Evaluation the Healthy Benefits of Consumption Aloe vera Supplemented Yoghurt on Albino Rats

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Abstract: For an in vivo study, six groups of rats fed for 14 days with either plain yoghurt or Aloe vera (AV) supplemented yoghurt (10% and 20%). Serum protein and lipid profiles were assessed. Results from in vivo studies showed a noticeable increase in weight gain, total proteins and globulins over a period of 14 days in experimental animals fed AV yoghurt. Results also showed a reduction of serum triglycerides, Low-density lipoprotein cholesterol (LDL-C), very low-density lipoprotein cholesterol (VLDL-C) and total cholesterol with increase in high-density lipoprotein cholesterol (HDL-C) levels in AV yoghurt fed rats.

Key words: Yoghurt • Aloe vera • Healthy Benefits • Albino Rats

INTRODUCTION

Many studies were carried on Medicinal efficacy of Aloe vera (AV) plant. The AV plant used for medicinal purposes in several cultures in wide part of the world; Greece, Egypt, India, Mexico, Japan and China [1-4]. The medicinal effects have been attributed to the polysaccharide found in the inner gel. Nevertheless, it is believed that synergistic action of the compounds contained in the whole leaf extracts is reason beyond the multiple and diverse beneficial properties of the plant [5].

The AV extracts have antibacterial and antifungal activities that may help in the treatment of minor skin infections, such as boils and benign skin cysts and antifungal effects [6, 7]. In higher concentration (60, 80 and 90%) whole leaf AV extracts can eliminate dozens of harmful microorganism [8, 9].

Many studies in animal models have reported that the body function of AV Gel could have a helpful impact by lowering blood cholesterol, triglycerides and phospholipids [10, 11]. Consequently, attempts made to develop alternative dietary ingredients that can manage blood cholesterol levels. Supplementation of the diet with fermented dairy products or foods containing Bifidobacteria and lactic acid bacteria have been reported for lowering serum cholesterol levels [12, 13].

Few years ago, intensive studies on isolates and other active phytochemicals extracted from AV species used in folkloric medicine for improvement of fertility [14-16]. Many studies have suggested that phytosterols may have effects on the reproductive system and they possess estrogenic activity [17, 18]. In addition, phytosterols reported for modulating the ovarian steroidogenesis, which then altered the ovarian structure-function [19].

Collins and Collins [20] described AV for the therapy of radiation burns and reported its potential efficacy in several dermatological problems. Investigations at the Linus Pauling Institute of Science and Medicine have provided evidence that AV gel is able to achieve various positive effects in the entire digestive tract. Apart from the improved faecal movement in the gut, an increased faecal density and reduced protein decomposition in the colon also became apparent. In addition, less flatulence observed, which is probably due to improved functioning of the microbiota of the large intestine. Number of in vitro and in vivo studies revealed the antiinflammatory activity of AV gel. The interaction of AV polysaccharides with stimulants such as plant growth factors and phytosterols activates macrophages and brings the body's own immune system into a heightened state of preparedness [21]. Willenburg’s study exhibited the antiinflammatory activity of mannose phosphate [22]. Moreover, specific
plant sterols may also contribute to the antiinflammatory activity of AV gel [23].

In the treatment of dry skin AV gel gloves improved the skin integrity, decrease appearance of acne wrinkle and decrease erythema where its moisturizing effects have been studied [24].

The AV was to be a highly effective treatment for diabetes [25-28]. Noor et al. [29] demonstrated the antidiabetic effect and reduction of degenerative changes in pancreatic tissues of rats fed with AV. The prevention of pancreatic islets destruction attributed to the radical scavenging activity of A. arborescens (Kidachi Aloe) [30]. Gowda demonstrated that mannose-6-phosphate is the significant constituent in the 50% AV extract [31]. At the same time, Morgan showed that the mannose-6-phosphate bind to the insulin like growth factor receptor [32].

Several clinical and experimental studies have demonstrated the hypoglycemic effects of AV [33, 34]. Additionally, there are some reports that AV derived extracts showed a preventive effect against insulin resistance [35], as well as the protective role of AV extracts in pancreatic β-cells of diabetic rats [36], were also reported in recent years.

The AV contains substantial amounts of antioxidants including α-tocopherol (vitamin E), carotenoids, ascorbic acid (vitamin C), flavonoids and tannins and it has been suggested that antioxidant action may be an important property of plant medicines used in treatment of various diseases [37]. One study determined that the total phenolic content of AV leaf skin extracts significantly correlated with the antioxidant capacity [38]. The methanol extracts of leaf skins and flowers of AV also screened for their antioxidant and antimycoplasmic activities and in vitro both extracts of leaf skin exhibited antioxidant activity [39].

In AV gel, glycoproteins are gift, posse growth and antitumor activity and to extend proliferation of normal human dermal cell [40]. Aloin, an anthraquinone being a natural compound and the main ingredient of Aloe vera, documented for its remarkable potential therapeutic options in cancer [41]. Aloin inhibits tumor angiogenesis with the potential of a drug candidate for cancer therapy [42]. In addition, Aloin treatment significantly inhibited in vitro Vascular Endothelial Growth Factor-induced angiogenic response of human endothelial cells, causing an inhibition of proliferation and migration of endothelial cells [43]. Aloe-emodin (AE) is also a subtype of anthraquinone, a natural compound that found to have diverse biological activities including anticancer functions [44, 45].

The AV is known for its antihyperlipidemic property because it has beneficial effects on the prevention of fatty streak development [46] and may help to reduce the development of atherosclerosis through modification of risk factors [27]. The AV leaf gel efficacy checked in hyperlipidemic type 2 diabetic patients wherein it reduced total cholesterol and low-density-lipoprotein (LDL) levels significantly (Huseini et al., 2012). Dried pulp of AV succotrina leaves produced a significant antihyperlipidemic effect in high-fat diet and fructose induced hyperlipidemic rats, with a significant reduction in serum levels of total cholesterol, total triglycerides, low-density lipoprotein cholesterol, very low-density lipoprotein and high-density lipoprotein cholesterol [47].

Others also suggested that Aloe vera gel treated polycystic ovarian syndrome (PCOS) rats exhibited a significant reduction in plasma triglyceride and LDL cholesterol levels, with an increase in high-density lipoprotein cholesterol [48]. The AV gel has phytocomponents with antihyperlipidemic effects and shown efficacy in management of PCOS but also the associated with metabolic complication [49]. The present study aimed to Evaluation the healthy benefits of Aloe Vera (AV) supplemented yoghurt on albino rats.

**MATERIALS AND METHODS**

**Collection of Plant Specimens:** Wild Aloe vera (AV) plants were collected freshly from El-Arish City, North Sinai, Egypt. The plant leaves used for extractions measured between 40 and 60 cm in length and taken from 3-year-old plants. To avoid biodegradation the AV leaf harvested and pulled carefully from the mother plant to avoid break of rind. Harvested leaves immediately kept in the icebox at 4±1°C to preserve their biological activity and transported to the laboratory.

**Preparation of AV Gel Aqueous Extract:** The fresh AV plant leaves was selected to derive crude gel. The gel collected as described in a previous study [50]. Briefly, the leaf surfaces were thoroughly washed with tap water and later with distilled water to remove traces of dirt and soil. The fleshy mass of the AV was carefully opened by cutting the sharp edges. The gel was funneled into a
sterile beaker. 10 and 20 g of AV gel were weighed using a digital microsensitive scale. Each of these was then diluted with 100 ml of distilled water to constitute 10 and 20 percentage (m/v) AV concentrations respectively. These were gently processed with a kitchen blender to achieve homogenous solution. This liquid was kept for 20 min to settle and later sieved using Whatman filter paper (No. 1) to obtain a particulate-free gel aqueous extract. The AV gel aqueous extract was freshly prepared every time before use. It contained all the functional ingredients of the crude gel in the same proportion as it appears in the leaf.

Collection of Milk: Fresh cow’s milk, obtained from private farm, in El-Arish City, North Sinai, Egypt.

Yoghurt’s Starter Culture: Commercial yoghurt culture (YC-X11, Thermophilic yoghurt culture - YoFlex®), containing Streptococcus thermophilus and Lactobacillus delbrueckii subsp. bulgaricus, was obtained from CHR-Hansen, Horsholm, Denmark.

Manufacturing of Yoghurt: Fresh cow’s milk pasteurized at 90°C for 20 min then cooled to 42°C and inoculated with 3% (v/v) Yoghurt’s starter culture (YC-X11). The plain yoghurt was prepared without addition of AV gel aqueous extract and was used as a control (0% Aloe vera). Aloe vera yoghurts were prepared by mixing milk and starter with different concentrations (5%, 10%, 15% and 20%) of AV extract, which was previously pasteurized (90°C for 20 min). Yoghurt samples were incubated at 42°C for 4 h until complete coagulation, cooled to refrigerator temperature (~6±1°C) and then stored for 14 days. Analyses were conducted at 7-day intervals.

Animal Feeding Experiments
Animals and Housing Conditions: Seventy-two male clinically healthy albino rats (Wistar strain) weighing 0.25 to 0.30 kg and aged from 12 to 16 weeks were obtained from animal house of Food Technology and Research Institute, Agriculture Research Center, Giza, Egypt. The animals were housed in cages under normal healthy conditions (23-25°C, 40-70% humidity and with 12-h light/12-h dark cycles) and they were kept under observation before the start of the experiment for two weeks. A standard laboratory diet and tap water were available ad libitum. Cages, bedding and glass water bottles were changed once or twice per week.

Animal Experimental Design: After a 2-week acclimatization period, the rats were randomly divided into six different groups, each group of rat using 2 replications (n = 6 rats). The rats were assigned to specific treatments as follow:

Group I: (Control - C): Rats were gavage orally with distilled water (2 ml/ 100 g body weight/day) [51] for two weeks and fed on standard chow diet.

Group II: (Yoghurt - Y): Rats were gavage orally with plain yoghurt (2 ml/ 100 g body weight/day) for two weeks and fed on standard chow diet.

Group III: (10% Aloe vera - AV 10%): Rats were gavage orally with 10% AV (2 ml/ 100 g body weight/day) for two weeks and fed on standard chow diet.

Group IV: (10% Aloe vera Yoghurt - AY 10%): Rats were gavage orally with AV Yoghurt 10% (2 ml/ 100 g body weight/day) for two weeks and fed on standard chow diet.

Group V: (20% Aloe vera - AV 20%): Rats were gavage orally with 20% AV (2 ml/ 100 g body weight/day) for two weeks and fed on standard chow diet.

Group VI: (20% Aloe vera Yoghurt - AY 20%): Rats were gavage orally with 20% AV Yoghurt (2 ml/ 100 g body weight/day) for two weeks and fed on standard chow diet.

Biochemical Assays
Determination of Serum Total Protein: Serum total protein concentration was determined calorimetrically according to the method described by Wootton and Freeman [52], using Biuret reagent.

Determination of Serum Albumin Concentration: Serum albumin concentration was determined by the method of Bartholomew and Delaney [53] using Bromocresol green (BCG).

Determination of Serum Globulins Concentration: The concentration of globulins was estimated according to Wuhrmann and Wunderly [54] by subtracting the albumin concentration from the total protein concentration.
Determination of Serum Lipids: Serum total cholesterol and triglycerides were determined according to the method of Schettler and Nüssel [55] using Enzymatic-Colorimetric. Serum high-density lipoprotein cholesterol (HDL-C) estimated according to the method of Gordon et al. [56] using Human kit (Germany) according to the instruction of the supplier. Serum low-density lipoprotein cholesterol (LDL-C) and very low Density Lipoprotein cholesterol (VLDL-C) estimated by the following equations:

(1) LDL-C = total cholesterol - triglyceride/5 - HDL-C
(2) VLDL-C = triglyceride/5

RESULTS

Physiological Effects of Aloe vera Yoghurt Feeding to Adult Rats

General Health and Mortality: Clinical or behavioral abnormalities were monitored immediately after gavage feedings in the rats. For the rest of the 14 days of the period of study, animals were monitored daily for mortality, abnormal clinical signs and any other adverse events. One rat given yoghurt died by drenching pneumonia at day 6 and two rats given 20% AV died by day 2 and 7. No mortality was recorded in the other groups throughout the experimental period (Fig. 1).

Body Weight Changes: The body weights of experimental rats were taken at daily interval for recording the average body weight change (%) throughout the experimental period (two weeks). The body weight change percent obtained from study are tabulated in Table 1.

At 7th day of study there were statistically significant ($P < 0.05$) differences in the body weight gain between the groups. There was weight loss in the control and yoghurt groups only and weight gain in the other AV groups. The 10% AV group had the highest weight gain compared to all other AV groups. However, the 10% AY group (IV) had the lowest weight gain compared to the other AV groups. The results showed a dose and duration dependent significance increase in the body weight gain in AV groups. Moreover, the percent reduction in body weights in yoghurt fed rats indicated comparatively greater susceptibility of weight loss probably by preventing the absorption of lipids.

At 14th day of study the average body weight change values of all experimental groups did not differ significantly amongst themselves. However, a drastic non significant reduction in body weight gain of animals in control and yoghurt groups (I and II) only can be observed. In contrast, there was a non significant increase in body weight gain of experimental animals in groups III, IV, V and VI compared to control and yoghurt groups (Fig. 2).

Serum Protein Profile

Total Serum Proteins: The details of mean ± SEM values of serum total proteins concentration (g/dL) of all the treatment groups at scheduled intervals of trial are presented in Table 2. The mean values of serum total proteins concentration in adult male albino rats studied ranged between (6.50±0.27 to 7.26±0.47) and (6.29±0.60 to 9.91±0.72) g/dL at 7th and 14th day of experiment respectively.

Fig. 1: Survival rate of rats during a 14-day experimental period. Values expressed as a percentage of rats alive compared with the initial group (n = 12). Control (C), Yoghurt (Y), 10% Aloe vera (AV 10%), 10% Aloe vera yoghurt (AY 10%), 20% Aloe vera (AV 20%) and 20% Aloe vera yoghurt (AY 20%).
Fig. 2: Body weight changes of rats during a 14-day experimental period. A. Normalized body weight. B. Percentage of body weight change. Data are presented as the mean ± SEM (n = 5-6 per group, independent t-test). *P < 0.05, vs. control (C) group, $P < 0.05, vs. yoghurt (Y) group, †P < 0.05, vs. day 7 within the same group.

Table 1: Percentage of body weight change of rats during a 14-day experimental period

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Body Weight Change (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Distilled Water (Control, C)</td>
<td>-0.87±1.06</td>
</tr>
<tr>
<td>II</td>
<td>Plain Yoghurt (Y)</td>
<td>-2.30±0.81</td>
</tr>
<tr>
<td>III</td>
<td>Aloe vera 10% (AV 10%)</td>
<td>3.29±1.13</td>
</tr>
<tr>
<td>IV</td>
<td>Y + AV 10% (AY 10%)</td>
<td>0.84±0.41</td>
</tr>
<tr>
<td>V</td>
<td>Aloe vera 20% (AV 20%)</td>
<td>3.03±1.20</td>
</tr>
<tr>
<td>VI</td>
<td>Y + AV 20% (AY 20%)</td>
<td>2.20±0.84</td>
</tr>
</tbody>
</table>

Values are means ± SEM for 5-6 rats per group.

The mean values of serum total proteins concentration in rats at 7th day of the study period found to be decreased non significantly in experimental rats of groups III IV, V and VI (6.50±0.27; 7.10±0.15; 7.02±0.31 and 6.58±0.51) respectively and increased non significantly in group II (7.26±0.47) when compared with mean values of control group I (7.20±0.50). Moreover, the serum total proteins concentration in rats of Group III showed the lowest value when compared to yoghurt group II had highest value.

However, the mean values of serum total proteins concentration in male rats at 14th day of the study period found to be increased gradually in experimental rats of groups II; III; IV, V and VI (6.77±0.62; 8.41±0.27; 8.71±0.34; 7.82±0.47 and 9.91±0.72) respectively when compared with mean values of respective control group I (6.29±0.60). Only, the serum total proteins concentration in rats of group VI showed significant (P < 0.05) increase when compared to control group I at 14th day.

The serum total proteins concentration in rats of group VI showed significant (P < 0.05) increase at 14th day, when compared to respective group values at 7th day respectively. Also, the serum total proteins concentration in rats of group VI showed significant (P < 0.05) increase, when compared to group V at 14th day respectively.

In present study, serum total proteins was found to be increased in the groups given AV compared to control group at 14th day of experiment but in the first week there is a non significant decrease between AV groups compared to control.

Serum Albumin: Table 3 shows the mean values of serum albumin concentration (g/dL) in different groups at 7th and 14th day of experimental period. The mean values of serum albumin concentration in male rats of groups I, II, III, IV, V and VI ranged between (4.39±0.58 to 5.12±0.19) and (4.12±0.57 to 5.16±0.26) g/dL after 7 and 14 days of feeding respectively. The results of the study showed
Table 3: Average values for the serum albumin concentrations

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Serum albumin conc. (g/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Day 7</td>
</tr>
<tr>
<td>I</td>
<td>Distilled Water (Control, C)</td>
<td>4.81±0.54</td>
</tr>
<tr>
<td>II</td>
<td>Plain Yoghurt (Y)</td>
<td>4.39±0.58</td>
</tr>
<tr>
<td>III</td>
<td>Aloe vera 10% (AV 10%)</td>
<td>4.54±0.51</td>
</tr>
<tr>
<td>IV</td>
<td>Y + AV 10% (AY 10%)</td>
<td>4.74±0.55</td>
</tr>
<tr>
<td>V</td>
<td>Aloe vera 20% (AV 20%)</td>
<td>5.12±0.19</td>
</tr>
<tr>
<td>VI</td>
<td>Y + AV 20% (AY 20%)</td>
<td>4.60±0.57</td>
</tr>
</tbody>
</table>

Values are means ± SEM for 5-6 rats per group.

Table 4: Serum globulins concentrations (Mean ± SEM, g/dL) in rats at 7th and 14th day of study

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Serum globulins conc. (g/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Day 7</td>
</tr>
<tr>
<td>I</td>
<td>Distilled Water (Control, C)</td>
<td>2.39±0.75</td>
</tr>
<tr>
<td>II</td>
<td>Plain Yoghurt (Y)</td>
<td>2.87±0.77</td>
</tr>
<tr>
<td>III</td>
<td>Aloe vera 10% (AV 10%)</td>
<td>1.96±0.65</td>
</tr>
<tr>
<td>IV</td>
<td>Y + AV 10% (AY 10%)</td>
<td>2.37±0.67</td>
</tr>
<tr>
<td>V</td>
<td>Aloe vera 20% (AV 20%)</td>
<td>1.89±0.44</td>
</tr>
<tr>
<td>VI</td>
<td>Y + AV 20% (AY 20%)</td>
<td>1.98±0.92</td>
</tr>
</tbody>
</table>

Values are means ± SEM for 5-6 rats per group.

that there were no statistically significant differences in serum albumin levels at the 7th and 14th day of the study between the groups.

At 7th day of study, The mean values of serum albumin concentration in male rats of groups I, II, III, IV, V and VI were 4.81±0.54; 4.39±0.58; 4.54±0.51; 4.74±0.55; 5.12±0.19 and 4.60±0.57 g/dL respectively. Control rats showed lowest levels (4.39±0.58). The results indicated that high level of AV alone could apparently increase serum albumin concentration in male rats at 7th day of study.

At 14th day of study, mean values of serum albumin concentration in male rats of groups I, II, III, IV, V and VI were 5.16±0.26; 4.32±0.54; 4.67±0.52; 4.61±0.52; 4.91±0.29; 4.12±0.57 g/dL respectively. Control rats showed the highest mean values (5.16±0.26) of serum albumin concentration, while yoghurt fed rats showed lowest levels (4.12±0.57). The results indicated that high level of AV could apparently decrease serum albumin concentration at 14th day of study in rats fed with 20% AV yoghurts.

The serum albumin concentration in rats of groups II, IV, V and VI showed non significant decrease at 14th day, when compared to respective group values at 7th day. However, the serum albumin concentration in rats of groups I and III showed non significant increase at 14th day, when compared to respective group values at 7th day. Interestingly, the serum albumin concentration in rats fed yoghurt and/or high levels of AV showed non significant decrease at 14th day, when compared to respective group values at 7th day with in contrast to groups I and III.

Serum Globulins: Table 4 shows the mean values of serum globulins concentrations (g/dL) in different groups at 7th and 14th day of experimental trial. The mean values of serum globulins concentrations in male rats of groups I, II, III, IV, V and VI ranged between (1.89±0.44 to 2.87±0.77) and (1.13±0.68 to 5.80±0.96) g/dL after 7 and 14 days of feeding respectively.

At 7th day of experiment, the mean values of serum globulins of male rats of groups I, II, III, IV, V and VI were 2.39±0.75; 2.87±0.77; 1.96±0.65; 2.37±0.67; 1.89±0.44 and 1.98±0.92 g/dL respectively. The rats fed yoghurt showed the highest mean value (2.87±0.77) of serum globulins, while rats fed 20% AV extract alone showed the lowest one (1.89±0.44) at 7th day. The mean serum globulins values in rats of different groups did not showed any significant alterations.

At 14th day of experiment, the mean values of serum globulins of male rats of groups I, II, III, IV, V and VI were 1.13±0.68; 2.45±0.85; 3.74±0.55; 4.10±0.73; 2.91±0.74 and 5.80±0.96 g/dL respectively. The rats fed 20% AV yoghurt showed the highest mean value (5.80±0.96) of serum globulins, while control rats showed the lowest one (1.13±0.68) at 14th day. The mean values of serum globulins in male rats of group VI showed marginal decrease at 14th day, when compared to respective group values at 7th day. However, the mean values of group II and V showed marginal increase at 14th day. However, the mean values of groups III, IV and VI showed a significant (P <0.05) increase compared to control group.

The serum globulins concentration in rats of groups III, IV, V and VI showed non significant increase at 14th day, when compared to respective group values at 7th day respectively. However, The serum total globulins concentration in rats of groups I and II showed no significant decrease, when compared to respective group values at 7th day respectively. In present study, serum total globulins was found to be significant (P <0.05) increased in the group given AV yoghurt compared to yoghurt and AV 20% group at 14th day.

Albumin/globulin Ratio: Table 5 shows the mean values of Serum albumin/globulin (A/G) ratio in different groups at 7th and 14th day of experimental trial. The mean values of serum A/G ratio in male rats of groups I, II, III, IV, V and VI ranged between (1.70±1.61 to 3.95±1.52) and (-0.33±1.92 to 2.32±0.71) after 7 and 14 days of feeding respectively.
Table 5: Albumin-globulin (A/G) ratio in rats at 7th and 14th day of study

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Day 7</th>
<th>Day 14</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Distilled Water (Control, C)</td>
<td>3.46±1.01</td>
<td>-0.33±1.92</td>
</tr>
<tr>
<td>II</td>
<td>Plain Yoghurt (Y)</td>
<td>2.31±0.78</td>
<td>1.63±2.88</td>
</tr>
<tr>
<td>III</td>
<td>Aloe vera 10% (AV 10%)</td>
<td>3.68±0.95</td>
<td>1.44±0.26</td>
</tr>
<tr>
<td>IV</td>
<td>Y + AV 10% (AY 10%)</td>
<td>2.74±0.55</td>
<td>1.37±0.28</td>
</tr>
<tr>
<td>V</td>
<td>Aloe vera 20% (AV 20%)</td>
<td>3.95±1.52</td>
<td>2.32±0.71</td>
</tr>
<tr>
<td>VI</td>
<td>Y + AV 20% (AY 20%)</td>
<td>1.70±1.61</td>
<td>0.84±0.21</td>
</tr>
</tbody>
</table>

Values are means ± SEM for 5-6 rats per group.

At 7th day of experiment, the mean values of serum A/G ratio of male rats of groups I, II, III, IV, V and VI were 3.46±1.01; 2.31±0.78; 3.68±0.95; 2.74±0.55; 3.95±1.52 and 1.70±1.61 respectively. The rats fed 20% AV extract alone showed the highest mean value (3.95±1.52) of serum A/G ratio, while rats fed 20% AV yoghurt showed the lowest one (1.70±1.61) at 7th day. The mean serum A/G ratio in rats of different groups did not show significant alterations.

At 14th day of experiment, the mean values of serum A/G ratio of male rats of groups I, II, III, IV, V and VI were -0.33±1.92; 1.63±2.88; 1.44±0.26; 1.37±0.28; 2.32±0.71 and 0.84±0.21 respectively. The control rats showed the lowest mean value (-0.33±1.92) of serum A/G ratio, while rats fed 20% AV extract alone showed the highest one (2.32±0.71) at 14th day. The mean values of A/G ratio in male rats of group I, III, IV, V and VI showed marginal decrease at 14th day, when compared to respective group values at 7th day. However, the mean values of group II showed marginal increase at 14th day, when compared to respective group values at 7th day.

Serum Lipid Profile

Serum Triglycerides: Table 6 summarizes the mean values of serum triglycerides concentrations (mg/dL) in different groups at 7th and 14th day of experimental duration. The mean values of serum triglycerides concentrations in rats of groups I, II, III, IV, V and VI after 7 and 14 days of feeding ranged between (151.19±4.79 to 166.15±5.57) and (106.13±8.73 to 152.86±2.11) mg/dL respectively.

At 7th day of experiment, the mean values of serum triglycerides of rats of groups I, II, III, IV, V and VI were 166.15±5.57; 155.69±3.58; 156.93±3.33; 157.14±6.24; 151.19±4.79 and 152.86±2.11 mg/dL respectively. The control rats showed the highest mean value (166.15±5.57) of serum triglycerides, while rats fed 20% AV extract alone showed the lowest one (151.19±4.79) at 7th day. The mean serum triglycerides values in rats of different groups did not show any significant alterations.

At 14th day of experiment, the mean values of serum triglycerides of rats of groups I, II, III, IV, V and VI were 152.86±2.11; 140.62±9.68; 106.13±8.73; 115.23±24.85; 122.37±7.55 and 106.23±9.96 mg/dL respectively. The control rats showed the highest mean value (152.86±2.11) of serum triglycerides, while rats fed 10% AV extract alone showed the lowest one (106.13±8.73) at 14th day. The mean values of serum triglycerides in rats fed 20% AV extract alone showed significant (P < 0.05) decrease when compared with rats in control group. In addition, the mean values of serum triglycerides in rats of groups III and VI showed significant (P < 0.05) decrease when compared with rats in control and yoghurt groups.

The serum triglycerides concentration in rats of groups I, III, V and VI showed significant (P < 0.05) decrease at 14th day, when compared to respective group values at 7th day. However, the serum triglycerides concentration in rats of groups II and IV showed non significant decrease at 14th day, when compared to respective group values at 7th day.

Serum Total Cholesterol: Table 7 shows the mean values of serum total cholesterol concentrations (mg/dL) in different groups at 7th and 14th day of experimental AV and/or Yoghurt feeding. The mean values of serum total cholesterol concentrations in rats of groups I, II, III, IV, V and VI after 7 and 14 days of treatment ranged between (90.52±3.89 to 109.12±20.85) and (90.17±7.59 to 112.93±18.03) mg/dL respectively.

At 7th day of experiment, the mean values of serum total cholesterol of rats of groups I, II, III, IV, V and VI were 90.63±5.29; 109.12±20.85; 96.30±12.28; 99.62±12.35; 100.52±3.89 and 103.40±8.66 mg/dL respectively. The yoghurt fed rats showed the highest mean value (109.12±20.85) of serum total cholesterol, while rats fed 20% AV extract alone showed the lowest one (90.52±3.89) at 7th day. The mean serum total cholesterol values in rats of different groups did not show any significant alterations.
Table 7: Serum total cholesterol concentrations (Mean ± SEM, mg/dL) in rats at 7th and 14th day of study

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Serum total cholesterol conc. (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Day 7</td>
</tr>
<tr>
<td>I</td>
<td>Distilled Water (Control, C)</td>
<td>90.63±5.29</td>
</tr>
<tr>
<td>II</td>
<td>Plain Yoghurt (Y)</td>
<td>109.12±20.85</td>
</tr>
<tr>
<td>III</td>
<td>Aloe vera 10% (AV 10%)</td>
<td>96.30±12.28</td>
</tr>
<tr>
<td>IV</td>
<td>Y + AV 10% (AY 10%)</td>
<td>99.62±12.35</td>
</tr>
<tr>
<td>V</td>
<td>Aloe vera 20% (AV 20%)</td>
<td>90.52±3.89</td>
</tr>
<tr>
<td>VI</td>
<td>Y + AV 20% (AY 20%)</td>
<td>103.40±8.66</td>
</tr>
</tbody>
</table>

Values are means ± SEM for 5-6 rats per group.

At 14th day of experiment, the mean values of serum total cholesterol of rats of groups I, II, III, IV and VI were 108.09±9.50; 90.17±7.59; 112.93±18.03; 97.66±5.19; and 100.41±5.21 mg/dL respectively. The rats fed 10% AV extract alone showed the highest mean value (112.93±18.03) of serum total cholesterol, while rats fed yoghurt alone showed the lowest one (90.17±7.59) at 14th day. The mean values of serum total cholesterol in rats of group III showed non significant increase when compared with control. However, the mean values of groups II, IV, V and VI showed non significant decrease compared to control group.

The yoghurt fed rats showed the lowest values of serum total cholesterol could be due to the consumption of yoghurt that decreased serum total cholesterol probably by preventing the absorption of cholesterol or lipids.

Also, the results showed a non significant decrease in serum total cholesterol levels in AV fed rats and this may be due to liver dysfunction and malnutrition. Also, as yoghurt consumption lead to a marked non significant decrease in serum total cholesterol levels, the results showed also decrease in serum total cholesterol levels in AV yoghurt fed rats.

Serum HDL Cholesterol: Table 8 shows the mean values of serum HDL cholesterol concentrations (mg/dL) in different groups at 7th and 14th day of experiment. The mean values of serum HDL cholesterol concentrations in male rats of groups I, II, III, IV, V and VI after 7 and 14 days of treatment ranged between (94.73±8.94 to 113.34±13.37) and (102.13±8.92 to 127.21±13.96) mg/dL respectively.

At 7th day of experiment, the mean values of serum HDL cholesterol of male rats of groups I, II, III, IV, V and VI were 113.34±13.37; 105.91±19.34; 105.74±13.80; 99.75±9.86; 94.73±8.94 and 97.66±5.56 mg/dL respectively. The control rats showed the highest mean value (113.34±13.37) of serum HDL cholesterol, while rats fed 20% AV extract alone showed the lowest one (94.73±8.94) at 7th day. The mean serum HDL cholesterol values in rats of different groups did not showed any significant alterations.

At 14th day of experiment, the mean values of serum HDL cholesterol of male rats of groups I, II, III, IV, V and VI were 108.21±10.07; 105.91±19.34; 102.13±8.92; 119.81±13.32 and 110.47±6.66 mg/dL respectively. The rats fed 10% AV yoghurt showed the highest mean value (127.21±13.96) of serum HDL cholesterol, while rats fed yoghurt alone showed the lowest one (102.13±8.92) at 14th day. The mean values of serum HDL cholesterol in male rats of all groups showed no significant difference compared with control group.

Table 8: Serum HDL-C concentrations (Mean ± SEM, mg/dL) in rats at 7th and 14th day of study.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Serum HDL cholesterol conc. (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Day 7</td>
</tr>
<tr>
<td>I</td>
<td>Distilled Water (Control, C)</td>
<td>113.34±13.37</td>
</tr>
<tr>
<td>II</td>
<td>Plain Yoghurt (Y)</td>
<td>105.91±19.34</td>
</tr>
<tr>
<td>III</td>
<td>Aloe vera 10% (AV 10%)</td>
<td>105.74±13.80</td>
</tr>
<tr>
<td>IV</td>
<td>Y + AV 10% (AY 10%)</td>
<td>99.75±9.86</td>
</tr>
<tr>
<td>V</td>
<td>Aloe vera 20% (AV 20%)</td>
<td>94.73±8.94</td>
</tr>
<tr>
<td>VI</td>
<td>Y + AV 20% (AY 20%)</td>
<td>97.66±5.56</td>
</tr>
</tbody>
</table>

Values are means ± SEM for 5-6 rats per group.

Interestingly, the serum HDL cholesterol concentration in rats fed high AV levels in groups V and VI showed a non significant decrease when compared to respective group values at 7th day. However, the serum HDL cholesterol concentration in rats of groups III, IV, V and VI showed non significant increase at 14th day, when compared to respective group values at 7th day.

Serum LDL Cholesterol: Table 9 shows the mean values of serum LDL cholesterol concentrations (mg/dL) in different groups at 7th and 14th day of experiment. The mean values of serum LDL cholesterol concentrations in male rats of groups I, II, III, IV, V and VI after 7 and 14 days of treatment ranged between (41.69±14.90 to 50.57±7.87) and (-13.08±24.28 to 37.14±8.55) mg/dL respectively.

At 7th day of experiment, the mean values of serum LDL cholesterol of male rats of groups I, II, III, IV, V and VI were 44.21±13.38; 41.69±14.90; 42.93±11.62;
Table 9: Serum LDL-C concentrations (Mean ± SEM, mg/dL) in rats at 7th and 14th day of study

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Day 7</th>
<th>Day 14</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Distilled Water (Control, C)</td>
<td>44.21±13.38</td>
<td>37.14±8.55</td>
</tr>
<tr>
<td>II</td>
<td>Plain Yoghurt (Y)</td>
<td>41.69±14.90</td>
<td>31.84±9.95</td>
</tr>
<tr>
<td>III</td>
<td>Aloe vera 10% (AV 10%)</td>
<td>42.93±11.62</td>
<td>-10.06±12.57</td>
</tr>
<tr>
<td>IV</td>
<td>Y + AV 10% (AY 10%)</td>
<td>48.50±12.20</td>
<td>-13.08±24.28</td>
</tr>
<tr>
<td>V</td>
<td>Aloe vera 20% (AV 20%)</td>
<td>47.78±4.98</td>
<td>-0.14±14.52</td>
</tr>
<tr>
<td>VI</td>
<td>Y + AV 20% (AY 20%)</td>
<td>50.57±7.87</td>
<td>-5.94±10.22</td>
</tr>
</tbody>
</table>

Values are means ± SEM for 5-6 rats per group.

48.50±12.20; 47.78±4.98 and 50.57±7.87 mg/dL respectively. The rats fed 20% AV yoghurt showed the highest mean value (50.57±7.87) of serum LDL cholesterol, while yoghurt rats showed the lowest one (41.69±14.90) at 7th day. The mean serum LDL cholesterol values in rats of different groups did not show any significant alterations.

At 14th day of experiment, the mean values of serum LDL cholesterol of male rats of groups I, II, III, IV, V and VI were 33.23±1.11; 31.14±0.72; 31.39±0.67; 31.43±1.25; 30.24±0.96 and 31.47±1.71 mg/dL respectively. The control rats showed the highest mean value (33.23±1.11) of serum LDL cholesterol, while rats fed 10% AV extract alone showed the lowest one (30.24±0.96) at 7th day. The mean serum LDL cholesterol values in rats of different groups did not show any significant alterations.

At 14th day of experiment, the mean values of serum LDL cholesterol of male rats of groups I, II, III, IV, V and VI were 30.57±0.42; 28.12±1.94; 21.23±1.75; 23.05±4.97; 24.47±1.51 and 21.25±1.99 mg/dL respectively. The control rats showed the highest mean value (30.57±0.42) of serum LDL cholesterol, while rats fed 10% AV extract alone showed the lowest one (21.23±1.75) at 14th day. The mean serum LDL cholesterol values in rats of different groups showed significant (P<0.05) decrease in the mean values of serum LDL cholesterol compared with control group.

The serum LDL cholesterol concentration in rats of groups II and IV showed no significant decrease compared with control group, while in groups III, V and VI showed significant (P<0.05) decrease in the mean values of serum LDL cholesterol compared with control group.

The serum LDL cholesterol concentration in rats of groups III, IV, V and VI showed significant (P < 0.05) decrease at 14th day, when compared to respective group values at 7th day respectively. However, rats of group II showed non significant decrease in the mean values of serum LDL cholesterol compared at 14th day, when compared to respective group values at 7th day respectively. Interstingly, the serum LDL cholesterol concentration in rats fed AV with yoghurt showed a marked decrease at 14th day, when compared to respective group values at 7th day respectively.

Serum VLDL Cholesterol: Table 10 shows the mean values of serum VLDL cholesterol concentrations (mg/dL) in different groups at 7th and 14th day of experiment. The mean values of serum VLDL cholesterol concentrations in male rats of groups I, II, III, IV, V and VI after 7 and 14 days of treatment ranged between (30.24±0.96 to 33.23±1.11) and (21.23±1.75 to 30.57±0.42) mg/dL respectively.

Table 10: Serum VLDL-C concentrations (Mean ± SEM, mg/dL) in rats at 7th and 14th day of study

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Day 7</th>
<th>Day 14</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Distilled Water (Control, C)</td>
<td>33.23±1.11</td>
<td>30.57±0.42</td>
</tr>
<tr>
<td>II</td>
<td>Plain Yoghurt (Y)</td>
<td>31.14±0.72</td>
<td>28.12±1.94</td>
</tr>
<tr>
<td>III</td>
<td>Aloe vera 10% (AV 10%)</td>
<td>31.39±0.67</td>
<td>21.23±1.75</td>
</tr>
<tr>
<td>IV</td>
<td>Y + AV 10% (AY 10%)</td>
<td>31.43±1.25</td>
<td>23.05±4.97</td>
</tr>
<tr>
<td>V</td>
<td>Aloe vera 20% (AV 20%)</td>
<td>30.24±0.96</td>
<td>24.47±1.51</td>
</tr>
<tr>
<td>VI</td>
<td>Y + AV 20% (AY 20%)</td>
<td>31.47±1.71</td>
<td>21.25±1.99</td>
</tr>
</tbody>
</table>

Values are means ± SEM for 5-6 rats per group.

At 7th day of experiment, the mean values of serum VLDL cholesterol of male rats of groups I, II, III, IV, V and VI were 33.23±1.11; 31.14±0.72; 31.39±0.67; 31.43±1.25; 30.24±0.96 and 31.47±1.71 mg/dL respectively. The control rats showed the highest mean value (33.23±1.11) of serum VLDL cholesterol, while rats fed 20% AV extract alone showed the lowest one (30.24±0.96) at 7th day. The mean serum VLDL cholesterol values in rats of different groups did not show any significant alterations.

At 14th day of experiment, the mean values of serum VLDL cholesterol of male rats of groups I, II, III, IV, V and VI were 30.57±0.42; 28.12±1.94; 21.23±1.75; 23.05±4.97; 24.47±1.51 and 21.25±1.99 mg/dL respectively. The control rats showed the highest mean value (30.57±0.42) of serum VLDL cholesterol, while rats fed 10% AV extract alone showed the lowest one (21.23±1.75) at 14th day. The mean values of serum VLDL cholesterol in rats of different groups showed significant (P<0.05) decrease in the mean values of serum VLDL cholesterol compared with control group.

The serum VLDL cholesterol concentration in rats of groups III, V and VI showed significant (P<0.05) decrease at 14th day, when compared to respective group values at 7th day respectively. However, rats of groups II and IV showed no significant decrease compared with control group, while in groups III, V and VI showed significant (P<0.05) decrease in the mean values of serum VLDL cholesterol compared with control group.

The serum VLDL cholesterol concentration in rats of groups III, V and VI showed significant (P < 0.05) decrease at 14th day, when compared to respective group values at 7th day respectively. However, rats of groups II and IV showed no significant decrease in the mean values of serum VLDL cholesterol compared at 14th day, when compared to respective group values at 7th day respectively. Interestingly, the serum VLDL cholesterol concentration in rats of group I showed significant (P < 0.05) decrease at 14th day, when compared to respective group values at 7th day respectively.

**DISCUSSION**

The results showed a dose and duration dependent significance increase in the body weight gain. Moreover, the percent of reduction in body weights in control rats
indicated comparatively greater susceptibility of weight loss.

The observations of Boudreau et al. [57], found that the body weights gain of rats exposed to AV which were decreased marginally & significantly at 7th and 14th days of study. The decline in body weights gain in AV could be preliminary attributed to a decrease in food intake, as they claim. However, in our study we found that there is an increase in weight gain of the AV fed experimental animals. The difference between our observation and finding of Boudreau et al. [57] could be attributed to several factors such as the difference in age of rats used, species of rats, concentrations of AV used, type of milk used and stage of lactation of animals where milk was obtained.

In present study, serum total protein was found to be increased in the groups given AV compared with other control groups at 14th day of experiment but in the first week there is a non significant decrease between AV groups compared to other control groups. The present observations of reduction in serum total protein at 7th day of experiment may suggest hepatotoxic effect and that may explained the change in the body weights of rats.

The results of the study showed that there were no statistically significant differences in serum albumin at the 7th and 14th day of the study between the groups. But we can still see reduction in serum albumin at 14th day of experiment, which may indicate the presence of liver injury and disturbance of water metabolism in rats fed AV.

The results of serum total globulins for all groups of rats which showed significant (P <0.05) increase in the groups given AV compared to other control groups suggesting that AV addition may induce tissue injury and increase the immunity response in rats fed AV.

Now serum lipid profile has become an approximate routine test and measured for cardiovascular risk evaluation. The test includes five basic parameters, triglycerides, total cholesterol, HDL cholesterol and VLDL cholesterol.

Triglycerides (TG) are fats that are present in foods like dairy products, cooking oils and meats. The present results clearly showed that fermented milk (yoghurt) in combination with AV lowered serum TG levels. Feeding rats 10% or 20% AV gel significantly reduced serum TG suggesting the adverse effects on lipid metabolism and liver function in rats fed AV. Our results are in agreement with the previous studies [58,59].

Agarwal [60] found a significant reduction in the level of TG in patients with type 2 diabetes that is consistent with our data. In another study, AV extract in dose of 300 mg given on a daily basis to patients with type 2 diabetes for two months. The oral administration of yoghurt resulted in maximum decreases of serum TG, whereas no change in cholesterol concentration observed [61].

In fact, AV extract can construct non-saturated fatty acids that remove free radicals from blood stream and control the metabolism of lipids in the body [62]. In addition, taking AV extract for 8 weeks in diabetic rats can lower the level of TG [59]. It was shown also that AV can significantly decrease the level of plasma TG by inhibiting activation of fat absorption mechanisms [63, 64]. Cholesterol is a fat-like matter that is present in all cells of the body. One of the important tools in the classification and diagnosis of lipemia is the determination of serum cholesterol. Also, one of the main risk factors for heart disease is high blood cholesterol [65]. The present results clearly showed that feeding AV extracts alone or with yoghurt slightly reduced serum total cholesterol (TC). Our study is in agreement with previous studies [58, 61].

In another study, it was shown that the use of AV extract as much as 200 mg/kg on a daily basis for as long as 100 days can significantly reduce the level of TC in normal mice [66]. Nonetheless, AV is beneficial even in short-term intakes (21 days) of 300 mg dose [11]. Some studies have mentioned that maximal dose of 50 mg AV could not improve the level of cholesterol in diabetic rats [67].

The high-density lipoprotein cholesterol (HDL-C) particles increase the removal of cholesterol from cells, especially those in atherosclerotic plaques and transport them to the liver. HDL-C particles also appear to have antioxidant and anti-inflammatory properties [68]. The present results clearly showed that feeding AV extract alone or with yoghurt slightly increased serum total cholesterol (TC), but not significant. Our study is in agreement with previous studies [58, 61].

High level of low-density lipoprotein cholesterol (LDL-C) is considered as the most important cause of atherosclerosis. Aggressive Serum LDL-C decrease strategies are recommended for secondary and primary inhibition of cardiovascular events [69]. The present results clearly showed that feeding AV extracts alone or with yoghurt significantly decreased serum LDL-C. Our study is in agreement with previous studies [58, 61].

Meyer et al. [70] observed that phenolic compounds extracted from fresh grapes were able to inhibit significantly the in vitro LDL-C oxidation at micro molar
phenol concentration. Catechin, epicatechin and gallic acid were particularly active as antioxidants in inhibiting LDL-C oxidation.

The serum very low-density lipoprotein cholesterol (VLDL-C) is synthesized in the liver for exportation of triglycerides to the extra-hepatic tissues [69]. The present results clearly showed that feeding AV extracts alone or with yoghurt significantly decreased serum VLDL-C. Our study is in agreement with previous reports [58, 61].

CONCLUSION

From the obtained results, it can be concluded that:

- Serum total protein was found to be increased in the groups given AV compared with other control groups at 14th.
- Serum total globulins for all groups of rats which showed significant \((P <0.05)\) increase in the groups given AV compared to other control groups suggesting that AV addition may induce increase the immunity response in rats fed AV.
- Feeding fermented milk (yoghurt) in combination with AV lowered serum TG levels.
- Feeding AV extract alone or with yoghurt slightly reduced serum total cholesterol (TC).
- The present results clearly showed that feeding AV extracts alone or with yoghurt significantly decreased serum LDL-C.
- Feeding fermented milk (yoghurt) in combination with AV increased high-density lipoprotein cholesterol (HDL-C).
- Feeding AV extracts alone or with yoghurt significantly decreased serum VLDL-C.

REFERENCES


