



Comparative evaluation of anti-obesity effect of *Aloe vera* and *Gymnema sylvestre* supplementation in high-fat diet fed C57BL/6J mice

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ABSTRACT

Background: The aim of the present study was to investigate, anti-obesity effect of *Aloe vera* (AV), and *Gymnema sylvestre* (GS) whole extract powders administration to high-fat diet (HFD) fed C57BL/6J mice for 12 weeks. **Materials and Methods:** At the end of experiment, different parameters such as body weight, feed intake, organ weights, fasting blood glucose, oral glucose tolerance test, plasma lipid levels, and expression analysis of adipocytokines were evaluated. **Results:** At the end of experimental period, oral administration of both herbs showed a significant ($P < 0.05$ and $P < 0.001$) decrease in the plasma glucose and lipid levels in HFD fed mice. In addition, increased in the epididymal fat (E. fat) weight in the HFD group was significantly ($P < 0.05$) reduced on GS administration alone. Finally, quantitative mRNA expression analysis of adiponectin gene was significantly up-regulated in AV supplementation. Further, no effect was observed with the both herbs on pro-inflammatory cytokines (interleukin 6 and tumor necrosis factor- α) in the E. fat tissue of HFD fed group. **Conclusions:** The anti-obesity and other metabolic studies depend on the type of diet, different parts of herbal extractions, and animal models used. Further studies are required in this area to strengthen the anti-obesity effects of herbs with active component, and it can be used a pro-drug instead of whole extract.

KEY WORDS: *Aloe vera*, *Gymnema sylvestre*, high-fat diet, inflammatory markers, obesity

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INTRODUCTION

Now-a-days, obesity is considered to be epidemic disease which leads toward a positive energy balance. It is associated with many health-related complications such as insulin resistance, Type-2 diabetes mellitus (T2DM), and other metabolic disorders. In obese individuals, the accumulation of more fat occurs in the adipose tissue and later stage fat deposition takes place other than non-adipose tissue (e.g., muscle and liver) [1]. Due to the accumulation of triglycerides (TG), the body does not respond to the action of insulin and results in insulin resistance, a hallmark of Type-2 diabetes [2-4]. Moreover, both familial and environmental factors play a significant part in obesity. The consumption of high-fat diet (HFD), an environmental factor is associated with the development of obesity and other metabolic diseases. Diet control, physical exercise, liposuction, and bariatric surgery are available to curb the obesity, but these are not effective with prolonged treatment duration. Hence, people are looking for alternative therapies such as herbal medicines and functional foods which are gaining the importance in treatment of obesity and its associated diseases. Nearly, 1200 plant species have been examined for their use in obesity and diabetes [5].

Herbs such as *Aloe vera* (AV) and *Gymnema sylvestre* (GS) showed antihyperglycemic and hypolipidemic effect in animal studies and clinical studies when administered through intragastric gavage [6,7]. *Aloe barbadensis* Miller (AV) belongs to the Liliaceae family, of which there are about 360 species and widely used in the manufacture of food and beverages, pharmaceuticals and cosmetics [8]. It has been used for centuries, treating obesity, diabetes, immunomodulator, anti-inflammatory, antiseptic, healing, and anti-tumor activities. Aloe gel contains predominantly polysaccharides such as glucomannan and acemannan possessing several medicinal values [9]. In a clinical trial, AV juice showed anti-diabetic activity performed by Mahidol University Research Group [10,11]. In a recent study, we demonstrated that AV gel powder (0.5% and 1% w/v) showed an increase in proteolysis and angiotensin I converting enzyme inhibitory activity in milk fermented with probiotics [12]. GS is another medicinal plant belonging to the Asclepiadaceae family. In the Ayurvedic system, it is referred as "Meshasringa," and it has potent anti-obesity and anti-diabetic activities. It is also used in the treatment of asthma, eye complaints, inflammation, family planning, and snake bite [13]. Its leaves contain gymnemic acids which are a potent inhibitor of glucose absorption in the

intestine [14]. In addition, gymnemic acids bind to the receptors present in the tongue that prevent the glucose intake [15-18]. Studies showed that GS have been found to increase insulin secretion from the pancreatic β -cells and causes lowering of blood glucose levels in animals and T2DM patients [19,20]. No reports are available with the addition of whole extracts of AV and GS herbs to the HFD for studying its anti-obesity effects. In this study, we investigated the comparative evaluation of AV and GS whole extract powders role in anti-hyperglycemic, hypolipidemic effect, and obesity-related gene expression analysis in HFD fed C57BL/6J mice.

MATERIALS AND METHODS

Animals and Experimentation

Twenty-four male C57BL/6J mice (age 5-6 weeks old) were obtained from National Institute of Nutrition, Hyderabad (Andhra Pradesh, India). All the experimental animals were housed in a group ($n = 6$) and fed ad libitum water and food, under 12 h light and dark conditions. The Institutional Animal Ethics Committee guidelines were followed for handling animals in this study. After 1 week of acclimatization period, mice were divided into four groups, namely, control diet, HFD, HFD + AV (1% w/w) (HFD + AV) and HFD + GS (1% w/w) (HFD + GS), and fed for 12 weeks. The HFD contains carbohydrate 35%, protein 20%, and fat 35% (7% soya bean oil and 28% lard). The complete list of diets composition is present at Pothuraju *et al.* [21]. Body weight was measured at weekly interval. At the end of the experimental period, mice were sacrificed by cervical dislocation under diethyl ether anesthesia and blood was collected from heart to determine plasma insulin and lipid levels. Organs such as epididymal fat (E. fat), liver, kidney, and spleen were separated and weighed. A portion of E. fat tissue was immediately transferred into RNA later and stored at -20°C for further expression analysis studies.

Fasting Blood Glucose Levels

The fasting blood glucose levels were determined by pricking the tail vein with needle gun and determined with GlucoDr[®] blood glucose meter at 0, 6, and 12 weeks in overnight fasting animals.

Oral Glucose Tolerance Test (OGTT)

The OGTT was performed after 12 weeks for which mice were fasted for overnight (12 h) and 20% glucose solution (1 g/kg body weight) was administered via intra-gastric gavage. Blood was collected before and after 30, 60, 90, and 120 min of glucose administration.

Plasma Insulin and Lipid Levels

Plasma insulin concentration was measured with sandwich ELISA kit (Crystal Chem. Inc., USA) while plasma total cholesterol (TC), TG, high-density-lipoprotein-cholesterol (HDL-C) levels were determined using enzymatic kit (M/s Span Diagnostics, India) and very low-density lipoprotein-cholesterol

(VLDL-C) was calculated using Friedewald's equation, i.e., $\text{VLDL-C (mg/dl)} = \text{triglycerides}/5$.

Isolation of Total RNA and Quantitative Analysis of Real-time Polymerase Chain Reaction (qRT-PCR)

Total RNA was isolated from E. fat using TRIzol (M/s Sigma-Aldrich, USA) method. RNA purity was determined by spectroscopy using an ultraviolet spectrophotometer and the ratio of absorbance values at 260 and 280 nm was calculated (A_{260}/A_{280}), and integrity was measured by subjecting to agarose gel electrophoresis. The cDNA template was synthesized by reverse transcription of 500 ng of total RNA using first strand cDNA synthesis kit (M/s Fermentas, India). SYBR green was used for real-time PCR detection and the primers used for qRT-PCR are listed at Pothuraju *et al.* [21].

Statistical Analysis

All the values are presented as mean \pm standard error of mean. The data were statistically analyzed using GraphPad Software Version 5 (San Diego, CA, USA). Tukey's post-test was used to define statistically significant differences ($P < 0.05$) among the groups.

RESULTS

Effect of AV and GS on Body Weight Gain

At the end of experiment, no statistically significant difference was observed in the body weight gain between control and HFD fed groups. However, supplementation of both herbal ingredients (AV and GS extracts) showed reduction in the body weight gain but not statistically significant ($P > 0.05$, Table 1).

Effect of AV and GS on Glucose and Insulin Levels

As shown in Figure 1, fasting blood glucose levels were determined at 0, 6, and 12 weeks. High-fat fed obese mice showed increase

Table 1: Body weight, plasma lipids, and insulin levels

Parameters	Control	HFD	HFD+AV	HFD+GS
Body weight (g)				
Initial	26.71 \pm 0.78	26.25 \pm 0.77	26.05 \pm 1.52	26.05 \pm 0.77
Final	32.57 \pm 2.2	35.08 \pm 1.95	36.59 \pm 3.4	34.22 \pm 1.16
TG (mg/dl)	38.79 \pm 3.79	52.68 \pm 8.53	48.72 \pm 8.10	39.86 \pm 4.00
TC (mg/dl)	83.35 \pm 3.51 ^a	128.50 \pm 6.41 ^b	83.41 \pm 6.90 ^{ac}	67.78 \pm 5.31 ^{ac}
HDL-C (mg/dl)	67.21 \pm 1.45 ^a	65.19 \pm 1.45 ^a	77.32 \pm 4.98 ^a	87.59 \pm 3.27 ^b
VLDL-C (mg/dl)	7.75 \pm 0.76	10.53 \pm 1.70	9.74 \pm 1.62	7.96 \pm 0.80
Insulin (ng/ml)	0.48 \pm 0.14	1.44 \pm 0.85	0.54 \pm 0.21	0.53 \pm 0.08

^{abc}Mean values within a row with at least one similar superscript letters do not differ significantly ($P > 0.05$, Tukey's test). TG: Triglycerides, TC: Total cholesterol, HDL-C: High-density-lipoprotein-cholesterol, VLDL-C: Very low-density lipoprotein-cholesterol, HFD+AV: High-fat diet+*Aloe vera*, HFD+GS: High-fat diet+*Gymnema sylvestre*

in glucose levels at 6 weeks (153 ± 8.00 mg/dl) and 12 weeks (168.5 ± 7.90 mg/dl), respectively. Administration of both herbs such as AV (122.0 ± 7.79 and 124.0 ± 10.36 mg/dl, $P < 0.05$) and GS (131.3 ± 7.34 , $P < 0.05$ and 104.2 ± 10.36 mg/dl, $P < 0.001$) to HFD fed mice, displayed a statistically lowering effect of blood glucose levels at the end of both 6 and 12 weeks, respectively. In OGTT levels were higher in HFD treatment and these glucose levels were lowered by both herbal extract supplementations without a significant difference (data not shown). On the other hand, HFD fed mice had higher levels of plasma insulin levels at the end of experiment. However, both AV and GS supplementation displayed lowering of plasma insulin concentrations without significant difference (Table 1). HFD fed mice exhibited increased fasting blood glucose and plasma insulin levels ($P < 0.05$). Both herbs showed significant decrease in the glucose levels; however, no effect was observed within insulin levels.

Effect of AV and GS on Organ Weights

After 12 weeks, E. fat, liver, kidney, and spleen were weighed. The E. fat weight was significantly increased in HFD group (1.48 ± 0.24 g, $P < 0.05$) in comparison to control (0.96 ± 0.13 g) group. Supplementation of GS had a significant reduction in the E. fat mass (0.61 ± 0.14 g, $P < 0.05$), whereas no effect was observed with AV fed group (0.89 ± 0.14 g, $P > 0.05$). No significant difference was found in the rest of the organ weights among different groups. Interestingly, significantly reduced E. fat mass weight without a marked reduction in body weight gain was observed by GS supplementation to HFD fed obese mice.

Effect of AV and GS on Plasma Lipid Profiles

The serum lipids (TC, TG, HDL-C, and VLDL-C) concentrations in a fasting condition of HFD fed obese mice were measured (Table 1). After 12 weeks, plasma TC levels were found to be higher in HFD compared to control group (128.5 ± 6.41 vs. 83.35 ± 3.51 mg/dl). Both herbal ingredients AV (83.41 ± 6.90) and GS (67.78 ± 5.31 mg/dl, $P < 0.05$) showed a significant reduction in the TC levels. Although in

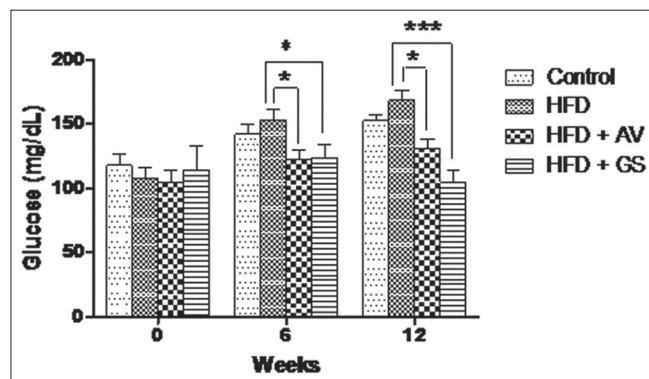


Figure 1: Effect of *Aloe vera* (AV) and *Gymnema sylvestre* (GS) on fasting blood glucose levels in mice fed with high-fat diet (HFD). Values are expressed as mean \pm standard error of mean ($n=6$). The bar over the histogram shows the significant difference of HFD+AV and HFD+GS with HFD group (* $P < 0.05$, *** $P < 0.001$)

HFD (65.19 ± 1.45 mg/dl) group along with AV (77.32 ± 4.98 , $P > 0.05$) and GS (87.59 ± 3.27 mg/dl, $P < 0.05$) fed groups, an increase in HDL-C levels was observed. However, a significant difference was observed only in HFD + GS group. No difference was observed in case of triglycerides and VLDL-C levels.

Effect of AV and GS on Expression Analysis

Expression analysis of genes such as adiponectin, leptin, and proinflammatory markers (interleukin 6 [IL-6] and tumor necrosis factor- α [TNF- α]) was done in E. fat tissue (Table 2). HFD fed mice showed downregulation of adiponectin expression levels. These adiponectin levels were significantly up-regulated by AV supplementation (4-fold change). No significant effect was observed in HFD + GS fed group. In addition, leptin and proinflammatory cytokines expression did not show any significant difference with the herbal ingredients.

DISCUSSION

In the present investigation, comparative evaluation of both herbal ingredients was studied for their role in anti-obesity effect in HFD fed C57BL/6/J mice for 12 weeks. Surprisingly, we did not observe any significant difference between control and HFD diet groups. The reason might be that the lard which is used in HFD preparation obtained from the local supplier. At the end of experiment, oral administration of AV and GS powders resulted in no significant difference in body weight and cumulative feed intake in comparison with the HFD fed group of mice (data not shown). Our results were consistently similar with Chihara *et al.* [22] who reported that AV gel extract did not show any significant difference in the reduction of body weight and food consumption after 7 weeks experimental period in HFD fed mice. Similarly, HFD supplementation of aloe formula such as processed AV gel (PAG), Aloe QDM, and Aloesin had no effect on body weight [23]. Administration of dried AV gel powder to diet-induced obesity rats did not display any significant effect on reduction in the body weight and food intake [24]. Misawa *et al.* [8,25] studied the oral ingestion of AV phytosterols (cycloartanol-Cy and lophenol-Lo) to Zucker diabetic fatty (ZDF) rats for 6 weeks and reported that no significant difference on body weight reduction and food intake. In contrary, administration of AV phytosterols (Cy and Lo) significantly reduced the body weight in diet induced obese (DIO) mice [26]. Few reports are available in relation to body weight and food intake by GS treatment. Our results are

Table 2: Effect of *Aloe vera* and *Gymnema sylvestre* on analysis of gene expression in epididymal fat mass of mice fed with HFD

Gene	Control	HFD	HFD+AV	HFD+GS
Adiponectin	1.03 \pm 0.15 ^a	0.63 \pm 0.14 ^a	5.5 \pm 2.04 ^b	0.61 \pm 0.05 ^a
Leptin	1.72 \pm 0.96 ^a	0.77 \pm 0.31 ^a	2.6 \pm 2.12 ^a	0.81 \pm 0.37 ^a
TNF- α	1.23 \pm 0.18 ^a	0.47 \pm 0.17 ^a	0.99 \pm 0.51 ^a	2.60 \pm 0.89 ^a
IL-6	1.32 \pm 0.53 ^a	1.26 \pm 0.58 ^a	2.31 \pm 0.91 ^a	1.21 \pm 0.12 ^a

^{a,b}Mean values within a row with different superscripts differ significantly ($P < 0.05$). Values are (fold in change) expressed as mean \pm standard error of mean ($n=3$). HFD: High-fat diet, HFD+AV: High-fat diet+*Aloe vera*, HFD+GS: High-fat diet+*Gymnema sylvestre*

consistent with the observations of Shigematsu *et al.* [27] who reported that no significant effect on reduction in the body weight and food intake when GS administration to HFD fed Wistar rats. In contrary to our results, Kumar *et al.* [28] reported that GS extract in the HFD-induced obese Wistar rats showed a significant decrease in the body weight while no effect on food intake for 28 days. On the other hand, saponins rich aqueous extract of GS also showed reduce gain in body weight, and no effect in food consumption in HFD fed obese rats [29]. The different AV and GS extract preparations used by the researchers had different effects on reduction in the body weight gain and food intake. In our study, we speculated that the less body weight in herbal fed groups might be due to the satiety effect that results reduction in fat tissue (E. fat) weight which is related to body weight gain. However, we could not measure the fat and lean mass parameters in all treatment groups.

Fasting blood glucose results of the present study were significantly reduced at both 6 and 12 weeks, respectively by both herbal supplementations in comparison to HFD fed group. Our results were corroborated by Shin *et al.* [23] demonstrating that dietary aloe components showed significant reduction in the blood glucose levels in obese mice. Kim *et al.* [6] observed that the fasting blood glucose and insulin levels were significantly reduced in a dose-dependent manner when PAC was orally administered. On the other hand, AV phytosterols (Cy and Lo) administration to ZDF rats showed no effect on fasting blood glucose and serum insulin levels after 5 weeks [25]. Similarly, Pérez *et al.* [30] observed that polyphenol-rich extract from AV gel did not show any effect on insulin levels in insulin resistance mice. However, GS administration of HFD fed Wistar rats showed a significant reduction in the blood glucose and serum insulin levels [28]. The possible mechanism underlying the lowering of blood glucose levels might be due to inhibition of its absorption in the intestine. Hypoglycemic effect of AV might be associated with pancreatic insulin synthesis and its secretion responsible for the lowering of circulatory glucose levels [31] whereas, gymnemic acids present in the GS binds to the glucose receptors to prevent the intestinal glucose absorption [14].

Herbal administration showed no effect on organ weights (liver, kidney, and spleen) whereas, E. fat weight was decreased significantly in case of GS fed group alone as compared to HFD fed obese mice. Our results are in agreement with several reports which showed no significant effect on liver and E. fat mass on AV treatment [8,24,25,32]. Reddy *et al.* [29] reported that the weights of liver, kidney, heart, and adipose tissue (peritoneal and perirenal fat) were lowered upon GS supplementation in HFD fed Wistar rats. In contrast, Shigematsu *et al.* [27] reported that epididymal and mesentery fat weight was not decreased by GS administration. In obesity, the pattern of body fat accumulation is thought to play a part in disease risk and body fat accumulation was closely associated with E. fat mass. In our study, gymnemic acids present in the GS might have been involved in increasing the energy expenditure through uncoupling protein-2 (UCP-2-involved in thermogenesis) expression. Nevertheless, we could not measure the expression of UCP-2 in adipose tissue and subcutaneous, mesenteric, retroperitoneal, and brown adipose tissue weights were also could not evaluate.

At the end of 12 weeks experimental period, plasma lipids were analyzed in HFD fed mice. Oral administration of both herbs showed a significant decrease in the plasma TC levels alone. Different results were observed by the researchers with the herbal extracts. Chihara *et al.* [22] reported that no significant effect on plasma TC and TG levels. Similarly, Misawa *et al.* [24] studied the oral administration of dried aloe gel (20 mg/kg body weight) and reported no effect on serum TC and TG levels in Sprague-Dawley rats. In contrary, serum TG levels were lowered by AV phytosterols (Cy and Lo) while no effect was observed in TC levels of ZDF rats [25]. Nomaguchi *et al.* [26] also reported that both serum TG and TC levels were significantly reduced by AV phytosterols in DIO C57BL/6J mice. In another study, reduction in the serum TC, TG, and LDL-C levels by GS administration in diabetic rats [7]. Reddy *et al.* [29] observed that decrease in the TG, LDL-C, VLDL-C, and increased in the HDL-C levels in HFD fed rats. Kumar *et al.* observed that GS extract showed a significant reduction in the serum total lipids (TC, TG, LDL-C, and VLDL-C) in HFD fed Wistar rats [28]. By considering above studies, it can be concluded that the differences in lipids levels depends on the type of animal models and extract preparations used. However, in our study, phytosterols present in the whole herbal extracts do not extensively absorb from the intestine rather it can bind to the cholesterol and prevent its absorption to show a hypolipidemic effect [33]. Furthermore, GS contains flavonoids, phenols, saponins, phenolic, and terpenoids might be responsible for lowering effect on lipids in high cholesterol fed rats [34].

Finally, expression analysis of adiponectin (role in regulating energy homeostasis) leptin, IL-6, and TNF- α genes were analyzed by qRT-PCR. In HFD fed mice, AV supplementation alone displayed up regulation of adiponectin mRNA levels. However, no significant difference was observed with the remaining genes. To the best of our knowledge, there are no such expression study reports available in this regard. However, further experiments are necessary to study the effect of AV and GS on the expression analysis of other obesity-related genes in animal models.

CONCLUSIONS

In conclusion, this study was carried out for comparative evaluation of anti-obesity effect and expression analysis of adiposity genes by AV gel and GS powders in high-fat fed C57BL/6J mice for 12 weeks. Both herbal extracts did not show any significant effect on body weight and food intake and further they displayed anti-hyperglycemic and hypolipidemic effects in HFD fed mice. In addition, E. fat pad was significantly decreased by GS administration alone. AV fed group alone showed a significant up regulation of adiponectin levels. Moreover, supplementation of both powder extracts did not show any effect on other genes. This study is the only preliminary experiment where we used 1% whole extract of herbs added to the HFD instead of active components. However, several researchers they did not show any significant effect on the supplementation of herbs using active component. For the better anti-obesity effects, using type of diet, herbal

extraction, treatment length, and animal models may be play crucial role.

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