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ANTI-OBESITY ACTIVITY OF *IPOMOEA SEPIARIA* EXTRACTS IN RATS FED WITH HIGH FAT DIET

Pavan Kumar Chadalawada*, B. Pooja, S. Manoharbabu, M. Prabhu Teja, M. Janbee, B. Himabindu and D. Srividya

India.

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***Corresponding Author**
Prof. Pavan Kumar Chadalawada
India.

ABSTRACT

Objective: Overweight and obesity represent a rapidly growing threat to the health of populations in an increasing number of countries. Indeed they are now so common that they are replacing more traditional problems such as undernutrition and infectious diseases as the most significant causes of ill-health so we have made an attempt for *in-vivo* anti- obesity activity. **Methods:** ***in-vivo*** effect of ***Ipomoea sepiaria*** methanol and aqueous on HFD induced Obesity by using *Wistar strain rats*. **Results:** To results from this study, test item *Ipomoea sepiaria* aqueous and methanol extract at a dose of 200 & 200 mg/kg and reference compound Atorvastatin (10 mg/kg) were effective in reducing Obesity and atherosclerosis induced by HFD in *Wistar* rats. Hence, the test item Methanolic extract of *Ipomoea sepiaria* could be considered for treatment of Obesity in human beings **Conclusion:** our findings on therapeutically properties of *Ipomoea sepiaria* aqueous extract on lipid levels progression showed that positive pharmacological effects of *Ipomoea sepiaria* aqueous and methanol extracts is not completely due to flavonoids and at least in part, it is attributed to the presence of higher amounts of other constituents such as polyphenols, tannins. Thus the plant can be further explored for its phytochemical profile to identify the active constituents for the above mentioned activities.

KEYWORDS: obesity, ***Ipomoea sepiaria***, HFD induced Obesity, analogous design.

INTRODUCTION

Herbal medicine is still the mainstay of about 75–80% of the world population, mainly in the developing countries, for primary health care because of better cultural acceptability, better compatibility with the human body and lesser side effects. However, the last few years have

seen a major increase in their use[1] in the developed world. The genus *Ipomoea*[2,3,4] since time immemorial has been in continuous use for medicinal uses. *Ipomoea* species is to treat constipation diabetes, aphrodisiac, astringent, Immunodeficiency Syndrome (AIDS) and hypertension.

Overweight and obesity represent a rapidly growing threat to the health of populations in an increasing number of countries.[5] Indeed they are now so common that they are replacing more traditional problems such as undernutrition and infectious diseases as the most significant causes of ill-health. Obesity comorbidities include coronary heart disease, hypertension and stroke, certain types of cancer, non-insulin-dependent diabetes, mellitus, gallbladder disease, dyslipidaemia, osteoarthritis and gout, and pulmonary diseases, including sleep apnoea, In addition, the obese suffer from social bias, prejudice and discrimination, on the part not only of the general public.

From the review of literature identified that folk-lore anti- obesity activity[6] was examined. So we have made an attempt for *in-vivo* anti- obesity activity. Methanol and aqueous extract of *Ipomoea sepiaria* was studied for the Anti-obesity activity in rats fed with High Fat Diet.

Experimental study protocol

There are different anti-obesity agents which are widely available in the market. But they are having more adverse effect.[7] As we know current scenario of Ayurveda therapy is growing rapidly. So we selected ***Ipomoea sepiaria*** *Which* consists of flavonoids as chemical constituents may have anti-obesity activity which is not evaluated till now. So our objective was to evaluate the anti-obesity ***Ipomoea sepiaria*** in high fat diet induced obese rats.

Preparation of extract

The plant material after collection was shade dried and powdered using a mechanical grinder and passed through 40 mesh sieve. Powder (100g) was defatted using 1.5L of petroleum ether (BP: 60-80ºC) and subjected to extraction in a Soxhlet apparatus using methanol for 12h. To obtain aqueous extract distilled water were used. The fraction was concentrated under reduced pressure and controlled temperature (40-50ºC). The yielding ratio of aqueous extract of was 19.65% w/w. The extract was stored in tightly closed container placed in refrigerator for further use.

Preparation of dosage formulation

The required quantity of compounds were taken and dissolved in a particular vehicle to prepare a formulation which can be given to the animals.

Acclimatization of animals

Animals were kept for one week, before enrolment into the study. Food, housing &water to the animals were taken care before the experimental study.

Study Design

The selected rats were divided into normal control, Disease control, and treatment groups in efficacy studies,[8] Disease induction is done for the efficacy models. Route and frequency of administration of formulation must be planed prior to the experiment.

Institutional Animal Ethics Committee (IAEC) Approval

The following study protocol has been reviewed and approved by the Institutional Animal Ethics Committee (IAEC) of Sims College of pharmacy. The recommendations as per the animal welfare guidelines regarding animal care and handling will be strictly adhered to. The approval has been documented in the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) specified 'Form B' protocol (*IAEC Protocol Approval Number: IAEC/SIMS 2015/004*). The procedures used in this protocol were designed to conform to the accepted practices and to minimize/avoid risk of causing pain, distress or discomfort to the animals. The number of animals selected for use in this study was considered to be the minimum requirement to meet rationale scientific endpoints.

Justification for Test System

Wistar rat was the species recommended for the assessment of obesity as they are easily prone to obesity and they are well reported in the literature.[9]

MATERIALS AND METHODS[10-13]

HFD induced Obesity: Effect of ***Ipomoea sepiaria*** on HFD induced Obesity.

Chemicals

a. Sodium carboxy methyl cellulose.
b. Atorvastatin (purchased from Aurobindo laboratories, Hyd.).
c. Double distilled water.
d. Normal saline. All other reagents used were of analytical grade.

Test item information: Information for *Ipomoea sepiaria* aqueous extract

Name of the test item		*Ipomoea sepiaria* aqueous extract
Color		Dark brownish powder
Flavor		Sweetish, chocolatey
% yield		95%
Storage condition		Room temperature

Vehicle Information: Information for Carboxy methyl Cellulose Sodium Medium

Product No	:	1123
Batch No.	:	9755
Manufactured by	:	Indian research products
Purity as per the COA	:	NA
Physical Appearance	:	Solvent
Storage Condition	:	Room Temperature

Induction Agent Information: Information for HFD:

Ingredient	:	Amount weighed
Coconut oil	:	100ml
Dalda	:	50gm
Bengal gram	:	50gm
Sugar	:	100gms
Storage Condition	:	Room temperature

Preparation of Dose Formulations

Ipomoea sepiaria aqueous extract

The required quantity of ***Ipomoea sepiaria*** aqueous extract was weighed by using an analytical balance and transferred to a mortar and pestle. The desired volume of 0.5% *w/v* carboxy methyl cellulose sodium medium viscosity (CMC-Na) in water was added and triturated to get the final concentration of 100,200 mg/mL suspensions. The suspension formulations were transferred to a centrifuge tube and subjected to vortexing for 2 minutes followed by sonication for another 2 minutes to obtain homogeneous suspensions.

ATORVASTATIN

The required quantity of Atorvastatin was weighed using an analytical balance and transferred to a centrifuge tube. The desired volume of 0.5% *w/v* CMC-Na in water was added to get the final concentration of 10 mg/mL suspension. The suspension formulation was subjected to vortexing for 2 minutes to obtain a homogeneous suspension.

TEST SYSTEM MANAGEMENT

Test system

Species	- Rat, *Rattus norvegicus*
Strain	*Wistar*
Sex	Male
Age at initiation of study	10-12 weeks old
Body weight range	150-200 g
Source	Animal Husbandry Divison, Mahaveer Enterprizes
Number of animals per group	6
Number of Groups	7
Number of Animals	42
Identification of Animals	By rat accession number and body Marking by 10% picric acid.

Husbandry

Environmental Conditions

Temperature	22 ±3 °C
Relative Humidity	40 - 70%
Photoperiod	12 hours light / 12 hours dark cycle

Housing

Rats were housed in solid bottom autoclaved polypropylene cages (Size: approximately L 425 x B 266 x H 185 mm), three rats per cage with stainless steel top grill having facilities for pellet feed and drinking water in polycarbonate bottle during acclimatization and throughout the study duration. Corn cob was used as bedding material and it was changed twice a week.

Diet: *ad libitum*

Rodent pellet feed (Animal Nutrition India *Pvt. Ltd.*,) was provided to the animals.

Water: *ad libitum*

The animals were given mineral water *ad libitum* by a water dispensing bottle.

Randomization and Grouping

A group of animals were examined for health and healthy *wistar* rats were selected for the study and were randomly assigned to different groups based on the body weight on the last day of acclimatization.

EXPERIMENTAL PROCEDURES

The selected rats were divided into different group as shown in the *Table* - below.

Study design

Group	Dose *(mg/kg, p.o. q.d.)*		Duration	No.of Rats
	HFD	Treatment		
G1 - Normal Control	-	-	30	6
G2 - HFD+ Vehicle	50	-	30	6
G3-HFD+***Ipomoea sepiaria*** aqueous extract 100mg/kg	50	100	30	6
G4-HFD+***Ipomoea sepiaria*** aqueous extract 200 mg/kg	50	200	30	6
G5-HFD+***Ipomoea sepiaria*** methanol	50	100	30	6
G6-HFD+***Ipomoea sepiaria*** methanol extract	50	200	30	6
G7-HFD+Atorvastatin	50	10	30	6

Acute toxicity study

The acute toxicity study in mice was performed as per the OECD guidelines (No. 423) to evaluate the undesirable effects or toxicity ***Ipomoea sepiaria*** aqueous extract. Swiss male albino mice were divided in to the groups of 3 animals per group and were administered once orally with dose of 2000 mg/kg of ***Ipomoea sepiaria*** aqueous extract. The mice were then critically observed for clinical signs, gross behavioral changes and mortality after 30min, 1hr, 2hr, 3hr and then after 24hr. These observations were continued for a period of 7 days.

Induction of obesity

High fat diet induced obesity

Obesity was induced in *wistar* rats by administrating the HFD (100 *mg/kg, p.o. q.d.*) consecutively for 30 days.

Administration of HFD

The freshly prepared HFD was placed in the cage carefully and was administered for 30 day to G2, G3, G4 and G5 groups. G1 group was administered *p.o.* 5mL/kg of normal saline. The dose volume was 5 mL/kg and it was calculated and adjusted according to the individual animal's body weight recorded on a weekly basis.

Administration of Ipomoea sepiaria aqueous extract **and Atorvastatin**

The freshly prepared suspension formulation of ***Ipomoea sepiaria*** aqueous extract was administered orally to G2 and G3, groups at a dose of 100 and 200*mg/kg* respectively and the Atorvastatin was administered orally to G5 group at a dose of 10*mg/kg*. G1 and G2 group were administered orally 10 mL/kg of 0.5% *w/v* CMC-Na. The dose volume was 10 mL/kg and it was calculated and adjusted according to the individual animal's body weight recorded on a weekly basis.

Route and Frequency of Administration HFD

Route of administration is oral as HFD has been known to cause Obesity and Atherosclerosis in animals' when taken orally. HFD will be administered to the respective groups for a period of 30 days.

Ipomoea sepiaria aqueous extract and Atorvastatin

Route of administration is oral as it is the intended route of administration in human beings. The ***Ipomoea sepiaria*** aqueous extract and Atorvastatin were administered to the respective groups for a period of 15 days.

7.6 OBSERVATIONS

General observation of Animals for Clinical Signs[14]

Animals were observed for general clinical signs once daily for 21 days throughout the study duration.

Body Weight

Body weight of animals were recorded prior to initiation of treatment and followed by weekly once during course of the study.

Dose preparation and administration of standard atorvastatin and *Ipomoea sepiaria* aqueous extract

Standard atorvastatin at a dose of 10mg/kg was prepared by suspending bulk atorvastatin in aqueous 0.5% methyl cellulose. ***Ipomoea sepiaria*** aqueous extract was dissolved in 2% gum acacia and at doses of 100, 200 mg/kg were given to the rats once in a day along with the high cholesterol diet orally. Treatment was given daily for 10 days.

Protocol for anti-obesity

The experimental animals were divided into five groups, 6 animals each group.

Group 1 normal.

Group 2 high cholesterol diet control.

Group 3 High cholesterol diet treated with ***Ipomoea sepiaria*** aqueous extract [100mg/kg b.w., p.o.]**.**

Group 4 High cholesterol diet treated with ***Ipomoea sepiaria*** aqueous extract [200 mg/kg b.w., p.o].

Group 5 High cholesterol diet treated with ***Ipomoea sepiaria*** methanol extract [100mg/kg b.w., p.o.]**.**

Group 6 High cholesterol diet treated with ***Ipomoea sepiaria*** methanol extract [200 mg/kg b.w., p.o].

Group 7 standard atorvastatin [10mg/kg body weight, orally (p.o).

Weight Measurements

The weight of the experimental animals has to be noted before and after the experiment and also on a weekly basis using an electronics balance to have clear information about the effect of the anti-obesity effect of ***Ipomoea sepiaria*** aqueous extract.

The net weight loss can be calculated as:

Net weight loss = Initial weight (W0) – New weight (W1)

% of weight loss= Total Weight loss x 100 / Initial weight

Blood sample collection and analysis[15]

Blood sample collection

The blood has to be collected from the animals initially and terminally to the experiments. Here the animals were anaesthetised using Isoflurane. Blood sample was collected by retro orbital puncture method, under mild Isoflurane anaesthesia after 8h fasting and allowed to

clot for 30 min at room temperature. Blood samples were centrifuged at 3000 rpm for 20 min and they will be used for biochemical estimations as shown in table.

List of biochemical and metabolic parameters for assessment of obesity

S.NO	TEST	PARAMETER
1.	Lipid profile	Total cholesterol. LDL, HDL,VLDL, total triglycerides
2.	Hormonal Assay	Insulin concentration and Leptin concentration
3.	Heart biomarker	CK-MB(Creatine kinase myocardial type),CRP(C Reactive protein)
4.	Lipid metabolism	Apolipoprotein B

Biochemical Measurements and Hormonal parameters

Serum was Prepared and stored at -20 ^{0}c until biochemical estimations were analyzed Spectrophotometrically for total serum cholesterol (TC),Triglycerides (TG) and high density lipoprotein, hormonal assay, lipid and heart biomarkers were estimated in Zed labs diagnostics, Guntur, India. Histopathological studies were carried out in Disha laboratories, Guntur, India.

RESULTS

Effect of *Ipomoea sepiaria* aqueous **extract on lipid profile**

Group II (disease control group) animals fed with HFD exhibited a significant ($p<0.001$) increase in Total cholesterol (TC), triglycerides' (TG), LDL and when compared to group I (Normal group) animals. Administration of CGM (200mg/kg) and Atorvastatin shows a significant reduction (<0.001) in TC,TG,LDL when compared with the group II animals, whereas decreased HDL levels observed in Group II animals were significantly($p<0.001$) increased in group V. Results were shown in the below tables.

Effect of *Ipomoea sepiaria* aqueous **extract on Serum Leptin, Insulin, CRP, CK-MB and Apolipoprotien-B**

Group II (disease control group) animals fed with HFD exhibited a significant ($p<0.001$) increase in levels of serum leptin, Insulin, CK-MB, Apo lipoprotien-B when compared to group I (Normal group) animals. Administration of CGM (200 mg/kg) and Atorvastatin shows a significant reduction (<0.001) in serum leptin, Insulin, CK-MB when compared with the Group II animals. Serum leptin levels were significantly ($p<0.001$) decreased in group III

and IV as compared with the group II. There is no considerable serum CRP levels are observed.

Effect of *Ipomoea sepiaria* aqueous **extract on lipid profile of high fat diet rats in 10 days**

S.no	Parameter	Group i	Group ii	Group iii	Group iv	Group v	Group vi	Groupvii
1.	TC	91±0.91	126±1.93	117±1.6	105±1.3	104±3.64	122±2.22	137±2.52
2.	TG	74.7±1.7	157±1.7	143±2.01	129.8±1.7	103±1.19	135±1.97	139±1.74
3.	HDL –C	45.2±1.49	26.7±2.79	36±2.19	45±1.32	48.5±1.12	136±2.24	138±2.33
4.	LDL	81.75±1.54	146±2.17	135.8±1.1	112.8±1.65	103.8±1.01	128±1.89	131±1.15

Values are given as mean ± standard error mean (S.E.M) for 7 groups of 6 animals each. Values are statically significant at p<0.001.Group II compared with group I and group III, IV,V,VI and VII were compared with group II.

Effect of *Ipomoea sepiaria* aqueous **extract on lipid profile of high fat diet rats in 30 days**

S.no	Parameter	Group i	Group ii	Group iii	Group iv	Group v	Group vi	Group vii
1.	TC	91.5±0.64	155±1.01	113±2.04	102.7±1.04	101.5±3.2	123±1.22	137±2.52
2.	TG	74.6±1.93	171±2.19	139.8±2.05	119.8±2.05	97.5±2.2	137±0.97	139±1.74
3.	HDL –C	50±1.95	33.2±1.37	43.2±1.84	45.5±1.32	51.25±0.85	137±2.24	138±2.33
4.	LDL	81.2±2.39	167±1.1	127±1.1	110.5±1.1	99.4±1.04	129±1.89	131±1.15

Values are given as mean ± standard error mean (S.E.M) for 5 groups of 6 animals each. Values are statically significant at *** p<0.001.Group II compared with group I and group III, IV,V,VI and VII were compared with group II.

DISCUSSION AND CONCLUSION

Ant-obesity was induced in Wistar rats by administrating HFD to G2, G3, G4 and G5 group at a dose of 100 gm. daily for 30 days. G1 group was administered *p.o.*5 mL/kg of normal saline. *Ipomoea sepiaria* aqueous extract (100 & 200*mg/kg)* and Atorvastatin (10mg/kg*)* were administered orally to G3, G4 G5, G6 and G7 group respectively. G1 and G2 group were administered orally 10mL/kg of 0.5% carboxymethyl cellulose (CMC). Antiobesity of test item and reference drug Atorvastatin was assessed on day 30 by measuring the changes in levels of lipids, hormones, biomarkers and plaque formation in aortas.

There was no treatment related morbidity and mortality observed during the study period and also no differences in body weight of animals were noticed between the treatment groups as compared to HFD control group. G2 animals administered with HFD at 100 mg/kg. Showed a significant increase ($^{***}p<0.001$) in the weights, lipid levels, hormones, biomarkers as compared to G1 group. Conversely oral administration of *Ipomoea sepiaria* aqueous and methanol extract at a dose of 200 & 200 mg/kg and Atorvastatin at a dose of 10 mg/kg

significantly (***$p<0.001$) decreased the mean Lipid levels, CK-MB, Apolipoprotein –B, Insulin and increased leptin levels in G3, G4 G5, G6 and G7 as compared to G2 group. No significant change was observed in heart rate.

To results from this study, test item *Ipomoea sepiaria* and methanol extract at a dose of 200 & 200 mg/kg and reference compound Atorvastatin (10 mg/kg) were effective in reducing Obesity and atherosclerosis induced by HFD in *Wistar* rats. Hence, the test item Methanolic extract of *Ipomoea sepiaria* could be considered for treatment of Obesity in human beings.

CONCLUSION

The results described here clearly confirmed the anti-obesity properties of *Ipomoea sepiaria* aqueous extract. Also, our findings on therapeutically properties of *Ipomoea sepiaria* aqueous extract on lipid levels progression showed that positive pharmacological effects of *Ipomoea sepiaria* aqueous and methanol extracts is not completely due to flavonoids and at least in part, it is attributed to the presence of higher amounts of other constituents such as polyphenols, tannins. Thus the plant can be further explored for its phytochemical profile to identify the active constituents for the above mentioned activities.

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