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Herbal Formulation development for Hypolipidemic and Anti-Obesity activity on Heartwood of *Caesalpinia sappan* Linn

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Abstract

Medicinal plants have been shown to play a major role in treating hyperlipidemia and obesity and considering this, the present study was designed to develop a herbal capsule formulation on heartwood of Caesalpinia sappan for hypolipidemic and anti-obesity activity. The hydro alcoholic extract (1:1) on heartwood of Caesalpinia sappan was prepared by cold maceration method and formulated into a hard gelatin herbal capsule. The quantitative analysis of phytoconstituents especially of flavonoids and phenolic content present both in extract and formulated herbal capsule was estimated by HPLC method showed the presence of rutin, quercetin, gallic acid, ascorbic acid and tannic acid. Hydroalcoholic extract of Caesalpinia sappan (HECS) herbal capsule was evaluated for acute toxicity studies in albino wistar rats and showed no toxicity upto 2000mg/kg. In-vivo evaluation of high fat diet (HFD) induced obesity in rats was carried out for HECS herbal capsule .The studies showed HECS herbal capsule(200mg/kg and 400mg/kg) significantly reduced the elevated levels of body weight, total cholesterol, triglycerides, LDL-cholesterol, VLDL-cholesterol, SGPT and SGOT and elevated the decrease level of HDL-cholesterol. These results suggest that, HECS capsule possess good hypolipidemic and anti-obesity activity, which may be due to its flavonoid, saponin and phenolic content.

Keywords: *Caesalpinia sappan, Atherosclerosis, Herbal capsule formulation, High fat diet, Hypolipidemic and Anti-obesity activity*

Introduction

Coronary artery disease (CAD), also known as ischemic heart disease (IHD) is a group of diseases that includes: coronary occlusion (**Atherosclerosis**), Angina pectoris, myocardial infarction and sudden coronary death. It is within the group of cardiovascular diseases of which it is the most common type. It is reported that almost 12 million people die of CHD disease each year all over the world. Risk factors of CAD includes: high blood pressure, diabetes, **high blood cholesterol obesity**, lack of exercise, smoking, lack of exercise, excessive alcohol, depression[1].
Brain receives blood from basilar artery and internal carotid artery. The obstruction of blood flow to the brain leading to permanent damage is called as **Cerebrovascular accident** (CVA) or cerebral infarction (stroke). If the symptoms are only temporary, it is referred to as a Transient ischemic attack (TIA). Rupture of an artery within the brain (cerebral haemorrhage) is also called a **stroke** or cerebrovascular accident. **Atherosclerosis** (plaque in the arteries) is the leading cause of cerebral ischemia[2]. Risk factors for cerebrovascular disease include smoking, coronary artery disease, hypertension, diabetes, **hyperlipidemia**, peripheral vascular disease, atrial fibrillation, carotid disease and valvular heart disease. Factors that may affect the rating of an applicant with a history of cerebrovascular accident include current neurological residuals, hypertension, **high cholesterol levels**, and generalized atherosclerosis[3].

Hyperlipidemia is one of the greatest risk factors which further lead to **coronary heart diseases, stroke, atherosclerosis** and **ischemic heart diseases**, which are the primary cause of death. Hyperlipidemia is a condition characterized by elevation of one or more lipids including cholesterol, cholesterol esters, phospholipids and triglycerides in the blood stream. It is also called as hyperlipoprotenemia, because these fatty substances travel in the blood attached to protein. Central to the pathogenesis of atherosclerosis is deposition of cholesterol in the arterial wall. Nearly all lipoproteins are involved in this process including cholesterol carried by very low density lipoproteins (VLDL), low density lipoproteins (LDL) and remnant lipoproteins. Hyperlipidaemia results in metabolic syndrome which is characterized by **obesity,** Insulin resistance and endothelial cell dysfunction which ultimately ends in Hypertension, Diabetes (or) Stroke[4].

Obesity is regarded as a social problem, associated with serious health risks and increased mortality. Obesity

is difficult to define in quantitative term. It refers to the above average amount of fat contained in the body this in turn is dependent on the **lipid content of each fat cell** and on the total number of fat cells. WHO says obesity is related to **cardiovascular disease, hypertension, diabetes mellitus, cancer, osteoarthritis, pulmonary diseases,** as well as psychological issues, including social bias, prejudice discrimination and over eating[5]. According to WHO more than half of the total mortalities are associated with cardio vascular diseases. It is estimated that 12 million deaths per year occur from cardiovascular diseases, while one million of death in the European country occur due to obesity per year[6].

Herbal Medicine sometimes referred to as Herbalism or Botanical Medicine, is the use of herbs for their therapeutic or medicinal value. An herb is a plant or plant part valued for its medicinal, aromatic or savoury qualities. Herb plants produce and contain a variety of chemical substances that act upon the body. The World Health Organization (WHO) estimates that 4 billion people, 80 % of the world population, presently use herbal medicine for some aspect of primary health care. Herbal medicine is a major component in all indigenous peoples' traditional medicine and a common element in Ayurvedic, homeopathic, naturopathic, traditional oriental, and Native American Indian medicine[7].

Herbal formulations enhances physical endurance, mental functions and non-specific resistance of the body and have been termed as Adaptogens. The potential utility of safer and cheaper herbal medicines as anti-obesity and hypolipidemic agents have been reported as they can withstand without altering the physiological functions of the body[8]. With this background the present study has been undertaken to prepare herbal capsule formulation.

Materials and Methods

Collection and authentication of plant material and chemicals

The Heartwood of *Caesalpinia sappan* Linn. Was collected from Kulesekaram in Kanyakumari district, Tamil Nadu in June-2015. The plant material was authenticated by Dr.V.Chelladurai, Research officer- Botany, Central Council for Research in Ayurveda and Siddha, Tirunelveli. The heartwood was shade dried, coarsely powdered and used for further studies. High cholesterol diet was prepared in college lab and 99% cholesterol (Analytical grade) from Fine chemicals, Chennai.

Extraction of plant material[9]

About 200gm of coarsely pulverized heartwood was taken in a closed bottle and it was defatted with petroleum ether. The defatting was continued for 9-10 days with occasional shaking. The petroleum ether extract was filtered. The marc left after petroleum ether defatting was taken out and dried under shade to get a dry mass, then extracted with ethanol and water (50:50) by using cold maceration extraction. The extraction was continued for 9-10 days with occasional shaking. The hydro alcoholic extract was filtered, concentrated under reduced pressure to a semisolid mass and was made free from solvent. The final obtained extract was weighed; percentage yield was calculated and stored in a cool place.

Evaluation of Quality Control Parameters for Raw Material

Raw materials are standardised such as determination of organoleptic evaluation, microscopical characters of heartwood, phytochemical constituents, then the quantitative amount of flavonoids content and phenolic compounds in hydro alcoholic extract was determined by HPLC method, and was expressed as milligrams of rutin equivalents (RE) per g and milligrams of Gallic acid equivalents (GAE) per g of Hydro alcoholic extract of *Caesalpinia sappan was* already summarised and reported[10]. Foreign organic matter determination (0.44%), physicochemical constant determination like ash values (total ash, water soluble ash, acid insoluble ash) and extractive values (alcohol soluble and water soluble extractives) were carried out for purity of plant material under WHO Guidelines and reported[11].

Quantitative analysis of heavy metals[12]

The raw material was analysed for its heavy metal limits and it was reported.

Instrument name: Inductive coupled plasma-Optical emission spectroscopy.

Detector system: Charge coupled detector, (UV-Visible detector which is maintaining at 40º C to detect the intensity of the emission line. Preparation of sample by acid digestion method. The results are reported in table 2.

Determination of foreign organic matter [13]

Procedure: 100g of the drug sample was weighed and then it was spread out in a thin layer. The foreign matter was detected by inspection with the use of a lens. Foreign matter found were separated and weighed and the percentage was calculated and the results are reported in table 6.

Microbial load analysis[14]

The following test is carried out for the estimation of number of viable aerobic microorganisms present and for detecting the presence of designated microbial species in the herbal medicines. The hydroalcoholic extract was analysed for its microbial load. The following test are

carried out for the estimation of number of viable aerobic microorganisms present and for detecting the presence of designated microbial species in the extract like Total aerobic viable counter, Yeast and moulds, *Escherichia coli, Salmonellae, Pseudomonas, Staphylococcus* and the results are reported in table 7.

HPTLC finger printing of extract

HPTLC is one of the versatile chromatographic method which helps in the identification of compounds and thereby authentication of purity of herbal drugs. The time required in this method for the demonstration of most of the characteristic constituents of a drug is very quick and short. In addition to qualitative detection, HPTLC also provides semi- quantitative information on major active constituents of a drug, thus enabling an assessment of drug quality.

Instrument: CAMAG HPTLC

Equipment: A Camag HPTLC system equipped with a sample applicator Linomat IV, Twin trough plate development chamber, TLC Scanner II.

Chromatographic conditions: Chromatography was performed on a 12 × 3 cm (H x W) pre-activated HPTLC silicagel 60 F254 plate.

Preformulation Studies[15]

To formulate any dosage forms, it is essential that fundamental physical and chemical properties of the drug powder are to be determined.

Definition*:* Preformulation involves the application of biopharmaceutical principles to the physicochemical parameters of drug substance are characterized with the goal of designing Optimum drug delivery system.

Selection of excipients[16]

For the formulation of capsules in addition to the active ingredients, excipients like diluents (filler), binder, disintegrating agent, lubricant and preservatives are required. The choice of excipients was made keeping in mind the current Food and Drugs Administration (FDA) regulations.

a) Diluents: Diluents/Fillers are added where the quantity of active ingredient is less (or) difficult to filling. Common tablet/capsule filler include Lactose, Dicalcium phosphate, Microcrystalline cellulose, etc.

b) Lubricants**:** They reduce friction during the filling process. In addition, they aid in preventing adherence of capsule material. Magnesium Stearate, Stearic acid, Hydrogenised vegetable oils and talc are commonly used lubricants.

c) Glidants**:** It is used to improve flow of the powder materials by reducing the friction between the particles. The most effective glidants are the Colloidal silicon dioxide, Talc and Starch.

E) Preservatives: The preservatives are added to herbal formulation to prevent contamination, deterioration and spoilage by bacteria, fungal and other microorganisms. The most effective preservatives are the sodium methyl paraben, sodium propyl paraben, sodium benzoate and bronopol. Selection of excipients in the formulation are given below:

- Microcrystalline cellulose
- Starch
- Colloidal sillicon dioxide
- Magnesium stearate
- Bronopol& sodium methyl paraben

Preparation of formulation[17-20]

The dry hyroalcoholic extract of *Caesalpinia sappan* were dried in tray drier at 60^{o}c for 20 minutes. All excipients used in this formulation except preservatives were dried separately in tray drier at 100^{o}c for 30 minutes. All active ingredients were weighed according to the formula, mixed and lubricated with magnesium stearate followed by diluents and preservatives were mixed well. The mixture was blended thoroughly for 30 minutes. Then the powder was transferred to the polythene bags and labelled for further studies.

From the 3 trial batches one optimized batch is selected for formulation based on above results. Trial batch 3 was found to be the perfect batch and it was selected for the consideration of further large scale manufacturing and the results was given in table 8&9.

Formulation of Capsules[21-26]

Capsules are small dosage form in which one or more medicinal and inert ingredients are enclosed in a small shell usually made of gelatin.

Capsule size and selection of filling method

The formulated granules were filled in “1” size capsules to an average net content the weight of 270 mg. The capsules were then de dusted, transferred into polybags, labelled and the Samples were evaluated as per the testing requirements. After approval from QAD the capsules were packed as per the packing instructions. A hand operated gelatin capsule filling machine (Chamunda pharm machinery) was used in this study for encapsulation of capsules from the final trial, samples were taken for accelerated stability studies as per the testing requirements.

Fig 1: Herbal capsule

Standardisation of herbal capsules[27,28]

The developed herbal capsules were standardized for its description, uniformity of weight, disintegeration time, moisture content, physicochemical parameters, phytochemical studies, fluorescence analysis. Standardization were carried out as per Indian pharmacopoeial procedures.

Quality control parameters

Description

The general appearance of a capsule, its visual identity and overall "elegance" is essential for consumer acceptance. The colour, shape, odour and surface texture are all noted for the capsules prepared.

Uniformity of weight: 20 individual units were selected at random and their content was weighed and their Average weight was calculated. Not more than two of the individual weights deviate from the average weight

Disintegration test*:* Disintegration test was performed using the digital microprocessor based disintegration test apparatus (Veego, Mumbai).One capsule was introduced into each tube and added a disc to each tube. The assembly was suspended in the water in a 1000 ml beaker. The volume of water was such that the wire mesh at its highest point is at least 25 mm below the surface of water, and at its lower point was at least 25 mm above the bottom of the beaker. The apparatus was operated and maintained the temperature at 37±2°C. (Indian Pharmacopoeia, 2010).

Determination of moisture content: The loss on drying test is important when the herbal substance is known to be Hygroscopic. An excess of water in medicinal plant materials will encourage Microbial growth, the presence of fungi, insects deterioration. In modern Pharmaceutical technology, the water content provides information concerning the Shelf life and quality of the drugs.

pH*:* 1 g of capsule powder was taken and dissolved in 100 ml demineralized water. The pH value of the solution was determined by means of a digital PH meter. The pH meter was calibrated using buffers of 4, 9 and 7 PH. The electrodes were immersed in the test solution and PH was measured results are reported in table 10&11.

Accelerated stability studies of the capsules[29-31]

Studies designed to increase the rate of chemical degradation or physical change of a drug substance or drug product by using exaggerated storage conditions as per of the formula stability studies. The ICH Harmonized Tripartite Guideline provides a general indication on the requirements for stability testing of new drug substances and products.

Accelerated stability condition : Accelerated stability study were carried out of storage condition at **40° C ± 2° C of humidity 70% RH for 30 days(time period covered).** The capsules found to be stable.

Pharmacological studies

Acute Toxicity Study (OECD 423 Guidelines) [32, 33]

Animals were kept in the lab for one week to acclimatize to laboratory conditions before starting the experiment, they were allowed to free access of water and standard rat feed. Organization for Economic co-operation and development (OECD) regulates guideline for oral acute toxicity study. It is an International Organization which work with the aim of reducing both the number of animals and the level of pain associated with acute toxicity testing. Organization for Economic co-operation and development (OECD) regulates guideline for oral acute toxicity study.

Toxicity – Acute toxic class method (OECD 423 guidelines)

The acute oral toxicity study was carried out as per the guidelines set by Organization for Economic Co-operation and Development (OECD), received from Committee for the Purpose of Control and Supervision of Experiments of Animals (CPCSEA), Ministry of Social justice and Empowerment, Government of India.

Healthy female albino rats were selected and the animal were procured from the animal house of the Institution were used. Animals were divided into 2 groups of 3animals each. The starting was deprived of diet for four hours and water was given *ad libitum*. The animals dose level 2000 mg/kg bw.,p.o of the HECS capsules was administrated.

The animals were kept under direct observation for first four hours and thereafter for 24 hours and were observed for mortality. The animals were then kept under observation for 14 days. Body weight of rats before and at the end of the termination was observed and any changes

in awareness, mood, motor activity , CNS excitation, Motor coordination, muscle tone , reflexes were noted. The onset of toxicity and signs of toxicity was also if any noted. There was no death as per the guidelines, so the study was repeated with the same dose to confirm the result.

The protocol for conducting the *In Vivo* study in either sex of adult *albino wistar* rat was approved by the Institutional Ethical Committee (ICE) of the Madras Medical College, Chennai - 600003, India **Approval no: Vide 14/243/CPCSEA. Dated :10.08.2015**

Experimental design[34-36]

***In-vivo* evaluation of hypolipidemic and anti-obesity activity (high fat induced obesity in rats model)**

Experimental animals

Thirty *albino wistar* rats of weighing 140-160 gm were randomly divided into 5 groups of six animals each and kept in their cages for 1 week prior dosing to allow for acclimatization to the laboratory conditions, with free access to food and water, *ad libitum*. The study was carried out after obtaining the Animal ethics committee (**14/243/CPCSEA. Dated :10.08.2015**).

Chemicals

Cholesterol, coconut oil and Atorvastatin, chemicals used for the study were of analytical grade.

Dose selection

The content of HECS herbal capsules was found to be safe at the dose of 2000mg/kg in the acute toxicity study. Hence for *In vivo* evaluation, two doses of the hydro alcoholic extract of *Caesalpinia sappan* (HECS) herbal capsule were selected as 200mg and 400mg/kg, p.o. Atorvastatin was calculated based on a human dose of 1mg /kg, p.o.

Experimental design

Rats were divided into five groups containing six animals each. The schedule of grouping and treatment is given below in table 1.

Group	Name of the group	Treatment schedule
Group 1	Normal control	Normal food and vehicle p.o for 60 days
Group 2	Disease control	HFD and vehicle p.o for 60 days
Group 3	Standard control	HFD and vehicle for p.o 60 days and atorvastatin 1mg/kg from 31-60th days.
Group 4	Test group 1	HFD and vehicle for 60 days and HECS herbal capsule 200mg/kg p.o from 31-60th days.
Group 5	Test group 2	HFD and vehicle for 60 days and HECS herbal capsule 400mg/kg p.o from 31-60th days.

Hyperlipidaemia was induced by feeding a high fat diet that consists of 58% fat, 25% protein and 17% carbohydrate, lard(13%), vitamins, minerals and cholesterol 400mg/kg in coconut oil to all healthy rats except group 1(normal control rats)for 60days.

Evaluation parameters

A) Body weight

The body weight (g) was recorded on day1, day 30 and day 60 using a digital weighing balance in each group animals. The changes in body weight were calculated.

B) Organs weight

The animals were sacrificed on 60th day by cervical dislocation and then the different organs (kidney, liver and heart) were removed and then weighed.

C) Biochemical lipid constituents parameters

2ml of the blood was collected from retro orbital sinus puncture, and all the animals were sacrificed by cervical dislocation, and then the organs were removed and weighed. The collected blood was allowed to clot for 30 minutes, centrifuged and then used for evaluating the lipid constituents and biochemical parameters.

Biochemical lipid constituents/parameters -The main biochemical parameters recommended by the National Cholesterol Education Program (NCEP) guidelines (2002) for lipid screening as follows.

- Total Cholesterol (TC)
- Low Density Lipoprotein Cholesterol (LDL)
- Very Low Density Lipoprotein
- Cholesterol (VLDL)
- High Density Lipoprotein Cholesterol (HDL)
- Triglycerides (TG)

They were evaluated from the serum. From the values, atherogenic index (TC:HDL) and LDL:HDL ratio were calculated using the formula.

Serum glutamate oxaloacetate transferase (SGOT) and serum glutamate pyruvate transferase (SGPT) by standard method were also evaluated from serum using standard methods.

Cardiac risk indicators — The cardiac risk ratios recommended by NCEP guidelines (2002) were estimated by calculating the TC: HDL ratio (Atherogenic Index) and LDL: HDL ratio. The Friedewald formula was used to calculate serum low-density lipoprotein cholesterol(LDL-C) values and atherogenic index as follows:

LDL-C= TC__ (HDL-C+TG/5)

Atherogenicindex(AI)=(total cholesterol__HDL-C)/ HDL-C

Histopathological studies

A small portion of aorta and liver was taken from each group and was immediately put in 10% Formasal

(formalin diluted to 10% with normal saline) and then it was processed. Sections were Stained with Ehrlich's haematoxylin and Eosin to find out the Atherosclerotic lesions in aorta and to find out cellular degeneration and necrosis in liver.

Statistical Analysis

Results were expressed as Mean ± SEM. The data was analyzed using one way analysis of variance (ANOVA) followed by Dennett's test. P values <0.01 were considered as Significant.

Results

Quantitative Estimation of Heavy Metals by ICP OES Method

Table 2: Quantitative estimation of Heavy metals

S.No	Element	Results (ppm)	Specification as per WHO Guidelines
1.	Mercury	Not detected	Not more than 0.5ppm
2.	Arsenic	Not detected	Not more than 5.0ppm
3.	Lead	0.002	Not more than 10ppm
4.	Cadmium	Not detected	Not more than 0.3ppm

Quantitative estmation of phytoconstituents

The *Caesalpinia sappan* Linn., was found to contain various phytochemical constituents and hence it is desirable to quantify few of them in order to establish a standard to maintain its quality. Among them the estimation of total Saponins , Flavonoids and phenolic content in the hydroalcoholic extract were decided to be taken as parameters. Samples were drawn from three random samples of *Caesalpinia sappan* Linn., and the total Saponins, Flavonoids and phenolic content present in them were estimated by HPLC method.

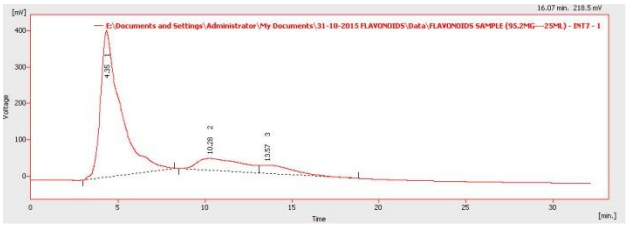

Fig 2: Graphical representation of Hydroalcoholic extract curve for flavonoids

Table 3: Quantitative estimation of flavonoids present in hydroalcoholic extract

Flavonoids	Hydoalcoholic extract per gm.
Rutin	0.934mg
Quercetin	0.184mg
Gallangin	0.158mg

Estimation of phenolic content by hplc method

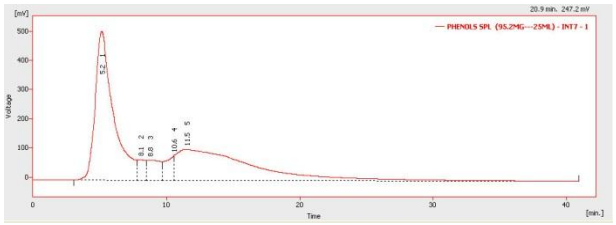

Fig 3: Graphical representation of aphenolic content

Table4: Quantitative estimation of phenolic content present in hydroalcoholic extract

Phenolic content	Sappan Extract Per gm.
Gallic acid	0.519mg
Tannic acid	0.083mg
Ascorbic acid	0.987mg

HPTLC Finger print Data of hydroalcoholic Extract of *caesalpinia sappan* Linn.,

High performance thin layer chromatography (HPTLC) finger printing was performed with the hydroalcoholic extract of ***Caesalpinia sappan* Linn.,**.

Solvent system

Table 5: Solvent system for HPTLC

Extract	Solvent System
Hydroalcoholic extract	Ethyl acetate : chloroform : methanol (5.3:1.5:0.5)

Fig 4: HPTLC Finger Print Data

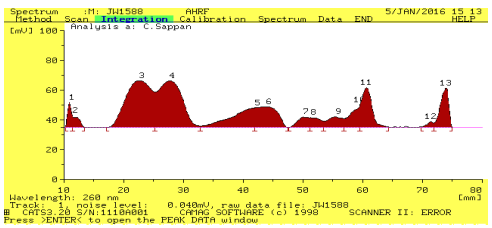

Fig 5: Graphical representation of chromatographic finger printing analysis of the extract

The HPTLC analysis of the extract showed 13 peaks of different Rf values and absorbance.

Preparation of formuulation

The raw materials were sampled, authenticated and analysed for their compliance to quality standards as established by WHO guidelines, pharmacopoeial and other standard reference books.

Raw material standardization

Table 6: Foreign organic matter determination

Plant name	Observation (w/w)	Limit
Caesalpinia sappan	0.44±0.01	NMT 2

Results are reported as mean ± standard deviation; (n=3): NMT- not more than.

The raw material standardization of organoleptic evaluation, microscopical evaluation and physico chemical evaluation were already given in the pharmacognostical and phytochemical studies of the plant.

Microbial load analysis

Tests carried out for the estimation of number of viable aerobic microorganisms present

Table 7: Microbial load analysis of the hydroalcoholic extract of Caesalpiinia sappan

S.no	Parameters	*Caesalpinia sappan* extract
1	Total aerobic count(NMT 1000cfu/g)	50cfu/g
2	Yeast and mould count(NMT 100 cfu/g)	1 cfu/g
3	*E.Coli*(To be absent)	Absent
4	*Salmonella*(To be absent)	Absent
5	*Pseudomonas*(To be absent)	Absent
6	*Staphylococcus*(To be absent)	Absent

Preformulation studies

Table 8: Development of formulation

Parameters	Trial-1	Trial-2	Trial-3
Bulk density(g/cm)	0.42±0.01	0.38±0.05	0.35±0.04
Tap density(g/cm)	0.45±0.03	0.47±0.01	0.50±0.04
Compressibility index(%w/w)	26.83±0.66	23.26±2.54	13.06±1.12
Hausner ratio	1.35±0.15	1.22±0.02	1.13±0.01
Angle of repose(^{o})	40.42±2.57	39.36±2.67	34.66±0.18

All values are expressed as standard mean deviation ±, where n=3.

As per the standards, the flow property of the blend to be filled in the capsules should be in good range and was confirmed by the above parameters. Trial batch- 3 showed excellent flow characters and that batch was taken for capsule filling.

The trial 3 flow properties were Excellent and all parameter were within the Specified limits. So, third trial was chosen for further studies.

Standardisation of finished formulation

The final batch was tested for organoleptic characters, physical and physic chemical parameters. The results observed are shown in table.

Table 10: Organoleptic characters

Name of test	Observations
Description	Pale brown powder contained in purple cap/ transparent body “1” size capsule
Colour	Reddish brown powder
Odour	Characteristic odour
Taste	Bitter

Table 11: Physical parameters

Name of the test	Observations
Moisture content	3.6%±0.22
Uniformity of weight	268mg±4.5mg
Disintegration time	3.32(min)±0.34
pH(1% aqueous solution)	7.33±0.21

results (n-=3) are reported as mean ± standard deviation.

- 1% aqueous solution of herbal formulation showed acidic pH.
- The average weight of the capsules was calculated as per I.P and the obtained value was with in the limit (±10%).
- Sample were taken randomly (3times) to specify quantity, the moisture content was calculated as per trail and error by KFR titration method. The result were given in the above table .
- Disintegration time of the herbal capsule was performed as per I.P and the obtained value showed that the capsule will be disintegrated within the prescribed time for the absorption.
- The uniformity of weight of the capsules was calculated as per the I.P and obtained value was within limit (±7.5).
- The formulated herbal capsule weight were the lower limit is noted as 248 mg and the upper limit is noted as 287mg.

Preliminary phytochemical screening of capsules

The herbal formulation was found to contain various phytochemical constituents and hence it is desirable to quantify few of them in order to establish a standard to

maintain its quality. Preliminary screening showed the presence of saponins, phenolic compounds, flavonoids, carbohydrates, proteins and terpenoids. Among them the estimation of total phenolics, Flavanoids in the aqueous extract were decided to be taken as parameters. Samples were drawn from three random samples of herbal capsules and the phenolics, Flavonoids content present in them were estimated by HPLC method and it is given below.

Estimation of flavonoids for HECS herbal capsule

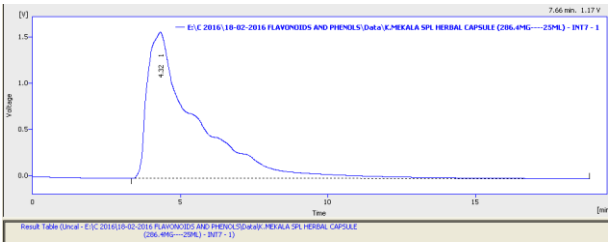

Fig 6: Graphical representation of HECS herbal capsule for flavonoid

Table 12: Quantitative estimation of flavonoids present in each herbal capsule

Flavonoids	HECS Herbal Capsule (250 MG)
Rutin	0.258mg
Quercetin	0.091mg
Gallangin	0.056mg

Estimation of phenolic compounds for HECS herbal capsule

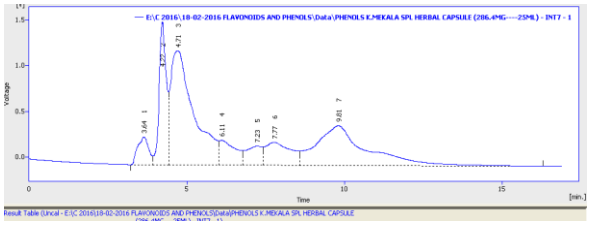

Fig 7: Graphical representation of Estimation of phenolic compounds

Table 13: Quantitative estimation of phenolic compounds in each capsule

Phenolic compounds	HECS herbal capsule (250mg)
Gallic acid	0.224mg
Ascorbic acid	0.481mg
Tannic acid	0.138mg

From the results obtained it is determined that the average content of phenolics, Flavonoids were present in the herbal formulation.

Pharmacological studies

Acute toxicity studies

Behavioural and physical observation of Caesalpinia sappan Linn., treated rats (2000mg/kg body weight) Acute toxicity studies were carried out as per the OECD Guidelines 423 and the HECS herbal capsules were found to be no morbidity and mortality upto 2000 mg/Kg body weight. Hence 1/10th and 1/20th of the dose (200 and 400mg/kg) were taken for the study.

In-vivo hypolipidemic and anti-obesity activity

Table 14: Changes in body weight

Groups	Changes in body weight			% increase in body weight
	1st day	30th day	60th day	
Group 1 (Normal control)	150±7.07	153±6.32	154±5.49	2.5%
Group 2 (Disease control)	154±8.01	183.3±12.11[a]	210±14.14[a]	36.36%
Group 3 (Standard control)	160±14.14	185±10.48[ab]	166±8.01[ab]	4.1%
Group 4 (low dose 200mg/kg)	168±14.71	202±14.71[ab]	188±17.51[ab]	11.9%
Group 5 (high dose 400mg/kg)	166±11.14	200±12.64[ab]	174.16±8.16[ab]	4.9%

Values are expressed as mean ± SEM(n=6)

'a' values are significantly different from normal control at P< 0.05

b' values are significantly different from disease control at P <0.05

Data are analysed by one way ANOVA followed by DUNNETT'S t-test.

It is seen that there was a considerable increase in the body weight of animals which was treated with HFD induced obesity. This increase in body weight was much reduced in animals concomitantly treated with Atorvaststin and HECS herbal capsule in 2 doses of 200mg/kg and 400mg/kg.

Effect of HECS herbal capsule on (different organs weight) HFD induced obesity in rats

Various different organs weight were evaluated for all five groups and tabulated in Table.

Table 15: Evaluation of organs weight

Groups	Different organ weights(g)			
	Heart	Liver	Kidney	
			Right	Left
Normal control	0.57±0.21	5.28±1.12	0.6±0.09	0.58±0.07
Disease control	0.69±0.02[a]	6.13±2.45[a]	0.66±0.7[a]	0.56±0.5[a]
Standard control	0.56±0.12[ab]	4.32±3.20[ab]	0.57±0.45[ab]	0.52±0.72[ab]
Test group 1	0.65±0.2[ab]	5.59±1.70[ab]	0.62±1.02[ab]	0.53±0.86[ab]
Test group 2	0.59±0.31[ab]	5.02±1.45[ab]	0.61±1.04[ab]	0.50±0.04[ab]

Table 16: Effect of HECS herbal capsule on lipid profile

Groups	Total cholesterol (mg/dl)	Triglycerides (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)
Group 1	62.55 ± 5.54	73.32±5.57	23.22±2.31	24.67±1.63	14.66±2.7
Group 2	195.2±10.56[a]	115±5.47[a]	17.71±6.10[a]	154.42±7.52[a]	23±2.01[a]
Group 3	65.43±2.62[ab]	72±11.12[ab]	24.30±3.10[ab]	25.66±3.32[ab]	14.4±2.10[ab]
Group 4	78.56±6.46[ab]	85.24±5.55[ab]	19.62±4.56[ab]	42.42±3.66[ab]	17.84±2.68[ab]
Group 5	69.72±5.62[ab]	76.5±5.69[ab]	24.14±3.13[ab]	30.33±3.51[ab]	15.3±2.11[ab]

Values represents mean ± SEM (n=6);

'a' values are significantly different from normal control at P< 0.05
'b' values are significantly different from disease control at P <0.05

Data are analysed by one way ANOVA followed by DUNNETT'S t-test.

It is seen that HECS herbal capsule remarkably decreases the organ weight of rats. Various lipid profile parameters were evaluated for all five groups and tabulated in table 16

Values are expressed as mean ± SEM

Data are analysed by one way ANOVA followed by DUNNETT'S t-test.

'a' values are significantly different from normal control at P< 0.05
'b' values are significantly different from disease control at P <0.05

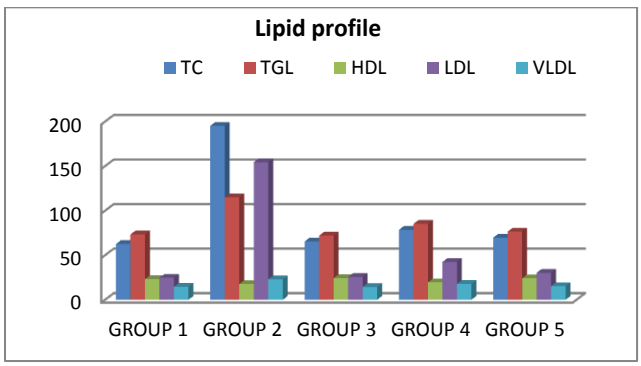

Fig 8: Graphical representation of lipid profile

It is seen that HFD treated groups, the lipid values were significantly higher than the control animals. Treatment with atorvastatin significantly reduced the lipid levels. Treatment with the HECS herbal capsule, at both doses also significantly reduced the lipid levels and increased the HDL level. At the high dose of 400mg/kg the protection offered was better.

Table 17: Effect of HECS herbal capsule on atherogenic index (AI) and LDL/HDL

GROUPS	AI	LDL/HDL
Group 1	1.69±0.23	1.06±0.70
Group 2	10.02±1.62[a]	8.69±1.23[a]
Group 3	1.69±1.01[ab]	1.05±1.07[ab]
Group 4	3.00±1.36[ab]	2.16±0.80[ab]
Group 5	1.88±1.03[ab]	1.25±1.12[ab]

Values are expressed as mean ± SEM

'a' values are significantly different from normal control at p < 0.05
'b' values are significantly different from diseased control at p < 0.05

Data are analysed by one way ANOVA followed by DUNNETT'S t-test.

The atherogenic index is an indicator of cardiovascular disease. The HFD treated group showed an increase level of atherogenic index compared to normal group. Herbal capsule treated groups showed decrease level of atherogenic index as compared to disease group.

Table 18: Effect of HECS herbal capsule of Liver function test

Values are expressed as mean ± SEM

'a' values are significantly different from normal control at $p < 0.05$
'b' values are significantly different from diseased control at $p < 0.05$

Data are analysed by one way ANOVA followed by DUNNETT'S t-test.

The atherogenic index is an indicator of cardiovascular disease. The HFD treated group showed an increase level of atherogenic index compared to normal group. Herbal capsule treated groups showed decrease level of atherogenic index as compared to disease group.

Table 18: Effect of HECS herbal capsule of Liver function test

Groups	SGPT (IU/L)	SGOT (IU/L)
Group 1	23.77±3.08	51.22±2.21
Group 2	62.84±2.17[a]	112.70±1.71[a]
Group 3	29.46±1.88[ab]	82.06±2.01[ab]
Group 4	38.03±2.19[ab]	84.44±1.45[ab]
Group 5	29.05±3.31[ab]	79.73±3.6[ab]

Values are expressed as mean ± SEM

'a' values are significantly different from normal control at $p < 0.05$
'b' values are significantly different from diseased control at $p < 0.05$

Data are analysed by one way ANOVA followed by DUNNETT'S t-test

Liver function parameters such as SGOT and SGPT also showed a significant increase in animals fed with cholesterol. These levels decreased significantly in the standard and extract treated groups.

Histopathology of Liver

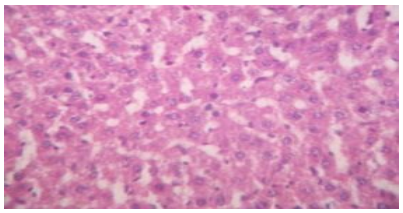

Fig 9: Normal control

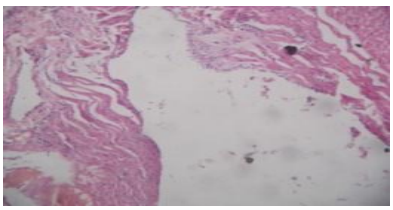

Fig 10: Disease control

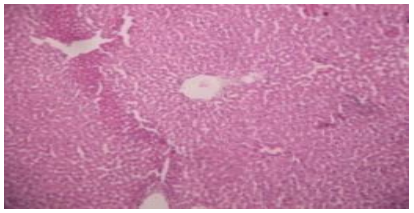

Fig 11: Standard group

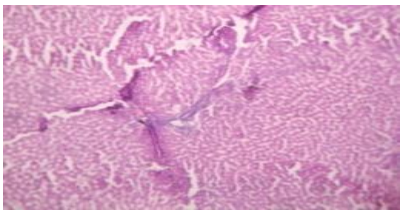

Fig 12: Test group 1

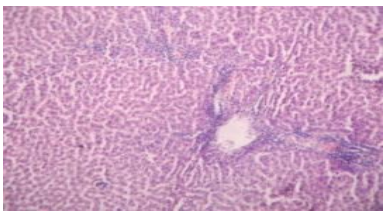

Fig 13: Test group 2

The high fat diet induced obesity and abnormal lipid metabolism all collectively are associated with inflammation , congestion, and non-alcoholic fatty liver disease leading to hepatic failure causing a boost in SGPT, SGOT level in the serum.

- Histopathological studies showed that the liver section was normal in normal control group.
- The HFD treated group showed marked ballooning, cellular degeneration and inflammation.
- These changes were absent in Atorvastatin treated standard group.
- HECS herbal capsule (200mg/kg) treated test group 1 showed decrease in cellular degeneration and inflammation when compared to control group.
- The test group 2 treated with HECS herbal capsule 400mg/kg showed maximum suppression of cellular degeneration and inflammation

Histopathology of Rat Aorta

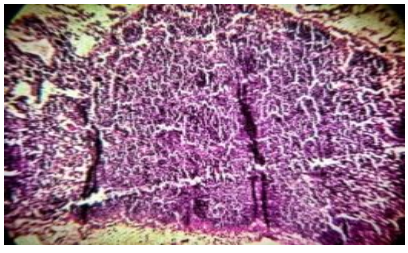

Fig14: Normal control

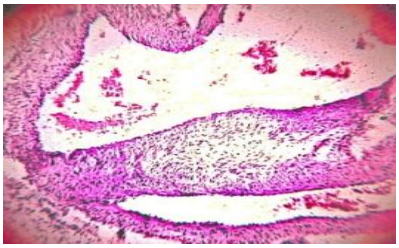

Fig15: Disease control

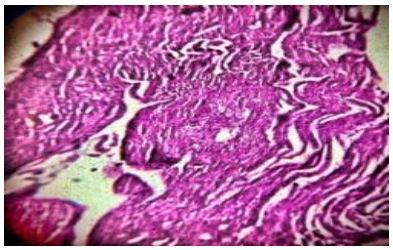

Fig 16: Standard control

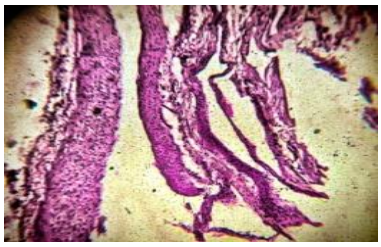

Fig 17: Test group 1

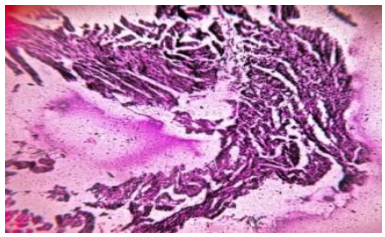

Fig 18: Test group 2

HFD intakes were shown to contribute to syndromes such as hyperlipidemia, glucose intolerance, hypertension and atherosclerosis. Atherosclerosis are main cause of cardiovascular and cerebro vascular diseases.

- Histopathological studies showed that the aorta section was normal in normal control group.
- The HFD treated group showed marked atheromatous thickening (plaque) in the intima and atheromatous inflammatory changes.
- These changes were absent in Atorvastatin treated group.
- The HECS herbal capsule (200mg/kg) treated group showed decrease in atheromatous plaque size and inflammatory changes as compared to HFD treated group.
- Wheareas 400 mg/kg of extract treated group showed maximum level of suppression of atheromatous plaque size and atheromatous inflammatory changes.

Discussion

The herbal raw materials were analyzed for their identity, quality and purity. The raw materials were standardized according to WHO Guidelines and Ayurvedic Pharmacopeia of India. Materials which complied with the specification were taken for further studies. The optimized herbal formulation was evaluated for various physiochemical and phytochemical parameters.

The formulation was optimized for it quality measures and its batch consistency by making three different trials (Trial I, II, III).The trails were subjected to pre formulation parameters to confirm the uniformity and quality. The result concludes that the trial III was excellent in all parameters and the values were found within the standard limits. Quantitative estimation of phytoconstituents were done for flavonoid, saponin and phenolic compounds .

Accelerated stability studies were carried out as per ICH Guidelines for a period of three month. The resultant stability data has shown that the formulation is stable under accelerated stability conditions.

Pharmacological studies were carried out for assessing the hypolipidemic and Anti- obesity of the plant *Caesalpinia sappan* Linn.,

Acute toxicity studies of the HECS herbal capsule were carried out for a period of 14 days. It did not produce any behavioural changes or mortality up to the dose of 2000 mg/kg of body weight of rat.

So the LD_{50} value in the range of 2000 to 5000 mg/kg of body weight .So the in vivo studies were carried out a dose of 200 mg/ kg and 400 mg /kg.

The high fat induced obesity in rats model and cholesterol 400mg/kg was used to induce hyperlipidemia and obesity in rats. The in-vivo studies showed that the hydroalcoholic extract of *Caesalpinia sappan* (HECS) herbal capsule, especially at a higher dose, improved the lipid profiles of the animals.The total cholesterol and LDL cholestertol levels were decreased whereas the HDL cholesterol level showed an increase.

The atherogenic index is an indicator of cardiovascular disease. A high atherogenic index indicates a higher risk of cardiovascular disease. The atherogenic index decreased in standard group and herbal capsule treated groups.The animals which were treated with high dose (400mg/kg) of the capsule showed a greater improvement in atherogenic index.

Human studies have revealed that increased fat intake is associated with body weight gain, which can lead to obesity and other related metabolic diseases. This study proves that rats exposed to high fat diet for 60 days cause significant increase of animals body weight, thus verifying the rats obese. The animals which were treated with high dose of 400mg/kg of the HECS herbal capsule showed a decrease in animals body weight and internal organs

weight. All the parameters reveal the potent hypolipidemic and anti- obesity activity of the HECS herbal capsule of *Caesalpinia sappan* Linn.,.

Conclusion

Sappan wood with high therapheutic effect and vast folklore uses is a rich natural resource of lead compounds for drug development. Based on literature review, *Caesalpinia sappan* heartwood has high potential for therapeutic and colouring use. Brazelien has potential pharmacological activity such as anti-tumour, anti inflammatory, anti-diabetic , immunostimulant properties and also anti thirst, blood purifying action and healing properties in Aurvedha and Unani beneficial to develop into a drug , neutraceuticals and cosmetics.

Future studies can be directed towards the Isolation, Characterization of individual compounds responsible for the hypolipidemic and Anti-obesity activity and mechanism of action responsible for this activity, so as to explore this plant for therapeutic purposes.In future Scope the developed herbal formulation may be taken up for clinical trials in the treatment of hyperlipidemic and obesity problems.

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