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**Research Article**

# ANTI-OBESITY PROPERTY OF HEXANE EXTRACT FROM THE LEAVES OF *GYMNEMA SYLVESTRE* IN HIGH FED CAFETERIA DIET INDUCED OBESITY RATS

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**ABSTRACT**
The hexane extract of leaves of *Gymnema sylvestre* was evaluated for its anti-obesity activity in the simplified high fed cafeteria diet induced obesity in Sprague dawley rats. Group-I (normal control) were fed on only basal diet without any treatment, Group-II (obesity control) were induced obesity and only on basal diet without any treatment, Group-III and Group IV were induced obesity and treated with hexane fraction of *Gymnema sylvestre* (150 mg/kg and 250 mg/kg body weight) respectively. Group V were induced obesity and treated with Atorvastatin as a standard drug. A significant (P>0.001) reduce in increased body weight, temperature due to obesity was observed after 45$^{th}$ day of treatment as compared to the normal and standard group. The extract also improved the cholesterol, triglyceride, LDL and HDL level. Blood samples were collected from retro-orbital plexus. Observed data was found statistically significant in reduction of extract-treated obesity rats. The effect of the extract was comparable to that of standard drug Atorvastatin (50mg/kg body weight).
**KEY WORDS**: Hexane extract, cholesterol, triglyceride, obesity, *Gymnema sylvestre,* atorvastatin.

## INTRODUCTION

Obesity greatly increases the risk for cardiovascular disease and Type-II diabetes especially when excess body fat is present in and around the abdomen[1]. It should be considered a serious medical condition that can lead to significant morbidity and mortality rather than a character flaw or personal weakness[2]. Obesity is difficult to define in quantitative terms. Obesity refers to the above average amount of fat contained in the body, this in turn being dependent on the lipid content of each fat cell and on the total number of fat cells[3]. Obesity is influenced by diet, developmental stage, age, physical activity and genes[4,5]. Genetic predisposition is a key contributing factor in obesity. Obesity, marked by an excess fat mass, is characterized by a high phenotypic heterogeneity linked most notably to differences in the stages of weight evolution[6,7]. Obesity is characterized by abnormal or excessive fat accumulation which may lead to hypertrophy and hyperplasia or adipose tissue[8]. India gives its most strange around in 50-55% of the country is obese, which is a critical world problem, so if we want to save the human civilization and to change everything, we have to start from our nutritional methods.

The *Gymnema sylvestre* found in climate that is moderate and it is spread throughout India, in dry forests up to 600m, Common throughout the district from January to November. Distributed in Asia, Tropical Africa, Malaysia and Srilanka[9]. The plant is Large climbers, rooting at nodes, leaves elliptic, acuminate, base acute to acuminate, glabrous above sparsely or densely tomentose beneath. The leaves of *G. sylvestre* contain triterpene saponins belonging to oleanane and dammarene classes[10]. Oleanane saponins are gymnemic acids and gymnemasaponins, while dammarene saponins are gymnemasides[11,12]. The leaves also contain resins, albumin, chlorophyll, carbohydrates, tartaric acid, formic acid, butyric acid, anthraquinone derivatives, inositole, alkaloids, organic acid (5.5%), parabin, calcium oxalate (7.3%), lignin (4.8%), and cellulose (22%)[13,15]. The roots, stems, leaves and fruits are used in indigenous system of medicine in diabetes [5] and plant is also reported to be bitter, astringent, acrid, thermogenic, anti-inflammatory, anodyne, digestive, liver tonic emetic,

diuretic, stomachic, stimulant, anthelmenthics, laxative, cardiotonic, expectorant, antipyretic and uterine tonic. Previous studies have reported some pharmacological activities (scientific studies) of the *Gymnema sylvestre* extracts[14-23].

However, despite this interesting health virtue, very few scientific studies have been carried out to determine the pharmacological activity involved in the anti-obesity effect. However, there is no scientific studies have been carried out using leaves of *Gymnema sylvestre* for anti-obesity activity in high fed cafeteria diet induced obesity in rats. Hence the objective of present study was to evaluate the anti-obesity activity in high fed cafeteria diet induced obesity in rats using hexane extract of *Gymnema sylvestre* leaves.

## MATERIALS AND METHODS

### Plant material

*Gymnema sylvestre*. is the materials of the present investigation. Leaves of *G. sylvestre* were collected in the month of collected in the month of November 2009 from Botanical garden, Guru Jambheshwar University, Hissar, India. The specimen plant (NISCAIR/RHMD/consult/-2009-10/1398/200) was identified with the help of literature and authenticated by Dr. H. B. Singh, Scientist F & Head, Raw Materials Herbarium and Museum, N.I.S.C.A.I.R, New Delhi, India.

### Preparation of plant powder and Extracts

Aqueous, ethanol, hexane and chloroform extract of leaves were prepared in accordance with the method of the National Institute of Health and Family Welfare (NIHFW), New Delhi, India. Dried and powdered plant material (1000 g) was successively Soxhlet extracted with hexane (60-80$^{o}$), chloroform, acetone, Methanol, aqueous for 72 h each according to their polarity. Crude aqueous extract of this plant was prepared separately by boiling the plant Material (25 g) with 200 ml of water for 15 min. The obtained extracts were evaporated in vacuum to give residues and their percentage yields (8.25%) was determined[24]. The extracts were stored in desiccators for use in subsequent experiments.

### Phytochemical analysis

Preliminary phytochemical screening[25]of aqueous, ethanol, hexane and chloroform extract of leaves revealed the presence of alkaloids, tannins, flavonoids and triterpenoids.

### Animals

Sprague dawley rats of either sex, weighing around 150gm-250gms were purchased from Punjab University, Chandigarh. Male animals were housed separately in groups 6 per cage (Polycarbonate cage size: 29x22x14 cm) under laboratory conditions with alternating light and dark cycle of 12 h each. The animals had free access to food and water. The animals were acclimatized for at least five days before behavioural experiments which were carried out between 09:00 and 17:00 h. The experimental protocol was approved by institutional animals ethics committee (IAEC) and animals care was taken as per guidelines of committee for the purpose of control and supervision of experiments on animals (CPCSEA), Govt. of India (Registration no. 828/ac/04/ CPCSEA). All studies were carried out using six rats in each group.

### Chemicals

Atorvastatin (Torrent Pharmaceutical Ltd., Gujarat), Diagnostic kits For Triglycerides, LDL (Transasia Bio-medical Ltd), Formalin solution (Ranchem, New Delhi). All solvents and chemicals were of analytical grade and procured from different companies like Petroleum ether (Ranbaxy Fine Che icals) and Methanol, Chloroform (Nice chemicals).

### Ant-obesity study

Induction of obesity - Obesity was induced in overnight fasted adult Sprague dawley rats of weighing around 150gm-250gms by the simplified high fed cafeteria diet which induces marked obesity in rats. This diet induces moderate degree of hyperphagia and obesity and is simple alternative to a conventional cafeteria diet. The induced obesity associated with a significant impairment of glucose tolerance and result in significantly raised basal leptin and insulin. The rats were found obesity on 7$^{th}$ day of treatment and were screened for anti-obesity study.

### Experimental design

Animals were divided into five groups of six rats each. Test groups were administered hexane extract at doses of 150 mg/kg bodyweight and 250 mg/kg bodyweight respectively by oral route. Standard and control animals were treated with standard drug atorvastatin at an oral dose of 1 mg/kg body weight and distilled water respectively. All doses were started 7$^{th}$ day after induction of obesity.. The experimental deigns are as follows

Group I – Normal operated + treated with Saline (2 ml/kg body weight)

Group II – Obesity control + treated with Saline (2 ml/kg body weight)

Group III – Obesity induced + hexane fraction of *Gymnema sylvestre* (150 mg/kg body weight)

Group IV - Obesity induced + hexane fraction of *Gymnema sylvestre* (250 mg/kg body weight)

Group V – Obesity + Atorvastatin as a standard drug (35 mg/kg body weight)

**Determination of anthropometric parameters**

An increase in body weight, abdomen circumference, was compared with control group was recorded as obesity. Body Mass index (BMI) i. e weight (g)/height ($cm^2$) were calculated before and after the treatment as an index of obesity[26]. Body weight was assessed biweekly. To evaluate the effect of high fat diet compared with normal group. To determine the Body weight of rats by standard weighing machine, Abdomen circumference was measured standard measuring tape and body temperature were measured through electrical Tele-thermometer by inserting probe in anus of rat at different day's interval

**Collection of blood and Determination of Biochemical Parameters**

Blood was withdrawn from the retro-orbital sinus under ether inhalation anesthesia and lipid levels were estimated using a commercially available kit. (Ascensia Entrust, Bayer Health Care, USA) at 7th, 14th , 21st, and 45th day. Values were expressed in mg/dl

**Statistical analysis**

Data were statistically calculated by utilizing one-way ANOVA and expressed as mean ± S.E.M. followed by Dunnett's t-test using computerized Graph Pad Instate version 3.05, Graph pad software, U.S.A.

**RESULTS AND DISCUSSION**

The initial phytochemical studies carried out in the laboratory for investigated the active constituents present in the plant. After extraction of whole plant of *Gymnema Sylvestre*, the dried crude extract has come out for different chemical test. The results indicated that various extracts of whole plant of *Gymnema Sylvestre* are having Alkaloids, Flavonoids, Tannins, Saponins after confirmation of positive test (+) of drangendroff & Mayer's reagent for alkaloids, lead acetate for flavonoids in chloroform and methanol, lead test, dil. nitric acid test and iodine test for tannins in pet ether and methanol and foam test for saponins in hexane, methanol and chloroform whereas Steroids was not give positive test in any extracts showing absence of steroids as shown in table 1.

The result of evaluation of anthropometric activity was expressed as the change in hexane extract of *Gymnema sylvestre* and was given in Table 2. It is clear from table that after a single dose the standard and hexane extract of *Gymnema sylvestre* showed significant reduction in body weight, organ weights, abdomen circumference, body temperature was compared with standard after 7th, 14th, 21st, and 45th day of treatment and the effect of reached its peak 45th day. Hypercholesterolemia and hypertriacylglycerolaemia are major risk factors for atherosclerosis and related occlusive vascular disease[26] Clinical complications as atherosclerosis could be diminished and life prolonged when blood lipids are lowered by hypercholesterolemia drugs27, 28. Experimental result also reflects that Hexane extract of *Gymnema sylvestre* significantly reduced serum cholesterol, triglyceride, LDL and HDL level in obesity induced albino rats. The result was given in Table 3.

**CONCLUSION**

It is therefore clear that the decoction of *Gymnema sylvestre* is not only good for overall physiological and biochemical disturbances due to obesity but also have physical effects like, effect on body weight in normal as well as diabetic animals. Lowering of serum lipid level through drug therapy seems to be associated with a decrease in the risk of vascular diseases and related complications. In conclusion, the results of the experiments above provide useful information regarding the lipid lowering activity of flavonoids and saponins probably from *G. Sylvestre* due to inhibition of pancreatic lipase activity. The beneficial effects of *G. Sylvestre* on serum lipids levels are time dependent. The current study supports, at least partly, the traditional use of this ethno medicinal plant. The mechanism responsible for this hypolipidemic effect of *G. Sylvestre* should be explored further in future studies. Further work is required to clarify the alteration of small intestinal enzymes in rat fed a high fed diet for longer term.

Hence the natural plants can be prescribed with modern drugs for better effect and reduce toxic effect.

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**REFERENCES**

1. Matsuzawa Y, Therapy insight: adipocytokines in metabolic Syndrome and related cardiovascular disease. Nat Clin Pract Cardiovasc Med 2006; 3(1): 35–42.
2. Oeser D. Epidemiology, Etiology and Pathophysiology and nonpharmacotherapeutic treatments. Internet J Acad Physician Assist 1997; 1(2).
3. Buskirk ER, Obesity: A brief overview with emphasis on exercise. Med Sci Sports Exerc 1974; 33(8):1948-1951.
4. Brockmann GA and Bevova MR. Using mouse models to dissect the genetics of obesity. Trends Genet 2002; 18: 367–376.
5. Friedman JM. A war on obesity, not the obese. Science 2003; 299: 856-858.
6. Friedman JM and Halaas JL. Leptin and the regulation of body weight in mammals. Nature 1998; 22: 763-770.

7. Stunkard AJ, Harris JR, Pedersen NL and McClearn GE. The body-mass index of twins who have been reared apart. N Engl J Med 1990; 322: 1483–1487.
8. Bjorntorp P. Adipose tissue distribution and function. Int J Obes 1991; 15: 67-81.
9. Keshavamurthy KR and Yoganarasimhan SN. Flora of Coorg – Karnataka, Vimsat publishers,Bangalore 1990; 282.
10. Dateo GP and Long L. Afr J Agric Food Chem 1973; 21: 899-903
11. Khramov VA, Spasov AA and Samokhina MP. Pharm Chem J 2008; 42 (1); 29-31.
12. Yoshikawa K, Nakagawa M, Yamamoto R, Arihara S and Matsura K. Chem Pharma Bull 1992; 40: 1779-82.
13. Sinsheimer JE and Rao GS. J Pharm Sci 1970; 59 (5): 622-628
14. Pati AK. Gurmar or Gymnema. Best Nutrition, Hayward, CA USA 2010
15. Nadkarni KM. Indian Materia Medica. Popular Prakashan, Bombay 1993; 596-599.
16. Chattopadhyay RR. J Ethnopharmacol 1999; 67:367-72
17. Pierce A. Gymnema Monograph: Practical guide to natural medicine, Stonesong Press Book, New York 1999; 324-26.
18. Preuss HG, Bagchi D, Bagchi M, Rao CV and Dey DK. Diabetes Obes Metab 2004;6(3): 171-80.
19. Preuss HG, Bagchi D, Bagchi M, Rao CV and Dey DK. Diabetes Obes Metab 2004;6(3): 171-80.
20. Bishayee A and Chatterjee M. Phyt Res 1994; 8(2): 118-120.
21. Rachh PR, Rachh MR, Ghadiya NR, Modi DC, Modi KP et al. Int J Pharmacol, 2010; 14.
22. Satdive RK, Abhilash P and Devanand PF. Fitoterapia 2003; 74; 699-701.
23. Malik JK, Manvi FV, Alagawadi KR and Noolvi M. Int J Green Pharm 2008; 2(2):114-15.
24. Naggapa AN, Thakurdesai PA and Singh VR. Anti diabetic activity of Terminalia catappa Linn fruits. J of Ethnopharmacol 2003; 88: 45-50.
25. Kokate CK, Practical Pharmacognosy, New Delhi, India, Vallabh Prakashan, 1994.
26. Novellie LB, Diniz YS, Galhardi CM, Ebaid GMX, Rodrigues HG et al. Anthropometrical parameters and markers of obesity in rats. Lab Animals 2007; 41: 111-119 .


**Table: 1. Response of extractives of plants *Gymnema Sylvestre* to qualitative tests for the presence of different phytoconstituents.**

| CHEMICAL TESTS | HEXANE | CHLOROFORM | METHANOL |
|---|---|---|---|
| **Alkaloids** | | | |
| Dragendroff's reagent | _ | _ | + |
| Mayer's reagent | _ | _ | + |
| Wagnor's reagent | _ | _ | + |
| **Flavonoids** | | | |
| Lead acetate acid | _ | + | + |
| **Tannin** | | | |
| Dil. Nitric acid test | + | _ | + |
| Iodine test | + | _ | + |
| Lead test | + | - | + |
| **Steroids** | | | |
| Salkowski reaction test | - | - | _ |
| Liebermann's reaction test | - | - | _ |
| **Saponin** | | | |
| Foam test | + | + | + |

**Table: 2. Effect of Atorvastatin and *Gymnema Sylvester* extracts on body weight of high-cafeteria diet at different day's interval.**

| Group(n=6) | Body Weight (g) | | | |
|---|---|---|---|---|
| | Initial (0 Day) | 7th Day | 21st day | 45th day |
| Normal | 184.8±3.8 | 186.1±2.1 | 187.5±2.6 | 189.1±1.7*** |
| Positive Control group (CD) | 180.6± 1.9 | 196.1±3.4 | 222.1± 2.7 | 234.7 ± 5.2 |
| Atorvastatin (50mg/kg)+CD | 183.7± 2.3 | 199.3 ± 6.1 | 189.1 ±4.1 | 178.6± 5.6*** |
| Hexane extract (150mg/kg) +CD | 188.1±1.4 | 202.8±4.4 | 196.1± 3.7 | 210.3± 7.0*** |
| Hexane extract (250mg/kg)+CD | 185.5 ± 3.6 | 210.2±7.3 | 198.20±4.3 | 182.23±4.9*** |

Values are Mean ± SEM
CD cafeteria diet
Data was analyzed by one-way ANOVA followed by Tukey's t test
Statistically significant value:
* $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$, ns- non significant

**Table: 2.1.Effect of Atorvastatin and *Gymnema Sylvester* extracts on body temperature of high - cafeteria diet at different day intervals**

| Group(n=6) | Body Temp | | | |
|---|---|---|---|---|
| | Initial (0 Day) | 7th Day | 21st day | 45th day |
| Normal | 36.4±1.3 | 37.1±1.1 | 37.8±1.2 | 33.3±1.5*** |
| Positive Control group (CD) | 37.5±1.4 | 38.3±1.2 | 39.6±1.5 | 40.3±1.8 |
| Atorvastatin (50mg/kg)+CD | 36.6±1.3 | 38.1±1.7 | 37.4±1.2 | 36.1±1.6*** |
| Hexane extract (150mg/kg) +CD | 36.9±1.8 | 37.4±1.3 | 37.1±1.5 | 36.7±1.1** |
| Hexane extract (250mg/kg) +CD | 35.7±1.4 | 36.2±1.6 | 35.4±1.2 | 34.2±1.3*** |

Values are Mean ± SEM, Data was analyzed by one-way ANOVA followed by Tukey's t test, Statistically significant value:,
* P ≤ 0.05, ** P ≤ 0.01, *** P ≤ 0.001, ns- non significant

**Table: 2.2. Effect of Atorvastatin and *Gymnema Sylvestre* extracts on abdomen circum-france of high-cafeteria diet at different day's interval.**

| Group(n=6) | Abdomen circumference | | | |
|---|---|---|---|---|
| | 0 Day | 7th | 21st day | 45th day |
| Normal | 14.9 ± 1.3 | 15.6 ± 0.6 | 15.8 ± 1.1 | 15.3 ±1.9 ** |
| Positive Control group (CD) | 15.1 ± 0.3 | 16.3 ± 1.7 | 20.4 ± 1.2 | 22.5 ± 3.2 |
| Atorvastatin (50mg/kg)+CD | 15.5 ± 3.4 | 18.9± 0.6 | 14.2 ± 1.9 | 16.8 ±1.4 * |
| Hexane extract (150mg/kg) +CD | 13.8 ± 0.8 | 19.0± 4.5 | 24.9 ± 3.7 | 21.5 ±3.9 ns |
| Hexane extract (250mg/kg) +CD | 15.5 ± 1.4 | 18.5 ± 0.6 | 18.1 ± 1.7 | 17.4 ±1.9 * |

Values are Mean ± SEM, Data was analyzed by one-way ANOVA followed by Tukey's t test, Statistically significant value:,
* P ≤ 0.05, ** P ≤ 0.01, *** P ≤ 0.001, ns- non significant

**Tabless: 2.3. Effect of Atorvastatin and *Gymnema Sylvester* extracts on weight of organs of high-cafeteria diet at different day's interval.**

| Group (n=6) | Weight of organ (g) | | | |
|---|---|---|---|---|
| | Left kidney | Right kidney | Spleen | Liver |
| Normal | 0.67±0.02 ns | 0.64±0.08 ns | 0.81±0.025*** | 6.32±1.32*** |
| Control(CD) | 1.66±1.08 | 1.62±1.2 | 1.37±1.5 | 12.14± 2.9 |
| Atorvastatin (50mg/kg) | 0.82±0.05 ns | 0.89±0.1 ns | 0.97±0.06*** | 7.11±1.08*** |
| Hexane extract (150mg/kg) +CD | 1.12±0.7 ns | 1.36±1.6 ns | 1.23±0.03*** | 9.09± 1.03* |
| Hexane extract (250mg/kg) +CD | 0.71±0.02 ns | 0.78±0.8 ns | 0.88±0.01*** | 8.10±0.23*** |

Values are Mean ± SEM, Data was analyzed by one-way ANOVA followed by Tukey's t test, Statistically significant value:
* P ≤ 0.05, ** P ≤ 0.01, *** P ≤ 0.001, ns- non significant

**Table: 3. Effect of Atorvastatin and *Gymnema Sylvester* extracts on biochemical para-meters of high-cafeteria diet at 45th day.**

| Group (n=6) | Serum Profile (mg/dl) | | | |
|---|---|---|---|---|
| | Serum Cholesterol | Triglycerides | LDL | HDL |
| Normal | 152± 7.3*** | 101±2.2*** | 80±2.7*** | 57.3±1.9*** |
| Obesity control | 210± 8.5 | 184.4±7.0 | 140±8.4 | 27±2.5 |
| Atorvastatin (50mg/kg) | 160±3.9*** | 131.51±2.6*** | 98.9±6.3*** | 45.5±2.1*** |
| Hexane extract (150mg/kg) +CD | 190±8.1*** | 170.2±10.4** | 120.1±4.2*** | 34.2±2.1*** |
| Hexane extract (250mg/kg) +CD | 163±5.8*** | 158±5.2*** | 105±2.9*** | 40.09±1.0 *** |

Values are Mean ± SEM, Data was analyzed by one-way ANOVA followed by Tukey's t test, Statistically significant value:
* P ≤ 0.05, ** P ≤ 0.01, *** P ≤ 0.001, ns- non significant

**Figure: 1. Gymneme Sylvestre Plant**

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