Original Article

Oral Co-administration of Soy-derived Extracts with Alcohol or with Sugar-sweetened Beverages Exerts Liver and Sugar Protective Effects

Tawfik Khoury*, Devorah Rotnemer-Golinkin, Yehudit Shabat, Lidya Zolotarovya and Yaron Ilan

Gastroenterology and Liver Units, Department of Medicine, Hadassah-Hebrew University Medical Center, Jerusalem, Israel

Abstract

Background and Aims: Both alcoholic drinks and high sugar-containing soft drinks cause major health problems worldwide. Oral administration of OS and M1 soy-derived extracts has been shown to alleviate liver injury in animal models. The aim of the present study was to determine the liver- and sugar-protective effect of OS and M1 soy-derived extracts when added to alcohol and sugar-enriched drinks. ***Methods:*** Mice were treated with alcohol or high sugar-containing drinks, with and without administration of a combination of OS and M1 soy extracts. Mice were observed for the effects on liver injury, glucose metabolism, and the immune system. ***Results:*** Co-administration of the soy extracts OS and M1 significantly alleviated the liver injury induced by acute alcohol, as evidenced by decreased liver enzymes. These beneficial effects were associated with promotion of subsets of regulatory T lymphocytes and with a trend towards a pro-inflammatory to an anti-inflammatory cytokine shift. Co-administration of OS M1 soy extracts with sugar-sweetened beverages significantly alleviated the increases in serum sugar levels. ***Conclusion:*** OS and M1 extracts exert a synergistic hepato- and glucose-protective effect in models of alcohol-induced liver damage and soft drinks-associated increases in serum glucose. These extracts may provide a solution to the two pressing health problems.
Citation of this article: Khoury T, Rotnemer-Golinkin D, Shabat Y, Zolotarovya L, Ilan Y. Oral co-administration of soy-derived extracts with alcohol or with sugar-sweetened beverages exerts liver and sugar protective effects. J Clin Transl Hepatol 2017;5(3):208–215. doi: 10.14218/JCTH.2017.00019.

Keywords: Soy; Soda drinks; Alcoholic liver disease; T regulatory cells.
Abbreviations: ALD, alcoholic liver disease; SSBs, sugar-sweetened beverages; GC, β-glucosylceramide; PBS, phosphate buffered saline; H&E, hematoxylin-eosin; AST, aspartate aminotransferase; ALT, alanine aminotransferase; MFI, mean fluorescence intensity; CrEL, Cremophor EL; NS, not significant; AUC, area under the curve; TNF-α, tumor necrosis factor-alpha; IFN-γ, interferon-gamma; IL-10, interleukin-10; ConA, concanavalin A; LAL, liver-associated lymphocyte; NF-κB, nuclear factor-kappa B.
Received: 9 March 2017; Revised: 14 April 2017; Accepted: 21 April 2017
***Correspondence to:** Tawfik Khoury, Gastroenterology and Liver Units, Department of Medicine, Hebrew University-Hadassah Medical Center, PO Box 1200, Jerusalem IL 91120, Israel. Tel: +972-509870611, E-mails: Tawfikkhoury1@hotmail.com, taufick@hadassah.org.il

Introduction

Excess alcohol use is the primary cause of liver-related mortality in western countries. Alcohol is the most commonly abused substance worldwide, and it remains a significant source of liver injury.[1] The prevalence of alcoholic liver disease (ALD) is increasing, and alcohol use is reportedly responsible for 4% of global mortality.[2,3] Alcoholic hepatitis is an inflammatory process of the hepatocytes accompanied by necrosis, and it is associated with a high rate of cirrhosis progression in 40% of cases.[4,5] The pathogenesis of alcohol-mediated liver injury involves interactions of several intracellular signaling pathways in different cell types of the liver.[6] The activation of innate immune cells and the inflammatory cascade also play a role in ALD.[7–9]

Sugar-sweetened beverages (SSBs) are also consumed globally and contribute to obesity.[10,11] The states of overweight and obesity, as well as diabetes have been found to be significantly linked to soft drink consumption worldwide.[12] A recent survey showed that SSBs are the single most modifiable component of diet that can impact preventable death and disability in adults in high, middle, and low-income countries.[13] Preventing alcohol and SSB overconsumption is the best way to stop the related target-organ damage, but it is difficult to achieve in many cases.

Soy is a basic diet nutrient. It contains soluble sugars, vegetable protein, complex carbohydrates, polyunsaturated fat and phytoestrogens.[14] It has been suggested that soy extracts exert a beneficial effect on various organs and on the control of serum lipids.[15] Dietary supplements of soy protein, isoflavones, and cotyledon fiber have demonstrated beneficial effects on cardiovascular risk markers in type 2 diabetes.[16] They have also shown therapeutic efficacy in immune-mediated diseases.[14] Moreover, soy, specifically isoflavones, improve the antioxidant capacity of hepatocytes and decrease oxidative stress.[17] Soy-derived β-glucosylceramide (GC) has been shown to exert an immune modulatory effect in animal models of immune-mediated disorders.[18–26]

Epidemiological studies have shown that soy products might decrease morbidity and improve anti-oxidant ability.[27] Soy protein was also shown to improve alcohol-induced lipid accumulation, oxidative stress and inflammation by decreasing pro-inflammatory cytokines and CYP2E1 protein expression.[27] Oral administration of two soy extracts, M1 and OS, was recently shown to exert an effect on the gut immune system, to alter the distribution of regulatory T cells

and alleviate immune-mediated liver injury, hyperlipidemia and insulin resistance in animal models.[28] The aim of the present study was, therefore, to determine the protective effect of these two soy-derived extracts when co-administered with alcohol and sugar compounds.

Methods

Animals

Mice were maintained in the animal core of the Hebrew University Hadassah Medical School (Jerusalem, Israel). The mice were given the diets described below (alcohol) with free access to water and were maintained in a 12-h light-dark cycle. All experiments were performed in accordance with the guidelines of the Hebrew University-Hadassah Institutional Committee for Care and Use of Laboratory Animals (IACUC protocol number: MD-15-14429-4).

Oral administration of soy fractions

Two soy extracts were obtained from Solbar Israel (CHS Inc., Ashdod, Israel) and studied in the animal models described below. The OS-fraction was derived from the solvent extraction of soybeans into oil and mainly contained tri and diglycerides, free fatty acids and phosphatides. The MI-fraction was derived from aqueous-ethanol extraction left after the solvent extraction and mainly contained isoflavones, sugars (oligo-, di-, mono-) and lipids (including phosphatides, phytosterols, saponins).

I. Assessment of the effect of oral soy extracts on liver damage in acute alcoholic liver injury model

Experimental groups: Approximately 11–12 week-old male mice were purchased from Harlan Laboratories (Jerusalem, Israel). Four groups were examined, and each group contained 10 mice. Mice in control group A were treated with phosphate buffered saline (PBS). To induce acute alcoholic liver injury, mice in group B were orally administered 300 μL of 70% ethanol, administered in three separate doses with 15 min intervals. Mice in group C were orally treated with a similar amount of ethanol, along with 3 mg of GC per mouse, a natural β-glycosphingolipid (Avanti Polar Lipids, AL, United States). Mice in group D were orally administered a similar amount of ethanol, along with 1.5 μg of each of the two soy extracts M1 and OS.

Histological examination of the liver: Livers of all the mice in all the experimental groups were cut into 4–5 μm thin slices, fixed in 10% formaldehyde solution, and kept at room temperature. Tissue blocks were made by embedding in paraffin. Sections were stained with hematoxylin-eosin (H&E) for morphological examination. Specimens were examined under a light microscope. Slides were scored by a blinded pathologist to assess the extent of liver damage.

Liver enzymes: Serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels were determined for all mice in all groups by use of an automatic analyzer.

Assessment of the effect of soy extracts on the systemic immune system: The immune modulatory effect of soy extracts was determined by FACS analysis and serum cytokines.

Flow cytometry: Flow cytometry was performed on splenocytes and hepatic lymphocytes, which were resuspended in 1 mL of FACS buffer (PBS + 1% bovine serum albumin + 0.1% sodium azide). Cells were stained with the diluted anti-LAP antibody (50 μL/sample), FITC-conjugated anti-CD4/CD8 (0.5 μL per sample), PE-conjugated anti-CD25/NK1.1 Pacific Blue–conjugated anti-CD3 (3 μL per sample) and PerCP-conjugated anti-CD45 (2 μL per sample). All stainings were performed after blocking the Fc receptor with anti-mouse CD16/CD32 (BD Fc Block). Flow cytometry was performed using a LSR-II flow cytometer and FCS Express software (BD Biosciences, CA, United States).

Cytokine measurement: Cytokine assessment was performed by MILLIPLEX® Analytes (EMD Millipore Corp., MO, United States) based on the Luminex xMAP® technology for performing immunoassays on the surface of fluorescent-coded magnetic beads MagPlex®-C microspheres. Acquiring and analyzing the data were performed using the Luminex analyzer (MAGPIX®) software. Cytokines assessment was measured by mean fluorescence intensity (MFI).

II. Assessment of the effect of soy extracts on glucose metabolism

Three experiments were performed to examine the effect of soy extracts on glucose metabolism. Effect of co-administration of soy extracts with SSBs:

a. Three groups of C57BL/6 mice (n = 7) that were 11–12 weeks old were studied. Group A was orally administered 350 μL of SSB-I. Groups B and C were orally administered 350 μL of SSB-I mixed with 3 μg soy extract M1 dissolved in 30% Cremophor EL (CrEL) or in double-distilled water, respectively.
b. Five groups of C57BL/6 mice (n = 6), 11–12 weeks old were studied. Group A was orally administered 350 μL of SSB-I. Group B was administered 350 μL of SSB-I and 6 μg of soy extract M1 dissolved in double-distilled water. Group C was given OS soy extract dissolved in double-distilled water and SSB-I. Group D was orally administered 350 μL of SSB-I and the combination of both soy extracts M1 and OS each of 6 μg per mouse dissolved in double-distilled water, and group E was administered 350 μL of SSB-I and 6 μg M1 dissolved in 30% CrEL.
c. Four groups of C57BL/6 mice (n = 2) that were 11–12 weeks old were studied. Mice in group A were orally administered chocolate milk (SSB-II) at 400 μL per mouse. Mice in group B were orally administered a mix of 400 μL of SSB-II and CrEL. Group C was orally administered 400 μL of SSB-II with 2.5 mg of each soy extracts (OS and M1), and group D was given 400 μL of SSB-II and 2.5 mg of each OS and M1 dissolved in CrEL.

Glucose levels in all three experiments were examined at baseline and 15, 30, 60, 90, 120 and 180 min after the SSB administration.

Statistical analysis

All analysis was performed using Excel 2003 (Microsoft, WA, United States). The variables were expressed as the mean ± standard deviation. The comparison of two independent groups was performed using Student's t-test. All tests applied were two-tailed. A p-value of 0.05 or less was considered to be statistically significant.

Results

Oral administration of soy-derived extracts alleviated alcohol-mediated liver injury in mice

Fig. 1A shows the effect of oral administration of the soy-derived extracts (OS, M1) on alcohol-mediated liver injury as measured by the effect on liver enzymes. Both the oral administration of OS and M1 extracts in group D and the oral administration of GC in group C were associated with a statistically significant alleviation of liver injury compared with the untreated controls in group B (33 vs. 132 vs. 32 vs. 57 IU for ALT levels; and 95 vs. 611 vs. 141 vs. 187 IU for AST levels, for groups A, B, C, and D, respectively, $p < 0.05$ between groups). Three mice in groups B and D died, but no mortality was observed in the other groups. The deaths seem to be related to the effect of acute alcohol administration and not to the effect of the drugs.

Fig. 1B shows the effect of soy extracts on the body weight of the mice. There was already a trend towards alleviation of the alcohol-related weight loss in group D compared to group B (27.17 vs. 26.84 g, $p = 0.05$).

Fig. 1C shows representative sections from liver biopsies performed at the end of the treatment period. Alleviation of liver apoptosis and improved hepatocyte architecture were both noted in the mice in groups C and D compared to group B.

Oral administration of soy-derived extracts altered the systemic immune response in mice

Fig. 2 shows the effects of oral administration of soy extracts on different subsets of lymphocytes. A statistically significant decrease in $CD4^+CD25^+$ lymphocytes was noted in the mice in group D compared to the healthy controls (group A) and to the untreated mice in group B (0.96% vs. 1.7% vs. 1.63%, for groups D, A and B, respectively, $p < 0.05$; Fig. 2A). A statistically significant increase in $CD8^+CD25^+Foxp3^+$ lymphocytes was noted in the mice of group D compared to groups B and C (2.45% vs. 1.69% vs. 1.82%, for groups D, B and C, respectively, $p < 0.05$; Fig. 2B). Fig. 2C shows effects on natural killer T lymphocytes. Overall, no significant change was noted in the soy-treated mice in group D compared to the control groups A and B (11.18% vs. 10.44% and 12.75%, p = NS). Similarly, Fig. 2D shows a significant increase in the level of $CD8^+CD25^+$ T lymphocytes in group D compared to group B (0.52% vs. 0.2%, $p < 0.05$). The overall CD4 to CD8 lymphocyte ratio was similar in all mice groups (p = NS). Of note, the ratio was only modestly higher in group D compared to group B (1.1% vs. 0.98%, $p = 0.1$; Fig. 2E)

Fig. 3 shows the beneficial effects on the liver damage associated with a pro-inflammatory to anti-inflammatory cytokine shift. A non-statistically significant trend towards suppression of the serum interferon-gamma (IFN-γ) (22.5 vs. 27.7 MFI; Fig. 3A) and tumor necrosis factor-alpha (TNF-α) (15.4 vs. 17.6 MFI; Fig. 3B) serum levels were noted in the mice of group D compared to group B ($p = 0.1$ and $p = 0.2$, respectively). A similar trend was noted for TNF-α for the mice treated with GC in group C compared to group B ($p = 0.1$). A trend toward a decrease in the IFN-γ to interleukin-10 (IL-10) ratio was noted for mice of groups C and D compared with the untreated controls in group B, reflecting the pro-inflammatory to anti-inflammatory cytokine shift in the treated mice (1.35 vs. 1.1 vs. 1.79 MFI; Fig. 3C).

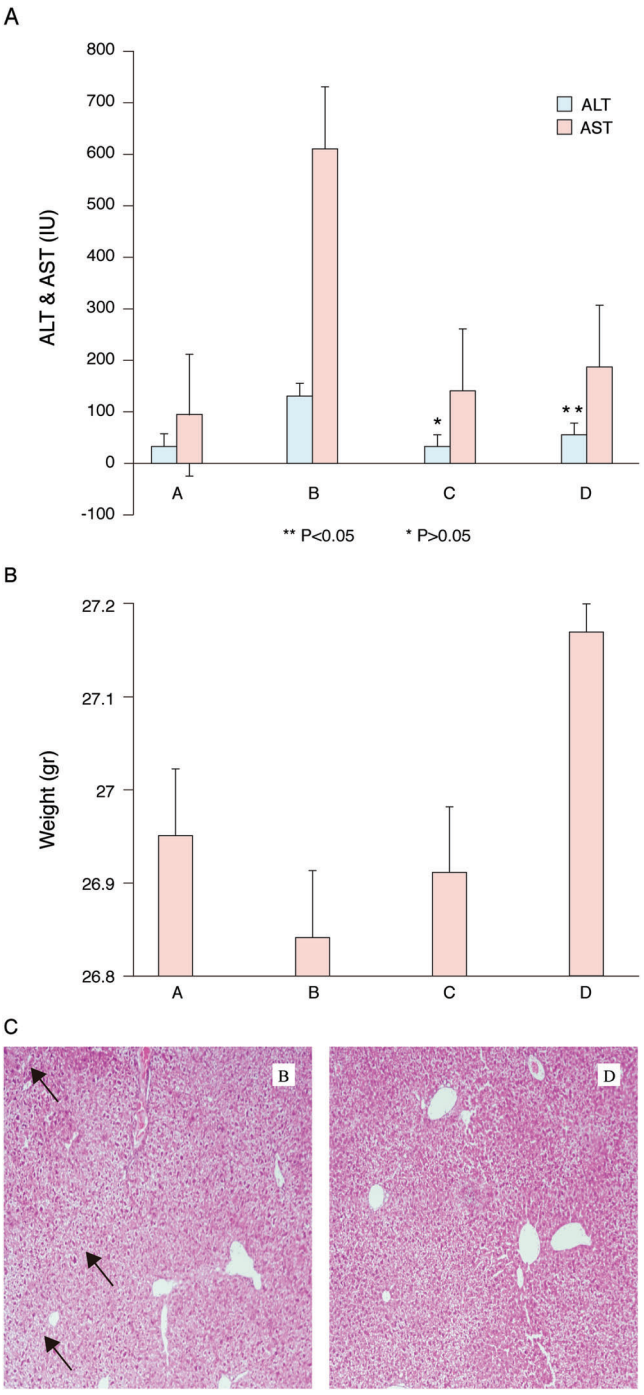

Fig. 1. Effect of oral administration of OS and M1 soy extracts on alcohol-induced liver damage in mice. A. Effects of soy extracts (OS and M1) on liver enzymes. There was a significant decrease in liver enzymes (ALT and AST) in group D compared to group B ($p < 0.05$). **B.** Effects of soy extracts on body weight of mice. A trend toward alleviation of the alcohol-related weight loss was noted in group D compared to group B. **C.** Effects of soy extracts on liver histology representative by H&E stain (magnification ×10) sections from liver biopsies. A decrease in apoptosis and hepatic necrosis in soy-treated group D compared to group B was noted.[47]

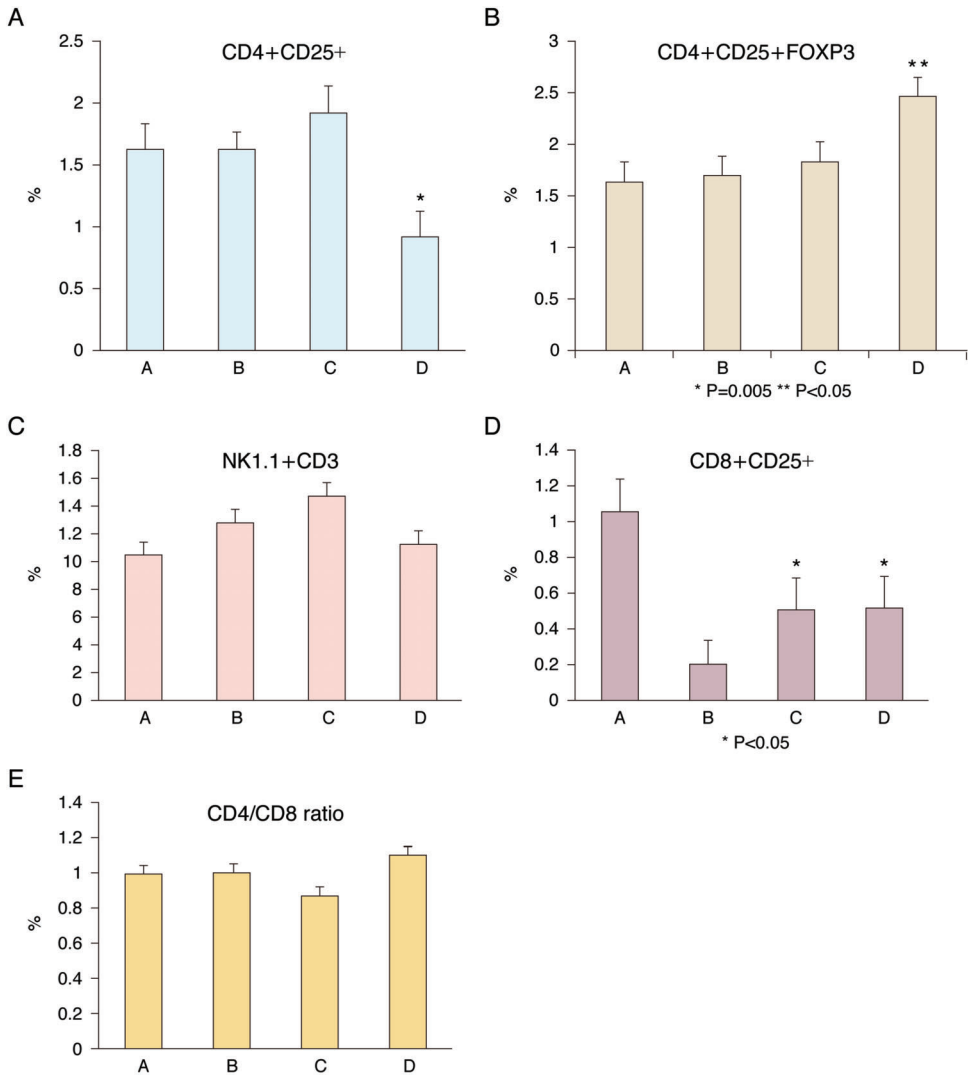

Fig. 2. Soy extracts alter the T regulatory cells in mice. FACS analysis was performed on lymphocyte subsets harvested from mice in all groups to detect: $CD4^+CD25^+$ (A); $CD8^+CD25^+FoxP3^+$ (B); $CD3^+CD56^+$ (C); $CD8^+CD25^+$ (D). The CD4/CD8 ratio was calculated for each group (D).

Oral administration of soy-derived extracts alleviated the SSBs-mediated increase in serum sugar levels in mice

Fig. 4A I show the beneficial effect of adding M1 to SSB-I. Mean blood glucose levels are shown for the 0, 15, 30 and 60 min time points after consumption of SSB-I alone or with supplementation of M1 dissolved in water or in 30% CrEL. A significant decrease in serum sugar levels was noted in the mice treated with soy extracts dissolved in CrEL and in water at 60 min compared to group A (110 vs. 106 vs. 113 mg/dL). A statistically significant decrease was noted in the glucose tolerance test area under the curve (AUC) in mice treated with soy extract M1 (groups B and C) compared to control group A at 60 min (3199 vs. 3101 vs. 3508 for C vs. A, $p < 0.05$, respectively; Fig. 4A II).

Fig. 4B I shows a similar sugar protective effect of soy extracts when given as OS soy extract dissolved in water and M1 dissolved in CrEL; groups C and E were co-administered with SSB-I. A decrease in glucose levels was noted in groups C and E compared to control group A (136.5 vs. 128.3 vs. 138.5 mg/dL at 15 min; 119.7 vs. 115.7 vs. 127 mg/dL at 30 min; and 104.5 vs. 108.5 vs. 111.2 mg/dL at 60 min, for groups C, E and A, respectively). Similarly, the co-administration of both M1 and OS extracts dissolved in water was associated with a decrease in glucose levels compared to controls in group A (104.3 vs. 111.2 mg/dL at 60 min; and 106.2 vs. 109.5 mg/dL at 90 min; and 81.7 vs. 87.5 mg/dL at 180 min, respectively; p = NS). Fig. 4B II shows a significant decrease in the AUC in mice given M1 dissolved in CrEL (group E) at 15 min compared to the control group A ($p = 0.04$), and a trend toward a decrease was noted at 30 min in group E compared to group A ($p = 0.06$). Similarly, a statistically significant decrease in the AUC was noticed in mice co-administered soy extracts M1 and OS dissolved in water (group D) at 90 and 120 min

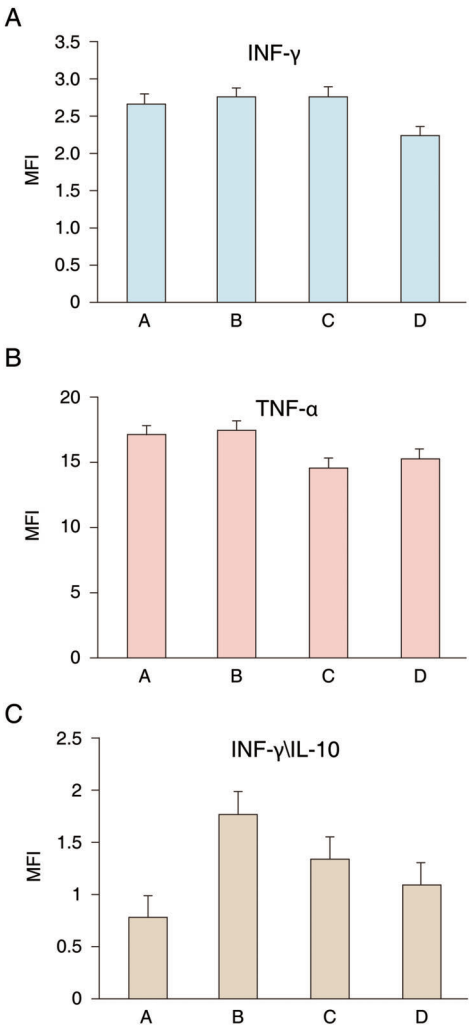

Fig. 3. Effects of soy extracts on the cytokine profile of mice. Serum cytokines were measured at the end of the study for: IFN (A); and TNF (B). The ratio of IFN to IL10 was calculated for all mice in all groups (C).

compared to mice administered soy extract M1 dissolved in water (group B) ($p = 0.05$).

Fig. 4C I shows a similar effect following the co-administration of the soy extracts with high sugar containing chocolate milk (SSB-II). A decrease in glucose levels was noted for the soy extracts-treated groups (groups C and D) compared to the mice in control group A (175.5 vs. 168.5 vs. 200 mg/dL at 15 min; 170 vs. 146 vs. 179 mg/dL at 30 min; 162 vs. 140.5 vs. 164 mg/dL at 60 min; 135.5 vs. 132.5 vs. 161.5 mg/dL at 90 min; 132 vs. 131 vs. 152 mg/dL at 120 min; and 128.5 vs. 125.5 vs. 136 mg/dL at 180 min, for groups C, D, and A, respectively). A significant decrease in glucose levels was noted in the mice in group C (treated with SSB-II mixed with OS and M1) at 90, 120 and 180 min compared to group A (135.5 vs. 132 vs. 128.5 mg/dL, compared to 161.5 vs. 152 vs. 136 mg/dL for groups C and A, respectively, $p < 0.05$). Similarly, there was a significant decrease in the glucose level in the group D mice (treated with SSB-II mixed with OS and M1 dissolved in CrEL) at 15, 30, 60, 90, 120 and 180 min compared to the control group A. The decrease was also significant when compared to group C ($p < 0.005$). There was a statistically significant decrease in group D compared to group C at 30 and 60 min. Fig. 4C II shows a statistically significant decrease in the AUC in the mice administered SSB-II dissolved in CrEL (group B) at 15, 30, 60 and 90 min following ingestion compared to the control group A ($p < 0.05$).

The oral administration of both combination of soy extracts OS and M1 (group C) and OS and M1 dissolved in CrEL (group D) was also associated with significant decrease in the AUC at 60 and 90 min for group C, and at 15, 30, 60, 90 and 120 min for group D ($p = 0.01$ and $p = 0.004$, respectively). Mice in group D had a statistically significant decrease in the AUC compared to mice in group C at 15, 30 and 60 min ($p = 0.05$, $p = 0.01$ and $p = 0.03$, respectively). The data support the notion that while CrEL has some glucose-protecting effect, the two soy extracts exert a synergistic effect both with each other and with CrEL.

Discussion

Over consumption of alcohol and SSBs are major global health problems. The results of the present study show that the co-administration of OS and M1 soy-derived extracts exerts protective effects on alcohol-induced liver damage and reduces serum sugar levels. The oral administration of soy-derived extracts alleviated alcohol-mediated liver injury, as noted by a decrease in liver enzymes, improvements in liver histology, and alleviation of alcohol-mediated weight loss. In addition, oral administration of soy-derived extracts alleviated SSB-mediated increases in serum sugar levels. OS, M1, and the combination of both extracts, when co-administered with two types of SSBs, alleviated the SSB-mediated increase in blood sugar levels. A synergistic effect was noted for the combination of both extracts, and for their combination with CrEL.

For both alcohol and SSB consumption, implementing a lifestyle change to eliminate the use of these compounds is a hard to achieve and to sustain for many. Current interventions, including education programs and new laws, have had some impact but are far from ideal.[29–32] There is a lack of evidence for recommending the implementation of alcohol advertising restrictions.[33] Similarly, interventions to reduce the consumption of SSBs have been only partially successful.[34] While it is possible to improve settings by making national recommendations, continuous actions are needed to decrease total consumption of sweet products among adolescents.[35] Dietary group interventions can moderately improve intake.[36] However, their effect on global health is limited due to the small number of participants. Moreover, the long-term effects of these interventions are unclear.[37]

Adding protective measures to the drinks which can reduce the risks associated with overconsumption of alcohol and SSBs may overcome some of the behavioral obstacles to reducing alcohol-mediated liver damage and the hyperglycemia and obesity induced by SSBs. Caffeic acid was shown to exert a beneficial effect and reduce the adverse effects of alcohol.[38] In a chronic alcohol model in rats, administration of caffeic acid along with alcohol significantly decreased the serum levels of liver and kidney markers to near-normal levels, and decreased the levels of lipid peroxidation markers.

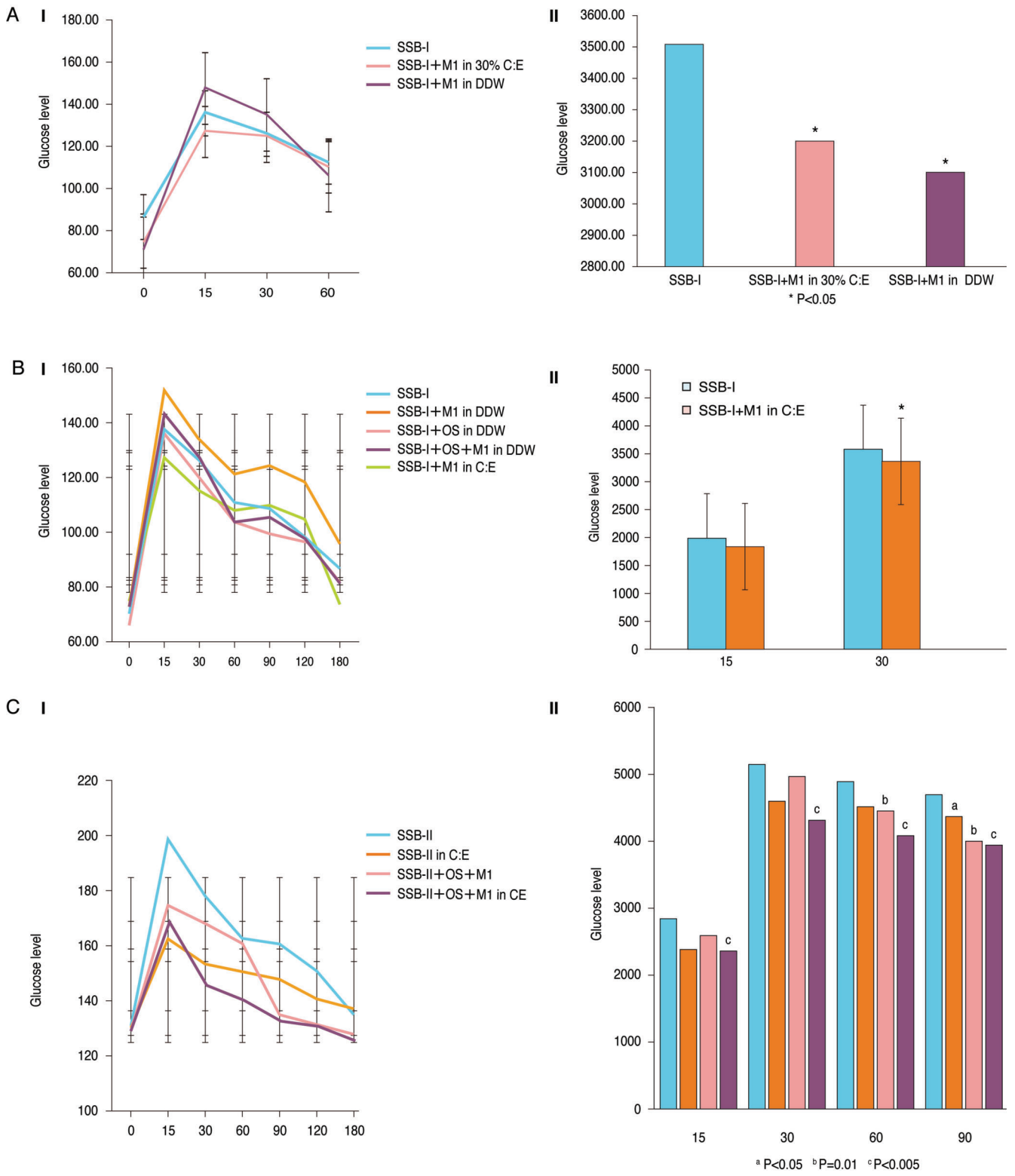

Fig. 4. Effects of soy extract on serum glucose level in mice orally administered SSBs. A. Mice were orally administered SSB-I with or without co-administration of M1 dissolved in water or in CrEL (panel I); the AUC at 60 min was calculated (panel II). **B.** The effect of co-administration of SSB-I with OS or M1 or the combination of both on serum glucose levels. **C.** The effect of co-administration of SSB-II with OS or M1 or the combination of both on serum glucose levels.

The data from the present study suggest that by co-administering safe naturally-derived soy extract, it is possible to alleviate the alcohol-mediated liver damage and to reduce serum sugar levels following the consumption of SSBs. Future studies will determine the effect of these compounds when used in combination with lethal dosages.

The results of the present study suggest that the immune modulatory effect of the OS and M1 soy extracts may underlie the noted beneficial effects. A decrease in $CD4^+CD25^+$, promotion of $CD8^+CD25^+$ and of $CD8^+CD25^+Foxp3^+$ regulatory lymphocytes, and normalization of natural killer T cells number were noted in the treated mice. Treatment was associated with normalization of the altered CD4/CD8 lymphocyte ratio. A trend toward a pro-inflammatory to an anti-inflammatory cytokine shift was observed in the soy extracts-treated mice. Oral administration of OS and M1 had an additive effect in alleviating concanavalin A (ConA) immune-mediated hepatitis, as evidenced by a decrease in liver enzymes serum levels. The hepatoprotective effect was associated with a reduction in pro-inflammatory cytokines. Similarly, oral administration of the OS and M1 soy-derived fraction ameliorated liver injury in the high-fat diet model of non-alcoholic steatohepatitis.[28] The beneficial effect was mediated by reduction of serum TNF-levels, along with the promotion of natural killer T and $CD8^+CD25^+Foxp3^+$ regulatory T cells.[28] Similar immune modulatory-mediated liver and insulin resistance protective effects were described for soy-derived glycosphingolipids.[18–20,24,26,39–41]

The immune system plays a role in the pathogenesis of both ALD and insulin resistance. The liver-associated lymphocyte (LAL) response to alcohol intake along with the immune stimulation determine the susceptibility to liver damage.[6,8] The CD8/CD4 ratio is higher in the LAL population than in peripheral blood. When LALs isolated from ethanol-consuming rats are transferred to non-ethanol-consuming rats, they induces ethanol-mediated damage.[6] Ethanol disrupts antigen presentation by dendritic cells and monocytes, and decreases proliferation of T cell lymphocytes.[42] Toll-like receptors are expressed and activated in innate immune cells and in liver parenchymal cells, thereby leading to ALD.[43] Alcohol-induced sensitization of liver macrophages to portal endotoxin/lipopolysaccharide contributes to the liver damage.[6] Alcohol-mediated activation of downstream signaling pathways including nuclear factor-kappa B (NF-κB) leads to increased pro-inflammatory cytokine production in ALD.[44] TNF-α and interleukin-6 production by LALs are increased by ethanol. Alcohol also shifts the oxidative reaction in the liver toward a pro-oxidant system, causing over-activation of the oxidative cascade, which produces reactive oxygen species and exerts a toxic effect on the hepatocytes.[45,46]

The results of the present study support the ability of OS and M1 soy extracts to normalize several immune derangements induced by alcohol that may be associated with liver damage. Previous studies have demonstrated the immune modulatory effect of OS and M1 soy extracts,[28] and these results support the notion that a similar anti-inflammatory effect may underlie the glucose protective effect noted in the present study. In summary, OS and M1 soy-derived extracts exert hepatoprotective and glucose protective effects in models of alcohol-induced liver damage as well as SSB-mediated increases in serum glucose levels. These beneficial effects were associated with an immunomodulatory effect. The strategy of adding protective compounds into potentially harmful drinks could provide an additional safety measure to educational interventions for the prevention of alcohol and SSB health-related problems.

Acknowledgements

The study was supported in part by the Ruth Epstein Foundation.

Conflict of interest

Ilan Y. is a Chief Marketing Officer of Natural Shield. The others have no conflict of interests related to this publication.

Author contributions

Contributed to the study conception and design (TK, YI), contributed to the conduction of the study (DRG, YS, LZ), contributed to the collection of data (TK, DRG, YS, LZ, YI), responsible for the analysis and interpretation of the data and drafting of the manuscript (TK, YI). All authors approved the final version to be published.

References

[1] European Association for the Study of Liver. EASL clinical practical guidelines: management of alcoholic liver disease. J Hepatol 2012;57:399–420. doi: 10.1016/j.jhep.2012.04.004.
[2] Testino G, Leone S, Borro P. Treatment of alcohol dependence: recent progress and reduction of consumption. Minerva Med 2014;105:447–466.
[3] Paula H, Asrani SK, Boetticher NC, Pedersen R, Shah VH, Kim WR. Alcoholic liver disease-related mortality in the United States: 1980–2003. Am J Gastroenterol 2010;105:1782–1787. doi: 10.1038/ajg.2010.46.
[4] Gao B, Bataller R. Alcoholic liver disease: pathogenesis and new therapeutic targets. Gastroenterology 2011;141:1572–1585. doi: 10.1053/j.gastro.2011.09.002.
[5] Lucey MR, Mathurin P, Morgan TR. Alcoholic hepatitis. N Engl J Med 2009;360:2758–2769. doi: 10.1056/NEJMra0805786.
[6] Batey RG, Cao Q, Gould B. Lymphocyte-mediated liver injury in alcohol-related hepatitis. Alcohol 2002;27:37–41. doi: 10.1016/S0741-8329(02)00213-6.
[7] Curtis BJ, Hlavin S, Brubaker AL, Kovacs EJ, Radek KA. Episodic binge ethanol exposure impairs murine macrophage infiltration and delays wound closure by promoting defects in early innate immune responses. Alcohol Clin Exp Res 2014;38:1347–1355. doi: 10.1111/acer.12369.
[8] Ward RJ, Lallemand F, de Witte P. Influence of adolescent heavy session drinking on the systemic and brain innate immune system. Alcohol Alcohol 2014;49:193–197. doi: 10.1093/alcalc/agu002.
[9] Szabo G, Mandrekar P, Petrasek J, Catalano D. The unfolding web of innate immune dysregulation in alcoholic liver injury. Alcohol Clin Exp Res 2011;35:782–786. doi: 10.1111/j.1530-0277.2010.01398.x.
[10] Qi Q, Chu AY, Kang JH, Jensen MK, Curhan GC, Pasquale LR, *et al*. Sugar-sweetened beverages and genetic risk of obesity. N Engl J Med 2012;367:1387–1396. doi: 10.1056/NEJMoa1203039.
[11] Malik VS, Popkin BM, Bray GA, Després JP, Hu FB. Sugar-sweetened beverages, obesity, type 2 diabetes mellitus, and cardiovascular disease risk. Circulation 2010;121:1356–1364. doi: 10.1161/CIRCULATIONAHA.109.876185.
[12] Basu S, McKee M, Galea G, Stuckler D. Relationship of soft drink consumption to global overweight, obesity, and diabetes: a cross-national analysis of 75 countries. Am J Public Health 2013;103:2071–2077. doi: 10.2105/AJPH.2012.300974.
[13] Singh GM, Micha R, Khatibzadeh S, Lim S, Ezzati M, Mozaffarian D. Estimated global, regional, and national disease burdens related to sugar-sweetened beverage consumption in 2010. Circulation 2015;132:639–666. doi: 10.1161/CIRCULATIONAHA.114.010636.
[14] Friedman M, Brandon DL. Nutritional and health benefits of soy proteins. J Agric Food Chem 2001;49:1069–1086. doi: 10.1021/jf0009246.
[15] Erdman JW Jr. Control of serum lipids with soy protein. N Engl J Med 1995;333:313–315. doi: 10.1056/NEJM199508033330511.
[16] Hermansen K, Søndergaard M, Høie L, Carstensen M, Brock B. Beneficial effects of a soy-based dietary supplement on lipid levels and cardiovascular

risk markers in type 2 diabetic subjects. Diabetes Care 2001;24:228–233. doi: 10.2337/diacare.24.2.228.
[17] Zhang HM1, Chen SW, Zhang LS, Feng XF. The effects of soy isoflavone on insulin sensitivity and adipocytokines in insulin resistant rats administered with high-fat diet. Nat Prod Res 2008;22:1637–1649. doi: 10.1080/14786410701869598.
[18] Ben Ya'acov A, Lalazar G, Livovsky DM, Kanovich D, Axelrod E, Preston S, *et al*. Decreased STAT-1 phosphorylation by a thio analogue of beta-D-glucosylceramide is associated with altered NKT lymphocyte polarization. Mol Immunol 2009;47:526–533. doi: 10.1016/j.molimm.2009.07.030.
[19] Zhang W, Moritoki Y, Tsuneyama K, Yang GX, Ilan Y, Lian ZX, *et al*. Beta-glucosylceramide ameliorates liver inflammation in murine autoimmune cholangitis. Clin Exp Immunol 2009;157:359–364. doi: 10.1111/j.1365-2249.2009.03971.x.
[20] Zigmond E, Preston S, Pappo O, Lalazar G, Margalit M, Shalev Z, *et al*. Beta-glucosylceramide: a novel method for enhancement of natural killer T lymphoycte plasticity in murine models of immune-mediated disorders. Gut 2007;56:82–89. doi: 10.1136/gut.2006.095497.
[21] Livovsky DM, Lalazar G, Ben Ya'acov A, Pappo O, Preston S, Zolotaryova L, *et al*. Administration of beta-glycolipids overcomes an unfavorable nutritional dependent host milieu: a role for a soy-free diet and natural ligands in intrahepatic CD8+ lymphocyte trapping and NKT cell redistribution. Int Immunopharmacol 2008;8:1298–1305. doi: 10.1016/j.intimp.2008.05.005.
[22] Lalazar G, Ben Ya'acov A, Lador A, Livovsky DM, Pappo O, Preston S, *et al*. Modulation of intracellular machinery by beta-glycolipids is associated with alteration of NKT lipid rafts and amelioration of concanavalin-induced hepatitis. Mol Immunol 2008;45:3517–3525. doi: 10.1016/j.molimm.2008.05.009.
[23] Lalazar G, Preston S, Zigmond E, Ben Yáacov A, Ilan Y. Glycolipids as immune modulatory tools. Mini Rev Med Chem 2006;6:1249–1253. doi: 10.2174/138955706778742722.
[24] Lalazar G, Ben Ya'acov A, Livovsky DM, El Haj M, Pappo O, Preston S, *et al*. Beta-glycoglycosphingolipid-induced alterations of the STAT signaling pathways are dependent on CD1d and the lipid raft protein flotillin-2. Am J Pathol 2009;174:1390–1399. doi: 10.2353/ajpath.2009.080841.
[25] Zigmond E, Zangen SW, Pappo O, Sklair-Levy M, Lalazar G, Zolotaryova L, *et al*. Beta-glycosphingolipids improve glucose intolerance and hepatic steatosis of the Cohen diabetic rat. Am J Physiol Endocrinol Metab 2009;296:E72–E78. doi: 10.1152/ajpendo.90634.2008.
[26] Lalazar G, Ben Ya'acov A, Eliakim-Raz N, Livovsky DM, Pappo O, Preston S, *et al*. Beta-glycosphingolipids-mediated lipid raft alteration is associated with redistribution of NKT cells and increased intrahepatic CD8+ T lymphocyte trapping. J Lipid Res 2008;49:1884–1893. doi: 10.1194/jlr.M800113-JLR200.
[27] Yang HY, Lin HS, Chao JC, Chien YW, Peng HC, Chen JR. Effects of soy protein on alcoholic liver disease in rats undergoing ethanol withdrawal. J Nutr Biochem 2012;23:679–684. doi: 10.1016/j.jnutbio.2011.03.016.
[28] Khoury T, Ben Ya'acov A, Shabat Y, Zolotarovya L, Snir R, Ilan Y. Altered distribution of regulatory lymphocytes by oral administration of soy-extracts exerts a hepatoprotective effect alleviating immune mediated liver injury, non-alcoholic steatohepatitis and insulin resistance. World J Gastroenterol 2015;21:7443–7456. doi: 10.3748/wjg.v21.i24.7443.
[29] Langford R, Bonell CP, Jones HE, Pouliou T, Murphy SM, Waters E, *et al*. The WHO Health Promoting School framework for improving the health and well-being of students and their academic achievement. Cochrane Database Syst Rev 2014:CD008958. doi: 10.1002/14651858.CD008958.pub2.
[30] Strobbe S. Prevention and screening, brief intervention, and referral to treatment for substance use in primary care. Prim Care 2014;41:185–213. doi: 10.1016/j.pop.2014.02.002.
[31] Kerr S, Lawrence M, Darbyshire C, Middleton AR, Fitzsimmons L. Tobacco and alcohol-related interventions for people with mild/moderate intellectual disabilities: a systematic review of the literature. J Intellect Disabil Res 2013;57:393–408. doi: 10.1111/j.1365-2788.2012.01543.x.
[32] Klimas J, Field CA, Cullen W, O'Gorman CS, Glynn LG, Keenan E, *et al*. Psychosocial interventions to reduce alcohol consumption in concurrent problem alcohol and illicit drug users: Cochrane Review. Syst Rev 2013;2:3. doi: 10.1186/2046-4053-2-3.
[33] Siegfried N, Pienaar DC, Ataguba JE, Volmink J, Kredo T, Jere M, *et al*. Restricting or banning alcohol advertising to reduce alcohol consumption in adults and adolescents. Cochrane Database Syst Rev 2014:CD010704. doi: 10.1002/14651858.CD010704.pub2.
[34] Bjelland M, Hausken SE, Bergh IH, Grydeland M, Klepp KI, Andersen LF, *et al*. Changes in adolescents' and parents' intakes of sugar-sweetened beverages, fruit and vegetables after 20 months: results from the HEIA study - a comprehensive, multi-component school-based randomized trial. Food Nutr Res 2015;59:25932. doi: 10.3402/fnr.v59.25932.
[35] Anttila J, Rytkönen T, Kankaanpää R, Tolvanen M, Lahti S. Effect of national recommendation on sweet selling as an intervention for a healthier school environment. Scand J Public Health 2015;43:27–34. doi: 10.1177/1403494814558150.
[36] Jancey JM, Dos Remedios Monteiro SM, Dhaliwal SS, Howat PA, Burns S, Hills AP, *et al*. Dietary outcomes of a community based intervention for mothers of young children: a randomised controlled trial. Int J Behav Nutr Phys Act 2014;11:120. doi: 10.1186/s12966-014-0120-1.
[37] Bingham CM, Lahti-Koski M, Puukka P, Kinnunen M, Jallinoja P, Absetz P. Effects of a healthy food supply intervention in a military setting: positive changes in cereal, fat and sugar containing foods. Int J Behav Nutr Phys Act 2012;9:91. doi: 10.1186/1479-5868-9-91.
[38] Pari L, Karthikesan K. Protective role of caffeic acid against alcohol-induced biochemical changes in rats. Fundam Clin Pharmacol 2007;21:355–361. doi: 10.1111/j.1472-8206.2007.00505.x.
[39] Margalit M, Shalev Z, Pappo O, Sklair-Levy M, Alper R, Gomori M, *et al*. Glucocerebroside ameliorates the metabolic syndrome in OB/OB mice. J Pharmacol Exp Ther 2006;319:105–110. doi: 10.1124/jpet.106.104950.
[40] Zigmond E, Tayer-Shifman O, Lalazar G, Ben Ya'acov A, Weksler-Zangen S, Shasha D, *et al*. β-glycosphingolipids ameliorated non-alcoholic steatohepatitis in the Psammomys obesus model. J Inflamm Res 2014;7:151–158. doi: 10.2147/JIR.S50508.
[41] Ilan Y, Maron R, Tukpah AM, Maioli TU, Murugaiyan G, Yang K, *et al*. Induction of regulatory T cells decreases adipose inflammation and alleviates insulin resistance in ob/ob mice. Proc Natl Acad Sci U S A 2010;107:9765–9770. doi: 10.1073/pnas.0908771107.
[42] Szabo G, Mandrekar P. A recent perspective on alcohol, immunity, and host defense. Alcohol Clin Exp Res 2009;33:220–232. doi: 10.1111/j.1530-0277.2008.00842.x.
[43] Szabo G, Petrasek J, Bala S. Innate immunity and alcoholic liver disease. Dig Dis 2012;30 Suppl 1:55–60. doi: 10.1159/000341126.
[44] Mandrekar P, Szabo G. Signalling pathways in alcohol-induced liver inflammation. J Hepatol 2009;50:1258–1266. doi: 10.1016/j.jhep.2009.03.007.
[45] Cederbaum AI. Cytochrome P450 2E1-dependent oxidant stress and upregulation of anti-oxidant defense in liver cells. J Gastroenterol Hepatol 2006;21 Suppl 3:S22–S25. doi: 10.1111/j.1440-1746.2006.04595.x.
[46] Dey A, Cederbaum AI. Alcohol and oxidative liver injury. Hepatology 2006;43:S63–S74. doi: 10.1002/hep.20957.
[47] Barrows IR, Ramezani A, Raj DS. Gut feeling in AKI: the long arm of short-chain fatty acids. J Am Soc Nephrol 2015;26:1755–1757. doi: 10.1681/ASN.2014111157.