Potential Effect of Olive Leaves Powder and its Extract on Streptozotocin-Induced Diabetic Rats

Dr. Hend M. Ali* and Dr. Zeinab Sayed Ahmed Ahmed*

*Home Economics Department, Nutrition and Food Science, Faculty of Specific Education, Assiut University
Hendma20@yahoo.com

مجلة البحوث في مجالات التربية النوعية

معرف البحث الرقمي DOI: 10.21608/jedu.2020.43113.1069

المجلد السادس . العدد التاسع والعشرين . يوليو 2020

الترقيم الدولي

P-ISSN: 1687-3424 **E- ISSN: 2735-3346**

موقع المجلة عبر بنك المعرفة المصري https://jedu.journals.ekb.eg/

موقع المجلة http://jrfse.minia.edu.eg/Hom

العنوان: كلية التربية النوعية . جامعة المنيا . جمهورية مصر العربية

التأثير المحتمل لمسحوق ومستخلص أوراق الزيتون على الفئران المصابة بالسكر المستحس بالأستربتوزتوسين

د.هند محمد على، د.زينب سيد أحمد أحمد

قسم الاقتصاد المنزلى . التغذية وعلوم الأطعمة . كلية التربية النوعية . جامعة أسيوط

الملخص العربى

تهدف هذه الدراسة إلي دراسة تأثير مسحوق ومستخلص أوراق الزيتون على ذكور الفئران المصابة بمرض السكر والمستحث بواسطة الأستربتوزوتوسين. تم تقسيم الفئران المستخدمة إلى ست مجموعات، كل مجموعة تحتوي على 6 فئران. وكانت الفئران من المجموعة الأولى تمثل المجموعة الضابطة السالبة. وكانت الفئران من المجموعة الثانية وهي المجموعة الضابطة الموجبة المصابة بمرض السكر بواسطة الأستربتوزوتوسين بجرعة 45 ملجم / كجم من وزن الفأر. وكانت المجموعات 3 ، 4 ، 5 ، 6 هي الفئران المصابة بالسكر والتي عولجت بواسطة مسحوق ومستخلص أوراق الزيتون والتي استخدم فيها جرعات منخفضة وعالية وهي 2,5 ٪ ، 5 ٪؛ 1 ، 3 جرام / كجم، على التوالي. تم دراسة تأثير مسحوق ومستخلص أوراق الزيتون على مستوى الجلوكوز ودهون الدم ووظائف الكبد والكلى وصورة الدم أيضا. كذلك تم تقدير بعض المركبات النشطة في أوراق الزيتون باستخدام جهاز التحليل الكروماتوجرافي عالي الكفاءة أظهرت النتائج أن مستخلص الإيثانول لأوراق الزيتون بالتركيزات المختلفة ادي إلى حدوث انخفاض كبير ومعنوي في نسبة الجلوكوز في الدم. كذلك انخفاض في دهون الدم، البروتينات الكلية، مستويات الكوليسترول والدهون الثلاثية، ووظائف الكبد (ALT ، AST، ALP) ووظائف الكلى (اليوريا ، حمض اليوريك والكرياتينين) وصورة الدم إلى مستويات قريبة من المستويات الطبيعية. يمكن أن تخلص النتائج أن استخدام مستخلص أوراق الزيتون خاصة تركيز 3 جم /كجم، أظهر نشاطًا مضادًا لارتفاع مستوى سكر الدم في الفئران المصابة بمرض السكر والمستحث بواسطة الأستربتوزوتوسين، مما قلل من الآثار الضارة للاستجابة للأكسدة ونشاط الحماية منها ويحسن التغييرات المرتبطة بمرض السكر ويرجع ذلك بسبب احتوائه على العديد من المركبات النشطة بيولوجيًا.

الكلمات المفتاحية

الفئران المصابة بالسكر، أوراق الزيتون ومستخلصه، المركبات النشطة، التحاليل الكيميائية الحيوية.

Potential Effect of Olive Leaves Powder and its Extract on Streptozotocin-Induced Diabetic Rats

Hend M. Ali and Zeinab Sayed Ahmed Ahmed

Abstract

The present study aimed to evaluate the effect of olive (*Olea europaea*) leaves powder and its extract on streptozotocin (STZ)-induced diabetic male rats. The experimental rats were divided into six groups; each group contained 6 rats. Rats of the first group served as normal control. Rats of the second group were diabetic control (STZ at a dose of 45 mg/kg bw.) and served as positive control. The 3, 4, 5 and 6 groups were diabetic rats, treated with olive leaves powder (OLP) and extract (OLE) at low and high doses 2.5 % & 5% and 1 & 3 g/kg; respectively. Effects of olive leave treatment on glucose, plasma parameters, liver, kidney functions and blood picture markers were determined. Also, some active compounds in olive leaves were determined using HPLC techniques. The results showed that the olive leaves ethanolic extract (OLE) of variety exhibited at termination, a significant reduction in blood glucose. The OLE tended to reduce plasma total proteins, cholesterol, triglycerides levels, liver functions (ALT, AST & ALP), kidney functions (urea, uric acid & creatinine) and blood picture toward the normal levels. It could be concluded that, the OLE showed an anti-hyperglycemic activity on STZ-diabetic rats, minimized the adverse effect of oxidative response, protective activity and improves changes associated with diabetes probably due to containment of many potentially bioactive compounds.

Key words

Diabetic rats, Olive leaves and extract, Active compounds, Biochemical analysis.

INTRODUCTION

Diabetes mellitus (DM) is defined as a metabolic disorder of multiple etiologies characterized by chronic hyperglycemia with disturbances of carbohydrate, protein and lipid metabolism obtaining from defects in insulin action, insulin secretion, or together **(Salam, 2014).** The majority of clinical diagnosis of diabetes is indicated by the presence of symptoms such as polyuria, unexplained weight, and polydipsia loss, and is allowed by measurement of abnormal hyperglycemia (**World Health Organization and International Diabetes Federation 2006**). The World Health Organization (WHO) expected that, over 300 million people worldwide will have DM by the year 2025 **(Park *et al.*, 2011)**.

Moreover, DM can be induced by selective destruction of insulin-producing b-cells of the pancreas with a single, rapid injection of streptozotocin (STZ), a glucose moiety with a reactive nitrosourea group which obtained from the mold such as *Streptomyces griseus*. STZ has been used as diabetogenic factor in experimental animals **(Emordi *et al.*, 2016)**.

Medicinal plants have usually an important source for finding new remedies for human health problems. Traditionally, numerous plants have been recommended for treatment of DM. Additionally, many researchers studied the effect of many plants on anti-diabetics **(Ghorbani, 2013)**.

The olive tree (*Olea europaea*, L.), family: *Oleaceae* and especially, its leaves have been used for the treatment of many diseases in traditional remedies in European and Mediterranean coast, as well as in Argentina, Saudi Arabia, Java, Norfolk Island, California and Bermuda **(Abd El-Rahman, 2016)**.

The leaves of the olive plant (*Olea europaea*, L.), have been used for centuries in folk medicine to treat diabetes. Previous reports on olive leaves extract (OLE) have demonstrated hypoglycemic, hypotensive, antimicrobial and antioxidant activities **(Sarbishegi *et al.*, 2017)**.

Soliman *et al.*, (2019) reported that OLE has a potential to inhibit hyperglycemia and oxidative stress induced by diabetes. In addition, our results suggest that administration of OLE may be helpful in the protection against diabetes associated male

reproductive disorders through up-regulating P450scc and 17b-HSD expression and via the enhancement of the antioxidant capacity.

Ben Salah *et al.*, (2019) mentioned that the administration of OLE significantly decreased glucose and increased insulin levels in diabetic rats when compared with diabetic control group. This decrease could be due to the loss or degradation of structural proteins that were known to contribute to the body weight and impairment in insulin action in the conversion of glucose into glycogen and catabolism of fats. We noted an increase in body weight in OLE-treated groups. Again, administration of OLE (100 mg/kg) had prevented the reduction in body weight on 21st day in diabetic rats.

Moghaddam *et al.*, (2013) investigated the effect of oral consumption of olive leaves on serum glucose level and lipid profile. Results showed that long-term oral consumption of olive leaves by streptozocin-induced diabetic rats has hypoglycemic effect, decreases serum triglyceride, total cholesterol and LDL-c and increases HDL-c level.

The hypoglycemic effect of olive leaves powder is presumably due to an increase in glucose consumption by the peripheral tissues. Olive leaves powder causes glucose consumption maintenance, probably due to continuing the response to insulin and inhibition of intestinal absorption of glucose **(Pari and Saravanan, 2002)**.

Eydi *et al.*, (2004) found that olive leaves alcoholic extract leads to a decrease in serum glucose level and an increase in serum insulin level in diabetic rats, but no effect was seen on healthy animals.

Jamei *et al.*, (2009) working on healthy and alloxan-induced diabetic rabbits for 4 months, found that hypoglycemic and antioxidant properties of olive leaves powder were approved and long-term administration of olive leaves led to a decrease in lipid peroxidation products like MDA.

Al-Attar *et al.*, (2019) studied the effect of olive (*Olea europaea*) leaves extract on streptozotocin (STZ)-induced diabetic male rats. The results indicated that the obtained results confirmed that the protective effects of olive leaves extract on diabetic rats, due to the antioxidant activities and their active compounds.

This work was conducted to study the effect of different concentrations 2.5 & 5% OLP and 1 & 3 g/kg OLE on biological and biochemical changes of diabetic rats.

Material and Methods

Materials

Olive leaves

Commercially fresh olive leaves (*O. europaea*) were obtained from the local farm El- Salheia City, El-Behara Governorate, Egypt in August 2019 from local market at Cairo Governorate, Egypt.

Chemicals and kits

Pure white crystalline cholesterol powder and saline solutions were purchased from SIGMA Chemical Co., (USA). Casein, cellulose, choline chloride powder, and DL-methionine powder, were obtained from Morgan Co. Cairo, Egypt. Chemical kits used in this study (TC, TG, HDL-c, ALT, AST, ALP, urea, creatinine, serum protein) were obtained from Al-Gomhoria Company for Drugs, Chemical and Medical Instruments, Cairo, Egypt. Folin-Ciocalteu reagent and standard substances including gallic acid, caffeic acid, oleuropein, protocatechuic acid and *p*-coumaric acid were purchased from Sigma Chemical Company (St. Louis, USA), synergic acid, ferulic acid, rutin and Quercetin from SIGMA Chemical Co., (USA). All reagents and standards were prepared using Milli-Q deionized water (Millipore, Bedford, USA). All other chemicals and reagents were of analytical reagent grade and purchased from Al-Ghomhoria Company, Egypt.

Experimental animals

A total of 36 adult normal male albino rats Sprague Dawley strain weighing 140 ±0 g was obtained from Vaccine and Immunity Organization, Ministry of Health, Helwan Farm, Cairo, Egypt.

Methods

Drying and extraction of olive leaves

Fresh leaves of olive were directly collected from the local farm El- Salheia City, El-Behara Governorate, Egypt. The leaves were thoroughly washed and dried at room temperature and ground to a fine powder using an air mill, high speed mixture

(Molunix, Al-Araby Company, Benha, Egypt) and then serving as powder size. The fine quality of dried leaves was kept in dry plastic container until use for extract processes. The ground leaves (1000 g) were extracted to exhaustion by percolation at room temperature with 90% ethanol and the extract was evaporated under reduced pressure to leave 160.82 g of the total extract. Additionally, the extract was stored in a refrigerator for subsequent experiments, according to the method described by **Soliman *et al.*, (2019)**.

Determination of phenolic compounds

Extraction, separation and quantification of phenolic compounds were determined according to the method described by **Goupy *et al.*, (1999)**. The HPLC system Perkin Elmer PE200 was composed of a binary pump, a column thermostat and an auto sampler. The mass spectrometer used was a 3200QTRAP MS/MS with ESI ionization (Applied Biosystems / MDSSciex, Foster City, USA). The experimental conditions where: Mobile phase A: 50%acetonitrile, 50%aceticacid (0.5%); mobile phase B: 2% acetic acid; gradient elution: 0 min 30% A,70%B; 10min 30% A,70%B; 30min100% A,0% B;35 min 100%A, 0% B; 40min30%A, 70% B for reconditioning of the system; flow rate: 0.7 mL/min; injection volume: 20 µL; ionisation: ESInegative; dwell time 50 ms; multi plereaction monitoring (MRM) transitions:gallic acid 169/125, dihydroxybenzoic acid153/109, sinapic acid 223/164, vanillic acid 167/123, caffeic acid 179/135, quercetin 301/151, chlorogenic acid 353/191, ferullic acid193/134, *p*-coumaric acid 163/119. Stock solutions of standards were diluted in the mobile phase to obtain working standard solutions. Concentrations of the compounds were calculated from chromatogram peak areas on the basis of calibration curves. The method linearity was assessed by means of linear regression of the mass of compounds injected vs. its peak area. All solvents were of HPLC grade and were filtered and degassed before use.

Induction of diabetics

DM was induced by a single intraperitoneal (ip) injection of freshly prepared STZ (Sigma-Aldrich Corp, St. Louis, MO, USA) in ice-cold citrate buffer (0.1 M, pH 4.5) at a dose of 45 mg/kg according to **Mestry *et al.*, (2017)**. Seventy-two h after STZ injection, DM induction was confirmed by determination of FBG levels in blood samples collected from the tail vein using a blood glucose meter (Accu-Check Performa, Roche Diagnostic, Germany). The fast blood glucose (FBG) levels over than 200 mg/dl were considered as diabetic model rats.

Experimental design

Thirty-six adult male white albino rats, Sprague Dawley Strain, 10 weeks' age, weighing (140±10g) were used in this experiment. All rats were fed on basal diet (casein diet) prepared according to **AIN (1993)** for 7 consecutive days for adaptation. After this adaptation period, rats were divided into 6 groups, six rats per each as follows:

Group (I): rats fed on basal diet as negative control.

Group (2): injected with streptozotocin at dose of 45 mg/kg were fed on standard diet only without any treatment and used as a positive control group.

Group (3): group diabetic rats fed on olive leaves as powder by 2.5% of diet.

Group (4): group diabetic rats fed on olive leaves as powder by 5% of diet.

Group (5): group diabetic rats fed on the olive leaves extract 1.5 g/kg of body weight.

Group (6): group diabetic rats fed on the olive leaves extract 3 g/kg of body weight.

During the experimental period, the experiment continued for 28 days, at the end of the experimental period each rat weight separately then, rats are slaughtered, and blood samples collected.

Blood sampling

After fasting for 12 hours, blood samples were obtained from hepatic portal vein at the end of each experiment. Two kinds of blood samples were taken. The blood samples were collected into a dry clean centrifuge glass tubes and left to clot in water bath

(37°C) for 30 minutes, then centrifuged for 10 minutes at 4000 rpm to separate the serum, which were carefully aspirated and transferred into clean cuvette tube and stored frozen in deep freezer till analysis according to method described by **Schermer (1967)**.

Biochemical analysis

Determination of blood glucose

Serum glucose was measured using the modified kinetic method according to **Kaplan (1984)** by using kit supplied by spin react. Spain.

Liver functions

Determination of Alanine Amino Transferase (ALT)

ALT activities were measured in serum using the modified kinetic method of **Hafkenscheid (1979)** by using kit supplied by Human, Germany.

Determination of Aspartate Amino Transferase (AST)

AST activities were measured in serum using the modified kinetic method of **Henry (1974)** by using kit supplied by human, Germany.

Determination of serum Alkaline Phosphatase (ALP):

Determination of serum ALP was carried out according to the method of **Moss (1982)**.

Kidney functions

Determination of urea nitrogen

Urea was determination in serum using the modified kinetic method or liquicolor of **Patton and Crouch (1977)** by using kit supplied by Human, Germany.

Determination of creatinine

Serum creatinine was measured using the modified kinetic method according to **Schirmeister (1964)** by using kit supplied by Human, Germany.

Determination of uric acid

Serum uric acid was measured using the modified kinetic method according to **While *et al.*, (1970)** by using kit supplied by Human, German.

Lipids profile

Determination of Total cholesterol (TC)

Serum cholesterol was measured using the modified kinetic according to **Richmond (1973)** by using kit supplied by Hu Germany.

Determination of Triglycerides (TG)

Serum triglycerides (TG) were measured using the modified kinetic method according to the method described by **Fossati and Prencipe (1982)** by using kit supplied by Spinreact, spain.

Determination of High-Density Lipoprotein cholesterol (HDL-c)

Serum high density lipoprotein cholesterol (HDL-c) was measured using the modified kinetic method according to **Allain (1974)** by using kit supplied by Human, Germany.

Determination of Very Low-Density Lipoprotein cholesterol (VLDL-c)

Serum very low-density lipoprotein cholesterol (VLDL-c) was calculated as mg/dl according to **Lee and Nieman (1996)** equation:

VLDL-c Concentration mg/dl = TG/5

Determination of Low-Density Lipoprotein cholesterol (LDL-c)

Serum low density lipoprotein cholesterol (LDI-c) was calculated as mg/dl according to **Castelli *et al.*, (1977)** equation:

LDL Concentration mg/dl = Total Cholesterol – HDL-c – VLDL-c

Determination of HGB, PLT, RBC and WBC:

The concentration of hemoglobin (HGB), Platelets (PLT), red blood cell count (RBC) and white blood cell counts (WBC) were estimated according to the method described by **Decie and Lewis (1998)**.

Statistical analysis

The data were analyzed using a completely randomized factorial design **(SAS, 1988)** when a significant main effect was detected; the means were separated with the Student-Newman-Keuls Test. Differences between treatments of ($P \leq 0.05$) were

considered significant using Costat Program. Biological results were analyzed by One Way ANOVA.

Results and discussion

Identification of phenolic compounds of olive leaves by HPLC

Data given in Table (1) showed the identification of phenolic compounds of olive leaves. It is clear to notice that the highest phenolic compounds of olive leaves recorded for oleuropein, gallic acid and rutin. The values were 48.70, 2.35 and 1.61 mg/g DW; respectively. While, the lowest value recorded for ferrulic acid, synergic acid and quercetin. The values were 0.03, 0.07 and 0.40 mg/g DW; respectively. These results are in agreement with ***Ghomari et al.*, (2019)**, they reported that dried olive leaves in ethanol resulted in the higher yield of phenolic compounds and flavonoids in term of total contents of these compounds and also demonstrated by the variety of phenolics identified by HPLC method. Also, **Cumaoglu *et al.*, (2011)** reported that the major compound of olive leaves extract (OLE) identified by LC-MS was the anti-diabetic secoiridoid glycoside oleuropein (14%). Oleuropein was identified via its MS spectra in both positive and negative mode. All compounds were identified by compare their MS data with literature.

Effect of olive leaves powder and its extract on glucose level of diabetic rats

Data presented in Table (2) results show the mean value of glucose level (mg/dl) of diabetic rats fed on various diets. It could be noticed that the mean value of glucose level (mg/dl) of control (+) group was higher than control (-) group, it was being 275±0.15 and 96.5±0.12 mg/dl; respectively. All diabetic rats fed on various diets, showed significant differences in mean values as compared to control (+) group. The values were 165±0.10, 131±0.13, 112.5±0.14, and 108±0.11 mg/dl; respectively. The best glucose level (mg/dl) was recorded for group 6 (diabetic rats fed on 3g/kg OLE) when compared to control (+) group. The results are in agreement with that obtained by **Al-Attar *et al.*, (2019)**, they reported that streptozotocin-induced diabetes treated with olive leaves extract leads to some changes at metabolic enzymes level due to absence or very low levels of insulin, therefore causes hyperglycemia. Also, the reduction of

DM levels due to the antioxidant activities and their active compounds found in olive leaves extract.

Effect of olive leaves powder and its extract on liver functions level of diabetic rats

Data of Table (3) show the mean value of ALT liver enzyme level (U/L) of diabetic rats fed on various diets. It could be noticed that the mean value of ALT liver enzyme level of control (+) group was higher than control (-) group with a significant difference, it was 20.50±0.13 and 12.50±0.10 U/L; respectively. All diabetic rats fed on various diets, showed significant differences in mean values as compared to control (+) group with a significant difference. The values were 16±0.14, 14±0.11, 15±0.16 and 12±0.12 U/L; respectively. The best treatment ALT liver enzyme level (U/L) was recorded for group 6 (diabetic rats fed on 3 g/kg OLE) when compared to control (+) group.

In case of AST liver enzyme, it could be noticed that the mean value of AST liver enzyme level (U/L) level of control (+) group was higher than control (-) group, it was being 95±0.11 and 39±0.15; respectively with a significant difference All diabetic rats fed on various diets, showed significant differences in mean values as compared to control (+) group. The values were 56±0.12, 49±0.13, 46±0.14 and 42±0.10 U/L; respectively. The best treatment AST liver enzyme level (U/L) was recorded for group 6 (diabetic rats fed on 3 g/kg OLE) when compared to control (+) group.

On the other hand, it could be noticed that the mean value of ALP liver enzyme level (U/L) level of control (+) group was higher than control (-) group, it was being 135.±0.10[a] and 102±0.16 U/L; respectively with a significant difference. All diabetic rats fed on various diets, showed significant differences in mean values as compared to control (+) group. The values were 121±0.10, 118±0.12, 110±0.13 and 104±0.10 U/L; respectively. Numerically, the best treatment for ALP liver enzyme level (U/L) was recorded for group 6 (diabetic rats fed on 3g/kg OLE) when compared to control (+) group. The results are in agreement with that obtained by many authors on theme. **Ben Salah *et al.*, (2019),** they reported that treatment with olive leaves extract (100

mg/kg) significantly reduced plasma total proteins, ALT, AST levels as compared with untreated diabetic rats. In addition, liver enzymes AST and ALT level increased in diabetic rats which is responsible for the liver damage. Also, **Mousa *et al.*, (2014)**, reported that oral administration of the olive leaves extract (0.1, 0.25 and 0.5 g/kg body wt) for 14 days significantly decreased the aspartate amino transferase (AST) and alanine amino transferase (ALT) activities.

Effect of olive leaves powder and its extract on lipid profile level (mg/dl) of diabetic rats

Table (4) results show the mean value of total cholesterol and triglycerides level (mg/dl) of diabetic rats fed on various diets. It could be noticed that the mean value of **total cholesterol** level (mg/dl) level of control (+) group was higher than control (-) group, it was 145±0.10 and 95±0.11 mg/dl; respectively with a significant difference. All diabetic rats fed on various diets, showed significant differences in mean values as compared to control (+) group. The values were 130±0.15, 125±0.13, 115±0.14 and 109±0.12 mg/dl; respectively. The best treatment total cholesterol level (mg/dl) was recorded for group 6 (diabetic rats fed on 3g/kg OLE) when compared to control (+) group.

In case of triglycerides, it could be noticed that the mean value of triglycerides level (mg/dl) level of control (+) group was higher than control (-) group, it was 95±0.16 and 71±0.10 mg/dl; respectively with a significant difference. All diabetic rats fed on various diets, showed significant differences in mean values as compared to control (+) group. The values were 88±0.12, 82±0.11, 84±0.10 and 79±0.15 mg/dl; respectively. The values between groups 5 and 6 showed non-significant differences between them were observed. Numerically, the best treatment triglycerides level (mg/dl) was recorded for group 6 (diabetic rats fed on 3g/kg OLE) when compared to control (+) group.

On the other hand, the mean value of **high density lipoprotein (HDL-c)** level (mg/dl) level of control (-) group was higher than control (+) group, it was 60±0.13 and 35±0.11 mg/dl; respectively with a significant difference. All diabetic rats fed on various diets, showed significant differences in mean values as

compared to control (+) group. The values were 41±0.10, 45±0.12, 50±0.15 and 54±0.16 mg/dl; respectively. The best treatment high density lipoprotein (HDL-c) level (mg/dl) was recorded for group 6 (diabetic rats fed on 3g/kg OLE) when compared to control (+) group.

In case of **low density lipoprotein (LDL-c),** it could be noticed that the mean value of level (mg/dl) of control (+) group was higher than control (-) group, it was 89±0.14 and 20.80±0.12 mg/dl; respectively with significant differences. All diabetic rats fed on various diets, showed a significant difference in mean values as compared to control (+) group. The values were 71.4±0.10, 63.6±0.13, 48.2 ±0.11 and 39.2±0.10 mg/dl; respectively. The best treatment of high-density lipoprotein (LDL-c) level (mg/dl) was recorded for group 6 (diabetic rats fed on 3g/kg OLE) when compared to control (+) group.

It could be noticed that the mean value of **very low density lipoprotein (LDL-c)** level (mg/dl) level of control (+) group was higher than control (-) group, it was 21±0.14 and 14.2±0.12mg/dl; respectively with a significant differences. All diabetic rats fed on various diets, showed significant differences in mean values as compared to control (+) group. The values were 17.6±0.10, 16.4±0.13, 16.8 ±0.11 and 15.8±0.10 mg/dl; respectively. The differences between groups 3, 4 and 5 showed non-significant difference between them. Numerically, the best treatment very low-density lipoprotein (VLDL-c) level (mg/dl) was recorded for group 6 (diabetic rats fed on 3g/kg OLE) when compared to control (+) group. These results are in agreement with that obtained by many authors on theme, **Hamzeh *et al.*, (2017)** reported that the hypolipidemic effect of *O. europaea* in diabetic rats is attributed primarily to hydroxytyrosol, oleuropein, and the polyphenols in the OLE. The lowered blood lipid profile is reported to be due to agonist actions of OLE on bile acid-activated Takeda G-protein-coupled receptor 5 (TGR5), a metabotropic G-protein-coupled receptor. Also, **El-Amin *et al.*, (2013)**, reported that rats with 3% OLE replacing an equivalent volume of water for 16 weeks. After 16 weeks feeding, TC, TG, and LDL-L were significantly reduced in the OLE- compared with placebo treated HCHF diet-fed rats. **Boaz *et al.*, (2011)** mentioned that when OLE

is administered long term to diabetes induced rats, it decreases serum TGs, TC, and LDL-C, and increases HDL-C levels because of the antioxidant properties of olive leaves. These animal study findings indicate that OLE is also effective in improving dyslipidemia caused by diabetes.

Effect of olive leaves powder and its extract on kidney functions (urea, uric acid and creatinine) level (mg/dl) of diabetic rats

Table (5) data show the mean value of on kidney functions (uric acid, urea and creatinine) level (mg/dl) of diabetic rats fed on various diets. It could be noticed that the mean value of **uric acid (UA)** (mg/dl) level of control (+) group was higher than control (-) group, it was 6.8±0.13 and 2.4±0.10 mg/dl; respectively with a significant difference. All diabetic rats fed on various diets, showed significant differences in mean values as compared to control (+) group. The values were 4.1±.0.15, 3.9±.0.16, 2.6 ±.0.11 and 2.5±0.11 mg/dl; respectively. The differences between groups 4 & 5 and 5 & 6 showed non-significant differences between them were observed. Numerically, the best treatment uric acid (UA) level was recorded for groups 5 and 6 (diabetic rats fed on 1 g/kg and 3g/kg OLE) when compared to control (+) group.

On the other hand, it could be noticed that the mean value of **urea (UR) level** (mg/dl) level of control (+) group was higher than control (-) group, it was 59.±0.13 and 23±0.10 mg/dl; respectively with a significant difference. All diabetic rats fed on various diets, showed significant differences in mean values as compared to control (+) group. The values were 39±.0.10, 36±0.15, 34 ±0.16 and 29±0.11 mg/dl; respectively. Numerically, the best treatment urea (UR) level (mg/dl) was recorded for group 6 (diabetic rats fed on 3g/kg OLE) when compared to control (+) group.

In case of **creatinine level (CR)**, it could be noticed that the mean value (mg/dl) of control (+) group was higher than control (-) group, it was being 1.75±0.13 and 0.73±0.10 mg/dl; respectively showing non-significant differences. All diabetic rats fed on various diets, showed significant differences in mean

values as compared to control (+) group. The values were 1.25±0.10, 1.01±.0.15, 0.92 ±0.16 and 0.84±0.11 mg/dl; respectively. Numerically, the best treatment creatinine (CR) level was recorded for group 6 (diabetic rats fed on 3g/kg OLE) when compared to control (+) group. The results are in agreement with that obtained by **Laaboudi *et al.*, (2016)**, they reported that the confirmed that the administration of OLE decreased the levels of creatinine, urea and uric acid in STZ diabetic male rats. Also, the study of **Al-Attar and Alsalmi (2017)** showed that, the administration of OLE can prevent severe alterations of renal haemato-biochemical markers and disruptions of its histological structure.

Effect of olive leaves powder and its extract on serum total protein level of diabetic rats

Table (6) show the mean value of total protein (TP) level (g/dl) of diabetic rats fed on various diets. It could be noticed that the mean value of total protein (TP) level (g/dl) level of control (-) group was higher than control (+) group, it was being 6.9±0.12 and 3.7±0.15 g/dl; respectively with a significant difference. All diabetic rats fed on various diets, showed significant differences in mean values as compared to control (+) group. The values were 5.8±.0.10, 6.2±.0.13, 5.5 ±0.14 and 5.7±0.11 g/dl; respectively. The differences between groups 3 & 4 and 5 & 6 showed non-significant differences between them were observed. Numerically, the best treatment total protein (TP) level (mg/dl) was recorded for group 6 (diabetic rats fed on 3g/kg OLE) when compared to control (+) group. The results are partly in agreement with that obtained by **Micol *et al.*, (2005)**, they reported that the increase in total protein level indicated the ability of olive oil and leaves to stimulate the regeneration of hepatic tissue which increased protein synthesis in damaged liver and improved the functional and status of the liver cells.

Effect of olive leaves powder and its extract on hematological level of diabetic rats

Table (7) showed the mean counts of blood picture of diabetic rats fed on various diets. It could be noticed that the mean

value of **red blood cell (RBCs)** level (10^6/mm^3) of control (-) group was higher than control (+) group, it was being 6.4±0.12 and 3.5±0.13 10^6/mm^3; respectively with a significant difference. All diabetic rats fed on various diets, showed significant differences in mean values as compared to control (+) group. The values were 6.1±.0.10, 6.5±.0.15, 4.9±0.10 and 4.6±0.12 10^6/mm^3; respectively. The counts between groups 3 & 4 and 5 & 6 showed non-significant differences between them. Numerically, the best treatment red blood cell (RBCs) level was recorded for group 6 (diabetic rats fed on 3g/kg OLE) when compared to control (+) group.

In case of **white blood cell (WBCs)** level, it could be noticed that the mean value of white blood cell (WBCs) level (10^3/mm^3) of control (-) group was higher than control (+) group, it was 10.5±0.10 and 7.7±0.14 10^3/mm^3; respectively showing a significant difference. All diabetic rats fed on various diets, showed significant differences in mean values as compared to control (+) group. The values were 10.7±.0.12, 11.1±.0.10, 8.6±0.12 and 8.8±0.14 10^3/mm^3; respectively. The values between groups 3, 4 and 5 showed non-significant differences between them. Numerically, the best treatment white blood cell (WBCs) level (10^3/mm^3) was recorded for group 5 (diabetic rats fed on 1g/kg OLE) when compared to control (+) group.

In case of **hemoglobin level** (g/dl)**,** it could be noticed that the mean value of control (-) group was higher than control (+) group, it was being 15.5±0.11 and 10.40±0.12 g/dl; respectively with a significant difference. All diabetic rats fed on various diets, showed significant differences in mean values as compared to control (+) group. The values were 12.6±.0.13, 13.5±.0.15, 13.9±0.12, 15±0.15 g/dl; respectively. The values between groups 3, 4 and 5 showed non-significant differences between them were observed. Numerically, the best treatment hemoglobin level (g/dl) was recorded for group 6 (diabetic rats fed on 3g/kg OLE) when compared to control (+) group.

On the other hand, the mean value of **platelet level** (10^6/mm^3) of control (-) group was higher than control (+) group, it was 567±0.16 and 147±0.14 10^6/mm^3; respectively showing significant differences. All diabetic rats fed on various diets,

showed a significant difference in mean values as compared to control (+) group. The values were 168±.0.10, 154±.0.13, 199±0.10 and 185±0.15 $10^6/mm^3$; respectively. The best treatment of platelet level was recorded for group 4 (diabetic rats fed on 5% OLP) when compared to control (+) group. The results of Table (7) were in line with that found by **Samet *et al.*, (2015)** indicating that long-term intake of olive leaves tea (OLT) may have favorable effects on human hematological parameters. We found that levels RBC count, Hb, and Ht were significantly increased in OLT group compared with those of in GT group. We reported in previous *in vitro* study that certain olive leaf components, namely Api7G and Lut7G induces hHSCs differentiation towards erythroid lineage, thus, have potential of ex vivo generation of blood cells.

Table (1): Identified phenolic compounds of olive leaves by HPLC

Phenolic compounds	Concentrations (mg /g)
Gallic acid	2.35
Caffeic acid	1.15
Quercetin	0.40
Coumaric acid	1.53
Rutin	1.61
Protocatechuic acid	1.05
Oleuropein	48.70
Ferulic acid	0.03
Synergic acid	0.07

Table (2): Effect of olive leaves powder and its extract on glucose level of diabetic rats

Treatment/Parameter	Glucose level (mg/dl)
Control group (-)	96.50 ^{f} ±0.12
Control group (+)	275.00 ^{a} ±0.15
Diabetic rats with 2.5 % OLP	165.00 ^{b}.±0.10
Diabetic rats with 5 % OLP	131.00 ^{c} ±.013
Diabetic rats with OLE 1g/kg	112.50 ^{d} ±0.14
Diabetic rats with OLE 3g/kg	108.00 ^{e} ±0.11
LSD ($p \leq 0.05$)	3.85

OLP= Olive leaves powder OLE= Olive leaves extract

Each value is represented as mean ± standard deviation (n = 3).

Mean under the same column bearing different superscript letters are different significantly ($p \leq 0.05$).

Table (3): Effect of olive leaves powder and its extract on liver functions of diabetic rats

Treatment/Parameter	ALT (U/L)	AST (U/L)	ALP (U/L)
Control group (-)	12.50 e ±0.10	39.00 e ±0.15	102.00 e ±0.16
Control group (+)	20.50 a ±0.13	95.00 a ±0.11	135.00 a ±0.10
Diabetic rats with 2.5 % OLP	16.00 b ±0.14	56.00 d ±0.12	121.00 b ±0.10
Diabetic rats with 5 % OLP	14.00 d ±0.11	49.00 b ±0.13	118.00 c ±0.13
Diabetic rats with OLE 1g/kg	15.00 c ±0.16	46.00 c ±0.14	110.00 d ±0.15
Diabetic rats with OLE 3g/kg	12.00 e ±0.12	42.00 d ±0.10	104.00 e ±0.12
LSD ($p \leq 0.05$)	0.75	1.85	3.50

OLP= Olive leaves powder OLE= Olive leaves extract

Each value is represented as mean ± standard deviation (n = 3).

Mean under the same column bearing different superscript letters are different significantly ($p \leq 0.05$).

Table (4): Effect of olive leaves powder and its extract on lipid profile of diabetic rats

Treatment / Parameter	Total cholesterol (mg /dl)	Triglycerides (mg /dl)	HDL-c (mg /dl)	LDL-c (mg /dl)	VLDL-c (mg /dl)
Control group (-)	95.00 e ±0.11	71.00 d ±0.10	60.00 a ±0.13	20.80 f ±0.12	14.20 d ±0.12
Control group (+)	145.00 a ±0.10	105.00 a ±0.16	35.00 f ±0.11	89.00 a ±0.14	21.00 a ±0.14
Diabetic rats with 2.5 % OLP	130.00 b ±0.15	88.00 b ±0.12	41.00 e ±0.10	71.40 b ±0.10	17.60 b ±0.10
Diabetic rats with 5 % OLP	125.00 b ±0.13	82.00 c ±0.11	45.00 d ±0.12	63.60 c ±0.13	16.40 b ±0.13
Diabetic rats with OLE 1g/kg	115.00 c ±0.14	84.00 c ±0.10	50.00 c ±0.15	48.20 d ±0.11	16.80 b ±0.11
Diabetic rats with OLE 3g/kg	109.00 d ±0.12	79.00 d ±0.15	54.00 b ±0.16	39.20 e ±0.10	15.80 c ±0.10
LSD ($p \leq 0.05$)	5.42	3.46	3.10	4.10	1.25

OLP= Olive leaves powder OLE= Olive leaves extract

Each value is represented as mean ± standard deviation ($n = 3$).

Mean under the same column bearing different superscript letters are different significantly ($p \leq 0.05$).

Table (5): Effect of olive leaves powder and its extract on (kidney function) uric acid, urea and creatinine of diabetic rats

Treatment/Parameter	Uric acid (mg/dl)	Urea (mg/dl)	Creatinine (mg/dl)
Control group (-)	2.40 c ±0.10	23.00 e ±0.10	0.73 b ±0.10
Control group (+)	6.80 a ±0.13	59.00 a ±0.13	1.75 a ±0.13
Diabetic rats with 2.5 % OLP	4.10 b ±0.15	39.00 b ±0.10	1.25 a ±0.10
Diabetic rats with 5 % OLP	3.90 b ±.0.16	36.00 b ±0.15	1.01 a ±0.15
Diabetic rats with OLE 1g/kg	2.60 c ±.0.11	34.00 bc ±.0.16	0.92 a ±.0.16
Diabetic rats with OLE 3g/kg	2.50 c ±.0.11	29.00 d ±.0.11	0.84 ab ±.0.11
LSD ($p \leq 0.05$)	1.15	3.40	0.83

OLP= Olive leaves powder OLE= Olive leaves extract

Each value is represented as mean ± standard deviation (n = 3).

Mean under the same column bearing different superscript letters are different significantly ($p \leq 0.05$)

Table (6): Effect of olive leaves powder and its extract on serum total protein level of diabetic rats

Treatment/Parameter	Serum total protein level (mg/dl)
Control group (-)	6.90 a ±0.12
Control group (+)	3.70 d ±0.15
Diabetic rats with 2.5 % OLP	5.80 a ±.0.10
Diabetic rats with 5 % OLP	6.20 a ±.0.13
Diabetic rats with OLE 1g/kg	5.50 bc ±0.14
Diabetic rats with OLE 3g/kg	5.70 a ±0.11
LSD ($p \leq 0.05$)	1.25

OLP= Olive leaves powder OLE= Olive leaves extract

Each value is represented as mean ± standard deviation (n = 3).

Mean under the same column bearing different superscript letters are different significantly ($p \leq 0.05$).

Table (7): Effect of olive leaves powder and its extract on hematological level of diabetic rats

Treatment/Parameter	RBC 106/mm3	WBC 103/mm3	Hemoglobin g/dl	Platelet 106/mm3
Control group (-)	6.40 a ±0.12	10.50 a ±0.10	15.50 a ±0.11	147.00 e ±0.14
Control group (+)	3.50 b ±0.13	7.70 c ±0.14	10.40 d ±0.12	567.00 a ±0.16
Diabetic rats with 2.5 % OLP	6.10 a ±.0.10	10.70 a ±.0.12	12.60 bc ±.0.13	168.00 c ±.0.10
Diabetic rats with 5 % OLP	6.50 a ±.0.15	11.10 c ±.0.10	13.50 c ±0.15	154.00 d ±.0.13
Diabetic rats with OLE 1g/kg	4.90 c ±0.10	8.60 b ±0.12	13.90 b ±0.12	199.00 b ±0.10
Diabetic rats with OLE 3g/kg	4.60 c ±0.12	8.800 b ±0.14	15.00 a ±.0.15	185.00 c ±0.15
LSD ($P \leq 0.05$)	1.05	1.06	1.20	5.14

OLP= Olive leaves powder OLE= Olive leaves extract

Each value is represented as mean ± standard deviation (n = 3).

Mean under the same column bearing different superscript letters are different significantly ($p \leq 0.05$).

REFERENCES

Abd El-Rahman, H.S.M. (2016). The effect of olive leaf extract and A-Tocopherol on nephroprotective activity in rats. J. Nutr. Food Sci., 6: 479.

AIN (1993): American Institute of Nutrition purified diet for laboratory Rodent, Final Report. J. Nutrition, 123: 1939-1951 and O. Compactum Benth. J. Essential Oil Res., 8 (6): 657-664.

Al-Attar, A.M. and Alsalmi, F.A. (2017). Influence of olive leaves extract on hepatorenal injury in streptozotocin diabetic rats. Saudi J. Biol. Sci.

Al-Attar, A.M.; Fawziah, A. and Alsalmi, F.A. (2019). Effect of *Olea europaea* leaves extract on streptozotocin induced diabetes in male albino rats. Saudi Journal of Biological Sciences, (26): 118-128.

Allain, C.C. (1974). Cholesterol enzymatic colorimetric method. J. of Clin. Chem., 20: 470.

Ben Salah, M.; Hafedh, A. and Manef, A. (2019). Anti-diabetic activity and oxidative stress improvement of Tunisian Gerboui olive leaves extract on alloxan induced diabetic rats. Journal of Materials and Environmental Sciences, 8 (4): 1359-1364.

Boaz, M.; Leibovitz, E.; Dayan, Y.B. and Wainstein, J. (2011). Functional foods in the treatment of type 2 diabetes: Olive leaf extract, turmeric and fenugreek, a qualitative review. Fun Foods Health Dis., (1): 472-481.

Castelli, W. P.; Doyle, J. T.; Gordon, T.; Hames, C. G.; Hjortland, M. C.; Halley, S. B.; Kagan, A. and Zuckel W. J. (1977). HDL cholesterol and other lipids in coronary hean disease. The cooperative lipoprotein phenotyping study Circulation, (55): 767-772.

Cumaogˇlu, A.; Rackova, L.; Stefek, M.; Kartal, M.; Maechler, P. and Karasu, Ç. (2011). Effects of olive leaf polyphenols against H2O2 toxicity in insulin secreting bcells. Acta Biochim. Pol, (58): 45-50.

Decie, A. and Lewis, J. (1998). Practical Hematology. Churchill Livingstone, New York.

El-Amin, M.; Virk, P. and Elobeid, M.A. (2013). Anti-diabetic effect of *Murraya koenigii*, (L) and *Olea europaea*, (L) leaf

extracts on streptozotocin induced diabetic rats.J.Pharm.Sci., (26): 359-365.

Emordi, J.E.; Agbaje, E.O.; Oreagba, I.A. and Iribhogbe, O.I. (2016). Antidiabetic and hypolipidemic activities of hydroethanolic root extract of *Uvaria chamae* in streptozotocin induced diabetic albino rats. BMC Complement. Altern. Med., 16: 468.

Eydi, A.; Eydi, M.; Oryan, M.; Flahian, F. and Darzi, R. (2004). Hypoglycemic effect of olea extract in diabetic rats. Journal of Medicinal Plants, (12): 36-40.

Fossati, P. and Prencipe, L. (1982). Determination of serum triglycerides. Clin. Chem., 28: 2077.

Ghomari, O.; Sounni, F.; Massaoudi, Y.; Ghanam,J ; Kaitouni, L.; Merzouki, M. and Benlemlih, M. (2019). Phenolic profile (HPLC-UV) of olive leaves according to extraction procedure and assessment of antibacterial activity. Biotechnology Reports, (23): 1-7.

Ghorbani, A. (2013). Best herbs for managing diabetes: A review of clinical studies Braz. J. Pharm. Sci., (49): 413-422.

Goupy, P.; Hugues, M.; Boivin, P. and Amoit, M.J. (1999). Antioxidant composition and activity of barley (*Hordeum vulgare*) and malt extracts and of isolated phenolic compounds. J. Sci. Food Agric., (79): 1625-1634.

Hafkenscheid, J.C. (1979). Determination of GOT. Clin. Chem., 25:155.

Hamzeh, A.; Wireen, L. and Sana, H. (2017). Effect of olive leaf extract on glucose levels in diabetes induced rats: A systematic review and meta-analysis. Journal of Diabetes, (9): 947-957.

Henry, R.J. (1974). Clinical Chemist: Principels and Techniques. 2nd, Edition, Hagerstoun (MD), Harcer, ROW, 882.

Jamei, H.; El-Feki, A. and Sayadi, S. (2009). Antidiabetic and antioxidant effects of hydroxytyrosol and oluropein from olive leaves in alloxan–diabetic rats. J. of Agric. Food Chem., (14): 1275-1284.

Kaplan, L.A. (1984). Clinical Chemistry, the C.V. Mosby Co. St. Louis, Toronto. Princent., 1032-1036.

Laaboudi, W.A.; Ghanam, J.A.; Ghoumari, O.U.; Sounni, F.A.; Merzouki, M.O. and Benlemlih, M.O. (2016). Hypoglycemic and hypolipidemic effects of phenolic olive tree extract in streptozotocin diabetic rats. Int. J. Pharm. Pharm. Sci., (8): 287-291.

Lee, R. and Nieman, D. (1996). Nutrition Assessment. 2nd Ed. Mosby, Missouri, USA, 591 - 594.

Mestry, S.; Dhodi, J.; Kumbhar, S. and Juvekar, A. (2017). Attenuation of diabetic nephropathy in streptozotocin-induced diabetic rats by *Punica granatum*, Linn. leaves extract. J. Tradit. Complement. Med., 7 (3): 273-280.

Micol, V.; Caturla, N.; Perez-Fons, L.; Estepa, A.; Mas, V. and Perez, L. (2005). The olive leaf extract exhibits antiviral activity against viral haemorrhagic septicaemia rhabdovirus (VHSV) Antiviral Res. (66): 129-136.

Moghaddam, M.; Masomi, Y.; Razavian, M. and Moradi, M. (2013). The effect of oral consumption of olive leaves on serum glucose level and lipid profile of diabetic rats. Basic and Clinical Pathol., 1 (2): 39-44.

Moss, D.W. (1982). Alkaline phosphatase isoenzymes. Clin. Chem., (28): 2007-2016.

Mousa, H. M.; Farahna, M.; Ismail, M. S.; Al-Hassan, A. A.; Ammar, A. S. and Abdel-Salam, A. M. (2014). Anti-diabetic effect of olive leaves extract in alloxan-diabetic rats. Journal of Agricultural and Veterinary Sciences, 7 (2): 181-196.

Pari, L. and Saravanan, G. (2002). Antidiabetic effect of Cogent db, an herbal drug in alloxan-induced diabetes mellitus. Comparative Biochemistry and Physiology Part C, (131): 19-25.

Park, M.K.; Jung, U. and Roh, C. (2011). Fucoidan from marine brown algae inhibits lipid accumulation. Drugs, (9): 1359-1367.

Patton, C.J. and Crouch, S.R. (1977). Enzymatic determination of urea. J. of Anal. Chem., (49): 464-469.

Richmond, W. (1973). Preparation and properties of a cholesterol oxidase from *Nocardia sp*. and its application to the enzymatic assay of total cholesterol in serum. Clin. Chem., 19 (12): 1350.

Salam, R. (2014). Perioperative management of diabetes mellitus, 28(1): 4-8.

Samet, I.; Villareal, M.O.; Motojima, H.; Han, J.; Sayadi, S. and Isoda, H. (2015). Olive leaf components apigenin 7-glucoside and luteolin 7-glucoside direct human hematopoietic stem cell differentiation towards erythroid lineage. Differentiation, 89 (5): 146-155.

Sarbishegi, M.; Gorgich, E.A.C. and Khajavi, O. (2017). Olive leaves extract improved sperm quality and antioxidant status in the testis of rat exposed to rotenone.Nephrourol. Mon., 9 (3): 47:127.

SAS (1988). SAS Users Guide: Statistics Version 5th Ed. SAS. Institute Inc., Cary N.C.

Schermer (1967). The Blood Morphology of Laboratory Animal. Longmans, Printed in Great Britain, Green and Co. Ltd.,.350.

Schirmeister, J. (1964). Creatinine standard and measurement of serum creatinine with picric acid. Deutsche Medizinische Wochenschrift., (89): 1018-1021.

Soliman, G.A.; Saeedan, A.S.; Abdel-Rahman, R.F.; Ogaly, H.A.; Abd-Elsalam, R.M. and Abdel-Kader, M.S. (2019). Olive leaves extract attenuates type II diabetes mellitus-induced testicular damage in rats: Molecular and biochemical study. Saudi Pharmaceutical Journal, (27): 326-340.

While, B. A.; Erickson, M.M. and Steven, S.A. (1970). Chemistry for Medical Theologiests. 3rd Ed., C.V. Mosby Company Saint Louis, USA, 662.

World Health Organization (WHO) and International Diabetes Federation (IDF) (2006). Definition and Diagnosis of Diabetes Mellitus and Intermediate Hyperglycemia, WHO, Geneva.