

## ***Hibiscus sabdariffa* extract alleviates vascular complications in streptozotocin-induced diabetic rats**

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§This paper is dedicated to the memory of Prof. Dr. Mohammed Zakaria, who recently passed away.

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### **ABSTRACT:**

Vascular diseases are the most important diabetic complications. Its pathophysiology involves abnormalities in vascular endothelium caused by sustained hyperglycemia, increased uric acid, increased oxidative stress, and inflammation mediated through toll-like receptor 4 (TLR 4) pathway. *Hibiscus sabdariffa* has been used traditionally in food products and it possesses anti-diabetic, anti-hyperlipidemic, anti-oxidative, anti-inflammatory, and anti-hypertensive effects. The present study aimed to investigate the effect of hibiscus extract against diabetic vascular complications. Diabetes was induced by a single injection of streptozotocin (STZ, 55 mg/kg, i.p.). Diabetic rats were treated with hibiscus extract (100, 200, or 400 mg/kg), lisinopril (5 mg/kg), or lisinopril plus hibiscus (2.5/100 mg/kg) daily for 8 weeks. Hibiscus extract significantly and dose-dependently improved abnormalities in serum lipids and blood pressure. Additionally, it suppressed oxidative stress, inflammation, particularly the upregulation of TLR 4 induced by STZ. There were no significant differences between the effects of hibiscus (400 mg/kg) and lisinopril (5 mg/kg). The effects of the combined administration of lisinopril plus hibiscus (2.5/100 mg/kg) were significantly better compared with either lisinopril or hibiscus alone. These results suggest the use of hibiscus as an alternative to the conventional use of angiotensin-converting enzyme inhibitors (ACEIs) or at least in combination with a small dose to enhance their effects in diabetic vascular complications.

**Key words:** Atherosclerosis; *Hibiscus sabdariffa*; Lisinopril; Toll-like receptor 4; Oxidative stress, Inflammation

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### **INTRODUCTION**

Diabetic patients are at increased risk of atherosclerotic vascular disease that manifests as coronary heart disease, cerebrovascular disease and peripheral arterial disease (Jarrett, 1984). The pathophysiology of diabetic vascular disease involves abnormalities in vascular endothelium and platelet function. (Kinlay *et al.*, 2001). Numerous metabolic derangements such as hyperglycemia, excessive free fatty acid liberation, and insulin resistance mediate endothelial dysfunction by affecting the synthesis or

degradation of nitric oxide (NO) (King, 1996). In addition, increased uric acid level, oxidative stress and inflammation are implicated in the vascular endothelial dysfunction. Michelsen *et al.* (2004) described the role of TLR 4 pathway in the pathogenesis of atherosclerosis. This pathway results in increased activity of the proinflammatory transcription factor, nuclear factor-kappa B (NF-κB), leading to expression of leukocyte adhesion molecules and production of chemokines and cytokines such as tumor necrosis factor-alpha (TNF-α) (Zeiher *et al.*, 1995).

These actions encourage monocyte migration into the intima and formation of macrophage foam cells, characterizing the initial morphological changes of atherosclerosis (Collins and Cybulsky, 2001).

*Hibiscus sabdariffa* has been used traditionally to prepare hot and cold beverages, as a flavoring agent, and as a herbal medicine. It has different pharmacological effects including anti-oxidative, anti-inflammatory, anti-diabetic, anti-hypertensive, anti-hyperlipidemic, and anti-tumor effects (Herrera-Arellano *et al.*, 2004; Onyenekwe *et al.*, 1999).

The present study was conducted to investigate the effect of the aqueous extract of hibiscus calyces against diabetic vascular complications. We tried also to explore the possible underlying mechanisms of hibiscus highlighting its effect on TLR 4 pathway as a new target for hibiscus in diabetic complications. Another target was to compare the effects of hibiscus with lisinopril, an ACEI, which is used clinically in the management of diabetic complications. In addition, we compared the effect of a combination of lisinopril and hibiscus extract with either treatment alone.

## MATERIALS AND METHODS

### Experimental animals

Adult male Wistar rats (180–205 g) were used in the present study. Animals were purchased from the Egyptian Organization for Biological Products and Vaccines (Cairo, Egypt). Rats were acclimatized for one week prior to experiments. The animals were kept at controlled temperature ( $23 \pm 2^\circ \text{C}$ ), humidity ( $60\% \pm 10\%$ ) and light/dark cycle (12/12 h). Rats were supplied with commercially available normal chow diet (El Nasr Company for Pharmaceutical Chemicals, Zagazig, Egypt) and allowed free access to water.

### Ethical statement

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. All procedures performed in studies involving animals were approved by the local authorities, Ethical Committee for Animal Handling at Zagazig University (ECAHZU), at the Faculty of Pharmacy, Zagazig University, Egypt in accordance with the recommendations of the Weatherall report. Every effort was done to minimize the number of animals used and their suffering during experiments.

### Drugs and chemicals

Streptozotocin (CAS Number: 18883-66-4) and lisinopril (CAS Number: 83915-83-7) were purchased from Sigma-Aldrich (St. Louis, MO, USA). STZ was dissolved in freshly prepared ice-cold sodium citrate buffer (10 mmol/L, pH 4.5), while lisinopril was dissolved in tap water immediately before administration.

### *Hibiscus sabdariffa* extract

#### Plant material

Dry dark-red calyces of *Hibiscus sabdariffa* (Family *Malvaceae*) were obtained from Faculty of Agriculture, Zagazig University, Egypt and identified by Dr. Mohammed Abd El-Kader, Department of Horticulture-Decoration Plants, Faculty of Agriculture, Zagazig University, Egypt. A voucher specimen has been kept for future reference at the Department of Pharmacology, Faculty of Pharmacy, Zagazig University, Egypt.

### Preparation of the aqueous extract

The collected calyces were powdered, 150 g of dried powder was macerated with hot distilled water ( $95^\circ \text{C}$ , 6 L) for 2h, the solution was then filtered and lyophilized to obtain 75 g of hibiscus extract, which was stored at  $4^\circ \text{C}$  (Wang *et al.*, 2011).

Aqueous solutions of the extract were prepared at a concentration of 80 mg/mL just before administration.

### **Induction of diabetes**

Following an overnight fasting, type 2 diabetes was induced by a single intraperitoneal injection of STZ (55 mg/kg). After 72 h, fasting tail-vein glucose level was measured using a glucometer (GM100, Bionime GmbH, Berneck, Switzerland) and only those animals having blood glucose level > 250 mg/dL were considered diabetic and used for the study (Wang et al., 2011).

### **Experimental design**

Rats were randomly divided into seven groups (6 rats each). Group I (Control): non-diabetic rats received the vehicle only. Group II (STZ): diabetic rats. Groups III, IV and V (H100, H200 and H400): diabetic rats received either 100, 200, or 400 mg/kg of hibiscus extract daily per oral gavage. Group VI (L5): diabetic rats received lisinopril (5 mg/kg) daily per oral gavage. Group VII (L2.5+H100): diabetic rats received a combination of lisinopril (2.5 mg/kg/day, p.o.) plus hibiscus extract (100 mg/kg/day, p.o.), where lisinopril was administered 2 h following hibiscus extract administration. All treatments were administered between 11:00 am and 1:00 pm. The administrations of hibiscus extract and lisinopril continued throughout the experimental period daily for 8 consecutive weeks. The doses of hibiscus extract and lisinopril were chosen based on previous studies in the literature (Kuno *et al.*, 2003; Wang et al., 2011; Yang *et al.*, 2013)

### **Methods**

#### **Non-invasive measurement of blood pressure (BP)**

Blood pressure was measured non-invasively using tail cuff method as described by Pasquié *et al.* (1999) with some modifications. The method depends

on measuring the pressure that has to be exerted on the wall of a blood vessel to stop the flow of blood in it.

### **Blood and tissue sampling**

Rats were fasted overnight; blood samples were collected from the retro-orbital sinus of rats using heparinized microcapillary tubes. The blood samples were allowed to clot for 30 min at room temperature. Serum was separated by centrifugation at 3000 rpm for 15 min and stored at -20 °C for further biochemical measurements. Rats were euthanized by cervical dislocation and thoracic aorta was carefully excised through opening in the abdomen and placed in a Petri dish filled with cold Krebs-Henseleit buffer containing NaCl (118.1 mM), KCl (4.69 mM), KH<sub>2</sub>PO<sub>4</sub> (1.2 mM), NaHCO<sub>3</sub> (25 mM), glucose (11.7 mM), MgSO<sub>4</sub> (0.5 mM) and CaCl<sub>2</sub> (2.5 mM). Each aorta was cleaned of excess connective tissue and fat and then divided into two parts. The first part was immediately snap frozen in liquid nitrogen and stored at -80° C for further biochemical measurements, while the other part was fixed in 10% neutral buffered formalin at room temperature for histopathological examination.

#### **Measurement of blood glucose and serum insulin level**

Tail-vein blood glucose level was measured using an electrochemical glucometer (GM100, Bionime GmbH, Berneck, Switzerland). Serum insulin level was measured by sandwich enzyme-linked immunosorbent assay (ELISA) using a diagnostic kit supplied by Millipore (Billerica, MA, USA).

#### **Measurement of serum lipid profile and the atherosclerotic index**

Serum total cholesterol (TC), triglycerides (TG), and high-density lipoprotein cholesterol (HDL-C) were measured by enzymatic colorimetric methods using diagnostic kits provided by Spinreact (Spain). Serum low-density

lipoprotein cholesterol (LDL-C) was calculated using the following formula as described by Friedewald *et al.* (1972): LDL-C (mg/dL) = TC – HDL-C – (TG/5). The atherosclerotic index (AI) was calculated as follows: AI = (TC-HDL-C)/HDL-C as described by Yang *et al.* (2008).

#### Measurement of serum uric acid

Serum uric acid level was measured by a colorimetric method using a diagnostic kit provided by Biodiagnostic (Cairo, Egypt)

#### Determination of oxidative stress markers in aorta tissue

Malondialdehyde (MDA) level and the activity of catalase (CAT) in the tissue homogenates of aorta were measured using kits provided by Biodiagnostic (Giza, Egypt).

#### Quantitative real-time PCR for the gene expression of inducible NO synthase (iNOS), endothelial NO synthase (eNOS), TLR 4, and TNF- $\alpha$ in aorta tissue:

Total RNA was extracted from aorta tissue using Trizol reagent (Invitrogen, Carlsbad, CA, USA). The RNA pellet was resuspended in DEPC-treated water. The quality and concentration of the RNA were assessed using the OD 260/280 ratio, and only samples with ratios above 1.5 were used. Total RNA was reverse transcribed

using RevertAid Premium Reverse Transcriptase-Kit (Fermentas International Inc., Burlington, Canada). Briefly, RevertAid H Minus M-MuLV Reverse Transcriptase was added to dNTP Mix (10 mM), 5x reaction buffer and random hexamer primers; the mixture was subjected to cDNA synthesis cycling conditions at 37 °C for 30 min and at 85 °C for 5 min.

Real-time quantitative polymerase chain reaction (qPCR) was performed using ABI PRISM 7500 sequence detector system (Applied Biosystems, Foster City, CA, USA), using the Maxima SYBR Green qPCR Kit (Fermentas International Inc., Burlington, Canada). The primer sequences used for iNOS, eNOS, TLR4, and TNF- $\alpha$  are listed in Table 1. Reaction mixtures contained 10 pmol/ $\mu$ L of each primer, 12.5  $\mu$ L Maxima SYBR mix and 5.5  $\mu$ L nuclease free water. An amount of 5  $\mu$ L of cDNA was added to each reaction mix. The thermal cycling protocol consisted of 2 min at 50 °C and 10 min at 95 °C, followed by 40 cycles of 95 °C for 30 s and 60 °C for 30 s and 72 °C for 30 s. Data from real-time assays were calculated using Sequence Detection Software from PE Bio systems v. 1.7 (Foster City, CA, USA). Relative expression of studied genes was calculated using the comparative C<sub>T</sub> method. All values were normalized to  $\beta$ -actin gene expression and reported as fold change from control group.

**Table 1:** Gene-specific primer sequences used for real-time polymerase chain reaction

Gene	Forward primer	Reverse Primer
iNOS <sup>a</sup>	5'-GGGCCACCTTTATGTTTGTG-3'	5'-CCTCAACCTGCTCCTCACTC-3'
eNOS <sup>b</sup>	5'-CTG CGG TGA TGT CAC TAT GG-3'	5'-AAA TGT CCT CGT GGT AGC GT-3'
TLR 4 <sup>c</sup>	5'-GCCGAAAGTTATTGTGGTGGT-3'	5'-ATGGGTTTTAGGCGCAGAGTTT- 3'
TNF- $\alpha$ <sup>d</sup>	5'-GAAAAGCAAGCAGCCAACCA-3'	5'-CGGATCATGCTTTCTGTGCTC-3'
$\beta$ -Actin	5'- TGT TTGAGACCTTCAACACC-3'	5'-TAGGAGCCAGGGCAGTAATC-3'

<sup>a</sup>iNOS, inducible NO synthase; <sup>b</sup>eNOS, endothelial NO synthase; <sup>c</sup>TLR 4, toll-like receptor 4; <sup>d</sup>TNF- $\alpha$ , tumor necrosis factor alpha.

## Histopathological examination

Aorta specimens were fixed using 10% neutral buffered formaldehyde for histopathological examination, collagen deposition & determination of intima media ratio. After proper fixation, the specimens were dehydrated in ascending grades of ethyl alcohol (70%, 90%, 100%), cleared in xylol, impregnated and then embedded in paraffin wax. Sections, 5  $\mu$ m thick, were cut using a rotatory microtome. The sections were then stained with hematoxylin and eosin (H&E) and Mallory's trichrome stains and examined under light microscope. H&E stain is used routinely for studying the general histological structure giving the cytoplasm red color and the nucleus blue color. On the other hand, Mallory's trichrome stain is used to visualize collagen fibers. Morphometric study was done to assess the tunica intima/media ratio in six randomly selected high power microscopic fields within the sections for each group using a computerized image system composed of a Leica Qwin 500 image analyzer which is connected to a Leica microscope.

## Statistical Analysis

All data are expressed as mean  $\pm$  standard error of the mean (SEM). Statistical analysis was performed using Graphpad prism software v.6 (GraphPad Software Inc., La Jolla, CA, USA). The statistical significance of differences between groups was tested using one-way analysis of variance (ANOVA) followed by Tukey's Post-test. A significant difference was assumed for values of  $P < 0.05$ .

## RESULTS

### Effect on blood glucose and serum insulin

As depicted in Fig. 1, diabetic rats had significantly elevated blood glucose level ( $523.5 \pm 25.89$  vs.  $102.3 \pm 3.301$  mg/dL,  $P < 0.05$ ) and significantly reduced serum

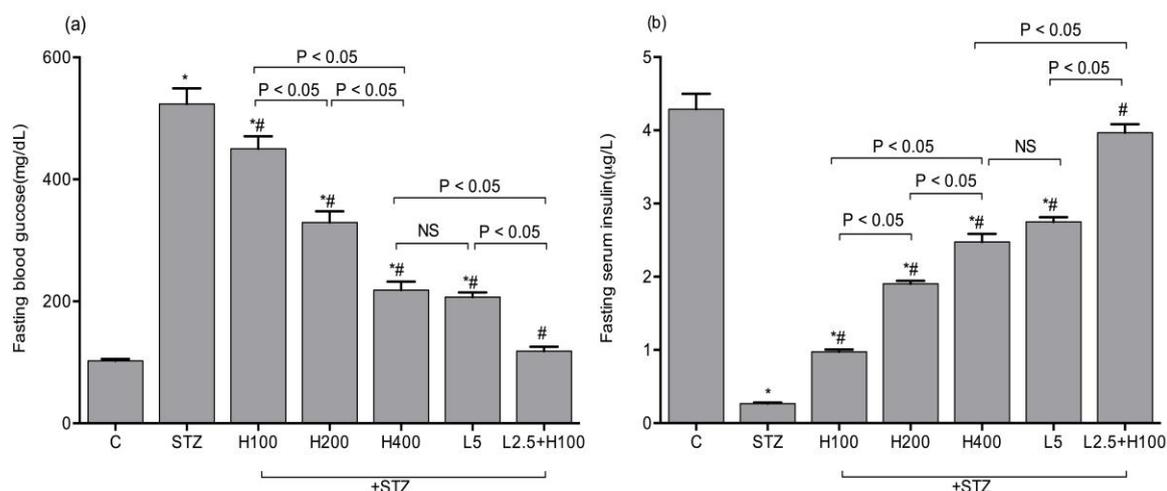
insulin level ( $0.2675 \pm 0.01377$  vs.  $4.288 \pm 0.2125$   $\mu$ g/L,  $P < 0.05$ ) compared with the control group. Treatment of diabetic rats with hibiscus extract at doses of 100, 200 or 400 mg/kg/day provoked a significant, dose-dependent reduction of blood glucose level by -14%, -37.1% and -58.3%, respectively ( $P < 0.05$ ), as well as a significant ( $P < 0.05$ ), dose-dependent elevation in serum insulin level by +264.5%, +611.4% and +825.2%, respectively ( $P < 0.05$ ), compared with diabetic rats. Likewise, L5 caused a significant reduction in blood glucose level by -60.5% and a significant increase in serum insulin level by +928 % compared with the STZ group ( $P < 0.05$ ). There were no significant differences in blood glucose or serum insulin levels between H400 and L5 groups. Furthermore, the combined administration of L2.5+H100 significantly ( $P < 0.05$ ) reduced blood glucose by -77.4% and significantly increased serum insulin level by +1383.4% compared with STZ diabetic group. Additionally, the improvement induced by the combined administration of L2.5+H100 was significantly better compared with H400 or L5 alone.

### Effect on seum lipid profile and atherogenic index

Fig. 2 demonstrates that STZ diabetic rats had significantly elevated serum TC ( $257.0 \pm 1.623$  vs.  $107.8 \pm 2.363$  mg/dL,  $P < 0.05$ ), TG ( $253.9 \pm 12.87$  vs.  $84.79 \pm 3.569$  mg/dL,  $P < 0.05$ ), LDL-C ( $183.9 \pm 2.566$  vs.  $32.25 \pm 1.919$  mg/dL,  $P < 0.05$ ) concentrations compared with the control rats. In addition, AI was significantly elevated ( $10.67 \pm 0.3995$  vs.  $0.8550 \pm 0.05309$ ,  $P < 0.05$ ) compared with the control rats. On the contrary, serum HDL-C level was significantly reduced in STZ diabetic rats ( $22.12 \pm 0.6731$  vs.  $58.57 \pm 2.552$  mg/dL,  $P < 0.05$ ) compared with the control rats. Oral administration of hibiscus extract caused significant dose-dependent reductions of serum TC level by -14.28%, -26.45% and -36.45%,

respectively, of serum TG level by -12.17%, -29.02 % and -42.69%, respectively, and of serum LDL-C level by -20.45%, -36.87% and -50.68%, respectively. Similarly, AI was reduced significantly by -39.1%, -60.88% and -74.11%, respectively. On the other hand, HDL-C level was significantly elevated by +33.09%, +66.91% and +96.83%, respectively, compared with STZ diabetic rats. L5 significantly reduced serum TC,

TG, LDL-C concentrations and AI by -39.72%, -51.20%, -53.33% and -76.5%, respectively, whereas it significantly increased HDL-C level by +100.40% compared with STZ diabetic rats. The effect of H400 was comparable to that of L5. Moreover, the combined administration of L2.5+H100 restored serum lipid profile close to normal levels seen with control rats and such effect was significant compared to H400 or L5 alone.

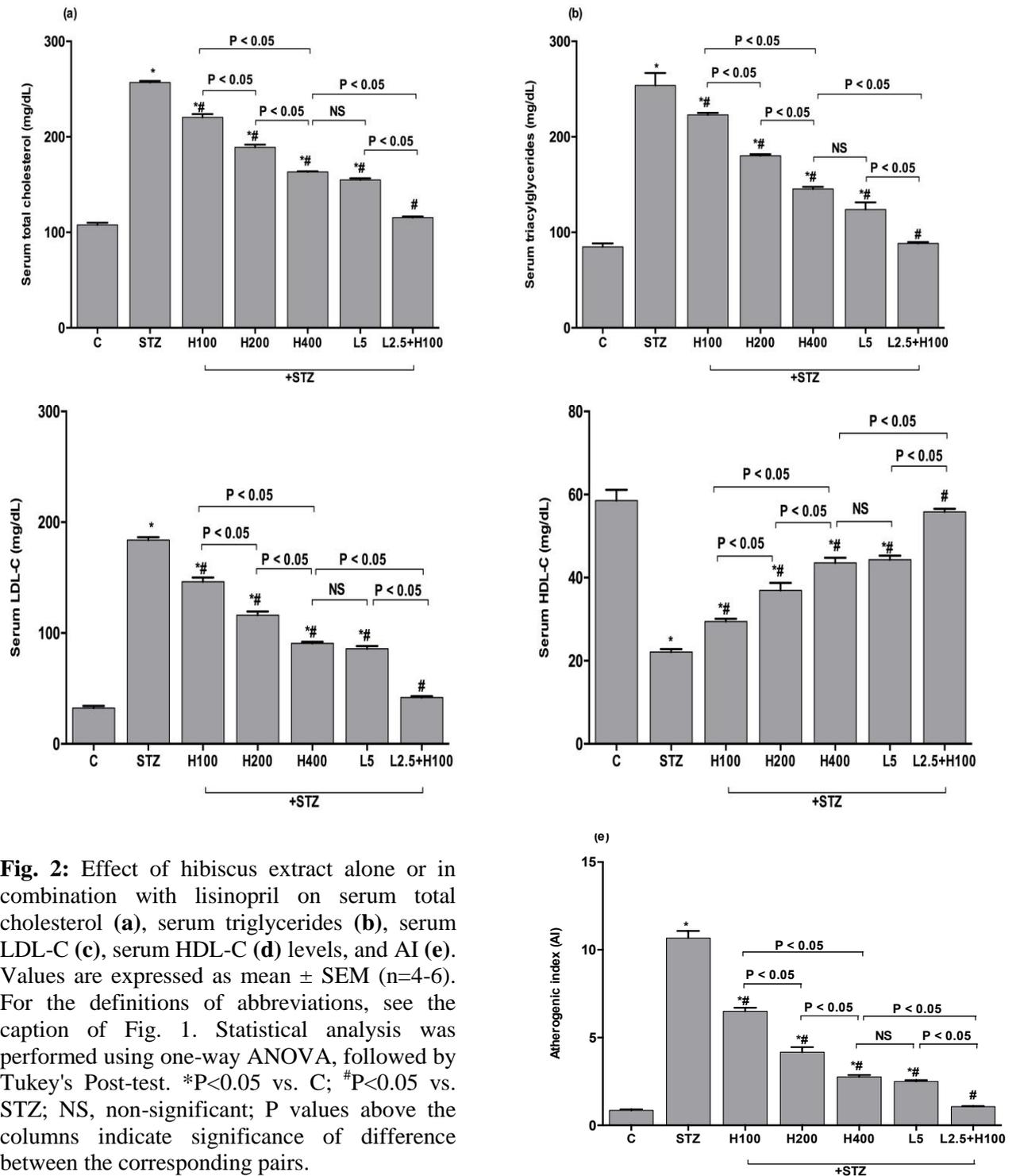


**Fig.1:** Effect of hibiscus extract alone or in combination with lisinopril on blood glucose level (a) and serum insulin level (b). Values are expressed as mean  $\pm$  SEM (n=4-6). C, control non-diabetic rats; STZ, diabetic rats received a single intraperitoneal injection of STZ (55 mg/kg); H100, H200, or H400, diabetic rats received hibiscus extract (100, 200, or 400 mg/kg, respectively) once daily by oral gavage; L5, diabetic rats received lisinopril (5 mg/kg) once daily by oral gavage; L2.5+H100, diabetic rats received lisinopril (2.5 mg/kg) plus hibiscus extract (100 mg/kg) once daily by oral gavage. Statistical analysis was performed using one-way ANOVA, followed by Tukey's Post-test. \* $P < 0.05$  vs. C; # $P < 0.05$  vs. STZ; NS, non-significant;  $P$  values above the columns indicate significance of difference between the corresponding pairs.

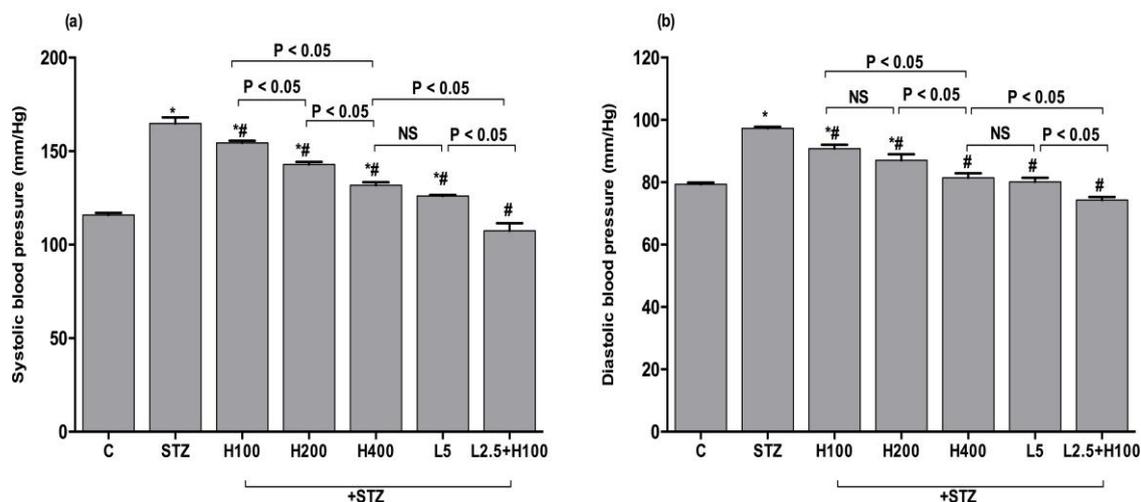
### Effect on blood pressure

As shown in Fig. 3, the administration of STZ caused significant increases in both systolic blood pressure (SBP) ( $164.9 \pm 3.2$  vs.  $115.9 \pm 1.1$  mm/Hg,  $P < 0.05$ ) and diastolic blood pressure (DBP) ( $97.33 \pm 0.5$  vs.  $79.33 \pm 0.6$  mm/Hg,  $P < 0.05$ ) compared with the control group. Hibiscus extract elicited significant, dose-dependent reductions of SBP by -6.37%, -13.34% and -20.07%, respectively, and DBP by -6.7%, -10.54% and -16.35%, respectively, compared with STZ group. Additionally,

L5 caused a significant reduction of both SBP and DBP by -23.59% and -17.67%, respectively compared with STZ group. The administration of L2.5+H100 significantly reduced both SBP and DBP by -34.87% and -23.65%, respectively. The hypotensive effect of H400 was comparable to that of L5. Importantly, the combination induced a significant reduction of SBP and DBP compared with either L5 or H400 alone.



**Fig. 2:** Effect of hibiscus extract alone or in combination with lisinopril on serum total cholesterol (a), serum triglycerides (b), serum LDL-C (c), serum HDL-C (d) levels, and AI (e). Values are expressed as mean  $\pm$  SEM (n=4-6). For the definitions of abbreviations, see the caption of Fig. 1. Statistical analysis was performed using one-way ANOVA, followed by Tukey's Post-test. \*P<0.05 vs. C; #P<0.05 vs. STZ; NS, non-significant; P values above the columns indicate significance of difference between the corresponding pairs.



**Fig. 3:** Effect of hibiscus extract alone or in combination with lisinopril on systolic blood pressure (a) and diastolic blood pressure (b). Values are expressed as mean  $\pm$  SEM (n=4). For the definitions of abbreviations, see the caption of Fig. 1. Statistical analysis was performed using one-way ANOVA, followed by Tukey's Post-test. \*P<0.05 vs. C; #P<0.05 vs. STZ; NS, non-significant; P values above the columns indicate significance of difference between the corresponding pairs

#### Effect on serum uric acid

STZ diabetic had a significant increase in serum uric acid level ( $5.9 \pm 0.29$  vs.  $1.5 \pm 0.19$  mg/dL,  $P < 0.05$ ) compared with the control group. The oral administration of hibiscus extract significantly reduced serum uric acid level by -18.28%, -34.9% and -48.68%, respectively, compared with STZ diabetic rats. Similarly, L5 significantly reduced serum uric acid level by -57.95%. The effect of H400 was comparable to that of L5. The combined administration of L2.5+H100 caused a significantly higher reduction in uric acid level compared to either L5 or H400 alone (Fig. 4a).

#### Effect on oxidative stress markers

As depicted in Fig. 4, STZ resulted in a significant increase in MDA content ( $424.9 \pm 8.2$  vs.  $117.1 \pm 2.6$  nmol/ g. tissue,  $P < 0.05$ ) and a significant decrease in CAT activity ( $5.7 \pm 0.3$  vs.  $18.14 \pm 0.32$  U/g. tissue,  $P < 0.05$ ) in aorta tissues compared with the control rats. Treatment with hibiscus extract induced a significant dose-dependent reduction in MDA content by -10.54%, -34.97% and -55.68%,

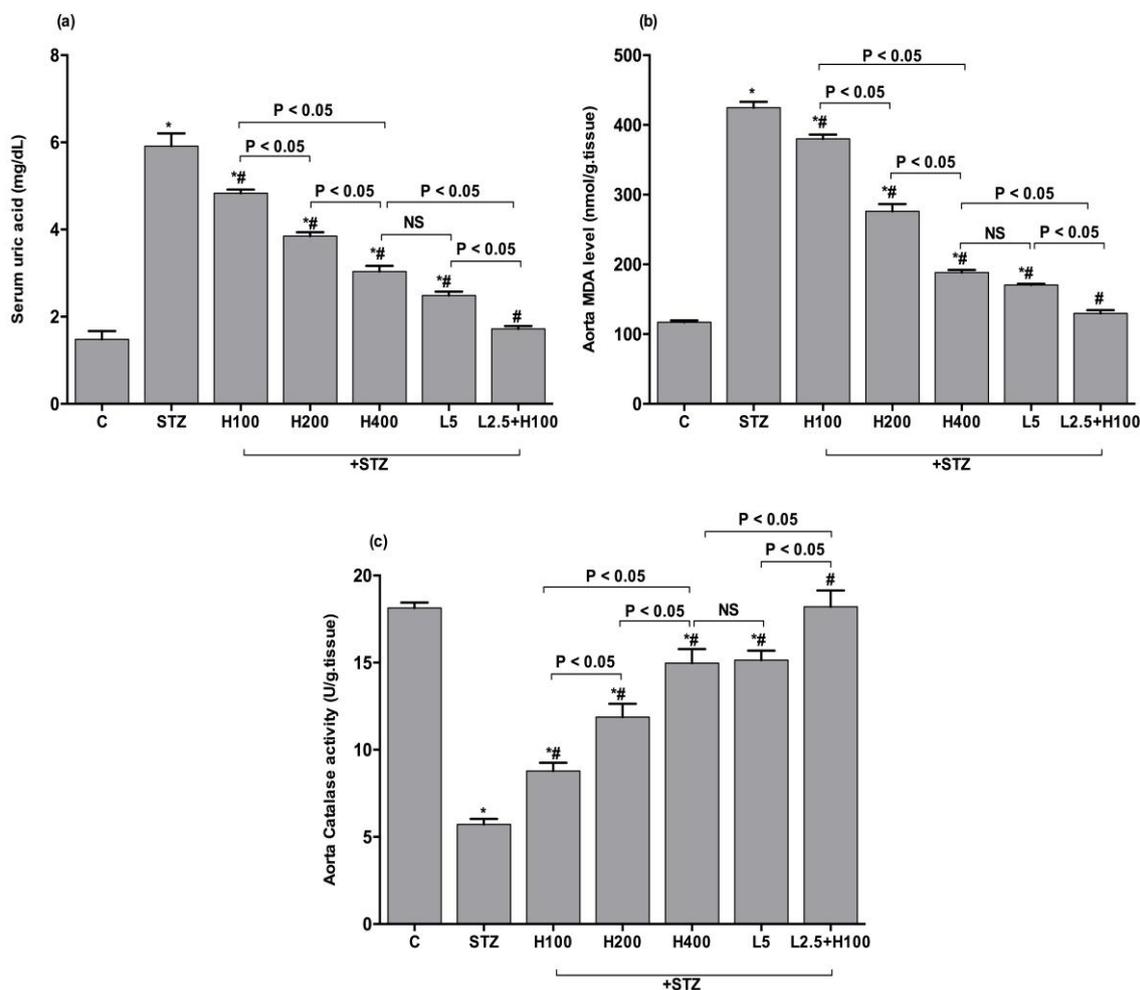
respectively, and elevation of CAT activity by +53.33%, +107.41% and +161.75%, respectively, compared with STZ diabetic rats. Similarly, the administration of L5 or L2.5+H100 significantly reduced MDA content and increased CAT activity compared with STZ diabetic rats. The difference between the effect of H400 and L5 was non-significant, whereas the effect of L2.5+H100 was significantly higher than L5 or H400 alone.

#### Effect on inflammatory markers and eNOS expression

As depicted in Fig. 5, STZ administration significantly upregulated the expression of iNOS (12.20-fold,  $P < 0.05$ ), TLR 4 (11.35-fold,  $P < 0.05$ ) and TNF- $\alpha$  (12.58-fold,  $P < 0.05$ ), whereas it significantly downregulated the expression of eNOS by -77.22% ( $P < 0.05$ ) in aorta tissues compared with the control rats. The administration of hibiscus extract at different doses, L5 or L2.5+H100 significantly improved the abnormalities of the expression of these genes induced by STZ administration. Like the effect on the other parameters, there was no significant difference between the effect of

H400 and L5, whereas the combined administration of L2.5+H100 provoked

a significant improvement compared with either L5 or H400 alone.



**Fig. 4:** Effect of hibiscus extract alone or in combination with lisinopril on serum AGEs (a) , serum UA (b), MDA content in aorta tissue (c) and CAT activity in aorta tissue (d) . Values are expressed as mean  $\pm$  SEM (n=4). For the definitions of abbreviations, see the caption of Fig. 1. Statistical analysis was performed using one-way ANOVA, followed by Tukey's Post-test. \*P<0.05 vs. C; #P<0.05 vs. STZ; NS, non-significant; P values above the columns indicate significance of difference between the corresponding pairs.

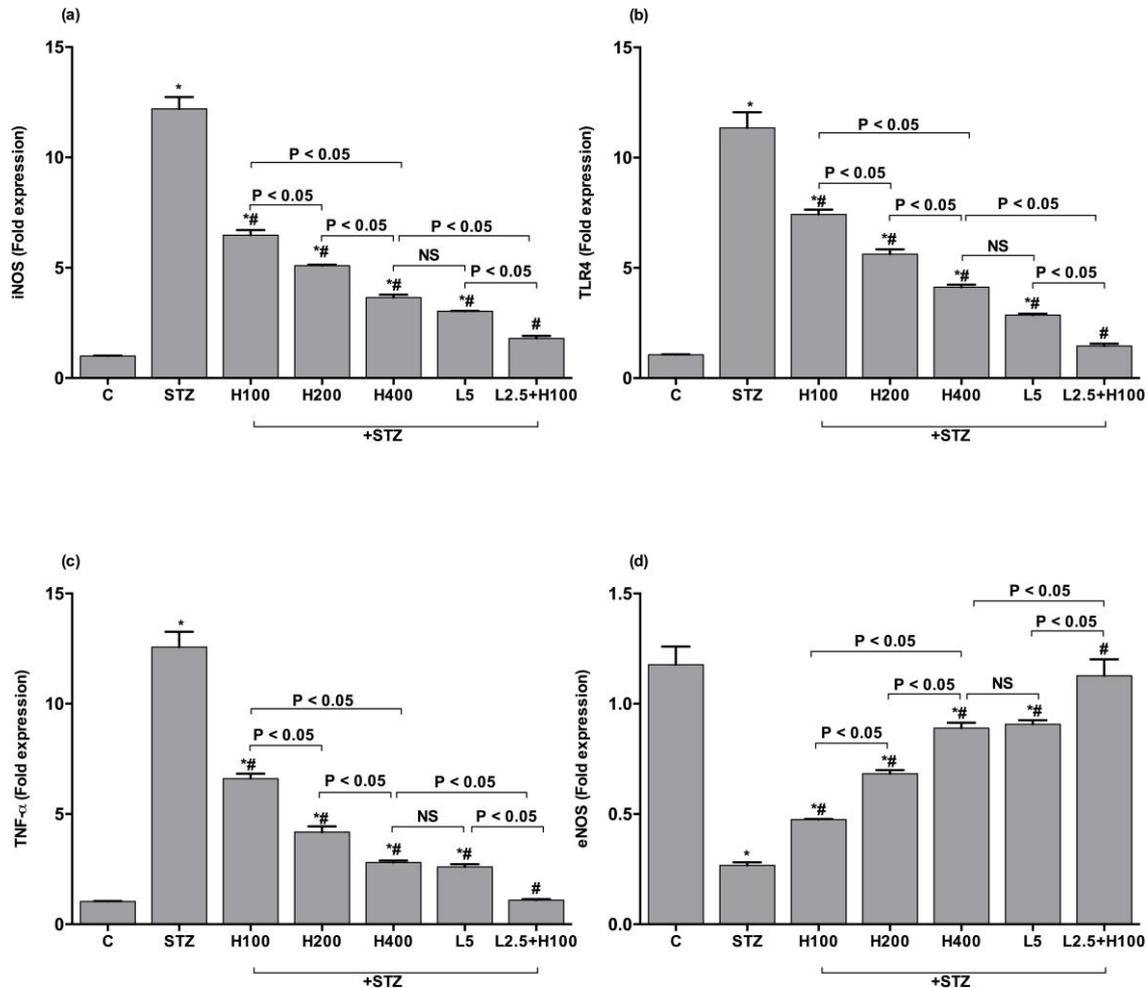
### Histopathological examination

Control rats showed normal structure of the aorta with flat endothelial layer of tunica intima, multiple oval nuclei of smooth muscle fibers in tunica media, and wavy elastic fibers (Fig 6a, b). Control rats showed also regular and normal collagen fibers distribution (Fig. 7a). On the other hand, aorta sections of STZ-treated rats showed multiple fatty streaks, irregular distribution of the elastic fibers, and marked increase in the thickness of the tunica media together with multiple

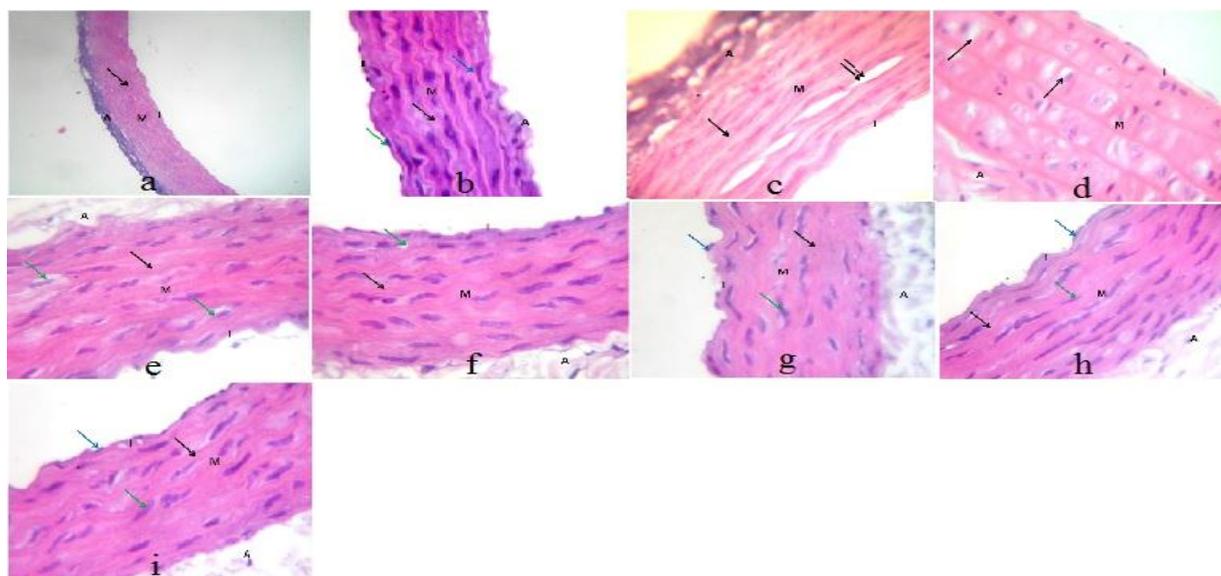
intracytoplasmic vacuoles of different sizes in smoothest muscle fibers of tunica media (fig. 6c, d). In addition, STZ-treated rats showed irregular and marked increase in collagen fibers content (Fig. 7b). H100 and H200 groups still show marked increase in the thickness of tunica media and many intracytoplasmic vacuoles of smooth muscle fibers. The elastic fibers are still irregularly arranged in the H100 rats (Fig. 6e) associated with a marked increase in collagen fibers content (Fig. 7c); however, H200 showed moderately

arranged elastic fibers (Fig. 6f) and only moderate increase in collagen fibers (Fig. 7d). Further, H 400 rats exhibited moderate increase in the thickness of tunica media, regular arrangement of the elastic fibers, and flat endothelial cells of tunica intima (Fig. 6g), as well as slight increase in collagen fibers content (Fig.

7e). L5 and L2.5+H100 rats showed mild increase in the thickness of tunica media and slightly thicker tunica media, respectively, (Fig.6h, i), as well as regular or little increase in collagen fibers content (Fig. 7f, g).



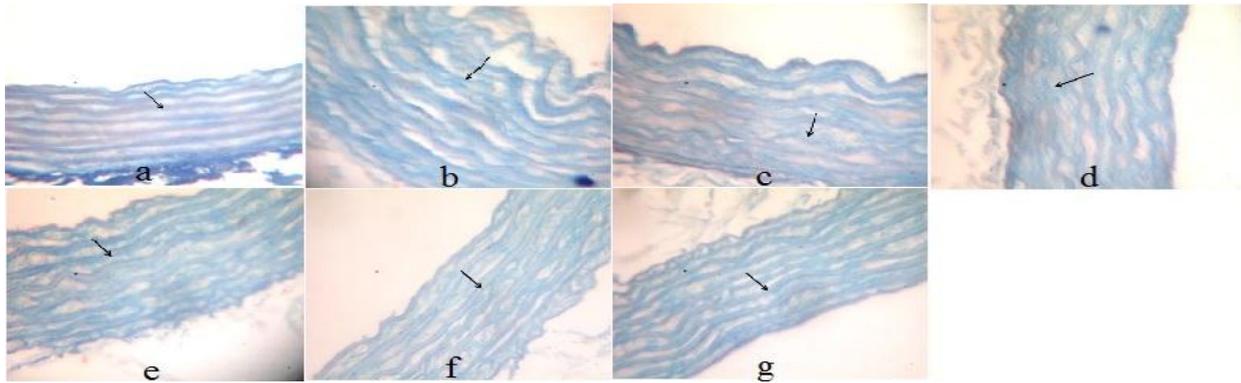
**Fig. 5:** Effect of hibiscus extract alone or in combination with lisinopril on the expression of iNOS (a), TLR 4 (b), TNF-α (c), and eNOS (d) in aorta tissue. Values are expressed as mean ± SEM (n=4). For the definitions of abbreviations, see the caption of Fig. 1. Statistical analysis was performed using one-way ANOVA, followed by Tukey's Post-test. \*P<0.05 vs. C; #P<0.05 vs. STZ; NS, non-significant; P values above the columns indicate significance of difference between the corresponding pairs



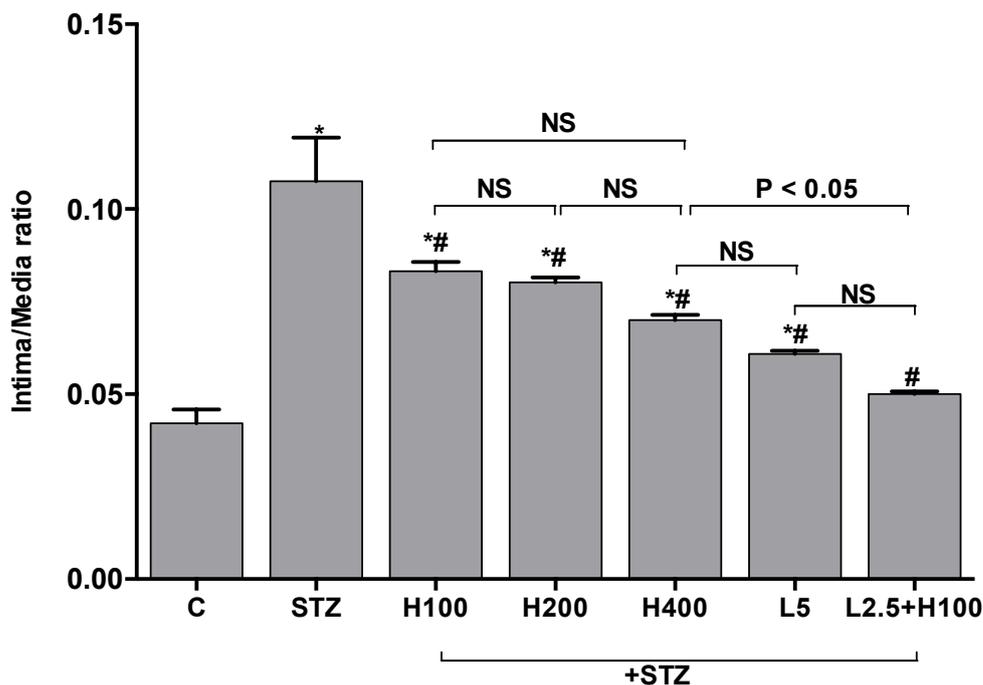
**Fig. 6:** H&E histopathological picture of aorta (x 200). Representative light photomicrographs are depicted from (a) control non-diabetic rats showing normal structure of the aorta: tunica intima (I), tunica media (M) with wavy elastic fibers (arrow) and tunica adventitia (A); (b) control non-diabetic rats showing normal structure of the aorta: flat endothelial layer (green arrow) of tunica intima (I), multiple oval nuclei of smooth muscle fibers (blue arrow) in tunica media (M) with wavy elastic fibers (black arrow) and tunica adventitia (A); (c) STZ diabetic rats showing multiple fatty streaks (double arrows), irregularly distributed elastic fibers (arrow) with marked increase in the thickness of the tunica media (M); (d) STZ diabetic rats showing multiple intracytoplasmic vacuoles of different sizes in most smooth muscle fibers of tunica media (arrows); (e) H100 rats showing marked increase in the thickness of tunica media (M), many intracytoplasmic vacuoles of smooth muscle fibers (green arrows), and irregularly arranged elastic fibers (black arrow); (f) H200 rats showing marked increase in the thickness of tunica media (M), intracytoplasmic vacuoles of smooth muscle fibers (green arrow), and regularly arranged elastic fibers (black arrow); (g) H400 rats showing moderate increase in the thickness of tunica media (M), intracytoplasmic vacuoles of smooth muscle fibers (green arrow), and regularly arranged elastic fibers (black arrow) with flat endothelial cells (blue arrow) of tunica intima (I); (h) L5 rats showing mild increase in the thickness of tunica media (M), flat endothelial layer (green arrow) of tunica intima (I), multiple oval nuclei of smooth muscle fibers (blue arrow) in tunica media (M) with wavy elastic fibers (black arrow) and tunica adventitia (A); (i) L2.5+H100 rats showing slightly thicker tunica media with a histological picture similar to that of the control group

Furthermore, Fig. 8 shows that STZ diabetic rats showed a significant increase in tunica intima/media ratio ( $0.11 \pm 0.01$  vs.  $0.04 \pm 0.003$ ,  $P < 0.001$ ) compared with the control rats. Treatment of diabetic rats with hibiscus extract at different doses, L5 or L2.5+H100 significantly decreased the

intima/media ratio compared with STZ group. The effects of H400 and L5 were comparable, whereas the combined administration of L2.5+H100 provoked a significant improvement compared with H400 alone, but the effect did not significantly differ from that of L5 group.



**Fig. 7:** Effect on the collagen deposition in aorta tissue. Specimens was stained with Mallory's trichrome stain (x 200). Representative photomicrographs are depicted from (a) control non-diabetic rats showing regular and normal collagen fibers distribution (arrow); (b) STZ diabetic rats showing irregular and marked increase in collagen fibers content (arrow); (c) H100 rats showing irregular and marked increase in collagen fibers content (arrow); (d) H200 rats showing irregular and moderate increase in collagen fibers content (arrow); (e) H400 rats showing slightly irregular and moderate increase in collagen fibers content (arrow); (f) L5 rats showing regular and little increase in collagen fibers content (arrow); (g) L2.5+H100 rats showing regular and little increase in collagen fibers content (arrow)



**Fig. 8**  
 Effect of hibiscus extract alone or in combination with lisinopril on intima/media ratio of aorta (n=4). For the definitions of abbreviations, see the caption of Fig. 1. Statistical analysis was performed using one-way ANOVA, followed by Tukey's Post-test. \*P<0.05 vs. C; #P<0.05 vs. STZ; NS, non-significant; P values above the columns indicate significance of difference between the corresponding pairs.

## DISCUSSION

The present study provides evidence of the therapeutic effect of hibiscus extract against diabetic vascular complications. In this study, type 2 diabetes was induced by STZ that specifically destroys  $\beta$ -cells and subsequently alters glucose homeostasis. This was manifested as alterations in glycemic parameters including hyperglycemia and reduction of serum insulin level. Diabetes was associated with aortic atherosclerotic changes after 8 weeks of induction including the elevation of SBP and DBP, irregular distribution of the vascular elastic fibers, increase in collagen fibers deposition, and increase in aortic intima/media ratio. Our results are consistent with the study of El-Bassossy *et al.* (2011), who reported also the significant elevation of both SBP and DBP in insulin deficiency and insulin resistance models. It has been suggested that the elevated SBP could be brought about by cardiac complications and/or aortic stiffness (El-Bassossy *et al.*, 2012), while the elevated DBP could be attributed to the altered vascular reactivity (O'Rourke and Nichols, 2005). In this regard, we observed a significant reduction in the expression of eNOS in aorta tissue, which is responsible for the synthesis of NO. Subsequently, reduced formation of endothelial NO can cause the vascular smooth muscle cells to become more sensitive to the effects of vasoconstrictors resulting in increased vascular tone and elevated blood pressure (Tran *et al.*, 2009).

Different factors might contribute to the vascular dysfunction associated with diabetes. Diabetic dyslipidemia represents a major risk factor for diabetic cardiovascular complications (Mooradian, 2009). Actually, in this study diabetes was associated with elevations of serum TC, TG, and LDL-C levels and a reduction of serum HDL-C level. These changes were reflected as an increase in the AI.

In addition, our results revealed a significant increase in MDA level, an indicator of lipid peroxidation, and a

significant reduction in CAT activity in aorta tissues in STZ diabetic rats. These alterations indicate a state of oxidative stress, which has been suggested as a main player in diabetes and diabetic complications. Oxidative stress during diabetes can be attributed to the excessive production of reactive oxygen species (ROS) directly via autoxidation of glucose or indirectly via the formation of advanced glycation end products (AGEs) during sustained hyperglycemic conditions (Sano *et al.*, 1998; Wolff, 1993). In addition, diabetes is associated with impairment of the activity of antioxidative enzymes leading to the reduction in their capacity to detoxify ROS, which can produce endothelial cell injury (Warren and Ward, 1986).

Furthermore, we observed a significant increase in serum uric acid level of STZ diabetic rats compared with control rats. Different reports described the implication of uric acid in vascular endothelial dysfunction through blocking acetylcholine-mediated arterial dilation and reducing endothelial NO bioavailability (Khosla *et al.*, 2005; Nakagawa *et al.*, 2006)

In addition, inflammation is a main factor in the pathogenesis of diabetic vascular complications. Our results demonstrated significant increases in aortic iNOS and TLR 4 gene expression associated with increased levels of aortic TNF- $\alpha$ . It has been suggested that AGEs formation is capable of inducing TLR activity (Jialal and Kaur, 2012). Others showed that AGE-modified LDL can induce TLR 4 expression (Hodgkinson *et al.*, 2008). The role of TLR 4 in the pathogenesis of atherosclerosis was described by Michelsen *et al.* (2004), who showed a decrease in lesion size, lipid content as well as macrophage infiltration in the plaques of TLR 4 knock out mice compared with wild-type mice. TLR 4 pathway involves downstream activation of nuclear factor-kappa B (NF- $\kappa$ B), which is a transcription factor that promotes the

expression of proinflammatory cytokines such as TNF- $\alpha$  and IL-1 (Akira and Takeda, 2004). Enhanced TNF- $\alpha$  production may also promote atherosclerosis through the expression of adhesion molecules. Others showed that specific agents that suppress production and/or activity of TNF- $\alpha$  may inhibit the development and exacerbation of chronic diabetic complications (Sato *et al.*, 2003).

Herbal drugs are now extensively prescribed owing to their effectiveness, fewer side effects and relatively low cost (Mukherjee *et al.*, 2006). *Hibiscus sabdariffa* has been widely used for the preparation of beverages, flavoring agents and sweets. The administration of hibiscus extract significantly improved the disturbances induced by STZ in glycemic parameters. These results are similar to many other studies showing the anti-diabetic effect of hibiscus extract (Farombi and Ige, 2007; Peng *et al.*, 2011). Hibiscus extract, dose-dependently, alleviated diabetic atherosclerotic changes in our study. The extract elicited significant improvements in blood pressure, elastic fiber distribution, collagen fiber deposition and intima/media ratio. A similar hypotensive effect of hibiscus extract has been described by other studies (Ajay *et al.*, 2007; Inuwa *et al.*, 2012). Different mechanisms have been suggested to explain the hypotensive activity of hibiscus including ACE inhibition (Ojeda *et al.*, 2010), acetylcholine-like and histamine-like mechanisms (Adegunloye *et al.*, 1996), diuretic effect (Mojiminiyi *et al.*, 2000), and direct vasorelaxant effects (Ajay *et al.*, 2007). The functional and structural improvement provoked by hibiscus extract can be explained based on different activities. The extract possesses anti-hyperlipidemic effect manifested by the alleviation of STZ-induced dyslipidemia we observed in this study. This effect has been described also by others (Chang *et al.*, 2006; Chen *et al.*, 2004).

In addition, hibiscus extract significantly reduced serum uric acid, which can help protect against STZ-induced vascular dysfunction mediated through uric acid. Similar results were observed in albino rats (Cáceres *et al.*, 1987). It has been demonstrated that hibiscus increases the activity of uricase enzyme, involved in uric acid degradation, thus promoting its clearance (Kuo *et al.*, 2012).

Moreover, the antioxidant and anti-inflammatory effects of hibiscus extract seem pivotal for its protective effects. Hibiscus extract significantly reduced MDA level and increased CAT activity, as well as it reduced the expression of iNOS, TLR 4 and TNF- $\alpha$ . Similarly, the extract has been shown by others to exert a strong scavenging effect on ROS (Farombi and Fakoya, 2005). Our results are also in harmony with others reporting hibiscus-induced reduction of iNOS protein expression in LPS-treated macrophages (Kao *et al.*, 2009).

The results of the present study revealed that hibiscus extract significantly suppressed the inflammatory TLR 4/TNF- $\alpha$  pathway. Previous studies pointed to the ability of hibiscus to suppress TNF- $\alpha$  (Fakaye, 2008; Fakeye *et al.*, 2008) in mice. However, this is the first study, to our knowledge, reporting the inhibitory effect of hibiscus extract on the expression of TLR 4 and its implication in the protection against diabetic vascular complications.

We compared the effects of hibiscus with the reference lisinopril, an ACEI that is widely used in managing vascular complications in diabetic patients (Amann *et al.*, 2003; Ravid *et al.*, 1996). Interestingly, the vasoprotective effects of the higher dose of hibiscus (H400) were comparable to that of lisinopril (5 mg/kg).

Another interesting finding in the present study was the augmentation of the vasoprotective effect of the low dose of lisinopril (2.5 mg/kg) when combined with the lowest dose of hibiscus (H100). This

combination resulted in significant improvement compared to the full-dose of lisinopril (5 mg/kg) or the highest dose of hibiscus (H400) alone. Such finding seems promising because it warrants achieving better therapeutic profile without exceeding the optimal therapeutic level of lisinopril, which would be a major concern particularly in high risk patients.

### CONCLUSION

In conclusion, the present study provides an evidence of the therapeutic potential of hibiscus for managing diabetic vascular complications. Such effect is likely mediated through the reduction of uric acid, as well as its hypolipidemic, antioxidative, and anti-inflammatory effects, particularly the suppression of TLR 4/ /TNF- $\alpha$  pathway.

### CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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### REFERENCES:

- Adegunloye B., Omoniyi J., Owolabi O., Ajagbonna O., Sofola O. and Coker H. (1996): Mechanisms of the blood pressure lowering effect of the calyx extract of *Hibiscus sabdariffa* in rats. *African Journal of Medicine and Medical Sciences*, 25(3): 235-238.
- Ajay M., Chai H., Mustafa A., Gilani A. H. and Mustafa M. R. (2007): Mechanisms of the anti-hypertensive effect of *Hibiscus sabdariffa* L. calyces. *Journal of Ethnopharmacology*, 109(3): 388-393.
- Akira S. and Takeda K. (2004): Toll-like receptor signalling. *Nat Rev Immunol*, 4(7): 499-511.
- Amann B., Tinzmann R. and Angelkort B. (2003): ACE inhibitors improve diabetic nephropathy through suppression of renal MCP-1. *Diabetes Care*, 26(8): 2421-2425.
- Cáceres A., Girón L. M. and Martínez A. M. (1987): Diuretic activity of plants used for the treatment of urinary ailments in Guatemala. *Journal of Ethnopharmacology*, 19(3): 233-245.
- Chang Y.-C., Huang K.-X., Huang A.-C., Ho Y.-C. and Wang C.-J. (2006): Hibiscus anthocyanins-rich extract inhibited LDL oxidation and oxLDL-mediated macrophages apoptosis. *Food and Chemical Toxicology*, 44(7): 1015-1023.
- Chen C. C., Chou F. P., Ho Y. C., Lin W. L., Wang C. P., Kao E. S., Huang A. C. and Wang C. J. (2004): Inhibitory effects of *Hibiscus sabdariffa* L extract on low-density lipoprotein oxidation and anti-hyperlipidemia in fructose-fed and cholesterol-fed rats. *Journal of the science of food and agriculture*, 84(15): 1989-1996.
- Collins T. and Cybulsky M. I. (2001): NF- $\kappa$ B: pivotal mediator or innocent bystander in atherogenesis? *J Clin Invest*, 107(3): 255.
- El-Bassossy H. M., El-Fawal R. and Fahmy A. (2012): Arginase inhibition alleviates hypertension associated with diabetes: effect on endothelial dependent relaxation and NO production. *Vascular pharmacology*, 57(5): 194-200.
- El-Bassossy H. M., Fahmy A. and Badawy D. (2011): Cinnamaldehyde protects from the hypertension associated with diabetes. *Food and chemical toxicology*, 49(11): 3007-3012.
- Fakaye T. (2008): Toxicity and immunomodulatory activity of fractions of *Hibiscus sabdariffa* Linn

- (family Malvaceae) in animal models. *African Journal of Traditional, Complementary and Alternative Medicines*, 5(4): 394-398.
- Fakeye T. O., Pal A., Bawankule D. U. and Khanuja S. P. (2008): Immunomodulatory effect of extracts of *Hibiscus sabdariffa* L. (Family Malvaceae) in a mouse model. *Phytother Res*, 22(5): 664-668.
- Farombi E. O. and Fakoya A. (2005): Free radical scavenging and antigenotoxic activities of natural phenolic compounds in dried flowers of *Hibiscus sabdariffa* L. *Mol Nutr Food Res*, 49(12): 1120-1128.
- Farombi E. O. and Ige O. O. (2007): Hypolipidemic and antioxidant effects of ethanolic extract from dried calyx of *Hibiscus sabdariffa* in alloxan-induced diabetic rats. *Fundam Clin Pharmacol*, 21(6): 601-609.
- Friedewald W. T., Levy R. I. and Fredrickson D. S. (1972): Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem*, 18(6): 499-502.
- Herrera-Arellano A., Flores-Romero S., Chavez-Soto M. A. and Tortoriello J. (2004): Effectiveness and tolerability of a standardized extract from *Hibiscus sabdariffa* in patients with mild to moderate hypertension: a controlled and randomized clinical trial. *Phytomedicine*, 11(5): 375-382.
- Hodgkinson C. P., Laxton R. C., Patel K. and Ye S. (2008): Advanced glycation end-product of low density lipoprotein activates the toll-like 4 receptor pathway implications for diabetic atherosclerosis. *Arterioscler Thromb Vasc Biol*, 28(12): 2275-2281.
- Inuwa I., Ali B. H., Al-Lawati I., Beegam S., Ziada A. and Blunden G. (2012): Long-term ingestion of *Hibiscus sabdariffa* calyx extract enhances myocardial capillarization in the spontaneously hypertensive rat. *Experimental Biology and Medicine*, 237(5): 563-569.
- Jarrett R. (1984): Type 2 (non-insulin-dependent) diabetes mellitus and coronary heart disease—chicken, egg or neither? *Diabetologia*, 26(2): 99-102.
- Jialal I. and Kaur H. (2012): The role of toll-like receptors in diabetes-induced inflammation: implications for vascular complications. *Current diabetes reports*, 12(2): 172-179.
- Kao E.-S., Hsu J.-D., Wang C.-J., Yang S.-H., Cheng S.-Y. and Lee H.-J. (2009): Polyphenols extracted from *Hibiscus sabdariffa* L. inhibited lipopolysaccharide-induced inflammation by improving antioxidative conditions and regulating cyclooxygenase-2 expression. *Bioscience, biotechnology, and biochemistry*, 73(2): 385-390.
- Khosla U. M., Zharikov S., Finch J. L., Nakagawa T., Roncal C., Mu W., Krotova K., Block E. R., Prabhakar S. and Johnson R. J. (2005): Hyperuricemia induces endothelial dysfunction. *Kidney Int*, 67(5): 1739-1742.
- King G. L. (1996): The role of hyperglycaemia and hyperinsulinaemia in causing vascular dysfunction in diabetes. *Ann Med*, 28(5): 427-432.
- Kinlay S., Libby P. and Ganz P. (2001): Endothelial function and coronary artery disease. *Curr Opin Lipidol*, 12(4): 383-389.
- Kuno A., Yamada T., Masuda K., Ogawa K., Sogawa M., Nakamura S., Nakazawa T., Ohara H., Nomura T. and Joh T. (2003): Angiotensin-converting enzyme inhibitor attenuates pancreatic inflammation and fibrosis in male Wistar Bonn/Kobori rats. *Gastroenterology*, 124(4): 1010-1019.
- Kuo C.-Y., Kao E.-S., Chan K.-C., Lee H.-J., Huang T.-F. and Wang C.-J.

- (2012): Hibiscus sabdariffa L. extracts reduce serum uric acid levels in oxonate-induced rats. *Journal of Functional Foods*, 4(1): 375-381.
- Michelsen K. S., Wong M. H., Shah P. K., Zhang W., Yano J., Doherty T. M., Akira S., Rajavashisth T. B. and Arditi M. (2004): Lack of Toll-like receptor 4 or myeloid differentiation factor 88 reduces atherosclerosis and alters plaque phenotype in mice deficient in apolipoprotein E. *Proceedings of the National Academy of Sciences of the United States of America*, 101(29): 10679-10684.
- Mojiminiyi F., Adegunloye B., Egbeniyi Y. and Okolo R. (2000): An investigation of the diuretic effect of an aqueous extract of the petals of *Hibiscus sabdariffa*.
- Mooradian A. D. (2009): Dyslipidemia in type 2 diabetes mellitus. *Nature clinical practice endocrinology & metabolism*, 5(3): 150-159.
- Mukherjee P. K., Maiti K., Mukherjee K. and Houghton P. J. (2006): Leads from Indian medicinal plants with hypoglycemic potentials. *J Ethnopharmacol*, 106(1): 1-28.
- Nakagawa T., Hu H., Zharikov S., Tuttle K. R., Short R. A., Glushakova O., Ouyang X., Feig D. I., Block E. R., Herrera-Acosta J., Patel J. M. and Johnson R. J. (2006): A causal role for uric acid in fructose-induced metabolic syndrome. *Am J Physiol Renal Physiol*, 290(3): F625-631.
- O'Rourke M. F. and Nichols W. W. (2005): Aortic diameter, aortic stiffness, and wave reflection increase with age and isolated systolic hypertension. *Hypertension*, 45(4): 652-658.
- Ojeda D., Jiménez-Ferrer E., Zamilpa A., Herrera-Arellano A., Tortoriello J. and Alvarez L. (2010): Inhibition of angiotensin convertin enzyme (ACE) activity by the anthocyanins delphinidin-and cyanidin-3-O-sambubiosides from *Hibiscus sabdariffa*. *Journal of ethnopharmacology*, 127(1): 7-10.
- Onyenekwe P. C., Ajani E. O., Ameh D. A. and Gamaniel K. S. (1999): Antihypertensive effect of roselle (*Hibiscus sabdariffa*) calyx infusion in spontaneously hypertensive rats and a comparison of its toxicity with that in Wistar rats. *Cell Biochem Funct*, 17(3): 199-206.
- Pasquie J. L., Herizi A., Jover B. and Mimran A. (1999): Chronic bradykinin infusion and receptor blockade in angiotensin II hypertension in rats. *Hypertension*, 33(3): 830-834.
- Peng C. H., Chyau C. C., Chan K. C., Chan T. H., Wang C. J. and Huang C. N. (2011): *Hibiscus sabdariffa* polyphenolic extract inhibits hyperglycemia, hyperlipidemia, and glycation-oxidative stress while improving insulin resistance. *J Agric Food Chem*, 59(18): 9901-9909.
- Ravid M., Lang R., Rachmani R. and Lishner M. (1996): Long-term renoprotective effect of angiotensin-converting enzyme inhibition in non-insulin-dependent diabetes mellitus. A 7-year follow-up study. *Arch Intern Med*, 156(3): 286-289.
- Sano T., Umeda F., Hashimoto T., Nawata H. and Utsumi H. (1998): Oxidative stress measurement by in vivo electron spin resonance spectroscopy in rats with streptozotocin-induced diabetes. *Diabetologia*, 41(11): 1355-1360.
- Satoh J., Yagihashi S. and Toyota T. (2003): The possible role of tumor necrosis factor- $\alpha$  in diabetic polyneuropathy. *Journal of Diabetes Research*, 4(2): 65-71.
- Tran L. T., Yuen V. G. and McNeill J. H. (2009): The fructose-fed rat: a review on the mechanisms of fructose-induced insulin resistance and hypertension. *Mol Cell Biochem*, 332(1-2): 145-159.

- Wang S.-C., Lee S.-F., Wang C.-J., Lee C.-H., Lee W.-C. and Lee H.-J. (2011): Aqueous extract from *Hibiscus sabdariffa* Linnaeus ameliorate diabetic nephropathy via regulating oxidative status and Akt/Bad/14-3-3 $\gamma$  in an experimental animal model. *Evid Based Complement Alternat Med*, 2011.
- Warren J. S. and Ward P. A. (1986): Oxidative injury to the vascular endothelium. *Am J Med Sci*, 292(2): 97-103.
- Wolff S. (1993): Diabetes mellitus and free radicals Free radicals, transition metals and oxidative stress in the aetiology of diabetes mellitus and complications. *British medical bulletin*, 49(3): 642-652.
- Yang R.-L., Shi Y.-H., Hao G., Li W. and Le G.-W. (2008): Increasing oxidative stress with progressive hyperlipidemia in human: relation between malondialdehyde and atherogenic index. *J Clin Biochem Nutr*, 43(3): 154.
- Yang Y.-S., Huang C.-N., Wang C.-J., Lee Y.-J., Chen M.-L. and Peng C.-H. (2013): Polyphenols of *Hibiscus sabdariffa* improved diabetic nephropathy via regulating the pathogenic markers and kidney functions of type 2 diabetic rats. *J Funct Foods*, 5(2): 810-819.
- Zeihner A. M., Fisslthaler B., Schray-Utz B. and Busse R. (1995): Nitric oxide modulates the expression of monocyte chemoattractant protein 1 in cultured human endothelial cells. *Circ Res*, 76(6): 980-986.

## خلاصة نبات الهيبسيسكس سابداريفا (الكرديه) تخفف حدة مضاعفات الأوعية الدموية في الجرذان

### المصابة بمرض السكري المحدث بالستربتوزوتوسين .

نهال سمير وهبه، عمرو عبد الرؤف عبد الغني محمود، مني فؤاد محمود، محمد نجيب محمد زكريا

أمراض الأوعية الدموية هي أكثر مضاعفات مرض السكري أهمية. تتطوي الفسيولوجيا المرضية لها على عيوب في بطانة الأوعية الدموية الناجمة عن ارتفاع سكر الدم المستديم، زيادة حمض اليوريك، زيادة الإجهاد التأكسدي، والالتهابات المتوسطة بمسار مستقبل شبيه التول ٤. يستخدم نبات الهيبسيسكس سابداريفا (الكرديه) تقليدياً في المنتجات الغذائية فهو يمتاز بتأثيرات مكافحة للسكري، مكافحة لزيادة دهون الدم، مكافحة للتأكسد، ومضادة للالتهابات، ومضادة لارتفاع ضغط الدم. تهدف هذه الدراسة إلى بحث تأثير خلاصة الكركديه ضد مضاعفات الأوعية الدموية لمرض السكري. تم احداث مرض السكري عن طريق حقنة واحدة من الستربتوزوتوسين (٥٥ مجم/كجم). تم علاج الفئران المصابة بالسكري بخلاصة الكركديه (١٠٠، ٢٠٠، ٤٠٠ مجم/كجم)، ليسينوبريل (٥ مجم/كجم)، أو ليسينوبريل مع الكركديه (١٠٠/٢،٥ مجم/كجم) يومياً لمدة ٨ أسابيع. حَسُنَت خلاصة الكركديه جوهرياً وبشكل تابع للجرعة عيوب نسبة الدهون في الدم وضغط الدم. بالإضافة إلى ذلك، كبحت خلاصة الكركديه الإجهاد التأكسدي، والالتهاب، وخاصة زيادة تعبير مستقبل شبيه التول ٤ الناجم عن الستربتوزوتوسين. لم يكن هناك فروق جوهريّة بين تأثير خلاصة الكركديه (٤٠٠ مجم/كجم) وليسينوبريل (٥ مجم/كجم). تأثيرات الاعطاء المشترك لليسينوبريل مع الكركديه (١٠٠ / ٢،٥ مجم/كجم) كانت أفضل جوهرياً مقارنة بالليسينوبريل أو الكركديه علي حدي. وتقتصر هذه النتائج استخدام الكركديه كبديل للاستخدام التقليدي لمثبطات الإنزيم المحول للأنجيوتنسين أو على الأقل بالاشتراك مع جرعة صغيرة لتعزيز تأثيرهم في علاج مضاعفات الأوعية الدموية لمرض السكري.