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Protective effect of rutin against carbon tetrachloride-induced hepatotoxicity in mice

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Abstract

Synthetic medicinal compounds used in the dealing of liver diseases are insufficient and can have severe undesirable effects. So there is an international trend to revisit to traditional way of treatment using plants or plant products. Several plant derived natural compounds are under research for the treatment of liver ailments. The aim of the current study was to evaluate the protective effect of rutin against carbon tetrachloride induced toxicity in Swiss albino mice. Rutin pretreatment to mice decreased the level of lipid peroxidation (LPO) significantly ($p < 0.001$) as compared to CCl_4 treated group. Rutin also restored the activity of liver antioxidant enzymes like superoxide dismutase (SOD) and catalse (CAT), glutathione-S-transferase (GST) significantly. Histopathological examinations of liver in rutin pretreated group showed protection against CCl4 induced injuries in liver. Over all rutin is able to reverse the altered biochemical profile towards normalization due to CCl4 exposure. Our results suggest that rutin works on the liver to maintain its standard performance and reduce cell membrane instability.

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Key words:
Rutin; carbon tetrachloride; CCl_4; Catalase; LPO

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Introduction:

Liver is the organ where most of the foreign compounds are metabolized first and that is why it is highly vulnerable to almost as many different diseases. Some are uncommon but there are a handful, including hepatitis, alcohol related disorders cirrhosis, and liver cancer. The most important

reason of these disorders is the exposure of different environmental pollutants and xenobiotics e.g., thioacetamide, paracetamol, carbon tetrachloride, alcohol, etc. These xenobiotic compounds mainly damage liver by producing the reactive oxygen species (ROS). Free radicals induce the toxicity by covalent binding and lipid peroxidation [1]. Scavenging of free radicals by antioxidants could reduce the fibrosis process in the tissues [2]. Free radicals may also be a contributory factor in a progressive decline in the function of immune system [3]. Cooperative defense systems that protect the body from free radical damage include the antioxidant nutrients and enzymes. The antioxidant enzymes include superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx). Their role as protective enzymes is well-known and has been investigated extensively with in vivo models. Conventional or synthetic drugs used in the treatment of liver diseases are inadequate and can have serious adverse effects. So there is a worldwide trend to go back to natural compounds, extracted from plats. Polyphenolic compounds are widely distributed in plants and known to be excellent antioxidants in vitro and have the capacity to scavenge free radicals and protect antioxidant defence [4,5,6,7].

Identification of a successful hepatoprotective agent will provide a useful tool for the treatment of hepatic diseases. In absence of reliable liver-protective drugs in modern medicine, a large number of medicinal preparations are recommended for the treatment of relief. Rutin is a member of bioflavonoids also called vitamin P with antioxidant, anti-inflammatory, antiallergenic, antiviral, and anti-carcinogenic properties and has been demonstrated to scavenge superoxide radicals [8,9,10]. In humans, it attaches to the iron ion (Fe2+), preventing it from binding to hydrogen peroxide, which would otherwise create a highly-reactive free radical that may damage cells [11]. It has shown many pharmacological benefits including anti-tumor, anti-inflammatory, anti-diarrhoeal, anti-mutagenic, myocardial protecting, immunomodulator [12]. Literatures revealed that rutin increased the antioxidant capacity of the kidney of normal mice as well as in liposomal models [13]. Additionally, cure of diabetic rats with rutin reduce lipid peroxides while total protein and reduced glutathione were augmented. On the basis of our acquaintance on free radicals and its participation in a number of diseases and the dependence on traditional medicine to replace ineffective medications, in this perspective, more studies should be carried out to explore the efficacy of natural antioxidants [14]. Therefore, we planned to investigate the role of rutin in CCl_4 induced hepatotoxicity in Swiss albino mice.

Materials and Methods

Chemicals:

Oxidized and reduced glutathione (GSSG and GSH), reduced nicotinamide adenine dinucleotide (NADPH), potassium dichromate, dithionitrobenzene (DTNB), 1-chloro2,4-dinitrobenzene (CDNB), Reduced Glutathione(GSH), Thiobarbituric acid (TBA),Trichloroacetic acid (TCA 10%), physiological normal saline (0.9%), Formalin (4%), Sulphosalicylic acid (4%), Glycine were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All other chemicals and reagents used were of highest purity commercially available.

Animal Maintenance

Swiss Albino mice weighing 20-30 g were housed in groups of six in polypropylene cages. Lighting was regulated to provide equal hours of light and dark. Animals were obtained from the central animal house facility of Jamia Hamdard, New Delhi, India. The animals were acclimatized to standard laboratory conditions (temperature 25±10 ◦C, relative humidity 50±15%) 1 week prior to the actual commencement of the experiment. They were provided with standard food pellets (Hindustan

Lever Ltd., India) and tap water ad libitum. The study was approved from the Committee for the purpose of control and supervision of experimental animals (CPCSEA). CPCSEA guidelines were followed for animal handling and treatment.

Experimental Design

Twenty four Swiss Albino mice were divided in four groups of six animals each. CCl_4 (1 ml/kg body weight) was administered to groups II and groups III animals by intraperitoneal injection which is well documented to induce acute hepatotoxicity in mice. Group I served as control group and received vehicle i.e olive oil. Group II served as CCl_4 control and received CCl_4:olive oil (1:1) on 7th day of the initiation of experiment. Group III was pretreated with rutin (80 mg/kg body weight) for 7 days and then CCl_4:olive oil (1:1) on the 7th day was injected (i.p.). Group IV received only rutin for 7 days. On day 8 all the animals were sacrificed and liver was collected for further assays.

Preparation of Homogenates

A 10 % homogenate of each liver was prepared separately in 0.1 M phosphate buffer (pH 7.4) using a motor driven Teflon-pestle homogenizer (Fischer) and centrifugation at 4000 rpm for 5 min at 4°C. The supernatant obtained was called "homogenate" and used for the assays.

Preparation of PMS (post mitochondrial supernatant)

The homogenate was centrifuged at 12000 rpm for 20 min at 4°C. The supernatant fraction obtained was called "PMS" and used for the assays.

Determination of lipid peroxidation

Briefly, 0.6 ml of homogenate was mixed with 0.4 ml of phosphate buffer pH 7.4 and 1 ml 10 % trichloroacetic acid (TCA) and finally kept for 5 min at room temperature. Then 1 ml 0.67 % thiobarbituric acid (TBA) was mixed and tubes were placed in boiling water bath at 90°C for 45 minutes and on ice bath for 15 minutes. Tubes were centrifuged at 2500x g for 10 minutes. Pellet was discarded and supernatant was collected to measure the absorbance at 535 nm in spectrophotometer. The result was expressed in nmoles of TBARS formed /min/g tissue at 37 °C by using a molar extinction coefficient of $1.56 \times 10^5 M^{-1}cm^{-1}$.

Determination of antioxidant enzymes:

Assay of Catalase

In brief, the assay mixture consisted of 1.950 ml phosphate buffer (0.1 M, pH 7), 1 ml hydrogen peroxide (0.34%) and 0.05 ml of PMS (10 %) in a final volume of 3.0 ml. Change in absorbance was recorded at 240 nm. The catalase activity was calculated in terms of mmoles H_2O_2 consumed/min/mg protein using a molar extinction coefficient of 43.6 M^{-1} cm^{-1}.

Assay of Super Oxide Dismutase (SOD)

The reaction mixture consisted of 1.6 ml hot glycine buffer (50 mM, pH 10.4), 0.2 ml of PMS (10 %) and 0.04 ml of chilled epinephrine in a final volume of 1.84 ml as described by Stevens et al., 1980. The change in absorbance was recorded at 480 nm and the enzymatic activity was calculated as nmol of epinephrine protected from oxidation/mg protein/min using a molar extinction coefficient of 4.02×10^3 M^{-1} cm^{-1}.

Assay of Glutathione S-transferase

The reaction mixture consisted of 2.4 ml phosphate buffer (0.1 M, pH 6.5), 0.2 ml reduced glutathione (1.0 mM), 0.2 ml CDNB (1.0 mM) and 0.2 ml of cytosolic fraction in a total volume of 3.0 ml. The changes in absorbance were recorded at 340 nm and the enzymatic activity was calculated as nmol CDNB conjugate formed/min/mg protein using a molar extinction coefficient of 9.6×10^3 M^{-1} cm^{-1}.

Histopathology:

Liver was removed, sliced and washed in saline and preserved in 10% formalin for histopathological studies. The pieces of liver were processed and embedded in paraffin wax. Sections were taken and stained with hematoxylin and eosin and.

Statistical analysis

Data from experiments were compared by one-way ANOVA followed by Turkey's *t*-test. All values were reported as mean ± SE for each group. The significant level was showed as $p<0.001$, $p<0.01$ and $p<0.05$.

Results:

Effect of rutin on CCl_4 -induced lipid peroxidation in mice liver:

Treatment of mice with CCl_4 (1 ml/kg body weight, i.p) caused a significant ($p< 0.001$) increase in TBARS, an estimation of lipid peroxidation in liver homogenate as compared to the control **(Table 1).** Pre-treatment of mice with rutin (80 mg/kg b.w.) resulted in significant ($p< 0.001$) decrease TBARS in liver homogenate **(Table 1).**

Effect of rutin on CCl_4 -induced suppression of antioxidant enzyme activities:

SOD, CAT and GST were measured as an index of antioxidant status of tissues. Significantly lower liver SOD, catalase, and GST activity were observed in mice from the CCl4 treated group as compared to the normal control group. Treatment with CCl4 significantly decreased the SOD, CAT and GST levels in the liver (19.80 ± 0.91, 316.40 ± 14.21,85.33 ± 1.78) as compared to the normal control group (48.32 ± 2.17,530.53 ± 12.06, 113.23 ± 4.15). Pretatment with rutin (80 mg/kg b.w.) significantly ($p < 0.001$) increased the levels of these antioxidant enzymes as compared to the CCl4-treated group **(Table 2).**

Effect of rutin on CCl_4 -induced hepatotoxicity:

The histopathological studies of the liver showed fatty changes, swelling and necrosis with loss of hepatocytes in CCl4 control mice in comparison with normal control. Treatment of rutin showed regeneration of hepatocytes, normalization of fatty changes and necrosis of the liver **(Fig. 1)**.

Discussion:

Liver is the organ where most of the xenobiotic substances are metabolized. CCl_4 is converted to glucuronide and sulphide conjugates in liver [15]. Many hepatotoxic agents induce generation of peroxy radicals directly or indirectly. These peroxy radicals damage liver by enhancing the membrane lipid peroxidation. It has been reported that lipid peroxidation of the plasma membranes is the primary source of CCl_4-induced hepatotoxicity, and is attributed by the generation of free radical derivatives of CCl_4 [16]. Superoxide dismutase (SOD), catalase (CAT), glutathione-S-transferase (GST) and glutathione peroxidase (GPx) constitute the cellular enzymatic antioxidants. These enzymes work together to neutralize superoxide anion and hydrogen peroxide (H_2O_2) of the cells and plays a significant role in the defense of cells against oxidative abuse. Current studies on flavonoids from various plant extracts disclosed their antioxidant properties and showed their stimulatory action on cellular enzymatic antioxidants [17]. Moreover it is reported that some flavonoids put forth a stimulatory act on the gene expression of certain cellular enzymatic antioxidants [18].

In this study, CCl_4-intoxication resulted in a significant increase in the amount of thiobarbituric acid reactive substances (TBARS), a measure of cellular lipid peroxidation. Pretreatment of rutin brought the augmented TBARS level near to normal. Rutin also restored the CCl_4 induced alterations in the cellular enzymatic antioxidants. Re-establishment of TBARS near to normal levels by these extracts may be because of an enhancement of antioxidant enzymes, such as superoxide dismutase, catalase, and glutathione-S-transferase.

In this study, histopathological observations showed huge fatty changes, tissue necrosis and loss of cellular boundaries in CCl4 intoxicated mice. Pretreatment of rutin showed a significant restoration of the hepatic lesions and architecture of the liver. Based on these observations it can be concluded that rutin acts by its

stabilizing effect on the cell membrane as was proved in case of silymarin [19].

Conclusion:

Carbon tetrachloride intoxication augments lipid peroxidation and alters the enzymatic antioxidants of the liver. Pretreatment of rutin is able to reverse the altered biochemical profile towards normalization due to CCl4-intoxication. Rutin stabilizes the plasma membrane as well as increases regenerative potential of the liver. Our data suggest that rutin can be used as a supplement for the treatment of hepatotoxicities.

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Table 1: Effect of rutin on carbon tetra chloride induced modification of lipid peroxidation (LPO)

Groups	Treatment	LPO (nmoles of TBARS formed/min/gm of tissue)
Group I	Control	1.66 ± 0.72
Group II	CCl_4	2.53 ± 1.10***
Group III	CCl_4 + Rutin	2.04 ± 0.89###
Group IV	Rutin	1.71 ± 0.74

Data are presented as mean ± SE of six mice per group. LPO is expressed as nmoles of TBARS formed/ min/gm of tissue. Significant differences are indicated by *** p< 0.001 when compared with control (Group I) and ###p< 0.001 when compared with CCl_4 treated animals (Group II).

Table 2: Effect of rutin on CCl_4 induced alterations in antioxidant enzymes

Groups	SOD (nmoles of epinephrine protected from auto-oxidation / min/ mg protein)	Catalase (mmoles of H_2O_2 oxidized/min/ mg protein)	GST (nmoles of CDNB conjugates/min/mg protein)
Control	48.32 ± 2.17	530.53 ± 12.06	113.23 ± 4.15
CCl_4	19.80 ± 0.91***	316.40 ± 14.21*	85.33 ± 1.78*
CCl_4 + Rutin	37.03 ± 1.18###	1589.85 ± 38.27###	151.30 ± 2.49###
Rutin	48.48 ± 1.18	858.92 ± 76.25	92.37 ± 12.58

Data are presented as mean ± SE of six mice per group. Significant differences are indicated by *<0.05; when compared with group I and with #<0.05; when compared with group II

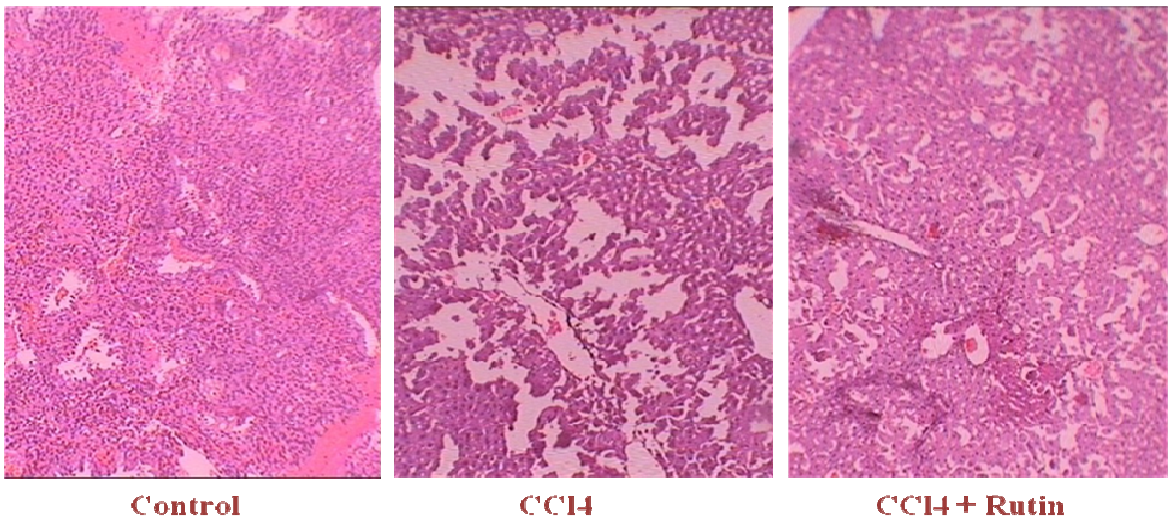

Figure 1: Histopathology of liver of mice from different groups. (A) Control group showing well defined cellular structure. (B) CCl4 exposure induced extensive hepato cellular damage as evidenced by the presence of inflammation and hyperplasmia showing cellular degeneration as compared with control group. (C) rutin treated group showed significant protection in cellular damage.

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