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Cardioprotective effect of grapeseed proanthocyanidin on doxorubicin induced myocardial injury in rats

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Abstract

Grapeseed Proanthocyanidin [GSP] seeds containing a significant antioxidant and cardioprotective capabilities were investigated against Doxorubicin induced cardiotoxicity in rats. Serum creatine phosphokinase [CPK] elevation was seen 15 days after intraperitoneal administration of DOX [10mg kg1 /b.wt] once daily. glycogen oxidase [GOX] An enzyme called aspartate transaminase [ASP] ALT, or alanine transaminase. Increasing levels of cardiac malondialdehyde [MDA] in response to elevated HDL cholesterol and triglycerides are linked to decreased levels of glutathione peroxidase [Gpx], glutathione-S transferase [GST], and glutathione reductase [GR]. Serum levels of cardiac marker enzymes normalized after 15 days of daily oral treatment of an aqueous preparation of GSP seeds extract at the dosage of (200 mg kg b.wt). In conclusion, the research confirms that GSP seeds have antioxidant and cardioprotective properties.

Key-Words: Grape seed proanthocyanidin, Doxorubicin, Myocardial Injury, Antioxidant

Introduction

Doxorubicin (also known as Adriamycin) is a medicine used to treat several types of cancer, including leukemia, lymphoma, and solid tumors [1, 2]. Doxorubicin is one of the most widely used anticancer drugs due to its effectiveness against a wide range of human cancers, including breast cancer, lung cancer, small cell carcinoma, and acute myeloid leukemia. Doxorubicin is most toxic to cardiac cells, then sarcoma cells, then melanoma cells, then normal muscle fibroblasts, and finally normal skin fibroblasts. Similarly, doxorubicin may dampen the immune system's response [2]. Myocardial injury was caused by dox [3-5]. During ischemia, free radicals may be generated in the endothelial cell due to the activity of xanthine oxidase or by the infiltration of white cells into the ischemic myocardium[6].Pathophysiological alterations after Dox treatment are compared to cardiac changes in humans[7].Ischemic heart disease is a kind of cardiac disease in which the organs do not get enough blood [8].Myocardial damage is now better understood, which has sparked the development of innovative treatments. Medicinal plants that have been found to have certain preventive measures in the treatment of [IHD]ischemic heart disease. Grape seed proanthocyanidins [GSP] have pained considerable attention due to their wide range of biological and pharmacologicalproperties].

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Material and Methods

Drugs and chemicals

Doxorubicin [Adrim] was purchased from Apollo Pharma Limited, Chennai, India. All other chemicals and solvents used were of the highest purity and analytical grade.

Collection of plant material

The seeds of Grapeseed Proanthocyanidins were collected manually during month of Feb 2011.

Extraction of plant material

The plant seed were freed of pericarp, shade dried and powdered in a mixer and the extract was prepared described earlier. The seeds were manually separated. 100gm of the seed powder was extracted with 70% ethanol at 50 to 60°c in a soxhlet apparatus for 72hrs.The liquid was cooled and concentrated by its liquid content in vacuum and freeze dried. An approximate yield of 15% was obtained. The extract of Grape seed Proanthocyanidin seed will be called as

Animals

Animals healthy Wister adult male rat between 100-180g were used for this purpose. The animals were housed in polypropylene cages and maintained at 24±2°under 12hrs light dark cycle and were fed with standard pellet diet and had free access to water maintenance and use of animals as per the experiments was approved by the institutional animal Ethics committee APCAS/IAEC/2011/11

Experimental protocol

The rats were divided in to six groups of six rats eachas follows:

GROUP I: Control rats received distilled water [1ml/kgbody weight] orally for 15 days.

GROUP II: Simultaneously IV administration of 5micro mole/kg of verapamil [IV] and DOX 10mg/kg

[IV] were given and sacrificed after 48hrs.

GROUP III: Rats were injected intra peritoneal with a single dose of DOX [10mg/kg IV] in normal saline and animals were sacrificed after 48hrs.

GROUP IV: Rats were administered DOX as in group II and pretreatment with GSP extract [200mg/kg body weight] before one hour DOX treatment by oral gavagefor 15 consecutive days. The blood samples were collected.

After the 15 days experimental period (i.e., on the 16th day), all the animals were anesthetized and were sacrificed and isolated heart. The heart tissue homogenates were prepared in 0.1M Tris HCL buffer PH 7.4 were used for the determination of lipid peroxides [Lpo] reduced glutathione [GSH] glutathione peroxidase [Gpx] glutathione s-transferase[GST]. The serum was used for the determination of aspartate amino transferase [AST] alanine amino transferase [ALT] lactatedehydrogenase [LDH] creatinine phosphokinase [CPK] glucose, total cholestrol, lipid profiles [LDL, HDL,VLDL] and triglycerides.

Statistical analysis

The statistical analysis was carried out using analysis one-way analysis of variance (ANOVA) followed by Dunnett's test using Graph pad.

P values <0.05 were considered as statistically significant condition.

Results and Discussion

Many plants derived drugs used in modern medicine are developed by ethno Medical leads and subsequent ethno pharmacological study. Plant containing flavanoids have been reported to possess a strong antioxidant properties [11]. Grape seed contain polyphenol of members of the proanthocyanidins structurally, the proanthocyanidin are a group of complex compounds made up of oligomers and polymers of poly hydroxy flavan-3-ol monomer units Several epidemiological studies have suggested that themortality rate from coronary heart disease can decreased by moderate conception of alcohol, particularly red wine [13] , proanthocyanidins found in seeds of the grapes and relatively abundant in red wine, have been thought to be the major components responsible for such epidemiological observations [14] . In the present study we explored the possible use of proanthocyandin from grape seed as a therapeutic drug in myocardial protection against Dox induced myocardial injury.

Table 1 shows the level of diagnostic marker enzyme [AST ALT, LDH, and CPK] in serum significantly increased in group III animal [Dox induced] when compared to control [group I].

The ethanolic extract of grape seed [group IV] at the dose of 200mg/kg body weight significantly [p<0.05] decreased in the enzyme level in serum when compared to DOX treated animal [groupIII].

Grape seed [group IV] at a dose of 200mg/kg body weight produced a non-significantly

decreased in the enzyme activity of [AST,ALT,LDH,CPK] and non- significantly increased activity of LDH when comparedto control animal].

Several studies reveled that generation of free radical decreased the antioxidant defense mechanism there by damage cellular constituent, glutathione (GSH), extensively found in cells, against electrophilic attacks provided by drugs such as free radicals and peroxides. GSH deficiency leads to cellular damage in kidney, liver and heart, the elevation of MDA levels, which is one of the end products of lipid per oxidation in the heart muscle tissue, and the reduction of cardiac GSH levels are important indicators in DOX-intoxicated rats. DOX is capable of generating super oxide free radicals, there by suppressed the GSH synthesis and increasing MDA levels due to per oxidation of polyunsaturated fatty acid of myocardial membranes [15]. DOX producesacute injury to the myocardial membrane which causes significant elevation of marker enzyme [AST, ALT, LDH, CPK] activities in serum could be regarded as a sign of damage to the heart muscle membrane, which suggest the event of enhanced lipid peroxidation. Several literature revealed that the elevation of lipid per oxidation lead to depletion of GSH in DOX induced rats. Leading to tissue damage and failure of antioxidant defense mechanism to prevent theformation of excessive free radicals[16-17].In present study grape seed proanthocyanidin extract prevent the leakage of marker enzymes by scavenging lipid peroxides and improve the GSH levels there by protecting integrity of membrane. The antioxidant enzymes, constituting the first line of defense mechanism to prevent and neutralize the reactive oxygen species (ROS) induced damage. This accomplished by a set of endogenous antioxidant enzymes such as (GR and GST), whose activities are dependant on the level of reduced glutathione(GSH),it as been well documented that depletion of GSH and elevation of lipid peroxide leads to decrease in antioxide enzyme DOX induced rats [18] .

Lipid consist of cholesterol (HDL and LDL cholesterol), triglyceride (neutral fat), In the presentstudies shows that DOX reduced the rate of lipolysis whereas markedly elevation of TG, HDL, LDL, levels in DOX induced cardiomyopathy, which is agreement with previous studies[18].Supplement of grape seed proanthocyanidin extract brought back near to normal lipid profile. It has been reported for its ability to scavenge the reactive oxygen species such as OH and superoxides also inhibits the lipidperoxydation [19-20] Sowe concluded that oral administration of grape seed proanthocyanidin seed have been shown to modulate the biochemical changes observed in DOX induced cardiotoxicity in animals. Flavanoids have been shown to inhibit lipid peroxidation formation in rat tissues and also inhibit the free radical production in the cells at various stages. In this context, we have reported that GSP treatment reduced the levels of heart TBARS in DOX induced lipid peroxidation. [21]

Lipids play an important role in cardiovascular disease,not only by way of hyperlipidaemia and the development of atherosclerosis, but also by modifying the composition, structure and stability of cellular membranes. Excess lipids in blood is considered to accelerate the development of arterosclerosis and are the major risk factor in myocardial infarction. High levels of circulating cholesterol and its circulation in heart tissue are well associated with cardiovascular damage[22] . An altered lipid metabolism can alter the cardiac function by changing the properties of cardiac membrane and these changes may contribute to the cell death that follows coronary artery occlusion [23]. The cardiac muscle generally utilizes fatty acid as the majorsource of energy of the total oxygen consumption: 60- 90% is utilized to oxidize fatty acid under aerobic condition. Under anoxic conditions, the cardiac muscle is not in a position to oxidize the available fatty acids, as a result of which there is an increase in the levels of these long chain fatty acyl coA derivatives [24].

DOX treated rats showed increased levels of total, free and ester cholesterol, triglycerides and acids in serumof rats. An increase in serum LDL and VLDL fraction, along with a decrease in HDL cholesterol, were also observed in DOX treated rats. These changes could beas a result of cyclic AMP's positive effect on lipid production in the heart [25]. There is a negative connection between high amounts of LDL in fractions and HDL cholesterol [26-29].

Histoarchaeological Research

Sections of hearts that had been preserved in 10% (w/v) buffered formalin were cut at 5 m in thickness, fixed in paraffin, and stained with hematoxylin and eosin. Histoarchitectural alterations were analyzed by looking at these sections using a light microscope.

Conclusion

The current work offers experimental proof that grape seed Proanthocyanidin (GSP) decides its many pharmacological actions also has high anti oxidant capabilities. This discovery may help explain why GSP protects the heart from DOX-induced myocardial damage**.**

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Table 1: Effect of Doxorubicin and GSP on the activities of cardiac marker enzyme in serum

S/ No.	PARAMETER	CONTROL	STANDARD	INDUCER[DOX]	TREATMENT
1.	AST	191.67±4.88	185±0.5	201.67±4.71	182.6±4.20
2.	ALT	28.04±2.06	20.12±1.084	76.39±7.03	20.5±1.83
3.	LDH	660.67±6.43	596±31.2	292±6.63	610.5±4.83
4.	CPK	52.12±7.035	58±3.401	185±4.070	56±8.035

Table 2: Effect of grape seed Proanthocyanidin on Gpx, GST, GR, in rat serum

GROUP	CONTROL	STANDARD	INDUCER[DOX]	TREATMENT
GPx (nmoles GSH oxidized min^{-1} mg^{-1} proten)	4.69±0.08	4.62±0.05	1.26±0.13	3.51±0.04
GST(nmoles CDNB conjugate formed min^{-1} mg^{-1} protein)	189.42±0.44	186.4±1.35	0.40±0.036	184.39±0.33
GR(nmoles NADPH oxidized min^{-1} mg^{-1} protein)	1.25±0.12	2.26±0.10	0.79±0.55	1.18±0.06

Table 2: Effect of grape seed Proanthocyanidin on Gpx, GST, GR, in rat serum

GROUP	CONTROL	STANDARD	INDUCER[DOX]	TREATMENT
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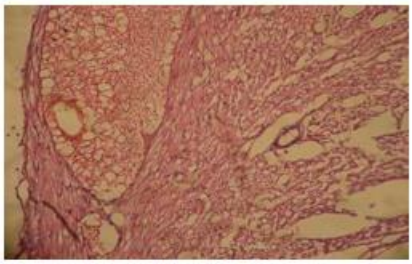
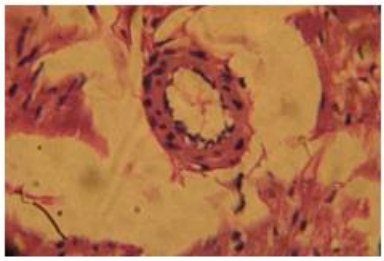
GR(nmoles NADPH oxidized min^{-1} mg^{-1} protein)	1.25±0.12	2.26±0.10	0.79±0.55	1.18±0.06

Table 3: Effect of Doxorubicin and GSP on the Lipid profile in serum

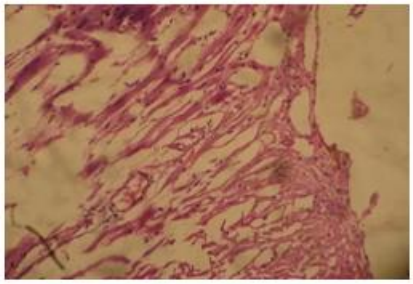
S/NO	PARAMETER	CONTROL	STANDARD	INDUCER[DOX]	TREATMENT
1.	TOTAL CHOLESTROL	94.71±8.95	135±2.01	139.36±9.97	90.15±8.31
2.	TG	105±2.073	89.9±3.45	105±2.073	96.2±1.075
3.	HDL	34.54±4.11	36.1±2.48	16.63±1.50	22.64±3.15
4.	LDL	78.41±5.22	65.6±1.96	98.60±10.62	61.31±9.11
5.	VLDL	6.54±0.84	3.53±3.20	15.14±1.26	9.64±1.87



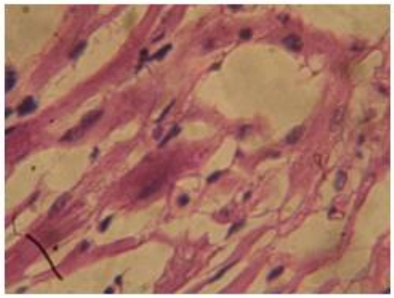
Control rats showing normal morphological appearance



DOX - treated rat showing focal fibrillar loss and cytoplasmic vacuolization



DOX – treated verapamil showing less focal fibrillar loss and cytoplasmic vacuolization



DOX – treated GSP (200 mg/kg) showing less focal fibrillar loss And sytoplasmic vacuolization