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Research Article

## Evaluation of Anti-Hyperlipidemic Activity of Ethanolic Extract of *Cassia Fistula* Leaf

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### ABSTRACT

The present study was designed to perform evaluate antihyperglycemic activity of ethanolic extract of *Cassia Fistula* leaf. Leaf of *cassia fistula* was extracted using ethanol as solvent by soxhlet apparatus. The evaluation of antihyperlipidemic activity was done using High Fat Diet induced hyperlipidemia models in Wistar albino rats. The work entitled evaluation of anti-obesity activity of leaf of *Cassia fistula* was to determine the efficacy and safety in experimental animals. Both aqueous and alcoholic extract of leaf of *Cassia Fistula* (Linn.) have shown significant reduction in weight of heart and both the kidney as compared to cafeteria diet fed animals confirming their anti-obesity property. The aqueous and alcoholic extract of leaf of *Cassia Fistula* (Linn.) has shown significant decrease in serum levels of Total-Cholesterol, LDL-Cholesterol, VLDL-Cholesterol and Triglyceride.

**Keywords:** Antihyperglycemic, *cassia fistula*, hyperlipidemia, High fat diet, alcoholic extract

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### INTRODUCTION

Globally, there are more than 1 billion overweight adults and at least 300 million of them are obese. Obesity and overweight pose a risk for chronic diseases including Type-II diabetes, cardiovascular disease, hypertension, stroke due to increased consumption of energy-dense foods containing high amount of saturated fat and sugars and reduced physical activity. The lipids of human plasma are transported in macromolecular complexes termed lipoproteins. A number of metabolic disorders that involve elevations in levels of any of the lipoprotein species are thus termed hyperlipoproteinemias or hyperlipidemias. The term hyperlipemia denotes increased levels of triglycerides in plasma. The two major clinical sequelae of the hyperlipoproteinemias are acute pancreatitis and atherosclerosis. The former occurs in patients with marked hyperlipemia. Control of triglycerides can prevent recurrent attacks of this life-threatening disease. Obesity is the state of excess body fat stores, which should be distinguished from overweight (i.e. excess body weight relative to a person's height). Obesity refers specially to having an abnormally high proportion of body fat or adipose tissue in relation to

lean muscle mass. Obesity is often simply defined "a condition of abnormal or excessive accumulation of adipose tissue, to the extent that health may be impaired". People with abdominal fat distribution or 'android obesity' are at greater risk to its consequences than those with the less serious 'gynoid' fat distribution where fat is more evenly and peripherally distributed around the body. Increasingly secondary lifestyle of modern living combined with the availability of tempting, high-fat (junk) foods has almost caused an epidemic of obesity in the affluent nation<sup>1,2</sup>.

*Cassia fistula* Linn also known as the golden shower tree (Bengali name: Sonali or Bandor lathi), belongs to the family *Caesalpinaceae*, is widely used for its medicinal properties, its main property being that of a mild laxative suitable for children and pregnant women. In traditional medicine, *Cassia fistula* is one of the most commonly used plants in Unani and Ayurvedic medicines, this plant has been described to be useful against skin diseases, liver troubles, tuberculous glands and its use in the treatment of haematemesis, pruritus, leucoderm and diabetes has been suggested. Traditionally, the plant is also used as an infusion, decoction, or powder, either alone or in combination with

other medicinal plants. In modern times, and in any controlled clinical trials, commercial preparations have tended to be standardized extracts of the whole plant. The plant has documented to possess analgesic, anti-inflammatory, antioxidant, antidiabetic, as well as hepatoprotective activity. Since many disease conditions commonly treated with *Cassia fistula* in traditional medical systems are considered self-limiting, its purported benefits need critical evaluation. This review summarizes current scientific findings and suggests areas where further research is needed and also to verify the therapeutic efficacy of *Cassia fistula* <sup>3,4</sup>.

## MATERIALS AND METHODS

### Collection of plant material

Herb was collected from the market place at Ghoda Nakkas from Moolchand and Foolchand Brothers, suppliers of Ayurvedic Medicinal plants in Bhopal, (M.P.) and leaf were purchased in the month of July-2011. Root part was separated and was made completely clean, dust free.

### Authentication of plant

Plant was identified and authenticated by Dr. Zea Ul Hasan Professor, Head of Department, H.O.D. Botany, Saifia Science College, Bhopal, (M.P.), and a voucher specimen no. 295/Bot/Saifia/11 was assigned.

### Preparation of extract

The powdered leaf was extracted with petroleum ether to remove lipids and then again extracted with ethanol in Soxhlet extractor. The extract was concentrated under

vacuum to get the residue. The residue was dried in vacuum desiccators. The extractive yield of ethanol was found to be more, and it was selected for antiobesity activity. All the test suspensions (100 mg/ml) were prepared in the vehicle, i.e., 5% w/v acacia mucilage and were administered in the dose of 200 mg/kg orally. Each time before extracting with alcohol, petroleum ether treated drug was air dried. For aqueous extract fresh untreated drug was taken<sup>5</sup>.

After the effective extraction, solvent was concentrated at room temperature in reduced pressure using a rotary evaporator and water was removed by heating on water bath. The color and consistency of the extract was noted. Extract were subjected to chemical investigation and pharmacological screening for its antiobesity activity.

### Evaluation of anti-obesity activity

#### Test drug

Both the aqueous and alcoholic extract was given as suspension prepared by dissolving of both extract in 2% acacia suspension to make test solution.

#### Cafeteria diet

The cafeteria diet consisted of 3 diets (condensed milk 48 g + bread 48 g), (chocolate 18 g + biscuit 36 g + dried coconut 36 g) and (cheese 48 g + boiled potatoes 60 g). The three diets were given to each group of 6 rats on day 1, 2 and 3 respectively and then repeated in same succession for 42 days in addition to normal pellet chow.

Table 1 Drug dose and route of administration

Drug	Dose	Route of drug administration
Orlistat	40 mg/kg	Orally
Alcoholic extract	200 mg/kg	Orally
Aqueous extract	200 mg/kg	Orally

### Lection of animals: - (Animal care and handling)

Wistar albino rats of either sex weight (75-175 g) were selected respectively, procured from institute's animal breeding house. The animals were acclimatized to the standard laboratory conditions in well cross ventilated

animal house at temperature 25±2°C relative humidity 44-56% and light and dark cycles of 10 and 14 hours respectively for 1 week before and during the experiments. The animals were fed with standard diet and water *ad libitum*. The experiments were approved by CPCSEA and the institutional ethics committee.

Table 2 Grouping and treatment of animal in high fat diet induced obesity in experimental rats

Diet	Group I (Normal pellet chow ad Libitum)	Group II (Cafeteria diets + Normal pellet chow)	Group III (Cafeteria Diet + Normal pellet chow)	Group IV (Cafeteria Diet + Normal pellet chow)	Group V (Cafeteria Diet + Normal pellet chow)
No. of animals used	6	6	6	6	6
No. of days studied	42	42	42	42	42
Treatment	2% Acacia suspension 1ml/100g body weight	—	Orlistat 40 mg/kg (p.o.)	Alcoholic extract of <i>Cassia Fistula</i> (200 mg/kg/p.o.)	Aqueous extract of <i>Cassia Fistula</i> (200 mg/kg/p.o.)
Evaluation parameters	Body weight, Body temperature, Biochemical analysis, Internal organ weight.				

### Procedure

Wistar Albino rats of either sex (75-175g) were given high fat diet for 42 days. Thirty rats were randomly divided into 5 groups of six animals each. The following schedule of dose, diet administration in experimental groups was followed. 6-10

- Group: I- The animals received 2% acacia suspension 1 ml/100 gm/p.o. and served as normal control.
- Group: II- The animals received only high fat diet and served as negative control.
- Group: III- The animals received Orlistat 40mg/kg/p.o. suspended in 2% acacia suspension and high fat diet. The above mentioned treatment schedule followed for the respective group of animals for 42 days.
- Group: IV- The animals received high fat diet and treated with alcoholic extract of *Cassia Fistula* (200mg/kg/ p.o) suspended in 2% acacia suspension.
- Group: V- The animals received high fat diet and treated with aqueous extract of *Cassia Fistula* (200 mg/kg/ p.o) suspended in 2% acacia suspension.
- Daily all experimental animals were given high fat diet with drug treatment of Orlistat, ethanolic and aqueous leaf extract of *Cassia Fistula*.

### Evaluation parameters

**Body weight:** The body weight (g) was recorded on 1<sup>st</sup> week and then on every week for 42 days.

**Body temperature:** On day 1<sup>st</sup> and on every week, the body temperature of each rat of all groups was recorded using digital temperature indicator, with a contact time of 1 min. after drug administration. The average reading of 6 rat animals of each group was calculated.

**Biochemical parameters:** On day 42 rats were anaesthetized with light chloroform, blood sample was directly withdrawn by heart puncture. Collected blood was poured slightly into tubes marked and after 30 min. centrifuged at 2000 rpm to obtain clear serum. The amount of Glucose, Total-Cholesterol, HDL-Cholesterol, LDL-Cholesterol, VLDL-Cholesterol and Triglycerides were estimated by using biochemical kits.

**Estimation of Glucose:** The glucose was determined by Aspen Diagnostics kit Gluco-Check (Glucometer) which reads the blood glucose level manually. The amount of glucose was calculated in mg/dl<sup>11-15</sup>.

### Estimation of total-cholesterol

Autospan Diagnostic's kit was used for estimation of Total-Cholesterol, which followed CHOD/POD method.

### Procedure

- (a) Taken 200µl of clear serum into tubes added 200 µl of reagent 3 and incubated at temperature for (15-30° C) for 10 min.
- (b) Kept all tubes in cooling centrifugation chamber at 2000 rpm for 15 minute for HDL Cholesterol estimation.
- (c) Added 100 µl of supernatant from step 1 reagent 4. Added 100 µl of reagent 1. Incubate at 37° C for 10 min. measured the absorbance of different sample.

**Working reagent preparation:** All the reagents are ready to use.

**Reagent storage and stability:** All the Reagents are stable at 2-8° C until the expiry date mentioned on the container label. Do not freeze the Reagent.

**Procedure:** Fresh clear serum with no hemolysis was used for estimation.

### Reaction parameters:

- Wavelength : 490-510 nm
- Flow cell temperature : 37° C
- Incubation : 10 min.
- Sample volume : 10 µl
- Reagent volume : 1ml
- Zero setting with : Reagent blank
- Light path : 1cm

**Table 3 Estimation of total-cholesterol**

Pipette into tube marked	Blank	Standard	Test
Serum/plasma	-----	-----	10µl
Reagent 2	-----	10µl	-----
Reagent 1	1000µl	1000µl	1000µl

Mix well; incubate at 37° C for 10 minute or at room temperature (15-30° C) for 30 minutes.

Programme the analyser as per assay parameters.

- Blank the analyser with reagent blank.
- Measured absorbance of standard followed by the test.
- Calculated the results as per given calculation formula.

### Calculation

$$\text{Cholesterol concentration (mg/dl)} = \frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \times 200$$

### Estimation of HDL-cholesterol

HDL-Cholesterol was estimated using Span Diagnostics kit, which follows the CHOD/POD method.

### Procedure

- (a) Taken 200µl of clear serum into tubes added 200 µl of reagent 3 and incubated at temperature for (15-30° C) for 10 min.
- (b) Kept all tubes in cooling centrifugation chamber at 2000 rpm for 15 minute for HDL Cholesterol estimation.
- (c) Added 100 µl of supernatant from step 1 reagent 4. Added 100 µl of reagent 1. Incubate at 37° C for 10 min. measured the absorbance of different sample.

**Table 4 Estimation of HDL-cholesterol, step-I**

Pipette into tube marked	Test
Serum/Plasma	200µl
Reagent 3	200µl

Mixed well and kept at room temperature (15-30° C) for 10 minutes. Centrifuge for 15 minutes at 2000 rpm and

separated clear supernatant. Use the supernatant for HDL-Cholesterol estimation.

**Table 5 Estimation of HDL-cholesterol, step-II**

Pipette into tube marked	Blank	Standard	Test
Supernatant from Step-1	-----	-----	100µl
Reagent-4	-----	100 µl	-----
Reagent-1	1000µl	1000µl	1000µl

Mix well incubate at 37 ° C for 10 minutes or at room temperature (15-30° C) for 30 minutes.

- Programmed the analyzer with reagent blank.
- Measured absorbance of standard followed by the test.
- Calculated results as per given calculation formula.

Calculation:

$$\text{HDL-Cholesterol concentration (mg/dl)} = \frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \times 50 \times 2^*$$

\*(2 dilution factor, as sample is diluted 1:1 in step 1)

#### Estimation of LDL-Cholesterol and VLDL-Cholesterol:

**The amount of LDL-Cholesterol and VLDL-Cholesterol were calculated using friedewald's equation\*\***

LDL-Cholesterol =

$$\frac{\text{Total-Cholesterol} - \text{Triglycerides} - \text{HDL-Cholesterol}}{5}$$

VLDL-Cholesterol = Triglycerides/5

LDL-Cholesterol = Total-Cholesterol - (HDL+VLDL)

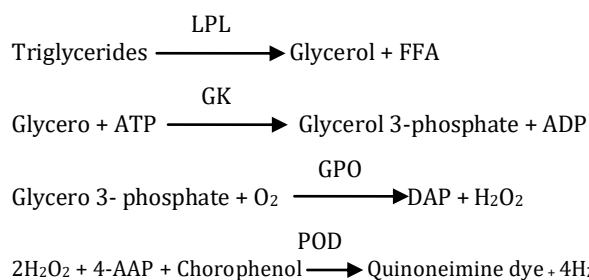
#### Estimation of triglycerides

Triglycerides was estimated using accurate Triglycerides kit of Span Diagnostics Pvt. Ltd. Accurately triglycerides estimation kit is formulated using GPO and peroxide for quantitative estimation of serum triglycerides. This method is more specific due to use of lipase to liberate glycerol which is estimated.

#### Principle

Triglycerides are hydrolysed by lipoproteins lipase (LPL) to produce Glycerol and Free Fatty Acids (FFA). In presence of Glycerol Kinase (GK), Adenosine and Adenosine Diphosphate (ADP). Glycero 3-Phosphate is further oxidized by Glycerol 3-Phosphate Oxidase (GPO) to produce Dihydroxyacetone Phosphate (DAP) and H<sub>2</sub>O<sub>2</sub>. In presence of Peroxidase (POD),

Hydrogen Peroxide couples with 4-aminoantipyrine (4-AAP) and 4-Cholorophenol to produce red Quioneimine dye. Absorbance of coloured dye is measured at 505 nm and id proportional to Triglycerides concentration in the sample.



**Reagent composition:** The kit contains 3 Reagents bottle:

- Enzyme reagents
- Diluent buffer
- Standard

#### Preparation of working reagent:

The contents of enzyme reagent were dissolved in 10 ml of diluent buffer. The working reagent is stable for 4 - 6 week at 2 - 8° C.

**Procedure:** Fresh clear serum with no hemolysis was used for estimation.

- Wavelength : 490-550 nm
- Flow cell temperature: 37° C
- Incubation : 10 minutes
- Sample volume : 10µl
- Reagent volume : 1000µl
- Zero setting with : Reagent blank

**Table 6 Estimation of triglycerides**

Pipette into tube marked	Blank	Standard	Test
Serum/Plasma	-----	-----	10µl
Reagent 2	-----	10µl	-----
Reagent 1	1000µl	1000µl	1000µl

Mix well. Incubate at 37° C for 10 minutes. Programme the analyser as per above assay parameters.

- Blank the analyser with Reagent Blank.
- Measure absorbance of standard followed by the test.

3. Calculate results as per given calculation formula.

**Calculation:**

$$\text{Triglycerides (mg/dl)} = \frac{\text{Absorbance of Test}}{\text{Absorbance of Standard}} \times 200$$

**\*\*For Glycerol free Triglycerides:**

Glycerol free Triglycerides = calculated Triglycerides - 10 mg/dl

**Organ weight:**

The animals were sacrificed by excess ether anesthesia and then midline abdominal incision was made. Different organ like live heart and both the kidney were isolated and weighed.

**Statistical analysis: -**

The results of each parameter in different groups were analyzed by the application of one-way analysis of variance-ANOVA. The multiple comparisons to elicit the significant differences between various groups were performed by means of Tukey test. Value of  $p < 0.05$ ,  $p < 0.01$ ,  $p < 0.001$  were considered statistically significant<sup>16-19</sup>.

**RESULTS AND DISCUSSIONS**

The alcoholic as well as the aqueous extract of the leaf of *Cassia Fistula* (Linn.) showed a significant antiobesity profile.

**Effect of cafeteria diet induced obesity:**

Cafeteria diet increase food intake and causes obesity in human as well as in animals. Further the composition and variety of cafeteria food exert synergistic effect on development of obesity. This model is of particular importance as it is much closer to human obesity, where in it explains various parameters like body weight, body temperature, serum biochemical parameters and internal organ weight.

**Body weight:**

It has been reported that variety of highly palatable energy rich carbohydrate cafeteria food elicited significant increase in body weight in as well as in animals due to increase in body fat content. As expected cafeteria fed animals showed significant increase in body weight when compared with normal group.

The standard drug Orlistat was significantly effective in reducing body weight. The aqueous extract showed moderate decrease in weight. Thus we may say that they exhibit appetite suppressing activity.

The mean body weight difference observed with standard, alcoholic and aqueous extract. (Table-7, Figure 1)

**Table 7 Effect of alcoholic and aqueous extract of leaf of *Cassia Fistula* (Linn.) on body weight (g) in cafeteria fed diet rats**

Sr. No	Groups	Body weight (g)							% Change in Body weight
		0 week	1 <sup>st</sup> week	2 <sup>nd</sup> week	3 <sup>rd</sup> week	4 <sup>th</sup> week	5 <sup>th</sup> week	6 <sup>th</sup> week	
1.	Control (1ml/100 g 2% acacia suspension)	77.89 ±2.37	113.92 ±3.67	115.72 ±0.92	94.87 ±1.27	113.24 ±1.15	116.91 ±3.17	118.11 ±0.83	34.05
2.	High fat cafeteria diet	88.01 ±2.29	130.93 ±2.26 a <sup>**</sup>	131.36 ±1.63 a <sup>***</sup>	138.42 ±0.91 a <sup>***</sup>	141.10±1.24 a <sup>***</sup>	142.69 ±2.93 a <sup>***</sup>	144.83 ±1.40 a <sup>***</sup>	39.23
3.	Cafeteria diet + Orlistat (40mg/kg)	156.11 ±2.71 a <sup>***</sup> , b <sup>***</sup>	171.21 ±2.75 a <sup>***</sup> , b <sup>***</sup>	184.77 ±0.63 a <sup>***</sup> , b <sup>***</sup>	194.34 ±1.54 a <sup>***</sup> , b <sup>***</sup>	197.40±3.02 a <sup>***</sup> ,b <sup>***</sup>	197.27 ±3.02 a <sup>***</sup> , b <sup>***</sup>	195.96 ±0.92 a <sup>***</sup> , b <sup>***</sup>	20.34
4.	Cafeteria diet + Alcoholic extract (200mg/kg/p.o.)	162.72 ±3.61 a <sup>***</sup> , b <sup>***</sup>	174.09 ±2.45 a <sup>***</sup> , b <sup>***</sup>	177.11 ±0.66 a <sup>***</sup> , b <sup>***</sup> ,c <sup>***</sup>	177.59 ±1.11 a <sup>***</sup> , b <sup>***</sup> ,c <sup>***</sup>	173.78±1.54 a <sup>***</sup> ,b <sup>***</sup> ,c <sup>***</sup>	165.41 ±3.68 a <sup>***</sup> , b <sup>***</sup> ,c <sup>***</sup>	163.06 ±1.40 a <sup>***</sup> , b <sup>***</sup> , c <sup>***</sup>	0.22
5.	Cafeteria Diet + Aqueous extract (200mg/kg/ p.o.)	175.53 ±3.48 a <sup>***</sup> ,b <sup>***</sup> , c <sup>***</sup> ,d <sup>*</sup>	183.22 ±2.78 a <sup>***</sup> ,b <sup>***</sup> ,c <sup>*</sup>	197.06 ±1.28 a <sup>***</sup> ,b <sup>***</sup> , c <sup>***</sup> ,d <sup>***</sup>	197.06 ±1.28 a <sup>***</sup> ,b <sup>***</sup> ,d <sup>***</sup>	196.43±0.72 a <sup>***</sup> ,b <sup>***</sup> ,d <sup>***</sup>	190.08 ±3.19 a <sup>***</sup> ,b <sup>***</sup> ,d <sup>***</sup>	180.70 ±1.47 a <sup>***</sup> ,b <sup>***</sup> , c <sup>***</sup> ,d <sup>***</sup>	2.86

Data are expressed in mean ± SEM, n= 6 (no. of six animals) in each groups,\* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$  compared with multiple group using One-way ANOVA followed by Tukey multiple comparison test.

a = significant difference in compared with vehicle treated group.

b= significance difference in compared with high fat diet group.

c= significance difference in compared with standard drug treated group.

d= significance difference in compared with test drug treated group.

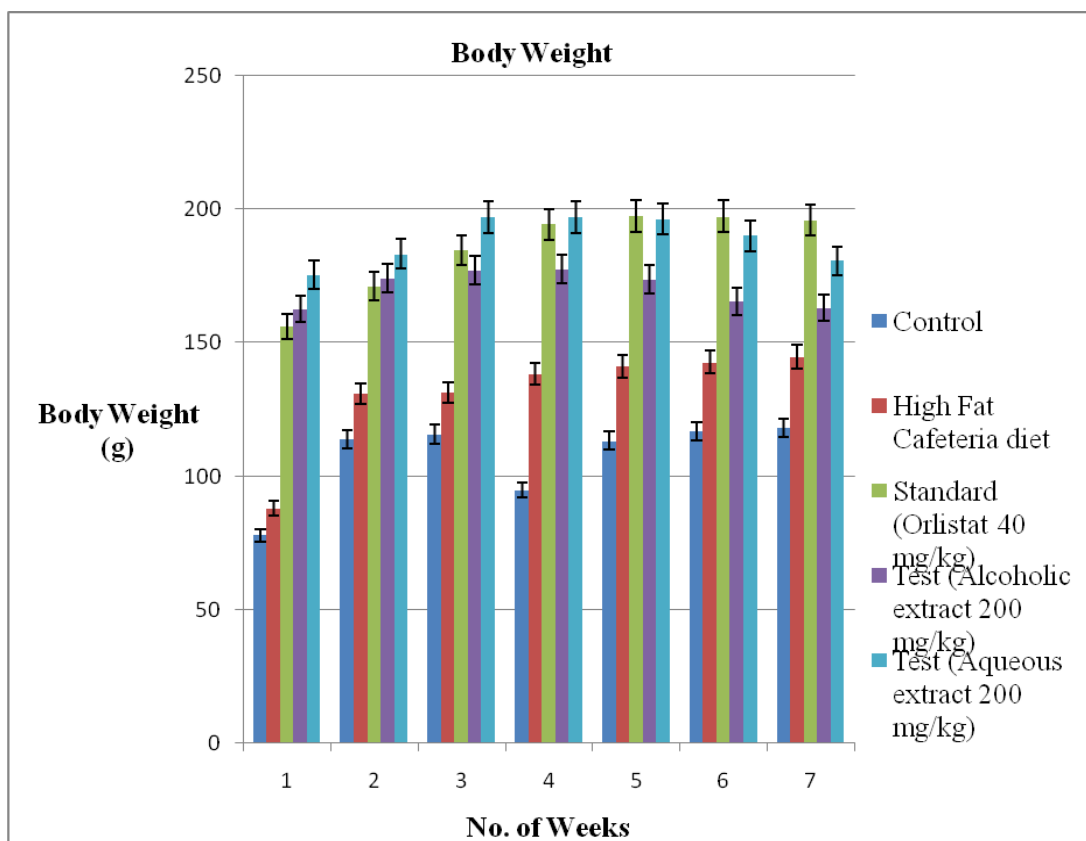


Figure 1 Effect of alcoholic and aqueous extract of leaf of *Cassia Fistula* (Linn.) on body weight (g) in cafeteria fed diet rats

**Body temperature**

In the animal model of obesity there will be decrease in diet induced thermogenesis due to decrease in sympathetic activation of brown adipose tissue. The main factor of this decrease in diet induced thermogenesis is the neurotransmitter NPY synthesized throughout the brain. NPY causes increase in food intake within 10-15 min.,

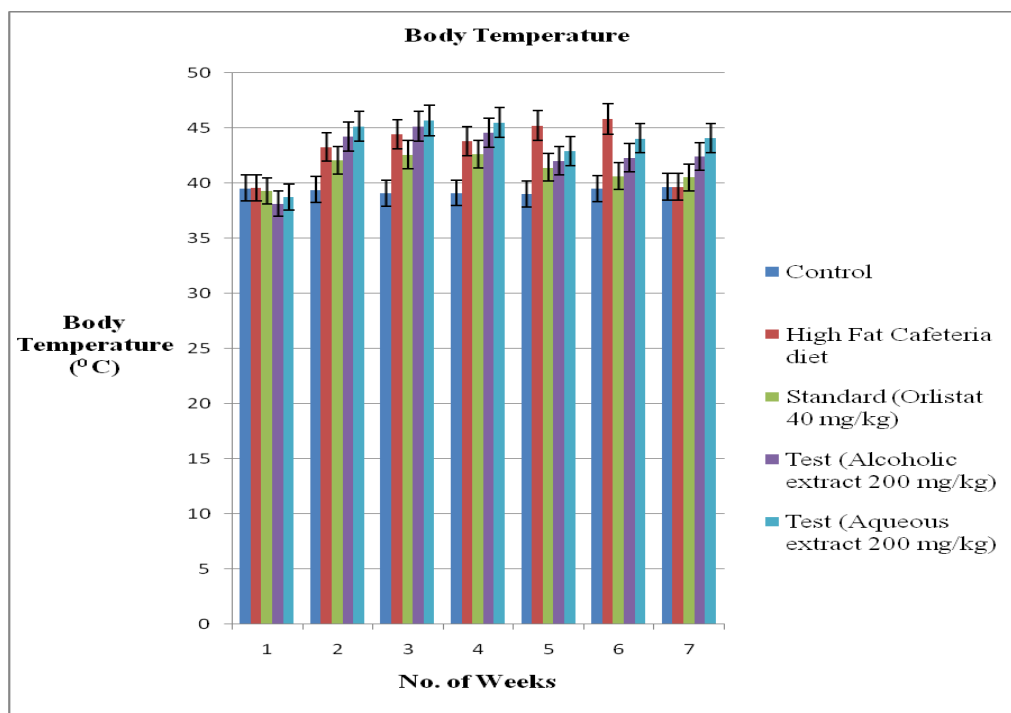
combined with a reduction in thermogenesis on its endogenous release. NPY inhibits thermogenesis by reducing activation of brown adipose tissue. Accordingly the significant decrease in body temperature was observed in cafeteria diet fed rats when compared with control animals. The standard drug Orlistat significantly reversed the decrease in body temperature thus showing a significant rise in temperature. (Table-8, Figure-2)

Table 8 Effect of alcoholic and aqueous extract of leaf of *Cassia Fistula* (Linn.) on body temperature in cafeteria fed diet rats

Sr. No	Groups	Difference in Body Temperature						
		0 week	1 <sup>st</sup> week	2 <sup>nd</sup> week	3 <sup>rd</sup> week	4 <sup>th</sup> week	5 <sup>th</sup> week	6 <sup>th</sup> week
1.	Control (1ml/100 g 2% acacia suspension)	39.52 ±0.61	39.38 ±0.53	39.06 ±0.27	39.10 ±0.80	39.00 ±0.57	39.50 ±0.29	39.65 ±0.33
2.	High fat cafeteria diet	39.55 ±0.43	43.28 ±0.73 a***	44.43 ±0.32 a***	43.8 ±0.57 a**	45.21 ±0.51 a***	45.81 ±0.40 a***	45.73 ±0.32 a***
3.	Cafeteria diet + Orlistat (40 mg/kg/p.o.)	39.3 ±0.73	42.06 ±0.50 a**,c*	42.55 ±0.55 a***, b**	42.60 ±1.35 a*	41.4 ±0.47 a***,b***	40.6 ±0.40 a***,b***	40.51 ±0.56 a*, b***
4.	Cafeteria diet + Alcoholic extract (200 mg/kg/ p.o.)	38.1 ±0.52	44.21 ±0.44 a***,c**	45.16 ±0.21 a***,c***	44.56 ±0.89 a***	42.01 ±0.47 a***,b***	42.3 ±0.46 a***,b***,c*	42.40 ±0.32 a***,b***,c*
5.	Cafeteria diet + Aqueous extract (200mg/kg/p.o.)	38.72 ±0.38	45.15 ±0.23 a***	45.66 ±0.22 a***,c***	45.5 ±0.80 a***	42.88 ±0.37 a***,b*	44.03 ±0.42 a***,b*c***,d*	44.09 ±0.39 a***,c,d*

Data are expressed in mean ± SEM, n = 6 (no. of six animals) in each groups, \*p<0.05, \*\*p<0.01, \*\*\*p < 0.001 compared with multiple group using One-way ANOVA followed by Tukey multiple comparison test.

- a = significant difference in compared with vehicle treated groups.
- b= significance difference in compared with high fat diet groups.
- c= significance difference in compared with standard drug treated groups.
- d= significance difference in compared with test drug treated groups.



**Figure 2** Effect of alcoholic and aqueous extract of leaf leaf of *Cassia Fistula* (Linn.) on body temperature in cafeteria fed diet rats

### Serum biochemical parameters

In obesity, the lipid disorder is characterized by increase in serum levels of Total-Cholesterol, LDL-Cholesterol, and Triglycerides along with low levels of serum HDL-Cholesterol. Accordingly the cafeteria fed rats displayed significant increase serum level of Total-Cholesterol, Triglycerides, LDL-Cholesterol and VLDL-Cholesterol. Along with moderate increase in serum HDL-Cholesterol levels as compared to cafeteria diet control. Treatment with standard drug Orlistat has resulted in reduction in serum level of Triglycerides, Total-Cholesterol, LDL-Cholesterol and VLDL-

Cholesterol. Along with moderate in serum HDL-Cholesterol levels as compared to cafeteria diet control. The aqueous and alcoholic extract of leaf of *Cassia Fistula* (Linn.) has shown significant decrease in serum levels of Total-Cholesterol, LDL-Cholesterol, VLDL-Cholesterol and Triglycerides. (Table-9, Figure-3). The aqueous as well as alcoholic extract significantly reduces the serum levels of HDL-Cholesterol. (Table-9, Figure-3) Obesity is associated with Hperinsulinemia and insulin resistance to increase in blood glucose levels. As expected the cafeteria diet fed rats showed significant increase in serum glucose levels when compared to normal group of rats. (Table-9, Figure-3)

**Table 9** Effect of alcoholic and aqueous extract of leaf of *Cassia Fistula* (Linn.) on serum biochemical parameters in cafeteria fed diet rats

Sr. No	Groups	Glucose (mg %)	Cholesterol (mg %)	HDL (mg %)	LDL (mg %)	VLDL (mg %)	TG <sub>s</sub> (mg %)
1.	Control (1ml/100 g 2% acacia suspension)	191.33 ±0.66	133.98 ±0.50	53.79 ±0.93	65.18 ±0.75	15.01 ±0.51	75.05 ±2.33
2.	High fat cafeteria diet	430.166 ±0.98 a <sup>***</sup>	146.81 ±0.88 a <sup>***</sup>	21.43 ±0.76 a <sup>***</sup>	106.28 ±0.29 a <sup>***</sup>	19.10 ±0.73 a <sup>**</sup>	95.53 ±2.12 a <sup>***</sup>
3.	Cafeteria diet + Orlistat (40mg/kg/p.o.)	227.83 ±1.10 a <sup>***</sup> , b <sup>***</sup>	117.29 ±0.74 a <sup>***</sup> , b <sup>***</sup>	30.98 ±0.79 a <sup>***</sup> , b <sup>***</sup>	82.3 ±0.11 a <sup>***</sup> , b <sup>***</sup>	4.99 ±0.61 a <sup>***</sup> , b <sup>***</sup>	24.98 ±2.27 a <sup>***</sup> , b <sup>***</sup>
4.	Cafeteria diet + Alcoholic extract (200mg/kg/p.o.)	367.66 ±1.45 a <sup>***</sup> , b <sup>***</sup> c <sup>***</sup>	131.40 ±1.47 b <sup>***</sup> , c <sup>***</sup>	33.57 ±1.02 a <sup>***</sup> , b <sup>***</sup>	86.47 ±0.33 a <sup>***</sup> , b <sup>***</sup> c <sup>***</sup>	11.36 ±0.71 a <sup>**</sup> , b <sup>***</sup> , c <sup>***</sup>	63.81 ±3.09 a <sup>*</sup> , b <sup>***</sup> , c <sup>***</sup>
5.	Cafeteria diet + Aqueous extract (200mg/kg/p.o.)	441.16 ±1.49 a <sup>***</sup> , b <sup>***</sup> , c <sup>***</sup> , d <sup>***</sup>	139.88 ±0.57 a <sup>***</sup> , b <sup>***</sup> , c <sup>***</sup> , d <sup>***</sup>	37.58 ±0.79 a <sup>***</sup> , b <sup>***</sup> , c <sup>***</sup> , d <sup>*</sup>	87.9 ±0.47 a <sup>***</sup> , b <sup>***</sup> , c <sup>***</sup>	14.4 ±0.59 b <sup>***</sup> , c <sup>***</sup> , d <sup>*</sup>	72.0 ±3.16 b <sup>***</sup> , c <sup>***</sup>

Data are expressed in mean ± SEM, n = 6 (no. of six animals) in each groups, \*p<0.05, \*\*p<0.01, \*\*\*p < 0.001 compared with multiple group using One-way ANOVA followed by Tukey multiple comparison test.

a = significant difference in compared with vehicle treated groups.

b= significance difference in compared with high fat diet groups.

c= significance difference in compared with standard drug treated groups.

d= significance difference in compared with test drug treated groups.

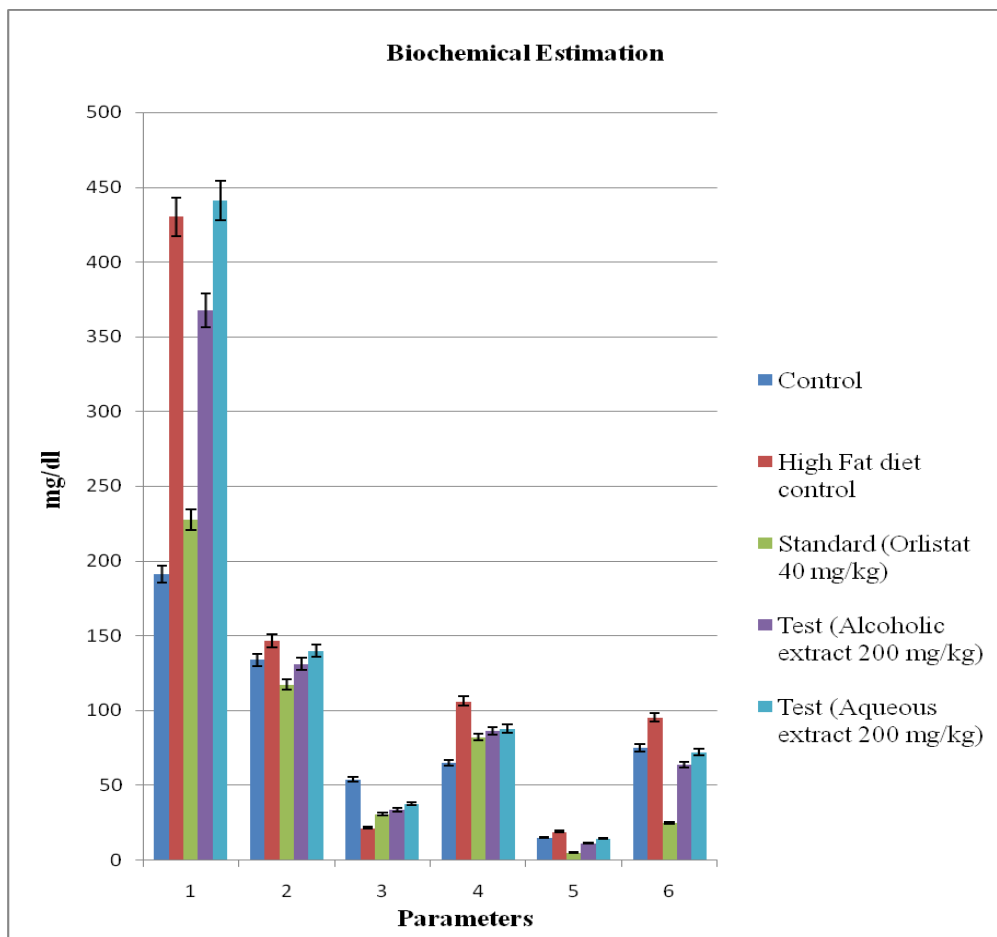


Figure 3 Effect of alcoholic and aqueous extract of leaf of *Cassia Fistula* (Linn.) on serum biochemical parameters on cafeteria diet fed rats

**Internal organ weight**

Obesity is associated with increased adipose tissue accumulation in the body. It is reported that cafeteria diet feeding in female rats leads to significant increase in internal organ weight, confirming to this, the cafeteria diet group of rats showed significant increase in weight of liver, and both kidney increase in weight of heart when compared to normal

groups. Orlistat has decreased consumption of diet and thus decrease in weight of kidney and liver and decrease weight of heart when compared to cafeteria diet fed animals. Both aqueous and alcoholic extract of leaf of *Cassia Fistula* (Linn.) have shown significant reduction in weight of heart and both the kidney as compared to cafeteria diet fed animals confirming their anti-obesity property. (Table-10, Figure-4)

Table 10 Effect of alcoholic and aqueous extract of leaf of *Cassia Fistula* (Linn.) on organ weight (g) in cafeteria diet fed rats

Sr. No	Groups	Liver	Heart	Kidney	
				Left	Right
1.	Control (1ml/100 g 2% acacia suspension)	0.90±0.06	0.79±0.00	0.87±0.06	0.73±4.21
2.	High Fat Cafeteria diet	1.38±0.14	0.96±0.02 a**	0.95±0.01	0.93±7.57
3.	Cafeteria Diet + Orlistat (40 mg/kg p.o.)	0.84±0.41	0.77±0.03 b**	1.04±0.03 a*	0.75±6.9
4.	Cafeteria Diet + Alcoholic extract (200 mg/kg p.o.)	0.79±0.46	0.69±0.04 b***	0.82±0.04 c**	0.80±1.68
5.	Cafeteria Diet + Aqueous extract (200 mg/kg p.o.)	0.76±0.04	0.78±0.05 b**	1.03±0.02 a*, d**	0.85±0.02

Data are expressed in mean ± SEM, n = 6 (no. of six animals) in each groups,\*p<0.05, \*\*p<0.01, \*\*\*p < 0.001 compared with multiple group using One-way ANOVA followed by Tukey multiple comparison test.

- a = significant difference in compared with vehicle treated groups.
- b= significance difference in compared with high fat diet groups.
- c= significance difference in compared with standard drug treated groups.
- d= significance difference in compared with test drug treated groups.



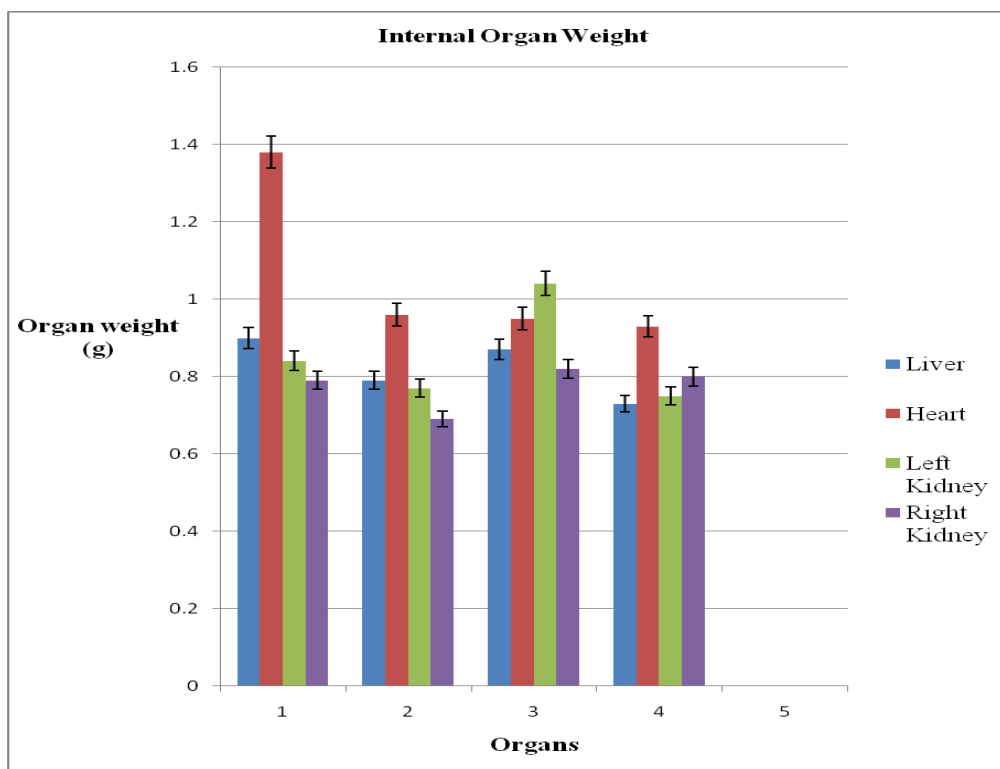


Figure 4 Effect of alcoholic and aqueous extract of leaf of *Cassia Fistula* (Linn.) on organ weight (g) in cafeteria diet fed rats

## CONCLUSIONS

The work entitled evaluation of anti-obesity activity of leaf of *Cassia fistula* was to determine the efficacy and safety of leaf extract in experimental animals. The plant was preliminary collected, authenticated and was subjected for extraction to obtain different extract. The crude extract was subjected to phytochemical analysis for presence of glycoside, flavonoids, saponins etc. Concluding that the alcoholic extract of leaf of *Cassia fistula* has comparatively shown better antiobesity activity than as compared to the aqueous extract of the leaf.

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**Conflicts of Interests:** The authors report no conflicts of interest.

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