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Original Article

GCMS/MS ANALYSIS AND CARDIOPROTECTIVE POTENTIAL OF *CUCUMIS CALLOSUS* ON DOXORUBICIN INDUCED CARDIOTOXICITY IN RATS

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ABSTRACT

Objective: The present investigations were undertaken to identify phytochemicals by GC MS/MS, to evaluate the cardioprotective and antioxidant activity of the ethanolic extract of leaves of *Cucumis Callosus* against DOX-induced cardiotoxicity in rats.

Methods: GC MS/MS method was carried out for identification of phyto-compounds present in the *Cucumis Callosus*. The Cardioprotective effect of *Cucumis Callosus* (CC) was determined by using Doxorubicin (DOX) intoxication of rats as experimental models. The extent of heart damage and effect of the plant extract was assessed by various biochemical parameters like alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total cholesterol (TC) and triglycerides (TGs) in blood serum and concentration of malondialdehyde (MDA), reduced glutathione (GSH), superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione-s-transferase (GST) in heart tissue were determined.

Results: In the *Cucumis Callosus extract* 32 compounds were identified by GC–MS/MS. Doxorubicin is used to bring significant changes in biochemical parameters and antioxidants in the heart. The pretreatment with *Cucumis Callosus* at two doses (250 mg/kg and 500 mg/kg) to DOX treated rats significantly prevented the altered enzymes SGPT, SGOT, CPK and LDH, lipid profile LDL, VLDL, TGs, HDL, TC and antioxidant SOD, GSH, CAT, GSH-Px and MDA to near normal level. Serum urea, uric acid, and ALP which are increased on DOX administration registered near normal values on pretreatment with *Cucumis Callosus*.

Conclusion: This study showed that the cardioprotective and antioxidant activity of *Cucumis Callosus*, therefore scientifically rampart the use of this plant in traditional medicine for treatment of heart diseases.

Keywords: Cardioprotection, *Cucumis Callosus,* Doxorubicin, Lipoprotein, Antioxidant

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INTRODUCTION

Myocardial infarction (MI) is characterized by necrosis of a portion of the heart muscle. It is one of the most frequent causes of death in the developing country. Coronary atherosclerosis has been frequently an underlying factor in the pathogenesis of MI. The acute event is often provoked by rupture of an atherosclerotic plaque, leading to the formation of an occlusive thrombus and vasospasm, which hinder the delivery of oxygen to the myocardial tissue Supplied by that artery [1].

Doxorubicin (DOX) is an anthracycline antibiotic that is rooted as a chemotherapeutic agent. The administration of DOX is known to induce numerous cardiotoxic effects, including transient arrhythmias, nonspecific electrocardiographic abnormalities, pericarditis, and acute heart failure [2, 3]. Increased level of low density lipoproteins (LDL) [4] and serum total cholesterol [5] and decreased levels of high density lipoprotein (HDL) [6] are associated with increased risk of MI. DOX-induced cardiotoxicity in rat was associated with increased lipid peroxide levels in the myocardium [7]. Oxidative stress refined by free radicals or reactive oxygen species (ROS), as evidenced by a marked increase in production of lipid peroxidative products and transient inhibition of endogenous antioxidant defense, such as superoxide dismutase (SOD), catalase (CAT) and reduced glutathione (GSH) has been shown to incline the myocardial damage during MI [8].

A number of herbs are conventionally used in different countries during drug or toxin induced hepatic, renal, and cardiac disorders [9]. *Cucumis callosus* (Rottle.) Cong. is commonly known as Bitter Cucumber belongs to the Cucurbitaceae family. It grows in desert regions. In India it mainly grows in dry districts. It is reported to be light, bitter, hot abortifacient, purgative, blood purifier and cathartic [10]. The fruit is useful in biliousness, jaundice, cerebral congestion, colic, constipation, dropsy, fever, worms and sciatica [11, 12]. Root is given in cases of abdominal enlargement, cough, asthma, and inflammation of the breast, ulcers, urinary diseases and rheumatism [13]. Oil from seeds is used for poisonous bits, bowel complaints, epilepsy and also for blackening the hair [14]. In India, ripe fruit eaten raw and used in curries, green fruit used as a vegetable, dried fruit rind and seeds used in curries.

Previous phytochemical investigations on this plant resulted in the discovery of the presence of flavonoids, alkaloids, steroids terpenoids and derivatives [15], a previous study reported the presence of alkaloids, Wilfortrine and Wilforine have been reported to possess immunosuppresive effects and cardioprotective activity [16]. Wilfortrine can inhibit leukemia cell growth in mice [17,18] and show anti-HIV activity [19].

Previous studies reported that the presence of alkaloids in the *Cucumis callosus extract which have cardioprotective activity,* however, no scientific claims are available on the cardioprotective activities of *Cucumis callosus*. Therefore, this study was designed to investigate the cardioprotective activity of *Cucumis callosus* extract against DOX-induced cardiotoxicity in rats.

MATERIALS AND METHODS

Plant material

Leaves of *Cucumis callosus* were collected, identified and authenticated by a Botanist, Dr. K. Shanthi, goverrntment arts college, Tiruvannamalai. Voucher specimen (KPCP-10/2016) was retained in the, PG Department of Pharmaceutical chemistry, Kamalakshi Pandurangan College of Pharmacy, Tiruvannamalai, Tamil Nadu, India.

Chemicals and reagents

Doxorubicin was purchased from Microlabs, Tamilnadu. India. ALT, AST, ALP, CPK kit were procured form span Diagnostics, Surat, India.

All other chemicals and solvent were of analytical grade and commercially available.

Acute toxicity studies

Acute oral toxicity study was performed as per OECD-423 guidelines (acute toxic class method), Albino rats (n=6) of single sex were selected for the acute toxicity study. Which received a single oral dose of 2000 mg/kg body weight of ethanol extract of combretum albidum. The dose was administered to overnight fasted rats and food was withheld fora further3-hours after administration of the drug and observed for signs of toxicity for a period of 14 d.

Extraction

Leaves (1000 g) of *Cucumis callosus* were cleaned with water and dried, then they were powdered using a mechanical grinder to obtain a coarse powder. The coarse powder (500 g) was passed through 40 mesh sieve and extracted with ethanol (90/10 v/v) in a Soxhlet apparatus at 25 °C. The extract was freeze-dried and stored in a vacuum desiccator and the yield was 7 g.

GC–MS/MS analyses of ethanol extract of *Cucumis callosus* for the determination of chemical composition

GC programme

Column BR-5MS (5% Diphenyl/95% Dimethyl poly siloxane), 30m x 0.25 mm ID x 0.25 mm df

Equipment Scion 436-GC Bruker,Carrier gas 1 ml per min, Split 10:1,Detector TQ Quadrupole Mass Spectrometer,Software MS Work Station 8,Sample injected 2 ml.

Oven temperature programme

110 °C hold for 3.50 min,Up to 200 °C at the rate of 10 °C/min-No hold,Up to 280 °C at the rate of 5 °C/min-9 min hold,Injector temperature 280 °C,Total GC running time: 37.50 min,

MS programme

Library used NIST Version-11,Inlet line temperature 290 °C, Source temperature 250 °C, Electron energy 70 eV, Mass scan (m/z) 50-500 amu,Solvent Delay 0-3.5 min,Total MS running time: 37.50 min

The identification of the constituents of the ethanolic extract of *Cucumis callosus* was performed using a mass spectrometer (Agilent 6890/Hewlett–Packard 5975) fitted with an electron impact (EI) ion source. The ethanolic extract (2.0 µl) of *Cucumis callosus* was injected manually in the split mode with a Hamilton syringe to the GC–MS for total ion chromatographic analysis. For quantitative analysis, the selected ion monitoring (SIM) mode was employed.

Experimental animals

Studies were carried out using Wistar male albino rats (150–200 g), obtained from the Institute of Veterinary Preventive Medicine (IVPM), Ranipet, and Tamilnadu, India. The animals were housed in polyacrylic cages (38 cm, 23 cm, and 10 cm) and maintained under standard laboratory conditions with dark/light cycle (12/12 h). The animals were fed with standard pellet diet (supplied by the poultry research station, Nandhanam, India) and fresh water *ad libitum*. All the animals were acclimatized to lab conditions for a week before commencement of the experiment. All animal studies were performed in accordance to guidelines of CPCSEA and Institutional Animal Ethical Committee (IAEC) of Kamalakshi Pandurangan college of Pharmacy, Tiruvannamalai (Tamilnadu). CPCSEA registration number was 745/03/ac/CPCSEA and all the procedures were followed as per rules and regulation.

Induction of experimental myocardial infarction

Doxorubicin was dissolved in sterile double distilled water and injected subcutaneously into rats

(20 mg/kg) in group II, IV and V respectively, after the last dose of the extract to induce Cardiotoxicity.

Experimental procedure

Group 1: (Normal). Saline (0.75 ml/animal), orally for 14 d.

Group 2: (Drug control). Saline (0.75 ml/animal)+DOX 20 mg/kg, single intraperitoneal injection after 14th day.

Group 3: (Extract control). *Cucumis callosus* (500 mg/kg), orally for 14 d.

Group 4: *Cucumis callosus* (250 mg/kg), orally for 14 d+DOX (20 mg/kg) single intraperitoneal injection after 14th day

Group 5: *Cucumis callosus* (500 mg/kg), orally for 14 d+DOX (20 mg/kg) single intraperitoneal injection after 14th day

Induction of experimental myocardial infarction

Doxorubicin was dissolved in sterile double distilled water and injected subcutaneously into rats (20 mg/kg) in group II, IV and V respectively, after the last dose of the extract to induce Cardiotoxicity.

Isolation of working heart preparation

The animals were anesthetized with chloroform after 72 h of DOX administration, and then heart was punctured with a sterile syringe and blood was stored with EDTA, which is an anticoagulant agent and was excised out. Cardiac muscle from the lower third of the ventricle was collected and stored in liquid nitrogen for antioxidant studies.

Biochemical analysis

Blood samples were collected into tubes pre-coated with EDTA by vein puncture at baseline and post intervention. Serum was separated by centrifuging for 10 min 3000×*g* at 4 °C. The serum used for the assay of urea, and uric acid which were being estimated by the methods of Natelson *et al.* [20] and Caraway *et al.* [21] respectively. The activities of serum glutamate-pyruvate transaminase (SGPT) and serum glutamate oxaloacetate transaminase (SGOT) were determined spectrophotometrically by the method of Mohun and Cook [22]. The lactate dehydrogenase (LDH), creatine phosphokinase (CPK) and alkaline phosphatase (ALP) were determined by the methods of King [23]. The levels of total cholesterol and triglycerides (TGs) were estimated by the methods of Zlatkis *et al.* [24], Foster and Dunn [25]. Serum high density lipoprotein (HDL) was determined according to the method of Wilson and Spiger [26]. Serum low density lipoproteins (LDL) and very low density lipoproteins (VLDL) were calculated as VLDL=triglycerides/5 and LDL = total cholesterol – (HDL cholesterol+VLDL cholesterol) respectively.

Antioxidant assay

The heart was dissected, immediately washed in ice-cold saline and a homogenate was prepared in 0.1 Mol L^{-1} Tris-HCl buffer (pH 7.4). The homogenate was centrifuged and the supernatant was used for the assay of antioxidant parameters. MDA content assayed by adding tissue homogenate to TBA aqueous solution and incubated, and then the MDA content was measured according to the method of Zhang *et al.* [27]. Superoxide dismutase (SOD) activity assayed by adding NADH and incubated, and then SOD activity was measured according to the method of Rai *et al.* [28]. CAT activity was determined from the rate of decomposition of H_2O_2 according to Bergmeyer, Gowehn, and Grassel [29]. Glutathione peroxidase (GSH-Px) activity was determined by measuring the decrease in GSH content after incubating the sample in the presence of H_2O_2 and NaN_3 according to Hafemann *et al.* [30]. GSH reductase activity was assayed according to Carlberg and Mannervik [31] and Mohandas *et al.* [32]. Bergmeyer *et al.* [29]. Glutathione peroxidase (GSH-Px) activity was determined by measuring the decrease in GSH content after incubating the sample in the presence of H_2O_2 and NaN3 according to Hafemann *et al.* [30]. GSH reductase activity was assayed according to Carlberg and Mannervik [31].

Statistical analysis

The obtained results were analyzed for statistical significance using one-way ANOVA followed by Dunnet test using graphpad prism software statistical software for comparison with control group and DOX-treated group. $P<0.05$ was considered as significant.

RESULTS

Acute toxicity

It was observed that the administration of single oral dose 2000 mg/kg/body weight of ethanol extract of *Cucumis callosus* to a rat, didn't induce drug-related toxicity and mortality in the animals, and it was safe up to the dose of 2000 mg/kg/body weight.

GC-MS/MS analysis

The ethanolic extract of *Cucumis callosus* is a complex mixture of many constituents, and 32 compounds were identified in this plant by GC–MS/MS (fig. 1, table 1). Glycerin, Hexadecanoic acid, methyl ester, Palmitoleic acid, n-Hexadecanoic acid, 9,12-Octadecadienoic acid (Z,Z)-, Oleic Acid, cis-13-Octadecenoic acid, Octadecanoic acid, 3,4-Dihydroisoquinoline, 1-[3-methoxybenzyl]-6-methoxy-,4H-Dibenzo[de,g]quinoline, 5,6,6a,7-tetrahydro-10,11-dimethoxy-6-methyl-, (R)-, 3,4-Dihydroisoquinoline, 1-[3-methoxybenzyl]-6-methoxy-,2,3,9,10-Tetrahydro-1,8-dioxa-7,12-diazadicyclopenta (b,j)phenanthrene, Furo (3,4-e)-1,3-benzodioxol-8(6H)-one, 6-(1,2,3,4-tetrahydro-6,7-dimethoxy-2-methyl-1-isoquinolinyl)-,(R-(R*,S*))-, Campesterol, and Stigmasterol were identified in the ethanol extract of *Cucumis callosus* by relating to the corresponding peak area through coupled GC–MS. Most of the phytochemical compounds possess medicinal properties (eg. Antioxidant, antimicrobial, antitumor, hepatoprotective, hypocholesterolemic, and anti-inflammatory properties, as identified by Dr. Duke's Phytochemical and Ethnobotanical Databases).

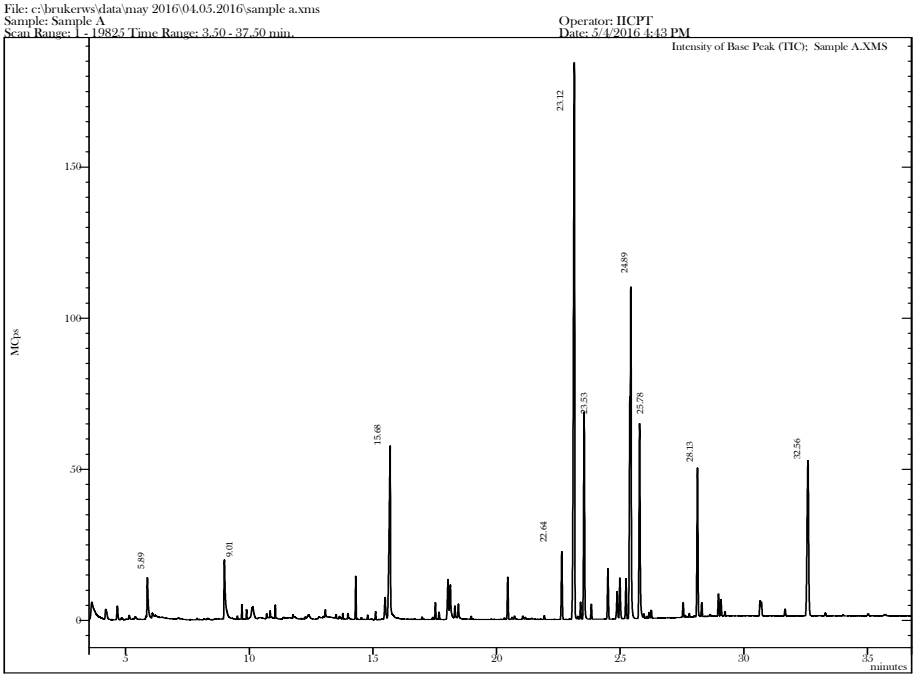

Fig. 1: The chromatogram showing n-Hexadecanoic acid (9.36), 4H-ibenzo[de,g]quinoline, 5,6,6a,7-tetrahydro-10,11-dimethoxy-6-methyl-, (R)-(30.82), and Dodecane, 1-cyclopentyl-4-(3-cyclopentylpropyl)-(7.37) peaks detected by GC MS/MS

Table 1: Phytocomponents identified in *Cucumis Callosus* (GC–MS/MS study)

S. No.	RT	Name of the compound	Molecular formulae	MW	Peak area %	Compound nature	**Activity
1.	3.68	p-Cresol	C7H8O	108	0.82	Phenolic compound	Antimicrobial, Anti-inflammatory Antioxidant, Analgesic
2.	4.19	Phenylethyl Alcohol	C8H10O	122	0.25	Aromatic compound	Ingredient in flavors and perfumery
3.	4.66	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	C6H8O4	144	0.27	Flavonoid fraction	Antimicrobial, Anti-inflammatory
4.	5.89	5-Hydroxymethylfurfural	C6H6O3	126	0.68	Aldehyde compound	Antimicrobial, Anti-inflammatory
5.	9.01	Benzeneethanol, 4-hydroxy-	C8H10O2	138	0.18	Phenolic compound	Antimicrobial, Anti-inflammatory Antioxidant, Analgesic
6.	9.70	Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl-	C15H22	202	0.39	Aromatic compound	No activity reported
7.	10.15	Lactose	C12H22O11	342	0.90	Sugar moiety	Preservative
8.	10.69	Dodecanoic acid	C12H24O2	200	0.22	Lauric acid	Arachidonic acid inhibitor, Urine acidifier
9.	13.07	Tetradecanoic acid	C14H28O2	228	0.33	Myristic acid	Antioxidant, Cancer preventive, Cosmetic, Hypercholesterolemic Lubricant, Nematicide
10.	13.99	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	C20H40O	296	0.44	Terpene alcohol	Antimicrobial, Anti-inflammatory
11.	15.10	Hexadecanoic acid, methyl ester	C17H34O2	270	0.15	Palmitic acid ester	Antioxidant, Hypocholesterolemic Nematicide, Pesticide, Lubricant

							Antiandrogenic, Flavor, Hemolytic 9.5-Alpha reductase inhibitor
12.	15.49	Palmitoleic acid	C16H30O2	254	1.34	Palmitoleic acid	No activity reported
13.	15.68	n-Hexadecanoic acid	C16H32O2	256	9.36	Palmitic acid	Antioxidant, Hypocholesterolemic Nematicide, Pesticide, Lubricant Antiandrogenic, Flavor, Hemolytic 9.5-Alpha reductase inhibitor
14.	18.03	9,12-Octadecadienoic acid (Z,Z)-	C18H32O2	280	1.89	Linoleic acid	Anti-inflammatory, Hypocholesterolemic Cancer preventive, Hepatoprotective, Nematicide Insectifuge, Antihistaminic Antieczemic, Antiacne, 5-Alpha reductase inhibitor Antiandrogenic, Antiarthritic, Anticoronary, Insectifuge
15.	18.12	Oleic Acid	C18H34O2	282	3.44	Oleic Acid	Cancer preventive, Flavor, Hypocholesterolemic, 5-Alpha reductase inhibitor, Antiandrogenic, Perfumery, Insectifuge, Anti-inflammatory Anemiagenic, Dermatitigenic Choleretic
16.	18.32	cis-13-Octadecenoic acid	C18H34O2	282	1.00	Unsaturated fatty acid	No activity reported
17.	18.46	Octadecanoic acid	C18H36O2	284	1.02	Stearic acid	No activity reported
18.	22.64	3,4-Dihydroisoquinoline, 1-[3-methoxybenzyl]-6-methoxy-	C18H19NO2	281	3.17	Alkaloid	Antimicrobial, Anti-inflammatory
19.	23.12	4H-Dibenzo[de,g]quinoline, 5,6,6a,7-tetrahydro-10,11-dimethoxy-6-methyl-, (R)-	C19H21NO2	295	30.82	Alkaloid	Antimicrobial, Anti-inflammatory
20.	23.53	3,4-Dihydroisoquinoline, 1-[3-methoxybenzyl]-6-methoxy-	C18H19NO2	281	5.83	Alkaloid	Antimicrobial, Anti-inflammatory
21.	24.49	(+)-Roemerine	C18H17NO2	279	1.54	Alkaloid	Cytotoxic Antimicrobial, Anti-inflammatory
22.	24.89	2,3,9,10-Tetrahydro-1,8-dioxa-7,12-diazadicyclopenta(b,j)phenanthrene	C16H12N2O2	264	0.81	Polyaromatic compound	No activity reported
23.	25.24	Spiro[2,5-cyclohexadiene-1,7'(1'H)-cyclopent [ij]isoquinolin]-4-one, 2',3',8',8'a-tetrahydro-5',6'-dimethoxy-1'-methyl-, (R)-	C19H21NO3	311	2.35	Alkaloid	Antimicrobial Anti-inflammatory
24.	25.42	Furo(3,4-e)-1,3-benzodioxol-8(6H)-one, 6-(1,2,3,4-tetrahydro-6,7-dimethoxy-2-methyl-1-isoquinolinyl)-,(R-(R*,S*))-	C21H21NO6	383	7.06	Alkaloid	Antimicrobial Anti-inflammatory
25.	25.78	(-)-1,2,3,4-Tetrahydroisoquinolin-6-ol-1-carboxylic acid, 7-methoxy-1-methyl-, methyl ester	C13H17NO4	251	3.06	Alkaloid	Antimicrobial Anti-inflammatory
26.	28.13	2-(p-Methoxyphenyl)-8-methyl-8H-thieno(2,3-b)indole	C18H15NOS	293	5.37	Alkaloid	Antimicrobial Anti-inflammatory
27.	28.99	Condyfolan-16-carboxylic acid, 2,14,16,19-tetradehydro-, methyl ester, (14E)-	C20H22N2O2	322	1.79	Nitrogen compound	Antimicrobial
28.	30.69	1,3,5-Cyclohexanetrione, tris (dimethylaminomethylidene)-	C15H21N3O3	291	2.45	Nitrogen compound	Antimicrobial
29.	31.65	Stigmastan-3-ol, 5-chloro-, acetate, (3β,5α)-	C31H53ClO2	492	1.89	Steroid	Antimicrobial, Anti-inflammatory Anticancer, Antiasthma, Hepatoprotective, Diuretic
30.	32.56	Dodecane, 1-cyclopentyl-4-(3-cyclopentylpropyl)-	C25H48	348	7.37	Hydrocarbon	No activity reported
31.	35.01	Campesterol	C28H48O	400	2.54	Steroid	Antimicrobial, Anti-inflammatory Anticancer, Antiasthma, Hepatoprotective, Diuretic
32.	35.70	Stigmasterol	C29H48O	412	1.28	Steroid	Antioxidant Anti-inflammatory Sedative Antihepatotoxic Caner-preventive Antiviral Ovulant Hypocholesterolemic Estrogenic Artemicide

**Source: Dr. Duke's Phytochemical and Ethnobotanical Databases

Biochemical parameters

The concentration of serum urea, uric acid, and alkaline phosphate was significantly increased in DOX-treated animals (group II) compared to normal animals (group I). Treatment with *Cucumis callosus* extract the levels of serum urea, uric acid, and alkaline phosphatase concentrations was decreased significantly (group IV and group V) compared with group II. (fig. 2).

The marker enzyme SGOT, SGPT, CPK, LDH were increased significantly in Group II compared with Group I (fig. 3 and 4). *Cucumis callosus* extract 250 and 500 mg/kg body weight (Group IV and V) treated animals marker enzymes levels were significantly reduced when compared with DOX-only treated group (group II).

The significant increase in levels of serum cholesterol, TGs, LDL and VLDL and decreased level in HDL were observed in DOX-treated rats when compared to the normal rats (group II). Pretreatment with *Cucumis callosus* (250 and 500 mg kg^{-1} d^{-1} for 14 d) to DOX-treated rats significantly altered the level of serum cholesterol, TGs, LDL and VLDL (fig. 5 and 6) and increased the serum HDL concentration when compared to normal rats.

SOD, CAT, GSH-PX and GSH levels in DOX-induced rats (group II) were significantly decreased (fig. 7) when compared to normal rats (group I,). But the Cucumis callosus extract (250 and 500 mg/kg/day) counteracted dose-dependent manner and the detrimental effect of DOX by increasing the content of antioxidants. Administration of *Cucumis callosus* alone (group III) did not show significant changes in antioxidants when compared to normal rats (group I).

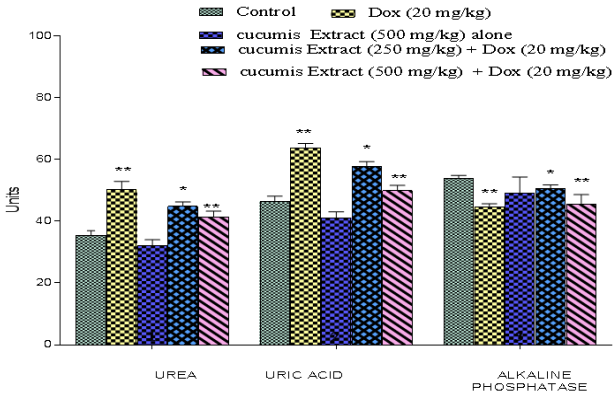

Fig. 2: Effect of ethanolic extract of cucumis callosus on urea (mg/l), uric acid (mg/l) and alkaline phosphatase (mg/l) in DOX intoxicated rats. Values are mean±SD (n=6) *P<0.01, **P<0.05 respectively

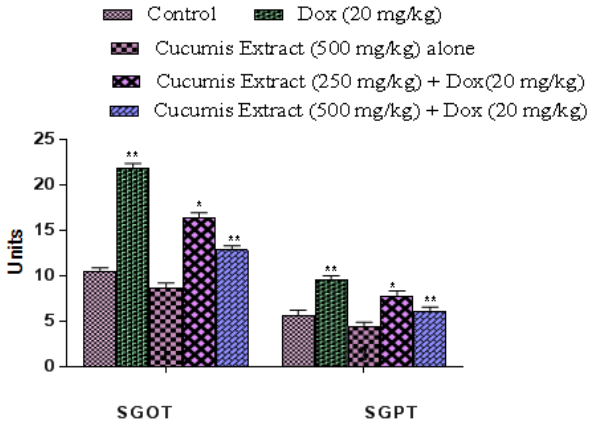

Fig. 3: Effect of ethanolic extract of cucumis callosus on SGOT (IU L^{-1}) and SGPT (IU L^{-1}) in DOX intoxicated rats. Values are mean±SD (n=6) *P<0.01, **P<0.05 respectively

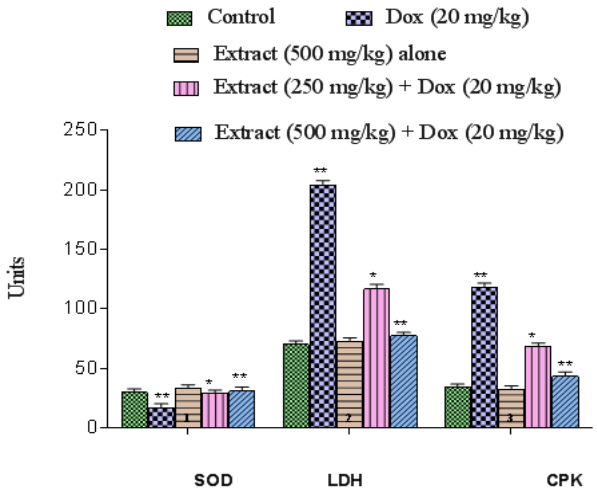

Fig. 4: Effect of ethanolic extract of cucumis callosus on SOD (U mg $protein^{-1}$), LDH (IU L^{-1}) and CPK (IU L^{-1}) in DOX intoxicated rats. Values are mean±SD (n=6) *P<0.01, **P<0.05 respectively

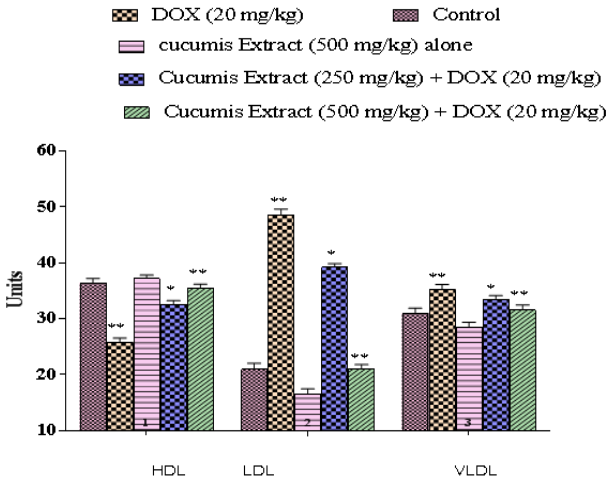

Fig. 5: Effect of ethanolic extract Cucumis on serum HDL (mg/l), LDL (mg/l), and VLDL (mg/l) in DOX intoxicated rats. Values are mean±SD (n=6) *P<0.01, **P<0.05 respectively

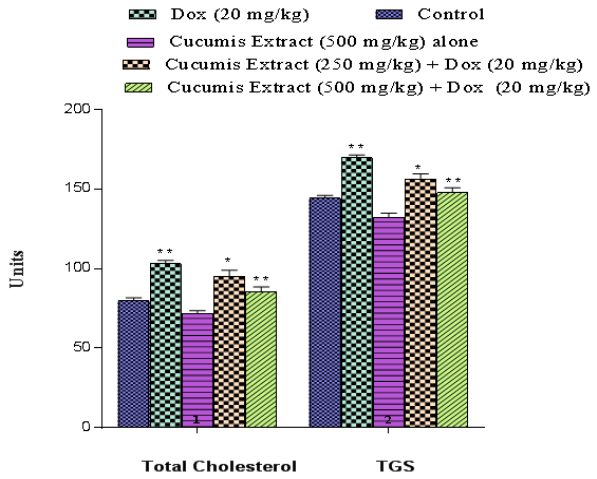

Fig. 6: Effect of ethanolic extract Cucumis on serum TGs (mg/l) and total cholesterol (mg/l) in DOX intoxicated rats. Values are mean±SD (n=6) *P<0.01, **P<0.05 respectively

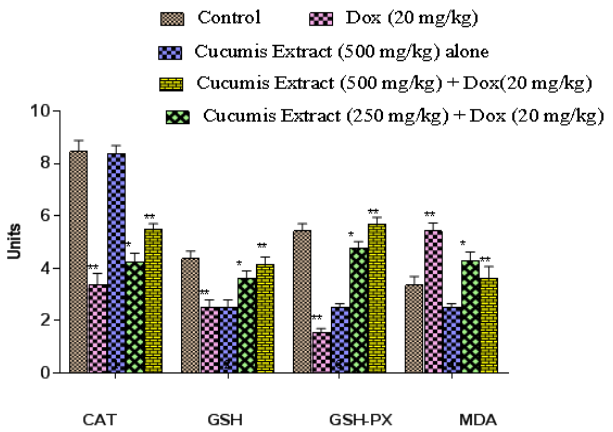

Fig. 7: Effect of ethanolic extract cucumis callosus on CAT (µmol of H2O2 consumed min^{-1} mg protein^{-1}), GSH (mM gram tissue^{-1}), GSH-Px (µg of GSH oxidized min^{-1} mg protein^{-1}), and MDA (nmol g tissue^{-1}) in DOX intoxicated rats. Values are mean±SD (n=6) *P<0.01, **P<0.05 respectively

DISCUSSION

This study entails the cardioprotective activity of ethanolic extract of *Cucumis callosus* against DOX-induced cardiotoxicity. The cardioprotective activity was proved by determining the level of serum marker enzymes (SGPT, SGOT, CPK and LDH) which are well known markers of myocardial infarction, When myocardial cells are damaged or destroyed due to deficient oxygen supply or glucose, the cardiac membrane becomes permeable or may rupture which results in leakage of enzymes.

These enzymes enter into the blood stream,thus increasing their concentration in the serum, it was showed, that oxygen-free radicals generated during Doxorubicin redox cycling are responsible for the damage that doxorubicin causes to the heart [33]. The level of these enzymes was decreased in the *Cucumis callosus* extract treated rats.

The cardioprotective activity of the *Cucumis callosus* was also proved by increased antioxidant enzyme activity and decreased lipid peroxidation. The existing experimental evidence suggests that doxorubicin oxidative stress is due to the generation of free radicals in the heart tissue [34]. The generated reactive oxygen species such as superoxide radicals and hydroxyl radicals are potential to cause damage to various intracellular components. Heart tissue is particularly susceptible to free-radical injury because it contains low levels of free-radical detoxifying enzymes/molecules like SOD, GSH and CAT. Further, doxorubicin also has high affinity for the phospholipid component of the mitochondrial membrane in the cardiac myocyte, leading to accumulation of doxorubicin in the heart tissue [35].

In our study the SOD activity was significantly decreased in DOX-treated animals and increase in the SOD activity was observed in *Cucumis callosus* pretreated animals in a dose-dependent manner. However, it has been reported that a rise in SOD activity, without a concomitant rise in the activity of catalase/GSH, might be detrimental [36] because the SOD activity increases the generation of hydrogen peroxide which is cytotoxic and needs to be scavenged by catalase/GSH. Thus a simultaneous increase in catalase/GSH activity is essential for an overall beneficial effect of an increase in SOD activity [37]. The oxidative stress and tissue injury caused by DOX was inhibited by the increased activity of GSH, SOD and catalase, following treatment of *Cucumis callosus.* The increase in catalase activity in DOX-treated animals supports the above hypothesis that this increase is possibly required to overcome excessive oxidative stress [38].

The increase in the level of plasma triglycerides, total cholesterol and low-density lipoproteins in the doxorubicin-treated group indicates doxorubicin may be interfering with metabolism or biosynthesis of lipids. Pretreatment with Cucumis callosus showed a reduction in blood lipid profile levels with a concomitantly increase in HDL cholesterol was observed. Decrease in the blood lipid profiles and increase in HDL cholesterol in Cucumis callosus treated group may be due to the presence of the synergetic effect of chemical compounds present in the extract.

Lipid-lowering effect of *Cucumis callosus* extract is due to inhibition of hepatic cholesterol biosynthesis, increased fecal bile acid secretion and stimulation of receptor-mediated catabolism of LDL cholesterol and increase in the uptake of LDL from the blood by liver [39].

Heart tissue injury induced by doxorubicin in rats was indicated by elevated levels of the marker enzymes such as serum LDH and CPK [40]. The increase of the LDH level in serum and extracellular fluid suggests an increased leakage of this enzyme from mitochondria as a result of toxicity induced by treatment with doxorubicin. This index has been recently used in other studies to test for cardiotoxicity [41].

Cucumis callosus was found to inhibit the doxorubicin-induced CPK and LDH release in the serum of rats. It is widely reported that doxorubicin-induced free-radical generation triggers membrane peroxidation and disruption of cardiac myocytes, which can lead to increased release of CPK in the serum. *Cucumis callosus* pretreatment led to inhibition of CPK and LDH release which resulted in either complete reversal or considerable recovery of the serum enzyme activities.

The Cardioprotective activity of *Cucumis callosus* was further supported by increased myocardial antioxidant enzyme activity and decreased the extent of lipid peroxidation. Lipid peroxidation is known to cause cellular damage and is primarily responsible for reactive oxygen species induced organ damage. Increased level of MDA and decreased levels of GSH, SOD and CAT were observed in heart tissue in doxorubicin-treated animals. Pretreatment with Cucumis callosus efficiently counteracted the doxorubicin-induced cardiac tissue damage by a significant decrease in MDA and the increase in GSH, SOD and CAT levels. The observed increase in CAT activity in doxorubicin-treated animals supports the above hypothesis that this increase is possibly required to overcome excessive oxidative stress [42].

CONCLUSION

In conclusion, the present results suggest that *Cucumis callosus* prevented the doxorubicin-induced myocardial toxicity by boosting the endogenous antioxidant activity. The cardioprotective property of *Cucumis callosus* could be due to lipid-lowering and antioxidant properties. Further studies are needed in isolation and characterization of chemical compounds present in the *Cucumis callosus* extract for the treatment of myocardial infarction.

CONFLICT OF INTERESTS

Declared none

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