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The Effect of Hydroalcoholic Seed Extract of *Securigera Securidaca* on the Hepatic Renin-Angiotensin System in the Streptozotocin-Induced Diabetic Animal Model

ABSTRACT

Background: The aim of the study was to investigate the effect of hydroalcoholic seed extract of *Securigera securidaca* (*S. securidaca*) (HESS) on liver local renin-angiotensin system (RAS) in streptozotocin-induced diabetic rats.

Methods: Three groups of diabetic male Wistar rats were treated with different doses of HESS (100, 200, 400 mg/Kg-BW), and the results were compared with diabetic and healthy control groups. To test the effects of HESS on liver local RAS as well as its alternative pathway, the tissue levels of renin, angiotensin-converting enzyme (ACE), ACE2, angiotensin-II (Ang-II), and Ang-(1-7) were measured. The oxidative state of liver tissue was evaluated by biomarkers of malondialdehyde (MDA), total oxidant status (TOS), and total antioxidant status (TAS). Due to the association between local RAS activity and tissue inflammation, the production of interleukins (IL) IL-1, IL-6, tumor necrosis factor alfa (TNF-α), and IL-10 in the liver was assayed in the experimental group.

Results: Dose-dependent effects of HESS showed that the highest dose of the extract had a reducing effect on the hepatic levels of local RAS components including angiotensinogen, ACE, and Ang-II. Surprisingly, despite the decrease in tissue level of ACE2, an increase in Ang-(1-7) tissue concentration was observed. Decreased local RAS activity through treatment with the highest dose of HESS was associated with decreased tissue levels of proinflammatory cytokines (IL-1, IL-6, TNF-α), and increased anti-inflammatory cytokine (IL-10). Most of the effects of the extract are attributed to its antioxidant properties.

Conclusions: *S. securidaca* seed can be suggested as a suitable drug supplement to prevent hepatic complications of diabetes. (Clin Diabetol 2022, 11; 2: 97–106)

Keywords: local renin-angiotensin system (RAS), angiotensin-converting enzyme, renin, angiotensin-II, angiotensin-(1–7), angiotensinogen

Introduction

It has been proved in addition to the systemic renin-angiotensin system (RAS), this system exists locally in various tissues including the pancreas, heart, kidneys, arteries, and fat as well as the nervous, reproductive,

and digestive systems [1]. The local RAS, in addition to maintaining tissue homeostasis, can induce other effects such as growth, differentiation, proliferation, apoptosis, production of reactive oxygen species, inflammation, and fibrosis under certain conditions in the same tissue.

The liver is one of the tissues equipped with autocrine and paracrine local RAS that is involved in the development of hepatic complications of diabetes. Although systemic RAS activity also increases in advanced diabetes, most studies have denied the effect of systemic RAS on localized RAS [2]. The liver local RAS has almost all the components of this system including renin, angiotensinogen, angiotensin-II (Ang-II), angiotensin-converting enzyme (ACE), angiotensin-type I receptor (AT1R). Evidence has shown an alternative pathway in the tissues whose effects are the opposite of local RAS [3, 4].

Vascular endothelium, hepatocytes, and bile duct epithelial cells are the main source of ACE. Basically, there are two types of receptors for Ang-II, AT1R, and AT2R of which the AT1R is present in all types of liver cells and is involved in proliferation, inflammation, and vascular complications of tissue. In pathological conditions, the production of components of the local RAS system is out of control. Ang-II secreted by cholangiocytes and stellate cells worsens the pathological condition of the liver by stimulating proliferation, fibrosis, and activation of Stella cells [5]. Hyperglycemia is one of the pathological conditions that can increase hepatic angiotensinogen expression [6]. The local liver RAS system increases tissue levels of inflammatory cytokines including interleukins (IL) IL-1, IL-6, and tumor necrosis factor alfa (TNF-α), but decreases anti-inflammatory cytokines such as IL-10. The elevated levels of such pro-inflammatory cytokines intensify insulin resistance and hyperglycemia as well as hepatic fibrosis [7, 8].

ACE2 is a type I integral protein-containing ectodomain that is released into blood circulation after cleavage by a metalloprotease. ACE2 catalyzes the removal of a single amino acid from the C-terminus of Agn-I and Agn-II which produces two important factors of Ang-1-7 and Ang-1-9, respectively. In the cirrhotic liver damages, ACE2 expression and consequently the production of Ang-1-7 production is increased. Ang-1-7 has a modulatory role in the systematic RAS. Furthermore, there are some inhibitors of local RAS that can prevent the progression of liver disease, heart failure, hypertension, and complications of diabetes by inhibiting Ang-II and AT1R [4, 9].

S. securidaca (L.) Degen & Dorfl with local names of Gande-Talkhe and Adasol-Molk is a herbal drug that is widely used in Iranian traditional medicine as well

as Egyptians and Indians for curing hyperglycemia in diabetic patients [10]. The presence of flavonoids, saponins, tannins, and alkaloids in the hydroalcoholic extract of *S. securidaca* was revealed by Garani et al. The phytochemical analysis by Aldal'in et al. showed a high percentage of aromatics derivatives, dodecanedioic acid derivatives, and L-Ascorbic acid as well as β-Sitosterol, and oxygenated hydrocarbons such as acyl glucuronides, α-D-glucopyranose, N-butylglycine, and 1,3-propanediol. In addition to the hypoglycemic effect, it is believed to be useful for the treatment of hyperlipidemia, and hypertension as well. In the present study, the effect of hydroalcoholic seed extract of *Securigera securidaca* (HESS) on diabetic liver tissue was investigated according to the local activity of RAS and its histological complications.

Materials and methods
Hydroalcoholic Extraction of Seed and Estimation of Total Phenolic and Flavonoid

The process of preparing hydroalcoholic extract from *S. securidaca* (herbarium code PMP-756) seed was performed using 70% ethanol and concentrated rotary evaporation method [11]. The total phenol and flavonoid contents of HESS were determined by the methods of Singleton and Rossi [12], and aluminum chloride colorimetric assay [13] in which Folin Ciocalteu was used as the phenol assay standard and Quercetin as the flavonoid assay standard.

Animal Models

The considered experimental models were 30 healthy male Wistar rats (240 g) obtained from the Laboratory Animal Center of Iran University of Medical Sciences. The animal management was approved by the Ministry of Health of Iran (ethical code: IR.IUMS. FMD.REC.1399.065 and performed according to the ethical instructions and guidelines of the laboratory animal care department of Iran University of Medical Sciences (https://ethics.research.ac.ir/docs/Ethics-Lab-Animal-Codes.pdf).

The hyperglycemic induction of animals performed by the standard method of streptozotocin intraperitoneal injection (STZ, 55 mg/kg-BW) and a blood sugar level of ≥250 mg/dL was considered as diabetic.

Experimental Design

Three doses of HESS (100, 200, and 400 mg/kg-BW) [14] were considered. Animals were randomly allocated to 5 groups of 6 rats each as follows: (I) normal (healthy) control (NC), (II) diabetic control (DC), (III) to (V) diabetic rats treated with HESS doses of 100, 200 and 400 mg/kg-BW as E-100, E-200, and E-400 groups,

respectively. Administration of HESS was carried out by gavage once a day for 35 days.

Sampling

At the end of the treatment period, the rats were anesthetized by exposure to chloroform vapor. The blood sample was collected through cardiac puncture and centrifuged (2000 g, 10 min, 23°C). The resultant serum was separated, and stored at −20°C until analysis. Immediately after sacrificing the animals, the liver was removed. The left part of the liver was placed in 10% neutral buffered formalin for histological evaluation and the right part was used for molecular and biochemical examination.

Biochemical Assay

The effects of various doses of HESS on body weight, cardiovascular indices, and serum biochemical parameters including, blood sugar, insulin, and lipid profiles as well as oxidant and antioxidant factors were assessed in our previous studies [11, 15]. Serum parameters of liver tests were determined as:

— **SGOT, SGPT, ALP, and GGT:** Serum activities of serum glutamic-oxaloacetic transaminase (SGOT), serum glutamic-pyruvic transaminase (SGPT), alkaline phosphatase (ALP), and gamma-glutamyl transferase (GGT) were determined by an enzymatic colorimetric method using the Pars Azmoon kits and an automated chemistry analyzer (Erba XL 200, Germany).

— **Preparation of liver tissue for biochemical tests:** The tissue was pulverized in liquid nitrogen using a mortar and pestle after immediate freezing. For each test, a certain amount of powdered tissue was homogenized in a special buffer containing antiprotease. Each sample was centrifuged at 4000 rpm at 4°C for 10 minutes and the supernatant was used for the following assays.

— **Total antioxidant status (TAS):** TAS level of the liver tissue was assayed by ferric reducing antioxidant power (FRAP) [16]. In this method, the level of ferric reduction to ferrous by the sample was considered as an indicator of antioxidant power. The complex between ferrous ion and tripyridyl-triazine (Fe^{2+}-TPTZ) forms a blue color (abs. 595 nm). The calibration curve was plotted by a serial dilution of Fe_2SO_4 in 1 mL of FRAP reagent (100 to 800 μM). The values were reported as μMol Fe^{2+}/g-tissue weight.

— **Total oxidant status (TOS):** The tissue generation of ROS was assayed based on oxidation level of ferrous ion to ferric by existent ROS such as superoxide anion radical (O2-), hydroxyl radicals

(OH-), hydrogen peroxide (H2O2), peroxyl (RO2-), alkoxyl (RO.), hypochlorous acid (HOCl), hydroperoxyl, singlet oxygen (1O2), (HO2-), and peroxynitrite (ONOO-). The test was calibrated by H_2O_2. The results were reported as μmol H_2O_2 Equiv/g-tissue weight.

— **Determination of lipid peroxidation (LPO):** LPO assay was carried out based on the conjugation of malondialdehyde (MDA) with 2-thiobarbituric acid (TBA) to form a pink product (abs. 535 nm). MDA content was reported as nmol/mL.

— **Local RAS and alternative route components assay:** The tissue levels of renin, ACE, ACE2, Ang-II, and Ang-(1-7) were assayed using Rat ELISA Kit (MBS041519, MBS2604372; MBS764117, MBS705139, MBS2604372) from MyBioSource Company and multi-plate ELISA reader (ELISA Reader-DANA-320., Japan). The tissue value of each parameter was reported based on pg/mg.pro.

Total RNA Extraction, cDNA Synthesis, and Quantitative Real-Time PCR (RT-PCR)

The study of gene expression levels of liver cytokines (IL-1β, IL-6, TNFα, and IL-10) and Angiotensinogen and Renin were performed by Quantitative Real-Time PCR (RT-qPCR) technique. About 50 mg liver tissue was frozen in liquid nitrogen and powdered via mortar and pestle. The powdered tissue was homogenized in TRIzol Reagent and total RNA was extracted according to the manufacturer's protocol (FAPDE050, Yektatajhize, Iran). The total RNA was reverse-transcribed into first-strand −cDNA(cat. no. YT4500, Yektatajhize, Iran). The glyceraldehyde 3-phosphate dehydrogenase (GAPDH) gene was considered as an internal control to quantify the expression of the target genes.

Histopathology

After fixation in neutral buffered formalin, the liver tissue was dehydrated in ascending ethanol concentrations, cleared in xylene, and infiltrated and embedded in paraffin. Then, using a rotary microtome (Leica RM2255, Germany), the tissue blocks were sectioned at 5–6 μm, stained with hematoxylin and eosin (H&E), and examined independently by a veterinary pathologist using a light microscope (Olympus CX41, Japan) equipped with a digital camera (Olympus DP25, Germany).

Statistical Analysis

The statistical analyses were performed using GraphPad Prism version 9 and SPSS software version 22. Differences between individual groups were determined by one-way ANOVA analysis and then Tukey

Table 1. Comparison of the Effects of HESS on Insulin, Blood Sugar, and Liver Tests in Study Groups

Parameters Groups	Insulin (ng/mL)	BS (mg/dL)	SGOT (U/L)	SGPT (U/L)	GGT (U/L)	ALP (U/L)
NC (a)	1.79 ± 0.14[b]	90.6 ± 5[b]	52.54 ± 4.4	68.30 ± 5.7	32.9 ± 4.6	95 ± 5.2
DC (b)	1.08 ± 0.08[a]	324.6 ± 14[a]	55.28 ± 6.7	71.86 ± 8.7	36.9 ± 7.2	102 ± 3.2
HESS-100 (c)	1.27 ± 0.05[a, b]	221.4 ± 13[a, b, d, e]	57.14 ± 3.1	74.28 ± 4	37.3 ± 8.3	101 ± 6.4
HESS-200 (d)	1.4 ± 0.07[a,b]	170.3 ± 8[a,b,c]	57.35 ± 7.3	74.55 ± 9.5	36.6 ± 4.1	98 ± 7.3
HESS-400 (e)	1.42 ± 0.08[a, b]	159.4 ± 14[a, b, c]	57.39 ± 5.5	74.60 ± 7.2	34.3 ± 3.8	99 ± 3.6
ANOVA/Pv	< 0.001	< 0.001	0.6187	0.6732	0.6794	0.6532

Superscript showed a statistically significant difference (p < 0.05) between the groups.
ALP — alkaline phosphatase; BS — blood sugar; GGT — gamma-glutamyl transferase; HESS — hydroalcoholic extract of *Securigera Securidac*a seeds; DC — diabetic control; NC — normal control; SGOT — serum glutamic-oxaloacetic transaminase; SGPT — serum glutamic-pyruvic transaminase
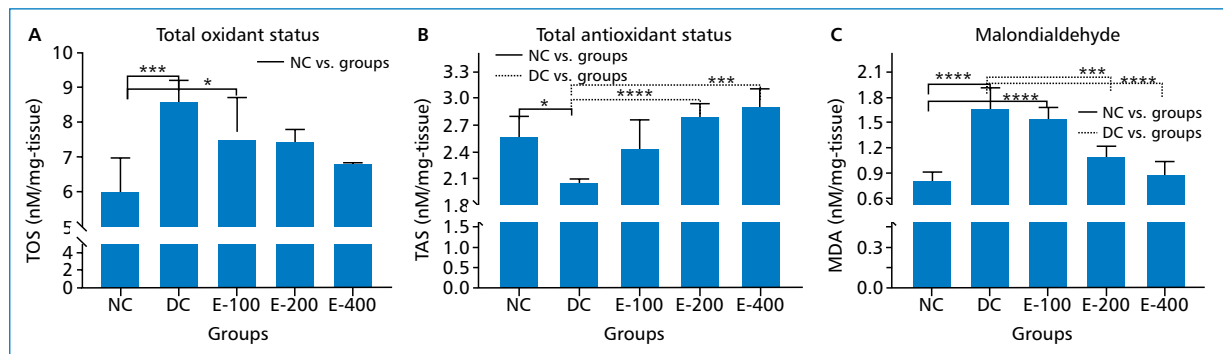

Figure 1. Comparison of Tissue Levels of Oxidative Stress Parameters in the Liver of the Study Groups; **A.** Total oxidant status (TOS); **B.** Total antioxidant status (TAS); **C.** Malondialdehyde (MDA)
DC — diabetic control; NC — normal control

test for paired comparison. The values were reported as the means ± standard deviation. P-value < 0.05 was reported to be statistically significant.

Results

Effects of HESS on Insulin, Blood Sugar, and Liver Tests

The dose-dependent effects of HESS on insulin secretion and blood glucose are shown in Table 1. One-way ANOVA analysis revealed significant differences in blood insulin concentration as well as blood glucose (p < 0.001) between groups. HESS treatment significantly increased insulin levels in all three groups compared to the diabetic control group (p < 0.05), but these levels were still significantly lower than the mean level in the healthy control group.

Serum activities of SGOT, SGPT, and GGT as sensitive indicators of liver damage showed almost uniform values among the studied groups. Therefore, treatment with HESS and/or STZ did not cause any significant toxic effects on the liver of treated animals.

Effects of HESS on Oxidative Stress in Liver Tissue

Figure 1 compares the liver levels of oxidative stress markers including TAS, TOS, and MDA among study groups. As expected, the tissue levels of all three parameters showed significant changes in diabetic control compared with the healthy group. Compared with the DC group, although HESS treatment dose-dependently decreased TOS and MDA levels and increased TAS levels, only moderate and the highest dose HESS made significant changes in the tissue levels of TAS and MDA (p < 0.05), and in the case of TOS, the changes were not significant (p > 0.05).

Effects of HESS on the Local RAS and Alternative Route Components

— **Local RAS components:** According to Figure 2, no significant difference was observed in renin tissue levels (Fig. 2A) between DC and NC groups (p < 0.05), but ACE and Ang-II levels (Fig. 2B, 2C, respectively) were significantly increased in the liver of DC group (p < 0.001). Administration of various doses of HESS did not alter tissue levels of renin (p > 0.05), but dose-dependently decreased tissue levels ACE and Ang-II in diabetic rats, so that both parameters became statistically significant at the highest dose of HESS (p < 0.05).

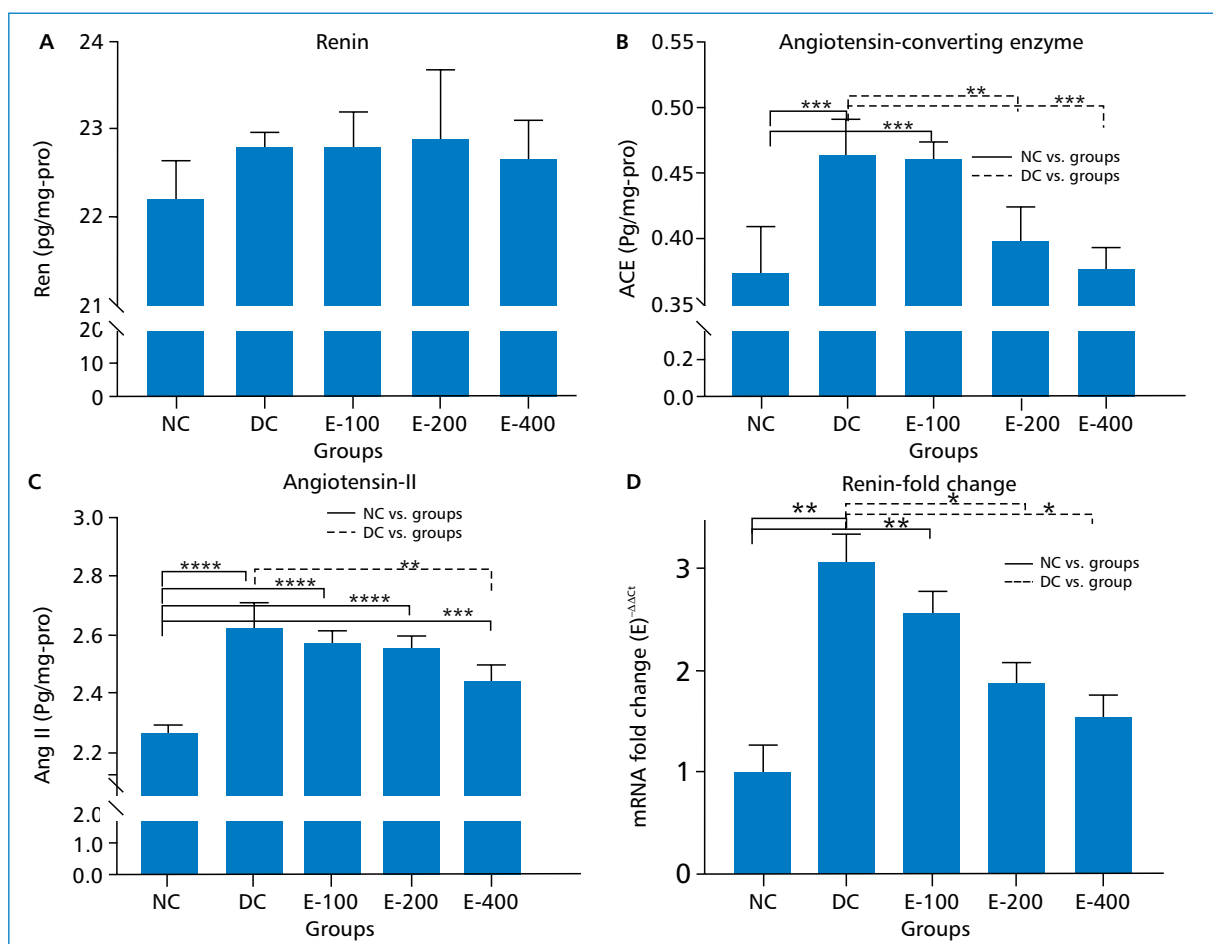Significant changes were expected to be detected in tissue levels of renin with increasing in tissue levels of

Figure 2. Comparison of Tissue Levels of Local Renin-Angiotensin System (RAS) Components in the Liver of the Study Groups:
A. Renin; **B.** Angiotensin-converting enzyme (ACE); **C.** Angiotensin II (Ang-II); **D.** mRNA fold changes
DC — diabetic control; NC — normal control

ACE and Ang-II, but no changes were observed in this regard. This discrepancy attributed to the short half-life of renin (~13 min) [17], was examined by measuring the level of renin gene expression. Based on Figure 2D, the level of renin gene expression in the liver of diabetic rats was significantly higher than in healthy rats ($p < 0.05$). HESS treatment dose-dependently decreased renin gene expression in diabetic rats, so that it was significant at doses of 200 and 400 ($p < 0.05$)

— **Alternative route components:** Compared with the NC group, a significant increase in the tissue levels of ACE2 ($p < 0.001$) (Fig. 3A) and a slight increase in Ang-(1-7) ($p > 0.05$) (Fig. 3B), the ACE2 enzyme product, was observed in the DC group. HESS administration slightly decreased ACE2 levels, but unexpectedly, significantly increased Ang-(1-7) in a dose-dependent manner, so that ACE2 level became non-significant and Ang-(1-7) became significant at the highest dose of HESS compared with the NC group.

— **Compression of tissue levels of Ang-II and Ang-(1-7):** Angiotensinogen gene expression level in liver tissue of DC group was significantly higher than that in NC group ($p < 0.05$) (Fig. 4A). HESS therapy dose-dependently decreased the expression of the angiotensinogen gene in diabetic rats which became significant at the highest dose of HESS ($p < 0.05$). Angiotensinogen is the precursor to Ang-II and Ang-(1-7) synthesis. Therefore, decreased Ang-II levels are expected to be associated with increased Ang-(1-7) levels and vice versa. For this purpose, the ratio of these two parameters was considered: Ang-(1-7)/Ang-II. A ratio higher than one indicates an increase in Ang-(1-7) and a ratio less than one indicates an increase in Ang-II. Figure 4B shows a lower concentration of Ang-(1-7) than Ang-II in all study groups. However, the ratio of Ang-(1-7)/Ang-II was significantly higher in the NC group in comparison with the DC group. Treatment with HESS
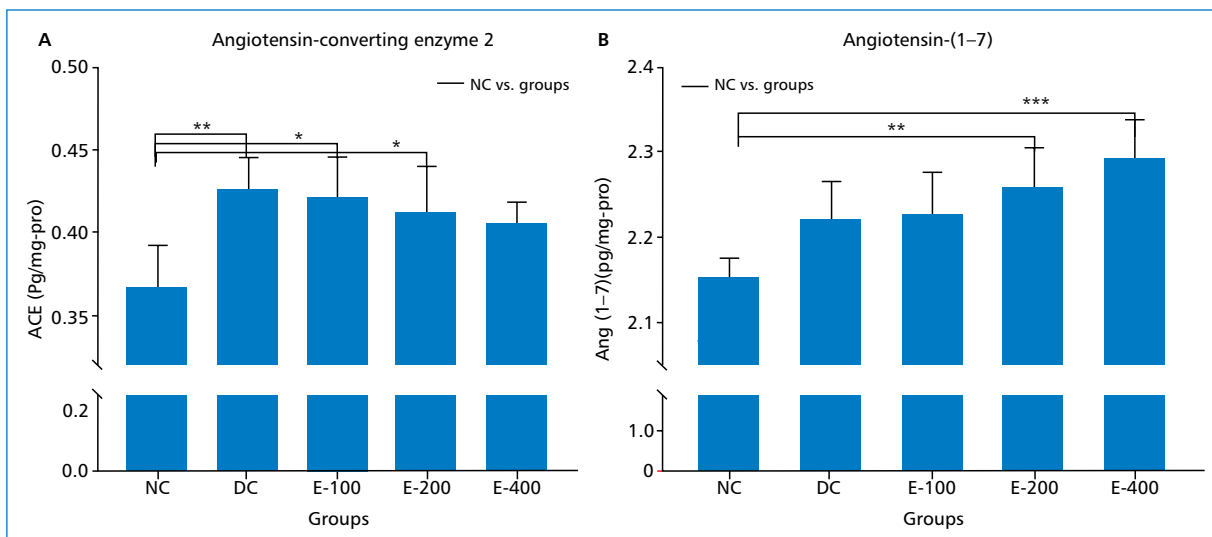
Figure 3. Comparison of Tissue Levels of Alternative Components in the Liver of the Study Groups: **A.** Angiotensin-converting enzyme2 (ACE2) and **B.** Ang-(1-7)
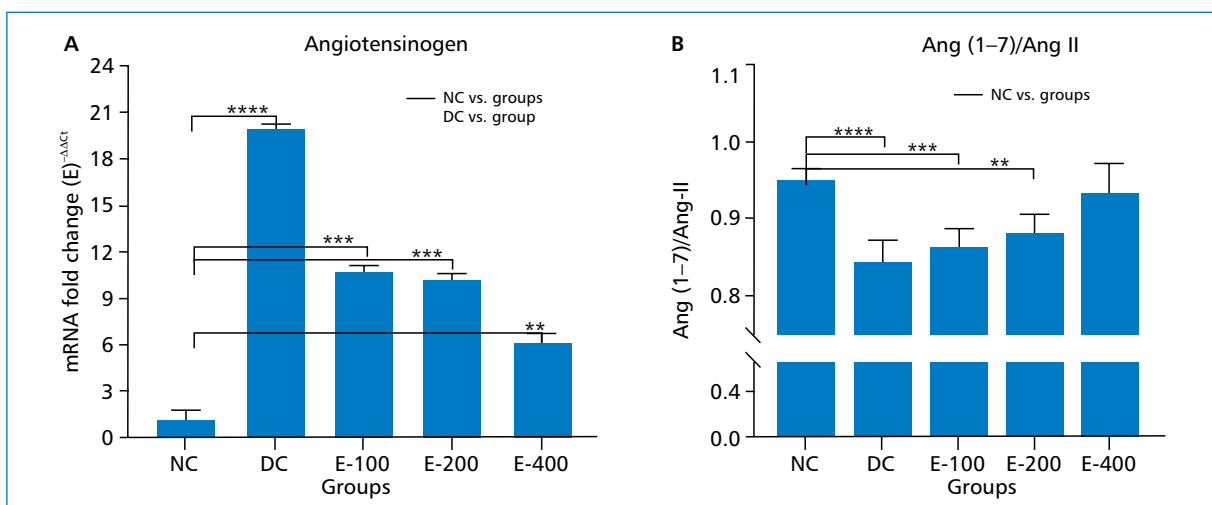DC — diabetic control; NC — normal control

Figure 4. Comparison of **A.** mRNA fold changes in the liver tissue of the study groups and **B.** tissue ratio of Ang-(1-7)/Ang-II
Ang — angiotensin; DC — diabetic control; NC — normal control

dose-dependently increased the ratio, which was non-significant at the highest dose of HESS compared with the NC group.

Effects of HESS on Pro-Inflammatory and Anti-Inflammatory Cytokines

Based on Figures 5A–5C, compared with the healthy group, the expression levels of IL-1, IL-6, and TNFα genes were increased in the liver tissue of diabetic rats ($p < 0.05$), however, treatment with HESS dose-dependently decreased the gene expression levels of these inflammatory factors in diabetic rats, which were significant at doses of 200 and 400

($p < 0.05$) for factors of IL-1, and TNFα. As it is indicated in Figure 5D, the gene expression level of IL-10 in liver tissue of healthy rats was significantly higher than that in diabetic rats ($p < 0.05$). HESS therapy increased the expression of the IL-10 gene in diabetic rats, which became significant at the highest dose of HESS ($p < 0.05$).

Effects of HESS on Liver Histology

As shown in Figure 6A, the histopathological examination of the liver of the NC group showed the normal tissue structure, with hexagonal or pentagonal lobules, circular central veins, and clear peripheral triads
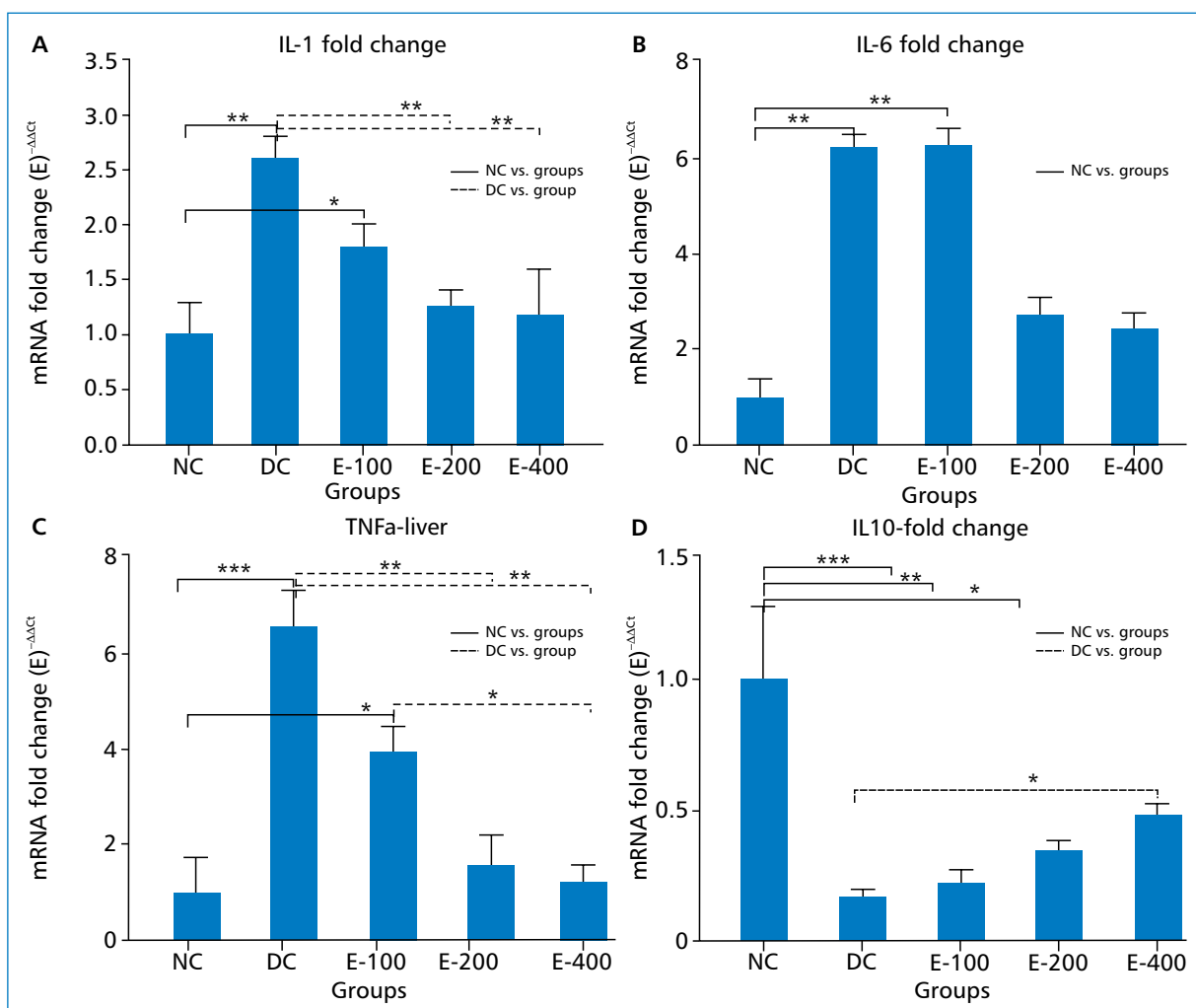
Figure 5. Comparison of gene expression levels of pro-inflammatory (A, B, and C) and anti-inflammatory (D) factors in liver tissue from study groups

DC — diabetic control; IL — interleukin; NC — normal control; TNF-α — tumor necrosis factor alfa

in the healthy control group. On the contrary, the DC group (Fig. 6B) showed the histopathologic complication of mild bile duct hyperplasia, moderate lobular inflammation, and hepatocellular ballooning. Treating with 100-dose HESS (Fig. 6C) slightly reduced the rate of inflammation and sinusoidal cell irregularities and hepatocellular ballooning. Mild ballooning of hepatocytes with slight lobular inflammation was observed in the group of the HESS-200 (Fig 6D). No pathological complications were seen in the liver tissue of the HESS 400 group (Fig 6E).

Discussion

The observed normal levels of liver enzymes in diabetic rats — treated and untreated with HESS — indicate the non-toxicity of HESS and STZ to the liver compared with healthy rats. TOS and TAS measurements showed a significant increase in tissue ROS and

MDA levels and a significant decrease in FRAP levels in diabetic control rats. HESS improved the above oxidative stress markers in a dose-dependent manner, such that at the highest dose of HESS, the levels of these markers became comparable to those of healthy rats. The decrease in ROS and MDA levels with a concomitant increase in FRAP in HESS-treated diabetic liver tissue may be attributed to the protective effects of phenolic and flavonoid compounds in the biological system [18]. The observed normal levels of liver enzymes in diabetic rats - treated and untreated with HESS - indicated the non-toxicity of HESS and STZ to the liver compared with healthy rats.

In the present study, along with hyperglycemia and increased oxidative stress in diabetic rats, the expression level of angiotensinogen also increased. Treatment with the highest dose of HESS reduced the level of angiotensinogen expression in diabetic rats,
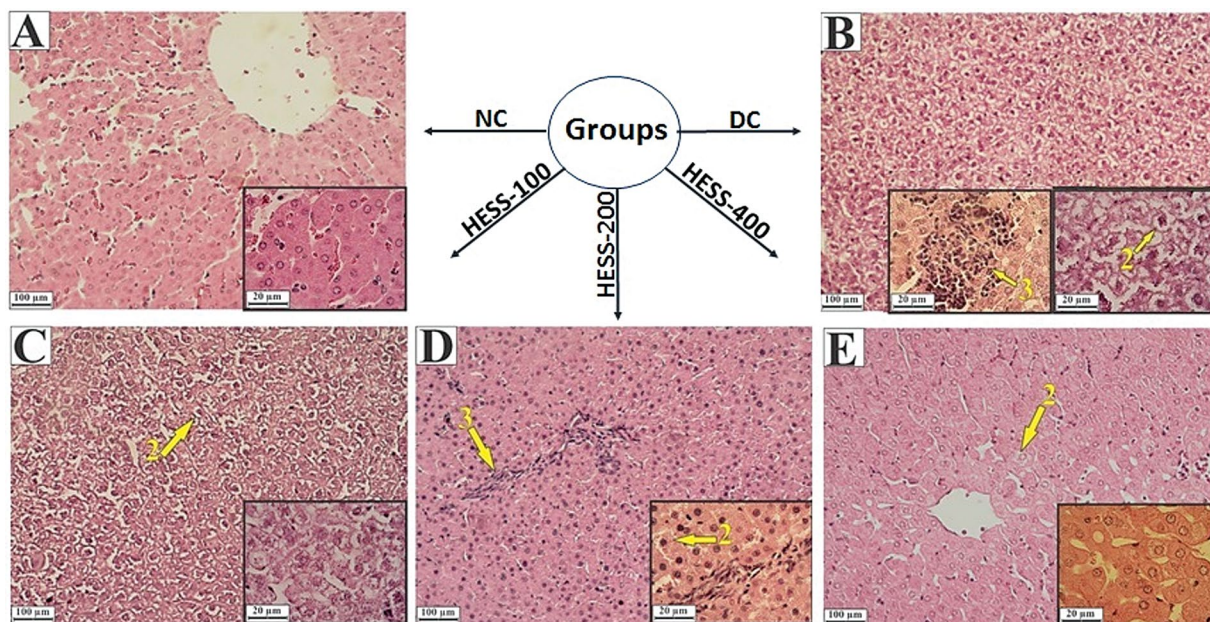
Figure 6. Morphological Assessment of Testicular Tissue; H&E staining. Magnification × 100. Arrow sign: 1: Bile duct hyperplasia; 2: Hepatocyte ballooning; 3: Lip inflammation. **A.** Normal control group with the normal tissue structure; **B.** Diabetic control group with bile duct hyperplasia, lobular inflammation, and hepatocellular ballooning; **C.** HESS-100 group with slightly sinusoidal cell irregularities and hepatocellular ballooning; **D.** HESS-200 group, and **E.** HESS-400 group with no pathological complication. HESS — hydroalcoholic extract of *Securigera securidaca* seeds; DC — diabetic control; NC — normal control

but there was still a significant difference from healthy rats in this regard. Despite higher levels of antioxidants in the group treated with the highest dose of the extract (due to the presence of phenolic and flavonoid compounds), the level of oxidants in this group was higher than in healthy controls. Therefore, the high expression of angiotensinogen in the HESS-400 group can be attributed to the effect of ROS.

In the present study, the ELISA assay for tissue levels of renin showed no significant difference between the study groups, but the real-time PCR assay revealed a significant increase in renin gene expression in the diabetic control compared with the healthy control. High doses of HESS reduced renin gene expression to levels that were not significantly different from those of the healthy control. Such a difference in renin tissue evaluation results may be due to the half-life of renin, although no reports of renin liver half-life were found [17]. Increased ROS and tissue inflammation, followed by increased concentrations of inflammatory cytokines such as IL-6, IL-1β, and TNF-α through the MAPK/STAT/NF-κB signaling pathways are among the tissue stimulators of renin gene expression [19, 20].

The present study showed a significant increase in the levels of ACE liver tissue in diabetic rats, which is confirmed by the results of previous studies [21]. Treatment with HESS significantly reduced the tissue levels of ACE in diabetic rats, which became compa-

rable to healthy rats at the highest dose of HESS. As expected, the increase in tissue ACE increased the tissue concentration of Ang- II in diabetic rats. However, the decrease in tissue ACE following administration of HESS did not significantly decrease the tissue concentration of Ang-II. High levels of Ang-II in the diabetic liver may be associated with other production pathways. One of these pathways is the enzymatic catalysis of chymase, a trypsin-like enzyme. Chymase produced in mast cells has a variety of functions, including an increase in Ang-II, a matrix of metalloproteinase-, and an increase in TGF-β, which are associated with oxidative stress, inflammation, and fibrosis, respectively [22, 23]. In the present study, liver tissue levels of ACE2 and consequently Ang-(1-7) were significantly higher in the diabetic control group than in the healthy group. Treatment with HESS caused a mild and dose-dependent decrease in liver ACE2 activity, in spite of a slight increase in Ang-(1-7) levels. This discrepancy may be related to the activity of other enzymes that partially mimic ACE2 activity. Neprilysin (NEP) is one of these enzymes capable of catalyzing Ang-I to Ang-(1-7) and through the Mas receptor, increases insulin sensitivity and beta-cell function [24, 25]. Studies have reported increased NEP activity in diabetic tissues, including the liver [24].

The tissue is affected by the balance between the RAS system and the alternative path, which was evalu-

ated by the Ang-(1-7) to Ang-II ratio. If the numerical ratio of 1 is considered as the equilibrium point, the lower this ratio is from 1, the greater the activity of the RAS system and the warning of tissue damage. The ratio of Ang-(1-7)/Ang-II levels in the liver tissue of diabetic rats was significantly lower than that in healthy rats. Treatment with the highest dose of HESS made this ratio somewhat comparable to healthy rats.

The main effects of Ang- II are mediated by AT1R, whereas Ang-(1-7) interacts only with AT2R and also the Mas receptor. AT2R is poorly expressed in hepatocytes and Stella cells, while AT1R is highly expressed in most of the liver cells, including hepatocytes, Stella cells, Kupffer cells, bile duct cells, myofibroblasts, and vascular endothelial cells of the liver [26]. High expression of AT1R in the liver justifies the liver to inflammation and fibrosis under pathological conditions, which activates pro-inflammatory pathways such as NF -κB and the expression and release of proinflammatory molecules IL-1β and IL-6, TNF-α [27].

As expected, the liver tissue expression of proinflammatory cytokines IL-1, IL-6, and TNF-α was significantly increased in diabetic control rats compared to the healthy control group. Treatment with moderate and high doses of HESS reduced the expression levels of such proinflammatory cytokines in the liver. However, the inhibitory effect of HESS on the expression of TNF-α and IL-1 genes was more significant than IL-6. IL-10 is an anti-inflammatory cytokine released by a variety of immune cells during inflammation. Studies show decreased levels of this cytokine in diabetic and obese individuals [28]. IL-10 inhibits the expression of IL-6 and TNF-α and can increase the sensitivity of beta cells to glucose and insulin secretion [29]. In this study, IL-10 gene expression levels were significantly decreased in the liver of diabetic rats, but treatment with the highest dose of HESS increased its expression level in liver tissue. Liver histological changes in the study groups were reasonably consistent with the tissue cytokine results. Histological examination showed moderate inflammation in the liver tissue of the diabetic control group. The central venous structure was somewhat damaged, lobules were markedly irregular, and sinusoidal irregularities and foaming of hepatocytes were seen. Treatment with a low dose of HESS was to some extent effective in reducing inflammation and liver tissue complications, but histological examination of the groups treated with doses of 200 and 400 HESS showed no specific pathological problems in the liver.

Conclusions

This study revealed that the highest dose of hydroalcoholic seed extract showed reducing effects on local RAS components including angiotensinogen, ACE enzyme, and Ang-II product. In the alternative route, although the highest dose of the extract increased Ang-(1-7) levels, it had less effect on tissue levels of ACE2 compared to lower doses. Such a discrepancy between Ang-(1-7) levels and ACE2 levels can be attributed to other tissue-converting enzymes such as neprilysin, although it needs to be investigated. By decreasing the activity of the local RAS system as well as levels of oxidative stress, a decrease in tissue levels of proinflammatory cytokines, and an increase in anti-inflammatory cytokine were observed. Therefore, in addition to the hypoglycemic effect, *S. securidaca* seeds can be considered as a suitable drug supplement to relieve and prevent the complications of diabetic liver tissue.

Acknowledgments

Conflict of interest

None declared.

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