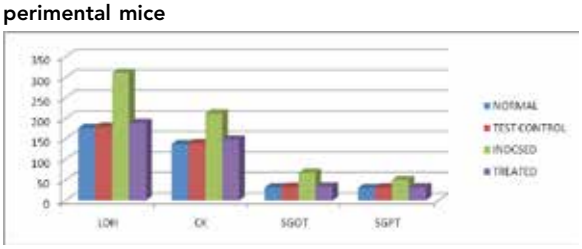


Table 5: Levels of serum LDH, CK, SGOT and SGPT in normal, test control, induced animals and caffeine induced with *Terminalia Arjuna* treated animals. The values are expressed as MEAN+SD for all groups of animals.

PARAMETER	LDH	CK	SGOT	SGPT
NORMAL	176.5 ±2.65	137.3±1.52	32.1±0.21	30.8±0.23
TEST CONTROL	180±3.12	139.5±1.65	33±0.25	32±0.24
INDUCED	311±5.32	211.8±2.35	67.6±0.45	50±0.35
TREATED	189±3.65	148±1.75	34±0.28	32±0.54

Values are expressed as MEAN+SD for all groups of animals.

Fig 3 Levels of serum LDH, CK, SGOT and SGPT in experimental mice



PARAMETER	TOTAL CHOLESTROL	TRIGLYCERIDE	HDL	LDL	TOTAL PROTEIN
NORMAL	152.6±1.02	125.6±1.34	31±0.65	86.5±0.32	6.3±0.52
TEST CONTROL	162.1±1.35	128.3±1.25	33.5±0.35	91.8±0.65	6.4±0.45
INDUCED	291±5.23	218±3.21	63.8±0.65	157.6±0.98	9.7±0.56
TREATMENT	169±1.89	131.1±1.20	34.1±0.45	94.2±0.75	6.7±0.42

Table 6: Levels of serum LDH, CK, SGOT and SGPT in normal, test control, induced animals and caffeine induced with *Terminalia Arjuna* treated animals. The values are expressed as MEAN+SD for all groups of animals.

Values are expressed as MEAN+SD for all groups of animals.

6. Result and Discussion

Terminalia Arjuna is a plant, well known for its cardio protective properties in the ancient Indian system of medicine. In the present study is a mainly focused cardio protective effect of oral administration *Terminalia Arjuna* against caffeine induced coronary heart disease. The result indicate that oral administration of caffeine in induced animals produced an increase in total serum cholesterol, Triglyceride, LDL – Cholesterol and VLDL cholesterol with a decrease in HDL cholesterol level relative to the control group animals. It show the significant of ($p<0.001$) when compared to control. Since the level of HDL cholesterol concentration has decreased and LDL cholesterol concentration has increase, these have been associated with increased risk of coronary heart disease. The mice treated with *Terminalia Arjuna* had a marked reduction in total cholesterol, triglycerides, LDL cholesterol and VLDL cholesterol .However, it also showed a increase HDL cholesterol with a significance of $p<0.005$ when compared to induced animals.

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6. Conclusion

Hyperlipidemic is metabolic disorder involving carbohydrate, Protein and fat metabolism due to a relative or an absolute elevation of cardiac enzymes. The body weight and weight of heart were corrected back to near normal levels on *Terminalia Arjuna* break extract treatment. The variation of biochemical parameter glucose, Urea, Cholesterol, Uric acid, Serum protein and activity of enzymes like serum Glutamate pyruvate transaminase (SGPT), CPK, LDH and live glutamate oxaloacetate transaminase (GOT) in caffeine induced cardiac mice have been corrected by *Terminalia Arjuna* bark extract treatment, when compared with normal and control animals.

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