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Protective Effect of Umbelliferone against Doxorubicin Induced Cardiotoxicity in Wistar Albino Rats

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ABSTRACT

Doxorubicin (DOX) is widely used as chemotherapeutic agent. Usefulness of this agent is limited due to its cardiotoxic effects. The aim of this study was to investigate the curative effect of Umbelliferone (UMB) against Doxorubicin (DOX) induced cardiotoxicity in albino rats. Biomarkers like LDH, AST, ALT, ALP, CK, along with heart weight index, antioxidant enzymes, lipid profiles and histological examination were assessed to determine the cardiotoxicity effect. DOX alone (25mg/kg bw intraperitoneally three times per week for two weeks) administered group exhibited significant increase in LDH, AST, ALT, ALP, CK, FC, FFA and TG levels and decrease in Enzymatic and Non enzymatic antioxidants activities when compared to control group. Oral administration of Umbelliferone (UMB) (30mg/kg bw) treatment daily for two weeks along with DOX significantly attenuated the increased serum biomarkers, reduced hyperlipidemia and restored the tissue antioxidant activities when compared to DOX alone treated group. Histology examination also clearly showed that UMB significantly inhibited the cardiac damage induced by DOX. Based on the results, this study clearly indicates that UMB used as a potent compound against cardiotoxicity induced by DOX.

Keywords: Doxorubicin, Umbelliferone, Cardiotoxicity

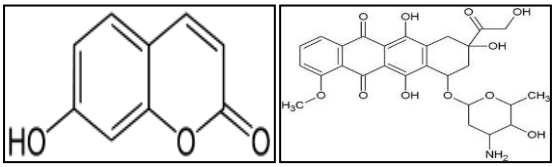
I. INTRODUCTION

Globally Doxorubicin (DOX) used as an anticancer drug because of its antineoplastic antibiotic effect, this has used to treat the several cancers including leukemia and solid tumours (1). Long term usage of this drug causes severe side effects, which includes predominantly cardio toxicity later that may lead to irreversible cardiomyopathy and heart failure (2). Administration of anticancer agents adversely affect the antioxidant haemostasis in the cellular levels thereby generation of abnormal free radicals which lead to oxidative stress that causes tissue damage (3).

Myocardial infarction or heart attack is the major disease that causes predominantly death from all over the world. When blood supply is insufficient to myocardium, death of cardiomyocytes occurs, a condition known as ischemia. Prolonged ischemia leads to necrosis, which is called as myocardial infarction. Currently many medicines are used to treat myocardial infarction that has so many side effects. Dietary occurring natural compounds play a key role in treating various diseases, including cardiac diseases. Herbal formulations are safer than modern drugs which lead to herbal preparations. World Health Organization also recommended the uses of herbal medicines as an alternative medicine in the developing countries (4).

Coumarin is a benzopyrene structured compound derived from the plant sources. Coumarin is extensively distributed in the entire parts of the plant, especially high amount in flowers, stem, and roots (5). Coumarins are vast group of natural compounds, among umbelliferone were predominantly found in the edible fruits of Golden apple *Aegle marmelos* (Rutaceae) (6). It is a yellowish-white crystalline solid, slightly soluble in hot water and high soluble in ethanol (7).

UMB contains wide spectrum of pharmaceutical effects including anti-lipidemic (8), anti-diabetic and anti-hyperglycemic (9), anti-inflammatory (10), radio protective (11), and antioxidant activity (12). In addition, recent studies revealed that UMB have significant hepatoprotective effect (13). But still, there is scarce information about the cardio protective effect of UMB. Hence this study was designed in such a way to elucidate the role of UMB on experimentally induced cardio toxicity.



A **B**

Figure 1. Structure of (A)Umbelliferone ($C_9H_6O_3$) and (B) Doxorubicin ($C_{27}H_{29}N0_{11}$)

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II. METHODS AND MATERIAL

Source of chemicals and drugs

Doxorubicin was purchased from Hi Media laboratories, India. Umbelliferone (7-Hydroxy coumarin) was purchased from Sigma-Aldrich, Bangalore. All other chemicals used which were of analytical grade obtained from SRL/TCI/HIMEDIA laboratories, India.

Experimental animals

Male Albino Wistar rats weighing about 150–160 g were procured from TANUVAS, Chennai, India and all the experiments were designed and conducted according to the Institutional Animal Ethics Committee approved guidelines. The animals were maintained in clean, sterile cages, well ventilated room with controlled temperature (25±2°C) and were acclimatized to 12-h light and dark cycles. Animals were fed with commercially available standard rat pelleted feed (M/S Hindustan Foods Ltd, Bangalore, India) throughout the experimental period. The animals were free access to food and water.

Experimental design

The rats were divided into four groups, each group consisting of six animals.

Group I Control rats were given 10% DMSO which served as a vehicle control through the experimental period along with standard diet and drinking water.

Group II Rats were injected DOX (25mg/kg bw, intraperitoneally using saline) three times per week for two weeks (14)

Group III Rats were administered with Umbelliferone orally (30mg/kg bw dissolved in 10% DMSO) daily for two weeks along with the same time DOX were injected intraperitoneally three times per week for two weeks period.

Group IV Rats were administered with UMB orally (30mg/kg bw dissolved in 10% DMSO) daily for two weeks.

At the end of the experimental period, the animals were fasted overnight and anesthetized with Ketamine (90 mg/kg bw) and Xylazine (10 mg/kg bw) and sacrificed followed by cervical decapitation. Blood were collected and allowed to coagulate at room temperature for 30 min. Serum were separated by centrifugation at 3000 rpm for 15 min at 4˚C. The heart were immediately excised and washed in ice-cold saline. The heart tissues were sliced and homogenized into 0.1 M Tris–HCL buffer (pH 7.4). The tissue homogenates were centrifuged at 1000 rpm for 10 min at 4˚C and the supernatants were collected and assessed for various parameters.

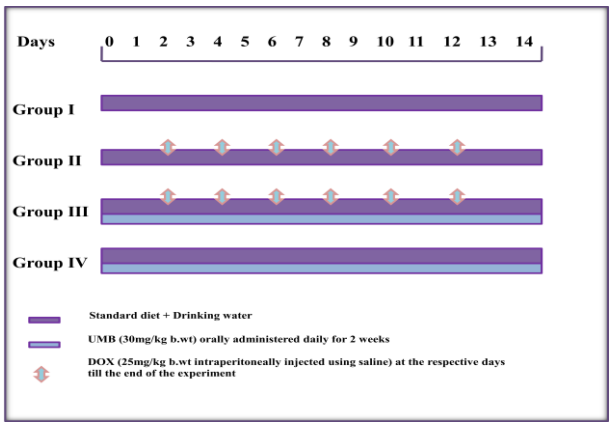


Figure 2. Schematic representation of experimental design Biochemical parameters

Estimation of marker enzymes

Activities of Lactate dehydrogenase (LDH) (15), Asparate transaminases (AST) (16), Alanine tansaminases (ALT) (16), alkaline phosphatase (ALP) (17) and Creatine kinase (18) were assayed in the serum of experimental animals.

Estimation of enzymic and non-enzymic antioxidants

The data were analysed with SPSS/10 Software. Hypothesis testing methods included one way analysis of variance (ANOVA) followed by least significant difference (LSD) test. P values of <0.05 were considered to indicate the statistically significance. All the results were expressed as mean ± standard error (SE) for six animals in each group.

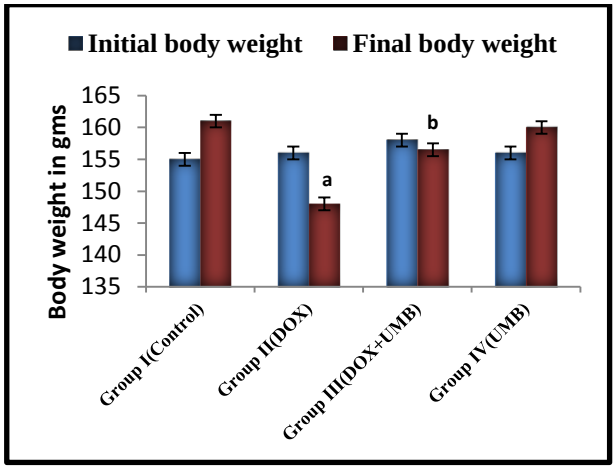
III. RESULT AND DISCUSSION

1. RESULT

Effect of UMB on body weight, Heart weight and relative heart weight

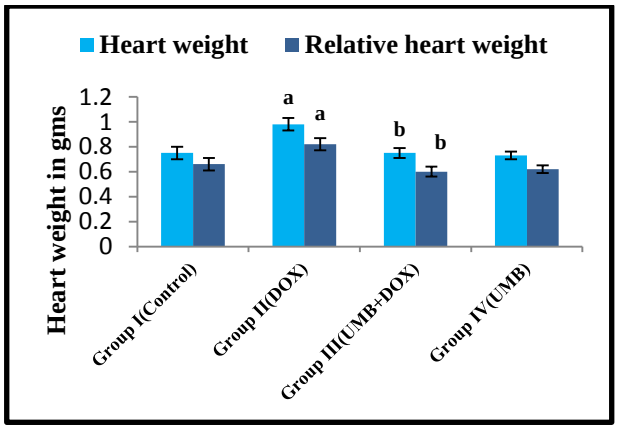
Fig 3 shows the initial and final body weight of the control and experimental group of animals. In DOX administered group II animals there is drastic reduction in the final body weight, whereas UMB+DOX administered group III animals were showed significant increase in the final body weight when compared with DOX alone group II animals. In addition there is no significant difference found in the final body weight of UMB alone treated animals when compared to control animals.

Fig 4 shows the heart weight and relative heart weight of control and experimental animals. DOX administered animals showed increase in the heart weight and relative heart weight. UMB+DOX treated animals exhibited the significant decrease in the heart weight and relative heart weight when compared with the DOX induced animals. There are no significant changes between heart weight of the control animals and UMB alone treated animals.



([a]Compared with control and [b]Compared with DOX)

Figure 3. Effect of UMB on body weight during experimental group of animals



([a]Compared with control and [b]Compared with DOX)

Figure 4. Effect of UMB on Heart weight and relative heart weight during experimental group of animals UMB decreased the levels of serum marker enzymes in DOX induced cardiotoxicity animals

Table 1 shows the effect of UMB on the levels of markers enzymes LDH, AST, ALT, ALP and CK in the serum of control and experimental group of rats. The marker levels were significantly increased in DOX induced animals when compared with control animals. Whereas UMB+DOX treated animals significantly decreased the levels of marker enzymes when compared with DOX induced animals. No significant changes observed between control and UMB alone treated animals.

UMB treatment increased the levels of HDL cholesterol

Table 2 shows the serum lipoprotein fractions such as LDL, HDL and VLDL of control and experimental groups of animals. DOX administered animals showed significant reduction in the HDL levels and the same increased in the LDL/VLDL when compared with control animals. Whereas UMB+DOX treated animals significantly restored the HDL levels and decreased in the LDL/VLDL levels when compared to DOX induced animals.

UMB alleviate the antioxidant activities during DOX induced cardiotoxicity

Table 3 and 4 indicates the enzymatic and non-enzymatic antioxidant activities in the heart of the control and experimental groups. DOX induced animals showed significant decrease in the activities of enzymatic antioxidants such as SOD, CAT, GPx, GR and GST when compared with control animals. Whereas UMB+DOX supplemented animals showed significant increase in the activities of these enzymes when compared with DOX induced animals.

Non enzymatic antioxidants such as GSH, G6PD, VIT C, VIT E and VIT A also found significantly decreased activities during DOX induced animals when compared with control animals. In UMB+DOX treated animals, there is significant increase in the activities of GSH, G6PD, VIT C, VIT E and VIT A when compared with DOX induced animals. No significant change was observed in UMB alone treated animals when compared with control animal.

Table 1: Effect of UMB and on the activities of marker enzymes in serum of control and experimental group of rats

Groups	LDH	AST	ALT	ALP	CK
Control	87.33 ± 6.32	108.6 ± 9.16	74.83± 5.26	19.42 ± 1.03	10.46 ± 0.97
DOX	178.32 ± 14.67 [a]	158.5 ±13.05 [a]	286.35 ± 21.84 [a]	73.53 ± 5.11 [a]	27.64 ± 1.98 [a]
UMB+DOX	111.23 ± 8.45 [b]	124.1 ±11.04 [b]	128.43 ± 7.62 [b]	48.13 ± 3.06 [b]	13.23± 0.73 [b]
UMB	89.11 ± 7.05	104.9 ± 8.99	72.32 ± 5.04	18.46 ± 1.69	10.14 ± 0.57

Results are expressed as mean ± S.D. for six rats in each group. Statistical significance at $p<0.05$ compared with [a]Compared with control and [b]Compared with DOX. Units CK: µmol of phosphorus/h/mg protein; LDH, AST and ALT: µmol of pyruvate/h/mg protein; ALP: KA U/l.

Table 2: Effect of UMB on the activities serum lipoprotein fraction of control and experimental group of rats

Groups	LDL	HDL	VLDL
Control	16.12 ± 1.05	40.77± 3.29	26.14 ± 2.11
DOX	32.16 ± 2.02 [a]	26.16 ± 1.80 [a]	42.66 ± 4.09 [a]
UMB+DOX	20.65 ± 1.01 [b]	38.14 ± 2.68 [b]	35.56 ± 2.68 [b]
UMB	15.67 ± 1.22	40.29 ± 3.11	25.62 ± 1.95

Results are expressed as mean SD for six rats each group. Statistically significance at $p<0.05$; Results are compared with [a]Compared with group 1 and [b]Compared with group 2.Units— mg/dl

Table 3. Effect of Umbelliferone on the activities of antioxidant enzymes in the heart of control and experimental groups of rats

Groups	SOD	CAT	GPx	GR	GST
Control	3.86 ± 0.17	69.06 ± 5.39	19.23 ± 1.41	2.99 ± 0.10	1.86 ± 0.12
DOX	1.68 ± 0.07[a]	37.45 ± 2.92[a]	11.96 ± 0.87[a]	0.74 ± 0.05[a]	0.59 ± 0.04[a]
UMB+DOX	2.32 ± 0.09[b]	54.84 ± 4.23[b]	16.28 ± 1.43[b]	1.46 ± 0.11[b]	1.43 ± 0.09[b]
UMB	3.67 ± 0.16	68.38 ± 4.96	18.56 ± 1.47	2.69 ± 0.10	2.11 ± 0.14

Results are expressed as mean ± S.D. for six rats in each group. Statistical significance at $p<0.05$ compared with [a]Compared with control and [b]Compared with DOX. Units: SOD in units/mg protein, CAT in µmol of H_2O_2 decomposed/min/mg protein, GPx in µmol of GSH utilized/min/mg protein, GR in µmol of NADPH oxidized/min/mg protein and GST in µmol of CDNB-GSH conjugate formed/min/mg protein. G6PD in µmol of NADPH oxidized/min/mg protein.

Table 4. Effect of Umbelliferone on the activities of non-enzymatic enzymes in the heart of control and experimental group of rats

Groups	GSH	G6PD	VITAMIN C	VITAMIN E	VITAMIN A
Control	5.92 ± 0.45	2.36 ± 0.13	1.84 ± 0.13	2.83 ± 0.25	3.16 ± 0.10
DOX	1.89 ± 0.07[a]	0.58 ± 0.04 [a]	0.66 ± 0.05[a]	1.43 ± 0.08[a]	0.88 ± 0.02[a]
UMB+DOX	3.75 ± 0.29[b]	1.83 ± 0.06 [b]	1.38 ± 0.07 [b]	2.32 ± 0.16 [b]	2.28 ± 0.18 [b]
UMB	5.66 ± 0.39	2.27 ± 0.12	1.71 ± 0.10	3.01 ± 0.20	3.54 ± 0.09

Results are expressed as mean ± S.D. for six rats in each group. Statistical significance at $p<0.05$ compared with [a] Compared with control and [b]Compared with DOX. Units: VitC in mg/g of wet tissue, VitE in mg/g of wet tissue, VitA in mg/g of wet tissue and GSH in µg/mg protein.

Table 5. Effect of UMB on cardiac lipids of control and experimental groups of rats

Groups	Free cholesterol	Esterified cholesterol	Phospholipids	Free fatty acids	Triglycerides
CONTROL	3.47 ± 0.29	2.34 ± 0.12	14.88 ± 1.21	3.19 ± 0.21	3.23 ± 0.18
DOX	6.34 ± 0.52[a]	0.54 ± 0.04[a]	09.37 ± 0.45[a]	7.11 ± 0.59[a]	6.89 ± 0.44[a]
DOX+UMB	4.08 ± 0.29[b]	1.78 ± 0.11[b]	12.87 ± 0.68[b]	4.31 ± 0.27[b]	4.96 ± 0.52[b]
UMB	3.39 ± 0.24	2.16 ± 0.16	14.66 ± 0.89	3.06 ± 0.29	3.17 ± 0.24

Results are expressed as mean ± S.D. for six rats in each group. Statistical significance at $p<0.05$ compared with [a]Compared with control and [b]Compared with DOX Units: mg/dL

Effect of UMB on cardiac lipid status

Table 5 shows the levels of free cholesterol, esterified cholesterol, phospholipids, free fatty acids and Triglycerides. DOX induced animals showed significant increase in the content of free cholesterol, free fatty acids, triglycerides and decreased levels of phospholipids, esterified cholesterol were observed. Whereas UMB+DOX administered animals found significant reduction in the levels of free cholesterol, free fatty acids, triglycerides and at the same time, increased in the levels of esterified cholesterol, phospholipids were observed when compared to DOX induced animals.

Histology examination

Haematoxylin and Eosin stained (H&E) sections of heart were assessed under a light microscope (40x). Control animals (Fig 5A) and UMB alone administered animals (Fig 5D) showed normal architecture of myocardial fibres and myocytes. (Fig 5B) Doxorubicin administered animals showed myocyte swelling, severe damage in myocardial fibres and hyalinization in the heart. Umbelliferone + Doxorubicin treated animals (Fig 5C) almost showed normal myocytes and less damage in myocardial fibres.

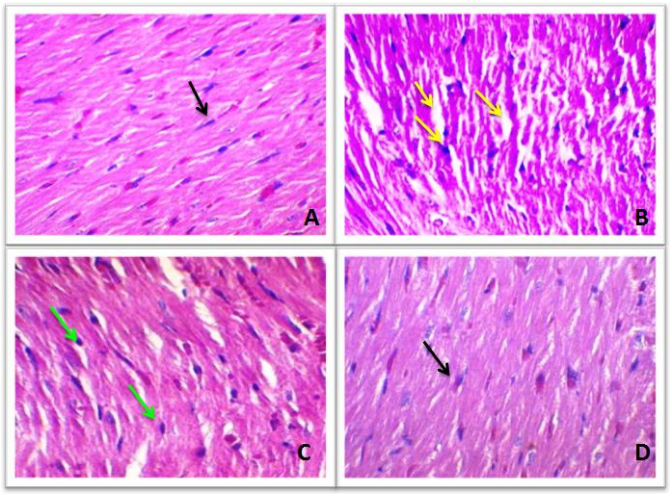


Figure 5. Photomicrographs (40x) obtained from the heart sections A. Control group, B. DOX alone group, C. UMB + DOX group, D. UMB alone group.(⟶) Shows normal myocytes and cardiomyofibres (⟶) Shows swollen myocytes and damaged myocardial fibres (⟶) Shows normal myocytes and less myocardial fibres damage

2. DISCUSSION

Doxorubicin is an anthracycline, which is used for the treatment of various malignant and non-malignant tumors. The use of doxorubicin as chemotherapeutic drug has been limited due to its diverse toxicities, including cardiac, renal, haematological and testicular toxicity (34). It has been reported doxorubicin induced cardio toxicity mediated by lipid peroxidation and inhibition of fatty acids and oxidation in cardiac tissues (35). Several studies reported, anticancer therapies adversely affect the physiological homoeostasis of different organ functions during cancer treatment. Doxorubicin induced myocardial damage have been well established in animal models.

The present study delineates the protective role of UMB in DOX induced cardio toxicity. Experimentally DOX challenged animal models severely causes oxidative stress mediated free radical generation that causes cardio toxicity. The metabolic action of DOX is initially converted into semiquinone form in the cardiac tissue, which is a toxic, short lived metabolite that interacts with molecular oxygen and initiates a cascade of reaction leading to ROS generation (36).

In this present study, administration of DOX drastically reduced in the final body weight and significantly increased the heart weight. Similarly DOX administration adversely affects the tissue by increase in the serum markers LDH, AST, ALT, ALP, and CK-MB. Umbelliferone challenged along with DOX animals significantly increase the body weight and attenuated the tissue damage thereby preventing the membrane enzymes leakage into the serum, this shows the protective effect of UMB in myocytes against cardiotoxicity (37).

Clinical investigations also suggest that increased oxidative stress associated with DOX causes cardiomyopathy that leads to heart failure (11). DOX and its mebolites reduced the oxidant level that leads to accumulate abnormal free radical generation in the heart tissue (38). In this study, the oxidative stress associated tissue injury caused by DOX results significant decreased in the activities of SOD, CAT, GPx, GR, GST and UMB along with DOX treatment restored the

enzymic antioxidant levels which clearly shows the beneficial effect against DOX induced cardiotoxicity (39) Non-enzymic antioxidants also play an excellent role in protecting the cells from oxidative damage. GSH an ubiquitous tripeptide which act as a free radical scavenger (39). Decreased level of GSH impairs the ability of cells thereby enhance in the levels of LPO. Lipid soluble antioxidant like Vitamin E which is present in the cellular membranes plays an important role in the suppression of free radicals (40). Vitamin C is a free radical scavenger, act as an antioxidant in recycling of Vitamin E. Cellular levels of vitamin C and vitamin E were maintained in the active form by GSH. In this study, DOX group shows reduced activities of Vitamin C, A, E and has pronounced GSH depletion were observed. UMB along with DOX treated animals showed significant increase in the levels of non enzymic antioxidants activities which may be due to ability of UMB quenched the free hydroxyl and superoxide radicals.

Hypercholestrolemia, hypertriglyceridemia is a well-known risk factor in cardiovascular disease. Heart attack risk can be reduced by lowering the cholesterol levels and the alteration in the lipoprotein transport and metabolism affects the physiological context of changes in plasma lipids (42). In this study DOX group significantly revealed increase in the levels of cholesterol and triglycerides. Interestingly UMB supplementation along with DOX completely attenuated the increased cholesterol and triglycerides levels. Biochemical data were further confirmed the histopathological studies from the cardiac tissues. UMB along with DOX treated animals showed the minimal damage by maintaining the cardiomyocytes and myofibres neatly to the normal architecture than the DOX animals (43). From the above, all the data revealed that the UMB has the potential to cure the cardiotoxic effect produced by the DOX.

IV. CONCLUSION

The present findings clearly shows the protective effect of UMB on DOX induced cardiotoxicity through maintaining systemic antioxidant activities, cellular membrane integrity and reducing hyperlipidimea conditions. Further studies are in the underway to investigate the intricate mechanism.

V. ACKNOWLEDGEMENT

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VI. REFERENCES

[1] Hrdina, R., Gersl, V., Klimtova, I., Simunek, T., Machackova, J, Adamcova, M (2000). Anthracycline induced cardiotoxicity Acta Medica; 43(3):75–82.

[2] Lefrak, EA.,Pitha, J., Rosenheim, S., Gottlieb, J.A (1973). A Clinicopathologic analysis of adriamycin cardiotoxicity Cancer; 32:302–314.

[3] Minotti, G., Ronchi, R., Salvatorelli, E., Menna, P., Gaetano, C.G (2001). Dox irreversibly inactivates iron regulatory proteins 1 and 2 in cardiomyocytes: Evidence for distinct metabolic pathways and implications for iron mediated cardio toxicity of antitumor therapy Cancer Res; (61): 8422-8428.

[4] Jain, PK., Joshi, H (2012). Coumarin: chemical and pharmacological profile J Appl Pharm Sci; 2:236 –40.

[5] Battino, M., (2001). Natural distribution and occurrence of coenzyme Q homologues in mammals In: Ebadi, M., Marwah, J., Chopra, R (Eds.), Mitochondrial Ubiquinone (Coenzyme Q10): Biochemical, Functional, Medial, and Therapeutic Aspects in Human Health and Diseases Prominent Press, Scottsdale, pp152 - 182.

[6] Venugopala, K.N., Rashmi, V., Odhav, B (2013). Review on natural coumarin lead compounds for their pharmacological activity Bio Med Research International doi.org/ 10.1155/ 2013/963248

[7] Dean, F.M (1963). Naturally occurring oxygen ring compounds Butter worths, London.

[8] Ramesh, B., Pugalendi, K.V (2005). Antihyperlipidemic and antidiabetic effects of umbelliferone in streptozotocin diabetic rats Yale J Biol Med; 78:189 – 96

[9] Ramesh, B., Pugalendi, K.V (2006). Antihyperglycemic effect of umbelliferone in streptozotocin - diabetic rats J Med Food; 9:562–6.

[10] Lino, C.S., Taveira, M.L., Viana, G.S.B., Matos, F.J.A (1997). Analgesic and antiinflammatory activities of Justicia pectoralis Jacq and its main constituents: coumarin and umbelliferone Phyther Res; 11:211–5.

[11] Jayakumar, S., Hari, N., Bhilwade, R.C.C (2013). Suppression of radiation-induced DNA damage and apoptosis in hematopoietic cells of mice by umbelliferone BARC Newsletter; p327–31.

[12] Hoult, J.R., Paya, M (1996). Pharmacological and biochemical actions of simple coumarins: natural

products with therapeutic potential Gen Pharmacol; 27:713–22

[13] Tamilarasi, S., Devaki, T(2016). Umbelliferone exetunates the abnormalities in lipid metabolism during galactosamine and lipopolysaccharide - induced fulminant hepatic failure in rats Int J Pharm Sci; 8: 79-84

[14] Dhankhar, S., Ruhil, S., Balhara, M., Dhankhar, S., Chhillar, AK (2011). Aegle marmelos (Linn.) correa: a potential source of phytomedicine; 5:1497–507

[15] King, J(1965). The dehydrogenases or oxidoreductases-lactate dehydrogense In: Van D editor Practical clinical enzymology; London: Nostrand; p83–93

[16] Mohun, A.F., Cook, L.J (1957). Simple method for measuring serum level of glutamateoxaloacetate and glutamate-pyruvate transaminases in laboratories J Clin Chem; 10:394–9

[17] King, J(1965). Van editor Practical clinical enzymology London: Nostrand; p363

[18] Okinaka, S., Sugita, H., Mamoi, T (1964). Cysteine-stimulated serum creatine kinase in health and disease J Lab Clin Med; 64:299–305

[19] Misra, H.P., Fridovich, J (1972). The role of superoxide anion in the auto-oxidation of epinephrine and a simple assay for superoxide dismutase J Biol Chem; 247:3170–5

[20] Bergmeyer, H.V., Gowehn, K., Grassel, M (1974). Methods of enzymatic analysis New York: Academic; p438

[21] Rotruck, J.T., Pope, A.L., Ganther, H.E (1973). Selenium: biochemical role as a component of glutathione peroxidase, purification and assay Science; 179:588–90

[22] Habig, W.H., Pabs, J (1974). Glutathione-S-transferase J Biol Chem; 249:7130–9

[23] Beutler, E., Matsumoto, F (1975). Ethinic variation in red cell glutathione peroxidase activity Blood; 46:103-10

[24] Maye, S.T., Turbull, T.P., Sauberchich, H.C (1979). Selected methods for determination of ascorbic acid in cells, tissues and fluids Methods Enzymol; 6:3-11

[25] Desai, I.D (1984). Vitamin E analysis methods for animal tissues Methods Enzymol; 105:138 47

[26] Bayfield, R.F., Cole, E.R (1980). Colorimetric estimation of vitamin A with trichloro acetic acid Methods Enzymol; 67:189-203

[27] Staal, G.E.J., Visser, J., Veger, C (1969). Purification and properties of glutathione reductase of human erythrocytes Biochim Biophys Acta; 185:39–48

[28] Wilson, D.F., Spiger, M.J (1973). A dual precipitation method for quantitative plasma lipoprotein measurement without ultracentrifugation J Lab Clin Med; 82:473-82

[29] Sperry, W.M., Webb, A (1950). A revision of schoenhemer-sperry method for cholesterol determination J Biol Chem; 187:97-106

[30] Barlett, G.R (1959). Phosphorous assay in column chromatography J Biol Chem; 234:466-8

[31] Rouser, G., Sidney F., Akira, Y (1970). Two dimensional thin layer chromatographic separation of polar lipids and determination of phospholipids by phosphorus analysis of spots Lipids; 494-96

[32] Horn, W.T., and Menahan, I.A (1981). A sensitive method for the determination of the fatty acids in plasma J Lipid Res; 22:377-81

[33] Rice, E.W (1970). Triglycerides ("neutral fats") in serum In: Standard methods of clinical chemistry MacDonald RP. Ed. Academic press: New York; 6:215-22

[34] YF1 Xin., Wan LL., Peng JL., Guo C (2010). Alleviation of the acute doxorubicin induced cardiotoxicity by Lycium barbarum polysaccharides through the suppression of oxidative stress. Food and Chemical Toxicology.; 9:259-264.

[35] Yilmaz S, Atessahin A, Sahna E, Karahan I, Ozer S. (2006). Protective effect of lycopene on adriamycin-induced cardiotoxicity and nephrotoxicity. Toxicology; 218:164-17.

[36] Oliveira, P.J., Bjork, J.A., Santos, M.S., Leino, R.L., Froberg, M.K., Moreno, A.J., et al., (2004). Carvedilol-mediated antioxidant protection against doxorubicin-induced cardiac mitochondrial toxicity. Toxicol Appl Pharmacol ; 200:159-168.

[37] Dorr, R.T. (1996). Cytoprotective agents for anthracyclines. Semin. Oncol. 23: 23-34.

[38] King, J (1965). The dehydrogenases or oxidoreductases-lactate dehydrogense. In: Van D. editor. Practical clinical enzymology. London: Nostrand; p. 83-93

[39] Singh, Ravinder, J (2002). Glutathione: a marker and antioxidant for aging. J Lab Clin Med; 140:380-1.

[40] Halliwell, B., Gutteridge, J.M.C (1984). Lipid peroxidation, oxygen radicals, cell damage and antioxidant therapy. Lancet; 1:1396–7

[41] Elberry, A.A., Abdel-Naim, A.B., Abdel-Sattar, E.A., Nagy, A.A., Mosli, H.A., Mohamadin, A.M., et al., (2010). Cranberry (Vaccinium macrocarpon) protects against doxorubicin-induced cardiotoxicity in rats. Food and Chemical Toxicology; 48:1178-1184.

[42] Mushlin, P.S., Cusack, B.J., Boucek, R.J., Andrejuk, T., Li, X and Olson, R.D (1993). Time-related increases in cardiac concentrations of doxorubicinol could interact with doxorubicin to depress myocardial contractile function. Br. J. Pharmacol., 110: 975–982.

[43] Mantawy, E.M (2014). Chrysin alleviates acute doxorubicin cardiotoxicity in rats via suppression of oxidative stress, inflammation and apoptosis'. Eur J Pharmacol; 728:107-18.