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Original Article

Effect of Genetically Modified Soybean Oil Consumption on Biochemical and Histological Changes of Liver and Kidney in Rats

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Abstract

Background: The use of transgenic foods has increased global food production and food security. However, there are concerns about their potential negative impacts on health. Studies conducted on the effect of transgenic products on humans and animals are limited, and they do not provide an answer regarding the possible health hazards of transgenic products. Therefore, this study aimed to examine the effects of a diet containing genetically modified soybean oil on organ health and biochemical changes in an experimental model.

Methods: The current study was conducted on 18 male Wistar rats in three different groups (6 rats per group). One group was fed a diet containing %10 genetically modified soybean oil for 90 days, while the other two groups served as control groups, receiving either non-genetically modified soybean oil or a standard diet, respectively. Body weight and food consumption were measured once and three times a week, respectively.

Results: Our findings indicated that transgenic soybean oil contributed to several histological derangements, including congestion, necrosis, and bile duct hyperplasia in the liver analysis. Similarly, congestion, hemorrhage, and glomerulosclerosis were observed in the kidney analysis. Moreover, transgenic soybean oil significantly increased gamma-glutamyl transferase (GGT) ($P=0.047$) and insulin ($P=0.048$) levels compared to a standard diet. Furthermore, urea and triglycerides (TG) were significantly higher in genetically modified (GM)-fed rats compared to rats fed with standard or non-GM diet ($P<0.001$).

Conclusion: According to the results, a 90-day treatment with transgenic soy-based oil caused significant organ changes in the liver and kidneys of rats. Further studies are needed to evaluate the long-term effects to better elucidate these impacts.

Keywords: Genetically modified, Soybean oil, Histology, Liver, Kidney, Rats

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Introduction

Diet is considered the most influential aspect of life.[1] However, significant advancements in biotechnology have provided several food choices and contributed to the cultivation of genetically modified (GM) crops, presenting a challenge in our food chain.[2] GM plants, in which novel genes have been put into the main genome, have been widely utilized worldwide.[3] These new genes are derived from a bacterium known as *Bacillus thuringiensis*,[4] which produces a protein that is noxious to pests and insects. This protein-producing gene is called the *Bt* gene.[4] The most abundant transgenic products are soybean, canola, corn, and cotton.[5] In the United States, herbicide-tolerant GM soybeans make up 94% of the cultivated soy.[6] Despite the potential benefits of GM products in addressing food insecurity, there are concerns about their safety.[7] Limited published data is available on GM plants.. Some studies have illustrated morphological and molecular changes in the tissues of animals fed with GM crops, including infertility, immune and gastrointestinal dysfunction, accelerated aging, and alterations in insulin balance.[9-12] Several pathways have been suggested for GM products to affect health.[2] Genetic engineering seems to cause unpredictable variations, regardless of the specific

gene transferred,[5] changing different parts of the plant genes.[13,14] Key et al conducted a study demonstrating negative effects on kidney and liver function and tissue in rats fed with GM maize for 91 days.[15,16] This study examined the effects of GM soybean oil on histological and biochemical parameters of the liver and kidney in 2-month-old male rats.

Methods

Diet Formulation

Table 1 presents the composition of the diets used in the study. The GM soybean oil and non-GM soybean oil were prepared. Meals were provided by mixing 10% of both types of oils. The laboratory diet contained 22% protein, 3.48% fat, and 3.71% fiber.

Animals and Housing

Eighteen male Wistar rats (two months old), with an average weight of 195 ± 5 g, were obtained from the animal house of Pasteur Research Institute in Tehran, Iran. The animals were adapted for one week on a normal diet. Then, they were subdivided and randomized into three groups (6 animals per group): one group was fed with a 10% GM-oil diet, another received a 10% non-GM-oil diet, and the third group was given a standard pellet diet for 13 weeks. The rats were housed in standard cages (six rats per cage) under controlled environmental conditions (22–25 ˚C temperature, 55%–60% humidity, and a 12-hour light/dark cycle). Diet and freshwater were prepared ad libitum. During the experimental period, general conditions were checked daily, body weight was recorded weekly, and dietary consumption was measured every two days. At the end of the study, the animals were euthanized by carbon dioxide inhalation and killed. All experimental procedures were conducted following the National Institutes of Health (NIH) ethical guidelines (Publication No. 85-23, revised 1985).

Table 1. Diet Formulation (%)

Ingredients	Standard	Control	GM
Protein (g)	22.5-23.5	22.5-23.5	22.5-23.5
Fat (g)	3.5	13.5	13.5
Carbohydrate (g)	52	52	52
Fiber (g)	4-5	4-5	4-5
Ash	Maximum10	Maximum10	Maximum10
Calcium	0.95-1	0.95-1	0.95-1
Phosphorus	0.65-7	0.65-7	0.65-7
Salt	0.5-0.55	0.5-0.55	0.5-0.55
Moisture	Maximum10	Maximum10	Maximum10
Lysine	1.15-1.2	1.15-1.2	1.15-1.2
Methionine	0.33-0.37	0.33-0.37	0.33-0.37
Methionine + Cysteine	0.63-0.65	0.63-0.65	0.63-0.65
Threonine	0.73-0.75	0.73-0.75	0.73-0.75
Tryptophan	0.25-0.32	0.25-0.32	0.25-0.32
Energy)kcal/g(	330	420	420

Note. GM: Genetically modified.

Biochemistry Examinations

Under anesthesia with ketamine (60 mg/kg BW)/ xylazine (6 mg/kg BW), blood samples were obtained from the animals after an overnight fast to reduce the variabilities of measured parameters. Heparin was used as an anticoagulant to preserve the blood samples. Plasma biochemical parameters, including, gamma-glutamyl transferase (GGT), alanine aminotransferase (ALT), aspartate aminotransferase (AST), total protein, albumin, creatinine, alkaline phosphatase (ALP), urea, uric acid, lipid profile, insulin, glucose, amylase, and lipase were measured using commercial enzymatic kits (Pars Azmun, Tehran, Karaj, and Hangzhou Eastbiopharm, Zhejiang, China).

Histological Examination and Organ Weights

At the end of the experimental period, the immune-related organs, liver, and kidneys were weighed, and a full set of tissues was collected. To preserve the tissues, they were immediately placed in 10% neutral buffered formalin. The preserved tissues were processed and placed in paraffin to make tissues hard enough for cutting. Using standard techniques, tissue sections approximately 4 mm thick were stained with eosin and hematoxylin.[17] These sections were examined by light microscope (Olympus, Japan) because light, or optical, microscopes make cells and morphological features of tissues visible. Light microscopes are essential for histological studies as they use glass lenses and visible light to magnify tissue samples.

Statistical Analysis

Statistical analyses were carried out by SPSS software. Statistical comparisons were made between three groups using a one-way analysis of variance (ANOVA) followed by Tukey's post hoc analysis. An independent sample t-test was performed when appropriate. Data were presented as mean ± standard error (SE), and differences with P values less than 0.05 were considered significant.

Results

The changes in body weight and food intake in the three treatment groups are presented in Figures 1 and 2, respectively. The results showed no significant changes in weight or food intake. Table 2 presents changes in the biochemical analysis for the study groups. The GM soybean oil group showed a significant increase in serum insulin and GGT levels compared to the standard diet group ($P = 0.047$). Urea, triglyceride (TG), and GGT concentrations were significantly higher in GM-fed rats compared to rats fed with standard and non-GM diets ($P < 0.001$), while no significant changes were observed in other parameters. In general, the GM-fed group exhibited higher but non-significant levels of AST, ALP, amylose, glucose, albumin, total protein, uric acid, and total cholesterol, but it exhibited lower levels of high-

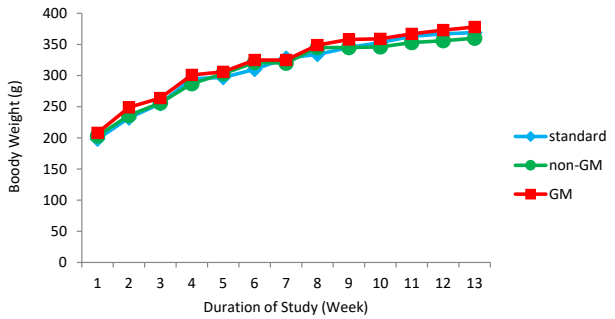

Figure 1. Growth Curves Based on Weekly Measurements of Body Weight During the Study. *Note*. The curves show group means based on 6 rats /group

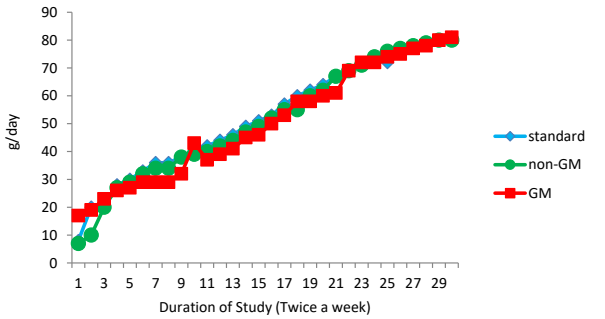

Figure 2. Food Intake Curves Based on Twice a Week Measurements During the Study. *Note*. The curves show group means based on 6 rats/group. Standard error bars are not shown for clarity

Table 2. Biochemical Findings Among Studied Groups

Parameters	Standard	Non-GM	GM	*P* Value
ALT (U/L)	28±87.7	16.4±90.2	32.6±72.91	0.060
AST (U/L)	13.4±65.78	8.7±62.18	3.9±66.98	0.232
ALP (U/L)	65.4±391.9	210±586.9	271±678.9	0.071
GGT (U/L)	0.98±3.16	1.3±6.58	3.4[a] *±8.50	0.047
Insulin (μIU/ml)	15.5±18.53	42.1±54.83	17.8 [a] *±63.75	0.048
Amylase (U/L)	60.3±545.2	52.8±542.53	54.8±575.4	0.110
Lipase (U/L)	38.0±156.0	97.8±216.3	75.8±143	0.231
Glucose (mg/dL)	39.1±171.51	9.8±148.68	11.6±178.68	0.111
Albumin (g/dL)	0.08±2.63	0.21±2.61	0.10±2.83	0.325
TP (g/dL)	0.6±8.01	0.2±7.90	0.4±8.20	0.059
Creatinine (mg/dL)	0.3±0.55	0.3±1.06	0.4±0.48	0.218
Uric acid (mg/dL)	0.4±1.48	0.3±1.33	0.5±1.68	0.061
Urea (mg/dL)	1.3±24.13	4.4±24.93	31.46±0.5 [a,b]**	<0.001
TG (mg/dL)	2.9±51.38	3.4±54.98	15.9 [a,b]*±71.40	<0.001
TC (mg/dL)	5.4±79.26	11.2±84.80	3.8±90.93	0.09
LDL-C (mg/dL)	1.7±6.73	3.2±6.93	3.3±6.36	0.270
HDL-C (mg/dL)	7.1±47.06	1±43.88	8.6±38.10	0.368

Note. GM: Genetically modified; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; ALP: Alkaline phosphatase; GGT: Gamma-glutamyl transferase; TG: Triglyceride; TC: Total cholesterol; LDL-C: Low-density lipoprotein cholesterol; HDL-C: High-density lipoprotein cholesterol. Each value is mean±standard error, and each group consists of five rats; [a] Significantly different versus standard group; [b] Significantly different versus non-GM group (*$P<0.05$, **$P<0.01$).

density lipoprotein (HDL) compared to the other two control groups. The absolute mean organ weights are provided in Table 3. There were no significant differences in the organ weights of rats among the study groups, but GM-fed rats had higher organ weights compared to other control groups. Figures 3 and 4 illustrate histological findings in the liver and kidneys of rats fed with GM soybean oil. In the liver, different levels of degeneration were observed, including vessel and sinusoid congestion, sporadic hepatocyte necrosis, sinusoidal space dilation, and biliary tract hyperplasia (Figure 3). In the kidneys, the effects of the GM oil diet were evident, with observable signs of hemorrhage, vessels and glomeruli congestion, glomerulosclerosis, degenerative changes in the duct cover, and focal presence of inflammatory cells (Figure 4).

Table 3. Absolute Organ Weights for Three Groups

Organ	Standard	Non-GM	GM
Liver (g)	27.03±1.8	27.86±6.5	30.96±1.6
Kidneys (g)	2.4±0.2	2.5±0.5	2.7±0.2

Note. GM: Genetically modified. Each value is mean±standard error.

Discussion

Animal models play a crucial role in assessing the safety of GM products for both livestock and human consumption.[18] The experimental substances used in the present study included a laboratory diet containing 10% GM soybean oil. Alteration in body weight can indicate dysfunction in various organs, so the final body and organ weights were examined among study groups, indicating no significant differences.[19] Similar results were found in a recent study,[20] as well as in a study containing 11% or 33% MON 810 corn, reporting no statistically significant differences in body or organ weights.[21] Likewise, a 90-day animal study on a GM-rice diet exhibited almost similar body and organ weights although the growth curve in the female rats decreased slightly in the GM-rice group in the 12th week of the study and finally returned to normal,

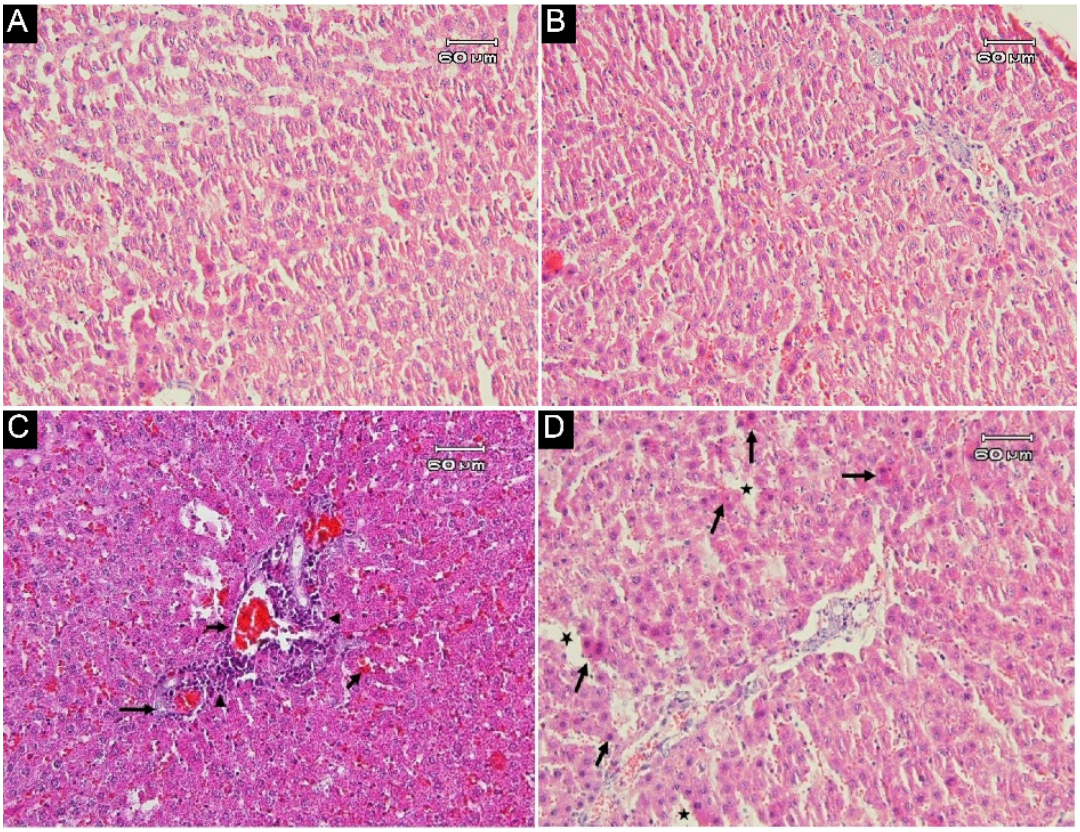

Figure 3. Photomicrographs of Liver Tissue of Rats Stained With H & E. (A) Standard diet shows the normal structure of liver tissue. (B) Non-GM diet for 90 days shows the normal structure of liver tissue. (C) GM diet for 90 days displays slight congestion in the central vein (small arrow), hyperplasia of the biliary tract (long arrow), and the presence of inflammation centers (arrowheads). (D) GM diet for 90 days exhibits sporadic necrosis in hepatocytes (arrows) and dilatation of the central vein (stars) (magnification: 100×)

possibly due to the stress of bleeding and fasting for blood donation.[22]

Histopathological examination showed that the diet containing GM soybean oil caused significant changes in the liver and kidneys of rats. The liver, responsible for biotransformation and detoxification, is particularly crucial, and changes in the liver can affect metabolic processes.[23] In our study, notable changes were observed in the liver tissue of the GM soybean oil group not in the other two groups. Previous studies have also reported significant modifications in hepatocyte nuclear properties in animals fed with GM diets.[24] Additionally, El-Shamei et al demonstrated histopathological changes in the kidneys, liver, and testis of rats fed with GM corn for 90 days.[4] Likewise, several studies reported significant histopathological alterations in the kidneys after GM corn feeding in an experimental model. In the study by Smith et al, MON 863 *Bt* corn caused significant kidney and liver inflammation and damage in rats.[25] In another study, male rats fed with 33% MON 863 *Bt* corn in a 90-day trial exhibited reduced kidney weight, tubular alterations, and inflammation.[8] Kiliç and Akay reported parietal layer extensions in Bowman's capsules and minimal tubule destruction at various ratios in their study groups.[23]

In the present study, an increase in the serum GGT levels was observed in the rats fed with GM soybean oil, indicating liver damage and bile ducts.[26-28] In line with our findings, El-Shamei et al demonstrated that feeding transgenic corn increased serum concentrations of ALT, ALP, and AST in rats. The amount of AST is associated with the extent of tissue damage, which can explain the increased levels of AST and ALP in the GM group in our study.[19] Several studies have reported increased serum ALT levels after the consumption of GM food products, with significantly higher plasma ALT activities observed in female rats fed GM soybean oil.[29] Furthermore, ALT levels increased after consuming transgenic rice in Alexander and colleagues' study.[18] In addition, a study on mice demonstrated slight changes in ALT and AST activity in response to GM soybean consumption.[30]

Moreover, the present study revealed an increase in serum cholesterol and TG levels in the GM soybean oil group, but this increase was not statistically significant ($P = 0.09$). Likewise, it has been indicated that higher cholesterol levels may lead to liver bile duct damage and biliary tract obstruction, resulting in the reduction of duodenal secretion and cholestasis subsequently.[19] In the present study, blood urea concentration significantly increased in rats fed the GM soybean oil, which is similar to the findings of the study by de Vendômois et al who reported increased levels of TG and urea concentrations in soybean-fed group.[31] The liver is responsible for the synthesis of urea, which is excreted by the kidneys. In addition to water and blood gases, urea is considered one of the most important substances in the body.[32] Increased serum urea levels may be due to kidney failure

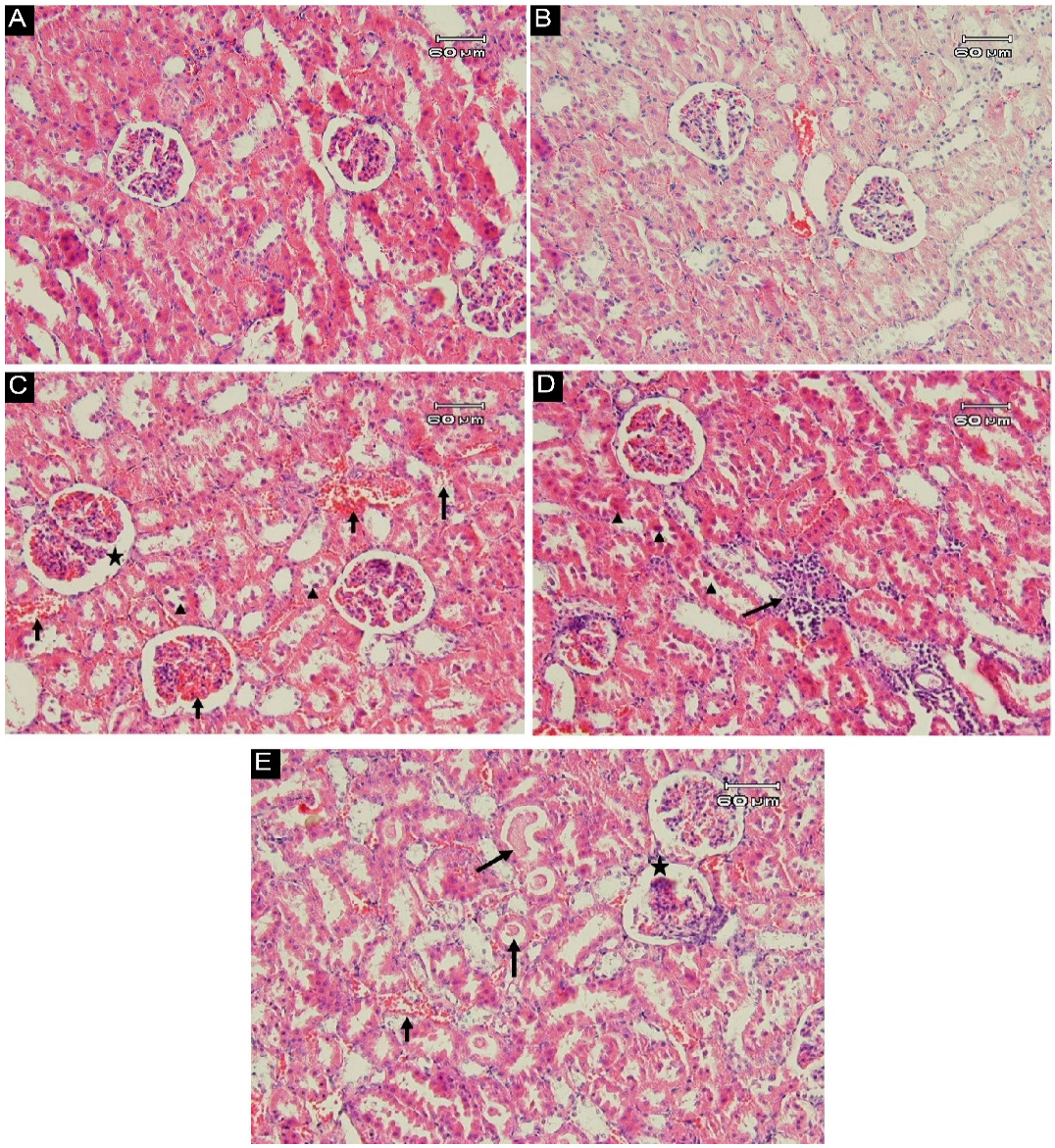

Figure 4. Photomicrographs of Kidney Tissue of Rats Stained With H & E. (A) Standard diet shows the normal structure of kidney tissue. (B) Non-GM diet displays the normal structure of kidney tissue. (C) GM diet shows severe congestion in the vessels and glomeruli (small arrows), hemorrhage in the tissue (long arrows), glomerulosclerosis (star), and degenerative changes in the duct cover (arrowheads). (D) GM diet exhibits focal presence of inflammatory cells (arrows) and degenerative changes in the duct cover (arrowheads). (E) non-GM diet shows congestion in the vessels (small arrow), the presence of hyaline cysts in ducts (long arrows), and glomerulosclerosis (star) (magnification: 100×)

and increased inflammation in the tissue, leading to the inability to excrete substances.[33] Increased blood TG has been reported to be likely due to alteration in liver function.[8] Studies by Oraby et al and Séralini et al displayed that rats consuming GM corn and soybeans have increased AST, ALT, creatinine, and uric acid levels, indicating liver and kidney damage.[8,28] Reduced blood creatinine levels in the GM group may indicate muscle problems, potentially muscle organs such as the heart.[31] Another 90-day study reported increased urea and decreased protein concentrations in male rats fed with *Bt* rice.[29] Other factors that may affect kidney function include inherent mutations in GM technology or new forms of mutated *Bt* gene.[31]

Furthermore, previous studies focused on glucose evaluation and did not measure insulin levels. However, the current study observed a significant difference in insulin levels in the transgenic group compared to the standard and control groups, which may be attributed to the increased number of islets in the pancreas of transgenic rats. In addition, the GM group tended to show higher glucose levels, showing the possibility of insulin resistance in this group. De de Vendômois et al[31] demonstrated that female rats in the GM feeding group exhibit increased circulating glucose and TG levels, indicating a pre-diabetic profile. Furthermore, they observed differential and specific alterations in the TG profile, creatinine, and urine chloride excretion over time in female animals, which are clear signs of toxicity.

The present study has several limitations similar to other studies. First, measuring inflammatory markers would be effective in explaining insulin resistance and other dysfunctions. Second, having direct access to transgenic plants and their oil would be better. Lastly, since our

study involved short-term feeding, we could only observe relatively acute and medium-term effects. Longer-term feeding experiments, extending up to 2 years, are necessary to gain a more complete understanding of the effects.

Conclusion

According to our findings, soy-based transgenic oil contributed to minor histopathological alterations in the liver (i.e, hyperemia, bile duct hyperplasia, inflammatory foci, and diffuse necrosis) and the kidneys (i.e., hyperemia, bleeding, glomerulosclerosis, and hyaline cysts) of rats. Additionally, it significantly increased GGT, insulin, urea, and TG levels while decreasing hemoglobin levels. Long-term feeding experimental models with GM food products are warranted to better clarify the effects of transgenic foods.

Ethics statement

The current study has been approved by the Ethics Committee of the Tabriz University of Medical Sciences (Identifier: IR.TBZMED.VCR.REC.1397.190) and registered on September 3, 2018.
The study was approved by the Veterinary Ethics Committee of the Tabriz University of Medical Sciences (Identifier: IR.TBZMED.VCR.REC.1397.190).

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Conflict of interests declaration

The authors declare that there is no conflict of interests.

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Data availability statement

All of the data are available upon reasonable request.

Author contributions

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Writing–original draft: Horiye Taheri.
Writing–review & editing: Mahdieh Abbasalizad Farhang, Alireza Rahimi Mamaghani.

Consent for publication

Not applicable.

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