Cardio-Nephroprotective Effects of Guava and Olive Leaves Extracts on Doxorubicin-Induced Toxicity in Rats

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Abstract: *Objective*: Doxorubicin (DOX) is an anticancer drug that is known to increase oxidative stress in several organs. Our objective was to evaluate the possible cardioprotective and nephroprotective effects of guava leaves extract (GLE) and olive leaves extract (OLE) on DOX-induced toxicity in rats.

Methods: Forty adult male albino rats were randomly divided into 4 groups of 10 rats each, as follows: a normal control group, a DOX group (a single dose of DOX; 30 mg/kg intraperitoneally), a GLE group (500 mg GLE/kg body weight), and an OLE group (500 mg OLE/kg body weight) for 12 d. DOX was administered in the DOX, GLE, and OLE rats, which were sacrificed 4 d after DOX administration.

Results: DOX injection resulted in a significant elevation in serum lactate dehydrogenase, creatinin kinase-MB (CK-MB), total protein, urea, and creatinine. Cardiac as well as renal glutathione (GSH) and catalase (CAT) were significantly decreased, whereas tissue lipid peroxidation significantly increased. Pretreatment with GLE and OLE significantly reduced the elevated concentrations of serum lactate dehydrogenase, KC- MB, urea, creatinine and total protein ($P < 0.05$). GLE and OLE increased cardiac as well as renal GSH and CAT concentrations and decreased malondialdehyde concentrations.

Conclusions: GLE and OLE showed promising protective effects against DOX-induced cardio-nephrotoxicity, which might be attributed to their antioxidant activities.

Keywords: Antioxidant, phenolic compounds, Flavonoids, Oxidative stress, Renal, Cardiac.

INTRODUCTION

Doxorubicin (DOX) is an anthracycline that has been used in anticancer therapy for several decades. However, Doxorubicin has a dose-dependent cardiotoxicity effect which limits its efficacy as an antitumor treatment. Doxorubicin (DOX) was found to cause early or Late cardiotoxicity [1].

Despite Doxorubicin is the of drug of choice for cancer treatment because of its efficacy but it has many acute and chronic side effects some of which are irreversible especially in the heart.

Doxorubicin cardiotoxicity and nephrotoxicity was reported to be due to oxidative stress.

It has been demonstrated that vegetables and fruits regular ingestion decreased the risk of certain malignancies [2]. Therefore, fruits and vegetable phytochemicals can be considered as promising chemopreventive agents.

Medicinal plants have been used as treatment for several human disorders. *Psidium guajava L.* commonly known as guava which considered as one of the common herbs used for long time in folk medicines as a therapeutic agent for the treatment of number of diseases, as an anti-inflammatory, diabetes, rheumatic pain, hypertension, wounds, ulcers and reducing fever. Guava leaves extract has various pharmacological effects [3].

Guava comprise many health benefiting components such as vitamins, phenolic compounds, tanins, flavonoids, sesquiterpene alcohols, essential oils and triterpenoid acids [4].

Olive leaves have been used for traditional therapies in Mediterranean countries. It has been illustrated that olive leaves have antihypertensive, antiatherogenic, antiinflammatory, hypoglycemic, and hypocholesterolemic actions [11–13]. These effects are attributed to the antioxidant components of olive leaves [5].

Oleuropein and its derivatives are considered to be the main phenolic components of olive leaves, which are thought to be responsible for their protective effects on oxidative stress-induced renal, cardiac and hepatic damage in experimental animals [6].

The objective of the currentt study was to examine the protective effects of Guava and Olive Leaves

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Extracts against DOX-induced cardiotoxicity and nephrotoxicity in rats.

MATERIALS AND METHODS

Chemicals

Doxorubicin (DOX was obtained from NOVARITS Co. Egypt. Guava and Olive Leaves were purchased from local markets, Cairo - Egypt. All other reagents and chemicals were of analytical grade.

Doxorubicin was dissolved immediately before use in saline and was intraperitoneally injected to rats with a single dose 30 mg/kg [6].

Animals

Forty adult male albino rats were obtained from the g) were purchased from Animal House of El-Salam-Farm, Giza, Egypt), and used for the research. Animals were acclimatized for one week to laboratory conditions before starting the experiment; they had free acceses to water and standard rat feed.

Preparation of Guava Leaves Extract

Guava leaves were purchased from local market. The leaves were identified and authenticated. GLE (Methanol extract) was prepared according to [7]. The leaves were dried and powdered using an electric blender. Guava leaves powder 100 g in 1.5 L pure methanol (purity 99.8%), was soaked in 1.5 L pure methanol for 3 days, then filtered and concentrated by evaporation under pressure at 40^{o}C. The resulting filtrates were dried using a freeze drier and stored at -18^{o}C for use.

Preparation of Olive Leaves Extract

The fresh leaves of olive were completely washed, air dried at room temperature, ground to obtain a fine powder and stored in a dry container until use for extraction processes for extraction of methanolic olive leaves extract according to [8]. 50 g olive leaves were macerated in 250 mL of pure methanol for 60 min in the dark at room temperature. The extract was separated by filtration, and the solvent was evaporated under vacuum.

Experimental Protocol and Treatment Schedule

After one week of acclimatization, the animals were randomly divided into 4 groups of 10 animals each as follows: Group 1 served as normal control, a DOX group (a single dose of DOX; 30 mg/kg intraperitoneally), a GLE group (500 mg GLE/kg body weight), and an OLE group (500 mg OLE/kg body weight) for 12 consecutive days, 8 days before and 4 days after the DOX administration. DOX was administered in the DOX, GLE, and OLE rats, which were sacrificed 4 d after DOX administration.

The OLE and GLE dosage used in this study was proven in previous studies to be safe and not relate to any organ injury.

At the end of experiments, rats were sacrificed under ether anesthesia and blood samples were collected in clear tubes, the blood was allowed to clot and serum was obtained from each sample by centrifugation at 3000R/Min for 10 min. Hearts and kidney tissue were quickly excised, removed and washed with normal saline solution to remove excess blood, dried with wattman filter paper, weighed and kept at 4 ^{0}C for antioxidant enzyme analysis.

Phytochemical Screening of Guava Leaves Extracts (GLE)

Guava leaves extract subjected to chemical analysis to determine total phenolic content (TPC) and Total flavonoids content (TFC).

Total phenolic content (TPC) were determined according to The Folin–Ciocalteu method [9] Results were expressed as mg caffeic acid/g extract.

Total Flavonoid content were determined by Moreno's method [10], and were assessed using quercetin as a standard.

The tests were run in triplicate and averaged.

Phytochemical Screening of Olive Leaves Extracts (OLE)

The total phenolic compounds content (TPC) were determined according to Thaipong *et al*. [11] Results were expressed as mg of gallic acid equivalents / g dry weight sample (mg GAE/g).

Total flavonoids content was determined according to Vuong *et al*. [12]. Results were expressed as mg of rutin equivalents per g dry weight sample (mg RE/g)

The tests were run in triplicate and averaged.

Biochemical Analysis

Serum lactate dehydrogenase (LDH) and creatinin kinase- MB (CK- MB) were measured according to

(Weishaar *et al*.) [13], and (Horder *et al*.) [14]) respectively. Total protein was estimated using the method of Doumas [15]. Serum Urea, and creatinine were also measured in serum according to [16, 17] espectively. Cardiac as well as renal glutathione were estimated according to [18]. Cardiac and renal catalase (CAT) were estimated according to [19] whereas tissue lipid peroxidation was estimated according to [20].

Statistical Analysis

Statistics were performed using the Statistical Package for the Social Science (SPSS) software version 17.0 for all statistical analyzes (SPSS Inc., Chicago, IL, USA). The results were expressed as means ± standard deviation (SD). Differences between treatment groups were analyzed by one-way analysis of variance (ANOVA). Differences were considered significant when ($P < 0.05$) [21].

RESULTS AND DISCUSSION

Treatment of cancer patients with DOX has many side and adverse effects which increase the burden and worse the situation on the cancer patient treated with drug.

Medicinal plants and herbs are great sources of antioxidants and have been used in the treatment of several diseases. The plant-derived small molecules (phytochemicals) have been shown to possess anti-inflammatory, antioxidant, and anticancer activities and so, they can be used to minimize the side effects of DOX in cancer patients.

The results presented in Table **1** indicated that the total phenolic content of OLE was 217.90 ± 8.15 mg GAE g-1 dried leaves while total flavonoid content of OLE was 339.03 ± 6.16 mg of rutine equivalents g-1 dried leaves indicating high TPC and flavonoids which correlated to its high antioxidant activity.

On the other hand Table **1** also showed that GLE contain 142.23 ± 6.5 mg caffeic acid g-1 extract as TPC and 92.28 ± 5.7 mg of quercetin equivalents g-1 extract as TFC.

Effects of Guava and Olive Leaves Extracts on Serum Lactate Dehydrogenase, Creatinin Kinase-MB (CK-MB) in Rats Treated with DOX

The heart is mostly vulnerable to free radical injury, because it contains less free radical detoxifying substances than do metabolic organs like liver or kidney (52, 53). Moreover DOX is known to have a higher affinity for cardiolipin, a major phospholipid component of the mitochondrial membrane in heart [22]

Some isoenzymes, including CK-MB, and LDH, are commonly used as indications for cardiac injury. CK-MB has been considered the core enzyme for the determination of neuromuscular diseases.

The results of the current study illustrated that DOX intraperitoneal administration at a dose of 30 mg/kg induces cardiovascular abnormalities by rise in free radical production as indicated by significant increase in CK-MB and LDH as shown in Table **2**. These results are in accordance with previous authors [23].

On the other hand Pretreatment with GLE and OLE significantly reduced the elevated concentrations of serum lactate dehydrogenase and serum creatinin kinase-MB (CK-MB) in rats treated with DOX as shown in Table **2**. The beneficial cardioprotective properties of GLE in DOX induced cardiotoxicity may be mainly attributed to their radical-scavenging activities. In addition, GLE possess high phenolic and flavonoid content as shown in Table **1**.

The cardioprotective effect of GLE could be attributed to Lycopene which prevent cardiovascular damage through its positive effects on dyslipidemia [24].

Table 1: The Total Phenolic Content (TPC) and Total Flavonoids Content (TFCC) of Guava and Olive Leaves Extracts (MEAN ±SD)

Constituent	
Guava leaves extracts	
Total phenolic compounds (mg caffeic acid/g extract)	142.23 ± 6.5
Total Flavonoids (mg of quercetin equivalents g-1 extract)	92.28 ± 5.7
Olive leaves extracts	
TPC are expressed as gallic acid equivalents (GAE)/g of dried leaves (mg GAE/g)	217.90 ± 8.15
Total Flavonoids expressed as rutin equivalents (RE)/g of dried leaves (RE/g)	339.03 ± 6.16

Table 2: Effects of Guava and Olive Leaves Extracts on Serum Lactate Dehydrogenase, Kinase-MB in Rats Treated with DOX

Parameters \ Groups	Group 1 Normal Control (C)	Group 2 Doxorubicin (DOX)	Group 3 GLE+DOX	Group 4 OLE+DOX
Serum lactate dehydrogenase (U/L)	1013.42 ± 6.25 [a]	2028.94 ± 7.14 [b]	1871.60 ± 7.81 [c]	1854.61 ± 6.39 [c]
Creatinin kinase-MB (U/L)	1120.94 ± 3.09 [a]	1756.40 ± 6.95 [b]	1575.62 ±3.74 [c]	1515.62 ± 5.07 [d]

Values are expressed as means ± SD. Means with similar superscript (a, b c, d) letters in rows indicate non-significant difference (P< 0.05).

The results of the current study agreed with previous researchers who reported that, aqueous extract of P. guajava had cardioprotective properties in myocardial ischemia-reperfusion injury in isolated rat hearts [25].

Previous studies exemplified the presence of Diversity of bioactive compounds in the leaves, seed and bark of Psidium guajava that are proficient of showing valuable effects on human health.

The effective cardioprotective properties of OLE on DOX-induced cardiotoxicity may be through obstructing lipid peroxidation products, decreasing oxidative stress and reducing inducible nitric oxide synthase (iNOS) in cardiomyocytes.

Oleuropein has a valuable influence on numerous aspects of cardiovascular disease via its vasodilatory, anti-platelet aggregation, anti-inflammatory and antioxidant properties.

Andreadou *et al*. [26] indicated that oleuropein fully restored the changes of metabolites to the normal levels.

Previous research revealed that oleuropein reduced Doxorubicin induced changes in serum levels of CPK, CPKMB and LDH which was associated with reduced lipid peroxidation [26].

OLE has with the most pronounced effect on lowering serum LD and CK-MB As total phenolic and total flavonoids of OLE are much higher than those of GLE which indicating the higher antioxidant activity of olive leaves extracts.

Our results are in harmony with the previous studies indicating the relation between TPC and antioxidant activity. Earlier research indicated that antioxidant capacity varied according to the phenolic compound profile [27]. Another study found that there are positive correlations between the phenolic compound concentration and antioxidant ability [28].

Effects of Guava and Olive Leaves Extracts on Kidney Function in Rats Treated with DOX

The kidney is greatly susceptible to damage caused by reactive oxygen species (ROSs), due to oxidative stress by polyunsaturated fatty acids in the composition of renal lipids, in addition this damage also can be caused by 1) high volume of blood flows through kidney and 2) large amounts of toxins filtered through kidney which can concentrate in kidney tubules [29, 30].

Doxorubicin administration in this study leads to the acute toxic effects on the heart and kidney. The results presented in Table **3** indicated that, DOX injection resulted in a significant elevation in serum total protein, urea, and creatinine, which may be due to the damage of kidney cells by Doxorubicin.

Nephrotoxicity is one of the most common side effects of Doxorubicin. The development of nephrotoxicity increases the burden and complications in cancer patients.

The DOX related alteration in kidney function demonstrated by the significant increase in serum urea, total protein and creatinine may be due to oxidative burden. DOX cause imbalance between free oxygen

Table 3: Effects of Guava and Olive Leaves Extracts on Kidney Function in Rats Treated with DOX

Parameters \ Groups	Group 1 Normal Control (C)	Group 2 Docxorubicin (DOX)	Group 3 GLE+DOX	Group 4 OLE+DOX
BUN (mg/dl)	24.71 ± 1.54[a]	77.09 ± 2.21 [b]	43.72 ±1.77 [c]	39.45 ± 1.45 [c]
Creatinine (mg %)	1.28 ± 0.023 [a]	6.80 ± 0.18 [b]	2.20 ± 0.076 [c]	2.49 ± 0.055 [d]
Total protein (mg %)	5.64 ± 0.21 [a]	13.79 ± 0.23 [b]	10.89 ± 0.29 [c]	10.44 ± 0.70 [c]

Values are expressed as means ± SD. Means with similar superscript (a, b c, d) letters in rows indicate non-significant difference (P< 0.05).

radicals and antioxidants, which resulted in tissue injury due to the disturbance in the oxidant – antioxidant system.

The semiquinone form of the anthracycline was previously shown to play a key role in DOX-induced nephrotoxicity by means of O_2^- generation [31].

On the other hand GLE and OLE supplementation significantly ($p<0.05$) decreased DOX – associated increase in serum total protein, urea, and creatinine as illustrated in Table **3**. OLE has a more significant effect than GLE due to its high phenolic and flavonoids content.

Results of the present study illustrated that GLE reduced DOX –induced kidney damage which may be attributed to its excellent antioxidant and free radical scavenging activity of GLE. several phenolic compounds are present in the leaves such as protocatechuic acid, ferulic acid, quercetin and guavin B [32, quercetin, ascorbic acid, gallic acid and caffeic acid Others include Terpenoids and flavonoids such as Oleanolic acid Nerolidiol, β-sitosterol, ursolic, crategolic and guayavolic acids as well as essential oil including α-pinene, β -pinene, limonene, menthol, terpenyl acetate, isopropyl alcohol, longicyclene, caryophyllene, β-bisabolene, cineol, caryophyllene oxide, β-copanene, farnesene, humulene, selinene, cardinene and curcumene[33] .

The enhancing influence of OLE on counteracting DOX – induced renal toxicity may possibly be associated to its multifunctional radical scavenging and anti-inflammatory actions.

Previous research demonstrated that, OLE significantly improved renal functions and prooxidant and antioxidant balance in gentamicin-induced nephrotoxicity in rats [34].

Some previous studies have shown adverse side effects of long term use of high dosage of OLE, so it is very important to use the right dosage of OLE which is precisely considered in the current research.

Effects of Guava and Olive Leaves Extracts on Antioxidant System in Organs

Doxorubicin (DOX) injection resulted in a significant elevation in MDA in kidney and heart, while cardiac and renal glutathione (GSH) and catalase (CAT) were significantly decreased due to DOX injection as shown in Table **4**.

Malondialdehyde (MDA) elevation may be related to the increase in the production of ROS and inflammatory cytokines. In addition DOX may stimulate inflammatory reactions, and apoptotic and necrotic changes in organs.

Our results are in accordance with previous studies which showed that DOX produced a pro-oxidant status in the organs of rats.

Several hypotheses have been postulated for The mechanism of DOX induced cardiomyopathy which include inhibition of nucleic acid [35] , protein synthesis [36], release of vasoactive amines [37] , alterations in sarcolemmal Ca^{++} transport [38], alterations in membrane bound enzymes [39], abnormalities in

Table 4: Effects of Guava and Olive Leaves Extracts on Cardiac and Renal Glutathione (GSH), Catalase (CAT) and Lipid Peroxidation (MDA) Levels in Rats Treated with DOX

Groups / Parameters	Group 1 Normal Control (C)	Group 2 Doxorubicin (DOX)	Group 3 GLE+DOX	Group 4 OLE+DOX
Cardiac GSH (nmoles/mg wet tissue)	2.25 ± 0.084 [a]	1.19 ± 0.0382 [b]	1.69 ± 0.056 [c]	2.08 ± 0.10 [c]
Cardiac CAT (U/mg wet tissue)	5.89 ± 0.262 [a]	0.62 ± 0.041 [b]	3.86 ± 0.188 [c]	4.75 ±0.191 [d]
Cardiac MDA (nmol/g)	13.85 ± 0.341 [a]	19.53 ± 0.828 [b]	12.64 ± 0.22 [c]	11.18 ± 0.21 [c]
Renal GSH (nmoles/mg wet tissue)	5.78 ± 0.22 [a]	3.56 ±0.12 [b]	4.27 ± 0.18 [c]	4.85 ± 0.35 [d]
Renal (CAT) (U/Mg wet tissue)	10.60 ± 0.23 [a]	1.73 ± 0.090 [b]	6.85 ± 0.23 [c]	7.84 ± 0.24 [d]
Renal (MDA) (nmol/g)	3.15 ± 0.094 [a]	6.61 ± 0.26 [b]	4.98 ± 0.19 [c]	3.86 ± 0.13 [d]

Values are expressed as means ± SD. Means with similar superscript (a, b c, d) letters in rows indicate non-significant difference ($P< 0.05$).

mitochondria and lysosomal alterations and an imbalance of myocardial electrolytes [40].

The major mechanism underlying DOX-induced renal and cardiotoxicity has been shown to be due to oxidative stress, leading to apoptosis and necrosis, which also transactivates p53 and increases proapoptotic proteins [41].

The generation of free radicals and other reactive oxygen species are considered the main DOX-associated side effects [42]. It has been demonstrated that DOX is transformed into a semiquinone through electron reduction by various NADPH-dependent reeducates in the complex 1 of the electron transport chain. This semiquinone reacts with molecular oxygen to produce the superoxide radical {O}and it converts DOX into quinone. This quinone–semiquinone cycle creates large amounts (O_2) , which subsequently give rise to ROS and RNS species such as hydrogen or peroxynitrite [43].

Table **4** results indicared that, pretreatment with GLE and OLE improves the biochemical marker levels indicating a decrease in oxidative stress as evident by increased levels of cardiac as well as renal GSH and CAT activity. In addition administration of GLE and OLE significantly ($P < 0.05$) reduced the elevated concentrations of MDA.

The protective effect of olive leaves extract may be through preventing the decline of antioxidant defense system and direct free radical scavenging activity.

The cardioprotective and antioxidant mechanism of OLE seems to be through modulation of various antioxidant parameters thereby improving the overall antioxidant defence of the myocardial tissue.

The antioxidant effect of OLE may be attributed to the antioxidant components of olive leaves. The major phenolic components of olive leaves including Oleuropein and its derivatives such as hydroxytyrosol and tyrosol, which are correlated to their pharmacologic effects. In addition, olive leaves contain caffeic acid, p-coumaric acid, vanilic acid, vanillin,luteolin, diosmetin, rutin, verbascoside, luteolin-7-glucoside, apigenin-7-glucoside, and diosmetin-7-glucoside [44] .

Several investigators have demonstrated the protective activity of OLE or its constituents, in several oxidative stress-induced pathologies such as atherosclerosis [45], diabetes [46], cerebral ischemia [47], and lead-induced neuropathy [48].

It has been documented that the oleuropein exert potent antioxidant activities, such as inhibition of low density lipoproteins oxidation and free radical scavenging. It modifies pathophysiological processes by inhibiting the production of superoxide anions [49]

Zari and Al-Attar [50] reported that, olive leaves extract ameliorates gentamicin nephrotoxicity via antioxidant activity, increase of renal glutathione (GSH) content, and increase of renal antioxidant enzymes activity.

OLE also decreased cardiac and hepatic injury together with the amelioration in metabolic and oxidative stress parameters in obese and diabetic rat model [51].

The beneficial effect of GLE in reducing oxidative stress observed in this study might be due to to the high level of Phenolic and flavonoids compounds.

Guava leaves has Hepatoprotive, antioxidant, anti-inflammatory, anti-spasmodic, anti-cancer, antimicrobial, antihyperglycemic, analgesic, these effects of guava leaves attributed to their high content of isoflavonoids, catechin, gallic acid, epicathechin, naringenin, rutin kaempferol and of phenolic copmounds in guava leaves [53, 54].

CONCLUSION

In conclusion, the results of the current research suggest that GLE and OLE treatment decrease oxidative stress and injury in the heart and kidneys of DOX treated rats which can be considered as a promising therapy to counteract DOX side effects in DOX –treated cancer patients. The ameliorating effect of GLE and OLE may be related to their multifunctional radical scavenging and anti-inflammatory actions. OLE has more pronounced cardiac and renal protective effects which could be due to its higher TPC and TFCC.

CONFLICT OF INTEREST

The author declares no conflict of interest.

ETHICAL APPROVAL

Bioethical clearance: This study was conducted in accordance with academic and ethical approval Experimental procedures conformed to the guidelines provided by the CPCSEA for studies and the ARV resolution on the use of animals in research and to institutional guidelines. The study performed according

to the recommendations of, Ain Shams University Research Ethical Committee.

REFERENCES

[1] Gianni L, Herman EH, Lipshultz SE, Minotti G, Sarvazyan N. Anthracycline cardiotoxicity: from bench to bedside. J Clin Oncol 2008; 26: 3777-3784. https://doi.org/10.1200/JCO.2007.14.9401

[2] Tang L, Lee AH, Su D and Binns CW. Fruit andvegetable consumption associated with reduced risk of epithelial ovarian cancer in southern Chinese women. Gynecol Oncol 2014; 132(1): 241-247. https://doi.org/10.1016/j.ygyno.2013.10.020

[3] Gutie´rrez RM, Mitchell S, Solis RV. Psidium guajava: a review of its traditional uses, phytochemistry and pharmacology. J Ethnopharma 2008; 117: 1-27. https://doi.org/10.1016/j.jep.2008.01.025

[4] Jang M, Jeong S W, Cho S K, *et al.* Anti-Inflammatory Effects of an Ethanolic Extract of Guava(Psidium guajava L.) Leaves *In vitro* and *In vivo*. J Med Food 2014; 17(6): 678-85. https://doi.org/10.1089/jmf.2013.2936

[5] Kontogianni VG, Gerothanassis IP. Nat. Phenolic compounds and antioxidant activity of olive leaves extrac. Prod Res 2012; 26: 186-189. https://doi.org/10.1080/14786419.2011.582842

[6] Liu Y N, Jung J. Effect of olive leaves extracts treatment on Doxorubicin-induced cardiac, hepatic and renal toxicity in rats. Pathophysiology 2015; 22(2): 117-23. https://doi.org/10.1016/j.pathophys.2015.04.002

[7] Njar VCO, Alao TO, Okogun JI, Holl HI. 2 methoxycathin-6-one: a new alkaloid from the stem wood of Quassiaamara. Plant Med 1993; 59: 259-61. https://doi.org/10.1055/s-2006-959664

[8] Sahin K, Sahin N, Kucuk O. Lycopene and chemotherapy toxicity. Nutrition and Cancer 2010; 62: 988-95. https://doi.org/10.1080/01635581.2010.509838

[9] Ainsworth EA, Gillespie KM. Estimation oftotal phenolic content and other oxidation substrates in plant tissues using Folin-Ciocalteu reagent. Nat Protoc 2007; 2: 875-877. ttps://doi.org/10.1038/nprot.2007.102

[10] Moreno MIN, Isla MI, Sampietro AR, Vattuone MA. Comparison of the free radical scavenging activity of propolis from several regions of Argentina. J Ethnopharmacol 2000; 71: 109-114. https://doi.org/10.1016/S0378-8741(99)00189-0

[11] Thaipong K, Boonprakob U, Crosby K, Cisneros-Zevallos L, Hawkins Byrne D. Comparison of abts, dpph, frap, and orac assays for estimating antioxidant activity from guava fruit extracts. J Food Compos Anal 2006; 19: 669-675. https://doi.org/10.1016/j.jfca.2006.01.003

[12] Vuong QV, Hirun S, Chuen TLK, *et al.* Physicochemical composition, antioxidant and anti-proliferative capacity of a lilly pilly (syzygium paniculatum) extract. J Herbal Med 2014; 4: 134-140. https://doi.org/10.1016/j.hermed.2014.04.003

[13] Weishaar D, Gossrou E, Faderl B. Ranges of alpha-HBDH, LDH, AP and LAP as measured with substrate optimated test charges. Med Welt 1975; 26: 387-92.

[14] Horder M, Elser R, Gerhardt W, Mathieum W, Simpson E. IFCC method for the measurement of catalytic concentration of enzymes. Part 7. IFCC method for creatine kinase (ATP: creatine N-phosphotransferase. J IFCC 1989; 1: 130-139.

[15] Doumas BT. Standards for total serum protein assays-A collaborative study. Clin Chem 1975; 21(8): 1159-1166.

[16] Tabacco A, Meiahimi F, Moda E, Tarli P. Simplified enzymatic colormetric serum urea nitrogen, determination. Clin Chem 1979; 25: 336-337.

[17] Fabing DL, Ertingshausen G. Automated reaction rate method for determination of serum creatinine with centrifichem. Clin Chem 1971; 17: 696-700.

[18] Beutler E, Duroun O, Kelly BM. Improved method for the determination of blood glutathione. J Lab Clin Med 1963; 61: 882-8.

[19] Aebi H. Catalase *in vitro*. Methods Enzymol 1984; 105: 121-6. https://doi.org/10.1016/S0076-6879(84)05016-3

[20] Draper HH, Hadley M. Malondialdehyde determination as an index of lipid peroxidation. Methods Enzymol 1990; 186: 421-430. https://doi.org/10.1016/0076-6879(90)86135-I

[21] Levesque R. SPSS Programming and Data Management: A Guide for SPSS and SAS Users 4th ed. SPSS Inc, Chicago, IL, 2007.

[22] Olson RD, Mushlin PS. Doxorubicin cardiotoxicity: analysis of prevailing hypotheses. FASEB Journal 1990; 4: 3076-86. https://doi.org/10.1096/fasebj.4.13.2210154

[23] Neri GC, Neri B, Bandinelli M, Tacca MD, Danesi R, Riccardi A. Anthracycline cardiotoxicity: *in-vivo* and *in-vitro* effects on biochemical parameters and heart ultrastructure of the rat. Oncology (Basel) 1991; 48: 327-333. https://doi.org/10.1159/000226952

[24] Sesso HD, Wang L, Ridker PM, Buring JE. Tomato-based food products are related to clinically modest improvements in selected coronary biomarkers in women. J Nutr 2012; 142: 326-333. https://doi.org/10.3945/jn.111.150631

[25] Chen KC, Chuang CM, Lin LY, Chiu WT, Chiu WT. The polyphenolics in the aqueous extract of Psidium guajava kinetically reveal an inhibition model on LDL glycation. Pharm Biol 2010; 48: 23-31. https://doi.org/10.3109/13880200903029365

[26] Andreadou I, Papaefthimiou M, Zira A, *et al*. Metabonomic identification of novel biomarkers in Doxorubicin cardiotoxicity and protective effect of the natural antioxidant oleuropein. NMR Biomed 2009; 22: 585-92. https://doi.org/10.1002/nbm.1370

[27] Kosinska A, Magdalena K, Estrella I, Hernandez T, Bartolome B, Dykes GA. Phenolic compound profiles and antioxidant capacity of Persea americana Mill. Peels and seeds of two varieties. J Agric Food Chem 2012; 60: 4613-4619. https://doi.org/10.1021/jf300090p

[28] Kim KM, Seguin P, Ahn JK, Kim JJ, Chun SC, Kim EH. Phenolic compound concentration and antioxidant activities of edible and medicinal mushrooms from Korea. J Agric Food Chem 2008; 56: 7265-7270. https://doi.org/10.1021/jf8008553

[29] Ozbek E. Induction of oxidative stress in kidney. Int J Nephrol 2012; 2012: 1-9. https://doi.org/10.1155/2012/465897

[30] Begum Q, Noori S, Mahboob T. Antioxidant effect of sodium selenite on thioacetamide-induced renal toxicity. Pak J Biochem Mol Biol 2011; 44: 21-26.

[31] Deman A, Ceyssens B, Pauwels M. Altered antioxidant defence in a mouse adriamycin model of glomerulosclerosis. Nephrology Dialysis Transplantation 2001; 16(1): 147-150. https://doi.org/10.1093/ndt/16.1.147

[32] Thaipong K, Boonprakob U, Cisneros-Zevallos L, Byrne DH. Hydrophilic and lipophilic antioxidant activities of guava fruit. Southeast Asian Journal of Tropical Medicine Public Health 2005; 36: 254.

[33] Gutiérrez RMP, Mitchell S, Solis RV. Psidium guajava: A review of its traditional uses, phytochemistry and pharmacology. Journal of Ethnopharmacology 2008; 117(1): 1-27. https://doi.org/10.1016/j.jep.2008.01.025

[34] Ahmadvand H, Toolabi P. Inhibitory effect of olive leaves extract on gentamicin-induced nephrotoxicity in rats. IJKD 2012; 6: 25-32.

[35] Arena E, Bionda F, DAlessndro N, Dusoncher L, Gebbia N, Gerbasi R. DNA, RNA protein synthesis in heart, liver and brain of mice treated with denaturation of adriamycin. Int Res Commun Syst Med Sci 1984; 2: 10543-61.

[36] Buja LM, Ferrans VJ, Mayer RJ, Roberts WC, Hinderson ES. Cardiac ultrastructural changes induced by daunorubucin therapy. Cancer 1973; 32: 771-78. https://doi.org/10.1002/1097-0142(197310)32:4<771::AID-CNCR2820320407>3.0.CO;2-A

[37] Bristow MR, Sagemen WS, Scot RH, Billingham ME, Bowden RE, Kernoff RS. Acute and chronic cardiovascular effects of Doxorubicin in the dog: the cardiovascular pharmacology of drug-induced histamine release. Cardiovasc Pharmacol 1980; 2: 487-15. https://doi.org/10.1097/00005344-198009000-00002

[38] Singal PK, Pierce GN. Adriamycin stimulates Ca2+- binding and lipid peroxidation but depress myocardial function. American Journal of Physiology 1986; 250: H419-24.

[39] Singal PK, Panagia V. Direct effect of adriamycin on the rat heart sarcolemma. Res Commun Chem Pathol Pharmacol 1984; 43: 67-77.

[40] Oslan HM, Young DM, Prieur DJ, LeRoy AF, Reagan RL. Electrolyte and oxidation products of certain lipids. J Biol Chem 1974; 174: 257-64.

[41] Octavia Y, Tocchetti CG, Gabrielson KL, Janssens S, Crijns HJ. Doxorubicin-induced cardiomyopathy: from molecular mechanisms to therapeutic strategies. J Mol Cell Cardiol 2012; 52: 1213-1225. https://doi.org/10.1016/j.yjmcc.2012.03.006

[42] Takemura G, Fujiwara H. Doxorubicin-Induced Cardiomyopathy from the Cardiotoxic Mechanisms to Management Progress in Cardiovascular Diseases 2007; 49(5): 330-352.

[43] Quiles JL, Ochoa JJ, Huertas JR, López-Frías M, Mataix J. Olive oil and mitochondrial oxidative stress: studies on adriamycin toxicity physical exercise and ageing. In: J.L. Quiles, M.C. Ramirez-Tortosa and P. Yaqoob, (Eds.), Olive Oil and Health. CABI Publishing, Oxford, Life Sci 2006; 50: 2111-2118. https://doi.org/10.1079/9781845930684.0000

[44] Kontogianni VG, Gerothanassis IP. Phenolic compounds and antioxidant activity of olive leaves extracts. Nat Prod Res 2012; 26: 186-189. https://doi.org/10.1080/14786419.2011.582842

[45] Wang L, Geng C, Jiang L, *et al.* The anti-atherosclerotic effect of olive leaves extract is related to suppressed inflammatory response in rabbits with experimental atherosclerosis. J Nutr 2008; 47: 235-243.

[46] Al-Azzawie HF, Alhamdani MS. Hypoglycemic and antioxidant effect of oleuropein in alloxan-diabetic rabbits. Life Sci 2006; 78: 1371-1377. https://doi.org/10.1016/j.lfs.2005.07.029

[47] Dekanski D, Selakovic V, Piperski V, Radulovic Z, Korenic A, Radenovic L. Protective effect of olive leaves extract on hippocampal injury induced by transient global cerebral ischemia and reperfusion in Mongolian gerbils. Phytomedicine 2011; 18: 1137-1143. https://doi.org/10.1016/j.phymed.2011.05.010

[48] Seddik TM, Bah, Aoues A, Slimani M, Benderdour M. Elucidation of mechanisms underlying the protective effects of olive leaves extract against lead-induced neurotoxicity in Wistar rats. Toxicol Sci 2011; 36: 797-809. https://doi.org/10.2131/jts.36.797

[49] Visioli F, Bogani P, Galli C. Healthful properties of olive oil minor components. In: Boskou D. (Ed.), Olive oil, Chemistry and Technology. AOCS Press, Champaign, IL, 2006; pp. 173-190.

[50] Zari TA, Al-Attar AM. Therapeutic effects of olive leaves extract on rats treated with a sublethal concentration of carbendazim. Eur Rev Med Pharmacol Sci 2011; 15: 413-426, 2011.

[51] Poudyal H, Campbell F, Brown L. Olive leaves extract attenuates cardiac, hepatic, and metabolic changes in high carbohydrate-, high fat-fed rats. J Nutr 2010; 140: 946-953. https://doi.org/10.3945/jn.109.117812

[52] Peng CC, Peng CH, Chen KC, Hsieh CL, Peng RY. The Aqueous Soluble Polyphenolic Fraction of Psidium guajava Leaves Exhibits Potent Anti-Angiogenesis and Anti-Migration Actions on DU145 Cells. Evid Based Complement Alternat Med 2011; 2011: 219069. https://doi.org/10.1093/ecam/neq005

[53] Chen KC, Peng CC, Chiu WT, Cheng YT, Huang GT. Action mechanism and signal pathways of Psidium guajava L aqueous extract in killing prostate cancer LNCaP cells. Nutr Cancer 62: 260-270. https://doi.org/10.1080/01635580903407130

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