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EVALUATION OF ANTIOBESITY EFFECT OF *GYMNEMA SYLVESTRE* AND *ACORUS CALAMUS* ON DIET INDUCED MODEL OF OBESITY IN RATS

Dheeraj Kumar Singh[*1], Anjula Sachan [2], Narendra Kumar [1], Preet Lakhani [1], Sachin Tutu [1], Pratap Shankar [1], R. Nath [1], Amod Kumar [1] and R. K. Dixit [1]

Department of Pharmacology [1], King George's Medical University, Lucknow - 226003, Uttar Pradesh, India.
Department of Pharmacology [2], Hind Institute of Medical Sciences, Mau Ataria, Sitapur Road, Lucknow - 261303, Uttar Pradesh, India.

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Correspondence to Author:
Dr. Dheeraj Kumar Singh

Department of Pharmacology, King George's Medical University, Lucknow - 226003, Uttar Pradesh, India.

E-mail: drdheerajsingh19@gmail.com

ABSTRACT: Introduction: Obesity is a morbid condition characterized by abnormal or excess deposition of body fat to an extent that it adversely affects health. The basic etiology behind obesity is the disparity between the energy intake and energy expenditure. Lifestyle modifications and drug treatment is the mainstay of treatment. Unfortunately the benefits are for short term that might be because of cessation of drugs use or side effects which ultimately leads to rebound weight gain. **Methods:** Experiment was conducted on 42 adult healthy female wistar rats weighing around 150-200g, divided into 7 groups with 6 rats in each group. Group 1 given normal chow while others were given High Fat Diet (HFD). From 5th week till the end of 8th week group 3 to 7 were given respective drugs (extracts of *Gymnema sylvestre* and *Acorus calamus*). Obesity parameters (body weight, BMI, locomotor activity) were recorded on day 0, at the end of 8th week and at the end of 8th week. Internal organs (kidney, liver, spleen and heart) and fat pads (perirenal, mesenteric and gonadal) were also removed and weighed. **Results:** Feeding rats on HFD significantly changed the obesity parameters and lead to development of obesity. Combination of GS and AC significantly reduced body weight, BMI and improved locomotor activity. It also significantly reduced the weight of internal organs and fat pads. Both GS and AC also improved the obesity parameters in a dose dependent manner. **Conclusions:** Extracts of *Gymnema sylvestre* and *Acorus calamus* has a potent antiobesity effect. Combination of both is superior to the individual drugs.

INTRODUCTION: Obesity is a morbid condition characterized by abnormal or excess deposition of body fat to an extent that it adversely affects health[1]. It is a multifactorial disease, with its causative agents ranging from genetic, metabolic, social, behavioural, and cultural factors [2]. In the last few decades the prevalence has increased to such an extent that can be attributed to psychological and behavioural factors instead of biological factors only. Overall in 2014, about 13% of the world's adult population was obese and 39% adults were overweight. Obesity affects the health adversely and can lead to variety of other co-morbid conditions like insulin resistance, type II diabetes

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mellitus, hypertension, dyslipidaemia, metabolic syndrome, coronary heart disease and even some cancers [3]. The basic etiology behind obesity is the disparity between the energy intake and energy expenditure [4]. The type of food that we eat plays a major role in disturbing the energy balance. Increase in energy density of foods, better palatability of food, portion size, and easy availability as well as low cost promote the development of overweight and obesity [5]. Decreased physical activity and sedentary lifestyle contributes equally in the occurrence of obesity [6].

Lifestyle modification is considered as the initial modality to control weight which includes increased physical activity, dietary and behavioural modifications [7]. However to continue these modification for long is itself a challenge. Pharmacotherapy is the other way to control the obesity but these are indicated in some particular conditions. Currently available drugs have their own side effects. Unfortunately the benefits are for short term that might be because of cessation of drugs use or side effects which ultimately leads to rebound weight gain [8]. *Gymnema sylvestre* is an indigenous herb widely distributed in India, tropical Africa, Australia, Japan, Indonesia, Vietnam, and some regions of China. The plant is known to be a good source of number of bioactive molecules [9]. Medicinal uses of this plant are well known since ages. Traditionally it has been found useful in constipation, dyspepsia, jaundice, renal calculi, haemorrhoids, asthma, bronchitis, conjunctivitis and number of other conditions [10].

Acorus calamus is a well known drug in traditional system of medicine and rhizomes of the plants are being used since ages [11]. In various studies it has shown antidiabetic activity, antiinflammatory and many other properties [12, 13]. Present study was designed to explore and compare the antiobesity effect of *Gymnema sylvestre* and *Acorus calamus* individually as well as in combination.

MATERIAL AND METHODS: Present work was started after getting ethical approval from Institutional Animal Ethics Committee of King George's Medical University.

Experimental Procedure: Experiment was conducted on 42 adult healthy female wistar rats weighing around 150-200g that were procured from Indian Institute of Toxicology Research, Lucknow. Animals were kept under standard condition of temperature (25 ± 2 °C), humidity (55 ± 5%) and twelve hour light- dark cycle at the institutional animal house of King George's Medical University. All the guidelines of CPCSEA were followed while conducting experiment. After an acclimatization period of 7 days animals were divided into 7 groups with 6 rats in each group. During this period all the animals were given normal chow and water *ad libitum*.

On day 0 all the parameters of obesity including body weight, Body mass index (BMI) and locomotor activity (ambulatory activity and rearing) was measured. During phase I of the study which lasted till 4 weeks obesity was induced in group 2 to 6 by feeding them on high fat diet (HFD) while group 1 was given normal chow. HFD was purchased from Bharat Science Solution Company, Lok Nagar, Unnao, Uttar Pradesh. Nutritional composition of HFD included carbohydrate 44%, crude fat 25%, crude protein 18%, fiber 13%, moisture 8 %, Vitamins, minerals and other ingredients in required quantity. All the parameters were measured again at the end of 4th week.

From 5th week phase II of the study commenced and lasted till the end of 8th week. During this phase group 1 was continued on normal chow while group 2 was continued with HFD. Group 3 to 6 were kept on HFD along with the respective drugs. Group 3 was given *Gymnema sylvestre* 100mg/kg and group 4 was given *Gymnema sylvestre* 200mg/kg. Group 5 was treated with *Acorus calamus* 100mg/kg while group 6 was given *Acorus calamus* 200mg/kg. All the drugs were given by oral route as a suspension using distilled water. At the end of 8th week all the parameters were measured. After that all the rats were sacrificed with high dose of anaesthesia. Their internal organs (kidney, liver, spleen and heart) and fat pad (perirenal, mesenteric and gonadal) were identified, removed and weighed.

Test Drugs:

***Gymnema sylvestre*:** Dried powdered extract of leaves of *Gymnema sylvestre* was obtained from Ekgaon Company, New Delhi.

The drug was administered in a dose of 100 and 200 mg/kg bw [14].

***Acorus calamus*:** Dried powdered extract of rhizomes of *Acorus calamus* was obtained from Sierra India Organics, Indore. It was administered orally in a dose of 100 and 200 mg/kg bw [15].

Measurement of Body Weight and Body Mass Index (BMI): Body weight was measured by digital weighing machine. BMI was calculated by using the following formula:

BMI = Body weight (kg) / (Naso-anal length in meter) ^{2}

Measurement of Locomotor Activity (Ambulatory Activity and Rearing): Locomotor activity (ambulatory activity and the frequency of rearing) was calculated by using open field behaviour test apparatus (68×68×45 cm). Rats were kept in the apparatus for a period of 5 minutes and their ambulatory activity (AA) and the frequency of rearing was recorded.

Removal of Different Organs and Fat Pads: Rats were sacrificed by using high dose of anaesthesia (sodium pentobarbitone 150mg/kg i.p.). Different organs (kidney, liver, spleen and heart) and fat pads (perirenal, mesenteric and gonadal) were identified, removed and weighed.

Statistical Analysis: Statistical analysis was done using SPSS Statistics 20 software (Armonk, NY: IBM Corp.). Data were expressed as mean ± standard error of mean (S.E.M.). Different groups were compared with one way analysis of variance (ANOVA) followed by Dunnett's t-test while to compare similar group at different time intervals, paired t test was used. A p-value < 0.05 was considered statistically significant.

RESULTS: At day 0 all the groups were compared by analysis of variance (ANOVA). All the groups were found comparable to each other for obesity parameters (body weight, BMI and Locomotor activity) as no significant difference was found (**Table 1**).

TABLE 1: COMPARISON OF ALL THE GROUPS AT DAY 0

Parameter	F value	p-value
Body weight	0.297	0.934
BMI	1.585	0.181
Ambulatory Activity	0.677	0.669
Rearing	0.673	0.672

Effect on Body Weight and BMI: HFD deleteriously affected the obesity parameters. Body weight and BMI of the groups on HFD increased significantly at week 4 from their previous values. After starting drug treatment, body weight and BMI reduced significantly in all the drug groups. AC at 100mg/kg caused 24.0% weight reduction while at 200mg/kg 32.8% reduction as compared to disease control group. GS 100mg/kg decreased body weight by 32.9% while at 200mg/kg it reduced weight by 38.1%. Combined GS 200mg/kg and AC 200mg/kg caused maximum reduction in body weight by 42.0%. Regarding BMI, AC 100mg/kg and 200mg/kg caused 32.6% and 37.9% reduction while GS 100mg/kg and 200mg/kg caused 34.7% and 42.1% decrease in BMI respectively. Combined dose of GS 200mg/kg and AC 200mg/kg caused maximum reduction by 49.5% (**Table 2** and **3**).

TABLE 2: PRE AND POST TREATMENT BODY WEIGHT (g) OF ALL THE GROUPS (MEAN ± SEM, n = 6)

Groups	Day 0	Week 4	Week 8	% change as compared to Group 2 at 8th week
Group 1	196.6 ± 3.1	198.2 ± 4.2	203.3 ± 2.7	38.7%
Group 2	197.1 ± 7.5	292.0 ± 3.7*	331.8 ± 5.0$^{\#\$}$	-
Group 3	188.2 ± 8.6	284.9 ± 7.4*	222.7 ± 3.3$^{\#\$}$	32.9%
Group 4	188.4 ± 11.7	283.8 ± 9.2*	205.5 ± 9.0$^{\#\$}$	38.1%
Group 5	193.3 ± 7.0	288.7 ± 4.8*	252.1 ± 4.2$^{\#\$}$	24.0%
Group 6	186.5 ± 6.8	280.0 ± 6.5*	222.9 ± 3.4$^{\#\$}$	32.8%
Group 7	188.7 ± 8.5	284.6 ± 8.1*	192.5 ± 6.8$^{\#}$	42.0%

*Significant as compared to day 0; $^{\#}$Significant as compared to week 4; $^{\$}$Significant as compared to day 0.

At the end of 8th week ANOVA revealed significant difference (p-value < 0.01) among the groups. Dunnett's post hoc test showed that all the drug groups differed significantly from the disease control group 2 for both body weight and BMI (**Table 4**).

TABLE 3: PRE AND POST TREATMENT BODY BMI OF ALL THE GROUPS (MEAN ± SEM, n = 6)

Groups	Day 0	Week 4	Week 8	% change as compared to Group 2 at 8th week
Group 1	5.6 ± 0.28	5.6 ± 0.25	5.8 ± 0.27	38.9%
Group 2	5.7 ± 0.33	8.4 ± 0.63*	9.5 ± 0.78#$	-
Group 3	5.2 ± 0.41	7.9 ± 0.59*	6.2 ± 0.52#$	34.7%
Group 4	5.1 ± 0.18	7.7 ± 0.31*	5.5 ± 0.18#$	42.1%
Group 5	4.9 ± 0.29	7.3 ± 0.57*	6.4 ± 9.45#$	32.6%
Group 6	5.0 ± 0.24	7.5 ± 0.40*	5.9 ± 0.39#$	37.9%
Group 7	4.8 ± 0.11	7.2 ± 0.26*	4.8 ± 0.17#	49.5%

*Significant as compared to day 0; #Significant as compared to week 4; $Significant as compared to day 0.

TABLE 4: ANOVA FOLLOWED BY DUNNETT'S POST HOC TEST (COMPARISON GROUPS- 2) AT THE END OF 8th WEEK FOR BODY WEIGHT AND BMI

	Body weight		BMI	
Y	Mean Difference (Y – Group 2)	p- value	Mean Difference (Y – Group 2)	p- value
Group 1	-128.5*	<0.01	-3.71667*	<0.01
Group 2	-	-	-	-
Group 3	-109.1*	<0.01	-3.31667*	<0.01
Group 4	-126.3*	<0.01	-3.91667*	<0.01
Group 5	-79.7*	<0.01	-3.10000*	<0.01
Group 6	-108.9*	<0.01	-3.53333*	<0.01
Group 7	-139.3*	<0.01	-4.65000*	<0.01

*Significant.

Effect on Locomotor Activity: Feeding rats with HFD lead to significant decrease in ambulatory activity and increase in frequency of rearing suggesting development of obesity by 4th week. Later on after giving drugs both the parameters improved *i.e.* AA increased and rearing decrease (**Table 5** and **6**). AC 100mg/kg increased AA by 22.7% and decreased rearing by 23.8%. Similarly AC 200mg/kg increased AA by 34.8% and decreased rearing by 19.4%. GS 100mg/kg increased AA by 38.3% and decreased rearing by 22.6%. At 200mg/kg GS improved AA by 46.5% and reduced rearing by 26.2%. Combined dose of GS and AC was found more effective and increased AA by 51.5% and decreased rearing by 36.5%.

TABLE 5: PRE AND POST TREATMENT AMBULATORY ACTIVITY OF ALL THE GROUPS (MEAN ± SEM, n=6)

Groups	Day 0	Week 4	Week 8	% change as compared to Group 2 at 8th week
Group 1	61.7 ± 1.9	60.3 ± 0.8	60.5 ±1.3	-41.4
Group 2	64.2 ± 2.6	48.7 ± 2.4*	42.8 ± 2.2#$	-
Group 3	66.8 ± 2.0	50.0 ± 1.6*	59.2 ± 1.2#	-38.3
Group 4	65.7 ± 2.3	52.7 ± 2.6*	62.7 ± 2.1#$	-46.5
Group 5	65.3 ± 2.3	47.5 ± 1.7*	52.5 ± 1.7#$	-22.7
Group 6	66.5 ± 1.8	51.3 ± 2.5*	57.7 ± 2.2#$	-34.8
Group 7	65.7 ± 1.7	52.2 ± 2.3*	64.8 ± 1.6#	-51.4

*Significant as compared to day 0; #Significant as compared to week 4; $Significant as compared to day 0.

TABLE 6: PRE AND POST TREATMENT VALUE OF REARING OF ALL THE GROUPS (MEAN ± SEM, n = 6)

Groups	Day 0	Week 4	Week 8	% change as compared to Group 2 at 8th week
Group 1	15.0 ± 0.9	15.3 ± 1.0	15.3 ± 1.3	39.3
Group 2	16.3 ± 1.3	22.6 ± 1.3	25.2 ± 1.1	-
Group 3	16.5 ± 1.3	23.0 ± 1.2	19.5 ± 1.0	22.6
Group 4	16.8 ± 1.1	23.3 ± 1.3	18.6 ± 1.2	26.2
Group 5	14.3 ± 1.1	20.3 ± 1.3	19.2 ± 1.3	23.8
Group 6	16.5 ± 0.8	21.5 ± 1.5	20.3 ± 1.4	19.4
Group 7	16.3 ± 1.3	22.3 ± 1.3	16.0 ± 1.0	36.5

*Significant as compared to day 0; #Significant as compared to week 4; $Significant as compared to day 0.

All the groups were compared by ANOVA at the end of 8th week. Significant difference was found among the groups (p-value < 0.01). Post hoc test, considering group 2 as comparison group, revealed that both the drugs significantly improved obesity parameter by increasing AA and decreasing rearing (**Table 7**).

TABLE 7: ANOVA FOLLOWED BY DUNNETT'S POST HOC TEST (COMPARISON GROUPS- 2) AT THE END OF 8th WEEK FOR AMBULATORY ACTIVITY AND REARING

	Ambulatory activity		Rearing	
Y	Mean Difference (Y – Group 2)	p- value	Mean Difference (Y – Group 2)	p- value
Group 1	17.7*	<0.01	-9.8*	<0.01
Group 2	-	-	-	-
Group 3	16.3*	<0.01	-5.7*	0.01
Group 4	19.8*	<0.01	-6.5*	<0.01
Group 5	9.7*	<0.01	-6.0*	0.01
Group 6	14.8*	<0.01	-4.8*	0.04
Group 7	22.0*	<0.01	-9.2*	<0.01

*Significant.

At the end of 8th week all the parameters were recorded again and then rats were sacrificed by high dose of anaesthesia. Their internal organs (kidney, liver, spleen and heart) and fats pads (perirenal, gonadal and mesenteric) were removed and then weighed for comparison. ANOVA revealed that there was significant difference between the groups regarding these parameters (**Table 8**).

TABLE 8: ANOVA AT THE END OF 8th WEEK FOR INTERNAL ORGANS

Internal organ/fat pad	F value	p-value
Kidney	30.16	<0.01
Liver	31.08	<0.01
Spleen	107.2	<0.01
Heart	12.77	<0.01
Perirenal	105.26	<0.01
Gonadal	170.38	<0.01
Mesenteric	73.57	<0.01

TABLE 9: ANOVA FOLLOWED BY DUNNETT'S POST HOC TEST (COMPARISON GROUPS-2) AT THE END OF 8th WEEK FOR INTERNAL ORGANS

	Kidney		Liver		Spleen		Heart	
Y	Mean Difference (Y– Group 2)	p- value	Mean Difference (Y – Group 2)	p- value	Mean Difference (Y – Group 2)	p- value	Mean Difference (Y – Group 2)	p- value
Group 1	-0.22*	<0.01	-0.53*	<0.01	-0.42*	<0.01	-0.11*	<0.01
Group 2	-	-	-	-	-	<0.01	-	-
Group 3	-0.11*	<0.01	-0.33*	<0.01	-0.23*	<0.01	-0.10*	<0.01
Group 4	-0.14*	<0.01	-0.46*	<0.01	-0.34*	<0.01	-0.11*	<0.01
Group 5	-0.10*	<0.01	-0.20*	<0.01	-0.12*	<0.01	0.00	1.00
Group 6	-0.13*	<0.01	-0.28*	<0.01	-0.25*	<0.01	-0.07*	0.02
Group 7	-0.22*	<0.01	-0.60*	<0.01	-0.42*	<0.01	-0.16*	<0.01

*Significant.

TABLE 10: ANOVA FOLLOWED BY DUNNETT'S POST HOC TEST (COMPARISON GROUPS-2) AT THE END OF 8th WEEK FOR INTERNAL FAT PADS

	Perirenal		Gonadal		Mesenteric	
Y	Mean Difference (Y– Group 2)	p- value	Mean Difference (Y – Group 2)	p- value	Mean Difference (Y – Group 2)	p- value
Group 1	-0.49*	<0.01	-0.35*	<0.01	-0.60*	<0.01
Group 2	-	-	-	-	-	-
Group 3	-0.16*	<0.01	-0.16*	<0.01	-0.17*	<0.01
Group 4	-0.31*	<0.01	-0.17*	<0.01	-0.30*	<0.01
Group 5	-0.16*	<0.01	-0.08*	0.02	-.19*	<0.01
Group 6	-0.28*	<0.01	-0.09*	<0.01	-0.30*	<0.01
Group 7	-0.52*	<0.01	-0.30*	<0.01	0-.52*	<0.01

*Significant.

Intergroup comparison was made by ANOVA followed by dunnett's post hoc test with group 2 as comparison group. As shown in **Table 9** weight of internal organs reduced significantly (p-value <0.01) by both the drugs as compared to the disease control group.

Treatment by both the drugs significantly (p-value < 0.01) reduced the weight of internal fat pads (perirenal, gonadal and mesenteric) as compared to disease control group suggesting the antiobesity and weight reducing properties of GS and AC (**Table 10**).

DISCUSSION AND CONCLUSION: Obesity is a condition characterized by abnormal or excess deposition of body fat to an extent that it adversely affects health. The basic cause behind obesity is the disparity between the energy intake and energy expenditure. The type of food that we eat plays a major role in maintaining the energy balance. The most widely employed method to assess overweight and obesity is by the calculation of body mass index (BMI).

In the present study obesity was induced by feeding rats with high fat diet. The parameters which were used to assess obesity were body weight, body mass index, ambulatory activity, frequency of rearing, weight of internal organs and internal fat pads. HFD for 4 weeks led to significant increase (p-value < 0.01) in the body weight, BMI and ambulatory activity along with significant reduction in frequency of rearing. This study supports the fact that although there are various models of obesity but HFD induced obesity can act as a rapid method that even lasts for long [16, 17].

After 4 weeks drug treatment was started along with HFD in groups 3-7 while group 1 was continued on normal chow and group 2 on HFD only. Both the test drugs significantly (p-value < 0.01) reduced the body weight. Highest reduction (42%) occurred by the combination of GS and AC when compared to the disease control group while GS alone at 200mg/kg caused 38.1% reduction. All the test drugs also significantly reduced the BMI with maximum improvement in the combination group (49.5%) as compared to disease control.

Locomotor activity has been used by few studies as a marker of obesity [18]. To assess the locomotor activity, ambulatory activity and frequency of rearing was observed. Both the drugs significantly increased the ambulatory activity and decreased the rearing. Combination was found superior than the individual drugs with 51.4% increase in the AA and 36.5% reduction in the rearing.

At the end of 8th week changes in the internal organs (kidney, liver, spleen and heart) and fat pads (perirenal, gonadal and mesenteric) were also measured and compared to the normal control group and the disease control group. Due to high fat diet the weight of internal organs and fad pads increased significantly in disease control group as compared to the normal control and other groups. Both *Gymnema* and *Acorus* significantly reduced the weight of internal organs and fat pads. Highest reduction occurred in the group taking combination of both drugs followed by GS 200mg/kg. Weight of liver, kidney, spleen and heart found comparable to the normal control group. Weight of internal fat pads also decreased significantly when compared with the disease control group with maximum reduction by the combination of drugs.

Both the plants, *Gymnema sylvestre* and *Acorus calamus*, are rich in phytochemicals and are well known in traditional system of medicine. Leaves extract of Gymnema is rich in gymnemic acids that help in the inhibition of fat and oil hydrolyzates absorption from the digestive tract [19]. Gymnemic acids not only reduce the craving for food but also increase the fecal excretion of bile acids and neutral steroids [20, 21]. Asarones are the phytochemicals that are thought to be responsible for the weight reducing property of *Acorus calamus*. Asarone inhibits the differentiation of adipocytes [22]. From the present study it can be concluded that leaves extract of both *Gymnema sylvestre* and *Acorus calamus* has a potent dose-dependent antiobesity activity as shown by reduction in all the parameters of obesity taken into account. Combination of both the drugs was found superior to the individuals drugs. Based on these findings it is suggested to extend the domain of this study onto higher animals and human beings to further substantiate the results.

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