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Research Paper

Hepatoprotective effect of chrysin on prooxidant–antioxidant status during ethanol-induced toxicity in female albino rats

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Abstract

Objectives To evaluate the effect of chrysin, a natural, biologically active compound extracted from many plants, honey and propolis, on the tissue and circulatory antioxidant status, and lipid peroxidation in ethanol-induced hepatotoxicity in rats.
Methods Rats were divided into four groups. Groups 1 and 2 received isocaloric glucose. Groups 3 and 4 received 20% ethanol, equivalent to 5 g/kg bodyweight every day. Groups 2 and 4 received chrysin (20 mg/kg bodyweight) dissolved in 0.5% dimethylsulfoxide.
Key findings The results showed significantly elevated levels of tissue and circulatory thiobarbituric acid reactive substances, conjugated dienes and lipid hydroperoxides, and significantly lowered enzymic and non-enzymic antioxidant activity of superoxide dismutase, catalase and glutathione-related enzymes such as glutathione peroxidase, glutathione reductase, glutathione-*S*-transferase, reduced glutathione, vitamin C and vitamin E in ethanol-treated rats compared with the control. Chrysin administration to rats with ethanol-induced liver injury significantly decreased the levels of thiobarbituric acid reactive substances, lipid hydroperoxides and conjugated dienes, and significantly elevated the activity of superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase, glutathione-*S*-transferase and the levels of reduced glutathione, vitamin C and vitamin E in the tissues and circulation compared with those of the unsupplemented ethanol-treated rats. The histological changes observed in the liver and kidney correlated with the biochemical findings.
Conclusions Chrysin offers protection against free radical-mediated oxidative stress in rats with ethanol-induced liver injury.
Keywords antioxidants; chrysin; ethanol; lipid peroxidation; liver disease

Introduction

Alcoholic liver disease remains one of the most common causes of chronic liver disease in the world.[1] Alcohol-related disorders are a challenging health problem with far-reaching medical, social and economic consequences. Long-term alcohol use potentially results in serious illnesses, including alcoholic fatty liver, hypertriglyceridaemia, cirrhosis, cardiovascular disease and pancreatic inflammation.[2] Ethanol is a fat-soluble non-electrolyte, which is readily absorbed from the gastrointestinal tract, diffuses readily into the circulation and is distributed uniformly throughout the body.[3] The main pathway for the hepatic oxidation of ethanol to acetaldehyde proceeds via alcohol dehydrogenase. In addition to alcohol dehydrogenase, ethanol can also be reduced by an accessory but inducible microsomal ethanol oxidizing system.[4]

Induction of the microsomal system results in enhanced acetaldehyde production, which in turn impairs the defence system during oxidative stress. The liver cytochrome P450 form CYP2E1, a major component of the microsomal ethanol oxidizing system, has been implicated in hepatotoxicity caused by ethanol.[5] This hepatic microsomal cytochrome P450 catalyses the oxidative metabolism of xenobiotics, including ethanol. Ethanol may also cause hepatotoxicity by activating macrophages and inducing the release of pro-inflammatory cytokines, such as tumour necrosis factor-α.[6,7]

Ethanol administration can elicit disturbances in the delicate balance between the prooxidant and antioxidant systems of the organism, thereby leading to oxidative stress.

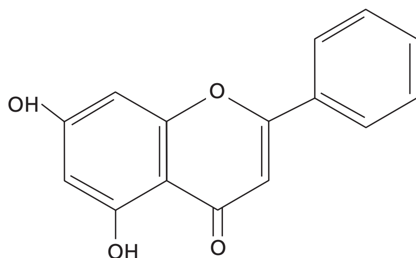

Figure 1 Chemical structure of chrysin (5,7-dihydroxyflavone)

Increased generation of oxygen- and ethanol-derived free radicals has been observed in the microsomes (particularly the ethanol-inducible cytochrome P450 isoform), the cytosolic xanthine and/or aldehyde oxidase, as well as through the mitochondrial respiratory chain.[8]

In chronic ethanol intoxication, increased lipid peroxidation could occur as a result of induction of microsomal membrane free radical generation.[9,10] Ethanol or its metabolite can alter the balance in the liver towards autooxidation, either acting as a prooxidant or reducing the antioxidant level, or both.[11] Lipid peroxidation mediated by free radicals is considered to be a primary mechanism of cell membrane destruction and cell damage.[12] To counteract these oxidants, cells have several antioxidant enzymes, including superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT).

Many antioxidants are present in the diet, vitamins E and C being the major ones. Poor nutrition leads to a deficiency of these vitamins, thus increasing the susceptibility of the liver to ethanol-induced oxidative stress. Flavonoids are plant polyphenolic compounds, that comprise several classes including flavonols, flavanones and flavans. Chrysin (5,7-dihydroxyflavone) (Figure 1) is a natural flavonoid contained in many plant extracts, honey and propolis.[13,14] Chrysin possesses antioxidant,[15] anti-allergic,[16] anti-inflammatory,[17] anticancer,[18] anti-estrogenic,[19] and anxiolytic[20] properties.

Chrysin has also been found to have tyrosinase inhibitory activity[21] and moderate aromatase inhibitory activity.[22] It can also inhibit estradiol-induced DNA synthesis.[23] In order to improve the biological activity of chrysin, a number of its derivatives have been prepared.[24–26] C-iso prenylated hydrophobic derivatives of chrysin are potential P-glycoprotein modulators in tumour cells.[27] In addition, it has been recently reported that chrysin exerts antihypertensive effects, and reduces left ventricular hypertrophy and endothelial dysfunction in spontaneously hypertensive rats.[28] Chrysin inhibits the activation of human immunodeficiency virus in models of latent infection.[29]

The present study aimed to evaluate the effect of chrysin on tissue lipid peroxidation and the antioxidant status in rats administered ethanol. The findings were compared with those of control and unsupplemented ethanol-treated rats.

Materials and Methods

Chemicals and reagents

Chrysin was purchased from Sigma Chemical Co (St Louis, MO, USA). Ethanol was obtained from E.I.D Parry India Ltd (Nellikuppam, Cuddalore District, India). All other chemicals used were of analytical grade and were obtained from Central Drug House Pvt. Ltd (New Delhi, India).

Animals

All the animal handling and experimental procedures were approved by the Institutional Animal Ethics Committee, Annamalai University (registration no: 160/1999/CPCSEA/483) and animals were cared for in accordance with the Indian National Law on Animal Care and Use.[30]

Female adult Wistar rats (160–180 g) were purchased from the Central Animal House, Rajah Muthiah Medical College and Hospital, Annamalai University, Tamil Nadu, India. Rats were housed in plastic cages with filter tops under controlled conditions of a 12-h light–dark cycle, 50% humidity and temperature of 28°C. All rats received a standard pellet diet (Lipton Lever Mumbai, India) and water *ad libitum*.

Study design

Animals were divided into four groups of eight rats each and all were fed the standard pellet diet. Rats in groups 1 and 2 received isocaloric glucose from a 40% glucose solution. Animals in groups 3 and 4 received 20% ethanol (2.5 ml in the morning and 2.5 ml in the afternoon) equivalent to 5 g/kg bodyweight as an aqueous solution by intragastric intubation for 30 days as described previously.[31] At the end of this period, the dietary protocol of group 1 and 3 animals was unaltered. In addition, animals in group 2 received chrysin (20 mg/kg bodyweight)[28] dissolved in 0.5% dimethylsulfoxide for the next 30 days, and animals in group 4 continued to receive ethanol every day by intragastric intubation along with chrysin as in group 2 for the next 30 days. The study design is shown in Figure 2.

The total duration of the experiment was 60 days, at the end of which the animals were fasted overnight, anaesthetized with an intramuscular injection of ketamine hydrochloride (30 mg/kg) and killed by cervical dislocation. Blood was collected from the carotid artery and allowed to coagulate at ambient temperature for 30 min.

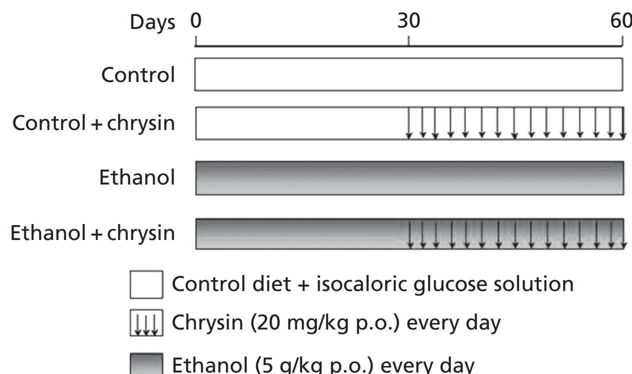

Figure 2 Experimental protocol

Preparation of tissue homogenate

Immediately after death, blood samples were collected in heparinized test tubes and plain tubes and centrifuged for the separation of plasma and serum, respectively. The erythrocytes were washed with 0.9% saline three times and distilled water was subsequently added for haemolysis. The haemolysate was then used for the estimation of total haemoglobin content, which we referred to as lysate. Liver, kidney and heart tissues were washed with ice-cold saline. The tissues were then cut into fragments and homogenized with 3 vols (w/v) of the appropriate buffer using a Potter-Elvehjam homogenizer with a Teflon pestle and centrifuged at 12 000*g* for 20 min at 4°C. The supernatant was used for the biochemical estimations.

Biochemical investigations

Lipid peroxidation was determined by measuring thiobarbituric acid reactive substances (TBARS) in the tissues using the method of Ohkawa *et al*.[32] The pink chromogen produced by the reaction of secondary products of lipid peroxidation such as malondialdehyde with thiobarbituric acid was estimated at 532 nm. The concentration of tissue lipid hydroperoxides (LOOH) was estimated by the method of Jiang *et al.*[33] Conjugated dienes were estimated by the method of Rao and Recknagel.[34] This method is based on the arrangement of double bonds in polyunsaturated fatty acids to form conjugated dienes with an absorbance maximum at 233 nm.

SOD (EC 1.15.1.1) was assayed according to the method of Kakkar *et al*.[35] The assay was based on the 50% inhibition of the formation of NADH-phenazine methosulfate nitroblue tetrazolium formazan at 520 nm. The activity of catalase (EC 1.11.1.6) was assayed according to the method of Sinha[36] based on the conversion of dichromate in acetic acid to perchromic acid and then to chromic acetate, when heated in the presence of H_2O_2. The chromic acetate formed was measured at 620 nm.

Reduced glutathione (GSH) in the tissues was assayed according to the method of Ellman.[37] GSH estimation was based on the development of yellow colour when 5,5-dithiobis (2-nitro benzoic acid) was added to compounds containing sulfydryl groups. The activity of GPx (EC 1.11.1.9) and glutathione reductase (EC 1.6.4.2; GR) were assayed according to the methods of Rotruck *et al.*[38] and Carlberg and Mannervik.[39] A known amount of enzyme preparation was incubated with H_2O_2 in the presence of GSH for a specified time. The amount of H_2O_2 utilized was determined by the method of Ellman.[37] The enzyme activity was expressed as mmol GSH consumed/min per mg protein. Glutathione *S*-transferase (EC 2.5.1.18; GST) activity was assayed according to the method of Habig *et al.*[40] The change in absorbance was recorded at 340 nm and enzyme activity was calculated as μmol of 1-chloro-2,4-dinitrobenzene conjugate formed/min per mg protein using a molar extinction coefficient of 9.6×10^3 min/cm.

Tissue ascorbic acid was estimated by the method of Roe and Kuether[41] and α-tocopherol by the method of Baker *et al*.[42] Proteins were estimated by the method of Lowry *et al*.[43] using bovine serum albumin as the standard.

Histological analysis

The remaining animals were subjected to whole-body perfusion with normal saline and 10% formalin under light ether anaesthesia. Liver and kidney were removed and immediately stored in 10% formalin. The tissues were subsequently embedded in paraffin, thinly sectioned with a microtome, stained with haematoxylin and eosin and examined by light microscopy.[44]

Statistical analysis

Data were analysed by one-way analysis of variance followed by Duncan's multiple range test using a commercially available statistics software package (SPSS for Windows, ver. 11.0; SPSS Inc., Chicago, IL, USA). Results were presented as mean ± SD. Values of $P < 0.05$ were regarded as statistically significant.

Results

Bodyweight changes, lipid peroxidation, enzymic and non-enzymic antioxidants in the liver, kidney, heart and circulation of rats in the control and experimental groups were examined and the data are given in the tables.

Table 1 shows the average weight gain, food intake and liver weight to bodyweight ratio of control and experimental rats during the experimental period. The food intake and weight gain were significantly reduced in ethanol-treated rats compared with control rats. The liver weight to bodyweight ratio was significantly decreased on chrysin supplementation to ethanol-treated rats (group 4) as compared to unsupplemented ethanol-fed rats (group 3; $P < 0.05$). Thus, significant weight gain, increased food intake and decreased liver weight to bodyweight were observed on chrysin supplementation.

Table 1 Effect of chrysin on bodyweight and liver weight to bodyweight ratio

Group	Bodyweight		Net gain (g)	Average food intake (g)	Liver weight × 100/bodyweight
	Day 1	Day 60			
Control	145.19	223.58	78.39 ± 4.53^a	9.20 ± 0.72^a	2.84 ± 0.12^a
Control + chrysin	143.11	222.87	79.78 ± 4.67^a	9.40 ± 0.83^a	2.89 ± 0.17^a
Ethanol	141.16	151.51	10.33 ± 1.01^b	7.51 ± 0.55^b	5.22 ± 0.35^c
Ethanol + chrysin	144.39	194.47	50.07 ± 3.42^c	8.86 ± 0.62^a	3.43 ± 0.26^b

Values are means ± SD for eight rats in each group. [a–c]Values with different superscript letters differ significantly at $P < 0.05$ (Duncan's multiple range test).

Table 2 Effect of chrysin on thiobarbituric acid reactive substances and lipid hydroperoxides

Group	Thiobarbituric acid reactive substances				Lipid hydroperoxides			
	Liver (nmol/g tissue)	Kidney (nmol/g tissue)	Heart (nmol/g tissue)	Plasma (nmol/ml)	Liver (mmol/g tissue)	Kidney (mmol/g tissue)	Heart (mmol/g tissue)	Plasma (mmol/ml)
Control	28.39 ± 2.00[a]	0.56 ± 0.03[a]	0.65 ± 0.04[a]	2.07 ± 0.14[a]	63.87 ± 4.50[a]	0.71 ± 0.05[a]	83.90 ± 5.92[a]	0.97 ± 0.06[a]
Control + chrysin	27.36 ± 1.93[a]	0.53 ± 0.03[a]	0.69 ± 0.04[a]	1.96 ± 0.13[a]	63.45 ± 4.47[a]	0.68 ± 0.04[a]	79.13 ± 5.8[a]	0.96 ± 0.06[a]
Ethanol	59.53 ± 4.20[c]	2.94 ± 0.20[b]	1.52 ± 0.10[c]	4.50 ± 0.31[c]	83.04 ± 5.86[b]	3.35 ± 0.23[c]	115.83 ± 8.17[b]	3.67 ± 0.25[b]
Ethanol + chrysin	32.83 ± 2.31[b]	0.62 ± 0.04[a]	0.80 ± 0.05[b]	2.84 ± 0.20[b]	59.16 ± 4.17[a]	0.92 ± 0.06[b]	79.69 ± 5.62[a]	0.84 ± 0.05[a]

Values are means ± SD of eight rats from each group. [a–c]Values with different superscript letters differ significantly at $P < 0.05$ (Duncan's multiple range test).

Table 3 Effect of chrysin on conjugated dienes

Group	Conjugated dienes			
	Liver (mmol/g tissue)	Kidney (mmol/g tissue)	Heart (mmol/g tissue)	Plasma (μmol/ml)
Control	102.86 ± 7.25[a,b]	97.71 ± 6.89[a]	39.97 ± 2.82[a]	0.66 ± 0.04[a]
Control + chrysin	94.17 ± 6.64[a]	97.03 ± 6.84[a]	38.32 ± 2.73[a]	0.65 ± 0.04[a]
Ethanol	137.72 ± 9.71[c]	136.52 ± 9.63[b]	81.10 ± 5.72[c]	2.65 ± 0.18[b]
Ethanol + chrysin	109.49 ± 7.72[b]	102.74 ± 7.25[a]	46.0 ± 3.24[b]	0.71 ± 0.05[a]

Values are means ± SD of eight rats from each group. [a–c]Values with different superscript letters differ significantly at $P < 0.05$ (Duncan's multiple range test).

The levels of TBARS, LOOH and conjugated dienes in the tissues and circulation of control and experimental animals are given in Tables 2 and 3. TBARS, LOOH and conjugated diene levels in the liver, kidney, heart and circulation of rats treated with ethanol (group 3) were significantly higher compared with those of the control rats (group 1) ($P < 0.05$). Chrysin supplementation to rats treated with ethanol (group 4) lowered the TBARS, LOOH and conjugated diene levels significantly compared with the unsupplemented ethanol-treated rats (group 3). Treatment with chrysin to control rats (group 2) did not alter the TBARS, LOOH and conjugated diene levels significantly.

The SOD and CAT activity in the tissues and circulation of control and experimental animals are given in Table 4. SOD and CAT activity in the liver, kidney, heart and the circulation of rats treated with ethanol (group 3) were significantly lowered compared with control rats (group 1) ($P < 0.05$), whereas administering chrysin to ethanol-treated rats (group 4) significantly elevated their activity compared with ethanol treatment alone (group 3). Administration of chrysin to control rats (group 2) did not significantly alter the SOD and CAT activity compared with the normal control rats (group 1).

The activities of GSH and glutathione-related enzymes such as GPx, GR and GST in the tissues and circulation of both the control and experimental animals are given in Tables 5 and 6. The levels/activities of GSH, GPx, GR and GST were significantly lowered in the liver, kidney, heart and circulation of animals treated with ethanol (group 3) compared with the control rats (group 1) ($P < 0.05$). Chrysin at a dose of 20 mg/kg together with ethanol treatment significantly elevated the activities of GSH and GPx, GR and GST in these tissues and circulation compared with those of the unsupplemented ethanol-treated rats (group 3). GSH, GPx, GR and GST values did not alter significantly on treatment with chrysin to control rats (group 2) compared with normal control rats (group 1).

Table 4 Effect of chrysin on superoxide dismutase and catalase

Group	Superoxide dismutase				Catalase			
	Liver	Kidney	Heart	Lysate	Liver	Kidney	Heart	Lysate
Control	6.69 ± 0.47[a]	3.97 ± 0.28[a]	7.53 ± 0.53[a]	9.28 ± 0.65[a]	76.78 ± 5.41[a]	44.89 ± 3.16[a]	48.05 ± 3.39[a]	43.32 ± 3.05[a]
Control + chrysin	7.18 ± 0.50[a]	3.88 ± 0.27[a]	7.55 ± 0.53[a]	9.29 ± 0.65[a]	84.93 ± 5.99[c]	45.36 ± 3.20[a]	47.91 ± 3.38[a]	42.98 ± 3.03[a]
Ethanol	3.85 ± 0.27[b]	2.57 ± 0.18[b]	5.03 ± 0.35[b]	5.04 ± 0.35[b]	49.90 ± 3.52[b]	33.40 ± 2.35[b]	36.9 ± 2.60[b]	24.79 ± 1.74[b]
Ethanol + chrysin	6.87 ± 0.48[a]	3.65 ± 0.25[a]	6.75 ± 0.47[c]	8.40 ± 0.59[c]	73.78 ± 5.20[a]	43.67 ± 3.08[a]	40.87 ± 2.88[c]	41.85 ± 2.95[a]

Values are means ± SD of eight rats from each group. [a–c]Values with different superscript letters differ significantly at $P < 0.05$ (Duncan's multiple range test). For superoxide dismutase the units are: 50% inhibition of NBT reduction/min per mg protein for liver, kidney and heart; enzyme required for 50% inhibition of NBT reduction/min per mg haemoglobin for lysate. For catalase the units are: μmol H_2O_2 utilized/min per mg protein for liver, kidney and heart; μmol H_2O_2 utilized/min per mg haemoglobin for lysate. NBT, nitroblue tetrazolium.

Table 5 Effect of chrysin on reduced glutathione and glutathione peroxidase

Group	GSH				GPx			
	Liver	Kidney	Heart	Plasma[a]	Liver	Kidney	Heart	Lysate
Control	18.36 ± 1.29[a]	16.36 ± 1.15[a]	96.38 ± 6.80[a]	30.66 ± 2.16[a]	14.70 ± 1.03[c]	13.26 ± 0.93[a]	7.32 ± 0.51[a]	14.50 ± 1.02[a]
Control + chrysin	19.15 ± 1.35[a]	17.7 ± 1.21[a,c]	98.92 ± 6.98[a]	30.05 ± 2.12[a]	15.75 ± 1.11[a]	13.76 ± 0.97[a]	7.45 ± 0.52[a]	14.28 ± 1.00[a]
Ethanol	11.78 ± 0.83[b]	10.25 ± 0.72[b]	75.1 ± 5.30[b]	17.4 ± 1.20[b]	6.81 ± 0.48[b]	9.89 ± 0.69[b]	4.70 ± 0.33[b]	7.66 ± 0.54[b]
Ethanol + chrysin	16.75 ± 1.18[c]	17.86 ± 1.26[c]	83.13 ± 5.86[c]	24.38 ± 1.72[c]	13.83 ± 0.97[c]	12.15 ± 0.85[c]	6.87 ± 0.48[a]	13.00 ± 0.91[c]

GSH, reduced glutathione; GPx, glutathione peroxidase. Values are means ± SD of eight rats from each group. [a–c]Values with different superscript letters differ significantly at $P < 0.05$ (Duncan's multiple range test). For GSH the units are: mmol/g tissue for liver, kidney and heart; mmol/mg protein for plasma. For GPx the units are: μg of GSH utilized/min per mg protein for liver, kidney and heart; μmol of GSH utilized/min per mg haemoglobin for lysate.

Table 6 Effect of chrysin on glutathione reductase and glutathione *S*-transferase

Group	GR				GST			
	Liver	Kidney	Heart	Lysate	Liver	Kidney	Heart	Lysate
Control	23.43 ± 1.65[a]	25.15 ± 1.77[a]	6.42 ± 0.45[a]	15.28 ± 1.07[a]	8.46 ± 0.59[a]	6.82 ± 0.48[a]	0.82 ± 0.06[a]	15.36 ± 1.08[a]
Control + chrysin	23.02 ± 1.62[a,c]	23.20 ± 1.63[d]	4.49 ± 0.31[d]	16.13 ± 1.1[a]	8.05 ± 0.56[a]	6.51 ± 0.46[a]	0.81 ± 0.06[a]	15.91 ± 1.12[a]
Ethanol	11.04 ± 0.77[b]	16.14 ± 1.13[b]	2.06 ± 0.14[b]	8.38 ± 0.59[b]	4.62 ± 0.32[c]	3.42 ± 0.24[b]	0.43 ± 0.03[b]	9.39 ± 0.66[b]
Ethanol + chrysin	21.40 ± 1.51[c]	21.00 ± 1.48[c]	3.90 ± 0.27[c]	10.19 ± 0.71[c]	4.03 ± 0.28[b]	4.98 ± 0.35[c]	0.62 ± 0.04[c]	10.16 ± 0.71[b]

GR, glutathione reductase; GST, glutathione *S*-transferase. Values are means ± SD of eight rats from each group. [a–d] Values with different superscript letters differ significantly at $P < 0.05$ (Duncan's multiple range test). For GR the units are: nmol of NADPH utilized/min per mg protein for liver, kidney and heart; μmol of CDNB-GSH conjugate formed/min per mg haemoglobin for lysate. For GST the units are: μmol of CDNB-GSH conjugate formed/min per mg protein; μmol CDNB-GSH conjugate formed/min per mg haemoglobin for lysate.

Table 7 Effect of chrysin on vitamins C and E

Group	Vitamin C				Vitamin E			
	Liver (mg/100 g tissue)	Kidney (mg/100 g tissue)	Heart (mg/100 g tissue)	Plasma (mg/dl)	Liver (mg/100 g tissue)	Kidney (mg/100 g tissue)	Heart (mg/100 g tissue)	Plasma (mg/dl)
Control	0.82 ± 0.05[a]	0.68 ± 0.04[a]	0.56 ± 0.03[a]	3.50 ± 0.24[a]	6.68 ± 0.47[a]	4.53 ± 0.31[a]	3.90 ± 0.27[a]	47.82 ± 3.37[a,c]
Control + chrysin	0.83 ± 0.05[a]	0.69 ± 0.04[a]	0.56 ± 0.04[a]	3.40 ± 0.24[a,c]	6.76 ± 0.47[a]	4.64 ± 0.32[a]	3.98 ± 0.28[a]	49.28 ± 3.47[a]
Ethanol	0.62 ± 0.04[b]	0.47 ± 0.03[b]	0.42 ± 0.02[b]	2.00 ± 0.14[b]	4.54 ± 0.32[b]	2.71 ± 0.19[b]	2.43 ± 0.17[b]	30.49 ± 2.15[b]
Ethanol + chrysin	0.89 ± 0.06[a]	0.65 ± 0.04[a]	0.57 ± 0.04[a]	3.21 ± 0.16[a]	6.49 ± 0.45[a]	4.17 ± 0.83[c]	3.62 ± 0.25[c]	44.91 ± 3.61[c]

Values are means ± SD of eight rats from each group. [a–c]Values with different superscript letters differ significantly at $P < 0.05$ (Duncan's multiple range test).

Table 7 shows the decrease in tissue and circulatory non-enzymic antioxidants such as vitamins C and E in ethanol-treated rats (group 3) compared with the control rats (group 1). Administering chrysin to ethanol-treated rats (group 4) significantly elevated their levels compared with rats with ethanol treatment alone (group 3). Vitamin C and E levels did not alter significantly on treatment with chrysin to control rats (group 2) compared with the normal control rats (group 1).

Histological changes

The histological changes of the liver and kidney tissues are shown in Figures 3 and 4. The liver of the rats that received ethanol showed hepatocytes with feathery degeneration around the central vein. In the rats treated with chrysin and ethanol, the liver showed normal histology with portal triad and the hepatocytes showed feathery degeneration. The control rats treated with chrysin showed Kupffer cell hyperplasia focally. The kidney of the rats that received ethanol showed cloudy swelling of the tubules. In the rats treated with chrysin and ethanol, the kidney showed normal histology with tubules containing fat vacuoles and normal glomeruli. The control rats treated with chrysin showed cloudy swelling of the lining cells.

Discussion

Ethanol-induced tissue damage may be a consequences of oxidative stress and nutritional deficiency.[45] Studies in our laboratory are in agreement with this hypothesis, with ethanol-treated rats showing decreased food intake and increased oxidative stress.[46] Alcohol is rich in calories (7.1 kcal/g) and devoid of nutrients. Thus, isocaloric substitution of carbohydrates by ethanol resulted in

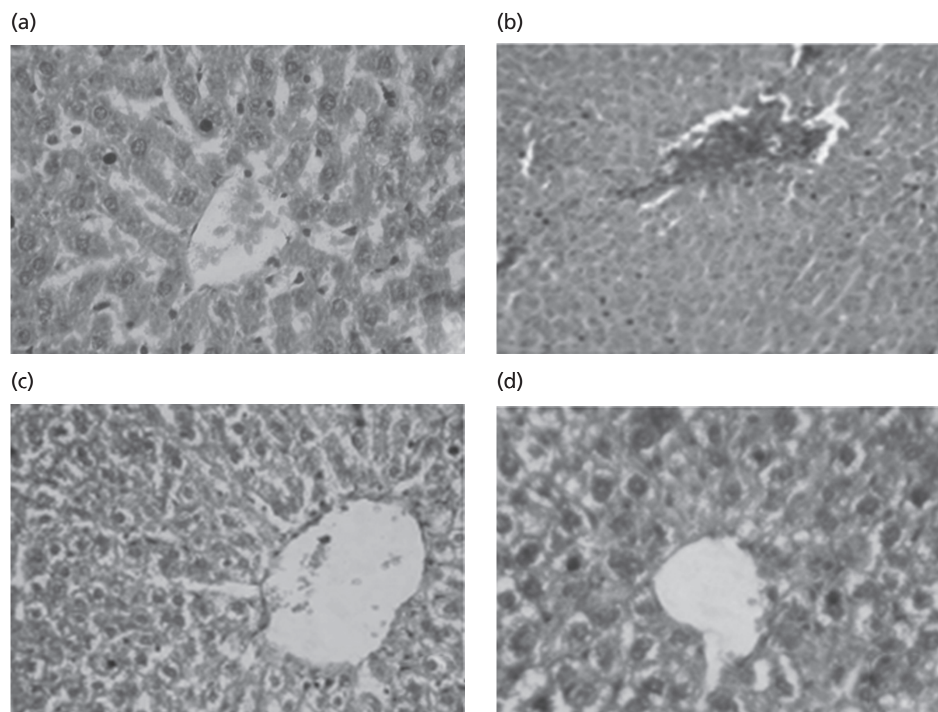

Figure 3 Histopathological changes in the rat liver. (a) Control liver: central vein surrounded by normal hepatocytes. (b) Control + chrysin treated liver: Kupffer cell hyperplasia is seen focally. (c) Ethanol treated liver: hepatocytes around the central vein show feathery degeneration. (d) Ethanol + chrysin treated liver: portal triad and hepatocytes showing feathery degeneration. Original magnification ×20.

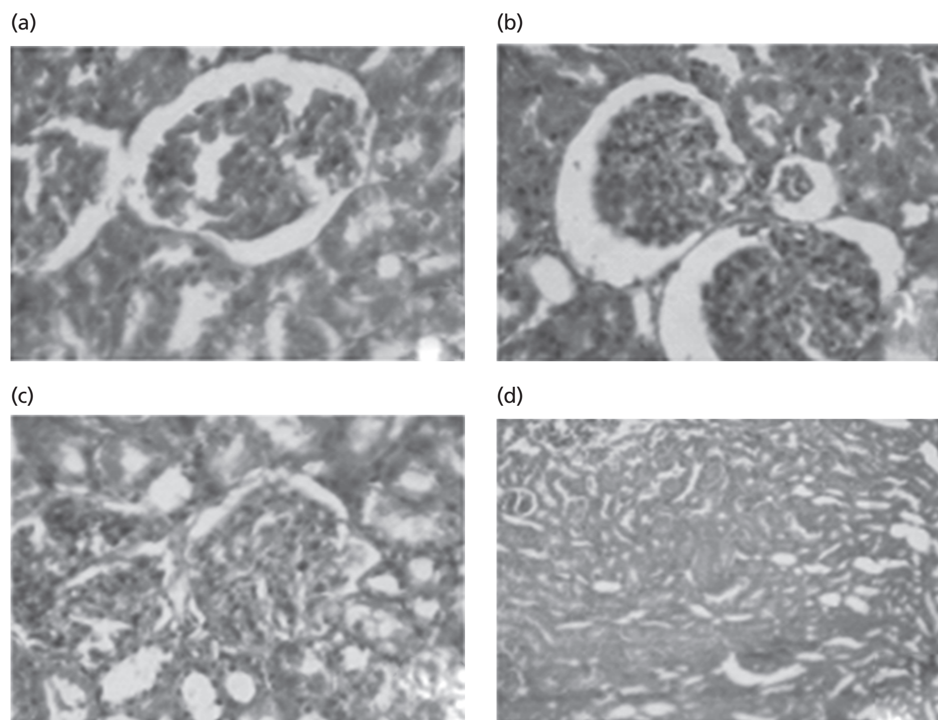

Figure 4 Histopathological changes in the rat kidney. (a) Control + chrysin treated kidney: glomerulus surrounded tubules. (b) Control + chrysin treated kidney: tubules show cloudy swelling of the lining cells. (c) Ethanol treated kidney: cloudy swelling of the tubules. (d) Ethanol + chrysin treated kidney: tubules contain fat vacuoles and normal glomeruli. Original magnification ×20.

decreased weight gain. Moreover, alcohol is known to reduce the absorption of foodstuffs and nutrients from the intestine.[47] Since bodyweight is considered to be a putative indicator of health, the increased weight gain in chrysin supplemented rats suggests the beneficial protective effect of chrysin against ethanol. The ratio between liver weights was significantly decreased and the total bodyweight showed a significant increase in chrysin supplemented ethanol-treated rats as compared with unsupplemented ethanol-treated rats. This may be due to the role of chrysin in eliminating ethanol directly from the intestines without absorption.

Ethanol affects almost all organs of the body because of its ability to permeate all tissues and its water- and fat-soluble properties. Ethanol administration results in excessive generation of free radicals such as hydroxyl ethyl radicals, superoxide radicals, hydroxyl radicals, peroxyl radicals and hydrogen peroxide.[48] All these radicals formed from ethanol-mediated processes have a great potential to react rapidly with lipids, which in turn leads to lipid peroxidation.[49] Enhanced lipid peroxidation is known to be one of the toxic manifestations of ethanol ingestion. Thus, extensive damage to tissues via free radical-mediated lipid peroxidation can result in membrane disorganization and subsequent decreased membrane fluidity.[50]

Excessive lipid peroxidation as measured by the formation of TBARS and/or lipid hydroperoxides and conjugated dienes in ethanol-treated rats has been reported by many researchers.[8] In agreement with these findings, ethanol-treated rats in the present study showed increased levels of TBARS, conjugated dienes and hydroperoxides in the circulation compared with control rats. The administration of chrysin to ethanol-treated rats significantly decreased the levels of these lipid peroxidation markers compared with ethanol-treated rats without chrysin supplementation. The decrease in lipid peroxidation on chrysin treatment can be correlated with elevated levels of antioxidants. This may be due to the free radical scavenging properties of the hydroxyl groups in the 5th and 7th position of chrysin. The ability of chrysin to enhance the levels of antioxidants along with its antilipid-peroxidative activity suggest that this compound might be potentially useful in counteracting the free radical-mediated injury involved in the development of liver damage caused by alcohol abuse.

SOD is a ubiquitous chain-breaking antioxidant found in all aerobic organisms. It is a metalloprotein widely distributed in all cells and plays an important protective role against oxidative damage induced by reactive oxygen species. SOD converts superoxide ion (O_2^-) to hydrogen peroxide (H_2O_2) and the hydrogen peroxide thus formed is degraded by CAT and GPx. CAT is present in all major body organs of animals and humans and is especially concentrated in the liver and erythrocytes. Superoxide ions (O_2^-) and hydroxyl radicals ($OH^\bullet$) are known to cause marked injury to the surrounding tissues and organs.[51,52] Any natural or synthetic compound with antioxidant properties may help to alleviate liver damage totally or partially. Therefore, scavenging superoxide ions and hydroxyl radicals is probably one of the most effective defence mechanisms against a variety of diseases.[52] The activities of SOD and CAT were significantly lowered in ethanol-treated rats as compared with those of the control rats. Reduced activity of SOD and CAT will result in the accumulation of these highly reactive free radicals, leading to deleterious effects such as loss of cell membrane integrity and function.[52] Previous studies from our laboratory reported a marked decrease in hepatic SOD and CAT activity on ethanol treatment.[31,53,54] Our present results are also in agreement with these observations. The decrease in the SOD and CAT activity may be associated with the elevation of the intracellular concentrations of H_2O_2.[55] CAT has been reported to be responsible for the detoxification of H_2O_2, which is an effective inhibitor of SOD.[56] The decrease in SOD activity could be due to oxidative inactivation of the enzyme due to excessive reactive oxygen species generation[57] or generation of the α-hydroxy ethyl radical from ethanol that inactivates SOD.[58] Chrysin supplementation to the ethanol-treated group elevated the SOD and CAT activity in the liver, kidney, heart and the erythrocytes, emphasizing the antioxidant and hepatoprotective activities of chrysin.

GSH is a tripeptide (L-γ-glutamylcysteinylglycine), an antioxidant and a powerful nucleophile, critical for cellular protection such as detoxification of reactive oxygen species, conjugation and excretion of toxic molecules, and control of the inflammatory cytokine cascade.[59] Depletion of GSH in tissues leads to impairment of the cellular defence against reactive oxygen species, and may result in peroxidative injury. The levels of GSH were significantly decreased in ethanol-treated rats. Our findings are consistent with previous reports, that showed that the GSH concentration is decreased during ethanol ingestion.[46,60,61] Moreover, in addition to being a direct free radical scavenger, GSH is known to function as a substrate for GPx and GST. The activities of GPx and GST in this study were reduced on ethanol treatment, which may be attributed to the unavailability of GSH. Administration of chrysin to ethanol-treated rats increased the levels of GSH and the activities of GPx and GST.

Chrysin is known to be a potent free radical scavenger. The hydroxyl groups present in the 5th and 7th positions may contribute to its potent antioxidant effects. Thus, the inherent antioxidant potential of chrysin may help in sparing the other antioxidants such as SOD, CAT, GSH, GPx, GST and GR. This may be the reason for the increased levels of these enzymic and non-enzymic antioxidants in the tissues and erythrocytes of chrysin and ethanol-treated rats.

GR helps to restore the levels of GSH by reducing the oxidized product of glutathione. The activity of GR was also lower upon ethanol treatment as compared with control rats, which in turn may inactivate many enzymes containing SH groups and inhibit protein synthesis.[62]

Non-enzymic antioxidants can also play a critical role in the defence against oxidative stress. In our study, we observed a significant decrease in the levels of the non-enzymic antioxidants vitamins C and E, which may be due to the enhanced oxidative stress. Our results are in agreement with previous studies,[46,63,64] reporting an 18% decrease in vitamin E concentration in alcoholic patients. Vitamin E can act as a chain-breaking antioxidant preventing lipid peroxidation, and any impairment in the antioxidant defence

results in free radical-induced cell injury. Vitamin E terminates lipid peroxidation by trapping free radicals, thereby getting itself converted to α-tocopheroxyl radicals, while vitamin C may have an important role in the regeneration of α-tocopherol from α-tocopheroxyl radicals.[65,66]

Alcohol administration produces a range of histological abnormalities in the liver.[67] The liver histology of the ethanol-treated rats showed pathomorphological alterations. These changes were predominant in the centrilobular region, having reduced oxygen perfusion. Hepatic damage may be partially attributed to cytochrome P450 dependent enzyme activity in the liver, which tends to be present in the greatest concentration near the central vein and lowest near peripheral sites.[68] Chrysin supplementation significantly reversed the alcohol-induced liver changes.

Kidney histology appeared normal in the control rats, whereas multiple areas of haemorrhage (rupture of a blood vessel and loss of blood) were observed in the ethanol-treated rats. Ethanol induces kidney cytochrome P450, which may enhance lipid peroxidation and tissue damage. Moreover the hyperlipidaemic action of ethanol can also lead to disposition of lipids in the kidney, producing fatty changes, as evidenced by the fatty infiltration in the kidney parenchyma. Administration of chrysin reduced the incidence of kidney pathology. Thus, our results show that chrysin protects the kidney against ethanol toxicity. There was no evidence of pathological changes in the chrysin-treated control rats.

Chrysin appears to alleviate the adverse effects of ethanol ingestion by enhancing hepatic antioxidants. Although chrysin supplementation was clearly beneficial for ethanol-treated rats, the detoxification mechanism at the pharmacological and biochemical level still needs to be elucidated. Further studies to identify the effective hepatoprotective mechanism of this compound are warranted.

Conclusions

Our results suggest that administration of chrysin during the entire experimental period significantly inhibited hepatotoxicity, decreased circulatory lipid peroxidation and enhanced enzymic and non-enzymic antioxidant concentrations. Chrysin at 20 mg/kg bodyweight offered marked hepatoprotection by reversing the changes produced by ethanol.

Declarations

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

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