ORIGINAL ARTICLE

Interrelationship of Circulating Biochemical Markers and Response of Lutein in Rats Receiving Carbon Tetrachloride (CCl_4) 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 (CCl_4)induced toxicity in rats.Thirty albinosWister 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 CCl_4 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 CCl_4 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 CCl_4 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 Clavazinhave potent defensive ability against CCl_4 induced liver toxicity in rats by their anti-oxidative and anti-inflammatory activity and may have protective effect on human liver.

Keywords: Carbon Tetrachloride (CCl_4,), Sodium oxide dismutase (SOD), Catalase (CAT), Glutathione (GSH), Total protein (TP), Malondialdehyde (MDA), Clavazin, Lutein.

INTRODUCTION

In the recent years, mostof halogenated alkane compounds have been banned due to their toxicity such as carbon tetrachloride (CCl_4), chloroform ($CHCl_3$) or idoform (CHI_3). CCl_4 induces their effects on different organs such as liver, heart, brain, kidney, lungs and testes (Weber *et al.,* 2003). Carbon tetra chloride (CCl_4) proved to be a lethal liver toxin resulting inproduction of free radicals that cause oxidative stress leading tocentri-lobular liver necrosis (Chen *et al.,* 2013).CCl_4 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).CCl_4 converts into CCl_3 by cytochrome P450, reactswith high partial pressure of oxygen and converts into trichloromethyl peroxyl radical ($Cl_3COO^{\cdot}$) (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 CCl_4 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.Anti-oxidative 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 carotenoidfamily, 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 (CCl_4) 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

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approved by the Ethics Committee for the Scientific Research at the University of Lahore.

Plant extract: The standardized extract of Luteinwas 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 CCl_4 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 asSilybum marianum (200mg), Picrorhiza kurroa (50mg), Glycyrrhiza glabra (50mg) and Cichorium intybus (75mg).

Group 1	Control rats, which received normal diet.
Group 2	Received CCl_4 4ml/ kg.B.wt
Group 3	Received CCl_4 + Lutein 200mg/kg.B.wt
Group 4	Received CCl_4 + Lutein 400mg/kg.B.wt
Group 5	Received CCl_4 + Clavazin 1tab/ kg.B.wt

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 -60ºC until further biochemical analysis.

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

RESULTS

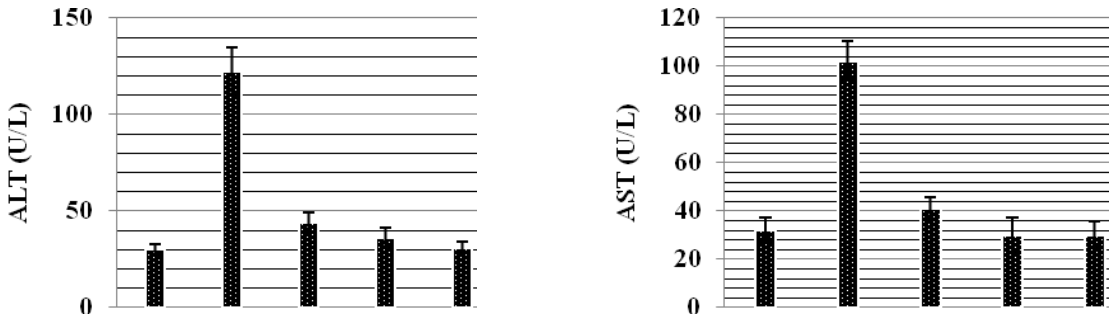
Serum biochemical parameters of ccl_4 induced toxicity in rats: The hepatic metabolic enzyme levels such as ALT, AST and ALP are significantly elevated and total protein (TP) level reduced in CCl_4 treated rats. The TP level reached 2.99IU/L in CCl_4 treated rats as compared to standard value (6.21IU/L), but observed TP level again increased (5.26, 6.25 and 5.21IU/L) in group C (CCl_4+Lutein 200mg/kg), group D (CCl_4+Lutein 400mg/kg) and group E (CCl_4+Lutein Clavazin 1Tab/kg) respectively(Figure-01 A, B, C and D).

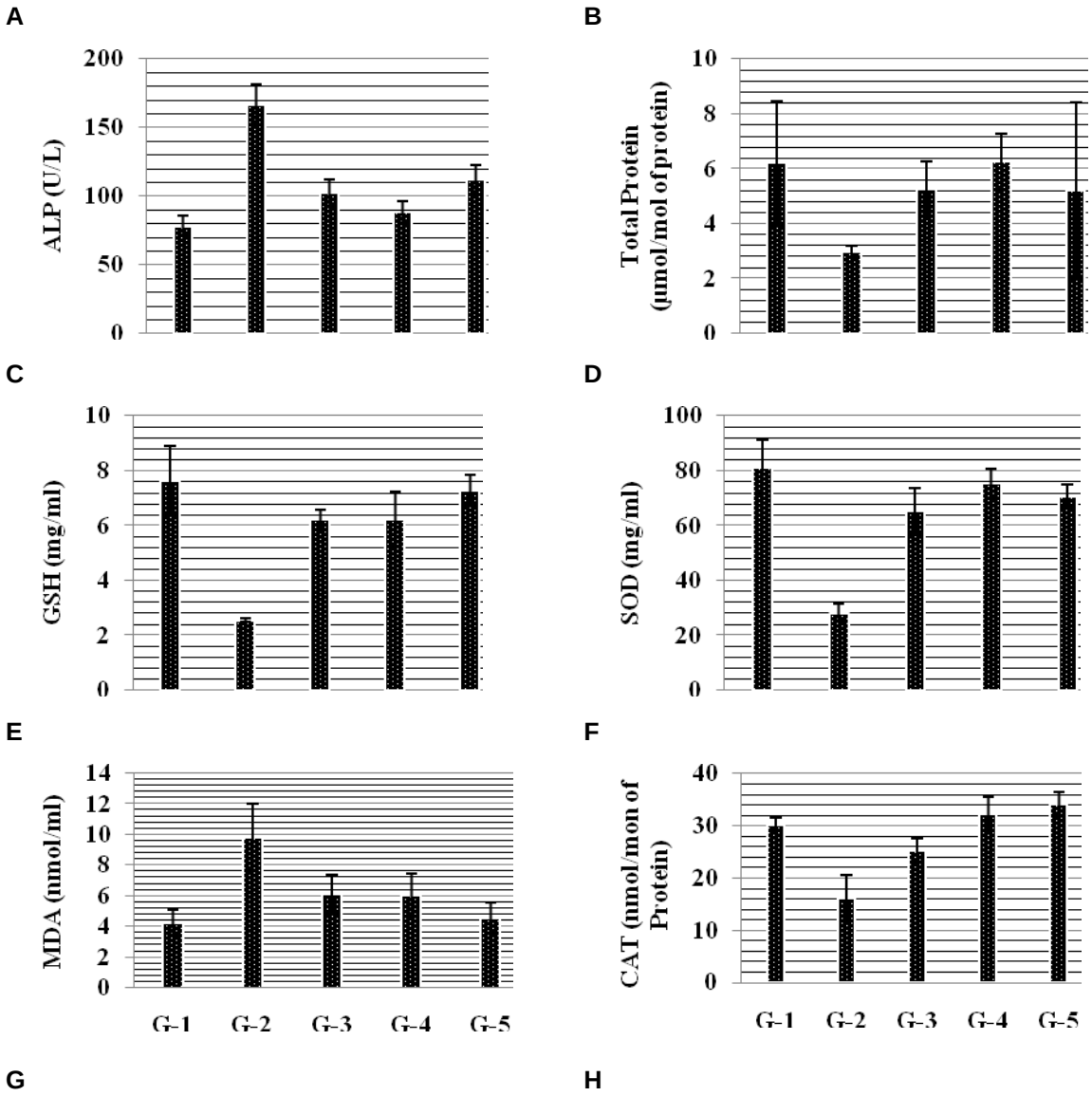
Consequences of lutein on enzymaticantioxidant activities in ccl_4 receiving rats: The antioxidant enzymes such as SOD (28.26mg/ml) and CAT (16.23µmol/mol of protein) counted low in CCl_4treated rats as compared to standard value (81.26mg/ml and 30.25µmol/mol of protein) but on another side, Lutein protects 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 ccl_4 receiving rats: The non-enzymatic antioxidant GSH activity reduced (7.65 mg/ml to 2.56mg/ml) in CCl_4 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 CCl_4 treat rats as compared to standard value (4.223nmol/ml). 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 (CCL_4) to induce hepatic injury G-1 (Control), G-2 (CCl_4 4ml/kg b.Wt), G-3 (CCl_4 4ml/kg b.Wt+Lutein @ 200 mg/kg b.Wt), G-4 (CCl_4 4ml/kg b.Wt+Lutein@400mg/kg b.Wt), G-5 (CCl_4 4ml/kg b.Wt+ CLAVAZIN @ 200 mg/kg b.Wt)





DISCUSSION

In the present study, firstly we induce CCL_4toxicity resulting in relative increasein aspartate amino-transferase (AST), alkaline phosphatase (ALP) and alanine amino-transferase (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 1mg/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).CCl_4 is highly reactive hepatotoxin, induce liver toxicity (Connor *et al.,* 1986)and its metabolism consists of two phases. In initial phase, CCl_4 converted into CCl_3 by cytochrome p450-dependent monooxygenease in hepatic parenchyma cells while in second phase, CCl_4 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 radicalsattack (Sagor *et al.,* 2015). After CCl_4 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 CCl_4 treated group B shows MDA increase but in group C and D Lutein therapy

decreases the oxidative stress, as MDA levelsare reduced. Group E that contain synthetic drug (Clavazin) also reduce the MDA levels**.**The primary antioxidantslike GSH, SODand CAT are also reduced by CCl_4 administration. Clavazintreated rats show highest levels of GSH and CAT while lowest levels of MDAand 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 ofguinea 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 CCl_4 toxicity.

CONCLUSION

The presentstudy concludes that CCl_4administration causes biochemical alterations while Lutein and Clavazin have potent anti-oxidant activity to recover the hepatic damage. Both of Lutein and Clavazin have potentialto 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.

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