

Hypoglycemic, Hypolipidemic and Anti-Oxidant Activities of Ethanolic Extract of *Olea Europaea* Linn

Husni S. Farah

College of Medicine, Biochemistry Department, Taif University, Kingdom of Saudi Arabia (KSA), P.O. Box (888).
Al-Hawaiya, Taif

Abstract: The effect of ethanolic extraction of olive leaves on alloxan – induced diabetic rabbits and lipid profile has been investigated. The maximum hypoglycemic activity (about 20% reduction in blood glucose level) was obtained from the treatment of diabetic rabbits with 600mg/ kg. There was a significant decrease in the concentrations of total cholesterol, LDL – cholesterol and HDL-cholesterol after treatment with 600 mg/kg for one week to the non-diabetic rabbits. However with this dose there was no effect on the level of triglycerides. The olive leaves extraction showed also significant antioxidant activity when compared with Ascorbic acid as standard anti-oxidant agent. The study suggests the use of alcoholic extract of olive leaves be beneficial in diabetic condition and in reducing the level of lipids. Further work on isolation and characterization of active constituents in olive is in progress in our laboratory.

Keywords: olea europaea ; LDL-cholesterol; Total cholesterol; Ethanolic Extract ; 2,2- Diphenyl 1-picryl hydrazine (DPPH).

1. INTRODUCTION

The ethanolic extract of leaf of *olea europaea* (oleaceae) was found to have many beneficial effects on experimental animals. It is also commonly known olive leaf and reported to have hypertensive and anti-atherosclerotic activity in rats (Somova et al, 2003; Cherif et al, 1996). Anti-oxidative activities were reported by Hasan and Mohamed, 2005; Gonzalez et al, 1992; Kaskos 2013). In fact the extract of *olea europaea* showed anti bacterial and anti inflammatory activities against bacteria and fungi (Esmailpour et al, 2010). It was suggested that the use of olive leaf polyphenols like oleuropein (OLP), hydroxytyrosol (HT), and others could decrease breast cancer cells viability significantly (Milanizadeh et al. 2013). Methanolic extract of *olea europaea* have been found to have anti-cancer activity (Nashwa et al. 2014). Animal studies have also demonstrated that dietary oleuropein reduces the plasma level of cholesterol and also reduces the oxidation of LDL (Coni et al, 2000; Visioli and Galli, 1998). The other important activities reported are vasodilation and inhibitory effect on platelets aggregation (Zarzuelo et al, 1991; Petroni, 1995). Recently it has been reported that the water extract of olive leaf inhibits the growth of several pathogenic bacteria and exhibits anti-HIV activities (Aziz et al, 1998; Markin et al, 2003; Lee-Huang et al, 2003; Dilek et al, 2012). In the present study the lipid profile, anti-diabetic and anti-oxidative activities of ethanolic extract of olive leaf of wild Jordan type was investigated on experimental rabbits.

2. MATERIALS AND METHODS

Plant Material: The plant material consisted of mature leaves of *olea europaea* which was collected from the trees growing in around Amman university campus in month of April. The leaves were completely dried and powdered.

Preparation of extract: The powdered leaves were placed in absolute (98%) ethanol over night with occasional shaking. The extract was filtered and ethanol was distilled off to get the total extract which was dried at room temperature and found to be 5.84% (w/w)

Anti-oxidant activity (DPPH free radical scavenging activity) of ethanolic extract: The ethanolic extract of olive leaf was tested for 2,2-Diphenyl 1-picrylhydrazine (DPPH) free radical scavenging activity by modified method of Braca et al. (2002). The dried extract was dissolved in methanol to get 1 mg/ml (1000 mg/ml) solution which was further diluted to get 10, 20, 50, 100, 250, 500 and 750 mg/ml solution in methanol. Ascorbic acid was used as standard in 1 - 100 mg/ml solution. 0.002% of DPPH was prepared in methanol and 1 ml of this solution was mixed with 1 ml of sample solution and standard solution to be tested. These solution mixtures were kept in dark for 20 minutes and absorbance was measured at 517 nm using Cecil-Elect. Spectrophotometer against methanol. The blank was used as 1 ml of methanol with 1 ml of DPPH solution (0.002%). The optical density (OD) was recorded and % inhibition was calculated using the following formula given by Bors et al. (1992).

$$\% \text{ inhibition of DPPH activity} = \frac{(\text{OD of blank} - \text{OD of sample})}{(\text{OD of blank})} \times 100$$

Biological assays:

a. Preparation of extract for Biological assay : The dried extract 5.84 g was suspended in 10 % gum acacia in 100 ml distilled water .

b. Animals: The experimental animals consisted of 12 rabbits weighing 1300 – 2000 gm . All animals were fed the normal standard diet . Blood glucose levels were determined in rabbits after a fasting period of 24 hours according to the method described by Kaplan (1984). These values of glucose were considered as control. Diabetes was induced in the animals by injecting 140 mg/kg of alloxan intravenously for five consecutive days . Blood glucose levels were determined on the fifth day after the end of alloxan administration . 400 mg and 600 mg /kg of the olive leaf extract were administered at 90 minutes as recommended by Gonzalez et al (1992) . Blood glucose levels were determined in diabetic rabbits treated with 600 mg/kg after 24 hour. In another experiment , eight rabbits weighing 1300-1900 gm were used . They were fasted for 24 hour and were allowed to drink water . Total cholesterol , LDL-cholesterol , HDL-cholesterol and Triglyceride concentrations were measured by the methods of Naito (1984) ; Wieland and Seidel (1983) ; Jacobs et al (1990) and Cole et al (1997) respectively.

3. RESULTS

As shown in table 1 the concentrations of glucose were significantly elevated from 115.8 ± 1.7 mg/100 ml to 135.0 ± 2.5 mg /100ml ($p < 0.01$) as a result of intravenous administration of rabbits with 140 mg/kg of alloxan for five consecutive days. This significant increase represents 17% change in the level of glucose . The maximum hypoglycemic activity was achieved at a dose of 600 mg/kg of extract where the glucose level was significantly reduced from 135 ± 2.5 mg / 10ml to 108.0 ± 7.8 mg/100ml ($p < 0.01$) and was maintained for 24 hours (99.0 ± 8.4 mg/100ml) . In Table 1, the treatment of non-diabetic rabbits with 600 mg/kg had no effect on glucose concentration ($p > 0.05$) . Treatment of non-diabetic rabbits with 600 mg/kg of extract shows a significant decrease in the concentration of total cholesterol from 137.9 ± 9.8 mg/100 ml to 100.8 ± 13.6 mg/100ml ($p < 0.01$) which represents 27% change as shown in Table 2 . Also in Table 2 the levels of LDL-cholesterol and HDL-cholesterol at 600 mg/kg were significantly reduced from 112.2 ± 21.9 to 78.7 ± 12.9 ($p < 0.05$) and from 104.2 ± 15.3 to 60.0 ± 6.8 ($p < 0.01$) respectively. However there was no significant effect in the level of triglyceride ($p > 0.05$) . In table 3 the maximum anti-oxidant activity was achieved at 600 µg/ml with the extraction of olive leaf which shows no significance difference with the standard anti-oxidant activity of ascorbic acid ($p < 0.05$) .

4. DISCUSSION

The present study is about the anti-diabetic activity which is related to the ethanolic extract of the olive leaf. In earlier studies water extract has been used by Gonzalez et al. The present study revealed the dose at which the maximum hypoglycemic and hypo-cholesteremia activity was observed. The other important observation was regarding the maintenance of blood sugar level to normal even after 24 hour. Other studies have observed the blood glucose level only after 90 and 150 minutes . It is therefore evident that the ethanolic extract contains some chemicals which possess anti-

diabetic activity. Elevation of cholesterol and LDL-cholesterol is associated well with cardiac problems. Therefore the dose at 600 mg/kg showed remarkable reduction in the level of total cholesterol and LDL-cholesterol. It has been reported that the cholesterol synthesis is inhibited by the inhibition of 3-Hydroxy-3-methylglutaryl (HMG)CoA reductase activity or by degradation of cholesterol in the intestine by certain bacterial metabolites (Champe et al. 2005). The ethanolic extract contains many constituents which are alkaloids, tannins, sugars, glycosides, fats and free amino acids which may be having the chemical structure similar to drugs which are used as anti-hypercholesterolemia which has to be established by chemical analysis of these constituents. The free radical scavenging activity of olive leaf extract was confirmed in the present investigation. It was observed that a very small dose (100mg/ml) of olive leaf extract was able to give 63.9% inhibition, whereas the maximum antioxidant activity was achieved at 600mg/ml which is comparable with the ascorbic acid as a standard antioxidant agent. Ethanolic extract of olive leaf used in this study seems to have high and significant antioxidant activity over the methanolic extract which was used by Naswa et al. 2014. Ethanolic extract of olive leaf shows significant anti-oxidant activity compared with the ascorbic acid as a standard anti-oxidant activity agent. However, the chemical present in the leaf extract which is responsible for this activity has still to be investigated and it is obvious that the constituents like alkaloids, tannins, reducing sugars and proteins present in the leaf extract may be responsible for such activity. The extract is being analyzed for separation of various active constituents in our laboratory for establishing this activity and to attribute the activity to chemical constituents present in the extract.

Thus from the results obtained in the present investigation, it may be concluded that the ethanolic extract of olive leaf possesses the chemical constituents which have remarkable anti-diabetic and anti-oxidative activities including the significant effect on the lipid profile. The work to isolate these chemical markers is under progress in our laboratory to pinpoint the constituents responsible for these activities.

REFERENCES

- [1] Aziz, N., Farag, S., Mousa, L., 1998. Comparative anti-bacterial and anti-fungal effects of some phenolic compounds. *Microbios.* 93, 43-54.
- [2] Bors, W., Saran, M., Eltsner, E., 1992. Screening for plant anti-oxidants. *Modern Methods of Plant Analysis.* 13, 277-295
- [3] Braca, A., Smtino, C., Politi, M., Morelli, I., Mendez, J., 2002. Anti-oxidant activity of flavonoids from *Licania licaniaeflora*. *J. Ethnopharmacology.* 79 (Suppl. 3), 379-381.
- [4] Champe, P., Harvey, R., Ferrier, D., 2005. *Lippincott's Illustrated Review: Biochemistry*, 3rd edit. Lippincott William and Wilkins, Baltimore, MD. pp. 221-222.
- [5] Cherif, S., Rahal, N., Haouala, M., 1996. A clinical trial of a titrated oleo extract in the treatment of essential arterial hypertension. *J. PharmBelg.* 51, 69-71.
- [6] Cole, T., Klotzsch, S., McNamara J., 1997. Measurement of Triglyceride concentration. In: Rifai N, Warnick G and Dominiczak M, eds. *Handbook of lipoprotein testing*. Washington: AACC Press. 115-126.
- [7] Coni, E., DiBenedetto, R., DiPasquale, M., Maslla, R., Modesti, D., Mattei, E., 2000. Protective effect of oleuropein, an olive oil biophenol, on low density lipoprotein oxidizability in rabbits. *Lipid.* 35 (Suppl. 1), 45-54.
- [8] Dilek K, Nur C, Aysel U. and Ayşe D. 2012. Antimicrobial activity and chemical constitutions of West Anatolian olive (*Olea europaea* L.) leaves. *Journal of Food, Agriculture and Environment*, Vol. 10, (2), 99-102.
- [9] Esmaeili-Mahani S, Rezaeezadeh-Roukerd M, Esmaeilpour K, Abbasnejad M, Rasouljan B, et al. (2010) Olive (*Olea europaea* L.) leaf extract elicits antinociceptive activity, potentiates morphine analgesia and suppresses morphine hyperalgesia in rats. *J Ethnopharmacol* 132: 200-205
- [10] Gonzalez, M., Zarzuelo, A., Gamez, M., 1992. Hypoglycemic activity of olive leaf. *Planta Med.* 5 (Suppl. 6) 513-515.
- [11] Hasan, F., Mohamed, A., 2005. Hypoglycemic and anti-oxidant effect of oleuropein in alloxan-diabetic rabbits. *Life Science.* 77, 23-26.

- [12] Jacobs, D., Kasten, B., Demott, L., Wolfson, W., 1990. In Laboratory and Test Handbook. Eds. Lexi- Complnc: Hudson (Cleveland). pp. 219-223.
- [13] Kaplan, A., 1984. Clinical Chemistry. The CV. Mosby Co. St. Louis,Toronto Princeton. pp. 1032-1036.
- [14] Kaskos A. 2013. Pharnacognostic specification of leaves of Olea europaea collected from Iraq. American J. phytomedicine and clinical Therapeutic. 2; 2. 153 – 160.
- [15] Lee-Huang, S., Zhang, L., Huang, P., Chang, Y., 2003. Anti-HIV activity of olive leaf extract (OLE) and modulation of host cell gene expression by HIV-1 infection and OLE treatment. Biochem.BiophysResCommun. 8 (Suppl. 307), 1029-1037. Markin, D., Duek, L., Berdicevsky, I., 2003. In vitro anti-microbial activity of olive leaves. Myeoses. 46 (Suppl. 3), 132-136.
- [16] Milanizadeh. S, . Bigdeli M. Rasoulia B and Amani D. 2013. The Effects of Olive Leaf Extract on Antioxidant Enzymes Activity and Tumor Growth in Breast Cancer. Thrita.; 3(1): 1 – 6.
- [17] Naito, H., 1984. Kaplan Clinical Chemistry. The CV. Mosby Co. St. Louis. Toronto Princeton. pp. 1194-1206
- [18] Nashwa, F. S. Morsy *M. E. Abdel-Aziz. 2014; Efficiency of olive (Olea europaea L.) leaf extract as antioxidant and anticancer agents. Journal of Agroalimentary Processes and Technologies 20(1), 46-53
- [19] Petroni, A., 1995. Inhibition of platelets aggregation and eicosanoid production by phenolic compounds of olive oil. Thromb. Res. 78, (Suppl. 2), 151-160.
- [20] Somova, L., Shode , F., Ramnanan , P., Nadar , A., 2003. Anti-hypertensive, anti-aterosclerotic and anti-oxidant activity of triteiprenoids isolated from oleo europea , subspecies African leaves. J. EthenoPharmamacology. 84 , 299-305.
- [21] Sudjana AN, D'Orazio C, Ryan V, Rasool N, Ng J, et al. (2009) Antimicrobial activity of commercial Olea europaea (olive) leaf extract. Int J Antimicrob Agents 33: 461-463.
- [22] Visioli, F., Galli, C., 1998. Oleuropein protects low density lipoprotein from oxidation. Life Science. 55 (Suppl. 24), 1965~1971.
- [23] Wieland, H., Seidel, D., 1983. Direct determination of LDL-Cholesterol in human serum. J.Lipid Res. 24, 904-908.
- [24] Zarzuelo, A., Duarte, J., Gonzalez, M., Utrilla, P., 1991. Vasodilator effect of olive leaf Planta Med. 57, 417-419.

APPENDIX - A

Table 1. Concentrations of glucose (mg/100ml) in non-diabetic rabbits treated with ethanolic olive leaf extract.

<u>Treatment</u>	<u>Concentration of glucose after 90 min. (mg/100ml)</u>	<u>SD</u>	<u>Concentration after 24hour (mg/100ml)</u>
Control (non-diabetic)	115.8 (a)	1.7	
Alloxan (diabetic)	135.0 (b)	2.5	
400ml/kg of extract	117.2 (c)	18.1	
600mg/kg of extract	108.0 (d)	7.8	99.0 ±8.4 (e)

Statistical comparison was based on independent sample t-test. The comparison was made as follows: p< 0.01 for (a) and (b) ; p> 0.05 for (b) and (c) ; p< 0.01 for (b) and (d) ; p> 0.05 for (d) and (e)

Table 2. The concentrations of lipids profile in non-diabetic rabbits treated with 600 mg/kg of ethanolic olive leaf extract.

<u>Lipids profile</u>	<u>Control</u>	<u>SD</u>	<u>Treatment with 600mg/kg</u>	<u>SD</u>	<u>t-test</u>
Total cholesterol	137.9	9.8	100.8	13.6	p < 0.01
LDL-cholesterol	112.2	21.9	78.7	12.9	p < 0.05
HDL-cholesterol	104.2	15.3	60.0	6.8	p < 0.01
Triglyceride	131.0	22.8	117.0	50.0	p > 0.05

Statistical comparisons were based on independent sample t-test. Tubular figures represent the mean ± SD of 8 values.

International Journal of Novel Research in Life Sciences

 Vol. 2, Issue 3, pp: (33-37), Month: May - June 2015, Available at: www.noveltyjournals.com
Table 3. DPPH free radical scavenging activity of standard ascorbic acid and ethanolic olive leaf extraction

Concentration ($\mu\text{g/ml}$)	Ascorbic acid (% inhibition)	SD	Olive leaf extraction (% inhibition)	SD
10	40.2	2.2	15.5	1.2
50	70.3	4.1	38.4	2.9
100	80.0	3.3	63.9	2.7
250	90.6	5.6	67.1	6.3
500	90.8	4.3	70.0	5.8
600	91.6	6.2	86.4	5.2

Tubular figure represents the mean \pm SD of 3 values. Statistical significance difference was found at 600 $\mu\text{g/ml}$ ($p < 0.05$).