Original Article

Toxic Effects of Doxorubicin on Cardiac Dysfuction and Reversal of it by Cassia Absus

Syeda Memoona Gillani[1], Jawad Mumtaz Sodhar[1], Alina Saqib[2], Fareeha Mushtaq[2], Qaiser Jabeen[3] and Fayaz Anjum[1]

ABSTRACT

Objective: Cassia absus is a seed for prophylaxis and treatment of cardiac dysfunction induced by Doxorubicin.
Study Design: Experimental Study
Place and Duration of Study: This study was conducted at CMH Institute of Medical Sciences Bahawalpur and Islamia University Bahawalpur from September 2017 to March 2018.
Materials and Methods: 70% methanolic (V/V) extract of Cassia absus (Ca.Cr) was prepared under reduced pressure. After development of crude extract for various phytoconstituents, In-vivo pharmacological tests were performed in Wistar albino rats. Animals were divided into six groups. Normal control group, Intoxicated groups, Treatment groups; administered different doses (30, 100 and 300 mg/kg of Ca. Cralong with intoxication) and Standard group. By cardiac puncture blood of each rat was collected after 72 h of Doxorubacin-intoxication, and sera were separated for analysis of (lactate dehydrogenase (LDH), creatinine kinase (CK-MB), aspartate transaminase (AST) and alanine transaminase (ALT)).For histopathology heart/body weight ratio was calculated and hearts were analyzed.
Results: By using Anti cancer drug that is Doxorubicin significantly increased the values of serum cardiac biomarkers,become less heart/bodyweight ratios and reveal marked changes in histological findings.Treatment with cassia absus Ca.Cr resulted in significant drop in serum cardiac biomarkersp<0.05),increased heart/bodyweight ratios and histological changesalso showed significant changes.
Conclusion: Cassia absus has cadioprotective effects against Doxorubicin on analysis of Cardiac biomarkers and histopathological.

Key Words: Cardiac Dysfunction, Cardiotoxicity, Cassia absus, Doxorubicin

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INTRODUCTION

Chemotherapeutic agent; doxorubicin produce toxicity by producing free radicals in association with decrease in endogenous antioxidants (superoxide dismutase, catalases and reduced glutathione) that lead to endocardium damage on repeated or large doses exposure leading to cardiac dysfuntion such as myocardial infarction[1] and Acute myocardial infarction (AMI) is a clinical condition that results from an injury to myocardial tissue due to an imbalance between oxygen supply and demand.

[1.] Department of Pharmacology / Anatomy[2], CMH Institute of Medical Sciences, Bahawalpur.
[3.] Department of Pharmacology, Islamia University Bahawalpur.

Correspondence: Syeda Memoona Gillani, Lecturer, Department of Pharmacology, CMH Institute of Medical Sciences, Bahawalpur, Pakistan.
Contact No: 0315-4087308
Email: duaagillani@gmail.com

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The death of myocytes is generally confluent; this pattern of injury distinguishes infarction pathologically from other forms of myocardial injury, which tend to destroy myocytes more diffusely[2]. Dose associated cardiotoxicity with doxorubicin (25mg/kg i.p.) is however kept as model to evaluate protective effects of natural products because for botanists, medicinal plants are a way to produce research theories and various medicinal plants have been used for their pronounced cardioprotective effects[3] and this study links the use of tradiotionally used plant e.g. Cassia absusdue to its reported antioxidant activity[4], by using beta blocker (atenolol) as standardas it reduces myocardial workload, by decreasing heart rate and blood pressure[5].

MATERIALS AND METHODS

Phytochemical Section

Plant Collection*:* On the basis of their data of local use Cassia absus seeds were selected. Seeds were washed and cleaned from extraneous substances.

Preparation of the Ca.Cr*:* By crushing and grinding seeds were obtained. For three days 70% methanolic extract of Cassia absus (Ca.Cr)was prepared by maceration of coarsely ground seeds.

By using Heidolph rotary evaporator the filtrate was evaporated under reduced pressure. The extract Ca.Crwas obtained in semi-solid form and the percentage yield was calculated.

Ca.Cr by Phytochemical Screening: For the presence of phytoconstituents such as alkaloids , carbohydrates, fats and oils, flavonoids, glycosides, phobatannins, phenols, proteins, quinones, sapponins, tannins and terpenes,bring Phytochemical analysis of Ca.Cr.[6]

Ca.Cr's Antioxidant Activity

Scavenging Assay DPPH Radical: spectrophotometric method was studied by DPPH scavenging activity of Ca.Cr described by Marsha and Lewis[7].

Acute Toxicity Assay: OECD guidelines reveals the acute toxicity of Ca.Cr[8]

Pharmacological Section

Animals: Animals are selected on the basis of their normal LDH level. Experiments were performed in Wistar albino rats of either sex (150-250g).

Induction of Cardiotoxicity: Doxorubicin were injected for induction of Cardiotoxicity(25mg/kg, i.p.) on 12th day of study.[9]

Experimental Protocol: Six groups, each comprising of six animals categorized as;

Normal Control Group: Administered physiological saline, 5ml/ kg orally for 14 days.

Intoxicated Group: Administered physiological saline for 14 days along with intoxication.

Treatment Groups: 3 groups were pretreated with Ca.Cr, orally for 14 days along with intoxication.

Standard Group: Administered atenolol (100mg/kg, orally) for 14 days.

By the end of the experimental period post doxorubicin-injection was given and[10] animals were anesthetized with ketamine xylazine (10:1) and by the cardiac puncture blood was collected and sera were separated for estimation of cardiac biomarker enzymes. The hearts were excised, stored in 10% formalin and evaluated for various histopathological parameters[11]. Percent heart/bodyweight ratio was calculated[12].

3.3.7. Statistical Analyses: By using One-way ANOVA data was analyzed by Graph Pad Prism version 5.

RESULTS

Cardioprotective effects of Cassia absus in Dox-induced cardiac dysfunction, was evaluated in two sections; phytochemical; for the presence of antioxidants and various phytoconstituents whereas, pharmacological section was carried out in rats.

Phytochemical section

Phytochemical Analysis ofCa.Cr: Phytochemical analysis of Ca.Cr is labeled in table1.indicates the presence of cardiac glycosides in the crude extract which serves as basis for this investigation.

Antioxidant assayof Ca.Cr: Fig 1 shows the antioxidant activity and shows that the increase in concentration of Ca.Cr and ascorbic acid.

Action of the Ca.Cr on Cardiac Marker Enzymes: Decreased the cardiac marker enzymes; CK-MB, LDH, AST and ALT in a dose dependent manner comparable with the effect of atenolol taken as standard. (table 2)

Actions of the Ca.Cr on Histological Parameters: In intoxicated group heart tissues showed remarkable structural disorganization of heart muscles which was identified by the introduction of vascular edema, as shown in table 3, while the treatment groups show deviation from this disorganization.

Table No.1. Phytochemical analysis of the crude extracts of Cassia absus (Ca.Cr)

Phytochemical Constituents	Ca.Cr
Alkaloids	+
Carbohydrates	+
Fats and oils	-
Flavonoids	+
Glycosides	+
Phlobatanins	+

(-) sign shows absence and (+) sign the presence of the constituent)

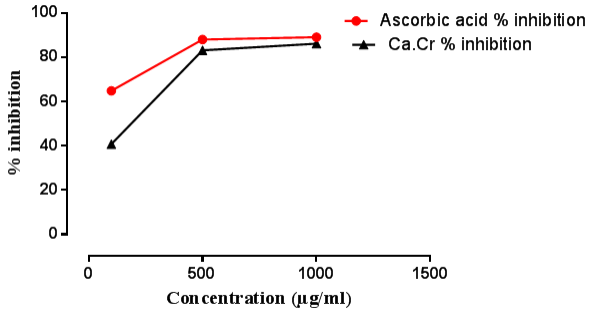

Figure No.1. Outcome of the Ascorbic acid alongwith the crude extract

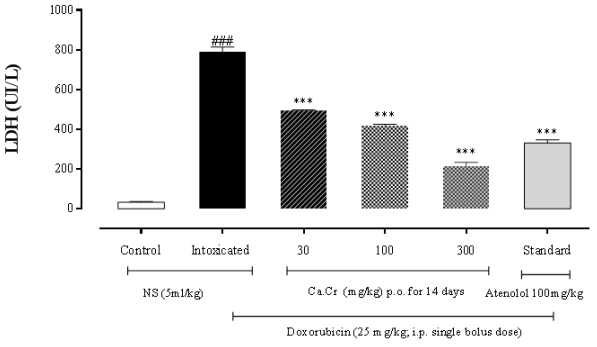

Figure No.2: The graphical representation of crude extract Cassia absus (Ca.Cr) on LDH (Lactate dehydrogenase) levels in Dox-induced cardiotoxic rats.. The intoxicated group is compared with control group ($^{###}$<0.001: highly significant) and the treatment groups (Ca.Cr+DOX) and atenolol standard group is compared with intoxicated group (ns: non-significant, * p<0.05: significant, **p<0.01: more significant and *** p<0.001: highly significant)

Table No.2: The effect of the crude extract of Cassia absus (Ca.Cr) on serum cardiac biomarker levels; CK-MB, LDH,AST, ALT and AST/ALT ratio.

		CK-MB (IU/l)	LDH (IU/l)	SGOT(AST) (IU/l)	SGPT(ALT) (IU/l)	AST/ALT
Control (N/S 5ml/kg)		65.39±16.20	33.09±3.45	24.23±5.422	54.56±4.434	0.444
Intoxicated (DOX 25mg/kg)		719.9±27.71$^{###}$	790.9±23.28$^{##}$	240.6±5.127$^{###}$	128.1±24.94$^{#}$	1.87
Treatment+ DOX	Ca.Cr (30mg/kg)	383.8±4.344***	493.7±4.09****	140.4±3.420***	147.0±11.12^{ns}	0.955
	Ca.Cr (100mg/kg)	300.4±31.90**	417.3±8.16***	113.1±4.751***	105.8±3.198^{ns}	1.06
	Ca.Cr (300mg/kg)	244.8±29.68***	215.7±18.24***	102.4±2.226***	40.39±6.817*	2.53
Standard + DOX	Atenolol (100mg/kg)	412.7±17.86***	330.4±16.35***	110.5±2.350***	44.17±5.029	2.50

The values are expressed as mean ± SEM of six animals in each group. The results of each group is compared, using one way ANOVA, with control group ($^{###}$ p<0.001) and with intoxicated group (*p<0.05, ** p<0.01 and*** p<0.001)

Table No.3: The effect of the crude extract of Cassia absus (Ca.Cr) on % change in heart weight/body weight ratio in doxorubicin-induced cardiotoxicity

		Body Weight	Heart Weight	Heart/Body Weight Ratio
Control (N/S 5ml/kg)		200±23.45	0.678±0.074	0.339
Intoxicated (DOX 25mg/kg)		158±5.750	0.578±0.031	0.376
Treatment+ DOX	Ca.Cr (30mg/kg)	137.7±3.84	0.518±0.019	0.376
	Ca.Cr (100mg/kg)	150.5±4.34	0.582±0.028	0.386
	Ca.Cr (300mg/kg)	120.8±2.07	0.473±0.022	0.391*
Standard+DOX	Atenolol (100mg/kg)	171.7±6.87	0.639±0.016	0.372

The results are expressed as mean and SEM of six animals in each group.

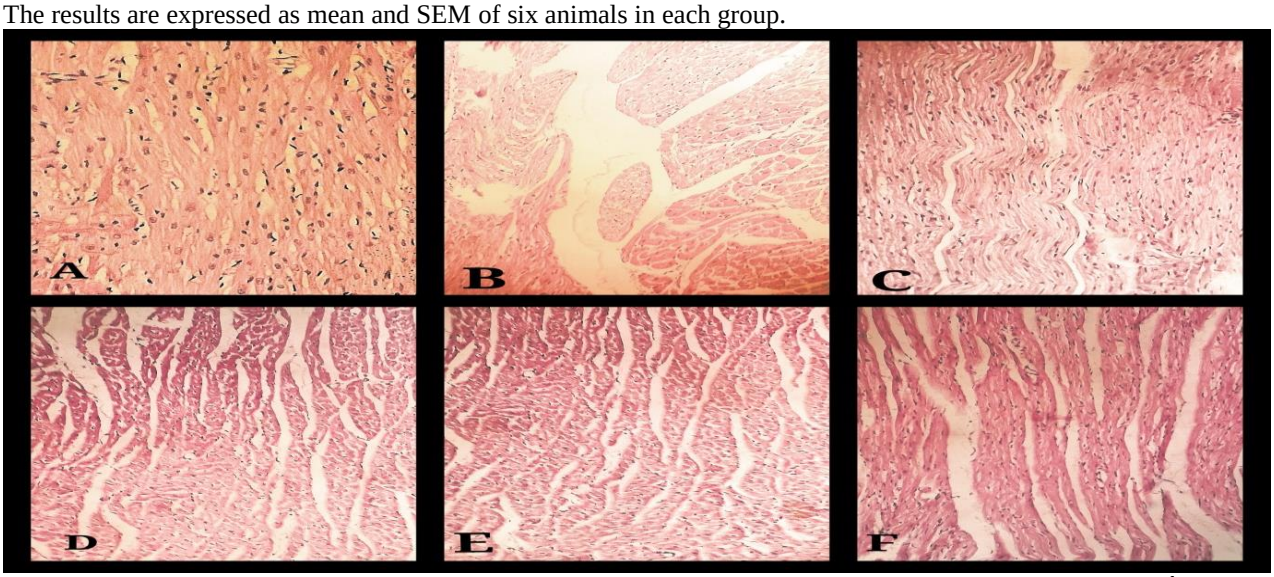

Figure No. 3: Histopathological changes in cardiac tissue; (A) control, (B) intoxicated (DOX 25mg/kg once at 12th day of pretreatment) (C) Atenolol (100mg/kg)+ intoxication (D) Ca.Cr (30 mg/kg)+intoxication, (E) Ca.Cr (100 mg/kg)+intoxication and (F) Ca.Cr (300 mg/kg)+intoxication in DOX-induced myocardial infarction in rats.

DISCUSSION

We conducted this study at CMH Institute of Medical Sciences Bahawalpur and Islamia University Bahawalpur to determine the Cardioprotective effects of Cassia absus against Doxorubicin that is anti cancer drug. Heart is a muscular organthatpumps blood via blood vessels of the circulatory system.Function of heart is supply the blood to the body with oxygen and nutrients and dismassal of metabolic wastes.

Cassia absus has the cardioprotective effects. The alkaloids, steroids, saponins, and flavonoids are present in different fractions of the seeds by chromatography of Cassia absus[13]. Cassia absus have antioxidant effect that combat oxidative lesions in cardiac diseases by the presence of phytoconstituents[14]. Prophylactic

administration of Cassia absus is because of compensatory counteraction of free radicals, which lowers the incidence of leaking and production of marker enzymes during cardiac injury, leading to decrease in cardiac workload.

Doxorubicin is anticancer drug that induces biochemical changes, as well as structural damage to myocytes[15]. However, histopathology of cardiac tissues according to other studies show that the cardiac tissues in normal animals are uniform in size and shape with no cellular infiltration of nectrotic cells is reported[15] while present study shows, dose dependent reduction in the cardiac lesions upon administration of Ca.Cr.Present study confirms dose dependent increase in enzymatic levels; i.e. CK-MB and LDH in intoxicated group while treatment groups show decreased levels of enzymes which can be assumed due to anti-scavenging effect of plants that detoxifies the free radicals produced in heart, leading to altered lipid peroxidation and myocardial necrosis. Present study also shows that superoxide radicals and hydroxyl radicals in the cardiomyocytes by administration of DOXinitiate cellular necrosis making cell membrane more permeable and cause leakage of enzymesthus a potent antioxidant[16].

It is evident from various studies that the levels of AST and ALT increase significantly as compared to normal animalsdue to DOX-intoxication. The ratio AST/ALT greater than 1 is considered as parameter to indicate cardiac injury upon exposure to larger doses of DOX[16]. Present study revels that ALT levels also have same pattern of increase in the intoxicated group and same effectiveness as that of Ca.Cr due to cardiotoxicity.

CONCLUSION

Cassia absus has cadioprotective effects against Doxorubicin on analysis ofCardiac biomarkers and histopathological parameters, when administered prophylactically via mechanism of antioxidant scavenging action. It has been proved that using natural products to prevent DOX-induced myocardial dysfunction.

Author's Contribution:

Concept & Design of Study:	Syeda Memoona Gillani
Drafting:	Jawad Mumtaz Sodhar, Alina Saqib
Data Analysis:	Fareeha Mushtaq, Qaiser Jabeen, Fayaz Anjum
Revisiting Critically:	Syeda Memoona Gillani, Jawad Mumtaz Sodhar
Final Approval of version:	Syeda Memoona Gillani

Conflict of Interest: The study has no conflict of interest to declare by any author.

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