

## Antioxidant and Antidiabetic Activities of the Methanolic Extract of *Olea europaea* L. Leaves in Streptozotocin Induced Diabetes in Rats

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### ABSTRACT

The present study was carried out to evaluate the effect of methanolic extract of *Olea europaea* L. leaves (MEO) on the antidiabetic and antioxidant activities in streptozotocin (STZ) induced diabetes in rats. Body weight, biochemical and histopathological changes were recorded. The results showed that MEO contained high polyphenolics and flavonoids contents with values of  $403.72 \pm 0.02$   $\mu\text{g}$  gallic acid equivalent/mg dry extract and  $52.54 \pm 0.018$   $\mu\text{g}$  quercetin equivalent/mg dry extract, respectively. Also, MEO showed a strong DPPH radical scavenging activity ( $\text{IC}_{50} = 0.040$  mg/ml), reducing power ( $\text{IC}_{50} = 0.093 \pm 0.014$  mg/ml) and inhibited the linoleic acid oxidation in ferric thiocyanate method with  $80.76 \pm 2.03\%$ . The administration of MEO at doses of 200 and 600 mg/kg increased catalase activity and GSH level and decreased lipid peroxidation, serum cholesterol and triglycerides levels. Furthermore, the histological damages in pancreas and liver tissues were reduced. These results indicated a good hypoglycemic and anti-oxidant activity of *Olea europaea* leaves. These results may explain its use in the folk medicine in the control of diabetes and preventing the diabetic complications by means of its antioxidant properties.

**Keywords:** *Olea europaea* L, antidiabetic, antioxidant, polyphenols

### INTRODUCTION

Diabetes mellitus is a chronic metabolic disorder of carbohydrates, proteins and fat metabolism which can be due to absolute or relative deficiency of insulin secretion or insulin resistance. It is characterised by high blood glucose level, which can cause various type of secondary complication associated with morbidity and mortality. The number of people suffering with diabetes worldwide is increasing at an alarming rate. It is predicated that, the number of diabetes person could reach up to 366 million by the year 2030<sup>1</sup>. Diabetes mellitus is mainly classified into: type 1 (T1D) and type 2 (T2D) diabetes mellitus. T1D involves the autoimmune destruction of insulin-producing pancreatic beta-cells via auto-aggressive T-cells and pancreatic macrophage infiltration<sup>2,3</sup>. Whereas, Type 2 diabetes mellitus (T2D) is the most common endocrine disorder worldwide, covering 90-95% of all diabetes cases. The classification and pathogenesis of T2D involves abnormalities in glucose and lipid metabolism, inadequate insulin secretion from pancreatic beta-cells and resistance to insulin activity<sup>4</sup>. The chronic hyperglycemia was found to increase the production of free radicals that is associated with long-term damage, dysfunction, and failure of various organs, especially the eyes, kidneys, nerves, heart, and blood vessels<sup>5,6</sup>. Free radical damage is one of the most prominent causes of devastating diseases that are responsible for killing many people in the world, such as

cardiovascular disease, which can manifest as heart attacks, and cancer<sup>7</sup>. Plants have always been an exemplary source of drugs and many of the currently available drugs have been derived directly or indirectly from them<sup>8</sup>. They produce an amazing diversity of secondary metabolites<sup>9</sup>. Among these are alkaloids, glycosides, galactomannan, polysaccharides, peptidoglycans, hypoglycans, guanidine, steroids, carbohydrates, glycopeptides, terpenoids, amino acids, inorganic ions and phenolic acid<sup>10,9</sup>. Over 4000 different flavonoids have been described as occurring in plants where polyphenol play role in conduction of water and also provide protection against biodegradation in plants<sup>11</sup>. The Olive tree (*Olea europaea* L. [Family: *Oleaceae*]) has been more than a thousand years. Not only the olive, but also the leaves have been used for medical purposes<sup>12</sup>. It appears among the plants in the past cultivated. The olive tree is mediterranean species cultivated in Algeria and the principal components of olive tree are : unsaturated fatty acids (acid oleic), protids, glucides, cellulose, flavones (oleoside, oleuro-peine), carotene, enzymes, many mineral elements (calcium, suffers, phosphorus), vitamins A, C ; whereas the sheets have tanins, triterpenes (mannitol), glucosides, saponins, lactone, choline, ole-astrol, oleoropine, leine<sup>13</sup>. It is well known that oleuropein and its derivatives such as hydroxytyrosol and tyrosol are the main phenolic constituents of olive leaves, which is

thought to be responsible for their pharmacological effects<sup>14,12</sup>. In many countries, they are known as a folk remedy for hypertension and diabetes<sup>12</sup> and as antiseptic, diuretic<sup>15</sup>. Like many natural herbs, olive leaves are also known to be an antioxidant and contain some of the most powerful known antioxidants<sup>16,17</sup>. The application of olive leaves leads to alleviation of disease symptoms<sup>18,19</sup>. There is little information about *in vivo* and *in vitro* effect of *Olea europaea L.* This work was undertaken to estimate the relationship between the antidiabetic activity and antioxidant activity with the contents of polyphenols in the methanolic extract of leaves.

## MATERIALS AND METHODS

### Plant material

For the period of sampling, spring is appointed because it is the season when the development and floristic diversity are maximum, in particular for the annual species<sup>20</sup>. So from this plant the parts used (leaves) were collected in April, 2014 and separated from the other parts and dried at room temperature in shadow and finely powdered. The powder was sieved through a 1 mm mesh to remove large fragments then was used for the extraction procedure.

### Extraction Procedure

The extract of plant was obtained following the extraction method described by Markham<sup>21</sup>. The powdered plant material (100g) was extracted with methanol (MeOH) (85% and 50%), at room temperature overnight. The MeOH fraction were combined and concentrated under reduced pressure on a rotary evaporator for obtaining methanolic extract of *Olea europaea L.* (MEO). Then, the extract were stored at -20 °C until use.

### Animals

Healthy male adult albino rats weighing 170–280 g were obtained from Pasteur Institute Algiers. They were housed in an air-conditioned animal room, with light/dark cycle photoperiod, and given free access to water and feeding *ad libitum*

### *In vitro* antioxidant activity

#### Determination of total flavonoid contents

The total flavonoid content in methanolic extract was determined using the method of Bahorun *et al*<sup>22</sup>. 1 ml of sample was mixed with 1 ml of aluminium chloride (AlCl<sub>3</sub>) solution (2% in methanol). After 10 min, the absorbance of the mixture was measured at 430 nm against methanol blank. Results were expressed as equivalent quercetin (mg quercetin /g dried extract).

#### Determination of total polyphenols

Total polyphenols were measured using Prussian blue assay described by Li *et al*<sup>23</sup> with slight modifications. Briefly, 100 µ L of methanolic extract of the plant (MEO) were mixed with 500 µ l folin-ciocalteu (2M) diluted 10 times. Then, 400 µ l of sodium carbonate (Na<sub>2</sub>CO<sub>3</sub>) with concentration of 7.5g/ 100ml were added. The absorbance was measured at 765 nm, after incubation for 1 hour and 30 min at ambient temperature against blank. Phenols were expressed as gallic acid equivalents (µg gallic acid/mg dried extract).

#### DPPH radical scavenging assay

The experiment was carried out according to the method described by Burits and Bucar<sup>24</sup>. 50µl of various concentrations of the extract were added to 5ml solution of the DPPH of concentration 0,004%. After 30 minutes of incubation at ambient temperature and in the darkness, the absorbance is read with a wavelength at 517nm. Inhibition of free radical DPPH in percent (I%) was calculated in following way:  $I\% = (A_{control} - A_{sample}) / A_{control} \times 100$  Where,  $A_{control}$  is the absorbance of the blank solution (containing all reagents except the test compound) and  $A_{sample}$  is the absorbance of the test compound. Extract concentration providing 50% inhibition (IC<sub>50</sub>) was calculated from the plot of inhibition percentage against extract concentration.

#### Reducing power

The reducing power of MEO was measured (dissolved in various organic solvents) using the method of Chung *et al*<sup>25</sup>. Briefly, 0.1 ml of various concentrations of the sample were mixed with the same volume of phosphate buffer (0.2 M, pH 6.6 =) and 0.1 ml of K<sub>3</sub>FeCN<sub>6</sub>. The mixture was incubated for 20 min at 50 ° C for the reduction of ferricyanide to ferrocyanide, after that, 0.25 ml (1%) of acid trichloroacetic were added to stop the reaction and then centrifuged process underway quickly cycle 3000 /minutes for 10 minutes. Then, 0.25 ml of the supernatant, were added to 0.25 ml of distilled water and 0.5 ml FeCl<sub>3</sub> (0.1%), and the absorbance was read at a wavelength of 700 nm to estimate the amount of ferric ferrocyanide formed (Prussian blue) by using a spectrophotometer.

#### Ferric thiocyanate (FTC) method

A modified method of Yen *et al*<sup>26</sup> was adopted for the FTC assay. In this method, the concentration of peroxide decreases as the antioxidant activity increases. 2.5 ml of 0.02M linoleic acid emulsion [pH=7; the emulsion prepared by 0.284 mg of linoleic acid plus 0.2804 g of Tween 20 and 50 ml of phosphate buffer] and 2 ml of 0.2 M phosphate buffer (pH= 7) were added to 0.5 ml of extract sample. The incubation was during five days. After that, 0.1 ml of the reaction mixture at 24 h intervals was added to 4.7ml (75% ethanol), 0.1 ml of ammonium thiocyanate (30%), and 0.1ml ferrous chloride (0.02 M in 3.5% HCl). After 3 min, the absorbance was measured at 500 nm. Each 24 h, the absorbance was taken until the absorbance of the control reached its maximum value. BHT was used as a positive control.

#### Induction of experimental diabetes

Diabetes was induced using the method described by Adolfo Andrade Cetto *et al*<sup>27</sup> with slight modifications; by a single intraperitoneal injection of a freshly prepared streptozotocin (STZ) solution (50 mg/kg in cold sodium nitrate (0.9%) to overnight fasted rats. Animals, which did not develop more than 250 mg/dl glucose levels, were rejected. The diabetic animals were classified into five groups, each of them with six rats. Group 1 as a control were administered 1 mL distilled water, group 2 received MEO at dose of 200mg/kg (200mg/kg+STZ) while group 3 received MEO at dose of 600mg/kg (600mg/kg +STZ), group 4 was given a standard oral hypoglycemic agent, glibenclamide or Glibil (3mg/kg) (Glibil+STZ) whereas

group 5 did not receive any thing or untreated group (Untreated).

#### *Effect of Olea europaea leaves methanolic extract on glucose loaded rats*

This study was performed on overnight fasting (14 h) in all five groups. Blood glucose level was measured after injection of SZT at 0, 60, 120, 180 and 24h min after to access the effect of extract on blood glucose levels. Blood glucose levels were determined by the glucose oxidase method using a reflective glucometer (ACCU-CHEK, Fast Clix, Germany). The bodyweight of the rats were also measured during the experiment.

#### *Blood collection and analytical methods*

After 18 days of treatment, rats were sacrificed. Blood was collected into 2 tubes: the first tubes, containing heparin and the second tubes containing EDTA, blood in tubes containing heparin was used to obtain the plasma which was separated by centrifugation (15 min, 3000rpm) and was separated into two one portion was analysed for: Triglycerides, total cholesterol, TGO, TGP levels using an automate apparatus and the second was used to assess the reducing power and DPPH assays. Whereas the tubes which containing EDTA was used to analyse the hematological parameters.

#### *Plasma antioxidant capacity using DPPH radical*

According to the method of Burits and Bucar<sup>24</sup> with some modifications, we the capacity of plasma to trap the DPPH radical was evaluated, this has the same principle as the test of DPPH *in vitro*. In brief, a volume of plasma was added to a solution of the DPPH (0.004%). After incubation for 30 min in the darkness followed by a centrifugation, the absorbance was measured at 517 nm and the plasmatic antioxydant capacity was calculated as follows:

% scavenging activity = [(Abs control-Abs sample)/Abs controls] X 100

A control: is the absorbance of the blank solution

A sample: is the absorbance in the presence of the test compound.

#### *2.7.2. Assessment of plasma reducing power*

According to the method of Chung *et al*<sup>25</sup>, the reducing power was determined. Briefly, 0.1 ml of plasma was mixed with 0.1 ml of sodium phosphate buffer (0.2 M, pH 6.6) and 0.1 ml of potassium ferricyanide (1 %). We incubated the mixture for 20 min at 50°C. After that, 0.250 ml of trichloroacetic (1%) was added. Then, the mixture was centrifuged for 10 min at 3000 rpm. An aliquot (0.250 ml) of the upper layer was mixed with 0.250 ml of distilled water and 0.5 ml of ferric chloride (0.1%), and the absorbance at 700 nm was measured. A higher absorbance indicated a higher reducing power.

#### *Histological sections*

The pancreas was rapidly removed to avoid its autolysis that occurs after a few moments of death of the animal also liver kidney was removed, put in formalin then removed from it and embedded in paraffin wax where finally cut into 1cm. The slides were selected from each group and were examined under light microscope by a histopathologist.

#### *Preparation of homogenate*

4.5 ml (1.15 M KCl buffer) were added to 0.5g of liver tissues, and homogenized using dounce homogenizer in ice-cold condition. The homogenate thus obtained was centrifuged at 4000 rpm for 15 min and the supernatant collected was used for the determination of lipid peroxidation (MDA), catalase, reduced glutathione(GSH) and total protein were assayed.

#### *Determination of total protein*

Protein concentration was measured by the method of Gornall *et al*<sup>28</sup> using the Biuret reagent and bovine serum albumin as a standard. Briefely, 1 ml biuret reagent was mixed with 25 µl sample or standard (albumin), and the absorbance of mixture was measured after 10 min of incubation at 37 °C at 540nm. Total protein level was calculated as follows:

Total protein (mg/ml) = (Abs of sample / Abs of standard) × n

Where n is standard concentration

#### *Determination of catalase activity*

Catalase activity was assayed according to Clairborne<sup>29</sup>. So, 2950 µl of 19 mM H<sub>2</sub>O<sub>2</sub> prepared in 0.1M phosphate buffer (pH 7.4) were added to 50 µl of each supernatant. The rate of the breakdown of H<sub>2</sub>O<sub>2</sub> by the catalase was measured spectrophotometrically in an interval of two minutes time against a blank at 240 nm. Catalase activity was determined as µmole/min/mg protein.

#### *Determination of lipid peroxidation levels*

MDA levels were measured according to Ohkawa *et al*<sup>30</sup>. Briefly, 0.5 ml of each homogenate were mixed with 0.5 ml of TCA (20 % w/v) and 1ml of TBA (0.67 % w/v). After that, the mixture was boiled in a water bath for 15 min. Leave to cool in a room temperature, and 4 ml of n-butanol were added to each sample and centrifuged at 3000 rpm for 15 min. The absorbance was measured at 532 nm with respect to the blank solution. The concentration of MDA was determined from a standard curve of 1,1,3,3 tetraethoxypropane in the same conditions and it was expressed as nmol/ g tissue.

#### *Determination of reduced glutathione (GSH)*

Total reduced glutathione (GSH) content was measured following the method of Ellman's<sup>31</sup>. 50 µl of supernatant were diluted in 10 ml phosphate buffer (0.1 M, pH 8). After dilution, 3 ml of the mixture were added to 20µl of DTNB (0.01 M). The absorbance was read at 412 nm after 5 min. A series of standards GSH were treated in a similar manner along with blank. Reduced glutathione was expressed as µmol/ g tissue.

#### *Statistical analysis*

Statistical analysis was performed using the Graph Pad Prism (version 5.01 for Windows). All *in vitro* results were calculated as mean ± SD and were analyzed by One-way analysis of ANOVA followed by Tukey's test. Whereas *in vivo* were calculated as mean ± S.E.M and were analysed by Student t-test.

## **RESULTS**

### *In vitro antioxidant activity*

#### *Total polyphenol and flavonoid contents of Olea europaea L.*

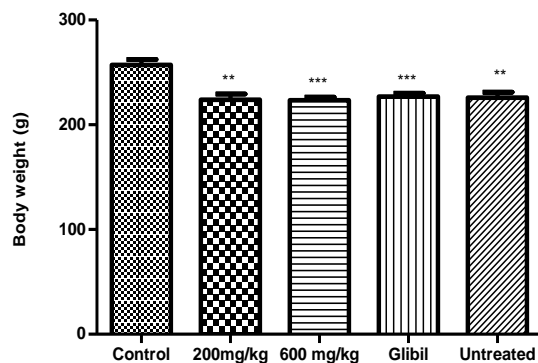


Figure 1: Change in mean body weight (g) of the 5 groups of rats in function of time. means  $\pm$  SD. (\*\*\*:  $P < 0.001$ , \*\*:  $P < 0.01$ ).

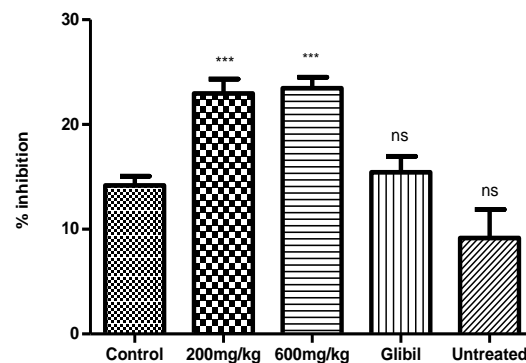


Figure 2: Comparison between different groups in plasma antioxidant capacity using DPPH radical. Data were presented as % means  $\pm$  SEM ( $n = 6$ ). (ns : no significant difference ; \*\*  $p < 0.01$ ) compared to control group.

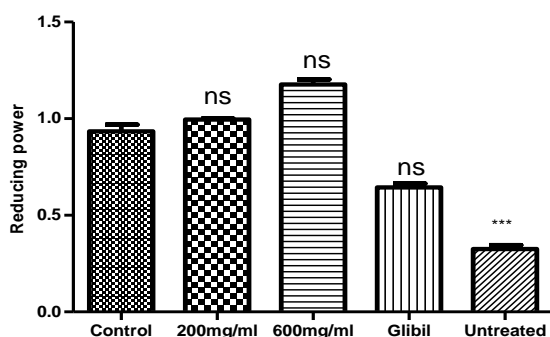


Figure 3: A comparison between different plant extracts in plasma reducing power. Data were presented as means  $\pm$  SEM ( $n = 6$ ). (ns : no significant difference ; \*  $p < 0.05$ ; \*\*\*  $p < 0.001$ ) compared to control group.

Total phenolics content of MEO was estimated as  $403.72 \pm 0.022$   $\mu\text{g}$  gallic acid equivalent /mg of extract whereas total flavonoids in the extract  $52.54 \pm 0.018$   $\mu\text{g}$  quercetin equivalent /mg extract.

#### DPPH scavenging activity of MEO

The DPPH free radical method determined the antiradical power of antioxidants. Regarding the  $IC_{50}$  values, the MEO and the commercial standards (BHT, Gallic acid, Quercetin, Rutin) depleted the initial DPPH concentration by 50% within 30min. The lower  $IC_{50}$  value is the higher of free radical scavenging activity of a sample. The free radical scavenging activities of MEO had the lowest free radical scavenging activity with the value of  $0.04 \pm 0.0001$  mg/ml. The effect of MEO show significant ( $p < 0.001$ ) and the scavenging activity decreased in the order of MEO > BHT > Rutin > Quercetin > Gallic acid.

#### Reducing power of MEO

MEO shows a dose-response curve for the reducing powers. Reducing power of MEO and standard compound (BHT) decreased in the following order: BHT < MEO.

#### Ferric thiocyanate (FTC) method

MEO exhibited good antioxidant potential with percent inhibition of ( $80.76 \pm 2.03\%$ ) as compared with BHT ( $89.18 \pm 1.88\%$ ). The result indicated that MEO exerted a

marked effect on inhibition of linoleic acid oxidation, which was as strong as the positive reference, BHT.

#### In vivo antioxidant and antidiabetic activities

##### Effect of Olea europaea leaves methanolic extract on glucose loaded rats

As shown in table1, an increase in blood glucose level was recorded in the first hour after treatment after the treatment with the two doses of 200 mg/kg and 60 mg/kg of MEO (4.53 to 4.75g/dl and 4.33 to 4.52 g/dl) and corrected at the second hour (from 4.75 to 3.08 g /dl and 4.52 to 3.94 g/dl). A diminution in blood level was noted after 24 h to stabilize around 3.20 and 3g/dl respectively for the both dose. Whereas, untreated rats showed no change in their blood level ( $> 6$  g/dl). While the rats treated with Glibinclamide showed an increase until the 2<sup>nd</sup> h and then decreased where it regulated after 24h to become 3.98 g/dl.

##### Body weight evolution of rats

The results obtained in this study showed that injection of STZ-induced significant loss of body weight in all groups of rats (\*\*\*:  $P < 0.001$ , \*\*:  $P < 0.01$ ) when compared to the control group ( $257.14 \pm 10.20$  g).

##### Blood analysis

##### Haematological analysis

As shown in table 2, not significant high levels of cholesterol, triglycerides and ALT, AST were observed in untreated rats ( $0.67 \pm 0.09$  g/l;  $1.67 \pm 0.25$  g/l;  $79.3 \pm 0.46$  UI/l and  $135.4 \pm 0.69$  UI/l) respectively when compared with control group. However, the rats treated with MEO at both doses (200 mg/kg and 600 mg/kg) showed a decrease in total cholesterol, triglycerides, ALT and AST while the group treated with Glibil showed approximately the same effect as group treated with 600 mg/kg. The olive leaves extract (at two doses: 200 mg/kg and 600 mg/kg) was found to be more effective than glibenclamide.

##### Plasma antioxidant capacity using DPPH radical

In this study, the obtained results (Figure 2) showed that the administration of MEO at doses of 200 and 600 mg/Kg in rats increased significantly the plasma antioxidant capacity with values of ( $33.46 \pm 17.50$  % and  $23.46 \pm 1.96\%$ , respectively) which were significantly higher than

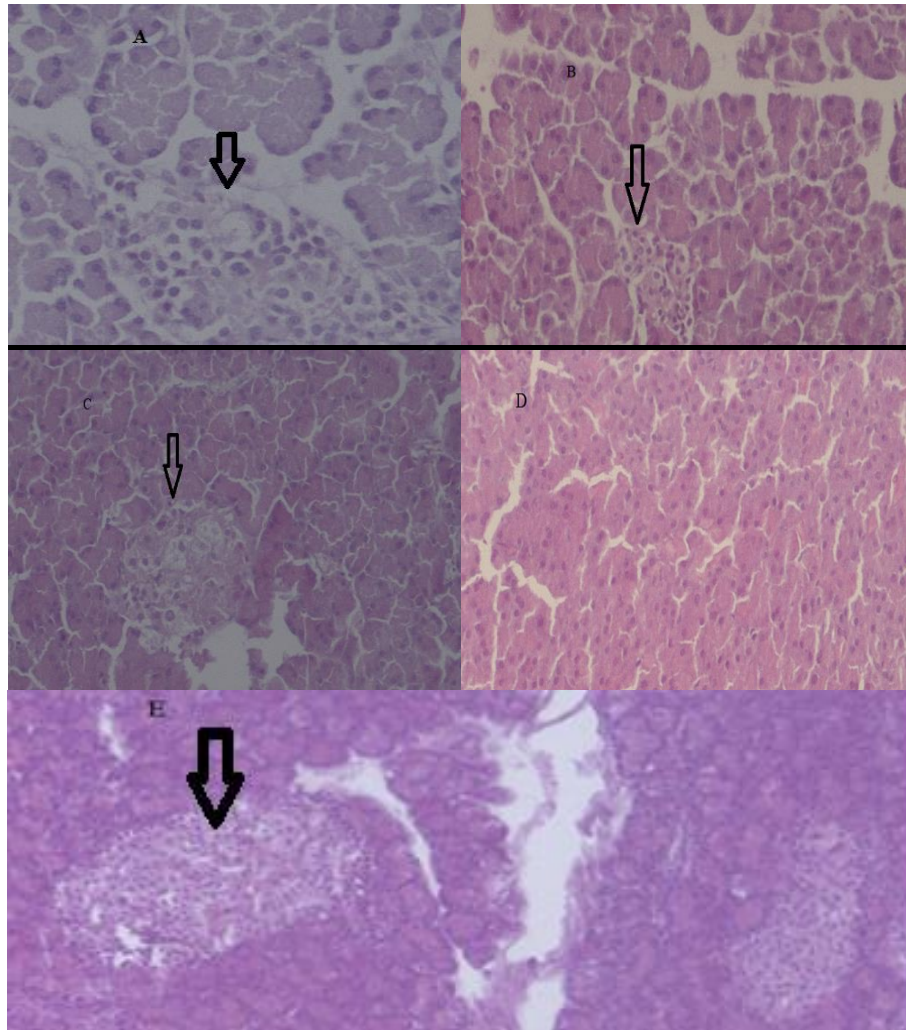


Figure 4: Microphotographs of pancreatic islets in rats treated with MEO after 18 days of the treatment. Severe islet cell necrosis is observed, X20. A: IL infiltrated by lymphocytes from the group (200mg/kg+STZ). B: severe inflammatory cells infiltration from group (600mg/kg+STZ). C: IL infiltrated by lymphocytes group (Glibil+STZ). D: Absence of IL (Untreated group). E : Normal IL (Control group).

that of control group ( $14.19 \pm 1.57\%$ ). On the other hand, the group treated with Glibil and untreated groups showed low scavenger activity compared to control group but this decrease is still statistically not significant.

#### Assessment of plasma reducing power

All the groups doses showed electron donation capacity, dose dependently. However, in rats that were orally treated with Glibil (3 mg/kg) their plasma reducing capacity values were  $0.64 \pm 0.01$  these values did not show any important alteration in plasma reducing power (no significant difference,  $p > 0.05$ ) when compared to control and positive groups which had values of  $0.93 \pm 0.03$  and  $0.32 \pm 0.03$ , respectively. Furthermore, the administration of MEO at two doses 200 and 600mg/kg showed significant difference ( $*** p < 0.001$ ) with the values of  $0.99 \pm 0.005$  and  $1.17 \pm 0.02$ , respectively.

#### Histological studies

As shown in figure 4, no histological changes showed in pancreatic tissue of control group (figure 4-E), however the pancreas were disappeared in groups (figure 4: A and D) were the destruction of Islets of Langerhans. Whereas, sections of pancreas from the treated animals groups

(figure 4: B and C) showed steaky inflammation by lymphocytes in the Islets region. The examination of the livers sections showed no change in the hepatocytes architecture except the sinusoidal spaces and portal veins which were congested in all the groups. When compared to control group, focal lymphocyte cells infiltration was abundant in untreated animals.

#### Effect of MEO on hepatic antioxidant status

##### Determination of total protein

In this study, the administration of the both doses of MEO and untreated rats showed a significant decrease (are all significant ( $P < 0.001$ , or  $P < 0.01$ ) on total proteins in liver. As seen in Figure 6, the total protein for MEO at doses of 200 mg/kg and 600 mg/kg were found to be ( $11.70 \pm 2.92$  mg/ml,  $13.79 \pm 1.46$  mg/ml, respectively) in liver.

##### Determination of catalase activity

*In vivo* effects of MEO on catalase activity in liver of rats were evaluated and shown in Figure 7. It was observed that the treatment at doses of 200 and 600 mg/kg exhibited increased activity of catalase in liver, but this increase didn't reach statistically significant difference ( $p > 0.05$ ) when compared to control group



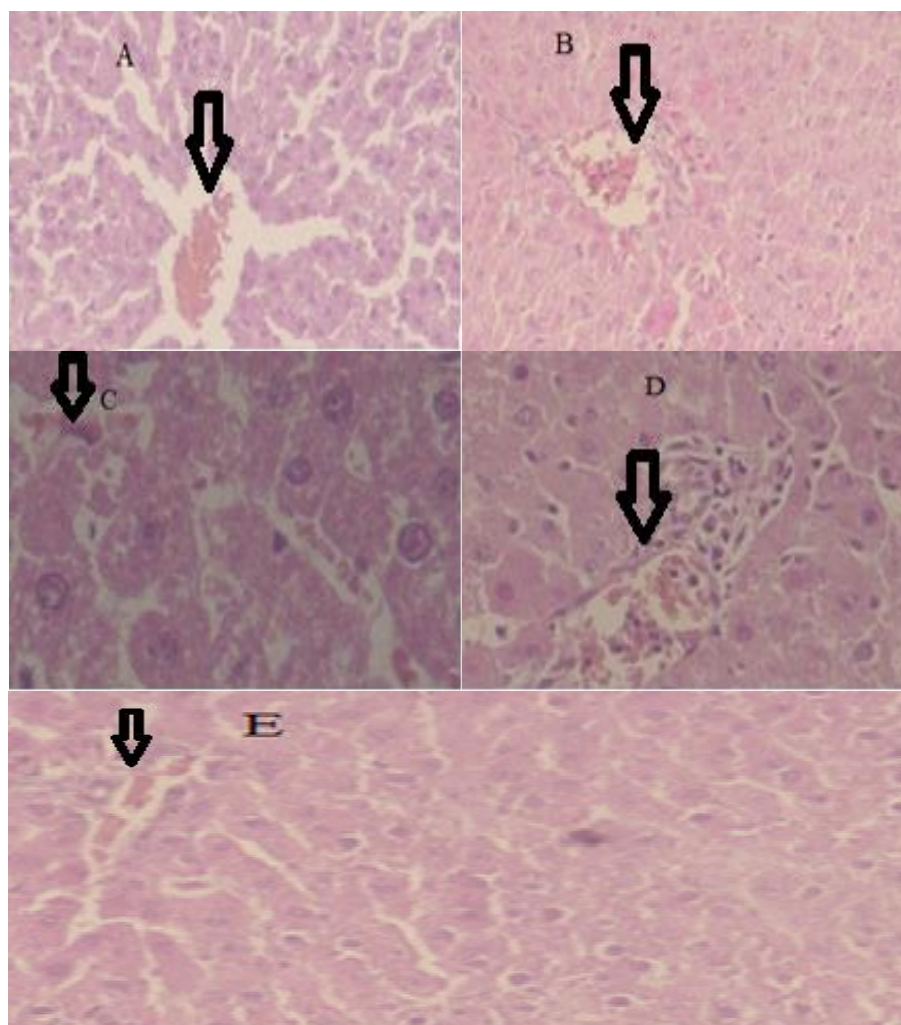


Figure 5: Photomicrographs of liver sections, X 10. A: normal hepatocytes showing normal architecture congestion in VCL (200mg/kg +STZ group). B: Congestion in portal vein (600mg/kg +STZ group). C: Congestion in VCL (Glibil +STZ group). D: lymphocyte infiltration (Untreated group). E: normal hepatocytes congested (Control group).

( $0.014 \pm 0.005 \mu\text{mol}/\text{min}/\text{mg}$  protein;  $0.01 \pm 0.0060 \mu\text{mol}/\text{min}/\text{mg}$  protein, respectively).

#### Determination of reduced glutathione (GSH)

MEO increased the level of GSH in liver ( $71.78 \pm 4.74 \mu\text{mol}/\text{g}$  tissue;  $74.61 \pm 1.35 \mu\text{mol}/\text{g}$  tissue) compared to control ( $53.36 \pm 2.52 \mu\text{mol}/\text{g}$  tissue).

#### Determination of MDA level

As shown in the figure 9, MEO at two doses of 200 and 600mg/kg, Glibil group decreased MDA formation in liver with level of  $41.40 \pm 3.82 \text{ nmol}/\text{g}$  tissue;  $35.98 \pm 1.40 \text{ nmol}/\text{g}$  tissue;  $39.95 \pm 4.79 \text{ nmol}/\text{g}$  tissue, respectively but this decrease was statistically not significant when compared to control group ( $38.62 \pm 9.16$ ) whereas, untreated groups showed an increase of the MDA level ( $202.38 \pm 2.38 \text{ nmol}/\text{g}$  tissue)

## DISCUSSION

In recent years, scientists have focused on the preventive effects of phenols against degenerative diseases mediated by the ROS. It has been reported that the phenolic compounds are able to interact with the biological systems and as bioactive molecules. They are particularly important inhibitors of lipid peroxidation<sup>32</sup>. In the present

study, we evaluated the antidiabetic effect of MEO in Sterptocytosin induced diabetic in rats and by three *in vitro* assays to evaluate antioxidant potency and assessment of the contents of phenolic compounds, the radical scavenging activity and the relationship between both of activities. MEO contains high level of phenolic compounds higher than that reported by Dekanski *et al*<sup>33</sup> who found that total phenols content was  $197.8 \mu\text{g AE}/\text{g}$  of dry extract. Total flavonoids and tannins content were 0.29 % and 0.52 % respectively. One similar study was performed by Abaza *et al*<sup>34</sup>, who extracted the phenolic compounds from olive leaves. They found a total phenolic content ( $24.36 \pm 0.85 \text{ mg GAE}/\text{g}$  dry weight). Olive flavonoids, phenols and oleuropeosides have been shown to possess an important antioxidant activity towards free radicals, which is principally based on the redox properties of their phenolic hydroxyl groups and the structural relationships between different parts of their chemical structure<sup>35</sup>. The redox properties of antioxidants play an important role in absorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides<sup>36</sup>. The DPPH free radical method determines the

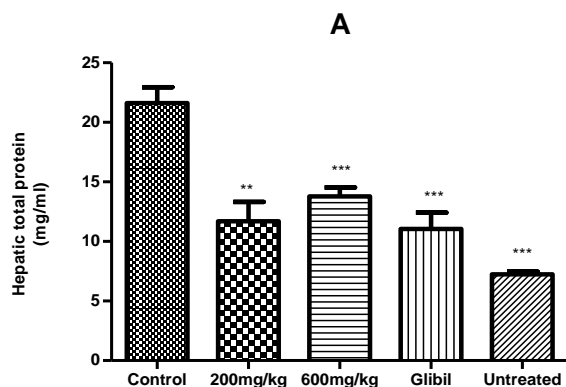


Figure 6: Effect of methanolic extract of *O.Europea* on total proteins level in the liver (A) of rats. Values are means  $\pm$  SEM (n=6). (\*\*\* :  $P < 0.001$ , \*\* :  $P < 0.01$ ).

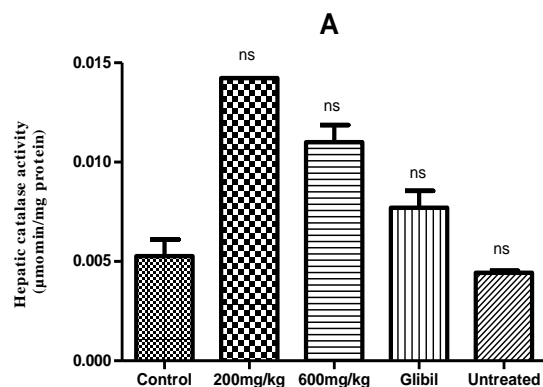


Figure 7: Effect of methanolic extract of *O. Europea* on catalase activity in liver (A) of rats. Values are given as means  $\pm$  SEM (n=6). (ns: no significant difference,  $p > 0.05$ ).

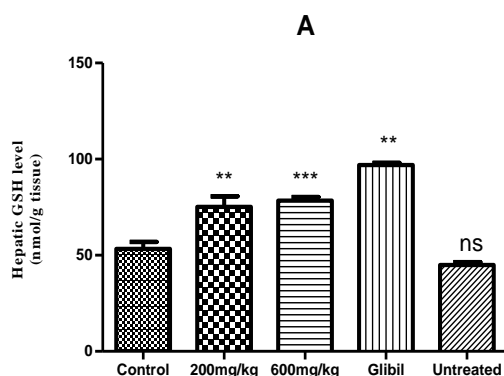


Figure 8: Effect of the methanolic extract of *O. Europea* on reduced glutathione level in liver (A) of rats. Values are given as means  $\pm$  SEM (n=6). (ns : no significance difference,  $P > 0.05$  ; \*\*\* :  $P < 0.001$ , \*\* :  $P < 0.01$ ).

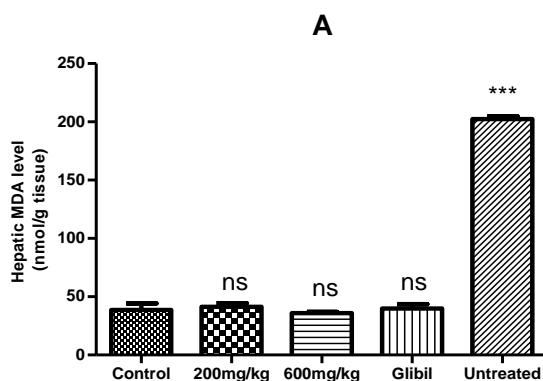


Figure 9: Effect of the methanolic extract of *O. Europea* on MDA level in liver (A) of rats. Values are given as means  $\pm$  SEM (n=6). (ns : no significant difference,  $p > 0.05$  ; \* :  $P < 0.01$ ).

antiradical power of antioxidants, they donate hydrogen to the free radicals so that the radicals remove the odd electron to turn to unreactive ones<sup>37</sup>. An antioxidant activity profiles showed that MEO had an antioxidant activity. Moreover a high correlation was showed between radical scavenging activity and total phenolic content by HPLC analyses of olive leaves ( $r = -0.9525$ ;  $< 0.05$ )<sup>38</sup>. A number of studies reported that a relation exists between the antioxidant activity and the reducing power<sup>39-41</sup>. The reduction of  $Fe^{+3}$  reagents is considered an important mechanism in the antioxidant activity of phenolic compounds<sup>42</sup>. Decker *et al* revealed that methanolic extracts from olive leaves showed a promising result even used at great extract concentration<sup>42</sup>. One of the indicators of antioxidant activity is an ability to inhibit self-oxygenation of unsaturated fatty acid<sup>43</sup>. The FTC method used to determine the amount of peroxide at the initial stage of lipid peroxidation. The peroxide reacts with ferrous chloride ( $FeCl_2$ ) to form a reddish ferric chloride ( $FeCl_3$ ) pigment<sup>44</sup>. In this method, the concentration of peroxide decreases as the antioxidant activity increases. The highest percent inhibition was shown in MEO means that MEO had a good antioxidant activity that is very

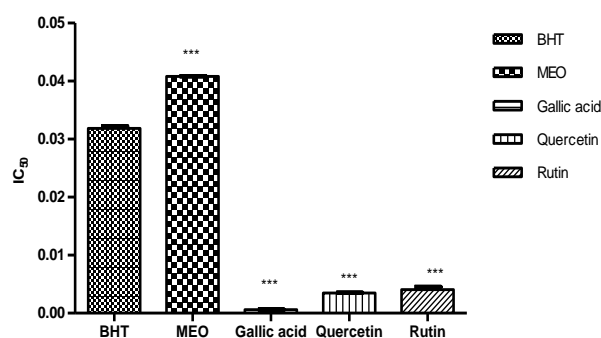


Figure 10: IC<sub>50</sub> values of methanolic extract of *Olea europaea L.* determined by DPPH assay. Bares are mean  $\pm$  SD (\*\*\* :  $P < 0.001$ ).

probably attributed to their high phenolic compounds and flavonoids. Further more, flavonoids are known to be related to antioxidant activity<sup>45-47</sup>. Additionally, methanolic extract have been reported to contain higher concentration of flavonoids<sup>45,46</sup>. Streptozotocin (STZ, 2-deoxy-2-(3-methyl-3-nitrosoureldo)-Dglucopyranose), is

Table 1: The Mean values of glucose.

Hours	Control (a)	200mg/kg	600mg/kg	Glibil	Untreated
0	1.19±0.13	4.53 ± 0.34***	4.33 ± 1.05***	4.13 ± 1.05**	HI***
1h	1.27 ± 0.15	4.75 ± 0.83***	4.33 ± 1.05***	4.31±0.87***	HI***
2h	1.32 ± 0.17	3.08 ± 1.88***	3.94 ± 1.35***	4.70 ± 1 ***	HI***
3h	1.41 ± 0.10	3.13 ± 1.42***	2.74 ± 1.54 ***	4.6± 0.35***	HI***
24h	1.30 ± 0.12	2.97±0.83**	2.73±0.76*	4.29±0.55***	HI***
10 days after	1.11 ± 0.09	3.20±0.15*	3 ± 2.05*	3.98± 1.74**	HI***

(a): dl/l. Value are mean ± SEM (n=6) (\*\*\*: P<0.001).

synthesized by *Streptococcus achroinogenes* and has long been used to generate animal models of diabetes<sup>48</sup> which leads to degeneration of the  $\beta$  cells of the islets of Langerhans. In this study, clinically the symptoms of diabetes are clearly observed after 48 h of injection of STZ as reported by Aldo *et al*<sup>49</sup>. STZ has acute toxic effects on many organs<sup>50,51</sup> as well as carcinogenic ones in pancreas, liver and kidney<sup>52-54</sup>. So, the STZ causes partial selective destruction of  $\beta$  cells of the islets of Langerhans and was identified by polydipsia and polyuria and loss in body weight. It induces in three phases response: acute elevation of blood glucose between the first and second hour (related glycogenolyse intense stress), then a deep hypoglycemia 7<sup>th</sup> to 10<sup>th</sup> hours (release of insulin by the  $\beta$  cells lysis way) and sustainable diabetes mellitus; resulting chronic hyperglycemia and altered lipid and protein metabolism, resulting from a defect in insulin secretion<sup>55</sup>. Diabetes has been associated with an increased generation of oxygen-derived free radicals through autoxidation of glucose<sup>56</sup>. According to the results above, the high-dose treatment induces a more rapid loss of insulin secretion (and therefore more rapid onset of diabetes). It was found that the two doses (200 mg/kg and 600 mg/kg) had a remarkable effect on glucose uptake by cells after 24h when compared with the standard drug Glibenclamide but the higher dose of 600 mg/kg of MEO showed the highest hypoglycemic effect in induced hyperglycemia in rats even at 24h and after 10 days of treatment. The antidiabetic effect of the extract was more effective than that observed with glibenclamide. The observed effect was comparable to a previous study conducted on diabetic rats<sup>57</sup> which 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 serum total cholesterol, triglycerides while it increased the serum insulin in diabetic rats but not in normal rats ( $p < 0.05$ ). Muhammad *et al*<sup>58</sup> showed that the aminotransferases (AST and ALT) levels were significantly increased in the kidney of STZ-treated animals. The increase in aminotransferases levels may be due to the cellular damage in the kidney caused by STZ-induced diabetes. In agreement with the present results, a few reports are available on the hypoglycemic effects of the leaves of *Olea europaea*<sup>59-61</sup>. One of the compounds responsible for this activity is oleuropeoside. The hypoglycemic activity of this compound may result from two mechanisms: (a) potentiating of glucose-induced insulin release, and (b) increased peripheral uptake of glucose<sup>62</sup>. Streptozotocin selectively destroys pancreatic insulin secreting  $\beta$ -cells<sup>63</sup>. In this study, histological sections revealed a severe islet cell necrosis by the

lymphocyte. MEO may act on the regenerative of the pancreatic cells via exocrine cells which lead to the positive effects of its compounds on the production of insulin. Liver and kidney are important organs of storage, detoxification, metabolism, and excretion of many metabolites, so they are particularly vulnerable to oxidative damage<sup>64</sup>. Infiltration of lymphocytes and congestion were observed in the sinusoidal spaces and portal veins that increased with the increase in streptozotocin-induced hyperglycemia or in untreated group. These findings of present study are in agreement with the findings of Ramesh *et al*<sup>65</sup> and as Muhammad *et al*<sup>58</sup> mentioned. The use of MEO for the treatment of diabetic rats, reduced the histological changes in the tissues, this may be due to the presence of phenolic in this plant. In the STZ-induced diabetic animals, lower levels of activities of endogenous antioxidant enzymes such as SOD and CAT were shown. Subsequently, these reductions can cause tissue degradations<sup>66</sup>. Chronic hyperglycemia induces carbonyl stress, which in turn can lead to increased lipid peroxidation. As mentioned above, there was a slight significant alteration of hepatic proteins level and this may be due to the protective and safety effects of the administered plant extracts on hepatic tissues. In addition, *O. europea* extract inhibits lipid peroxidation and induces the activity of antioxidant enzymes such as catalase, and GSH. In this study, MEO administered at dose of 200 and 600 mg/kg increased significantly the hepatic reduced glutathione and catalase activity, where the increase in catalase which was not found significant ( $p > 0.05$ ). However, there was a decrease in MDA level for the both doses of MEO. Although, there is controversy about the antioxidant status in diabetes, in several studies have reported decreased plasma or tissue concentrations of superoxide dismutase, catalase, glutathione and ascorbic acid in both clinical and experimental diabetes<sup>67</sup>. These observed results may be due to the high polyphenolic and flavonoids contents of the plant rich extract and could be due to the increase in the antioxidant status, resulting in an increase in the activity of catalase and concentration of the GSH.

## CONCLUSION

As conclusion, MEO of the leaves had high content of total polyphenols and flavonoids and also an antihyperglycemic effect. This suggests that the MEO play a protective role from any alteration of tissues and corrected the blood glucose in diabetic rats. Data of this study indicates that *Olea europea* can either increase antioxidant power or reduce the oxidative stress or both. These results provide



Table 2: The Mean values of serum triglycerides, total cholesterol, AST, ALT .

Groups	Triglycerides (g/l)	Total cholesterols (g/l)	AST (UI/l)	ALT (UI/l)
Control	1.26 ± 0.28	0.53 ± 0.02	128.06 ± 0.21	51.48 ± 0.93
200mg/kg	1.26 ± 0.33 <sup>ns</sup>	0.54 ± 0.06 <sup>ns</sup>	134.5 ± 0.32 <sup>ns</sup>	51.65 ± 0.21 <sup>ns</sup>
600mg/kg	1.29 ± 1.07 <sup>ns</sup>	0.58 ± 0.08 <sup>ns</sup>	129.33 ± 0.31 <sup>ns</sup>	56.35 ± 0.91 <sup>ns</sup>
Glibil	1.46 ± 0.25 <sup>ns</sup>	0.61 ± 0.06 <sup>ns</sup>	130.3 ± 0.4 <sup>ns</sup>	61.6 ± 0.69 <sup>ns</sup>
Untreated	1.67 ± 0.25 <sup>ns</sup>	0.67 ± 0.09 <sup>ns</sup>	135.4 ± 0.69 <sup>ns</sup>	79.3 ± 0.46 <sup>**</sup>

Values are mean ± SEM (n=6) (ns : no significant difference ;\*\* : P<0.01).

support about the beneficial utilization of this plant as natural antioxidants in food and in folk medicine which prevents excessive production of free radicals due to reduction glucose level in diabetic rats.

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