

Research

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Volume 2 : Issue 1

Article Ref. #: 1000TFMOJ2114

Article History

Received: February 1st, 2017

Accepted: April 17th, 2017

Published: April 18th, 2017

Citation

Renuka M, Suneetha Y, Srinivasulu Reddy M. Cadmium induced oxidative stress in wistar rats: Ameliorative effect of quercetin and *embilica officinalis* plant extracts. *Toxicol Forensic Med Open J*. 2017; 2(1): 26-35. doi: [10.17140/TFMOJ-2-114](https://doi.org/10.17140/TFMOJ-2-114)

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Cadmium Induced Oxidative Stress in Wistar Rats: Ameliorative Effect of Quercetin and *Embilica Officinalis* Plant Extracts

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ABSTRACT

Background: Cadmium is a naturally occurring metal that is widely distributed throughout the biosphere. The present investigation is aimed to assess the antioxidant potential of Quercetin and *Embilica officinalis* (amla) extracts, which are reported to have a wide range of pharmacological properties including the efficacy of these selected substances on antioxidants and lipid peroxidation status in the liver tissue of rats during cadmium intoxication.

Methods: After three weeks of cadmium oral administration, certain specific enzymes of the hepatic tissue of Wistar rats were assayed.

Results: After three weeks of cadmium oral administration, certain specific enzymes of the hepatic tissue including aspartate aminotransferase (AST), alanine aminotransferase (ALT), acid phosphatase (ACP), γ -glutamyl transferase (GGT), and lactate dehydrogenase (LDH) were significantly elevated, thus indicating cellular damage. The concentration of lipid peroxidation markers represented by thiobarbituric acid reactive substances (TBARS), lipid hydroperoxides and protein carbonyl contents of the liver tissue were significantly elevated, whereas vitamin C and E levels were found to be significantly reduced. But the activity of the antioxidant enzymes including superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione S-transferase (GST) activities were found to be significantly inhibited.

Conclusion: The results obtained in the present investigation clearly demonstrates the antioxidant potential of Quercetin and *E. officinalis* extract, by ways of decreasing lipid peroxidation against cadmium induced oxidative stress in rats.

KEY WORDS: Quercetin; *Embilica officinalis*; Antioxidant enzymes; Oxidative stress.

ABBREVIATIONS: AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; ACP: Acid phosphatase; GGT: γ -glutamyl transferase; TBARS: Thiobarbituric acid reactive substances; SOD: Superoxide dismutase; CAT: Catalase; GPx: Glutathione peroxidase; GST: Glutathione S-transferase.

INTRODUCTION

Heavy metals are the most dangerous group of anthropogenic environmental pollutants which are highly toxic and persistent in the environment. The major anthropogenic sources of environmental pollution include smelting operations, phosphate fertilizers, pigments, cigarette smoke, automobiles, etc., which constitutes almost 90% of cadmium levels in the environment leading to its bioaccumulation in humans and animals. Cadmium has been reported to exert deleterious effects in organisms at low levels of exposure,¹ which are nephrotoxic, cytotoxic, genotoxic and carcinogenic.²⁻⁴

The literature is replete with the reports on metal induced oxidative stress that has been recently implicated in the pathogenesis of metal toxicities. It is established that metals

can generate reactive oxygen species (ROS), which in turn overwhelms the cell's innate antioxidant defences leading to oxidative stress. Studies have demonstrated that cadmium stimulates free radical production, resulting in oxidative damage to lipids, proteins and DNA, eventually resulting in membrane damage, protein dysfunction and DNA damage. These conditions can further culminate into pathological conditions both in humans and animals,^{4,5} including diabetes, cardiovascular diseases, cancer, etc. Antioxidants are compounds that mop up the free radicals and prevent cellular damage.⁶⁻¹¹ Although, several chelating agents and antagonistic compounds are available which helps to reduce cadmium toxicity, some of them are burnt with undesirable side effects. Due to the intrinsic limitations and variability of the effectiveness of heavy metal chelating agents, research is being undertaken to apply cadmium intoxication therapy with the development of new therapeutic agents especially from flavonoids and other related phytometaterials. In the recent years, phytometaterials have been addressed as potent free radical scavengers and have attracted tremendous interest as possible therapeutics, against free radical material diseases.^{12,13} The efficacy of a wide variety of phytocompounds has been reported to have a wide spectrum of pharmacological properties including anti-inflammatory,¹⁴ anti-allergic,⁹ anti-tumour³ and anti-oxidant characteristics.¹⁵⁻¹⁷ With this premise, the present investigation was designed to study the ameliorative effect of phytocompounds like Quercetin and *Embilica officinalis* (amla) extracts on Cadmium-induced oxidative stress in Wistar rats.

MATERIALS AND METHODS

Quercetin ($C_{15}H_{14}O_9$; MW 338.27; 2-(3,4-dihydrophenyl)-3,5,7-trihydroxy-4H-chromen-4-one; a plant derivative of Rutin) and shade dried amla fruit were obtained from the local market. Ten grams of dried amla fruits were ground to powder form in an electric grinder and is dissolved in 50 ml of distilled water and was room evaporated for further use in experimentation.

Experimental Animals

Male albino rats weighing 100 ± 5 g were used for the present investigation. The animals were fed with commercial standard pellet diet (Lipton India Ltd, Mumbai, MH, India) and had free access to water under well ventilated conditions of 12 h (day:night). The animals were acclimatized to laboratory conditions (temperature 24 ± 2 °C) for at least one week before the experiments, were performed, maintained on standard diet, and given free access to food and water. The animals were housed in specially designed plastic rodent cages in the animal house in Sri Venkateswara University, Tirupati, Andhra Pradesh. This study and all procedures were approved by the Animal Care and Bio-ethical Committee. Cadmium chloride was dissolved in distilled water and administered orally to rats. The animals were divided into six groups each comprising of six rats. The experimental period was three weeks (21 days) and the group were as follows:

Group 1: Control group (kept under standard laboratory conditions);
Group 2: Received cadmium chloride (5 mg/kg Body weight/day);
Group 3: Received cadmium chloride (5 mg/kg Body weight/day) along with Quercetin (100 mg/kg Body Weight/day orally) prior to the administration of $CdCl_2$;
Group 4: Received Quercetin (100 mg/kg Body weight/day orally) alone for 21 days;
Group 5: Received cadmium chloride (5 mg/kg Body weight/day) along with *E. officinalis* extract (150 mg/Kg Body weight/day);
Group 6: Received *E. officinalis* extract (150 mg/kg Body weight/day orally) alone for 21 days.

At the end of the experimental period, all the animals were starved overnight and then they were killed by cervical decapitation with mild ether anaesthesia. The liver tissue was dissected out, weighted and washed using chilled saline solution. For all the enzyme assays, the liver tissue was preserved at $-80^\circ C$ till further analysis was performed.

Biochemical Analysis

Liver tissue homogenates from each experimental group were prepared in accordance with the standardized protocols. The methodologies adopted in the present investigation have been presented in the following tabulation:

1	Lipid peroxidation (Thiobarbituric acid reactive substances TBARS)	Fraga et al ¹⁸
2	Lipid hydroperoxides	Jiang et al ¹⁹
3	Protein carbonyl content	Levine et al ²⁰
4	Reduced Glutathione (GSH)	Ellman ²¹
5	Vitamin C	Omaye et al ²²
6	Vitamin E	Desai ²³
7	Superoxide dismutase (SOD)	Kakkar et al ²⁴
8	Catalase (CAT)	Sinha ²⁵
9	Glutathione peroxidase (GPx)	Rotruck et al ²⁶
10	Glutathione S-transferase (GST)	Habig et al ²⁷
11	Aspartate amino transferase (AST)	Reitman & Franke ²⁸
12	Alanine amino transferase (ALT)	Reitman & Franke ²⁸
13	Reduced GSH assay	Moron et al ²⁹
14	Alkaline phosphatase (ALP)	Bodansky ³⁰
15	Lactate dehydrogenase (LDH)	Srikanthan & Krishnamoorthy ³¹
16	γ -Glutamyl transferase (GGT)	Spectrophotometrically
17	MDA	Ohkawa et al ³²
18	Protein	Lowry et al ³³

All the chemicals and reagents used in the present study were analytical grade and were obtained from Sigma Chemical Company, Himedia Laboratories and local firms.

Statistical Analysis

The results were expressed as the Mean $\pm$ SD of six individual observations. The data was subjected to statistical analysis by ANOVA by using Statistical Packages (SPSS) Program.

RESULTS

The results presented in Table 1, indicate that the body weight of the wistar rats was increased during the three weeks of experimental period in both control and experimentally treated rats of all groups. The liver tissue weights were also shown to be increased, both in control and experimentally treated rats of all groups. Table 2, presents the changes in the level of lipid peroxidation products including thiobarbituric acid reactive substances (TBARS), lipid hydroperoxides and protein carbonyl contents were significantly higher, whereas the levels of vitamin C and vitamin E were significantly lower in both control and experimentally treated rats (Figure 1). The administration of Quercetin and *E. officinalis* plant extracts significantly decreased the levels of lipid peroxidation products, lipid hydroperoxides and protein carbonyl contents in liver tissue of experimentally treated rats. Table 3, shows the levels of serum hepatic marker enzymes in control and experimentally treated rats of all groups. The oral administration of cadmium for 21 days induced several

abnormalities in the levels of serum hepatic marker enzymes. The levels of serum hepatic marker enzymes such as aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline Phosphatase (ALP), lactate dehydrogenase (LDH), and γ -glutamyl transferase (GGT) were found to be significantly increased in cadmium treated rats. Consequently, the administration of quercetin and plant extract of *E. officinalis* significantly decreased the activity levels of serum hepatic marker enzymes compared to cadmium treated rats (Table 3). Table 4, presents changes in hepatic antioxidants including superoxide dismutase (SOD), catalase (CAT), glutathione peroxide (GPx), glutathione s-transferase (GST) and reduced glutathione (GSH) which were found to be increased significantly with the administration of Quercetin and *E. officinalis* plant extract to the cadmium-treated experimental rats (Figures 2 and 3).

DISCUSSION

Cadmium is one of the main environmental and occupational pollutants in the industrialized countries. Exposure to cadmium is linked with serious health hazards and unlike other heavy metals, is unable to generate free radicals by itself; however, reports have indicated that the superoxide radical, hydroxyl radical and nitric oxide radicals could be generated indirectly.³⁴ A study by Watnabe et al¹⁰ showed the generation of non-radical hydrogen

Table 1: Changes in Body Weight (g) and Liver Tissue (g) During Cadmium Induced Toxicity in Rats.

Groups	Body weight (g)		Liver weight (g)	
	Initial	Final	Initial	Final
Group-I	100.25 $\pm$ 2.88 PDC	122.45 $\pm$ 3.12 (+22) $p < 0.05$	6.45 $\pm$ 0.28 PDC	7.08 $\pm$ 0.31 (+9.8) $p < 0.05$
Group-II	100.08 $\pm$ 2.84 PDC $p > 0.05$	120.12 $\pm$ 2.75 (+20) $p > 0.05$	6.45 $\pm$ 0.24 PDC $p > 0.05$	6.59 $\pm$ 0.28 $p < 0.05$
Group-III	100.49 $\pm$ 2.72 PDC $p > 0.05$	122.58 $\pm$ 3.15 (+22) $p > 0.05$	6.45 $\pm$ 0.25 PDC $p > 0.05$	6.89 $\pm$ 0.29 (+6.8) $p > 0.05$
Group-IV	100.54 $\pm$ 2.59 PDC $p > 0.05$	123.19 $\pm$ 3.42 (+ 23) $p > 0.05$	6.45 $\pm$ 0.26 PDC $p > 0.05$	7.13 $\pm$ 0.24 (+10.5) $p > 0.05$
Group-V	100.72 $\pm$ 2.89 PDC $p > 0.05$	120.13 $\pm$ 2.82 (+19) $p > 0.05$	6.45 $\pm$ 0.27 PDC $p > 0.05$	6.94 $\pm$ 0.28 (+8) $p > 0.05$
Group-VI	100.45 $\pm$ 2.85 PDC $p > 0.05$	123.34 $\pm$ 3.015 (+23) $p > 0.05$	6.45 $\pm$ 0.26 PDC $p > 0.05$	7.15 $\pm$ 0.26 (+11) $p > 0.05$

Values are Mean $\pm$ SD of six individual observations.
PDC: Percent Deviation over Control
Group-I : Control
Group-II : Received Cadmium Chloride 5 mg/Kg Body weight /day
Group-III : Received Cadmium Chloride 5 mg/Kg Body weight/day along with Quercetin 100 mg/Kg Body weight/day
Group-IV : Received Quercetin (100 mg/Kg Body weight/day orally for 21 days.
Group-V : Received Cadmium Chloride 5 mg/Kg Body weight/day along with *Embilika officinalis* extract (150 mg/Kg Body weight/day)
Group-VI : Received *Embilika officinalis* extract alone for 21 days.(150 mg/Kg Body weight/day orally

Table 2: Changes in the Lipid Peroxidation levels in the Tissue of Rats in Different Treatments.

Groups	TBARS ^a	Hydroperoxide ^a	Protein Carbonyls ^b	VitaminC ^c	VitaminE ^c
Group-I	2.88±0.12	114.42±6.13	204.18±10.12	2.48±0.12	2.13±0.14
Group-II	6.13±0.34 (+112) <i>p</i> <0.001	274.14±10.12 (+90) <i>p</i> <0.001	415.42±15.44 (+103) <i>p</i> <0.001	0.94±0.05 (+163) <i>p</i> >0.05	1.11±0.10 (+92) <i>p</i> <0.001
Group-III	3.72±0.25 (+30) <i>p</i> >0.05	175.13±8.12 (+54) <i>p</i> <0.001	315.49±10.75 (+54) <i>p</i> <0.001	2.19±0.15 (+13) <i>p</i> >0.05	1.45±0.12 (+47) <i>p</i> <0.001
Group-IV	3.13±0.22 (+9) <i>p</i> >0.05	134.18±7.42 (+17) <i>p</i> <0.05	249.45±10.13 (+22) <i>p</i> <0.001	2.77±0.18 (+12) <i>p</i> >0.05	1.94±0.11 (+10) <i>p</i> <0.01
Group-V	4.13±0.28 (+43) <i>p</i> >0.05	163.42±6.77 (+42) <i>p</i> <0.001	285.77±8.85 (+40) <i>p</i> <0.001	2.11±0.12 (+18) <i>p</i> >0.05	1.58±0.12 (+26) <i>p</i> <0.001
Group-VI	3.05±0.22 (+6) <i>p</i> >0.05	128.42±6.75 (+12) <i>p</i> >0.05	238.41±7.77 (+17) <i>p</i> <0.001	2.68±0.12 (+8) <i>p</i> >0.05	1.96±0.12 (+8) <i>p</i> <0.05

Values are Mean±SD of six individual observations.

Values in parenthesis are Percent deviation over their respective Control

Group-I : Control

Group-II : Received Cadmium Chloride 5 mg/Kg Body weight /day)

Group-III : Received Cadmium Chloride 5 mg/Kg Body weight/day along with Quercetin 100 mg/Kg Body weight/day

Group-IV : Received Quercetin (100 mg/Kg Body weight/day orally for 21 days.

Group-V : Received Cadmium Chloride 5 mg/Kg Body weight/day along with *Embilika officinalis* extract (150 mg/Kg Body weight/day)

Group-VI : Received *Embilika officinalis* extract alone for 21 days. (150 mg/Kg Body weight/day orally)

^amM/100 g weight of tissue

^bnmoles/mg protein

^cµmoles/g weight of tissue

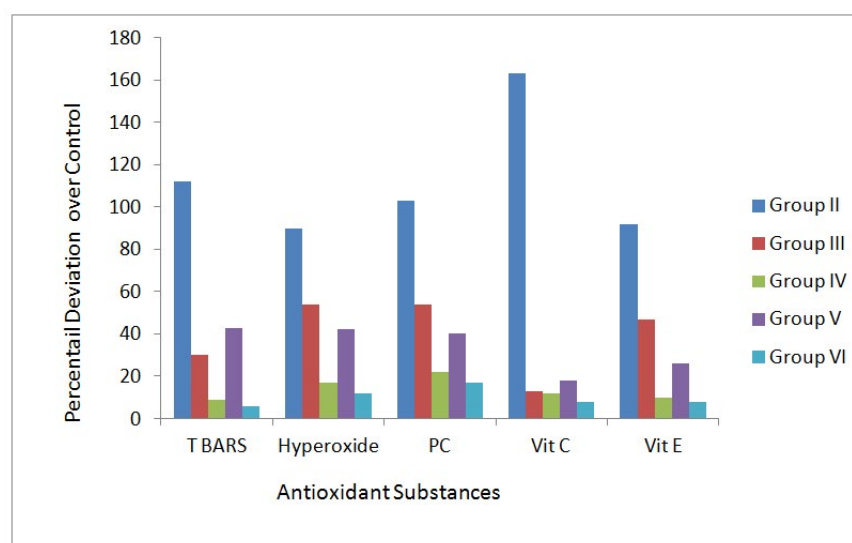
Figure 1: Changes in Percent Deviation Over Control Values of Selected Antioxidant Substances in Different Groups of rats Treated with Cadmium.

Table 3: Changes in the Activities of Hepatic Marker Enzymes during Cadmium Induced Toxicity.

Groups	AST ^a	ALT ^a	ACP ^b	LDH ^c	GGT ^d
Group-I	65.72±2.45	39.42±1.94	102.45±3.78	145.44±6.42	102.41±6.75
Group-II	95.14±3.78 (+45) <i>p</i> <0.001	72.41±2.74 (+84) <i>p</i> <0.001	175.48±5.94 (+72) <i>p</i> <0.001	184.49±6.18 (+27) <i>p</i> <0.001	175.48±5.72 (+72) <i>p</i> <0.001
Group-III	175.48±5.72 (+72) <i>p</i> <0.001	46.43±1.78 (+18) <i>p</i> <0.001	159.42±5.12 (+56) <i>p</i> <0.001	174.58±5.13 (+21) <i>p</i> <0.001	168.41±5.15 (+65) <i>p</i> <0.001
Group-IV	68.49±2.75 (+5) <i>p</i> <0.001	42.49±2.05 (+8) <i>p</i> <0.001	128.49±4.75 (+26) <i>p</i> <0.001	155.45±4.88 (+7) <i>p</i> <0.001	142.41 ± 3.99 (+40) <i>p</i> <0.001
Group-V	76.74±3.49 (+17) <i>p</i> <0.001	42.74±2.05 (+9) <i>p</i> <0.001	165.48±5.15 (+62) <i>p</i> <0.001	165.48±5.15 (+62) <i>p</i> <0.001	172.43±5.42 (+69) <i>p</i> <0.001
Group-VI	70.43±3.45 (+8) <i>p</i> <0.001	40.11±1.42 (+2) <i>p</i> <0.05	125.44±5.12 (+23) <i>p</i> <0.001	150.42±4.88 (+4) <i>p</i> <0.001	138.74±4.88 (+36) <i>p</i> <0.001

Values are Mean±SD of six individual observations.

Values in parenthesis are Percent deviation over their respective Control

Group-I : Control

Group-II : Received Cadmium Chloride 5 mg/Kg Body weight /day)

Group-III : Received Cadmium Chloride 5 mg/Kg Body weight/day along with Quercetin 100 mg/Kg Body weight/day

Group-IV : Received Quercetin (100 mg/Kg Body weight/day orally for 21 days.

Group-V : Received Cadmium Chloride 5 mg/Kg Body weight/day along with *Embliika officinalis* extract (150 mg/Kg Body weight/day)Group-VI : Received *Embliika officinalis* extract alone for 21 days. (150 mg/Kg Body weight/day orally)^aμmoles of Pyruvate liberated/mg protein/hr^bμmoles of Pi liberated/mg protein/hr^cμmoles of Formazon formed/mg protein/hr^dunits/mg protein/hr**Table 4:** Changes in the Antioxidant Levels in the Liver Tissue of Control and Experimental Rats.

Groups	SOD ^a	CAT ^a	GPx ^a	GST ^b	GSH ^c	MDA ^d	LPO ^d
Group-I	16.18±0.72	105.42±5.38	18.45±0.38	42.45±1.38	75.18±3.42	6.78±0.25	12.14±0.35
Group-II	10.13±0.62 (+38) <i>p</i> <0.001	60.38±2.49 (+43) <i>p</i> >0.05	13.12±0.29 (+29) <i>p</i> <0.001	28.34±1.04 (+34) <i>p</i> <0.001	53.44±2.42 (+29) <i>p</i> >0.05	18.14±0.78 (+167) <i>p</i> >0.05	26.72±1.28 (+120) <i>p</i> <0.001
Group-III	12.42±0.58 (+24) <i>p</i> <0.001	70.13±2.84 (+34) <i>p</i> >0.05	14.15±0.32 (+24) <i>p</i> <0.001	32.41±1.04 (+24) <i>p</i> <0.001	60.13±2.41 (+20) <i>p</i> >0.05	13.19±0.68 (+94) <i>p</i> >0.05	16.42±0.72 (+36) <i>p</i> <0.001
Group-IV	14.18±0.59 (+13) <i>p</i> <0.001	88.42±3.49 (+17) <i>p</i> >0.05	16.89±0.34 (+9) <i>p</i> <0.05	40.78±1.19 (+4) <i>p</i> <0.001	74.79±2.55 (+05) <i>p</i> >0.05	10.45±0.66 (+55) <i>p</i> >0.05	13.77±0.58 (+14) <i>p</i> <0.01
Group-V	13.38±0.59 (+18) <i>p</i> <0.001	95.14±3.25 (+10) <i>p</i> >0.05	15.42±0.34 (+17) <i>p</i> <0.001	34.84±1.42 (+18) <i>p</i> <0.001	62.44±2.38 (+17) <i>p</i> >0.05	16.43±0.72 (+42) <i>p</i> >0.05	17.49±0.75 (+44) <i>p</i> <0.001
Group-VI	16.42±0.59 (+2) <i>p</i> >0.05	112.14±4.98 (+7) <i>p</i> >0.05	19.05±0.42 (+4) <i>p</i> >0.05	43.49±1.13 (+3) <i>p</i> <0.01	77.49±2.59 (+4) <i>p</i> >0.05	8.79±0.68 (+30) <i>p</i> >0.05	14.15±0.32 (+17) <i>p</i> <0.001

Values are Mean±SD of six individual observations.

Values in parenthesis are Percent deviation over their respective Control

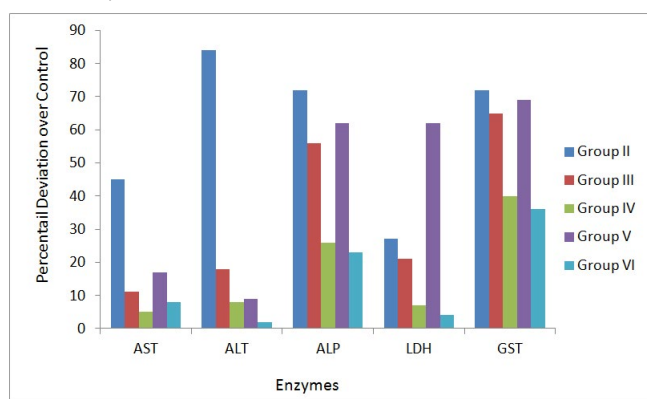
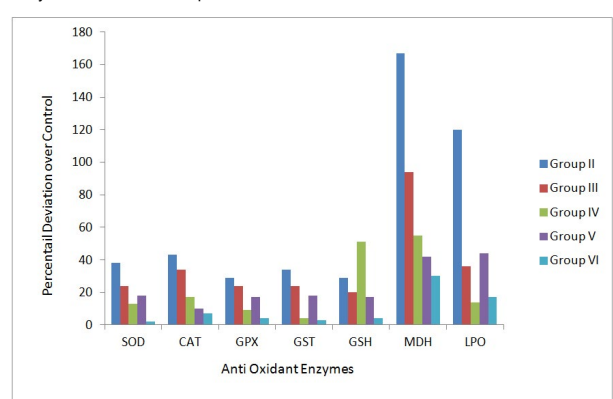
Group-I : Control

Group-II : Received Cadmium Chloride 5 mg/Kg Body weight /day)

Group-III : Received Cadmium Chloride 5 mg/Kg Body weight/day along with Quercetin 100 mg/Kg Body weight/day

Group-IV : Received Quercetin (100 mg/Kg Body weight/day orally for 21 days.

Group-V : Received Cadmium Chloride 5 mg/Kg Body weight/day along with *Embliika officinalis* extract (150 mg/Kg Body weight/day)Group-VI : Received *Embliika officinalis* extract alone for 21 days. (150 mg/Kg Body weight/day orally)

Figure 2: Changes in Percent Deviation Over Control Values of Selected Enzymes in Different Groups of Rats Treated with Cadmium.**Figure 3:** Changes in Percent Deviation Over Control Values of Selected Antioxidant Enzymes in Different Groups of Rats Treated with Cadmium.

peroxide, which by itself became a significant source of free radicals *via* the Fenton reaction. The replacement of iron and copper by cadmium from a number of cytoplasmic and membrane proteins like ferritin, in turn would release and increase the concentration of unbound iron or copper ions. These free ions participate in causing oxidative stress *via* the Fenton reactions.³⁵ The generation of reactive oxygen species (ROS) has been attributed to cadmium induced pathotoxicity that leads to a disruption in the pro-oxidant/anti-oxidant balance, a condition coined as oxidative stress. A quest for safe phytoconstituents as antioxidants has been the main stay of recent research in phytotherapy to alleviate the pathologies that are associated with oxidative cellular damage. Studies have indicated that cadmium is an inducer of cell oxidative stress, either in a variety of cell culture systems³⁶ or *in vivo* models through all routes of exposure.³⁷ Lipid peroxidation is one of the consequences of oxidative damage and is found to play an important role in the toxicity of cadmium.³⁸⁻³⁹ Cadmium-induced oxidative stress is caused due to the production of hydroxyl radicals, superoxide anions, nitric oxide and hydrogen peroxide.⁴⁰⁻⁴²

The hepatic TBARS content was shown to be significantly increased in the cadmium-treated rats which may be attributed to excessive formation of free radicals which subsequently led to changes in the biological macromolecules.^{4,5,37} Several authors reported that lipid peroxidation is a sensitive marker of cadmium hepatic toxicity in vertebrate species.^{4,5} In the present study, cadmium treatment resulted in an excessive production of free radicals such as hydroxyl radical, superoxide radical, peroxyl radical and hydrogen peroxide. All the above mentioned radicals have a great potential to react rapidly with lipids, which in turn leads to lipid peroxidation.⁴ Decomposition products of lipid hydroperoxide such as malonaldehyde and 4-hydroxynonenal, can cause chaotic cross-linkage with proteins and nucleic acids, which plays an important role in the process of carcinogenesis. In the present investigation, hepatic lipid peroxidation (LPO) activities show significant increase due to cadmium intoxication. Furthermore, extensive damage to tissues in a free radical mediated LPO results in membrane damage and subse-

quently decrease the membrane fluid content. Consequently, the treatment of cadmium exposed rats to both Quercetin and plant extract of *E. officinalis* resulted in a significant decrease in the levels of TBARS, hydroperoxides and protein carbonyl contents compared with cadmium treated rats. This observation clearly demonstrates that the free radical scavenging efficiency of both Quercetin and *E. Officinalis* extract may be associated with the presence of two hydroxyl groups in the β -ring of its molecule.⁴¹ The presence of polyunsaturated substitution on the β -ring of Quercetin together with 2,3 double bond, a free 3-hydroxyl substitution and a 4-ketogroup confer potent anti-peroxidative properties of Quercetin.⁴² Several authors also reported that LPO was either blocked or prevented due to administration of Quercetin.⁴³ Thus, Quercetin effectively quenches free radicals, inhibits LPO and protects the hepatic tissue from cadmium-induced oxidative damage. Literature has reported that the antioxidative potential of amla fruit has been attributed to its high vitamin C content. However, recent studies report the presence of bioactive tannoid principles in the fruit, comprising of emblicanin A, emblicanin B, punigluconin and pedunculagin, which have been shown to exhibit antioxidant activity *in vitro* and *in vivo* and are responsible for preventing the oxidation of ascorbic acid.⁴⁴

The results obtained in the present investigation showed a marked increase in lipid peroxidation in the hepatic tissue of rats after cadmium treatment, which is in consensus with the previous reports on cadmium-induced oxidative stress in rats,^{45,46} in poultry⁴⁷ and in fish.⁴⁸ In addition to cellular lipids, studies have shown that cellular proteins may also be affected by radical accumulation. It is well established that cadmium does not directly generate free radicals like other heavy metals, but it is capable of generating non-radical hydrogen peroxide that eventually acts as a source of free radicals through the Fenton chemistry. The formation of carbonyl derivatives of proteins is suggested to be a useful measure of oxidative damage to proteins. The carbonyl derivatives of proteins may result from oxidative modification of amino acid side chains and reactive oxygen-mediated rapid cleavage.⁴⁹ Stolis and Bagchi³⁷ have reported that the primary target of the oxygen radical attack,

promoted by cadmium, is represented on cellular proteins. The results obtained in the present investigation also showed an increased protein carbonyl content in the cadmium treated rat liver tissue. When cadmium-exposed rats were subjected to treatment with both Quercetin and *E. officinalis* extracts, protein carbonyl contents were significantly decreased, which may be attributed to the antioxidant properties of the selected compounds in the present study. Both the compounds selected in the present study, by its free radical scavenging action, prevents the attack of free radicals on amino acids and this reduces the production of the protein carbonyl groups thus driving cadmium intoxication of rats.

GSH, is a tripeptide (L- α -glutamylcysteinol glycine), an antioxidant and a powerful nucleotide, critical for cellular protection such as detoxification of ROS, conjugation with xenobiotics, excretion of toxic molecules and control of inflammatory cytokine cascade.⁵⁰ General depletion of GSH in the tissues of animals leads to the impairment of cellular defence against ROS and may result in peroxidative tissue injury. In the present investigation, a significant reduction in the hepatic reduced glutathione (GSH) levels in the cadmium-treated rats and reduced levels of Vitamin C and Vitamin E in the liver tissue, portrays the implications of cadmium-induced stress condition in rats.

Vitamin C is the most potent water soluble antioxidant that scavenges a wide variety of ROS and nitrogen, including superoxide radical.⁵¹ Vitamin E, is a major chain breaking antioxidant, found in the lipid phase of membrane, and acts as a powerful terminator of LPO.⁵² In the present investigation, the non-enzymatic antioxidants levels were found to be significantly depleted in cadmium-intoxicated rats, signifying that increased levels of free radical generation by cadmium which is effectively managed by both Vitamin C and E, are considered as the most effective free radical scavengers. Several authors also reported reduced production of hepatic antioxidants including GSH, Vitamin C and Vitamin E during cadmium intoxication in rat.⁵³

The results obtained in the present study demonstrated that cadmium exposure showed an increased activity level of markers such as ALT, AST, ALP in the liver tissue of rats. The increase in these enzymatic activities may be due to cellular necrosis of hepatocytes, which causes increase in the permeability of the cell. Lactate dehydrogenase (LDH) is an index of cell damage including hepatotoxicity and the endothelial disruption in blood vessel. In the present study, increased level of LDH was substantially detected in all cadmium-treated rats, compared with the control group. This result is suggestive of the beginning of cytolysis, which is a possible indication of membrane damage including the endothelial membranes of blood vessels. This disruption of endothelial membrane, directly or indirectly, as reported earlier, includes the generation of reactive oxygen species in endothelial cells.

Generally under normal conditions, the animals tend to maintain a balance between generation and neutralization of

ROS in the tissues. But, when the organisms are subjected to Xenobiotic stress, the rate of production of ROS including O_2^- , H_2O_2 , $OH^\cdot$, $ROO^\cdot$, exceeds their scavenging capacities. All the organisms have their own cellular antioxidant defence system composed of both enzymatic and non-enzymatic components. Enzymatic antioxidant pathway consists of SOD, CAT and GPx. Superoxide anion O_2^- is dismutated by SOD to H_2O_2 , which is reduced to water and molecular oxygen by CAT or is neutralized by GPx, that catalyses the reduction of H_2O_2 to water and organic peroxide to alcohols using GSH as a source of reducing equivalent. GR regenerates GSH from oxidized glutathione (GSSG), which is a scavenger of ROS as well as a substrate for other enzymes. GST conjugates xenobiotics with GSH for exclusion. One non-enzymatic pathway components consists of small organic molecules such as β -carotene, GHS, Vitamin C and Vitamin E.⁵⁴ Some of these parameters could serve as stress indicators in animals when exposed to environmental pollutants. Superoxide dismutase is considered to be its first line of defence against the deleterious effects of oxygen radicals in the cells and it scavenges ROS by catalysing the dismutation of superoxide to H_2O_2 . Several authors also reported that SOD activities was considerably reduced during cadmium intoxication.⁵⁵ The inhibition of SOD activity may be associated with an increased flux of superoxide into the cellular compartments which may be the reason for decreased lipid peroxide indices in the present investigation. Catalase acts as a preventive antioxidant and plays an important role in protection against the deleterious effects of LPO, GPx and GST. Several authors have also reported that hepatic antioxidant enzyme activities were significantly reduced during cadmium intoxication, which may be due to excessive production of ROS. Glutathione peroxidase is also a first line of defence against oxidative damage due to H_2O_2 or lipid hydroperoxides, thus protecting the membrane from oxidative damage. GST is considered to be the second line of defence against xenobiotics on account of its direct conjugation with the expense of GST, both being associated with glutathione dependent activities. In the present investigation, both GST and GPx activities were significantly reduced during cadmium intoxication due to excessive production of H_2O_2 by Cadmium. Thus, the analysis of antioxidant status in the present study, indicates that the levels of both non-enzymatic and enzymatic antioxidants were significantly reduced due to cadmium reduced oxidative stress. Administration of both Quercetin and *E. officinalis*, extract significantly modulates the antioxidant status in the liver tissue of rats, suggesting the enhancing effect of the above substances on cellular antioxidant defences. The antioxidant role of the above selected substances may include the following interventions- scavenging of O_2^- , $OH^\cdot$ peroxy radical and peroxyxynitrite.⁴ Several authors also reported that the above substances were known to prevent DNA damage during cadmium intoxication, enhanced the GSH dependent protection and prevented the depletion of thiols during oxidative stress.⁵ Thus, it was indicated that both the substances selected might have played a role in quenching the free radicals, inhibiting LPO and ultimately reducing the build up of antioxidants during Cadmium intoxication.

CONCLUSION

The results of the present study clearly indicate that heavy metal cadmium causes oxidative stress in rats and concluded that, Quercetin and phytonutrient rich plant extract from *E.officinalis* possess antioxidant property, which may be attributed to its protective action on lipid peroxidation and to the enhancing effect on cellular antioxidant defences which might be further implicated in its protective role against the damage due to cadmium toxicity in rats. The plant extracts selected in the present investigation were known to contain rich phytochemicals that bring about free radical quenching effect. Thus, the use of Quercetin and phytonutrients to counter oxidative damage serves as a therapeutic approach to restore normal body function. The current results also contribute towards improving our knowledge on the possible development of oxidative stress induced by cadmium treatment in rats indicating a possible role of antioxidant systems in the prevention of induced damage in rats.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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