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COMPARATIVE STUDY OF GARLIC AND CURCUMIN FOR ITS CARDIOPROTECTIVE PROPERTY

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ABSTRACT

The combination of garlic and curcumin showed a significant increase in total protein value as compared to cadmium control, thus showing decrease in oxidation of total protein. Garlic and curcumin combination also showed significant reduction in serum enzymes i.e SGOT, SGPT, as compared to other antioxidants and combination. Current study has shown that exposure to cadmium had significant effects on rats blood parameters. In our present study treatment of garlic and curcumin in combination decreased the toxic effects of Cadmium on the hematological values and had a protective role in anaemia induced by cadmium as compared to other antioxidants. The combination of garlic and curcumin was found to be most significantly effective in the treatment of cadmium induced oxidative stress. In current study garlic and curcumin in combination decreased the activity of SOD and Catalase significantly as compared to other treatment groups.

Keywords: SGOT, SGPT, Cadmium, Serum Protein, Antioxidants, Garlic, Curcumin, Cardioprotective.

INTRODUCTION

Antioxidants are the compounds Prevents the transfer of electron from O_2 to organic molecules which Stabilizes free radicals and terminates free radical reactions. The contractile function of the heart dictates its high metabolic demand. This, in turn, requires the heart be equipped with a rich supply of mitochondria. The mitochondrial respiratory chain is the primary energy-releasing system in the cell. A series of oxidation-reduction reactions are involved in the energy generation. special concern for oxidative heart injury is the potential formation of highly reactive oxygen species during electron transport. Accumulation of these toxic oxygen species can result in exacerbation of damage to the heart. Cadmium (Cd) is an industrial and environmental pollutant, arising primarily from battery electroplating, plastic, fertilizer industries, and cigarette smoke. A variety of experiments have suggested that Cd causes oxidative damage to cells. Cadmium has been demonstrated to stimulate free radical production, resulting in oxidative deterioration of lipids, proteins and DNA, and initiating various pathological conditions in

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humans and animals. It has been shown that various antioxidants and antioxidant defense systems (AOS) protect cells from Cd-induced toxicity.

Objective

1) To evaluate the role of various antioxidants (Vitamin C, Vitamin E, Garlic, and Curcumin) in cadmium induced cardiac oxidative stress.
2) To compare the role of various antioxidants and their combination in cadmium induced cardiac oxidative stress.

Plan of Work:

a) Literature survey and Review of study undergone on oxidative stress
b) Procurement of Animals and their diet
c) Required Chemicals.
d) Chemical reagent kit for the estimation of SGOT, SGPT, and total protein.
e) Induction of Oxidative stress by Cadmium Chloride.
f) Evaluation of Antioxidants and their combinations in cadmium induced oxidative stress with help of following parameters.
   i. Blood Pressure evaluation
   ii. Biochemical evaluation
   iii. Blood evaluation
   iv. Antioxidant enzyme estimation
   v. Interpretation of result by statistical analysis.
   vi. Compilation of data.

MATERIALS AND METHODS

Materials:

Procurement of Animals:

a) The female albino rats (Wistar strain) weighing 170-200g, bred in the animal house of Institute of Pharmaceutical Education and Research (IPER), Wardha, were procured.
b) All animals were maintained under controlled conditions of temperature (22±2 ◦C), and illumination (12 h light–dark cycle), with free access to food (lab diet) and water.
c) All animal experiments were approved by the Institutional Animal Ethical Committee (Reg. No.535/02/a/CPCSEA /Jan 2002) of IPER, Wardha.

Procurement of Diagnostic Kits and Chemicals:

a) Diagnostic kits for estimation SGOT, SGPT, and total protein were obtained from Merck Ltd.
b) Cadmium chloride and antioxidants (Vitamin C, Vitamin E and Curcumin) were obtained from Loba Chemie, (Mumbai).
c) Garlic extract was procured from Zeus Biotech.Ltd. (Mysore).
d) All the other chemicals used for experimental purpose were of laboratory grade.

Drugs:

a) Cadmium Chloride (1.5 mg/kg)
b) Vitamin C (500 mg/kg)
c) Vitamin E (200 mg/kg)
d) Garlic (500 mg/kg)
e) Curcumin (50 mg/kg)

Instruments Used for Parameter Testing:

a) Biopac (Biopac systems MP35)

b) Autoanalyzer (Merck microlab 300)
c) Auto Cell Counter (Swelab 920)
d) UV Spectrophotometer (Shimadzu UV2401 PC)

Experimental Design:

All animals were divided into eight experimental groups of six each. 1.5 mg/kg, cadmium as cadmium chloride was administered through gastric gavage daily for 10 days for induction of oxidative stress and six animals received plain water and served as normal. The cadmium exposed animals were randomly divided into six groups and treated by gastric gavage daily for 10 days as follows.

Table 1: Classification of group of animal

Group	Treatment
Group I	Normal
Group II	Cadmium Control
Group III	Vitamin C (500mg/kg)
Group IV	Vitamin E (200mg/kg)
Group V	Garlic Extract (500mg/kg)
Group VI	Curcumin (50mg/kg)
Group VII	Vitamin C (500 mg/kg) + Vitamin E (200 mg/kg)
Group VIII	Garlic extract (500 mg/kg) + Curcumin (50 mg/kg)

Following Parameters were been evaluated:

a) Blood Pressure
b) Biochemical Parameters like (SGOT, SGPT, Total Protein)
c) RBC Count
d) Enzyme Estimation.

RESULT AND DISCUSSION:

Table 2: Antihypertensive effect of antioxidants and their combination

Group	**0 day**	**5th day**	**10th day**
Normal	113.33 ± 3.44	110.50 ± 3.61	111.83 ± 5.45
Cadmium Control	131.50 ± 1.99	144.33 ± 1.11	144.66 ± 0.49
Vitamin C	128.66 ± 1.97	140.16 ± 1.01	141.16 ± 0.94
Vitamin E	126.66 ± 1.80	135.00 ± 1.86	140.33 ± 0.66
Vitamin C + Vitamin E	123.66 ± 1.96	131.00 ± 1.36	134.83 ± 1.74
Garlic Extract	121.00 ± 1.06**	123.83 ± 1.66**	125.00 ± 1.23**
Curcumin	123.33 ± 1.72*	125.00 ± 0.89*	127.66 ± 0.98*
Garlic + Curcumin	116.00 ± 1.15***	118.66 ± 0.80***	119.66 ± 0.61***

Values expressed as mean ± SEM., n=6, One way ANOVA followed by Dunnett t test. All groups were compared with Cadmium control, (*** P <0.001, **P <0.01,*P <0.05).

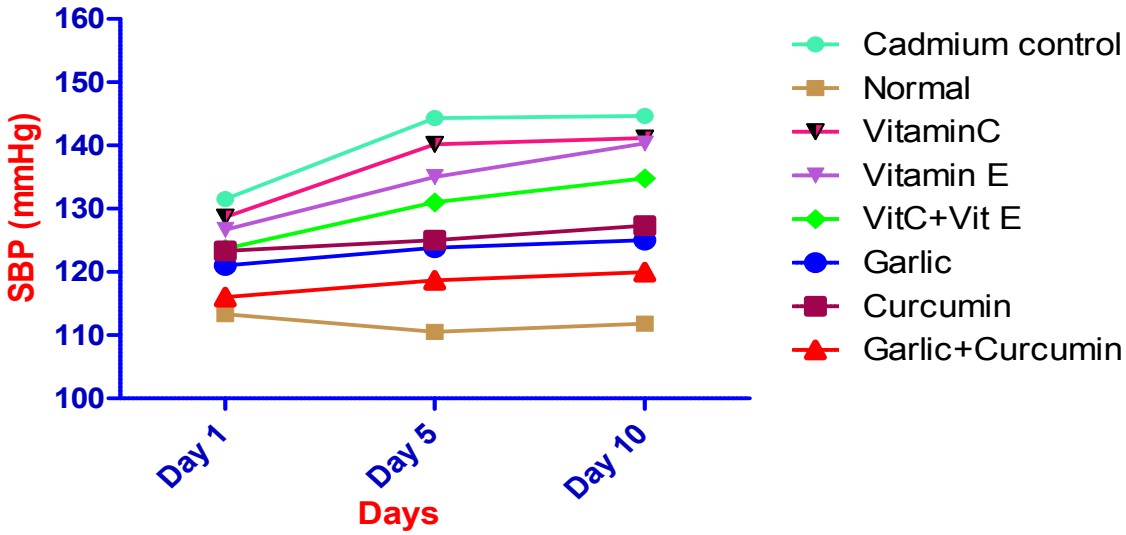

Figure 1: Diagrammatic representation of effect of antioxidants and their combination

Table 3: Effect of antioxidants and their combinations on Blood Catalase levels

Gr	Treatment	Catalase (U/mg protein)
I	Normal	70.38 ± 0.03
II	Cadmium Control	261.85 ±1.02
III	Vitamin C	245.52±0.92*
IV	Vitamin E	238.78 ± 0.52*
V	Garlic Extract	232.33±0.12**
VI	Curcumin	236.66 ±0.23**
VII	Vit C + Vit E	234.5 ±0.17**
VIII	Garlic + Curcumin	176.16 ±0.75***

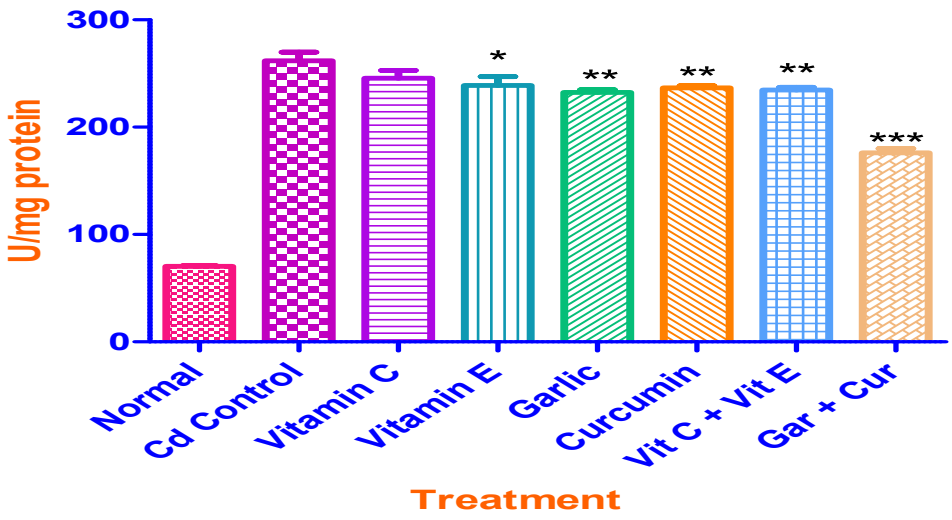

Figure 2: Diagrammatic representation of effect of antioxidants and their combinations on Blood Catalase levels

Table 4: Effect of antioxidants and their combinations on Blood SOD levels

Gr	Treatment	SOD (U/mg protein)
I	Normal	5.41 ±0.72
II	Cadmium Control	9.56 ±0.97
III	Vitamin C	8.87 ± 0.67**
IV	Vitamin E	8.93 ± 0.54*
V	Garlic Extract	8.81 ± 0.49**
VI	Curcumin	8.94 ± 0.56*
VII	Vit C + Vit E	8.86 ± 0.66**
VIII	Garlic + Curcumin	8.10 ± 0.54***

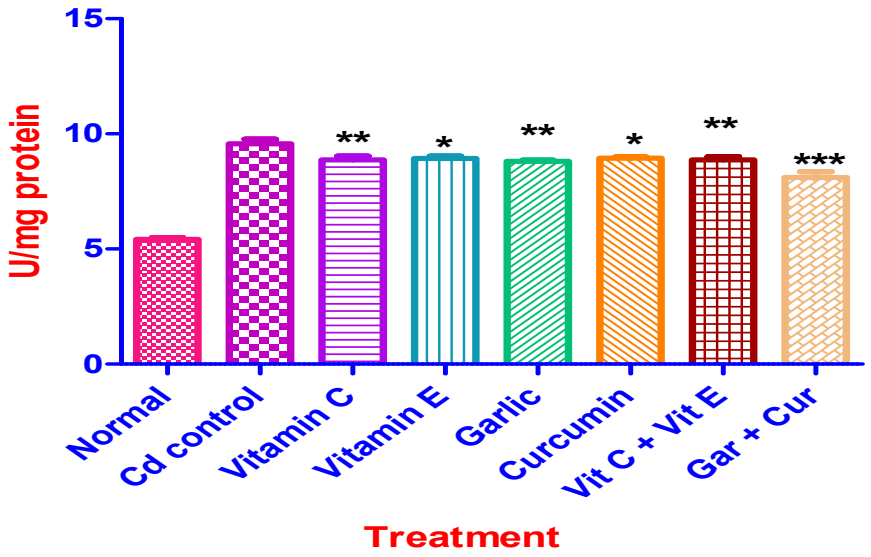

Figure 3: Diagrammatic representation of effect of antioxidants and their combinations on Blood SOD levels

Table 5: The effect of antioxidants on Blood RBC Count

Gr	Treatment	RBC Count
I	Normal	6.60 ± 0.10
II	Cadmium Control	4.00 ± 0.12
III	Vitamin C	4.10 ± 0.48
IV	Vitamin E	4.29 ± 0.12*
V	Garlic Extract	4.34 ± 0.08**
VI	Curcumin	4.32 ± 0.11*
VII	Vit C + Vit E	4.39 ± 0.17**
VIII	Garlic + Curcumin	4.94 ± 0.14***

Values expressed as mean ± SEM., n=6, One way ANOVA followed by Dunnett t test. All groups were compared with Cadmium control, (*** P <0.001, **P <0.01,*P <0.05).

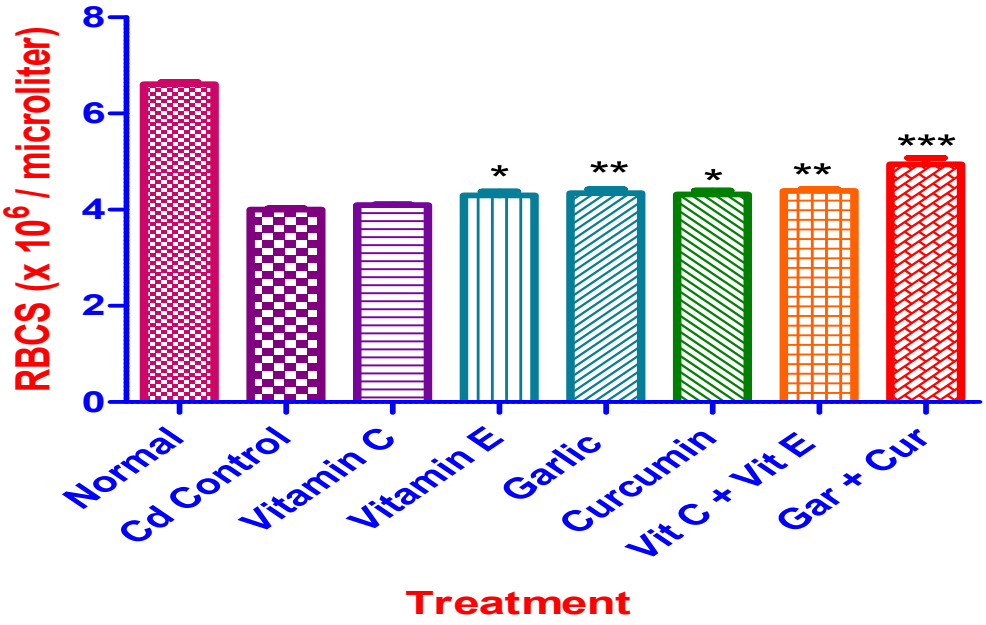

Figure 4: Diagrammatic representation of effect of antioxidants on Blood RBC Count

Table 6: Effect of Antioxidants and their combinations on Total Serum Protein

Gr	Treatment	Serum Total Protein (g/dl)
I	Normal	7.32 ± 0.02
II	Cadmium Control	3.96 ± 0.03
III	Vitamin C	4.60 ± 0.07*
IV	Vitamin E	4.65 ± 0.10*
V	Garlic Extract	4.69 ± 0.08*
VI	Curcumin	4.62 ± 0.06*
VII	Vit C + Vit E	4.79 ± 0.07**
VIII	Garlic + Curcumin	5.31 ± 0.11***

Values expressed as mean ± SEM., n=6, One way ANOVA followed by Dunnett t test. All groups were compared with Cadmium control, (*** P <0.001, **P <0.01,*P <0.05).

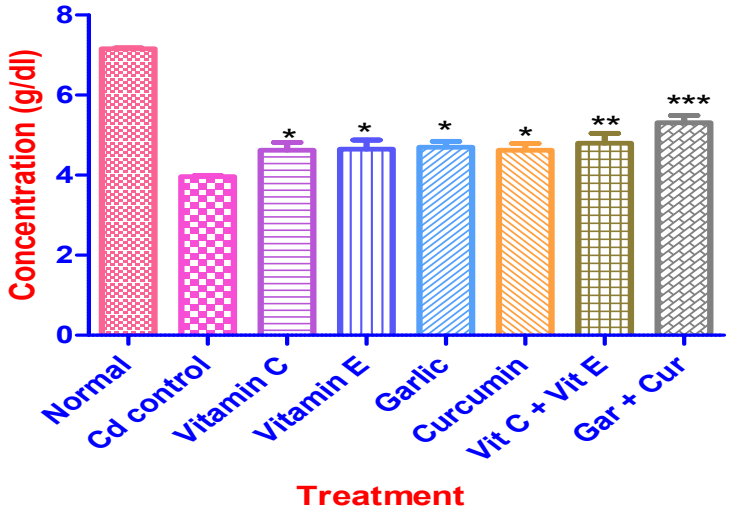

Figure 5: Diagrammatic representation effect of Antioxidants and their combinations on Total Serum Protein.

Table 7: Effect of Antioxidants and their combinations on Serum SGOT level

Gr	Treatment	SGOT (U/L)
I	Normal	57.10 ± 0.42
II	Cadmium Control	100.38 ± 0.72
III	Vitamin C	87.62 ± 0.87*
IV	Vitamin E	86.45 ± 0.91*
V	Garlic Extract	83.19 ± 0.76**
VI	Curcumin	85.69 ± 0.81*
VII	Vit C + Vit E	82.46± 0.58**
VIII	Garlic + Curcumin	74.83 ± 0.98***

Values expressed as mean ± SEM., n=6, One way ANOVA followed by Dunnett t test. All groups were compared with Cadmium control, (*** P <0.001, **P <0.01,*P <0.05).

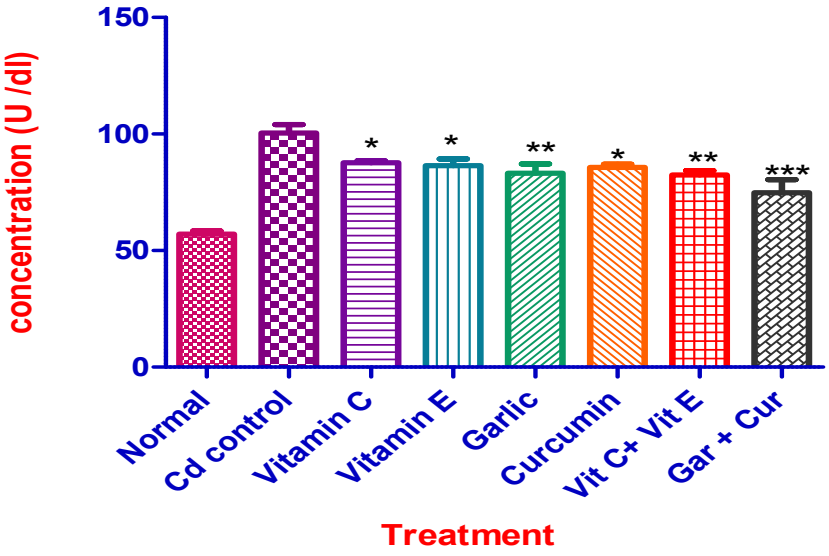

Figure 6: Diagrammatic representation effect of Antioxidants and their combinations on Serum SGOT level

Table 8: Effect of Antioxidants and their combinations on Serum SGPT level

Gr	Treatment	SGPT (U/L)
I	Normal	38.95 ± 0.44
II	Cadmium Control	82.99 ± 0.56
III	Vitamin C	53.06 ± 0.70*
IV	Vitamin E	58.97 ± 0.98*
V	Garlic Extract	47.83 ± 0.78*
VI	Curcumin	54.74 ± 0.32*
VII	Vit C + Vit E	55.44 ± 0.50*
VIII	Garlic + Curcumin	42.38 ± 0.59*

Values expressed as mean ± SEM., n=6, One way ANOVA followed by Dunnett t test. All groups were compared with Cadmium control, (*** P <0.001, **P <0.01,*P <0.05).

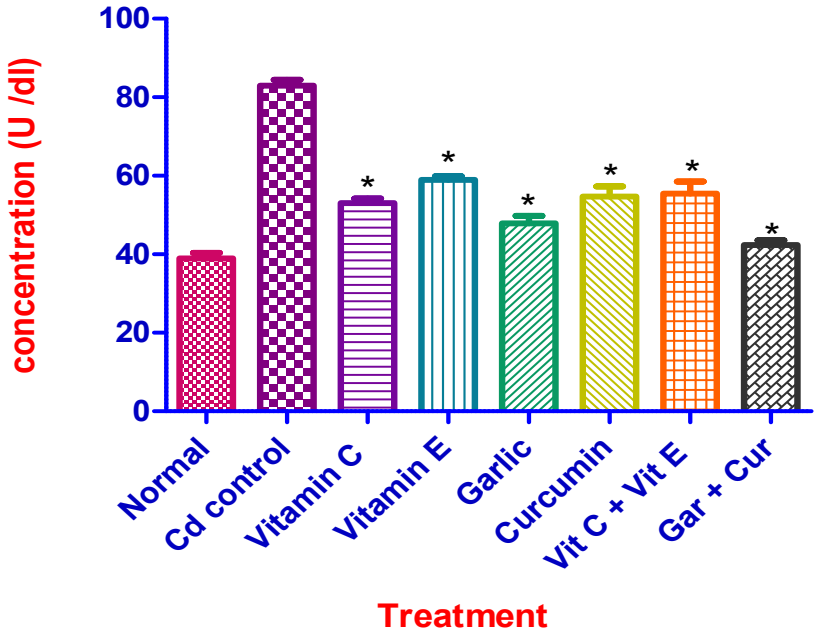

Figure 7: Diagrammatic representation effect of Antioxidants and their combinations on Serum SGPT level.

DISCUSSION

Cadmium is a potent toxic metal and exerts its adverse cardiovascular effects by promoting atherosclerosis and by inducing disadvantageous cardiac functional and metabolic changes.

In this study oral administration of cadmium chloride (1.5 mg/kg) resulted in an increase in SBP and increase in activity of catalase and SOD in blood which indicates alteration of vascular endothelium and enhanced lipid peroxidation in these cadmium exposed animals. The results are in accordance with Eybl *et al*, (2004) S.K. Tandon *et al*., (2003) suggesting that cadmium toxicity can cause oxidative stress by generation of reactive oxygen species (ROS).[66,54]

Cadmium induced Hypertension:

The possible mechanism for hypertension in cadmium induced rats was suggested to be oxidative stress. i.e. increase in free radicals which oxidizes NO to peroxynitrate and subsequently nitrite to nitrate.

This result in loss of bioactive NO-mediated vasodilatation an increase in vasoconstriction and subsequently leading to hypertension. Also oxidation of LDL leading to atherogenisis can lead to hypertension. In our study amongst all four antioxidants garlic showed significant reduction in systolic blood pressure in cadmium induced hypertension

Blood Analysis:

The decrease in hematological parameters (RBCs,and Hb) is in agreement with Karmakar *et al.* (2000).[64]The significant reduction in RBCs indicated the fact that Cd adversely affected the erythropoiesis, Recently, Luchese *et al.* (2007) demonstrated that Cd^{2+} inhibits aminolevulinate dehydratase (ALAD), who catalyses the asymmetric condensation of two molecules of aminolevulinic acid to porphobilinogen in the initial steps of heme biosynthesis.[65].

Biochemical Assays:

The data presented in this work show significant changes in the activities of all examined antioxidant defense enzymes in blood during the treatment of rats with cadmium.

It is known that cadmium induces oxidative stress by producing superoxide anion radical and nitric oxide and it is reasonable to expect an increased activity of SOD and Catalase.

In our study garlic and curcumin in combination decreased the activity of SOD and Catalase significantly as compared to other treatment groups.

CONCLUSION

Our study suggests that generation of reactive oxygen species (ROS) by cadmium is responsible for alterations in prooxidant - antioxidant balance and induction of oxidative stress. The comparative study demonstrates that garlic is more potent antioxidant than vitamin C, vitamin E, and curcumin in cadmium induced oxidative stress. The results of our work suggest that garlic acts as a potent antioxidant in combination with curcumin in protection of rats against oxidative stress induced by cadmium.

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