Alterations of triglyceride and cholesterol in response to Aloe vera gel extract in HepG2 cells and hyperlipidemic guinea pigs

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Abstract

Aloe vera is well-known for its pharmacological and nutritional properties. The aim of the present study was to determine the effect of A. vera gel extracts on the secretion and cell content of triglyceride (TG) and cholesterol (TC) in HepG2 cells and their short-term effects on the dietary hyperlipidemic guinea pig model. The effects of increasing concentrations of A. vera crude gel and its alcoholic and hydro-extract were compared to HepG2 cells in both basal and TG induced conditions with 20 mM glucose for 24 h. In addition, 24 male guinea pigs were randomly separated into six experimental groups as follows: control, hyperlipidemic control, levostatin control and A. vera receiving groups (fed with lipid-rich diet supplemented with A. vera crude gel, alcoholic or hydro-extracts of A. vera gel). Treatments were carried out for 10 d TG and TC levels were measured in both collected fluid (sera and media) and extracted tissue (HepG2 and liver). Although basal and stimulated conditions of crude gel and its hydro-extract decreased the secretion and cell content of TG, compared to the control ($p<0.05$). This pattern was not seen with the alcoholic extract. Furthermore, A. vera did not have any effect on the serum or liver contents of TG or TC. Our results suggest that A. vera could be a beneficial supplement to modulate the levels of TG and TC. However, it does not appear to be a short-term lipid modulator for hyperlipidemia.

Introduction

It is well-recognized that lipids and the lipoprotein family play a significant role in the formation and progression of atherosclerotic plaques. The National Cholesterol Education Program (NCEP) and the European Atherosclerosis Society Guidelines continue to emphasize the importance of adequate lipid control in the treatment of coronary heart disease. Although specific target levels have not been identified as yet, higher blood plasma level of triglycerides (TG), cholesterol and LDL-C are often cited as markers for increased risk of coronary artery disease (Grundy et al., 2000; American Heart Association, 2002).

Hydroxy-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors are most often prescribed for lowering plasma cholesterol levels in patients with primary hypercholesterolemia. These agents are very effective for this indication, but have a limited ability to decrease triglyceride (TG) levels. For the patient who presents with a concomitant elevation in total cholesterol (TC) and TG levels, treatment options are limited. In this case, the co-administration of an HMG-CoA reductase inhibitor with another drug agent that can reduce TG in this population has been recommended (Alaupovic et al., 1997). A single dose of pitavastatin a potent (HMG-CoA) reductase inhibitor, lowered postprandial triglyceride levels in rats by decreasing chylomicron-triglyceride secretion, probably through a reduction of intestinal microsomal triglyceride transfer protein (MTP) activity and triglyceride droplet formation in the endoplasmic reticulum (Aoki et al., 2002).

However, this combination therapy results in higher costs to the patient, more difficulties with compliance, and an increased risk of myositis and renal failure.

In order to treat patients with hyperlipidemia, several classes of drugs and traditional medicines are available that lower blood serum TC and TG levels. Nowadays, there is an increasing usage of traditional or herbal medicine to lower blood serum TC and TG levels, such as fig tree leaf (Asadi et al., 2006).

To date, different therapeutic effects have been attributed to Aloe vera gel, including a traditional supplement for the treatment of burns and wounds

(Grindlay et al., 1986; Reynolds et al., 1999), anti-inflammation, anti-cancer, and anti-diabetes properties, and the ability to activate macrophages (Reynolds et al., 1999). Furthermore, extracts of A. vera leaves are used for the treatment of eye infections and hepatomegaly and splenomegaly (Chandan et al., 2007).

The ethanolic extract from stem of the plant has antibacterial activity against Escherichia coli . Its leaf extract is active against Mycobacterium tuberculosis and its pulp has both antifertility and oxytocic activities (Vogler et al., 1990). It has been shown that blood glucose level in streptozotocin- (STZ-) induced diabetic rats was significantly lower after the oral administration of an ethanolic extract of A. vera gel (Rajasekaran et al., 2006).

In spite of consuming the A. vera as a vegetable and as a traditional medicine in single and compound prescriptions, few reports have characterized the bioactive constituents of A. vera . Moreover, despite its wide use as a remedy over a long period of time, the biochemical details of its action in terms of physiological and pathological functions have not been systematically investigated (Lime et al., 2003). The objective of the present study was to investigate the effect of A. vera on the lipid state in cell culture and animal models. For this purpose, we used A. vera gel extract and its alcoholic and aqueous extract solutions to detect their effect on the secretion and cellular content of TG and TC in HepG2 cells. Moreover, the effect of short-term consumption of A. vera extracts on the dietary hypercholesterolemic guinea pigs model.

Materials and Methods

A. vera plant was kindly obtained from the garden of University of Zabol (Sistan and Balochestan, Iran; June 2007), and its filets were taken by skinning the leaf, with care to avoid contamination of the gel from the outer layers. At first, fresh extract was used to find the effects of the A. vera crude gel at three different concentration (2, 10, 20 µl/ml) on the lipid states. Then, the crude gel was dried under the flow of nitrogen gas (N_2). Again, 1 g of the dried gel was extracted with distilled water (100 mL) and 1 g prepared with ethanol alcohol (100 mL) for 1 h, using the Soxhlet apparatus.

HepG2 cells maintained at a low passage number were grown in DMEM supplemented with 10% FBS, 1% L-glutamine and penicillin/streptomycin. At 80% confluence, the cells were plated at 12,000 cells/well on 24-well plates for experiments. On the seventh day after plating (at 100% cell confluence), HepG2 cells were switched to DMEM serum-free medium for 2 h, followed by incubation with crude gel in various concentrations of hydro-extract (HE; 0.05%, 0.075%, 0.6%) and alcoholic extract (AE; 0.05%, 0.075%, 0.17%), for 24 h, in both basal and TG-stimulated condition with 20 mM glucose; in this case, TG production was stimulated by 20 mM glucose. Appropriate vehicle controls were used in the experiment. After the incubation period, the medium was removed and cells were washed twice in ice-cold PBS. The lipid component of the cells was extracted after 1 h in 1 mL of mixed hexane: isopropanol (3:2), and were then rinsed with an additional 1 mL of the above solvent mix. Lipid extracts were evaporated to dryness under N_2 gas flow and re-dissolved in 0.1 mL of chloroform-methanol (2:1, V/V) (Maslowska et al., 2006). Then, TG and TC were measured by the method of Neri and Frings (Neri and Frings, 1973) and the lipid component of the medium was extracted according to the method of Bligh and Dyer (Bligh and Dyer 1959). Briefly, lipids of the 1 mL medium were extracted for 15 min in 2 mL chloroform: methanol (1:2, V/V), vortexed, and 1 mL chloroform was added and mixed by vortex for 1 min. Then, 1 mL NaCl solution (0.9%) was added and mixed for 1 min. After centrifugation, the lower phase was collected. The upper phase was re-extracted by the addition of a further 1 mL of chloroform. These collected extracts were pooled and dried under the flow of N_2 gas to dryness and dissolved in 1,500 µL hexane. TG and TC were measured as mentioned previously.

All procedures involving animals were approved by the Animal Care Committee at the School of Veterinary Medicine, University of Tehran, Iran. Twenty-four male white guinea pigs were purchased from Institute of Razi (Karaj, Iran). All guinea pigs were acclimatized in the animal house (12 h light: dark cycle at 22 ± 2°C) for 10 d and received a standard guinea pig diet ad libitum . After the adaptation period, guinea pigs were randomly divided into six separate groups with four guinea pigs in each group, as follows: the control group (fed with the regular diet), the hyperlipidemic control group (fed with a lipid-rich diet containing 1.6% cholesterol and 15% corn oil), the levastatin control group (fed with a lipid-rich diet supplemented with 0.045 g levastatin/day/guinea pig), and the A. vera receiving group (fed with lipid-rich diet supplemented with ACG (0.25 mL/kg/guinea pig), AE (0.075 g/day/guinea pig) or HE (0.2 g/day/guinea pig). After 10 d, guinea pigs that were fasted for 12 h were bled by a needle inserted into the heart after a light anesthesia with petroleum ether, and the sera were collected. Guinea pigs were then sacrificed using deep anesthesia and their livers were extracted.

Serum TG and TC levels were measured using the glycerol-phosphate oxidase -aminophenazone (GPO-PAP) and the cholesterol oxidase -aminophenazone (CHOD-PAP) methods, respectively (Neri and Frings, 1973). Lipid levels in 1 g liver samples were extracted and TG and TC concentrations were measured as described previously.

Statistical analysis was done by a one-way

ANOVA between groups using Sigma Stat 2 software (Systat Software Inc, Point Richmond, CA, USA).

Results

As it is shown in Figure 1, the A. vera crude gel (CG) showed a significant decrease in TG secretion from HepG2 cells compared to both basal condition and induced TG secretion stimulated by 20 mM glucose.

Treatment with AE resulted in a biphasic pattern for both baseline and induced TG secretion. The lowest concentration (0.05%) caused a significant decrease ($p<0.001$) and the highest concentration (0.17%) showed a significant increase ($p<0.001$) in levels of TG secretion from HepG2 cells. As it is also shown in Figure 1, induced TG secretion significantly decreased in response to HE. In this regard, HE decreased TG secretion below the basal level when

Figure1: Cell TG content (€g/mg cell protein, Mean•SD) The effect of different concentrations of Aloe vera crude gel (ACG), hydro-extract (HE) and alcoholic extract (AE). I(ACG ; 2€L/mL), II(ACG;10€L/mL), III(ACG;20€L/mL), IV(HE-0.05%), V(HE-0.075%), VI(HE-0.6%), VII(AE-0.05%), VIII(AE-0.075%), IX(AE-0.17%), X (Control).

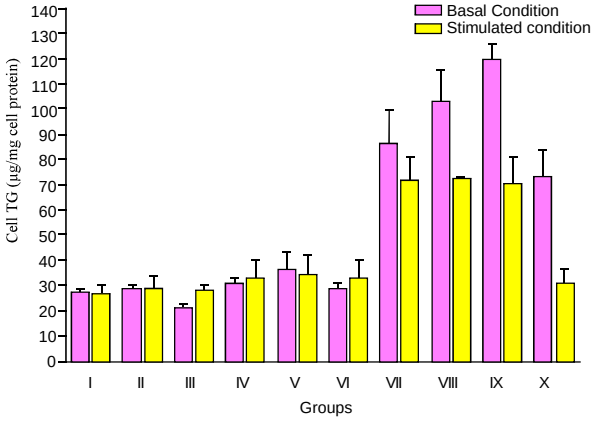


Figure 2: Cell TC content (€g/mg cell protein, Mean•SD) The effect of different concentrations of Aloe vera crude gel (ACG), hydro-extract (HE) and alcoholic extract (AE). I(ACG ; 2€L/mL), II(ACG;10€L/mL), III(ACG;20€L/mL), IV(HE-0.05%), V(HE-0.075%), VI(HE-0.6%), VII(AE-0.05%), VIII(AE-0.075%), IX(AE-0.17%), X (Control).

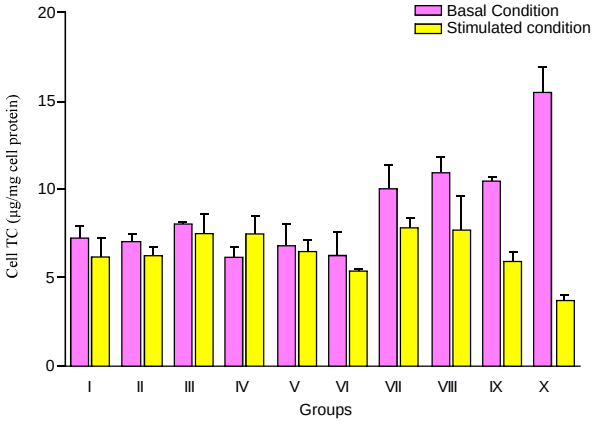


Figure 3: Serum TG concentration (Mean•SD) .The effect of Aloe vera crude gel (CG) , its hydroextract (HE), alcoholic extract (AE) , lovastatin on, negative control (neg. CTRL) and Hyperlipidemic control (Hyp. CTRL).

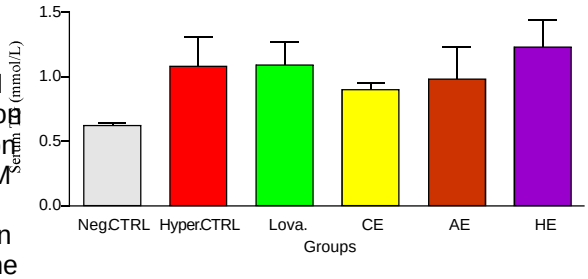
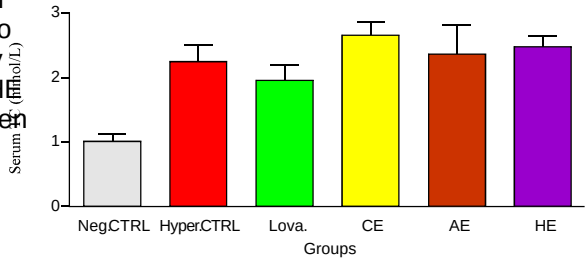


Figure 4: Serum TC concentration (Mean•SD) .The effect of Aloe vera crude gel (CG), its hydroextract (HE), alcoholic extract (AE), lovastatin on, negative control (neg. CTRL) and Hyperlipidemic control (Hyp. CTRL).



treatment was performed in basal conditions.

Induced cell TG content was significantly decreased in response to all three concentrations of CG. In addition, the same pattern was shown during basal conditions. However, cell TG content in response to AE showed a significant increase in both basal and induced cell TG content. Our results showed that HE had a significant effect on the cell TG content of basal states but not on the induced states.

TC secretion in response to CG showed an apparent trend towards a concentration-dependent decrease in both the basal and induced states. In this respect, Figure 2 shows that major effect was due to the HE. Our analyses showed that CG, HE and AE decreased cell TC levels in the basal state but increased it in induced conditions, as compared to the corresponding controls.

The effect of CG, HE, AE and levastatin on serum TG and TC levels (Figures 3 & 4) and liver TG and TC levels (Figures 5 & 6) on the dietary hyperlipidemic guinea pig model. Our analysis showed that A. vera extracts had no significant effects on serum or liver TG and TC levels when compared with the hyperlipidemic guinea pigs (Table 1).

As it is also shown in Figure 7 & 8, the effect of CG, HE, AE and levastatin on media TG and TC levels were the same as effects on cells.

Discussion

Previously, it has been shown that hypolipemic effect of a combination of hydrosoluble chitosan of A. vera gel has been attributed to its capacity to bind dietary lipids, particularly cholesterol, in the stomach. This then forms a

Table 1: The effect of Aloe vera crude gel (ACG; 0.25 mL/kg/day) and its hydroextract (HE; 0.2 g/day/guinea pig), alcoholic extract (AE; 0.075 g/day/guinea pig) and levostatin (0.045 g/day/guinea pig) on the serum and liver total cholesterol (TC) and triglyceride (TG) levels in dietary hyperlipidemic guinea pigs. Experiments were done for 10 d in all treatments, the negative control and hyperlipidemic groups. TC and TG concentrations in the serum and liver were expressed as mean • SD. n=4 separate experiments.

Groups	Serum TG (mmol/L)	Serum TC (mmol/L)	Liver TG (mg/g liver)	Liver TC (mg/g liver)
I (negative control)	0.62 ± 0.02	1.01 ± 0.11	44.88 ± 9.64	5.20 ± 1.05
II (Hyperlipidemia)	1.08 ± 0.23	2.25 ± 0.27	47.18 ± 5.53	14.12 ± 1.05
III (Levostatin)	1.09 ± 0.18	1.96 ± 0.24	46.08 ± 8.05	12.26 ± 1.97
IV (CE)	0.9 ± 0.05	2.66 ± 0.21	38.28 ± 1.61	10.77 ± 2.54
V (AE)	0.98 ± 0.25	2.37 ± 0.46	59.48 ± 2.09	11.64 ± 0.77
VI (HE)	1.23 ± 0.21	2.48 ± 0.17	53.88 ± 13.83	11.59 ± 1.87
p-value (differences among the groups)	I,VI (0.002); I, III (0.018); I,II (0.021)	I,IV; I,VI; I,IV; I,II; I,III (<0.001); III,IV(0.017)	No significant differences	I,II; I,III; I,V; I,VI (<0.001); I,IV (0.002)

Figure 5: Liver TG content (mg/g protein of liver, Mean•SD).The effect of Aloe vera crude gel (CG), its hydroextract (HE), alcoholic extract (AE), lovastatin on, negative control (neg. CTRL) and Hyperlipidemic control (Hyp. CTRL).

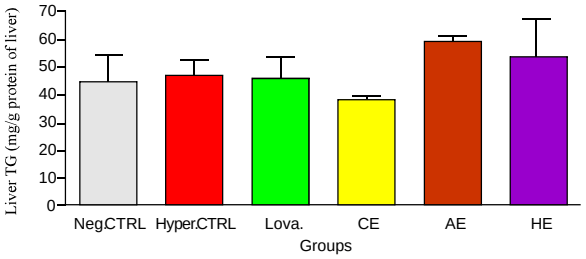


Figure 6: Liver TC content (mg/g protein of liver ,Mean•SD). The effect of Aloe vera crude gel (CG) , its hydroextract (HE), alcoholic extract (AE), lovastatin on, negative control (neg. CTRL) and Hyperlipidemic control (Hyp. CTRL).

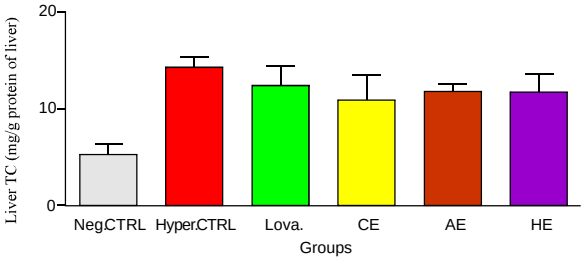


Figure 7: Media TG (€g/mL, Mean•SD) The effect of different concentrations of Aloe vera crude gel (ACG), hydro-extract (HE) and alcoholic extract (AE). I(ACG ; 2€L/mL), II(ACG;10€L/mL), III(ACG; 20€L/mL), IV(HE-0.05%), V(HE-0.075%), VI(HE-0.6%), VII(AE-0.05%), VIII(AE-0.075%), IX(AE-0.17%), X (Control).

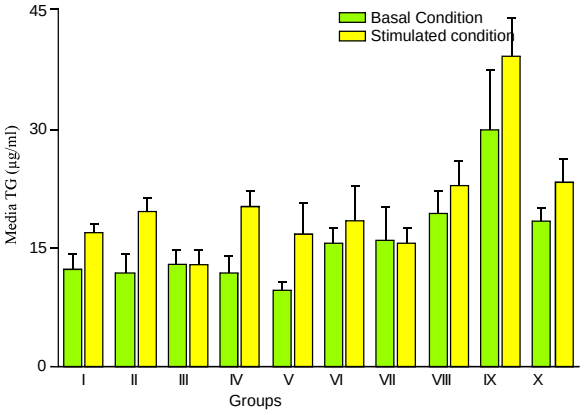
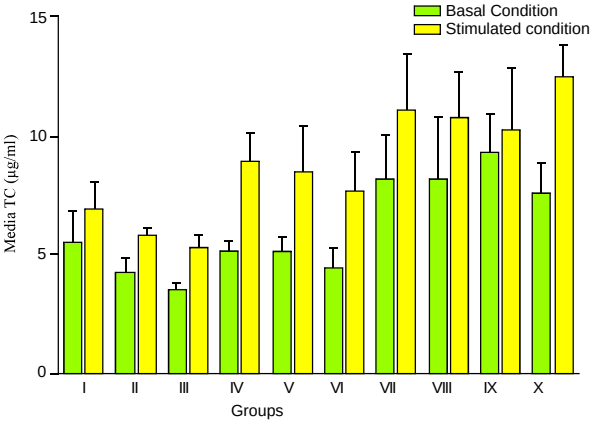


Figure 8: Media TC (€g/mL, Mean•SD) The effect of different concentrations of Aloe vera crude gel (ACG), hydro-extract (HE) and alcoholic extract (AE). I(ACG ; 2€L/mL), II(ACG;10€L/mL), III(ACG; 20€L/mL), IV(HE-0.05%), V(HE-0.075%), VI(HE-0.6%), VII(AE-0.05%), VIII(AE-0.075%), IX(AE-0.17%), X (Control).



gel in the gastrointestinal tract, which is excreted through the feces (Geremias et al., 2006). On the other hand, the effect of A. vera gel extract on the blood lipid profiles of streptozotocin-induced diabetic rats has been shown (Rajasekaran et al., 2006); these results demonstrated that A. vera gel extract normalized lipid profiles after 21 d in these diabetic rats. However, we have shown the novel findings of the insulin-independent effects of A. vera constituents on the lipid status in a cell culture model. In this regard, we have shown that A. vera extract, especially its HE, decreased both the secretion and cell content of TG and TC in HepG2 cells.

Rajasekaran et al. (2006) showed the effect of A. vera extract on the plasma lipoprotein status in the STZ-induced diabetic rats. They argued that treatment with extract normalized plasma lipid status, presumably by the control of lipid metabolism. Moreover, decreases in liver cholesterol, TG, phospholipid and free fatty acids in diabetic rats were shown after treatment with A. vera extract. They argued that A. vera extract has effects on fatty acid synthesis, which means that phenolic compounds and saponins in the gel extract of A. vera may be responsible for its anti-hyperlipidemic effect. Moreover, clinical trials were performed on the diabetic patients using dietary A. vera (Vogler et al., 1990). In all of these treatments, A. vera corrected and improved both the blood sugar and serum lipid (TG and TC) states.

However, in the present study, we have not found any effect of A. vera gel extracts on serum and liver TG and TC concentrations. This may be due to the applied model that was used. To date, the effect of A. vera was

studied in models of diabetes. In addition, all previous studies have shown that the effect of A. vera extracts on the serum lipid profile is due to the insulinogenic effect of A. vera through the activation of pancreatic € cells. In this respect, human clinical trials on the anti-diabetic actions of A. vera are consistent with those of the animal studies (Rajasekaran et al., 2006). It appears that the hypoglycemic, and consequently hypolipidemic, effects of A. vera are mediated through the stimulation of synthesis and/or release of insulin from the € cells of the langerhans islets (Ajabnoor et al., 1999).

The discrepancy between the findings in cell culture and the animal model may be due to the dietary hyperlipidemic model and the duration of supplementation. In all of the above-mentioned studies, supplementation was performed for at least 6 wk, with different dosages, while we have studied short-term effect of A. vera extracts in the non-diabetic dietary hyperlipidemic guinea pigs.

The main chemical constituents of A. vera include amino acids, anthraquinones, enzymes, minerals, vitamins, lignins, monosaccharides, polysaccharides, salicylic acid, saponins and sterols. However, A. vera also contains tannins, resins, mannins, lectins, monosulfonic acid and gibberlin (Khan et al., 2010). Tannic acid (a commercial form of tannin) is a polyphenolic compound, which appears to have direct plasma lipid-lowering effects in rats fed with high levels of cholesterol. The hypolipidemic effect of tannins has been reported by Park et al. (2002) in rats after intraperitoneal injection of tannic acid for 3 wk. In this regard, tannic acid lowered both the plasma lipid concentrations (cholesterol and TG) and hepatic HMG-CoA reductase activity.

This study suggests that A. vera could be a beneficial supplement to modulate the levels of TG and TC. However, it is not appear to be a short-term lipid modulator for hyperlipidemia. More investigation is needed to conduct a suitable usage of this plant for treatment and prevention of hyperlipidemia in human.

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