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Research Article

Preventive Role of Soy Protein in Fructose Induced Metabolic Dysfunction in Rats via Inhibition of Nuclear Factor kappa B Pathway

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Nahla El-Ashmawy[1], Eman Khedr[1], Hoda Elbahrawy[1], Reham Abdelhamid[1]*

[1]*Department of Biochemistry, Faculty of Pharmacy, Tanta University, Tanta 31111, Egypt*

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ABSTRACT

Soy protein is an important component of soybeans that has a beneficial role in improving insulin resistance. Nuclear factor-kappa B (NF-κB) is a crucial pathway that has been implicated in the development of metabolic syndrome. Our study aimed to examine the protective effect of soy protein isolate - as a natural NF-κB inhibitor - and its possible mechanism of action in amelioration of inflammatory and metabolic disorders induced in male albino rats by high fructose diet (10% w/v) via using synthetic NF-κB inhibitor (IMD-0354). Rats were randomized into normal control group, soy group, NF-κB inhibitor (IMD-0354) group, high fructose group, high fructose with soy group, high fructose with NF-κB inhibitor group, high fructose with soy and NF-κB inhibitor group. Serum glucose, serum insulin, serum-free fatty acids, insulin resistance (HOMA-IR), NF-κB, phosphorylated insulin receptor (pISR), carbohydrate-responsive element-binding protein (ChREBP) were determined and histopathological examination of liver tissue was performed. The concurrent administration of IMD-0354 and/or soy protein with high fructose significantly increased pISR and decreased FFAs, NF-κB, glucose, insulin, HOMA-IR, and ChREBP, as well as improved the pathological conditions of the livers. The metabolic and inflammatory disorders induced by chronic consumption of fructose could be inhibited by co-administration of soy protein through the regulation of the NF-κB signaling pathway.

Keywords: fructose; metabolic dysfunction; soy protein; NF-κB; insulin resistance.

1. INTRODUCTION

Fructose is a highly lipogenic, ketonic monosaccharide that is found in several fruits and is used as a sweetener in the food industry. The over-consumption and chronic consumption of fructose results in increased levels of reactive oxygen species (ROS) and free fatty acids (FFAs) as well as reduced antioxidant capacity.[1] Fructose consumption is associated with the dysregulation of adipokines and augmentation of proinflammatory processes. Additionally, there are many metabolic disorders induced by high fructose consumption due to increased levels of FFAs that activate the inhibitor kappa kinase β (IKK-β) and protein kinase C theta (PKCθ), which are related to the dysregulation of insulin signalling.[2]

Soy protein is a protein isolated from the soybean. It is a rich source of dietary protein which provides the cells with essential amino acids and valuable macronutrients as shown in **Table (1).**[3] The unique importance of soy protein could be attributed to that it contains isoflavones that responsible for many biological and beneficial properties for maintenance of health[4]. Isoflavones are known as phytoestrogens that have estrogen-like structures and effects that protect postmenopausal women from bone loss and maintain a

**Department of Biochemistry, Faculty of Pharmacy, Tanta University, Tanta, Egypt. 31527, Tel.: (202) 040-3336007; fax: (202) 040-3335466.*
E-mail address: rehamabdelhamed@hotmail.com

healthy heart.[5] Consumption of soy protein has been found to reduce serum concentrations of total cholesterol, low-density lipoproteins (LDLs), and triglycerides. Soy protein also contributed to the control of hyperglycemia, body weight, hyperlipidemia, and hyperinsulinemia.

Genistein, one of the phytochemicals found in soy protein, has a role in cancer prevention by preventing the angiogenesis process.[6] Much research has been conducted to study the effect of soy protein and its isoflavones in the reduction of body fat mass and weight. Isoflavones could reduce the obesity through lowering triglycerides and decrease the insulin resistance.[7]

A novel NF-κB inhibitor, IMD-0354 (N-(3,5-bis-trifluoromethylphenyl) – 5 - chloro -2-hydroxybenzamide), inhibits IKK-β and blocks IκB phosphorylation in the NF-κB pathway. The NF-κB signalling pathway plays an essential role in both inflammation and angiogenesis. Inhibition of NF-κB by IMD-0354 has been studied in several preclinical models, including models of cancer, reperfusion injury, allergy, and lung fibrosis.[8,9]

Accordingly, the present work aimed to study the different metabolic disorders induced by chronic consumption of fructose. This work also aimed to evaluate the role and possible mechanism of action of soy protein in amelioration of inflammatory and metabolic disorders induced by over-consumption of fructose in rat models.

Table (1): Components of soy protein

Composition or element determined	Amount determined in soy protein isolate
Protein (weight percent)	93.45
Crude fat (weight percent)	<0.1
Fat by acid hydrolysis (weight percent)	2.32
Ash (weight percent)	5.51
Sodium (ppm)	21.165
Potassium (ppm)	1461
Calcium (ppm)	856
Phosphorus (ppm)	11.684
Magnesium (ppm)	516
Isoflavones (microgram/gram total dry matter)	121.1
Daidzin	14.62
6''-O-malonyldaidzin	24.02
6''-O-acetyldaidzin	2.09
Daidzein	4.18
Genistin	25.06
6''-O-malonylgenistin	36.55
6''-O-acetylgenistin	5.22
Genistein	4.18
Glycitin	2.09
6''-O-malonylglycitin	2.09
Glycitein	1.04
Nitrogen Solubility Index (NSI) (%)	81.2
Viscosity (10% Dispersion) (cP)	86

2. METHOD

2.1. Experimental design

This study was performed in accordance with the guidelines for the care and use of laboratory animals and was approved by the Research Ethics Committee (Faculty of Pharmacy, Tanta University, Egypt). In our study, 140-160 g male albino rats were utilized. The rats were purchased from the National Research Center (NRC) Dokki, Giza, Egypt. The rats were weighed and housed in separated aluminium cages for two weeks under identical environmental conditions and a 12-hour light-dark cycle. The animals were allowed free access to a standard pellet diet and water ad libitum. After the acclimatization period, rats were weighed and randomly divided into seven groups: Group 1 [the normal control group; n=10], the rats were given a normal diet with a daily i.p. injection of the vehicle for eight weeks. Group 2 [the soy protein group; n=10], the rats fed on a diet containing 20% soy protein isolate powder (Solae Company®, USA) daily for eight weeks.[10] Group 3 [the inhibitor (IMD) group; n=10], the rats received a daily i.p. injection of 10 mg/kg IMD-0354 (Selleckchem®, USA) for eight weeks.[11] Group 4 [the high fructose (HF) group; n=10], the rats were given 10% w/v fructose in their drinking water (UNIPHARMA®, Egypt) daily for eight weeks.[12] Group 5 [the high fructose with soy (HF-S) group; n=10], the rats received 10% w/v fructose in their drinking water along with a diet containing 20% soy protein isolate powder daily for eight weeks. Group 6 [the high fructose with inhibitor (HF-IMD) group; n=10], the rats received 10% w/v fructose in their drinking water along with a daily i.p. injection of 10 mg/kg IMD-0354 for 8 weeks. Group 7 [the high fructose with soy and inhibitor (HF-S-IMD) group; n=10], the rats received 10% w/v fructose in their drinking water along with a diet containing 20% soy protein isolate powder and a daily i.p. injection of 10 mg/kg IMD-0354 for eight weeks.

After the experiment (8 weeks), the rats were weighed, anaesthetized by halothane (Delta Pharma®, Egypt), and sacrificed. Blood was drawn from the heart through the cardiac puncture, and serum was separated for determination of glucose, insulin, and FFAs. Serum samples were stored at -20°C until the analytical methods were performed. The rats were dissected under completely sterile conditions to collect liver samples. The fresh liver was washed twice with ice-cold saline, dried on a clean paper towel, and weighed. The liver index was calculated as liver weight (g)/final body weight (g) × 100. The liver was divided into four portions: one portion was preserved in 10% formalin for histopathological examination, and the other parts were immediately frozen in liquid nitrogen and stored at -80°C for biochemical analysis.

2.2. Biochemical analysis of blood and serum

The fasting blood glucose level was determined in rat blood using an Accu-Chek glucometer (Roche diagnostics Deutschland GmbH®, Germany), and the level of blood glucose is expressed as mg/dL. Fasting serum insulin levels were measured using a rat insulin enzyme-linked immunosorbent assay (ELISA) kit (Glory Science®, USA). The concentration of serum insulin was determined according to the manufacturer's procedure. The level of insulin was obtained from the standard curve and is expressed as mIU/L. Insulin resistance (HOMA-IR) was calculated using the following formula: HOMA-IR = [fasting insulin level (μIU/mL) × fasting glucose level (mg/dL)] / 405.[13] Serum

FFAs were assayed using a rat FFA ELISA kit (Sunred Biological Technology®, China). The concentration of serum FFAs was determined according to the manufacturer's procedure using a standard curve and is expressed as μmol/L.

2.3. Biochemical analysis of liver tissue

NF-κB concentration was determined in liver tissue using a rat NF-κB ELISA kit (Sunred Biological Technology®, China) according to the manufacturer's procedure. The level of NF-κB was obtained from a standard curve and is expressed as ng/mL. Phosphorylated insulin receptor (pISR) concentration was determined in liver tissue using a rat pISR ELISA kit (Sunred Biological Technology®, China) according to the manufacturer's procedure. The level of pISR was obtained from a standard curve and is expressed as ng/mL. Carbohydrate-responsive element-binding protein (ChREBP) concentration was determined in liver tissue using a rat ChREBP ELISA kit (Sunred Biological Technology®, China) according to the manufacturer's procedure. The level of ChREBP was obtained from a standard curve and is expressed as ng/mL.

2.4. Histopathological examination

The liver was fixed in 10% formalin and embedded in paraffin to form blocks that were serially sectioned (3-5 mm thick) and stained with haematoxylin and eosin (H&E). The slides were examined blindly by a pathologist under a light microscope. Images were viewed and recorded using an Olympus microscope equipped with a spot digital camera using the computer program MATLAB software in the Histochemistry and Cell Biology Department, Medical Research Institute, Alexandria University, Egypt.

2.5. Statistical analysis

All measured biochemical parameters were analyzed using SPSS software version 20.0. Quantitative data are presented as the mean ± standard deviation (SD) of the mean. Statistical comparisons between more than two groups were performed by one-way analysis of variance (ANOVA) followed by post hoc Tukey's test. The significance of the obtained results was obtained at $p<0.05$.

3. RESULTS

3.1. Body weight

Final rat weight was significantly increased in the HF group (1.05-fold increase, $p<0.05$) compared to the normal control group, while concurrent use of soy protein with a high fructose diet significantly decreased the weight of rats (1.05-fold decrease, $p<0.05$) compared to the weight of rats in the HF group. Additionally, in the HF-S-IMD group, rat weight significantly decreased (1.06-fold decrease, $p<0.05$) compared to the HF group (Table 2). The change in rat weight is expressed as the percentage change in weight, and the results are shown in **Figure (1).**

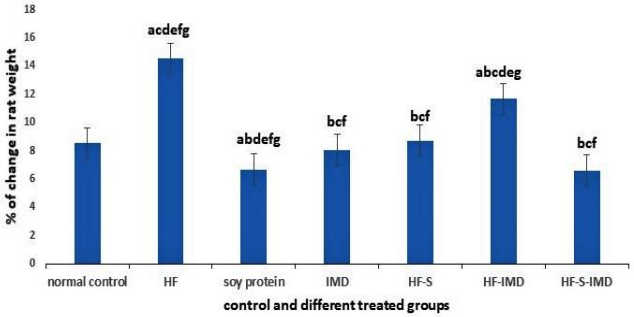


Fig. 1: Effects of fructose, soy protein, and IMD-0354 on the percent change in rat weight in the studied groups. HF: high fructose diet, IMD: inhibitor of NF-κB, HF-S: high fructose diet with soy protein, HF-IMD: high fructose diet with NF-κB inhibitor, HF-S-IMD: high fructose diet with soy protein and NF-κB inhibitor. a: significant versus normal control. b: significant versus HF. c: significant versus soy protein. d: significant versus IMD. e: significant versus HF-S. f: significant versus HF-IMD. g: significant versus HF-S-IMD. Significance was set at $p < 0.05$.

3.2. Liver weight and liver index

Liver weight and liver index were significantly increased in the HF group (1.50- and 1.42-fold increase, respectively, $p<0.001$) compared to the normal control group. Liver weight and liver index were significantly decreased in the HF-S

Table 2: Changes in rat weight, liver weight, and liver index in different studied groups

Data are presented as mean ± SD, n=10 rats. HF: high fructose diet, IMD: inhibitor of NF-κB, HF-S: high fructose diet with soy protein, HF-IMD: high fructose diet with NF-κB inhibitor, HF-S-IMD: high fructose diet with soy protein and NF-κB inhibitor. a: significant versus normal control. b: significant versus HF. c: significant versus soy protein. d: significant versus IMD. e: significant versus HF-S. f: significant versus HF-IMD. g: significant versus HF-S-IMD. Significance was set at $p < 0.05$.

Groups	Weight of rats (g) (before)	Weight of rats (g) (after)	Liver weight (g)	Liver index (%)
Normal control	152.00 ±2.54	165.00 ±2.94	4.36 ±0.25	2.64 ±0.0018
HF	152.00 ±2.49	174.10 ±5.10 ^{acdeg}	6.55 ±0.45 ^{acdefg}	3.76 ±0.0018 ^{acdefg}
Soy protein	152.70 ±3.16	162.90 ±2.56^{b}	4.69 ±0.58^{b}	2.88 ±0.0033^{bg}
IMD	151.87 ±2.59	164.13 ±4.32^{b}	4.44 ±0.29^{be}	2.70 ±0.0062^{b}
HF-S	152.40 ±2.84	165.70 ±2.11^{b}	5.07 ±0.22^{abdf}	3.06 ±0.0027^{abfg}
HF-IMD	152.50 ±.90	170.30 ±0.79	4.43 ±0.55^{be}	2.60 ±0.0014^{be}
HF-S-IMD	153.10 ±3.28	163.20 ±7.79^{b}	4.04 ±0.38^{b}	2.48 ±0.0031^{bce}

group (1.29- and 1.23-fold decrease, respectively, p<0.001), the HF-IMD group (1.48- and 1.45-fold decrease, respectively, p<0.001) and the HF-S-IMD group (1.62- and 1.52-fold decrease, respectively, p<0.001) compared to the HF group **(Table 2)**.

3.3. Fasting blood glucose

The level of fasting blood glucose was significantly increased in the HF group (2.25-fold increase, p<0.001) compared to the normal control group. Blood glucose levels were significantly decreased in the soy protein group (1.07-fold decrease, p<0.001) compared to the normal control group. Fasting blood glucose levels in the HF-S, HF-IMD, and HF-S-IMD groups were significantly decreased compared with the levels in the HF group [1.84-, 2.27- and 2.29-fold decrease, respectively, p<0.001, **(Table 3)**.

3.4. Fasting serum insulin and HOMA-IR

Levels of fasting serum insulin and HOMA-IR were significantly increased in the HF group (1.77- and 3.98-fold increase, respectively, p<0.001) compared to the normal control group. Fasting serum insulin and HOMA-IR were significantly decreased in the soy protein group (1.44- and 1.09-fold decrease, p<0.001) compared to the normal control group. However, fasting serum insulin and HOMA-IR were decreased in the HF-S group (1.58- and 2.91-fold decrease, respectively, p<0.001) compared to the HF group. Additionally, fasting serum insulin and HOMA-IR were significantly decreased in the HF-IMD (1.80- and 4.08-fold decrease, respectively, p<0.001) and HF-S-IMD groups (1.79- and 4.12-fold decrease, respectively, p<0.001) compared to the HF group **(Table 3)**.

Table 3: Changes in fasting blood glucose, fasting serum insulin, and HOMA-IR in different studied groups

Groups	FBG (mg/dL)	FSI (mIU/L)	HOMA-IR
Normal control	92.00 ±2.40	17.36 ± 0.40	3.94 ± 0.17
HF	206.90 ±5.82^{acdefg}	30.69 ±1.36^{acdfg}	15.69 ± 0.98^{acdfg}
Soy protein	86.00 ±2.67^{abef}	12.02 ±0.46^{abe}	2.55 ± 0.10^{abefg}
IMD	89.88 ±3.91^{be}	17.16 ± 0.41^{be}	3.81 ± 0.14^{be}
HF-S	112.10 ±5.52^{abcdfg}	19.45 ±0.89^{abcdfg}	5.39 ±0.44^{acdfg}
HF-IMD	91.30 ±2.41^{bce}	17.02 ± 0.40^{be}	3.84 ± 0.12^{abce}
HF-S-IMD	90.40 ±1.84^{be}	17.07 ± 0.44^{be}	3.81 ± 0.11^{abce}

Data are presented as mean ± SD, n=10 rats. HF: high fructose diet, IMD: inhibitor of NF-κB, HF-S: high fructose diet with soy protein, HF-IMD: high fructose diet with NF-κB inhibitor, HF-S-IMD: high fructose diet with soy protein and NF-κB inhibitor, FBG: fasting blood glucose, FSI: fasting serum insulin. a: significant versus normal control. b: significant versus HF. c: significant versus soy protein. d: significant versus IMD. e: significant versus HF-S. f: significant versus HF-IMD. g: significant versus HF-S-IMD. Significance was set at p < 0.05.

3.5. Serum free fatty acids

The level of serum FFAs was significantly increased in the HF, HF-S, HF-IMD, and HF-S-IMD groups (↑169.28%, 149.38%, 142.77%, and 144.19% respectively, p<0.001) compared to the normal control group. Serum FFAs were significantly decreased in the HF-S, HF-IMD, and HF-S-IMD groups (↓7.39%, 9.84%, 9.31% respectively, p<0.001) compared with the HF group, **(Table 4)**.

3.6. Carbohydrate responsive element binding protein (ChREBP) in liver tissue

The level of ChREBP in liver tissue was significantly increased in the HF, HF-S, HF-IMD, and HF-S-IMD groups (↑158.53%, 154.86%, 154.86%, and 155.38% respectively, p<0.001) compared to the normal control group. The soy protein group showed an insignificant decrease in ChREBP level (↓22.57%, p<0.05) compared to the normal control group. The decrease in levels of ChREBP in liver tissue was insignificant in the HF-S, HF-IMD, and HF-S-IMD groups (↓1.42%, 1.42%, and 1.22% respectively, p<0.05) compared to the HF group **(Table 4)**.

3.7. Nuclear factor-kappa B in liver tissue

In the HF group, there was a significant increase in NF-κB in liver tissue (2.17-fold increase, p<0.001) compared to the normal control group. There was a significant decrease in NF-κB level in IMD group (1.26-fold decrease, p<0.001) and in the soy group (1.25-fold decrease, p<0.001) compared to the normal control group. On the other hand, NF-κB in liver tissue was significantly decreased in the HF-S, HF-IMD, and HF-S-IMD groups (2.43-, 2.97- and 2.95-fold decrease respectively, p<0.001) compared to the HF group **(Table 4)**.

3.8. Phosphorylated insulin receptor (pISR) in liver tissue

The level of pISR measured in liver tissue was significantly decreased in the HF group (1.64-fold decrease, p<0.001) compared to the normal control group. pISR in liver tissue was significantly increased in the HF-S, HF-IMD, and HF-S-IMD groups (1.98-, 1.76- and 1.66-fold increase respectively, p<0.001) compared to the HF group. However, the pISR level was significantly higher in the HF-S group than in the normal control group (1.21-fold increase, p<0.01) **(Table 4)**.

3.9. Histopathology

Histopathological analysis of liver sections in the normal control group revealed normal hepatocytes arranged in cords around the portal area, as shown in Figure (2A), and a normal histological structure of the hepatic lobule (Figure 2B). The

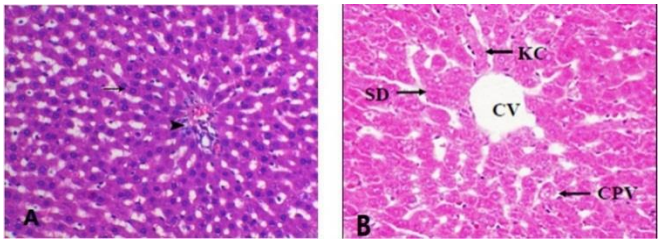
livers of animals fed a soy protein-containing diet showed normal hepatocytes arranged in cords (Figure 3A). The livers of animals injected with IMD showed normal hepatic vacuolation associated with quiescent Ito cells (Figure 3B). Microscopic examination of liver sections of rats supplemented with 10% fructose showed swollen hepatocytes associated with vacuolation of their cytoplasm, consistent with increased glycogen storage and TGs accumulation **(Figure 4A)**.

Table 4: Changes in free fatty acids, Carbohydrate responsive element binding protein, Nuclear factor-kappa B, and Phosphorylated insulin receptor in different studied groups

Groups	FFA (µmol/L)	ChREBP (ng/mL)	NFκB (ng/mL)	PIR (ng/mL)
Normal control	275.40 ± 9.87	3.81 ± 0.77	6.97 ±0.64	4.96 ±0.64
HF	741.60 ±56.77^{acdefg}	9.85 ±0.48^{acd}	15.11 ±0.70^{acdefg}	3.03 ±0.27^{acdefg}
Soy protein	265.40 ±11.05^{befg}	2.95 ±0.201^{abdefg}	5.56 ±0.49^{abdfg}	5.76 ±0.39^{bg}
IMD	270.62 ±3.11^{befg}	4.32 ±0.66^{befg}	5.54 ± 0.38^{abc}	5.30 ±0.48^{b}
HF-S	686.80 ±25.86^{abcd}	9.71 ±0.61^{acd}	6.21 ±0.77^{abfg}	6.00 ±0.82^{b}
HF-IMD	721.60 ±11.82^{acd}	9.71 ±0.35^{acd}	5.13 ±0.27^{abce}	5.33 ±0.33^{b}
HF-S-IMD	672.50 ±9.61^{abcd}	9.73 ±0.52^{acd}	5.08 ±0.23^{abce}	5.02 ±0.40^{bc}

Data are presented as mean ± SD, n=10 rats. HF: high fructose diet, IMD: inhibitor of NF-κB, HF-S: high fructose diet with soy protein, HF-IMD: high fructose diet with NF-κB inhibitor, HF-S-IMD: high fructose diet with soy protein and NF-κB inhibitor. FFA: free fatty acids, ChREBP: carbohydrate responsive element binding protein, NFκB: nuclear factor kappa B, PIR: phosphorylated insulin receptor. a: significant versus normal control. b: significant versus HF. c: significant versus soy protein. d: significant versus IMD. e: significant versus HF-S. f: significant versus HF-IMD. g: significant versus HF-S-IMD. Significance was set at $p < 0.05$.

Additionally, periportal inflammatory cell infiltration was associated with hepatic steatosis **(Figure 4B)**. Liver sections of animals supplemented with 10% fructose showed a marked appearance of a mosaic pattern in the liver, which is associated with increased periportal glycogen storage **(Figure 4C)**.



The livers of rats fed with 10% fructose and soy protein showed a mild degree of hepatic vacuolation but were mostly within the normal limits **(Figure 5A)**. Histopathological examination of the livers of rats fed with 10% fructose and injected with IMD showed normal-sized hepatocytes arranged in hepatic cords and a normal degree of hepatic vacuolation **(Figure 5B)**. Histopathological changes induced by concurrent administration of fructose with soy protein and IMD showed a normal degree of hepatic vacuolation and steatosis **(Figure 6A, B)**.

Fig. 2: Photomicrographs of liver sections (H&E, X200) from the normal control group (normal diet with i.p. injection of the vehicle showing A: normal hepatocytes arranged in cords (arrow) around the portal area (arrowhead). B: normal histological structure of hepatic lobules. CPV: Cytoplasmic vacuolation, CV: Central vein, KC: Kupffer cell, SD: Sinusoidal dilatation.

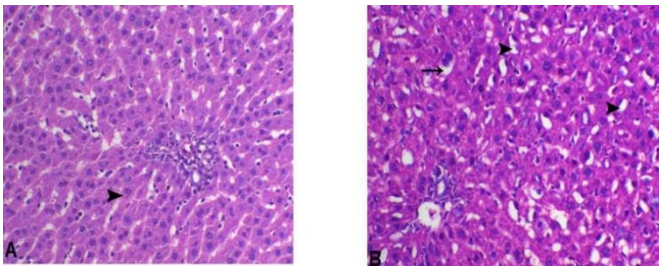


Fig. 3: Photomicrographs of liver sections (H&E, X200) from (A): the soy group (20% soy protein-containing diet) showing normal hepatocytes arranged in cords (arrowhead). (B): the IMD group (i.p. injection of 10 mg/kg IMD-0354) showing normal hepatic vacuolation (arrow) associated with quiescent Ito cells (arrowhead).

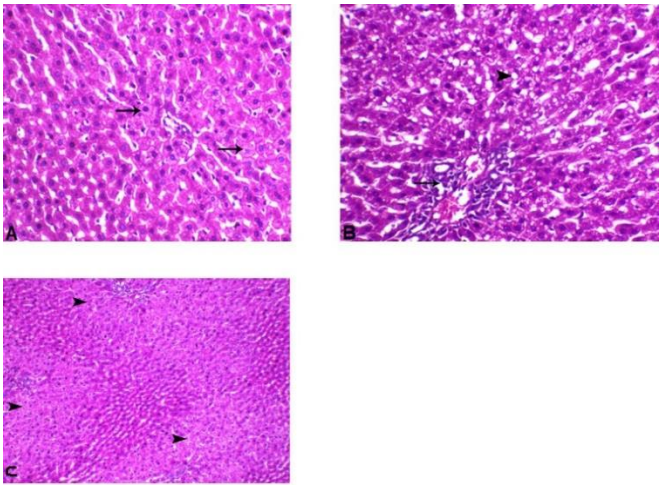


Fig. 4: Photomicrographs of liver sections (H&E, X200) from the HF group (10% w/v fructose in drinking water) showing (A): swelling of the hepatocytes associated with vacuolation of their cytoplasm, consistent with increased glycogen storage (arrow). (B): periportal inflammatory cell (macrophage and lymphocyte) infiltration (arrow) associated with hepatic steatosis (round fat vacuoles within the hepatocytes) (arrowhead). (C): a marked appearance of a mosaic pattern in the liver, which is associated with periportal increased glycogen storage (arrowheads).

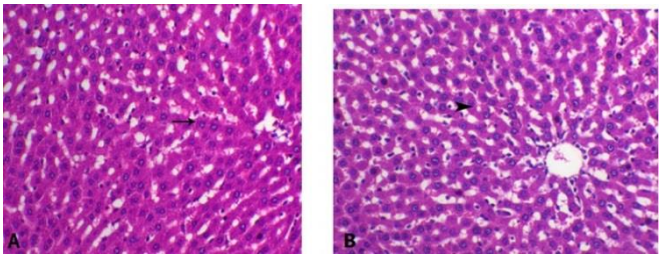


Fig. 5: Photomicrographs of liver sections (H&E, X200)

from (A): the HF-S group (10% w/v fructose along with 20% soy protein) showing a mild degree of hepatic vacuolation and mostly within the normal limits (arrow). (B): the HF-IMD group (10% w/v fructose along with i.p. injection of 10 mg/kg IMD-0354) showing a normal degree of hepatic vacuolation (arrow).

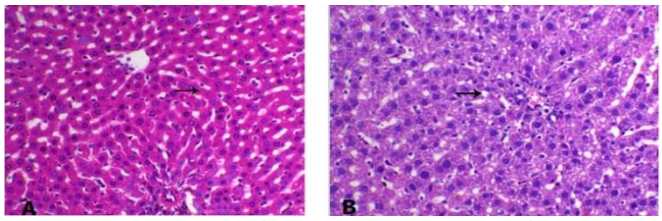


Fig. 6: Photomicrographs of liver sections (H&E, X200) from the HF-S-IMD group (10% w/v fructose in drinking water along with 20% soy protein-containing diet and i.p. injection of 10 mg/kg IMD-0354) showing (A): a normal degree of hepatic vacuolation (arrow). (B): a normal degree of hepatic steatosis (arrow)

4. DISCUSSION

Non-alcoholic fatty liver disease (NAFLD) is the most common liver disease worldwide, with a prevalence ranging from 25% to 45%, increasing in parallel with obesity and diabetes.[14] Because of the disease burden, effective prevention is a significant unmet need.[15]

This work aimed to examine the protective effect of soy protein isolate as a natural NF-κB inhibitor in male rats exposed to induction of NAFLD by a high fructose diet (10% w/v) and to evaluate the anti-inflammatory, anti-hyperlipidaemic, and anti-diabetic properties of soy protein. High fructose diet-induced NAFLD has been used in experimental rat models because fructose is a highly lipogenic sugar, and fructose is phosphorylated by fructokinase, forming fructose-1-phosphate, which can then be converted to several three-carbon molecules, including glyceraldehyde, dihydroxyacetone phosphate, and glyceraldehyde-3-phosphate. Some of these three-carbon molecules can be converted to glucose through gluconeogenesis, or they can be used to generate other products such as triglycerides (TGs). TGs can be hydrolyzed by lipoprotein lipase to form FFAs and monoacylglycerol.[16] Excessive fructose intake leads to high levels of FFAs, causing fatty liver, inflammation, and metabolic disorders.[17]

In the present study, NAFLD was manifested by a significant increase in body weight, an enlarged liver, a greater liver index, and elevated levels of blood glucose, serum insulin, serum FFAs, NF-κB, and ChREBP, as well as decreased levels of pISRs in liver tissue. This effect was also manifested histologically by swelling of the hepatocytes associated with cytoplasm vacuolation, consistent with increased glycogen storage and TG accumulation in the HF group. Moreover, the HF group showed periportal inflammatory cell infiltration associated with hepatic steatosis and a marked mosaic pattern in the liver, which is associated with increased periportal glycogen storage. The present findings were in agreement with those by Sreeja et al.[18] who reported that high fructose diet-induced metabolic disorders were associated with significant histopathological changes in liver tissue.

The histopathological changes induced by a high fructose diet in the current work were partially corrected by the administration of soy protein and/or IMD-0354, where the liver sections showed an area of recovering hepatocytes with a mild degree of hepatic vacuolation that was mostly within the normal limits and normal-sized hepatocytes arranged in hepatic cords. Our results were in accordance with the findings of Zhou et al.[19] They observed a significant decrease in the severity of hepatocellular vacuolation and improved pathological conditions in the livers of rats that received a soy protein-containing diet.

The body weight, liver weight, and liver index were significantly increased in the HF group compared to those in the normal control group because fructose enhances the accumulation of fats in the liver, resulting in higher liver weight. These findings were in line with the results by Toop et al.[20] 21 who reported that a high fructose diet induces metabolic disorders with fatty liver in animal models. However, the concurrent use of soy protein with a high fructose diet significantly decreased the body weight, liver weight, and liver index compared to the use of a high fructose diet alone as it reduces hepatic lipid accumulation and improves liver function. These results were in agreement with the findings of Won et al.[21], who reported that the use of soy protein could maintain normal liver weight.

Determination of serum FFAs is regarded as a good method to quantify NAFLD and to evaluate the effectiveness of new anti-inflammatory agents.[22] FFAs represent the major source of hepatic fat accumulation in NAFLD. FFAs are also involved in the pathogenesis of different metabolic disorders that are associated with insulin resistance by activation of the c-Jun-terminal kinase (JNK) pathway, contributing to the development of hepatic steatosis and insulin resistance.[23]

Our results showed that serum FFAs were significantly increased in the HF group compared to the normal control group and the other groups that received either soy protein alone or IMD-0354 alone. Serum FFAs were significantly decreased in the HF-S, HF-IMD, and HF-S-IMD groups compared to the HF group. These results were in agreement with those by Deol et al.[24]

Free fatty acids can activate the NF-κB pathway, which is a vital mediator of NAFLD and insulin resistance in fructose-fed models. NF-κB activation regulates a variety of cytokines involved in inflammation and transcription initiation of many genes such as TNF-α, IL-1, IL-6, and IL-8.[25] These findings are supported by our results, which revealed higher levels of NF-κB in the HF group compared with the normal control group. NF-κB activation by the high fructose diet may be due to the accumulation of lipids in the liver, resulting in increasing levels of fatty acid oxidation, thus stimulating inhibitor kappa kinase β (IKK-β), which activates NF-κB. On the other hand, concurrent administration of soy protein and/or IMD-0354 with fructose showed a marked reduction in NF-κB when compared with the administration of the high fructose diet alone, supporting the anti-inflammatory effect of soy protein.

Proinflammatory cytokines are the main players in promoting insulin resistance because they are activators of signalling pathways, such as the JNK and the inhibitor kappa

kinase (IKK) pathways, which negatively interfere with the early steps in the insulin signalling cascade. Therefore, chronic administration of fructose induces metabolic disorders and insulin resistance due to increased levels of FFAs and NF-κB.[26] This was confirmed in our study by a significant increase in blood glucose and serum insulin levels, a higher HOMA-IR score, and a significant decrease in pISRs in the HF group compared to the normal control group.

Our results show a significant decrease in blood glucose and serum insulin levels in the HF-IMD, HF-S, and HF-S-IMD groups compared to the HF group. Additionally, the pISR levels in the liver tissue of these groups were approximately near the normal level in the control group. These results indicate that receiving soy protein and/or IMD-0354 can properly maintain pISR levels by their effects on insulin resistance. These results confirm the effects of soy protein and/or IMD-0354 on NAFLD and insulin resistance.

Kim et al.[27] demonstrated that ChREBP is a transcriptional activator of glycolytic and lipogenic genes that plays a critical role in insulin resistance and de novo lipogenesis. Fructose consumption was associated with activating ChREBP production, as fructose ingestion directly transactivates glucose-6-phosphatase expression, a mechanism by which activating ChREBP might contribute to impaired glucose homeostasis, which is supported by our results, where the level of ChREBP in the liver tissues of the HF group was significantly increased compared with that in the normal control group. On the other hand, ChREBP was decreased significantly in the soy group compared with all other groups, indicating that the use of flavonoids present in soy protein significantly decreased lipogenic gene expression and suppressed ChREBP signalling.

5. CONCLUSIONS

The current study shows that a high fructose diet is associated with metabolic and inflammatory diseases, which could lead to metabolic syndrome and insulin resistance. After the addition of an NF-κB inhibitor to the high fructose diet, the levels of insulin, insulin receptors, glucose, ChREBP, and FFAs were significantly decreased compared with those in the control group, which indicates that the mechanism of fructose induction of metabolic diseases is NF-κB dependent. The use of soy protein as a natural NF-κB inhibitor significantly reduced the deleterious effects of the high fructose diet. Thus, the use of soy protein could be beneficial in patients with high fructose-induced metabolic disorders and may be used in combination with other medications for decreasing the risks of metabolic diseases as well as hepatic steatosis. Further studies are warranted to investigate the therapeutics efficacy of soy protein against hepatic steatosis in clinical settings.

CONFLICTS OF INTEREST

The authors declare no conflict of interest

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