LIPID LOWERING EFFECTS OF ALOE VERA – EFFECT ON HDL-CHOLESTEROL PROTEIN EXPRESSION

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Abstract

Aim: The effect of aqueous extracts of Aloe vera on body weight, lipid accumulation in adipose tissues and HDL protein expression was evaluated in obesity induced female swiss albino mice.

Experimental design: The study was conducted in four sets of mice. One set was maintained as control under normal conditions of temperature, humidity and light. Second set of mice was made obese by the administration of cholesterol rich diet for 35 days. The third set of mice was made obese by the feeding them with cholesterol rich diet for 35 days and then treated with Aloe veraextract for 15 days. The fourth set of mice was control mice administered with Aloe vera extract for 15 days.

Results: Extracts of Aloe vera significantly reduced the body weight in obese mice. Also, the lipid accumulation in the adipose tissues of obese mice was found to be decreased by treatment with Aloe vera extract. Furthermore, Aloe vera elevated the HDL protein expression in the obese mice.

Conclusion: These results show that Aloe vera possesses anti-obesity effects and could be used for weight loss.

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1. Introduction

Aloe vera is commercially used in the form of gels, juice, health drinks etc [1]. It acts as a high interest herb in the field of cosmetology and pharmacology due to its easy availability and vast health benefits [2]. Aloe vera is known for its potential to reduce cholesterol, detoxify the body, protect body from stress, stabilize blood sugar, reduce hypertension, prevent renal calculi, rejuvenate skin and body, heal wounds and infections etc [3-5]. However, its protective role in major events of obesity including lipolysis in adipose tissues and

HDL protein expression is not known. Adipose tissues are the primary sites for body fat accumulation and hence they play an important role in body fat metabolism [6]. Various metabolic and cell signaling pathways, adipocytokines etc are involved in the lipogenesis in adipose tissues. HDL-cholesterol on the other hand scavenges the fat deposits from adipose tissues and other organs by promoting reverse cholesterol transport [7-15]. In the present study, we investigated the anti-obesity effects of Aloe vera by evaluating its potential to promote lipolysis and HDL-cholesterol protein expression.

2. Materials and Methods

2.1. ANIMALS

90 days old healthy, female Swiss Albino Mice (Mus musculus) were procured and individually housed in polycarbonate cages. They were kept in a room which is maintained under constant temperature ($24°C$) with a 12-h light/ dark cycle. The mice were allowed to acclimatize for 1 week after delivery. Then, they were randomly divided into the following four groups with each group consisting of 6 animals:

GROUP I: Control mice.

GROUP II: Mice orally fed with High fat diet containing 45kCal% fat, 20kCal% protein and 35 kcal% carbohydrate

GROUP III: Mice orally fed with high fat diet for 60 days to induce obesity and treated with Aloe vera extract (0.3mL/Kg body weight of mice) for another 30 days.

GROUPIV:Mice orally fed with Aloe vera extract (0.3mL/Kg body weight of mice) for 30 days.

All the mice were given free access to food and distilled water was made available ad libitum. Body weight of the animals were measured weekly. At the end of the experimental period, all the mice were anesthetized with isofluorane (5mg/Kg body weight) after a 12-h fast and blood samples were collected from inferior vena cava in a tube for the estimation of hematology and serum parameters. The blood was centrifuged at 3000rpm for 10 minutes and the serum was isolated and stored at -80C until use.

2.2. PREPARATION OF AQUEOUS EXTRACT OF ALOE VERA

5g of Aloe vera powder was mixed with 50mL water and this solution was shaken in mechanical shaker at 290rpm for 3 days at $37°C$. This solution was then filtered twice using muslin cloth and was stored in a sterile container under refrigeration.

2.3. SDS-PAGE AND EXPRESSION OF HDL

After the estimation of serum protein using Bradford reagent, they were subject to SDS-PAGE using 10% main separating gel and 5% stacking gel. 20□g of protein was loaded onto the wells and the electrophoresis was carried out at 50V. 70kDa marker (Genei Laboratories, Bangalore) was used to tract the molecular weight of the separated proteins. The gel was then stained with Coomassie Brilliant Blue R-250 and then destained with methanol, acetic acid and water. The gel photo was then documented and analyzed.

2.4. HISTOCHEMICAL STAINING OF ADIPOSE TISSUE WITH 'OIL RED O' STAIN

After obtaining the abdominal white fat tissue, they were washed in ice cold Phosphate Buffered Saline solution and immediately frozen in liquid nitrogen and kept at $-80°C$. Then cryostat sections of each tissue were taken at 10□ on clean glass slides and fixed in 10% cold acetone. The slides were washed in tap water, rinsed in distilled water and the excess water was drained off. The slides were then subjected to two changes of propylene glycol each change with an interval of 5 minutes. The slides were stained with Oil Red O for 7 minutes under agitation. 85% propylene glycol was then added to the slides for 3 minutes. The slides were rinsed in distilled water and stained with hematoxylin (Gill) for one minute. The slides were then washed with tap water, rinsed with distilled water, and mounted with aqueous mounting media, glycerin jelly and viewed under light microscope to view the lipid deposition in adipose tissue.

2.5. STATISTICAL ANALYSIS

The data obtained are presented as mean ± Standard Deviation and the statistical analysis was performed using SPSS software version 10.0. Tukey's post-test was used to determine the statistically significant differences ($p<0.05$) between the four groups of mice under study.

3. RESULTS

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3.1. Effect of Aloe vera extract on changes in body weight in high fat diet-induced mice

Table 1 represents the body weight of all the experimental animals during the beginning and the end of the experimental period. There is no significant change in the body weight of group I mice during the course of the study. However, HFD has significantly increased body weight in group II ($p<0.007$) and group III ($p<0.0007$) mice. Aloe vera has dramatically reduced the body weight of HFD fed group III mice from $33.1+0.8$g to $29.6+1.6$g ($p<0.05$). This in turn indicates that administration of Aloe vera promotes weight loss in obese mice. Interestingly, Aloe vera did not induce any weight loss in group IV mice with normal body weight.

Body weight (g)	GROUP I	GROUP II	GROUP III	GROUP IV
Initial	27.6+2.9	29.8+1.3	28.1+0.63	28.6+2.4
Final	29.2+1.4	33.8+2.6	29.6+1.6	27.4+0.8
P value	0.25	0.007	0.05	0.45

Table 1: Effect of Aloe vera extract on changes in body weight in high fat diet-induced Swiss Albino Mice. Each value represents mean±S.D of 6 animals.

3.2. Effect of Aloe vera extract on HDL protein expression by SDS-PAGE

SDS-PAGE was used to study the expression of HDL protein. Figure 1 represents the expression of serum proteins in different groups of experimental animals. Lane 1 represents the protein marker, lane 2 represents the protein expression in group I mice, lane 3 represents the protein expression in group II mice, lane 4 represents the protein expression in group III mice, and lane 5 represents the protein expression in group IV mice. The denser the band intensity in a particular region of the gel, the more is the expression of that particular protein. The gel photograph shows that the HDL protein expression decreases in the experimental animals in the following manner:
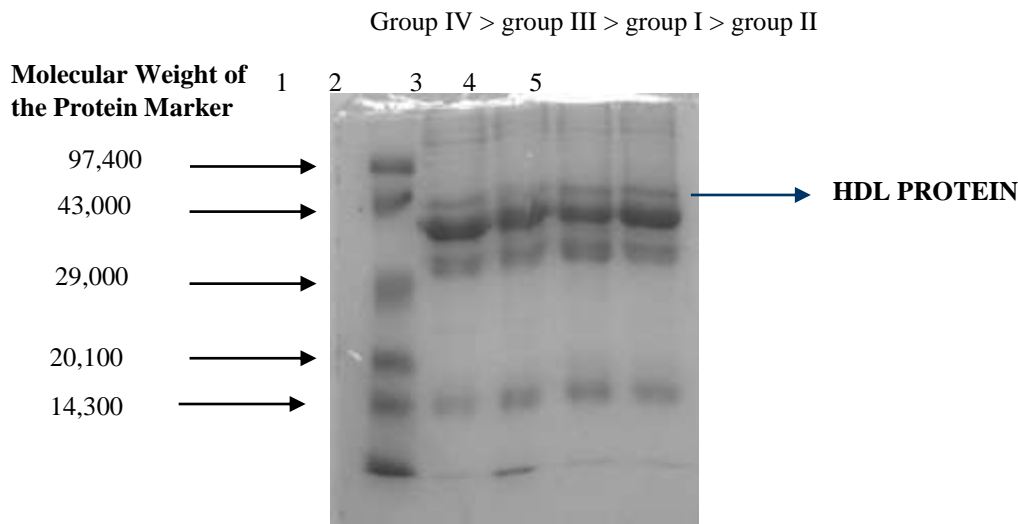
Group IV > group III > group I > group II

Figure 1: HDL protein expression using SDS- PAGE

3.3. Effect of Aloe vera extract on oil red O staining of adipose tissue

Fig 2A shows the sections of the adipose tissue of normal mice stained with Oil Red O. The cell morphology is normal with the nucleus being present at the centre which indicates that there is only a moderate lipid deposition in these cells. Fig 2B shows the sections of the adipose tissue of the obese mice stained with Oil Red O. Significant change in the morphology of the adipose tissue is observed in the animals belonging to this group. The increase in the size and the color (dark orange) intensity of the cell and the shift in the nucleus of the cell towards the periphery confirm the excess deposition of lipid in the adipose tissue of the overweight mice.
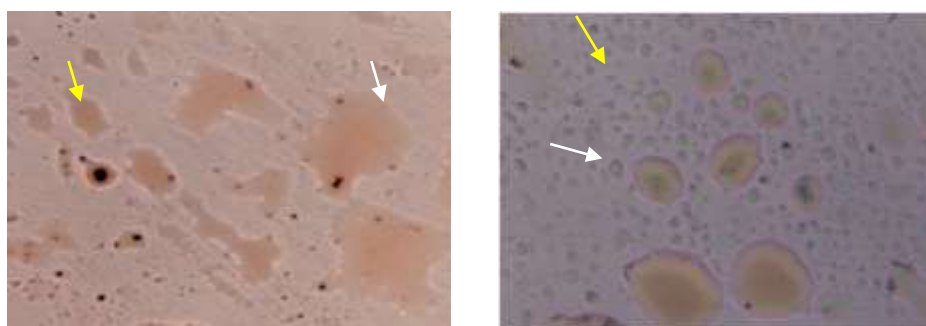
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Figure 2 A: Normal Mice
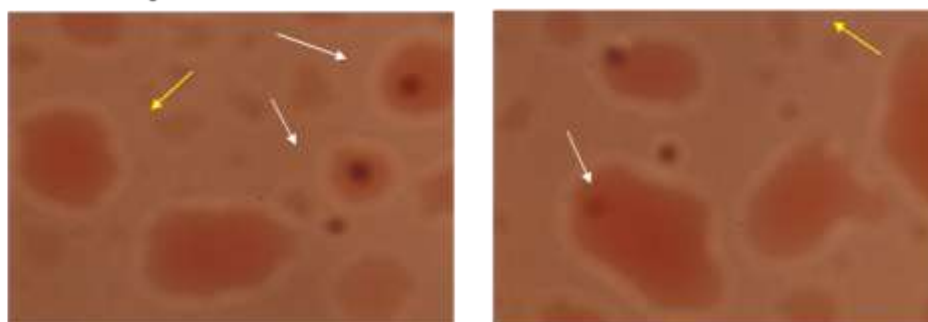Figure 2B: obese Mice

Figure 2 C: Transiently Overweight Mice treated with Aloe Vera

Figure 2 D: Normal Mice Treated with Aloe Vera

Fig.2 A,B C and D. Effect of Aloe vera extract on oil red O staining of adipose tissue. Yellow arrow indicates Matured Cells while white arrow indicates immature cells.

Fig 2C. represents the sections of the adipose tissue of the obese mice treated with Aloe vera. The cell morphology of these mice is similar to that observed in obese mice, which suggest that Aloe vera does not exert any significant influence over the morphology of the adipose cells. These cells are mild orange in color, which indicates that Aloe vera has reduced the lipid content in these cells by bringing about lipolysis. Fig 2D represents the sections of the adipose tissue of the normal mice treated with Aloe vera. The cell morphology of this set of mice is similar to that observed in normal mice. The cells are mild orange in color and the color intensity is lesser than that observed in normal mice which indicates that Aloe vera brings about lipolysis in the adipose cells of normal mice also. The presence of more number of immature cells in these mice suggests that Aloe vera bring about weight loss by inhibiting the maturation of adipose cells. All these observations thus made suggest that Aloe vera is effective in bringing about lipolysis in adipose tissue thereby leading to reduction in body weight.

4. Discussion

Number of reports show that Aloe vera possess anti-diabetic and anti-proliferative effects [16, 17]. Our objective was to investigate the anti-obesity effects of Aloe vera in HFD mice and this has yielded satisfactory results. There was an increase in BMI in HFD fed mice then normal diet fed mice. Treating obese mice with Aloe vera has significantly reduced their body weight. HDL, a 70,000 kilodalton protein is also called as 'good cholesterol" because it reduces the risk for cardiovascular disease by scavenging the lipid deposits from the organs like heart, pancreas, etc., by promoting reverse cholesterol transport [18]. Expression of HDL protein in blood is usually decreased in the obese subjects when compared to that in the lean subjects. In the present study, very less HDL protein expression is seen in HFD fed mice whereas more HDL protein expression is seen in Aloe vera treated mice. These observations suggest that Aloe vera can bring about weight loss by promoting the expression HDL protein, enhancing the reverse cholesterol transport and thereby clearing the lipid deposits in the cardiovascular system.

Lipogenesis is the deposition of fat. This process occurs in the adipose tissue at cytoplasmic and mitochondrial sites [19]. Energy ingested as

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carbohydrate can be stored as glycogen in the liver and muscle. Carbohydrate can also be converted to triglycerides primarily in the liver and transferred to adipose tissue for storage. Thus, adipose tissue are the fat depots which consists of excess fats during positive energy balance and vice versa. Lipids deposited in the adipose tissue can take up the Oil Red O stain and appear orangish red in color. The more the lipid content in the cell the more is the intensity of the color and vice versa. In the present study, the adipose tissues of the HFD fed rats show more lipid accumulation than control rats. However, treatment of HFD fed rats with Aloe vera significantly reduced the lipid accumulation in the adipose tissues. These observations show that Aloe vera is very effective in bringing about lipolysis in adipose tissue thereby leading to reduction in body weight.

5. Conclusion

Taken together, our results show that Aloe vera could reduce the body weight, lipid deposition in adipocytes and increase the HDL protein expression. However, more detailed studies at molecular level has to be carried out in assessing the role of Aloe vera in the prevention of cardiovascular disease.

CONFLICT OF INTERESTS
None declared.

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