Effect of Vitamin E on Chemically Induced Hepatic Fatty Degeneration and Portal Inflammation in Albino Mice

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ABSTRACT

Objectives: To determine the antioxidant effect of vitamin E against carbon tetrachloride (CCl_4) induced fatty degeneration and portal inflammation in the liver of albino mice.

Study design: Analytical experimental study. This study was performed on different groups of albino mice to determine the antioxidant effect of vitamin E against carbon tetrachloride (CCl_4) induced fatty degeneration and portal inflammation in the liver.

The study duration was 5 months. The animals were randomly distributed into five groups A, B, C, D and E .The group A served as control group, group B was administered only with CCl_4 (no vitamin E) and groups C, D and E received test drug (vitamin E) in different doses along with CCl_4. At the end of experiment, the livers were dissected for microscopic study, carried out in research laboratory of Khyber Medical College Peshawar.

Results: The results revealed highly significant difference (p value < 0.05) in degree of fatty changes and portal inflammation in groups treated with CCl_4. The animals administered with 50 mg of vitamin E showed reduction in severity of these parameters.

Conclusions: This study reveals that fatty changes and portal inflammation in liver caused by CCl_4 can be reduced by vitamin E in dose of 50 mg/kg body weight.

Key words: Antioxidant, Fatty degeneration, Portal inflammation.

INTRODUCTION

Liver is the second largest organ of the body after skin. It has an extraordinary ability of regeneration [1]. The liver is located in a very cardinal and strategic position as all the nutrients from intestine after absorption are transported to the liver through hepatic portal vein before its entry into general circulations. Liver plays very vital roles in our body such as secretion of bile, metabolism of carbohydrates, lipids and proteins and storage of glycogen and various vitamins [2,3].

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It is also involve in synthesis of clotting factors, detoxification and elimination of harmful substances [4]. Liver is responsible for maintaining metabolic homeostasis in the body therefore, it is a target of many hazardous insults leading to impaired hepatic homeostasis and causing liver diseases. A loss of liver functions will result in a fatal morbidity or even death in minutes revealing its great importance.

Liver pathology is worldwide health problem[5].It is now considered to be one of the major health hazard in Pakistan [6]. Liver diseases are usually caused by toxic substances, various drugs, infections, obesity, diabetes and alcohol consumption. The hepatotoxic chemicals harm liver cells primarily by inducing lipid peroxidation and other oxidative damages [7]. Different kinds of antioxidant offer effective and natural therapy by constraining the progression of degenerative processes and play a defensive role against toxic effects of reactive oxygen species [8]. They simply convert active oxygen molecules into non-toxic molecules by donating electrons to free radicals to diminish their reactivity and thus

maintaining the cellular pro-oxidant/antioxidant balance[9]. The antioxidants are produce endogenously and can be received from exogenous sources. Essential antioxidants include various kinds of vitamins such as vitamin A, C and E. Minerals like copper, zinc and manganese and some compounds like glutathione and flavonoids have also remarkable antioxidant properties[10].

In present study the vitamin E has been used to demonstrate its effectiveness in reducing the hepatic inflammation and fatty degeneration induced by CCl_4 in animal model.

MATERIALS & METHODS

Study design: Analytical experimental study conducted on different groups of albino mice.
Setting: Research laboratory of Khyber Medical College (KMC) Peshawar.
Duration of study: five months after approval by the ethical committee.

35 healthy albino mice of same age weighing 25 to 30 g were used in present study. The animals were lodged in well ventilated (temperature 20±25 ^{0} C) animal house with 12hr light and dark photo cycle [11]. They were fed on standard pelleted feed. The body weight of mice was recorded regularly throughout the experimental period. The animals were randomly grouped in to group A (control group), group B (administered with CCl_4) and group C, group D and group E. They received Vitamin E orally in dose of 1, 10 and 50mg/kg body weight respectively along with injection CCl_4.The CCl_4 hepatotoxicity was induced in mice at a dose of 1 ml/kg subcutaneously mixed with olive oil .The dose of CCl_4 was repeated weekly.

At the end of experimental period all animals were killed by cervical dislocation to induce painless death. Animals were dissected and liver was separated with care. The specimens were fixed in formalin. According to conventional techniques the tissues were processed for routine microscopy [12]. The specimens were stained with Haematoxylin and Eosin[13] and Reticulin stain. The slides were examined for fatty changes and portal inflammation under light microscope, at magnification of 10× and 40×. Scoring and grading system was applied in order to evaluate the degree of portal inflammation and fatty degeneration. Changes were marked as no change, mild, moderate and severe.

Statistical Analysis

Statistical data was recorded and analysed using SPSS version 20.The results were expressed as mean and standard deviation of variables. One way ANOVA was performed and the level of significance was set at p-value < 0.05. Further comparisons among groups were made according *post hoc* Tuckey's test.

RESULTS

The CCl_4 treated groups showed change in behaviour immediately after administration of CCl_4 injection. The animals became little drowsy and their physical activities got sluggish.
The livers of all animals of control group depicted no gross abnormalities. The obvious change in colour of liver was noticed in groups B, C, D and E. The tissues were examined under light microscope for following parameters.

Fatty Changes

Fatty changes in hepatocytes were graded as no change = 0, mild change as 1, moderate as 2 and severe as 3. One way ANOVA shows highly significant p value (0.0001<0.05). It was observed that CCl_4 treated experimental groups B, C, D and E developed fatty changes as compared to control group A. The results are mentioned in table 1. The representative photograph is mentioned in figure 2.

Portal inflammation

Portal inflammation was recorded according to Knodel histological activity [14,15]. Statistical analysis one way ANOVA showed highly significant p value (0.0001 < 0.05). The results are shown in table 2.
The microscopic study of the liver sections from a control group showed normal parenchymal architecture and no remarkable lesions were observed.

Table 1: Effects of vitamin E on fatty degeneration.

Groups	n	Mean	Std/Dev
Group A (Control)	7	0.00	0.000
Group B (CCl_4 only)	7	2.3	0.1224

Group C (CCl_4 + 1 mg/kg vit E)	7	2.52	0.1303
Group D (CCl_4 + 10 mg/kg Vit E)	7	2.26	0.1949
Group E (CCl_4 + 50 mg/kg Vit E)	7	1	0.000

Table 2: Effects of vitamin E on Portal inflammation.

Groups	N	Mean	Std/dev
Group A (Control)	7	0.00	0.000
Group B (CCl_4 only)	7	2.42	0.2387
Group C (CCl_4 + 1 mg/kg Vit E)	7	2.36	0.2701
Group D (CCl_4 + 10 mg/kg Vit E)	7	2.28	0.1788
Group E (CCl_4 + 50 mg/kg Vit E)	7	0.74	0.3714

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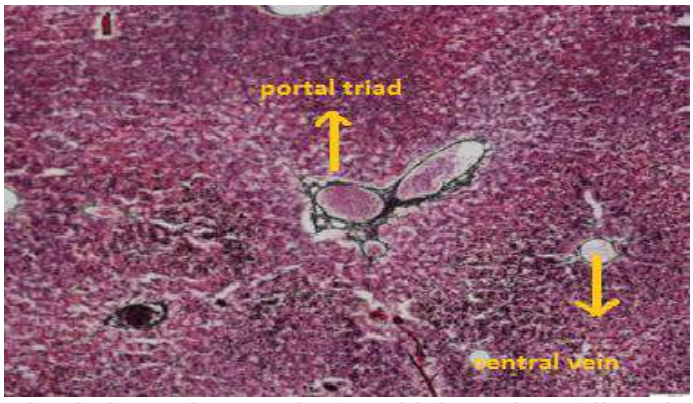

Figure 1: Reticulin stained Photomicrograph of 5µ thick section of liver tissue showing normal parenchymal architecture in control group (10×)

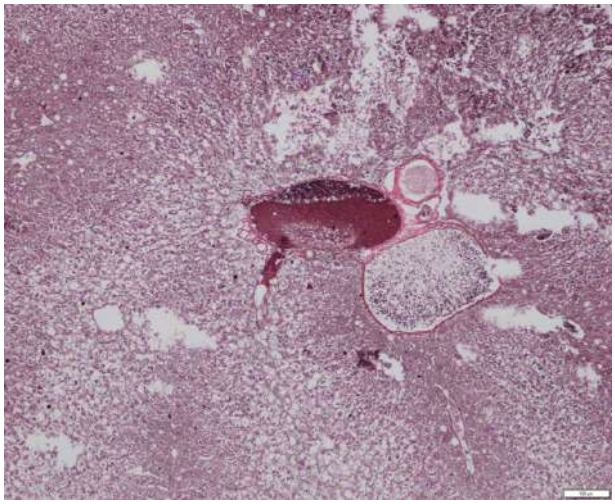

Figure 2: Reticulin stained Photomicrograph of 5µ thick section of liver tissue showing fatty changes in hepatocytes and portal inflammation (10×).

DISCUSSION

Present study was conducted to demonstrate the protective ability of vitamin E against hepatic damage induced by CCl_4 in albino mice. Liver plays significant role in detoxification of various chemicals so it is considered to be one of the target organs of toxic chemicals .The portal inflammation and fatty degeneration of liver were induced by CCl_4 as it is one of

the potent hepatotoxic chemical [16].The carbon tetra chloride caused significant liver injury in group B ,C , D and group E as manifested by fatty degeneration and portal inflammation, it has been reported by previous studies that CCl_4 causes inflammatory cell infiltration and fatty degeneration [17,18].

Several studies had been done regarding CCl_4 inducing fatty changes in liver. CCl_4 poisoning fat accumulation is the result of covalent binding of CCl_4 metabolites to cell structures and disturbance of balance between lipid synthesis and degradation[19].

The fatty changes noticed in group B, C and group D were of moderate degree .On other hand it was noticed that fatty degeneration was of mild degree in group E (treated with 50 mg/kg of vitamin E). These findings indicate the therapeutic significance of vitamin E in reducing the fatty changes in dose of 50mg/kg body weight. Previous studies have suggested the hepatoprotective role of herbs rich in vitamin A and vitamin C against chemically induced hepatotoxicity in animal models [20].

Additionally, portal inflammation was noticed in all groups treated with CCl_4. A moderate degree of portal inflammation was observed whereas the group E showed a drastic improvement in inflammatory cells infiltration. These findings are in line with observation of study conducted by Mohamed SA. El-Gerbed in 20015, who reported the effectiveness of vitamin C in reducing inflammation of liver insulted by Capecitabine [21].

In summary, the findings in this study revealed that treatment of mice with the vitamin E in dose of 50mg/kg body weight causes marked improvement in fatty degeneration and portal inflammation of liver. The possible reason could be due to its antioxidant ability.

CONCLUSION

This study reveals that fatty changes and portal inflammation in liver caused by CCl_4 can be reduced by vitamin E in dose of 50 mg/kg body weight.

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