Research Article

Protective Role of Hydroalcoholic Extract of *Vitis vinifera* against Adriamycin Induced Cardiac, Renal and Hepatic Toxicities in Rat

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ABSTRACT

Adriamycin (ADR) causes toxicities in heart, liver and kidneys via inducing the peroxidative alterations in organ tissues. Recent studies showed that *Vitis vinifera* exerts beneficial effects on heart, liver and kidney injuries induced by different pathological conditions. We hypothesize that *Vitis vinifera* extract (VVE) have a protective effect on ADR induced cardiac, renal and hepatic toxicities by inhibiting the peroxidative alterations in organ tissues. Total six groups of six animals each were divided into control, ADR (15mg/kg i.p. on 28th day), Vitamin E + ADR and VVE groups (100, 200 and 400 mg/kg for 30 days + ADR). After 4 weeks, the serum biochemistry variables were determined. ADR caused significant cardiac, renal and hepatic toxicities indicated by the serum biochemistry variables. The tissue MDA level in heart, kidney and liver in rats treated with ADR were markedly elevated, while GSH, SOD, CAT content in these tissues were significantly reduced. *Vitis vinifera* administration induced significant reduction of MDA level and increase of GSH, SOD, CAT content in all tissues. This study suggests that *Vitis vinifera* play an overall protective effect on ADR-induced toxicities in heart, liver and kidneys. Inhibition of tissue peroxidative alterations and detoxification of free radicals that are generated, might contribute to this beneficial effect.

Keywords: Adriamycin, heart, kidney, lipid peroxidation, liver, *Vitis vinifera*

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INTRODUCTION

Adriamycin, an anthracycline antibiotic, is widely used in the treatment of a variety of human malignancies, including breast cancer, small cell carcinoma of the lung and acute leukemia's [1]. Like most of the anticancer drugs, Adriamycin also causes various toxic effects, including cardiotoxicity which leads to acute and chronic heart failure [2], hepatotoxicity [3] and nephrotoxicity [4].

Black grapes (*Vitis vinifera*) is one of the most widely grown fruit crops in the world. Most phenolics in Black grapes are located in the seeds [5]. Gallic acid, catechin and epicatechin are the main phenolics found in Black grapes seeds, while ellagic acid and myricetin are the major ones in the skins. Black grapes well known for their high levels of antioxidants and polyphenols, have also shown promise as novel antimicrobial agents [6], anti-cancer properties [7], anti-inflammatory activity [8] and antimicrobial activity against *Escherichia coli* O157:H7 [9], antiulcerative, antiarthritic, anti-viral, prevent skin aging, scavenge free radicals [10] and inhibit UV-radiation induced peroxidation activity [11, 12]. Resveratrol, Quercetin, Catechin, Flavone, Flavonols, Procyanidin, Anthocyanin, gallic acid, Epicatechin are the phenolic compounds isolated from the black grapes [13].

Therapeutic strategies, designed to augment cellular endogenous defense systems have been identified as a promising approach to combat oxidative stress-associated disease conditions [14, 15]. The hypothesis proposed was that if ADR induced cardiotoxicity, hepatotoxicity and nephrotoxicity are related to free radical formation and oxidative stress, an

antioxidant such as *Vitis vinifera* may protect against adriamycin-induced toxicity in the heart, liver and kidney.

In view of these facts the present study was designed to test the hypothesis whether a nutritional strategy like chronic administration of hydroalcoholic extract of *Vitis vinifera* could prevent adriamycin-induced cardiotoxicity, hepatotoxicity and nephrotoxicity in terms of oxidative stress.

MATERIALS AND METHODS

Collection of plant material:

The black grapes were collected from the local market in Ranga Reddy District and the botanical authentication was done by Dr. Ram Chandra Reddy, Head of Botany Department, Osmania University, Hyderabad.

Preparation of the extract

The skin, seed and pulp were separated from black grapes and were shade dried individually. The dried skin, seed and pulp were ground to powder. This dried powder was used for soxhlet extraction. Extraction was done by using the soxhlet apparatus at a temperature below 60^{o}C for 24 hours. Powder was extracted with methanol. The solvent thus obtained was evaporated under vaccum to get a semi-solid form of the extract. Percentage yield was 23.5% with respect to dried powder.

Animals

Adult male Sprague–Dawley rats (150 ± 10 g body weight) were obtained from the departmental animal facility where they were housed under standard husbandry conditions (25 ± 2 °C temp., 60–70% relative humidity and 12 h photoperiod) with standard rat feed and water *ad libitum*. Experiments were conducted in accordance with the guidelines set by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), India and experimental protocols were approved by the Institutional Animal Ethics Committee (CPCSEA/1217/2008/a).

Experimental design

Thirty six Wistar Albino male rats of weight 150gms-250gms were selected for this study. Animals were divided into six groups of six animals each.

Group 1: Control group (0.9% Normal saline 1ml)

Group 2: Positive control (Intraperitoneal injection of adriamycin 15 mg/kg body weight)

Group 3: Standard (Vitamin E) 100 mg/kg body weight p.o. + adriamycin 15 mg/kg body weight i.p.

Group 4: *Vitis vinifera* extract 100 mg/kg body weight p.o. + adriamycin 15 mg/kg body weight i.p.

Group 5: *Vitis vinifera* extract 200 mg/kg body weight p.o. + adriamycin 15 mg/kg body weight i.p.

Group 6: *Vitis vinifera* extract 400 mg/kg body weight p.o. + adriamycin 15 mg/kg body weight i.p.

Animals were grouped into six groups as explained above. The control group animals were given 0.9% normal saline 1ml for 30 days. Group 2 animals were given normal saline water until 30th day and a single dose of adriamycin 15 mg/kg body weight i.p. on 28th day. Group 3 animals were given Vitamin E 100 mg/kg body weight p.o. until 30th day and a single dose of adriamycin 15 mg/kg body weight i.p. on 28th day^{28}. Group 4 animals were given *Vitis vinifera* extract 100 mg/kg body weight p.o. until 30th day and a single dose of adriamycin 15 mg/kg body weight i.p. on 28th day. Group 5 animals were given *Vitis vinifera* extract 200 mg/kg body weight p.o. until 30th day and a single dose of adriamycin 15 mg/kg body weight i.p. on 28th day. Group 6 animals were given *Vitis vinifera* extract 400 mg/kg body weight p.o. until 30th day and a single dose of adriamycin 15 mg/kg body weight i.p. on 28th day.

Serum sample preparation:

The animals were sacrificed 48 h after the injection of adriamycin using ether anesthesia, blood was collected by cardiac puncture method. Blood was centrifuged using Remi cool centrifuge at 4000 rpm for 15 mins. Serum was separated for the estimation of various biochemical parameters like lactate dehydrogenase, serum troponin, SGPT and SGOT, alkaline phosphatase, total protein and blood urea nitrogen and serum creatinine according to the standard procedures given in the kits

Tissue sample preparation:

At the end of the experiment, animals were sacrificed by cervical dislocation with light ether anesthesia. Heart, liver and kidney

tissues were separated and washed with phosphate buffer saline (0.05M, ph7.4). The heart, liver and kidney later were taken and minced into small pieces and homogenized in ice cold phosphate buffer saline (0.05M, ph7.4) using tissue homogenizer to obtain 1:9 (w/v) (10%) whole homogenate. A part of the homogenate was taken and mixed with equal volume of 10% Trichloroacetic acid (TCA) for the estimation of malondialdehyde. Homogenate was centrifuged using Remi cool centrifuge at 8000 rpm for 30 mins. The supernatant was separated and used for estimation of anti-oxidant levels of different enzymes i.e. Super oxide dismutase, catalase, glutathione and malondialdehyde in all the three tissues–heart, liver and kidney [16, 17].

Statistical Analysis

The experimental results were expressed as the Mean ± SEM with six rats in each group. The intergroup variation between various groups were analyzed statistically using one-way analysis of variance (ANOVA) using the Graph Pad Prism version 5.0, followed by Dunnett's multiple comparison test (DMCT). Results were considered statistically significant when $P < 0.05$.

RESULTS

Effect of *Vitis Vinifera* extract on Serum Troponin:

For control group a distinct pinkish purple line in the control region 'C' appeared which indicates non-reactive for Troponin-I. For adriamycin treated group a purple coloured line is appeared in the test region 'T' and pinkish purple line in the control region 'C' which indicates increase in the levels of the Troponin-I when compared to the control group. Standard Vitamin E 100 mg/kg and *Vitis vinifera* 400 mg/kg has shown only a distinct pinkish purple line in the control region'C' and no colour in the test region 'T' which indicates the significant decrease in levels of Troponin-I when compared to the adriamycin treated group.

Serum biochemical variables

Table 1 showed the serum biochemical variables in all rats 4 weeks after *Vitis vinifera* and Vitamin E administration. These biochemical variables are commonly used for the assessment of heart, liver and kidney damages. ADR treatment induced significant increases in the LDH, Cr, BUN, SGPT, SGOT, ALP and decrease in total protein levels compared to those in the control group. This means that ADR administration caused systemic organ toxicities in rats. In rats from ADR+ Vitamin E, ADR+ *Vitis vinifera* treated groups, the serum LDH, Cr, BUN, SGPT, SGOT, ALP were significantly lowered and total protein levels were significantly raised 4 weeks after the *Vitis vinifera* administration, when significant differences were seen compared with those in rats from ADR group.

Table 1: Effect of hydroalcoholic extract of *Vitis vinifera* on serum biochemical variables of all rats

GROUPS	SGPT (IU/L)	SGOT (IU/L)	ALP (IU/L)	TP (g/dl)	Creatinine (mg/dl)	BUN (mg/dl)	LDH (IU/ml)
Control	25.5±6.95	35.34±7.73	44.22±11.56	6.86±0.39	0.6±0.1	17.41±0.51	224.42 ± 39.26
Doxorubicin	96±4.97^	80.94±9.60^	110.56±12.36^	4.55±0.19^	1.4±0.1^	51.63±1.60^	609 ± 78.53^
Vitamin-E	30±5.30#	41.04±4.90**	60.808±10.34*	6.78±0.199#	0.7±0.12#	19.41±1.03#	288.54 ± 59.97*
VVE I+ADR	49.5±8.07*	61.56±9.08	93.97±6.77	5.03±0.2	1.09±0.122	39.42±1.03	448.84 ± 93.47
VVE II+ADR	43.5±8.68*	57.1±6.49	77.392±10.34	5.61±0.13*	0.9±0.11*	29.62±1.07*	384.72 ± 81.73
VVE III+ADR	42±6.09#	47.88±5.28**	66.336±14.09*	6.55±0.33#	0.8±0.122#	20.21±0.86#	320.6 ± 71.68*

Values are expressed as mean ± SEM., Data analyzed by one way ANOVA followed by Dunnet's Multiple Comparison Test. #p<0.001, **p<0.01,*p<0.05 significant as compared with doxorubicin group. ^p<0.05 as compared to that of control group.

Effect of *Vitis Vinifera* extract on tissue MDA, GSH, SOD and CAT levels:

Fig. 1 shows the tissue MDA levels, Fig. 2, 3, 4 shows the GSH, SOD and CAT content in the heart, the liver and the kidneys during a time course of 4 weeks. ADR administration induced dramatic, constant increases of MDA production, GSH, SOD and CAT content in ADR treated rats decreased dramatically in these tissue samples. After administration of vitamin E the MDA levels declined dramatically, the GSH, SOD and CAT levels increased dramatically. Four weeks later, the tissue MDA levels, the GSH,

SOD and CAT content in rats from the ADR+VVE treated groups reached their minimums, with a significant difference compared to those of ADR group respectively.

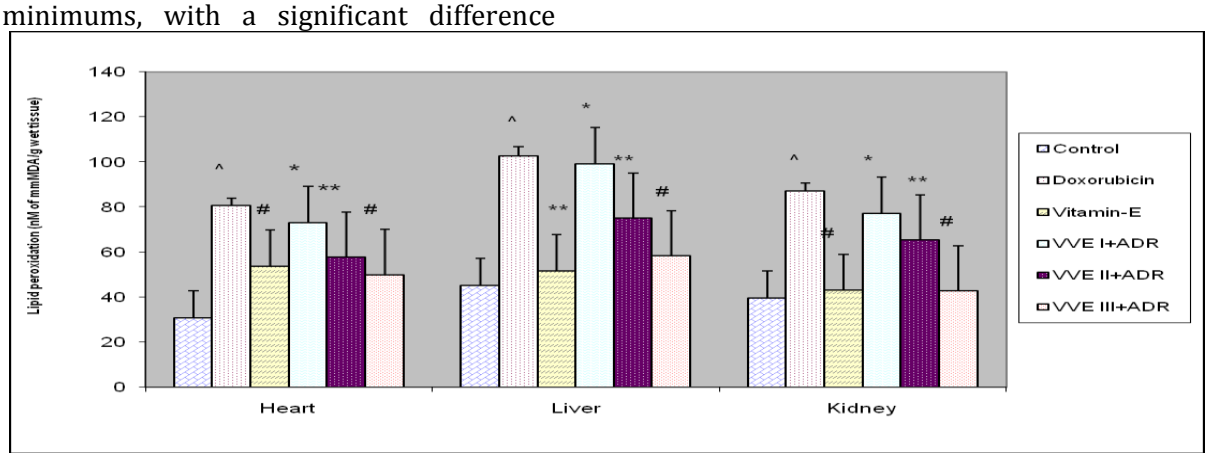

Fig. 1: Effect of Hydroalcoholic Extract of *Vitis vinifera* on cardiac, hepatic and renal MDA levels

Values are expressed as mean ± SEM., Data analyzed by one way ANOVA followed by Dunnet's Multiple Comparison Test. #p<0.001,**p<0.01,*p<0.05 significant as compared with doxorubicin group. ^p<0.05 significant as compared with control group

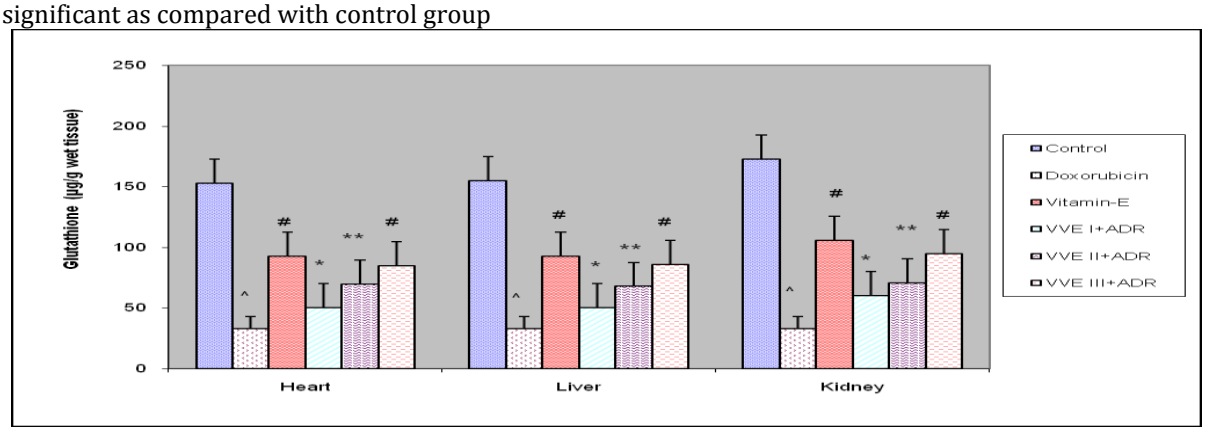

Fig. 2: Effect of Hydroalcoholic Extract of *Vitis vinifera* on cardiac, hepatic and renal glutathione levels

Values are expressed as mean ± SEM., Data analyzed by one way ANOVA followed by Dunnet's Multiple Comparison Test. #p<0.001,**p<0.01,*p<0.05 significant as compared with doxorubicin group. ^p<0.05 significant as compared with control group

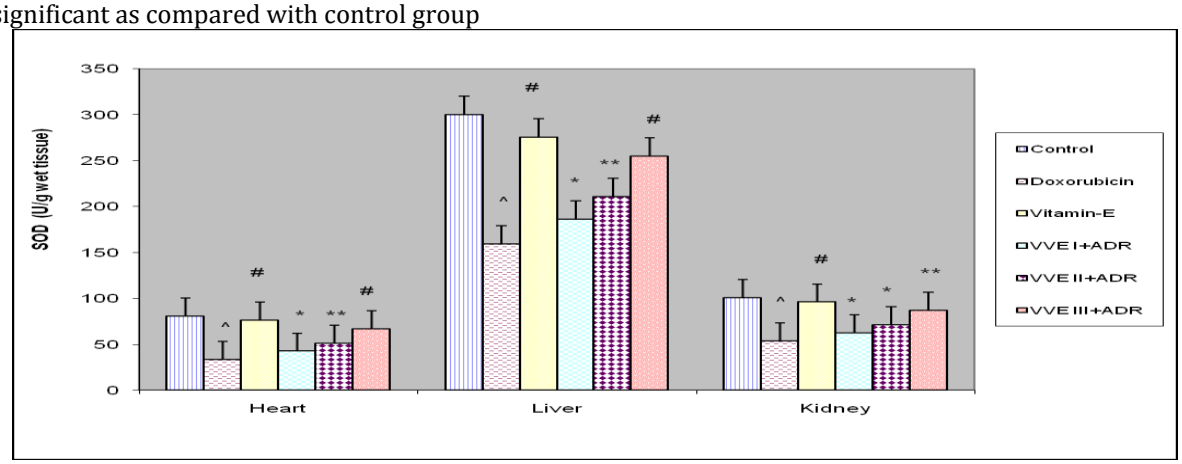

Fig. 3: Effect of Hydroalcoholic Extract of *Vitis vinifera* on cardiac, hepatic and renal SOD levels

Values are expressed as mean ± SEM., Data analyzed by one way ANOVA followed by Dunnet's Multiple Comparison Test. #p<0.001,**p<0.01,*p<0.05 significant as compared with doxorubicin group. ^p<0.05 significant as compared with control group

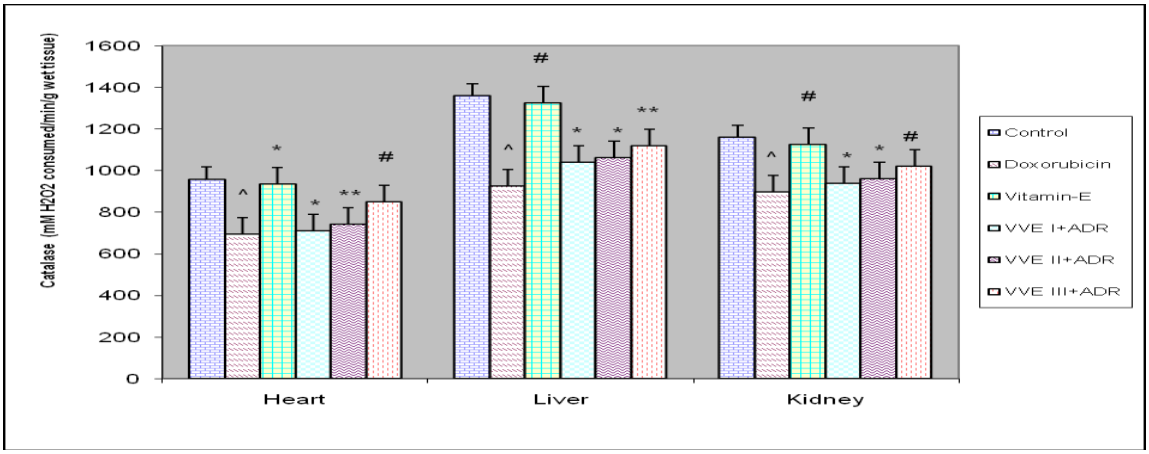

Fig. 4: Effect of Hydroalcoholic Extract of *Vitis vinifera* on cardiac, hepatic and renal catalase levels

Values are expressed as mean ± SEM., Data analyzed by one way ANOVA followed by Dunnet's Multiple Comparison Test. #p<0.001,**p<0.01,*p<0.05 significant as compared with doxorubicin group. ^p<0.05 significant as compared with control group

DISCUSSION

ADR is a broad spectrum antibiotics used as a chemotherapeutic drug for the treatment of different forms of human neoplastic disease [18]. However, the clinical use of anticancer drug is greatly limited by its dose-dependent toxicity [19]. Free radicals generation and lipid peroxidation have been suggested to be responsible for ADR-induced toxicity in different tissues including heart, liver and kidney [20]. This oxygen derived radicals' causes severe damage to plasma membrane and interferes with cytoskeleton assembly [21].

Free radicals ROS and RNS are generated by our body by various endogenous systems, exposure to different physiochemical conditions, or pathological states. A balance between free radicals and antioxidants is necessary for proper physiological function. If the free radicals overwhelm the body's ability to regulate them, a condition known as oxidative stress ensues. Free radicals thus adversely alter lipids, proteins, and DNA and trigger a number of human diseases. Hence, application of an external source of antioxidants can assist in coping this oxidative stress [22].

The present study is to evaluate the potential protective effect of *Vitis vinifera* against adriamycin induced oxidative stress in heart, liver and kidney tissues. It is evident from the results of the present investigation that supplementation of *Vitis vinifera extract* with adriamycin protected the animals from toxic effects of adriamycin. Black grapes are an important dietary antioxidant; it significantly decreases the adverse effect of reactive species such as reactive oxygen and nitrogen species that can cause oxidative damage to macromolecules such as lipids, DNA and proteins. Black grapes possess potent antioxidant capacity, capable of scavenging/neutralizing an array of reactive oxygen species hydroxyl, alkoxyl, peroxyl, superoxide anion, hydroperoxyl radicals and reactive nitrogen radicals such as nitrogen dioxide, nitroxide, peroxynitrite at very low concentration [23].

ADR-induced toxicity includes one electron reduction of ADR lead to the formation of corresponding semi-quinone free radicals in heart, liver and kidney tissues by CYP-450 and flavin monoxigenase. In the presence of oxygen, these free radicals rapidly donate their electron to oxygen or react with molecular oxygen and initiate cascade of reaction producing ROS. Free radical generation and lipid peroxidation have been suggested to be responsible for ADR-induced toxicity [20].

Moreover, heart tissue especially is susceptible to the free radical injury because of the low level of free radical detoxifying enzymes such as SOD, CAT, and GSH and less oxygen reserve. Further, ADR also has a high affinity for the phospholipids component of mitochondrial membrane in

cardiac myocytes, leading to accumulation of ADR in heart tissue. The cellular GSH level is closely related to lipid peroxidation and disturbances of Ca^{++} influx induced by toxic agents. ADR administration induced oxidative stress in cardiac tissue as manifested by the alteration observed in the cardiac antioxidant defense system both enzymatic and nonenzymatic.

Alanine transaminase and aspartate transaminase are regarded as markers of liver injury, since liver is the major site of metabolism. Decline in the activities of liver alkaline phosphatase in adriamycin injected animals' noticeably demonstrated cellular damage which correlates with the present findings. Elevation of serum levels of alanine transaminase and aspartate transaminase in adriamycin injected animals is attributed by lipid peroxidation in the liver. Influence of adriamycin toxicity, therefore, reveals leakage of these enzymes from damaged liver cells.

The results of the renal function test revealed that adriamycin administration produced intrinsic acute renal failure, which was evident from the elevated levels of serum urea and creatinine. Anticancer therapy usually demolishes the physiological homoeostasis and affects multiple organs during treatment process. Effective anticancer therapy with anthracyclines is limited because of its toxicity to various organs including kidneys [24]. The toxicity has been attributed to radical formation and oxidant injury. Nephrotoxic action of ADR is also considered to be via drug-induced free radical generation [25, 26]. The formation of free radicals as well as an increase in response to ADR treatment has already been documented.

Adriamycin administration alters the levels of various biochemical parameters like increase of LDH, Troponin- I, SGPT, SGOT, ALP, BUN, Serum creatinine, decrease of total protein levels and also alters the antioxidant levels like decrease of SOD, GSH, CAT and increase in the lipid peroxidation. These alterations lead to cardiotoxicity, hepatotoxicity and nephrotoxicity. But the pretreatment of hydroalcoholic extract of *Vitis vinifera extract* significantly reduced levels of various biochemical parameters like LDH, Troponin- I, SGPT, SGOT, ALP, BUN, Serum creatinine and lipid peroxidation, and significantly increased total protein levels and antioxidants levels like SOD, GSH and CAT as that of the standard Vitamin E.

The protecting effect of hydroalcoholic extract of *Vitis vinifera* is due to free radical scavenging and iron-chelating properties, hydrogen-donating radicals, scavenger by the scavenging lipid alkoxyl and peroxyl radical. On the basis of these findings, it may be worthy to suggest the subsequent consumption of black grape prior to the ADR use in cancer chemotherapy. Further studies are required to isolate the other potential phytoconstituents present in the extract.

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