Cadmium acts as a silent killer of liver by inducing oxidative stress and hepatocellular injury and a possible amelioration by vitamin B_{12} and folic acid in rat model

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ABSTRACT

Purpose: To investigate the involvement of oxidative stress in Cadmium (Cd) induced alteration in the functional status of the liver. And to assess the efficacy of folic acid and vitamin B_{12} in preventing Cd-induced damage in the same.

Materials and methods: The experiment was carried out for four weeks. For the experiment, 25 healthy male adult Wistar albino rats were randomly selected and were divided into five equal groups and treated as control, treated with Cd, supplemented with vitamin B_{12} and folic acid and in the combination of these two. After 28 days the liver function enzymes and oxidative stress parameters were measured.

Results: Cd is the silent killer of the hepatic system through the induction of oxidative stress in male rats. From this investigation, it is evident that the folic acid+vitamin B_{12} possess significant hepatoprotective and antioxidant activity against Cd-induced hepatotoxicity in the rat model. In addition, results revealed that the folic acid alone and or in combination with vitamin B_{12} blunted the hepatotoxic effect significantly.

Conclusions: Based on results obtained, it can be concluded that folic acid and vitamin B_{12} offer a protective effect in Cd-induced oxidative stress associated with hepatocellular injury. Folic acid and vitamin B_{12} can be considered as a potent natural antioxidant which has the capacity to provide protection against Cd-induced oxidative stress in the liver in rats. However, to elucidate the exact mechanism of this modulatory effect and to examine its potential therapeutic effects further studies are essential.

Keywords: Cadmium, liver, hepatocellular injury, oxidative stress.

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INTRODUCTION

Cadmium (Cd) is one of the environmental pollutants arising from electroplating, fertilizers, pigments and plastic accumulation [1]. Therefore, it can easily contaminate the soil, plant, water, air, and water. Cd is absorbed and accumulates in various tissues. Cd is considered as toxic metal that induces oxidative damage by disturbing the prooxidant-antioxidant balance in the tissues. Cd poisoning can also cause bone damage and kidney failure. Cd was released into rivers by mining companies. Cd exposure has increased during the last century. Cd exposure mainly occurs by two main routes, inhalation, and ingestion. Cadmium intoxication arose in Japan, as a result, Itai-itai disease was epidemic. The major symptoms were bone and renal damage which was caused by rice which was polluted by cd. Itai-itai disease is one of the four big diseases in Japan. Excess Cd exposure may affect several organisms by their toxic action. Nowadays, we all aware of the fact that occupational and environmental cadmium exposure can result in hepatotoxicity. The liver is the main target organ of cadmium toxicity which follows both acute and chronic exposure. The mechanism by which cadmium is absorbed, transported and taken up by cells is still not fully understood. Cd is a non-essential metal because of its extensive use in industry and agriculture presents it a worldwide health hazard. Cd can be released into the atmosphere by smelting or other processes and travel long distances. Once deposited onto the ground it is taken up into the food chain by cereals and root crops. Cd is taken up more rapidly by plants than other metals such as lead and mercury. Cd can also enter the food chain via [1] environmental contamination arises from industrial emissions, soil, and contaminated food as well as smoking [2].

Cd retention is generally higher in women than men, and the severe Cd–induced Itai-itai disease is mainly a women's disease. Cigarette smoking is the most significant source of human Cd exposure. Blood and kidney Cd levels are consistently higher in smokers than non-smokers. Inhalation due to industrial exposure can be significant in occupational settings, for example, welding or soldering and can produce severe chemical pneumonitis. Different route of exposure of Cd are as follows (Table 1).

Table 1. Exposure of Cd through different routes

Route of exposure and inhalation Cd is more efficiently absorbed from the lung than from gastrointestinal tract. The absorption efficiency is a function of specific Cd compound (soluble compounds are absorbed rapidly), its exposure concentration and route [3].	
1. Inhalation:	Inhalation and oral ingestion are the most common routes of entry of Cd into the body. Once absorbed, Cd binds to plasma proteins, primarily albumin and two macroglobulin [4].
2. Absorption:	Absorption of Cd via inhalation is dependent on solubility and the particle size and hence the site of deposition in the respiratory tract (Risk Assessment Information System,1991).
3. Oral:	Orally ingested Cd is absorbed from the intestine because of its suitable pH (pH-6). Only 5% of the Cd is absorbed [5]. High fiber diet, lack of iron, higher intake of calcium, zinc can increase the Cd uptake, whereas low intake of calcium, zinc, vitamin D can cause lower resorption of Cd [6].
4. Dermal:	In 1991 experimented on their sorption from Cd-contaminated soil and water solutions by human cadaver skin in a diffusion cell-model. The skin showed hyperkeratosis and acanthosis with occasional ulcerative change, and an increase of the mitotic index of the skin cells. Also Cd concentration in blood, liver and kidney increased, thus indicating percutaneous absorption [7].
5. Distribution:	Cd is transported throughout the body, usually bound to a sulfhydryl group containing protein like metallothionein. About 30% deposits in the liver and 30% in the kidneys, with the rest distributed throughout the body, with a clearance half-life of twenty-five years. The half life of Cd in the blood has been estimated at 75 to 128 days, but this half life primarily represents deposition in organs, not clearance from the body [8].

Route of Excretion	
1. Urinary Excretion:	Little Cd is normally excreted in the urine. The rate of excretion increases slowly with increasing body burden, but, as renal dysfunction develops, it increases sharply and the hepatic and renal Cd concentrations fall [9].
2. Faecal Excretion:	The mechanism of faecal excretion may involve both sloughed mucosal cells and excretion in the bile. After an initial rapid phase, biliary excretion represents 0.02–0.04% of the body burden, and most is associated with a fraction of low relative molecular mass [10].
3. Biliary Excretion:	Biliary excretion is a complex process involving uptake into liver cells, intracellular sequestration and/or biotransformation, and transport into bile. Enterohepatic circulation interferes with the biliary elimination of from the body [11].
Effect of Cd on human	
1. Acute effect of Cd:	Inhalation of Cd fumes and dust may result in a wide range of effects including a metallic taste, headache, dyspnoea, chest pain, cough pain with foamy or bloody sputum and muscular weakness [12]. The respiratory system is affected severely by the inhalation of Cd-contaminated air: Shortness of breath, lung edema and destruction of mucous membranes as part of Cd-induced pneumonitis [13]. Cd exposure through oral route may cause nausea, vomiting, diarrhoea, weakness etc. [14].
Chronic effect of Cd	
1. Cardio vascular system:	Cd decreases excitability of cardiac conduction system, myocardial contractile activity, thus decreases heart rate and impairs energy metabolism. It causes blocking of calcium ion channels leading to congested heart failure [15]. Lowering of pulse rate velocity and pressure throughout the arterial system has also been reported chronic cd atherosclerosis [16].
2. Respiratory system:	The major source of inhalative Cd intoxication is cigarette smoke. The human lung resorbs 40–60% of the Cd in tobacco smoke. Workers exposed to Cd-containing fumes have been reported to develop acute respiratory distress syndromes [17].
3. Excretory system:	Cd is accumulated in the proximal convoluted tubule (PCT) of kidney bound to metallothionein and act as nephrotoxicant. It decreases PCT cell membrane fluidity and alters transport process by inhibiting Na+K+ATPase and carbonic anhydrous resulting in glycosuria [18].
4. Reproductive system:	Cd appears to interfere with the ovarian steroidogenic pathway in rats. Piasek et al. evaluated the direct effects of in vitro Cd exposure on steroidogenesis in rat ovaries. The most affected were productions of progesterone and testosterone [19]. Low dosages of Cd are reported to stimulate ovarian progesterone biosynthesis, while high dosages inhibit it [20].
5. Immune system:	The immune system suffers from Cd-induced impairment at several levels. Prenatal Cd exposure may impair postnatal T cell production and response to immunization [21] as well as dysregulates thymocyte development [22]. Cd induces increased rates of autoimmunity, increased production of nonspecific antibodies, and decreased production of antigen-specific antibodies [23]. Lymphocyte proliferation and natural killer cell activity are also suppressed by Cd [24]. Metallothionein protects against Cd immune toxicity.
6. Nervous system:	Cd plays a critical role in neurobiology; a growing number of clinical investigations have pointed to Cd intoxication as a possible etiological factor of neurodegenerative diseases, including Parkinson's disease, Alzheimer's disease, and Huntington's disease [25].
7. Effects on skin:	The skin showed hyperkeratosis and acanthosis with occasional ulcerative change, and an increase of the mitotic index of the skin cells. Only a very high concentration of Cd can affect the skin [7].

8. Apoptosis:	Apoptosis is a genetically regulated form of cell death, which plays an important role in the development and maintenance of tissue homeostasis in multicellular organism Cell death resulting from Cd intoxication has been confirmed to occur through apoptosis by morphological and biochemical studies [26].
9. Haematopoetic effect:	Hematopoeisis is adversely affected, most notably in itai-itai disease where severe anaemia is observed, in association with marked suppression of erythropoietin production. Hemolysis may also be a factor in producing Cd-associated anaemia [27].
10. Oxidative stress:	Cd has been observed to cause oxidative stress and histologically visible membrane disturbances in the central nervous system [28].
11. **Carcinogenicity:**	The United States Environmental Protection Agency considers Cd to be a Class B1 carcinogen. There is contradictory evidence linking Cd exposure to breast cancer [29] and denying that link [30].

Folate, also known as folic acid, folacin, and vitamin B. The recommended daily intake of folate in the US is 400 micrograms from foods or dietary supplements. Folate in the form of folic acid is used as treatment of anaemia caused by folic acid deficiency. Folic acid is also used as a supplement by pregnant women to prevent neural tube defects (NTD) in the baby. Deficiency of folic acids in early pregnancy are believed to be the cause of more than half of babies born with neural tube defects. Long term intake may reduce the risk of stroke and cardiovascular disease. It may be taken by injection or by mouth. Cobalamin and Folic acid act as potentially very useful agent for inhibiting nitric oxide synthase and nitric oxide production i.e. ROS production, controlling nuclear factor-kappa β activation, elevation of GSH/GSSG ratio, and down regulation of active caspase-3 expression. Thus, vitamin B_{12} and folic acid may be considered as antioxidant. Lack of either vitamin B_{12} or folic acid or both can be the cause of megaloblastic anaemia. Nicotine users tend to have lower levels of the folic acid and vitamin B_{12} and both of which affect homocysteine levels by acting as co-enzyme. Vitamin B_{12} might help protect our body and organisms against chronic disease and neural tube defects, but more research, particularly in the area of nutritional genomics, is needed to determine how vitamin B_{12} might augment the benefits of folic acid. Some consideration should be given to the potential value of fortifying foods with vitamin B_{12} in addition to the current mandatory folic acid fortification of grains. Folate (vitamin B_9) is an essential nutrient that is required for DNA replication and as a substrate for a range of enzymatic reactions involved in amino acid synthesis and vitamin metabolism. Folic polyglutamate, the principal dietary form of folate, consists of folic acid bound to one to six glutamic acid residues in a gamma peptide linkage. Folic acid absorption is an active process that occurs primarily in the duodenum and jejunum. Following absorption, folic acid present in human and canine portal blood is not methylated, although methylation may occur later in the liver following reduction [31]. Folic acid is distributed into milk [32]. Folate is excreted in the urine as folate cleavage products. Intact folate enters the glomerulus and is reabsorbed into the proximal renal tubule. Very little intact folate is excreted in the urine. Folate is excreted in the bile and much of it is reabsorbed via the enterohepatic circulation [33].

Folic acid is converted (in the presence of ascorbic acid) in the liver and plasma to its metabolically active form (tetrahydrofolic acid) by dihydrofolatereductase [34].

It has been proposed that folic acid has many beneficial effects on human body, such as anti-carcinogenic effect [35] cardio-protective effect [36], anti-depressive effect [37], reno-protective effect [38], free radical scavenging properties and antioxidant activity [39], neuro-protective effect [40], hepato-protective effect [41], etc. A folate-rich diet can reverse age-related changes in T-cell proliferation and cytokine production in rats [42].

Vitamin B_{12} is a water-soluble vitamin that is naturally present in some foods, added to others, and available as a dietary supplement and a prescription medication. Vitamin B_{12} exists in several forms and contains the mineral cobalt [43] so compounds with vitamin B_{12} activity are collectively called "cobalamins".

Methyl-B_{12} is absorbed by two processes. The first is an intestinal mechanism using intrinsic factor through which 1–2 micrograms can be absorbed every few hours. The second is a diffusion process by which approximately 1% of the remainder is absorbed. The human physiology of vitamin B_{12} is complex, and therefore is prone to mishaps leading to vitamin B_{12} deficiency [44]. Vitamin B_{12} is distributed into the liver, bone marrow, and other tissues, including the placenta. Vitamin B_{12} is secreted in bile and reabsorbed via the enterohepatic circulation by ileal receptors which require if the development of vitamin B_{12} deficiency is likely to be more rapid in patients

with pernicious anaemia. Vitamin B_{12} is excreted via the faeces. It is estimated that daily vitamin B_{12} loss are in proportion to body stores with approximately 0.1% excreted per day [45].

Vitamin B_{12} is believed to be converted to coenzyme form in the liver and is probably stored in tissues in this form [32].

It has been proposed that vitamin B_{12} has so many beneficial effects on human body, such as lung protective effect [46] cardio-protective effect, hepatoprotective effect [47], etc. Researchers have long been interested in the potential connection between vitamin B_{12} deficiency and dementia [48].

Vitamin B_{12} has various effects on biological processes in vivo. It is well known that megaloblastic anaemia and peripheral nerve disturbances are caused by lack of vitamin B_{12} in the immune system, an important role of vitamin B_{12} has been reported [49].

Folates and vitamin B_{12} have fundamental roles in the central nervous system (CNS) function at all ages. Folic acid and vitamin B_{12} may have roles in the prevention of disorders of CNS development, mood disorders, and dementias, including Alzheimer's disease and vascular dementia in elderly people [40].

Oxidative stress is a feature of many chronic inflammatory diseases. Such diseases are associated with up regulation of a vitamin B_{12} blood transport protein and its membrane receptor, suggesting a link between cobalamin and the cellular response to inflammation. The ability of cobalamin to regulate inflammatory cytokines suggests that it may have antioxidative properties [50].

The aim of the present study is therefore to investigate the involvement of oxidative stress in Cd-induced alteration in the functional status of the liver and to assess the efficacy of folic acid and vitamin B_{12} in preventing Cd-induced damage in this organ.

MATERIALS AND METHODS

Experimental animals:

Eight weeks of old male albino rats (Wister strain) of body weight 120-140 g were used for the study.

The rats were acclimatized in the experimental animal house for 7 days before the commencement of experiment.

The animals housed in stainless steel cages under standard laboratory condition of temperature (25±2ºC) and humidity (55±5%) and in 12 hours light-dark cycle schedule with free access to water supply.

Animals were fed with normal rat pellets. A standard diet containing approximately 130 mmol/kg sodium, 160 mmol/kg potassium, 23% protein and 5% fat. All the rats were allowed free access to food and water and ad libitium, throughout the experimental period.

Good hygiene was maintained by constant cleaning and removal of faeces and spilled feed from the cages daily.

All the experiments were performed according to the ethical guidelines suggested by the Institutional Animals Ethics Committee (IAEC)

Experimental design: The experiment was carried out for four weeks. For the experiment, 25 healthy male adult Wistar albino rats were randomly selected and were divided into five equal groups (n=5) and treated as follows (Table 2).

The animals of all groups were provided with a control diet composed of 71 % carbohydrate, 18% protein/ 7% fat and 4% salt mixture [51].

Table 2. Experimental design

Group/s	Supplied material/s and concentration/s
Group A (Control)	Received Normal saline 10 ml/kg body weight/day, orally.
Group B	Cd treated group (Cd) at 1 mg/ kg body wt. i.p.
Group C	Cd + Folic acid (36µg/kg body wt. orally) treated group.
Group D	Cd+Vitamin B_{12} (0.63 µg/kg body wt. orally) treated group.
Group E	Cd + Folic acid + Vitamin B_{12} treated group.

Preparation of Cd chloride solution: Cd was administered to rats in the form of Cd chloride ($CdCl_2$). Thus, 25 mg of anhydrous $CdCl_2$ was dissolved in 50 ml of distilled water (1 mg/kg body wt.) [52].

Preparation of Folic acid solution: 0.1 ml of stock folic acid solution (STOCK=0.5 mg in 0.1 ml) was mixed with 49.9 ml of distilled water (36µg/kg body wt.) [53].

Preparation of Vitamin B_{12} solution: 0.1 ml of stock vitamin B_{12} solution (STOCK=0.0125 mg in 0.1 ml) was mixed with 49.9 ml of distilled water (0.63 µg/kg body wt.) [53].

Animal Treatment: After acclimatization to laboratory environment, the animals of the control group (A) received the vehicle (0.9% NaCl) only. The animals of the Cd treated groups were administered Cd chloride intraperitoneally (i.p.) at a dose of 1mg/kg body weight per day for a period of 28 days.

The animals of the Cd + folic acid treated group (Cd+F.A) were administered Cd chloride i.p. at a dose of 1mg/kg body weight and were administered folic acid orally at a dose of 36µg/kg body weight per day for a period of 28 days.

The animals of Cd + vitamin B_{12} treated group (Cd + Vit.B_{12}) were administered Cd chloride i.p. at a dose of 1mg/kg body weight and were administered vitamin B_{12} orally at a dose of 0.63µg/kg body weight per day for a period of 28 days.

The animals of the Cd+ folic acid + Vitamin B_{12} treated group (Cd+F.A.+Vit.B_{12}) were administered Cd chloride i.p. at a dose of 1mg/kg body weight and were administered folic acid orally at a dose of 36µg/kg body weight and were administered Vitamin B_{12} orally at a dose of 0.63 µg/kg body weight per day for a period of 28 days.

Blood collection, serum preparation and tissue collection: After the experimental period is over (day-29), the animals of all groups were anaesthetized and sacrificed by cervical dislocation, which is one of the recommended physical methods of euthanasia by the IAEC.

Blood was drawn from heart and serum was separated for the biochemical assay; liver was collected for the analysis of enzymatic and nonenzymatic antioxidants and various biochemical parameters and histopathological study.

Preparation of Tissue extract: Liver was isolated from all animals for estimation of enzymatic and nonenzymatic antioxidant and oxidative stress biomarkers. 250 mg of each tissue was placed in ice cold phosphate buffer (pH 7.4) and homogenized immediately in a glass homogenizing tube equipped with a Teflon pestle [54].

Evaluation of Oxidative Stress parameters

Estimation of lipid peroxidation (LPO): LPO was detected by measuring thiobarbituric acid reactive substance (TBARS). Absorbance was measured at 530nm [55]. Results were expressed as mM/mg protein.

Estimation of Nitric oxide (NO) production: NO activity was determined by Griess reaction [56] which was expressed in the form of nitrite accumulation. Absorbance was measured at 550nm.Results were expressed as µM/ mg protein.

Estimation of antioxidant enzymes

Superoxide dismutase (SOD): The nitrobluetetrazolium (NBT) method of Beauchamp and Fridovich, which is based on the inhibition of NBT reduction by SOD, was used for the determination of SOD activities [57]. Absorbance of blue formazan was recorded at 560 nm and 25 °C. Results were expressed as U /mg protein.

Catalase (CAT)*:* Catalase activity was determined according to the of Beer's method [58]. Absorbance of blue formazan was recorded at 240 nm and 25 °C. Results were expressed as U/ mg protein.

Estimation of Glutathione (GSH): GSH was estimated according to the method of Ellman (1959). Results were expressed as mM/mg protein [59].

Estimation of Protein: Protein in the homogenate of liver tissue was estimated by the method of Lowry [60] using bovine serum albumin (BSA) as standard.

Histopathological examinations: After the experimental period is over (day 29), liver from all groups of animals were selectively taken and were fixed in Formol reagents. Paraffin blocks were prepared and 5 µm thin sections were cut by using rotary microtome and routine microscopic slides were prepared and stained with haematoxylin and eosin and finally mounted in DPX. Stained slides were light microscopically examined for changes if any and their photomicrographs were taken.

Statistical analysis: Data were expressed as Mean ± SE. Kruskal–Wallis nonparametric ANOVA test was performed to find whether or not scores of different groups differ significantly. To test inter-group significant difference, Mann–Whitney U multiple comparison test was performed to find correlation between the study variables. Statdirect 3.0 was used for statistical analysis. Differences were considered significant if $p<0.05$.

RESULTS AND DISCUSSION

Cd has been linked with altered liver metabolism and liver damage. ALT (Alanine transaminase), AST (Aspartate transaminase) and ALP (Alkaline phosphatase) are considered among the most sensitive markers of hepatocellular injury.

Results of our study (Figure 1) indicate that folic acid and vitamin B_{12} administration could blunt Cd induced increase in activities of different marker enzymes of hepatocellular injury, viz. ALT ($p<0.01$), AST ($p<0.05$) and ALP ($p<0.01$), suggesting that folic acid and vitamin B_{12} possibly has a protective influence against Cd induced hepatocellular injury and degenerative changes.

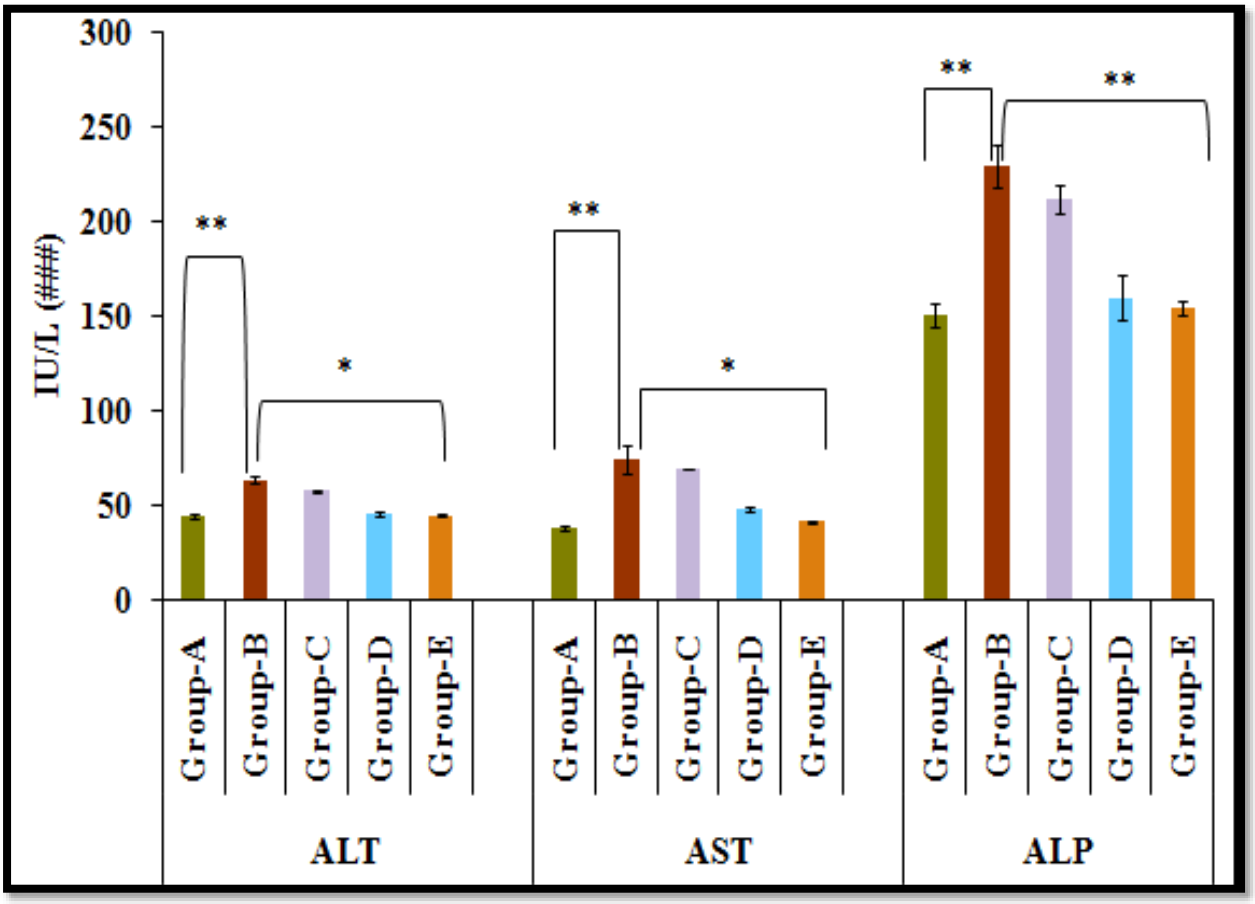

Figure 1: Effect of folic acid (36µg/kg body weight/day for a period of 28 days, orally) and vitamin B_{12} (0.63 µg/kg body weight/ day for a period of 28 days, orally) on Cd (1mg/kg body weight/day for a period of 28 days, i.p.) induced changes in liver functional enzymes on male rat model. Error bar represent mean ± S.E (n=5). Significance level based on Kruskal Wallis test [###p <0.001]. Significance based on Mann-Whitney U multiple comparison test: Group A vs Group B:**p<0.01, Group B vs Group C/D/E: **p<0.01, *p<0.05

LPO is a major indicator of oxidative damage initiated by ROS (reactive oxygen species) and causes impairment of membrane function.

The increase in LPO observed in this study may be attributed to a direct effect of increased generation of ROS resulting from Cd treatment.

In our study, Cd significantly ($p<0.001$) induce LPO and NO and also significantly ($P<0.001$) reduce the level of GSH, SOD and CAT in the liver of the experimental animals (Figure 2).

Administration of folic acid and vitamin B_{12} alone and both in combination increase the activity of SOD, CAT, GSH and reduce the LPO and NO activity significantly ($p<0.01$) suggests that the folic acid and vitamin B_{12} have an efficient protective activity in response to ROS. These finding also indicate that folic acid and vitamin B_{12} may be associated with decreased

oxidative stress and free radical mediated tissue damage and to prevent the accumulation of excessive free radicals and protect liver from Cd induced damage.

Histological findings are also discussed in the following diagram (Figure 3).

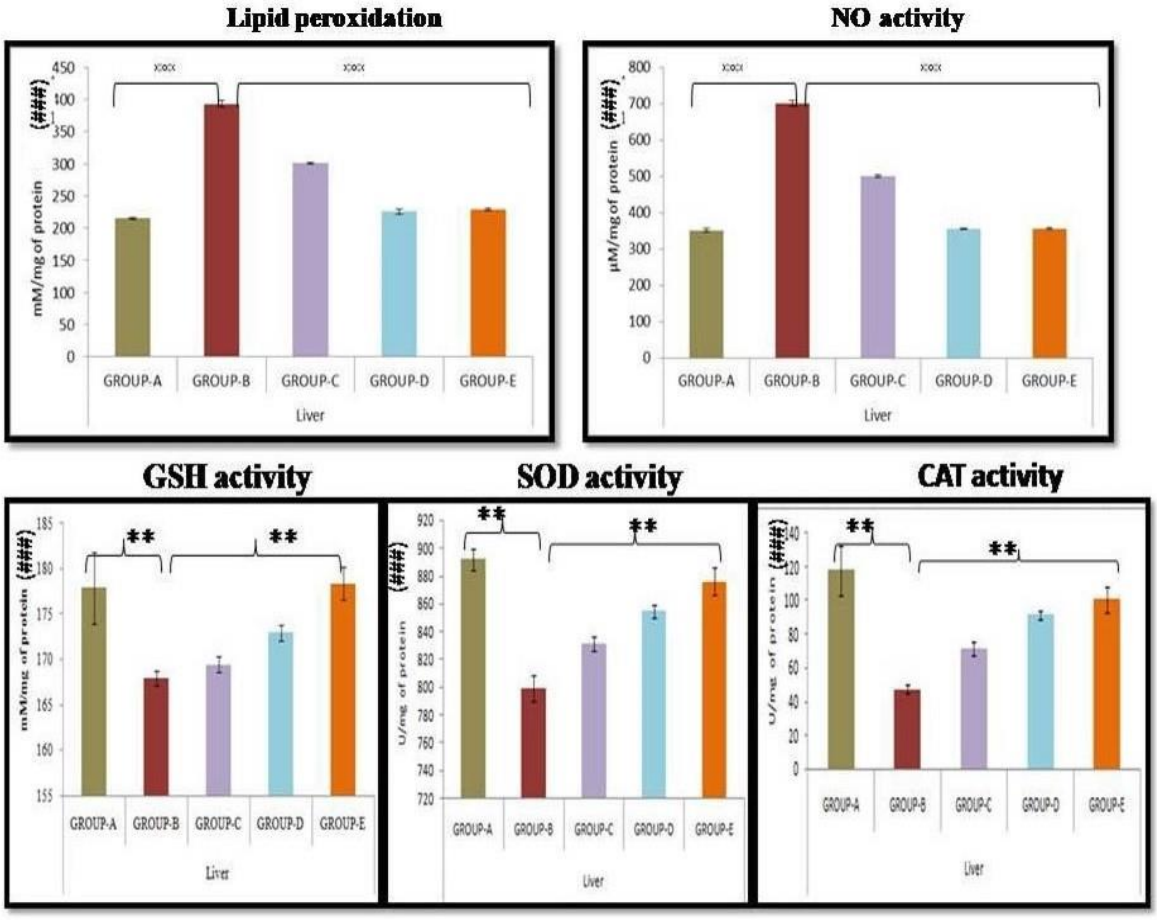

Figure 2: Effect of folic acid (36µg/kg body weight/day for a period of 28 days, orally) and vitamin B_{12} (0.63 µg/kg body weight/ day for a period of 28 days, orally) on cadmium (1mg/kg body weight/day for a period of 28 days, i.p.) induced changes in oxidative stress parameters on male rat model. Error bar represent mean ± S.E (n=5). Significance level based on Kruskal Wallis test [###p <0.001]. Significance based on Mann-Whiteny U multiple comparison test: Group A vs Group B:**p<0.01, Group B vs Group C/D/E: **p<0.01.

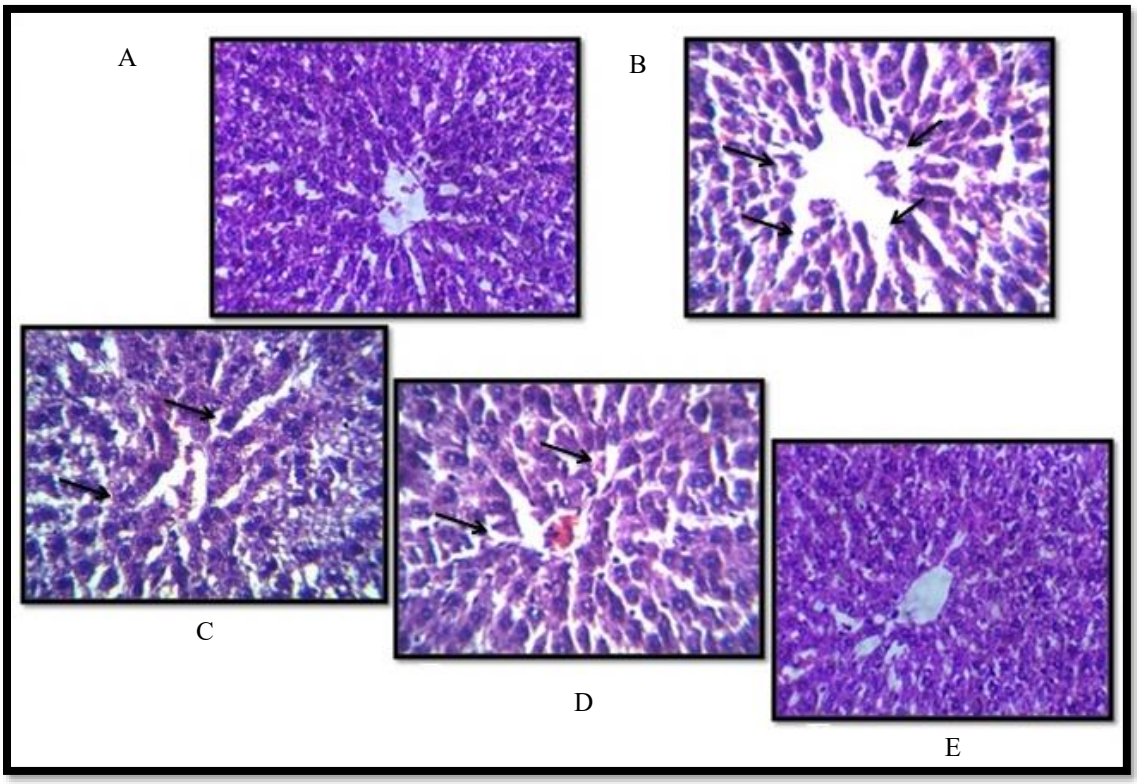

Figure 3: Effect of Cd on histopathological changes in rat liver

- **A.** Representative section from control rat, normal architecture of the liver was observed.
- **B.** Representative section from rat treated with Cd typical characteristics of injury are seen disorganization of normal radiating pattern of cell plates around central vein, showing disruption (marked by the arrow) of cords and sinusoidal network with degenerative changes in the form of atrophy of cells and disintegration of nuclei, ruptured endothelial lining which was invaded by lymphocytic infiiltration and few inflammatory cells, hemorrhage increased vacuolation.
- **C.** Representative section from rat treated with Cd+folic acid: gradually improve the pattern of the central vein and radiating pattern of cell plates.
- **D.** Representative section from rat treated with Cd+ vitamin B_{12}: Normal pattern of the central vein and radiating pattern of cell plates.
- **E.** Representative section from rat treated with Cd+folic acid+ vitamin B_{12}: Gradual improvement of normal architecture of the liver was observed.

ALT, AST and ALP are considered as the most sensitive markers for detecting hepatocellular injury. Formation of ROS, oxidative stress and hepatocellular injury has been implicated to liver disease. It has been documented that hepatocyte is the major source of generating ROS during regular Cd exposure; this is primed and activated for enhanced production of pro-inflammatory factors. Moreover, Cd induced liver injury has been associated with increased amount of LPO. Thus, in the present study it may be possible that, an elevated level of marker enzymes AST, ALT and ALP are accompanied with disruption of membrane structure and integrity by LPO. The result seemingly agrees with the earlier reports which was monosodium glutamate induced toxicity [61] that the activity of serum ALT increased in male rats were fed Cd probably due to the Cd induced oxidative stress in the liver.

LPO is a major indicator of oxidative damage initiated by ROS and causes impairment of membrane function. The increase in LPO observed in this study may be attributed to a direct effect of increased generation of ROS resulting from Cd treatment. All human cells protect themselves against free radical damage by enzymes like superoxide dismutase (SOD) and catalase (CAT). CAT is a major antioxidant enzyme that primarily catalyses the degradation of H_2O_2 to H_2O and O_2 [62]. In our study, Cd significantly

induces LPO and the level of NO and significantly ($p<0.001$) reduces the level of GSH and activities of SOD, CAT in the liver of the experimental animals ($p<0.001$). The decrease in the activity of these enzymes could result from their inactivation by ROS or by their glycation. Protein glycation is caused by the products of glucose auto-oxidation. Reduction in the activity of these enzymes might have contributed to the increased level of LPO and decreased concentration of GSH. As these enzymes become inactivated, more GSH would be utilized in neutralizing acyl radicals and other ROS.

Increased LPO level resulted in decreased level of GSH. GSH acts as an antioxidant in many ways. It can function as a direct radical scavenger and stabilize membrane structure through the removal of acyl peroxides formed during LPO reaction. Glutathione depletion is a positive indicator of tissue degeneration and the magnitude of depletion parallels the severity of the damage.

Besides, results obtained from the histopathological images showed typical disorganization of normal radiating pattern of cell plates around central vein, showing disruption of cords and sinusoidal network with degenerative changes in the form of atrophy of cells and disintegration of nuclei, ruptured endothelial lining which was invaded by lymphocytic infiltration and few inflammatory cells, haemorrhage increased vacuolation in Cd treated animals and gradual improvement was notified by supplementation with folic acid and or in combination with vitamin B_{12}.

In our study, we found that folic acid and vitamin B_{12} were potent enough in protecting Cd induced liver damage. Considering the relationship between Cd exposure and oxidative stress, it is reasonable that administration of some antioxidant should be an important therapeutic study on metal–induced oxidative stress is an emerging area of research. Antioxidants property of the ameliorative substances (vitamin B_{12} and folic acid) protect against oxidative stress and thus prevent organs from getting damaged.

CONCLUSION

Antioxidant therapeutic approaches by folic acid and vitamin B_{12} may be directly or indirectly protecting cell from adverse effect of Cd induced oxidative damages in liver cells. Based on results obtained, it can be concluded that folic acid and vitamin B_{12} offers protective effect in Cd induced oxidative stress associated with hepatocellular injury. folic acid and vitamin B_{12} can be considered as a potent natural antioxidant which has the capacity to provide protection against Cd induced oxidative stress in liver tissue in experimental rats.

Conflicts of interest

The authors declared no conflicts of interest.

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