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Research article

# PHARMACOLOGICAL EVALUATION OF *BETA VULGARIS* ON DEXAMETHASONE INDUCED CARDIOTOXICITY IN RATS

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**ABSTRACT**

The methanolic-HCl extract of *Beta vulgaris* was studied for the cardioprotective activity using dexamethasone induced cardiotoxicity. Methanolic-HCl extract of *Beta vulgaris* was shown significant cardioprotective activity by elevating antioxidant enzymes superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GSH) and Lipid peroxidation (LPO). The phytochemical present in *Beta vulgaris* like Alkaloids, glycosides, tannin, flavonoids and betacyanin which is confirmed from qualitative analysis may be responsible for its cardioprotective activity.

**Keywords**: Dexamethasone, *Beta vulgaris*, Cardiotoxicity.

## INTRODUCTION

Dexamethasone used therapeutically for inflammatory disease, respiratory disorders like asthama and in auto immune disorders [1]. Dexamethasone is synthetic Glucocorticoid cause remarkable increase in heart weight to body weight ratio, fibrosis with left ventricular remodeling. Dexamethasone has potent immunosuppressive and anti inflammatory property. It caused cardiac toxicity by pathologic remodeling of cardiac tissue that later impairs cardiac functioning and diastolic dysfunction. The cardiac abnormality caused by improper calcium handling along with calcineurin signaling pathway activation by affecting expression of many proteins at molecular level [2]. Similarly doxorubicin, a chemotherapeutic agent that caused dose dependent cardiotoxicity that may end up to heart failure [3].

Adult male albino Wistar rats of either sex, weighting 150-200 g, obtained from Bharat Serum and Vaccines Ltd., Thane, India, were used for the study. They were housed in polypropylene cages lined with husk, renewed every 48h under 12:12 h light dark cycle at around 25 ± 5°C. They were fed with commercial pellet rat chow and given water *adlibitum*. The experiment was carried out according to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, India, and approved by the MGV's Institutional Animal Ethics Committee (Protocol number: MGV/PC/XXVI/01/2011-12).

Roots of *Beta vulgaris* were obtained from local market, Nasik and were identified by Dr. J. Jayanti, Scientist, Botanical Survey of India, Pune where a voucher specimen (DRP-1) has been retained.

The extraction was carried out by maceration process. Roots of *Beta vulgaris* (1 kg) were purchased from local market, cleaned with water. The roots were uniformly chopped. The chopped pieces were subjected to maceration with 1 % methanolic HCl for two days with frequent shaking the macerating flask [4]. The macerated product was then air dried for removal of methanol (Yield: 5.2% w/w). The methanolic extract shown the presence of Alkaloids, glycosides, tannin, flavonoids. The extracted samples were heated in 2 M HCl for 5 min at 100º C. The colour was fade away, it indicated the presence of betacyanin. To the extracted sample solution 2 M NaOH was added dropwise and the colour changed to yellow, which shows the presence of betacyanin.

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The animals were divided into six main groups. Each group contains 5 animals. Group I received vehicle, group II received dexamethasone injection (20 mcg/kg/day, s.c.) for 14 days , group III received extract of *Beta vulgaris* (100 mg/kg/day, p.o.) for 14 days, group IV received extract of *Beta vulgaris* (300 mg/kg/day, p.o.) for 14 days , group V received dexamethasone injection (20 mcg/kg/day, s.c.) and extract of *Beta vulgaris* (100 mg/kg/day, p.o.) for 14 days and group VI received dexamethasone injection (20 mcg/kg/day, s.c.) and extract of *Betavulgaris* (300 mg/kg/day, p.o.) for 14 days.

After 14 days of dosing period the heart tissue of animals of all groups was excised and subjected to estimation of antioxidant enzyme -superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GSH) and Lipid peroxidation (LPO).

Various Parameters are estimated at the end of study

**Heart Weight**

The animal heart was excised immediately after the completion of vascular reactivity and was weighed and heart weight in each group was noted.

**Biochemical Analysis**

**Determination of *in vivo* antioxidant status**

On the 14th day of dosing, the animals were sacrificed by decapitation immediately after behavioral assessments. The hearts were removed, and rinsed with isotonic saline and weighed. A 10% (w/v) tissue homogenate was prepared in 0.1 M phosphate buffer (pH 7.4), the post nuclear fraction for catalase assay was obtained by centrifugation of homogenate at 1000 rpm for 20 min, at 4°C and for other enzyme assays centrifuged at 12000 rpm for 60 min at 4°C.

**Estimation of superoxide dismutase activity (SOD)**

The assay of SOD was based on the ability of SOD to inhibit spontaneous oxidation of adrenaline to adrenochrome [4].

**Epinephrine solution (3X 10 $^{-4}$M)**

Epinephrine (3.664 mg) was dissolved in 1 ml of 0.1N hydrochloric acid. Then 0.884 ml diluted upto 10 ml with distilled water.

**Procedure**

To 0.05 ml supernatant, 2.0 ml of carbonate buffer and 0.5 ml of EDTA were added. The reaction was initiated by addition of 0.5ml of epinephrine and the auto-oxidation of adrenaline (3 X $10^{-4}$ M) to adrenochrome at pH 10.2 was measured by following change in optical density at 480 nm. The change in optical density every minute was measured at 480 nm against reagent blank. The results were expressed as SOD U/mg wet tissue.

**Estimation of Catalase (CAT) activity**

The Catalase activity assay was based on the ability of CAT to induce the disappearance of hydrogen peroxide [5].

**Procedure**

The reaction mixture consisted of 2 ml phosphate buffer (pH 7.0), 0.95 mL of hydrogen peroxide (0.019 M) and 0.05 ml supernatant in final volume of 3 ml absorbance were recorded at 240 nm every 10 sec for 1 min. The results were expressed as units of CAT activity (mg/protein). The results were expressed as catalase U/mg wet tissue.

**Estimation of reduced glutathione (GSH)**

DTNB (5, 5'-dithiobisnitro benzoic acid) solution: 1.98 mg of DTNB was dissolved in 0.1 % sodium citrate.

**Procedure**

Reduced glutathione was determined by the method of Ellman [6]. To the homogenate add 10% TCA, centrifuged, 1.0 ml of Ellman reagent (19.8 mg of 5, 5'-dithiobisnitro benzoic acid (DTNB) in 100 ml of 1.0% sodium citrate and 3 ml of phosphate buffer (pH 8.0). The color develop was measured at 412 nm. The results were expressed as nmol GSH/mg wet tissue.

**Estimation of lipid peroxidative indices (LPO)**

Lipid peroxidation as evidenced by the formation of TBARS was measured by the method of Niehaus [7].

**Thiobarbituric acid solution (TBA)**

Solution of 1% was prepared by dissolving 1 g of TBA in 0.25 N HCL (2.125 ml of concentrated HCl diluted to 100ml of distilled water)

**Procedure**

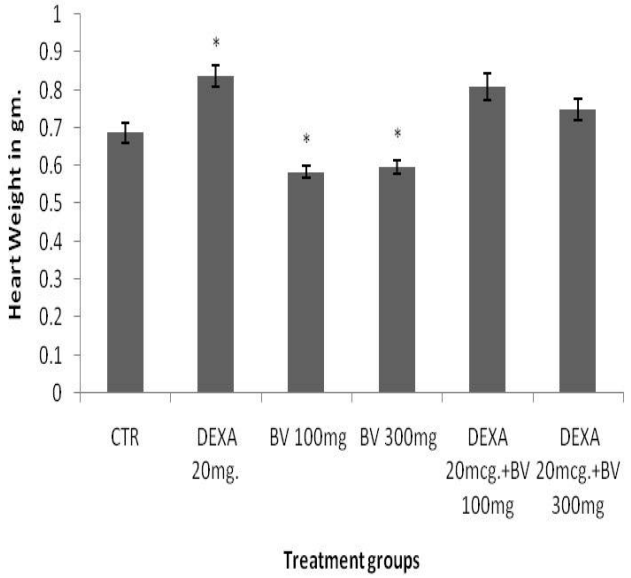
In brief, 0.1 ml of homogenate (Tris-HCl buffer, pH 7.5) was treated with 2 ml of (1:1:1 ratio) TBA-TCA-HCl reagent (Thiobarbituric acid 0.37%, 0.25N HCl and 15% TCA) and placed in water bath for 15 min, cooled and centrifuged at room temperature for 10 min at 1000 rpm. The absorbance of clear supernatant was measured against reference blank at 535 nm. The results were expressed as LPO nmol/mg wet tissue.

The Results were estimated for above mentioned parameters

**Heart Weight**

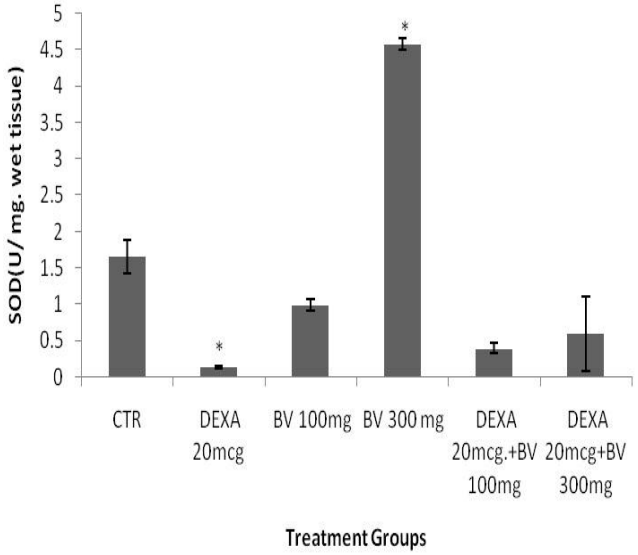
The heart weight in control animals was recorded as 0.688 ± 0.027 gm.. The DEXA administered group significantly increase in heart weight as compared to control animals. Animals treated with *Beta vulgaris* (100 and 300 mg/kg, p.o. for 14 days) showed a significant ($p<0.05$) decrease in heart rate compared to control treated group.

**Fig 1. Effect of *Beta vulgaris* on heart weight in dexamethasone induced cardiotoxicity in rats**



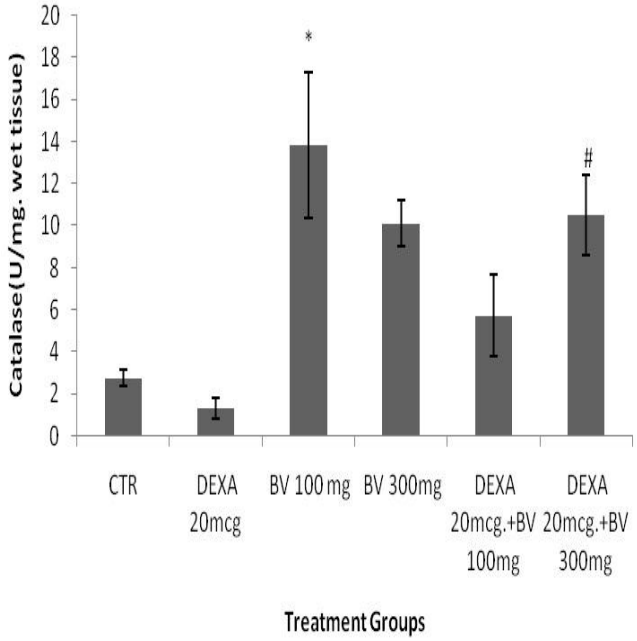

All values are expressed as mean ± SEM, n=5. All data are subjected to One-Way ANOVA followed by Dunnett's test. * p<0.05 when compared to control and # p<0.05 when compared to DEXA group. Vertical lines represent SEM. CTR: Control. DEXA: Dexamethasone. BV: *Beta vulgaris*.

**Fig 2. Effect of *Beta vulgaris* on superoxide dismutase levels in dexamethasone induced cardiotoxicity in rats**



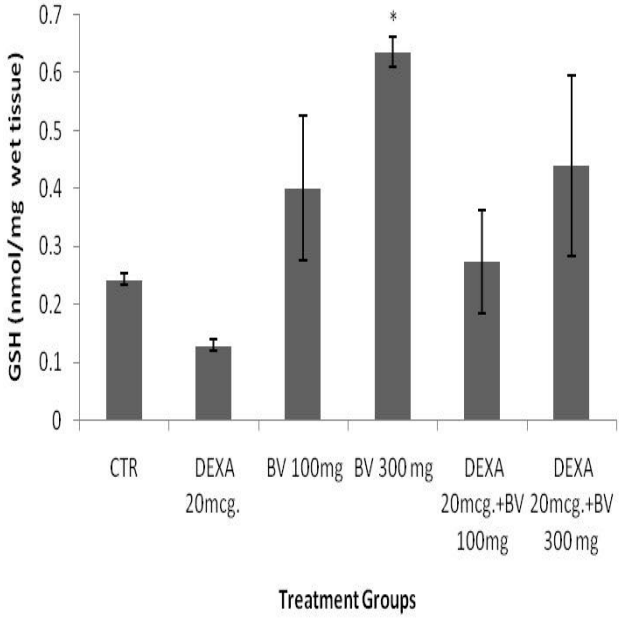

All values are expressed as mean ± SEM, n=5. All data are subjected to One-Way ANOVA followed by Dunnett's test. * p<0.05 when compared to control and # p<0.05 when compared to DEXA group. Vertical lines represent SEM. CTR: Control. DEXA: Dexamethasone. BV: *Beta vulgaris.* SOD: Superoxide dismutase

**Fig 3. Effect of Beta *vulgaris* on catalase levels in dexamethasone induced cardiotoxicity in rats**



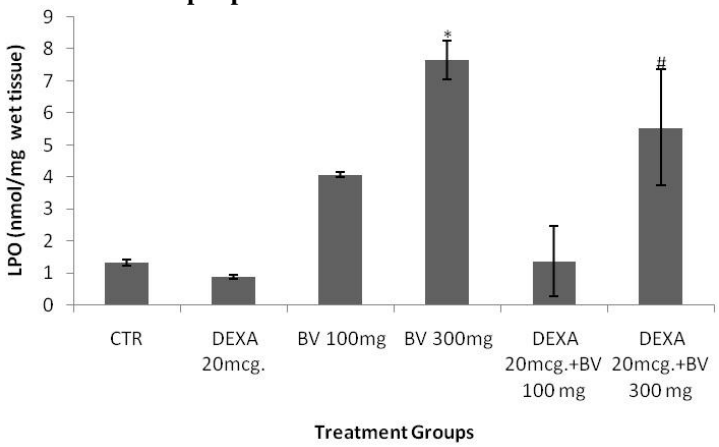

All values are expressed as mean ± SEM, n=5. All data are subjected to One-Way ANOVA followed by Dunnett's test. * p<0.05 when compared to control and # p<0.05 when compared to DEXA group. Vertical lines represent SEM. CTR: Control. DEXA: Dexamethasone. BV: *Beta vulgaris*. CAT: Catalase.

**Fig 4. Effect of *Beta vulgaris* on GSH level in dexamethasone induced cardiotoxicity in rats**




All values are expressed as mean ± SEM, n=5. All data are subjected to One-Way ANOVA followed by Dunnett's test. * p<0.05 when compared to control and # p<0.05 when compared to DEXA group. Vertical lines represent SEM. CTR: Control. DEXA: Dexamethasone. BV: *Beta vulgaris.* GSH: Glutathione.

**Fig 5. Effect of Beta *vulgaris* on extent of lipid peroxidation in dexamethasone induced cardiotoxicity in rats**




All values are expressed as mean ± SEM, n=5. All data are subjected to One-Way ANOVA followed by Dunnett's test. * $p<0.05$ when compared to control and # $p<0.05$ when compared to DEXA group. Vertical lines represent SEM. CTR: Control. DEXA: Dexamethasone. BV: *Beta vulgaris.* LPO: Lipid peroxidation.

**BIOCHEMICAL ANALYSIS**

**Estimation of Superoxide dismutase activity (SOD)**

The SODlevel in control animals was recorded as 1.657 ± 0.234 U/gm wet tissue. The DEXA administered group significantly decreased the SOD level compared to control animals. Animals treated with *Beta vulgaris* (100 mg/kg p.o. for 14 days) per se did not significantly alter SOD level when compared to control group. Animals treated with *Beta vulgaris* (300 mg/kg p.o. for 14 days) per se significantly increased SOD level when compared to control group. Animals treated with DEXA along with *Beta vulgaris* (100 and 300 mg/kg p.o. for 14 days) did not showed a significant ($p<0.05$) change in SOD level when compared to DEXA treated group.

**Estimation of Catalase (CAT) activity**

The CATlevel in control animals was recorded as 2.783 ± 0.391 U/gm wet tissue. The DEXA administered group did not significantly alter the CAT level compared to control animals. Animals treated with *Beta vulgaris* (100 mg/kg p.o. for 14 days) per se significantly increased CAT level when compared to control group. Animals treated with *Beta vulgaris* (300 mg/kg p.o. for 14 days) per se did not significantly alter CAT level when compared to control group. Animals treated with DEXA along with *Beta vulgaris* (100 mg/kg p.o. for 14 days) did not showed a significant ($p<0.05$) change in CAT level when compared to DEXA treated group. Animals treated with DEXA along with *Beta vulgaris* (300 mg/kg p.o. for 14 days) showed a significant ($p<0.05$) increase in CAT level when compared to DEXA treated group.

**Estimation of Glutathione (GSH) activity**

The GSH level in control animals was recorded as 0.2431 ± 0.0095 nmol/mg wet tissue. The DEXA administered group did not significantly alter the GSHlevel compared to control animals. Animals treated with *Beta vulgaris* (100 mg/kg p.o. for 14 days) per se did not significantly alter GSH level when compared to control group. Animals treated with *Beta vulgaris* (300 mg/kg p.o. for 14 days) significantly increased GSH level when compared to control group. Animals treated with DEXA along with Beta *vulgaris* (100 and 300 mg/kg p.o. for 14 days) did not showed a significant ($p<0.05$) change in GSH level when compared to DEXA treated group.

**Estimation of extent of lipid peroxidation (LPO)**

The LPOlevel in control animals was recorded as 1.32 ± 0.085 nmol/mg wet tissue. The DEXA administered group did not significantly alter the LPO level compared to control animals. Animals treated with *Beta vulgaris* (100 mg/kg p.o. for 14 days) per se did not significantly alter GSH level when compared to control group. Animals treated with *Beta vulgaris* (300 mg/kg p.o. for 14 days) per se significantly increased LPO level when compared to control group. Animals treated with DEXA along with *Beta vulgaris* (100 mg/kg p.o. for 14 days) did not showed a significant ($p<0.05$) change in LPO level when compared to DEXA treated group. Animals treated with DEXA along with *Beta vulgaris* (300 mg/kg p.o. for 14 days) showed a significant ($p<0.05$) increase in LPO level when compared to DEXA treated group.

*Beta vulgaris* (Beet root) is claimed to possess potential antioxidants betanin and also the inorganic nitrite which are claimed to have a good antihypertensive and cardioprotective activity.

Protective antioxidant enzyme activity determination like SOD, CAT, GSH and LPO, the DEXA administered group showed a significant reduction in

their activity when compared to control group while the *Beta vulgaris* treated group showed significant elevation in these enzyme activity.

Thus in conclusion the methanolic-HCl extract of *Beta vulgaris* has cardioprotective activity in dexamethasone model.

**ACKNOWLEDGEMENT**

Nil

**CONFLICT OF INTEREST**

No interest.

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