EFFECT OF SILYMARIN ON CADMIUM INDUCED TOXICITY IN RATS

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ABSTRACT

Introduction: Cadmium is an environmental pollutant that affects various organs in humans and experimental animals especially by inducing oxidative stress. Oxidative stress is a common mechanism contributing to initiation and progression of hepatic damage in a variety of liver disorders. As a result, there is an enormous demand for the development of agents with potent antioxidant effect. Silymarin is extracted from the herb milk thistle, is widely known as antioxidant, hepato protective and anti-carcinogenic. **Objective:** The aim of this present study is to assess the efficacy of silymarin as a hepato protective and antioxidant against cadmium induced oxidative damage in the liver of rats. **Methods:** In an experimental study, 24 male Albino rats were divided into four equal groups as follows: Group A: The Control group (normal water), Group B: Cadmium treated (10mg/kg/ 30 days, orally), Group C: Silymarin treated (40mg/Kg/ 30 days, orally), Group D Cadmium plus Silymarin (10mg/kg +40mg/kg 30 days, orally). The lipid peroxidation, (TBARS – Thiobarbituric acid) and antioxidant status (Super oxide dismutase (SOD), Catalase (CAT), Glutathione peroxidase (GPx) and reduced Glutathione (GSH)) were evaluated for the control and experimental rat's liver tissue. **Results:** The result of this study specify that after the oral administration of cadmium, there is a significant increase in the level of lipid peroxidation marker (TBARS) and decrease in the level of antioxidant enzymes, like SOD, CAT, GPx and GSH in liver when compared to normal Control. Oral administration of Silymarin

significantly reduced the level of lipid peroxidation marker (TBARS) and also restored the antioxidant defence in the liver when compared to cadmium treated rats. **Conclusion:** The result from the present study indicates that Silymarin exhibited a remarkable protective effect against cadmium induced oxidative hepatic injury in rats.

KEYWORDS: Cadmium chloride, Silymarin, Lipid peroxidation, antioxidant enzymes, oxidative stress.

INTRODUCTION

Heavy metals are found in increasingly hazardous concentration in Air, Food and water. The increase in pollution is a major and global problem. This is due to the use of toxic chemicals or xenobiotics or by certain synthetic compounds such as heavy metallic compounds.[1]

Cadmium is a naturally acquiring group II B element found in the earth's crust. Approximately 30,000 tons of cadmium is released into the atmosphere each year, with an estimated 4,000 to 13,000 tons coming from human activities. Since Cd^{2+} does not breakdown in the environment, the risk of human exposure is increasing constantly.[2] The importance of cadmium as an industrial and Environmental pollutant has become increasingly apparent in recent years; it currently ranks 7th on the Agency, for Toxic Substances and Disease Registry (ATSDR)/ EPA list of hazardous substances.[3] During the past century, Cd and its compounds have been used extensively in the smelting and electroplating industries, and in the manufacturing of batteries, dyes, paints and plastics. Large amount of Cd have also been released into the environment through the burning of refuse materials that contains Cd and through the use of Cd- contaminated sludge and phosphate salts as fertilizers.[4] Cadmium exposure has been shown to have adverse effects on a variety of tissues and is linked with various chronic diseases in both animals and humans. Cadmium can cause osteoporosis, anaemia, non-hypertrophic emphysema, irreversible renal tubular injury, eosinophilia, chronic rhinitis, hepatic damage, central nervous system injury, hyper tension, atherosclerosis and cardiomyopathy.[5]

Cadmium may induce oxidative damage in different tissues by enhancing peroxidation of membrane lipids in tissues and altering the antioxidant defense system of the cells. This defense system includes the glutathione (GSH), and the enzymes Catalase (CAT), Super oxide dismutase (SOD) and Glutathione peroxidase (GPx).The peroxidative damage to the cell membrane may cause injury to the cellular components due to the interaction of metal

ions with the cell organelles.[6] Cadmium depletes glutathione and protein bound sulfhydryl groups resulting in enhanced production of reactive oxygen species such as super oxide ions, hydroxyl radical and hydrogen peroxides. These reactive oxygen species result in increased lipid peroxidation.[7] Biological compounds along with antioxidant properties added for the fortification of cells and tissues against harmful effects of reactive oxygen species and other free radicals. Protective Agents from plant origin with antiperoxidative and antioxidant properties play an important role in protecting the liver against toxicity.[8]

Although several chelating agents and antagonists are established to reduce the Cd toxicity. Some of them are burned with undesirable side effects. Due to the intrinsic limitation and variability of efficacy of heavy metal chelating agents, cadmium intoxication therapy is looking for the development of new therapeutic agents with different mode of actions especially from phytochemicals.[9]

Now a days, plant extract herbal medicines are being widely used to treat extensively many varieties of clinical diseases. More attention has been paid to the protective effects of natural antioxidant against chemically induced toxicities.[10]

Silymarin, a polyphenolic flavonoid isolated from the seeds of the milk thistle (Silybum marianum), primarily consists of four isomeric compounds of the active flavanoligans: silychristin, Silydianin, silibin and isosilibin. It has been reported as having multiple pharmacological activities including antioxidant, hepatoprotectant, anti-inflammatory, antibacterial, antiallergic, antimutagenic, antiviral, antineoplastic, antithrombotic agents and vasodilatory actions.[11] Silymarin have also been used as a natural remedy for liver diseases. Results of studies in experimental animal model suggest that silymarin has a broad spectrum of the hepato protective effects. It protects animals against the multiple type of experimental liver injury such as carbon tetrachloride, acetaminophen, ethanol, iron overload, bile obstruction and amanita mushroom poisoning. Some positive result have been reported in humans; indeed silymarin has been claimed for the clinical applications in the treatment of viral hepatitis, fatty liver cirrhosis, and radiation toxicity due to its antioxidative, antilipid proxidative, antifibrotic and even liver regenerating effect.[12]

Silymarin has been reported to act as an excellent antioxidant in scavenging free radicals and inhibiting lipid peroxidation there by protecting cells against oxidative stress. It augments the non-enzymatic and enzymatic antioxidant defense systems of cells involving reduced

glutathione, SOD, CAT, GPx. In addition, it protects the liver, brain, heart, and other vital organs from the oxidative damage for its ability to prevent lipid peroxidation and also refills the reduced glutathione levels.[13] The antioxidant effect of silymarin against carbon tetrachloride[14], Arsenic[15], Acetaminophen[16], cisplatin[17] and thioacetamide[18] induced toxicity in rats have been studied. However the antioxidant and protective effect of silymarin against cadmium induced toxicity in respect to antioxidant status in liver tissues remains unexplored.

Therefore the present study has been carried out to delineate its antioxidant potential against cadmium induced oxidative damage especially in the liver tissue of rats.

MATERIALS AND METHODS

Chemicals

Cadmium chloride (Cdcl2), thiobarbituric acid (TBA), nitro blue tetrazolium (NBT), reduced glutathione (GSH), 5,5'-dithio-2-nitrobenzoic acid (DTNB) and silymarin were purchased from Sigma-Aldrich chemical co. (St. Louis, MO, USA). The rest of the chemical utilized were obtained from a local firm and were of analytical grade.

Experimental Animals

Adult Male albino rats (Wistar strain) weighing around 175-200 g obtained from the Central animals house Rajah Muthiah Medical College and Hospital(RMMC and H), Chidambaram, Tamilnadu, India were used in this study. They were housed (6 animals per each cage) in polypropylene cage over husk bedding under standard conditions of humidity(55±10%) control temperature(27$^°$±2℃) and light with 12: 12hr L:D cycle (12 hr of light:12hr of darkness) was maintained throughout the study. Animals were free access to water and standard pellet diet (Hindustan lever Ltd; India). They were given a week time to get acclimatized with laboratory conditions. The experiments were conducted according to the ethical norms approved by CPCSEA, and institutional animal ethics committee guidelines.

Experimental Design

After acclimatization the animals were randomly divided into the following four groups of six rats in each group. The duration of the study was 30 days.

Group A: The control

Group B: Cadmium treated

Group C: Silymarin treated

Group D: Cadmium + Silymarin treated.

Animal in the Group A served as control and received only normal tap water and normal diet. The rats in the Group B were orally received cadmium as cadmium chloride (10mg/kg body weight /day) for 30 days. While rat in the group C were treated with silymarin (40mg/kg body weight/day) orally for 30 days. Finally the animals in the group D were treated with cadmium chloride as in the group B (10mg/kg body weight) and silymarin as in the group C (40mg/kg body weight) orally for 30 days. At the end of the experimental period (30 days) the rats were deprived of food over night and sacrificed by the light ether anesthesia. Blood was collected in the tubes for the separation of serum. The liver tissue was dissected out, weighed and washed using chilled saline solution.

Tissue preparation

A known weight of hepatic tissue was minced and homogenized (10%W/V) in ice cold phosphate buffer (0.1M, P^H 7.4) using potter Elvehjem Teflon homogenizer. The homogenate was centrifuged at 5000rpm at 4ºC for 30 minutes and supernatant obtained was used for the assay of various enzymes.

Biochemical Assays

Determination of lipid peroxidation

Lipid peroxidation in the liver was estimated spectro photometrically by measuring thiobarbituric acid reactive substances (TBARS) by the method of Ohkawa *et al.,* 1979.[19]

Determination of enzymatic and Non-enzymatic antioxidant

Super oxide dismutase (SOD) activity was determined by the method of Misra and Fridoivch 1972.[20] The activity of Catalase (CAT) was determined by the method of Sinha 1972.[21] Glutathione peroxidase (GPx) activity was estimated by the method of Rotruck *et al.,* 1973.[22] Reduced glutathione (GSH) activity was assayed by the method of Moron *et al.,* 1979.[23] The proteins was quantified as per lowery *et al.,*[24] using bovine serum albumin as standard.

STASTICAL ANALYSIS

Statistical analysis was performed by one way analysis of variance (ANOVA). Critical difference (CD) was calculated at 1% level according to the method of Gomez *et al.,*[25] and result were expressed as mean ± SD of 6 rats in each group.

RESULT

Effects on hepatic oxidative stress marker

The level of lipid preoxidation in the liver tissue of control and experimental rats are illustrated in the below Table. The level of lipid peroxidation product, thiobarbituric acid reactive substances (TBARS) significantly ($P<0.01$) increased in Cd intoxicated rats (Group B) when compared with control rats (Group A). Simultaneous administration of silymarin along with cadmium (Group D) were significantly decreased ($P<0.01$). The level of TBARS in the liver tissue of rat when compared to rats treated with cadmium alone (Group B).

Table 1: Effect of Silymarin against Cadmium induced toxicity in Liver

(Values of mean ± SEM of 6 rats)

Groups	Treament	TBARS (nmol/mg protein)	SOD* (units/mg protein)	CAT# (units/mg protein)	GPxδ (units/mg protein)	GSH (nmole/mg protein)
A	Normal control	0.97 ± 0.06^a	7.99 ± 0.61^a	71.75 ± 5.46^a	13.33 ± 1.02^a	5.83 ± 0.44^a
B	Cadmium treated	2.71 ± 0.75^b	4.35 ± 0.33^b	35.80 ± 2.73^b	8.82 ± 0.67^b	2.89 ± 0.22^b
C	Silymarin alone	0.94 ± 0.07^a	8.32 ± 0.67^a	72.42 ± 5.52^a	13.58 ± 1.03^a	6.01 ± 0.46^a
D	Cadmium + silymarin	1.40 ± 0.11^c	6.81 ± 0.52^c	64.21 ± 4.89^c	10.31 ± 0.79^c	4.97 ± 0.38^c

* = Units/mg protein (amount of enzyme required to exhibit 50% reduction of NBT)

\# = Units/mg protein (μm of H_2O_2 decomposed /mg protein/min)

δ = Units/mg protein (μg of GSH consumed /min/mg/protein)

Values with same superscript did not differ significantly ($P<0.01$)

Effects on Hepatic Enzymatic and Non-Enzymatic Antioxidants

Changes in the levels of hepatic enzymatic and non-enzymatic antioxidants in control and experimental animals are shown in the above Table. In cadmium treated animals (Group B), there was a significant decrease ($P<0.01$) in the activities of SOD, CAT, GPx when compared to normal control (Group A). Administration of silymarin along with cadmium treated rats (Group D) exhibited a significant increase ($P<0.01$) in the activities of enzymatic antioxidants (SOD, CAT, GPx) when compared to rats treated with cadmium alone (Group B). The level of GSH (non-enzymatic antioxidant) was significantly decreased ($P<0.01$) in liver of rats treated with cadmium (Group B) as compared to normal control (Group A) table. When administration of silymarin to cadmium intoxicated rats (Group D) shows a significant increase ($P<0.01$) in the level of non-enzymatic antioxidant, GSH when compared to rats treated with cadmium alone (Group B).

DISCUSSION

Cadmium induces a broad spectrum of toxicological effects and biochemical dysfunctions constituting serious hazards to health. Cadmium interferes with antioxidant defense mechanisms together with the production of ROS, which may act as a signaling molecule in the induction of cell death. Liver is one of the most critical organs for the toxicity of cadmium and also is the target organ for cadmium toxicity. It can subject to distinct pathological and morphological changes under Cd effect. Because of its oxidative stress inducing nature the cadmium induced toxicity can be restored by the treatment of various antioxidants.[26]

Many mechanisms have been suggested for the protective effects of silymarin, which include antioxidation, prevention of lipid peroxidation, enhancing detoxification and retarding glutathione depletion.[27] In this content, the present study also confirmed that administration of silymarin (40mg/kg/day) significantly restored the liver enzymatic and non-enzymatic antioxidants against the toxicities induced by cadmium.

Lipid peroxidation is one of the key manifestations of oxidative damage and it plays a major role in the toxicity of cadmium. Cadmium induces oxidative stress by producing hydroxyl radicals, superoxide anions, nitric oxide and hydrogen peroxide. Significant increase in the level of hepatic TBARS in Cd intoxicated rats could be possibly due to excessive formation of free radicals, which leads to the deterioration of biological macro molecules.[28] Manca *et al.,*[29] have reported that lipid peroxidation is considered as a sensitive marker of cadmium exposure. Hussain *et al.,*[30] have also reported the elevated level of lipid peroxidation in various tissue of Cd intoxicated rats.

In the present investigations Cd intoxicated rats pretreated with silymarin showed a marked decrease in the levels of TBARS. This may be attributed to the presence of phenolic structure, flavanoids and antioxidant activity.

Administration of silymarin to cadmium treated rats, significantly decrease lipid peroxides due to the ability of silymarin to scavenge free radicals, suggesting the bioactivity of silymarin to directly react with reactive oxygen species (ROS). The free radical scavenging property of silymarin has been already well established in experimental rats.[31] Many invitro studies have also revealed that silymarin has antilipoperoxidative activity against benzoyl peroxide[32], carbon tetra chloride[33], 1, 2 dimethyl hydrazine[34] induced oxidative stress.

The components of silymarin interacts with the polar head groups of phospholipids at the lipid water interface of the membrane and offers protection against membrane lipid peroxidation and further breakdown of membrane integrity caused by oxidative mechanisms.[35]

GSH is a tripeptide and a cysteine rich proteins participate in the maintenance of cytoplasmic and membrane thiol status. It is an antioxidant and a powerful nucleophile, critical for cellular protection such as detoxification of ROS. Depletion of GSH in tissues leads to the impairment of cellular defense against ROS and may result in peroxidative tissue injury.[36] In the present study the cadmium treated rats showed a significant depletion of GSH in hepatic tissue compared to normal control. This could be probably due to either increased utilization of GSH by the cells act as scavengers of free radicals produced by cadmium or enhanced utilization of GSH for the activity of GPx forming oxidized glutathione (GSSG) due to increased generation of ROS.[37] Significantly depletion of hepatic GSH has also been reported by Jeyaprakash and Chinnaswamy[38] (2005) in cadmium intoxicated rats. Administration of silymarin to cadmium treated rats shows significant increase in the level of GSH near to control definitely revealed the protective nature of the silymarin against cadmium hepato toxicity. This possible protective effect may be due to the silymarin directly scavenges the free radicals through chelating cadmium and moderate the expenditure of Non-enzymatic antioxidant endogenously. Chelation of cadmium reduces oxidative stress, reverses the antioxidation level and glutathione metabolizing enzymes activities due to the presence of hydroxyl groups in the Silymarin and it reacts with free radical.[39] Silymarin enhances hepatic glutathione generation by elevating cysteine availability and inducing cysteine synthesis while inhibiting its catabolism to taurine.[40]

Silymarin has been reported to maintain the GSH homeostasis in the system and this might be the reason for elevated glutathione levels observed during Silymarin treatment. Increase in GSH levels in turn contribute to the recycling of other antioxidant such as vitamins E and vitamin.[41] This shows that Silymarin maintains the level of antioxidant vitamins by maintaining GSH homeostasis thereby protecting the cells from further oxidative stress.

Silymarin restores the level of GSH possibly by directly scavenging the ROS or through the transcriptional up regulation of ɣ-GCS gene expression[42] or by acting as an iron chelating agent.[43] Replenishment of GSH by Silymarin in the present study provides multiple protective actions against cadmium induced toxicity.

Super oxide dismutase (SOD), Catalase (CAT), and Glutathione peroxidase (GPx) constitute a mutually supportive team of antioxidant defense against ROS. SOD is a metalloenzyme that catalyses the dismutations of super oxide radical. CAT is a heme protein which catalyses the reduction of H_2O_2 to water and oxygen and thus protects the cells from the oxidative damage of H_2O_2 and OH^-. GPX is a Seleno enzyme which plays a major role in the reduction of hydrogen peroxide and hydro peroxides. In the present study Cd intoxicated rats showed a significant decrease in the activities of SOD, CAT and GPX in liver tissue when compared to normal control. This may be due to either the antagonistic effect of cadmium with copper and zinc which are important metals for the activity of SOD molecules[44] or inactivation of SOD by cadmium induced lipid peroxidation.[45] Whanger *et al.,*[46] reported that the decrease in catalase activity may reflect decreased absorption of iron, an essential trace element required for the activity of catalase.

Cadmium decreased the availability of metal selenium which is important for GPX activity.[47] These findings support the present study. Administration of silymarin to cadmium treated rats shows the significant elevated levels of enzymatic antioxidant in the liver. Silymarin inhibits the membrane lipid peroxidation, which induce the expression of SOD in astrocytes.[48] Krithiga *et al.,* (2014)[49] who found that Silymarin administration normalized the activities of SOD, CAT, GPx, GR, GST as well as TBARS level in hydrogen peroxide treated erythrocytes.

The level of CAT, SOD significantly increased during Silymarin treatment shows that Silymarin might be involved in the removal of superoxide anions, hydrogen peroxide and further help in the protection of liver cells from oxidative injury. Silymarin is reported to protect against H_2O_2 induced cell injury through its superoxide radical scavenging capacity.[50]

The free radical scavenging and antioxidant properties of Silymarin demonstrated by (a) restoration of the endogenous antioxidant enzymes (SOD, CAT, GPX, GST, GR) and non-enzymatic antioxidant in the liver and the other tissues of stressed animals. (b) Increased intracellular concentration of GSH in liver and other tissues. (c) Decreased lipid peroxidation, detected as reduced TBARS.[51]

CONCLUSION

In conclusion, this study demonstrates that Silymarin has the protective effect against Cd-induced oxidative damage in the liver of rats. The mechanism contributing to its effectiveness involves the quenching of free radicals, anti oxidation and metal chelating ability of Silymarin. Recently much attention has been focused on the protective biochemical system against toxic heavy metals. The present study therefore provides biological evidence supporting the usefulness of silymarin against cadmium induced toxic oxidative stress on the liver tissue of rat.

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