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ORIGINAL ARTICLE

Turcuron: A Standardized Bisacurone-Rich Turmeric Rhizome Extract for the Prevention and Treatment of Hangover and Alcohol-Induced Liver Injury in Rats

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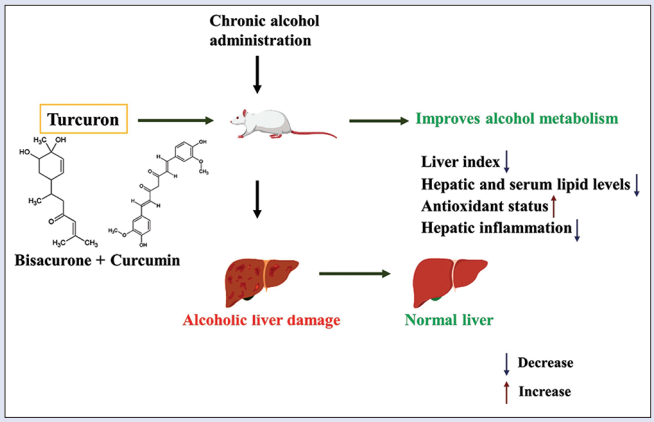
ABSTRACT

Objectives: The present work investigated the hepatoprotective effects of Turcuron, a first-of-its-kind extract from *Curcuma longa* tubers containing 8% bisacurone, with respect to alcohol-induced liver injury in rats. We have further studied its efficacy in improving alcohol metabolism in rats. **Materials and Methods:** *In vitro* studies were performed using nitric oxide (NO) scavenging and xanthine dehydrogenase activity. Alcohol-induced liver injury was established in male Sprague Dawley rats by oral administration of 4 g/kg/day of 40% ethanol for 6 weeks. Turcuron (150 and 300 mg/kg) was administered to the rats after 3 weeks of alcohol treatment till the end of the study. In alcohol-induced hangover model, male Wistar rats were administered with single oral dose of 5 ml/kg b.w. of alcohol, 1 h after the treatment with Turcuron (200, 300, and 400 mg/kg). **Results:** In our preliminary study, Turcuron markedly inhibited the NO production and xanthine dehydrogenase activity *in vitro*. In the *in vivo* model rats, Turcuron restored the liver architecture and biochemical parameters and reduced the expression of inflammatory proteins in the liver. Furthermore, Turcuron reduced the blood alcohol and acetaldehyde levels in alcohol hangover model rats. It also increased the activity of Aldehyde dehydrogenase (ALDH) significantly in the liver. **Conclusion:** Here, we report for the first time, Turcuron-mediated liver protection attributing to the presence of high content of bisacurone in the turmeric extract. Collectively, our data provide valuable evidence for the application of bisacurone-rich turmeric extract as a functional food and/or medicine.

Key words: Alcoholic liver disease, bisacurone, hangover, rats, turmeric

SUMMARY

- The present study reports the hepatoprotective effects of a first-of-its-kind turmeric extract standardized to 8% bisacurone (Turcuron)
- Turcuron restored the normal liver architecture, lipid levels, and liver function parameters in alcohol-induced liver injury model rats
- Turcuron significantly reduced the alcohol-induced hepatic inflammation in rats
- In alcohol hangover model rats, Turcuron reduced the blood alcohol and acetaldehyde levels and enhanced the activity of aldehyde dehydrogenase (ALDH) activity significantly in the liver
- Overall, our study validates the potential hepatoprotective effects of a bisacurone-containing turmeric extract that can be explored as a functional food ingredient.



Abbreviations used: ALD: Alcoholic liver disease; ADH: Alcohol dehydrogenase; ALDH: Aldehyde dehydrogenase; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; TG: Triglyceride; TC: Total cholesterol; TNF-α: Tumor necrosis factor-α; Cox-2: Cyclooxygenase-2; HPLC: High-performance liquid chromatography; IAEC: Institutional Animal Ethics Committee; TBARS: Thiobarbituric Acid Reactive Substances; MDA: Malondialdehyde; GSH: Reduced glutathione; EDTA: Ethylenediaminetetraacetic acid; H and E: Hematoxylin and eosin; RT: Retention time; iNOS: Inducible nitric oxide synthase.

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INTRODUCTION

Alcoholic liver disease (ALD) is one of the serious public health concerns worldwide, with a mortality rate of 20%.[1,2] ALD is the consequence of chronic alcoholism associated with oxidative stress and inflammation.[3,4] The byproducts of alcohol metabolism encourage the development of simple steatosis progressively leading to hepatosteatosis, fibrosis, cirrhosis, and liver cancer.[5] Current understanding of ALD suggests that oxidative stress and associated inflammatory phenomenon play a key role in alcohol-induced liver damage.[6] The common medications for ALD such as tiopronin, bifendate, and potenline are associated with side effects including diarrhea, dizziness, and leukopenia.[1] Hence,

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researchers have drawn their attention toward hepatoprotective agents of herbal origin as potential choices for treating ALD.

Chronic alcohol consumption can be detrimental to health causing adverse effects. Alcohol hangover is the most frequently experienced condition characterized by the unpleasant signs and symptoms that start a few hours after the consumption and may last for up to 24 h.[7] The physical symptoms of alcohol hangover include dehydration, dizziness, weakness, nausea, vomiting and stomach pain, low blood sugar, and disturbance of sleep.[8] Following consumption, alcohol is absorbed through the small intestine and oxidized by alcohol dehydrogenase (ADH) to an intermediate product, acetaldehyde which is then oxidized to acetic acid by aldehyde dehydrogenase (ALDH) in the liver.[9] Accumulation of acetaldehyde in the body leads to the occurrence of hangover symptoms. Due to hangover job performance, the productivity of an individual could be affected alongside the adverse economic concerns.[10,11] Several studies been conducted in the past to develop an effective treatment for hangover. These studies are focused on the efficacy of both herbal (e.g., *Opuntia ficus-indica, Cynara scolymus,* and *Borago officinalis*) and nonherbal products (e.g., propranolol, tolfenamic acid, tropisetron, paracetamol, aspirin, and chlormethiazole).[12,13]

Curcuma longa L. (family Zingiberaceae) is a perennial plant cultivated in tropical and subtropical regions of the world. The yellow turmeric powder resulting from the boiling and drying of rhizomes of the plant is greatly valued as a spice and medicine in Asia. Several researchers have previously demonstrated the pharmacological significance of curcumin, a major bioactive component of turmeric. The anti-inflammatory, antioxidant, anti-stress, neuroprotective, and anticancer activities of turmeric extract are attributed to curcumin. There is substantial evidence on the protective role of curcuminoids in liver diseases.[14] In addition to curcuminoids, there are several other active principles identified in turmeric. Till date, more than 200 compounds which include phenolic compounds and terpenoids have been identified in turmeric.[15] Bisacurone, a terpenoid, present in turmeric is shown to have potent anti-inflammatory activity.[16] Bisacurone is reported to have a plausible synergistic role in liver protection along with curcumin.[17] Here, we hypothesize that the enriched content of a terpenoid, bisacurone, could enhance the hepatoprotective effects of turmeric extract. Hence, this study was conceived to investigate the hepatoprotective activity of a terpenoid-rich (8% bisacurone) turmeric rhizome extract, Turcuron on alcohol-induced liver injury in rats. Further, we have assessed the effect of Turcuron on the pharmacodynamics of alcohol metabolism in rats. Our research provides the first evidence on the potential benefits of turmeric extract in liver protection and improvement in alcohol metabolism, attributable to its higher content of bisacurone.

MATERIALS AND METHODS

Turcuron™

Turcuron™ is a terpenoid-rich turmeric rhizome extract. Turcuron was obtained from the Department of Phytochemistry, R and D Center for Excellence, Vidya Herbs Pvt Ltd., Bangalore, India. Turcuron was dissolved in 10% polysorbate for animal experiments.

Chemicals and reagents

The diagnostic kits for biochemical analysis of alanine aminotransferase (ALT), aspartate aminotransferase (AST), total cholesterol (TC), and triglycerides (TGs) were purchased from Randox Laboratories, UK. All other chemicals were purchased from Sigma-Aldrich. Antibodies for tumor necrosis factor (TNF)-α, cyclooxygenase-2 (Cox-2), nuclear factor kappa-light-chain-enhancer of activated B-cells (NF-kB), inducible nitric oxide synthase (iNOS), glyceraldehyde 3-phosphate dehydrogenase (GAPDH), and HRP-conjugated IgG antibody were obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA).

High-performance liquid chromatography analysis

The appropriate amount of Turcuron was mixed and dissolved in methanol to obtain a concentration of 400 ppm. Before analysis, the solution was filtered through 0.20 µm nylon membrane filters. High-performance liquid chromatography (HPLC) was performed on a Shimadzu LC2030 C Prominence-i (Japan) system equipped with a quaternary low-pressure gradient solvent delivery LC2030 pump with high-pressure switching valves, online LC2030 degasser unit, a high-sensitivity LC2030 ultraviolet (UV) detector, and large capacity column oven. A separation was performed in Kinetex C-18 column (100 Aº, 250 mm × 4.6 mm, 5 µm pore size). For the analysis of bisacurone, the mobile phase consisted of isocratic elution with a low-pressure gradient using 0.2% formic acid:acetonitrile (65:35) with a flow rate of 0.8 ml/min and the injection volume of 10 µl. All solutions were degassed and filtered through a 0.45 µm pore size filter. The column was maintained at 26°C throughout analysis, and the UV detector was set at 254 nm. HPLC was performed for the analysis of curcuminoids using mobile phase of 0.1% formic acid:acetonitrile (50:50) with a flow rate of 1.0 ml/min and the injection volume of 5 µl; UV detector was set at 420 nm. 100% methanol was used as a diluent for assay by HPLC analysis, and the total liquid chromatography runtime was 15 min. The retention time (RT) of bisacurone and curcuminoids was confirmed by injection of corresponding reference standards separately.

In vitro assays

The nitric oxide (NO)-scavenging effect of Turcuron was demonstrated by measuring the NO (Nitric oxide [NOx]) generated from sodium nitroprusside using Griess reaction.[18] The appearance of pink color due to the chromophore was measured at 546 nm (UV-visible spectrophotometer, Shimadzu). Percentage inhibition of NOx was calculated.

Xanthine dehydrogenase assay was performed using rat liver S9 fraction as a source of enzyme. Briefly, the reaction mixture consisted of 40 mM phosphate buffer pH 7.0, 93 µM NADPH, 10 µM xanthine, 200 µg S9 fraction, and different concentrations of Turcuron, incubated for 10 min at 37°C. The absorbance was recorded at 340 nm, and the results were expressed as the percentage of relative activity. Allopurinol was used as a reference compound.

Animals

Male Sprague Dawley rats (8–9 weeks old) and Wistar rats (250–275 g) were purchased from Biogen, Bangalore, India (Reg No. 971/PO/RcBiBt/S/2006/CPCSEA). The animals were acclimatized to humidity (30%–70%)- and temperature (22 ± 3°C)-controlled room environment with a 12-h light/dark cycle. During the 7-day acclimatization, the rats were fed a commercial rodent diet and tap water *ad libitum*. The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) of Vidya Herbs Pvt Ltd (ALD model: VHPL/PCL/IAEC/11/18 and alcohol-induced hangover model: VHPL/PCL/IAEC/16/18).

Alcoholic liver disease model

After a week of acclimatization, all rats were randomly divided into four groups (six rats in each group) including one normal control group, one model control (alcohol) group, and two dose groups treated with Turcuron. Briefly, all rats except the normal control group were orally administered with 4 g/kg of 40% ethanol once a day for 6 weeks. Turcuron treatment started from the 4th week. At the end of week 6,

all the rats were euthanized. Blood samples were collected by heart puncture and serum separated for analysis of cholesterol, TG, AST, and ALT using a biochemical analyzer (Randox RX Imola, Co Antrim, UK). Liver was harvested, weighed and homogenized in tissue lysis buffer (50 mM Tris pH 7.5, 150 mM NaCl, and 1% Triton X-100) for further analysis.

Determination of liver antioxidant status

Hepatic measurement of lipid peroxidation was performed by quantifying the malondialdehyde (MDA) levels using Thiobarbituric Acid Reactive Substances (TBARS) method.[19] The reaction mixture contained 0.2 mL of liver homogenate, 0.2 mL of 8% sodium dodecyl sulfate, and 3.0 mL of 0.8% thiobarbituric acid in 20% acetic acid, and the solution was made up to 4.0 mL using distilled water. Solution was incubated at 95°C in water bath for 60 min. After incubation, solution was cooled and absorbance was measured at 532 nm. Total TBARS were expressed as MDA, using a molar extinction coefficient for MDA of 1.56×10^5/cm/M. Results were expressed as nmol MDA/mg protein.

Reduced glutathione (GSH) level in the liver homogenates was determined by the method of Ellman[20] with some modifications. Briefly, 200 µL of liver homogenate was mixed with 100 µL of 10 mM Ellman's reagent. The volume was made up to 1 mL using 0.1 M phosphate buffer with 5 mM ethylenediaminetetraacetic acid, pH 7.4, and the yellow color developed was read at 412 nm. The concentration of GSH in the sample was determined GSH standard curve.

Peroxidase assay was performed as follows: 100 µL of 10 mM KI solution was added to 100 µL of the liver homogenate and 780 µL of 100 mM potassium phosphate buffer (pH 6.0). The reaction was initiated by addition of 20 µL of 0.5% H_2O_2. The change in absorbance at 353 nm was measured for 5 min. The enzyme activity was expressed as units per milligram of proteins.

Histopathological examination

The liver paraffin-embedded tissue samples were examined for histological changes using hematoxylin and eosin (H and E) staining. Photomicrographs were taken using a light microscope (Leica, Germany).

Western blotting

The liver tissue homogenates were measured for total protein using Bradford assay. Aliquots of protein samples (50–100 µg) were resolved using sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred on to Polyvinylidene difluoride (PVDF) membrane. The membranes were blocked for 1 h in 5% skimmed milk solution and then incubated with primary antibodies, including anti-Cox-2 and anti-TNF-α overnight at 4°C. The membranes were incubated with secondary antibody (1:5000 dilution) for an hour at room temperature. The protein bands were detected on ImageQuant™ LAS 500 (GE Healthcare Life Sciences) and quantified using Image J software (version 1.46, National Institutes of Health, Bethesda, Maryland). GAPDH was used as a loading control.

Alcohol-induced hangover model

Pharmacodynamics of alcohol metabolism

Twenty-four male Wistar rats (250–275 g) were allocated to four groups of six animals each. Group I rats were given 10 ml/kg b.w. of vehicle (10% Tween 20 in water) and served as control. Rats of Groups II, III, and IV were orally administered with 200, 300, and 400 mg/kg body weight respective doses of Turcuron. 5 ml/kg b.w. of alcohol was administered to all the groups 1 h after respective treatment. After 0.5, 1, 2, and 4 h of alcohol treatment, blood samples were collected from the retro-orbital plexus into heparinized tubes and immediately stored at −80°C for gas chromatography–mass spectrometry (GCMS) analysis.

About 0.5 ml of blood sample was mixed with 1 ml of 0.01% isopropanol (in 10% perchloric acid). The vial was then placed in the headspace analyzer. The blood alcohol and acetaldehyde levels were estimated by Shimadzu gas chromatography (GCMS-QP2010) AOC-20i, autoinjector; column, ZEBRON with helium gas as a carrier; and run rate 20 ml per min.

Determination of alcohol dehydrogenase and Aldehyde dehydrogenase (ALDH) activities in liver

Following a washout period of 15 days, male Wistar rats used previously for the pharmacodynamic study were randomly assigned to four groups ($n = 6$). Group I animals were given 10 ml/kg b.w. of vehicle (10% Tween 20 in water) and served as control. Rats of Groups II, III, and IV were administered with respective doses of Turcuron, as mentioned previously. One hour later, the animals were given 5 ml/kg b.w. alcohol orally. After 2 h, the animals were euthanized using isoflurane gaseous anesthesia and the liver excised. Liver tissues were homogenized in Tris-buffered saline (50 mM Tris pH 7.5, 150 mM NaCl) with 1% Triton X-100. The liver homogenates were used further for evaluating the ADH and ALDH activities. The enzyme assays were performed using the pooled samples from each group.

Alcohol dehydrogenase activity

The reaction mixture for alcohol dehydrogenase assay contained 767 µL of sodium pyrophosphate buffer (50 mM, pH 8.8), 33 µL ethanol (95 %), 100 µL nicotinamide adenine dinucleotide (NAD) solution (2.4 mM) mixed in a cuvette. Solution was mixed by inversion. Cuvette was placed in a suitably thermostated spectrophotometer and equilibrated to 25°C. Then, 100 µL of liver homogenate (200 µg) was added and mixed immediately. Bovine serum albumin (1 mg/ml) in 10 mM sodium phosphate buffer (pH 7.5) was used as blank. The absorbance was read at 340 nm for ~6 min. ΔA_{340}/min was calculated using the 1–6-min range for both the tests and blank. The ADH enzyme activity was expressed in mU/mg protein.

Aldehyde dehydrogenase activity

For aldehyde dehydrogenase assay, the following reagents were pipetted into suitable cuvette: 875 µL of sodium pyrophosphate buffer (50 mM, pH 8.8), 15 µL of NAD solution (1.5 mM), and 10 µL of acetaldehyde (100 mM). Solution was mixed by inversion and equilibrated to 25°C. Then, 100 µL of liver homogenate (200 µg) was added and immediately mixed by inversion. Bovine serum albumin (1 mg/ml) in 10 mM sodium phosphate buffer (pH 7.5) was used as blank. Absorbance was measured at 340 nm for ~6 min. ΔA_{340}/min was calculated using the maximum linear rate for both the tests and blank. The ALDH enzyme activity was expressed in mU/mg protein.

Statistical analysis

The data were analyzed by one-way analysis of variance and Tukey's test using GraphPad Prism (Version 5.0) (San Diego, CA). Values are mean ± standard deviation. $P < 0.05$ was considered significant.

RESULTS AND DISCUSSION

High-performance liquid chromatography analysis of Turcuron

An optimized HPLC chromatogram of standard bisacurone and Turcuron is shown in Figure 1. The RT of bisacurone reference standard was found to be 6.242. The sample (Turcuron) chromatogram confirmed the presence of bisacurone RT at 6.242 without any interference.

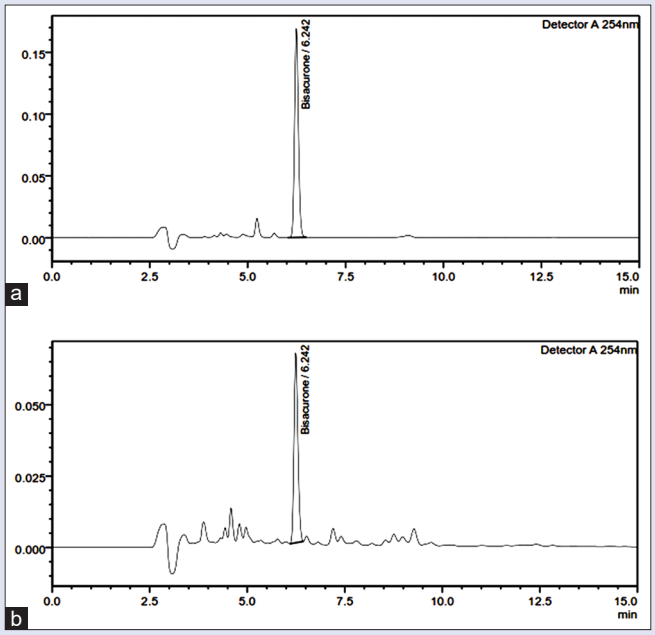

Figure 1: High-performance liquid chromatography chromatogram of Turcuron (a) bisacurone and (b) Turcuron

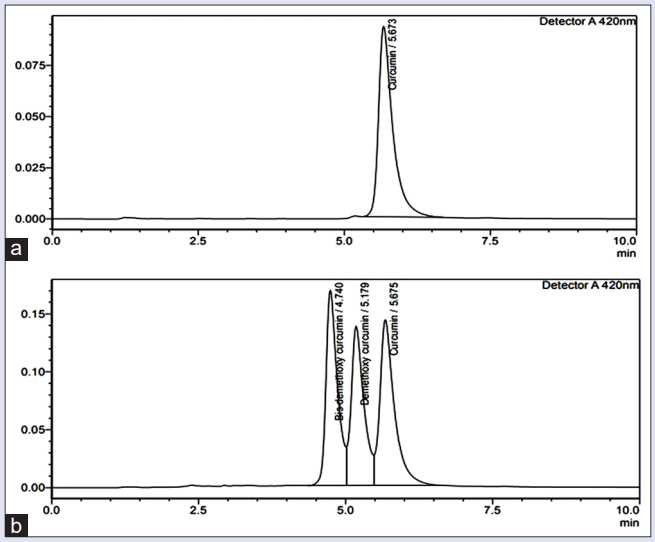

Figure 2: High-performance liquid chromatography chromatogram of (a) curcumin and (b) Turcuron

Further HPLC analysis confirmed the presence of curcuminoids in Turcuron [Figure 2]. The RT for curcumin, bisdemethoxycurcumin, and demethoxycurcumin was found to be 5.673, 4.740, and 5.179, respectively. HPLC analysis revealed that Turcuron contained 8% of bisacurone and 12% curcuminoids.

Inhibitory effect of Turcuron on nitrogen oxide and xanthine dehydrogenase activity

The results of *in vitro* assays are presented in Figure 3. Turcuron at different concentrations (12.5–200 µg/mL) exhibited appreciable NOx-scavenging activity [Figure 3a]. The percentage inhibition of NOx increased in a concentration-dependent manner from 8.94 ± 1.46 at 12.5 µg/mL to 66.9 ± 1.44 µg/mL at 200 µg/mL, with an IC_{50} value of 135.9 ± 3.22 µg/mL.

Further, Turcuron showed a concentration-dependent inhibitory effect on xanthine dehydrogenase activity, with an IC_{50} value of 140 ± 1.56 µg/mL. The IC_{50} value for reference compound Allopurinol was found to be 73.92 ± 1.49 µg/mL [Figure 3b].

Effect of Turcuron on body weight and liver index in alcohol-induced model rats

The changes in body weight and liver index of alcohol-induced rats are represented in Table 1. The initial body weights of rats were not altered significantly. However, at the end of experiment, the untreated alcohol group showed a significant decrease in the body weight compared to control ($P < 0.05$). A 4-week administration of Turcuron (150 mg/kg and 300 mg/kg) markedly increased the mean body weight of alcohol-treated rats. With the high dose of Turcuron, the body weight increased by 13.87%, while it was 17.8% at the low dose when compared with the body weight increase in the alcohol group (3.59%). Following treatment with low and high doses of Turcuron, the liver indices were decreased by 35.19% and 39.56%, respectively, when compared to the alcohol group.

Table 1: Effect of Turcuron on body weights and liver index in alcoholic liver disease model

	Body weight (g)		Liver weight (g)	Liver index (%)
	Initial	Final		
Groups				
Control	241.7±36.93	282.5±31.95	6.91±1.06	2.47±0.48
Alcohol	217.3±9.99	225.1±14.83$^{\#}$	9.31±1.87	4.12±0.7$^{\#\#\#}$
Turcuron (mg/kg)				
150	221.3±30.86	260.7±29.83	6.97±1.17	2.67±0.33***
300	248.8±39.71	283.3±51.19*	6.98±0.99	2.49±0.34***

The values are mean±SD of 6 rats in each group. Data were analyzed by one-way ANOVA, followed by Tukey's test. $^{\#}P<0.05$ and $^{\#\#\#}P<0.001$ versus control group. *$P<0.05$ and ***$P<0.001$ versus alcohol group. SD: Standard deviation; ANOVA: Analysis of variance

Effect of Turcuron on liver histology and hepatic lipid levels

The effect of Turcuron on liver histology of rats in alcohol-induced liver injury was examined by H and E staining [Figure 4a]. The untreated alcohol-induced group showed pathological changes such as inflammatory infiltration of lymphocytes, necrosis, and fat droplets. On the contrary, Turcuron treatment demonstrated a significant liver protection by restoring normal liver architecture.

The hepatic lipid levels of alcohol-induced rats are shown in Figure 4b. Rats in the alcohol-treated group exhibited a significant increase in the liver TG and TC level when compared with the control group. However, treatment with low and high doses of Turcuron reduced the TC level by 16.25% and 17.26%, respectively. In comparison with the alcohol group, the 150 and 300 mg/kg Turcuron treatment groups showed a 24.14% and 36.16% decrease in TG levels, respectively.

Effect of Turcuron on serum biochemical markers of liver injury

The effect of Turcuron treatment on serum biochemical markers of alcohol-induced model rats is presented in Figure 5. Rats in the

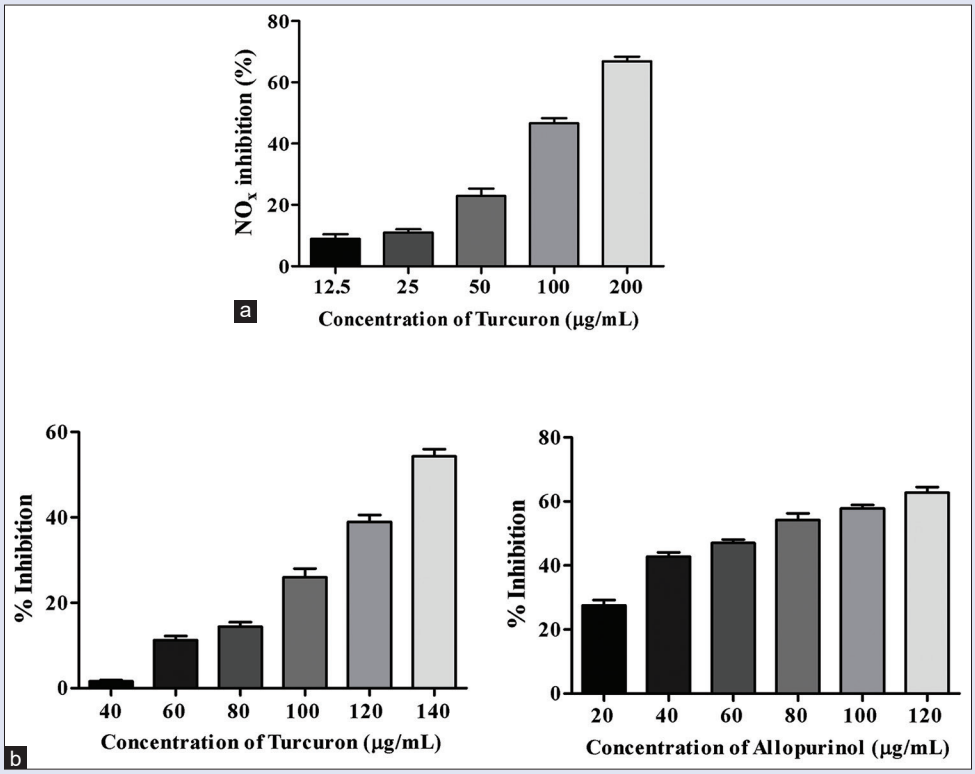

Figure 3: Effect of Turcuron on nitric oxide scavenging (a) and xanthine dehydrogenase activity *in vitro* (b). The values are mean ± standard deviation of three independent experiments

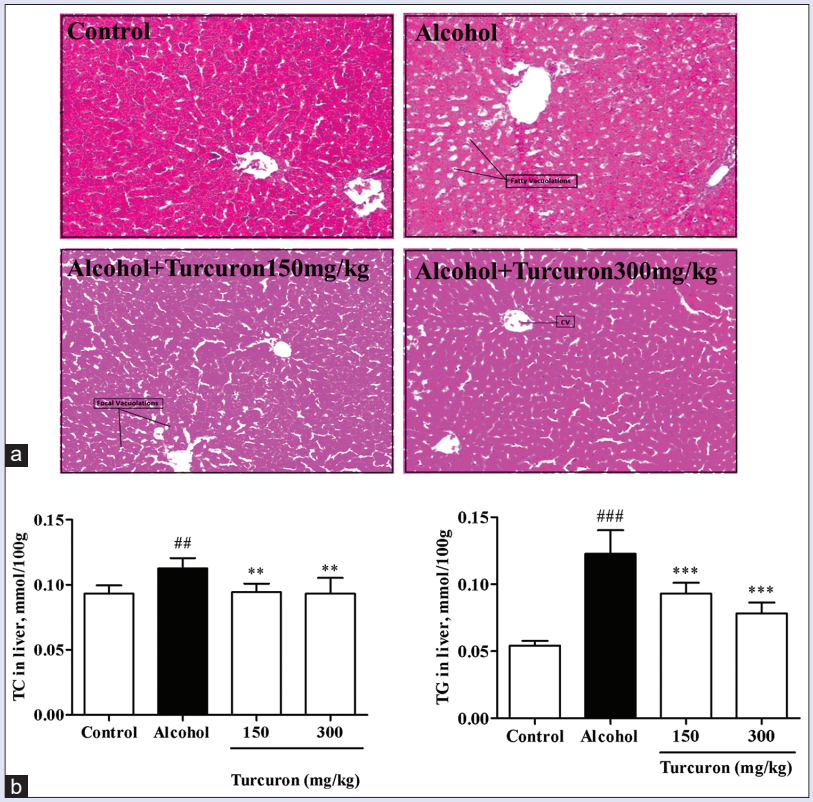

Figure 4: Effect of Turcuron on liver histomorphology and lipid levels. Representative photomicrographs of liver histopathology (a), hepatic lipid levels (b). The values are mean ± standard deviation ($n = 6$ for each group). Data were analyzed by one-way analysis of variance followed by Tukey's test. $^{\#\#}P < 0.01$ and $^{\#\#\#}P < 0.001$ versus control group; $^{**}P < 0.01$ and $^{***}P < 0.001$ versus alcohol group

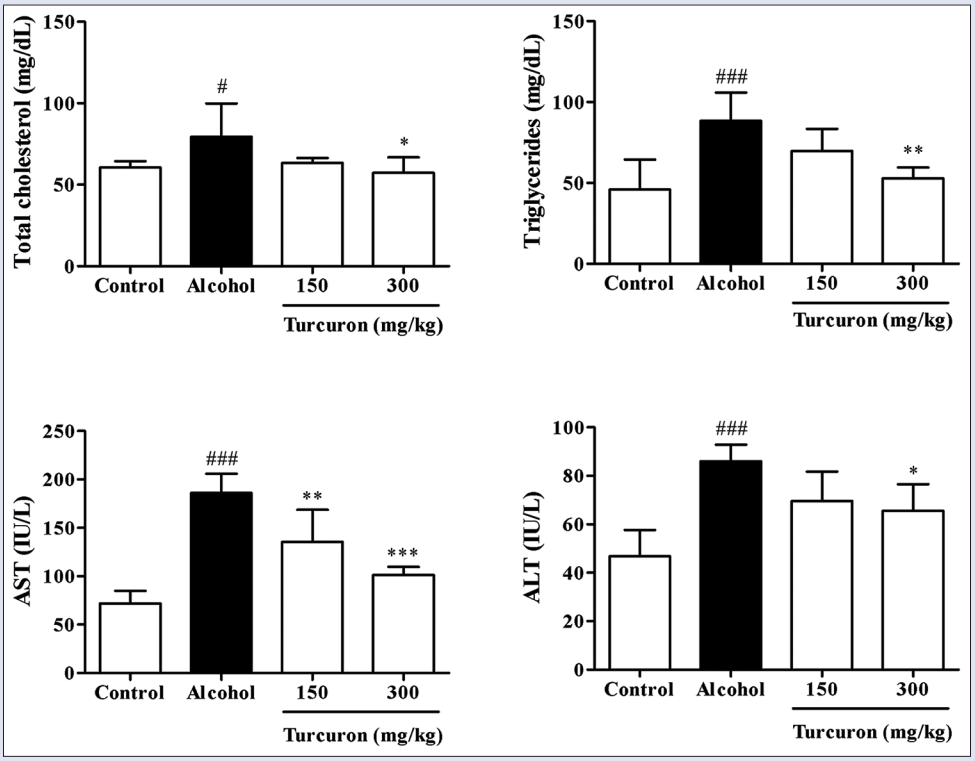

Figure 5: Effect of Turcuron on serum lipid levels and enzyme activities. The values are reported as mean ± standard deviation (n = 6 for each group). The data were analyzed by one-way analysis of variance followed by Tukey's test. $^{\#}P < 0.05$ and $^{\#\#\#}P < 0.001$ versus control group. $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$ versus alcohol group

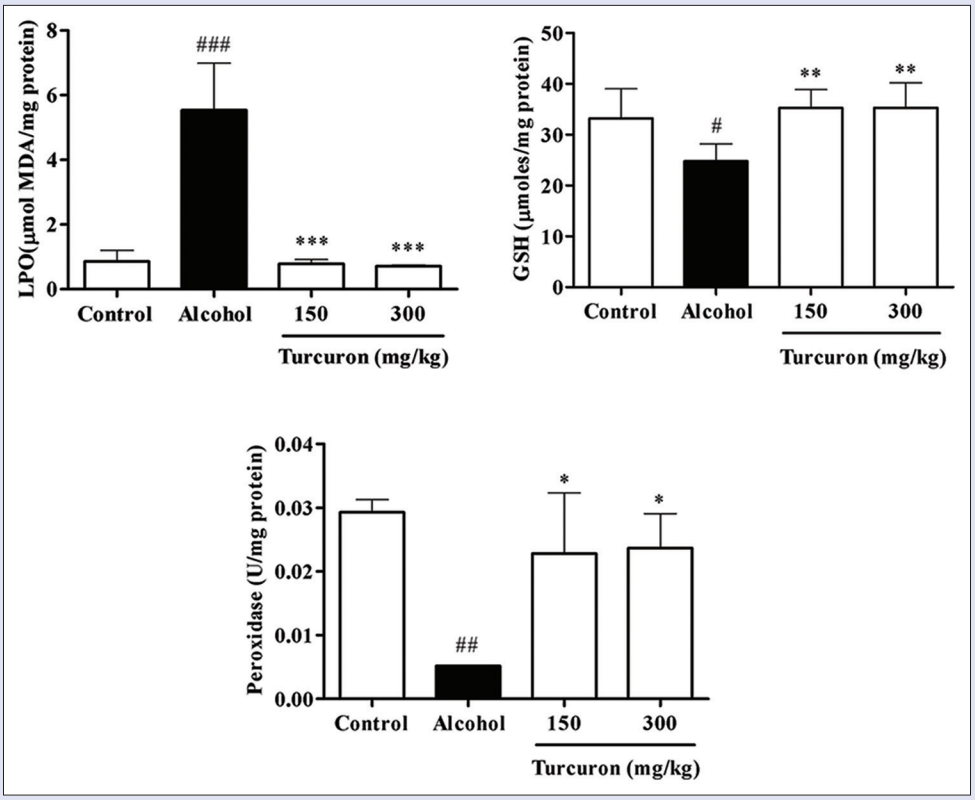

Figure 6: Effect of Turcuron on hepatic antioxidative status. The values are reported as mean ± standard deviation (n = 6 for each group). The data were analyzed by one-way analysis of variance followed by Tukey's test. $^{\#\#}P < 0.01$ and $^{\#\#\#}P < 0.001$ versus control group. $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$ versus alcohol group

untreated alcohol group showed a significant rise in TC ($P < 0.05$) and TG ($P < 0.01$) compared to the control group. The administration of Turcuron significantly restored the serum lipid levels in alcohol-treated rats. Briefly, the TC levels were reduced by 20.14% and 27.9% following Turcuron treatment at 150 mg/kg and 300 mg/kg, respectively. The serum TG levels were 21.16% and 40.23% lower than those in the alcohol group at 150 mg/kg and 300 mg/kg Turcuron treatments, respectively. Furthermore, the AST and ALT activities in the high-dose Turcuron treatment group were significantly reduced as compared to the alcohol group. We could observe a dose-dependent effect of Turcuron.

Effect of Turcuron on antioxidative status

Reduced antioxidant status is an indication of early liver damage due to alcohol. In the present study, the untreated alcohol group showed significantly lower hepatic peroxidase enzyme activity ($P < 0.01$) and GSH ($P < 0.05$), while there was an increase in MDA levels ($P < 0.001$) compared to the control group. Turcuron at the tested doses (150 mg and 300 mg/kg) significantly restored the antioxidant status of the alcohol-treated rats. Turcuron exhibited a dose-dependent effect in improving the antioxidant activity [Figure 6].

Effect of Turcuron on expression of inflammatory proteins in the liver of alcohol-induced model rats

We have performed the Western blot analysis to investigate the expression of inflammatory markers in alcohol-induced liver injury rats. There was a considerable increase in the hepatic levels of NF-kB, Cox-2, TNF-α, and iNOS in alcohol administered rats. Low- and high-dose treatment of Turcuron showed a marked reduction in the levels of these inflammatory markers [Figure 7].

Effect of Turcuron on alcohol metabolism

Prophylactic treatment with Turcuron decreased the blood alcohol levels in a dose-dependent manner at the tested time points. The decreasing trend in blood alcohol concentration was significant at 30 min after alcohol treatment as compared to control ($P < 0.001$) [Figure 8a]. Interestingly, Turcuron was effective in reducing the levels of acetaldehyde at the time points tested [Figure 8b]. The data were significant at the 300 mg and 400 mg/kg treatment groups ($P < 0.001$).

Hepatic alcohol dehydrogenase and ALDH activity

Turcuron dose dependently increased the ADH activity in comparison with the control group. However, the data were not significant. However, Turcuron at all the tested doses significantly increased the ALDH activity ($P < 0.05$) [Figure 9]. These data clearly suggest that Turcuron effectively reduces acetaldehyde accumulation in alcohol-treated rats, ameliorating the alcohol hangover.

C. longa contains curcumin, the wonder molecule known for its protective functionalities. The therapeutic potentials of turmeric are mainly contributed by curcuminoids.[21] However, there are other major groups of secondary metabolites which attribute to the health benefits of turmeric. In addition to curcuminoids, several terpenoids are identified in turmeric.[22] These terpenoid compounds exhibit several pharmacological properties such as antiviral, anticancer, antidiabetic, anti-inflammatory, and immunomodulatory activities.[23-26] These terpenoids may exert pharmacological activities alone or in synergy with curcuminoids.[27]

Bisacurone is a bioactive terpenoid present in turmeric at a low concentration. In the present study, we have used a standardized turmeric extract, Turcuron with enriched content of bisacurone, to evaluate the potential liver health benefits in chronic alcohol-induced liver injury model rats. Turcuron treatment significantly reduced the liver index of rats. Our study revealed that Turcuron treatment showed appreciable improvement in the liver function parameters such as AST

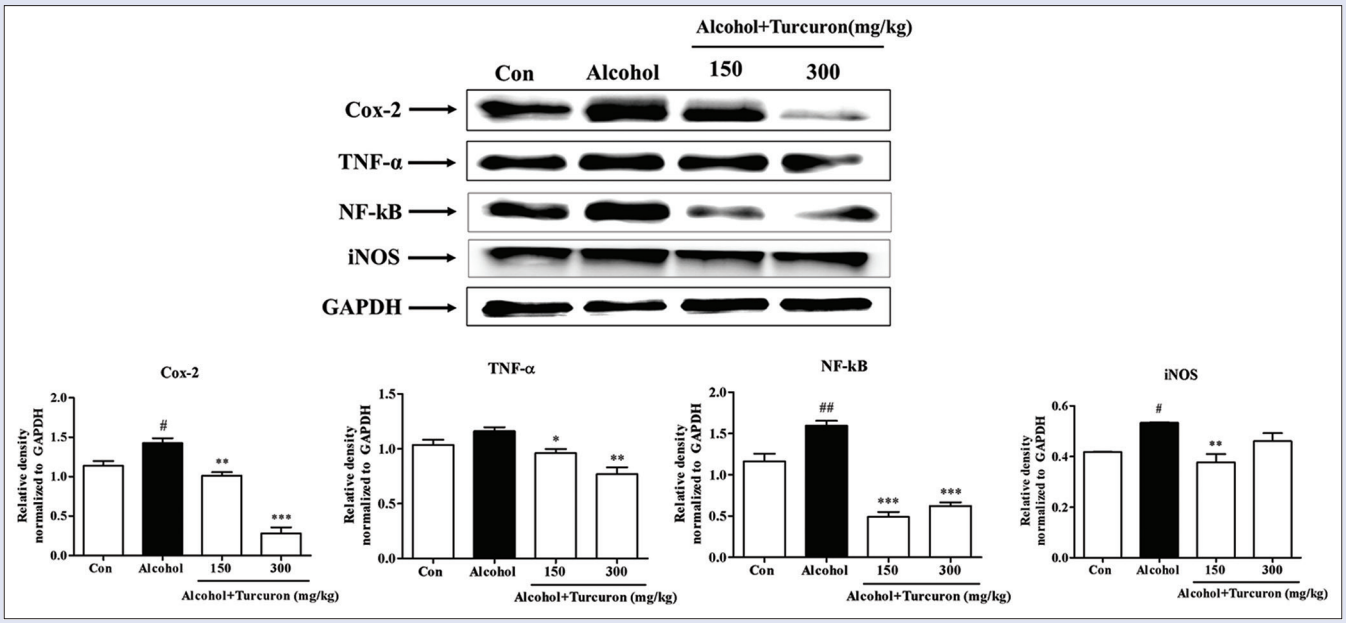

Figure 7: Effect of Turcuron on hepatic expression of inflammatory proteins in alcohol-induced liver injury rats. Western blot analysis was performed to determine the regulation of inflammatory marker proteins in the liver. The protein bands were detected by Western blot analysis and measured using densitometry. Data were analyzed by one-way analysis of variance followed by Tukey's test. $^{\#}P < 0.05$ and $^{\#\#}P < 0.01$ versus control group. $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$ versus alcohol group

and ALT compared to the untreated alcohol group. Furthermore, the hepatic and serum levels of lipids (TC and TG) were markedly reduced in the treatment groups. Turcuron at 150 mg and 300 mg/kg significantly restored the hepatic antioxidant status indicating that the extract is highly effective in oxidative stress management. These functionalities of Turcuron can be attributed to the high content of bisacurone, which plausibly has a synergistic effect with curcumin and other turmerones in the extract. Our results agree with the earlier findings from Uchio *et al.* who demonstrated that a hot water extract containing 0.3% of bisacurone and 0.1% curcumin showed a protective effect against ethanol-induced liver injury.[17]

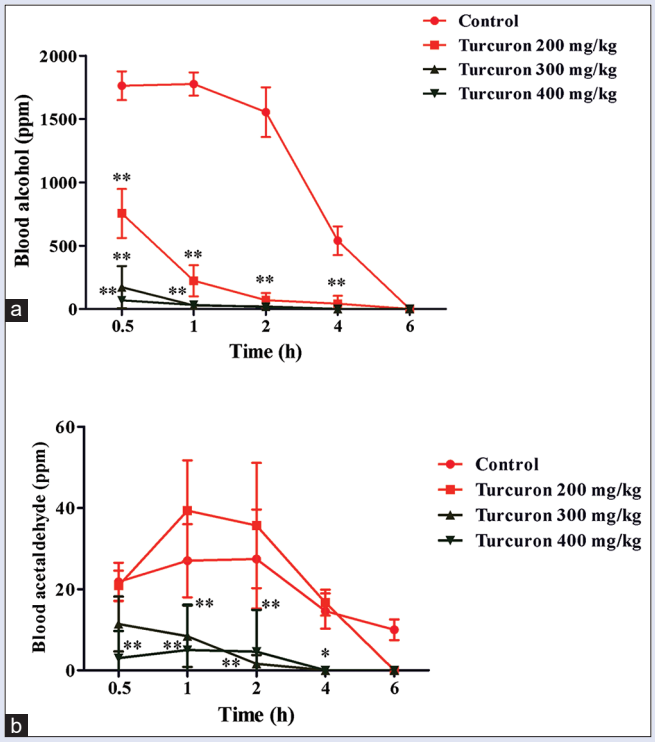

Figure 8: Effect of Turcuron on kinetic profile of blood alcohol (a) and acetaldehyde (b) in rats. The values are mean ± standard deviation ($n = 5$). The data were analyzed by two-way analysis of variance followed by Bonferroni posttest using GraphPad Prism (5.0). $^{**}P < 0.001$ and $^{*}P < 0.05$ compared to control group

It is well-established that ALD complications are associated with inflammation.[28] The progressive inflammation in the liver of alcoholics may lead to more serious pathological condition called alcoholic hepatitis.[29,30] In the present study, Turcuron substantially reduced the hepatic levels of inflammatory proteins Cox-2, iNOS, and TNF-α in rats as compared to the untreated alcohol group. Correlation of elevated NF-kB expression in the liver during ALD and inflammation has been previously reported.[31] Our results demonstrate that the alcohol-induced hepatic NF-kB activity was reduced in Turcuron-treated rats, thus mitigating the degree of inflammation. These effects could be attributed to the synergistic effect of curcuminoids and high content of bisacurone. Previously, Sun *et al.* have demonstrated the anti-inflammatory effects of bisacurone in human umbilical vein endothelial cells through the downregulation of vascular cell adhesion molecule-1.[16]

In this study, results of *in vitro* screening support the hepatoprotective nature of Turcuron. NO-scavenging activity indicates the ability of extract in ameliorating the oxidative stress and inflammation. Xanthine dehydrogenase contributes to reactive oxygen species generation in the liver, and the upregulation of the enzyme is correlated to inflammation.[32,33] Turcuron could inhibit the enzyme activity in a concentration-dependent fashion.

In the present study, we have assessed the effects of Turcuron on alcohol hangover in rats. We have measured the alcohol and acetaldehyde levels in blood at several time points after the alcohol administration in rats. Heavy alcohol drinking leads to the accumulation of acetaldehyde in blood causing hangover symptoms.[34] Our results showed that pretreatment with Turcuron (200, 300, and 400 mg/kg) significantly lowered the blood levels of alcohol and acetaldehyde in comparison with control. Further, there was a significant increase in the hepatic ALDH activity at 200, 300, and 400 mg/kg doses of Turcuron in rats. These data clearly suggest that Turcuron with high bisacurone content has the potential to act as an active ingredient in hangover relief beverages.

CONCLUSION

Here, we have demonstrated potential benefits of turmeric rhizome extract (Turcuron) containing higher content of bisacurone in mitigating the alcohol-induced liver damage. This study provides the first-ever evidence on the potential benefits of bisacurone in reducing the alcohol hangover. Turcuron is a first-of-its-kind extract which can be explored as a prospective functional food ingredient for the prevention of ALD and hangover symptoms.

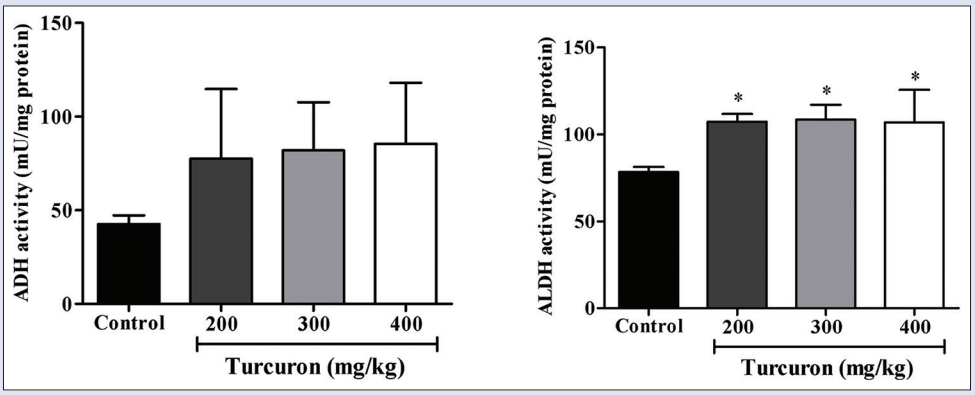

Figure 9: Effect of Turcuron on hepatic alcohol dehydrogenase and aldehyde dehydrogenase (ALDH) activities. The values are expressed as mean ± standard deviation of three independent experiments. Data were analyzed by one-way analysis of variance followed by Dunnett's multiple comparison test using GraphPad Prism (5.0). $^{*}P < 0.05$ compared to control

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Conflicts of interest

There are no conflicts of interest.

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