Algerian Journal of Environmental Science and Technology
Avril edition. Vol.2. N°2. (2016)
ISSN : 2437-1114
www.aljest.webs.com

Preliminary effects of olive mill wastewater on the glucose absorption in mice

N. Senani-Oularbi[1]*, D. El benna[2], J. C. Marie[2], F. Moulti-Mati[1]

[1]Laboratoire de Biochimie Analytique et Biotechnologies, Université M. Mammeri, BP N◦17 RP, 15000 Tizi Ouzou, Algeria.
[2]INSERM, U1149, Center for research on inflammation, Paris, France.

*Corresponding author: senanibiochem@yahoo.fr ; Fax & Tel : +213 26 18 61 43

ARTICLE INFO

Article History:

Received ***: 29/01/2016***
Accepted ***:05/07/2016***

***Key Words*:**

Olive mill wastewater;
Intestinal glucose absorption; mice;
Oral glucose tolerance;
Diabetes.

ABSTRACT

Abstract: *Olive mill wastewater (OMWW) is the liquid by-product generated during olive oil production. The aim of this study is to investigate the effect of OMWW on intestinal glucose absorption in mice, notably for treating diabetes. Oral glucose tolerance test (OGTT) is a medical test in which glucose is given and blood samples taken afterward to determine how quickly it is cleared from the blood. This test was performed in Male C57BL/6J mice weighing 20–25 g. Gavages of mice with glucose were performed after a 16-h fast using a D-glucose solution (1 g/Kg) without or with OMWW, in dose range of 0.5– 4 g/kg of body weight. The bleeds were further taken at 15, 30, 60, and 120 min after oral glucose administration. These experiments were performed at least with 6 individual animals. Oral glucose tolerance test carried out in mice showed that oral administration of OMWW decreased glycemia at all tested concentration, which is significant at concentration of 4g/Kg of body weight. These results suggest OMWW have potentially hypoglycemic effect in mice.*

I. Introduction

The olive oil extraction is one of the ancient agricultural industries all over the Mediterranean area and up to day it is of fundamental economic importance for many countries. The olive oil industry generates large amounts of a liquid waste effluent named olive mill wastewater (OMWW) or vegetation water [1]. The annual world OMWW production is estimated from 10 to over 30 million m^3 [2]. The great variety of components found in OMWW (carbohydrates, polysaccharides, sugars, lipids and phenolic compounds) [3]. On other hand, the health benefits arising from a diet containing olive oil have been attributed to its richness in phenolic compounds that act as natural antioxidants and are thought to contribute to the prevention of heart diseases and cancers [4, 5]. The beneficial health effects of OMWW have been mainly attributed to its elevated phenols content [6]. Excessive oxidative stress is implicated in the pathology and complications of Diabetes mellitus (DM) and polyphenols with antioxidant properties exert beneficial antidiabetic effect by correcting the disturbed oxidative milieu in diabetic conditions [7]. Diabetes is a chronic disease characterized by disordered metabolism and inappropriately high blood sugar (hyperglycaemia). DM is sharply increasing globally, including in many parts of the developing world, in major part as a consequence of the world wide “epidemic” of obesity, characterized by a loss of glucose homeostasis with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both [8, 9]. The use of the drugs is restricted by their pharmacokinetic properties, secondary failure rates and accompanying side effects [10]. Thus searching for a new class of compounds is essential to overcome diabetic problems. There is continuous search for alternative drugs [11]. The present investigation is directed to characterize OMWW and the exploration its antidiabetic effect in mice, show inhibitory effect of glucose utilization and, are in use as hypoglycemic agent in traditional system of medicine.

II. Materials and methods

II.1. Materiels

II. 1.1. Chemicals

D-glucose (180.16, Prolabo). All other chemicals and reagents were of analytical grade and were purchased from Sigma Chemical Co. (St Louis, MQ, USA), Aldrich Chemical Co. (Steineheim, Germany), Merck (Darmstadt, Germany).

II.1.2. Plant resources

Fresh olive mill waste (OMWW) was supplied by a discontinuous three phase olive processing mill from from the area of kabylia in Algeria. This sample was taken at the middle of the olive harvest season, fractioned and conserved at -40°C.

II.1.3. Animals

Male C57BL/6J mice weighing 20–25 g were from Centre Elevage Janvier, Le Genest-St. Isle, France. The animals had free access to tap water and standard food and were treated in accordance with European Community guidelines concerning care and use of laboratory animals.

II.2. Methods

II.2.1. Physicochemical characteristics of OMWW

The electrical conductivity (EC) and the pH were directly measured in the sample using a pH meter 211(HANNA instruments, Romania). The OMWW water content and the total solids (TS) were determined before and after drying the sample overnight at 105°C respectively. The volatile solids (VS) content was gravimetrically deduced after the incineration of the dry sludge at 550°C for 4 h. The total suspended solids (TSS) were assessed gravimetrically after centrifugation of the crude OMWW at 400rpm for 15 min [12]. Protein concentration was measured by Bradford method [13]. The total *lipid* content *determined* following the method of Knight *et al.* [14]. Total sugar content was measured following the method of Dubois *et al*. [15]. Reducing sugars were measured by DNS method [16]. Analyses were performed in triplicate, and the results are given as mean values.

2.2. Extraction of phenolic compounds from OMWW

Liquid–liquid extraction with ethyl acetate was carried out on OMWW [17]. The mixture (solvent-OMWW) was vigorously shaken for 10 min to achieve equilibrium and was then allowed to settle for 30 min. The phases were separated and the extraction was repeated successively four times. All the runs were performed at ambient temperature (25 ◦C). The ethyl acetate was evaporated in a rotary evaporator at 40°C to obtained dry residue.

2.3. Total phenol content determination

The total phenol content determined by the Folin–Ciocalteu method [18] with calibration curves for gallic acid. Absorption was determined data at wavelength of 750 nm with a spectrophotometer (UV-1700 Shimadzu, Tokyo, Japan). Data were expressed as gallic acid equivalents (mg gallic acid /mL OMWW).

2.4. Glucose tolerance test

Gavages of mice with 1 g of glucose per kilogram of body weight were performed after a 16-h fast using a D-glucose solution without (control) or with OMWW. The total bolus volume for mice was 0.25 ml. results in dose range of 0.5–4 g/kg of body weight. Preliminary experiments showed that the OMWWgiven by gavage was found in proximal small intestine after 5 min with a recovery of 70 % [19]. Before starting the OGTT, blood samples were taken from the tail and fasting blood glucose levels (milligram per deciliter) were determined (ACCU-CHEK; Roche Diagnostic, Meylan, France). The bleeds were further taken at 15, 30, 60, and 120 min after oral glucose administration. These experiments were performed at least with 6 individual animals.

III. Results and discussion

III.1. Olive mill wastewater characterization

OMWW contain an enormous supply of organic matter, Main characteristic properties obtained for these OMWW sample are given in Table1. Olive mill wastewater (OMWW) is an acidic effluent with a high nutrient content. The characteristic brown–black color of this effluent is chemically related to polymers of low molecular weight phenolic compounds and lignin derivatives. The precise color mainly depends on the age and type of oil processed and also the type of the technology used. Fresh OMWW has a strong and characteristic smell. The wastewater is slightly acidic, having pH values from 3 to 5. OMWW is generally composed of water (83–94 %), organic matter (4–16 %) and mineral salts (0.4–2.5 %) [20. Oils (1–14 %), polysaccharides (13–53 %), proteins (8–16 %), organic acids (3–10 %), polyalcohols (3–10 %) and polyphenols (2–15 %) are listed as its main organic contents. More than 50 phenolic compounds, many alcohols, aldehydes and other low molecular weight compounds have been reported in the literature. Mineral salts of OMW are mainly carbonates (21 %), phosphates (14 %), potassium (47 %) and sodium (7 %). Total suspended solid (TSS) is principally derived from the olive pulp and contains mainly cellulose and pectins [21]. Both quality and quantity of OMWW are highly variable as a result of several factors such as: type of production process, type of olives, area under cultivation, use of pesticides and fertilizers, climatic conditions, and harvesting time (i.e. stage of olive maturity) [22]. However arabinose and galactose were found to be the major neutral sugars in OMWW [23]. Main characteristic properties obtained for OMWW sample are given in Table 1.

Table 1. *Characteristics of olive mill waste water sample.*

Parametrs	OMWW
pH	04.80 ± 0.00
Electrical conductivity (25°C) (dS/m)	09.70 ± 0.06
Dry matter (%)	11.85 ± 01.50
Mineral solids (g/L)	11.75 ± 10.03
Volatile solids (g/L)	106.76 ± 02.36
Total suspended solids (g/L)	34.30± 0.25
Mineral suspended solids (g/L)	01.40 ± 0.10
Volatile suspended solids (g/L)	32.87 ±0.15
Lipids (g/L)	01.32 ± 0.08
Total phenols (g/L)	06.27 ± 1.67
Total sugars (g/L)	13.50 ± 2.12
Reducing sugar (g/L)	04.38±0.90
Total proteins (g/L)	01.24 ± 0.06

III.2. Effect of OMWW on glucose tolerance tests.

Oral administration of OMWW decrease glycemia during OGTT carried out in mice (Figure1.) as compared with control groups. The area under the curve was significantly (P <0.05).

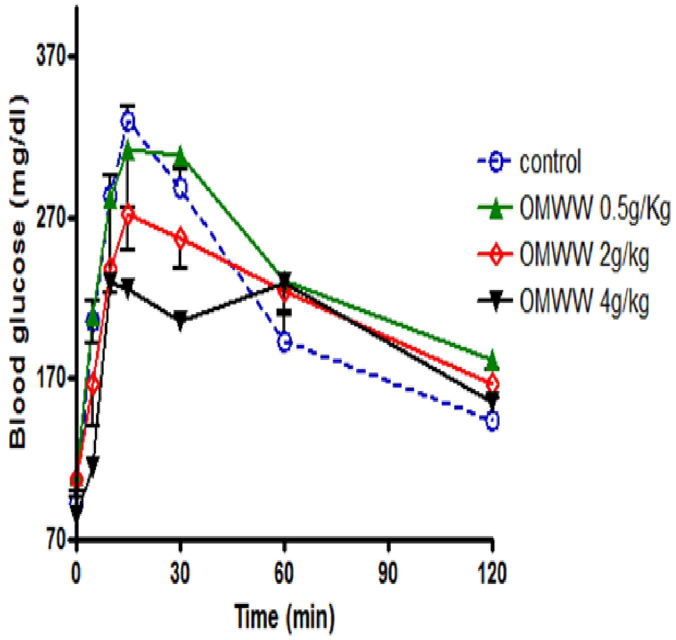

Figure 1. *OGTT in mice. OGTT (1 g/kg) was performed in overnight-fasted mice or with a 15 % D-glucose solution without (control) or with OMWW (0.5- 4 g/kg).*

The present work is the first investigation dealing with the protective effects of OMWW on diabetes in insulin deficiency and/or resistance conditions, the hyperglycemia is a consequence of the combination of increased endogenous glucose production, related to the higher rates of glycogenolysis and gluconeogenesis processes, and reduced glucose uptake by peripheral tissues. It has been proposed that the search and/or development of new compounds that correct these glucose metabolism disturbances will be important to highlight new options for diabetes treatment [24, 25]. Crescent attention has been given to the investigation of the antidiabetic activity of many plant species all around the world, using different experimental models and several methodologies. However, information about the safety of OMWW is essential, considering that medicinal plants are easily acquired by the population and that many toxicological effects may be observed after the use of herbal preparations. In this way, it is common the use of mice in the experimental investigation of the acute toxic effects of plant extracts orally administered in high doses [26]. Data from this study showed that the OMWW did not have any acute toxicity in animals, at every tested dose. In front of the lack of toxicity, the doses of 0.5 and 4 g/kg were chosen to the evaluation of the antidiabetic activity of OMWW in mice.

The present study indicated that OMWW has antihyperglycemic activity. The antidiabetic effect of OMWW can be attributed, at least in part to phenolic compounds (PC). PC can reach levels of 1.5 and 10.2 g/L in OMWW [27, 28]. Therefore, it can be suggested that polyphenols are potential candidates of natural origin to diabetes treatment [29]. In the past few years, polyphenolics substances have received widespread attention because of their potential for preventing some highly prevalent chronic diseases. In fact it has been reported that polyphenols are endowed with interesting biological activities such as anti-inflammatory, antioxidant, antiallergic, hepatoprotective, antithrombotic, antiviral, and anticarcinogenic activities [5]. In addition, OMWW biophenols are efficient components for the low incidence of cardiovascular diseases in the Mediterranean area [5]. A hen, olive mill waste is potentially a rich source of a diverse range of biophenols with a wide array of biological activities [30, 5]. The beneficial health effects of OMWW have been mainly attributed to its elevated phenols content [6]. Among them, hydroxytyrosol (3,4-dihydroxyphenylethanol) stands out as a compound of high added-value, due to its interesting antioxidant and potential beneficial human health properties [31]. Hydroxytyrosol is an effective hypoglycemic and antioxidant agent in alleviating oxidative stress and free radicals as well as in enhancing enzymatic defenses in diabetic rats [4]. OMWW is the liquid by-product generated during olive oil production. Although, olive oil, have been claimed as being highly efficacious in the treatment of type 2 diabetes mellitus [34] little is known about the effect of OMWW. Thus, research efforts have been directed toward the valorization of olive by-products for production of various valuable and actif compounds such as phenolic compounds and polysaccharides [30]. Further investigations are ongoing in our laboratory to detail the mechanisms of action that explains the antidiabetic effect of this by-product.

IV. Conclusion

This study demonstrated the beneficial effect of OMWW as an effective hypoglycemic agent. The use of OMWW may be of prophylactic value in reducing diabetes mellitus. Future studies will need to elucidate the active principles as well as molecular sites of action responsible for hypoglycemic effect of OMWW.

V. References

1. Cassano, A. ; Conidi, C. ; Giorno, L. ; Drioli, E. Fractionation of olive mill wastewaters by membrane separation techniques. *Journal of Hazardous Materials* (248–249) (2013) 185–193.
2. Eroğlu, E. ; Eroğlu, I., Gündüz, U.; Türker, L.; Yücel, M. Biological hydrogen production from olive mill wastewater with two-stage processes. *International Journal of Hydrogen Energy* (31) (2006) 1527–1535.
3. Dermeche, S.; Nadour, M., Larroche, C., Moulti-Mati, F., Michaud, P. Olive mill wastes: biochemical characterizations and valorization strategies. *Process Biochemistry* 48 (2013) 1532–1552.
4. Hamden, K.; Allouche, N.; Damak, M; Elfeki, A. Hypoglycemic and antioxidant effects of phenolic extracts and purified hydroxytyrosol from olive mill waste in vitro and in rats. *Chemico-biological Interactions* 180 (3) (2009) 421–432.
5. Obeid, H.K.; Allen, M.S.; Bedgood, D.R.; Prenzied, P.D., Robards, K.; Stockmann, R. Bioactivity and analysis of biophenols recovered from olive mill waste. *Journal of Agricultural and Food Chemistry* 53 (2005) 823–837.
6. Casalino, E. ; Calzaretti, G. ; Sblano, C. ; Landriscina, V. ; Tecce, M.F. ; Landriscina, C. Antioxidant effect of hydroxytyrosol (dpe) and mn2+ in liver of cadmium intoxicated rats. *Comparative biochemistry and physiology part c*.133 (2002) 625–632.
7. Abdelmoaty, M.A.; Ibrahim, M.A.; Ahmed, N.S.; Abdelaziz, M.A. Confirmatory studies on the antioxidant and antidiabetic effect of quercetin in rats. *Indian Journal of Clinical Biochemistry* 25(2010) 188–92.
8. Rajiv Gandhi G.; Sasikumar P. Antidiabetic effect of merremiae marginata burm. F. In streptozotocin induced diabetic rats. *Asian Pacific Journal of Tropical Biomedicine* 2 (2012) 281-286.
9. Abo, K.A.; Fred-Jaiyesimi, A.A.; Jaiyesimi, A.E. Ethnobotanical studies of medicinal plants used in the management of diabetes mellitus in south western nigeria. *Journal of Ethnopharmacology* 115 (2008) 67–71.
10. Vishwakarma, S.L.; Rakesh, S.; Rajani, M.; Goyal, R.K. Evaluation of effect of aqueous extract of enicostemmalittorale blume. In streptozotocin induced type 1 diabetic rats. *Indian Journal of Experimental Biology* 48 (2010) 26-30.
11. Syamsudin. Standardization of extract of leucaenaleucocephala (lmk) de wit seeds by α-glucosidase inhibitor. *International Journal of Phytomedicine* 2 (2010) 430-435.
12. El-abbassi, A.; Hajar, K.; Abdellatif, H. Phenolic profile and antioxidant activities of olive mill wastewater. *Food Chemistry* 132 (2012) 406–412.
13. Bradford,; MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry 72* (1976) 248-254.
14. Knight J.A.; Shauna A,; James, M. R.(1972), Chemical basis of the sulfo-phospho-vanillin reactionfor estimating total serum lipids. *Clinical Chemistry*, 18(3), 199-202.
15. Dubois, M.; Gilles, K.A; Hamilton, J. K. ; Rebers, P. A. ; Smith, F. Colorimetric method for determination of sugars and related substances. *Analytical Chemistry* 28 (3) (1956) 350-356.
16. Miller, G. L. Use of dinitrosaiicyiic acid reagent for determination of reducing sugar. *Analytical Chemistry* 31(3) (1959) 426-428.
17. De marco, E. ; Savarese, M. ; Paduano, A. ; Sacchi R. Characterization and fractionation of phenolic compounds extracted from olive oil mill wastewaters. Analytical Nutritional and Clinical Methods. *Food chemistry* (2007) 858–867.
18. Folin, O., & Ciocalteau, V. On tyrosine and tryptophan determination in protein. *Journal of Biological Chemistry* 73(1927) 627–650.
19. Belharbi, K. R. ; Lettéron, P. ; Chedid, P. ; Nazaret C, Ducroc, R. ; Marie, J-C. Resistin-like molecule_

inhibits sglt-1 activity and enhances glut2-dependent jejunal glucose transport. *Diabetes* 58 (2009) 2032–2038.
20. Eroglu, E.; Gunduz, U.; Yucel, M.;Turker, L.; Eroglu, I. Photobiological hydrogen production by using olive mill wastewater as a sole substrate source. *Internantional Journal of Hydrogen energy* 29(2) (2004) 163–71.
21. Hamdi, M. Toxicity and biodegradability of olive mill wastewaters in batch anaerobic digestion. *Applied biochemistry and biotechnology* 37(1992) 155–63.
22. Eroglu, E.; Eroglu, I.; Gunduz, U.; Yucel, M. Treatment of olive mill wastewater by different physicochemical methods and the utilization of their liquid effluents for biological hydrogen production. *Biomass and Bioenergy* 334 (2009) 701–5.
23. Vierhuis, E.; Korver, M.; Schols, H. A. ; Voragen, A. G. Structural characteristics of pectic polysaccharides from olive fruit (*olea europaea* cv moraiolo) in relation to processing for oil extraction. *Carbohydrate Polymers* 51(2) (2003) 135-148.
24. Leney, S. E, Tavaré, J.M. The molecular basis of insulinstimulated glucose uptake: signaling, trafficking and potential drug targets. *Journal of Endocrinology 203* (2009) 1-18.
25. Wu, C.; Okar, D.A. ; Kang, J.; Lange, A.J . Reduction of hepaticglucose production as a therapeutic target in the treatment of diabetes. *Current drug targets- immune endocrine metabolic disorders 5* (2005) 51-59.
26. Mu, L.H. ; Huang, Z.X, Liu, P.; Hu,Y.; Gao, Y. Acute and subchronic oral toxicity assessment of the herbal formula kai-xin-san. *Journal of Ethnopharmacology 138* (2011) 351-357.
27. Nesseris, G. K.; Stasinakis, A. S. Investigation of municipal and olive mill wastewater co-treatment in activated sludge–powdered activated carbon (as-pac) systems. *Journal of Chemical Technology and Biotechnology* 87 (4) (2012) 540–545.
28. Filidei, S. ; Masciandaro, G. ; Ceccanti, B. Anaerobic digestion of olive oil mill effluents: evaluation of wastewater organic load and phytotoxicity reduction. *Water, Air, & Soil Pollution* 145(1) (2003) 79-94.
29. Hwang J.T.; Kwon D.Y.; Yoon, S.H. Amp-activated protein kinase: a potential target for the diseases prevention by natural occurring polyphenols. *New Biotechnology* 26 (2009) 17-22.
30. Nadour, M. ; Michaud, P. ; Moulti-Mati, F. Antioxidant activities of polyphenols extracted from olive (olea europaea) of chamlal variety. *Appl Biochem Biotechnol* 167 (6) (2012) 1802 -1810.
31. Dudley, J.I.; Lekli, I.; Mukherjee, S.; Das, M. ; Bertelli, A.A. ; As, D.K . Does white wine qualify for french paradox? Comparison of the cardioprotective effects of red and white wines and their constituents: resveratrol, tyrosol, and hydroxytyrosol. *Journal of Agricultural and Food Chemistry* 56 (2008) 9362–9373.
32. Zhang, X.; Jiang, L.; Geng, C.; Yoshimura, H.; Zhong, L. Inhibition of acrylamide genotoxicity in human liver-derived hepg 2 cells by the antioxidant hydroxytyrosol. *Chemico-Biological Interactions* 176 (2008) 173–178.
33. Deiana, M. ; Incani, A. ; Rosa, A. ; Corona, G. ; Atzeri, A., Loru, D. ; Melis, M.P. ; Dessì, M.A. Protective effect of hydroxytyrosol and its metabolite homovanillic alcohol on h2o2 induced lipid peroxidation in renal tubular epithelial cells. *Food and Chemical Toxicology* 46 (2008) 2984–2990.

Please cite this Article as:
Senani-Oularbi N., El benna D., Marie J.C., Moulti-Mati F., *Preliminary effects of olive mill wastewater on the glucose absorption in mice*, ***Algerian J. Env. Sc. Technology**, **2**:***2 (2016) 10-14**