ISSN Print: 2664-6536
ISSN Online: 2664-6544
Impact Factor: RJIF **5.4**
IJBB 2025; 7(1): 126-131
www.biosciencejournal.net
Received: 01-02-2025
Accepted: 04-03-2025

Mohd Danish
Section of Genetics,
Department of Zoology,
Aligarh Muslim University,
Aligarh, Uttar Pradesh, India

Riaz Ahmad
Professor, Section of Genetics,
Department of Zoology,
Aligarh Muslim University,
Aligarh, Uttar Pradesh, India

Corresponding Author:
Riaz Ahmad
Professor, Section of Genetics,
Department of Zoology,
Aligarh Muslim University,
Aligarh, Uttar Pradesh, India

Experimental evidence of hepatoprotective action of trigonelline during chemically-induced liver injury in rodents

Mohd Danish and Riaz Ahmad

DOI: https://www.doi.org/10.33545/26646536.2025.v7.i1b.108

Abstract
Liver is an extremely important organ of the body, primarily responsible for detoxification. Liver contains several types of cells including liver sinusoidal endothelial cells, hepatocytes, hepatic stellate cells and Kupffer cells. It possesses a remarkable property of regeneration and hence diseases associated with the liver may take time to fully develop. Adulterated food, intake of toxins, alcohol, parasites, viruses, and life style are some of the important factors which may trigger liver diseases. Further, liver may be overloaded with toxins if synthetic medicines are utilized for a long time. Therefore, it becomes imperative to find some suitable alternatives which may be equally efficacious, challenging and can protect the liver from damage. In this context, the present study was undertaken to examine anti-inflammatory property of a plant-based alkaloid, trigonelline. In one group of animals (Albino rats), intraperitoneal injections of Nitrosodiethylamine were given to induce inflammation while in another group these injections followed a subsequent treatment with trigonelline to investigate protection. In this study, parallel controls were also run to compare the scientific data. Primary liver function test parameters such as levels of alkaline phosphatases (ALP), alanine transaminase (ALT) and aspartate transaminase (AST) and bilirubin, as the established biomarkers, were examined. Further, quantity of liver collagen and anatomical alterations in the tissue were also taken into consideration to establish the effect of trigonelline. It was noted that trigonelline significantly declines the augmented levels of investigated parameters within two weeks and refurbished the architectural changes in the tissue. It is thus concluded that trigonelline may an alternative choice to invigorate liver cells and to correct hepatic functioning.

Keywords: Collagen, liver injury, nitrosodiethylamine, trigonelline

Introduction
Liver being the essential organ of human body performs a large number of vital functions including metabolism, detoxification, protein synthesis and regulation of various life processes. On a daily basis, liver is exposed to a number of toxicants that may lead to injury. In recent years, liver diseases have become a significant challenge for both developed and developing nations with a significant increase in number of cases contributing to inflated global mortality rate (Ahmad and Ahmad, 2012; Dunbar and Crombie, 2011) [3, 7]. Liver injury may occur due a variety of causes, including viral infections (i.e. hepatitis B and C), parasitic infections, exposure to toxins, drugs, and alcohol. In case of delayed attention, the liver may transform into more aggressive form and undergoes fibrotic stage. Liver fibrosis, at cellular level involves transformation of quiescent hepatic stellate cells into activated myofibroblast-like cellular state, which produces extracellular matrix proteins contributing to scarring and impaired liver function. Over time, this dynamic process disrupts the normal structure and function of liver, potentially progressing later to cirrhosis and ultimately to liver failure (Friedman, 2003; George and Chandrakasan, 1996) [10, 13]. However, on one side researches have shown that liver damage at the fibrosis stage is reversible if appropriate interventions are put into action; while on the other hand it is suggested that if proper care is taken during initial stage of damage then the risk may be potentially reduced. This highlights the importance of early detection and treatment to restore the liver to a fully functional state. Present study utilises nitrosamine (i.e. N'-Nitrosodiethylamine; NDEA) for induction of hepatic injury *in-vivo*. N'-Nitrosodiethylamine which is a known hepatotoxicant with mutagenic and

carcinogenic potential found in a variety of food items like smoked fish and meat, dairy products, soft drinks, cigarette smoke and alcoholic beverages (Dutra *et al.*, 2007; Inami *et al.*, 2010) [8, 18] is taken directly or indirectly by human beings. Further, modern synthetic medicines possessing more cons than pros in long term exposure, therefore there is a dire need to explore alternative and body-friendly medicines with least or no side effects and maximum efficacy. In this context, trigonelline (TG), a plant-based alkaloid, was utilised to investigate its protective potential in the liver of injured rodents. Trigonelline is a strong polar hydrophilic compound found in coffee, soybeans, onion, corn etc (Evans and Tramontano, 1984; Viani and Horman, 1974) [9, 36] and reported to possess anticancer, neuroprotective, anti-diabetic and anti-dyslipidemic properties (Bakuradze *et al.*, 2010; Hamden, 2013; Hirakawa *et al.*, 2005; Liang and Kitts, 2014; Morani *et al.*, 2012; Özçelik *et al.*, 2011; Sathiyaseelan *et al.*, 2020; Tohda *et al.*, 2005) [5, 16, 17, 20, 22, 29, 35]. The present study is a preliminary attempt to examine the effect of trigonelline utilizing some primary biochemical parameters which are routinely performed in such type of studies and are utilized to establish hepatoprotective potential of any bioagent.

Material and methods

Description of animals: The study uses Albino rats (Wistar strain) weighing around 200-250g. Study plan on animal use was approved by Institutional Animal Ethics Committee (IAEC). Animals in this study were provided with a standard diet (sterilized) and free access to water. To help them adjust to laboratory conditions, animals were acclimatized for seven days before the experiments commenced. They were housed in clean, sterile plastic cages, ensuring proper hygiene, and were kept on a consistent and standard light-dark exposure. Later, the animals were randomly grouped into four for treatment and further investigations.

Treatment plan

The experimental animals were divided into four groups for treatment and examination. Group-I: received normal saline in doses of 2ml/Kg body weight for 2 weeks on alternate days.

- **Group-II:** received single dose of NDEA (1% in 0.15M NaCl) in dose of 10ml/Kg body weight.
- **Group-III:** received single dose of NDEA (1% in 0.15M NaCl) in dose of 10ml/Kg body weight along with aq. solution of trigonelline in doses of 50mg/Kg body weight on alternate days for 2 weeks.
- **Group-IV:** received only aq. aq. solution of trigonelline in doses of 50mg/Kg body weight on alternate days for 2 weeks.

Extraction of sera and preparation of tissue homogenates: After completion of the duration of fourteen days, animals were carefully anesthetized before being humanely sacrificed for further analysis. Blood was collected using sterilized syringe and kept at room temperature for about an hour. This was then allowed to centrifuge at 3000rpm for 10min at 4°C to obtain the pale-yellow to golden colour sera. Sera from different groups were collected in the same way and analysed fresh for estimation of protein, enzyme activity and other analyses. Liver from the sacrificed animals were excised and washed in normal saline (chilled) to remove the surrounding debris. A small piece of $1cm^3$liver tissues was collected and fixed in phosphate buffered formalin (10%) for histopathological assessments. A small part of tissue from different treated groups was also kept for estimating collagen content in liver tissue. Liver tissue was also analysed for estimation of protein for which the homogenate was prepared in 50mM Tris-HCl, pH 7.5 in the ratio of 1 part tissue and 3-part buffer. Clear homogenate was prepared by centrifuging the homogenised tissue at 8000rpm for 20min at 4°C.

Sera and tissue protein content and liver function test

To estimate concentration of protein in serum and tissue, method of Lowry *et al.*, (1951) [21] was followed. The sample (unknown) were incubated with alkaline copper reagents for 10min at RT followed by reaction with Folin's reagent and further incubation for 25min at RT. Optical density of the mixture was measured at 660nm against standard of known concentrations of bovine serum albumin.

Biomarkers for liver function were examined using standard commercial kits purchased from Erba Diagnostics Mannheim Gmbh (Mumbai, India). Liver function parameters assayed during the study includes alanine aminotransferase (ALT), alkaline phosphatase (ALP), aspartate aminotransferase (AST) and bilirubin (total and direct).

Liver collagen content

Estimation of collagen was done by indirect means *via* estimating hydroxyproline (an imino acid) contents using the standard procedure as described by Woessner (1961) [37]. According to this method, portion of fresh tissue (0.1g)was processed by heating it with 0.3mL of 6M hydrochloric acid at 110°C for 24 hours to facilitate hydrolysis. This was followed by heating the mixture carefully in a boiling water bath to evaporate any acid residues. The resulting dried material was then allowed to dissolve in 20mL of distilled water; out of which, approximately 1 mL content was taken and mixed with 1 mL of 1.4% chloramine-T. It was allowed to react for 20min and later 1mL of perchloric acid was added slowly to the mixture. After a brief waiting period of 5min, 1mL of freshly prepared p-dimethylaminobenzaldehyde (20%) was added. The mixture was finally incubated at 60°C for 20min to complete the reaction. The absorbance of the resulting solution was then measured using a UV-Visible spectrophotometer at a wavelength of 560nm. After calculation of hydroxyproline value, collagen contents were extrapolated by multiplying the hydroxyproline value with a constant of 7.46 as recommended by Neuman and Logan (1950) [26].

Histopathological analysis

Previously fixed liver tissues in 10% phosphate buffered formalin were processed for preparing section, of up 5µm thickness, using microtome. Prepared sections were stained with Haematoxylin and Eosin (H&E) and picrosirius red stain (Ahmad and Ahmad, 2014) [1] and observed under light microscope Nikon 80i/MoticBA210 with attached camera and confocal microscope. The observations were made to examine architectural changes in tissue structure as well as to visualize the collagen accumulation in the liver, which stain red in presence of picrosirius red stain.

Statistical analysis
Results of all biochemical parameters taken during the present study were presented as mean value ± standard deviation. The obtained data were drawn in MS excel ver. 2021 and statistically tested by one-way ANOVA using graph pad prism (version 9.5.1) and considered significant at $p<0.05$, $p<0.01$ or $p<0.001$.

Results
Changes in liver phenotype and hepato-somatic indices (HSI): After the excision of liver from animals, morphological changes were examined and liver tissues were photographed. Liver of control and TG treated rats showed normal appearance with brownish colour. Liver of NDEA treated animals were slightly yellowish with apparent scars. However, liver tissues of animals treated with NDEA and TG showed almost similar morphology as of normal liver. The study also evaluates the hepato-somatic index of animal where the liver to body weight ratio changed significantly than control group, indicating altered energy reserves in the liver and disrupted metabolic activity in injured animals. Treatment with TG significantly normalizes the hepato-somatic index in the given duration of treatment (Fig. 1A).

Status of sera / tissue protein content and liver function biomarkers: Exposure to NDEA caused significant biochemical disruptions highlighting its toxic effects on liver functioning. The total protein levels in the initial duration of treatments in the liver were markedly reduced and elevated in serum. Additionally, NDEA exposure led to a substantial increase in serum levels of liver enzymes such as AST, ALP, and ALT, along with augmented bilirubin direct level. These changes in liver biochemistry are an indication of altered liver function which is provoked by NDEA in two weeks of treatment. These altered levels of liver biochemistry parameters were significantly restored in animals given TG within the experimental duration (Fig.1B-C).

Changes in liver collagen content
Hydroxyproline contents were notably elevated in NDEA-treated injured rats thereby suggesting increased collagen synthesis and deposition in the liver. However, these levels of collagen were observed reduced in the liver of animals treated with TG for two weeks (Fig.1D). These findings are indicative of hepatoprotective potential of trigonelline as the collagen deposition halts proper liver functioning and disrupts liver anatomy.

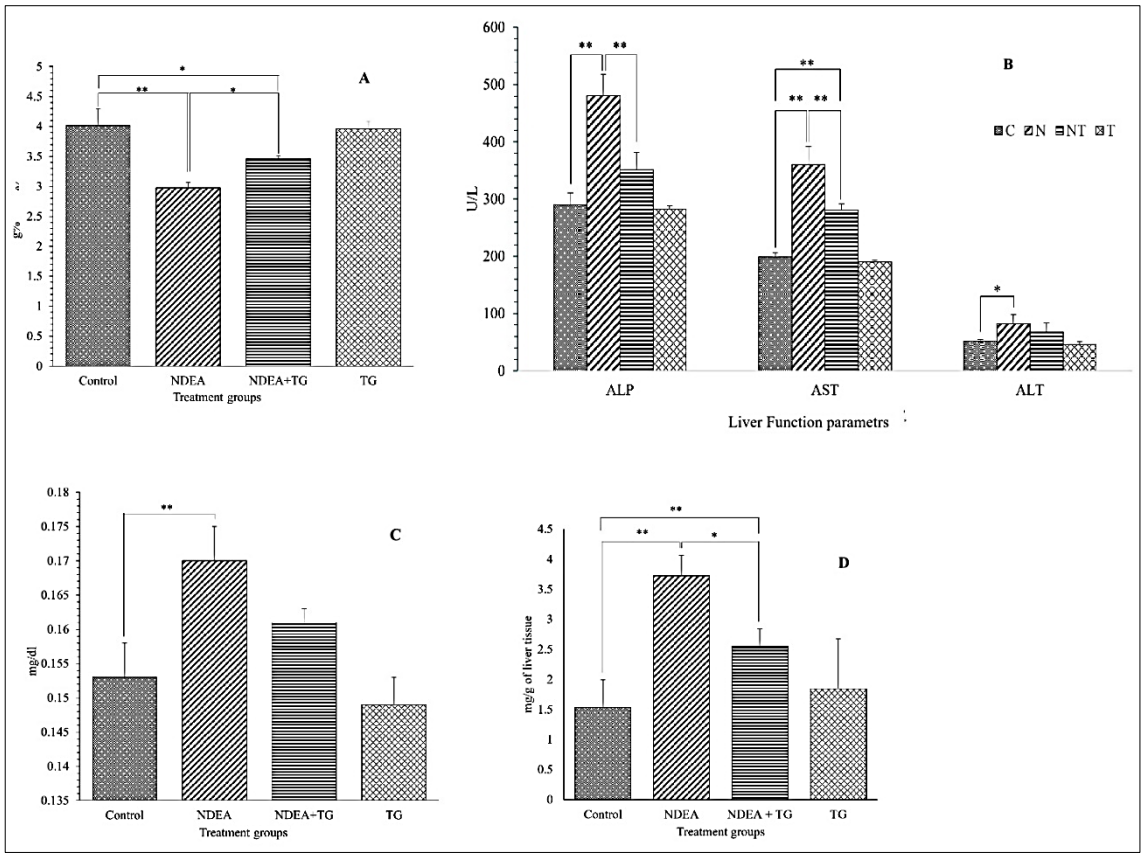

Fig 1: Liver function variations across groups

Figure 1: Bar graph showing variations in the (A) liver hepato-somatic index (g%); (B) variations in the liver function parameters; (C) serum bilirubin direct (mg/dl) and (D) liver collagen content (mg/g of liver tissue) in different groups of rats (Control, NDEA, NDEA + Trigonelline (TG) treated, Trigonelline (TG) treated).(ALP=alkaline phosphatase; AST=aspartate aminotransferase; ALT=alanine aminotransferases; Bil D= Direct Bilirubin). Each bar represents the mean ± SD value (n=3) of experiments performed in duplicates *p<0.05 and **p<0.01.

Histopathological analysis
Light microscopy examination of liver tissue stained with Haematoxylin and Eosin (H&E) revealed distinct differences between control and NDEA+TG treated groups. In the animals belonging to control and TG treated group, the liver displayed normal architecture with unbroken

central veins, healthy hepatocytes with clear nuclei, and no signs of haemorrhage or cellular infiltration (Fig.2A, D). However, livers from NDEA-treated group of animals showed severe structural damage characterized by dilated central veins, infiltration of neutrophils, haemorrhage (Fig.2B). It is noteworthy that TG administration significantly restored hepatic structure which was damaged by NDEA treatment (Fig.2C).Confocal microscopy further revealed collagen deposition in the liver tissues of NDEA treated animals (Fig. 3). This collagen contributed to obstruct liver functioning and disrupted anatomy, indicating generation of liver fibrosis. However, two weeks treatment of TG notably declined the presence of collagen which was evident from both, the light and confocal microscopy data.

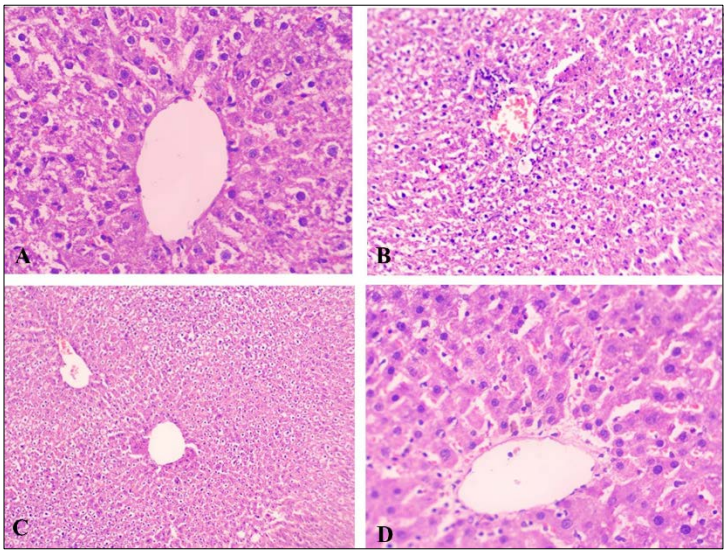

Fig 2: Histological liver changes with treatment

Figure 2: Haematoxylin and Eosin (H&E) staining of sections of liver sections during NDEA-induced hepatic fibrosis and treatment with Trigonelline (TG). (A) Control liver (10X); (B) NDEA treated group (10X); (C) treatment of NDEA treated group with TG (40X); (D) showing liver section treated with TG only (10X).

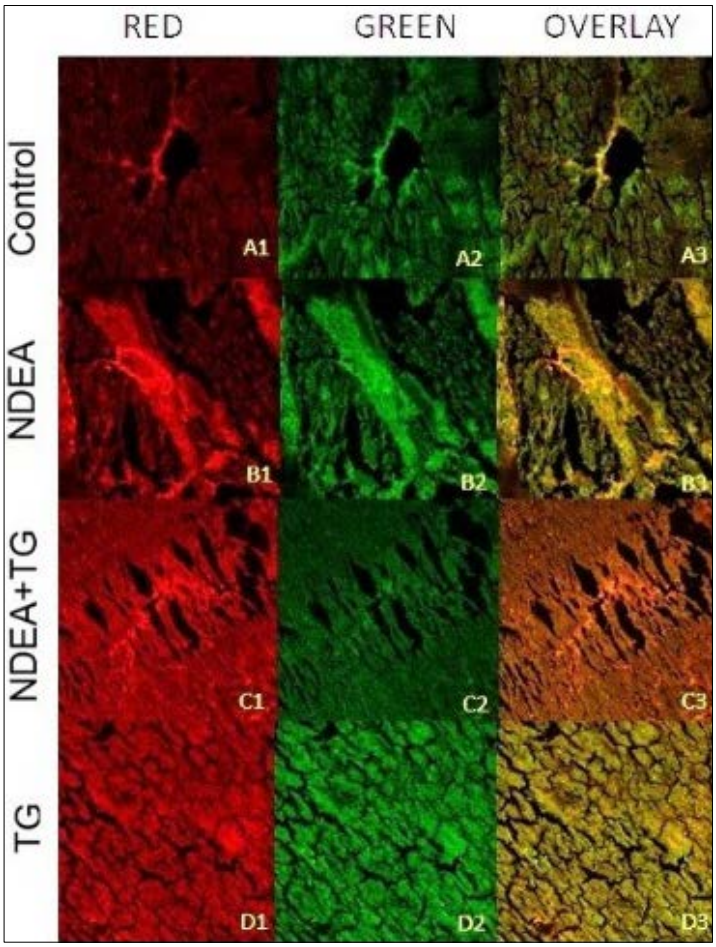

Fig 3: Confocal microscopy reveals liver fluorescence

Figure 3: Confocal; microscopy of rat liver specimen observed under red and green filter with corresponding overlay image in liver sections of control (A1, A2, A3); (B1, B2, B3) NDEA-treated; (C1, C2, C3) NDEA + TG -treated and (D1, D2, D3) TG-treated.

Discussion

A number of studies demonstrated that Nitrosamines show detrimental effect in time and dose dependent fashion in rodents (Ahmad and Ahmad, 2014; Latief *et al.*, 2016; Mukherjee and Ahmad, 2015a; Snodin *et al.*, 2024) [1, 19, 23, 32], and hence they have been used to generate animal model for studying pathology and drug discoveries. Metabolic activation of nitrosamines occurs primarily in liver which leads to liver injury (Ahmad *et al.*, 2014; Mukherjee and Ahmad, 2015b; Sheweita, 2000) [1, 24, 30]. Hepatic injury usually affects organ weight and dimensions along with structural alterations (Simon *et al.*, 2020) [31]. Though rarely specific, changes in weight of liver and HSI may be important to detect and evaluate the effect of hepatotoxins. Decrease in weight of liver signifies atrophy or severe hepatocellular injury (Cattley and Cullen, 2013) [6]. In the present study, NDEA causes gross phenotypic changes as well as altered hepato-somatic indices (HSI), which may due to the loss of stored material and atrophy due to NDEA induced damage. Changes in hepatic morphology were also substantiated by the amended liver function markers clearly demonstrating that the NDEA disrupts liver biochemistry and functioning leading to significant elevation in sera ALT, ALP, AST enzymes and bilirubin. In addition to it, total protein contents were also affected indicating impaired protein synthesis and leakage of proteins into the bloodstream, as evidenced by elevated serum liver function biomarkers (Mukherjee *et al.*, 2022; Teloh, 1978) [25, 34]. However, the levels of investigated proteins and tissue phenotype were significantly refurbished by the treatment of trigonelline (TG) within two weeks, suggesting that TG potentiates liver cells to cope with the damage induced by NDEA in animals.

Levels of hydroxyproline were also analysed as a measure of collagen content. Hydroxyproline, a key component of collagen, plays a critical role in maintaining lobular architecture to maintain tissue integrity. NDEA triggers activation of hepatic stellate cells (HSC) which produces collagen-rich extracellular matrix (ECM), with hydroxyproline serving as a biochemical footprint of this process (Gabr *et al.*, 2017) [12]. An increase in the levels of collagen was found in NDEA-treated group of animals indicating collagenesis and subsequent generation of fibrosis in the liver (Fuchs *et al.*, 2013; Ricard-Blum *et al.*, 2018) [11, 28]. These findings were also confirmed by histopathology of tissue belonging to different treatment group of animals. Haematoxylin and eosin staining is a routine histopathology procedure that represents one of the best technique to study liver architecture. Liver sections treated with NDEA showed gross damage to the central vein along with haemorrhage and immune cell infiltration. Picrosirius Red staining sections when observed under confocal microscope, under red and green filters, also showed collagen deposition in NDEA-treated group of animals. These observations based on light and confocal microscopy clearly demonstrate collagen accumulation in the liver that led to fibrosis and obstructed function of the liver (George *et al.*, 2001; Hall *et al.*, 2021; Takahashi *et al.*, 2010) [14, 15, 33]. However, animals when treated with trigonelline demonstrated significant reduction in the levels of collagen deposition which is also concomitant with improved cellular function and lobular anatomy. These findings were also corroborated with previous studies where nitrosamine induced fibrosis has been studied in rodents and the possible alteration in function and structure were established (Ahmad and Ahmad, 2014; Badria *et al.*, 2019; Mukherjee *et al.*, 2022) [1, 4, 25]. Treatment with TG in selected doses declines collagen levels and restores hepatic architecture, as also evident by light and confocal microscopy.

Conclusions

Nitrosodiethylamine induces damage to the liver within two weeks, even if given in a single dose. This damage includes both the functional and structural, which was examined by primary functional parameters of liver such as levels of ALT, AST, ALP, bilirubin, total proteins, hydroxyproline, collagen and histopathology. Trigonelline (TG) when given to the NDEA-treated animals reduces its toxic effects and refurbished liver biochemistry and anatomy. Thus, it is concluded that TG in its selected doses corrects the NDEA-induced changes in the liver of rodents and offers strong protective effect to invigorate hepatic cells.

Acknowledgement

Authors are grateful the Chairperson, department of Zoology for providing necessary laboratory facilities. University sophisticated Instrumentation Facility (USIF), established under DBT-Builder Program, facilitated the confocal microscopy. Support extended by Akbar Hussain, Research Scholar is also thankfully acknowledged. MD expresses thanks to the Aligarh Muslim University for providing UGC-Non NET Fellowship.

References

1. Ahmad A, Afroz N, Gupta UD, Ahmad R. Vitamin B12 supplement alleviates N′-nitrosodimethylamine-induced hepatic fibrosis in rats. Pharmaceutical Biology. 2014;52:516-523. https://doi.org/10.3109/13880209.2013.864682
2. Ahmad A, Ahmad R. Resveratrol mitigate structural changes and hepatic stellate cell activation in N′-nitrosodimethylamine-induced liver fibrosis via restraining oxidative damage. Chemico-Biological Interactions. 2014;221:1-12.
3. Ahmad A, Ahmad R. Understanding the mechanism of hepatic fibrosis and potential therapeutic approaches. Saudi Journal of Gastroenterology. 2012;18:155. https://doi.org/10.4103/1319-3767.96445
4. Badria F, Hassan MM, Abd-Al Moneim MM. Trigonelline: a new natural hepatoselective fibrosuppressive agent. Polymorphism. 2019;2:51-65.
5. Bakuradze T, Lang R, Hofmann T, Stiebitz H, Bytof G, Lantz I, *et al.* Antioxidant effectiveness of coffee extracts and selected constituents in cell-free systems and human colon cell lines. Molecular Nutrition & Food Research. 2010;54:1734-1743. https://doi.org/10.1002/mnfr.201000147
6. Cattley RC, Cullen JM. Liver and gall bladder. In: Haschek and Rousseaux's Handbook of Toxicologic Pathology. Elsevier; 2013. p. 1509-1566. https://doi.org/10.1016/B978-0-12-415759-0.00045-5
7. Dunbar JK, Crombie IK. The rising tide of liver cirrhosis mortality in the UK: Can its halt be predicted?

Alcohol and Alcoholism. 2011;46:459-463. https://doi.org/10.1093/alcalc/agr042
8. Dutra CB, Rath S, Reyes FGR. Nitrosaminas voláteis em alimentos. Alimentos e Nutrição. 2007;18:111-120.
9. Evans LS, Tramontano WA. Trigonelline and promotion of cell arrest in G2 of various legumes. Phytochemistry. 1984;23:1837-1840. https://doi.org/10.1016/s0031-9422(00)84927-4
10. Friedman SL. Liver fibrosis - from bench to bedside. Journal of Hepatology. 2003;38:38-53. https://doi.org/10.1016/S0168-8278(02)00429-4
11. Fuchs BC, Wang H, Yang Y, Wei L, Polasek M, Schühle DT, *et al.* Molecular MRI of collagen to diagnose and stage liver fibrosis. Journal of Hepatology. 2013;59:992-998. https://doi.org/10.1016/j.jhep.2013.06.026
12. Gabr SA, Alghadir AH, Sherif YE, Ghfar AA. Hydroxyproline as a biomarker in liver disease. In: Patel VB, Preedy VR, editors. Biomarkers in Liver Disease. Dordrecht: Springer Netherlands; 2017. p. 471-491. https://doi.org/10.1007/978-94-007-7675-3_26
13. George J, Chandrakasan G. Molecular characteristics of dimethylnitrosamine-induced fibrotic liver collagen. Biochimica et Biophysica Acta - Protein Structure and Molecular Enzymology. 1996;1292:215-222. https://doi.org/10.1016/0167-4838(95)00202-2
14. George J, Rao KR, Stern R, Chandrakasan G. Dimethylnitrosamine-induced liver injury in rats: the early deposition of collagen. Toxicology. 2001;156:129-138. https://doi.org/10.1016/S0300-483X(00)00352-8
15. Hall A, Cotoi C, Luong TV, Watkins J, Bhathal P, Quaglia A. Collagen and elastic fibres in acute and chronic liver injury. Scientific Reports. 2021;11:14569. https://doi.org/10.1038/s41598-021-93566-1
16. Hamden K. Inhibition of key digestive enzymes related to diabetes and hyperlipidemia and protection of liver-kidney functions by Trigonelline in diabetic rats. Scientia Pharmaceutica. 2013;81:233-246. https://doi.org/10.3797/scipharm.1211-14
17. Hirakawa N, Okauchi R, Miura Y, Yagasaki K. Anti-invasive activity of niacin and trigonelline against cancer cells. Bioscience, Biotechnology, and Biochemistry. 2005;69:653-658. https://doi.org/10.1271/bbb.69.653
18. Inami K, Ishimura S, Akaike Y, Suzuki E, Tsutsumi N, Takeda K, *et al.* Oxidation products of N-nitrosodialkylamines generated by Fenton's reagent in the presence of copper are direct acting mutagens. Journal of Health Science. 2010;56:576-580. https://doi.org/10.1248/jhs.56.576
19. Latief U, Husain H, Mukherjee D, Ahmad R. Hepatoprotective efficacy of gallic acid during nitrosodiethylamine-induced liver inflammation in Wistar rats. The Journal of Basic & Applied Zoology. 2016;76:31-41.
20. Liang N, Kitts DD. Antioxidant property of coffee components: assessment of methods that define mechanisms of action. Molecules. 2014;19:19180-19208.
21. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the folin phenol reagent. Journal of Biological Chemistry. 1951;193:265-275. https://doi.org/10.1016/S0021-9258(19)52451-6
22. Morani AS, Bodhankar SL, Mohan V, Thakurdesai PA. Ameliorative effects of standardized extract from *Trigonella foenum-graecum* L. seeds on painful peripheral neuropathy in rats. Asian Pacific Journal of Tropical Medicine. 2012;5:385-390.
23. Mukherjee D, Ahmad R. Dose-dependent effect of N′-nitrosodiethylamine on hepatic architecture, RBC rheology and polypeptide repertoire in Wistar rats. Interdisciplinary Toxicology. 2015;8:1-7. https://doi.org/10.1515/intox-2015-0001
24. Mukherjee D, Ahmad R. Glucose-6-phosphate dehydrogenase activity during N′-nitrosodiethylamine-induced hepatic damage. Achievements in the Life Sciences. 2015;9:51-56.
25. Mukherjee D, Ahmad R, Nayeem S. Molecular interplay promotes amelioration by quercetin during experimental hepatic inflammation in rodents. International Journal of Biological Macromolecules. 2022;222:2936-2947.
26. Neuman RE, Logan MA. The determination of hydroxyproline. Journal of Biological Chemistry. 1950;184:299-306.
27. Özçelik B, Kartal M, Orhan I. Cytotoxicity, antiviral and antimicrobial activities of alkaloids, flavonoids, and phenolic acids. Pharmaceutical Biology. 2011;49:396-402. https://doi.org/10.3109/13880209.2010.519390
28. Ricard-Blum S, Baffet G, Théret N. Molecular and tissue alterations of collagens in fibrosis. Matrix Biology. 2018;68-69:122-149. https://doi.org/10.1016/j.matbio.2018.02.004
29. Sathiyaseelan A, Saravanakumar K, Jayalakshmi J, Gopi M, Shajahan A, Barathikannan K, *et al.* Trigonelline-loaded chitosan nanoparticles prompted antitumor activity on glioma cells and biocompatibility with pheochromocytoma cells. International Journal of Biological Macromolecules. 2020;163:36-43. https://doi.org/10.1016/j.ijbiomac.2020.06.165
30. Sheweita SA. Drug-metabolizing enzymes mechanisms and functions. Current Drug Metabolism. 2000;1:107-132.
31. Simon G, Heckmann V, Tóth D, Pauka D, Petrus K, Molnár TF. The effect of hepatic steatosis and fibrosis on liver weight and dimensions. Legal Medicine. 2020;47:101781. https://doi.org/10.1016/j.legalmed.2020.101781
32. Snodin DJ, Trejo-Martin A, Ponting DJ, Smith GF, Czich A, Cross K, *et al.* Mechanisms of nitrosamine mutagenicity and their relationship to rodent carcinogenic potency. Chemical Research in Toxicology. 2024;37:181-198. https://doi.org/10.1021/acs.chemrestox.3c00327
33. Takahashi A, Abe K, Yokokawa J, Iwadate H, Kobayashi H, Watanabe H, *et al.* Clinical features of liver dysfunction in collagen diseases. Hepatology Research. 2010;40:1092-1097. https://doi.org/10.1111/j.1872-034X.2010.00707.x
34. Teloh HA. Serum proteins in hepatic disease. Annals of Clinical and Laboratory Science. 1978;8:127-129.
35. Tohda C, Kuboyama T, Komatsu K. Search for natural products related to regeneration of the neuronal network. Neurosignals. 2005;14:34-45. https://doi.org/10.1159/000085384
36. Viani R, Horman I. Thermal behaviour of trigonelline. Journal of Food Science. 1974;39:1216-1217. https://doi.org/10.1111/j.1365-2621.1974.tb07357.x
37. Woessner JF Jr. The determination of hydroxyproline in tissue and protein samples containing small proportions of this imino acid. Archives of Biochemistry and Biophysics. 1961;93:440-447.