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Protective Effects of Ruitn and / or Hesperidin Against Doxorubicin-Induced Hepatotoxicity

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Abstract The present study was conducted to evaluate the protective role of rutin and hesperidin on experimental doxorubicin induced hepatotoxicity. Doxorubicin (DXR) administered rats (25 mg / kg; three times intraperitoneally / week for two weeks) were pretreated with rutin, hesperidin, or their mixture (50 mg / kg body weight) three times per week for three weeks. Results showed that DXR caused a marked rise in serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and gamma glutamyl transferase (GGT) activities alongside an increase in serum total bilirubin, α-fetoprotein (AFP) and sialic acid levels Concerning oxidative stress and antioxidant defense system, the depleted hepatic glutathione content of DXR-administered rats was increased above normal levels as a result of pretreatment with rutin, hesperidin and both rutin and hesperidin. However, while elevated lipid peroxidation was noticed in DXR treated rats, pretreatment with rutin, hesperidin or both produced a detectable decrease in the lipid peroxidation level. Taken these data together, it can be concluded that natural plant components such as Rutin and Hesperidin could protect the liver against DXR-induced liver toxicity.

Keywords: *rutin, hesperidin, hepatotoxicity, oxidative stress*

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1. Introduction

Doxorubicin (DXR, trade name Adriamycin; also known as hydroxy daunorubicin) has been widely used over the past several decades to treat patients with various cancers, including hepatocellular carcinoma based on its ability to kill transformed liver cells [1]. Liver damage is a relatively common adverse effect in patients with other cancers who are treated with DXR [2]. DXR hepatotoxicity has been also reported in a number of studies on animals [3,4,5,6].

The mechanisms of DXR cytotoxicity, in cancer cells include: (i) intercalation into DNA with inhibition of DNA replication and RNA transcription; (ii) generation of free radicals with DNA damage and lipid peroxidation; (iii) DNA binding and arylation; (iv) DNA crosslinking; (v) interference with DNA unwinding, DNA strand separation, and helicase activity; (vi) direct membrane damage due to oxidation of lipids and (vii) inhibition of topoisomerase II. The liver may play an essential role in heart damage by forming DXR metabolites that are more cardiotoxic than DXR [7,8,9,10,11]. NADPH-dependent cellular reductases convert DXR to semiquinone free radicals which can generate reactive oxygen species (ROS) including superoxide, hydroxyl radicals and hydrogen peroxide. These free radicals are critical to DXR medicated cytotoxicity [12]. Such ROS can damage liver membranes to produce ALT release. DXR can also activate phospholipases via lipid peroxide which can elevate intracellular Ca^{2+} and can also lead to ALT release and liver cell death [13].

The clinical usefulness of DXR is limited by its cardiotoxicity, hepatotoxicity and cardiotoxicity, excessive exposure also causes nephrotoxicity [4,14,15].

The reduction of oxidative DNA damage by antioxidants has been evaluated as a chemotherapeutic approach for reducing damage caused by chemotherapy agents such as doxorubicin [16]. So, recent studies hypothesized that the combination of the chemotherapeutic drug together with a potent antioxidant may be the appropriate approach to reduce the toxic side effects of anticlastogenic agents; but some properties of synthetic antioxidant drugs limited their therapeutic application [17].

Rutin (quercetin rutinoside) is a glycoside of the flavonoid quercetin. Rutin is used in as a medication for blood vessel protection and is an ingredient of numerous multivitamin preparations and herbal remedies. It can combine with cations, supplying nutrients from the soil to the cells in plants [18]. In humans it is a potent antioxidant where its actions include: attaching to the iron ion Fe^{2+}, preventing it from binding to hydrogen peroxide, which

would otherwise create a highly-reactive free-radical that may damage cells [19]. Rutin also shows anti-inflammatory activities in some animal and invitro models [20]. Rutin and ferulic acid, can reduce the cytotoxicity of oxidized (Low density lipoprotein) LDL cholesterol and lower the risk of heart disease. Rutin is the strongest antioxidant when compared with quercetin, acacetin, morin, hispidulin, hesperidin, and naringin [21].

In a rat model, quercetin reduced preneoplastic hepatic foci in 2-amino-3-methylimidazo [4, 5-f] quinoline (IQ)-induced hepatocarcinogenesis [22]. Quercetin prevented the development of gamma glutamyl transferase (GGT)-positive preneoplastic foci in a diethyl nitrosamine-induced hepatocarcinogenesis model [23]. Quercetin has also been shown to reduce glutathione S transferase (GST)-P-positive foci in a choline-deficient, L-amino acid-defined diet model [24].

Hesperidin is a flavanone glycoside (flavonoid $C_{28}H_{34}O_{15}$) found abundantly in citrus fruits. Its aglycone form is called hesperetine. Hesperidin is believed to play a role in plant defense. It acts as an antioxidant according to *in vitro* studies. In human nutrition, it contributes to the integrity of blood vessels [25]. Hesperidin also has anti-inflammatory effects [26].

Dietary hesperidin exerts anti-carcinogenic actions in the tongue, colon, esophagus, and urinary bladder in rat carcinogenesis models [27].

Therefore, the present investigation was undertaken to test the anticlastogenic or clastogenic effects of rutin and hesperidine on the liver tissue in the rat to determine their protective effect on the liver enzyme level and oxidative stress induced by DXR.

2. Materials and Methods

2.1. Experimental Animals

Male Wistar albino rats weighing about 140-180 g were used as experimental animals in the present investigation. They were obtained from the animal house of Research Institute of Ophthalmology, El-Giza, Egypt. They were kept under observation for about 15 days before the onset of the experiment to exclude any intercurrent infection. The chosen animals were housed in plastic cages with good aerated covers at normal atmospheric temperature (25 ± 5°C) as well as 12 hours daily normal light periods. Moreover, they were given access of water and supplied daily with standard pellet diet ad libitum. All animal procedures are in accordance with the recommendations of the Canadian Committee for Care and Use of animals (Canadian council on Animal care [CCAC], 1993) [28].

2.2. Chemicals

Doxorubicin (Adriblastina® produced by Carlo Erba) was purchased from local pharmacy in the form of 10 mg / ampoule. Rutin and hesperidin were purchased from Sigma Chemical Company (St. Louis, MO, USA). All other chemicals used for the investigation were of analytical grade.

2.3. Doses and Treatment

The dose of doxorubicin (DXR) used in this study was 25 mg / kg body weight. This dose was previously reported to induce an increase in the frequency of cell damage in mammalian systems [29]. The animals were treated with doxorubicin by an intraperitoneal route. The chosen dose of DXR was adjusted to 0.2 ml / 25 g b.wt in distilled water prior to use and was given three times per week for two weeks. Rutin and hesperidin doses were adjusted to 50 mg / kg and were given three times per week for three weeks.

2.4. Experimental Design

The animals of the present experiment were allocated into 4 groups:

1-Normal control: The rats of this group were given the equivalent volume of vehicle (0.9 % Na Cl) for 35 days.

2-Doxorubicin–administered control: The rats of this group were intraperitoneally administered doxorubicin at a dose of 25 mg / kg b.wt for 2 weeks.

Group 3: Doxorubicin-administered group treated with Rutin at a dose level of 50 mg / kg [30]: This group was intraperitoneally administered with doxorubicin at a dose of 25 mg / Kg b.w. / week for 2 weeks and was orally treated (by oral gavage) with Rutin extract at dose level of 50 mg /kg b.w. / day for 3weeks.

Group 4: Doxorubicin-administered group treated with Hesperidin at a dose level of 50 mg / kg [31]: This group was intraperitoneally administered with doxorubicin at a dose of 25 mg / Kg b.w. / week for 2 weeks and was orally treated (by oral gavage) with Hesperidin extract at dose level of 50 mg / kg b.w. / day for 3weeks.

Group 5: Doxorubicin-administered group treated with mixture of Rutin and Hesperidin at a dose level of 50 mg / kg [31]: This group was intraperitoneally administered doxorubicin at a dose of 25 mg / Kg b.w. / week for 2 weeks and was orally treated (by oral gavage) with Rutin and Hesperidin extract at dose level of 50 mg / kg b.w. / day for 3weeks.

2.5. Sampling

Under diethyl ether anesthesia, 5 ml of blood sample was collected from jugular vein of each animal in a centrifuge tube and left to clot at room temperature for 45 minutes. Sera were separated by centrifugation at 3000 r.p.m at 30°C for 15 minutes and kept frozen at -30°C for various physiological and biochemical analyses.

Liver from each animal was rapidly excised after dissection. 0.5 g liver tissue was homogenized in 5 ml 0.9 % Na Cl (10 % w / v) using a teflon homogenizer (Glass-Col, Terre Haute, USA).

2.6. Biochemical Analyses

Serum total bilirubin concentration was determined by a colorimetric procedure using kits obtained from Biodiagnostic (Egypt) [32]. Alpha-fetoprotein levels in serum [33] using ELISA micro wells purchased from Monobind, INC. (USA). Hepatocellular leakage of the enzyme ALT reflects the degree of liver injury and serves as a reliable marker of hepatotoxicity involving necrosis. Serum ALT and AST activities were determined by using kits obtained from Biodiagnostic (Egypt) [34]. Serum ALP and GGT were determined by using kits obtained from Biodiagnostics [35,36]. Serum albumin was measured by colorimetric method using kits obtained from

Biodiagnostics [37]. Serum sialic acid concentrations were measured by Warren's thiobarbituric acid method [38]. Liver oxidative stress and antioxidant defense parameters were estimated using chemicals purchased from Sigma Chemical Company (USA) and using Jenway Spectrophotometer (Germany), Glutathione activity in homogenates was determined according to the chemical method of Beutler et al. (1963) [39] with little modification. Lipid peroxidation in homogenates was determined according to the chemical method of Preuss et al [40]. Peroxidase (POX. EC 1.11.1.7) activity in homogenates was estimated according to the modified chemical method of Cohen [41]. Glutathione peroxidase (GSHPx EC I.11. 1.9) activity in homogenates was determined according to the chemical method of Marklund and Marklin [42]. Glutathione-S-transferase (GST. EC 2.5.1.18) concentration in homogenates was determined according to the chemical method of Mannervik and Guthenberg [43].

2.7. Statistical Analysis

The data were analyzed using the one-way analysis of variance (ANOVA) [44] followed by Fisher's least significant difference (LSD) analysis to compare various groups with each other. Results were expressed as mean ± standard error (SE). F-probability, obtained from one-way ANOVA, expresses the effect between the groups.

3. Results

3.1. Biochemical Changes

Changes in different serum variables related to liver function are presented in Table 1 and Table 2.

Table 1. Protective effect of rutin and / or hesperidin on serum liver function activities in doxorubicin treated rats

Parameters / Treatments	ALT (U / ml)	% change	AST (U / ml)	% change	ALP (IU / L)	% change	7-GT (U / L)	% change
G1 Normal	89.60 ± 3.80^{a}	-	88.90 ± 2.5^{b}	-	101.66 ± 0.40^{b}	-	27.91 ± 0.10^{e}	-
G2 Doxorubicin	124.80 ± 3.80^{a}	39.29	128.00 ± 0.00^{a}	43.98	105.55 ± 2.20^{c}	3.82	101.98 ± 2.00^{a}	265.39
G3 Rutin + Doxorubicin	93.30 ± 1.40^{a}	-25.24	89.90 ± 3.0^{b}	-29.77	102.29 ± 0.60^{a}	-3.09	92.46 ± 2.00^{b}	-9.34
G4 Hesperidin + Doxorubicin	97.50 ± 3.50^{b}	-21.88	90.50 ± 4.30^{c}	-29.30	102.52 ± 0.30^{e}	-2.87	89.05 ± 1.10^{c}	-12.67
G5 Rutin + Hesperidin + Doxorubicin	109.30 ± 3.70^{b}	-12.42	102.40 ± 2.80^{b}	-20.00	104.29 ± 0.26^{d}	-1.20	48.28 ± 1.40^{d}	-52.66
F-Probability	P < 0.001		P < 0.001		P < 0.001		P < 0.001	
LDS at 5 % level	9.90		8.39		8.14		4.27	
LDS at 1 % level	13.39		11.35		11.01		5.77	

Data are expressed as mean ± standard error
Number of animals in each group is six
Means, which have the same superscript symbol (s), are not significantly different
Percentage changes (%) are calculated by comparing normal with doxorubicin treated group and pre-treated doxorubicin groups with doxorubicin treated group

Table 2. Protective effect of rutin and / or hesperidin on serum total bilirubin, α-fetoprotein, albumin and sialic acid levels in doxorubicin treated rats

Parameters / Treatments	Total bilirubin (mg / dl)	% change	α-fetoprotein (ng / ml)	% change	Albumin (g / dl)	% change	Sialic acid (nmol)	% change
G1 Normal	2.56 ± 0.15^{c}	-	0.23 ± 0.02^{b}	-	1.97 ± 0.04^{d}	-	0.039 ± 0.002^{bc}	-
G2 Doxorubicin	3.32 ± 0.43^{abc}	29.69	0.29 ± 0.02^{a}	26.09	1.54 ± 0.15^{c}	-21.83	0.053 ± 0.002^{a}	35.90
G3 Rutin + Doxorubicin	2.59 ± 0.43^{ab}	-21.98	0.23 ± 0.01^{b}	-20.69	1.96 ± 0.23^{b}	27.27	0.086 ± 0.002^{b}	62.26
G4 Hesperidin + Doxorubicin	2.77 ± 0.39^{a}	-16.57	0.23 ± 0.01^{b}	-20.69	1.85 ± 0.05^{c}	20.13	0.059 ± 0.002^{c}	11.32
G5 Rutin + Hesperidin + Doxorubicin	2.90 ± .21^{bc}	-12.65	0.27 ± 0.01^{a}	-6.90	1.95 ± 0.11^{a}	26.62	0.057 ± 0.002^{b}	7.55
F-Probability	P < 0.01		P < 0.001		P < 0.001		P < 0.01	
LDS at 5 % level	1.00		0.034		0.397		0.0070	
LDS at 1 % level	1.35		0.045		0.537		0.0098	

Data are expressed as mean ± standard error
Number of animals in each group is six
Means, which have the same superscript symbol (s), are not significantly different
Percentage changes (%) are calculated by comparing normal with doxorubicin treated group and pre-treated doxorubicin groups with doxorubicin treated group

Concerning serum enzymes related to liver function, the doxorubicin-administered rats exhibited significant increase ($p < 0.01$; LSD) in ALT, AST, ALP and γ-GT activities. The pre-treatment with rutin, hesperidin and their mixture successfully ameliorated the elevated activities of AST, ALT, ALP and γ-GT.

Serum total bilirubin concentration was highly significantly elevated ($p < 0.01$; LSD) in doxorubicin-administered rats, recording a percentage increase of

29.69 %. The pre-treatment with rutin and / or hesperidin produced no significant effect ($p > 0.05$; LSD).

Serum α-fetoprotein level was significantly increased as a result of administration of DXR alone, while it was significantly decreased in animals pretreated with either rutin or hesperidin. However, the pre-treatment of rats with a mixture of rutin and hesperidin has a non-significant effect on serum α-fetoprotein level as compared with doxorubicin-administered control. Serum albumin level was significantly decreased in DXR administered rats. The pretreatment of doxorubicin-administered rats with hesperidin had no significant effect ($p > 0.05$; LSD) while the pre-treatment with rutin alone or rutin and hesperidin in a mixture induced a significant elevation of the albumin concentration as compared to the doxorubicin-administered control.

Serum sialic acid level was highly significantly increased ($p < 0.01$; LSD) in doxorubicin-administered rats. The pre-treatment with rutin and / or hesperidin induced an elevation of the serum sialic acid level and the recorded percentage changes were 62.26, 11.32 and 7.55 % as a result of rutin, hesperidin and their mixture respectively when compared with DXR-administered control.

Table 3. Protective effect of rutin and / or hesperidin on liver glutathione level, glutathione peroxidase and glutathione-s-transferase activities in doxorubicin treated rats

Parameters / Treatments	Glutathione (nmol /gm)	% change	Glutathione Peroxidase (U / min)	% change	Glutathione-s-transferase (U / gm)	% change
G1 Normal	1188.21 ± 9.50^{e}	-	26.47 ± 0.73^{a}	-	690.90 ± 17.90^{a}	-
G2 Doxorubicin	1011.08 ± 5.30^{c}	-14.91	21.52 ± 0.54^{c}	-18.70	463.18 ± 8.50^{b}	-32.96
G3 Rutin + Doxorubicin	1236.88 ± 31.60^{a}	22.33	25.98 ± 0.58^{a}	20.72	476.82 ± 7.00^{c}	2.94
G4 Hesperidin + Doxorubicin	1252.08 ± 20.70^{b}	23.84	25.79 ± 0.97^{a}	19.84	485.46 ± 9.30^{c}	4.81
G5 Rutin + Hesperidin + Doxorubicin	1124.67 ± 17.80^{d}	11.23	25.24 ± 1.30^{bc}	17.29	487.99 ± 10.70^{c}	5.36
F-Probability	P < 0.0001		P < 0.0004		P < 0.0001	
LDS at 5 % level	56.21		2.51		33.08	
LDS at 1 % level	76.05		3.39		44.75	

Data are expressed as mean ± standard error
Number of animals in each group is six
Means, which have the same superscript symbol (s), are not significantly different
Percentage changes (%) are calculated by comparing normal with doxorubicin treated group and pre-treated doxorubicin groups with doxorubicin treated group

Table 4. Protective effect of rutin and / or hesperidin on liver peroxidase activity and lipid peroxidation level in doxorubicin treated rats

Parameters / Treatments	Lipid Peroxidaion (nmol / g / hr)	% change	Peroxidase (U / gm)	% change
G1 Normal	1.61 ± 0.06^{b}	-	158.68 ± 4.50^{a}	-
G2 Doxorubicin	1.89 ± 0.06^{a}	17.39	144.97 ± 4.00^{b}	-8.64
G3 Rutin + Doxorubicin	1.35 ± 0.09^{c}	-28.57	154.10 ± 2.60^{a}	6.30
G4 Hesperidin + Doxorubicin	1.69 ± 0.05^{b}	-10.58	148.89 ± 3.10^{b}	2.70
G5 Rutin + Hesperidin + Doxorubicin	1.87 ± 0.04^{a}	-1.06	146.79 ± 3.80^{b}	1.26
F-Probability	P < 0.0001		P < 0.0001	
LDS at 5 % level	0.176		10.69	
LDS at 1 % level	0.238		14.46	

Data are expressed as mean ± standard error
Number of animals in each group is six
Means, which have the same superscript symbol (s), are not significantly different
Percentage changes (%) are calculated by comparing normal with doxorubicin treated group and pre-treated doxorubicin groups with doxorubicin treated group

Table 3 and Table 4 show the effect of the tested rutin agent on the liver oxidative stress markers and the antioxidant defense system of normal and doxorubicin-administered rats. The pre-treatment of these animals with rutin, hesperidin and / or their mixture produced a potential increase ($p < 0.01$; LSD) of the glutathione level which was elevated above the normal level; the percentage changes were 22.33 %, 23.84 % and 11.23 % respectively as a result of rutin, hesperidin and their mixture as compared to DXR treated group (-14.91) which exhibited significant decrease in GSH level when compared to normal control group. Glutathione-S-transferase and glutathione peroxidase activities exhibited a non-significant change in all pretreated groups in comparison to the DXR treated group that showed a significant decrease in the activities of glutathione-S-transferase ($p < 0.01$; LSD). The tested agents induced a significant decrease of the elevated value ($p < 0.05$; LSD) of GSH, glutathione-S-transferase and glutathione peroxidase in animals pre-treated with either of rutin and hesperidin or their mixture.

In contrast, liver lipid peroxidation was increased significantly as a result of DXR administration while, peroxidase activities exhibited a non-significant change in

all pretreated groups in comparison, to the DXR treated group that showed a significant decrease in the activities of peroxidase ($p < 0.05$; LSD). One-way ANOVA revealed that the effect on the variables of oxidative stress and antioxidant system was of $p < 0.001$ between groups.

DXR cytotoxicity and genotoxicity may be mediated by free radicals derived from this drug and its capability to induce apoptosis through a wide variety of mechanisms including production of reactive oxygen species (ROS), alkylation of cellular macromolecules, DNA intercalation and cross-linking, lipid peroxidation, cell membrane damage, ceramide production and p 53 induction in various tissues [16,45,46].

The current results show that doxorubicin administration caused a significant increase in alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), gamma glutamyl transferase (GGT) activities and total bilirubin concentrations. Doxorubicin administration also caused a significant decrease in serum albumin and an increase of the sialic acid level. The present results are in agreement with many authors who show that doxorubicin treated rats showed significant increases in ALT, AST and bilirubin serum levels when compared with the untreated control group [47,48,49].

The higher activities of AST and ALT recorded in the present study may be as result of drastic conditions caused by the toxic activity of doxorubicin accumulations in the liver and in turn this might provoke cellular destruction or increase the permeability of hepatic cells [50,51].

Serum total bilirubin concentration exhibited a relative increase. This higher value may be attributed to defense mechanisms against free radical induced oxidative damage including a reduction of free radicals by increasing electron donors, such as bilirubin [52]. In fact, there is a strong evidence of the presence of hepatic injury as revealed by the increase in the activities of AST, ALT, ALP and total bilirubin level.

The present study indicated that the serum enzyme (ALT, AST, ALP and γ-GT) activities of rats pretreated with rutin, hesperidin and / or their mixture were successfully ameliorated. However, while the mixture is the most potent in improving γ-GT activity, hesperidin seemed to be the most effective in reducing elevated serum AST activity. In addition, hesperidin and the mixture of rutin and hesperidin produced a significant decrease in serum ALT and ALP activities.

In an N-nitrosodiethylamine-induced hepatocarcinogenesis model, quercetin accelerated the remodeling of preneoplastic foci and significantly decreased the number of persistent foci, hyperplastic nodules and hepatocellular carcinoma (HCC) cells [53]. Despite this promising evidence, other authors have found dietary quercetin to enhance GST-P-positive focus formation in a rat model of heterocyclic-amine-induced hepatocarcinogenesis. Their studies, however, analysed GST-P foci as the surrogate endpoint rather than tumor development [54,55].

In vitro, quercetin inhibits proliferation and induces apoptosis of human HCC cells [56,57,58]. Quercetin has also been shown to enhance radiation-induced cell death in human HCC cells [59]. In BALB / c mice inoculated with hepatoma cells, liposomal quercetin significantly inhibited tumor growth in a dose-dependent manner [60].

The present study shows that doxorubicin treatment caused a marked decrease in peroxidase, glutathione peroxidase activities as well as glutathione-S-transferase activities. On the other hand, doxorubicin treatment caused a marked increase in lipid peroxidation level. Inversely, doxorubicin administered rats pretreated with rutin and hesperidin exhibited amelioration (increase) in glutathione level, peroxidase, glutathione peroxidase and glutathione-S-transferase activities, while a significant reduction in lipid peroxidation level was recorded.

The present results are in agreement with those of several authors who reported that oxidative stress leads to lipid peroxidation, which is the result of an interaction between free radicals of diverse origin and unsaturated fatty acids typically in membrane lipids. The net result of these events is the accumulation of a variety of toxic lipid peroxides and malondialdehyde (MDA). The level of tissue MDA is reported to be a reliable marker of lipid peroxidation [19].

Oxidative stress has been implicated as a factor that contributes to various forms of cell death. Vehicle Control and SMN alone groups demonstrated comparably low levels of oxidative stress. DXR alone produced a 30-fold increase in MDA, whereas SMN treatment reduced the DXR-induced increase in MDA to 4-fold. The pattern of MDA accumulation paralleled changes in ALT and the levels of observed tissue injury. Mitochondria are positioned to play a decisive role in DXR toxicity as principle generators of superoxide for initiating ROS cascades. Agents that inhibit mitochondrial complex-I, such as MPP+ or NAPQI, and reactive metabolites of MPTP and acetaminophen respectively, induce oxidative stress accompanied by apoptosis [59,60] as an inhibitor of mitochondrial complex-I, DXR produce ROS which can result in oxidative stress and subsequent apoptosis [61,62,63]. Opening of the mitochondrial permeability transition pore (MPT) and the disruption of mitochondrial transmembrane potential are also central steps in the apoptotic cell death signaling pathway [19]. In producing hepatocyte apoptosis, tumor necrosis factor α (TNF α) induces the MPT and cytochrome c release [64,65]. DXR generates oxidant radicals, such as, HO and O_2-as well as H_2O_2 and induces cytochrome c release [65,66,67].

Mitochondria are also natural targets of phytochemical antioxidant protection [10,68]. SMN could protect against DXR toxicity through a number of mitochondrial actions including the up-regulation of specific anti-ROS proteins, prevention of mt DNA damage, stimulation of replication, inhibition of membrane active lipases, and protection of the electron transport chain for optimal ATP production during energy depletion. Silymarine (SMN) has been shown to protect the intracellular microenvironment by conserving the mitochondria-dependent antioxidant components [69].

4. Conclusions

In conclusion, the present results demonstrate that rutin and hesperidin play an important role in the protection against doxorubicin induced hepatotoxicity, by improving the activities of liver enzymes (ALT, AST, and ALP & GGT) in addition to the amelioration in the levels of total bilirubin, albumin and sialic acid. Doxorubicin

administered rats pretreated with rutin and hesperidin recorded a significant increase in glutathione level, glutathione peroxidase, glutathione-S-transferase and peroxidase activities, and a reduced lipid peroxidation level. Pretreatment with rutin and hesperidin may protect the liver from the hepatotoxic effect caused by doxorubicin. Further clinical studies are required to assess the benefits and safety of these flavonoids and their effects on the therapeutic activity of doxorubicin in cancer treatment before their use in human beings and approval by Food and Drug administration (FDA).

Conflicts of Interest

The authors declare they have no conflicting interests.

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Abbreviations

(DXR) Doxorubicin
(ROS) Reactive Oxygen species
(ALT) Alanine transferase
(LDL) Low density Lipoprotein
(IQ) 2-amino-3-methylimidazo [4, 5-f] quinoline
(AST) aspartate transaminase
(ALP) alkaline phosphatase
(GGT) gamma glutamyl transferase
(GSH) Liver glutathione
(GST) Glutathione S transferase
(TBARS) thiobarbituric acid reactive substances
(MDA) malondialdehyde
(HCC) hepatocellular carcinoma cells
(SMN) Silymarine
(LSD) Fisher's least significant difference

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