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Co-therapeutic effects of metformin and Sidr fruits and leaves in experimental diabetic rats

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Abstract

Many diabetic patients prefer to use herbal medicine and treated with conventional drugs, concurrently. The main objective of this study was to evaluate the co-therapeutic effects of Sidr(*Zizyphusspina-christi* L.) fruits or leaves and metformin in diabetic rats. Forty two adult male albino rats were used and divided into seven groups (G1-G7). G1 was kept on basal diet (negative control), while other groups were subcutaneously injected with alloxan (120 mg/kg body weight).G2 was kept untreated as a positive control, while the other alloxan -injected groups were treated with metformin (100 mg/kg body weight) as a reference drug (G3), or fed on basal diet supplemented with 5% of either Sidr fruits (SFP) or leaves powder (SLP) (G4 and G5, respectively). G6 and G7 received combined treatments (metformin + SFP and metformin + SLP, respectively). The curative period lasted for 4 weeks. By its end, body weight gain (BWG) and relative weights of liver, kidneys and pancreas were calculated. Fasting glucose, insulin, amylase activity, liver glycogen, lipid profile as well as liver and kidney functions were determined. The typical manifestations of diabetes were noticed in untreated diabetic group including hyperglycemia and hypoinsulinemia.Moreover, a significant increase in serum amylase activity and relative liver and kidney weights was noticed associated with dyslipidemia, liver and kidney dysfunction and a significant decrease in BWG and absolute pancreas weight along with marked histopathological abnormalities in pancreas tissue.SFP was more effective than SLP in normalizing body weight, pancreas weight and liver antioxidant status, while the capability of SLP was higher in correcting the level of serum low density lipoprotein cholesterol (bad cholesterol). In general, receiving combined treatments was more efficient than receiving metformin alone.In conclusion, Sidr fruits and leaves can help metformin in the treatment of diabetes mellitus**.**

Key words: Diabetes mellitus, alloxan, *Zizyphusspina-christi* L.,metformin, experimental rats.

Introduction

All over the world, there is a profuse increase in diabetics and pre-diabetics due to several risk factors including aging, unhealthy diet, hyperlipidemia, sedentary life style, and smoking***(Wild et al., 2004)***. Based on the report cited by International Diabetes Federation, diabetes mellitus is the mammoth cause of mortality and morbidity, with a projected range of 382 million adults being affected and 5.1 million people killed in 2013. In 2035, the incidence of the disease is ordained to be about 592 million, and the prevalence will be higher (at least 80%) in developing countries with low and middle-incomes ***(International Diabetes Federation, 2013).***

Diabetes is portrayed by minified glucose homeostasis which may prelude to an elevated blood glucose level with an alteration in lipid parameters ***(International Diabetes Federation, 2013).***

Meanwhile, extended accumulation of elevated blood glucose may lead to the overproduction of reactive oxygen species accompanied with oxidative stress. This noxious state may be involved in the progression of fatal pathological event like atherosclerosis, cardiovascular disease and other diabetic complications. Further, uncontrolled elevated glucose level may impose a greater burden in the form of restricted daily activity and work which result in high economic costs ***(Juárez-Reyes* et al.*, 2015).***
A large number of antidiabetic drugs are available. Of which, metformin is the mostly prescribed ***(Rojasand Gomes, 2013).***

In general, allopathic medicines, currently used in the management of diabetes mellitus, exert serious adverse effects and affect the quality of life ***(Aicheret al., 2010).*** Hence, herbal medicines encompassing anti hyperglycemic potential may serve as a suitable and safer alternative or as an adjunct candidate in the management of hyperglycemia.

Zizyphusspina-christi L. (Christ's thorn or Jerusalem thorn in English and Sidr or Napk in Arabic) is a plant belonging to the Rhamnaceae family and is indigenous to warm and subtropical areas including North Africa, the South and Middle East, East of Asia, Mediterranean region, South Europe, Australia, and tropical America ***(Yossefet al., 2011).*** In folk medicine, Sidr is used as an antimicrobial, a demulcent, an astringent for toothaches and a mouthwash. It is also used to heal several ailments such as liver complaints, urinary issues, digestive syndromes, weakness, obesity, diabetes, skin infection, appetite loss, fever, bronchitis, pharyngitis, anemia, insomnia and diarrhea ***(Abalakaet al., 2010; Ghafooret al., 2012)***. Although all parts of Sidr plant are of medicinal value, fruits and leaves were the most important traditionally. A lot of phenolic compounds that exert antioxidant properties are present in Sidr fruits and leaves including ferulic acid, rutin, p-hydroxybenzoic acid and chlorogenic acid in fruits***(Yossefet al., 2011; Ghafooret al., 2012),*** while leaves are rich in ceanothic, betulinic acids, saponins, various flavonoids, triterpenes, tannins and flavonoids ***(Asgarpanah and Haghighat, 2012).***

The present study was carried out to: (1) compare the anti- diabetic effects of Sidr fruits and leaves with each other and with metformin, as a reference drug used widely, (2) evaluate the therapeutic effects resulted from the interaction between Sidr fruits or leaves and metformin, and (3) investigate the mechanisms behind all these effects.

Materials and Methods

Materials:
FreshSidr (*Zizyphusspina-christi* L.) fruits and leaves were purchased from the local market, Tanta City, Al-Gharbia Governorate, Egypt. The herb was identified by the Department of Flora, Agricultural Museum, Ministry of Agriculture and the Herbarium of the Department of Botany, Faculty of Science, Cairo University.

A total of 42 normal male albino rats (Sprague_ Dawley strain) weighing 160 ± 5g were obtained from the animal colony, Helwan Farm, Vaccine and Immunity Organization, Cairo, Egypt.
Casein, vitamins, minerals, cellulose, choline chloride, DL-methionine and other required chemicals were obtained from El-Gomhoreya Company for trading drugs, chemicals and medical appliances, Cairo, Egypt.Alloxan(Sigma-Aldrich Chemical Co.)as well as all kits used for biochemical determinations were obtained from Gama Trade Company for chemicals, Cairo, Egypt. Metformin tablets were purchased from a local pharmacy in Tanta City, Al-Gharbia Governorate, Egypt. Sucrose,

soybean oil and corn starch were obtained from the local market, Tanta City, Al-Gharbia Governorate, Egypt.

Methods:

Drying of plant samples

Fresh Sidr fruits and leaves were washed thoroughly and allowed to drain.After that,fruits were cut and seeds were removed. Then, fruits and leaves were spread thinly on clean aluminum trays in a well ventilated room at 25°C away from sunlight for seven days. Natural current of air was used for shadow drying and the leaves were constantly turned to avert fungal growth according to ***Vanderhulstetal., (1990)*** with some modification.

Milling and storage of driedplant samples

After drying, Sidr fruits and leaves were milled separately to a fine powder using a hammer mill (Thomas Willey mills, model Ed-5, Germany). After that, they were sieved with a screen of 2 mm pore size and stored at room temperature in airtight glass containers in the dark until used.

Preparation of experimental diet

The basal diet was formulated according to ***Reeves et al., (1993)*** with some modification.Each 100 g of the formulated basal diet consisted of 14, 4, 5, 3.5, 1, 0.25, 0.3 and 10 g of casein, soybean oil, cellulose, mineral mixture, vitamin mixture, choline chloride, DL-methionine and sucrose, respectively, while corn starch was added up to 100 g.

Preparation of the reference drug

Metformin tablets were freshly dissolved in saline (0.9%)at a concentration of 10 mg/ml for oral administration

Animals & study design

Male albino rats (n = 42) of Sprague Dawley strain weighing (160± 5g) were housed in well-aerated cages under hygienic conditions "22-25°C and a 12 h light-dark cycle" and fed on basal diet for one week for adaptation. After that, rats were weighed and divided into two main groups. The first group (G1, n = 6) was fed on basal diet as a negative control group for four weeks, while the second group (diabetic group, n = 36) were injected subcutaneously with alloxan (120mg/kg body weight) according to the method described by ***Mostafavinia et al., (2016).***Six diabetic rats died 3 days after injection.Diabetes was identified by measuring fasting serum glucose levels five days after injection. The mean value of serum glucose in diabetic group was 300.33mg/dl, while it was 100.40 mg/dl in the negative control group. After diagnosis, the diabetic group (n= 30) was divided into six equal groups, one of them was kept untreated and fed on basal diet only as a positive control group (G2), while the other diabetic groups were treated daily by metformin (100 mg/kg body weight) as a reference drug (G3) or fed on basal diet supplemented with 5% of either Sidr fruits (SFP) or leaves powder (SLP) (G4 and G5, respectively). G6 and G7 were fed on basal diet supplemented with 5% of either SFP or SLP, respectively, and treated with 100 mg metformin/kg body weight.The curative period lasted for four weeks. Meanwhile, diet and water were provided *ad-libitum* and body weight was recorded once a week.

Blood sampling

At the end of the curative period, animals were weighed and fasted overnight before sacrificing under very light ether anesthesia. Blood samples were collected from hepatic portal vein of each rat into dry clean centrifuge tubes. Sera were carefully separated by centrifugation of blood

samples at 3000 rpm (round per minute) for 10 minutes at room temperature, then transferred into dry clean Eppendorf tubesand kept frozen at - 20˚C till analyzed. Adequate quantities of serum were used for glucose determination in 2 hours after separation.

Tissue sampling and histopathological examination

After sacrificing, liver, kidneys and pancreas were removed from each rat by careful dissection, washed in ice-cold NaCl (0.9%), dried using filter paper and independently weighed.A specimen from each liver was stored at -80ºC until homogenate preparation.On the other hand, a specimen from each pancreas was immersed in 10% buffered neutral formalin solution, then the fixed specimens were trimmed, washed and dehydrated in ascending grades of alcohol. They were then cleared in xylol, embedded in paraffin, cut in sections of 4-6 microns thickness and stained with haematoxylin and eosin according to ***Drury and Wallington, (1980).***

Preparation of liver homogenate

In order to prepare liver homogenate, one gram of liver tissue was homogenized in ice-cold 1.15% solution of potassium chloride in 50 mmol L-1 potassium phosphate buffer solution (pH 7.4) to yield a liver homogenate10% (W/V). Homogenization was performed using Sonicator, 4710 Ultrasonics Homogenizer (Cole- Parmer Instrument Co., USA).The homogenate was centrifuged at 4000 rpm for 5 minutes at 4°C. The supernatant was collected and stored at -80ºC until used.

Body weight gain and relative organ weight calculation

Body weight gain (BWG) was calculated by subtracting the initial weight of each rat from its final weight. As for relative weights of liver, kidney and pancreas (RLW, RKW and RPW), they were calculated according to ***Angervall and Carlström, (1963).***

Determination of glycemia–related markers

In serum, glucose was determined according to the method described by ***Trinder, (1969),*** while the concentration of insulin was estimated using Enzyme-Linked Immunoabsorbent Assay (ELISA) method using insulin kit from Syntron Bioresearch (USA). Moreover, Serum alpha-amylase activity was determined following the standard biochemical protocol of ***Hansawasdiet al., (2000)*** with slight modifications by ***Dineshkumaret al., (2010).*** On the other hand, glycogen content in liver tissue homogenates was measured calorimetrically using anthrone reagent according to the method of ***Chun and Yin, (1998).***

Determination of lipid profile in serum

Triglycerides(TG) and total cholesterol (TC) were determined in serum according to the methods described by ***Jacobs and VanDenmark, (1960) andRichmond, (1973),*** respectively. In addition, high density lipoprotein cholesterol (HDL-c) was determined according to the method proposed by ***Friedwaldet al., (1972),*** while low and very low-density lipoprotein-cholesterols, (LDL-c and VLDL-c) were calculated according to the equations of ***Friedwald et al., (1972*** .

Determination of serum transaminases and oxidant/antioxidant biomarkers in liver tissue homogenate

In serum, aminotransferases (AST and ALT) were determined according to the method described by ***Reitman and Frankel, (1957).***Lipid peroxidation, expressed as malondialdehyde (MDA), and total antioxidant capacity (TAC) were determined in liver tissue homogenate following the methods of ***Ohkawaet al., (1979)*** and ***Koracevic et al., (2001),***respectively.

Determination of kidney function markers

The concentrations of urea and creatinine in serum were determined according to ***Patton and Crouch, (1977) and Houot, (1985),*** respectively.

Statistical analysis

Statistical analysis was carried out using the programme of statistical package for the social sciences (SPSS), PC statistical software (Version 20; Untitled – SPSS Data Editor). The results were expressed as mean ± standard deviation (mean ± SD). Data were analyzed using one-way classification, analysis of variance (ANOVA) test. The differences between means were tested for significance using Duncan test at $p<0.05$ ***(Sendcor and Cochran, 1979).***

Results

Body weight gain & relative weight of internal organs

Table (1) showed no significant differences in body weight were found among all groups at the beginning of the experiment. By the end of the experiment,body weight gain (BWG) of untreated diabetic group was significantly ($p<0.05$) less than that of negative control group. All treated diabetic groups recorded a significant elevation in their final weight,and hence BWG, compared with untreated diabetic group. Among the three single treatments,Sidr fruits powder (SFP)was the best, followed by Sidr leaves powder (SLP), while metformin alone resulted in the lowest final weight and BWG values.

On the other hand, the relative weights of liver and kidneys (RLW and RKW, respectively) were significantly increased in untreated diabetic group compared to the negative control one. The best results of RLW were noticed in the groups fed on SFP singly or in combination with metformin, as other treatments led to no significant decrease compared with untreated diabetic group. All treated diabetic groups recorded a significant decrease in RKW compared with untreated diabetic group. Like RLW, the best results were noticed in the groups fed on SFP singly or in combination with metformin. In contrast, the mean values of relative pancreas weight (RPW) of all experimental groups did not differ significantly.

Table (1)

Effects of Sidr fruits and leaves versus metformin on body weight gain and the relative weights of liver, kidney and pancreas in alloxan -induced diabetic rats

Droups / Parameters	Normal control	Untreated diabetic	Metformin -treated	SFP -fed	SLP -fed	Metformin + SFP	Metformin + SLP
Initial weight (g)	170.00±22.80	172.80±23.44	176.80±23.76	175.20±25.56	175.60±19.62	175.60±26.77	175.60±19.57
Final weight (g)	$262.00±18.06^{d}$	$202.00±12.57^{a}$	$226.60±16.52^{b}$	$256.40±18.47^{cd}$	$234.40±12.84^{bc}$	$256.40±19.23^{cd}$	$245.60±18.19^{bcd}$
BWG (g)	$92.00 ±12.88^{e}$	$29.20±4.09^{a}$	$49.80±7.26^{b}$	$81.20±8.50^{d}$	$58.80±7.29^{b}$	$80.80±8.32^{d}$	$70.00±5.83^{c}$
RLW (%)	$1.94±0.09^{a}$	$2.30±0.27^{b}$	$2.20±0.31^{ab}$	$1.90±0.04^{a}$	$2.11±0.27^{ab}$	$1.90±0.04^{a}$	$2.13±0.30^{ab}$
RKW (%)	$0.71±0.04^{a}$	$0.95±0.11^{c}$	$0.85±0.08^{b}$	$0.73±0.06^{a}$	$0.82±0.08^{ab}$	$0.73±0.05^{a}$	$0.80±0.09^{ab}$
RPW (%)	0.11±0.01	0.10±0.01	0.10±0.01	0.11±0.01	0.10±0.01	0.10±0.01	0.10±0.02

-Values that have different letters in each row differ significantly, while the difference among those with similar letters is not significant ($p<0.05$).

Glycemia –related markers

In table (2), it could be noticed that untreated diabetic group recorded a significant increase ($p<0.05$) in serum glucose level and amylase activity compared with negative control group. In contrast, serum insulin level as well as glycogen content in liver tissue homogenate were significantly decreased. Compared to the untreated diabetic group, all treated groups showed a significant decrease in serum glucose and amylase activity as well as a significant increase in both serum insulin and liver glycogen. While metformin was more effective compared with SFP and SLP as single treatments regarding glucose, insulin and glycogen results, they were more efficient in decreasing amylase activity. In general, receiving combined treatments (metformin+SFP or metformin+SLP) was more efficient than receiving metformin alone, i.e. herbal treatments enhanced the hypoglycemic effects of metformin.

Table (2)

Effects of Sidr fruits and leaves versus metformin on serum levels of glucose, insulin, amylase activity and glycogen content in liver tissue homogenate in alloxan -induced diabetic rats

Groups / Parameters	Normal control	Untreated diabetic	Metformin -treated	5 % SFP -fed	5 % SLP -fed	Metformin + SFP	Metformin + SLP
Glucose (mg/dL)	95.75±15.48^{a}	283.00±27.69^{f}	170.40±17.62^{cd}	200.40±18.37^{e}	186.67±17.80^{de}	157.00±17.25^{bc}	140.67±14.65^{b}
Insulin (mIU/mL)	43.20±3.27^{f}	8.60±1.14^{a}	23.00±2.92^{c}	18.20±2.49^{b}	19.20±2.59^{b}	29.00±3.24^{d}	33.00±3.32^{e}
Amylase (ng/mL)	3.34±0.33^{ab}	4.96±0.45^{d}	4.10±0.54^{c}	3.06±0.41^{a}	2.98±0.42^{a}	4.00±0.45^{c}	3.88±0.35^{bc}
Glycogen (mg/g tissue)	38.00±3.37^{e}	13.00±1.58^{a}	24.00±2.55^{c}	20.00±2.26^{b}	20.20±2.28 ^{b}	29.40±2.77^{d}	32.00±2.85^{d}

- Values that have different letters in each row differ significantly, while the difference among those with similar letters is not significant ($p<0.05$).

Lipid profiles

Table (3) showed that untreated diabetic group recorded a significant increase ($p<0.05$) in serum levels of triglycerides (TG), total cholesterol (TC) as well as low density and very low density lipoprotein cholesterol (LDL-c and VLDL-c, respectively) compared with negative control group. In contrast, serum high density lipoprotein cholesterol (HDL-c) was significantly decreased. Compared to the untreated diabetic group, all treated groups showed a significant decrease in serum TG, TC, LDL-c and VLDL-cas well as a significant rise in serum HDL-c. While metformin was more effective compared with SFP and SLP as single treatments regarding TG and HDL-c results, they were more efficient in decreasing TC and LDL-c levels, especially SLP. In general, receiving combined treatments was more efficient than receiving metformin alone, i.e. herbal treatments improved the hypolipidemic effects of metformin.

Table (3)

Effects of Sidr fruits and leaves versus metformin on serum lipid profiles in alloxan -induced diabetic rats

Groups / Parameters	Normal control	Untreated diabetic	Metformin -treated	5 % SFP -fed	5 % SLP -fed	Metformin + SFP	Metformin + SLP
TG (mg/dL)	49.60±6.50^{a}	112.60±14.54 ^{e}	79.33±9.60 ^{cd}	90.75±9.01 ^{d}	85.75±9.01 ^{d}	69.00±9.62^{bc}	58.25±7.50 ^{ab}
TC (mg/dL)	121.00±15.56^{a}	187.80±26.86c	161.60±20.82b	156.60±19.09b	141.60±18.66ab	136.60±19.36ab	124.40±15.63a
HDL-c (mg/dL)	56.60±7.80 ^{f}	24.80±3.03^{a}	43.00±5.24 ^{cd}	34.00±3.32^{b}	38.00±3.67 ^{bc}	49.00±5.76^{de}	53.00±6.52 ^{ef}
LDL-c (mg/dL)	54.48±6.86^{a}	140.48±18.86 f	102.73±15.04de	104.45±15.28e	86.45±11.60cd	73.80±10.34 bc	59.57±8.31^{ab}
VLDL-c (mg/dL)	9.92±1.30^{a}	22.52±2.91^{e}	15.87±1.92^{cd}	18.15±1.80d	17.15±1.80^{d}	13.80±1.92^{bc}	11.65±1.50^{ab}

- Values that have different letters in each row differ significantly, while the difference among those with similar letters is not significant (p<0.05).

Liver functions and liver antioxidant status

In table (4),it could be noticed that untreated diabetic group recorded a significant increase (p<0.05) in the activities of serum transaminases (AST and ALT) compared with negative control group. On the other hand, while total antioxidant capacity (TAC) was significantly decreased, malondialdehyde (MDA) concentration was significantly increased in liver tissue homogenate of untreated diabetic group compared to negative control group. Compared to the untreated diabetic group, all treated groups showed a significant decrease in the activities of serum transaminases and liver MDAas well as a significant rise in liverTAC level, except for the group treated with metformin alone as it recorded no significant decrease in serum ALT activity. Herbal treatments, especially SFP,were more efficient than metformin in decreasing serum ALT activity and improving the antioxidant status in liver tissue homogenate. In general, receiving combined treatments was more efficient than receiving metformin alone, i.e. herbal treatments improved the hepato –curative effects of metformin.

Table (4)

Effects of Sidr fruits and leaves versus metformin on liver functions and liver antioxidant status in alloxan -induced diabetic rats

Groups / Parameters	Normal control	Untreated diabetic	Metformin -treated	5 % SFP -fed	5 % SLP -fed	Metformin + SFP	Metformin + SLP
AST (U/L)	131.00±16.94^{a}	197.79±27.89^{c}	154.20±17.14^{ab}	164.40±21.47^{b}	169.40±21.47^{b}	131.33±15.12^{a}	149.20±17.14^{ab}
ALT (U/L)	56.00±7.38^{a}	116.00±15.87 ^{c}	105.00±9.92^{c}	59.50±7.76^{a}	65.00±9.92a	84.25±9.23 ^{b}	89.45±8.90^{b}
MDA (nmol/g)	2.00±0.25^{a}	15.00±1.58^{g}	11.00±1.27^{f}	7.00±0.79^{d}	9.00±0.79e	4.00±0.35^{b}	5.46±0.73^{c}
TAC (ng/mL)	0.97±0.14^{e}	0.15±0.02^{a}	0.34±0.04^{b}	0.57±0.08 ^{c}	0.48±0.04c	0.82±0.10^{d}	0.76±0.08^{d}

- Values that have different letters in each row differ significantly, while the difference among those with similar letters is not significant (p<0.05).

Kidney functions

Table (5) showed that untreated diabetic group recorded a significant increase (p<0.05) in serum levels of urea and creatinine compared to negative control group. Compared to the untreated diabetic group, all treated groups showed a significant decrease in both markers. Receiving combined

treatments were more efficient than receiving metformin alone, i.e. herbal treatments improved the renal curative effects of metformin.

Table (5)

Effects of Sidr fruits and leaves versus metformin on kidney functions in alloxan -induced diabetic rats

Groups / Parameters	Normal control	Untreated diabetic	Metformin -treated	5 % SFP -fed	5 % SLP -fed	Metformin + SFP	Metformin + SLP
Urea (mg/dL)	40.60 ±6.39^{a}	74.67±10.54^{d}	56.25±8.17^{bc}	61.25±8.17^{c}	55.20±7.16^{bc}	50.20±7.16^{ab}	46.00±5.87^{ab}
Creatinine(mg/dL)	0.84±0.10^{ab}	1.55±0.19^{c}	0.80±0.11^{ab}	0.85±0.11^{b}	0.76±0.09^{ab}	0.73±0.09^{ab}	0.68±0.09^{a}

- Values that have different letters in each row differ significantly, while the difference among those with similar letters is not significant ($p<0.05$).

Histopathological findings

Findings of histopathological examination of pancreas specimens of rats from different experimental groups were illustrated in the following figures:

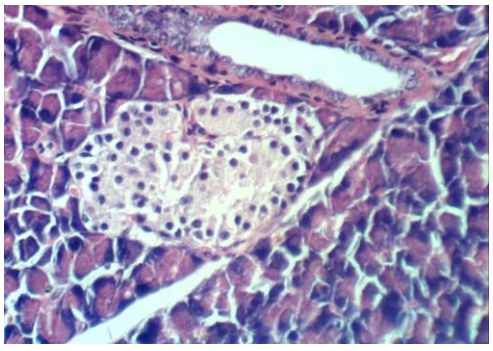

Fig. 1:
Pancreas tissue of rat from negative control group shows no histopathological changes (H & E X 400).

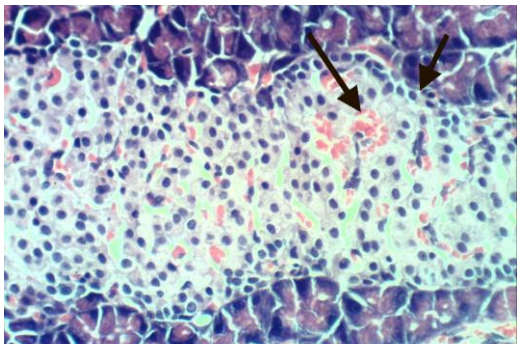

Fig. 2:
Pancreas tissue of rat from untreated diabetic group shows hypertrophy of islets of Langerhan's and congested blood capillaries (H & E X 400).

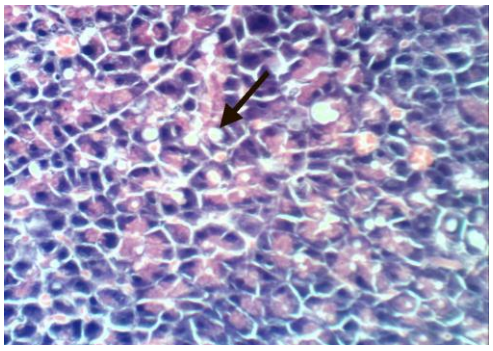

Fig. 3:
Pancreas tissue of another rat from untreated diabetic group shows vacuolation of epithelial lining pancreatic acini (H & E X 400).

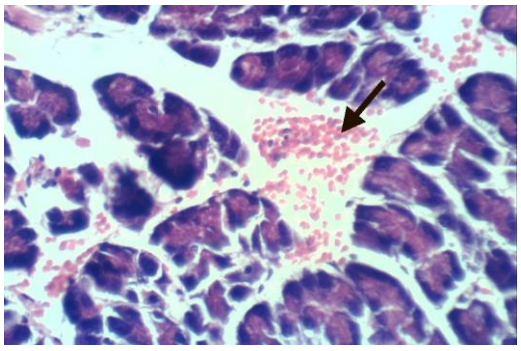

Fig. 4:
Pancreas tissue of another rat from untreated diabetic group shows focal pancreatic haemorrhage (H & E X 400).

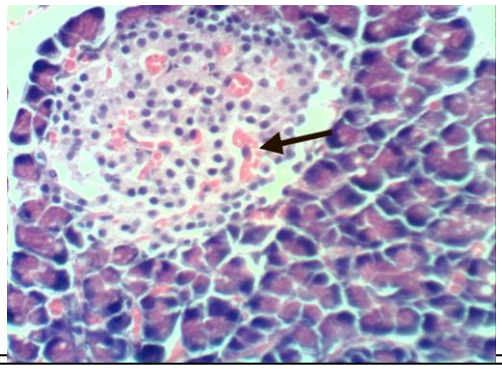

Fig. 5:
Pancreas tissue of rat from metformin –treated diabetic group shows congested blood capillaries (H & E X 400).

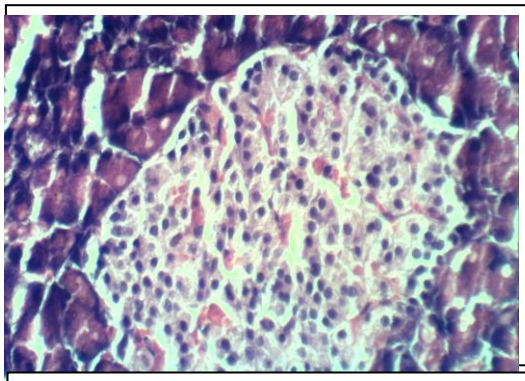

Fig. 6:
Pancreas tissue of another rat from metformin - treated diabetic group shows no histopathological changes(H & E X 400).

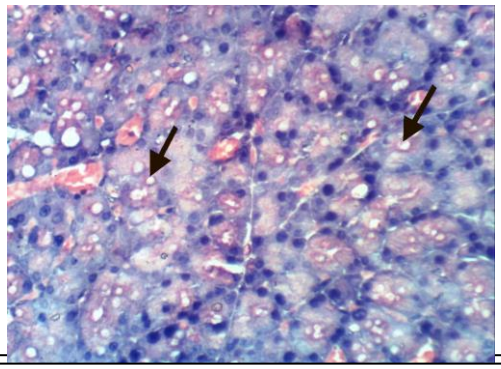

Fig. 7:
Pancreas tissue of rat from SFP –fed diabetic group shows vacuolation of epithelial lining pancreatic acini (H & E X 400).

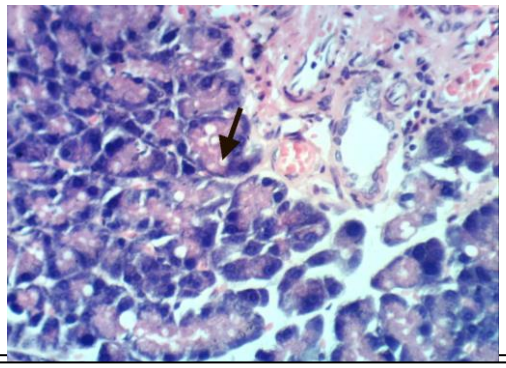

Fig. 8:
Pancreas tissue of rat from SLP- fed diabetic group shows vacuolation of epithelial lining pancreatic acini (H & E X 400).

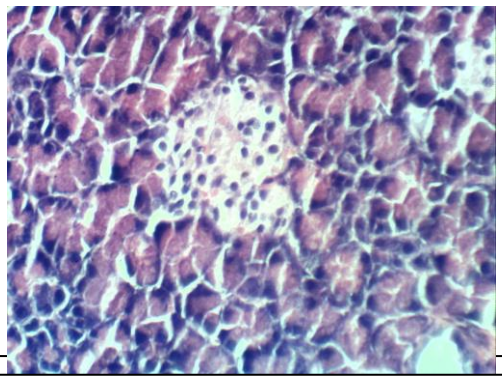

Fig. 9:
Pancreas tissue of rat from the group received metformin and SFP shows no histopathological changes (H & E X 400).

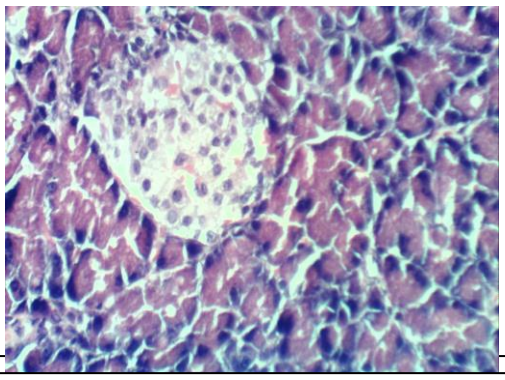

Fig. 10:
Pancreas tissue of rat from the group received metformin and SLP shows no histopathological changes (H & E X 400).

DISCUSSION

Nowadays, lots of people thought that herbal products are inherently safe, owing to their natural origin not based on experimentally approved evidence***(Ekor, 2013).*** Moreover, despite the fact that there are limited studies in the field of herb-drug interactions, some of patients prefer to use conventional drugs with herbal medicine, concurrently***(Agbabiakaet al., 2016; Gopalakrishnaet al., 2017).*** In fact, interactions between herbs and drugs may increase or decrease the pharmacological or toxicological effects of either components***(Fugh-Berman, 2000).*** Therefore, it is imperative to promote credible research on safety and possibility interaction of herbal medicine with synthetic drugs.In the present study, the therapeutic effects resulted from the interaction between Zizyphusspina-christi L. fruits or leaves and metformin were evaluated.The mechanisms behind these effects were also investigated.

The present results revealed that BWG of untreated diabetic group was significantly lower than that of negative control group. This result agrees with both ***Ewenighiet al., (2015)and Udiaet al., (2016).*** Diabetes is accompanied with dehydration, hyperglycemia, insulin deficiency or resistance, increased glycogenolysis, lipolysis, gluconeogenesis and these biochemical activities result in muscles wasting and loss of tissue protein ***(Ewenighiet al., 2015).Virdiet al., (2003)*** explained thatdiabetics suffer from dehydration and catabolism of fats and proteinsdue to unavailability of carbohydrates for utilization as an energy source.Since metformin increases insulin sensitivity***(Li et al., 2019),*** it increased body weight of diabetic rats in the present study. However, because of itslowering effect on food and caloric intake as confirmed by***Leeand Morley, (1998)***,its weight gain effect was the lowest compared with this induced by herbal treatments.SFP and SLP were found to decrease insulin resistance and have hypoglycemic effect. These effects along with their efficiency in healing appetite loss***(Abalakaet al., 2010; Yossefet al., 2011; Ghafooret al., 2012)***can account for their effects on body weight of diabetic rats in the present study.

Increased glucose levels in the blood have been shown to lead to oxidative stress, which is considered as one of the causative factor for diabetes complications including organs enlargement. In this regard,it was found that nonalcoholic fatty liver disease (NAFLD) was extremely common in people with type 2 diabetes***(Targheret al., 2007).***These findings agree with the present study in whichalloxaninjection resulted in a significant increase in the relative weight of both liver and kidneys. The present results also are in agreement with those of ***Berredjemet al., (2015) and Muhamed, (2019). Zafar and Naeem-Ul-Hassan Naqvi, (2010)*** demonstrated that STZ-induced diabetes caused a significant reduction in the body weight while the relative weights of kidneys and liver were increased and the weight of pancreas was unaffected. Increased relative liver weight in diabetics could be attributed to increased triglyceride accumulation which could be due to the increased influx of fatty acids into the liver induced by hypoinsulinemia and the low capacity of excretion of lipoprotein secretion from liver resulting from a deficiencyof apolipoprotein B synthesis. On the other hand, the increased relative kidney weight in alloxan –induced diabetic rats may be due to enlargement of lining cells of tubules, fatty infiltration, large area of hemorrhage and lymphocyte infiltration ***(Evan et al., 1984).*** Moreover, a key morphological change associated with sustained hyperglycemia was the accumulation of glycogen granules in distal tubules which leads to the renal hypertrophy ***(Kang et al., 2005).***

Metformin and herbal treatments received singly or in combination reversed the increase of RKW and improved RLW. In general, the hypoglycemic and hyperinsulinemic effects of metformin and herbal treatments can account for their effects on organs weight. Because SFP, either received singly

or in combination with metformin, induced the best results of BWG, it accordingly resulted in the best results regarding organs weight.

The manifestations of diabetes mellitus – hyperglycaemia and hypoinsulinemia – were observed in the untreated diabetic group. This agrees with the reports of several workers that alloxan exerts a cytotoxic effect on pancreatic β-cells, resulting in type 1 diabetes mellitus ***(Lenzenet al., 1996; Szkudelskiet al., 1998). Szkudelski, (2001)*** indicated that the mechanism of cytotoxic action of alloxan on β-cells involve oxidation of essential sulphydryl (-SH) group, inhibition of glucokinase, generation of toxic free radicals and disturbances in intracellular calcium homeostasis. The resulting damage to β -cells, responsible for reduced secretion of insulin, results in a decrease in insulin release and the attendant hyperglycaemia with metabolic and other associated diabetic complications.

Amylase activity increased significantly in serum of untreated diabetic group compared with negative control group.In contrast, glycogen level in liver tissue homogenate was significantly decreased. In harmony with these results, ***Belhadj et al., (2018)*** found that α-amylase activity in serum of diabetic group was raised compared to control group. In contrast, liver glycogen storage was reduced.

Alpha-amylase is an endoglucosidase that is involved in the breakdown of long chain carbohydrates (starch and glycogen) into smaller oligosaccharides ***(Shori, 2015).*** In the duodenum lumen, the α-amylase hydrolysis is supplemented by the reduction of oligosaccharides by α-glucosidase into glucose and galactose readily transferred into enterocytes cytosol through the Na^+-dependent glucose transporter 1 (SGLT1) situated in the brush border membrane ***(Leturqueand Brot-Laroche, 2013).*** The transfer of simple sugars to the blood is mediated through the glucose transporter 2 (GLUT2) of the duodenum enterocytes basolateral membrane ***(Wright et al., 2007).*** The hyperphagia, and the continuous feeding of energetic diet to alloxan –induced diabetic rats would increase the number and transport rate of SGLT1 and GLUT2 per cell, leading to an accelerated absorption of glucose and then to hyperglycemia ***(Bhoret al., 2004).*** The augmented rates and numbers of these transporters would explain the increased activity of α-amylase, the fasting blood glucose and the reduced hepatic glycogen storage recorded in diabetic rats in this work. On the other hand, hypoinsulinemia recorded by untreated diabetic group is responsible for decreased liver glycogen content as insulin stimulates glycogen synthesis as well as inhibits glycogenolysis and gluconeogenesis in the liver ***(Ranget al., 2012).***

The hypoglycemic effect of metformin, noticed in the present study, is attributed to its effect on insulin as it improves peripheral sensitivity to insulin. Insulin, in turn, stimulates glucose uptake by muscle and liver and decreases its production. It has been reported also that metformin strengthens the antioxidant status, inhibits gastrointestinal absorption of glucose and restores inflammatory parameters in diabetic patients ***(Davidson and Peters, 1997;Chakrabortyet al., 2011).***

The antidiabetic effect of SFP and SLP was reported in several studies.***Niamatet al., (2012)*** stated that phenolics, alkaloids, flavonoids, terpenoids and glycosides are among the frequently cited phytochemicals responsible for the anti hyperglycemic properties of the fruits of *Z. spina-christi*. In addition, a phloretin,a knownhexose transport inhibitor across the basolateral membrane of the small intestine, derivative has been identified in Sidr fruits ***(Pawlowska et al., 2009).Ibrahim et al., (2016)***concluded that Sidr fruits could prevent postprandial hyperglycemia via inhibition of the activities of intestinal maltase and sucrase. Hence, it is logical to hypothesize the involvement of inhibition of

sugar transportin addition to enzyme inhibition in the mechanism of action of Sidr fruits.More over, the tannins in *Ziziphus*fruits have antioxidative effect. Oxidative stress is one of the important factors in tissue injury in diabetes mellitus ***(Baynes, 1991).*** These potent antioxidants may protect beta cells and increase insulin secretion in diabetic patients. Also, tannins may inhibit insulin degradation and improve glucose utilization by stimulation of GLUT4 (Glucose Transporter 4) protein content of the muscle ***(Mohamadin et al., 2003).*** Christinin-A, a main saponin glycoside found in Sidr fruits, was also reported to have hypoglycemic and hyperinsulinemic effects ***(Adzu et al., 2002). Michel et al., (2011)*** revealed that oral administration of *Z. spina-christi* leaf extract, plain and formulated for 28 days reduced blood glucose level with significant increase in serum insulin and C-peptide levels. Marked elevation in total antioxidant capacity with normalization of percentage of glycated hemoglobin (HbA1C%) was reported. Moreover, they succeeded to reduce the elevated blood lactate level and to elevate the reduced blood pyruvate content of diabetic rats. In line with amelioration of the diabetic state, *Zizyphus*extract, plain and formulated restored liver and muscle glycogen content together with significant decrease of hepatic glucose-6-phosphatase and increase in glucose-6-phosphate dehydrogenase activities. In vitro experiments showed a dose-dependent inhibitory activity of *Zizyphus* extract against α-amylase enzyme. All these effects may be due to both saponin and polyphenols content.***Abdel-Zaheret al., (2005)*** suggested that the safe insulinotropic and subsequent hypoglycemic effects of *Z. spina-christi*leaves may be due to a sulfonylurea-like activity. Recently, ***Al-Ghamdiet al., (2019)***showed that α-amylase and α-glucosidase enzymes are considerably inhibited by Sidr leaves methanol extract and its ethyl-acetate fraction.

The synergistic hypoglycemic effects of both metformin and herbal treatments, observed in this study, might be due to the similar antidiabetic mechanisms.In the present study, the dyslipidemia observed in untreated diabetic group is supported by ***Abubakar et al., (2018).***This dyslipidemia may result from the disturbance in the regulation of the activity of the enzyme, hormone sensitive lipase, by insulin due to its deficiency or absence, caused by the alloxan-induced destruction of β- islet cells ***(Tessier, 1994).***

The hypolipidemic effect of metformin, noticed in the present study, was supported by the results of ***Zhang et al., (2017)*** which implied that metformin may help ease diabetic nephropathy symptoms by modulating lipid metabolism and dyslipidemia. Metformin may counter the derangements in lipid metabolism in type 2 diabetes mellitus through several pathways ***(Han and Kaufman, 2016).*** Through increasing insulin sensitivity, metformin reduces the rate of lipolysis, thereby slowing the conversion of free fatty acids to lipoprotein precursors in the liver ***(Melmed et al., 2016).*** By reducing plasma glucose levels, metformin lowers the fraction of irreversibly glycated LDL-c, which is removed less efficiently from the body ***(Sima et al., 2010).*** On the other hand, the hypolipidemic effects of both SFP and SLP, in this study, tend to support the trend of the use of plants in the management of hyperlipidemia resulting from diabetes. Regarding SLP, its hypolipidemic effect is in harmony with ***Othman et al., (2009)*** and ***Parsaeyan and Rezvani, (2015).*** In general, the hypolipidemic effect of different *Ziziphus* parts may be due to the presence of saponins as these phytochemicals form an insoluble complex with cholesterol and increase fecal lipid excretion ***(Zhao et al., 2005).*** Saponins also increase liver LDL receptor activity and also decrease synthesis of triglycerides ***(Yugarami et al., 1992).*** Effects of alloxan on liver and kidney function markers, in the present study,are in agreement with ***Khadre et al., (2011).*** The increased activities of AST and ALT in serum may be mainly due to the leakage of these enzymes from the liver cytosol into the blood stream which give an indication on the hepatotoxic effect of alloxan ***(Navarro et al., 1993).*** On the other hand, it was found that diabetic hyperglycemia induces elevation of the plasma levels of urea and creatinine which are considered as significant markers of renal dysfunction ***(Almadal and Vilstrup, 1988).***This may result from failure of

the body to excrete the metabolic end products of proteins because proteins metabolic rate increased in diabetics as a result of increased gluconeogenesis rate***(Guyton and Hall, 2000).***

The effect of metformin on AST and ALT activities in the present study was supported by ***Hassanzadeh-Taheriet al., (2018*****)** who revealed that metformin particularly in the high dose (100 mg/kg) ameliorated AST levels and microvesicular alterations while it could not affect ALT levels significantly.***Ghadgeet al., (2016)***reported that metformin had no beneficial effects on elevated ALT levels in diabetic rats. As for kidney function indices, ***Zhanget al., (2017)*** reported that treatment of streptozotocin-induced diabetic rats with metformin alone reduced the elevated levels of serum creatinine and blood urea nitrogen as well as successfully counteracted the morphological alterations noticed in kidney tissue.

Not only AST, but also ALT activity was significantly lowered by herbal treatments (SFP and SLP). So,they can be considered more efficient than metformin in alleviating liver dysfunction associated with diabetes. This efficiency may be attributed to the high content of phenolic compounds which exert antioxidant potential on liver tissue evidenced by increased total antioxidant capacity and decreased level of lipid peroxidation marker, malondialdehyde, in liver tissue homogenate of SFP and SLP –fed diabetic groups compared with metformin –treated group. In general, the present results agree with those of ***Al-Ghamdiet al., (2019)*** who found that pretreatment with methanol and aqueous Sidr leaves extract significantly decreased the serum levels of AST, ALT and ALP toward normal levels in carbon tetrachloride –intoxicated rats.Similarly, ***Othman et al., (2009)*** demonstrated that administration of the crude extract of Sidr leaves significantly ameliorated the oxidative stress evidenced by lowering hepatic thiobarbituric acid-reactive substance and protein carbonyl as well as increasing the activities of hepatic antioxidant enzymes as compared with streptozotocin –injected rats. SFP showed more efficiency than SLP in decreasing malondialdehyde level in liver tissue homogenate. A correlation may be noticed with the previously mentioned effects of the two powders on BWG.
As for kidney function markers, ***Al-Ghamdiet al., (2019)*** found that methanol and aqueous extracts of Sidr leaves significantly decreased serum creatinine and uric acid. In addition, the extract evidently enhanced Non-protein sulfhydryls and protein depletion in kidney tissue, and significantly reduced malondialdehyde concentration. ***Okasha et al., (2017)*** suggested that addition of Sidr fruit extract to hyperthermia slightly reduce the renal damage induced by Ehrlich Ascites Carcinoma load in experimental animals.Like liver, the antioxidant potentials of Sidr fruits and leaves are the cause behind their renal curative effects noticed in the present study.

Conclusion

According to the obtained results, it was concluded that Sidr fruits and leaves can help metformin in the treatment of diabetes mellitus**.**

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التأثيرات العلاجية التعاونية للميتفورمين وثمار وأوراق السدر فى جرذان التجارب المصابة بمرض البول السكري

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الملخص العربي

يفضل كثير من مرضى البول السكري استخدام الأعشاب الطبية مع العلاج الدوائي في نفس الوقت. الهدف الرئيسي من هذه الدراسة هو تقييم التأثيرات العلاجية التعاونية لكل من ثمار وأوراق نبات السدر مع الميتفورمين فى الجرذان المصابة بالبول السكري. أجريت الدراسة باستخدام 42 جرذ ألبينو ذكر بالغ ، حيث تم تقسيمهم إلى سبع مجموعات تركتإجداهن لتتناول الغذاء القياسى فقط وهى المجموعة الضابطة السالبة ، فى حين تم حقن المجموعات الأخرى تحت الجلد بمادة الألوكسان بجرعة 120 ملجم/كجم من وزن الجسم ، حيث تم الإبقاء على المجموعة الثانية بدون علاج بينما عولجت المجموعة الثالثة بالميتفورمين (100 ملجم/كجم من وزن الجسم) ، وتم تغذية المجموعات الرابعة والخامسة على الغذاء القياسي مدعما بمسحوق ثمار وأوراق السدر بنسبة 5% على التوالي. تم علاج المجموعات السادسة والسابعة بالميتفورمين وفى نفس الوقت تم تغذيتهن على الغذاء القياسي مدعما بمسحوق ثمارأو أوراق السدر على الترتيب.استمرت فترة العلاج أربعة أسابيع ، وفى نهايتها تم حساب الزيادة المكتسبة فى وزن الجسم والأوزان النسبية للكبد والكلى والبنكرياس ، كما تم تقدير كل من الجلوكوز وهرمون الإنسولين ونشاط إنزيم الأميليز والدهون بالسيرم بالإضافة إلى وظائف الكبد والكلى وتركيز الجليكوجين بالكبد.لوحظت المظاهر النمطية للبول السكري وتشمل ارتفاع تركيز الجلوكوز بالدم وانخفاض مستوى هرمون الانسولين به فى المجموعة المصابة غير المعالجة. علاوة على ذلك ، لوحظ حدوث ارتفاع معنوي في نشاط إنزيم الأميليز بالسيرم والأوزان النسبية للكبد والكلى مع اختلال دهون الدم ووظائف الكبد والكلى بالإضافة إلى حدوث نقص معنوي فى وزن الجسم والبنكرياس مقترنا بظهور تغيرات مرضية غير طبيعية واضحة فى نسيج البنكرياس. أظهرمسحوق الثمار كفاءة أكبر من مسحوق الأوراق في إعادة وزن الجسم والبنكرياس وحالة مضادات الأكسدة بالكبد لمستواهم الطبيعي ، في حين كانت قدرة مسحوق الأوراق أكبرفي تصحيح مستوى كوليسترول البروتينات الدهنية منخفضة الكثافة (الكوليسترول الضار) فى السيرم.وبشكل عام ، فإن التداوي بالميتفورمين مع الأعشاب كان أكثر فاعلية من التداوي بالميتفورمين وحده. وبناءا عليه يمكن استنتاج أن ثمار وأوراق السدر يمكنها مساعدة الميتفورمين فى علاج مرض البول السكرى.

الكلمات المفتاحية: البول السكرى - الألوكسان –نبات السدر - الميتفورمين –جرذان التجارب.