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PHARMACOLOGICAL INVESTIGATION OF ANTI-OBESITY ACTIVITY OF SEEDS OF *CUCUMIS MELO* LINN. IN RATS

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ABSTRACT
Obesity is a condition in which excess body fat is accumulated to an extent that health may be negatively affected. The prevalence and severity of obesity and its associated co-morbidities are rapidly increasing for the management of obesity ailments. The use of herbal medicines became the subject of interest for the management of obesity due to its natural origin, cost effectiveness and minimal side effects. The present study designed to investigate the effect of Ethanolic extract of *Cucumis melo* (EECM) 250 and 500mg/kg b.w on energy balance disease like obesity in wistar albino rats using two models namely High fat diet (HFD) and Monosodium glutamate induced (MSG). Obesity was induced by administration of hypercaloric diet for 49 days in HFD model and 15 days in MSG. From the observations of the study, it could be predicted that methanolic extract of *Cucumis melo* Linn exerted significant antiobesity due to hypophagic. Over the course of study, it was found that *Cucumis melo* Linn significantly reduced body weight, food intake and lipid profile parameters like LDL, TGs, TC, VLDL, and marked increase in HDL level was observed and significantly decrease SGOT and SGPT levels. In MSG model it showed significant decrease in body weight and food intake no significant changes was observed in water intake. Dose dependent antiobesity activity (250mg/kg, 500mg/kg) was observed with EECM seeds due to presence of phytoconstituents. These results suggest that seeds of *Cucumis melo* Linn possess Antiobesity activity.

KEYWORDS: Antiobesity; Cucumis melo Linn; High fat diet; Monosodium glutamate; Orlistat.

INTRODUCTION

The world health organization (WHO) has declared obesity as the world's largest chronic health problem in adults, describing it as "Globesity", which is increasingly becoming a more serious problem than malnutrition.[1] It is defined as a medical condition with an abnormal accumulation of body fat and is associated with excessive growth and expansion of adipose tissue due to an imbalance between energy intake and expenditure.[2] Individual is considered obese when the amount of fat tissue is increased to such an extent that physical and mental health are affected and life expectancy reduced.[3] It is now clear that overweight and obesity result from an interaction of many factors, including genetic, metabolic, behavioural, and environmental influences.[4]

Among these causes excessive of calorie intake is the most common risk factor of obesity.[5] The prevalence of obesity is increasing worldwide; Numerous diseases are caused or made worse by obesity. These include type-2 diabetes, hypertension, dyslipidemia; ischemic heart disease; stroke; obstructive sleep apnea; asthma; nonalcoholic steatohepatitis; gastroesophageal reflux disease; degenerative joint disease of the back, hips, knees and feet; infertility and polycystic ovary syndrome; various malignancies; and depression.[6]

Globally, it is estimated that over 205 million men and 297 million women were obese, which account for a total of more than 600 million adults worldwide.[7] This disease has many factors which contribute to its Etiology including sedentary lifestyle such as white-collar jobs, lack of physical work out, increase in calorie consumption, endocrine disorders, and psychiatric issues among others.[8] Obesity prevalence has reached global epidemic proportions not only in industrialized countries, but has radiated its effect to developing countries.[9] It is reported to constitute the 16th leading cause of global mortality.[10] The treatment of choice for obesity (behaviour weight loss treatment) only results in moderate and transient reduction in body weight.[11] At present, just a few FDA (Food and drug administration)-approved anti-obesity drugs like orlistat, lorcaserin, phentermine- topiramate and naltrexone-bupropion are on hand, but they have remarkable adverse effects. On the contrary, herbal medicines are safer, efficacious and cost effective which make them a drug of choice in today's era for treating various ailments.[12]

Cucumis melo fruit is round in shape, tan to greenish tan with a rough texture and orange pink flesh. It is well known for its sweet taste and fragrance. It is native to Persia, Armenia, etc. Many phytochemicals having potential benefits are present in *C. melo*. It is rich in carbohydrates, Proteins, fibre, citric acid, vitamin K, vitamin A and folate.[13]

Cucumis melo commonly known as Kharbuzah in Hindi and musk melon or cantaloupe in English belongs to Cucurbitaceae family. Musk melon is beautiful, juicy, delicious fruit popular for its nutritive and medicinal value. Musk melon is growing in tropical and subtropical areas of the world C. melo acts as purgative. It is used in dysuria, regulate the kidney functions, reduced blood pressure, dyspepsia, flatulence, leprosy, fever, jaundice, diabetes, obesity, cough, bronchitis, ascites, anaemia, constipation, other abdominal disorders, amentia and menorrhagia. The fruit is used as cooling agent, cleansing agent or moisturizer for the skin. It acts as demulcent and stomachic. The seeds have antitussive, febrifuge and vermifuge properties. Fruit pulp is employed as a lotion for chronic and acute eczema.[14]

MATERIALS AND METHODS

Plant materials

Fruit of *Cucumis melo* Linn were purchased from local market Chitradurga, Karnataka and they were washed, cut and took out the seeds and dried under shade. The fruit material was identified and authenticated by botanist, Chitradurga, Karnataka.

Preparation of plant extracts[15]

The seeds of the fruits were cleaned and shade dried at room temperature and pulverized. Powdered seeds will be macerated in 3.5 L ethanol: water (70:30) for 7 days with frequent shaking. Soaked material will be filtered, the mixtures were decanted and filtered through Whatman No.1 filter paper The yield was about 8%w/w crude extracts was stored in airtight container in desiccator and used for further studies.

The seed extract was dissolved in distilled water and subjected to the following studies.

1. Preliminary phytochemical screening
2. Acute oral toxicity study
3. Antiobesity activity

Preliminary phytochemical screening

Preliminary phytochemical investigations were carried out on the ethanolic extract of *cucumis melo* seeds for detection of various phytochemicals by using standard methods prescribed in practical pharmacognosy by C K Kokate and R K Khandelwal.

Experimental animals

Healthy adult Wistar rats of either sex weighing about 150 to 200g and Female albino mice weighing 20 -25g were used throughout the experiment. The animals were procured from Biogen Laboratory animal facility Bangalore - 562107. Before initiation of experiment, the animals were acclimatized for 10 days. Standard environmental conditions such as temperature (26±2^{0}C), relative humidity (45-55%) and 12hrs light/dark cycle were maintained. All the animals were allowed free access to standard laboratory pellets and drinking water *ad libitum.* Ethical clearance for performing the experiments on animals was obtained from Institutional Animals Ethics Committee. (IAEC) (**Ref.No.02SJMCP/IAEC/2020-21**).

Determination of acute toxicity (LD_{50})

The acute toxicity for extract of seeds of *cucumis melo* was determined in albino mice. The animals were fasted overnight prior to the experiment, fixed dose method was adopted as per OECD Guidelines No.423. 1/10th, 1/5th LD_{50} cut off values of the EECM extract were selected as screening doses for antiobesity activity. Group of 3 mice were taken for each test dose.

EVALUATION OF ANTI-OBESITY ACTIVITY

a. High fat diet induced obesity[16]

Preparation of the diet[17]

High fat diet is a hyper caloric diet was prepared by mixing Indian Vanaspati ghee and coconut oil in the ratio of 3:1 (v/v). The feed was prepared and administered orally (3ml/kg) every morning to animals along with normal pellet chow and water *ad libitum* for 49 days.

Experimental Design

The obtained wistar rats weighing 150-250g were randomly divided into 5 groups each containing 6 animals. Group 1 was fed with normal diet and remaining groups fed with high fat diet along with standard and EECM extract for 49 days. The schedule of dose and diet administration in experimental groups was followed as:

The various groups used in experiment

Group I: The animals received normal chow pellet and did not receive any treatment served as negative control group.

Group II: The animals received high fat diet for 49days and served as positive control group.

Group III: The animals received high fat diet and treated with standard drug Orlistat(30mg/kg) from 16th day and continued for 49th day.

Group IV: The animals received HFD with EECM (250mg/kg) from 16th day and continued up to 49th day.

Group V: The animals received HFD with EECM (500mg/kg) from 16th day and continued up to 49th day.

In vivo Pharmacological evaluation

The animals were observed for Body weight, Food intake, Biochemical parameters.

Body weight: The body weight (gm) was recorded on day one and then on alternate days for 49 days using digital weighing balance.

Food intake: The daily food intake for group of 6 rats was measured daily for 49 days and expressed as mean daily food intake for group of 6 rats.

Biochemical parameters
On day 50th animal blood samples were collected by retro orbital puncture the serum was separated at 3000rpm for 15min and was used for the estimation of Total cholesterol, Triglycerides, HDL, VDL, VLDL, and liver function test SGOT, SGPT.

a. MONOSODIUM GLUTAMATE INDUCED OBESITY[18]

Experimental design
A stock solution was prepared by dissolving 250g of MSG powder in 250ml saline. Orlistat (30mg/kg) is used as standard and EECM extract is used for treatment. Dosages were calculated as per the body weight of animals (5g/kg). The obtained wistar rats were randomly divided into 5 groups each containing 6 animals. Group 1 was fed with normal diet and remaining groups fed with monosodium glutamate along with standard and EECM extract for treatment groups for 15 days. The schedule of dose and diet administration in experimental groups was followed as:

The various groups used in experiment
Group I: The animals received normal chow pellet and did not receive any treatment served as negative control group.
Group II: The animals received MSG for 15days and served as positive control group.
Group III: The animals received MSG and treated with standard drug Orlistat(30mg/kg).
Group IV: The animals received MSG with EECM (250mg/kg) daily for 15 days.
Group V: The animals received MSG with EECM (500mg/kg) daily for 15days.

In vivo Pharmacological evaluation
The animals were observed for Body weight, Food intake, and water intake.

Body weight: The body weight (gm) was recorded on day one and for 15 days daily using digital weighing balance.

Food intake: The daily food intake was measured daily for 15days and expressed as mean daily food intake for group of 6 rats.

Water intake: The daily water intake was measured daily for 15days and expressed as mean daily food intake for group of 6 rats.

STATISTICAL ANALYSIS
The data obtained from the above findings was subjected to statistical analysis using one-way ANOVA followed by Tukey's Kramer Multiple Comparison Test to assess the statistical significance of the result.

RESULTS AND DISCUSSION
Percentage yield of ethanolic extract: 8%.

Preliminary phytochemical screening

Constituents	Ethanolic extract of *Cucumis melo* L
Carbohydrates	+
Triterpenoids	-
Flavonoids	+
Tannins	+
Glycosides	+
Proteins	+
Resins	-
Steroids	-

(+) Present, (-) Absent

Acute toxicity study LD_{50}
The ethanolic extracts was studied for acute toxicity at the dose of 2000mg/kg oral in albino mice. The extracts were found devoid of mortality of the animals. Hence 2500mg/kg was considered as LD_{50} cut off value for the extract.

The screening dose chosen for the evaluation of antiobesity activity as per OECD guidelines No.423, Fixed doses are stated as below.
i. 250mg/kg EEMC (1/10th of 2500mg/kg).
ii. 500mg/kg EEMC (1/5th of 2500mg/kg).

Model 1: High fat diet
Effect of feed intake: Group II animals fed with HFD rats showed significant ($p<0.01$) increase in daily food intake when compared with group I animals. Treatment with EECM (250mg/kg and 500mg/kg) and orlistat showed significant ($p<0.01$) decrease in daily food intake as compared with group II animals.

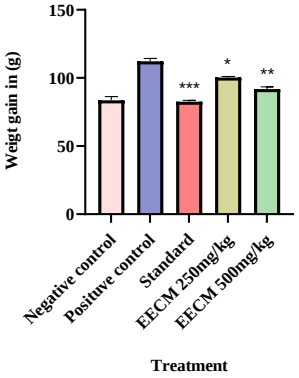
Effect on body weight: Group II animals fed on high fat diet (HFD) exhibited significant ($p<0.01$) increase in body weight between day 1 and day 49 as compared to group 1 animals. Treatment with EECM ($p<0.01$, $p<0.05$) and orlistat shows decrease in body weight as compared with group II animals. The EECM extract at two dose levels resulted in dose dependent decrease body weight.

Lipid Profile
The oral administration of EECM for a period of 33 days showed the significant changes in the Biochemical parameters. Group II (positive control) animals fed with HFD exhibited a significant increase in TC, TG, LDL, and VLDL when compared to Group I (Negative control) animals. Administration of EECM (250mg/kg and 500 mg/kg) and Orlistat (30 mg/kg) shows a significant reduction in TC, TG, LDL, and VLDL, when compared with the Group II animals. Whereas decreased HDL levels observed in Group II animals and significantly increased in Group III, IV, V. The liver marker enzyme profile SGOT and SGPT were found to be significantly increased in the Group II (positive control) over the

Group I (Negative control). Administration of EECM (250mg/kg and 500 mg/kg) and Orlistat (30 mg/kg) shows a significant reduction in SGOT and SGPT when compared with the Group II animals.

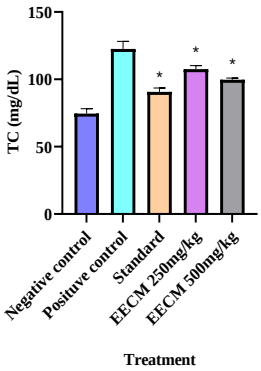
Effect of EECM on different parameters

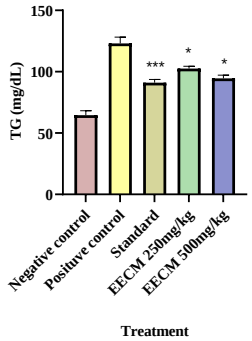
Groups	Treatment	Body weight gain during treatment	Food intake
I	Negative control	57.00 ± 2.53	79.12 ± 0.77
II	Positive control	73.67 ± 1.78	80.22 ± 0.74
III	Standard	43.83 ± 1.64 ***	63.46 ± 2.71 **
IV	EECM 250mg/kg	64.00 ± 1.46 *	73.00 ± 1.02 *
V	EECM 500mg/kg	54.33 ± 1.60 **	72.38 ± 0.76 *

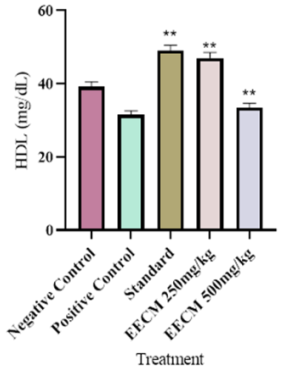


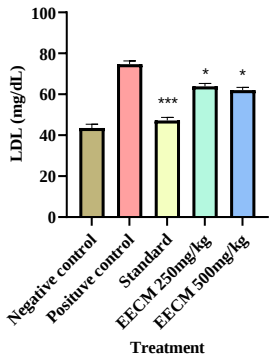
Effect of EECM on Lipid profiles

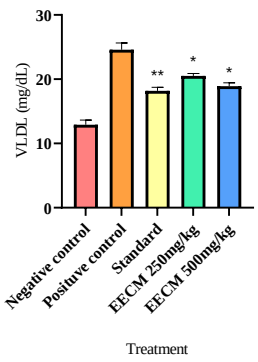
Groups	Treatment	TC	TGs	HDL	LDL	VLDL
I	Negative control	74.50±3.73	64.50±3.73	39.17±1.37	43.50±1.89	12.90±0.74
II	Positive control	122.5±5.72	123.0±5.24	31.50±1.11	74.67±1.64	24.60±1.04
III	Standard	90.67±2.88*	91.00±2.47***	33.50±1.11**	47.17±1.53***	18.20±0.54**
IV	EECM 250mg/kg	107.5±2.71*	102.5±1.94*	49.00±1.52**	63.83±1.44*	20.50±0.38*
V	EECM 500mg/kg	99.67±1.30*	94.50±2.68*	47.00±1.52**	62.00±1.36*	18.90±0.53*





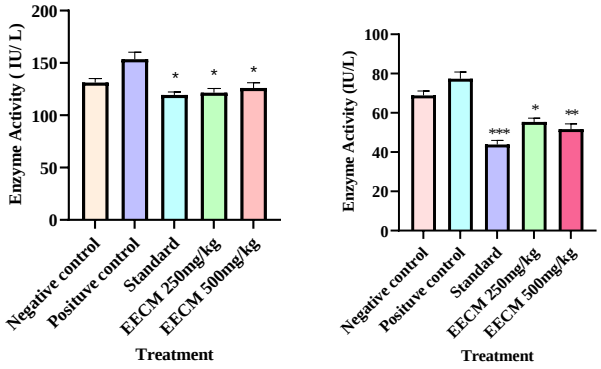






Effect of EECM on liver function test

Groups	Treatment	SGOT	SGPT
I	Negative control	131.2 ± 3.91	68.83 ± 2.33
II	Positive control	153.5 ± 6.70	77.33 ± 3.44
III	Standard	119.3 ± 2.92 *	43.83 ± 2.15 ***
IV	EECM 250mg/kg	121.5 ± 4.04 *	55.17 ± 2.65*
V	EECM 500mg/kg	126.0 ± 5.12 *	47.17 ± 2.83 **

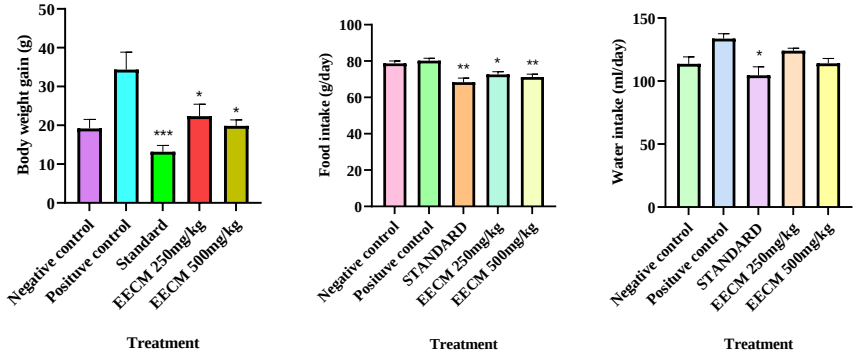


Model 2: Monosodium glutamate induced

Animals fed with MSG showed significant gain (P<0.001) in body weight (g) when compared to those in negative control group. Animals fed with MSG + Orlistat exhibited significant decrease (P <0.0001) in weight gain. Animals fed with MSG + EECM (250mg/kg and 500mg/kg) exhibited dose dependent reduction in body weight gain. Food intake and water intake did not show any significant changes on the day zero in all the groups. MSG treated rats (Group II) showed a significant increase in the food intake, and water intake when compared to Group I, Group III, Group IV and Group V. Administration of orlistat (30 mg/kg) along with MSG (Group III) significantly decreased the food intake, and water intake when compared to Group II. Administration of EECM (250mg/kg and 500 mg/kg) along with MSG significantly showed the dose dependent reduction of food intake, and no significant changes was observed in water intake when compared to Group II.

Effect of EECM on different parameters

Groups	Treatment	Body weight gain during treatment	Food intake	Water intake
I	Negative control	19.17 ± 2.35	78.67 ± 1.39	113.7 ± 5.48
II	Positive control	34.33 ± 4.50	80.23 ± 1.29	133.7 ± 3.94
III	Standard	13.17 ± 1.64 ***	68.33 ± 2.30 **	104.5 ± 6.83 *
IV	EECM 250mg/kg	22.33 ± 3.08 *	72.61 ± 1.51*	124.0 ± 2.02
V	EECM 500mg/kg	19.83 ± 1.57 *	71.24 ± 1.54**	114.0 ± 3.87



Despite decades of treating obesity with traditional methods of dieting and exercise, progress has not been made. It is becoming clear that obesity is less an ongoing personal choice than a fact of biology once a certain level of adiposity dysfunction is established, the person with obesity rarely escapes its consequences.[19]

Medicinal plants play a vital role in health care system of human being and animals. They have medicinal values

due to substances present in various plant tissues. These substances used as therapeutic agent or an active ingredient for medical preparation & they are rich sources of bioactive compounds & thus serves as important raw material for drug production.[20]

The present research study was aimed at evaluating the antiobesity activity of ethanolic extract of *Cucumis melo* Linn in wistar albino rats using high fat diet induced models of obesity and Monosodium glutamate induced obesity that bear close resemblance to the human obesity.

Phytochemical screening of EECM was performed it shows the presence of bioactive substances like alkaloids, flavonoids, tannins, Glycosides, Proteins, amino acids.

In HFD model, Obesity was induced in wistar albino rats by administrating High fat diet (HFD) to Group II, III IV, and V at a dose of 3ml/kg by daily oral administration, for 49 days along with the treatment from 16th day. The study showed that EECM at dose levels significantly exerted effects on body weight and lipid profile parameters, and liver function test. This shows that EECM has a definite influence on body fat metabolism.

From the observations of the study performed, it could be predicted that ethanolic extract of seeds of Cucumis melo exerted significant dose dependent anti-obese activity due to its presence of flavonoids, and tannins.

Flavonoids inhibit weight gain by reducing food intake and increasing the feeling of satiety. Both adipocytes express UCP-1 protein, which is mainly responsible for thermogenesis and energy consumption. Thermogenesis is mainly regulated by various mechanisms and improves the activity of the sympathetic nervous system, which results in the secretion of norepinephrine, which ultimately results in energy consumption and reduction of fat accumulation.[21]

Tannins reduces the body weight through the reduction of feed intake. However, it is still not clear whether this happens through the reduction in palatability or digestibility.[22]

In MSG model obesity was induced in wistar albino rats by administrating Monosodium Glutamate (MSG) to Group II, III, IV, and V at a dose of 5 g/kg daily for 15 days. The present pharmacological investigation revealed that MSG elicited significant increase in body weight and food intake.

Treatment with EECM (250 mg/kg & 500 mg/kg) resulted dose dependent reduction of body weight and food intake in MSG fed rats indicating that the extracts possess weight reducing property. MSG might also increase the palatability of food by disrupting the hypothalamic signalling cascade of leptin action. In our study, MSG might have inhibited the leptin signalling leading to overfeeding. Whereas, EECM might mediate the stimulatory effect on leptin signalling causing the antiobesity effect. Further, no significant changes in water intake was observed in the Group II vs Group III, IV, V.

CONCLUSION

The ethanolic extract of *cucumis melo* was found to be effective in countering High fat diet and Monosodium glutamate induced obesity. The presence of flavonoids and tannins in seed extract of cucumis melo Linn maybe responsible for Antiobesity activity**.**

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