Heliyon 9 (2023) e16031

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

Assessing the hepatoprotective effects of hesperidin on liver-associated disorders in albino rats with experimentally induced obesity and type II diabetes: A histological and biochemical study

Ihab Shafek Atta [a,f,*], Mohamed R. Elnady [b], Ali G. Alghamdi [c], Ahmed Hassan Alghamdi [d], Alaa A. Aboulata [e,g], Ibrahim M. Shatla [b,f]

[a] Pathology Department, Faculty of Medicine, Al-Azhar University, Assuit, Egypt
[b] Physiology Department, Damietta Faculty of Medicine, Al-Azhar University, Egypt
[c] Surgery Department, Faculty of Medicine, Al Baha University, Saudi Arabia
[d] Paediatric Department, Faculty of Medicine, Al Baha University, Saudi Arabia
[e] Microbiology and Immunology Department, Faculty of Medicine, Al-Azhar University, Egypt
[f] Pathology Department, Faculty of Medicine, Al Baha University, Saudi Arabia
[g] Biomedical and Dental Sciences, Faculty of Dentistry, Al Baha University, Saudi Arabia

ARTICLE INFO

Keywords:
High-fat diet
Hesperidin
Rat
Steatosis
Streptozotocin
T2DM

ABSTRACT

Introduction: Hesperidin (HSP) has multiple beneficial effects in verities of clinical situations including type 2 diabetes mellitus (T2DM). Aim: Determination of curative effects of HSP on the liver in T2DM rats through biochemical and histopathological studies. Methods: Animals. Fifty rats were enrolled. 10 rats were fed a normal diet (control group), and the remaining 40 rats received a high-fat diet (HFD) for 8 weeks. The HFD-fed rats were grouped into Group II: 10 rats, and Group III: 10 rats received HSP 100 mg/kg. Group IV: 10 rats received a single dose of streptozotocin (STZ), 30 mg/kg, and Group V: 10 rats received STZ and HSP. Body weight, Blood glucose, insulin level, liver enzymes, lipid profile, oxidative stress, TNF-α, NF-κB, and liver biopsy were estimated. Results: there is improvement in the histological profile of the steatosis in HFD-fed rats treated with HSP either in group III or in group V (received STZ) along with amelioration in blood glucose, insulin, liver enzymes, lipid profile, oxidative profile, TNF-α, and NF-κB. Conclusion: HSP in this STZ model revealed an improvement in steatosis, biochemical markers, and histologic findings. By studying these factors, we expected to identify the prospective targets for intervention that could help improve outcomes for individuals with obesity and diabetes-related liver diseases.

1. Introduction

Type 2 Diabetes is one of the most prevalent metabolic diseases caused by decreased production of insulin by pancreatic beta cells, as well as a loss in the tissues' ability to respond to insulin, resulting in hyperglycemia [1]. According to the International Diabetes

* Corresponding author. Pathology Department, Faculty of Medicine, Al-Azhar University, Assuit, Egypt. , ,
E-mail addresses: Ihab.bassyouny@Azhar.edu.eg, Ibassyouny@bu.edu.sa (I.S. Atta).

https://doi.org/10.1016/j.heliyon.2023.e16031
Received 15 February 2023; Received in revised form 27 April 2023; Accepted 3 May 2023
Available online 6 May 2023

Federation Diabetes Consultation, more than 463 million people worldwide had diabetes in 2019, and 700 million were expected to have it by 2045 [2]. Diabetes not only affects the quality of life for patients and communities but also places a significant economic burden on society. The International Diabetes Federation estimates that the direct and indirect costs related to diabetes in 2019 are USD 760 billion and will reach approximately USD 825 billion in 2030 [3].

Diabetes can have significant effects on various organs throughout the body, including the liver. The high blood sugar levels, abnormal levels of fats in the blood, oxidative stress, and inflammation that accompany diabetes can result in liver-related complications. One such complication is a non-alcoholic fatty liver disease (NAFLD), which occurs when fat accumulates in the liver, leading to inflammation and fibrosis that can progress to cirrhosis and liver failure. Additionally, diabetes may increase the risk of developing hepatosclerosis and liver cancer [4,5].

Several studies have found a clear correlation between liver disease, diabetes, and obesity. For instance, Machado and colleagues revealed that non-alcoholic fatty liver disease (NAFLD), a form of liver disease mainly linked to obesity and diabetes, was seen in up to 70% of type 2 diabetes patients [6]. Furthermore, Younossi et al. revealed that up to 75% of individuals with NAFLD were either obese or diabetic [7]. Hesperidin has been shown to be highly safe, as obtained by Rizza et al. which revealed no side effects have been obtained from healthy adult volunteers who took hesperidin supplements at dosages of up to 500 mg per day for 12 weeks [8]. Morand et al. revealed that hesperidin had no considerable negative effects in any of the eight human volunteers they investigated [9].

There is a substantial overlap between the triad of obesity, diabetes, and liver disease, which emphasizes the necessity for patients to be experienced about the increased risk of liver disease. Fortunately, the administration of these natural products including hesperidin can be extremely effective and safe in manging these conditions. Consequently, considering hesperidin may be the key to managing this situation.

Hesperidin (HSP) is a type of plant pigment naturally occurring as flavanone glycoside found in citrus [10]. Hesperidin has varied effects as an analgesic, diuretic, anti-inflammatory, antihypertensive, antibacterial, antiviral, analgesic, diuretic, hypolipidemic, anti-cancer, antioxidant effects and anti-edematous [10,11]. However, hesperidin has not been commonly used clinically, because it has low water solubility. Well-developed strategies have been implemented to overcome this barrier, including the design of efficient delivery systems for hesperidin-containing drugs [11–13]. The safety margin of HSP in herbal formulations is high, with minimum or no adverse or toxic effects because its lethal dose (LD50) in acute oral toxicity testing is $>$ 2000 mg/kg [14,15].

In addition, hesperidin can improve metabolic and vascular health in overweight and obese rats [16–19]. Furthermore, hesperidin can activate Nrf2/ARE pathway and up-regulates mRNA and protein levels of the downstream target genes in some diseases [19–21].

Recent studies revealed the role of hesperidin as a part of flavonoid phytochemicals in modulating Immune checkpoint inhibitors (ICIs) therapy for certain cancers including hepatocellular carcinoma, breast cancer and bladder cancer [22–24]. In addition, flavonoids have a vital role as prognostic and predictive factors including tumors especially hepatocellular carcinoma. Rizzo et al. suggest more research on phytochemicals as flavonoids on ICIs including PD-L1 expression, tumor-infiltrating lymphocytes (TILs), tumor mutational burden (TMB), and several other biomarkers [23,24].

2. The aim

The aim is to determine the possible curative effects of HSP on the liver of experimentally induced type 2 diabetes mellitus model (T2DM) by low dose STZ in high-fat diet-fed rats through biochemical and histopathological study. In addition, the current study aimed to examine the role of ameliorating inflammation, oxidative stress, and insulin resistance in mitigating the negative effects of these conditions on the liver. By studying these factors, we hoped to identify potential targets for intervention that could help improve outcomes for individuals with obesity and diabetes-related liver diseases.

3. Material and methods

3.1. Chemicals and reagent kits

Hesperidin (HSP) and streptozotocin (STZ) were purchased from Sigma chemicals Co., St. Louis, MO, and stored at 4 °C. Biochemical kits for estimation of biochemical markers as total cholesterol, triglycerides, HDL-cholesterol, LDL-cholesterol, aspartate aminotransferase (AST), alanine aminotransferase (ALT) and antioxidants were purchased from Bio-Diagnostic Co. Cairo, Egypt.

3.2. HFD preparation

Daily fresh preparation of HFD by thoroughly mixing 1.0% of cholesterol and 9% of saturated fat (palm oil) and 90% of the standard commercial diet (Alwatanya Company, Cairo, Egypt) [25].

3.3. Experimental animals

Fifty adult male Sprague-Dawley rats were purchased from Serum and Antigen Laboratories at Helwan with weights ranging between 170 and 190 g. The animals were housed in a 22 $\pm$ 2 °C temperature and humidity (40–60%)-controlled area with 12/12 h light-dark cycle.

3.4. Experimental design and procedures

The experiment was done after fulfilling the criteria of "The Guide for the Care and Use of Laboratory Animals produced by the National Institute of Health, USA [26]. The steps of research were done according to guidelines of the Committee on the Ethics of Animal Experiments of Al-Azhar University. After a one-week adaptation period, prior to the start of the current experiment, the 50 SD male rats were enrolled in the study. 10 rats were fed a normal diet, received distilled water by oral gavage for 4 weeks, and were taken as a control group (group I), the remaining 40 rats were randomly allocated into the high-fat diet (HFD) regimen for 8 weeks regimen, HFD (HFD, n = 40) for induction of obesity.

After 8 weeks, the rats' weights were 280–320 g. The rats were grouped into the following: 10 obese rats received distilled water by oral gavage for 4 weeks, and 10 obese rats were designed as group III and received HSP only 100 mg/kg by oral gavage for 4 weeks. The remaining 20 rats were intraperitoneally injected with a single low dose of STZ (30 mg/kg) to induce partial destruction of beta cells to be T2DM. Rats with a fasting blood glucose of ≥200 mg/dL were further subdivided into 2 groups; 10 diabetic rats (group IV; not treated with HSP) and 10 diabetic rats treated by HSP for 4 weeks (group V).

3.5. Measurement of body weight and blood glucose level

Measurement of the body weight of rats was done weekly. The fasting blood glucose (FBG) of overnight fasting rats was measured after injection with STZ with a glucose strip (Accu-Check, Roche, New Cairo, Egypt) using a tail-vein blood sample. Before the estimation of fasting blood sugar, all rats fasted for 12 h. Following 1 week from streptozotocin injection, the blood glucose was estimated using glucose test strips. Fasting blood glucose of ≥200 mg/dL was deemed to diagnose T2DM. In addition, insulin was also estimated, and Homeostatic Model Assessment for Insulin Resistance (HOMA-IR) was also calculated.

3.6. Estimation of biochemical parameters in the blood

Plasma samples were collected from all overnight fasting rats. The blood samples were obtained from the retro-orbital venous plexus using a capillary tube method. The blood was collected into three tubes; the first tube contained EDTA, the second tube contained sodium fluoride, and the third tube was plain.

The serum glucose level was determined calorimetrically following the protocol described by Tietz [27]. Insulin concentration in serum samples was quantified using an enzyme-linked immunosorbent assay (ELISA) with rat insulin ELISA kits following the protocol described by Carlsson et al. [28]. The Homeostatic Model Assessment of Insulin Resistance (HOMA-IR) index was calculated as [Insulin concentration in μIU/L] multiplied by [glucose concentration in mmol/L] divided by 22 [29]. Total Cholesterol and Triglycerides, High-density lipoprotein-C, Low-density lipoprotein C, alanine aminotransferase (ALT), aspartate aminotransferase (AST), using a biochemical analyzer (Molecular Devices MD2800, San Francisco, CA, USA) and calculated using Friendewald's equation [30,31].

The clear supernatant of liver tissue homogenates was used for assaying the activities of antioxidant enzymes SOD using the method of Nishikimi et al. [32], catalase using the spectrophotometric method of Aebi [33] and GSH using the method of Prins and Loose, [34]. Inflammatory cytokine TNF-α [R&D Systems Inc., USA; Catalog Nos. RTA00], NF-κB [MyBioSource, Inc., USA; Catalog Nos. MBS722386], was assessed using an ELISA kit according to the manufacturer's instructions.

3.7. Histopathological examination of liver biopsies

A histopathological microscopic examination was performed. The rats were euthanized with a ketamine-xylazine mix at a ratio of 1: 1 and a dosage of 0.1 ml/100 g at the end of the study. The tissue of the liver was excised, preserved in 10% formalin for 72 h at room temperature, cut into 4 mm thick, stained with Hematoxylin/eosin (H&E) [35,36], and examined using light microscopy.

3.8. Statistical analysis

The data analysis uses SPSS version 23 (IBM, Chicago, IL, USA). The mean and standard error of the Mean was used to represent the data. Comparisons were made using a one-way analysis of variance (one-way ANOVA) followed by Tukey's post hoc test for multiple comparisons. p-value was deemed statistically significant when $p < 0.05$.

Table 1
Effect of HFD, STZ, and Hesperidin on body weight, FBG, insulin, and insulin resistance.

Parameters	Control (Group I)	HFD (Group II)	HFD + HSP (Group III)	HFD + STZ (30 mg/kg) (Group IV)	HFD + STZ + HSP (Group V)
Body weight (g)	225 ± 10.3	368 ± 16.4[a]	255 ± 14.8[b]	284 ± 11.1[ab]	247 ± 12.5[b]
FBG (mg/dL)	87 ± 5.6	157 ± 7.7[a]	109 ± 6.1[b]	338 ± 17.3[ab]	203 ± 12.6[abd]
Insulin (μU/mL)	6.32 ± 0.41	12.37 ± 0.92[a]	8.48 ± 0.74[b]	6.73 ± 0.58[b]	7.34 ± 0.65[b]
HOMA-IR	1.4 ± 0.09	4.8 ± 0.23[a]	2.3 ± 0.13[ab]	5.6 ± 0.29[a]	3.7 ± 0.21[abd]

a = significance with control group.
b = significance with HFD group.
d = significance with HFD + STZ group.

4. Results

4.1. Effect of HFD, STZ, and hesperidin on body weight

As shown in Table 1, by the end of the experiment all HFD-fed groups showed an increase in body weights, but it was significant in groups 2 and 4 and insignificant in groups III and V as compared to the control group receiving a normal diet. In HFD-fed rats, administration of Hesperidin as in group III, induction of diabetes by STZ in group IV, or administration of Hesperidin after induction of diabetes in group V, all showed a significant decrease in body weights in comparison to group II fed on HFD only. Diabetic obese rats in group 5 presented insignificant lower body weight as compared to group IV (Table 1).

4.2. Effect of HFD, STZ, and hesperidin on blood glucose

Table 1 shows significantly higher blood glucose levels in groups II, IV, and V and amelioration in group III to a normal level as compared to the control group. In HFD-fed animals, HSP-treated group III showed a significant reduction in blood glucose while animals received STZ in groups 4, STZ, and HSP in group V showed a significant increase as compared to group 2 received HFD only. Also, group V showed a significant decrease when compared to group 4 (Table 1).

4.3. Effect of HFD, STZ, and hesperidin on serum insulin

The results showed a significant increase in serum insulin levels of group II with no significant changes in groups III, IV, and V in comparison to the control group. Also, groups III, IV, and V showed a significant decrement in serum insulin compared to HFD group II. There are no significant changes between groups V, and IV (Table 1).

4.4. Effect of HFD, STZ, and hesperidin on HOMA-IR (insulin resistance)

The results show a significant increase in HOMA-IR of groups II, III, IV, and V as compared to the control group. Also, HOMA-IR showed a significant decrease in groups III, and V and insignificant changes in groups IV in comparison to group II received HFD only. There is a significant decrease in HOMA-IR in group V compared to group IV (Table 1).

4.5. Effect of HFD, STZ, and hesperidin on liver enzymes

Table 2 shows significantly higher levels of liver enzymes ALT, and AST in HFD-fed groups II, and IV with Interestingly normalization of HSP-treated group III as compared to the control group. Group V presented an elevation of liver enzymes which was significant in ALT and AST levels as compared to the control group. Liver enzymes ALT and AST significantly lowered in group III and elevated in groups IV and V in comparison to group II. There is also a significant decrease in diabetic HSP-treated group V when compared to diabetic group IV (Table 2).

4.6. Effect of HFD, STZ, and hesperidin on lipids profile

This study has shown a significant increase in TC, TG, and LDL in HFD-fed groups II, IV, and V with intriguing normalization of HSP-treated group III as compared to the control group. HDL showed a significant increase in groups II and IV and amelioration to the extent that the changes are not significant in groups III and V as compared to the control group. TC, TG, and LDL significantly decreased in HSP-treated group III and significantly elevated in group IV which received STZ, and insignificant changes in diabetic HSP-treated group V in comparison to the HFD group while HDL showed a significant increase in HSP-treated group III and a significant decrease in group IV which received STZ and insignificant changes in diabetic HSP treated group V in comparison to HFD group. Group V presented a significant decrease in TC, TG, and LDL and a significant increase in HDL when compared to group IV (Table 2).

Table 2
Effect of HFD, STZ, and Hesperidin on liver enzymes and lipids profile.

Parameters	Control (Group I)	HFD (Group II)	HFD + HSP (Group III)	HFD + STZ (30 mg/kg) (Group IV)	HFD + STZ + HSP (Group V)
ALT (U/L)	36.1 ± 1.86	59.7 ± 2.59[a]	42.3 ± 2.06 [b]	82.9 ± 6.77 [ab]	63.7 ± 5.41 [ad]
AST (U/L)	25.7 ± 2.19	54.3 ± 3.26[a]	33.1 ± 2.54 [b]	76.8 ± 4.83 [ab]	60.2 ± 4.15 [ad]
ALP (U/L)	49.2 ± 4.15	86.3 ± 5.27[a]	59.7 ± 4.18[b]	127.6 ± 7.33 [ab]	69.8 ± 6.01[d]
TC (mg/dL)	133 ± 9.6	248 ± 10.3[a]	167 ± 7.8 [b]	364 ± 18.6 [ab]	179 ± 13.1 [ad]
TG (mg/dL)	125 ± 6.47	213 ± 9.9[a]	163 ± 7.3[b]	295 ± 15.3[ab]	178 ± 11.8 [ad]
LDL (mg/dL)	44.6 ± 2.78	93.3 ± 6.35[a]	67.9 ± 3.66 [b]	152.8 ± 9.13 [ab]	104.8 ± 6.2 [ad]
HDL (mg/dL)	52.2 ± 3.19	35.3 ± 2.05[a]	59.5 ± 3.61 [b]	19.6 ± 2.49 [ab]	40.5 ± 3.46[d]

a = significance with control group.
b = significance with HFD group.
d = significance with HFD + STZ group.

4.7. Effect of HFD, STZ, and hesperidin on the activity of antioxidant enzymes

As shown in Table 3 there is a significant decrement in the antioxidant activity of SOD, catalase, and GSH enzymes in groups II, IV, and V while group III showed insignificant changes in comparison to the control group. There is a significant increase in the antioxidant activity of SOD, catalase, and GSH in groups III, and V and a significant decrement in group IV as compared to HFD group II. Group V displayed a significant increase in the antioxidant activity of SOD, catalase, and GSH correlated to group IV (Table 3) (Figs. 1–3).

4.8. Effect of HFD, STZ, and hesperidin on inflammation mediators

This study has shown an increase in TNF-α and NF-κB which was significant in groups II, IV, and V and insignificant in group III as compared to the control group. In correlation to group II, TNF-α and NF-κB decreased significantly in group III and increased significantly in group IV with no significant changes in group V. Group 5 displayed a significant decrease of TNF-α and NF-κB as compared to group IV (Table 3) (Figs. 4 and 5).

4.9. The histopathological alterations in the liver tissue of the studied groups

4.9.1. Histological examination of group I

Histologic examination of liver biopsy in group I (control group) revealed well-preserved hepatic lobules, and uniformity of the size of hepatocytes. The portal triad is normal. and the hepatic cords were normal and well-oriented from the central vein to the boundaries of the hepatic lobules. The hepatocytes appear polygonal in shape and contain rounded vesicular nuclei and prominent nucleoli, some hepatocytes were binucleated. The hepatocyte has acidophilic cytoplasm with scattered fine basophilic granules. Blood sinusoids were seen as a network in between the plates of hepatocytes radiating toward the central vein (Fig. 6).

4.9.2. Histological examination of group II (HFD)

The histology revealed several histological changes in the form of disturbed hepatic architecture, marked dilatation of central veins, dilatation and congestion of portal veins, and blood sinusoids. In addition to mild to moderate cellular infiltrations mainly around central veins bile ductules. Most of the hepatocytes reveal cytoplasmic vacuolations, and cell ballooning (Fig. 7a, b, c).

4.9.3. Histological examination of group III

The cytoplasmic vacuoles are less observed with the restoration of hepatic architecture. The hepatocytes retain their acidophilic cytoplasm, and the sinusoids appear less dilated. Inflammatory infiltrates are scarce (Fig. 8 A, B).

4.9.4. Histological examination of group IV

Rats in the group IV (HFD-STZ) group had severely damaged liver structures, including numerous necrotic hepatocytes that had lost their normal shape and architecture, marked cytoplasmic vacuolations, presence of pyknotic nuclei, and apoptosis. In addition, the liver sinusoids were enlarged and congested. Heavy lymphocytic infiltration and other inflammatory cells were marked in most of the lobules giving the appearance of a lobular pattern of inflammation. In other fields, the inflammation ranged from mild with minor infiltrates and spotty necrosis of single hepatocytes, to widespread necrosis with architectural disturbance and lobular disarray (Fig. 9 A, B).

4.9.5. Histological examination of group V

In group V, the treated group (HFD-STZ-HSP) restoration of hepatic lobules to great extent with evidence of hepatocyte regeneration more marked at the periportal area than in the pericentral area with markedly reduced vacuolization and sinusoidal congestion in the liver. The inflammatory infiltrates were less evident with increased ductular proliferation and mitosis (Fig. 10a and b).

Table 3
Effect of HFD, STZ and Hesperidin on the activity of antioxidant enzymes (oxidative stress) and inflammation.

	Control	HFD	HFD + HSP	HFD + STZ (30 mg/kg)	HFD + STZ + HSP
SOD (U/L)	46.5 ± 2.01	27.3 ± 1.14[a]	42.9 ± 1.36[b]	17.4 ± 0.69 [ab]	38.6 ± 1.43 [abd]
Catalase (U/min)	21.3 ± 0.81	13.7 ± 0.49[a]	19.6 ± 0.93[b]	8.5 ± 0.52[ab]	16.9 ± 0.78 [abd]
GSH (μg/mg protein)	24.2 ± 0.97	15.4 ± 0.84[a]	23.5 ± 1.04 [b]	11.3 ± 0.56 [ab]	19.6 ± 1.23 [abd]
TNF-α [Pg/ml]	51.6 ± 2.51	70.5 ± 2.8[a]	57.2 ± 2.48[b]	89.4 ± 4.66 [ab]	74.8 ± 3.31 [ad]
NF-κB [ng/mg protein]	0.74 ± 0.029	1.55 ± 0.067[a]	1.11 ± 0.053 [b]	2.54 ± 0.126 [ab]	1.73 ± 0.091 [ad]

a = significance with control group.
b = significance with HFD group.
d = significance with HFD + STZ group.
F for HFD P for Hesperidin Z for STZ.

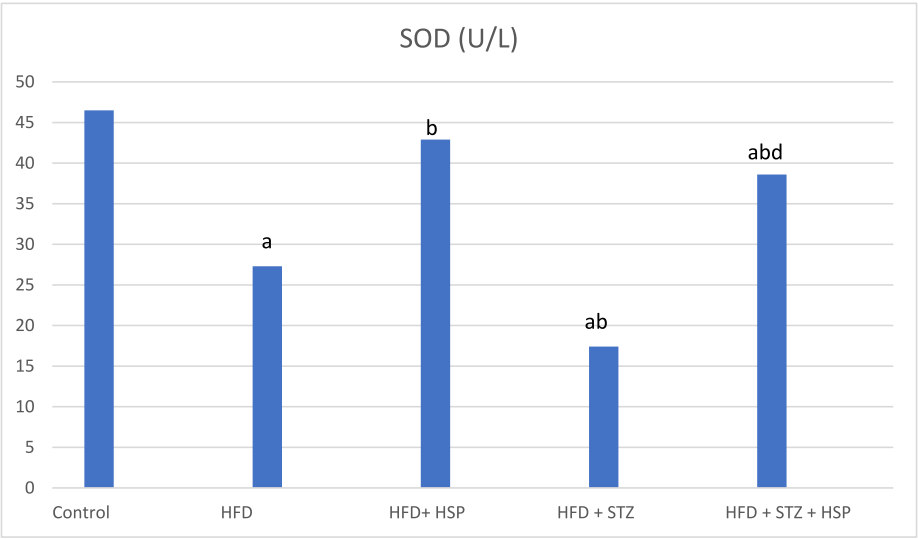

Fig. 1. Shows the results of SOD and its correlation among the five groups in the current study. a = significance with control group b = significance with HFD group. d = significance with HFD + STZ group.

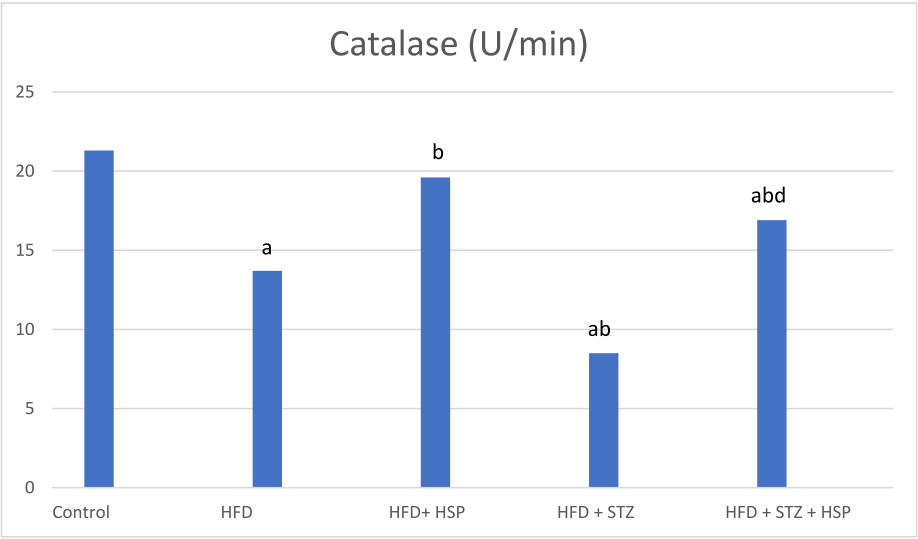

Fig. 2. Shows the results of catalse and its correlation among the five groups in the current study. a = significance with control group b = significance with HFD group. d = significance with HFD + STZ group.

5. Discussion

In the present study, we utilized a low dose of STZ with HFD in the experimental rats to induce obesity. This model is identified in many studies [37–39] to describe the early stage in the pathogenesis of T2DM because the animals never acquire beta cell failure as may be observed in humans in the late stage of T2DM [40–42]. For the initiation of beta cell dysfunction, a combination of HFD with a single injection of STZ was introduced [43,44].

Regarding blood glucose and insulin, we found that the HSP ameliorates hyperglycemia in both HFD and HFD-STZ-treated diabetic

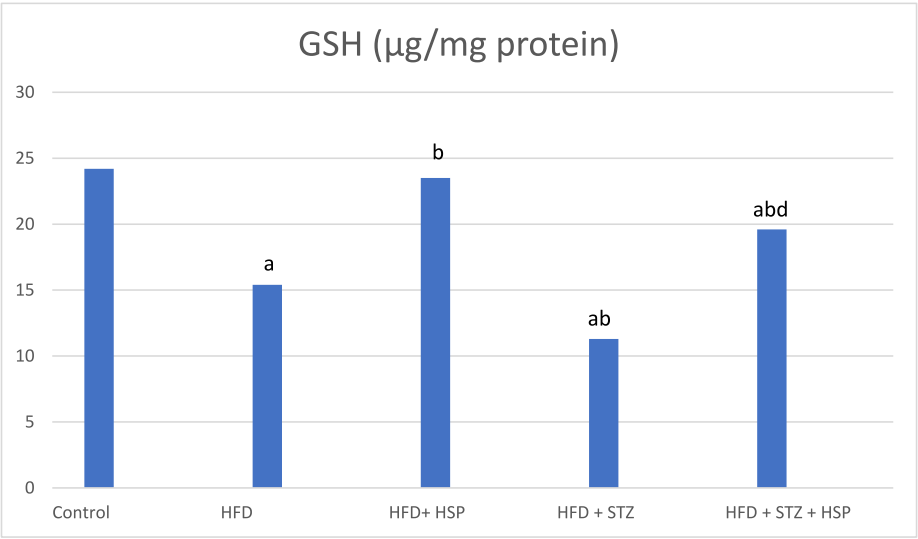

Fig. 3. Shows the results of GSH and its correlation among the five groups in the current study. a = significance with control group b = significance with HFD group. d = significance with HFD + STZ group.

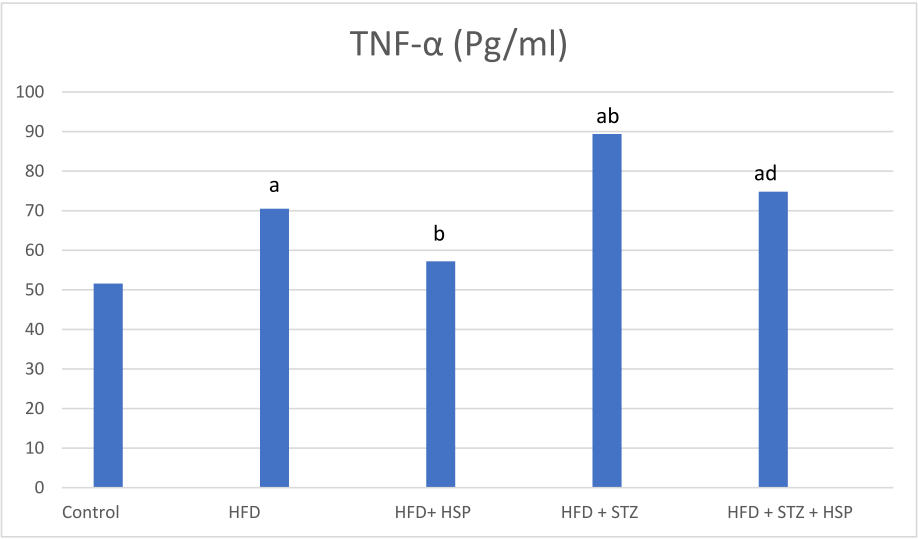

Fig. 4. Shows the results of TNF-α and its correlation among the five groups in the current study. a = significance with control group b = significance with HFD group d = significance with HFD + STZ group.

rats by increasing the production of insulin from beta cells (Table 1). In group V insulin is slightly elevated compared to the control group (group I) but is considered low relative to high FBG. This denotes partial destruction of beta cells. This is documented by the results of HOMA –IR which indicates the presence of insulin resistance. So, a diagnosis of T2DM was made. In treated groups (III, V) there is marked improvement in all parameters including FBG, insulin, and HOMA-IR.

Many studies demonstrated that blood glucose level is decreased with HSP treatment [45–47]. El-Shahawy et al. revealed improvement in diabetes, increasing the liver glycogen content, as well as lipogenesis adding to increase both insulin sensitivity and glucose uptake by lessening the oxidative stress in rats treated with the HSP formula [13]. The mechanism of lowering blood glucose

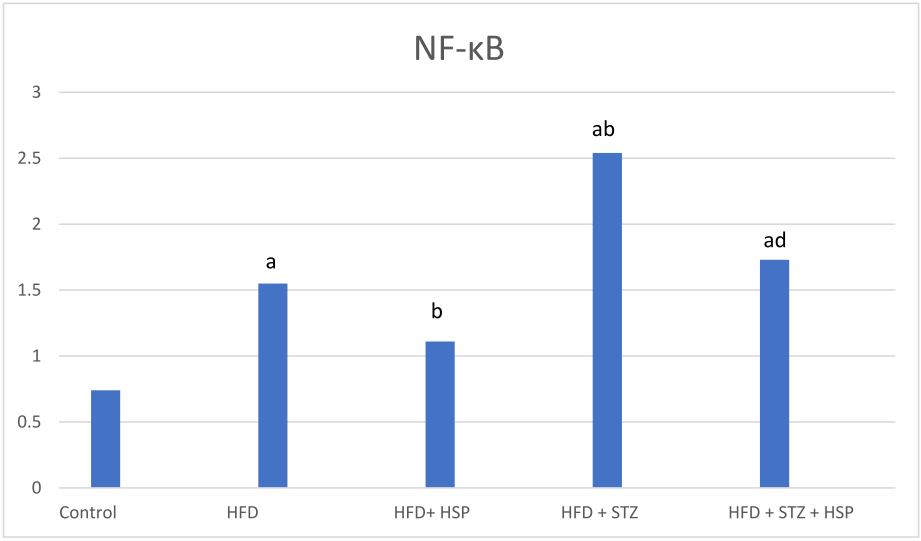

Fig. 5. Shows the results of NF-κB [ng/mg protein] and its correlation among the five groups in the current study. a = significance with control group b = significance with HFD group d = significance with HFD + STZ group.

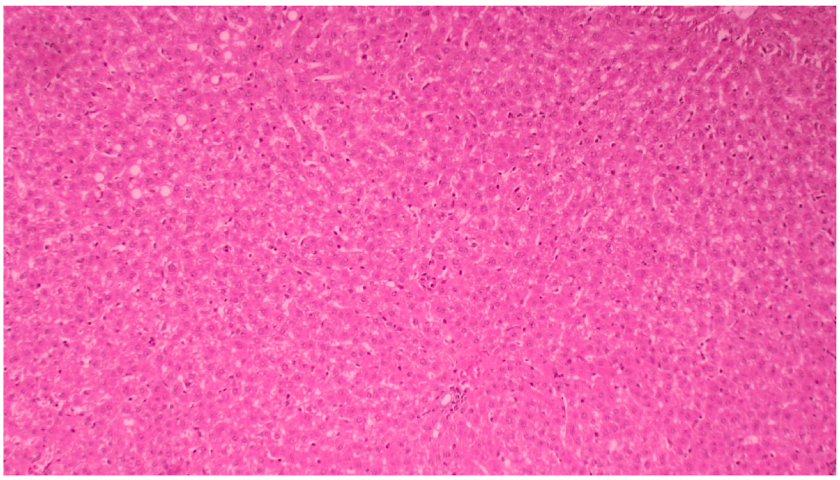

Fig. 6. Group I (Control group; rat fed normal diet). The photomicrograph showing normal lobular architecture, hepatocytes are arranged in cords separated by normal sinusoids. The hepatocytes have an acidophilic cytoplasm and central dark stained nucleus. (H&E stain, ×100).

with HSP is best explained by increasing the level of mRNA glucokinase which is the key enzyme in the cycle of glucose catabolism, as well as decreasing the level of gluconeogenic enzyme G6Pase, in T2DM mouse liver [48]. In addition, another study found that increased level of insulin with HSP treatment in the HFD-STZ group [49].

Regarding liver enzymes and lipid profile, in non-treated groups (II, IV) (HFD-STZ), the level of lipid profiles as the serum TG, TC, LDL, and HDL concentrations have been significantly increased (Table 2). This is compatible with many studies in which a low dose of STZ was used and the dose ranged from 35 to 40 mg/kg single dose [50–53].

In another way, these results in HSP-treated groups (III, V) revealed marked improvement in non-treating groups (II, IV). This coincides with the study of Horcajada et al. [54]. This agrees with the study of Akiyama et al. [55] and in contrast with the study of Miyake et al. which revealed no significant role of HSP in serum lipid levels [47].

The mechanism of HSP in lowering cholesterol and TG has been demonstrated by Bok et al. who explained it by inhibiting both β-Hydroxy β-methylglutaryl-CoA (HMG-CoA) reductase and acyl CoA: cholesterol acyltransferase (ACAT) [56].

Furthermore, hesperidin enhances the expression of the gene encoding the LDL receptor [57]. Horcajada et al. [54] revealed that STZ injection leads to a decrease in the serum level of adiponectin (anti-diabetic, anti-inflammatory properties) which returned to its

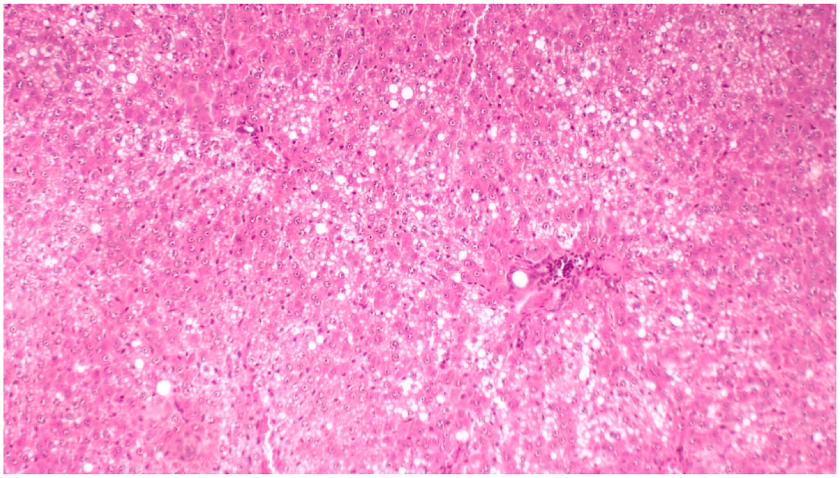

Fig. 7a. Group II (HFD). The photomicrograph reveals disarray of lobular architecture, cell steatosis and cell ballooning. The sinusoids appear dilated with ductular proliferation and dispersed scarce inflammatory cell all over hepatocytes and around the ductules. Multiple small vacuoles are seen between the hepatocytes (H& E, ×200).

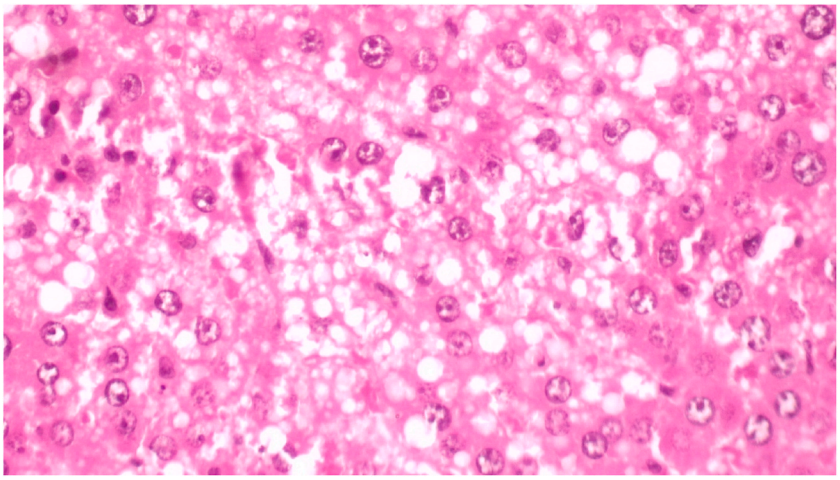

Fig. 7b. Group II (HFD). The section showing signs of liver steatosis, the hepatocytes show variable cytoplasmic vacuolation which is either small and multiple vacuoles or may be coalescent to form large one, some hepatocytes are ballooning. Ballooned hepatocytes containing one large vacuole, some hepatocytes with darkly stained nuclei, others are binucleated, and fragmented nuclei. Some hepatocytes show clearing up of the nuclei with an empty appearance (H&E × 400).

normal level with HSP administration. Therefore, this observation was considered indirect evidence for the effects of hesperidin.

Regarding oxidative stress, our results revealed that oxidative stress was decreased in the non-treated groups and improved in the treated groups (Table 3). This coincides with many studies [58–60].

TNF-α and NF-κB were found high in the HFD and STZ-HFD (non-HSP-treated groups) and improved in the HSP-treated groups (Table 3). The elevation of IL-6 occurred because of the up-regulation of the NF-κB pathway by IL-17, thus promoting insulin resistance, which leads to T2DM development [61]. With hesperidin treatments, the level of TNF-α and IL-17 is lowered that suppresses the NF-kB and consequently reduces the secretion of IL-6 and enhances insulin resistance and T2DM is improved [62,63].

Regarding histopathological changes in non-treated groups, we found an alteration in the hepatocytes and hepatic lobules, and portal triad together with vacuolization and lymphocytic infiltration. These changes were mild to moderate in the HFD group and more marked in the STZ-HFD group (groups II, and IV). These changes coincided with other studies [64–66].

The improvement in the histological profile of the treated groups with HSP (group III and group V is supported by previous studies utilizing hesperidin in different situations. Various studies have investigated the effects of HSP on damaged hepatocytes. They found that the key point in the process of regeneration is the activation of the PI3K/Akt pathway through the summative effects of HSP [67–70]. Another observation comes from the study of Li et al. [71] which revealed that HSP plays a role in the protection against liver tissue injury by improving oxidative stress, inhibiting the inflammatory responses, and reducing apoptosis via activation of the Akt

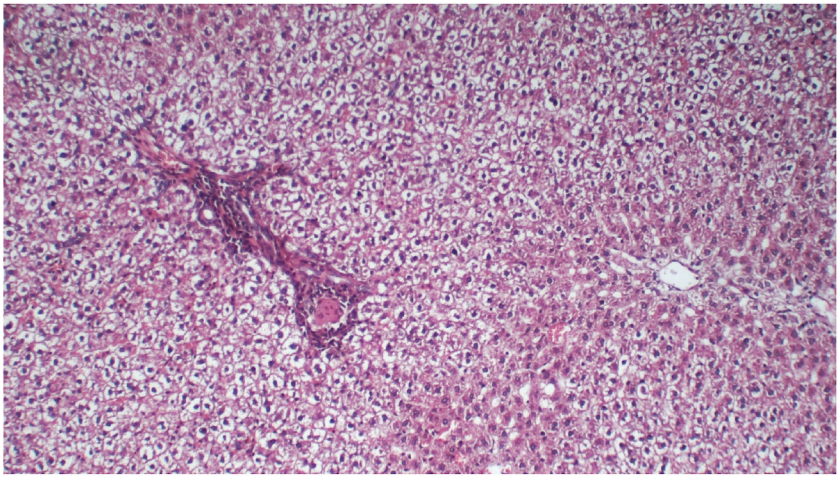

Fig. 7c. Group II (HFD). Another photomicrograph revealed that most hepatocytes have vacuolated cytoplasm with marked ductular proliferation. Some central veins are dilated, and others are congested (H&E, ×200).

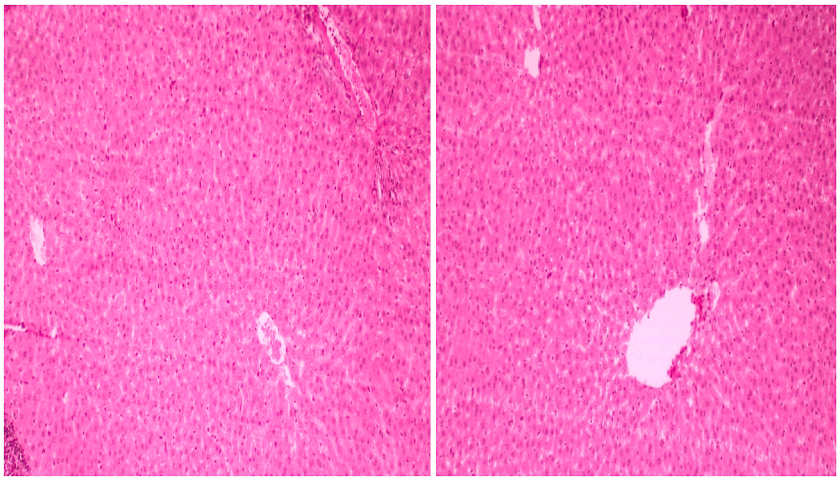

Fig. 8. Group III. (HFD + HSP) the photomicrograph showing the histological effect of HSP treatment in HFD rat. The hepatocytes restore its acidophilic cytoplasm, the central vein and sinusoids are still congested (figure A) and dilated (figure B). The lobular architecture is preserved (H&E stain, ×100).

pathway. However, the mechanism of hepatocytes and hepatic lobule recovery after injury in SD rats is still unknown in the study of Park et al. [72]. In the present study, the regenerative process (disappearance of vacuoles and presence of mitoses) was evidently more in the periportal area than in the pericentral area, this coincides with many studies [73–75].

Despite significant advances in research and medicine, the prevalence of obesity and diabetes-related liver diseases remains high, and the underlying pathogenesis of these conditions is still not fully understood. Considering this, our study was designed to investigate potential protective and therapeutic strategies for these diseases.

Based on the findings of our study, hesperidin appears to possess a range of beneficial properties that make it a promising candidate for the prevention and treatment of liver disease related to obesity and diabetes. Specifically, our results demonstrate that hesperidin has anti-inflammatory and antioxidant effects, as well as hypoglycemic properties, which contribute to its ability to improve insulin resistance and preserve liver tissue.

Given these findings, we believe that hesperidin could be used as a protective and curative agent for individuals with liver disease related to obesity or type 2 diabetes. This may involve using hesperidin alone or in combination with regular antidiabetic drugs, depending on the state of the patient and the severity of their condition.

Overall, our study has important implications for the field of liver disease research and suggests that hesperidin may offer a safe and effective alternative or adjunct to existing treatment options. Future research should focus on exploring the mechanisms underlying the beneficial effects of hesperidin and further investigating its potential as a therapeutic agent for liver diseases.

Our study provides compelling evidence that administration of hesperidin at a dosage of 100 mg/kg can significantly improve

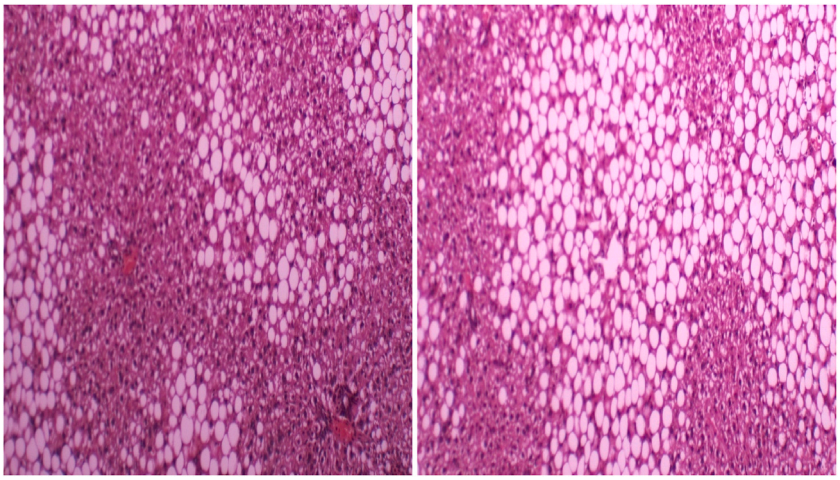

Fig. 9. Group IV. (Single low dose STZ). The photomicrograph showing disruption of hepatic architecture. The hepatocytes showing severe degree of inflammatory infiltrates, pyknosis, apoptosis and steatosis as seen in figure A (Figure A) that are more obvious in figure B (Figure B). Presence of darkly stained fragmented nucleus together with apoptotic changes, marked inflammatory cells with dilated congested portal trac and ductular proliferation diagnose the hepatotoxic effects of STZ. (H&E stain ×400).

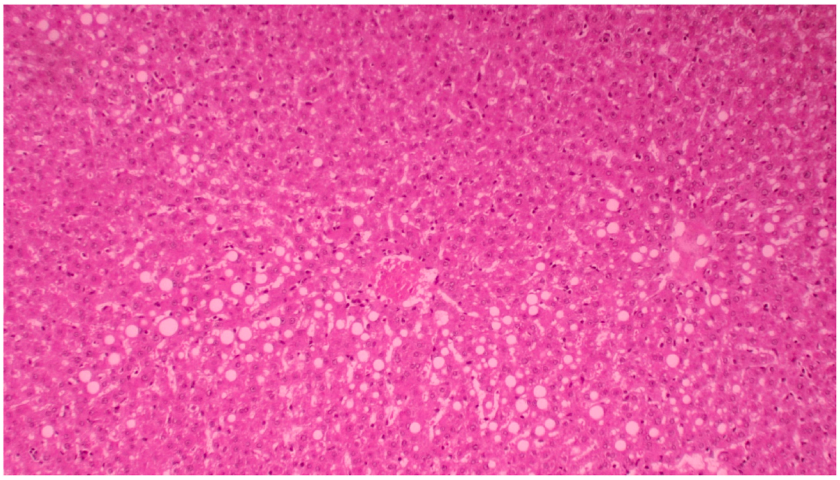

Fig. 10a. (Group V: receiving HSP + STZ low single dose). The photomicrograph showing the histological effect of HSP treatment on the injected single low dose STZ rat. Presence of regenerative process as the number of cytoplasmic vacuoles is less marked in the hepatocytes compared with that seen in Fig. 9 together with restoration of some of lobular architecture. Sinusoidal dilatation is present with congestion and dilation of the central vein the regenerative process is more marked at the periphery of the lobules. (H&E stain, ×200).

inflammation, oxidative stress, and insulin resistance with consequent amelioration of liver enzymes and reducing steatosis and restoring histologic hepatocyte abnormalities in rats with obesity and diabetes-related liver diseases. These results have important implications for the development of new therapeutic strategies for individuals with these conditions.

Further research is needed to confirm and expand upon our findings, but our study represents an important step forward in understanding the potential benefits of hesperidin for individuals with liver diseases related to obesity and diabetes. Given the high prevalence of these conditions and the lack of effective treatment options, identifying new therapies that can improve outcomes for affected individuals is of critical importance. We believe that our study provides a strong foundation for future research in this area and hope that it will ultimately lead to better health outcomes for individuals with these conditions.

Based on the good results that emerged from this study, it is necessary to expand the study of flavonoids in all liver diseases and the effect of these substances on the drugs used in all liver diseases.

5.1. Limitation of the study

Despite the positive results observed in this study, there are several limitations that should be considered when interpreting the

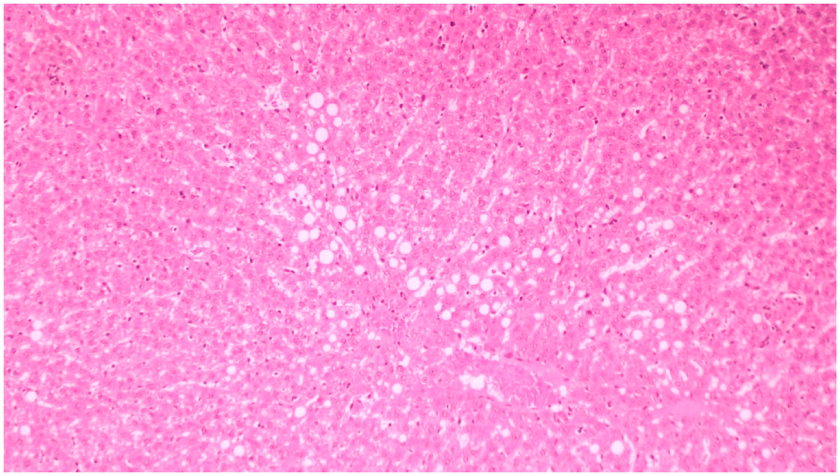

Fig. 10b. (Group V: receiving HSP + STZ low single dose). The photomicrograph showing the histological effect of HSP treatment on the injected single low dose STZ rat. the photomicrograph show that the regenerative process is more evident in the periphery of hepatic lobule than in the pericentral area, the number of cytoplasmic vacuoles is markedly decreased compared with that seen in Fig. 9 with restoration of lobular architecture. Sinusoidal dilatation is still present. (H&E stain, ×200).

findings. Firstly, the sample size used in this study was relatively small, with only 10 rats in each group. This small sample size may limit the generalizability of the findings to larger populations of rats or other animal species. Secondly, the duration of the treatment was only four weeks, which may not be sufficient to fully capture the effects of the treatment, particularly if the treatment has a long-term impact or takes time to accumulate in the body. Additionally, the outcomes measured in this study were limited to a specific set of histological and biochemical measures, which may not fully capture the impact of the treatment on other relevant outcomes. It is important to consider these limitations when interpreting the findings of this study and to replicate the study in larger and more diverse samples with longer treatment duration and more comprehensive outcome measures.

6. Conclusion

The obtained results reveal that a high-fat diet combined with a low dose of STZ 35 mg/kg at a single dose demonstrated to be a better way for creating a steady animal model of type 2 diabetes mellitus, and this model may be appropriate for varieties of pharmaceutical settings. This low-dose STZ model is sufficient to aggravate steatosis in steatosis in HFD-induced rats. In these circumstances, administration of HSP alone or in the low dose STZ model will be effective in the improvement of steatosis and restore the histologic hepatocyte abnormalities to normal. Herbal compounds containing HSP is highly recommended in improving lipid profile and must be addressed in more research related to human.

Author contribution statement

Ihab Shafek Atta: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.
Mohamed R. Elnady: Performed the experiments; Contributed reagents, materials, analysis tools or data.
Ali G. Alghamdi, Ahmed Hassan Alghamdi, Ala A. Aboulata: Analyzed and interpreted the data.
Ibrahim M. Shatla: Conceived and designed the experiments; Performed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Funding statement

This work did not receive any specific funding or grant.

Data availability statement

Data included in article/supp. Material/referenced in article.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

[1] U. Galicia-Garcia, A. Benito-Vicente, S. Jebari, A. Larrea-Sebal, H. Siddiqi, K.B. Uribe, H. Ostolaza, C. Martín, Pathophysiology of type 2 diabetes mellitus, Int. J. Mol. Sci. 21 (17) (2020) 6275, 10.3390/ijms21176275. PMID: 32872570; PMCID: PMC7503727.

[2] P. Saeedi, I. Petersohn, P. Salpea, et al., Global and regional diabetes prevalence estimates for 2019 and projections for 2030 and 2045: results from the international diabetes federation diabetes atlas, in: 9th Edition. Diabetes Research and Clinical Practice, 2019, p. 157, https://doi.org/10.1016/j.diabres.2019.107843. Article ID: 107843.

[3] R. Williams, S. Karuranga, B. Malanda, P. Saeedi, A. Basit, S. Besançon, C. Bommer, A. Esteghamati, K. Ogurtsova, P. Zhang, S. Colagiuri, Global and regional estimates, and projections of diabetes-related health expenditure: results from the international diabetes federation diabetes atlas, in: 9th Edition. Diabetes Res Clin Pract, 162, 2020 Apr, 108072, https://doi.org/10.1016/j.diabres.2020.108072. Epub 2020 Feb 13. PMID: 32061820.

[4] A.E. Hamed, N. Elwan, M. Naguib, R. Elwakil, G. Esmat, M. El Kassas, S. Abd-Elsalam, S. Moussa, Diabetes association with liver diseases: an overview for clinicians, Endocr., Metab. Immune Disord.: Drug Targets 19 (3) (2019) 274–280, https://doi.org/10.2174/1871530318666181116111945. PMID: 30444204.

[5] P. Loria, A. Lonardo, F. Anania, Liver, and diabetes. A vicious circle, Hepatol. Res. 43 (1) (2013 Jan) 51–64, https://doi.org/10.1111/j.1872-034X.2012.01031.x. PMID: 23332087; PMCID: PMC3733501.

[6] M.V. Machado, H. Cortez-Pinto, A. Gutierrez, Hepatic steatosis: a role for mitochondria in the pathogenesis of nonalcoholic fatty liver disease, J. Lipid Res. 56 (11) (2015) 2233–2245.

[7] Z.M. Younossi, A.B. Koenig, D. Abdelatif, Y. Fazel, L. Henry, M. Wymer, Global epidemiology of nonalcoholic fatty liver disease—meta-analytic assessment of prevalence, incidence, and outcomes, Hepatology 71 (6) (2019) 1913–1923.

[8] W. Rizza, N. Veronese, L. Fontana, E. Rejc, C. Sieber, F. Fanelli, L. Ferrucci, Evaluation of the safety and efficacy of Hesperidin supplementation in human: a systematic review of clinical trials, Phytother Res. 32 (2) (2018) 219–225.

[9] C. Morand, C. Dubray, D. Milenkovic, D. Lioger, J.F. Martin, A. Scalbert, A. Mazur, Hesperidin Contributes to the Vascular Protective Effects of Orange Juice: a Randomized Crossover Study, 2011.

[10] P. Pandey, U. Sayyed, R.K. Tiwari, M.H. Siddiqui, N. Pathak, P. Bajpai, Hesperidin induces ROS-mediated apoptosis along with cell cycle arrest at G2/M phase in human gall bladder carcinoma, Nutr. Cancer 71 (2019) 676–687.

[11] S.H. Jo, M.E. Kim, J.H. Cho, Y. Lee, J. Lee, Y.D. Park, J.S. Lee, Hesperetin inhibits neuroinflammation on microglia by suppressing inflammatory cytokines and MAPK pathways, Arch Pharm. Res. (Seoul) 42 (2019) 695–703.

[12] O. Ciftci, C. Ozcan, O. Kamisli, A. Cetin, N. Basak, B. Aytac, Hesperidin, a Citrus flavonoid, has the ameliorative effects against experimental autoimmune encephalomyelitis (EAE) in a C57BL/J6 mouse model, Neurochem. Res. 40 (6) (2015) 1111–1120.

[13] A.A.G. El-Shahawy, A. Adel Abdel-Moneim, S. Abdelazim, M. Ebeid Asm, Z.E. Eldin, M.I. Zanaty, A novel layered double hydroxide-hesperidin nanoparticles exert antidiabetic, antioxidant and anti-infammatory efects in rats with diabetes, Mol. Biol. Rep. 48 (2021) 5217–5232, https://doi.org/10.1007/s11033-021-06527-2.

[14] A.A. Zanwar, S.L. Badole, P.S. Shende, M.V. Hegde, S.L. Bodhankar, Chapter 76 - cardiovascular effects of hesperidin: a Flavanone glycoside, in: R.R. Watson, V. R. Preedy, S. Zibadi (Eds.), Polyphenols in Human Health and Disease, Academic Press, San Diego, 2014, pp. 989–992.

[15] Y. Li, A.D. Kandhare, A.A. Mukherjee, S.L. Bodhankar, Acute and sub-chronic oral toxicity studies of hesperidin isolated from orange peel extract in Sprague Dawley rats, Regul. Toxicol. Pharmacol. 105 (2019) 77–85.

[16] M. Xue, M.O. Weickert, S. Qureshi, N.B. Kandala, A. Anwar, M. Waldron, A. Shafie, D. Messenger, M. Fowler, G. Jenkins, N. Rabbani, P.J. Thornalley, Improved glycemic control and vascular function in overweight and obese subjects by glyoxalase 1 inducer formulation, Diabetes 65 (8) (2016) 2282–2294.

[17] M. Xue, N. Rabbani, H. Momiji, P. Imbasi, M.M. Anwar, N. Kitteringham, B.K. Park, T. Souma, T. Moriguchi, M. Yamamoto, P.J. Thornalley, Transcriptional control of glyoxalase 1 by Nrf2 provides a stress-responsive defence against dicarbonyl glycation, Biochem. J. 443 (1) (2012) 213–222.

[18] Y.W. Liu, Y.Q. Cheng, X.L. Liu, Y.C. Hao, Y. Li, X. Zhu, F. Zhang, X.X. Yin, Mangiferin upregulates glyoxalase 1 through activation of Nrf2/ARE signaling in central neurons cultured with high glucose, Mol. Neurobiol. 54 (6) (2017) 4060–4070.

[19] Ya-Jing Chen, Li Kong, Zhuang-Zhuang Tang, Yu-Meng Zhang, Yue Liu, Tao-Yun Wang, Yao-Wu Liu, Hesperetin ameliorates diabetic nephropathy in rats by activating Nrf2/ARE/glyoxalase 1 pathway, Biomed. Pharmacother. 111 (2019) 1166–1175.

[20] M.C. Chen, Y.Y. Ye, G. Ji, J.W. Liu, Hesperidin upregulates heme oxygenase-1 to attenuate hydrogen peroxide-induced cell damage in hepatic L02 cells, J. Agric. Food Chem. 58 (6) (2010) 3330–3335.

[21] H. Ren, J. Hao, T. Liu, D. Zhang, H. Lv, E. Song, C. Zhu, Hesperetin suppresses inflammatory responses in lipopolysaccharide-induced RAW 264.7 cells via the inhibition of NF-kappaB and activation of Nrf2/HO-1 pathways, Inflammation 39 (3) (2016) 964–973.

[22] J. Lee, Y. Han, W. Wang, H. Jo, H. Kim, S. Kim, K.M. Yang, S.J. Kim, D.N. Dhanasekaran, Y.S. Song, Phytochemicals in cancer immune checkpoint inhibitor therapy, Biomolecules 11 (8) (2021) 1107, https://doi.org/10.3390/biom11081107. PMID: 34439774; PMCID: PMC8393583.

[23] A. Rizzo, A. Cusmai, G. Gadaleta-Caldarola, G. Palmiotti, Which role for predictors of response to immune checkpoint inhibitors in hepatocellular carcinoma? Expet Rev. Gastroenterol. Hepatol. 16 (4) (2022) 333–339, https://doi.org/10.1080/17474124.2022.2064273. Epub 2022 Apr 10.

[24] A. Rizzo, A.D. Ricci, Biomarkers for breast cancer immunotherapy: PD-L1, TILs, and beyond, Expet Opin. Invest. Drugs 31 (6) (2022) 549–555, https://doi.org/10.1080/13543784.2022.2008354. Epub 2021 Nov 25. PMID: 34793275.

[25] M. Esmail, S. Anwar, M. Kandeil, A.M. El-Zanaty, M. Abdel-Gabbar, Effect of Nigella sativa, atorvastatin, or L-Carnitine on high fat diet-induced obesity in adult male Albino rats, Biomed. Pharmacother. 141 (2021), 111818, https://doi.org/10.1016/j.biopha.2021.111818. Epub 2021 Jun 11. PMID: 34126354.

[26] National Research Council (US), Committee for the Update of the Guide for the Care and Use of Laboratory Animals. Guide for the Care and Use of Laboratory Animals, eighth ed., National Academies Press (US), Washington (DC), 2011 https://doi.org/10.17226/12910. Available from: https://www.ncbi.nlm.nih.gov/books/NBK54050/.

[27] N.W. Tietz, Clinical Guide to Laboratory Tests, third ed., WB. Saunders, Philadelphia, 1995, pp. 268–273. Toxicol Appl Pharmacol 362:86–94.

[28] A. Carlsson, I.B. Hallgren, H. Johansson, S. Sandler, Concomitant enzyme-linked immunosorbent assay measurements of rat insulin, rat C-peptide, and rat proinsulin from rat pancreatic islets: effects of prolonged exposure to different glucose concentrations, Endocrinology 151 (10) (2010 Oct) 5048–5052, https://doi.org/10.1210/en.2010-0433.

[29] D.R. Matthews, J.P. Hosker, A.S. Rudenski, B.A. Naylor, D.F. Treacher, R.C. Turner, Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man, Diabetologia 28 (7) (1985 Jul) 412–419.

[30] W.T. Friedewald, R.I. Levy, D.S. Fredrickson, Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge, Clin. Chem. 18 (1972) 499–502.

[31] P. Krishnaveni, V.M. Gowda, Assessing the validity of friedewald's formula and anandraja's formula for serum LDL-cholesterol calculation, J. Clin. Diagn. Res. 9 (12) (2015 Dec) BC01, https://doi.org/10.7860/JCDR/2015/16850.6870. Epub 2015 Dec 1. PMID: 26816879; PMCID: PMC4717736.

[32] M. Nishikimi, N.A. Rao, K. Yagi, The occurrence of superoxide anion in the reaction ofreduced phenazinemethosulfate and molecular oxygen, Biochem. Biophys. Res. Commun. 46 (1972) 849, 8543.7.

[33] H.E. Aebi, Catalase, in: H.U. Bergmeyer, J. Berg- meyer, M. GraBI (Eds.), Methods of Enzymatic Analy- Sis, third ed., Verlag Chemie, Weinheim, 1983, pp. 273–286.

[34] H.K. Prins, J.A. Loos, in: J.J. Yunis (Ed.), Glutathione, Bio-Chemical Methods in Red Cell Genetics, Academic Press, New York, 1969, pp. 127–129.

[35] R. Drury, Theory and practice of histological techniques, J. Clin. Pathol. 36 (5) (1983) 609.

[36] M. Torbenson, Biopsy Interpretation of the Liver, Wolters Kluwer Health, 2014.

[37] M. Albersen, et al., Functional, metabolic, and morphologic characteristics of a novel rat model of type 2 diabetes-associated erectile dysfunction, Urology 78 (476) (2011) e471–e478, https://doi.org/10.1016/j.urology.2011.03.024.

[38] K. Sahin, et al., Effect of chromium on carbohydrate and lipid metabolism in a rat model of type 2 diabetes mellitus: the fat-fed, streptozotocin-treated rat, Metab., Clin. Exp. 56 (2007) 1233–1240, https://doi.org/10.1016/j.metabol.2007.04.021.

[39] K. Srinivasan, B. Viswanad, L. Asrat, C.L. Kaul, P. Ramarao, Combination of high-fat diet-fed and low-dose streptozotocin-treated rat: a model for type 2 diabetes and pharmacological screening, Pharmacol. Res. 52 (2005) 313–320, https://doi.org/10.1016/j.phrs.2005.05.004.

[40] H.M. El-Bassossy, M.L. Watson, Xanthine oxidase inhibition alleviates the cardiac complications of insulin resistance: effect on low grade inflammation and the angiotensin system, J. Transl. Med. 13 (2015) 82, https://doi.org/10.1186/s12967-015-0445-9.

[41] J.S. Lee, et al., Histologic and metabolic derangement in high-fat, high-fructose, and combination diet animal models, TheScientificWorldJOURNAL (2015), 306326, https://doi.org/10.1155/2015/306326(2015.

[42] Y.H. Pyo, K.W. Lee, Preventive effect of Monascus-fermented products enriched with ubiquinones on type 2 diabetic rats induced by a high-fructose plus high-fat diet, J. Med. Food 17 (2014) 826–829, https://doi.org/10.1089/jmf.2013.3001.

[43] M.J. Reed, et al., A new rat model of type 2 diabetes: the fat-fed, streptozotocin-treated rat, Metab., Clin. Exp. 49 (2000) 1390–1394, https://doi.org/10.1053/meta.2000.17721.

[44] M. Zhang, X.Y. Lv, J. Li, Z.G. Xu, L. Chen, The characterization of high-fat diet and multiple low-dose streptozotocin induced type 2 diabetes rat model, Exp. Diabetes Res. (2008), 704045, https://doi.org/10.1155/2008/704045.

[45] B. Sharma, C. Balomajumder, P. Roy, Hypoglycemic and hypolipidemic effects of flavonoid rich extract from Eugenia jambolana seeds on streptozotocin induced diabetic rats, Food Chem. Toxicol. 46 (2008) 2376–2383.

[46] H. Cao, I. Hininger-Favier, M.A. Kelly, R. Benaraba, H.D. Dawson, S. Coves, A.M. Roussel, R.A. Anderson, Green tea polyphenol extract regulates the expression of genes involved in glucose uptake and insulin signaling in rats fed a high fructose diet, J. Agric. Food Chem. 55 (2007) 6372–6378.

[47] Y. Miyake, K. Yamamoto, N. Tsujihara, T. Osawa, Protective effects of lemon flavonoids on oxidative stress in diabetic rats, Lipids 33 (1998) 689–695.

[48] U.J. Jung, M.K. Lee, Y.B. Park, M.A. Kang, M.S. Choi, Effect of citrus flavonoids on lipid metabolism and glucose-regulating enzyme mRNA levels in type-2 diabetic mice, Int. J. Biochem. Cell Biol. 38 (2006) 1134–1145.

[49] A. Abdel-Moneim, M.B. Ashour, A.M. Mahmoud, O.M. Ahmed, Insulin sensitizing effects of hesperidin and naringin in experimental model of induced type 2 diabetes in rats: focus on tumor necrosis factor-alpha and resistin, Nat. Sci. 7 (2011) 134–141.

[50] S.H. Hu, T. Jiang, S.S. Yang, et al., Pioglitazone ameliorates intracerebral insulin resistance and tau-protein hyperphosphorylation in rats with type 2 diabetes, Exp. Clin. Endocrinol. Diabetes 121 (2013) 220–224.

[51] D.M. Abo-elmatty, S.S. Essawy, J.M. Badr, et al., Antioxidant and anti-inflammatory effects of *Urtica pilulifera* extracts in type 2 diabetic rats, J. Ethnopharmacol. 145 (2013) 269–277.

[52] H.B. Khan, K.S. Vinayagam, B.T. Moorthy, et al., Anti-inflammatory and anti-hyperlipidemic effect of *Semecarpus anacardium* in a high fat diet: STZ-induced type 2 diabetic rat model, Inflammopharmacology 21 (2013) 37–46.

[53] G.R. Gandhi, A. Stalin, K. Balakrishna, et al., Insulin sensitization via partial agonism of PPAR gamma and glucose uptake through translocation and activation of GLUT4 in PI3K/p-Akt signaling pathway by embelin in type 2 diabetic rats, Biochim. Biophys. Acta 1830 (2013) 2243–2255.

[54] M.N. Horcajada, V. Habauzit, A. Trzeciakiewicz, C. Morand, A. Gil-Izquierdo, J. Mardon, P. Lebecque, M.J. Davicco, W.S. Chee, V. Coxam, E. Offord, Hesperidin inhibits ovariectomized-induced osteopenia and shows differential effects on bone mass and strength in young and adult intact rats, J. Appl. Physiol. 104 (2008) 648–654.

[55] S. Akiyama, S. Katsumata, K. Suzuki, Y. Ishimi, J. Wu, M. Uehara, Dietary hesperidin exerts hypoglycemic and hypolipidemic effects in streptozotocin-induced marginal type 1 diabetic rats, J. Clin. Biochem. Nutr. 46 (1) (2010 Jan) 87–92, https://doi.org/10.3164/jcbn.09-82. Epub 2009 Dec 29. PMID: 20104270; PMCID: PMC2803138.

[56] S.H. Bok, S.H. Lee, Y.B. Park, K.H. Bae, K.H. Son, T.S. Jeong, M.S. Choi, Plasma and hepatic cholesterol and hepatic activities of 3-hydroxy-3-methyl-glutaryl-CoA reductase and acyl CoA: cholesterol transferase are lower in rats fed citrus peel extract or a mixture of citrus bioflavonoids, J. Nutr. 129 (1999) 1182–1185.

[57] L.J. Wilcox, N.M. Borradaile, L.E. Dreu, M.W. Huff, Secretion of hepatocyte apoB is inhibited by the flavonoids, naringenin and hesperetin, via reduced activity and expression of ACAT2 and MTP, J. Lipid Res. 42 (2001) 725–734.

[58] K. Azuma, Y. Minami, K. Ippoushi, J. Terao, Lowering effects of onion intake on oxidative stress biomarkers in streptozotocin-induced diabetic rats, J. Clin. Biochem. Nutr. 40 (2007) 131–140.

[59] R. Jalal, S. Majid, S.M. Bagheri, A. Moghimi, M.B. Rasuli, Hypoglycemic effect of aqueous shallot and garlic extracts in rats with fructose-induced insulin resistance, J. Clin. Biochem. Nutr. 41 (2007) 218–223.

[60] P. Stanley Mainzen Prince, N. Kamalakkanna, Rutin improves glucose homeostasis in streptozotocin diabetic tissues by altering glycolytic and gluconeogenic enzymes, J. Biochem. Mol. Toxicol. 20 (2006) 96–102.

[61] C. Chen, Y. Shao, X. Wu, C. Huang, W. Lu, Elevated Interleukin-17 levels in patients with newly diagnosed type 2 diabetes mellitus, Biochem Physiol 5 (2016) 206.

[62] K. Ohshima, M. Mogi, F. Jing, et al., Roles of interleukin 17 in angiotensin II type 1 receptor-mediated insulin resistance, Hypertension 59 (2) (2012) 493–499.

[63] N. Marx, J. Froehlich, L. Siam, et al., Antidiabetic PPAR gamma-activator rosiglitazone reduces MMP-9 serum levels in type 2 diabetic patients with coronary artery disease, Arterioscler. Thromb. Vasc. Biol. 23 (2) (2003) 283–288.

[64] Y. Wang, R. Singh, J.H. Lefkowitch, R.M. Rigoli, M.J. Czaja, Tumor necrosis factor-induced toxic liver injury results from JNK2-dependent activation of caspase-8 and the mitochondrial death pathway, J. Biol. Chem. 281 (22) (2006) 15258–15267, https://doi.org/10.1074/jbc.M512953200. Epub 2006 Mar 29. PMID: 16571730; PMCID: PMC3668334.

[65] T. Kohl, N. Gehrke, A. Schad, M. Nagel, M.A. Wörns, M.F. Sprinzl, T. Zimmermann, Y.W. He, P.R. Galle, M. Schuchmann, J.M. Schattenberg, Diabetic liver injury from streptozotocin is regulated through the caspase-8 homolog cFLIP involving activation of JNK2 and intrahepatic immunocompetent cells, Cell Death Dis. 4 (7) (2013), e712, https://doi.org/10.1038/cddis.2013.228. PMID: 23828575; PMCID: PMC3730402.

[66] D.A. Barrière, C. Noll, G. Roussy, et al., Combination of high-fat/high-fructose diet and low-dose streptozotocin to model long-term type-2 diabetes complications, Sci. Rep. 8 (2018) 424, https://doi.org/10.1038/s41598-017-18896-5.

[67] Y.S. Mo'men, R.M. Hussein, M.A. Kandeil, Involvement of PI3K/Akt pathway in the protective effect of hesperidin against a chemically induced liver cancer in rats, J. Biochem. Mol. Toxicol. 33 (6) (2019), e22305, https://doi.org/10.1002/jbt.22305. Epub 2019 Feb 19. PMID: 30779474.

[68] W. Qi, C. Lin, K. Fan, Z. Chen, L. Liu, X. Feng, H. Zhang, Y. Shao, H. Fang, C. Zhao, et al., Hesperidin inhibits synovial cell inflammation and macrophage polarization through suppression of the PI3K/AKT pathway in complete Freund's adjuvant-induced arthritis in mice, Chem. Biol. Interact. 306 (2019) 19–28.

[69] A. Justin-Thenmozhi, M. Dhivya Bharathi, R. Kiruthika, T. Manivasagam, A. Borah, M.M. Essa, Attenuation of aluminum chloride-induced neuroinflammation and caspase activation through the AKT/GSK-3β pathway by hesperidin in wistar rats, Neurotox. Res. 34 (2018) 463–476.

[70] X. Li, X. Hu, J. Wang, W. Xu, C. Yi, R. Ma, H. Jiang, Short-term hesperidin pretreatment attenuates rat myocardial ischemia/reperfusion injury by inhibiting high mobility group box 1 protein expression via the PI3K/Akt pathway, Cell. Physiol. Biochem. 39 (2016) 1850–1862.

[71] S. Li, Q. Qin, D. Luo, W. Pan, Y. Wei, Y. Xu, J. Zhu, L. Shang, Hesperidin ameliorates liver ischemia/reperfusion injury via activation of the Akt pathway, Mol. Med. Rep. 22 (2020) 4519–4530.

[72] H.K. Park, S.W. Kang, M.S. Park, Hesperidin ameliorates hepatic ischemia-reperfusion injury in Sprague-Dawley rats, Transplant. Proc. 51 (2019) 2828–2832.

[73] D. Cassiman, C. Denef, V.J. Desmet, T. Roskams, Human and rat hepatic stellate cells express neurotrophins and neurotrophin receptors, Hepatology 33 (2001) 148–158.

[74] G.K. Michalopoulos, Z. Khan, Liver regeneration, growth factors, and amphiregulin, Gastroenterology 128 (2005) 503–506.

[75] R. Gebhardt, A. Baldysiak-Figiel, V. Krugel, E. Ueberham, F. Gaunitz, Hepatocellular expression of glutamine synthetase: an indicator of morphogen actions as master regulators of zonation in adult liver, Prog. Histochem. Cytochem. 41 (2007) 201–266.