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Full Length Research Paper

Hypoglycemic and antioxidant effects of *Hibiscus rosa-sinensis* L. leaves extract on liver and kidney damage in streptozotocin induced diabetic rats

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Hibiscus **is commonly used traditionally for the treatment of some diseases such as hypertension and as antidiabetic herbal medicine. The objective of the present study is to investigate the effect of the oral administration of aqueous methanolic extract of *Hibiscus rosa-sinensis* leaves (400 mg/Kg) on streptozotocin (STZ) induced diabetic rats and alteration in liver and kidney functions. The treatment of diabetic rats with hibiscus leaves extract reduced levels of plasma glucose, cholesterol, aspartate aminotransferase (AST), alanine aminotransferase (ALT), uric acid and creatinine and hepatic malondialdehyde that was elevated in diabetic rats. Moreover, the *Hibiscus* leaves extract mitigates the decrease in hepatic superoxide dismutase and plasma protein levels due to STZ injection. The treatment of rats with STZ only results in some pathological effects in liver and kidneys as degeneration in most of hepatocyte and glomeruli. The extract of *H. rosa-sinensis* leaves reduced the pathological changes. The treatment of diabetic rats with *Hibiscus* extract was shown to have hepatic and renal protective effects in diabetic rats induced experimentally. Here, two compounds, that is, orientin (Luteolin-8-C-glucoside) and verbascoside (phenylpropanoids glycoside) were isolated from *H. rosa-sinsensis*. The two compounds were identified by spectral analysis (UV, ^{1}H and ^{13}C-NMR). These results clearly indicate that aqueous leaves extract of *H. rosa-sinensis* possess antidiabetic and hypolipidemic effects in diabetic rats which may be due to antioxidant properties of the hibiscus extract.**

Key words: *Hibiscus rosa-sinensis*, hypoglycemic, antioxidant, orientin, verbascoside.

INTRODUCTION

The plant kingdom represents a large reservoir of biologically active compounds not only as drugs, but also as unique templates that could serve as a starting point for synthetic analogs. Numerous biologically active plants are discovered by evaluation of ethnopharmacological data, and these plants may offer accessible therapeutic products (Aquino et al., 1995). Numerous medicinal plants are used traditionally for treatment and

management of diabetes (Verspohl. 2002).Various active principles of plants with hypoglycemic activity have been identified, including alkaloids, flavonoids, glycosides and polysaccharides (Day 1990). Among the herbal remedies, hibiscus is used in folk medicine. It has antihypertensive (Ojeda et al., 2010), antiatherosclerosis (Chen et al., 2003), anti-inflammatory (Tomar et al., 2010) and analgesic (Sawarkar et al., 2009) activities. Moreover, the extract of *Hibiscus* can be used effectively in the treatment of peptic ulcer (Mandade et al., 2012) and Leukaemia (Arullappan et al., 2013).

The leaves of *Hibiscus rosa-sinensis*, a well-known member of the family Malvaceae, were found to contain large amounts of phenolic and flavonoid compounds. Methanolic extract of *H. rosa-sinensis* possessed significant antioxidant activity as compared to aqueous extract (Garg et al., 2012). It is concluded that *Hibiscus* cannabinus extract has significant antidiabetic activity, which lowered blood glucose level in diabetic rats (Rajkumar et al., 2011). Diabetes mellitus is one of metabolic syndrome that alter carbohydrate, lipid and protein metabolism and additionally increased risk of complications of various vascular diseases. Hyperlipidemia associated atherosclerosis is the most common cause of death in diabetes (Chait and Bornfeld, 2009). Insulin-dependent diabetes mellitus or type 1 diabetes is an autoimmune disorder characterized by destruction of insulin producing β-cells because auto-aggressive T-lymphocytes infiltrate the pancreas that leads to–hypoinsulinemia and thus hyperglycemia (Bach, 1995). Hyperglycemic condition results in an increased glycosylation and biochemical and morphological abnormalities which over a period of time develops diabetic complications such as nephropathy, retinopathy, neuropathy and cardiomyopathy (Arky, 1982). Moreover, diabetes altered liver and kidney functions (Elgazar et al., 2013). The numbers of people with diabetes will be more than double over the next 25 years, to reach a total of 366 million by 2030. Most of this increase will occur in developing countries (WHO, 2015). Preliminary phytochemical screening of *H. rosa sinensis* stem and leaves revealed the presence of several classes of compounds including flavonoids, flavonoids, glycosides and tannins (Ajay et al., 2007).

In this study, the efficacy of *H. rosa -sinensis* leaf extract in relieving the hepatic oxidative stress, hypercholesterolemia and kidney damage associated with STZ-induced diabetic rat model were investigated. Here, two compounds of *H. rosa-sinsensis*, that is, orientin (Luteolin-8-C-glucoside) were isolated from EtOAc fraction and (phenylpropanoids glycoside) verbascoside from BuOH fraction, these two compounds were isolated for the first time from this plants.

MATERIALS AND METHODS

Leaves of *H. rosa-sinensis* were collected during the flowering period from the garden of the National Research Centre, Cairo, Egypt in April 2010. The plant was kindly identified by, Mrs. Tersea Labib, a taxonomist at Orman Botanical garden, Giza, Egypt and Dr. Mona Marzok, Researcher at the Herbarium of National Research Centre, Giza, Egypt. The voucher specimens were deposited at the herbarium of the National Research Centre, Cairo, Egypt.

Preparation of the extract and isolation of compounds

1200 g of *H. rosa-sinensis* (HRS) leaves were cleaned and dried completely under the mild sun and then ground with an electric grinder. The powdered material was extracted with 80% methanol at room temperature for 7 days with shaking and stirring. The extract was filtered and then concentrated to dryness in the rotary evaporator to obtain crude extract. The yield was 9.42% (w/w, in terms of dried starting material). 20 g from the dry extract were dissolved in distill water and partitioned successfully in chloroform ($CHCl_3$), ethyl acetate (EtOAc) and n-butanol (BuOH). The EtOAc and BuOH fractions were subjected to different column chromatography on Silica gel and Sephedex L-H-20 column led to the isolation of orientin from the EtOAc fraction and verbascoside from the BuOH fraction. The two compounds were identified by spectral analysis ^{1}H and ^{13}C-NMR (Tables 1 and 2) and compared with published data (Ismail et al., 1995; kim et al., 2010).

Animals

Adult male albino rats (Sprague Dawley rats) weighing 140 to 190 g were obtained from animal house, National Research Centre, Dokki, Giza. The rats had free access to standard rodent chow and water *ad libitum*. All animals received human care in compliance with guidelines of Ethical Committee of National Research Center and followed the recommendations of The National Institute of Health Guide for care and use of Laboratory animals (Eighth edition). The animals were housed throughout the experiment in polypropylene cages (each cage housing six animals) and allowed to acclimatize to the laboratory environment for seven days before the beginning of the experiment. Animals were maintained under controlled conditions of temperature (25 ± 1°C), humidity (50±15%) and normal photoperiod (12 to 12 h light-dark cycles).

Acute toxicity studies (LD_{50})

Acute toxicity studies of the plants extracts were conducted for doses of the plant extracts administered to animals in this study. Oral LD_{50} determination was done by the method of Lorke (1983) using rats. No mortality in the rats was observed up to 3000 mg/kg.

Induction of diabetes

Diabetes was induced by intraperitoneal injections of streptozotocin

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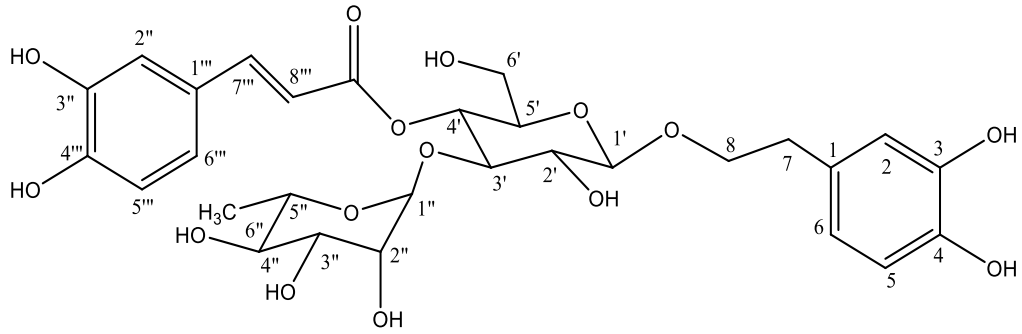
Table 1. NMR of Verbascoside.

C No.	δ_H	δ_C	C No.	δ_H	δ_C
1		131.3	1"	5.29 d (1.2)	101.9
2	6.77 d (1.8)	115.9	2"	3.89 brs	71.5
3		144.3	3"	Not resolved	72.0
4		146.3	4"	3.32 t-like (9.3)	73.6
5	6.72 d (8.1)	116.9	5"	Not resolved	69.5
6	6.45 dd (8.1, 1.8)	121.1	6"	1.11d	18.5
7	2.67 t-like (7.8)	36.2	1'''		127.6
8a,b	3.99 m	72.2	2'''	7.17 d (2.1)	115.3
1'	4.42 d (7.8)	103.8	3'''		145.7
2'	Not resolved	75.9	4'''		148.9
3'	3.44 t-like (8.1)	79.7	5'''	6.87 d (8.1)	116.4
4'	4.90 t-like (9.6)	70.3	6'''	7.05 dd (8.1, 2.1)	122.8
5'	3.9-3.5 m	76.3	7'''	7.58 d (15.9)	146.8
6'a	4.38 br d (11.4)	62.4	8'''	6.29 d (15.9)	115.2
6'b	4.21 dd (12.5, 5.7)		9'''		167.1



Structure of verbascoside.

(Sigma Co.) at dose of 50 mg/kg b.w. which was freshly prepared in aqueous solution of citrate buffer (pH 4.5) as vehicle. The animals were allowed to fast for 24 h before streptozotocin (STZ) injection. After two days, rats with moderate diabetes (blood glucose concentration, >250 mg/dl) were selected for the experiment, while rats with blood glucose levels lower than the previous level were excluded from the study. All treatments were carried out two days post STZ treatment. The values of recorded glucose levels were considered for zero time.

Experimental design

Group I: Control rats were injected with citrate buffer (pH 4.5) intraperitoneally;
Group II: Rats were made diabetic by a single intraperitoneal injection of STZ (50 mg/kg b.w.);
Group III: Diabetic rats treated with leaf extract of *H. rosa sinensis* (400 mg/kg b.w.) daily by oral administration for 3 weeks;
Group IV: Normal rats treated with leaf extract of *H. rosa sinensis* (400 mg/kg b.w.) daily by oral administration for 3 weeks;
Group V: Diabetic rats treated with glibenclamide (0.3 mg/kg b.w.) (Ezekwesili et al., 2012) daily by oral administration for 3 weeks.
Alcoholic extract of the plant was dissolved in one drop of Tween (80%, Prolabo, France) and distilled water.

Samples

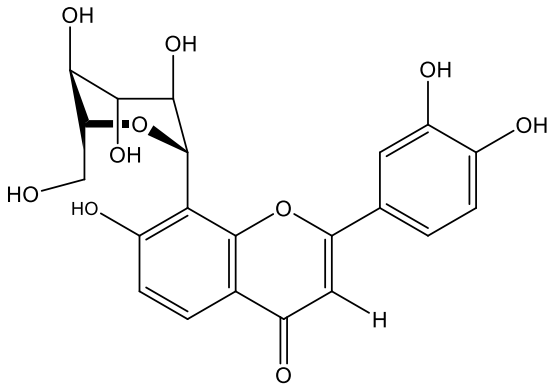
After overnight starvation, blood samples were withdrawn after one, two and three weeks. The blood samples were withdrawn from the retro-orbital venous plexus into centrifuge tube containing anticoagulant, ethylene diamine tetra acetic acid (EDTA). Samples were subjected to centrifugation at 4000 rpm for 15 min. The separated plasma was used for the different biochemical analysis. The plasma glucose and body weight results were recorded at the three previous intervals beside zero time, but the other parameters were evaluated at the third week only. At the end of the experiment, the treated animals were killed and their livers were collected, quickly blotted with filter paper and kept at -40°C until analysis. The livers were homogenized by using potter-Elvehjem homogenizer with Teflon pestle. The supernatant of homogenates was separated by centrifugation.

Biochemical analysis

Blood glucose (Trinder, 1969), alanine aminotransferase (ALT), aspartate aminotransferase (AST) (Reitman and Frankel, 1957), uric acid (Fossati et al., 1980), creatinine (Larsen, 1972), total protein (Gornal et al., 1949) and cholesterol (Allain et al., 1974) were evaluated by using Biodiagnostic kits, Egypt. Also, hepatic

Table 2. NMR of orientin (luteolin-8-C-glucoside).

C No.	δ_H	δ_C
2		165.31
3	6.48	104.50
4		182.82
5	7.68	128.52
6	6.88	113.00
7		168.27
8		113.32
9		161.75
10		119.45
1'		122.25
2'	6.50	119.23
3'		145.72
4'		149.59
5'	6.52	122.31
6'	7.34	128.25
1"	4.85	74.01
2"		70.99
3"		78.96
4"	3.93-3.16	71.49
5"		81.58
6"		61.90



Structure of orientin (Luteolin-8-C-glucoside).

malondialdehyde (MDA) (Uchiyama and Mihara, 1978), reduced glutathione (GSH) (Beutler et al., 1963), superoxide dismutase (SOD) (Nishikimi et al.,1972) and catalase (Aebi, 1984) were assayed by using Biodiagnostic kits, Egypt.

Histological study

The liver and kidney of different groups were removed and fixed in 10% formol saline, 5 µm thick paraffin sections were stained with haematoxylin and eosin and investigated by light microscope.

Statistical analysis

All values were expressed as mean ± S.E. Statistical analysis of data was performed using one-way ANOVA followed by least significant difference (LSD).

RESULTS

NMR spectroscopy

The NMR spectra (Tables 1 and 2) are recorded in methanol deutreated (CD_3OD) on a Bruker DRX-400 spectrometer (Bruker Biospin GmbH, Rheinstetten, Germany) operating at 400.13 MHz for ^{1}H and at 100.61 MHz for ^{13}C.

Body weight

Diabetic control rats lost a significant amount of weight over the 3 week duration of the study, whereas control rats gained weight (Table 3).

The percent of changes relative to zero time for diabetic control (weekly for three weeks) were -24, -29 and -24%, while they were 24, 45 and 50% for normal control rats. The body weight of diabetic rats treated with hibiscus extract did not change significantly as compared to values of zero time or values of glibenclamide group. On the other hand, the body weight of diabetic control rats decreased significantly as compared to diabetic rats treated with reference drug.

Blood glucose level

The treatment of diabetic rats with hibiscus extract led to a significant decrease in plasma glucose level after one, two and three weeks post-treatment as compared to zero time values (Table 4). No significant change in glucose level was observed in STZ+ hibiscus group as compared to the reference drug group.

Hepatic oxidative stress parameters

There was a significant increase ($p<0.01$) in hepatic MDA or GSH levels of diabetic control rats as compared to normal control rats or reference drug group (Table 5). The values of MDA and GSH of diabetic rats treated with hibiscus extract were significantly less than those of diabetic control ones and they did not change significantly as compared to the reference drug. The concentration of SOD for the diabetic control rats was significantly less than those of normal control rats. SOD level of diabetic rats given hibiscus extract was significantly higher than those of the diabetic control rats. The treatment of normal rats with hibiscus led to a significant increase in hepatic GSH or CAT levels. However, the diabetic rats given glibenclamide exhibited a significant increase in hepatic GSH, SOD and catalase

Table 3. Body weight (g) of diabetic rats treated with *H. rosa-sinensis* leaves extract.

Treatment	Time in week			
	0	1	2	3
Normal control	144.5±1.52	178.83±9.80$^{@}$	210.00±8.56$^{@}$	216.66±8.03$^{@}$
Diabetic control	140.2±2.30	106.67±6.67$^{@g}$	100.00±8.94$^{@g}$	106.67±6.67$^{@g}$
Diabetic rats+HRS (400 mg/kg)	156.7±2.14	138.33±13.76	135.00±12.04	151.67±11.67
HRS (400 mg/kg)	129±1.73	153.33±8.43	140.00±5.16	143.33±8.03
Diabetic rats+Glib. (0.3 mg/kg)	175±6.71	152.50±5.74	161.67±4.77	186.66±12.82

Each value is the mean±SE, n=6. The values of body weight of all groups before streptozotocin treatment are considered zero time.Values marked with @, differ significantly from zero value; $P^{@}<0.01$. Values marked with letter g, differ significantly from glibenclamide group; $P^{g}<0.01$ Statistical analysis of data was performed using ANOVA followed by least significant difference (LSD). Glib: Glibenclamide

Table 4. Blood glucose level (mg/dl) of diabetic rats treated with *H. rosa-sinensis* leaves extract.

Treatment	Time in week			
	0	1	2	3
Normal control	84.47±3.16	95.97±3.39	91.9±5.47	93.27±4.76
Diabetic control	389.83±18.72	389.4±27.80^{g}	354.25±33.40^{g}	292.3±24.37^{g}
Diabetic rats + hibiscus (400 mg/kg)	326.67±25.76	205.73±24.06^{*A}	210.05±20.22 ^{*A}	154.11±17.91 ^{*A}
Hibiscus (400 mg/kg)	91.5±4.56	95.5±5.69	85.06±5.30	61.94±5.98
Diabetic rats+Glib. (0.3 mg/kg)	291.8±19.80*^{A}	201.92±25.92 ^{*A}	173.72±9.78 ^{*A}	131.67±13.52 ^{*A}

Each value is the mean±SE, n=6; Values marked with asterisks differ significantly from control value; P*<0.01; Values marked with letter A, differ significantly from diabetic control group; $P^{A}<0.01$; Values marked with letter g, differ significantly from glibenclamide group; $P^{g}<0.01$; Statistical analysis of data was performed using Anova followed by least significant difference (LSD). Glib: Glibenclamide

Table 5. Hepatic oxidative stress parameters of diabetic rats treated with *H. rosa-sinensis* leaves extract.

Parameter / Treatment	MDA (nmol/mg)	GSH (mg/g tissue)	SOD (U/g tissue)	Catalase (U/g tissue)
Normal control	4.98 ± 0.62	5.12 ± 0.53	223.67 ± 13.38	0.53 ± 0.03
Diabetic control	10.05 ± 0.39^{*g}	17.47 ±2.53*^{g}	177.9 ± 10.78*^{g}	0.56 ± 0.02
Diabetic rats+HRS (400 mg/kg)	7.48 ± 0.45*^{A}	8.18 ± 0.85*^{A}	246.00 ± 8.63^{Ag}	0.63 ± 0.02*
HRS (400 mg/kg)	4.77 ± 0.87	9.81 ± 1.59*	211.97 ± 11.82	0.60 ± 0.02*
Diabetic rats+Glib. (0.3 mg/kg)	7.57 ± 0.19*^{A}	11.08 ± 1.51*^{A}	313.20 ± 7.66^{*A}	0.66 ± 0.02*^{A}

Each value is the mean±SE, n=6; Values marked with asterisks differ significantly from the control value; P*<0.01. Values marked with letter A, differ significantly from diabetic control group; $P^{A}<0.01$. Values marked with letter g, differ significantly from glibenclamide group; $P^{g}<0.01$. Statistical analysis of data was performed using ANOVA followed by least significant difference (LSD). Glib: Glibenclamide

as compared to the normal control.

Hepatic and renal biochemical parameters

The concentrations of ALT, AST, uric acid and creatinine of diabetic rats administered hibiscus extract exhibited a significant decrease as compared to the diabetic control, while they did not change significantly as compared to the reference drug (Table 6). The treatment of diabetic rats with hibiscus extract preserved the protein level in normal range. The cholesterol concentration showed a significant increase in diabetic control rats and it did not change significantly in diabetic rats administered hibiscus

Table 6. Effect of *H. rosa-sinensis* leaves extract on liver and kidney function parameters.

Parameter / Treatment	ALT (U/l)	AST (U/l)	Uric acid (mg/dl)	Creatinine (mg/dl)	Total protein (g/dl)	Cholesterol (mg/dl)
Normal control	24.83±1.96	38.33±4.07	3.68±0.26	1±0.17	7.27±0.24	88.28±2.20
Diabetic control	60.33±1.05*	67.33±1.65*	6.47±0.65*	2.29±0.17*	4.5±0.31*	110.67±7.18*
Diabetic rats+HRS (400 mg/kg)	47.00±4.63^{*A}	55.67±6.59	5.22±0.48^{*Ag}	1.11±0.33^{A}	7.65±0.29^{Ag}	99.67±11.07
HRS (400 mg/kg)	47.17±4.28^{*A}	57.62±3.86*	4.57±0.17*	0.55±0.14*	10.03±0.24*	92.43±3.96
Diabetic rats+Glib. (0.3 mg/kg)	32.5±4.02^{*A}	40±1.29^{A}	3.85±0.16^{A}	1.00±0.06^{A}	6.17±0.35^{*A}	93.17±4.85

Each value is the mean±SE, n=6. Values marked with asterisks, differ significantly from control value; $P^{*}<0.01$. Values marked with A, differ significantly from STZ group; $P^{A}<0.01$. Values marked with g differ significantly from glibenclamide group ,$P^{g}<0.01$. Statistical analysis of data was performed using ANOVA followed by least significant difference (LSD).

extract as compared to the normal control.

Histological study

Liver sections of the rats treated with streptozotocin only (Figure 1B and C) demonstrated signs of degeneration in most of the hepatocyte in the form of karyolysis and karyorrhexis. Foci of necrosis, an area of hemorrhage in blood sinusoid, dilated congested portal vein and thickening in its wall, cellular infiltration around it, dilated and congested blood sinusoid were seen. Most hepatocyte of liver tissue from rats treated with STZ along with hibiscus extract (Figure 1 D) appeared normal but some signs of degeneration appeared in the form of karyolysis, pyknosis dilated and congested blood sinusoid. Concerning rats treated with streptozotocin in combination with glibenclamide showing improvement in pathological changes in the form of normal hepatocyte, but dilated, congested portal vein and thickening in its wall were seen (Figure 1E). The liver tissue of a rat subjected to hibiscus extract only showed normal histological structure (Figure 1F).

Kidney sections of rats treated with STZ only (Figure 2B) manifested degeneration in most of glomeruli with wide urinary space. On the other hand, decreased pathological changes were recorded in diabetic rats treated with hibiscus extract (Figure 2C). Kidney of diabetic rats treated with glibenclamide had some pathological alterations such as the necrosis in tubular epithelial cells and vacuolar degeneration (Figure 2D).

DISCUSSION

Medicinal plants have always been an important source for the treatment of many diseases including diabetes owing to their minimal adverse effects. The present experiment indicated the decreased blood glucose level significantly in diabetic rats treated with hibiscus leaves extract compared with diabetic control rats. No significant difference was observed between diabetic rats treated with hibiscus extract and diabetic rats treated with glibenclamide, a reference drug. The hypoglycemic effect of hibiscus leaves extract is likely due to the enhancement of insulin secretion and the increase of the β-cell number of pancreas islets (Moqbel et al., 2011). The study of Garg et al. (2012) indicated the presence of high content of phenolics, flavonoids and tannins in aqueous and methanolic extracts of *H. rosa-sinensis* leaves. Jadhav and Puchchakayala (2012) revealed that the blood glucose lowering activity of flavonoid compounds may be by stimulating β-cells to release more insulin or by enhancement peripheral glucose utilization through the skeletal muscle.

Since phenolics and flavonoids are responsible for the antioxidant activity, their presence in the leaf extract of hibiscus indicates good antioxidant activity (Garg et al., 2012). The present results indicated that hibiscus leaves extract reduced oxidative stress in diabetic rats as manifested by decreased hepatic malondialdehyde and enhancement of SOD, an antioxidant enzyme. Antioxidant property of hibiscus leaves extract has been reported (Moqbel and Naik, 2011; Saravanan et al., 2011). This potent antioxidation is thought to form the basis of many of the other healing activities of hibiscus leaves extract including its hepatoprotective activities. This investigation showed increased hepatic glutathione level of diabetic control rats significantly, suggesting a compensatory defense mechanism.

The extract of hibiscus leaves significantly inhibited the increase in the activities of AST and ALT in diabetic rats and it reduced the pathology of the liver. The results of this study showed a significant increase in lipid peroxidation in the liver of STZ diabetic rats. The ability of lipid peroxidation to generate free radicals was confirmed (Szkudelski, 2001) which may lead to the pathological changes observed in liver and kidney of the present work. The activities of AST and ALT are cytosolic marker enzymes, indicating hepatocellular necrosis as they are released into the blood after cell membrane damage. The current study revealed that diabetic control group has significantly higher level of serum AST and ALT as compared to that of the normal control group. Liver

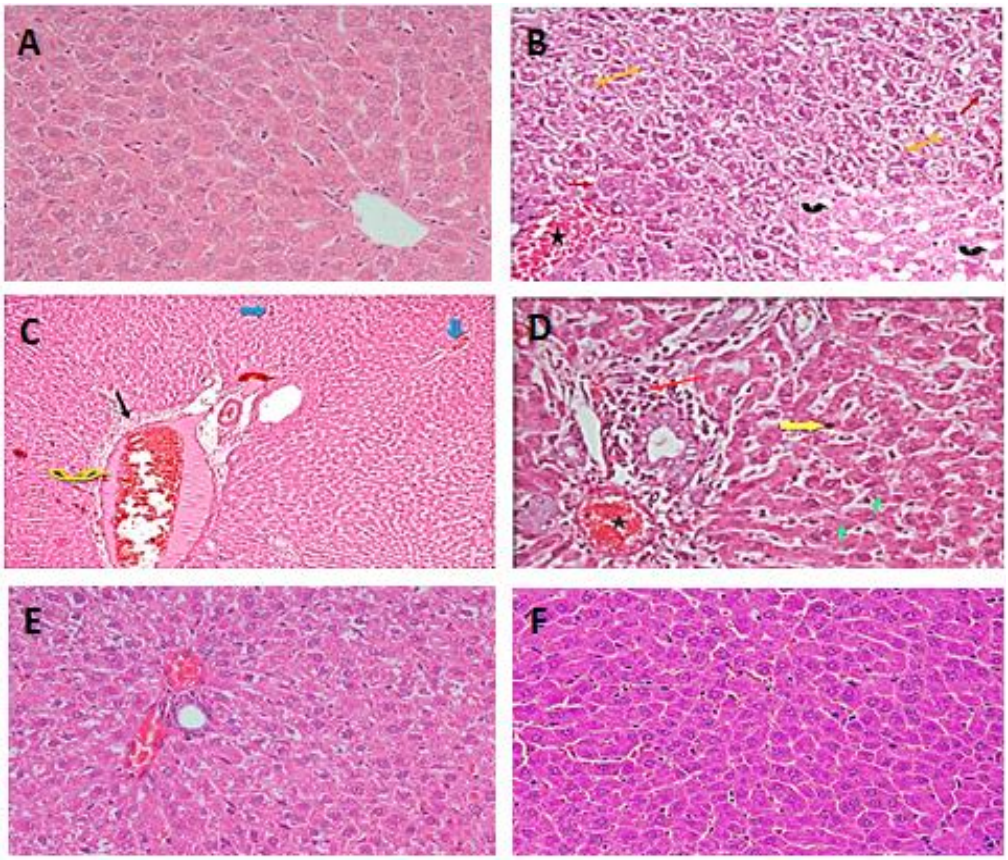


Figure 1. Liver sections of diabetic or normal rats treated with hibiscus leaves extract. A: Normal control. B: Liver of a rat treated with STZ only showing signs of degeneration in most of hepatocyte in the form of karyolysis (yellow arrow), karyorhexis (black arrow), foci of necrosis and area of hemorrhage in blood sinusoid were seen (star), vacuolar degeneration could be observed at right of figure. C: Another field of previous group (STZ only)showing dilated, congested, edematous, vacuolated portal vein, thickening in the wall (yellow curved arrow). Dilated and congested blood sinusoid (light blue arrow) and ceullar infiltration around (black arrow). D: Liver of diabetic rat treated with hibiscus extract. Most of hepatocyte appeared normal but some exhibited signs of degeneration in the form of karyolysis (green arrow), pyknosis (yellow arrow). Dilated congested portal vein (star) and cellular infiltration (red arrow) were observed. E: Section in the liver of diabetic rat treated with glibenclamide showing minimal pathological changes. F: Liver of normal rat given hibiscus extract showing normal structure. All the sections are Hx&E x400 except C is Hx&E x200.

sections of diabetic control rats showed congestion of hepatic sinusoid, vacuolization of hepatocytes and necrosis of hepatocytes. These results agreed with Hamadi et al. (2012) who reported that elevated activities of serum AST and ALT is a common sign of liver diseases among diabetic rats. Hyperglycemia increases the generation of free radicals by glucose auto-oxidation and the increment of free radicals may lead to cell damage. The present study indicated that diabetic control rats had significant increase in plasma levels of uric acid and creatinine and had significant decrease in concentration of protein as compared to that of the normal control rats.

These results assured, by Parvizi et al., (2014) who revealed that hyperglycemia is associated with kidney dysfunctions in the diabetic rats which may be related to the generation of reactive oxygen species and lipid peroxidation that led to tissue injury. In addition, Shah et al. (2007) reported that increased oxidative stress and reduced antioxidative ability in diabetes results in renal tubular injury, proteinuria and leads to gradual loss of renal function.

Hypercholesterolemia has been reported to occur in diabetes (Samarghandian et al., 2014). In this study, TC level increased significantly in diabetic control rats when compared with normal rats. Treatment of diabetic rats with hibiscus extract significantly reduced TC level. The hypolipidemic effect of hibiscus was due to the action of its different constituents, including sitosterol-β- D-galactoside (Mironova and Kalashnikova, 1982) and flavonoids (Liu et al., 2010). Moreover, Yang et al. (2010) reported that polyphenols of hibiscus exhibited more

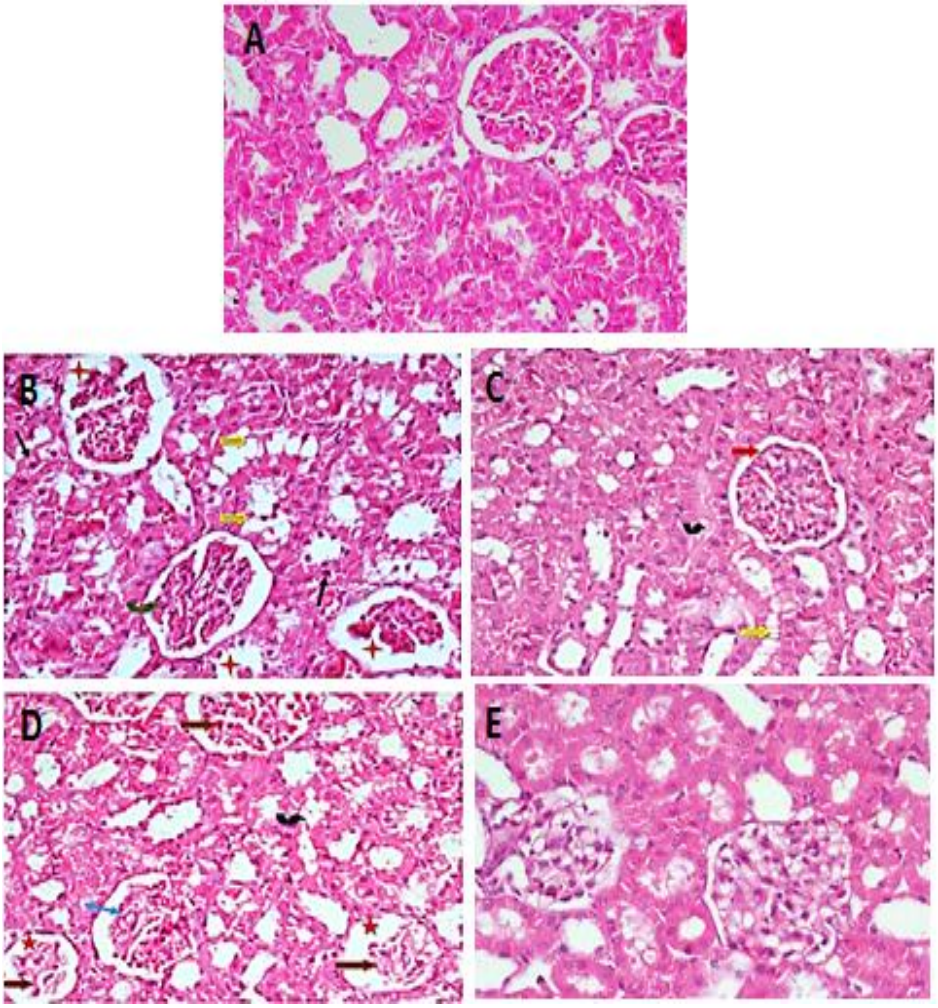


Figure 2. Kidney sections of diabetic or normal rats treated with hibiscus leaves extract. A: Normal control. B: Kidney of a rat treated with STZ only showing degeneration in most of glomeruli with wide urinary space (red star), while others showed lobulation (olive green). Vacuolar degeneration in some tubular epithelial cells (yellow arrow) was observed. C: Diabetic rat treated with hibiscus extract, most tubule appeared normal. D: Diabetic rat treated with glibenclamide showing wide urinary space (star), necrosis in tubular epithelial cells and vacuolar degeneration (black curved arrow). E: Normal rats treated with hibiscus extract, most renal tubules and glomerulus appeared normal (Hx&E x400).

potency to decrease plasma cholesterol and LDL cholesterol than the crude extract, and increased HDL cholesterol.

In the LD_{50} study, no mortality was recorded in rats except 3000 mg/kg of the methanolic extract of *H. rosa-sinensis*. It has been established that any substance with LD_{50} estimate greater than 2000 mg/kg body weight by oral route may be considered to have low toxicity and safe (Bruce, 1987).

Conclusion

H. rosa-sinensis could have great importance as a safe therapeutic agent in diabetes mellitus. *Hibiscus* leaves extract has a significant hypoglycemic and hypocholestrolemic effects in diabetic rats which may led to a decrease in oxidative stress and improvement of liver and kidney functions. *H. rosa-sinensis* could have great importance as safe therapeutic agent.

CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

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