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Investigation on protective effect of wheat germ oil in doxorubicin-induced cardiotoxicity in rats

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ABSTRACT

Doxorubicin (DOX) is a potent anticancer drug used in the treatment of various cancer, but its clinical use is limited for its marked cardiotoxicity. The aim of this study was to investigate the possible protective effect of wheat germ oil (WGO) on doxorubicin-induced cardiotoxicity and its impact on oxidative stress and lipid peroxidation. Wheat germ oil (WGO) contains richest amount of tocopherols among all vegetable oils and also polyphenols. Incorporation of WGO in our diet is the best way to ameliorate the DOX-induced cardiotoxicity and for enhancing its clinical use. DOX in a single dose (10 mg/kg i.p.) was used to produce oxidative stress and lipid peroxidation. Pretreatment with WGO was initiated in dose-dependent (125 mg/kg, 250 mg/kg and 500 mg/kg) and time-dependent (15 days, 30 days and 45 days) manner to evaluate the cardioprotective activity. DOX produced elevation in heart malodialdehyde (MDA) levels, decreased the levels of glutathione (GSH), lactate dehydrogenase (LDH), total proteins and decreased the activities of superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px). Plasma levels of aspartate amino transferase (AST), alanine amino transferase (ALT), nitric oxide (NO) increased and serum levels of creatine kinase isoenzyme MB (CK-MB) were also increased. All doses of WGO were capable of preventing oxidative stress and lipid peroxidation. Cardiac histopathological changes were observed in the DOX alone treated group as compared to the control group. In contrast, these histopathological changes appeared nearly normal in the groups pretreated with WGO. The ameliorating effect of WGO is possibly due to the presence of tocopherols, polyphenols, linolenic acid, linoleic acid, octacosanol and polycosanol.

Keywords: Doxorubicin, Cardiotoxicity, Oxidative stress, Lipid peroxidation, Wheat germ oil, Tocopherols, Histopathlogy.

INTRODUCTION

Cardiotoxicity is damage to the cardiac muscle inhibiting the normal function of the heart. It is commonly seen with chemotherapy and medications taken to control existing diseases. The physiological mechanisms of cardiotoxicity are not completely understood. Overabundance of free radicals leads to oxidative stress causing the death of cardiac muscle cells. A minor loss of left ventricle pumping efficiency causes shortness of breath. More serious cases can result in congestive heart failure (CHF), heart attack, or death. Symptoms of this effect include shortness of breath, fatigue, and anemia. If at risk for CHF symptoms include chronic coughing, swelling of

the ankles and feet, and weight gain.(1) Several antineoplastic agents can induce cardiac toxicity. However, the incidence is low, mostly unpredictable and the occurrence is not related to the cumulative dose in most cases. Cyclophosphamide Fluorouracil is associated with cardiotoxicity, with symptoms varying from cardiac arrhythmias, silent myocardial ischemia, angina, congestive heart failure and even sudden death [2].

Doxorubicin (anthracycline group of drug) induced cardiotoxicity is a major public health concern within the exposed population as it may not be manifested for many years and remains a lifelong threat. Cardiotoxicity was the particular problem in children treated for cancer because it is hoped that they will survive for several decades after treatment. Due to their high antitumor activity and to improve the gold standard anthracyclines, there is urge to develop antioxidant supplements to counter the cardiotoxicity caused by the anthracyclines. Polyphenols can be considered as potential protectors against toxicity caused by doxorubicin due to their antioxidant capacities (radical scavenging). Natural products have been the starting point for the discovery of many important modern drugs. This has led to the worldwide search for pharmacologically important oils from plants source to utilize their antioxidant properties on cardioprotection. Dietary antioxidants such as phenolic compounds showed protective effect against doxorubicin toxicity. Antioxidants such as vitamin–E provide protection from cardiac cell damage with a simultaneous decrease in lipid peroxidation.

Wheat germ oil is obtained from the kernals of wheat germ and is the natural richest source of tocopherols, fat-soluble vitamins, linolenic acid, linoleic acid, polyphenols, octacosanol and polycosanol which possess antioxidant activity. Cardiotoxicity induced by anthracyclines is largely mediated through the generation of free radicals. Incorporation of antioxidant rich agents might be capable of preventing damage to the cardiac muscle. Therefore, the present study was designed to investigate the impact of wheat germ oil (WGO) on doxorubicin-induced cardiotoxicity.

MATERIALS AND METHODS

Drugs

1. Doxorubicin Hydrochloride – HETERO DRUGS PVT LTD, HYDERABAD.
2. Wheat germ oil - Katyani Exports, New Delhi.

Animals

Healthy male albino rats of Wistar strain weighing about 150-200 g were procured from National Institute of Nutrition, Hyderabad and acclimatized in our own animal house for two weeks prior to the experiment. The animals were maintained at 22 ± 3^0 C under 12 h/ 12 h light dark cycle conditions and fed on standard diet with free access to distilled water. This experimental protocol has been approved by the Institutional Animal Ethical Committee and the experiments have been conducted as per guidelines laid down by Committee for the Purpose and Control of Supervision of Experiments on Animals (CPCSEA), Chennai, India.

EXPERIMENTAL DESIGN

Dose Dependent Study

Male albino rats of Wistar strain divided in to seven groups (n=6). The dose selection of wheat germ oil is based on previous studies [3]. 125 mg/kg, 250 mg/kg and 500 mg/kg were selected for our study. The dose of doxorubicin HCL selected as 10 mg/kg from the previous studies [4]. Group I (Control group): This group was treated with saline. Group II (Positive conrol group): This group was treated with doxorubicin HCL at a dose of 10mg/kg b.w. Group III: WGO (125mg/kg, b.w.) & Doxorubicin HCL (10 mg/kg, b.w.) Group IV: WGO (250mg/kg) & Doxorubicin HCL (10 mg/kg, b.w.) Group V: WGO (500mg/kg) & Doxorubicin HCL (10 mg/kg, b.w.). WGO was administered daily once by oral route for a period of 15 days after 2 hours Doxorubicin HCL was administered by intraperitoneal route and animals were sacrificed after 24 hours.

Determination of Tocopherols [5]

Tocopherols contentwas determined according to Bunge Europe Research and Development Center–in house method. Oil samples dissolved in

hexane (0.5000 g in 5 mL) were injected (5–20µL) on a LiChrospher 100 Diol (125mm×4mm, 5µm particle size, Agilent Technologies) column and analyzed by an Agilent 1100 HPLC system with autosampler and fluorescence detector. The mobile phase was hexane with tetrahydrofuran (96:4 vol/vol%) and a flow rate of 0.8 mL/min. The excitation and emission wavelengths at 280 and 340 nm were used respectively. The concentrations were calculated from the calibration curves prepared for α-, β-, γ - and δ -tocopherol isomers.

Estimation of Total Phenol Content [6]

A calibration curve of gallic acid in methanol was performed in concentration range 0.04±0.7 mg/ml. The solutions for the spectrophotometric analysis were per- formed as follows: In a 50 ml volumetric flask, 1 ml of a standard solution of gallic acid, 6 ml of methanol, 2.5 ml of the Folin-Ciocalteau reagent, 5 ml of 7.5% Na_2CO_3 were added, reaching the final volume with purified water. The solutions were stored overnight and the spectrophotometric analysis was performed at λ=765 nm.

The determination of polyphenols was performed as follows: 2.5 g of oil was diluted with 2.5 ml of n-hexane and extracted three times by 5 min centrifugation (5000 rpm) with CH_3OH:H_2O 80:20 v/v. The extract was added to 2.5 ml Folin-Ciocalteau reagent, 5 ml of Na_2CO_3 (7.5%), in a 50 ml volumetric flask reaching the final volume with purified water. The samples were stored overnight, and the spectrophotometric analysis was performed at λ =765 nm.

Estimation of Malondialdehyde [7]

To 0.2 ml of tissue homogenate, 0.2 ml of 8.1% sodium dodecyl sulphate (SDS), 1.5 ml of 20% acetic acid (pH 3.5) and 1.5 ml of 0.8% TBA were added. The mixture was made up to 4.0 ml with water and then heated in a water bath at 95.8^{o}C for 60 min using glass ball as a condenser. After cooling, 1.0 ml of water and 5 ml of n-butanol and pyridine mixture were added and shaken vigorously. After centrifugation at 4000 rpm for 10 min, the organic layer was taken and its absorbance was measured at 532 nm. The level of lipid peroxides was expressed as nmoles of MDA formed/g tissue.

Estimation of Glutathione [8]

0.1 ml of tissue homogenate was precipitated with 5% trichloroacetic acid (TCA). The contents were mixed well for complete precipitation of proteins and centrifuged. To 0.1 ml of supernatant, 2.0 ml of 0.6 mM 5, 5 dithiobis (2-nitrobenzoic acid) (DTNB) reagent and 0.2 M phosphate buffer (pH 8.0) were added to make up to a final volume of 4.0 ml. The absorbance was read at 412 nm against a blank containing TCA (trichloroacetic acid) instead of sample. A series of standards treated in a similar way also run to determine the glutathione content. The amount of glutathione was expressed as µmoles/mg protein.

Estimation of Glutathione Peroxidase [9]

The reaction mixture consisted of 0.2 ml of 0.8 mM ethylene diamine tetra acetic acid (EDTA), 0.1 ml of 10 mM sodium azide, 0.1 ml of 2.5 mM H_2O_2, 0.2 ml of reduced glutathione, 0.4 ml of 0.4 M phosphate buffer pH 7.0, and 0.2 ml of tissue homogenate and was incubated at 37.8^{o}C for 10 min. The reaction was arrested by the addition of 0.5 ml of 10% TCA (trichloroacetic acid) and the tubes were centrifuged at 2000 rpm. To the supernatant 3.0 ml of 0.3 mM disodium hydrogen phosphate and 1.0 ml of 0.04 % DTNB were added and the colour developed was read at 420 nm immediately. The activity of GSH-Px was expressed as µmoles of glutathione oxidized/min/mg protein.

Estimation of Catalase [10]

To 1.2 ml of 50 mM phosphate buffer pH 7.0, 0.2 ml of the tissue homogenate was added and reaction was started by the addition of 1.0 ml of 30 mM H_2O_2 solution. The decrease in absorbance was measured at 240 nm at 30 s intervals for 3 min. The enzyme blank was run simultaneously with 1.0 ml of distilled water instead of hydrogen peroxide. The enzyme activity was expressed as units/mg protein.

Estimation of Superoxide Dismutase [11]

0.1 ml of tissue homogenate was added to the tubes containing 0.75 ml ethanol and 0.15 ml chloroform (chilled in ice) and centrifuged. To 0.5 ml of supernatant, added 0.5 ml of 0.6 mM EDTA solution and 1 ml of 0.1 M carbonate-bicarbonate

(pH 10.2) buffer. The reaction was initiated by the addition of 0.5 ml of 1.8 mM epinephrine (freshly prepared) and the increase in absorbance at 480 nm was measured in a Shimadzu UV-1601 spectrophotometer. One unit of the SOD activity was the amount of protein required to give 50% inhibition of epinephrine autoxidation. The enzyme activity was expressed as units/mg protein.

Estimation of Total Protein [12]

Different dilutions of bovine serum albumin (BSA) solutions are prepared by mixing stock BSA solution (1 mg/ ml) and water in the test tube as given in the table. The final volume in each of the test tubes is 5 ml. The BSA range is 0.05 to 1 mg/ ml. From these different dilutions, pipette out 0.2 ml protein solution to different test tubes and add 2 ml of alkaline copper sulphate reagent (analytical reagent). Mix the solutions well. This solution is incubated at room temperature for 10 mins. Then add 0.2 ml of reagent FolinCiocalteau solution (reagent solutions) to each tube and incubate for 30 min. Zero the colorimeter with blank and take the optical density (measure the absorbance) at 660 nm. Plot the absorbance against protein concentration to get a standard calibration curve. The amount of total protein was expressed as mg protein/mg tissue.

Estimation of Plasma Nitrite [13]

The production of nitric oxide (NO) was assessed indirectly by measuring the nitrite/ nitrate concentration in plasma using the Griess reagent (1% sulfanilamide in 3N HCl and 0.1% napthylethlenediamine dihydrochloride, in a ratio of 1 : 1). Blood was obtained and centrifuged at 3000 rpm for 5 min. Plasma samples were stored overnight in a freezer. The day of the experiment, an aliquot of 750 µl of plasma was mixed with 750 µl of the Griess reagent, protected from light, and maintained at room temperature for 15 min. The concentration of nitrite/nitrate in the samples was determined spectrophotometrically at 540 nm. For every NO assay, a standard curve was performed, using sodium nitrite ($NaNO_2$) as a NO source. The plasma nitrite levels were expressed as µM.

Estimation of Lactate Dehydrogenase [14]

The reaction mixture contained 1 ml of 0.1 M Tris – HCl pH 8.0, 0.2 ml of 1mM sodium pyruvate and 0.2 ml of 0.15 mM nicotenamide adenine dinucleotide reduced (NADH). The reaction was started by the addition of 0.1 ml of tissue homogenate. The change in the absorbance due to the oxidation of NADH was followed at 340 nm for 2 minutes. The enzyme activity was calculated on the basis of molar extinction coefficient of NADH 6.22 10^{-3} $M^{-}CM^{-}$ and expressed as µmol NADH oxidized per min per mg of protein.

Parameters Estimated Using Diagnostic Kits [14]

The plasma Aspartate amino transferase (AST) and Alanine amino transferase (ALT) levels were determined according to the manufacturer's instructions. The serum creatine kinase isoenzyme MB (CK-MB) levels were assayed using CK-MB test kit (Erba diagnostics). CK-MB activity was measured in freshly separated serum according tlo the manufacturer's instructions.

Statistical Analysis

Data are expressed as mean ± SEM. Analysis of data was done by One-way ANOVA followed by Dunnett comparison test. Graph Pad In Stat version 3.10 for Windows 2009 (Graph Pad Software) was used. The statistical significance was set as 0.05 ($p<0.05$).

RESULTS

Total Phenolic Content of WGO

The total phenolic content of wheat germ oil was found to be 299.06µg GAE/ kg oil. (Figure-1)

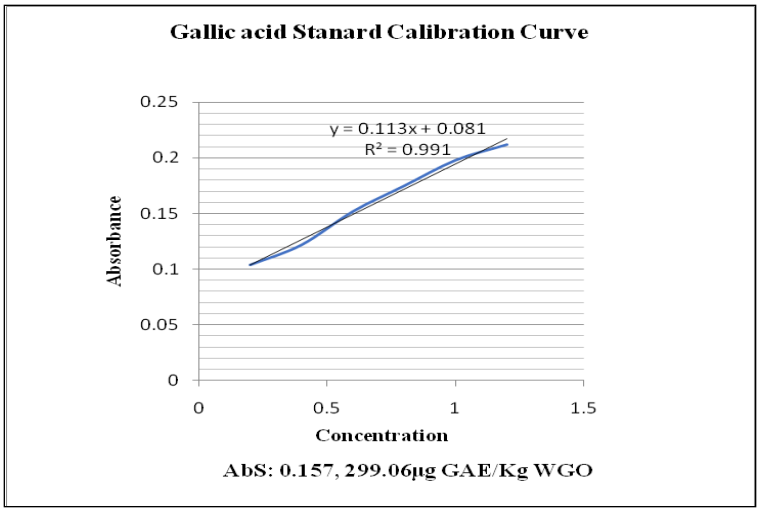


Figure-1: Estimation of total phenol content in wheat germ oil

Estimation of Tocopherol content in WGO

Three types of tocopherol isomers were found in wheat germ oil. α-tocopherols-525 ppm, γ-tocopherols-963 ppm, δ-tocopherols-334 ppm. Total tocopherol content was 1822 ppm/0.5 gm. (Table-1)

Table-1: Estimation of Tocopherol content in wheat germ oil

Type of isomers	Value in ppm
α-tocopherol	525 ppm
γ-tocopherol	963 ppm
δ-tocopherol	334 ppm
Total tocopherols	1822 ppm

Effect of WGO on Malondialdehyde levels in Doxorubicin induced cardiotoxicity

A significant elevation was noticed in the levels of MDA in ADR treated groups compared with the control ($p<0.05$). Pretreatment with WGO dose dependently reduced the levels of MDA compared with ADR alone treated group ($p<0.05$). (Table-2)

Table-2: Effect of WGO on MDA levels in Doxorubicin-induced cardiotoxicity.

Group	Treatment	Duration	MDA (nmol/g tissue)
G-I	Control (Saline)	15 days	190.13±14.905
G-II	Doxorubicin 10mg/kg	15 days	281.63±15.329*
G-III	WGO 125mg/kg+DOX 10mg/kg	15 days	278.7±13.111*
G-IV	WGO 250mg/kg+DOX 10mg/kg	15 days	268.76±17.425*
G-V	WGO 500mg/kg+DOX 10mg/kg	15 days	249.71±11.78*
G-VI	WGO 250mg/kg+DOX 10mg/kg	30 days	226.39±12.181#
G-VII	WGO 250mg/kg+DOX 10mg/kg	45 days	205.32±14.292#

Values are expressed as Mean±SEM, N=6,*$p<0.05$ significant compared to control, #$p<0.05$ significant compared to doxorubicin, Statistics: Dunnett Multiple comparision test, WGO: Wheat germ oil, DOX: Doxorubicin.

Effect of WGO on Glutathione and Glutathione Peroxide in Doxorubicin induced cardiotoxicity

WGO pretreatment in all three doses & duration significantly increased the GSH and GSH-Px levels in cardiac tissue compared with DOX alone treated group. An enhanced level of GSH and GSH-Px was observed following 30 days treatment with WGO (Table-3 & 4).

Table-3: Effect of WGO on GSH levels in Doxorubicin-induced Cardiotoxicity

Group	Treatment	Duration	GSH (µmol/mg protein)
G-I	Control (Saline)	15 days	133.72±7.956
G-II	Doxorubicin 10mg/kg	15 days	61.11±3.287*
G-III	WGO 125mg/kg+DOX 10mg/kg	15 days	72.553±3.313*
G-IV	WGO 250mg/kg+DOX 10mg/kg	15 days	81.6±5.277*
G-V	WGO 500mg/kg+DOX 10mg/kg	15 days	95.13±6.199*#
G-VI	WGO 250mg/kg+DOX 10mg/kg	30 days	103.09±6.395*#
G-VII	WGO 250mg/kg+DOX 10mg/kg	45 days	129.59±7.895#

Values are expressed as Mean±SEM, N=6, *p<0.05 significant compared to control, #p<0.05 significant compared to doxorubicin, Statistics: Dunnett Multiple comparision test, WGO: Wheat germ oil, DOX: Doxorubicin.

Table-4: Effect of WGO on GSH-Px in Doxorubicin-induced cardiotoxicity.

Group	Treatment	Duration	GSH-Px (µmol/mg protein)
G-I	Control (Saline)	15 days	116.53±8.108
G-II	Doxorubicin 10mg/kg	15 days	54.677±4.951*
G-III	WGO 125mg/kg+DOX 10mg/kg	15 days	54.91±5.196*
G-IV	WGO 250mg/kg+DOX 10mg/kg	15 days	65.28±5.643*
G-V	WGO 500mg/kg+DOX 10mg/kg	15 days	77.01±7.054*
G-VI	WGO 250mg/kg+DOX 10mg/kg	30 days	85.7±7.781*#
G-VII	WGO 250mg/kg+DOX 10mg/kg	45 days	103.28±8.095#

Values are expressed as Mean±SEM, N=6,*p<0.05 significant compared to control, #p<0.05 significant compared to doxorubicin, Statistics: Dunnett Multiple comparisiontest, WGO: Wheat germ oil, DOX: Doxorubicin.

Effect of WGO on Superoxide Dismutase and Catalase in Doxorubicin-induced Cardiotoxicity

The levels of antiperoxidative enzymes, superoxide dismutase (SOD) and catalase (CAT) were significantly lowered in DOX alone treated group compared to control group (p<0.05). Pretreatment with WGO, dose dependently elevated the levels of catalase compared in DOX alone treated group (p<0.05). Similarly treatment with WGO for 30 and 45 days also elicited a significant improvement in the levels of CAT compared with DOX alone treated group (p<0.05). The CAT levels of groups treated with WGO in doses of 500 mg/kg and 250 mg/kg for 45 days showed restoration in the levels of CAT in relation to the control. With pretreatment 250 mg/kg for 45 days group showed significant increase in the levels of DOX alone treated group. (Table 5 & 6)

Table-5: Effect of WGO on CAT in Doxorubicin-induced cardiotoxicity.

Group	TREATMENT	DURATION	CAT(U/mg protein)
G-I	Control (Saline)	15 days	20.8±0.691
G-II	Doxorubicin 10mg/kg	15 days	13.96±0.6553*
G-III	WGO 125mg/kg+DOX 10mg/kg	15 days	14.52±0.9482*
G-IV	WGO 250mg/kg+DOX 10mg/kg	15 days	15.4±0.9027*
G-V	WGO 500mg/kg+DOX 10mg/kg	15 days	16.52±1.059*
G-VI	WGO 250mg/kg+DOX 10mg/kg	30 days	17.93±0.8091#
G-VII	WGO 250mg/kg+DOX 10mg/kg	45 days	20.87±0.8692#

Values are expressed as Mean±SEM, N=6,*p<0.05 significant compared to control, #p<0.05 significant compared to doxorubicin, Statistics: Dunnett Multiple comparisiontest, WGO: Wheat germ oil, DOX: Doxorubicin.

Table-6: Effect of WGO on SOD in Doxorubicin-induced cardiotoxicity.

Group	TREATMENT	DURATION	SOD(U/mg protein)
G-I	Control (Saline)	15 days	11.043±0.3403
G-II	Doxorubicin 10mg/kg	15 days	5.34±0.5424*
G-III	WGO 125mg/kg+DOX 10mg/kg	15 days	5.34±0.3441*
G-IV	WGO 250mg/kg+DOX 10mg/kg	15 days	6.82±0.432*
G-V	WGO 500mg/kg+DOX 10mg/kg	15 days	7.84±0.3141*#
G-VI	WGO 250mg/kg+DOX 10mg/kg	30 days	8.91±0.5064*#
G-VII	WGO 250mg/kg+DOX 10mg/kg	45 days	10.52±0.6262#

Values are expressed as Mean±SEM, N=6,*p<0.05 significant compared to control, #p<0.05 significant compared to doxorubicin, Statistics: Dunnett Multiple comparision test, WGO: Wheat germ oil, DOX: Doxorubicin.

Effect of WGO on Total Protein in Doxorubicin-induced Cardiotoxicity

The levels of total protein significantly lowered in DOX alone treated group compared to control group (p<0.05). Pretreatment with WGO dose dependently elevated the levels of total protein compared in DOX alone treated group (p<0.05). Similarly treatment with WGO for 30 and 45 days also produced a significant improvement in the levels of total protein compared with ADR alone treated group (p<0.05).

Table-7: Effect of WGO on total protein levels in Doxorubicin-induced cardiotoxicity.

Group	Treatment	Duration	Total Protein (mg protein/mg tissue)
G-I	Control (Saline)	15 days	0.0348±0.0028
G-II	Doxorubicin 10mg/kg	15 days	0.03183±0.0032
G-III	WGO 125mg/kg+DOX 10mg/kg	15 days	0.0273±0.002808
G-IV	WGO 250mg/kg+DOX 10mg/kg	15 days	0.0333±0.00278
G-V	WGO 500mg/kg+DOX 10mg/kg	15 days	0.03887±0.002678
G-VI	WGO 250mg/kg+DOX 10mg/kg	30 days	0.0463±0.00278*#
G-VII	WGO 250mg/kg+DOX 10mg/kg	45 days	0.0581±0.002936*#

Values are expressed as Mean±SEM, N=6, *p<0.05 significant compared to control, #p<0.05 significant compared to doxorubicin, Statistics: Dunnett Multiple comparision test, WGO: Wheat germ oil, DOX: Doxorubicin.

Effect of WGO on Plasma AST and ALT in Doxorubicin-induced cardiotoxicity

The amount of diagnostic marker enzymes present in plasma is directly correlated with the necrotic lesions present in the myocardium. The plasma AST and ALT levels were increased in DOX alone treated group. WGO produced a significant decrease in the levels of AST and ALT at three dose levels and duration of treatment compared with DOX alone treated group (p<0.05). A significant difference in the levels of these enzymes was not observed with 250 mg/kg of WGO administered for a period of 30 and 45 days. (Figure-2& 3)

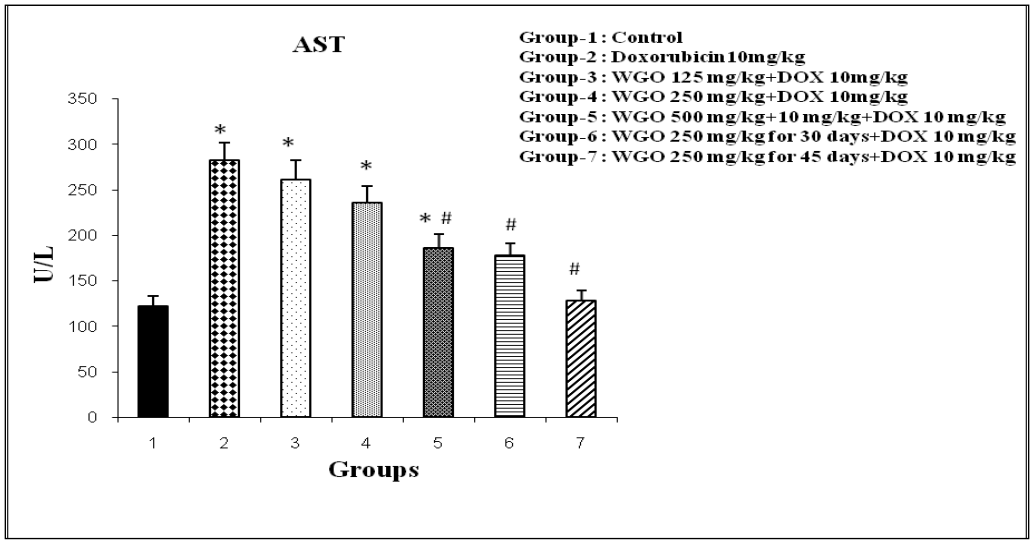


Figure-2: Effect of WGO on plasma AST level in Doxorubicin-induced cardiotoxicity

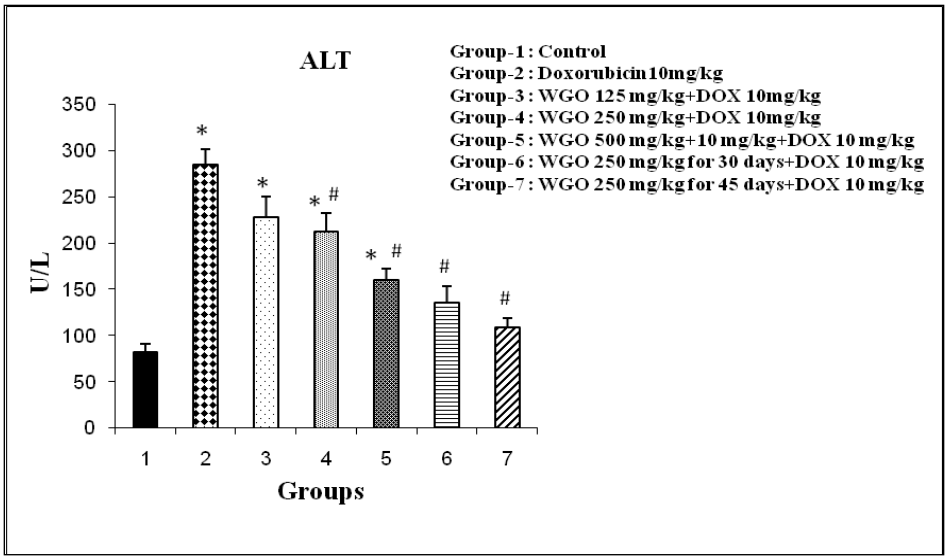


Figure-3: Effect of WGO on plasma ALT level in Doxorubicin-induced cardiotoxicity

Effect of WGO on Creatine Kinase Isoenzyme MB levels in doxorubicin-induced cardiotoxicity

DOX alone treated groups exhibited a significant elevation in the levels of serum CK-MB compared to control ($p<0.05$). WGO in a dose of 250 mg/kg for 45 days showed a marked reduction in the levels of CK-MB which is comparable to control. The serum levels of CK-MB significantly declined with WGO treatment at all dose levels as well as with 30 and 45 days treatment compared with DOX treated group ($p<0.05$). (Figure-4)

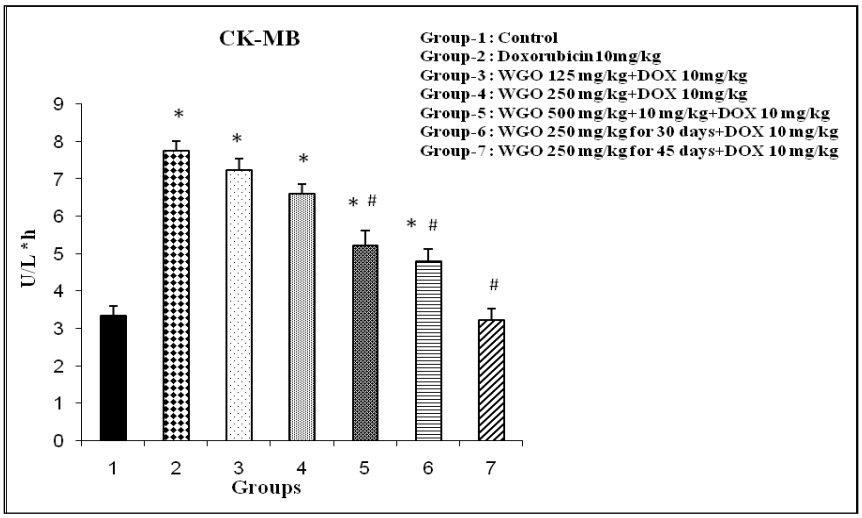


Figure-4: Effect of WGO on serum creatine kinase-MB levels in doxorubicin-induced cardiotoxicity.

Values are expressed as Mean±SEM, N=6, *$p<0.05$ significant compared to control, #$p<0.05$ significant compared to doxorubicin, Statistics: Dunnett Multiple comparision test, WGO: Wheat germ oil, DOX: Doxorubicin.

Effect of WGO on lactate dehydrogenase levels in Doxorubicin induced cardiotoxicity

The levels of LDH significantly lowered in DOX alone treated group compared to control group ($p<0.05$). Pretreatment with WGO of dose dependently elevated the levels of LDH compared in DOX alone treated group ($p<0.05$). Similarly treatment with WGO for 30 and 45 days also produced a significant improvement in the levels of LDH compared with DOX alone treated group($p<0.05$). (Figure-5)

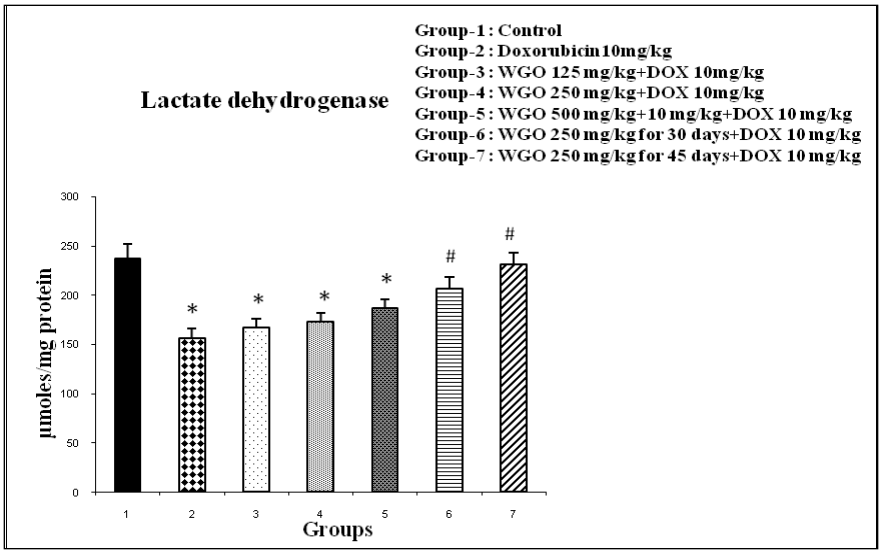


Figure-5: Effect of WGO on lactate dehydrogenase levels in doxorubicin-induced cardiotoxicity.

Effect of WGO on plasma nitrite levels in Doxorubicin induced cardiotoxicity

A significant elevation was noticed in the DOX alone treated group when compared to control ($p<0.05$). Pretreatment with WGO significantly decreased the levels of plasma nitrite levels as the dose and duration of treatment increases. (Figure-6)

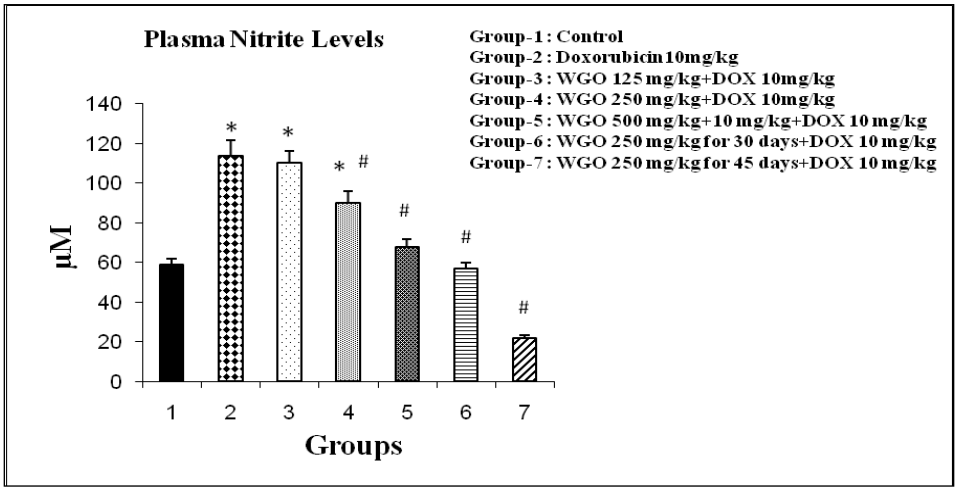
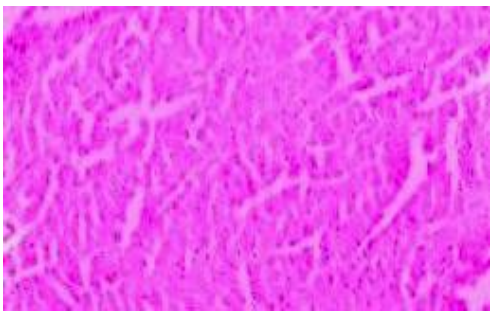


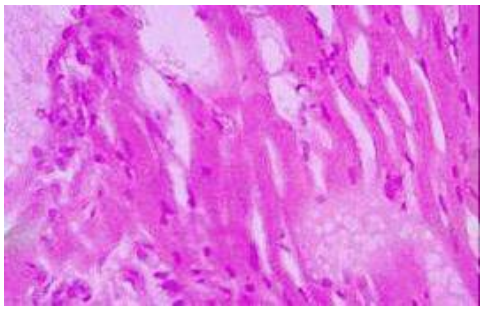
Figure-6: Effect of WGO on plasma nitrite levels in Doxorubicin induced cardiotoxicity

Histological Assessment of Myocardial Tissue in Doxorubicin induced cardiotoxicity

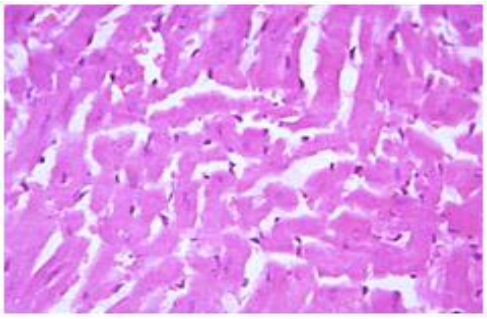
The control group showed even distribution of mononuclear cells with normal organization of myocardium. DOX alone treated group showed myocardial degeneration, disorganization and mononuclear cell infiltration in the space between damaged myocardial cells. As the dose of WGO increases, the mononuclear cell infiltration, myocardial degeneration and disorganization was prevented. (Figure-7).



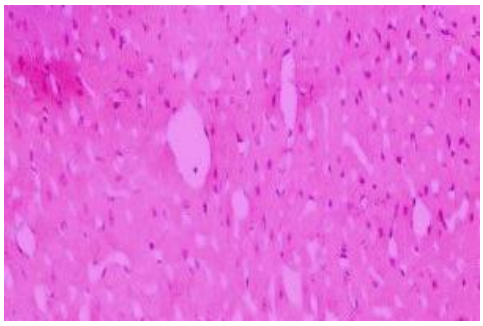
Control group



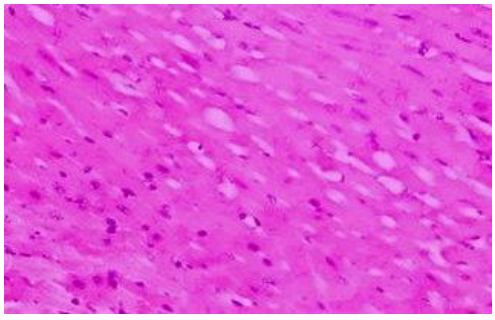
Doxorubicin group (10mg/kg)



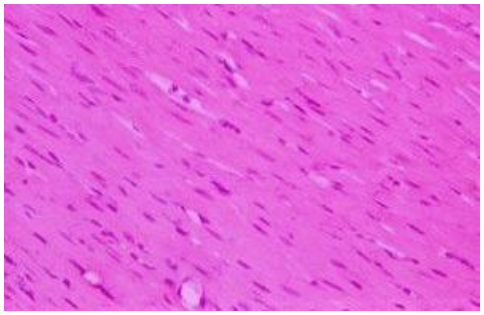
WGO 125 mg/kg for 15 days+DOX10mg/kg



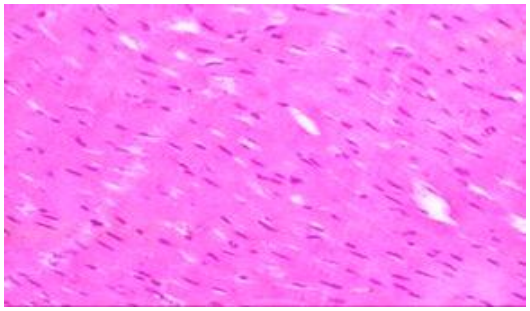
WGO 250 mg/kg for 15 days+DOX10mg/kg



WGO 500 mg/kg for 15 days+DOX10mg/kg



WGO 250 mg/kg for 30 days+DOX 10mg/kg



WGO 250 mg/kg for 45 days+10mg/kg

Figure-7: Histological Assessment of Myocardial Tissue in Doxorubicin induced cardiotoxicity

DISCUSSION

In the present study tocopherol content and total phenol content of wheat germ oil (WGO) was established. Further investigation of the ability of WGO to abrogate doxorubicin-induced cardiotoxicity in rats was carried out. Doxorubicin (DOX) belongs to anthracycline group of drugs which is potent antitumor antibiotic in the treatment of haemopoietic malignancies as well as for breast, thyroid, lymphoma and leukemia. Due to its high anti tumor activity, DOX was included as the gold standard chemotherapy regimens for all stages of breast cancer treatment. Oxidative stress and the production of free radicals are involved in DOX action in relation to both anticancer effects and cardiotoxicity. Thus it leads to direct oxidative injury to DNA and generates lipid peroxidation. [15].

Oxidative stress is an imbalance between the production of oxidants and antioxidants of an organism. Oxidants includes oxygen free radicals, reactive nitrogen species, sulphur centred radicals and various other radicals [16]. Reactive oxygen species (ROS) are biologically important, damaging molecules, such as lipids, DNA or proteins, and are involved in the pathobiochemistry of degenerative diseases. Pretreatment with WGO for 30 and 45 days showed elevated levels of SOD and CAT, this indicates protection against oxidative stress by counteracting superoxide radicals and hydroxyl radicals.

Decreased GSH concentration may also contribute to decreased GPx activity because GSH is one of the substrates for GSH-Px. Decreased GSH-Px activity prevents the peroxide reduction which utilizes GSH as the substrate, this phenomenon occurred in case of DOX treated group. But on pre-treatment with WGO for 30 and 45 days with 250 mg/kg contributed to the increased levels of GSH-Px activity. `

Total protein levels were decreased in DOX treated group compared to the control group. As the dose increases, the total protein levels were increased. The dose of 250 mg/kg for a period of 30 and 45 days showing a significant increase in the total proteins levels when compared to the DOX treated group.

A single dose of DOX caused an elevation in the diagnostic marker levels such as AST and ALT in plasma. The increased levels of these enzymes are directly correlated with the necrotic lesions present in the myocardium. On pre-treatment with WGO decreased the levels of AST and ALT. In myocardial injury condition, the heart LDH levels were decreased. In DOX treated group LDH levels were decreased this is an indicative of myocardial injury. In the time dependent study increase in the LDH levels were observed. Therefore WGO facilitated protection of cardiac tissue against DOX-induced cardiotoxicity. [17]

CK-MB determination in the serum was regarded as the reference standard for myocardial infarction caused by the DOX. Serum CK-MB levels were increased in DOX administered group of rats. WGO treatment significantly decreased the serum CK-MB levels at a dose of 500 mg/kg and dose of 250 mg/kg for a period of 30 days and 45 days. Production of NO (via constitutive NOS isoforms) modulates cardiomyocite contractility and blood flow distribution; high levels of NO production (via iNOS) are associated with dilated cardiomyopathy and congestive heart failure.

Doxorubicin is able to evoke synthesis of NO and reactive oxygen species such as $O_2^{\bar{}}$. Furthermore, these two radicals can react together, leading to the synthesis of peroxinitrite ($ONOO^{\bullet\bar{}}$), a powerful cellular oxidant molecule, which may be an important contributor to doxorubicin-induced cardiac dysfunction. DOX produced significant increase in plasma nitrite levels. WGO treatment with 250 mg/kg (15, 30 and 45 days) and 500 mg/kg showed a great decrease in plasma nitrite levels. This indicates the cardioprotective effect of WGO.

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