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Antihypertensive, antiatherosclerotic and antioxidant activity of triterpenoids isolated from *Olea europaea*, subspecies *africana* leaves

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Abstract

For the first time a biossay-directed study of triterpenoids isolated from the leaves of *Olea europaea* from Greece, from wild African olive and from a cultivar of *O. europaea* grown in Cape Town was reported. The experiment was undertaken since our preliminary analyses showed that the African wild olive leave is rich in triterpenoids and contain only traces of the glycoside oleuropein, which is typical for the European olive leaves. The isolate of the African wild olive leaves (AO) used in the experiments was found to contain 0.27% 1:1 mixture of oleanolic acid and ursolic acid, named oleuafricein. The isolate of Greek olive leaves (GO) was found to contain 0.71% oleanolic acid, and the Cape Town cultivar (CT) contained 2.47% oleanolic acid. No ursolic acid was found in either GO or CT. The antihypertensive, diuretic, antiatherosclerotic, antioxidant and hypoglycemic effects of authentic oleanolic and ursolic acid and the three isolates (GO, AO and CT) were studied on Dahl salt-sensitive (DSS), insulin-resistant rat genetic model of hypertension. All three isolates, in a dose 60 mg/kg b.w. for 6 weeks treatment, prevented the development of severe hypertension and atherosclerosis and improved the insulin resistance of the experimental animals. GO, OA and CT isolates could provide an effective and cheap treatment of this particular, most common type of salt-sensitive hypertension in the African population.

Keywords: Triterpenoids; European olive leaves; Cape Town cultivar; African wild olive leaves

1. Introduction

Olea europaea L., (Oleaceae) has been used widely in folk medicine in European Mediterranean islands and countries such as Spain, Italy, France, Greece, Israel, Morrocco, Tunisia, Turkey, etc. In Potter's New Encyclopaedia (Wren, 1994) are cited at least nine biblical references for the medicinal use of the plant in ancient times. Apart from the Mediterranean region, the plant is widespread in the Arabian peninsula, the Indian subcontinent and Asia (Kirtikar and Basu, 1991) and other tropical and subtropical parts of the world. Several subspecies are recognized, one of which is the small-fruited subspecies *africana* (formerly *Olea africana*). Although *O. europaea* is thought to be derived from subspecies *africana* (Mabberly, 1999), in early 80s the African wild olive was defined as *O. europaea*, *subspecies africana* (*Mill*) *P.S. Green* (Van Wyk et al., 1997).

In Southern Africa the wild olive is one of the most popular plants used by Sotho (named *motholoari*), Xhosa and Zulu (named *umnquma*, *isadlulambazo*) tribes (Watt and Breyer-Brandwijk, 1962; Hutchings, 1989; Van Wyk and Gerike, 2000). Recently it was reported (Dold and Cocks, 1999) that from 120 plant species, *umnquma* was designated 'the most important plant' in use in the traditional medicine. There are reports for the use of the wild olive in folk medicine of Mauritius (Fakim, 1990), Reunion and Rodrigues islands (Gurib-Fakim et al., 1993; Adsersen and Adsersen, 1997). The plant is widespread throughout

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Southern Africa and northwards through East Tropical Africa into Erithrea (Neuwinger, 1994). In traditional medicine the plant is used as a diuretic, hypotensive, emollient, febrifuge and tonic, for urinary and bladder infections and for headaches (Hutchings et al., 1996).

The hypotensive and hypoglycemic effects of olive leaves from Mediterranean *O. europaea* have been well documented. Reports of the experimental and clinical studies of these effects have been published as far back as in 1942 (Manceau et al., 1942) and 1949 (Capretti and Bonaconza, 1949), and more recently (Ribeiro et al., 1986; Zarzuelo et al., 1991; Fehri et al., 1994; Cherif et al., 1996).

Studies on the active principles of the European olive leaf, the two secoiridoids oleuropein and oleacein, have been conducted for decades. It was reported that the bitter glycoside oleuropein had a hypotensive, coronary dilating and antiarrhythmic action (Petkov and Manolov, 1972). Recently, a bioassay-directed fractionation showed that another component of European olive leaf, beta-(3,4 dihydroxyphenyl) ethanol was a potent calcium-antagonist (Rauwald et al., 1994). The isolate by fractionation from the olive leaf, secoiridoid oleacein, was reported to have distinct angiotensin converting enzyme (ACE) inhibitory effect (Hansen et al., 1995) and anti-oxidant activity (Bruneton, 1995). Oleuropein has been marketed in USA as Roex® Oleuropein olive leaf herbal extract as a nonspecific immunostimulant.

In 1985 it was reported (Cortesi et al., 1985) that HPLC of leaf extracts of *O. europaea* yielded, in addition of oleuropein as a main constituent, tetra and pentacyclic triterpenes, sterols, erythroidol, uvaol and oleanolic acid but no bioassay-directed phytochemical study has been published. Recently, in a bioassay-directed phytochemical study of wild African olive leaves, we found that the main component of the extracts was a 1:1 mixture of oleanolic acid and its isomer ursolic acid. We named this component oleuafricein. It displayed very low toxicity, significant and sustained hypotensive and diuretic effects in acute and chronic experiments in rats and increased coronary blood flow. All described effects were similar to the reported effects of pure oleanolic and ursolic acid. It was previously anticipated (Hutchings et al., 1996; Casanovas et al., 1997) that the chemical components and bioactivity of the African wild olive might differ from that of the European olive leaves due to geographic differences of climate, soil, etc.

The objectives of the present study were to compare the cardiovascular effects of isolates from African wild olive leaves containing triterpenoids, with isolates from Greek olive leaves and cultivars of Greek olive trees grown in South Africa As positive controls, authentic pure oleanolic and ursolic acids were used.

2. Materials and methods

The procedures followed were approved by the Ethics Committee of the University of Durban, Westville. The principles of laboratory animal care (WIH publication 85–23, revised in 1985) were observed. Dahl salt-sensitive (DSS) genetically hypertensive and control normotensive Dahl salt-resistant (DSR) rats were imported from Sprague Dawly Inc., USA. Male weanling (4 weeks old) rats, weighing 35–40 g at the beginning of the experiment, were used. They were housed in the University Biomedical Resource Center, exposed to a 12-h light:12-h dark cycle and constant humidity. Water and standard food were provided ad libitum. The animals were treated daily in blind-study manner with phytochemicals in a dose 60 mg/kg b.w., intraperitoneally (i.p.) for 6 consecutive weeks (42 days). In preliminary acute experiments on the mean arterial pressure in the carotid artery of rats, the reference substances, oleanolic and ursolic acids were tested after a single i.p. injection of different doses: 20, 40, 60 and 80 mg/kg b.w. The maximal hypotensive effect was established at a dose 60 mg/kg. At the same dose, both acids showed very low toxicity (see results). For that reason, the two acids and the phytochemicals were applied in a dose of 60 mg/kg, i.p., during the chronic experiments. The phytochemicals were dissolved originally in DMSO (stock solution), and in 0.9% saline before application. DMSO was proven indifferent to any of the studied parameters.

2.1. Plant material

Three types of air dried olive leaves were used in this study: wild African olive leaves from a tree grown behind the green house of the Department of Botany, University of Durban-Westville, Natal province, South Africa; leaves from Kalamata olive trees grown in Volos area, Greece; leaves from Kalamata olive trees cultivated in Paarl, West Cape province, South Africa. The material was collected during the summer period in South Africa (January 2000) and Greece (July, 2000). Voucher, specimens of the plant materials were assigned collector's numbers F.O. Shode/1; F.O. Shode/2 and F.O. Shode/3, preserved and housed at Ward Herbarium, Department of Botany Herbarium Unit, UDW.

Authentic oleanolic and ursolic acid, used as positive controls, were purchased from Africa International Food and Cosmetic Technologies, South Africa. Their purity (about 98%) was confirmed by spectroscopic techniques (^{1}H and ^{13}C-NMR).

2.2. Extractives of O. europaea and africana

The dried leaves (1.5 kg wild African olive leaves, 56.7 g Greek olive leaves and 518 g Paarl/Cape Town

olive leaves) were crushed and successively extracted with hexane, ethyl acetate, methanol, and 80% aqueous methanol to give hexane solubles (OAH), ethyl acetate solubles (13.9, 1.3 and 25.6 g, respectively) (OAE), methanol solubles (OAM), and aqueous methanol solubles (OAW). From preliminary experiments we knew that the OAE fraction contained the active material oleuafricein, so this fraction was further purified by repeated silica gel column chromatography with gradient elution (100% hexane to 80% hexane/EtOAc) to give powdery substance (OAE/1) as the major fraction used in the biological experiments.

2.3. Tests for toxicity

Acute toxicity of the extracts was evaluated using brine shrimp (*Artemia salina*) bioassay (Meyer et al., 1982). LC_{50} and 95% confidence intervals were determined from the 24 h counts of the survived naupii by intersection on log dose-response curves by using the same doses: 20, 40, 60 and 80 mg. For qualitative/semiquantitative determination of toxicity, the hippocratic test on rats was used in a 5 day follow-up period after a single intraperitoneal injection (60 mg/kg b.w.; Malone, 1983).

2.4. Hemodynamic screening

Six DSS rats were used per compound, in addition to six untreated control DSR and six untreated DSS rats. A tail-cuff computerized blood pressure monitor (IITC Life Sciences 31, USA) was used. The method was standardized and used routinely in our laboratory and is described in details elsewhere (Somova et al., 1999).

Two types of measurements were performed:

- after a single intraperitoneal (i.p.) application of the drug, systolic and diastolic blood pressure and heart rate were monitored for 60 min in 10 min intervals;
- the same parameters were monitored for 6 consecutive weeks (42 days) after a single i.p. daily application. The drugs were applied after early morning measurement of blood pressure.

2.5. Diuretic and saluretic activity in rats

The Lipschitz test was used (Vogel and Vogel, 1997). The 'Lipschitz value' of diuretic activity is the quotient between excretion by the test animals (six per group) and excretion by the urea control. The rats were placed in individual Nalene metabolic cages at standard conditions. The test compound was applied i.p. in a dose of 60 mg/kg b.w. in 5 ml distilled water per kg b.w., and the urea—in a dose of 1 g/kg b.w. Urine excretion was recorded after 5 and after 24 h. The electrolyte content of the urine was analyzed by using Beckman Synchron EL-ISE Electrolyte System (Germany).

The sum of Na^+ and Cl^- excretion was calculated as a parameter of saluretic activity. The ratio Na^+/K^+ was calculated for natriuretic activity. The ratio $Cl^-/Na^+ + K^+$ (ion quotient) was calculated to estimate carbonic anhydrase inhibition.

2.6. Biochemical determinations

At the end of 6-week experiment the animals were fasted overnight and sacrificed after anesthesia (40 mg/kg b.w., i.p., sodium thiopentone, Rhone-Poulenc, SA) by exsanguination via cannulated carotid artery. The collected heparinazied blood was used for the biochemical determinations.

Blood glucose was estimated by Glucometer Elite, Bayer Diagnostics, in whole blood. Lipid analysis (plasma tryglycerides, total cholesterol and its lipoprotein fractions) was asayed using commercially available Boehringer–Mannheim (Germany) monotest kits.

Glutathione peroxidase (GPx) in whole blood was assayed using Ransel–Randox (Britain) kit, based on the original method of Paglia and Valentine (1967). Superoxide dismutase (SOD) was assayed using Ransod–Randox (Britain) kit, based on the original method of Winterbourn et al. (1975). In both cases of estimation of GPx and SOD, before introduction of the kit, the method was standardized in our laboratory by comparing with the original biochemical method. Both enzymes, GPx and SOD are routinely used to determine the therapeutic efficacy and antioxidant potential of drugs.

2.7. Statistical analysis

Values are expressed as mean ± S.E.M. For statistical analysis, where applicable, the Instat V2.04 programme was used, including one-way Student's t test. A P value of < 0.05 was considered statistically significant.

3. Results

The spectroscopic analysis (^1H and ^{13}C-NMR) of the fraction **OAE/1** from wild African olive leaves revealed it to be a 1:1 mixture of oleanolic acid and ursolic acid. The quantification was estimated 1:1 mixture using the relative intensities of the olefinic proton signals at δ 5.26 (height 12.6) and δ 5.23 (height 14.0). Various attempts to separate this mixture failed. It was, therefore, named oleuafricein and its yield was 0.27%. The same analysis of the samples of OAE/1 from Greek olive leaves and European olive leaves cultivated in Paarl/Cape Town, revealed that they were oleanolic acid, 0.71 and 2.47%,

Table 1
Blood glucose, plasma lipids and antioxidants in control, normotensive DSR, untreated DSS hypertensive rats, and DSS rats treated with oleanolic, ursolic acid and phytochemicals for 6 weeks

Control/parameter	Blood glucose (mmol/l)	Total cholestrol (mg/dl)	HDL cholestrol (mg/dl)	LDL cholestrol (mg/dl)	Triglycerides (mg/dl)	Gluthathione peroxidase (U/ml)	SOD (U/ml)
Control DSR	5.00±0.28	1.57±0.09	0.92±0.04	0.45±0.07	0.44±0.05	103.03±2.3	278.07±20.54
Untreated DSS	6.80±0.20[b]	3.27±0.09[b]	1.06±0.10	1.88±0.13[b]	1.45±0.23	80.47±1.67[b]	210.40±5.25[b]
Oleanolic acid	4.58±0.08[a]	2.65±0.18[a]	1.76±0.22[a]	0.86±0.19[a]	0.77±A[a]	90.3±5.1[a]	236.68±11.32[a]
Ursolic acid	4.60±0.23[a]	1.59±0.23[a]	1.82±0.12[a]	0.52±0.04[a]	0.40±0.07[a]	88.5±3.1[a]	257.41±12.09[a]
O. europaea (Wild African)	4.20±0.18[a]	1.76±0.17[a]	0.91±0.21[a]	0.65±0.06[a]	0.60±0.10[a]	88.4±3.7[a]	246.85±31.61[a]
O. europaea (cultivated in CT)	4.00±0.09[a]	1.97±0.21[a]	0.98±0.30[a]	0.73±0.10[a]	0.78±0.12[a]	95.0±3.2[a]	270.20±19.40[a]
O. europaea (Greece)	4.22±0.15[a]	1.91±0.31[a]	1.17±0.30[a]	0.56±0.08[a]	0.51±0.07[a]	105.4±4.1[a]	251.80±22.32[a]

Mean±S.E.M. The phytochemicals were applied i.p, in a dose 60 mg/kg b.w.
[a] The difference is significant compared with the DSS untreated group.
[b] The difference is significant between DSR normotensive and DSS hypertensive untreated rats.

Table 2
Follow-up changes in blood pressure (mmHg) and heart rate (beats per min) after a single intraperitoneai injection of the phytochemicals (60 mg/kg b.w. i.p.)

Group/parameter	Baseline			10 min			20 min			30 min			60 min		
	SBP	DBP	HR	SBP	DBP	HR	SBP	DBP	HR	SBP	DBP	HR	SBP	DBP	HR
Oleanolic acid	145±3.1	102±1.6	460±16.2	141±1.4	95±1.3	490±17.2	145±3.6	98±3.8	470±14.6	145±3.4	98±4.2	480±18.2	140±4.2	96±3.7	406±14.6[a]
Ursolic acid	147±3.6	104±5.8	480±14.8	150±6.6	103±4.2	432±16.2	142±1.6	102±2.0	377±16.6	140±3.4	102±4.8	450±14.6	140±4.4	100±2.8	406±18.2[a]
O. europaea (Wild African)	143±2.0	104±4.2	463±12.2	139±2.2	101±1.6	358±12.0	137±1.5[a]	99±1.5	373±18.6	4 -[a]$139±3.0	100±2.6	400±16.2	135±2.3[a]	100±2.6	422±12.4[a]
O. europaea (Cultivated in CT)	145 ±2.5	98 ±3.2	462±12.6	140±4.3	97±1.5	424±18.2	146 ±2.0	96±1.6	480±12.6	148±2.8	102±3.0	461 ±8.0	138±1.6[a]	98±2.2	300±8.0[a]
O. europaea (Greece)	145 ±2.3	102±2.9	465±13.6	144±2.0	104±2.1	443±14.3	138±2.2[a]	102±2.2	448±14.6	144±4.3	106±4.6	450±14.6	135±4.2[a]	101 ±3.2	406±10.9[a]
Vehicle (DMSO)	144±4.6	100±2.6	438±11.0	141±2.2	98±2.4	440±13.0	143±3.2	99±3.0	445±10.0	141±2.6	102±3.4	400±10.2	143±3.2	102±3.0	445±16.0

Mean±S.E.M.; SBP, systolic blood pressure; DBF, diastolic blood pressure; HR, heart rate.
[a] Significant compared with baseline value.

Table 3
Changes in blood pressure (mmHg) and heart rate (beats per min) of DSS hypertensive rats treated with the phy to chemicals for 6 weeks

Group/parameter	Control			1 week			2 weeks			3 weeks			4 weeks			5 weeks			6 weeks		
	SBP	DBP	HR	SBP	DBP	HR	SBP	DBP	HR	SBP	DBP	HR	SBP	DBP	HR	SBP	DBP	HR	SBP	DBP	HR
Control untreated	124±2.8	82±2.1	332±14.6	121±2.5	95±4.4	404±27.0	140±3.5	102±4.3	451±16.2	140±5.4	115±5.8	483±20.4	148±2.3	106±2.0	458±18.7	155±2.2	120±2.8	466±14.7	167±2.7	128±3.5	462±14.3
Oleanolic acid	123±1.9	83±2.6	405±22.0	122±3.6	88±4.1	402±24.4	140±2.9	104±3.2	480±14.6	143±4.6	100±1.5	471±16.4	135±2.9*	100±2.0	403±16.0*	143±1.5*	110±2.7*	417±20.0	144±1.0*	116±1.3*	304±10.2*
Ursolic acid	129±3.4	82±1.6	437±19.8	123±3.5	89±4.1	358±26.4	140±3.2	98±3.0	448±12.0	136±5.6	94±2.2	362±12.8*	138±1.0*	102±2.1	408±12.2*	145±1.6*	114±2.6	427±12.8	140±4.0*	109±4.0*	313±10.0*
O. europaea (Wild Africa)	129±3.7	89±2.2	477±11.6	126±3.8	88±4.0	393±22.6	139±3.9	102±3.0	484±12.8	141±3.4	104±2.6	424±18.6	140±1.8*	105±1.6	444±10.8	148±2.6	120±2.2	462±12.0	145±3.6*	116±2.3*	403±9.6*
O. europaea (Cultivated CT >	131±3.7	89±2.2	478±12.6	120±4.5	92±3.7	442±22.5	140±1.2	102±2.6	483±22.2	142±4.6	97±3.3	340±22.4*	142±2.0	109±2.2	460±17.1	138±1.3*	108±1.8*	440±8.9	144±4.6*	114±3.3*	401±14.2*
O. europaea (Greece)	131±3.6	90±4.0	500±16.2	130±6.4	104±6.6	492±16.8	128±4.2*	101±2.8	485±12.0	140±2.3	102±2.9	335±26.6*	142±2.2	108±3.7	408±16.0*	137±3.1*	108±2.8*	456±8.0	140±4.8*	110±6.0*	422±8.8*

Mean±S.E.M; SBP, systolic blood pressure; DBF, diastolic blood pressure; HR, heart rate. The phytochemicals were applied i.p. in a dose 60 mg/kg b.w.

respectively. In both Greek and Paarl (Cape Town) extracts there was no ursolic acid.

In the further description of the results the following abbreviations will be used: AO for oleuafricein from wild African olive leaves; GO for the extract from Greek olive leaves and CT for the extracts from leaves of Paarl/Cape Town cultivar.

The brine shrimp test showed that both oleanolic and ursolic acid have low toxicity with LC_{50} 0.10 and 0.95 mg/ml, respectively. The AO, GO and CT showed similarly low toxicity with LC_{50} 1.25, 1.95 and 1.70 mg/ml, respectively. During the hyppocratic test, apart from slight transient diarrhea of the animals treated with authentic oleanolic and ursolic acid on day 4 and 5, no toxicity at a dose 60 mg/kg b.w. was recorded.

The results of the in vivo experiments are presented in Tables 1–4. At the age of 10 weeks (the end of 6-week experiment), DSS untreated rats developed spontaneously hypertension with significantly increased heart rate. Since this strain of rats are insulin resistant (Somova et al., 1999) they displayed significantly increased blood glucose by 26% and they were prone to develop an early atherosclerosis with significantly increased total cholesterol by 108%, increased more than four times LDL cholesterol and triglycerides. DSS rats showed a compromised antioxidant status with significantly decreased blood GPx by 22% and red blood cells SOD by 24%, compared with the control normotensive DSR rats. All of the above biochemical parameters normalized almost completely after 6 weeks treatment with authentic oleanolic and ursolic acid and the extractives (AO, GO, and CT). They showed a potent hypoglycemic, antihyperlipidemic (antiatherosclerotic) and antioxidant activity (Table 1).

The results of the blood pressure and heart rate follow-up after a single i.p. application (60 mg/kg b.w.) are presented in Table 2. They showed a trend of decreased blood pressure on the 20 and 60 min after a single i.p. application of AO, GO and CT. All five phytochemicals, i.e. oleanolic acid, ursolic acid, AO, GO and CT, produced significant bradicardia 60 min after application.

The results of blood pressure and heart rate after 6 weeks daily i.p. (60 mg/kg b.w.) treatment showed that from the 4th week onward all drugs (the two acids and three phytochemicals) prevented the development of hypertension in DSS rats, with significant bradicardia.

The two acids and the three phytochemicals showed potent diuretic activity, and comparable natriuretic and saluretic activity to that of hydrochlorothiazide, suggesting inhibition of Na^+ and K^+ reabsorption in the early portion of the distal tubule. No carbonic anhydrase inhibition was detected (Table 4).

4. Discussion

As described in the Section 1, the cardiovascular effects of extracts from *O. europaea* leaves have been well researched and attributed to the main components of the European leaves, the two secoiridoids oleuropein and oleacein (Lasserre et al., 1983). No research on the biological effects of the triterpenoids present in the leaves was reported, probably because of their relatively low quantity (0.71% as it was found in the present study). But some European authors (Zarzuelo et al., 1991) suspected that in addition to the hypotensive effect of the oleuropeoside, there might be at least one other active principle which is either a vasodilator itself or else potentiates the relaxant effect of the oleuropeoside.

We found that African wild olive lives contain 0.27% mixture of oleanolic and ursolic acid (named oleuafricein) with potent antihypertensive, diuretic/natriuretic, antihyperlipidemic, hypoglycemic and antioxidant activity. The most surprising finding was that a cultivar of Greek *O. europaea*, grown in Cape Town Province, contained a high level of pure oleanolic acid (2.47%), and displayed the best of the above effects.

The DSS model of genetic hypertension was used in the present experiment, since it is the closest model of the salt-sensitive, insulin resistant type of hypertension typical for the African population (Mufunda and Somova, 1993). Hypertension is only one component of a multifacetal metabolic-hemodynamic complex that also includes obesity, subtle and overt glucose intolerance, dyslipidemia, enhanced vascular resistance and accelerated atherosclerosis. Number of studies in the past several years have shown that even nonobese, nondiabetic individuals with hypertension display insulin resistance, which is located in peripheral tissues, is limited to nonoxidative pathways of glucose disposal, and appears to be directly correlated with the severity of hypertension. Insulin resistance and associated hyperinsulinemia in hypertensive individuals are also associated with increased plasma triglyceride levels and decreased high-density lipoprotein concentrations, which likely contributes to enhanced atherosclerosis (Sowers, 1992).

The potent antihypertensive, antihyperlipidemic (antiatherosclerotic), hypoglycemic and antioxidant effects of all three examined phytochemicals (AO, CT and GO), suggested that they may be used as a potent, non-toxic and very cheap treatment of salt-sensitive, insulin resistant type of hypertension. Another important suggestion from the present experiment is that AO and especially CT, containing significant level of oleanolic acid, might be used in hypertension complicated with cardiac failure, since it was reported (Duke, 1992) that oleanolic acid might have a cardiotonic effect unlike oleuropeoside which has an opposite, negative inotropic

Table 4
Diuretic, salurelic and natriuretic activity of oleanolic acid, ursolic acid and phy to chemical extracts of olive leaves, compared with the effects of urea and hydrochlorothiazide

	5 h after administration					5 h after administration				
	Diuresis (ml/100 g b.w.)	Lipschitz value (T/U)	Na+Cl (mmol/l)	Na/K (mmol/l)	Cl/Na+Cl (mmol/l)	Diuresis (ml/100 g b.w.)	Lipschitz value (T/U)	Na+Cl (mmol/l)	Na/K (mmol/l)	Cl/Na+Cl (mmol/l)
Urea (lg/kg b.w.)	1.4±0.2	–	440±12.6	1.67±0.17	0.654±0.02	7.0±0.6	–	281±10.0	1.32±0.12	0.648±0.01
Hydrochlorothiazide 25 mg/kg b.w.	3.5±0.6	2.5	520±8.4	3.44±0.10	0.834±0.02	14.6±0.5	2.08	382±7.6	2.43±0.12	0.672±0.01
Oleanolic acid	1.4±0.2[b]	1.0	376±15.7[a,b]	1.07±0.12[b]	0.618±0.05[b]	7.0±0.4[b]	1.00	269±8.4[b]	0.92±0.13[b]	0.539±0.01[b]
Ursolic acid	1.6±0.3[b]	1.1	442±12.8[b]	1.47±0.17[b]	0.466±0.03[a,b]	6.8±0.4[b]	0.97	227±12.2[b]	0.83 ±0.10[b]	0.566±0.02[b]
O. europaea (Wild African)	1.7±0.3[b]	1.2	482±17.5	1.93±0.26[b]	0.501±0.04[a,b]	6.8±0.5[b]	0.97	243±10.1[b]	0.88±0.10[b]	0.551±0.02[b]
O. europaea (Cultivated in CT)	1.1±0.2[b]	0.80	482±20.0	1.56±0.21[b]	0.621±0.05[b]	5.5±0.4[b]	0.78	268±14.6[b]	0.85 ±0.11[b]	0.575±0.04[b]
O. europaea (Greece)	0.9±0.1[b]	0.60	426±15.2[b]	1.33±0.15[b]	0.887±0.09[a]	5.6±0.3[b]	0.80	246±7.4[b]	0.86±0.10[b]	0.526±0.01[b]

Mean±S.E.M.; The phitochemicals were applied i.p. in a dose 60 mg/kg b.w.
[a] The difference is significant compared with the effect of urea.
[b] The difference is significant compared with the effect of hydrochlorothiazide.

effect (Duarte et al., 1993). In our current study we are exploring this possibility.

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