Effect of Syrian *Capparis Spinosa* Leave Extract on Alloxan-Induced Diabetes in Rats

Jinan Hussain[1*], Hala Sarhan[2], Mustafa Bassal[3]

[1]Ph.D. student, Pharmacology Laboratory, Department of Pharmaceutical Industry Research at Scientific Studies and Research Center (SSRC), Damascus, Syria.
[2]Dr. Hala Sarhan: Senior Researcher in Pharmacology, Pharmacology Laboratory, Department of Pharmaceutical Industry Research at Scientific Studies and Research Center (SSRC), Damascus, Syria.
[3]Professor in Animal Physiology, Department of Animal Biology, Faculty of Sciences, Damascus University, Syria.

ABSTRACT

The present study aims to determine the beneficiary effects of Syrian *Capparis spinosa* (CS) leave extract on the diabetic rats. The primary qualitative chemical analysis demonstrates the presence of flavonoids and their glycosides in CS leave extract. A CS leave ethanolic extract rich in flavonoids and glycosides has been used in this study. Diabetes mellitus was induced in rats by intraperitoneal (150 mg/kg b.w.) single dose injection of alloxan monohydrate. Animals showing a blood glucose level ≥ 200 mg/dl after 3 days of alloxan injection were selected as diabetic rats. Leave ethanolic extract at a single dose of 2000 mg/kg/po did not show any clinical signs of toxicity in mice, nor a change in blood biochemical parameter levels. After 3 days of alloxan injection, treatment of the diabetic rats with a single dose of the extract (400 or 800 mg/kg/po) reduced significantly the blood glucose level after 8 and 24 hour ($p \leq 0.05$, $p \leq 0.01$) respectively, while treatment with a single dose of 200 mg/kg/po did not affect blood glucose level. The dose of 400 mg/kg/po of extract has been chosen as the effective dose for subsequent treatments of diabetic rats. Chronic treatment of diabetic rats with 400 mg/kg/po/d of CS leave extract, for 4 weeks, reduced significantly blood glucose level. It also reduced the concentrations of serum SGPT, creatinin and total cholesterol to normal values in comparison with the untreated diabetic rats. Prophylactic treatment of normal rats with 400 mg/kg/po of CS leave extract before one hour of alloxan injection, protected against the rise of blood glucose level induced by alloxan, and allowed continuous gradual increment of body weight which was equal to normal untreated rats. In conclusion, our study demonstrates an antidiabetic activity of Capparis spinosa leave extract, and supports its traditional usage in controlling diabetes.

Key Words: animal model, diabetes mellitus, alloxan, *Capparis spinosa*, hypoglycaemic effect.

eIJPPR 2017;7(6):*31-43*

HOW TO CITE THIS ARTICLE: Jinan Hussain*, Hala Sarhan , Mustafa Bassal,(2017). "Effect of Syrian Capparis Spinosa Leave Extract on Alloxan-Induced Diabetes in Rats ",*International Journal of Pharmaceutical and Phytopharmacological Research, 7*(6), pp.31-43.

INTRODUCTION

Diabetes Mellitus (DM) is a chronic metabolic disease, which occurs either when pancreatic beta-cells are not able to produce and secrete sufficient insulin (type1 diabetes), or when the is not able to use the insulin effectively (type2 diabetes)[1, 2]. That leads to prolonged hyperglycemia conjugated with disturbances in

Corresponding author Jinan Hussain
Address: Ph.D. student, Pharmacology Laboratory, Department of Pharmaceutical Industry Research at Scientific Studies and Research Center (SSRC), Damascus, Syria.
e-mail✉hussain75.jinan@gmail.com
Relevant conflicts of interest/financial disclosures: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.
Received: 14 July 2017; **Revised:** 26 October 2017; **Accepted:** 03 December 2017

most metabolic processes in human body[3]. Untreated cases of diabetes show severe tissue and vascular damages leading to serious complications such as retinopathy[4], neuropathy [5], nephropathy[6] cardiovascular complications [7], and ulceration [8, 9]. Mortality in case of cardiovascular complications concomitant with diabetes is approximately doubled compared to non- diabetic cardiovascular patients, and tripled in case of renal failure patients with diabetes in comparison with non- diabetic ones[10].

Diabetes may cause alteration in thyroid hormone system through reduction of TSH activity in the pituitary gland leading to thyroid gland dysfunction, and so reduction of T4 hormone conversion to T3[11, 12]. DM also affects the nervous system of our body [13].

The current available therapy for type 2 diabetes includes various oral antidiabetic agents such as the sulfonylureas, biguanides, thiazolidinediones and α-glucosidase inhibitors, and insulin at the last stage of the disease. These drugs are used as monotherapy or in combination to achieve better glycemic control. Each of the oral antidiabetic agents is however associated with a number of serious adverse effects [14, 15, 16]. Thus, these drugs treat one of the key symptoms of type2 diabetes, hyperglycemia, but exacerbate the condition of being overweight or obese, one of the leading causes of type2 diabetes. Therefore, while these drugs are beneficial over the short term of therapy, but they are not optimal for a long term health of type2 diabetic patients [17]. It would be advantageous to develop new types of antidiabetic drugs that are either hypoglycemic or anti-hyperglycemia without inducing side effects or promoting weight gain (adiposity) [17].

Herbal medicines known to be useful in diabetes treatment may be able to lead to compound(s) with such a combination of ideal therapeutic properties and minor side effects [18,19]. Recent scientific investigation and clinical studies have confirmed the efficacy of many medicinal plants and herbal preparations in the improvement of normal glucose homeostasis [9, 20]. Biological actions of these plants are related to their chemical composition. Herbal products that are rich in phenolic compounds, alkaloids, flavonoids, terpenoids, coumarins and glycosides usually show positive effects on glucose homeostasis [21, 22].

Capparis spinosa L. (CS) is belonging to Capparidaceae family. It is a common perennial shrub in the Mediterranean regions, growing both wild and cultivated, with medicinal and aromatic properties [23]. CS is an evergreen shrub growing to 1 m (3ft, 3in) by 2 m (6ft). It grows on rocky cliffs and stone walls in the sea-spray zone. Leaves are alternates, rounds to ovates, thicks, and glistening. It has beautiful flowers that are hermaphrodite. Flowers are about 2 inches in diameter, white with numerous violet stamens, and very pleasing in appearance [2]. Different flavonoids were identified in caper bush and capers: rutin (quercetin rutinoside), quercetin-7-rutinoside, quercetin-3-glucoside-7-rhamnoside, kaempferol-3rutinoside, kaempferol-3-glucoside, and kaempferol-3-rhamnorutinoside. Flavonoids are potent antioxidants, and are known to modulate the activity of various enzymes due to their interaction with various biomolecules, and they regenerate the damaged ß-cells in alloxan diabetic rats [2, 24, 25]. CS has been subjected to many phytomedicinal studies. Its extract can be used as antioxidants[26], antihepatotoxic[27], antifungal, antidiabetic, anti-leishmania, anti-inflammatory[28], antiallergic[29] and anticancer[30]. It is also used for the treatment of cardiovascular diseases [31]. Capers have been used or still being used in reducing flatulence, treating rheumatism, anemia, arthritis and gout [32, 33, 34].

The objective of this study was to examine the influence of the oral administration of CS leave extract on the blood glucose concentration in diabetic rats, and the role of the extract in protecting from alloxan-induced diabetes in rats. The present study aimed also to determine the beneficiary effects of CS leave extract on alanine aminotransferase (ALT) which is an indicator of safe liver and normal function, and on creatinine level as a critical marker for kidney damage.

MATERIALS: Alloxan monohydrate ≥98 purity, A7413, from Aldrich Company. Metformin HCL from MENARINI, RM0387. All other biochemical kits were obtained from LABKIT assay and used according to the manufacturer's protocols. Glucose was measured with an autoanalyzer glucometer Accu-Check Active, Roche, Germany. All other chemicals are purchased from Merck Company with 99% purity.

METHODS

Plant material preparation: *Capparis spinosa* was classified in Damascus University, Faculty of Sciences, Department of Plant Classification. Leave were collected between May and June since 2016, from local area (Barza), Damascus city, Syria. They were dried at ambient temperature till 5% humidity, crashed and preserved in opaque containers till extraction.

Leave extract preparation:

Extraction: 25 g of dried and crashed *Capparis spinosa* leaves were extracted by Soxhlet apparatus with 250 ml ethanol 80%. The extract was evaporated by rotavapor under low pressure, washed several time with methanol until obtaining the smallest volume. The extract was then transmitted to a plate to be submitted to more evaporation in a water bath at 40 °C, until full extract dryness. After that, the extract on plate was

incubated in a desiccator containing silica gel until obtaining the stably weight [35].

Primary qualitative chemical analysis: Flavonoids, phenols and glycosides were qualitatively determined in accordance with our latest research publication [35], where the test methods are carried out.

Animal experiments: Westar albino rats and Swiss albino mice bred in the Scientific Studies and Research Center (SSRC), department of pharmaceutical industry research-Syria, were used in this study. Toxicity experiments were carried out on female mice weighting between 20-24g and aged between 8-12 weeks old. Pharmacological experiments were carried out on male rats weighting between 130-160 g. Animals were kept under standard conditions with temperature 23 ± 2 °C, relative humidity 55 ± 10%, 12 h light/dark cycle, and provided a standard diet and water. All experiments on animals were carried out in accordance with the National Ministry of Health Guide for the Care and Use of Laboratory Animals, and were approved by the Animal Ethics Committee of Damascus University of Medical sciences.

Acute oral toxicity of leave extract: The acute toxicity study was carried out on female Swiss albino mice using the "Fixed dose" method, according to the OECD (Organization for Economic Cooperation and Development) guideline no. 420. Test procedure with fixed dose of 2000 mg/kg/po was adopted. Fifteen mice fasted overnight, but allowed free access to water, were randomly divided into 3 groups of 5 mice per group. Control group received orally 0.2 ml distilled water. The second group received orally 0.2 ml CMC 0.5% solution (carboxy methyl cellulose). The third group was administered orally up to 0.2 ml of CS leave extract suspended in 0.5% CMC solution, which corresponded to a dose of 2000 mg/kg. Eventual signs of toxic effects were observed during 3 h after extract administration, and mortality was recorded for 48 h after treatment. Mice body weight was recorded weekly, for four weeks after treatment. SGPT and creatinin were measured the last fourth week [2, 36, 37].

Determination of blood glucose concentration and other biochemical parameters: Blood was collected from the animal tails after 12 h fasting. A drop of blood was used for the blood glucose test with the help of a Glucometer Accu-check Active Roche, Germany. For the measurement of serum SGPT or ALT, creatinin and total cholesterol, blood was collected at the time of harvest, allowed to clot at room temperature for 5 min, then centrifuged at 4,000 rpm, at 4 °C for 10 min. Serum was removed and stored at −80°C until analysis. Serum was analyzed using a LABKIT assay kit according to the manufacture protocol.

Diabetes induction: Diabetes mellitus (DM) was induced in rats by intraperitoneal injection of alloxan (150 mg /kg body weight), which was dissolved in sodium citrate buffer (0.1 M, pH 4.5). Animals were fasted for 12 h before diabetes induction. Control group was injected a similar volume of vehicle (citrate buffer, 1 ml/kg). After 3 days of alloxan injection where diabetes disease would be stabilized, blood glucose of rats was measured. The rats with blood glucose level above 200 mg/dl were selected as diabetic rats, and were used in the future experiments [2, 22, 17, 35, 38, 39].

Treatment of diabetic rats with different single doses of leave extract: Diabetic rats were divided into four groups for ten rats per group, and treated orally once as follows: normal group (non-diabetic rats) didn't receive any treatment. The first diabetic rat group received extract vehicle (CMC 0.5%). The second, third and fourth diabetic groups received 200, 400, 800 mg/kg leave extract respectively. The volume of extract or extract vehicle given orally was up to 1ml/kg. Blood glucose was measured at 0, 2, 4, 8 and 24 hours after leave extract treatment. The most effective dose of leave extract in reducing the blood glucose level in diabetic rats, was chosen to be used in the next experiments.

Effect of repeated oral dose of CS leave extract in the treatment of diabetic rats (sub- chronic treatment):

To evaluate the sub-chronic treatment of diabetic rats with leave extract, one group of normal rats and three groups of diabetic rats were prepared and treated during 30 days, with ten rats per group. The normal rat group didn't receive any treatment. The first diabetic group received orally CMC (0.5%) solution. The second diabetic group received CS leave extract (400 mg/kg/po), and the third diabetic group received metformin (20 mg/kg/po). The volume of oral doses was up to 1ml/kg. Rat body weight and blood glucose level were monitored every week. SGPT, creatinin and total cholesterol were also measured at the end of the experiment.

Prophylaxis effect of a single oral dose of CS leave extract against alloxan-induced diabetes:

Twenty normal rats were divided into two groups. The first group was injected alloxan (150mg/kg/ip), and the second was treated orally with CS leave extract (400 mg/kg) one hour before alloxan injection (150 mg/kg/ip). Blood glucose was monitored on the 1, 2, 3 days and 1, 2, 3, 4 weeks after treatment. Rats body weight was also monitored on the 1, 2, 3, 4 weeks.

Statistical analysis: Statistical analysis was performed using the software Graph Pad Prism 6. Results were expressed as mean ± SEM. Multiple t-test was applied to compare several groups at several times; one way unpaired t-test was used to

compare one treated group with control group. Probability ($p<0.05$) was considered statistically significant.

RESULTS AND DISCUSSION

Primary qualitative chemical analysis: The primary qualitative chemical analysis demonstrated the presence of flavonoids and glycosides in ethanolic CS leave extract. All tests for detecting flavonoids were clearly positives, so CS leave extract is going to present a rich source of flavonoids. Borntrager test was negative, meaning that anthraquinone glycosides are absents in the extract. In contrast, Legal and Keller-Killiani tests were positives, meaning that non-saturated lactone glycosides are presents. Concerning the simple phenol tests, only ferric chloride test was positive, while lead acetate and diluted nitric acid tests were negatives, meaning the absence of simple phenol in the extract (table 1).

Table -1: Qualitative chemical tests for detection of flavonoids, glycosides and simple phenols in *Capparis spinosa* leave extract.

Simple phenol tests	Glycoside tests	Flavonoïd tests
FeCl3 +++ Lead acetate - Dil.HNO3 -	Borntrager - Legal + Keller-Killiani ++	FeCl3+++ Lead acetate +++ Shinoda +++ Sodium hydroxide +++

Acute oral toxicity: Ethanolic CS leave extract, at a dose of 2000 mg/kg/po, didn't show any toxicity signs. All animals included in this test were healthy after 14 and 30 days of observation period. There was no significant differences in the means of body weight, SGPT and creatinin, between the normal control group, CMC control group, and the extract treated group, that throughout all the experiment period (table 2,3 figure 1). The LD50 value of CS leave extract was considered greater than 2000 mg/kg/po.

Table -2:Effect of single dose of Capparis spinosa leave extract on mice body weight increment.

Treated group (n=5)	Time after treatment with extract/ weeks				
	Initial weight	1	2	3	4
Normal control	20.92±0.64	21.82±0.78	22.18±0.52	22.21±0.56	22.25±0.62
CMC control	21.08±0.47	21.56±0.40	22.04±0.53	22.00±0.67	22.12±0.79
CS Extract treated group (2000 mg/kg/po)	20.08±0.23	20.18±1.10	20.93±0.78	21.28±0.85	21.34±0.56

No significant differences between all animal groups ($p>0.05$). Values are expressed as mean ± SEM. n = number of mice; p.o.= per oral.

Table -3: Effect of single dose of *Capparis spinosa* leave extract on mice liver and kidney parameters (SGPT, creatinin) at the fourth week of treatment.

Animal Groups (n=5)	SGPT(U/L)	Creatinin (mg/dl)
Normal control	21.72 ± 3.95	1.104 ± 0.28
Vehicule (CMC 0.5%) control	24.13 ± 4.79	1.012 ± 0.24
CS extract treated group (2000 mg/kg/po)	21.58 ± 5.56	1.124 ± 0.22

No significant differences between all animal groups ($p>0.05$). Values are expressed as mean ± SEM. n = number of mice; p.o.= per oral

.

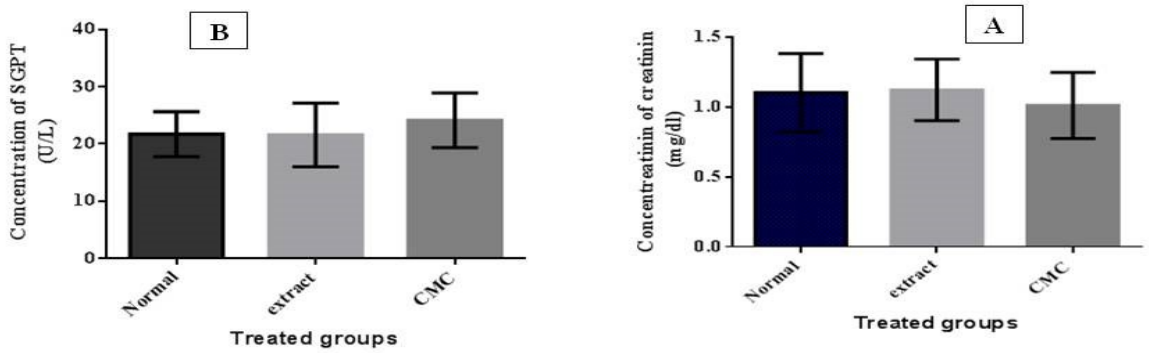

Figure 1. Effect of single dose of *Capparis spinosa* leave extract (2000mg/kg/po) on the level of creatinin (A) and SGPT (B) in mice serum, (n=5).

Treatment of diabetic rats with different single doses of CS leave extract: The effect of different single oral doses of CS leave extract on blood glucose level in diabetic rats, are shown in table 4. Blood glucose levels didn't change significantly between the start and the end of observation (24 hours) after extract treatment, either in the untreated diabetic rats or those treated with a single dose of 200 mg/kg CS extract. Although there was a graded reduction in blood glucose levels throughout the 24 hours of observation after rat treatment with a single dose of 400 mg CS extract/kg/po, but no significant differences were noted between the untreated and treated diabetic rat groups after 2 and 4 hours of treatment. Differences in blood glucose levels were registered significants after 8 and 24 hours ($p<0.05$, $p<0.01$ respectively), table 4. The same results were found with diabetic rat treated with a single dose of 800 mg CS extract/kg/po. It has been noted a graded fall in blood glucose levels throughout the 24 hours of observation after treatment, but only at the 24th hour that has been registered a significant difference between the untreated and treated diabetic rat groups ($p<0.05$, table 4).

Table -4: Effect of different single oral dose of CS leave extract (200, 400, 800mg/kg) on the blood glucose level in diabetic rats at different times after treatment.

Animals groups (n= 10)	Blood glucose concentration (mg/dl) at different times after treatment				
	0 hr	2 hrs	4 hrs	8 hrs	24 hrs
Non-diabetic rats	99.50 ±20.20	96.20 ±18.90	93.80 ± 20.50	99.70± 18.30	92.96± 25.20
Diabetic rats	399.17±66.08	384.83±71.27	391.18±73.36	441.33±55.87	556.33±32.87
Diabetic rats treated with 200 mg/kg CS extract	505.33±40.32	454.50±58.11	451.83±42.95	443.83±48.79	470.00±43.51
Diabetic rats treated with 400 mg/kg CS extract	404.83±72.10	347.17±62.51	281.67±52.66	239.50±50.73*	213.33±50.44**
Diabetic rats treated with 800 mg/kg CS extract	342.00±83.42	349.50±76.17	292.50±66.85	268.17±59.25	360.00±69.52*

Statistics: Unpaired multiple t-test by row was used to compare data in each time between treated and untreated diabetic groups.*: p<0.05, **: P<0.01. Unpaired t-test was used to compare data between treated diabetic groups: No significant difference between groups treated with 400 and 800 mg extract/kg/po. Significant difference between groups treated with 400 and 200 mg extract/kg/po, p=0.0018. Values are expressed as mean ± SEM. n = number of rats; p.o.: per oral.

The comparison between the three treated diabetic rat groups was performed by using the one way unpaired t-test analysis. Statistical analysis didn't show any significant difference in lowering the blood glucose level between the two diabetic rat groups treated with a single dose of 400 or 800 mg CS extract/kg/po (p>0.05). But an important and significant difference was registered between the two diabetic rat groups treated with 200 or 400 mg CS extract/kg/po (P=0.0018). The dose of 400 mg/kg/po of leave extract was chosen as the most effective dose to reduce blood glucose level in diabetic rats, and it will be used in the next experiments (table 4, figure 2).

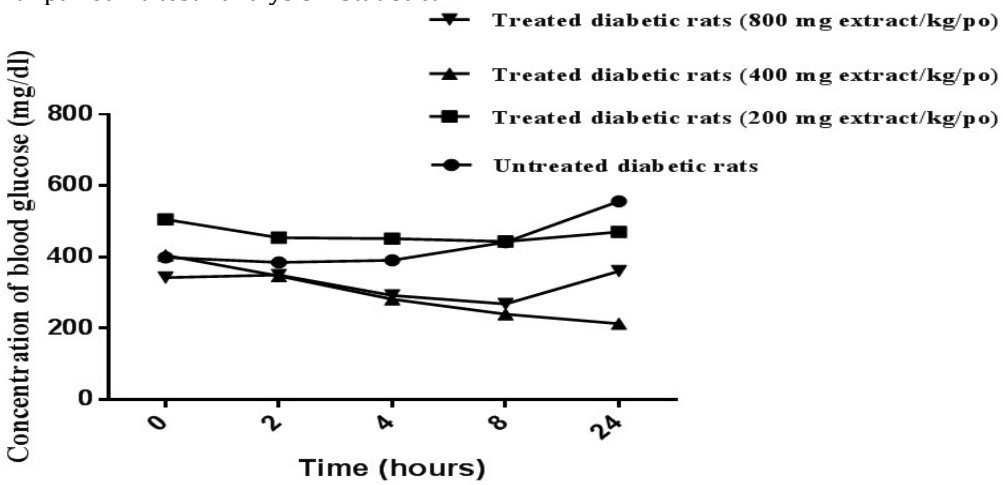

Figure 2. Effect of different single oral dose of CS extract on blood glucose level in diabetic rats, at different time after treatment.

Effect of repeated oral dose of CS leave extract in treatment of diabetic rats (sub- chronic treatment): Daily treatment of diabetic rats with the dose of (400 mg/kg/po) of CS leave extract, reduced significantly the blood glucose level at the 1rst, 2nd and 4th week of treatment (p≤0.05, p≤0.01, p≤0.05 respectively) in comparison with saline treated group, table 5. But unfortunately no significant effect of the extract was observed at the 3rd week. Daily diabetic rat treatment with metformin was very effective in normalizing the blood glucose level at the 1rst, 2nd, 3rd and 4th week, as reduction was very significant (p≤0.01) in comparison with saline treated group, table 5.

By comparing the blood glucose levels between diabetic rat groups treated chronically either with metformin or CS extract, we didn't observe any significant difference at every observed time, except at the 3rh week where metformin has shown better effect in normalizing the blood glucose level than extract P≤0.05, ((table 5, figure 3).

Table -5: Effect of chronic treatment with daily oral dose of CS leave extract (400mg/kg) or metformin (20mg/kg), for 30 days, on blood glucose level in diabetic rats.

Diabetic groups (n=10)	Concentration of blood glucose after different times (weeks) of treatment				
	0	1	2	3	4
Saline treatment	444.86 ± 6.65	506.86 ± 30.36	471.43 ± 29.27	337.71 ± 29.44	472.86 ± 14.43
Metformin treatment (20mg/kg/po)	378.14 ± 59.16	235.29 ± 24.40**	208.86 ± 23.93**	211.71 ± 11.59**	184.29 ± 14.58**
CS leave extract treatment (400mg/kg/po)	393.40 ± 60.30	334.80 ± 81.19*	261.400 ± 62.56**	371.80 ± 64.93¤	285.00 ± 88.37*

Time 0: after 3 days of alloxan injection. Values are expressed as mean ± SEM. n = number of rats; p.o.: per oral.

1- Multiple t-test for comparison of metformin or extract treated diabetic groups with saline treated diabetic group; *p≤0.05, **p≤0.01.

2- Multiple t-test for comparison between diabetic rats treated with metformin or extract. No significant differences were shown at 1, 2 and 4 weeks of treatment, but there was a significant difference at 3 weeks; ¤ p≤0.05.

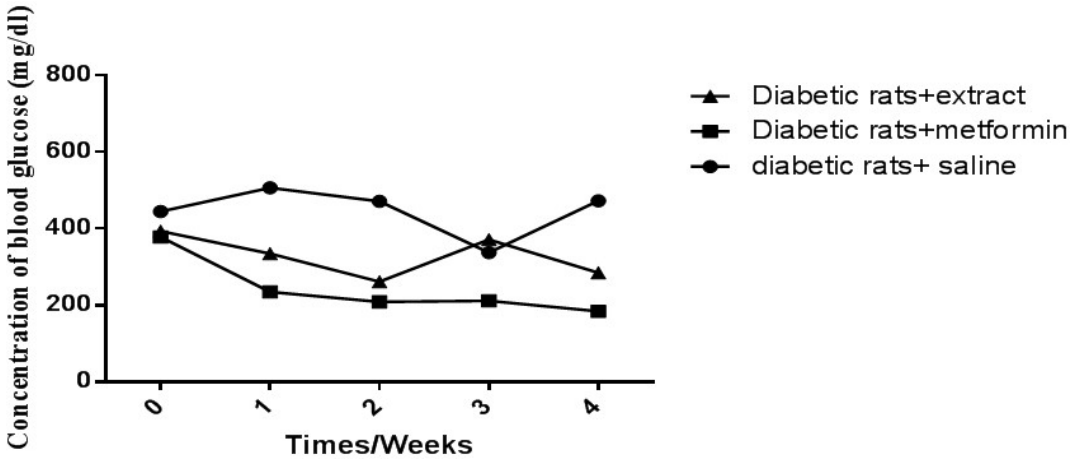

Figure3. Effect of chronic treatment with daily oral dose of CS leave extract (400mg/kg) or metformin (20mg/kg), for 30 days, on blood glucose level in diabetic rats. n=10

Alloxan affected severely the body weight increment in saline treated diabetic group, where it was decreased from 141.14± 4.44 g to 128.43± 8.30 g at the end of the fourth week. The mean body weight of normal control group was subjected to an increase from 147.29± 4.44 to 223.86± 9.32, table 6. Although there was an increase in body weight of treated diabetic rats with extract (from 144.29± 4.00 g to 183.00± 8.28 g), or metformin (from 144.86± 5.03g to 162.86± 3.60g) at the end of the fourth week treatment, but the relative increment was minor in comparison with body weight increase of normal non-diabetic group (from 147.29± 4.44 to 223.86± 9.32), table 6, figure 4. On the other hand, there was no statistic difference in body weight means between metformin or extract treated diabetic groups (p≥0.05), that through the four weeks of treatment (table 6, figure 4).

Table 6: Effect of daily treatment with oral dose of CS leave extract (400mg/kg) or metformin (20mg/kg), for 4 weeks, on the body weight of diabetic rats.

Animals groups (n= 10)	Body weight (g) at different times (weeks) of treatment					
	Initial weight	Start of treatment	1	2	3	4
Normal rats	147.29 ± 4.44	167.43± 6.44	187.57 ± 8.73	199.57 ± 9.16	212.43 ± 10.14	223.86 ± 9.32
Diabetic rats treated with saline	141.14 ± 4.44	130.00± 5.25	130.14 ± 3.23	128.43 ± 4.15	125.00 ± 4.74	128.43 ± 8.30
Diabetic rats treated with metformin (20mg/kg/po)	144.86 ± 5.03	141.57± 4.94	152.29 ± 1.86	154.71 ± 1.78	161.00 ± 1.73	162.86 ± 3.60
Diabetic rats treated with CS leave extract (400 mg/kg/po)	144.29 ± 4.00	140.14± 3.66	139.14 ± 5.08	146.00 ± 7.96	165.43 ± 7.72	183.00 ± 8.28

Start of treatment: 3 days after alloxan injection. Values are expressed as mean ± SEM. n = number of rats; p.o.: per oral.

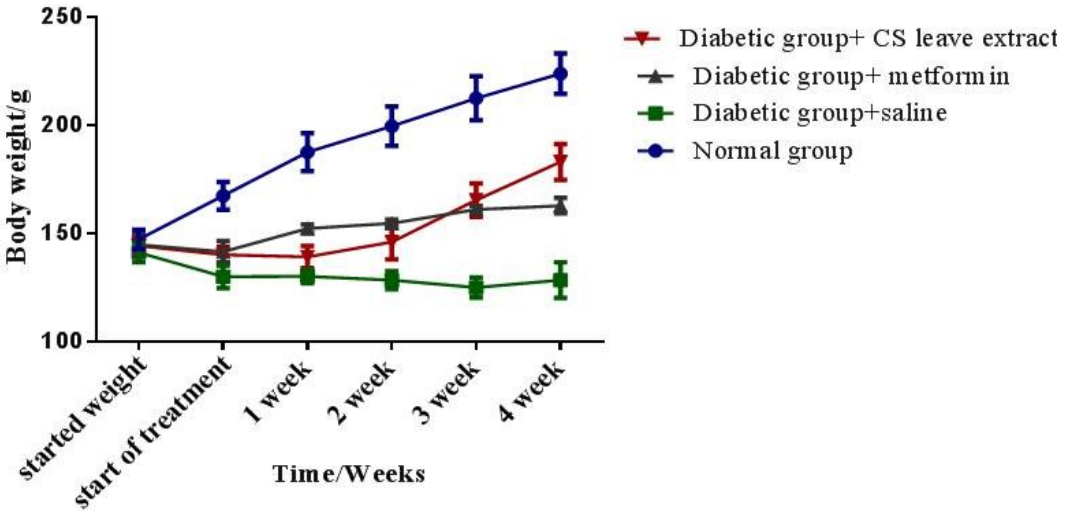

Figure 4. Effect of daily treatment with oral dose of CS extract (400mg/kg) or metformin (20mg/kg), for 4 weeks, on the body weight of diabetic rats.

Alloxan-induced diabetes leads to liver dysfunction, so concentration of alanine aminotransferase (ALT) or SGPT was increased significantly from 26.54 ± 1.72 U/L in normal rats to 58.63 ± 5.51 U/L in diabetic rats (p≤0.01). Daily treatment of diabetic rats with 400 mg/kg/po of CS leave extract, for four weeks, decreased SGPT values to 38.99 ± 5.44 U/L, which were not significantly different from SGPT values of normal non-diabetic rat group, p>0.05 (table 7).

Alloxan-induced diabetes affects also kidney function, that the creatinin concentration was increased significantly from 0.95 ± 0.06 mg/dl in normal rats, to 1.27 ± 0.10 mg/dl in diabetic rats (p≤0.05). But daily treatment of diabetic rats with 400 mg/kg/po of CS leave extract, for four weeks, protected the kidney function on the base that creatinin means were not significantly different between the normal and treated diabetic groups, p>0.05 (table 7).

Alloxan didn't show any effect on total cholesterol level when comparing means of normal non-diabetic rat group and saline treated diabetic group (table 7). But the chronic treatment of diabetic rats with CS leave extract reduced significantly the total cholesterol concentration from 75.91 ± 1.22 mg/dl in normal rat group, to 55.36 ± 1.42 mg/dl in treated diabetic rat group (p≤0.01).

Table -7: Effect of chronic treatment with daily oral dose of CS extract (400 mg/kg) for 4 weeks, on SGPT, creatinin and total cholesterol.

Animal Groups (n=10)	SGPT (U/L)	Creatinin (mg/dl)	Total cholesterol (mg/dl)
Normal control rats	26.54 ± 1.72	0.95 ± 0.06	75.91 ± 1.22
Diabetic rats treated with saline	58.63 ± 5.51**	1.27 ± 0.10*	70.03 ± 2.48
Diabetic rats treated with CS leave extract (400mg/kg/po)	38.99 ± 5.44	0.84 ± 0.08	55.36 ± 1.42**

Values are expressed as mean ± SEM. n= number of rats; p.o.: per oral. Unpaired t-test for comparison between treated group and normal group: *P≤0.05, **P≤0.01.

Prophylaxis effect of single oral dose of CS leave extract against alloxan-induced diabetes: The effect of a single oral dose of CS leave ethanolic extract (400 mg/kg) administered one hour before alloxan injection (150 mg/kg/ip), on rat blood glucose levels is shown in table 8, figure 5. Blood glucose levels in the extract treated

diabetic group were significantly less than levels in the untreated diabetic group at all times of observation, which was started a day after treatment. One day after alloxan injection, the blood glucose level raised from 112.0 ±4.8 mg/dl to 261.0 ±63.1 mg/dl in the untreated diabetic group, while it raised from 106.2 ±5.8 mg/dl to 176.8 ±19.8l mg/dl in the diabetic group treated with CS extract. Blood glucose concentration continued to rise till the last day of observation (end of the 4th week), to attain 462.2 ±58.5 mg/dl in the untreated diabetic group, and which is considered a critical level; while the blood glucose concentration raised to only 214.1 ±43.3 in the extract treated group (table 8, figure 5).

Table -8: Protective effect of CS leave extract on blood glucose level in alloxan-induced diabetic rats**.** Diabetes was induced in rat by alloxan (150mg/kg/ip). One of the two groups was administered CS leave extract (400mg/kg/po) one hour before alloxan injection.

Time/Day	Blood glucose concentration of (mg/dl)	
	Alloxan treated group (n=10)	*Cs* leave extract + alloxan treated group (n=10)
0	112.0 ±4.8	106.2 ±5.8
1	261.0 ±63.1	176.8 ±19.8
2	418.8 ±51.6	265.2 ±48.0*
3	432.0 ±85.3	186.0 ±28.7**
7	418.3 ±67.5	214.1 ±50.1**
14	416.2 ±58.1	244.9 ±43.6*
21	335.4 ±38.1	228.2 ±53.5
28	462.2 ±58.5	214.1 ±43.3**

Values are expressed as mean ± SEM. n = number of rats

Statistic: Unpaired multiple t-test to compare in time the blood glucose levels between the two groups, *p<0.05, **p<0.01. Unpaired t-test to compare curves of blood glucose rise in the two groups, P=0.033

Prophylaxis effect of single oral dose of CS leave extraction body weight increment in diabetic rats: Table 9 shows the mean body weights of rats through the four weeks of observation after inducing diabetes with alloxan (150 mg/kg/ip). Statistic data comparison was performed between two groups of rats: One group was induced diabetes with alloxan without any prophylaxis treatment (alloxan group), the other group was treated with CS leave extract (400 mg/kg/po) one hour before alloxan injection (extract treated group). It was registered a significant difference in mean body weight increment between the two groups (p=0.033). The mean body weight in alloxan groups was decreased from 141.6±4.7 g to 137.9±5.2 g at the end of the fourth week of observation, whereas the mean body weight in extract treated group was increased from 141.1±3.4 g to 204.5±14.9 g at the fourth week, table 9.

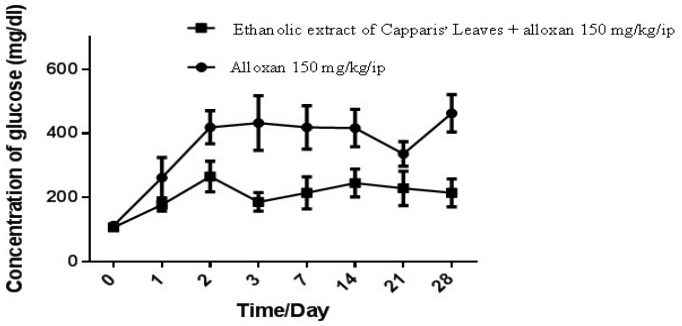

Figure 5: Protective effect of CS leave extract (400 mg/kg/po) on blood glucose level in alloxan-induced diabetic rats.

Table -9: Prophylaxis effect of single oral dose of CS leave extract on body weight increment in diabetic rats.

Animal Groups	Increment of rat body weight with time (g/week)				
	Initial weight	1st week	2 rd week	3 rd week	4th week
Alloxan group	141.6 ± 4.7	132.8 ± 4.8	134.3 ± 4.3	135.7 ± 4.7	137.9 ± 5.2

Extract treated group	141.1 ± 3.4	142.6 ± 10.2	166.7 ± 11.7	191.8 ± 13.8	204.5 ± 14.9

Values are expressed as mean ± SEM. n = number of rats. Unpaired t-test for comparison between extract treated group and alloxan group: P=0.034 significant.

Diabetes mellitus is a chronic metabolic disease, which occurs either when pancreatic beta cells are not able to produce and secrete sufficient insulin (type 1 diabetes), or when the body is not able to effectively use insulin (type 2 diabetes). According to the World Health Organization, the global prevalence of diabetes was approximately 9% among adults in 2014, and in 2030 diabetes will be the 7th leading cause of mortality in the world[1]. Many studies reported the benefits of natural flavonoids in the control of blood glucose level and in the management of diabetic complications [40].

Our study demonstrated clearly that *Capparis spinosa* (CS) leave extract is a rich source of flavonoids and it could be beneficial in the control of diabetic complications induced by alloxan. Also, we proved that ethanolic CS leave extract, at a dose of 2000 mg/kg/po/mice, didn't induce any clinical signs of toxicity and didn't affect the liver and kidney function, nor the body weight relative increment. All mice included in toxicity studies were healthy after 14 and 30 days of observation period**.** The LD_{50} value of CS leave extract was considered greater than 2000 mg/kg/mice/po according to OECD guideline n° 420, and that agree with (Mishra et al. 2012) results [2].

Rat treatment with alloxan (150 mg/kg/ip) caused a critical rise in glucose levels (250- 600 mg/dl) after three days of injection. It also caused a reduction in body weight and an increase in values of certain biochemical parameters, such as serum SGPT and creatinin. Alloxan didn't affect the total cholesterol level. In the present study, we used the alloxan-induced diabetes in rat to investigate the effect of CS leave extract in normalizing the blood glucose level, the serum SGPT or ALT levels as a marker of safe liver function, the serum creatinin level as a marker of renal dysfunction, and the total cholesterol level and body weight increment.

We investigated the effect of different single oral doses of CS leave extract in normalizing the blood glucose level in diabetic rats. The dose of 400 mg/kg/po had shown the most effectiveness in reducing the blood glucose concentration, so it was chosen in subsequent experiments. It reduced significantly the blood glucose concentration from 404.83±72.10 mg/dl to 239.5±50.73 mg/dl after 8 hours of diabetic rat treatment ($p<0.05$), and to 213.33±50 mg/dl after 24 hours of treatment ($p<0.01$, table 4).

Daily chronic treatment of diabetic rats with a dose of (400 mg/kg/po) of CS leave extract, for 4 weeks, reduced significantly the blood glucose level at the 1rst, 2nd and 4th week of treatment ($p\leq0.05$, $p\leq0.01$and $p\leq0.05$) respectively, that in comparison with blood glucose level in non treated diabetic rats, (table 5, figure 3). The cause why the blood glucose level didn't continue to fall at the 3rd week of chronic treatment with CS leave extract, is still obvious to explain. It is important to notice that untreated diabetic rats had shown a highly rise in blood glucose concentration at the 1rst week after alloxan treatment (from 445±7 mg/dl one day after alloxan treatment, to 507±30 mg/dl at the 1rst week of treatment). But this raise was refallen to 338±29 mg/dl at the 3rd week, and rerisen to 473±15 at the 4th week. Perhaps there is a sort of biological mechanism of defense in the body against the pathologic rise of blood glucose concentration. The same way of discussion could be applied to explain the sudden rise of blood glucose level in chronic treated diabetic rats with CS leave extract. The extract succeeded to stop the biological events induced by alloxan leading to rise of the blood glucose concentration. But at the 3rd week of chronic treatment, it happened a sort of competition between the reciprocal biological effects of alloxan and CS leave extract, and the effect of extract in reducing the blood glucose level was gained over alloxan effects at the 4th week of treatment. Concerning the chronic treatment of alloxan-induced diabetic group with metformin, there was no gap in blood glucose concentration fall during the four weeks, where blood glucose fallen gradually from 378 mg/dl at the 1rst day to 184 mg/dl at the 4th week, which could be rebacked to the purity of the compound.

Alloxan-induced diabetes affects also the body weight, liver and kidney functions. The body weight of untreated diabetic group was fallen from 141± 4 g to 128± 8 g after 4 weeks of alloxan treatment, table 6. It also caused a raise of serum SGPT from 26.54 ± 1.72 U/L to 58.63 ± 5.51 U/L, and serum creatinin from 0.95 ± 0.06 mg/dl to 1.27 ± 0.10 mg/dl after 4 weeks of treatment, table 7. Alloxan-induced diabetes didn't show any effect on serum cholesterol level. Daily chronic treatment of diabetic rats with a dose of CS leave extract (400 mg/kg/po), for four weeks, improved their health. It improved the gradual body weight increment, which was raised from 144.29± 4.00 to 183.00± 8.28 g, table 6. It reduced the serum SGPT and creatinin till normal values respectively (38.99 ± 5.44 U/L, 0.84 ± 0.08 mg/dl, table 7). Those results demonstrate that CS leave extract beside possessing a therapeutic effect on diabetes, it could have a benefit effect on liver and kidney function.

Although alloxan didn't affect the normal value of total cholesterol, but daily treatment of diabetic rats with CS leave extract reduced significantly the concentration of total cholesterol, from 75.91 ± 1.22 mg/dl in serum of normal untreated group to 55.36 ± 1.42 mg/dl ($p \leq 0.01$) in serum of extract treated diabetic group, table 7.

Our results proposed also CS leave extract as a probable protecting product from diabetes induced by alloxan. The treatment of rats with a dose of 400 mg/kg/po of extract, 1 hour before alloxan injection, protected them from alloxan-induced diabetes. The blood glucose level didn't increase significantly comparing to alloxan treated group, table 8; and the body weight increment was normal comparing to alloxan treated group, where there was a gradual decrease in body weight throughout 4 weeks after treatment, table 9.

There are several hypotheses explaining the antidiabetic activity of *Capparis spinosa* L. leave and other medicinal plants. Some researchers refer that to the presence of polyphenolic compounds which possess a hypoglycemic activity[20]. Phenolic acids and flavonoids are well known as antioxidants which their antidiabetic activities have been reported in many studies [41, 42, 43]. Other researchers believe that flavonoids can regenerate the damaged ß-cells in alloxan diabetic rats[2, 44]. Other kind of hypothesis suggests that certain herbs, including caper fruits and leaves will reduce the digestion and absorption of carbohydrates from the digestive system, causing a progressive entry of glucose into blood stream, and that preventing a sudden increase in blood glucose after food intake [45, 46]. On the basis of the above evidences, it is possible that the flavonoids present in CS leave extract may be responsible for the observed antidiabetic activity [43, 47].

CONCLUSION

Finally, our study clearly demonstrates a significant antidiabetic activity of *Capparis spinosa* leave extract. It acts effectively against diabetes and maintains normal glucose level. It also maintains biochemical parameters of creatinin and SGPT to their normal levels. Our study supports the traditional usage of Capparis spionsa in controlling diabetes.

REFERENCES

[1] Hosseini A, Mollazadehb H, et al" Effects of a standardized extract of *Rheum turkestanicum janischew* root on diabetic changes in the kidney, liver and heart of streptozotocin-induced diabetic rats" Biom. Pharmacothera., 2017, 86: 605-611.

[2] Mishra RP, Panda K P, et al" Antidiabetic and antihypelipidaemic activity of Capparis spionsa extract" Int. J. Pharm. Sci. Rev. Res., 2012, 14 (1): 38-43.

[3] Bastaki A," Diabetes mellitus and its treatment" Int. J. Diabetes Meta., 2005, 13: 111-134.

[4] Bearse M A, Han Y, et al" Local multifocal oscillatory potential abnormalities in diabetes and early diabetic retinopathy" Inv ophthalmol visual sci, 2004, 45: 3259-3265.

[5] Seki M, Tnanka T, et al" Involvement of brain-derived neurotrophic factor in early retinal neuropathy of streptozotocin induced diabetes in rats therapeutic potential of brain-derived neurotrophic factor for dopaminergi amacrine cells" Diabetes, 2004, 53: 2412-2419.

[6] Looker HC, Campagna A F, et al" Homocystein as a risk factor for nephropathy and retinopathy in Type 2 diabetes, 2003, Diabetologia, 46: 766-772.

[7] Sevensson M, Eriksson J W, et al" Early glycemic control, age at onset, and development of microvascular complications in childhood-onset type 1 diabetes a population-based study in Northern Sweden" Diabetes Care , 2004, 27: 955-962.

[8] Wallace C, Reiber G E, et al" Incidence of falls, risk factors for falls, and fall-related fractures in individuals with diabetes and a prior foot ulcer" Diabetes Care, 2002, 25: 19831986.

[9] Singab A N, Youssef F S, et al" Medicinal plants with potential antidiabetic activity and their assessment" Med. Aromat. Plants , 2014, 3 (1): 151.

[10] Mohan V, Shanthirani C S, et al" Mortality rates due to diabetes in a selected urban south indian population the Chennai urban population study " JASSOC Physic. India , 2006, 54: 113-7.

[11] Calvo R, Escobar M G, et al" Maternal non thyroidal illness and fetal thyroid hormone status as studied in the streptozotocin-induced rat model" Endocrinol ,1997, 138: 1159-1169.

[12] Udiong C E J A, Udoh E, et al" Evaluation diabetes mellitus in calabar, Nigeria, India " J.Clin. Biochem, 2007, 22: 74-78.

[13] Kumar R, Iqubal A, et al" Hypoglycemic and Nephropotective effect of Cinomamum cassia on alloxan induced diabetic mice " Int. J. Diabetes Res.,2014, 3(3): 36-40.

[14] Moller D E,"New drug targets for type 2 diabetes and metabolic syndrome " Nature, 2001, 414:821-825.

[15] Nwaegerue E, Nweke I N, et al" Glucose lowering effect of leaf extracts of

Viscum album in normal and diabetic rats" J. Res. Med. Sci., 2007, 12: 235-240.

[16] Osadeb O P, Odoh U E, et al" The search for new hypoglycemic agents from plants" Afri. J. Pharm. Pharma., 2014, 8(11): 292-303.

[17] Zaman U R, " Glycaemic evaluation of folk recipe (medicinal plants) in alloxan induced diabetic rabbits " British J. Medic. Res., 2011, 1(2): 67-84.

[18] Samane S, Noel J, et al" Insulin-sensitizing and anti-proliferative effects of *Argania spinosa* seed extracts" Evid Based Complement Alternat med., 2006, 3(3): 317-327.

[19] Iwalokun A B and Iwalokun O S," Association between erythrocyte Na+K+-ATPase activity and some blood lipids in type 1 diabetic patients from Lagos Nigeria" BMC Endocr Disord., 2007, 7: 7.

[20] Lemhadri A and Eddouks M," Anti-hyperglycaemic and Anti-obesity Effects of *Capparis spinosa* and Chamaemelum nobile Aqueous Extracts in HFD Mice" Amer. J. Pharmaco. Toxico., 2007, 2 (3): 106-110.

[21] Malazy T O, Larijani B, " A Systematic Review of in vitro Studies Conducted on Effect of Herbal Products on Secretion of Insulin from Langerhans Islets " J Pharm Pharmaceut Sci., 2012,15 (3): 447-466.

[22] Hashemnia M, Oryan A, et al" Blood glucose levels and pathology of organs in alloxan-induced diabetic rats treated with hydro-ethanol extracts of Allium sativum and Capparis spionsa" Afric. J. Pharm. Pharmaco., 2012, 6 (21): 1559-1564.

[23] Baghiani A, Ameni D, et al" Studies of antioxidants and xanthine oxidase inhibitory potentials of root and aerial parts of medicinal plant *Capparis spinosa* L." Americ. J. Med. Medic. Sci., 2012, 2(1): 25-32.

[24] Mohammad M S, Kashani H H, et al" Capparis spinosa L. propagation and medicinal uses" Life Sci.J., 2012, 9: 684-686.

[25] Taghavi M M, Nazari M, et al" Outcome of *Capparis spinosa* fruit extracts treatment on liver, kidney, pancreas and stomach tissues in normal and diabetic rats " Med chem., 2014, 4(10): 717-721.

[26] Germano M P, De Pasquale R, et al" Evaluation of extracts and isolated fraction from *Capparis spinosa* L. buds as an antioxidant source" J. Agricult. Food Chemist., 2002, 50: 1168-71.

[27] Gdgoli C and Mishra S H," Antihepatotoxic activity of p-methoxy benzoic acid from Capparis spionsa" J. Ethnopharmacol, 1996, 66: 187-192.

[28] Rajesh P, Selvamani P, et al" A review on chemical and medicobiological applicantions of Capparidaceae family" J. Pharmacy, 2009, 3: 378-387.

[29] Trombetta D, Occhiuto F, et al" Antiallergic and antihistaminic effect of two extracts of *Capparis spinosa* L. flowering buds" Phytother. Res, 2005, 19: 29-33.

[30] Esiok D, Otles S, et al" Herbs as a food source in Turkey" Asian Pacific J. Cancer Prev., 2004, 5: 334-339.

[31] Eddokus M, Lemhadri A, et al " Hypolipidemic activity of aqueous extract of Capparis spinosa L. in normal and diabetic rats " J. Ethnopharmacol, 2005, 98: 345-350.

[32] Peter K V," Handbook of Herbs and Spices" Woodhead Publishing, Cambridge, England, 2004, 2: 360.

[33] Maghaddasian, Behnaz, et al" Caper the mystique of the recent century" Int. J. Agricult. Crop Sci., 2012, 4(10): 604-608.

[34] Sumalatha B V, Devprakash, et al" Isolation of flavonol of Trephrosia purpurea" Res. J. Pharmaceut., Biologic. Chemi. Sci., 2012, 3(3): 105-110.

[35] Hussain J, Bassal M, et al" Quantitative and Quantitative comparison of rutin, quercetin and gallic acid in Syria Capparis spionsa L. leaves " J. Pharmacog. Phytochemist., 2017, 6: 407-415.

[36] Agarwal V, Sharma A, et al" Hypoglycemic effects of Citrulus colocynthis roots"Acta Poloni. Pharmaceutica-Drug Res, 2012, 69(1): 75-79.

[37] Nkono L B, Sokeng D S, et al" Antihyperglycemic and antioxidant properties of Alstonia boonei De Wild. (Apocynaceae) stem bark aqueous extract in dexamethasone-induced hyperglycemic rats" Int. J. Diabetes Res., 2014, 3(3): 27-35.

[38] Abu-zaiton A and Abu-albasal M," Water decoction of olive leaf reduces blood glucose in normal and alloxan diabetic rats" Int. Confer. Medic., Biologic. Pharmaceu. Sci., 2012: 100102.

[39] Nurdiana S, Shukri M K, et al" Antidiabetic activity of *Azadirachta excelsa* extract on alloxan induced diabetic rats" The Open Conference Proceedings Journal, 2013, 4: 49-52.

[40] Chen J, Mangelinckx S, et al" Natural flavonoids as potential herbal medication for the treatment of diabetes

mellitus and its complications" Nat. Prod. Commun, 2015, 10: 187– 200.

[41] Mukherjee P K, Maiti K, et al" Leads from Indian medicinal plants with hypoglycemic potentials" J Ethnopharmacol, 2006, 106: 1-28.

[42] Aslan M, Deliorman-Orhan D, et al" In vivo antidiabetic and antioxidant potential of Helichrysumplicatum ssp. plicatumcapitulums in streptozotocin induced-diabetic rats" J Ethnopharmacol, 2007, 109: 54-59.

[43] Oudah S K, Al-Salih R H, et al" Study the role of polyphenolic extract of Capparis spinosa L. leaves as a hypoglycemic agent" Int. J. Scientif. Engin. Res., 2014, 5(5): 1561-1575.

[44] Chakravathy B K, Gupta S, et al" Pancreatic β cell generation- A novel antidiabetic mechanism of *Pterocarpus marsupium Rox*" Ind.J. Pharmacol,1980, 12: 123-127.

[45] Jenkins D J, Wolever T M, et al" Improved glucose tolerance four hours after taking guar with glucose" Diabetologia, 1980, 19: 21-4.

[46] Rahmany R, Mahmoodi M, et al" Effect of hydroalchoholic extract of *Capparis spionsa* fruit in blood sugar and lipid profil of diabetic and normal rats" Zahedan J. Res. Medic. Sci., 2013,15: 34-38.

[47] Suba V, Murugesan T, et al" Antidiabetic potential of *Barleria lupulina* extract in rats" Fitoterapia, 2004,75:1–4.