Effects of Olive Leaves Extract on Urea, Uric Acid and Creatinine Concentrations in Serum of Heat Stressed Male Rabbits

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تأثيرات مستخلص أوراق الزيتون على تركيز اليوريا وحامض اليوريك والكرياتنين في مصل ذكور الأرانب المجهدة حرارياً

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الخلاصة

أجريت الدراسة الحالية للتحري عن التأثيرات الايجابية لمستخلص أوراق الزيتون على التغايرات لبعض اختبارات وظائف الكلى (اليوريا، حامض اليوريك والكرياتنين) في مصل ذكور الأرانب المرباة تحت ظروف الإجهاد الحراري.

30 من ذكور الأرانب البيضاء (بعمر 12-14 اسبوع و بوزن 1500-2000 غرام) وضعت في أقفاص فردية لمدة 30 يوم. قسمت الحيوانات إلى ستة مجاميع (5 حيوانات لكل مجموعة) مجموعة سيطرة وخمس مجاميع تجريبية(ربيت تحت ظروف الإجهاد الحراري). المجموعة التجريبية الأولى تعرضت للإجهاد الحراري فقط، واثنين من المجاميع الأخرى استلمت كل يوم 1000/ملغم/كلغم و2000/ملغم/كلغم من وزن الجسم على التوالي من المستخلص الجاف لأوراق الزيتون وضعت في كبسولات وجرعت فمويا، أما المجموعتان الباقيتان فقد استلمت 200 ملغم/ مل و 400 ملغم/مل على التوالي من المستخلص المائي لأوراق الزيتون مع ماء الشرب يوميا خلال مدة التجربة.

أظهرت النتائج زيادة معنوية في مستويات كل من اليوريا وحامض اليوريك والكرياتنين في الأرانب المجهدة حراريا، بينما كان هناك تحسن واضح في هذه المعايير الثلاثة في الحيوانات المعاملة بالمستخلص الجاف والمائي لأوراق الزيتون اعتمادا على التركيز. وبذلك يمكن الاستنتاج إن هذه النتائج تؤكد الخواص الصحية الايجابية للزيتون كمنتج مضاد للإجهاد الحراري.

Abstract

The purpose of this study is to investigate the positive effects of olive leaves extract on the alteration of some renal function test (urea, uric acid and creatinine) in serum of male rabbits that have been reared under heat stress conditions.

Thirty , New Zealand white male rabbits,(12-14 weeks old and 1500-2000 gm in weight) are individually caged, for 30 days. The animals are divided into six groups(5 animals for each) control group and 5 experimental groups (reared under heat stress environment). The first experimental group heat stressed only, the other two groups have received every day, 1000mg/kg/bw and 2000mg/kg/bw respectively of dried extract of olive leaves put in capsules and fed orally, the rest two groups have received 200 mg/ml and 400mg/ml respectively of aqueous extract of olive leaves with drinking water every day of the experiment.

The results show a significant increase of urea, uric acid and creatinine levels in sera of heat stressed rabbits, while there is an improvement of these three parameters in the animal treated with the dried and aqueous extracts in concentration dependent manner. In conclusion, these results confirm the positive healthful properties of olive as anti-heat stress products.

Introduction

The adverse effects of hot weather on health are of increasing public health concern, particularly for urban areas. With climate change, warmer climates are expected to result in higher mean summer temperatures and fluctuations will likely result in more frequent and intense heat waves and their associated health risks [1,2]. Among the potential direct risks that global warming presents to human health is the increase of heat-related deaths during intermittent hot weather, as predicted by WHO [3].

An increase in mortality related to heat waves has been reported from various industrialized countries in Europe and Asia [4,5],these studies indicate that especially elderly are at highest risk of heat-related mortality.

Most of the animal research which is concerned with heat stress issues deals with cattle and poultry because of economic reasons. Many studies have suggested that hyperthermia associated with heat stress (HS) has been proven that energy from nutrient intake is critical because of the decline in feed intake that occurs [6,7], also milk has a lower solids content [8] , and animals show impaired reproductive performance and greater susceptibility to ill-health [9,10,11]. High environmental temperature induces physiological stress in rabbits leading to production losses [12], also because of their quite poor thermoregulation ability, some consequences of heat stress affect digestive system functions, with impaired appetite, growth and feed conversion, but also with increased disease incidence [13].

Several methods are available to alleviate the negative effects of high environmental temperature, such methods are mostly focused on the natural products since these products have served as an important source of drugs since ancient times and a significant part of today's drugs are somehow derived from natural sources, therefore, many people in developing countries use traditional drugs derived from medicinal plants to meet their primary health care needs [14].

In this respect, olive leaves are used because of the nutritional and healthful properties of olive products, which are actively being explored, also there are no reports on the toxicological properties of this plant in literatures [15].

Olive tree (*Olea europaea*) is a small evergreen tree, member of the family Oleaceae, native to coastal areas of Southern Europe, the Mediterranean region and Asia Minor. The olive tree had been domesticated during the early Neolithic in the Near East, and more than 1000 different cultivars have been identified to date, however, the Mediterranean region accounts for almost 98% of the world's olive tree plantation [16] .

Evidence of olive use for oil production can be found in historical and sacred texts, such as the Holy Koran, Holy Bible, and the Odyssey [17]. It is traditionally used to treat abdominal colic, baldness, paralysis, rheumatic pains, hypertension, atherosclerosis and improvement of health (17,18), besides olive products are used as an effective hypoglycemic and antioxidant agent in alleviating oxidative stress and free radicals as well as in enhancing enzymatic defenses in diabetic rats (19). Furthermore recent studies evaluate the potential anti-cancer effects of olive leaves *Olea europaea* extracts on human leukemia cell [20.21].

Thus, this study is designed to investigate the positive effects of olive leaves extract on the alteration of some renal function test (urea, uric acid and creatinine) in serum of male rabbits that have been reared under heat stress conditions.

Materials and Methods

Plant Material and Preparation of Extracts

Fresh samples of *O. europaea* L. leaves are collected from olive tree in winter of 2010 . The sample, are carefully washed with water and put under mild sunlight with continuous overturn, to prevent moldiness, until they completely dried. These

parts are then pulverized into powder. Tow concentrations of this leaves powder have been used in this experiment (1000mg/kg/body weight, 2000mg/kg/body weight).

The dried powder material of *O. europaea* L. leaves is extracted with methanol in a Soxhlet apparatus. The solvent is completely removed under reduced pressure and a semisolid mass is obtained and stored in a refrigerator at 4°C. until use. Two concentrations of this stock extract are prepared by dissolving in distill water (200 mg/mL, 400 mg/mL).

Animals and Experimental Design

Thirty, New Zealand white male rabbits,(12-14weeks old and 1500-2000 gm in weight) are individually caged, for 30 days (from 1/7/2011-1/8/2011) . The animals are divided into six groups(5 animals for each): control group and 5 experimental groups (reared under heat stress environment and additional heat is applied through a heating lamp, if necessary, to maintain the rabbits at continuous heat stress). The first experimental group heat stressed only (T1), the other two groups have received every day 1000mg/kg/bw (T2), and 2000mg/kg/bw (T3)respectively of dried extract of olive leaves put in capsule and fed orally. the rest two groups have received 200 mg/ml (T4) and 400mg/ml(T5) respectively of aqueous extract of olive leaves with drinking water every day of the experiment.

Blood samples of 2 ml were obtained from the rabbits via venepuncture of an antecubital vein. Blood was collected into polypropylene tubes for biochemical analysis.

Assessment of Serum Uric acid, Urea and Creatinine

The uric acid and creatinine levels in all the sample sera are estimated by modified methods of Henry *et al.*, (1982) [(22)] and Bonsnes & Taussky, (1982) [(23)], respectively, on standard diagnostic test kits (Spinreact Inc., Spain) ,(Randox Laboratories, U.K.), while urea levels are estimated by standard diagnostic test kits (Spinreact Inc., Spain) following the procedures described in the kits. The urea, uric acid and creatinine levels in serum are expressed as mg/dl.

Statistical Analysis

The results are expressed as mean values ±S.D. (The decimal values are rounded off to 2 digits). Differences between groups are assessed by one-way ANOVA using the SPSS (Statistical Package for Social Sciences) computer package for windows (version 10). Least Significance Range(L.S.R.)of Duncan's Multiple Range Test is performed for inter-group comparisons using the significance at 0.05.

Results

Data that are presented in table (1) show a significant increase ($P<0.05$)of urea, uric acid and creatinine levels in sera of heat stressed rabbits (T1) as compared to unstressed animals (control group), also the results which are summarized in tables (1) show the effects of the dried and aqueous extracts on the three parameters, they are statistically significant ($P<0.05$) lower than untreated rabbits (heat stressed rabbits only T1), especially in higher concentrations of both olive leaves extracts (T3, T5) and reach in levels near to the control significantly ($P<0.05$). Also the results revealed that the aqueous extract was more effective in improve the negative effects of heat stress conditions.

Table (1): Effects of dried and aqueous extracts of olive leaves on urea, uric acid and creatinine concentrations in serum of heat stressed male rabbits for 30 days.

Parameters / Groups	Urea (mg/dL)	Uric acid (mg/dL)	Creatinine (mg/dL)
Control (unstressed)	**21.48± 0.41 e**	**3.44 ± 0.10 dc**	**1.42 ± 0.01 b**
T1 (heat stressed only)	**32.44 ± 1.27 a**	**6.18 ± 0.31 a**	**2.23 ± 0.19 a**
T2 (heat stress + 1000mg/kg/bw of dried extract of olive leaves)	**27.68 ± 0.48 bc**	**4.84± 0.37 a**	**1.93 ± 0.04 a**
T3(heat stress + 2000mg/kg/bw of dried extract of olive leaves)	**28.45 ± 1.36 ab**	**4.28 ± 0.04 ab**	**1.87 ± 0.06 a**
T4(heat stress + 200mg/ml of aqueous extract of olive leaves)	**25.74 ± 0.26 cd**	**3.70± 0.12 cd**	**1.60 ± 0.04 a**
T5(heat stress + 400mg/ml of aqueous extract of olive leaves)	**23.82 ± 0.30 de**	**3.54 ± 0.13 dc**	**1.48 ± 0.02 b**
	*	**	***

-Numbers represent means ± standard error.
- Different letters means significance difference at ($P<0.05$).
***L.S.R (Duncan's value): 4.72 , 4.44 , 4.21 , 3.93 , 3.78**
****L.S.R (Duncan's value): 1.85 , 1.40 , 0.81 , 0.58 , 0.36**
*****L.S.R (Duncan's value): 0.74 , 0.63 , 0.49 , 0.31 , 0.28**

Discussion

High temperature is of one the most extensively studied of the various environmental conditions can cause severe organ disorders through many mechanisms such as the metabolic activation to highly reactive free radical, besides heat stress and other forms of stress, such as hypoxia/ ischemia, oxidative stress, or exposure to heavy metals, is overwhelming the various inherent defense mechanisms such as the antioxidant defense mechanisms, intracellular concentration of glutathione, superoxide dismutase (SOD) and catalase (CAT) activities become significantly impaired and insufficient [(24)].

As a measure of renal function status, serum uric acid ,urea and creatinine are often regarded as reliable markers [(22,23)]. Urea is the detoxification product of the ammonia derived from deamination of amino acids, thus urea considered to be the end product of protein catabolism [(25)]. Creatinine is a catabolic end product ,an anhydride of creatine(or phosphocreatinine) produced

by loss of water (or phosphoric acid) from the molecule in an irreversible reaction [26]. The catabolism of the purines (adenine and guanine) product uric acid by xanthine oxidase.

Thus, elevations in the serum concentrations of these markers are indicative of renal injury simply because the kidneys excrete them. Elevation in uric acid and creatinine in agreement with results of previous studies in broiler chicken [7,27,28,]. *Gudev* and colleagues [28] have suggested that increasing plasma urea level in heat stressed buffaloes closely related with the dynamic of cortisol and blood volume fluctuation in animals under heat. Furthermore Atlan et al., (2003) [29] mention that increase cortisol level in heat stress broiler leads to increase of catabolism rate of proteins to generate glucose in gluconeognesis process.

These biochemical alterations are corroborated by the histological findings of glomerular and tubulo-interstitial necrosis in the heat stress animals in other studies [7,30].

As mention previously in results that there is an improvement of the three parameters in the heat stressed animals treated with the dried and aqueous extracts of olive leaves in concentration dependent manner. These results are accepted since this plant produce a great diversity of substances that could be of therapeutic significance in many areas of medicine.

Olive leaves are rich in biophenols such as oleuropein, verbascoside, ligstroside, tyrosol, and hydroxytyrosol, these compounds have shown biological activities such as anti-oxidation [21,31,32]. However, tyrosol is effective in preserving and inducing survival proteins, probably through intracellular accumulation [33]. Japón-Luján et al. (2006) [34] showed that olive leaves are considered to have the most radical scavenging power of different parts of olive trees [35], but the phenol content and antioxidant capacity of them significantly changed between seasons [36] . Therefore we used olive leaves collected in winter because according to Gonzalez et al., (1992) [37] phenol content and antioxidant capacity of these substances are more concentrated.

The difference between the effects of two extracts of olive leaves that have been used in current study may be explained as bioavailability of a compound refers to the degree in which it is extracted from a food matrix and absorbed by the body [38]. Research has shown that the phenolic compounds, hydroxytyrosol and tyrosol are absorbed after ingestion in a dose-dependent manner [39,40].

Literature have shown olive leaves with nephroprotective properties to mediate their protection via antioxidant and/or free radical scavenging activities due to the high concentration of biophenols they contain. In this aspect Tavafi et al (2012) [41] found that olive leaf extract

protects from gentamicin-induced nephrotoxicity possibly by inhibition of lipid peroxidation, enhancing renal glutathione content and antioxidant enzymes activity. In addition, (Visioli et al., 2009) [35] found that olive phenolics increase glutathione levels in healthy volunteers. Also the effective role of the extracts may partially explained by hypotensive effects of olive leaf extract that make kidney work normally [42]. Again, the histological findings of almost normal renal histological architecture corroborate the decreased levels of urea and creatinine confirmed protection effects by the extract within the stipulated time interval, especially at the maximum oral dose the extract [15].

In conclusion the results of the current study confirm the positive healthful properties of olive as anti-heat stress products, especially the aqueous extracts in concentration dependent manner.

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