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Research Paper

Anti-apoptotic effects of tamarind leaves against ethanol-induced rat liver injury

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Abstract

Objectives The leaf decoctions of *Tamarindus indica* (TI) have long been traditionally used in liver ailments. The aim of this study was to investigate the anti-apoptotic activity of TI leaf extract against acute ethanol (EtOH)-induced liver injury. The major constituents of the extract were also examined for standardization purposes.
Methods Rats ($n = 5–7$) were orally pretreated with TI leaf extract (25, 50 and 100 mg/kg) for seven days. Silymarin was used as a positive control. Liver tissue biochemical assays included key markers of apoptosis and its redox signalling. Serum enzyme levels were also determined.
Key findings All graded doses of TI leaf extract mitigated the EtOH-induced liver caspase-3 activation (42, 57 and 64%) as well as DNA fragmentation (32, 47 and 50%), respectively. The highest dose of the extract demonstrated membrane-stabilizing (38%) in addition to glutathione-replenishing (88%) effects. Also, the leaves improved the liver histopathological alterations. Moreover, major plant bioactive polyphenolics, that might be responsible for the extract's observed effects, were isolated and identified.
Conclusions TI leaf extract demonstrated promising anti-apoptotic hepatoprotective effects in rats. The use of TI leaves in different liver diseases, having apoptosis as the underlying pathology, hence warrants further clinical investigation.

Introduction

Liver disorders, caused by various agents like alcohol and other chemical, environmental and biological toxins, remain one of the major public health problems.[1] Apoptosis, or physiologically programmed cell death, is recognized as an important pathologic feature in the development of most liver diseases. It is the first and central cellular response to many toxic events, and accompanies viral hepatitis, alcohol-induced liver disease and cholestatic liver diseases.[2] The outstanding characteristics of apoptosis are the fragmentation of cellular DNA, and the activation of caspases that are responsible for the major steps in the process of apoptosis. Particularly, caspase-3 (casp-3) is the central effector caspase leading to apoptotic cell death.[3] Considerable circumstantial evidence suggests that reactive oxygen species (ROS), lipid peroxidation (LP) and glutathione (GSH) oxidation may provide an alternate signalling pathway for apoptosis, including ethanol (EtOH)-induced liver apoptosis.[4,5] Indeed, EtOH-induced liver apoptosis has been widely recognized in rodents and humans.[6] Inhibiting liver apoptosis holds promise as a relevant therapeutic strategy to target.[4]

Recently, crude plant extracts have been accepted worldwide as an important source of complex phytochemicals having a wide variety of biological activity, including being an essential source to screen for hepatoprotective agents.[1] The tamarind tree, *Tamarindus indica* (TI) Linn., family Fabaceae, is a large tree indigenous to tropical Africa. Tamarind may have originated in Africa and was introduced to India by humans in early times. The Arabs called it 'tamarihindi', meaning 'date of India', and it is the basis of a popular drink in many African countries, including Egypt.[7] Cultivation of tamarind in Egypt and India has been documented between 1200 and 200 BC. Almost every part of the plant is reported to possess biological activity in various traditional pharmacopoeias, and it has high diversity of medicinal uses. Tamarind is a well-known effective and reliable source of African home medicine in the treatment of constipation, abdominal pains,

wounds, fever, malaria and dysentery and is used as an aphrodisiac. Pharmacological studies of the plant revealed that tamarind possesses antimicrobial, antidiabetic, antioxidant and hepatoprotective activity.[7–10] It is a plant with wide usage and availability but the leaves may sometimes be discarded. Otherwise, TI leaves have long been used in jaundice, hepatitis and other liver complaints in African folk medicine.[7,10] The aqueous extract of leaves contains ascorbic acid and β-carotene that are proven to be anti-hepatotoxic and anti-lipoperoxidant, against hepatocellular necrosis, using elevated doses of the extract (350 mg/kg).[7,9] However, the protective activity of much lower doses of the leaves against the more prominent liver cell death by apoptosis has yet to be investigated. Therefore, this study set out to investigate for the first time the anti-apoptotic and anti-oxidant activity of tamarind leaves standardized extract.

Materials and Methods

Chemicals

EtOH was purchased from SDS (Peypin, France). Trichloroacetic acid (TCA) was supplied by Park Scientific Ltd (Northampton, UK). Other chemicals and reagents used in the experiments were obtained from Sigma-Aldrich (St Louis, MO, USA) or Merck (Darmstadt, Germany), or were of analytical and fine grades from commercial suppliers.

Plant material and extraction protocol

Tamarind leaves were collected from Zoo garden, Giza, Egypt. They were authenticated by Prof. Dr Abdel Salam El Noyehy, Prof. of Taxonomy, Faculty of Science, Ain Shams University, Cairo, Egypt. A voucher specimen was deposited at the herbarium of the Pharmacognosy department, Faculty of Pharmacy, Ain Shams University, Cairo, Egypt. The plants were dried in shade and reduced to a fine powder. The air-dried leaves of tamarind (1 kg) were extracted by 70% ethanol on cold. The solvent was distilled off in Rota vapor at 55°C until dryness (62 g). The extract was dried to constant weight in a vacuum desiccator over anhydrous calcium chloride.

Phytochemical standardization of the extract

Phytochemical screening and standardization were performed using standard procedures.[11]

LC-ESI

LC-ESI was conducted on an Agilent 1100 HPLC coupled to an Agilent LC/MSD (HP 1101) (Figure 1). Chromatographic separation of all samples was conducted using a C-18, reversed phase 5 μm column (25 cm × 2 mm i.d.; Latex, Eppelheim, Germany) using a mobile phase consisting of 2% acetic acid in double-distilled water (solvent A) and methanol (solvent B) and gradient with a flow rate of 0.5 ml/min. The analyses were conducted in the negative-ion mode under the following conditions: drying gas (nitrogen) flow = 101/min; nebulizer pressure = 30 psi, drying gas temperature = 350°C, capillary voltage = 2500 V; fragmentor voltage = 100 V; mass range 50–3000 D.

Ultraviolet spectrophotometric analysis

Chromatographically pure materials, 1 mg each, were dissolved in analytically pure methanol then subjected to UV spectroscopic investigation in 4 ml capacity quartz cells 1 cm thick using a Carl Zeiss spectrophotometer PMQ II. $AlCl_3$, $AlCl_3$/HCl, fused NaOAc/H_3BO_3 and NaOMe reagents were separately added to the methanolic solution of investigated material and UV measurements were then carried out.

Nuclear magnetic resonance spectroscopic analysis

The NMR spectra were recorded on a Varian Mercury VX-300 NMR spectrometer. ^{1}H spectra were run at 300 MHz and ^{13}C spectra were run at 75.46 MHz in deuterated dimethyl sulfoxide (DMSO-*d*6). Chemical shifts are quoted in δ and were related to those of the solvents.

Isolation and identification

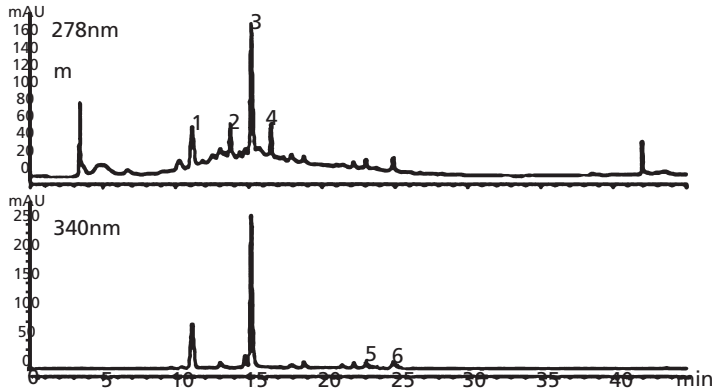
The hydroethanolic extract (40.0 g) was chromatographed over silica gel (*n*-hexane/EtOAc/MeOH) to obtain different fractions that were further chromatographed to obtain the major constituents as detailed in Table 1 having their ^{1}H and ^{13}C NMR consistent with previously published data.

Animals

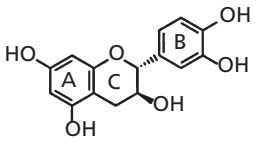
Experiments were carried out on adult male albino rats, 250–300 g, that were obtained from The Nile Co. for Pharmaceuticals and Chemical Industries (Cairo, Egypt). Rats were maintained in stainless-steel cages under standard environmental conditions (25 ± 5°C, 12-h light/dark cycle) and had free access to standard diet of pellets and tap water. Rats were routinely acclimatized for at least two weeks before any experiments. Animal welfare and experimental procedures were carried out in accordance with international guidelines for the care and use of laboratory animals and related local ethical regulations of Damanhour and Ain Shams Universities, Egypt.

Experimental design

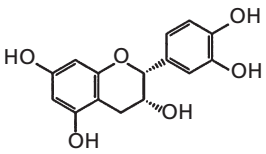
Rats were randomly assigned into three treatment groups receiving high, medium and low doses of TI leaf extract (100, 50 and 25 mg/kg, respectively). Doses and route of administration were chosen as intermediate taking guidance from published data of tamarind leaves in experimental animals,[8,9] as well as our own preliminary experimental trials.



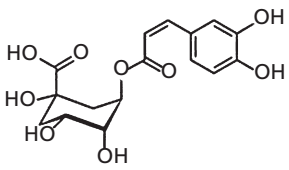
	Compound	mg/kg
1.	Chlorogenic acid isomer	485
2.	Catechin	1209
3.	Chlorogenic acid	1215
4.	Epicatechin	955
5.	Kaempferol glucoside	112



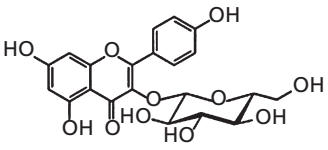
(+)- Catechin



(–)- Epicatechin



Chlorogenic acid



Kaempferol 3-glucoside

Figure 1 HPLC chromatogram and chemical structures of the compounds isolated from *Tamarindus indica* with their yield in mg/kg.

Each experimental group consisted of 5–7 rats along with appropriate controls: the normal negative control group of rats that received the same volume of water, and the positive control group that received silymarin (Sil) (50 mg/kg), a standard agent being applied clinically to treat liver disease and that protects against experimental rodent liver apoptosis.[9,12] Control, test and standard agents were orally administered once daily for seven successive days. On the last day, rats were administered 25% (w/v) ethanol (5 g/kg, by gavage). Alcohol was given every 12 h for 24 h, as a human binge drinking model that was followed for acute induction of liver apoptosis in rodents,[6] with some minor variations. Twelve hours after the last alcohol treatment, blood was first obtained from the rat under light anaesthesia through the orbital vessels using capillary tubes. Blood was allowed to clot and the serum was separated by centrifugation at 3000 rpm for 20 min at 4°C. Fresh serum samples were used to analyse the activity of the liver enzymes alanine transaminase (ALT) and aspartate transaminase (AST). Then, the rats were sacrificed under ether anaesthesia to perform the liver tissue biochemical assays. Liver was homogenized in ice-cold phosphate buffer (pH 7.2) and the protein concentration was determined by the method of Lowry *et al*.[13] using bovine serine albumin as a standard.

Determination of serum parameters (ALT and AST)

Plasma activity of ALT and AST was determined by commercial assay kits (Randox Laboratories Ltd, Crumlin, UK) based on Reitman and Frankel's method according to the manufacturer's protocol.[14]

Determination of glutathione

Liver homogenate was mixed with an equal volume of a precipitating solution (10% trichloroacetic acid/0.2% EDTA) followed by centrifugation. To the resulting supernatant, phosphate buffer (0.1 M, pH 8) and Ellman's reagent were added, and the optical density was measured at 412 nm.[15]

Table 1 ^{1}H and ^{13}C NMR data of the compounds isolated from tamarind leaves

	(+) Catechin		(–)-Epicatechin		Astragalin		Chlorogenic acid	
	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C
1								76.1
2	4.48,d,*J* = 7.4	81.0	4.80[a]	77.6		158.51	2.00–2.2 m	38.2
3	3.90 , m	67.4	4.10, br s	63.3		135.46	4.15, m	71.3
4	Ax:2.76,dd,*J* = 16.0&5.6, Eq:2.4,dd,*J* = 16.0&8.1	28.3	Ax:2.78,dd,*J* = 16&2.9, Eq:2.6,dd,*J* = 16.0& 2.9	29.0		179.53	3.71,dd,*J* = 3.1, 8.4	73.5
5		156.2		156.0		163.09	5.32, m	72.0
6	5.82, d, *J* = 2.1	95.1	5.91, d, *J* = 1.9	94.6	6.2,d,*J* = 1.9	99.87	2.00–2.2 m	38.8
7		155.3		155.0		165.97		
8	5.87, d, *J* = 2.1	93.8	5.93, d, *J* = 1.9	93.6	6.4,d,*J* = 1.8	94.74		
9		156.5		155.7		159.09		
10		99.0		98.0		105.75		
1′		131.6		130.1		122.80		127.8
2′	6.84, d, *J* = 1.7	114.5	6.8, d,*J* = 1.7	114.3	8.0,d,*J* = 8.9	132.27	7.0,d,*J* = 2.0	115.2
3′		144.8		144.0	6.8,d,*J* = 8.8	116.07		146.8
4′		144.8		144.0		161.56		149.6
5′	6.7, m	115.1	6.75, m,	114.3	6.8,d,*J* = 8.8	116.07	6.7,d,*J* = 8.2	116.5
6′	6.7, m	118.4	6.75, m,	117.5	8.0,d,*J* = 8.9	132.27	6.9,dd,*J* = 2.1,8.1	123.0
7′							7.5,d,*J* = 15.9	147.1
8′							6.2,d,*J* = 15.9	115.3
9′								168.7
1″					5.4,d,*J* = 7.2	104.07		
2″						75.74		177.0 (COOH)
3″						78.05		
4″						71.37		
5″						78.43		
6″						62.64		

[a]Submerged H_2O.

Determination of malondialdehyde

Hepatic lipid peroxidation was quantified by measuring thiobarbituric acid-reactive substance (TBARS).[16] Liver tissue homogenate was added to 1% ortho-phosphoric acid and 0.6% TBA, mixed, and then the mixture was boiled for 45 min. After cooling, n-butanol was added and then the n-butanol layer was separated by centrifugation. The absorbance of the pink-coloured product was measured at 535 and 520 nm. The difference in optical density between the two readings (Δ A 535-520) was taken as the level of TBARS in the sample.

Detection of liver caspase-3 activity

Caspase-3 protease activity in the liver tissue was measured using a casp-3 colorimetric assay kit (Sigma, St Louis, USA), according to the manufacturer's instructions. Briefly, liver tissues were homogenized with a Teflon homogenizer in cell lysis buffer, and incubated on ice for 20 min. Homogenates were centrifuged at 20 000*g* for 10 min at 4°C. The supernatants were transferred to new tubes, frozen in liquid nitrogen, and stored in small volumes at −20°C. Then, proteins extracted from samples were added to microtitre plates with 100 µl of assay buffer and 10 µl of Asp-Glu-Val-Asp-*p*-nitroanilide (pNA), the caspase substrate (200 µmol/l). Cleavage was monitored colorimetrically at 405 nm in a microtitre plate reader (Hyperion, Miami, FL, USA) and recorded at 60-min intervals for 180 min at 37°C.

Analysis of DNA fragmentation

The diphenylamine method was adopted as modified by Jafari and Rabbani.[17] Briefly, the liver was homogenized in ice-cold lysis buffer. Portions of the homogenate were centrifuged at 10 000 rpm for 20 min at 4°C, to separate intact chromatin in the pellet from fragmented DNA in the supernatant. To the pellets (P) and the supernatants (S), 25% trichloroacetic acid (TCA) was added and incubated at 4°C for 24 h. The samples were centrifuged for 20 min at 10 000 rpm at 4°C and the pellets were suspended in 5% TCA, followed by incubation at 90°C for 15 min. Subsequently, to each sample DPA solution (DPA in a mixture of acetaldehyde and acetic and sulphuric acids) was added and incubated at room temperature for 24 h. DNA was determined spectrophotometrically at 600 nm upon staining with dipheny-

lamine that reacts with the sugar residues combined with the DNA purines. The proportion of fragmented DNA was calculated from absorbance reading at 600 nm using the formula: % Fragmented DNA = [OD(S)/(OD(S) + OD(P))] × 100.

Histopathological examination

Liver samples were fixed in 10% buffered formalin solution and embedded in paraffin. Microtome sections of 5 μm thickness were stained with hematoxylin and eosin (H&E) for patholgical examination by light microscopy.

Statistical analysis

Results are expressed as means ± standard error of mean (SEM). Data was analysed using one-way analysis of variance (ANOVA), followed by Tukey-Kramer as post-hoc test for multiple comparisons. Statistical significance was set at $P < 0.05$. Statistical analysis was performed using GraphPad InStat (GraphPad Software. V2.04a).

Results

Isolation and identification of the hydro-alcoholic extract of tamarind leaves

LC-ESI was conducted on an HPLC coupled to an LC/MSD for chromatographic separation[18] of all samples to yield an HPLC chromatogram (Figure 1). Chromatographically pure materials subjected to UV spectroscopic investigation and NMR spectroscopic analysis for ^{1}H and ^{13}C spectra were identified (Table 1 and Figure 1). The hydroethanolic extract (40.0 g) chromatographed over silica gel (*n* hexane/EtOAc/MeOH) yielded thirty-two fractions. Fraction 10, eluted from *n*-hexane–EtOAc (1 : 1), was further chromatographed on silica gel elution with EtOAc–MeOH (20 : 1) to obtain catechin (18 mg) and epicatechin (15 mg). Fraction 18, eluted with *n*-hexane–EtOAc (1 : 8), was further separated and purified by silica gel column chromatography to yield kaempferol 3-glucoside (astragalin) (9 mg). Fraction 30 eluted with methanol was subjected to column chromatography over silica gel eluting with $CHCl_3$–MeOH (95 : 5, 90 : 10, 80 : 20, 50 : 50) in increasing order of polarity. The fraction that was obtained from $CHCl_3$–MeOH (50 : 50) was then subjected to repeated column chromatography over silica gel eluting with $CHCl_3$–MeOH (4 : 6) to offer chlorogenic acid (20 mg) (Figure 1).

Effect of *Tamarindus indica* leaf extract on serum alanine transaminase and aspartate transaminase activity

The serum activity of ALT and AST was used as a biochemical marker for the EtOH-induced acute hepatic damage. Serum AST showed a normal control value of 28.14 ± 2.31 U/l, which was not statistically different from a modest rise by EtOH (32.92 ± 3.78 U/l). On the other hand EtOH slightly, but significantly, increased the ALT activity by 67.7% over that in normal control rats. TI (100 mg/kg) pretreatment offered significant ($P < 0.01$) protection in acute alcohol-intoxicated rats by attenuating (38%) the ALT elevation (Table 2). Neither the 50 mg/kg nor the 25 mg/kg dose of TI showed similar protection of cell membranes against the enzymatic leakage.

Table 2 Effect of *Tamarindus indica* leaf extract on serum alanine aminotransferase activity and liver glutathione content in EtOH-intoxicated rats

Group	Alanine aminotransferase (U/L)	Glutathione (nmol/mg protein)
Control	17.7 ± 1.3	67.9 ± 2.7
EtOH	29.7 ± 2.7*	34.1 ± 1.9*
TI 25 + EtOH	22.2 ± 1.7	42.8 ± 4.4*
TI 50 + EtOH	21 ± 2.3	58.8 ± 3.9#
TI 100 + EtOH	18.3 ± 1.6#	64 ± 3.6#
Sil + EtOH	19.7 ± 2.5#	47 ± 2.2*#

Rats were orally administered *Tamarindus indica* (TI) leaf extract as 25, 50 and 100 mg/kg or silymarin (Sil) (50 mg/kg) once daily for 7 days before ethanol (EtOH) (5 g/kg, twice for 24 h). Serum and liver tissue samples were collected 12 h after last challenge for determination of transaminase activity and glutathione (GSH) content, respectively. Values are expressed as means ± SEM, $n = 5–7$. *$P < 0.05$ vs control group and #$P < 0.05$ vs EtOH group. Statistical analysis was done using analysis of variance followed by Tukey–Kramer as post-hoc test.

Effect of *Tamarindus indica* leaf extract on glutathione content in liver tissue

The basal hepatic antioxidant GSH level was decreased to about half of its initial value after alcohol treatment. Pretreatment with the high (100 mg/kg) and medium (50 mg/kg) doses of the TI extract exhibited significant protection by 88 and 72%, respectively, against the alcohol-induced GSH depletion (Table 2). The protection afforded by the medium dose of TI was even more statistically significant than that of the positive control standard drug, Sil, at the same dose level of 50 mg/kg ($P < 0.001$ vs $P < 0.05$, respectively).

Effect of *Tamarindus indica* leaf extract on liver malondialdehyde level

The level of lipid peroxidation (LP) was taken as an index for oxidative stress. The TBARS calculated as malondialdehyde (MDA) content in liver tissues were assessed (Figure 2). Data revealed that administration of EtOH caused an augmentation in control LP by more than two fold. Conversely, no protection could be demonstrated by any of the tested doses of the extracts used.

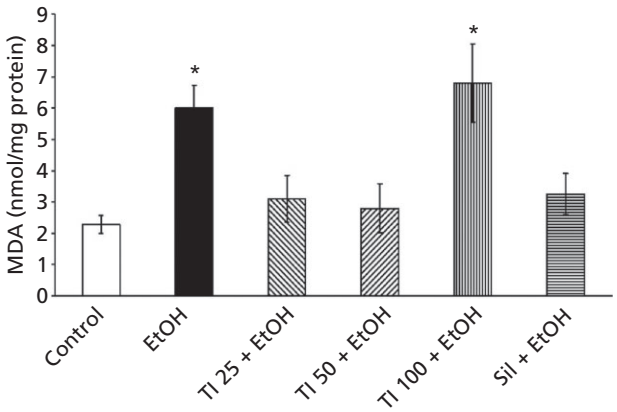


Figure 2 Effect of *Tamarindus indica* leaf extract on liver MDA level in EtOH-intoxicated rats. Rats were orally administered TI leaf extract 25, 50 and 100 mg/kg, or silymarin (Sil) 50 mg/kg once daily for 7 days before ethanol (EtOH) (5 g/kg, twice for 24 h). Liver tissue samples were collected 12 h after last challenge, and malondialdehyde (MDA) level was determined. Values are expressed as means ± SEM, $n = 5–7$. *$P < 0.05$ vs control group. Statistical analysis was done using analysis of variance followed by Tukey–Kramer as post-hoc test.

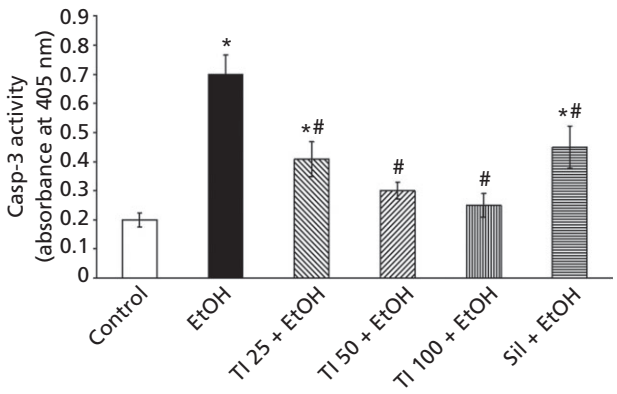


Figure 3 Effect of *Tamarindus indica* leaf extract on liver casp-3 activity in EtOH-intoxicated rats. Rats were orally administered TI leaf extract 25, 50 and 100 mg/kg, or silymarin (Sil) 50 mg/kg once daily for 7 days before ethanol (EtOH) (5 g/kg, twice for 24 h). Liver tissue samples were collected 12 h after last challenge, and caspase-3 (casp-3) activity was determined. Values are expressed as means ± SEM, $n = 5–7$. *$P < 0.05$ vs control group and #$P < 0.05$ vs EtOH group. Statistical analysis was done using analysis of variance followed by Tukey–Kramer as post-hoc test.

Effect of *Tamarindus indica* leaf extract on liver casp-3 activity

Key apoptotic markers were assessed in liver tissue – in particular, the effector Casp-3 enzyme that plays a central role in apoptosis execution. EtOH increased the control Casp-3 enzyme activity value by more than three fold (Figure 3). In contrast, the TI extract at its high, medium and low doses significantly lessened this activation (64, 57 and 42%, respectively) in a dose-dependent manner, reversing it to near approximate basal level. Of note, the protection exhibited by the lowest dose of TI (25 mg/kg) was even more statistically significant than that of Sil at double the dose (i.e. 50 mg/kg; $P < 0.01$ vs $P < 0.05$, respectively).

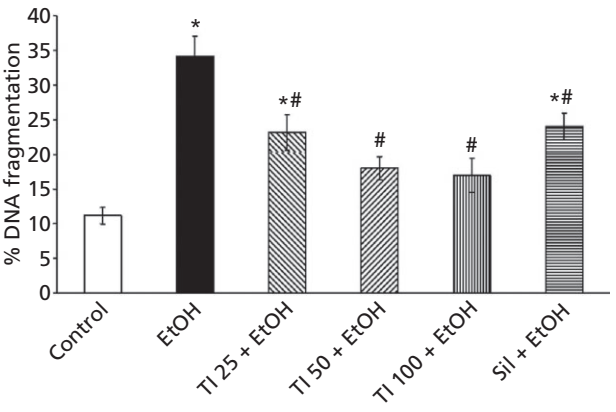


Figure 4 Effect of *Tamarindus indica* leaf extract on liver DNA fragmentation in EtOH-intoxicated rats. Rats were orally administered TI leaf extract 25, 50 and 100 mg/kg, or silymarin (Sil) 50 mg/kg once daily for 7 days before ethanol (EtOH) (5 g/kg, twice for 24 h). Liver tissue samples were collected 12 h after last challenge and %DNA fragmentation was determined. Values are expressed as means ± SEM, $n = 5–7$. *$P < 0.05$ vs control group and #$P < 0.05$ vs EtOH group. Statistical analysis was done using analysis of variance followed by Tukey–Kramer as post-hoc test.

Effect of *Tamarindus indica* leaf extract on DNA fragmentation in liver tissue

DNA fragmentation is considered a downstream late event in apoptosis. The percentage of DNA fragmentation was determined by staining with diphenylamine. The proportion of fragmented DNA was considerably elevated (206%) by EtOH as compared with the control level. On the contrary, TI extract dose-dependently counteracted the EtOH-induced elevation by 50, 47 and 32% for the high, medium and low doses, respectively (Figure 4).

Effect of *Tamarindus indica* leaf extract on liver histopathology

Pathological changes, such as liver cell death, chromatin condensation, fatty changes and inflammation with oedema, were observed in the EtOH group (Figure 5b), compared with the normal histological control (Figure 5a). There was a clear improvement in these pathological changes after treatment with the plant extract (Figure 5c).

Discussion

The model of acute alcohol-induced liver injury has been extensively used to screen for hepatoprotective

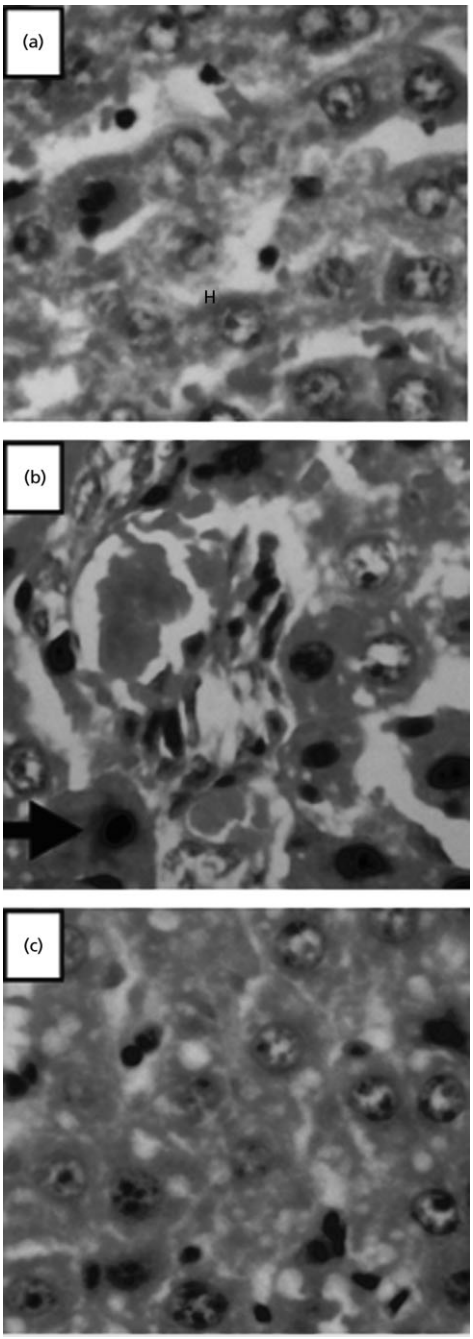


Figure 5 Effect of *Tamarindus indica* leaf extract on liver histopathology in EtOH-intoxicated rats. Rats were orally administered TI leaf extract 25, 50 and 100 mg/kg, or silymarin (Sil) 50 mg/kg once daily for 7 days before ethanol (EtOH) (5 g/kg, twice for 24 h). Liver tissue samples were collected 12 h after last challenge, and histopathological changes were photographed. (a) Representative photomicrograph of sections taken from control normal livers. (b) Representative photomicrograph of section taken from EtOH-intoxicated livers. (c) Representative photomicrograph of the effect of *Tamarindus indica* leaf extract on EtOH-intoxicated livers. H refers to a normal hepatocyte in (a), whereas, a hepatocyte undergoing cell death is indicated by a black arrow surrounded by several pathological changes in (b).

phytochemicals.[19,20] The results of this study support those of previous reports by using an experimental model of acute EtOH-induced liver cell death in rats. Rat body and liver weights in the experimental model did not offer significantly different information (±10%), most probably due to the short treatment duration of the acute toxicity study (data not shown). Reduction in the level of ALT towards the normal value was an indication of the stabilization of plasma membrane and the repair of hepatic tissue by the highest dose used (100 mg/kg) of the TI leaf extract. This effect is in agreement with the argument that serum levels of transaminases return to normal with the healing of hepatic parenchyma and regeneration of hepatocytes.[19] The serum activity of ALT and AST was used as a biochemical marker for early acute liver necrosis. These enzymes may not be elevated although minor cell death, particularly by apoptosis, is progressing in the liver cells with membranes still intact. A new term – necroptosis – describes the implication of both apoptosis and necrosis in liver cell injuries, makes it difficult to determine the relative contribution of the two processes.[4,20] ALT is reported to be a better index of liver injury than AST, as liver ALT represents 90% of total enzyme present in the body.[1] The protection afforded by TI was even more than that of the positive control standard drug, Sil, at the same dose level of 50 mg/kg. This finding may suggest the extract's ability to restore the balance between generation and clearance of ROS, and the stability of the hepato-biliary function during injury.[19] Conversely, no protection against the EtOH-induced LP could be demonstrated by any of the tested doses of the extracts used. Thus, one could presume that reducing oxidative injury may not be a major therapeutic pathway for the effect of TI leaf extract. On the other hand, a direct causal relationship between liver cell death and LP has long been disputed by several scientists.[20]

Concerning key apoptotic markers in liver tissue, the casp-3 enzymatic activity is considered a very specific and sensitive marker of apoptosis.[3] DNA fragmentation, as a main final event in apoptosis, as well as liver histopathological changes, has also been relied on in many hepatotoxicity models.[12,21,22] All three key markers were significantly altered by the acute administration of EtOH and restored by the plant extract pretreatment. The TI extract at its high, medium and low doses decreased the casp-3 activation and the DNA fragmentation in a dose-dependent manner. It is worth mentioning that the protection exhibited by the lowest dose of TI (25 mg/kg) was even more statistically significant than that afforded by the positive hepatoprotective anti-apoptotic drug, Sil. The inhibitory effect of TI leaf extract on the mechanistic expression of Fas/Fas ligand system-mediated casp-3 activation was neither clear nor significant (unpublished observations). Fas-mediated apoptosis can be blocked by overexpression of Bcl-2 or Bcl-xL in type II cells, such as hepatocytes. Moreover, downregulation of Fas is ubiquitous

in tumours, including hepatocellular carcinoma, and is associated with increased resistance to Fas-mediated apoptosis.[4] Then, the lack of a significant downregulation by the TI extract may be advantageous in this respect.

The overall hepatoprotective activity of TI against EtOH-induced toxicity may be explained in terms of the chemical composition of TI. The phytochemical screening of the leaf extract afforded varieties of polyphenolic components; condensed tannin [(−)-epicatechin (+)-catechin], glycosylated flavonoid (kaempferol 3-*O*-β-D-glucoside) and phenolic acid (chlorogenic acid). Altogether, major individual structures were identified via conventional methods of analysis.[11,18] Tamarind is also an important source of food in tropical regions, but at present the leaves are sometimes discarded. These waste products, which contain a lot of phenolics, demonstrated antioxidant and anti-apoptotic hepatoprotective effects in the current experiments on a rat model. These findings are in frank agreement with previous effects of polyphenols as demonstrated by our groups[20,23] and by others.[24] It has been reported that chlorogenic acid has a strong antioxidant activity, as well as catechin and epicatechin.[25] Besides, green tea catechins, chlorogenic acid and kaempferol-3-*O*-β-D-glucoside (astragalin) ameliorate the serum activity of AST, ALT and LDH and the antioxidant parameters, including hepatic MDA and GSH, as well as the antioxidant enzymes and transcriptional factors in different rodent models of liver injuries.[26–28] Regarding apoptosis, epicatechin and kaempferol strongly inhibit casp-3-like protease activity in neurons protecting the central nervous system against oxidative and excitotoxic stress.[29] Furthermore, epicatechin has been shown to promote cell proliferation, whereas chlorogenic acid increases cellular GSH levels.[30]

Conclusions

In summary, our results suggest that TI leaf extract has a potent hepatoprotective effect on acute alcohol-induced liver apoptosis in rats. The primary mechanisms underlying the protective effects of TI might be its membrane stabilization and its alleviation of GSH depletion. Besides, TI inhibited casp-3 activation and DNA fragmentation in a dose-dependent manner, and ameliorated liver histopathological changes. It may warrant further investigation to examine other extrinsic pathways of apoptosis, including the Fas/Fas ligand system, in addition to the intrinsic pathway that might have been the key player during the present experiments. Further studies are recommended to better elucidate the mechanism of action and evaluate the hepatoprotective and anti-apoptotic activity of isolated ingredients of TI leaf extract, so that the poly phenolic content may be used as marker compounds for the qualitative and quantitative estimation of the plant. Future research should give better insight into its usefulness in the clinical therapy of different liver diseases having apoptosis as the first and foremost underlying pathology. Furthermore, the consumption of tamarind leaves, as a popular drink besides the fruits, warrants further clinical investigation into its possible liver supporting effect. This is based on the presently shown promising hepatoprotective anti-apoptotic effects of low doses of the tamarind leaf extract in rats.

Declarations

Conflict of interest

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

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