Interrelationship of Circulating Biochemical Markers and Response of Lutein in Rats Receiving Carbon Tetrachloride (CCl₄) to Induce Hepatic Injury

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ABSTRACT

This study was conducted to evaluate the hepato-protective effects of Lutein and Clavazin in different dose pattern to reduce the oxidative stress against carbon tetrachloride (CCl₄) induced toxicity in rats. Thirty albinos Wister rats were included, divided into five groups and every group had six rats. Group A was control while group B, C, D and E were treated with 4ml/kg CCl₄ for 10 days with body weight of 160g to 200g. Further group C and D were administered with Lutein 200mg/kg and 400mg/kg respectively for 1 month. Infected CCl₄ group E was treated with a synthetic drug (Clavazin 1Tab/kg). The elevation of ALT, AST, ALP and MDA level in group B while reduced the levels of TP, SOD, CAT and GSH were recorded due to CCl₄ administration. Therapy with Lutein and synthetic drug (Clavazin) in different dose pattern for 1 month significantly decreases the levels of ALT, AST, ALP and MDA. On the other hand, increased levels of TP and anti-oxidants such as GSH, CAT and SOD were observed. The present study demonstrated that Lutein and Clavazin have potent defensive ability against CCl₄ induced liver toxicity in rats by their anti-oxidative and anti-inflammatory activity and may have protective effect on human liver.

Keywords: Carbon Tetrachloride (CCl₄), Sodium oxide dismutase (SOD), Catalase (CAT), Glutathione (GSH), Total protein (TP), Malondialdehyde (MDA), Clavazin, Lutein.

INTRODUCTION

In the recent years, most halogenated alkane compounds have been banned due to their toxicity such as carbon tetrachloride (CCl₄), chloroform (CHCl₃) or idoform (CHI₃). CCl₄ induces their effects on different organs such as liver, heart, brain, kidney, lungs and testes (Weber et al., 2003). Carbon tetrachloride (CCl₄) proved to be a lethal liver toxin resulting in the production of free radicals that cause oxidative stress leading to centrilobular liver necrosis (Chen et al., 2013). CCl₄ mediated cell damage by covalent binding of reactive intermediates to cellular components which initiate the lipid per-oxidation, leading to apoptosis (Boll et al., 2001). CCl₄ converts into CCl₃ by cytochrome P450, reactivation with high partial pressure of oxygen and converts into trichloromethyl peroxyl radical (Cl₂COO) (Weber et al., 2003). Liver is the main organ for nourishment, metabolism and detoxifying external chemical mediators as it is the center of most important activities like Cytochrome P450 generation that suppress due to CCl₄ toxicity (Sagor et al., 2015).

Antioxidants play crucial role in protecting the cells against inflammation caused by oxidative damage or free radicals which generate protective response to cellular injury, eliminating the injured tissue and elevating the tissue repairing. Antioxidative effects of several biomedical and herbal chemicals have been observed to work against the hepato-toxic effects (Chen et al., 2013). Lutein belongs to the carotenoid family, present in dark green leafy plants like kale and spinach and recommended daily intake is 1.7 mg (Koushan et al., 2013). The transport mechanism of lutein from intestine to tissue is not clearly understood. The process of carotenoids transportin blood and distributed to different tissue is done by the help of low density lipoprotein (LDL). 67% of beta carotenoids are distributed by LDL and 53% of lutein was present in HDL for transportation. The present study was designed to assess the interrelationship of circulating biochemical markers and response of Lutein and Clavazin in rats receiving carbon tetrachloride (CCl₄) to induce hepatic injury.

MATERIALS AND METHODS

Adult Wister male albino rats were taken from the National Institute of Health, Islamabad, Pakistan and its ranging from 160 to 200g. The study was...
approved by the Ethics Committee for the Scientific Research at the University of Lahore.

**Plant extract**: The standardized extract of Lutein was purchased from the Sigma-Aldrich Corporation (St. Louis, MO, USA).

**Experimental design**: Thirty (30) albino rats divided into five groups. Every group had six rats. For fourteen days every group administrate with CCl₄ to induce toxicity. To check the hepatoprotective effects give lutein to group three and group four for one month in different doses pattern. Group five treated with synthetic drug Clavazin 1tab/kg that contains several extracts such as Silybum marianum (200mg), Picrorhiza kurroa (50mg), Glycyrrhiza glabra (50mg) and Cichorium intybus (75mg).

<table>
<thead>
<tr>
<th>Group 1</th>
<th>Control rats, which received normal diet.</th>
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<tbody>
<tr>
<td>Group 2</td>
<td>Received CCl₄ 4ml/kg.B.wt</td>
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<tr>
<td>Group 3</td>
<td>Received CCl₄ + Lutein 200mg/kg.B.wt</td>
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<tr>
<td>Group 4</td>
<td>Received CCl₄ + Lutein 400mg/kg.B.wt</td>
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<tr>
<td>Group 5</td>
<td>Received CCl₄ + Clavazin 1tab/kg.B.wt</td>
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**Blood and serum separation**: During the dissection 5mL blood was collected from the rat heart and serum was separated by centrifugation at 1500 rpm for 10 min. After the separation of serum it was stored at -60°C until further biochemical analysis.

**Biochemical assays**: The aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and total protein (TP) were determined by using commercial kits (Biomerieux, USA). The levels of GSH, CAT, SOD and MDA were measured by Moron et al., (1979), Aebi, (1974), Kakkar et al., (1984), Ohkawa et al., (1979).

**RESULTS**

**Serum biochemical parameters of ccl₄ induced toxicity in rats**: The hepatic metabolic enzyme levels such as ALT, AST and ALP are significantly elevated and total protein (TP) level reduced in CCl₄ treated rats. The TP level reached 2.99IU/L in CCl₄ treated rats as compared to standard value (6.21IU/L), but observed TP level again increased (5.26, 6.25 and 5.21IU/L) in group C (CCl₄+Lutein 200mg/kg), group D (CCl₄+Lutein 400mg/kg) and group E (CCl₄+Lutein Clavazin 1Tab/kg) respectively (Figure-01 A, B, C and D).

**Consequences of lutein on enzymatic antioxidant activities in ccl₄ receiving rats**: The antioxidant enzymes such as SOD (28.26mg/ml) and CAT (16.23µmol/mol of protein) counted low in CCl₄ treated rats as compared to standard value (81.26mg/ml and 30.25µmol/mol of protein) but on another side, Lutein protects the alteration of SOD and CAT. So, antioxidant activity is enhanced for SOD and CAT in group C (65.26mg/ml, 25.26±2.35), group D (75.26mg/ml, 32.25±3.26) and group E (70.26mg/ml, 34.26±2.25) respectively (Figure-01 F and H).

**Consequences of lutein on non-enzymatic antioxidant activity in ccl₄ receiving rats**: The non-enzymatic antioxidant GSH activity reduced (7.65 mg/ml to 2.56mg/ml) in CCl₄ treated rats. But Lutein recovered the GSH activity in group C (6.23mg/ml), group D (6.25mg/ml) and group E (7.26mg/ml) respectively (Figure-01 E).

**Consequences of lutein on hepatic oxidative stress marker in ccl₄ receiving rats**: The lipid peroxidation end product MDA is significantly high (9.823nmol/ml) in CCl₄ treated rats. However, Lutein recovered the MDA level and in group C observed the (6.123nmol/ml), in group D (6.023nmol/ml) and in group E (4.526nmol/ml) respectively (Fig.1 G).

Fig. 1: Response of lutein in rats receiving carbon tetrachloride (CCL₄) to induce hepatic injury G-1 (Control), G-2 (CCl₄ 4ml/kg b.Wt), G-3 (CCl₄ 4ml/kg b.Wt+Lutein @ 200 mg/kg b.Wt), G-4 (CCl₄ 4ml/kg b.Wt+Lutein@400mg/kg b.Wt), G-5 (CCl₄ 4ml/kg b.Wt+ CLAVAZIN @ 200 mg/kg b.Wt)
DISCUSSION

In the present study, firstly we induce CCl₄ toxicity resulting in relative increase in aspartate aminotransferase (AST), alkaline phosphatase (ALP) and alanine aminotransferase (ALT) and decrease in total protein (TP) in group B. The levels of these biomarkers were reversed, AST and ALP were lowest and TP was highest in rats receiving Lutein at a rate of 400 mg/kg of body weight, ALT was more close to normal in rats administering Clavazin at a rate of 1 mg/kg of body weight. The elevated levels of ALT and AST indicate the disturbance in K⁺ and Na⁺ ions that diminish the cell membrane integrity (Naik and Panda, 2008). ALP elevation levels suggest obstruction of liver sinusoids (Giannini et al., 2005). CCl₄ is highly reactive hepatotoxin, induce liver toxicity (Connor et al., 1986) and its metabolism consists of two phases. In initial phase, CCl₄ converted into CCl₃ by cytochrome p450-dependent monoxygenase in hepatic parenchyma cells while in second phase, CCl₄ activates the Kupffer cells that produce pro-inflammatory mediators (Eidi et al., 2013).

Lutein, a xanthophyll are natural carotenoids having ability to reduce the oxidative stress. Lutein have protective efficacy for lipid membrane against free radicals attack (Sagor et al., 2015). After CCl₄ administration, the Lutein therapy recovers the hepatic damage. During liver damage, increased ROS production is determined by the end product of lipid peroxidation (MDA). The CCl₄ treated group B shows MDA increase but in group C and D Lutein therapy
decreases the oxidative stress, as MDA levels are reduced. Group E that contain synthetic drug (Clavazin) also reduce the MDA levels. The primary antioxidants like GSH, SOD and CAT are also reduced by CCl₄ administration. Clavazintreated rats show highest levels of GSH and CAT while lowest levels of MDA and also represent higher SOD levels only after group D treated with Lutein at a rate of 400 mg/kg of body weight. Kim (Kim et al., 2012) has demonstrated the same positive effects of Lutein on oxidative stress in liver of guinea pigs. Thus, by comparing Lutein and Clavazin, it has been shown that if both are used as a co-treatment it can be more beneficial in neutralizing the deleterious effects produced by CCl₄ toxicity.

CONCLUSION

The present study concludes that CCl₄ administration causes biochemical alterations while Lutein and Clavazin have potent anti-oxidant activity to recover the hepatic damage. Both of Lutein and Clavazin have potential to treat the liver injury in rats but still more research is required to explore their protective effects on human liver that is the major problem of current days.

REFERENCES