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Phytochemical screening and Invivo Antihyperlipidemic activity of hydroalcoholic extract of leaves of plant Cassia fistula

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ABSTRACT: The present investigation was carried out to evaluate the hypolipidemic activity of hydroalcoholic leaf extracts of Cassia fistula Linn (Family: Fabaceae). The rats receiving the treatment with leaf hydroalcoholic extract of Cassia fistula showed significant reduction in total cholesterol, triglycerides, High density lipoprotein, and other parameters at dose dependent manner but the effect was less than the standard drug Orlistat. The results revealed the effectiveness of CF plant against hyperlipidemic activity.

I. INTRODUCTION

Hyperlipidemia-Hyperlipidemia disease has afflicted humankind since antiquity. In 2002, coronay heart Epidemiological evidence strongly supported the positive correlation between blood lipids, hyperlipidemia and its complications, mainly CHD. This relationship has been shown between and within cultures . The hyperlipidemia is traditionally defined as conditions in which the concentration of cholesterol or triglyceride-carrying lipoproteins in plasma exceeds an arbitrary normal limit. These lipoproteins deposit in the interstitial space of arteries arising from aorta, restricting the blood supply to the heart. This phenomenon is known as atherosclerosis. Higher deposition of lipoproteins completely blocked the blood supply to the heart, and thus myocardial infarction (MI) occurs, which is commonly known as heart attack.

Hyperlipidemia classification

Hyperlipidemia generally can be classified in:

Primary

It is also called **familial** due to a genetic defect, it may be monogenic: a single gene defect or polygenic: multiple gene defects. Primary hyperlipidemia can usually be resolved into one of the abnormal lipoprotein patterns. (Tripathi, 2008).

Secondary

It is **acquired** because it is caused by another disorder like diabetes, chronic alcoholism, hypothyroidism and with use of drugs like corticosteroids, beta blockers and oral contraceptives. Secondary hyperlipidemia together with significant hypertriglyceridemia can cause pancreatitis(Joseph, 2011).

Causes and Risk Factors of Hyperlipidemia

Dietary Causes Dietary Fats and Fatty Acids: Dietary fatty acids are divided into three major classes (saturated, monounsaturated and polyunsaturated fatty acids). The foods that contribute to saturated fatty acids (e.g. myristic acid, palmitic acid, stearic acid, etc) meats (e.g. beef, pork, processed meat products, poultry), milk and other dairy products (e.g. butter, cheese, ice cream, yoghurt), tropical fats (e.g. Coconut, palm oils) and egg (contain proportionately less saturated fat compared to other animal food sources). Monounsaturated fatty acids are present as oleic acid in olive oil, avocado, animal fats, etc. Polyunsaturated fatty acids are the omega-3 fatty acids (e.g. linoleic acid) and omega-6 fatty acids (e.g. linolenic acid) (Fauci et al., 2008)..

Dietary Cholesterol: Like other sterols, cholesterol is a sterol i.e. a combination of steroid and alcohol) and lipid (a type of fat). It is found in foods such as eggs and dairy products and is also manufactured in the body, especially the liver. Cholesterol also stabilizes a cell against temperature changes. It is a major part of the membranes of the nervous system, the brain, the spinal cord and the peripheral nerves. In particular, it is incorporated into the myelin sheath that insulates the nerves from the surrounding tissue. Cholesterol is al so the forerunner of important

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hormones such as the female sex hormone, oestradiol and the male sex hormone, testosterone and of vitamin D. Cholesterol is also used to produce the bile which is required to digest the fats in food. Nearly most of the body tissues are capable of making cholesterol, but the liver and intestines make the most.

Other Dietary Factors

Carbohydrates: Dietary recommendations to lower the total fat intake include increasing dietary carbohydrate intake because favorable plasma lipid and lipoprotein levels have been reported for populations and individuals whose habitual diet is rich in carbohydrates. High carbohydrate consumption being associated with a decrease in HDL cholesterol levels. Plasma triglyceride levels are not elevated in these individuals, possibly because obesity is rare (Charney, 1999).

Fiber: Studies have shown that only water-soluble fiber plays a role in lipoprotein metabolism in humans. A meta-analysis of 20 studies found that intake of oat products reduces serum cholesterol levels. The mechanism by which dietary fiber affects plasma lipid levels is unknown. Insoluble fibers in wheat and vegetables do not to reduce cholesterol, but they do have other beneficial effects.

Protein: Soy protein also lowers serum cholesterol levels in animals and in hypercholesterolemic individuals when compared with casein (a dairy protein) and beef proteins. The mechanism underlying these changes is unknown but it has been stated that soy protein affects cholesterol absorption, bile acid absorption, the insulin-glucagon ratio, serum thyroxine levels and hepatic LDL-receptor activity.

Obesity: For a given level of body mass index (BMI), obesity is associated with hyperlipidemia, insulin resistance and hypertension and independent predictor of coronary artery disease (CAD). A meta-analysis of 70 studies indicated that weight reduction was related to increases in HDL cholesterol levels and significant decreases in total, LDL and VLDL cholesterol and triglyceride levels (Woollett et al., 1992). Although they are not always coincident, obesity is also often accompanied by hyperlipidemia. Both obesity and hyperlipidemia are independently associated with atherosclerosis, non-alcoholic fatty liver disease and insulin resistance (Cortse et al., 1983).

Diabetes and Insulin Resistance: Insulin resistance (type II diabetes) is associated with a number of lipid and lipoprotein abnormalities (Keys et al., 1985). The lipid abnormality is associated with insulin resistance and hyperinsulinemia is hypertriglyceridemia. VLDL and total triglycerides are elevated in individuals with type II diabetes although the exact roles of insulin resistance and hypertriglyceridemia are disputed.

Physical Exercise/Activity: Sedentary lifestyles contribute to the development and maintenance of obesity (Keys et al., 1985). Diet can also change in plasma lipoprotein concentrations that occur with exercise.

Alcohol Intake: Low dose ethanol consumption in healthy volunteers modestly activates hepatic de novo lipogenesis and that the major quantitative fate of ethanol is acetate produced in the liver. The acetate released into the plasma which inhibits lipolysis in peripheral tissues by 53% and whole body lipid oxidation is decreased by 73%. .

Contraceptives and Other Pharmacologic Agents: Premenopausal women, using oral contraceptives containing a relatively low dose of estrogen combined with a medium or high dose of progestin had a 24 % higher median concentration of LDL cholesterol than who are not using hormones. Glucocorticoids and estrogens elevate triglycerides and raise levels of HDL cholesterol (Hegsted et al., 1985).

Antihypertensives have variable effects on lipids and lipoproteins. Although short-term use of thiazide raises cholesterol, triglycerides and LDL cholesterol, long-term usage is not associated with significant alterations in lipid levels (Bananome and Grundy, 1988)

Complications of hyperlipidaemia

I. **Atherosclerosis:** It is a common disorder and occurs when fat, cholesterol and calcium deposits in the arterial linings. This deposition results in the formation of fibrous plaques. A plaque normally consists of three components:

1) atheroma which is a fatty, soft, yellowish nodular mass located in the centre of a larger plaque that consists of macrophages, which are cells that play a role in immunity;

2) a layer of cholesterol crystals.

3) calcified outer layer. Atherosclerosis is the leading cause of cardiovascular disease.

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II. **Coronary Artery Disease (CAD):** Atherosclerosis is the major cause of CAD. It is characterised by the narrowing of the arteries that supply blood to the myocardium and results in limiting blood flow and insufficient amounts of oxygen to meet the needs of the heart. The narrowing may progress to the extent that the heart muscle would sustain damage due to lack of blood supply. Elevated lipid profile is correlated to the development of coronary atherosclerosis.

III. **Myocardial Infarction (MI):** MI is a condition which occurs when blood and oxygen supplies to the cardiac arteries are partially or completely blocked, resulting in damage or death of heart cells. The blockage is usually due to the formation of a clot in an artery. This condition is commonly known as heart attack. The studies show that one-fourth of survivors of myocardial infarction were hyperlipidemic.

IV. **Angina Pectoris:** Angina is not a disease but a symptom of an underlying heart condition. It is characterised by chest pain, discomfort or a squeezing pressure. Angina occurs as a result of a reduction or a lack of blood supply to a part or the entire heart muscle. Poor blood circulation is usually due to CHD when partial or complete obstruction of the coronary arteries is present.

V. **Ischemic stroke or Cerebrovascular Accident (CVA):** It occurs when blood circulation in part of the brain is blocked or diminished. When blood supply, which carries oxygen, glucose, and other nutrients, is disrupted, brain cells die and become dysfunctional. Usually, strokes occur due to blockage of an artery by a blood clot or a piece of atherosclerotic plaque that breaks loose in a small vessel within the brain. Clinical trials revealed that lowering of LDL and total cholesterol by 15% significantly reduced the risk of first stroke.

Causes of hyperlipidemia

• A diet rich in saturated fat and cholesterol increases blood cholesterol and triglyceride levels.
• Other disorders as obesity, diabetes mellitus and hypothyroidism increase the risk of hyperlipidemia.
• Smoking and not exercising may lead to hyperlipidemia.
• Excessive use of alcohol also increases the risk of hyperlipidemia.
• Certain drugs as steroids and β–blockers may cause hyperlipidemia.
• Hereditary factor is also one of the common causes for hyper-lipidemia.
• In some cases hyperlipidemia occurs during pregnancy.
• Lipoprotein lipase mutations .

Symptoms of hyperlipidemia

Hyperlipidemia usually has no noticeable symptoms and tends to be discovered during routine examination for atherosclerotic cardiovascular disease.
• Symptoms may include chest pain (angina), heart attack or stroke.
• When levels are exceedingly high, cholesterol may be deposited in tendons or just beneath the skin under the eyes.
• Swelling of organs such as liver, spleen or pancreas.
• Blockage of blood vessels in brain and heart.
• Higher rate of obesity and glucose intolerance.
• Pimple like lesions across the body.

Treatment of hyperlipidemia

In 1987 the National Institute of Health (NIH) established the National Cholesterol Education Program (NCEP) to be directed by the Adult Treatment Panel (ATP) for the purpose of issuing information for health professionals and the general public concerning testing, evaluating, monitoring and treating hyperlipidemia. An important criterion of ATP guidelines is the development of treatment goals for hyperlipidemia based on patient's risk of CHD.
ATP recommends two methods of treatment:
1) Therapeutic lifestyle changes
2) Drug therapy.

Therapeutic lifestyle changes

Diet modification, regular physical activity, smoking cessation, and weight reduction should be tried as initial treatment, especially in mild cases of hyperlipidemia and in persons without CHD or CHD risk equivalent and<2 risk factors. It should be kept in mind that when dieting, cholesterol intake is reduced. At the same time, production of cholesterol, especially by the liver, increases. It is recommended that the intake should be restricted to 25%-35% of energy intake and that saturated fatty acids make up less than 7% of energy intake and that cholesterol intake should be less than 200 mg daily. The intake of plant sterol esters and soluble fibre is advisable. A healthy diet

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can result in 10% to 15% reduction of cholesterol blood level.

Drug therapy

High LDL, the presence of risk factors, and documentation of CHD should qualify initiating drug therapy along with TLC. During the early stages of the hyperlipidemia, blood monocytes and platelets attach to a vessel wall at the sites of endothelial damage. The release of the mediators such as platelet derived groth factors leads to a proliferation of smooth cells in the intimal and medial lining of the vessel, collagen synthesis, cholesterol uptake and the beginning of the hyperlipidemic plaque results. Plaque ruptures are resulting in the acute syndromes of unstable angina, myocardial infarction and sudden cardiac death (Scott, 1991).

Diagnosis of hyperlipidemia

Hyperlipidemia typically shows no symptoms and can only be detected by a blood test. Screening for hyperlipidemia is done with a blood test called a lipid profile. According to National Cholesterol Education Program (NCEP) screening (National cholesterol education program, 1994) should start at age 20, and if the report is normal, it should be repeated at least every five years. Normal levels for a lipid profile (AAFP, 2013) are listed below table 1.1.

Table 1.1: Normal levels for a lipid profile

Lipids	Desirable value	Borderline	High Risk
Cholesterol	Less than 200 mg/dl	200-239 mg/dl	240 mg/dl
Triglycerides	Less than 140 mg/dl	150-199 mg/dl	200-499 mg/dl
HDL cholesterol	60 mg/dl	40-50 mg/dl	Less than 40 mg/dl
LDL cholesterol	60-130 mg/dl	130-159 mg/dl	160-189 mg/dl
Cholesterol/HDL ratio	4.0	5.0	6.0

Pharmacological treatment

Numbers of hypolipidemic drugs are available in the market for the treatment of hyperlipidemia. In 1975, the results of the Coronary Drug Project indicated that the drugs are relatively ineffective for preventing myocardial infarction in patients with preestablished CHD.

This project examined the effects of estrogens, D-thyroxin, clofibrate and nicotinic acid. The high-dose estrogens were discontinued in 1970 because of an increased number of fatal cardiovascular events without any indication of benefit.

The low-dose estrogens were discontinued in 1975 because of suggestion of an excess incidence of mortality from cancer. Dthyroxin was discontinued in 1971 because of increased mortality in this group (The coronary drug project, 1957).

Ayurvedic treatment

Ayurvedic medicine is one of the world's oldest medical systems. Ayurvedic therapeutics is based on the "laws" of nature. Its approach to health-care is based on understanding the interrelationship of body, mind and spirit. The aim of ayurveda medicine is to integrate and balance these elements to prevent illness and promote wellness through diet, nutrition, herbs, yoga, meditation and daily seasonal routines (Tarabilda, 1998).

Ayurvedic medicine has been used for thousands of years for treatment of various metabolic disorders. However, few studies have been conducted to evaluate the effectiveness of Ayurveda herbal medicine formulae on hyperlipidemia. Higher quality studies, such as randomised clinical trials, are lacking (Singh et al., 2007).

Herbal medicines and their preparations have been widely used traditionally, for the thousands of years in developing and developed countries owing to its natural origin and lesser side effects or dissatisfaction with the results of synthetic drugs. One of the characteristics of oriental herbal medicine preparations is that all the herbal medicines, either presenting as single herbs or as collections of herbs in composite formulae. The traditional preparations comprise medicinal plants, minerals, organic matter, etc. Herbal drugs constitute mainly those traditional medicines which primarily use medicinal plant preparations for therapy. These drugs are made from renewable resources of raw materials by eco-friendly processes and will bring economic prosperity to the masses growing these raw materials.

India is known as the "Emporium of Medicinal plants" due to availability of several

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thousands of medicinal plants in the different bioclimatic zones. Medicinal plants continue to provide valuable therapeutic agents, both in modern medicine and in traditional systems of medicine. Attention is being focused on the investigation of efficacy of plant based drugs used in the traditional medicine because they are economy, have a little side effects and according to W.H.O, about 80% of the world population rely mainly on herbal remedies.

The World Health Organization has recently defined traditional medicine (including herbal drugs) as comprising therapeutic practices that have been in existence, often for hundreds of years, before the development and spread of modern medicine and are still in use today. Herbal medicines are being used by about 80% of the world population primarily in the developing countries for primary health care. They have stood the test of time for their safety, efficacy, cultural acceptability and lesser side effects.

Ancient literature also mentions herbal medicines for age-related diseases namely memory loss, osteoporosis, osteoarthritis, diabetes, immune and liver disorders, etc. for which no modern medicine or only palliative therapy is available. The chemical constituents present in them are a part of the physiological functions of living flora and hence they are believed to have better compatibility with the human body.

II. MATERIALS AND METHODS

Plant material collection Leaves ofCassia fistulawere collected from local area of Bhopal. The plant was collected in the month of August, 2017. It was made completely clean, dust free and allowed to get dried under the shade.The plants have been selected on the basis of its availability and Folk use of the plant.The herbarium sheet of plant of Cassia absuswas submitted in the department of botany under the Senior Botanist Dr. Jaswinder Mehta. These herbs Ref.No./Bot./Herb/04/2017/09 is submitted Career College, Bhopal (M.P.). Given which was preserved for future reference.

Extraction procedure

Following procedure was adopted for the preparation of extracts from the shade dried and powdered herbs(Mukherjee, 2007;Kokate, 1994):

Defatting of plant material

Leaves of Cassia fistulawas shade dried at room temperature. The shade dried plant material was coarsely powdered and subjected to extraction with petroleum ether by maceration. The extraction was continued till the defatting of the material had taken place.

Extraction by maceration process

100 gm of dried powdered leaves of Cassia fistulahas been extracted with hydroalcoholic solvent (80:20: ethanol: water) using maceration process for 48 hrs, filtered and dried using vacuum evaporator at 40^0C.

Total Phenolic Content Estimation

The total phenolic content of the extract was determined by the modified Folin-Ciocalteu method (Olajuyigbe and Anthony, 2011).

Preparation of Standard: 50 mg Gallic acid was dissolved in 50 ml Methanol, various aliquots of 5-25µg/ml was prepared in Methanol

Preparation of Extract: 10mg of dried extracts of were dissolved in 10 ml Methanol and filter. Two ml (1mg/ml) of this solution was used for the estimation of phenol.

Procedure: 2 ml of each extract or standard was mixed with 1 ml of Folin-Ciocalteu reagent (previously diluted with distilled water 1:10 v/v) and 1 ml (7.5g/l) of Sodium Carbonate. The mixture was vortexed for 15s and allowed to stand for 15 min for colour development. The absorbance was measured at 765 nm using a spectrophotometer.

Total Flavonoids Content Estimation

Determination of total flavonoids content was based on Aluminium Chloride method(Olajuyigbe and Anthony, 2011).

Preparation of standard: 50 mg Quercetin was dissolved in 50 ml methanol, and various aliquots of 5- 25µg/ml were prepared in methanol.

Preparation of extract: 10mg of dried extracts of were dissolved in 10 ml methanol and filter. Three ml (1mg/ml) of this solution was used for the estimation of flavonoid.

Procedure: 1 ml of 2% $AlCl_3$ Methanolic solution was added to 3 ml of extract or standard and allowed to stand for 15 min at room temperature; absorbance was measured at 420 nm.

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Pharmacological studies:

Acute oral toxicity studies

Acute oral toxicity was conducted according to the method of Organisation for Economic Co-operation and Development (OECD) (OECD, 2001). Animals were kept fasting providing only water, hydroalcoholic extract of Cassia fistula (50,100,150,200,300 mg/kg/day) was administered orally for 4 days of six groups of rats (n=6) and the animals were kept under observation for mortality as well as any behavioral changes for evaluation of a possible antihyperlipidemic effect.

Table No. 1.2 Grouping of Animals

Group No.	Animals	Sex	Treatment	Dose (mg/kg body weight)
I	Wistar Rat	Male	Test group	50mg/kg
II	Wistar Rat	Male	Test group	100mg/kg
III	Wistar Rat	Male	Test group	150mg/kg
IV	Wistar Rat	Male	Test group	200mg/kg
V	Wistar Rat	Male	Test group	300mg/kg

Dose Selection:

60mg/kg/day of Orlistat was selected as a standard drug. Hydroalcoholic extract of leaves of plant Cassia fistula was used to treat hyperlipidemia and the dose of hydroalcoholic extract of leaves of plantCassia fistula was 100mg/kg.

Dose Preparation:

The dose were given according to the body weight of animal, Orlistat, hydroalcoholic extract of leaves of plant Cassia fistulawere diluted in the water for injection. The dose of hydroalcoholic extract of leaves of plant Cassia fistula (0.6,0.88,0.8mg/kg)

In vivo Antihyperlipidemic activity of hydroalcoholic extract ofCassia fistula.

Animals:-

Wistar rats (180–250 g) were group housed (n= 6) under a standard 12 h light/dark cycle and controlled conditions of temperature and humidity (25±2 °C, 55–65%). Rats received standard rodent chow and water ad libitum. Rats were acclimatized to laboratory conditions for 7 days before carrying out the experiments. All the experiments were carried in a noise-free room between 08.00 to 12.00 h. Separate group (n=6) of rats was used for each set of experiments. The animal studies were approved by the Institutional Animal Ethics Committee (IAEC), constituted for the purpose of control and supervision of experimental animals by Ministry of Environment and Forests, Government of India, New Delhi, India.

Induction of hyperlipidemia

Rats with an average body weight were made hyperlipidemic by giving high-fat diet (HFD) for 15 days. The HFD contained Cholesterol (2%), Cholic acid (1%), Dalda (20%), and Coconut oil (6%) as major constituents. Hyperlipidemia was confirmed by measuring the levels of serum lipids and lipoproteins in the rats.

Experimental designs

Table No. 1.3 Groups division for induce drugs

Group	No. of animals in each group	Group (drug dose mg/kg I.P.)
I	6	Normal (vehicle alone)
II	6	Hyperlipidemic rats treated with vehicle alone
III	6	Hyperlipidemic rats treated with hydroalcoholic extract of Cassiafistula(100mg/kg, p.o.)
IV	6	Hyperlipidemic rats treated with hydroalcoholic extract of Cassia fistula(200mg/kg, p.o.)
V	6	Hyperlipidemic rats treated with Orlistat (60 mg/kg/day p.o.)

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Animals were divided into five groups of 6 animals each. The first group treated normal vehicle alone. The group II received hyperlipidemic rats treated with vehicle alone (positive control). The groups III, IV and V received 100 mg/kg and 200 mg/kg of hydroalcoholic extract of Cassia fistulaand Orlistat (60 mg/kg/day p.o.) respectively for 15 days (Ahmadet al., 2018; Onyeike et al., 2012).

III. RESULTS AND DISCUSSION

Result of Percentage Yield

Table 1.4

S. No.	Solvents	% Yield
1.	Hydroalcoholic	4.59

Result of Phytochemical screening of hydroalcoholic extract of leaves of plant Cassia fistula

Table 1.5

S. No.	Constituents	Hydroalcoholic extract
1.	**Alkaloids**	
	Mayer's Test	-ve
	Wagner's Test	-ve
	Dragendroff's test	-ve
	Hager's test	-ve
2.	**Glycosides**	
	Modified Borntrager's Test	-ve
	Legal's test	-ve
3.	**Flavonoids**	
	Lead acetate	+ve
	Alkaline test	-ve
4.	**Phenolics**	
	Ferric Chloride Test	+ve
5.	**Proteinsand Amino acids**	
	Xanthoproteic test	+ve
	Ninhydrin Test	-ve
6.	**Carbohydrates**	
	Molisch's Test	-ve
	Benedict's Test	-ve
	Fehling's test	-ve
7.	**Saponins**	
	Froth Test	+ve
	Foam test	-ve
8.	**Diterpins**	
	Copper acetate test	+ve

Results of estimation of Total Phenolic Contents

Total Phenolic Content estimation (TPC)

The content of Total Phenoli content(TPC) was expressed as mg/100mg of Gallic acid equivalent of dry extract sample using the equation obtained from the calibration curve: **Y = 0.042X+0.002, R^2= 0.999,** where X is the gallic acid equivalent (GAE) and Y is the absorbance.

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Calibration curve of Gallic acid

Table 1.6: Preparation of calibration curve of Gallic acid

S. No.	Concentration (µg/ml)	Absorbance
0	0	0
1	5	0.197
2	10	0.430
3	15	0.6417
4	20	0.851
5	25	1.029

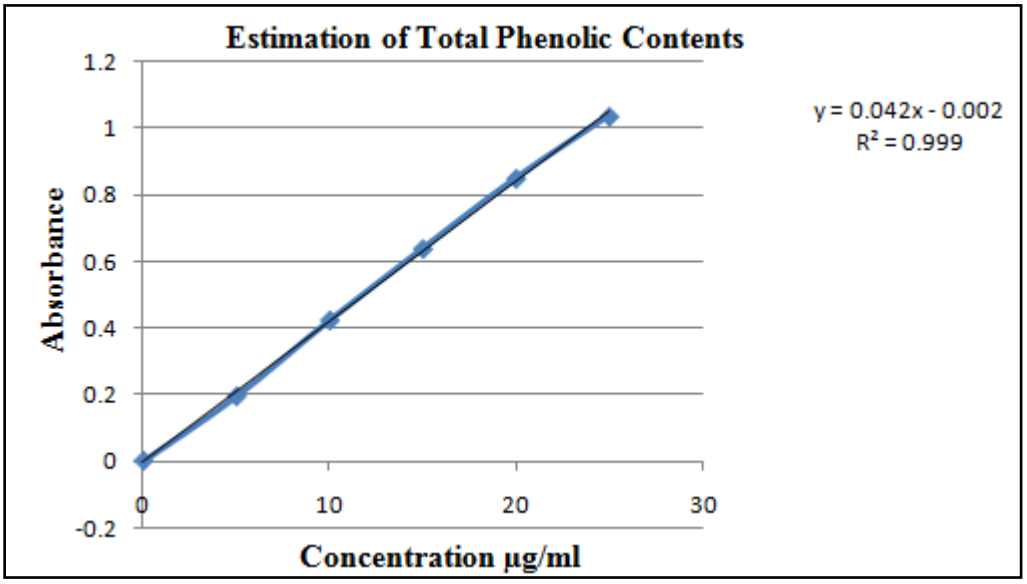

Figure 1.1: Graph of estimation of Total Phenolic Content

Total Flavonoid Content estimation (TFC)

The content of Total Flavonoid Compounds (TFC) Content was expressed as mg/100mg of quercetin equivalent of dry extract sample using the equation obtained from the calibration curve: **Y= 0.06X+0.019, R^2= 0.999,** where X is the quercetin equivalent (QE) and Y is the absorbance.

Calibration Curve of Quercetin

Table 1.7: Preparation of calibration curve of Quercetin

S. No.	Concentration(µg/ml)	Absorbance
0	0	0
1	5	0.349
2	10	0.691
3	15	0.921
4	20	1.211
5	25	1.519

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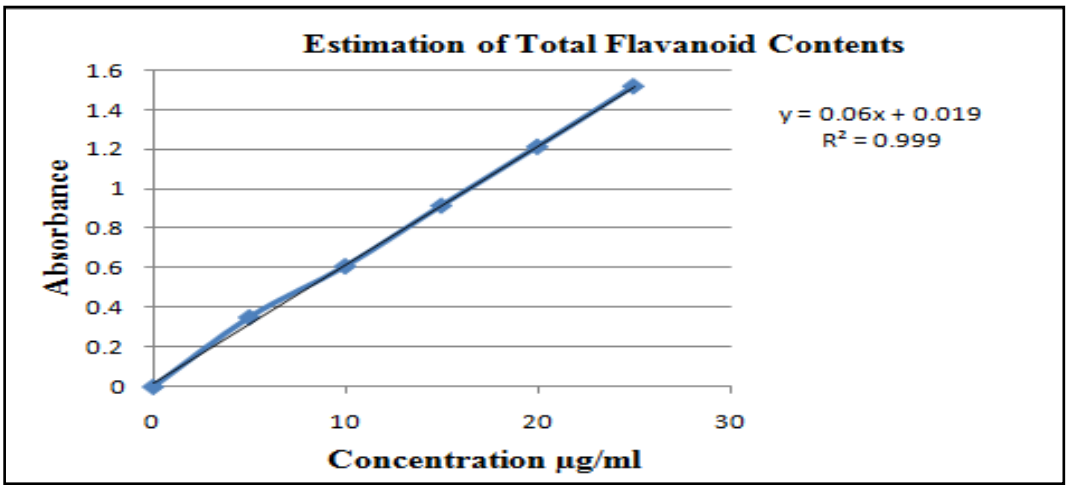

Figure 1.2: Graph of Estimation ofFlavonoid Content

Table 1.6: Total Phenolic and Total Flavonoid Content of hydroalcolic extract of leaves of plant Cassia fistula

Table 1.8

S. No.	Extract	Total Phenol (mg/100mg)	Total flavonoid (mg/100mg)
1.	Hydroalcoholicextract	0.748	0.897

Results of In vivo Antihyperlipidemic activity hydroalcoholic extract of leaves of plant Cassiafistula extract

Antihyperlipidemic effect of the hydroalcoholic leaves extract of Cassiafistula on the high fat diet induced rats. The mean body weight as shown in Table 6.6. The activity levels of serum total cholesterol (TC), triglycerides (TG) and Serum high density lipoprotein (HDL) were observed in normal and experimental animals. In group II animals, the activity levels of serum total cholesterol (TC) and triglycerides (TG) were significantly elevated when compared to that of normal groups (Table 6.6). On the other hand the serum level of Serum high density lipoproteins (HDL) were significantly depleted in the HFD fed rat. In group III, IV and V animals, the activity levels of serum total cholesterol (TC) and triglycerides (TG) were significantly decreased when compared to that of normal groups (Table 6.7). Also HDL level was significantly increased in the same groups.

Table 1.9: Mean Body Weight Change

Group	Drug	Dose	Body weight (g)	
			Onset of study	End of study
I	Normal	Normal Saline	181	194
II	control	HFD	186	245
III	Cassia fistula	100mg/kg	193	188
IV	Cassia fistula	200mg/kg	199	190
V	Orlistat	60mg/kg	197	184

Values are expressed as the mean ± SEM of six observations. $^{***}P<0.001$ vs. control treatment (One-way ANOVA followed by Dunnett's test)

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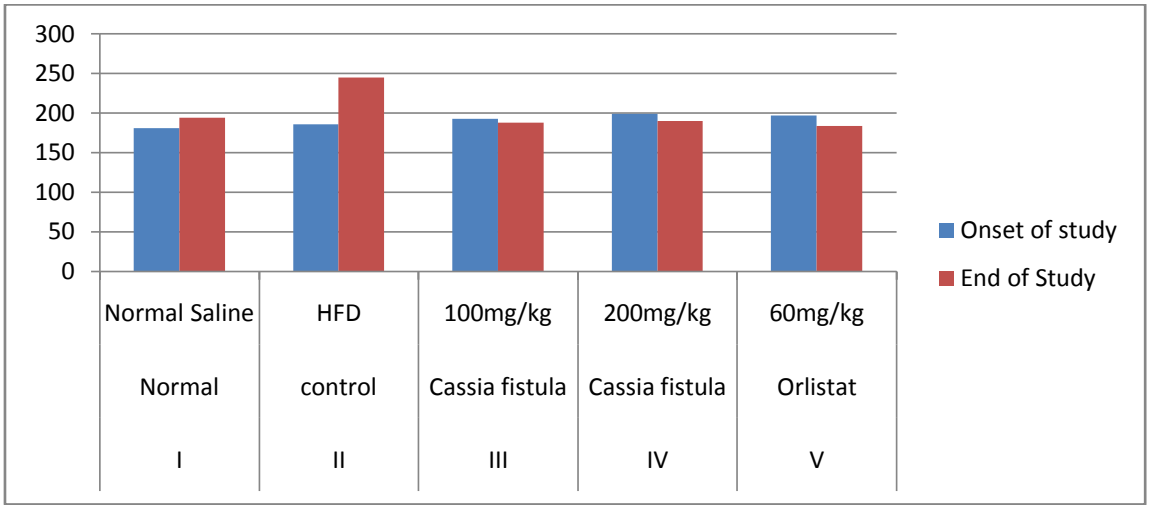

Figure 1.3: Effect of the hydroalcoholic extract of leaves of plant Cassia fistulaon body weight in HFD induced rat

Table 1.10: Effect of the hydroalcoholic extract of leaves of plantCassia fistulaon serum lipid profile levels (mg/dL) in HFD induced rat

Treatment	Dose	Total cholesterol (mg/dL)	Triglycerides (mg/gL)	High density lipoproteins (mg/dL)
Normal	Normal Saline	83	86	38
Control	HFD	155	152	29
Cassia Fistula Extract	100mg/kg	97	99	34.7
Cassia Fistula Extract	200mg/kg	91	92	36
Orlistat	60mg/kg	88	87	37.5

Values are expressed as the mean ± SEM of six observations. $^{***}P<0.001$ vs. control treatment (One-way ANOVA followed by Dunnett's test)

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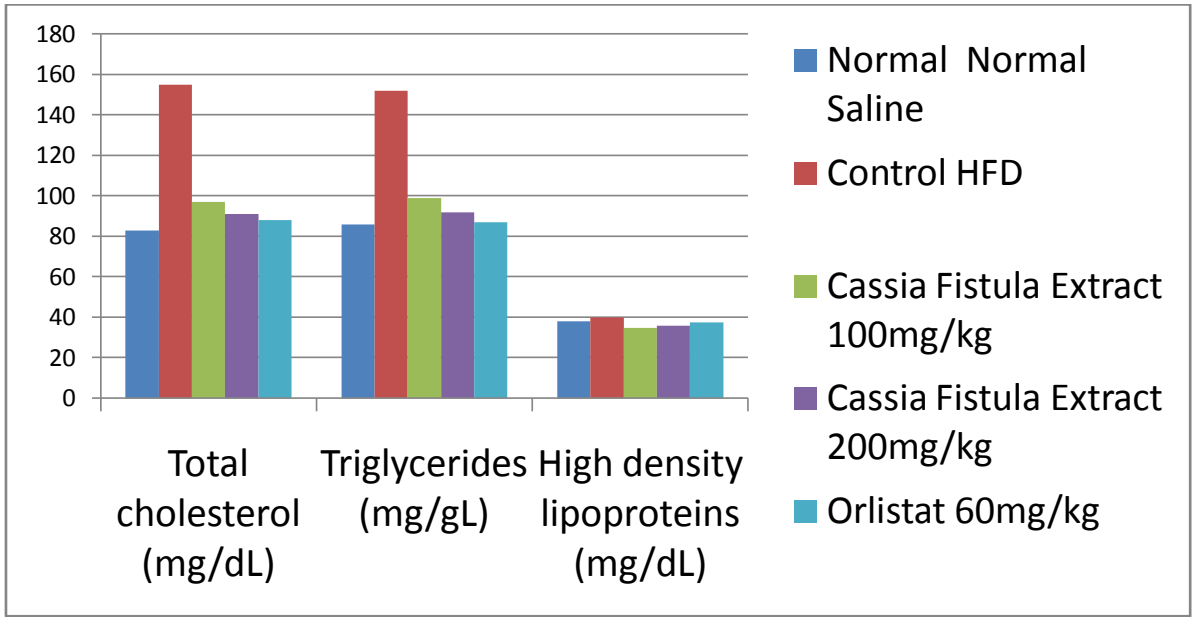

Figure 1.4: Effect of the hydroalcoholic extract of leaves of plant Cassia fistulaon serum lipid profile levels- Total Cholesterol(mg/dL), Triglycerides (mg/dL), High density lipoproteins (mg/dL) in HFD induced rat

IV. DISCUSSION

The results regarding feed intake of different groups of rats found that HFD had higher feed intake due to high-fat diet given to them that increased their energy intake and energy storage. While the hyperlipidemic rats that received hydroalcoholicleaves extract of Cassia fistulaconsumed less feed due to presence of active constituents in it that may not stimulate the appetite of rats. Cassia fistulawas shown a well known traditional medicinal plant possesses diverse biological activities and pharmacological function including reducing blood glucose and serum lipids. The body weight gain of different groups of rats showed that hyperlipidemic group gained higher body weight due to high fat diet (cholesterol) used to induce hyperlipidemia in the rats that increased energy intake and energy storage. When hyperlipidemic rats were given hydroalcoholic leaves extract of Cassia fistulaat doses of 100 and 200 mg/kg b. wt then their body weight gain decreased. The decrease in body weight gain was due to capability of active constituents in hydroalcoholic leaves extract of Cassia fistula that decreased the food intake of rats.Cholesterol is synthesized in all animal tissue. It is important to relate to its role in the stabilization of membrane structures because of its rigid planar structure. It also as a precursor for the synthesis of steroid hormones. The results of total cholesterol levels of different groups of rats depicted that TC and TG level had highest value. Conversely, addition of Cassia fistulaat doses of 100 and 200 mg/kg b. wt at different levels lowered the TC and TG levels. Hydroalcoholic leaves extract of Cassia fistulacontained antioxidant that significantly lowered total cholesterol level due to its ability to increase the bile acid excretion by preventing reabsorption from small intestine through disruption of micelle formation of bile acid. The increase in excretion of bile acid and cholesterol activates cholesterol 7α-hydroxylase that enhances the conversion of liver cholesterol to bile acid thus resulting in cholesterol reduction.This model was used to study the potential of hypolipidemic effect of hydroalcoholic leavesextract of Cassia fistula that contained significant amounts of antioxidants properties. From this study, we found that daily oral administration hydroalcoholic leaves extract of Cassia fistulashows significantly reduced total cholesterol levels in plasma after 15 days of administration. This result agrees with literature where depleted level of HFD fed hyperlipdemia. HDL is directly anti-androgenic and it is believed to remove cholesterol from the developing lesions. The intense interest in this area results in part from the generally low toxicity of antioxidants and the hope that treatment with antioxidants might be additive with cholesterol lowering regimes. In the present study serum TG levels were significantly

elevated in HFD rat. The excess of fat diet increased the TG level which is one of the causes of hardening of arteries. In conclusion, it could be said that the hydroalcoholic leaves extract of Cassia fistulaexhibited a significant hypolipidemic activity. Administration of HFD produced a highly significant increase in weight mesenteric fat pads. A reduction in the raised weight in the fat pads as observed in the groups of animals treated with hydroalcoholic leaves extract of Cassia fistula may be attributed to increased thermogenesis and decreased lipogenesis.

V. CONCLUSION

The Phytochemical screening revealed the presence of Carbohydrates, Proteins, Amini acids, Flavonoids, Saponins, Diterpenes and Phenolic compounds in the hydroalcoholic extract of leaves of plant Cassia absus.These findings show that the hydroalcoholic extract of leaves of plant Cassia fistulapossesses antihyperlipidemic activity.In the present study serum TG levels were significantly elevated in HFD rat. The excess of fat diet increased the TG level which is one of the causes of hardening of arteries. In conclusion, it could be said that the hydroalcoholic extract of Cassia fistulaexhibited a significant hypolipidemic activity. Administration of HFD produced a highly significant increase in weight mesenteric fat pads. A reduction in the raised weight in the fat pads as observed in the groups of animals treated with hydroalcoholic extract of Cassia fistulamay be attributed to increased thermogenesis and decreased lipogenesis.It is concluded that hydroalcoholic extract of Cassia fistulahas anti-hyperlipidemic effects in albino rats, and therefore could be a promising nutraceutical therapy for the management of hyperlipidemia and its associated complications.

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