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Original Article

PHARMACOLOGICAL SCREENING FOR ANTI-OBESE ACTIVITY OF AQUEOUS ROOT EXTRACT FROM *IPOMOEA SEPIARIA* KOENING EX. ROXB IN HIGH FAT DIET-INDUCED OBESE MALE WISTAR RATS.

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ABSTRACT

Effect of *Ipomoea sepiaria* aqueous root extract on high cholesterol diet rats was investigated. Obesity was induced in rats by giving high cholesterol diet (coconut oil, grains, sugar, etc) for seven days in standard rat chow diet. The aqueous extract of *Ipomoea sepiaria* (100,200 mg/kg body weight) was orally administered once a day to rats fed a high cholesterol diet for 30 days. High cholesterol-fed diet rats exhibited a significant increase in total serum cholesterol, triglycerides, low-density lipoproteins, very low-density lipoproteins and a significant decrease in high-density lipoproteins. Treatment with aqueous extract of *Ipomoea sepiaria* significantly decreased total serum cholesterol, triglycerides, low-density lipoproteins, very low-density lipoproteins and increased the high-density lipoproteins in obese rats and was comparable with that of standard Atorvastatin. After completion of experiment rats were anesthetized with Isoflurane and sacrificed and the atherosclerotic lesions in the aorta were assessed. The obtained results of the investigation of all 5 groups were compared and found with significant inhibition of atherosclerotic plaque formation.Although a clear dose-dependent relationship was observed at the applied doses. Hence, it was concluded that significant anti-obese and atherosclerotic activities of *Ipomoea sepiaria* may be due to the presence of carbohydrates, tannins, phenols, flavonoids, proteins, amino acids and saponins found in the preliminary phytochemical screening.

Keywords: *Ipomoea sepiaria,* aqueous root extract, high cholesterol diet fed rats

INTRODUCTION

Obesity is one of the most widespread metabolic disorders in contemporary society. It is associated with the development of Type-II diabetes mellitus, coronary heart disease, cancer, respiratory complications and osteoarthritis[1].It is the most common nutritional disorders in humans, is the major problem not only in Asia but also in all over the world. According to world health organization, the prevalence of obesity is rapidly rising at an alarming rate to epidemic proportion globally [2]. Recently, natural and alternative anti-obesity agents, in the form of beverages or teas, have been used for the treatment of obesity. These could attenuate the clinical adverse effects of chemical anti-obesity agents.[3] Leptin is a hormone secreted by adiposities that provide a negative feedback signal to the brain to decrease energy intake. Deficiency in leptin as well as genetic defects in the leptin receptor are known to cause obesity in mice and have been proposed to play a role in obesity in humans[4].The persistence of hyper choles -teromic state causes enhanced oxidative stress, leading to the development of atherosclereosis, coronary artery disease and other complications of obesity[5]. Obese persons are at increased risk for developing serious medical conditions such as diabetes mellitus, hypertension, and cardiovascular disease, and obese persons are at increased risk of death from cardiovascular disease, diabetes, kidney disease and cancer [6]. Due to clinical limitations and adverse effect, there is critical interest in the development of efficient and safe drugs for the treatment of obesity and atherosclerosis.

Ipomoea sepiaria Koening ex. Roxb., is a source of the classical Ayurvedic medicinal plant Lakshmana. It is a glabrous or occasionally pubescent or hirsute, slender twinning climber with a slightly thickened or tuberous perennial root [7]. The root system consists of a fairly long, somewhat thickened taproot and several slightly thinner or slender branches, arising from its base with very few wiry rootlets. Leaves are a simple alternate, entire, blotched with brownish patches towards the middle [8]. Roots are delicate purple or white with a purple eye, along with short to long peduncles and short pedicels [9]

MATERIALS AND METHODS

Chemicals

Sodium Carboxy methyl cellulose**,** Atorvastatin (Gift sample from Aurobindo Pharma, Hyd) Double distilled water**,** Normal saline and all other reagents used were of analytical grade.

Plant Material

The plant of *Ipomoea sepiaria* for the present investigation was collected from the University College of Pharmaceutical Sciences, Acharya Nagarjuna University, Guntur, A.P. The plant was authenticated by a professor in Dept. of Botany Dr.S.M.Khasim, and a specimen was deposited in the herbarium for future reference.

Preparation of extract

The roots after collection were shade dried and powdered using a mechanical grinder and passed through 40 mesh sieve. Powder (100 g) was defatted using 1.5L of petroleum ether and subjected to extraction in a Soxhlet apparatus using double distilled water for 12h.The fraction was concentrated under reduced pressure and controlled temperature (40-50 ºC). The yielding ratio of aqueous extract was 19.65% w/w. The thick dark brown/dark green paste of aqueous extract of *Ipomoea sepiaria* roots was stored in air tight container at 4ºC till further use. These extracts were used for the evaluation of anti-obese activity.

Preliminary evaluation tests

Phytochemical Screening

The aqueous extract of *Ipomoea sepiaria* plant was subjected to preliminary phytochemial tests and analysed for the presence of

various bioactive chemical constituents such as glycosides, alkaloids, steroids, proteins and triterpenoids, carbohydrates, flavonoids[10]. Results were reported in Table 1.

1. Test for steroids

Salkowski test: Few drops of H_2SO_4 is added to the plant extract, shaken the lower layer turns red in color it indicates the presence of steroids.

2. Test for triterpenoids

Libermann buchards test: To the chloroform solution of extract, few drops of acetic anhydride added from the sides of test tube a reddish brown ring is observed at the junction of two layers indicates the presence of triterpenoids.

3. Test for saponins

Foam test: Small amount of extract is shaken with little quantity of water, and then foam was produced, persists for 10min it confirms the presence of saponins.

4. Test for alkaloids

Wagners test: The acid layer when mixed with few drops of Wagner's reagent (solution of iodide in potassium iodide) gives brown to red precipitate indicates the presence of alkaloids.

5. Test for carbohydrates

Benedicts test: The extract on heating with Benedict's reagent, brown precipitate was observed indicating the presence of sugar.

6. Test for cardiac glycosides

Keller-killiani test for cardiac glycosides: Chloroform extract of plant and glacial acetic acid with ferrous chloride and 0.5ml of Conc. H_2SO_4 the acetic acid layer shows blue color indicating the presence of glycosides**.**

7. Tests for Phenolic compound

Ferric chloride test: Treated the extract with ferric chloride solution and blue color appeared indicating the presence of hydrolysable tannins.

Experimental section

Animals

Male Wistar rats (weighing around100-150g) obtained from Mahaveer enterprises, Hyderabad were used in the study. They were maintained at 22 ± 3 ºC, relative humidity 40-70% with free access to food and water *ad libitum*, under a 12:12 light /dark cycle (light on at 8:00 h). All manipulations were carried out between 9:00 and 15:00 h. with each animal used only once. The experimental protocol was approved by the Institutional Animal Ethics committee of the institute as per the direction of the Committee for the Purpose of Control and Supervision of Experiments on Animals (IAEC/ANUCPS/2016/008). The animals were acclimatized for a period of 7 days before the study. All efforts were made to reduce the number of animals used and treated humanely to minimize their pain and discomfort.

Table 1: Grouping of Animals

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+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 into the groups of 3 animals per group and were administered once orally with a 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.

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 methylcellulose 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.

Blood sample collection and analysis:

The blood has to be collected from the animals initially and terminally to the experiments. Here the animals were anesthetized 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

Statistical analysis

Experimental results were Mean±SEM (standard error mean) of 6 animals. The results were statistically analysed using one-way Analysis of Variance (ANOVA) followed by Tukey's multiple tests to determine the level of significance. Data were considered statistically significant only when the value of $p<0.001$.

RESULTS

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

Group II (disease control group) animals fed with HFD exhibited a significant ($p<0.001$) increase in Total cholesterol (TC), Tryglycerides (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.

Effect of *Ipomoea sepiaria* aqueous **root** extract **on Serum Leptin, Insulin, CRP, CK-MB and Apolipoprotein-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 lipoprotein-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.

Table 2: Effect of Ipomoea *sepiaria* aqueous root 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
1.	TC	93±0.91	126±1.93	117±1.6	107±1.3	106±4.64
2.	TG	76.7±1.7	158±1.7	143±2.01	131.8±1.7	105±2.19
3.	HDL –C	46.2±1.49	27.7±1.79	38±2.19	45±1.32	49.5±1.32
4.	LDL	82.75±1.54	148±3.17	135.8±1.1	116.8±1.65	104.8±1.03

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 and V were compared with group II.

Table 3: Effect of *Ipomoea sepiaria* aqueous root 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
1.	TC	93.5±0.64	157±1.04	113±1.04	102.5±1.04	102.5±3.2
2.	TG	76.7±1.93	170±2.19	139.8±2.05	119.8±2.05	97.5±2.2
3.	HDL –C	51±1.95	33.2±1.37	43.5±1.84	47.5±1.32	51.25±0.85
4.	LDL	82.2±2.39	167±1.1	127±1.1	110.5±1.1	99.5±1.04

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 and V were compared with group II.

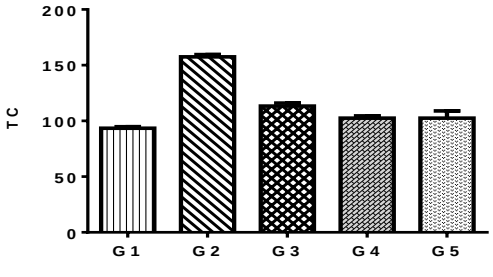

Fig.1: Effect of *Ipomoea sepiaria* aqueous root extract on TC for 30 days

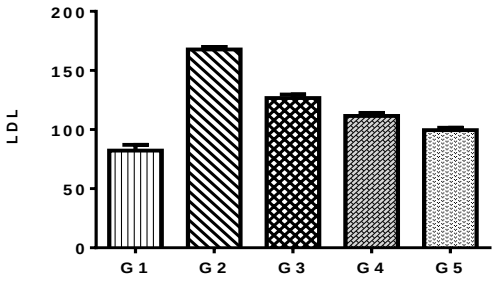

Fig. 3: Effectof *Ipomoea sepiaria* aqueous root extract on LDL for 30 days

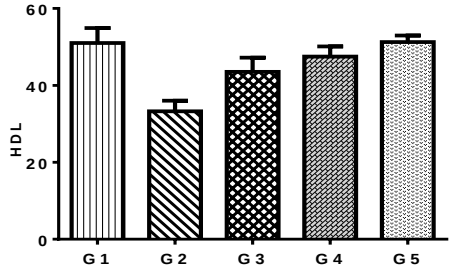

Fig.2: Effect of *Ipomoea sepiaria* aqueous root extract on HDL for 30 days

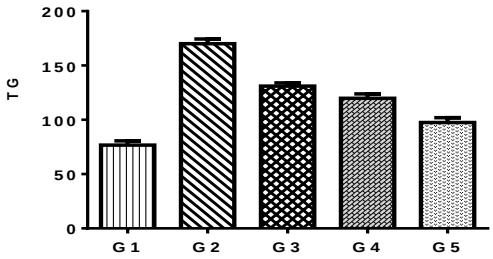

Fig. 4: Effect of *Ipomoea sepiaria* aqueous root extract on TG for 30 days

Table 4: Effect of *Ipomoea sepiaria* aqueous root extract on serum leptin, Insulin,CRP, CK-MB, and apolipoprotein B in wistar rats

S.NO	PARAMETER	GROUP I Normal (Mean±std)	GROUP II HFD	GROUP III HFD + Extract100 mg/kg	GROUPIV HFD+extract 200 mg/kg	GROUP V HFD+Atr 10mg/kg
1.	Serum leptin (ng/ml)	1.85±0.06	2.85±0.10	1.87±0.12	1.45±0.06	1.47±0.08
2.	Serum insulin(Iu/ml)	1.15±0.06	3.37±0.17	2.37±0.12	1.87±0.12	1.45±0.13
3.	Serum CRP	_	_	_	_	_
4.	Serum CK-MB(mg/dl)	3.27±0.16	8.32±0.2	7.15±0.14	5.7±0.2	4.8±0.36
5.	Serum apolipoprotien B(g/dl)	4.42±0.13	21.6±0.10	5.47±0.1	5.7±0.2	4.8±0.36

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

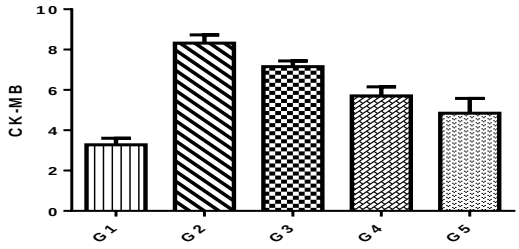

Fig .5: Effect *Ipomoea sepiaria* aqueous root extract on CK-MB for 30 days

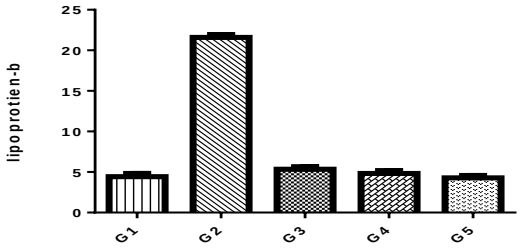

Fig .6: Effect of *Ipomoea sepiaria* aqueous root extract on CK-MB for 30 days on Lipoprotien-b for 30 days

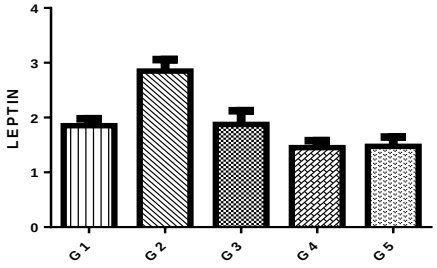

Fig .7: *Effect of Ipomoea sepiaria* aqueous root extract *on leptin*

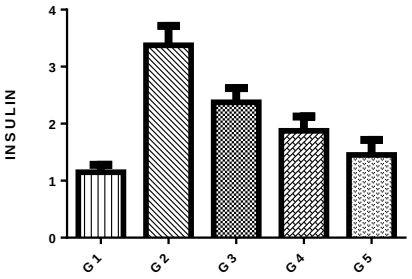

Fig .8: Effect of *Ipomoea sepiaria* aqueous root extract on Insulin

Table 5: Effect of *Ipomoea sepiaria* aqueous extract on body weight HFD rats.

WEEK	Initial	GROUP I	GROUP II	GROUP III	GROUP IV	GROUP V
I	159.6±2.19	161.34±3.01	163.45±2.56	168.72±4.13	173.78±5.13	179.43±2.65
II	160.01±2.12	172.89±2.45	185.8±2.23	222.63±2.33	233.46±3.21	238.18±4.78
III	158.71±2.09	162.82±2.40	168.84±2.57	181.09±3.05	183.24±3.22	186.66±3.77
IV	159.56±2.55	162.93±2.75	168.57±3.38	191.78±3.57	201.81±2.33	216.77±3.68
V	158.91±2.32	161.78±2.77	168.84±2.70	182.95±2.19	189.65±2.84	196.53±1.79

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 and V were compared with group II.

Histopathology of arota

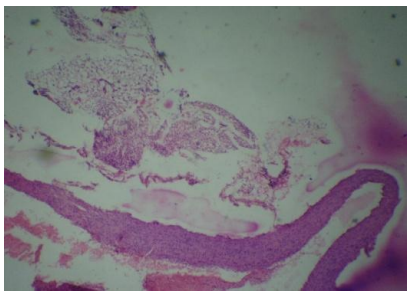

Fig.9: PLATE-I

Arota of Normal rat

Plaque formation and local vascular inflammation was significantly greater in the placebo versus wild-type group

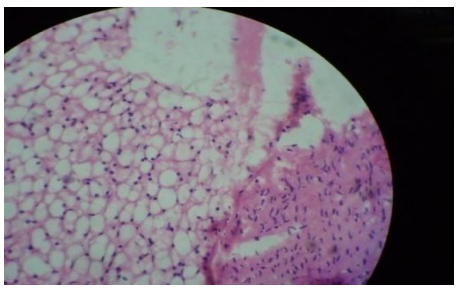

Fig.10: PLATE II

Arota of DC rat

Macrophage uptake into atherosclerotic plaques was significantly greater.A big atheromatous plaque mostly obstructing the lumen.

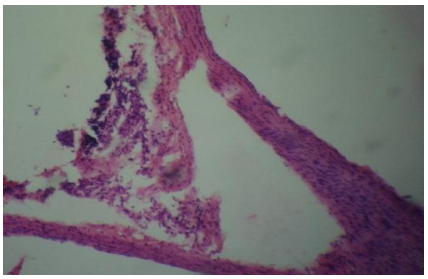

Fig.11: PLATE.III

Arota of STD rat

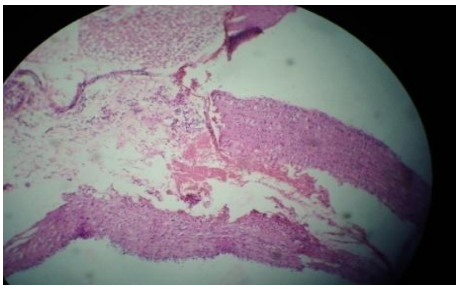

Fig.12: PLATE.IV

Tiny atheromatous plaque with few fat globules are observed. But the fat globules are very less when compared to disease control. This Standard drug was showing a significant effect.

Arota of T1

Big atheromatous plaque mostly obstructing with congestion and hemorrhage at the base. This test dug having the dose of 100 mg/kg was not that much effective when compared to standard and normal groups.

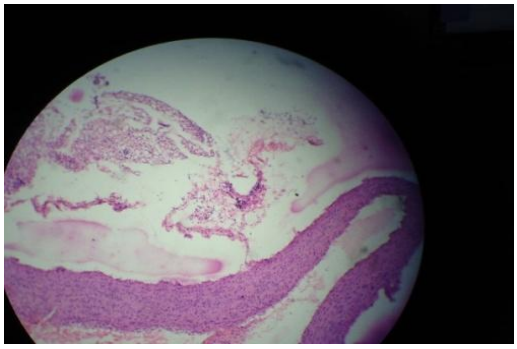

Fig.13: PLATE.V

Arota of T2

A small plaque with congestion, hemorrhage and rupture at the base. This test dose was somewhat effective when compared to lower dose[T1], but not that much as the effect shown by the standard drug(Atorvastatin).

DISCUSSION

Obesity is one of the most important environmental factors associated with the incidence of cardiovascular disorders. Metabolic syndrome, a combination of medical disorders that increase the risk of developing cardiovascular disease and is associated with abdominal obesity, blood lipid disorders, inflammation, and insulin resistance. Animal models are useful tools for obesity research as they readily gain weight when fed high-fat diets. The rats fed with high fat develop obesity, hyperphagia, hyperleptinemia and hypertriglyceridemia[10]. Hyperliporoteinaemias with a significant increase in serum cholesterol and its carrier LDL are known to be associated with an increased risk of heart coronary diseases. In human, a 15% higher LDL-C can increase the risk of coronary heart diseases by about 15-45%. Moreover, the formation of ox datively-modified LDL is proposed as a critical risk factor involved in the soknown “oxidation hypothesis” of atherosclerosis.

Rats switched from a diet low in carbohydrates and high in protein to a high intake of HFD, develop an acute hypertriglyceridemia. The abnormalities and the disease progression in HFD fed rats resemble the human condition of metabolic syndrome. Leptin is important adipocyte-derived hormone that interacts with a putative receptor(s) in the hypothalamus to regulate body weight and involved in the pathophysiology of obesity as well as atherosclerosis. Leptin release is increased upon intake of carbohydrates. Leptin regulates the over consumption of carbohydrate rich foods. Leptin has also been reported to decrease food intake in HFD fed rats. This increased level of leptin caused by CGE reduces the food intake and suppresses the appetite. Thus increased leptin and decreased other parameters might be the connecting link between antiobesity and atherosclerosis like activity of CGE. Free radicals are recognized to have crucial importance in mechanisms of many pathological processes including atherosclerosis via lipid peroxidation of LDL. It has been shown that flavonoids in CGE are large group naturally occurring antioxidants that could inhibit lipid peroxidation of LDL by scavenging free radicals. Based on some clinical studies, after the administration of CGE, the antioxidant enzyme activities reversed to near normal. Mariappan Premanathan et al, (2011). The present study showed that polyphenolics, flavonoids can positively modify lipoprotein profiles and antihypercholesterolemic effects of this extract were dose-dependent. Based on our results, CGE caused a mild increase in HDL-C, a property of this component which is beneficial to treatment of atherosclerosis. Similar to Radjabian observations, in this trial we showed that CGE has strong anti-atherosclerotic activity [11]. Recent data suggest that the inhibition of cholesterol absorption caused by CGE could be a mechanism contributing to the positive change in plasma lipoprotein profile. CGE can act as an anti-inflammatory factor through inhibition of 5-lipoxygenase. Recently, atherosclerosis is more and more being recognized as a chronic inflammatory process, and some experimental findings showed that this chronic disease is due to an imbalance between synthesis of reactive oxygen species and antioxidative defective mechanisms. Consequently, it is proposed that biomolecules such as flavonoids with anti-inflammatory and anti-oxidative properties could have beneficial effects on atherosclerosis. Because of the complex mechanism(s) action of CGE, it may be a natural multifunctional and multi-target medicinal herbal drug. An interesting feature of our experiment was evaluation of anti-hyperlipidemic and antiatherosclerotic properties of CGE as compared to Atorvastatin as a standard drug. Our results confirmed the hyperlipidemic and anti-atherosclerotic effects of CGE. Although, the main active compound is believed to be flavonoids, polyphenols such as other plant extracts contain many compounds and it is not completely clear that which constituents is largely responsible for the medical benefits attributed to CGE. There are Fig.Presentation of atherosclerotic plaques formation in aorta sections of rats in different tested groups; Disease control (II), CGM (III), CGM (1V) that at the dose of 100mg/kg, 200mg/kg respectively and Standard control (V) at a dose of 10 mg/kg.

CONCLUSION

The results described here clearly confirmed the anti-obesity properties of *Ipomoea sepiaria* aqueous root 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 extract 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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