Efficacy of curcumin to reduce hepatic damage induced by alcohol and thermally treated oil in rats

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Summary

The authors investigated the effect of curcumin on markers of oxidative stress and liver damage in rats that chronically ingested alcohol and heated oil. Nine groups of ten Wistar male rats received combinations of curcumin 100 mg/kg body weight daily, ethanol 5 mg/kg, 15% dietary sunflower oil and 15% heated sunflower oil for 12 weeks. Serum and liver tissue were collected. Groups 4-6, which had received compounds causing oxidative stress, showed increased serum aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, total bilirubin, cholesterol, triglycerides, low density lipoprotein, very low density lipoprotein and reduced high density lipoprotein, protein and albumin, compared with the controls. Reductions were observed in glutathione peroxidase and reductase gene expression, superoxide dismutase activity, glutathione peroxidase activity, glutathione reductase activity, reduced glutathione concentration and catalase enzyme activity. Groups 7, 8 and 9 which received curcumin with heated oil, ethanol or both, showed lower elevations in serum and oxidative damage markers compared with the corresponding non-curcumin treated groups. It can be concluded that curcumin reduces markers of liver damage in rats treated with heated sunflower oil or ethanol.

Keywords

Alcohol, Curcumin, Egypt, Liver, Oil, Rat, Stress.

Efficacia della curcumina nella riduzione del danno epatico in ratti trattati con alcol e olio sottoposto a trattamento termico

Riassunto

Gli autori hanno indagato l'effetto della curcumina sui marcatori dello stress ossidativo e del danno epatico in ratti trattati cronicamente con alcol e olio riscaldato. Nove gruppi di dieci ratti Wistar maschi hanno ricevuto giornalmente combinazioni di curcumina (100 mg/kg di peso corporeo), etanolo (5 mg/kg), olio di girasole dietetico (15%) e olio di girasole riscaldato (15%) per 12 settimane. Sono stati raccolti campioni di siero e tessuto epatico. Rispetto al gruppo di controllo, i gruppi 4-6 trattati con composti responsabili dello stress ossidativo hanno evidenziato aumento dei livelli sierici di aspartato aminotransferasi, alanina amino-transferasi, fosfatasi alcalina, bilirubina totale, colesterolo, trigliceridi, lipoproteina a bassa e bassissima densità e riduzione dei livelli di lipoproteina ad alta densità, proteine e albumina. Sono state osservate riduzioni, nell'espressione genica, del glutatione-reduttasi e del glutatione-perossidasi, nell'attività della superossido dismutasi, del glutatione-perossidasi e del glutatione-reduttasi, nella concentrazione di glutatione e nell'attività dell'enzima catalasi. I gruppi 7, 8 e 9 trattati con curcumina e olio riscaldato, etanolo o entrambi, hanno evidenziato aumenti inferiori dei marcatori sierici e del danno ossidativo rispetto ai gruppi corrispondenti non trattati con curcumina. È possibile concludere che la curcumina riduce i

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livelli dei marcatori del danno epatico nei ratti trattati con olio di girasole riscaldato o etanolo.

Parole chiave
Alcol, Curcumina, Egitto, Fegato, Olio, Ratto, Stress.

Introduction

Oxidative stress occurs with an imbalance between pro-oxidants and antioxidants (1). In a rat model, the administration of alcohol or thermally treated oil for 45 days caused excess generation of free radicals and oxidative injury to the liver (38). The mechanism for alcohol oxidative damage to the liver has been reported to be produced by the nuclear ethanol metabolising system which is capable of bioactivating ethanol to acetaldehyde and 1-hydroxyethyl radicals. These reactive metabolites covalently bind to nuclear proteins and lipids, and provoke oxidative stress to nuclear components (11).

The newer vegetable oils which are termed 'heart friendly' are rich in polyunsaturated fatty acids. Deep frying of these oils has a deleterious effect on liver cells through the production of certain toxic products which damage hepatocytes (3, 50). The increased intake of polyunsaturated fatty acids increases the degree of unsaturation of biomembranes, making them more susceptible to lipid peroxidation (13). Thermally treated sunflower oil intake, with ethanol, has also been shown to aggravate hepatic toxicosis (4).

Turmeric, derived from the rhizome of the herb *Curcuma longa*, has been used for centuries as a dietary spice in Asia. Diferuloylmethane is an active principle present in curcumin which is the main active ingredient of turmeric; it exerts potent biological effects *in vitro* and *in vivo* (2, 43), particularly attenuating oxidative stress (15, 20). Curcumin has been used to treat various diseases, including hepatic disorders (32) and exhibits antioxidant, anti-inflammatory, anti-viral, antibacterial, antifungal, and anticancer activities (2).

Material and methods

Animals

A total of 90 Albino Wistar strain male rats (100-120 g body weight) were acquired from an animal farm in Helwan, Egypt. The animals were housed individually 15 days before the study in stainless steel wire-mesh cages with free access to water and food (casein 180 g/kg, corn starch 460 g/kg, sucrose 220 g/kg, mineral mixture 50 g/kg, vitamin mixture 10 g/kg, cellulose 40 g/kg, sunflower oil 49 g/kg) according to Degrace *et al.* (10). All ingredients were obtained from Sigma-Aldirsh in Cairo.

Experimental design

The 90 rats were equally divided into nine groups. All treatments were administered for 12 weeks, as follows:

- Group 1 received normal saline 5 ml/kg body weight daily via intragastric intubation
- Group 2 received curcumin (curcumin from *Curcuma longa*) (Sigma Chemical, St Louis, Missouri) 100 mg/kg body weight daily via intragastric intubation according to Reyes-Gordillo *et al.* (36)
- Group 3 received 15% sunflower oil in the diet and normal saline 5 ml/kg bodyweight daily via intragastric intubation
- Group 4 received 15% thermally treated sunflower oil in the diet (180°C for 30 min, twice) according to Rukkumani *et al.* (38) and normal saline 5 ml/kg body weight daily via intragastric intubation
- Group 5 received ethanol (Hayman Limited, Witham, Essex,) 25% 5 ml/kg body weight daily via intragastric intubation according to Hussein *et al.* (21)
- Group 6 received 15% thermally treated sunflower oil in the diet and ethanol 5 ml/kg body weight daily via intragastric intubation
- Group 7 received curcumin 100 mg/kg bodyweight daily via intragastric intubation and 15% thermally treated sunflower oil in the diet
- Group 8 received curcumin 100 mg/kg bodyweight dissolved in ethanol 5 ml/kg body weight daily via intragastric intubation
- Group 9 received curcumin 100 mg/kg bodyweight dissolved in ethanol 5 ml/kg

bodyweight daily via intragastric intubation and 15% thermally treated sunflower oil in the diet.

Blood samples were collected by heart puncture (after anaesthetising the animal with ether in a cage/mask). Serum was obtained by centrifugation and the following analytes measured:

- aspartate aminotransferase and alanine aminotransferase (34)
- alkaline phosphatase (22)
- total bilirubin (28)
- total protein (17)
- albumin (53)
- serum globulins were calculated as the difference between total protein and albumin
- cholesterol (37)
- triglyceride (51)
- high-density lipoprotein cholesterol (52)
- low-density lipoprotein cholesterol and very low-density lipoprotein cholesterol (14)

Two liver samples were collected after anaesthetising the animal; these were immediately placed in liquid nitrogen. The first was used to determine glutathion peroxidase and glutathione reductase gene-expression according to Meadus (25). Liver homogenate was used to measure reduced glutathione concentrations according to Grunet and Phillips (19), glutathion peroxidase activity (23), glutathion reductase activity (5), superoxide dismutase activity (27) and catalase enzyme activity (44). Euthanasia was performed at the end of the experiment by freezing the rats while anaesthetised.

Samples were collected from all animals in each group and data were analysed using the MSTAT-C computer program (42). The design used to analyse the data was one-way analysis of variance (ANOVA) (F test) and comparison between means by least significant difference (LSD) values were considered statistically significant when $p \leq 0.05$.

Results

Groups 2 and 3 revealed no alterations of all measured parameters. Groups 4, 5 and 6 showed a significant increase in serum aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP) and total bilirubin, together with a significant decrease in the serum concentrations of total protein and albumin, when compared with the control group. In addition, a significant decrease in the albumin/globulin ratio was reported in the group to which both ethanol and thermally treated oil had been administered (Table I). Groups 7, 8 and 9 which received curcumin in addition to liver oxidants, showed significantly less alterations in the various analytes when compared with the corresponding groups that had not been treated with curcumin.

Serum concentrations of cholesterol, triglycerides, low-density lipoprotein and very low-density lipoprotein had increased significantly, while a marked decrease in the high-density lipoprotein was recorded in all groups except Groups 2 and 3 which had received curcumin and oil, respectively (Table II). Groups 7, 8 and 9 showed significantly less alterations in the various analytes when compared with the corresponding non-curcumin-treated groups and this improvement was more marked in groups treated with heated oil or ethanol than Group 9 which was treated by both.

Reduced glutathione concentrations, glutathione-peroxidase activity, glutathione-reductase activity, superoxide-dismutase activity and catalase enzyme concentration, glutathione peroxidase and glutathione reductase gene expression were significantly decreased in Groups 4, 5, 6, 7, 8 and 9 (Table III; Figs 1, 2 and 3). The groups that received curcumin with either thermally treated sunflower oil, ethanol, or both, showed significantly less alterations in the various analytes when compared with the corresponding non-curcumin treated groups.

Table I
Biochemical parameters measured in serum of the rats
(mean values ± standard error)

Group parameters	Normal control	Curcumin	Oil	Heated oil	Ethanol	Heated oil plus ethanol	Curcumin plus heated oil	Curcumin plus ethanol	Curcuminplus heated oil plus ethanol	LSD
AST (U/l)	20.50$^{(a)}$ ±0.58	20.20$^{(a)}$ ±0.79	21.20$^{(a)}$ ±0.53	29.00$^{(b)}$ ±0.70	31.50$^{(c)}$ ±0.40	38.70$^{(d)}$ ±0.58	24.80$^{(e)}$ ±0.57	26.10$^{(e)}$ ±0.41	29.00$^{(b)}$ ±0.65	1.66
ALT (U/l)	15.20$^{(a)}$ ±0.51	15.20$^{(a)}$ ±0.41	16.20$^{(a)}$ ±0.63	22.70$^{(b)}$ ±0.84	25.40$^{(c)}$ ±0.91	29.00$^{(d)}$ ±0.70	19.90$^{(e)}$ ±0.59	20.50$^{(e)}$ ±0.64	23.30$^{(b)}$ ±0.67	1.88
Bilirubin (mg/dl)	0.58$^{(e)}$ ±0.04	0.57$^{(e)}$ ±0.04	0.60$^{(e)}$ ±0.03	0.87$^{(c)}$ ±0.05	0.90$^{(c)}$ ±0.04	1.02$^{(d)}$ ±0.03	0.72$^{(b)}$ ±0.05	0.75$^{(b)}$ ±0.05	0.87$^{(c)}$ ±0.03	0.1189
ALP (U/l)	71.20$^{(f)}$ ±1.35	70.20$^{(f)}$ ±1.60	73.00$^{(f)}$ ±0.89	96.50$^{(b)}$ ±1.65	101.90$^{(c)}$ ±0.71	114.60$^{(d)}$ ±1.05	78.60$^{(a)}$ ±1.93	79.00$^{(a)}$ ±2.76	91.20$^{(e)}$ ±1.14	4.48
Total protein (g/dl)	7.03$^{(d)}$ ±0.05	7.00$^{(d)}$ ±0.04	6.92$^{(d)}$ ±0.09	5.73$^{(b)}$ ±0.13	5.55$^{(b)}$ ±0.08	5.25$^{(e)}$ ±0.11	6.81$^{(c, d)}$ ±0.09	6.61$^{(c)}$ ±0.13	5.82$^{(b)}$ ±0.10	0.275
Albumin (g/dl)	4.748$^{(d)}$ ±0.07	4.72$^{(d)}$ ±0.05	4.64$^{(d)}$ ±0.06	3.588$^{(b, e)}$ ±0.08	3.392$^{(e)}$ ±0.07	3.12$^{(a)}$ ±0.04	4.548$^{(c, d)}$ ±0.09	4.38$^{(c)}$ ±0.09	3.63$^{(b)}$ ±0.05	0.197
Globulin (g/dl)	2.28$^{(d)}$ ±0.04	2.28$^{(d)}$ ±0.05	2.28$^{(d)}$ ±0.06	2.14$^{(d)}$ ±0.05	2.16$^{(d)}$ ±0.05	2.13$^{(d)}$ ±0.07	2.26$^{(d)}$ ±0.03	2.23$^{(d)}$ ±0.06	2.19$^{(d)}$ ±0.05	NS
A/G ratio	2.08$^{(c, d)}$ ±0.06	2.07$^{(d)}$ ±0.06	2.05$^{(d)}$ ±0.05	1.68$^{(c)}$ ±0.02	1.58$^{(b, c)}$ ±0.05	1.47$^{(b)}$ ±0.03	2.01$^{(d)}$ ±0.05	1.98$^{(d)}$ ±0.05	1.66$^{(c)}$ ±0.03	0.13

Data are presented as mean ± SE, n = 10 and values within the same row which carrying different superscripts are significant at $p \leq 0.05$ using the analysis of variance (ANOVA) test

AST aspartate aminotransferase
U mean unit
ALT alanine aminotransferase
ALP alkaline phosphatase
A/G albumin globulin ratio
LSD least significant difference
NS not significant

Table II
Lipogram in the serum of the rats
mean values ± standard error)

Group parameters	Normal control	Curcumin	Oil	Heated oil	Ethanol	Heated oil plus ethanol	Curcumin and heated oil	Curcumin plus ethanol	Curcumin plus heated oil plus ethanol	LSD
Cholesterol (mg/dl)	105.40$^{(a)}$ ±1.08	103.90$^{(a)}$ ±2.81	107.00$^{(a)}$ ±2.45	132.00$^{(b)}$ ±0.77	124.60$^{(c)}$ ±0.97	141.90$^{(d)}$ ±0.98	115.80$^{(e)}$ ±1.89	114.30$^{(e)}$ ±2.18	122.10$^{(c)}$ ±1.81	5.25
Triglyceride (mg/dl)	81.70$^{(f)}$ ±0.86	81.10$^{(f)}$ ±1.64	82.30$^{(f)}$ ±1.48	116.90$^{(b)}$ ±0.70	109.50$^{(c)}$ ±0.79	123.60$^{(d)}$ ±1.17	92.70$^{(a)}$ ±1.94	88.90$^{(a)}$ ±3.86	103.20$^{(e)}$ ±0.80	5.05
HDL (mg/dl)	34.90$^{(b, d)}$ ±0.35	36.00$^{(d)}$ ±0.77	33.50$^{(b)}$ ±0.85	26.80$^{(d)}$ ±0.77	27.40$^{(d)}$ ±0.54	23.50$^{(a)}$ ±0.73	31.10$^{(c)}$ ±0.60	31.40$^{(c)}$ ±0.96	28.50$^{(e)}$ ±0.81	2.05
LDL (mg/dl)	54.16$^{(a)}$ ±1.08	51.68$^{(a)}$ ±2.98	57.04$^{(a)}$ ±2.31	81.82$^{(b)}$ ±1.08	75.30$^{(c)}$ ±1.31	93.68$^{(d)}$ ±1.27	66.16$^{(e)}$ ±1.77	65.12$^{(e)}$ ±2.15	72.96$^{(c)}$ ±1.50	5.128
VLDL (mg/dl)	16.34$^{(f)}$ ±0.17	16.22$^{(f)}$ ±0.33	16.46$^{(f)}$ ±0.30	23.38$^{(b)}$ ±0.15	21.90$^{(c)}$ ±0.16	24.72$^{(d)}$ ±0.23	18.54$^{(a)}$ ±0.39	17.78$^{(a)}$ ±0.77	20.64$^{(e)}$ ±0.29	1.011

Data are presented as mean ± SE, n = 10 and values within the same row which carrying different superscripts are significant at $p \leq 0.05$ using the analysis of variance (ANOVA) test

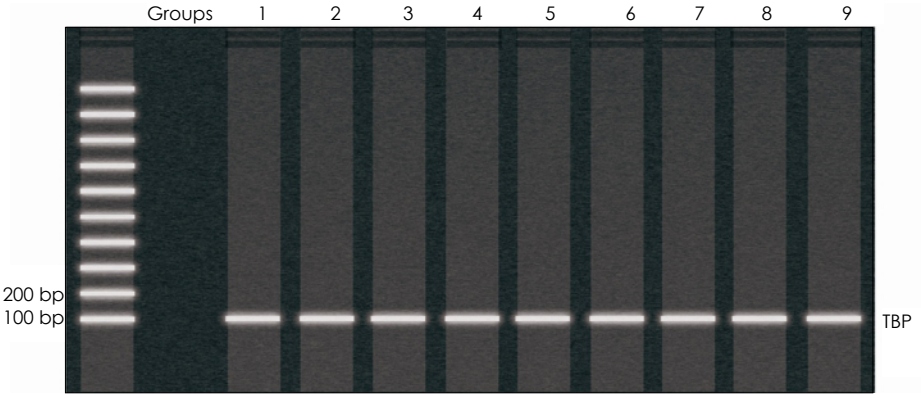
LSD least significant difference
HDL high-density lipoprotein cholesterol
LDL low-density lipoprotein cholesterol
VLDL very low-density lipoprotein cholesterol

Table III
The antioxidants in hepatic tissue of rats
(mean values ± standard error)

Group parameters	Normal control	Curcumin	Oil	Heated oil	Ethanol	Heated oil plus ethanol	Curcumin plus heated oil	Curcumin plus ethanol	Curcumin plus heated oil plus ethanol	LSD
GSH (mg/gmT)	18.86$^{(a)}$ ±0.61	19.70$^{(a)}$ ±0.88	18.16$^{(a)}$ ±0.92	14.60$^{(b)}$ ±0.40	14.31$^{(b)}$ ±0.45	12.34$^{(c)}$ ±0.43	16.40$^{(d)}$ ±0.31	16.23$^{(d)}$ ±0.37	14.50$^{(b)}$ ±0.50	1.62
GPX (µmol NADPH /mg protein)	56.40$^{(a)}$ ±0.67	57.30$^{(a)}$ ±0.67	55.70$^{(a)}$ ±0.89	46.50$^{(b)}$ ±1.09	46.10$^{(b)}$ ±0.92	40.60$^{(c)}$ ±0.82	51.50$^{(d)}$ ±0.92	50.20$^{(d)}$ ±0.79	44.20$^{(b)}$ ±0.76	2.38
GRD (IU/g tissue)	10.17$^{(a)}$ ±0.28	10.29$^{(a)}$ ±0.33	9.67$^{(a)}$ ±0.25	6.64$^{(b)}$ ±0.17	6.47$^{(b)}$ ±0.20	5.56$^{(c)}$ ±0.17	8.75$^{(d)}$ ±0.21	8.12$^{(d)}$ ±0.20	6.61$^{(b)}$ ±0.26	0.66
SOD (µg/mg protein)	0.47$^{(a)}$ ±0.02	0.48$^{(a)}$ ±0.02	0.44$^{(a)}$ ±0.02	0.27$^{(b)}$ ±0.01	0.26$^{(b)}$ ±0.02	0.21$^{(c)}$ ±0.01	0.37$^{(d)}$ ±0.01	0.33$^{(d)}$ ±0.01	0.28$^{(b)}$ ±0.02	0.043
CAT µmol H_2O_2 decomposed /g tissue	1.56$^{(a)}$ ±0.07	1.59$^{(a)}$ ±0.06	1.55$^{(a)}$ ±0.08	1.10$^{(b)}$ ±0.04	1.10$^{(b)}$ ±0.06	0.85$^{(c)}$ ±0.06	1.33$^{(d)}$ ±0.05	1.30$^{(d)}$ ±0.05	1.06$^{(b)}$ ±0.05	0.169
GPX gene expression (relative OD)	31.00$^{(a)}$ ±1.64	31.20$^{(a)}$ ±1.07	29.60$^{(a)}$ ±0.81	20.40$^{(b)}$ ±0.81	20.00$^{(b)}$ ±1.00	16.40$^{(c)}$ ±1.12	25.00$^{(d)}$ ±1.14	24.40$^{(d)}$ ±1.50	19.80$^{(b)}$ ±1.24	3.406
GRD gene expression (relative OD)	4.74$^{(a)}$ ±0.21	4.78$^{(a)}$ ±0.15	4.62$^{(a)}$ ±0.27	3.26$^{(b)}$ ±0.18	3.20$^{(b)}$ ±0.11	2.40$^{(c)}$ ±0.16	3.98$^{(d)}$ ±0.32	3.90$^{(d)}$ ±0.18	3.00$^{(b)}$ ±0.17	0.59

Data are presented as mean ± SE, n = 10 and values within the same row which carrying different superscripts are significant at $p \leq 0.05$ using the analysis of variance (ANOVA) test

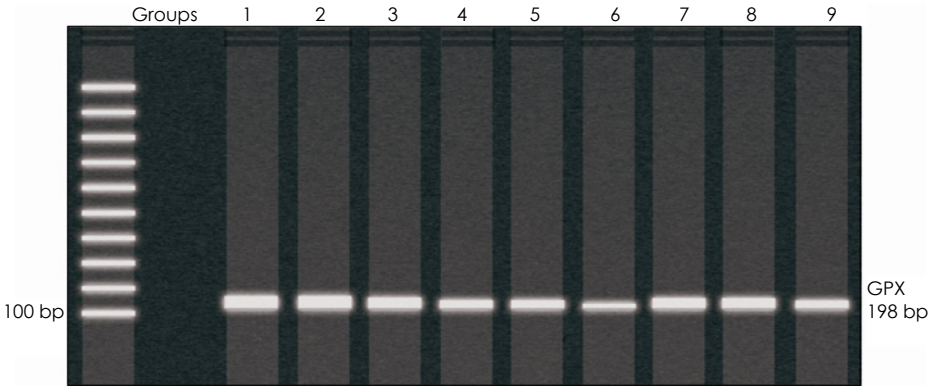
LSD least significant difference
GSH reduced glutathione concentration
GPX glutathione peroxidase activity
NADPH nicotinamide adenine dinucleotide phosphate
GRD glutathione reductase activity
SOD superoxide dismutase activity
CAT catalase enzyme activity
OD optical density



TBP Tata box binding protein

Groups
1. Negative control
2. Curcumin
3. Oil
4. Heated oil
5. Ethanol
6. Heated oil plus ethanol
7. Curcumin plus heated oil
8. Curcumin plus ethanol
9. Curcumin plus heated oil plus ethanol

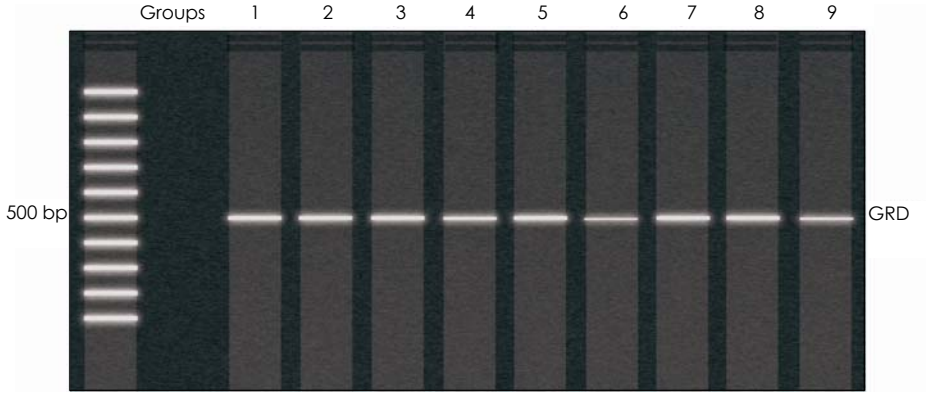
Figure 1
Polymerase chain reaction product of Tata box binding protein gene expression (internal control)



GPX glutathione peroxidase activity

Groups
1. Negative control
2. Curcumin
3. Oil
4. Heated oil
5. Ethanol
6. Heated oil plus ethanol
7. Curcumin plus heated oil
8. Curcumin plus ethanol
9. Curcumin plus heated oil plus ethanol

Figure 2
Polymerase chain reaction product of glutathion peroxidase gene expression



GRD glutathione reductase activity

Groups
1. Negative control
2. Curcumin
3. Oil
4. Heated oil
5. Ethanol
6. Heated oil plus ethanol
7. Curcumin plus heated oil
8. Curcumin plus ethanol
9. Curcumin plus heated oil plus ethanol

Figure 3
Polymerase chain reaction product of glutathion reductase gene expression

Discussion

In this study, the administration of liver oxidants thermally treated oil and ethanol were associated with increased serum levels of aminotransferases (AST and ALT), ALP and total bilirubin caused by hepatocellular damage (46). The liver is the main organ susceptible to damage by oral ethanol because it receives the portal blood directly from the intestinal tract. Ethanol administration may cause injury to the liver through different mechanisms, including microsomal damage (24) and the release of metabolites from ethanol, such as malondialdehyde and acetaldehyde, which synergistically bind to proteins to form malondialdehyde-acetaldehyde modified proteins (48). These modified proteins induce pro-inflammatory and pro-fibrotic responses by liver endothelial

cells (47). Furthermore, the formation of 1-hydroxyethyl free radicals from ethanol also damage the liver (33), induce cytochrome P450 2E1 (16) and sensitise Kupffer cells to endotoxin (lipopolysaccharide). These cells then produce tumour necrosis factor-α which is critical for progression of alcoholic liver injury (12).

Heating oil rich in polyunsaturated fatty acids has a deleterious effect on liver cells through different pathways, including the production of toxic products which damage hepatocytes (3, 50). The increased intake of poly-unsaturated fatty acids increases the degree of unsaturation of biomembranes, making them more susceptible to lipid peroxidation (13), the generation of various cytotoxic aldehyde species (18) and the production of various lipid peroxidative as end-products of heating (4).

Another explanation for the elevation in bilirubin may be the presence of ethanol that increases erythrocyte deformability (7), subsequently increasing the risk of haemolysis. Similarly, consumption of high levels of n-3 polyunsaturated fatty acids may also lead to enhanced membrane lipid peroxidation by free radicals (40). Previous studies on rats have reported similar findings (4, 41) with Pari and Karthikesan (31) also reporting increased cell wall permeability, damage and/or necrosis of hepatocytes.

A significant decrease was found in the total protein and albumin in groups that received thermally treated oil, ethanol or both. Similarly, the albumin globulin ratio significantly decreased in the group to which both thermally treated oil and ethanol had been administered. The hypoproteinaemia and decreased albumin globulin ratio were due to the hypoalbuminaemia which may be due to the loss of albumin through the gastro-intestinal tract (6), increased excretion through damaged kidneys or disturbed production by the liver (31). Our findings were consistent with results obtained by Hussein *et al.* (21) who recorded hypoproteinemia and hypo-albuminaemia in rats after ingestion of ethanol. Similarly, Aruna *et al.* (4) found that the intake of thermally treated sunflower oil, together with alcohol, aggravated hepatic toxicity.

The lipogram revealed a significant increase in the levels of cholesterol, triglycerides, low density lipoprotein and very low density lipoprotein as well as a significant decrease in high density lipoprotein associated with the groups to which thermally treated oil, ethanol, or both had been administered. This is consistent with the findings of Aruna *et al*. and Rukkumani *et al*. (4, 39) who reported that levels of cholesterol, triglycerides, phos-pholipids and free fatty acids increased in the plasma of rats to which alcohol, thermally oxidised oil, or alcohol and thermally oxidised oil had been administered.

The antioxidant defence systems play an important role in protecting living organisms from the deleterious effects of reactive oxygen metabolites (38) produced by hepatic oxidant chemicals. Among these protective systems, superoxide dismutase, glutathione peroxidase, glutathione reductase and catalase play an important role. Our study showed that superoxide dismutase activity, glutathione peroxidase activity, glutathione reductase activity, reduced glutathione concentration and catalase enzymes significantly decreased following chronic ethanol and thermally treated oil ingestion in rats. The decreased glutathione peroxidase and glutathione reductase gene expression support our findings. Oxidative stress following the ingestion of thermally treated oil and ethanol caused increased use and weakened protective effect of the antioxidant enzymatic system in the rats in our study.

Similar results of oxidant damage to the liver have been reported by Rukkumani *et al.* (38) who recorded a significant decrease in reduced glutathione concentration, glutathione peroxidase activity, superoxide dismutase activity and catalase activity in rats after ingestion of thermally treated oil, ethanol, or both. Hussein *et al.* (21) also reported that the mean value of reduced glutathione and superoxide dismutase activities decreased significantly following thee administration of ethanol to rats.

In our study, the administration of curcumin with thermally treated oil, alcohol, or both, caused a significant decrease in serum and gene markers of liver damage when compared to groups which had not been treated with curcumin. These findings support the ability of curcumin to counteract some of the oxidative stress produced by the ingestion of thermally treated oil, ethanol, or both, in rats.

Curcumin is believed to prevent necrosis factor -kappaβ activation and therefore suppress the secretion of pro-inflammatory cytokines (35), inhibition of cyclooxygenase-2, lipoxygenase and inducible nitric oxide synthase, which are important enzymes that mediate inflammatory processes (26). Previous studies have indicated that curcumin attenuates hepatic oxidative stress in rats (15, 20) and inhibits liver cirrhosis through multiple biological effects on hepatic stellate cells. The latter cells play a central role in the pathogenesis of hepatic fibrosis (29) and may prevent and reverse cirrhosis by reducing transforming growth factor-beta (TGF-beta) expression (36) which affects development, homeostasis and tissue repair. Furthermore, the antioxidant effect of curcumin may occur through chelating metal ions which stimulate lipid peroxidation as iron (9, 45), scavenging of free radicals (49) and strong inhibitory properties towards cytochrome P450 and glutathione S-transferase activities (8, 30).

Conclusions

It can be concluded that curcumin partially protects the liver by ameliorating the oxidative stress and activating the antioxidant defence systems.

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