

EFFECT OF APPLE CIDER VINEGAR ON PLASMA LIPIDS (MODEL EXPERIMENT IN MICE)

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ABSTRACT

Model experiment was carried out to investigate the effect of apple cider vinegar (ACV) on the blood and liver cholesterol (Ch), triglycerides (TG) and one of a marker of antioxidant status of blood (FRAP) in laboratory mice. Animals consumed a basal mice diet (Control) served as the control group. The same diet was supplemented either 1% cholesterol (Ch) or 1% edible sunflower oil (SFO). All groups were duplicated and their animals were supplied drinking water containing ACV (50 mg l⁻¹)(groups: Control+ACV, Chol+ACV, SFO+ACV).The feeding and drinking was ad libitum for 21 days. At the end of experiment the animals were exterminated. Blood and liver samples were analyzed for total cholesterol (tCh), triglycerides (TG) and ferric reducing antioxidant power (FRAP). The results show that the Ch supplemented group stored higher concentration of tCh in the liver (P<0.01) than SFO treated animals. The cholesterol reserves were less in ACV treated groups. The alterations of plasma tCh showed no significant changes by cholesterol or SFO supplementation and drinking ACV containing water. The concentration of plasma and liver TG remained in the same range in all groups independently by different treatments. Animals of SFO supplemented groups (SFO and SFO+ACV) got more fatten than Control and Ch groups and their liver/body mass ratio (%) decreased (P<0.05). The ACV exerted a decreasing effect on the level of plasma tCh and TG markedly (P<0.05) but only in that group (Control+ACV) which consumed the basal diet. This lowering effect could be demonstrated only in the case of TG in the liver. The groups receiving ACV showed decreasing FRAP values. This means a lower antioxidative capacity of plasma. The ACV can helps in the lowering of plasma lipids (tCh and TG) and can depress their liver storage in the case of normal level of lipid consumption. When the lipid input was elevated this benefit not occurred.

Keywords: apple cider vinegar, cholesterol, triglyceride, FRAP, mice

INTRODUCTION

The modern pharmaceutical industry based on synthetic chemistry severed the historical connection between plants, food and medicines. Nowadays food and feed additives of natural origin, used in natural and folk medicine with a partial predilection are coming more and more into the front. Multicomponent botanical therapeutics that comprise functional foods, dietary supplements and botanical drugs hold several advantages over conventional drugs that may earn them a more prominent place in the medicine of the future (Raskin and Ripoll, 2004). One of these natural substances known for hundred years and nowadays living its renaissance is the apple cider vinegar (ACV) which has been helping people to healthier lives. This is claimed by advertisements in different media (journals, TV, InterNet). They argue that ACV can help maintain blood sugar levels in weight management, along with a low calorie diet, by helping to lower the amount of body fat and also helps break down the cholesterol formations that build up on walls of blood vessel.

In the propagating literature can be found that ACV is an essential source for several vitamins and trace elements. It improves renal function and stops multiplication and colonilization of harmful bacteria (Vijayakumar and Wolf-hall, 2002). It has a corrective effect on circulation; it is "blood thinner", helps healing wounds, and speeds up metabolism.

Beneficial effects of ACV have been proved by several practical observations, but there are only a few scientific evidences to prove these facts right. The search for publications in scientific data base surprisingly has only a few scores about the biological experiments with ACV. Practical evidences confirmed that this substance is an outstanding fodder additive for farm animals, based on its vitamin, free amino acid and rich mineral element content. Apart from these and its vinegar (acetic acid) content the substance has other acid components too, such as: citric acid, malic acid and soluble dietary fiber: pectin (Hellmiss, 1997) and sorbose (McComb, 1975) a non-fermentable hexose too. Due to its pectin content ACV has a decreasing effect on the plasma LDL cholesterol level. Specific components in the apple juices and extracts that contributed to antioxidant activity have found that both fresh apple and juices inhibited copper-catalyzed LDL oxidation (Pearson, et al., 1999).

Based on our previous experiments with Japanese quails, which are regularly used test animals for fowls (Wilson et al, 1961), and on turkeys getting 1:100 dilutions of the ACV in drinking water we could state that total cholesterol (tCh) and triacyl-glycerols (TG) had decreased in blood (Bárdos, Kiss, 2000a, and b; Czirle and Bárdos, 2000). Since these are primary factors in applying ACV as an additive for foodstuffs or as a medicinal substance of natural origin. We decided to start a model experiment on

Table 1 Experimental set-up

| Groups | Control | Ch | SFO | Control+ACV | Ch+ACV | SFO+ACV |
|----------------|-----------|----|-----------------------------|-------------|--------|---------|
| Feed | 1 | 2 | 3 | 1 | 2 | 3 |
| Drinking water | Tap water | | Tap water containing 1% ACV | | | |

1. Basal mice feed, 2. Diet 1 containing 1% cholesterol, 3. Diet 1 containing 1% sunflower oil; ACV apple cider vinegar

mammals. This model experiment was carried out to investigate the effect of ACV on the blood and liver cholesterol (Ch) and triglycerides (TG) and one of a marker of antioxidant status of blood (FRAP) in laboratory mice.

MATERIAL AND METHODOLOGY

Animals and experimental set-up

CFLP inbred (Charles River Ltd, Isaszeg, Hungary) laboratory male mice were used in the experiment. Six groups were arranged with ten-ten animal (average weight: 25 g) in each. Animals were fed ad libitum with a basic and/or supplemented feed. Basal diet used for the mice was laboratory mice feed. We mixed the additives with it. After grinding this feed we mixed it with 1% cholesterol (w/w) and with sunflower oil (v/w), respectively. From the mixture we formed scones using cooking gelatin so we could apply them for feeding after dehydration. The control diet without any supplementation but it was reformulated with gelatin too. The drinking water of ACV treated groups was mixing with apple cider vinegar in the ratio of 100 (water) to 1 (ACV) resulted concentration of 500 mg.l⁻¹. The animals were fed for 21 days. Table 1 contents the experimental and feeding set-up.

Feed additives

The experimental feed was supplemented with cholesterol (Fluka, Germany), sunflower oil (purchased in pharmacy) as additives. Commercial apple cider vinegar containing 5% (v/v) acetic acid (Almaacet 5%, Buszesz Ltd., Budapest, Hungary) was added to the drinking water. The used gelatin for the making feed scones was commercial edible grade.

Sampling

Six mice from each group were picked out and lege artis sacrificed at the end of experiment. Blood samples were drawn into tubes containing heparin. The body and liver weights were measured. Blood plasma and liver samples were refrigerated (-20 oC) until the analyzes.

Analytical methods

Total cholesterol (tCh) and triglyceride (TG) concentrations of plasma were measured by enzymatic (GPO-PAP) colorimetric methods with reagents kits (Reanal Ltd., Budapest). Removing the total lipid content from the tissue (Floch et al., 1957) the Ch and TG concentrations from the homogenized liver tissue were measured using the same methods as above. The antioxidant capacity of plasma was characterized by FRAP method (ferric reducing ability of plasma) (Benzie and Strain, 1996).

Statistic

One-way ANOVA with Dunnett's post test was performed using GraphPad Prism version 5.00 for Windows, (GraphPad Software, San Diego California USA, www.graphpad.com).

RESULTS AND DISCUSSION

Our first result is that the basal diet mixed with additives and glued with gelatin results a solid nutrient (feed scones) again. Gelatin is a substantially pure protein food ingredient, obtained by the thermal denaturation of collagen, which is most common protein in the animal kingdom. This meet with our requirements that the additives (cholesterol and sunflower oil) must be dissolved uniform so they can be dosed accurately. The mice consumed this feed readily. Gelatin is not a complete protein source because it is deficient in tryptophan and low in methionine content, however the digestibility is excellent. We could not calculate with the deficiency of these amino acids because the animals were fed for three weeks only.

The literary facts and figures concerning animals reflected only production effects were presented with ACV application in the diet until now. In the present experiments we tried to find a different approach to evaluate the beneficial physiological effects of the ACV in the point of view of lipid metabolism. Mice treated with ACV (Control+ACV, Ch+ACV and SFO+AVC) and its control groups without ACV supplementation (Control, Ch and SFO) were compared.

The group of mice consuming the feed containing Ch and drinking ACV containing water had a little bit smaller bodyweight and liver weight than those of the control and Ch groups (Table 2). During dissection we found in the mice consuming feed enriched with cholesterol large quantities of deposited fat under the skin and in the abdominal cavity i.e. in the mesentery, too. This is the explanation for the smaller weight but bigger size, since fat is lighter weight than other tissues. This phenomenon is an evidence for the weight-reducing effect of ACV, since the group consuming it (Control+ACV) with normal feed had a smaller body weight than those of the control group. Acetic acid administration inhibited the accumulation of body fat and hepatic lipids without changing food consumption or skeletal muscle weight. In conclusion, Acetic acid suppresses accumulation of body fat and liver lipids by upregulation of genes for fatty-acid-oxidation-related proteins by mediation in the liver (Kondo et al. 2009).

In case of the group consuming cholesterol, we found that the mice became extensively fatty. This reflects in the weight ratio of liver/body. Animals of SFO supplemented groups (SFO and SFO+ACV) got more fatten than Control and Ch groups and their liver/body mass ratio (%) decreased compared to Control (P<0.05) (Table 2).

Table 2 Body weight and liver weight (mean \pm SEM)

| Groups | Control | Control+ACV | Ch | Ch+ACV | SFO | SFO+ACV |
|-----------|------------------|-------------------|------------------|-------------------|------------------|--------------------|
| Body (g) | 26,62 \pm 1,22 | 26,19 \pm 1,20 | 26,47 \pm 2,06 | 26,09 \pm 1,16 | 28,12 \pm 2,04 | 26,53 \pm 1,91 |
| Liver (g) | 1,71 \pm 0,21 | 1,45 \pm 0,23** | 1,86 \pm 0,46 | 1,44 \pm 0,15** | 1,82 \pm 0,37 | 1,08 \pm 0,13*** |
| Liver % | 6,45 \pm 0,98 | 5,55 \pm 0,88 | 7,06 \pm 1,82 | 5,52 \pm 0,60** | 6,43 \pm 1,10 | 4,09 \pm 0,59** |

** $p < 0.01$; *** $p < 0.001$ compared to control by Dunnett's Multiple Comparison Test

Table 3 Cholesterol triglyceride and FRAP values of plasma and liver (mean \pm SEM)

| Groups | Control | Control+ACV | Ch | Ch+ACV | SFO | SFO+ACV |
|--------|---------------|-----------------|-----------------|--------------------|---------------------|-------------------|
| Plasma | FRAP (mmol/L) | 0.64 \pm 0.16 | 0.51 \pm 0.1 | 0.66 \pm 0.11 | 0.66 \pm 0.11 | 0.47 \pm 0.25 |
| | tCh (mmol/L) | 1.9 \pm 0.61 | 1.65 \pm 0.32 | 1.47 \pm 0.19 | 1.86 \pm 0.42 | 2.11 \pm 0.23 |
| | TG (mmol/L) | 1.51 \pm 0.5 | 1.19 \pm 0.16 | 0.69 \pm 0.12*** | 0.69 \pm 0.2*** | 0.74 \pm 0.1*** |
| Liver | tCh (mmol/g) | 6.76 \pm 2.55 | 6.8 \pm 1.26 | 25.8 \pm 5.08*** | 24.49 \pm 3.84*** | 8.36 \pm 3.49 |
| | TG (mmol/g) | 11.8 \pm 4.26 | 9.02 \pm 1.57 | 9.32 \pm 2.69 | 10.84 \pm 3.7 | 9.37 \pm 4.16 |

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ compared to control by Dunnett's Multiple Comparison Test

The alterations of plasma tCh showed no significant changes by cholesterol or SFO supplementation and drinking ACV containing water (Table 3). The plasma Ch decreased in Control+ACV group. The results of our experiment show that the Ch supplemented groups stored higher concentration of Ch in the liver ($P < 0.01$) than Controls and SFO treated animals. The storage of Ch was somewhat less in ACV treated groups (Table 3). These findings can be explained by sorbose and pectin content of ACV. One of a non-fermentable (Tamura et al., 1991) carbohydrate constituent of ACV is the L-sorbose (McComb, 1975). Sorbose significantly reduced plasma cholesterol and VLDL by approximately 50%. Absolute and relative abdominal fat weights were and fat content in the pectoral muscle also decreased as dietary sorbose increased (Beyers and Jensen, 1993). It was concluded that dietary sorbose can be used as a potential regulator of lipid deposition in broilers (Furuse et al., 1991). The TG levels of plasma in all supplemented groups were markedly lower ($p < 0.001$) than in animals receiving basal diet (Table 3). The concentration liver TG remained in the same range in all groups independently by different treatments (Table 3). According to Aprikian et al. (2001) the lipoprotein profile was markedly altered in apple-fed rats. The reduction of cholesterol in the triglyceride rich lipoprotein fraction, together with a rise in the HDL fraction was described. This was parallel by effects of the apple on cholesterol absorption, which was markedly depressed, whereas bile acid digestive balance was unaffected. Others have demonstrated that water soluble components of fruits have influence on lipid metabolism. Sugar beet pulp and apple pomace dietary fibers hindered the rise of plasma lipids in rats fed cholesterol (Leontowicz et al., 2001). We have only information of acidity of commercial ACV which was used in our experiment. But there are data in the literature that among the main organic acidic components (acetic, propionic, malic and lactic acid) ACV contains free amino acids, non-fermentable sugar and roughage in the forms of potash and apple pectin (McComb, 1975; Hellmiss, 1997). One of the water soluble dietary fibers is the pectin (Linder 1991). The supplementation of diet with apple dietary fiber from extraction juices or alcohol-insoluble substances had

minor effects on blood serum lipids but the fecal excretion of bile acids increased (Sembries et al., 2004). The ACV which containing this materials exerted a decreasing effect on the level of plasma Ch and TG markedly ($P < 0.05$) but only in that group (Control+ACV) which consumed the basal diet in presented experiment. This lowering effect could be demonstrated only in the case of TG in the plasma. The groups receiving ACV showed decreasing FRAP values compared with the same supplementation without ACV (Table 3). This means a lower antioxidative capacity of plasma because of the direct reduction of the color-forming reagent (ferric tripyridyltriazine), so the antioxidant capacity is proportional to the reducing ability of plasma. These findings are against to others' results. According to Aprikian et al. (2001), there was a positive effect of the apple diet on parameters of oxidative stress prevention: higher FRAP plasma levels than in controls, together with a reduced MDA excretion in urine. In conclusion, their work indicates that the supply of apples elicits interesting effects on lipid and peroxidation parameters. Others found that acetic acid was considered to have an impact on the release of PUFA. For example lipid oxidation products increased in the acid-treated oyster digestive organs. PUFA and lipid oxidation products after treatment with the acid were higher than that in PBS-treated ones at 37 °C (Sajiki et al.1995). In our case presumably these effects occurred in all ACV treated and especially in the SFO+ACV and cholesterol+ACV supplemented groups.

CONCLUSION

The problem of the application of dry matter additives to laboratory mice was solved by gelatin gluing of components. The ACV can help in the lowering of plasma Ch and TG and can depress their liver storage of TG in the case of normal level of lipid consumption. When the lipid input was elevated this benefit not occurred in the blood, but a decreasing tendency of cholesterol and triglyceride contents were determined in the liver.

We hope that our experimental results will bring us nearer to understand of a better and more determined utilization of the ACV, as a natural food additive, concerning both human and animal nutrition.

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