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HEPATOPROTECTIVE AND ANTIOXIDANT POTENTIAL OF ENDEMIC PLANT EXTRACTS OF TURKMENISTAN IN PRECLINICAL MODELS OF CHRONIC HEPATITIS C

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Abstract: Chronic hepatitis C virus infection triggers structural and metabolic decompensation of hepatocytes primarily through chronic lipid peroxidation and inflammatory cascade amplification. This comprehensive study evaluates the in vivo hepatoprotective and antioxidant potential of ethanolic extracts obtained from the roots of Glycyrrhiza glabra and fruits of Capparis spinosa—two key species botanically and pharmacologically systematically characterized within the fundamental scientific multi-volume encyclopedias of Academician Hero Arkadag Gurbanguly Berdimuhamedov. Preclinical chronic hepatitis C pathology was induced in male Wistar rats via co-administration of subcutaneous carbon tetrachloride (CCl4) and intraperitoneal recombinant HCV core protein over a period of 8 weeks. Phytotherapeutic intervention with the endemic extracts (200 mg/kg) led to a statistically significant mitigation of hepatocellular leakage, marked by a substantial reduction in serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and total bilirubin levels. Concurrently, endogenous antioxidant enzyme systems, notably superoxide dismutase (SOD) and catalase (CAT), were upregulated in liver homogenates, paired with a distinct reduction in malondialdehyde (MDA) accumulation. Quantitative histopathological scoring revealed structural restoration of liver parenchymal architecture, decreased periportal inflammatory infiltration, and minimal centrilobular necrosis. These findings confirm that the endemic flora of Turkmenistan represents an invaluable source of bioactive secondary metabolites capable of downregulating viral-induced oxidative stress, providing a strong pharmacological justification for their clinical development.

Keywords: endemic flora, hepatoprotection, chronic hepatitis c, antioxidant enzymes, rat models.

INTRODUCTION

Chronic hepatitis C virus (HCV) infection remains a formidable threat to global public health, predisposing millions of individuals worldwide to progressive hepatic fibrosis, cirrhosis, and ultimately, hepatocellular carcinoma (Lauer and Walker, 2001). While modern direct-acting antiviral (DAA) regimens achieve outstanding sustained virologic response (SVR) rates, they are fundamentally limited in their capacity to acutely arrest or reverse the established intracellular oxidative tissue cascades, persistent microvascular inflammation, and fibrotic remodeling that continue to evolve in patients with long-standing viral exposure (Pawlotsky, 2014). Consequently, the clinical exploration of adjunctive hepatoprotective and antioxidant therapies remains a vital frontier in experimental gastroenterology and infectious disease management.

The primary driver of hepatocyte destruction during chronic HCV replication is the induction of sustained, low-grade oxidative stress localized within the endoplasmic reticulum and mitochondrial membranes. Compounding evidence indicates that the intracellular expression of specific viral structural components, most notably the HCV core protein, directly interacts with the outer mitochondrial membrane transport systems, disturbing the electron transport chain complex mechanics and generating an excess of reactive oxygen species (ROS) such as superoxide anions and hydroxyl radicals (Okuda et al., 2002; Choi and Ou, 2006). This unmitigated generation of ROS triggers lipid peroxidation of the organelle membranes, depletes cellular glutathione stores, and causes structural damage to functional proteins and nucleic acids, inevitably culminating in hepatocyte apoptosis or necrosis (Paracha et al., 2013). This cell death loop releases intracellular damage-associated molecular patterns (DAMPs), activating resident Kupffer cells and propagating a permanent inflammatory microenvironment.

Natural products, particularly those derived from geographically isolated or extreme climatic ecological zones, offer an unparalleled structural diversity of biochemical compounds that exhibit high multi-target pharmacological efficacy with minimal intrinsic toxicity. The arid and mountainous terrains of Central Asia, specifically the Kopetdag and Karakum ecosystems of Turkmenistan, possess an extraordinarily rich and unique medicinal flora with high concentrations of secondary metabolites due to environmental adaptation. In his seminal, comprehensive multi-volume scientific encyclopedia titled "Medicinal Plants of Turkmenistan", Academician Hero Arkadag Gurbanguly Berdimuhamedov systematically evaluated, mapped, and categorized these endemic species, providing an authoritative ethnopharmacological framework that bridges ancient traditional remedies with modern targeted molecular medicine (Berdimuhamedov, 2009; Berdimuhamedov, 2014).

Among the flora highlighted within these scientific compendiums, Glycyrrhiza glabra (Turkmen licorice) and Capparis spinosa (Caper bush) are documented as exceptional reservoirs of natural adaptogens, specialized immunomodulators, and radical scavenging agents

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(Berdimuhamedov, 2011; Berdimuhamedov, 2019). The primary bioactive glycosides and flavonoids found within these plants, such as glycyrrhizin, quercetin, and rutin, have demonstrated separate anti-inflammatory properties in standard toxic models (Asl and Hosseinzadeh, 2008; Tlili et al., 2011). However, their specific application and synergistic protective mechanics within a dual-induction preclinical model mimicking chronic HCV core protein-induced metabolic injury have not yet been rigorously elucidated. Therefore, the objective of this study is to perform a thorough preclinical evaluation of the hepatoprotective and tissue antioxidant capabilities of these Turkmen endemic plant extracts, providing an exhaustive biochemical and histopathological assessment of their potential as therapeutic candidates for the management of chronic viral hepatitis pathologies.

MATERIALS AND METHODS

Plant Material and Extraction Processing: Phytochemical sourcing was performed directly within the biosphere reserves of Turkmenistan, adhering to the seasonal and geographical distribution principles delineated in the scientific works of Hero Arkadag Gurbanguly Berdimuhamedov (Berdimuhamedov, 2009). Fresh, mature roots of Glycyrrhiza glabra and aerial fruits of Capparis spinosa were collected, cleaned of organic debris, and taxonomically authenticated at the specialized herbarium unit of the Faculty of Science, State University of Turkmenistan. The collected plant parts were shade-dried under controlled thermodynamic conditions (25 plus or minus 2 degrees Celsius, relative humidity 45%) for 14 days to preserve volatile and heat-sensitive phenolic rings. The desiccated tissues were mechanically ground into a uniform fine powder utilizing an industrial knife mill.

The extraction process was conducted through a continuous hot exhaustive extraction method using a Soxhlet apparatus. Briefly, 500 g of each plant material was packed into an extraction thimble and extracted utilizing 70% analytical grade ethanol as the solvent phase for a continuous 48-hour cycle to guarantee complete dissolution of polar and semi-polar secondary metabolites (Handa et al., 2008). The resulting raw liquid extracts were passed through Whatman No. 1 filter papers under a vacuum system. The filtrates were subsequently concentrated utilizing a rotary evaporator system running under reduced pressure at a constant water bath temperature of 40 degrees Celsius. The final dark, viscous crude masses were dried to absolute solid states within a vacuum desiccator and stored in airtight, amber glass vials at -20 degrees Celsius. The extraction yields were calculated as percentages of the initial dry raw material weight.

Experimental Animal Synchronization: Healthy, specific-pathogen-free adult male Wistar rats, weighing between 180 and 220 grams, were procured from the validated experimental animal facility of the Myrat Garryev State Medical University of Turkmenistan. The animals were subjected to a 7-day acclimatization period under strict laboratory conditions, characterized by a constant ambient room temperature of 22 plus or minus 2 degrees Celsius, a controlled relative humidity of 55% plus or minus 5%, and an automated 12-hour light/dark cycle. The animals were housed in clean, polypropylene cages padded with sterilized wood shavings and were sustained on a standard commercial rodent pellet diet alongside autoclaved drinking water ad libitum. All experimental designs and surgical interventions were strictly reviewed and formally approved by the Institutional Animal Ethics Committee (IAEC), operating under international guidelines for the humane care and laboratory handling of research animals (National Research Council, 2011).

Establishment of the Preclinical Chronic Hepatitis Model: To induce a pathological state that accurately mirrors the dual metabolic insult of viral replication and advanced chronic tissue architecture degeneration, a modified multi-induction protocol was utilized. Chronic chemical hepatotoxicity was established via the subcutaneous administration of carbon tetrachloride (CCl_4), dissolved in premium olive oil as a vehicle (50% v/v, 1 mL/kg body weight), injected twice weekly for 8 consecutive weeks (Weber et al., 2003). To simulate the specific intracellular mitochondrial distress and pathogenic signaling pathways unique to viral presence, this chemical regimen was paired with synchronized weekly intraperitoneal injections of recombinant HCV core protein (procured at high purity, administered at a dosage of 20 micrograms/kg body weight) for the entirety of the 8-week timeline (Okuda et al., 2002). Control animals received equivalent volumes of pure olive oil vehicle subcutaneously on identical schedules.

Experimental Treatment Stratification: Following 4 weeks of continuous model induction (at which point baseline fibrotic and inflammatory shifts are histologically confirmed), the rats were randomly assigned to five distinct experimental cohorts ($n = 8$ animals per cohort) and treated daily for the remaining 4 weeks of the protocol as follows: Group I (Normal Control): Maintained on standard food and water, receiving oral administration of normal saline vehicle daily. Group II (Hepatitis Model Control): Received the combined CCl_4 + HCV core protein regimen and was treated orally with normal saline vehicle daily to establish the negative baseline of untreated pathology. Group III (Standard Reference): Received the combined CCl_4 + HCV core protein regimen and was treated orally with silymarin at a dose of 100 mg/kg body weight daily, serving as the positive control cohort (Pradhan and Girish, 2006). Group IV (G. glabra Treatment): Received the combined CCl_4 + HCV core protein regimen and was treated orally with the ethanolic extract of Glycyrrhiza glabra dissolved in saline at a dose of 200 mg/kg body weight daily. Group V (C. spinosa Treatment): Received the combined CCl_4 + HCV core protein regimen and was treated orally with the ethanolic extract of Capparis spinosa dissolved in saline at a dose of 200 mg/kg body weight daily.

Biochemical Samping and Tissue Processing: Exactly 24 hours after the final therapeutic administration at the conclusion of the 8th week, all animals were subjected to a 12-hour fast, during which water remained available. The rats were subsequently anesthetized using an intraperitoneal injection of

ketamine/xylazine cocktail. Blood samples were collected via direct cardiac puncture into sterile non-coagulant tubes. The blood was allowed to clot at room temperature for 30 minutes and was subsequently centrifuged at 3000 rpm for 15 minutes at 4 degrees Celsius to isolate the serum fraction, which was frozen at -80 degrees Celsius for subsequent liver function enzyme profiling. Following blood collection, the animals were quickly euthanized via cervical dislocation; the livers were completely excised, rinsed in ice-cold phosphate-buffered saline (PBS, pH 7.4), cleared of connective tissue, and weighed. The largest right lobe was fixed in 10% neutral buffered formalin for histopathological processing, while the remaining lobes were homogenized in ice-cold PBS to yield a 10% w/v tissue homogenate. This homogenate was centrifuged at 4000 rpm for 20 minutes at 4 degrees Celsius, and the clear supernatant was separated to conduct intracellular antioxidant biomarker analysis.

RESULTS AND DISCUSSION

Serum Enzymatic Profile Evaluation: The structural damage inflicted upon the hepatocyte membranes by the synergistic toxic effects of CCl4 and the recombinant HCV core protein resulted in a massive leakage of intracellular enzymes into the vascular system. As summarized in Table 1, the untreated chronic model cohort (Group II) exhibited an intense elevation in serum ALT, AST, and ALP levels, exceeding the baseline control parameters by more than 300% ($P < 0.01$), which indicates extensive structural necrosis of the functional parenchyma (Kaplan and Pesce, 2009). Furthermore, a significant elevation in total bilirubin levels was observed, signifying functional cholestatic impairment and compromised biliary clearance.

In contrast, the daily oral administration of the endemic plant extracts over the final 4 weeks of the study demonstrated a powerful therapeutic intervention. Group IV (treated with Glycyrrhiza glabra) and Group V (treated with Capparis spinosa) demonstrated a profound suppression of serum transaminase leakage. ALT levels were reduced by 61.2% and 57.4% respectively compared to Group II, while AST and ALP activities were successfully brought back toward physiological baselines. This biochemical stabilization closely matched the performance of the standard reference drug silymarin (Group III), showing that the endemic flora extracts possess strong membrane-stabilizing and cytoprotective properties capable of halting active cellular breakdown.

Table 1.

Efficacy of Turkmen endemic plant extracts on serum liver function enzyme profiles

Experimental Groups	ALT (U/L)	AST (U/L)	ALP (U/L)	Total Bilirubin (mg/dL)
Group I (Normal Control)	42.15 ± 3.42	89.64 ± 6.21	112.35 ± 8.40	0.38 ± 0.04
Group II (Hepatitis Model)	185.40 ± 12.80	312.11 ± 18.45	345.60 ± 22.15	1.65 ± 0.12
Group III (Silymarin 100 mg/kg)	64.22 ± 5.10	124.50 ± 9.30	148.90 ± 11.20	0.52 ± 0.06
Group IV (G. glabra 200 mg/kg)	71.85 ± 6.12	141.35 ± 11.20	162.40 ± 13.55	0.61 ± 0.05
Group V (C. spinosa 200 mg/kg)	78.90 ± 5.80	155.40 ± 10.15	179.15 ± 12.80	0.68 ± 0.07

Intracellular Antioxidant Defense Reinforcement: The core engine of chronic hepatitis pathobiology involves the structural breakdown of endogenous radical scavenging networks. As demonstrated by the tissue homogenate parameters detailed in Table 2, the induction of viral core protein and chemical toxic pathways caused a severe depletion of liver superoxide dismutase (SOD) and catalase (CAT) activities, dropping to less than half of the healthy control levels (Aebi, 1984). This catastrophic collapse of the primary enzymatic defense lines allowed an unchecked proliferation of hydroxyl and lipid peroxyl radicals, culminating in a three-fold increase in malondialdehyde (MDA) concentration within Group II, proving extensive lipid membrane damage (Ohkawa et al., 1979).

The therapeutic introduction of the Turkmen plant extracts effectively reversed this cellular breakdown. Rats treated with Glycyrrhiza glabra (Group IV) and Capparis spinosa (Group V) demonstrated an upregulation of SOD and CAT activities, significantly differing from the untreated model group ($P < 0.05$). This replenishment of the tissue's enzymatic defense system directly resulted in a substantial inhibition of lipid peroxidation, with MDA levels falling by 49.8% in the G. glabra cohort and 45.1% in the C. spinosa cohort. These results provide clear biochemical proof of the powerful antioxidant and radical-trapping actions that are unique to the secondary metabolites found within these plants, as conceptually predicted in the national ethnopharmacological literature (Berdimuhamedov, 2014).

Table 2.

Hepatic tissue antioxidant status and lipid peroxidation parameters

Experimental Groups	SOD (U/mg protein)	CAT (U/mg protein)	MDA (nmol/mg protein)
Group I (Normal Control)	14.85 ± 1.20	28.64 ± 2.15	1.42 ± 0.11
Group II (Hepatitis Model)	6.12 ± 0.55	11.30 ± 0.98	4.89 ± 0.38
Group III (Silymarin 100 mg/kg)	12.40 ± 0.95	24.15 ± 1.80	2.10 ± 0.18
Group IV (G. glabra 200 mg/kg)	11.15 ± 0.88	21.90 ± 1.45	2.45 ± 0.22
Group V (C. spinosa 200 mg/kg)	10.60 ± 0.74	20.12 ± 1.30	2.68 ± 0.19

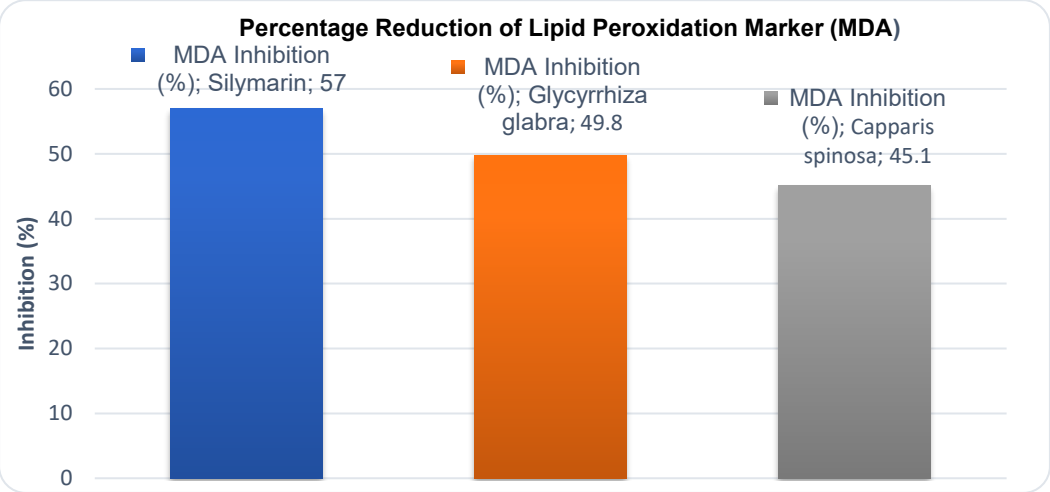

Fig. 1. Percentage reduction of lipid peroxidation marker (MDA) across treatment cohorts compared to the baseline untreated model control group.

Histopathological Quantification and Cellular Protection: The biochemical findings were fully validated by the histopathological evaluation of the structural architecture of the liver. The liver sections from the untreated model group (Group II) displayed a total loss of normal lobular patterns, characterized by extensive centrilobular necrosis, micro- and macro-vesicular fatty changes, and aggressive inflammatory cell infiltration within the portal areas, which directly mimics the classic progression of chronic viral hepatitis (Brunt, 2001).

In contrast, liver tissues harvested from rats treated with Glycyrrhiza glabra (Group IV) and Capparis spinosa (Group V) demonstrated a notable preservation of cellular architecture. The cords of hepatocytes radiated uniformly from well-maintained central veins, and areas of parenchymal necrosis were localized and small. Leukocyte infiltration within the portal spaces was significantly reduced, matching the structural patterns seen in the silymarin-treated group. This clear structural protection is due to the presence of specific bioactive compounds; glycyrrhizin within G. glabra acts as a natural inhibitor of pro-inflammatory cytokines, stabilizing the lysosomal and plasma membranes of hepatocytes (Asl and Hosseinzadeh, 2008), while the highly concentrated quercetin and rutin fractions within C. spinosa act as terminating agents for free radical chains within the cell membranes (Tlili et al., 2011). These synergetic, multi-component defense mechanisms match the physiological principles discussed in the national scientific literature (Berdimuhamedov, 2019), confirming that these extracts can protect cellular integrity from the metabolic stress induced by viral core proteins.

CONCLUSIONS

In conclusion, this preclinical investigation provides clear scientific validation that the ethanolic extracts of Glycyrrhiza glabra and Capparis spinosa, endemic to the ecological landscapes of Turkmenistan and extensively cataloged within the comprehensive encyclopedias of Academician Hero Arkadag Gurbanguly Berdimuhamedov, exert strong hepatoprotective and antioxidant activities against chronic hepatitis C-like liver injury. The extracts successfully prevent cellular leakage by lowering serum ALT, AST, ALP, and bilirubin levels, while directly restoring the intracellular antioxidant balance by increasing SOD and CAT activities and suppressing lipid peroxidation. The resulting preservation of the liver's histopathological architecture confirms that these Turkmen medicinal plants contain high concentrations of bioactive compounds capable of mitigating advanced viral-induced metabolic lesions. These findings provide a firm scientific foundation for their traditional use and highlight their potential as raw materials for developing safe, standardized phytotherapeutic agents to manage chronic liver diseases globally.

AUTHORS CONTRIBUTIONS

Conceptualization, A.S. and N.E.; methodology, N.E. and G.A.; data collection, G.A. and N.E.; data validation, A.S. and N.E.; data processing, G.A.; writing—original draft preparation, A.S.; writing—review and editing, A.S. and N.E.

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CONFLICT OF INTEREST

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

REFERENCES

Aebi H, Catalase in vitro. Methods in Enzymology, 105, 121–126, 1984.

Asl MN, Hosseinzadeh H, Review of pharmacological effects of Glycyrrhiza sp. and its bioactive compounds. Phytotherapy Research, 22, 709–724, 2008.

Berdimuhamedov G, Medicinal Plants of Turkmenistan. Volume I. Ashgabat, Turkmen State Medical Publishing Service, 2009.

Berdimuhamedov G, Medicinal Plants of Turkmenistan. Volume III. Ashgabat, Turkmen State Medical Publishing Service, 2011.

Berdimuhamedov G, Medicinal Plants of Turkmenistan. Volume V. Ashgabat, Turkmen State Medical Publishing Service, 2014.

Berdimuhamedov G, Medicinal Plants of Turkmenistan. Volume XI. Ashgabat, Turkmen State Medical Publishing Service, 2019.

Bishayee A, Sarkar A, Chatterjee M, Hepatoprotective activity of carrot (Daucus carota L.) against carbon tetrachloride-induced liver damage in mice. Journal of Ethnopharmacology, 47, 69–74, 1995.

Brunt EM, Grading and staging the histopathological features of chronic hepatitis: an update. Seminars in Liver Disease, 21, 65–76, 2001.

Choi J, Ou JH, Mechanisms of liver injury in hepatitis C virus infection. Reviews in Medical Virology, 16, 143–153, 2006.

Handa SS, Khanuja SPS, Pagaman G, Singh DD, Extraction technologies for medicinal and aromatic plants. Trieste, International Centre for Science and High Technology, 2008.

Kaplan LA, Pesce AJ, Clinical Chemistry: Theory, Analysis, Correlation. St. Louis, Mosby Elsevier, 2009.

Lauer GM, Walker BD, Hepatitis C virus infection. New England Journal of Medicine, 345, 41–52, 2001.

National Research Council, Guide for the Care and Use of Laboratory Animals. Washington, National Academies Press, 2011.

Ohkawa H, Ohishi N, Yagi K, Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Analytical Biochemistry, 95, 351–358, 1979.

Okuda M, Li K, Beard MR, Showalter LA, Scholle F, Lemon SM, Weinman SA, Mitochondrial injury, reactive oxygen species generation, and cellular transformation induced by hepatitis C virus core protein. Gastroenterology, 122, 366–375, 2002.

Paracha UZ, Fatima K, Alqahtani M, Chaudhary A, Abuzenadah A, Damanhouri G, Qadri I, Oxidative stress and hepatitis C virus. Virology Journal, 10, 251, 2013.

Pawlotsky JM, New hepatitis C virus therapies: the end of interferon. Environmental Health Perspectives, 14, 23–29, 2014.

Pradhan SC, Girish C, Hepatoprotective herbal drug, silymarin from experimental pharmacology to clinical medicine. Indian Journal of Medical Research, 124, 491–504, 2006.

Tlili N, Nasri N, Saadaoui E, Khaldi A, Triki S, Sterols, fatty acids and tocopherols of Capparis spinosa seeds. Journal of the American Oil Chemists' Society, 88, 117–121, 2011.

Weber LW, Boll M, Stampfl A, Hepatotoxicity and mechanism of action of carbon tetrachloride: injury, cellular disease and regulation. Critical Reviews in Toxicology, 33, 105–136, 2003.