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Research Article

Evaluation of Anti-Obesity Activity of *Lantana camara* Var Linn. by Progesterone Induced Obesity on Albino Mice

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ABSTRACT
The anti-obesic activity of Lantana camara was studied on progesterone induced models of hyperlipedemia in mice. Hyperlipidemia condition established by progesterone, which changed various parameters in the body . An increase in food consumption and water consumption usually accompanies the body weight gain, which is the characteristic nature of progesterone stimulation. Increased consumption of food and water generally leads to elevated parameters like LDL,VLDL, serum cholesterol etc. Accumulation of fat in areas like inguinal, epididymal , neck etc. was observed . Ethanobotanical knowledge of medicinal plants is one of the most prominent source of new drugs and has shown potential results for treatment of obesity. Preliminary phytochemical analysis of *Lantana camara* revealed the presence of phyto constituents such as steroids , flavinoids , alkaloids, etc.

Keywords: Adipocyte, progesterone, Obesity

INTRODUCTION

Obesity is a chronic metabolic disorder caused by an imbalance between energy intake and expenditure. Over weight and obesity are defined as abnormal or excessive fat accumulation that presents a risk to health. Obesity is one of the greatest health threats of this century. Chronic obesity is a problem of epidemic proportions, and is rapidly increasing in prevalence in both the West and the Asia-Pacific region[1 ¯²] which has an important impact on life style-related diseases such as coronary heart disease, dyslipidemia, glucose intolerance, diabetics, hypertension and some cancers[3] . Several factors, including lack of exercise, sedentary lifestyles and the consumption of energy rich diets are contributory to the etiology of obesity[4]. Despite the urgent need for safe and efficient therapeutics and the potential size of the market for anti-obesity drugs, the current status for the development of such drugs are still unsatisfactory [5]. Some edible medicinal plants have been used as dietary supplements for body-weight management and control in many countries[6-7] .

In the literature survey, it was found that flavonoids, sterols, tannins, and alkaloids have shown promising effects to tackle obesity by various mechanisms, *Lantana camara* whole plant has shown the presence of sterols, triterpenoids, flavonoids alkaloids and saponins, and others in the extracts. Moreover, traditional Indian medicine also claims for its antiobesity activity. With this back ground, this plant has been selected for its phytochemical analysis and screening of its antiobesity activity against progesterone-induced obesity in female mice. The neuroactive steroid, progesterone is a female reproductive hormone. Its level increases during the later phase of the menstrual cycle and controls the secretory phase of the endometrium. Substantial evidence links progesterone excess in pathophysiology of eating and affective disorders. Some reports suggest the use of progesterone-containing preparations as contraceptive or for the hormone replacement therapy to cause sufficient weight gain by causing hyperphagia and increased fat deposition in the body[8]. Reports also suggest that progesterone can produce these effects by inducing myriad of neurotransmitter changes of which alterations of serotonin level can have important. With this setting neuroactive to induce obesity in female mice has been chosen.

MATERIALS AND METHODS

Collection of plant material: Lantana camara whole plants materials were collected from Tirupathi . The plant authentication was done by Department of Botany,Sri Venkateshwara University , Tirupathi dist. Chittoor , Andhra Pradesh, and the voucher was preserved.

Preparation of the extract: Leaves of Lantana camara were shade dried at room temperature for 2-3 days. These dried leaves were then powdered in a mixture. The extraction process was done in a Soxhlet extractor. The fine powder (100 grams) was suspended in (200 ml) of methanol for 24 hours at room temperature. After extraction , the solvent was evaporated by rotary evaporator and the residue was dried.[9]

Experimental animals: Female albino mice (20–25 g) were used in this study. Mice bred at GSN Pharmaceuticals PVT.LTD animal house were used in this experiment. Animals were housed in a standard controlled animal care facility in cages (5 mice/cage).

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The following table consists of the data derived from the results of the histopathology of the various slides consisting of the organs(liver) sections of various groups. The table consists of the inducing agent, size of the organs, changes observed, and conclusions drawn.

S.NO	GROUP	SIZE	COLOR	INFERENCE	CONCLUSION
1.	INDUCING AGENT	Slight increase	normal	Mild fatty change	The liver is prone to fatty diet,as there is increase in the fat cells.
2.	INDUCING AGENT + TREATED DRUG EXTRACT	normal	normal	Positive necrosis,focal necrosis of hepatocytes,swelling found	Extract found to show effect on liver.fatty change is abscent ,drug extract found to show its action on the fatty liver.
3.	INDUCING AGENT + TREATED DRUG EXTRACT(DOUBLE DOSE)	normal	normal	Focal necrosis,swelling of hepatocytes with change in the cells and degenation in some places,	Extract found to show its action on liver,fatty change is abscent,drug extract found to show its action on the fatty liver
4.	INDUCING AGENT + STANDARD DRUG	normal	normal	Mild necrosis found. Less extent when compared to 2nd and 3rd conditions.	Action of the drug is mild when compared to 2nd and 3rd conditions,drug having mild properties

The animals were maintained in a temperature-controlled room (22°C–25°C, 45% humidity) on a 12:12 h dark:light cycle. The animals were maintained under standard nutritional and environmental conditions throughout the experiment. All the experiments were carried out between 9:00–16:00 hours at ambient temperature. Nations CPCSEA guidelines were strictly followed and all the studies were approved by the Institutional Animal Ethics Committee (IAEC).

Preliminary Phytochemical analysis: The methanol extract of Lantana camara was subjected to preliminary phytochemical analysis to assess the presence of various phytoconstituents; it revealed the presence of flavonoids, alkaloids and glycosides. All these tests were performed at GSN Pharmaceuticals PVT.LTD.

Induction of progesterone-induced obesity: Progesterone vial contents were dissolved in arachis oil and a dose of 10 mg/kg was administered subcutaneously in the dorsal neck region to mice for 28 days, control group received the vehicle. All drugs were given at a dose of 0.4 mL/100 g body weight. The test drugs were injected 30 min before progesterone administration.[10]

Test drug preparation: the extract and standard sibutramine are soluble in water, so distilled water was used as media to dissolve. For progesterone, arachis oil was used as a vehicle and diluent for appropriate doses. All the drug concentrations were prepared freshly just before administration. All the test drugs, including the standard were given by oral gavages by p.o. route.

EVALUATION

Body weight: The body weights of mice (g) were recorded every week for 28 days in each group just before dosing by using precision balance of 10 mg sensitivity.

Biochemical parameters: Preparation of serum: On day 29 of the study, that is, after the last test drug administration, the mice were anesthetized under light ether anesthesia and blood for serum preparation was collected by retro orbital puncture, using 10 μL×20 mm (L) × 0.8 mm (2R) glass capillary into sterile EDTA-coated tube (3 mg/mL) for the estimation. Blood was kept in wet ice for 30 min, centrifuged for 5 min at 4000 rpm at 4°C (REMIMAK, Remi Instruments Ltd, Mumbai, India) and plasma was aspirated out for the analysis of lipid profile. The serum was stored in the refrigerator for the analysis of biochemical parameters. All analyses on serum were completed within 24 h of sample collection. Serum samples were analysed at AZ Laboratory(Hyderabad).

STATISTICAL ANALYSIS

The results are expressed as mean±SEM. Comparisons between the treatment groups and positive control; positive control and control were performed by one-way analysis of variance (ANOVA) followed by Dunnett's test. In all the tests the criterion for statistical significance was $P<0.05$ (95% level). P value<0.05 is considered as significant ($*P<0.05$, $**P<0.01$).

Hystopathology of Liver : The animals were sacrificed on 30th day and the animals were dissected and the liver was isolated ,and kept in containers filled with formalin(10% formalin). The organs were then studied to find out the various changes that have occurred. The hystopathology was carried out at AZ Labs.

RESULTS

Hystopathology of Liver: MECP – Methanolic extract of Lantana camara; TC – Total cholesterol; TG –

Triglycerides; HDL-C – High density lipoprotein cholesterol; LDL-C – Low density lipoprotein cholesterol; VLDL – Very low density lipoprotein cholesterol; SGOT – Serum glutamate oxaloacetate transaminase; SGPT – Serum glutamate pyruvate transaminase; Progesterone induced group have shown significant increase in TG, LDL-C, VLD-C, SGOT and SGPT level and reduction in HDL-C level compared to normal control mice. Treatment with MECP 200 and 400 mg/kg have shown significant increase in HDL-C and reduction in other biochemical parameters. Sibutramine have shown most significant results by increasing in HDL-C level and reduction in TG, LDL-C, VLDL-C, SGOT and SGPT level compared with progesterone induced and any Lantana camara treated group. *Comparison of test and disease control with normal control; #Comparison test with disease control

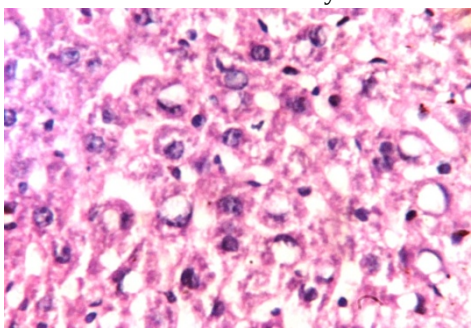
DISCUSSION

Obesity is a medical condition in which excess body fat has accumulated to the extent that it may have an adverse effect on health, leading to reduced life expectancy and/or increased health problems[11]. Body mass index (BMI), a measurement which compares weight and height, defines people as over weight (pre-obese) if their BMI is between 25 and 30 kg/m2, and obese when it is greater than 30 kg/m2.

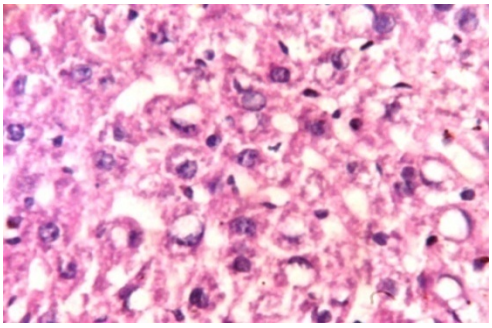
Obesity increases the likelihood of various diseases ,

The following are the microscopic images of the histopathology of various groups of liver samples:

Progesterone induced: The presence of the fatty change in the liver cells shows that the progesterone has successfully responsible for the induction of obesity and the occurance of fat globules

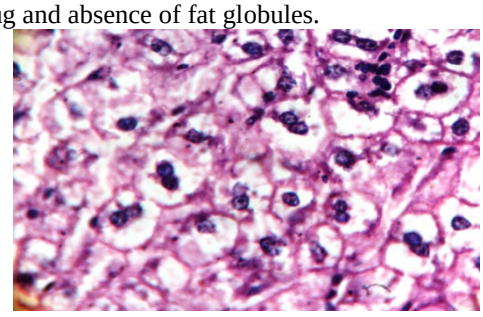


Fatty change in hepatocytes

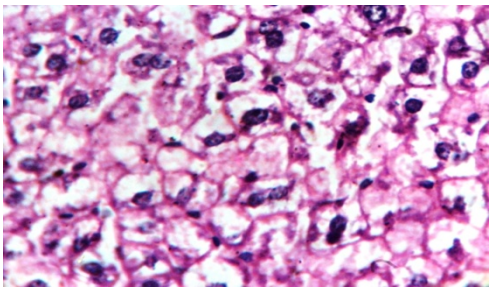


Fatty change in hepatocytes

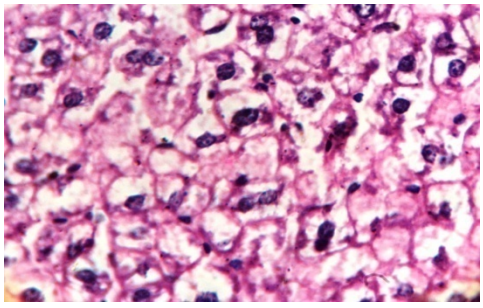
MELc 200mg/kg+pro: The methanolic extract of 200mg of Lantana camara brought changes like focal necrosis, and slight hepato toxicity, which proves that there is action opf the drug and absence of fat globules.



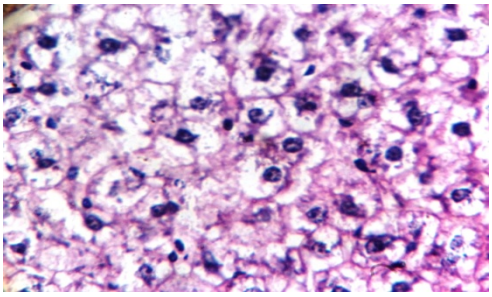
Swelling of hepatocytes



focal Necrosis +

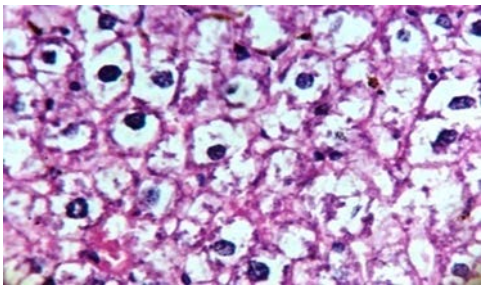


Swelling with focal necrosis

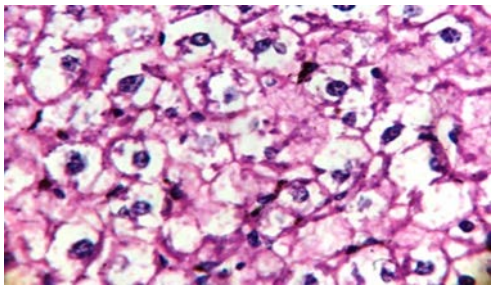


Focal necrosis

MELc 400mg+pro: In this extract, there is significant and increased focal necrosis and total absence of fat globules and this extract shows perfect decrease in the fat cells which were induced by progesterone.

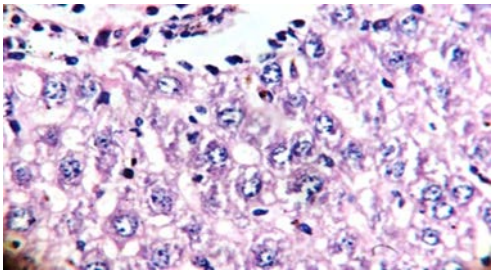


Swelling of hepatocytes

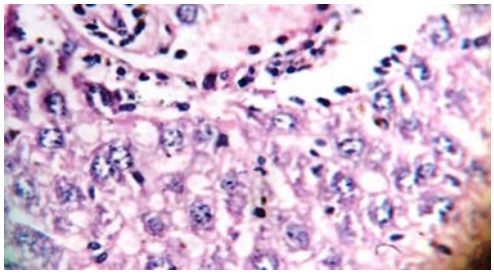


Swelling of hepatocytes

Sibutramine 10mg/kg+pro: In this case there was mild necrosis found.



Mild necrosis–central vein



Few damaged cells

Treatment	Glucose (mg/dl)	TC (mg/dl)	TG (mg/dl	HDL-C (mg/dl)	LDL-C (mg/dl)	VLDL-C (mg/dl)	SGOT (IU/L)	SGPT (IU/L)
Control (Vegetable oil)	135.67±14.01	100.16±16.65	79.905±7.29	26.06±0.555	57.29±16.61	15.81±1.457	137.23±6.51	68.88±6.99
Progesterone	188.43±10.19	137.17±11.37	147.15±13.23	19.28±0.713**	84.45±13.01	29.43±2.646**	153.68±11.77	97.25±9.31
MELc (200 mg/kg) + Pro	144.35±9.65	143.11±14.62	113.43±9.28	21.31±1.024**	103.18±14.63	22.686±1.85	144.2±14.04	141.58±14.54
MELc (400 mg/kg) + Pro	118.3±5.69	126.01±7.67	94.8±6.11*	26.89±1.045##	80.165±8.00	18.96±1.22	109.1±21.06	107.03±18.35
Sibutramine 10mg/kg+pro	136.33±6.66*	66.4±4.74**	57.37±5.95**	30.97±0.841**##	30.97±0.841#	11.47±1.18##	114.7±12.16	80.97±7.56

Values are expressed as mean ± SEM. Levels of significance- Group II compared with Group I,III and IV. **p_0.01 and ***p_0.001.

particularly heart disease , type2 diabetis, obstructive sleep apnea, certain types of cancer, and osteoarthritis. Obesity is most commonly caused by a combination of excessive food energy intake, lack of physical activity, and genetic susceptibility, although a few cases are caused primarily by genes, endocrine disorders, medications or psychiatric illness. Evidence to support the view that some obese people eat little yet gain weight due to a slow metabolism is limited; on average obese people have a greater energy expenditure than their thin counterparts due to the energy required to maintain an increased body mass.[12]

In medical sciences many attempts have been made to correct this disorder, producing a number of agents including fibrates , sibutramine, and drugs like orlistat but unfortunately they are teamed with lots of adverse effects[13-14]. Due to various reasons like cost factor, adverse effects etc.. the essentiality of devolopment of herbal formulations are been enhanced. Due to this effort, herbal formulations may be regarded as an excellent

alternative strategy for devoloping future effective , and safe anti-obesity drugs. A variety of natural products including crude extracts and isolated compounds from plants , can add up the effort to body weight reduction and prevent diet induced obesity . Therefore they have been widely used in treating obesity.[15-16]

Several plant extracts in traditional medicine are been used to treat obesity with deleted adverse effects. Plants like Camellia sinensis(L), Citrus aurantium L. , Salix matsudana Koidzumi, Nelumbo nucifera, Hibiscus sabdariffa L. are been used traditionally.[17-18]

Lantana camara Linn.(Verbenaceae) is a plant which is commonly known as wild sage- notorious weed. It is an annual plant which grows upto 1.2-2.4 m high and has various uses in folklore medicine in many parts of the world[19-20]. The leaves are reported to be useful in the treatment of urinary diseases, tetanus, malaria, epilepsy. They are also used as carminatives and antipasmodics. The plant has also been found to have hypoglycemic activity. This plant was also named as wonder drug in various places.

In our study, initial phytochemical analysis was carried out and it was evident that Lantana camara contains alkaloids, flavanoids, tanins, and glycosides. Based on this phytochemical screening and ethanobotanical claims this plant was selected to carry out this study. And also it has been reported that chemical constituents like flavanoids , alkaloids were reported for anti-obesity effect in many plants, and thus in the present study such attestations lead to the initiation of this anti-obesity activity.

In the present study, we have studied the effects of methanolic extract of Lantana camara var Linn against progesterone for 28 days. Various parameters are been recorded like blood lipid levels, histopathology of liver etc.

The neuro active steroid progesterone is a female reproductive hormone. Its level increases during the second part of the menstrual cycle and control the secretory phase of endometrium. As the name suggests, (Pro = for, gest= gestation), the higher endogenous levels of progesterone and its metabolites in pregnant women are reported to enhance food ingestion throughout pregnancy and conserve energy for the growing fetus. Progesterone also exerts anti-esterogenic effects, which also been shown to increase in food intake. Further, some reports suggest that use of progesterone containing preparation as contraceptive or for hormonal replacement therapy to cause significant weight gain by increasing fat deposition. Furthermore, progesterone has been reported as the most fattening of steroids hormone that promotes synthesis and storage of fats. Therefore, progesterone-induced hyperphagia causes weight gain and fat deposition is useful as animal model of drug-induced obesity. Our results demonstrated that progesterone (10mg/kg) induced hyperphagia the results are consistent with the reported dose dependent increase in food intake with progesterone and maximum effect at 10mg/kg dose.[21]

It is very much believed that progesterone producing hyperphagia via progestin receptors, which have been reported to be expressed on the serotonergic neurons[22] and sibutramine suppresses the progesterone-induced hyperphagia by inhibiting reuptake of 5-HT (serotonin) at the hypothalamic site which regulate the food intake, which suggests the possible interaction exists between the neurosteroid and serotonin receptor system in regulating food intake and body weight. Further, these data implicate that disturbances in the ovarian hormone levels may predispose females to eating disorders by causing alterations in the serotonin level or serotonergic receptor function[23]. The reduction in the food intake by the administration of MELc at medium and high dose is may be due to its saponin and flavonid content; these phytoconstituents are present in abundant quantity which is confirmed by total saponin and total flavonoid contents of the extrac. Crude saponin and flavonoid has been reported for its the appetite suppressant property[24]. From this study we are predicting that saponin and flavonoids after absorption from GIT it cross the blood brain barrier (BBB) and enter in the brain and amplify signaling in the basal hypothamus energy sensing function, which is the master regulator of food intake and energy expenditure or it may also possible that saponin inhibits the re-uptake of 5-HT in the hypothalamus. Some flavonoides also causes to activate β-adrenergic receptors which are involved in the burning of fats[25].

Progesterone is also reported to exert various metabolic effects such as rising basal insulin levels, stimulating lipoprotein lipase activity and enhancing fat storage in the body. In this study progesterone modulated various bio-chemical parameters in female mice. It caused significant increase in the serum glucose, Triglycerides (TG) and very low density lipoprotein cholesterol (VLDL-C) levels and decrease in HDL-C levels as compared to the normal control animals which were significantly reversed by co-administration of MELc 100, 200 and 400mg/kg as well as standard sibutramine.

CONCLUSION

In culmination with these results, the study was initiated due to the presence of phytoconstituents such as flavanoids, glycosides and alkaloids in the methanolic extract, it could be responsible for the possible significant anti-obesic activity. This activity initially was assessed with the ethano-pharmacological survey, but finally confirmed with the above animal model. The present study proves that the methanolic extract of Lantana camara exhibited a significant Anti-obesic activity. Oral administration of extracts reduced the level of circulating lipids as well as the size of adiposite diameter, resulting in the decrease of body weights in female albino mice, which bearing close resemblance to human obesity. Extracts has also shown significant changes in the liver which has been evident in the hystopathology of the liver,and there were noticeable results like focal necrosis,swelling of cells etc. There was an assumtion that there may be inhibition of pancreatic lipase activity. From this we also proposed that use of MELc along the

progesterone might be useful as a supplement to attenuate hyperphagic effect of progesterone. And hence, we finally conclude that Lantana camara is found to have significant anti-obesity activity.

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