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Alleviation of Fatty Liver by Using Soy Proteins in Rat

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Abstract

The purpose of the current investigation is to determine how soy isoflavone (ISF) and genistein (GS) affects oxidative stress, IL1-ß and PPARγ signaling pathways in liver of obese rats and how this pathway is involved in controlling the formation of hepatic fat. The study included 60 male Sprague Dawley rats that were allocated to six groups (n = 10rats) :(I) Fatty liver was induced in rats were daily fed (60% fat energy, high fat diet (HFD)) for 12 weeks ; (II) (Fatty liver + ISF group) rats were daily fed with HFD and 10 mg/Kg ISF intragastrical for 12 weeks ;(III) (Fatty liver + GS group) rats were given HFD and 16 mg/Kg GS in 0.1% DMSO once daily by intragastric tube for 12 weeks; (IV) (Normal control group) rats were fed with normal balanced diet for 12 weeks; (V)(Normal diet + ISF group) rats were fed with normal diet and 10 mg/Kg ISF intragastrical for 12 weeks; (VI) (Normal diet + GS group) rats were fed with normal diet and 16 mg/Kg GS for 12 weeks. All rats allowed water whereas rats got HFD were accompanied by 18% sucrose solution freely. Also, weight was measured weekly. At the end of the experiments lipid profile and liver function were analyzed. Moreover, the levels of MDA, SOD, CAT, and GSH, and the gene expression of IL1-ß and PPARγ genes were detected. Our study showed that fat content was significantly lowered in the liver of ISF and GS -fed obese rats, accompanied by a reduction in hepatocellular vacuolation when compared to the fatty liver control. In ISF and GS fatty liver treated groups SOD, CAT and GSH activities were significantly increased in comparison to the Fatty liver untreated group in addition to that MDA level decreased in ISF and GS groups.IL1-ß expression and PPARγ expressions was dowenregulated in Fatty liver + ISF and Fatty liver+ GS treated rats when compared with Fatty liver one,however the results in Fatty liver+ GS treated rats was significantly Improved over ISF + Fatty liver.Genistein administration alleviated fatty liver through the down-regulation of PPARγ and IL-1 β and up-regulation the activity of oxidative stress marker (SOD , CAT and GSH).

KEYWORDS

Soy isoflavone, Genistein, Oxidative stress.

INTRODUCTION

Chronic diseases are responsible for 35 million deaths annually. Non-alcoholic fatty liver disease (NAFLD) is a major cause of chronic liver disease worldwide (Bedossa, 2017). The estimates of the worldwide prevalence of NAFLD range from 6.3% to 33%, with a median of 20% in the general population, based on the assessment method. Its prevalence rate was estimated to be ranged from 20%–25% in Western countries and 25%–40% in the Middle East (Bellentani, 2017).

It is characterized by the accumulation of triglyceride in the hepatocytes, exceeding 5–10% of liver weight. Most NAFLD patients may suffer only from increased liver fat and this condition called simple steatosis while others may suffer from simple steatosis plus inflammation and ballooning degeneration which defined as nonalcoholic steatohepatitis (NASH). Up to 20% of patients have the ability to progress to advanced fibrosis, cirrhosis, and ultimately hepatocellular carcinoma (HCC) (Mahmoud *et al.*, 2015).

The pathogenesis of NAFLD is complex and multifactorial. A critical role in NAFLD progression is attributed to the imbalance between pro- and anti-inflammatory stimuli (Świderska *et al.*, 2017). Inflammation develops as a result of steatosis by the recruitment and activation of inflammatory cells in the liver. It was demonstrated that increased lipolysis of the fatty tissue, higher intake of dietary fat and enrichment of serum fatty acids increase the hepatic and systemic inflammation. Indeed, increased FAs amounts were observed in NAFLD patients leading to excessive accumulation of triglycerides and long-chain acyl-coenzyme A. Moreover, it was shown that lipid accumulation in the liver may induce oxidative stress via disturbances in antioxidant systems, mitochondrial activity, and mitochondrial function (Świderska *et al.*, 2019).

Hepatocyte apoptosis can be considered as one of the critical features of NAFLD patients. Different studies proved that active caspases 3 and 7 as well as the strong expression of Fas receptors in NASH specimens were strongly correlated with hepatocyte apoptosis and the progression of NASH. Caspase 3 activation and hepatocyte apoptosis are prominent features of different experimental models of NAFLD as well as human NAFLD and have been shown to be correlated with disease severity (Kanda *et al.*, 2018a). BCL-2 family modulates the checkpoint of apoptosis and has two major category members: apoptosis members like Bax, and anti-apoptosis members like BCL-2. The anti-apoptotic BCL-2 expression has been proved to be diminished in both hepatocytes and serum in accordance with stage of NAFLD/NASH (Li *et al.*, 2020).

Recently, various researches have been discussed and clarified the mechanisms of NAFLD development, from liver steatosis to more advanced liver inflammation and fibrosis. The peroxisome proliferator-activated receptor (PPAR)β/δ has inserted as a therapeutic target to alleviate NAFLD. PPARβ/δ has receptors in

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different liver cell kinds such as hepatocytes, Kupffer cells, cholangiocytes and hepatic stellate cells and it has an important role in tissue development and repair, regeneration, insulin sensitivity, inflammation, mitochondrial function, energy expenditure, and both lipid and carbohydrate metabolism (Zarei *et al*., 2021).

Soy isoflavone is the common soy phytoestrogen. It principally consists of genistein, daidzein and glycitein. There is various evidence on its abilities to prevent osteoporosis, cancer, cardiovascular disease and relieving menopausal syndrome. Different studies documented that soy isoflavone has the ability to prevent microsomal lipid peroxidation and decrease the malondialdehyde level in injured liver. Also, it has hypolipidemic effect and can increase insulin sensitivity that can lead to development of NAFLD. The protective role of soy isoflavone on liver is linked with the anti-oxidative, hypolipidemic effect and improvement of insulin resistance (Liu *et al*., 2017).

Genistein (4,5,7-trihydroxyisoflavone) is the principle active isoflavone isolated from soybean products. It can be called a tyrosine kinase inhibitor. It has powerful antioxidant and anti-inflammatory effects. It has been documented to affect a few pathophysiologic pathways that are normally deregulated in cancer, metabolic syndrome, and obesity (Xin *et al*., 2019).

This study aimed to detect the efficacy of isoflavone and Genistein to eliminate and alleviate nonalcoholic fatty liver rat induced by high fat diet.

MATERIALS AND METHODS

Animal and treatment

The animals used in this study were 60 of male Sprague–Dawley (SD) rats, weighting approximately 200±20 g. They were housed under standard conditions in the animal house at The Faculty of Veterinary medicine, Mansoura University, Egypt. The temperature was kept at 20°C, and relative humidity at 60-80% with a 12-h light/12-h dark cycle. All care and procedures adopted for the present investigation were in accordance with the approval of the Institutional Animal Ethics Committee of the National Research Center. After adaptive feeding, these rats were randomly divided into 6 groups as follow: group 1(fatty liver group, N= 10): the group fed with D12492 (60% fat energy, high fat diet (HFD)), group 2 (N=10): the group fed with HFD and 10 mg/Kg soy isoflavone (ISF) intragastrical. group 3 (N=10): the group were given adlibitum HFD and 16 mg/Kg genistein (GS) once daily by intragastric tube, group 4 (Control, N=6): the group fed with D12450B (10% fat energy), group 5 (N=10): the group fed with normal diet and 10 mg/Kg ISF intragastrical and group 6 (N=10): the group fed with normal diet and 16 mg/Kg GS. Soy isoflavone (Sigma Aldrish, USA) was dissolved in dist. Water in a volume of 0.5 ml/100 g while genistein (Sigma Aldrish, USA) was dissolved in 0.1% DMSO. The study was conducted for 12 weeks, all rats allowed water ad libitum, and rats got HFD were accompanied by 18% saccharose solution freely. Weight was measured weekly.

Sample collection

After 12 weeks, all rats were fasted overnight. Under anesthesia, 4–5 ml blood was withdrawn from the abdominal aorta rapidly. Then the serum was collected after clotting the blood sample and centrifuged at 2,000 rpm. and 4°C for 10 min, then saved at −80°C till used. After sacrifice, liver was rapidly removed and weighted, then immediately frozen at −80°C or fixed in 10% formaldehyde for the subsequent experiments.

Investigation of liver function tests and lipid profile

Alanine Aminotransferase (ALT) and Aspartate transferase (AST) activities, Albumin concentration, Cholesterol concentration and triglyceride concentration were assayed in serum samples according to manufacture instruction using a commercially available assay kits (Vitro Scient Company, Elmontaza St. Heliopolis, Cairo, Egypt) (Reitman and Frankel, 1957).

Detection of oxidative stress in liver tissues

The activity of superoxide dismutase (SOD), reduced glutathione (GSH), Catalase (CAT) and level of malanodialdehyde (MDA) were assayed in liver tissue homogenates according to manufacture instruction using a commercially available assay kits (Biodiagnostic Company, Dokki, Giza, Egypt) (Aebi, 1984; Nishikimi *et al*., 1972; Sedlak and Lindsay, 1968).

Apoptotic assay

Liver tissues were prepared according to Tribukait *et al*. (1975), briefly; specimens were washed with EDTA buffer ,3.029g of 0.1M tris,1.022g of 0.07Msodium chloride , and 0.47g of 0.005M EDTA. They were dissolved in 250 ml of distilled water and pH was adjusted at 7.5 by using 1 N HCL. The cell suspension was centrifuged at 1800 rpm for 10 min, whereupon supernatant was aspirated. After the centrifugation and the aspiration of the supernatant, the cell is fixed in ice-cold 96-100% ethanol in approximately 1 ml of each sample.

Then liver cells were resuspended in PBS and stained by a combination of antibodies: PE-conjugated BcL2, caspase 3 and incubated in the dark for 30 min. at room temperature. Stain buffer was added to cells and the expression of the corresponding cell-surface antigen was assayed by using FACS caliber (Becton Dickinson, Sunnyvale, CA, USA).

Gene expression for PPARγ and IL-1 β by RT-PCR

Total RNA from liver tissue was extracted by Rneasy Puls Mini Kit (Thermo Fisher Scientific, Waltham, Massachusetts, United States) according to the manufacturer's guidelines. Subsequently reverse transcription of total RNA (5 µg) was conducted by using Reverse transcriptase quantitative real time –PCR kit (Thermo Fisher Scientific, Waltham, Massachusetts, United States). The primer sequences for IL-1β, PPARγ, and β-actin are listed in Table 1. After reverse transcription, PCR was conducted using SYBR-Green Master Mix kit (Thermo Fisher Scientific, Waltham, Massachusetts, United States). The relative expression ratio (R) was obtained according to the following equation: $R = 2^{-\Delta\Delta Ct}$ (Negrin *et al*., 2014; Viswanathan and Grace, 2018).

Table 1. List of primer sequences.

Gene	Primer sequence	Accession number
ß-actin	F: TCCTCCTGAGCGCAAGTACTCT R: GCTCAGTAACAGTCCGCCTAGAA	>NM_031144.3
PPARγ	F: CATTTCTGCTCCACACTATGAA R: CGGGAAGGACTTTATGTATGCG	>NM_001145367.1
IL1-ß	F: CTCTGTGACTCGTGGGATGATGAC R: TCTTCTTCTTTGGGTATTGTTTGG	>NM_031512.2

Histopathological examination

For histological examinations, liver specimens were stored in buffered formalin fixative. Paraffin sections with a thickness of 5

µm were stained with haematoxylin and eosin (H&E), dehydrated, mounted and cover slipped (Liu *et al*., 2017). On the other hand, liver sections were stained with Oil red O stain to confirm lipid droplet accumulation. The prepared slides were examined using a light microscope (Olympus, Japan). Depending on fat accumulation, slides were classified into four categories as previously described (Zheng *et al*., 2018).

Statistical analysis

The comparison between mean ± SD of baseline and endpoint measures in each group was tested using paired sample t test. The comparison of the changes in mean ± SD of biochemical and molecular markers between different groups were performed using one way ANOVA test with Scheffe's post hoc test. The histopathological parameters scores were reported as median. Differences in median were performed by Mann-whitney U test. All statistical analysis was performed using SPSS version 20 (IBM Corp., Armonk, NY, USA)., with p value <0.05 considered significant.

RESULTS

Serum lipid profile and liver function

In comparison to the control group, there was a significant elevation in total cholesterol (TC), triglycerides (TG), Low-density lipoprotein cholesterol (LDL-C), and Very Low-density Lipoprotein cholesterol (VLDL-C) and a decrease in high-density lipoprotein cholesterol (HDL-C) concentration in Fatty liver group ($p < 0.05$). Fatty liver + ISF and Fatty liver +GS treated groups showed marked reductions in TC, TG, LDL-C, and VLDL-C concentrations and marked improvement in HDL-C compared with Fatty liver non-treated rats ($p < 0.05$). Whereas ISF and GS groups showed reduced TC and LDL-C but no static difference in TG, HDL-C, and VLDL-C concentrations than control values (Table 2).

Liver enzyme levels (ALT, AST) were significantly higher in the Fatty liver group than in the control group ($p < 0.05$). At the same time, they had insignificant changes in ISF and GS groups compared to the control group. Fatty liver + ISF and Fatty liver + GS treated groups showed a significant decrease in ALT activity.

Table 2. Comparison of the serum lipid profile within the study groups.

	Control group	Fatty liver group	ISF group	GS group	Fatty liver + ISF group	Fatty liver+ GS group
			Cholesterol			
Mean ± S. E	163.35±0.66 b	187.63±3.63 a	149.56±4.93 b	137.37 ± 2.77 c	165.76±0.20 b	163.36±2.43 b
Range (Min-Max)	158.60-166.60	183.70-194.90	141.60-158.60	133.70-142.80	165.40-166.12	162.20-164.50
			TGs			
Mean ± S. E	55.38 ± 6.34c	173.25±12.47a	53.62 ±3.71 c	49.78 ±4.3 c	121.90±12.02 b	118.06±8.18 b
Range (Min-Max)	47.80-69.60	165.16- 200.60	51.60-63.00	39.30-57.40	98.60-138.70	106.08-133.70
			HDL			
Mean ± S. E	60.96±2.90 a	41.10±2.84 cd	54.93±2.62 ab	56.56±0.64 ab	47.73±2.15 c	53.43±1.35 bc
Range (Min-Max)	55.60 -65.60	37.40 - 46.70	51.20-60.00	55.30-57.40	44.70-51.90	51.10 -55.80
			LDL			
Mean ± S. E	91.32±1.22b	111.88 ±1.49 a	84.31± 2.94 c	70.86±2.13 d	93.65± 0. 62 b	86.32±2.24 b
Range (Min-Max)	89.00-93.80	100-115.00	79.5 – 86.00	69.80-73.80	90.20 – 94.80	84.90-91.00
			VLDL			
Mean ± S. E	11.07±1.26 c	34.65± 1.44 a	10.32±1.35 c	9.95 ±1.67c	24.38±2.40 b	23.61±1.63b
Range (Min-Max)	9.58- 13.92	33.03 - 37.52	8.92-13.60	8.86-11.48	19.72-27.74	21.22-26.74

Table 3. Comparison of the liver functions within the study groups

	Control group	Fatty liver group	ISF group	GS group	Fatty liver + ISF group	Fatty liver+ GS group
			Cholesterol			
Mean ± S. E	163.35±0.66 b	187.63±3.63 a	149.56±4.93 b	137.37 ± 2.77 c	165.76±0.20 b	163.36±2.43 b
Range (Min-Max)	158.60-166.60	183.70-194.90	141.60-158.60	133.70-142.80	165.40-166.12	162.20-164.50
			TGs			
Mean ± S. E	55.38 ± 6.34c	173.25±12.47a	53.62 ±3.71 c	49.78 ±4.3 c	121.90±12.02 b	118.06±8.18 b
Range (Min-Max)	47.80-69.60	165.16- 200.60	51.60-63.00	39.30-57.40	98.60-138.70	106.08-133.70
			HDL			
Mean ± S. E	60.96±2.90 a	41.10±2.84 cd	54.93±2.62 ab	56.56±0.64 ab	47.73±2.15 c	53.43±1.35 bc
Range (Min-Max)	55.60 -65.60	37.40 - 46.70	51.20-60.00	55.30-57.40	44.70-51.90	51.10 -55.80
			LDL			
Mean ± S. E	91.32±1.22b	111.88 ±1.49 a	84.31± 2.94 c	70.86±2.13 d	93.65± 0. 62 b	86.32±2.24 b
Range (Min-Max)	89.00-93.80	100-115.00	79.5 – 86.00	69.80-73.80	90.20 – 94.80	84.90-91.00
			VLDL			
Mean ± S. E	11.07±1.26 c	34.65± 1.44 a	10.32±1.35 c	9.95 ±1.67c	24.38±2.40 b	23.61±1.63b
Range (Min-Max)	9.58- 13.92	33.03 - 37.52	8.92-13.60	8.86-11.48	19.72-27.74	21.22-26.74

Still, a reduction in AST activity was not statistically crucial compared with the Fatty liver group. Albumin level was significantly decreased in the Fatty liver group compared to the control group. However, there was no change in albumin levels in ISF and GS groups compared to the control group. Also, the Fatty liver + ISF and Fatty liver + GS groups showed a significant increase toward normal levels compared to the Fatty liver group ($p < 0.05$) (Table 3).

Oxidative Stress levels in Liver Tissues

There was a significant increase in MDA level in the Fatty liver group and a decrease in ISF and GS groups compared to the control group ($p < 0.05$). In the Fatty liver + ISF and Fatty liver + GS groups, hepatic MDA was significantly declined than that in the Fatty liver group ($p < 0.05$).

In the Fatty liver group, SOD, CAT, and GSH activities were diminished considerably compared to the control group ($p < 0.05$). The ISF and GS groups had significantly increased in SOD, CAT, and GSH activities compared to the control ($p < 0.05$). In the Fatty liver + ISF treated group and Fatty liver + GS treated group, SOD, CAT, and GSH activities were significantly increased compared to the Fatty liver group ($p < 0.05$) (Table 4).

Protein expression of bcl2 and caspase

BCL-2 and caspase-3 protein level in liver tissues as apoptosis marker was measured in the different groups. Fatty liver revealed significantly decreased in BCL-2 and significantly elevation in caspase-3 proteins expressions compared to control group ($p < 0.05$). In contrast, BCL-2 protein significantly increased while caspase-3 protein expressions significantly decreased in ISF , GS groups and Fatty liver + ISF and Fatty liver + GS treated groups when compared to control and Fatty liver groups respectively ($p < 0.05$; Figs. 1 and 2).

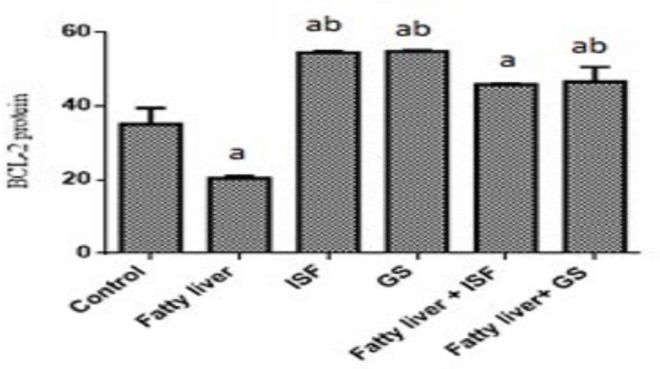

Fig. 1. BCL2 protein in different studied groups.

Gene expression level of hepatic IL1-ß and PPARγ

The expressions of IL1-ß and PPARγ were assessed in liver

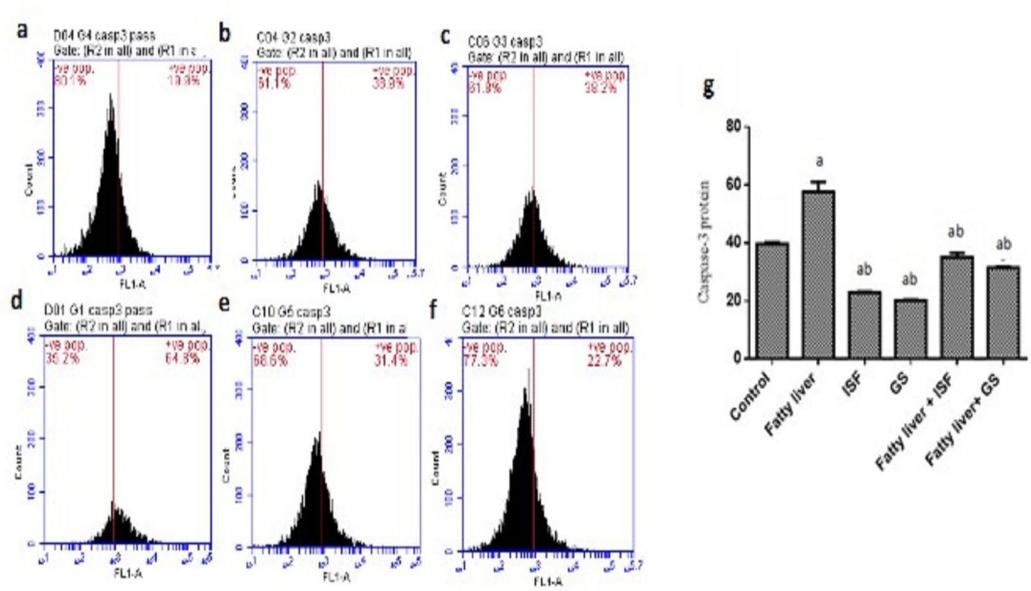

Fig. 2. Caspase-3 protein in different studied groups.a) control group, b) ISF group, c) GS group, d) fatty liver group, e) fatty liver + ISF group, f) fatty liver + GS group, and g) scorring of caspase-3.

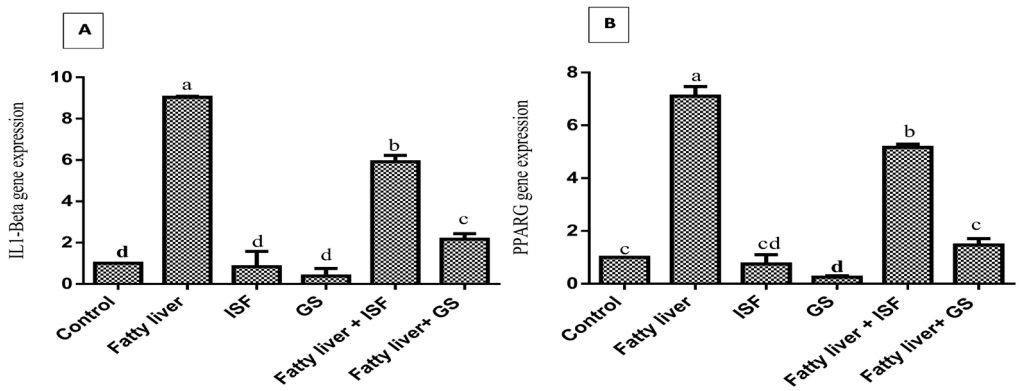

Fig. 3. Relative expression of PPARγ and IL1-ß genes by RT-PCR of different studied groups

tissue. The presentation of IL1-ß and PPARG was significantly upregulated in the Fatty liver group compared to the control group ($p < 0.05$). There was no significant difference in the level of IL1-ß and PPARγ between the ISF and control group. There was no significant expression of IL1-ß between GS and the control group. In contrast, the expression level of PPARγ expression was significantly downregulated in the GS group compared to the control group ($p < 0.05$).IL1-ß expression and PPARG expressions were downregulated in Fatty liver + ISF and Fatty liver+ GS treated rats compared to Fatty liver one (Fig. 3).

Changes in the hepatic histopathological

Histopathological quantification scoring showed Grade 0 of mean fatty infiltration in control, ISF, and GS treated groups, Fatty liver group showed Grade 3 which decrease to Grade 2 in fatty liver+ISF group and Grade 1 in fatty liver+ GS group (Table 5). As shown in Fig.4, H&E staining revealed normal hepatic histological architecture with homologous distribution of hepatocytes throughout the hepatic parenchyma in control, ISF, and GS treated groups (Fig. 4A, 1B, 1C). In contrast, fatty liver group showed disturbance in hepatocytes arrangement with marked hepatocytes diffuse vacuolation with pushed nucleus at the peripheral forming signet ring appearance (Fig.4D). With ISF and GS treatment, an improvement was observed in the hepatocytes arrangement with low vacuolation (Fig. 4 E, 4F). On the other hand, Oil Red-O stain was detected among different treated groups (Fig. 5). Control, ISF, and GS groups showed absences of oil red stain, while fatty liver group displayed abundant accumulation of fat droplets in hepatocytes. Moreover, fatty liver + ISF and fatty liver+ GS groups revealed moderate to mild oil red stain respectively.

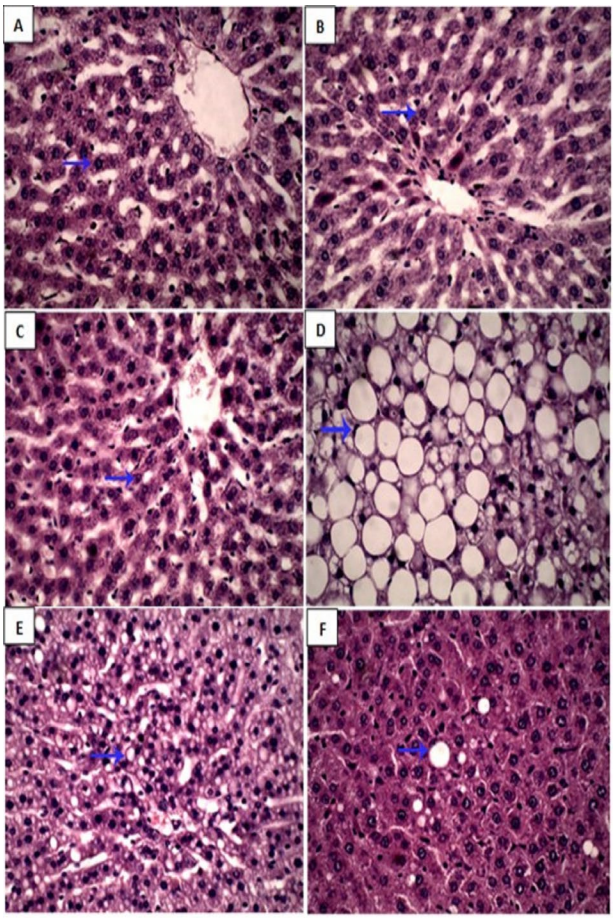

Fig 4. Microscopic imagining of Liver tissue showing normal hepatocytes and normal hepatic architecture (arrow) in control group (A), normal hepatocytes in ISF group (B), normal hepatic morphology in GS group (C), liver displays clear vacuolation of the hepatocytes with pushed nucleus at the peripheral forming signet ring appearance (arrow) in fatty liver group (D), low vacuolation of the hepatocyte in fatty liver+ISF group (E), normal hepatocytes and normal hepatic architecture (arrow) in fatty liver+ GS group (F). (HE, 400x).

Table 4. Comparison of the enzymes and oxidative stress markers in liver homogenates within the study groups.

	Control group	Fatty liver group	ISF group	GS group	Fatty liver + ISF group	Fatty liver+ GS group
			MDA concentration (nmol/g tissue)			
Mean ± S. E	65.00±2.30 d	190.33±9.73 a	33.66±1.20 e	29.33±1.20 e	118.66±2.96 b	89.00±2.64 c
Range (Min-Max)	61.00-69.00	171.00-202.00	32.00-36.00	22.00-31.00	113.00-123.00	84.00-93.00
			SOD activity (U/g tissue)			
Mean ± S. E	30.50±0.76 c	6.00±0.86 e	34.83±1.20 b	43.33±1.20 a	17.16±0.60 d	29.50±1.32 c
Range (Min-Max)	29.50-32.00	4.50-7.50	32.50-36.50	41.00-45.00	16.00-18.00	27.50-32.00
			CAT activity (U/g tissue)			
Mean ± S. E	52.66±1.76 c	16.66±2.02 f	63.33±1.45b	77.50±1.44 a	30.00±1.15 e	47.33±1.45 d
Range (Min-Max)	50.00-56.00	13.00-20.00	61.00-66.00	75.00-80.00	28.00-32.00	45.00-50.00
			GSH (mmol/g tissue)			
Mean ± S. E	8.46±0.40c	1.22± 0.17 f	10.58± 0.30 b	12.26± 0.37 a	2.66 ±0.22 e	7.00±0.57d
Range (Min-Max)	7.75-9.15	0.91 – 1.50	10.00 – 11.00	11.80 – 13.00	7.22 – 3.00	6.00-800

Table 5. Histopathological scoring in different rerated groups

	Steatosis (0-3)	Hepatocellular ballooning (0-3)	Lobular inflammation (0-2)	Fibrosis (0-4)	Steatosis (0-3)
Normal control group	0.0±00	0.0±00	0.0±00	.0.0±00	.0.0±00
Fatty liver group	3.00±00	3.00±00	3.00±00	3.00±00	3.00±00
ISF group	0.0±00	0.0±00	0.0±00	0.0±00	0.00±00
GS group	0.0±00	0.0±00	0.0±00	0.0±00	0.00±00
Fatty liver + ISF group	2.00±00	2.00±00	2.00±00	2.00±00	2.00±00
Fatty liver+ GS group	1.00±00	1.00±00	1.00±00	1.00±00	1.00±00

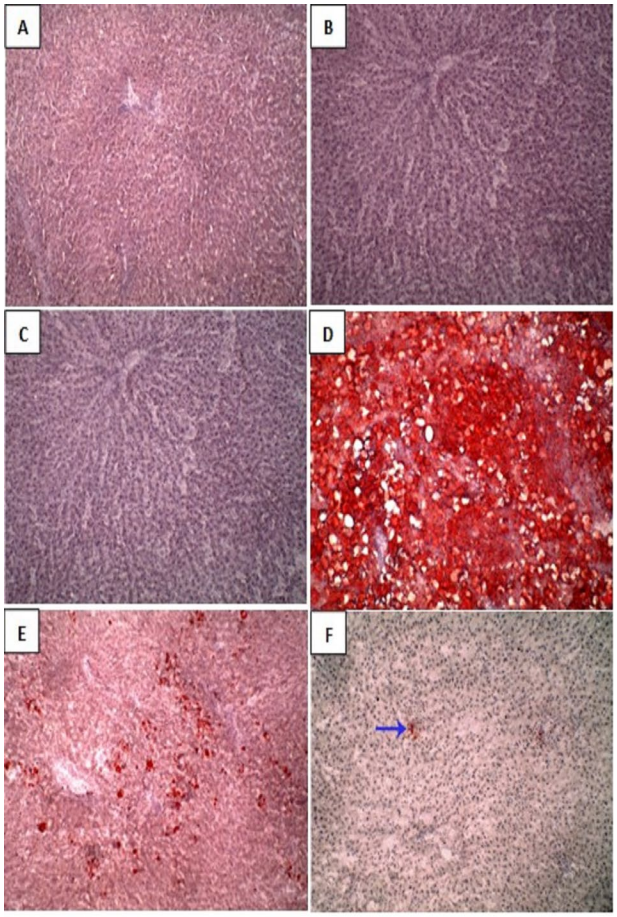

Fig. 5. Microscopic imagining of oil red-O-stained liver tissue showing absence of oil red O-stained hepatocytes in control group (A), absence of oil red O-stained hepatocytes in ISF group (B), absence of oil red O-stained hepatocytes in GS group (C), intense red stained hepatocytes against oil red O stain in fatty liver group (arrow) (D), moderate red-stained hepatocytes against oil red O stain in fatty liver+ IS group (E), mild red-stained hepatocytes against oil red O stain in fatty liver+ GS group (arrow) (F). (100x).

DISCUSSION

Dietary interventions may play a significant role in preventing or treating metabolic disorders such as fatty liver disease. Animal investigations and clinical trials have demonstrated that soy protein has hypolipidemic effects. Soy protein also contains bioactive peptides and isoflavones (genistein, glycitein, daidzein), contributing to these positive effects (Illesca *et al*., 2017). Based on that, the present study aimed to investigate how ISF and GS affect oxidative stress, IL1-ß, and PPARγ signaling pathways in the liver of obese rats and how this pathway is involved in controlling the formation of hepatic fat.

Two essential functions of lipids in the body are energy storage and membrane structure. Moreover, the lipid is a crucial ingredient for the biosynthesis of steroids and bile acids. Due to their insolubility, lipids require protein in the form of lipoproteins for transport (Kaneko, 1996). The lipid measurements in blood showed that TG , LDL , VLDL and TC in the fatty liver group were higher than those in the control group. However, their levels decreased in GC and ISF intervention groups. The rise in lipid profile causes an increase in hepatic fat and nonalcoholic fatty liver disease, which in turn causes hepatic lipotoxicity and liver injury (Pummoung *et al*., 2020). GC and ISF affect hepatic fat accumulation in rats with NAFLD induced by a high-fat diet (HFD) by decreasing serum TG, LDL, V-LDL and prevented the buildup of lipids in the liver (Liu *et al*., 2017).

Consuming a high-fat diet on a long-term basis can lead to abnormal lipoprotein metabolism. ALT and AST are vital liver function indicators. ALT and AST elevations play a crucial role in the development of NAFLD. Research documented that ALT and AST were elevated in obese individuals and correlated with the presence of NAFLD (Hakkak *et al*., 2018). which is in accordance with our study. Serum levels of ALT and AST were decreased using ISF and GS. These results are in line with the data obtained by previous study (Kim and Kang, 2012) .

Oxidative stress is one of the mechanisms that trigger the progression of simple steatosis to NASH. MDA is derived from lipid peroxidation, which frequently occurs due to oxidative damage (Esterbauer *et al*., 1992). In fact, NASH patients suffered from higher MDA level than healthy people (Koruk *et al*., 2004), these findings are in parallel with our result that showed a significant increase of MDA level in Fatty liver group as compared with normal control. However, this increase was diminished by GC and ISF, the greatest antioxidant among isoflavones. GC and ISF were reported to act as an antioxidant directly or indirectly by scavenging free radicals and activating antioxidants (Susutlertpanya *et al*., 2015).

In contrast, SOD, CAT and GSH are major enzymes that are responsible for ROS detoxification in mitochondria. The present data showed that the levels of SOD, CAT and GSH were decreased in fatty liver group and their levels were restored after intervention of GC and ISF. Lee (2006) showed that the administration of isolated soy protein increased the activity of hepatic antioxidant enzymes (SOD, CAT and GSH) associated with reduced levels of lipid peroxidation in streptozotocin-induced diabetic rats.

Since the original description that caspase activation and apoptotic cell death are characteristic pathologic features in the liver of NASH patients, increasing amount of data have demonstrated that hepatocyte cell death is a key process involved in NASH pathogenesis (Thapaliya *et al*., 2014). Caspase 3 plays a role in lipid-induced hepatocyte apoptosis and is related to the production of apoptosis-associated fibrogenic factors. Additionally, liver free caspase 3 content was shown to be reduced in mice with NASH (Kanda *et al*., 2018b).

In the current study, Fatty liver revealed a significantly increase in Caspass-3 proteins level. While the level of Caspase-3 in Fatty liver + ISF and Fatty liver + GS treated groups was respectively decreased, suggesting the role of ISF and GS as antiapoptotic agents as reported by (Lin *et al*., 2014) who found that soy isoflavone and Genistein attenuated the elevated level of caspases-3 in ipopolysaccharide (LPS)/D-galactosamine (D-GalN)-induced acute hepatic failure.

Panasiuk *et al*. (2006) reported that there is a negative correlation between Bcl-2 and non-alcoholic fatty liver disease as advanced stage of liver steatosis had a statistically significant decrease in Bcl-2 expression in NASH. Our results are in accordance with these findings, as there was a significant decrease in BCL2 expression in fatty liver group. In contrast, treatment with soy isoflavone and Genistein manifested a significant remodeling in BCL2 expression. This antiapoptotic role was previously mentioned by Lin *et al*. (2014), who found that Genistein augmented the expression of Bcl-2 in ipopolysaccharide (LPS)/D-galactosamine (D-GalN)-induced acute hepatic failure.

IL1-ß enhances hepatic steatosis by promoting the accumulation of triglycerides and cholesterol in primary liver hepatocytes and the formation of lipid droplets (Negrin *et al*., 2014). In our findings there was a statistically significant upregulation of IL1-ß expression in Fatty liver group when compared to control group. In contrast IL-1β expression was changed decreasingly regulated when treated with ISF and GS. These results are in parallel with the findings of Iwase *et al*. (2020) providing that ISF and GS repressed almost all inflammatory gene expression.

PPARγ is a ligand-activated nuclear receptor transcription factor, which has an important glucose metabolism, role in lipid storage, and adipocyte differentiation. Whether PPAR protects against or exacerbates NAFLD remains debatable. A number of animal studies have demonstrated that PPAR overexpression is associated with adipogenic transformation of hepatocytes and that hepatic PPAR deletion ameliorates hepatic steatosis (Gavrilova *et al*., 2003). In this study, GS and ISF significantly reduced hepatic PPARγ expression in treated group compared to fatty liver group. These results are in line with the results obtained by

Pummoung *et al*. (2020).

In a harmony with the changes observed in the biochemical parameters, pathological examination of liver tissue showed marked hepatocytes diffuse vacuolation with pushed nucleus at the peripheral forming signet ring appearance with intense red stained hepatocytes against oil red O stain indicating fatty infiltration in Fatty liver group. This observation is agreed with the findings of Brunt *et al*. (1999), who reported that Steatohepatitis is a morphological pattern of liver injury, which in nonalcoholic patients, may represent a form of chronic liver disease known as nonalcoholic steatohepatitis (NASH). Treatment with soy isoflavone and Genistein showed a significant improvement in liver tissue and hepatocytes arrangement and reduces vacuolation and fatty infiltration. These results are in parallel with previous study reported that it is possible that for NAFLD rats, the main target organ of genistein is the liver, and the genistein can improve liver injury and decrease liver index in genistein intervention groups (Yin *et al*., 2019).

CONCLUSION

The results show the hypolipidemic effect of GS and ISF, which is probably related to the decreasing expression of apoptotic markers, PPARγ and IL-1β and increasing the activity of antioxidant markers (CAT, SOD, GSH) which in turn enhanced the liver metabolism. The findings suggest that GS and ISF effectively attenuate the emergence of fatty liver, which may be helpful in further studies and applications for protection from fatty liver.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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