

Pharmacological Evaluation of *Decalepis hamiltonii* Seed Extract: Dose-Dependent Hepatoprotective and Antioxidant Mechanisms in Experimental Models of Liver Toxicity

Gauranga Trivedi

gaurangatrivedi@gmail.com

Janardan rai Nagar Rajasthan Vidyapeeth, deemed to be university, Udaipur

Gajendra Singh Rathore

Janardan rai Nagar Rajasthan Vidyapeeth, deemed to be university, Udaipur

Parikshit Nagda

dr.parikshitnagda@gmail.com

Janardan rai Nagar Rajasthan Vidyapeeth, deemed to be university, Udaipur

Research Article

Keywords: Hepatoprotection, *Decalepis hamiltonii*, Antioxidant Mechanisms, Xenobiotic-Induced Liver Injury, Pharmacological Evaluation, Phytotherapy, Dose-Response

Posted Date: May 26th, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-9797505/v1>

License:   This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Additional Declarations: The authors declare no competing interests.

Abstract

Liver toxicity, often mediated by oxidative stress and xenobiotic metabolism, remains a critical pharmacological challenge. While *Decalepis hamiltonii* roots are traditionally used for hepatoprotection, the pharmacological potential of its seeds is largely unexplored. This study investigates the mechanistic basis of the methanolic seed extract of *D. hamiltonii* in mitigating carbon tetrachloride (CCl₄)-, paracetamol-, and ethanol-induced hepatotoxicity in Wistar rats, with a focus on its antioxidant and cytoprotective pathways.

Phytochemical profiling revealed the presence of bioactive constituents, while acute oral toxicity studies confirmed its safety. In vitro antioxidant assays (DPPH, ABTS, FRAP, phosphomolybdenum, superoxide radical scavenging, H₂O₂ scavenging, and metal chelation) demonstrated robust free radical scavenging activity. In vivo, the extract (50, 100, and 200 mg/kg) exhibited dose-dependent attenuation of liver injury, as evidenced by normalized serum biomarkers (ALT, AST, ALP, total bilirubin) and restored hepatic antioxidant enzymes (SOD, CAT, GSH). Histopathological analysis further validated its efficacy, with the 200 mg/kg dose showing hepatic architectural preservation comparable to silymarin, a standard hepatoprotective agent.

These findings elucidate the pharmacological mechanisms underlying the hepatoprotective effects of *D. hamiltonii* seed extract, positioning it as a potential phytotherapeutic candidate for drug development targeting oxidative stress-mediated liver disorders.

1. Introduction

The liver, the largest internal organ in the human body, maintains physiological homeostasis through over five hundred biochemical actions, including nutrient metabolism, plasma protein synthesis, detoxification, bile production, and immune surveillance [1]. Its unique anatomical position—receiving blood directly from the gastrointestinal tract—and high metabolic workload render it particularly vulnerable to hepatotoxic agents [2].

Toxin-induced liver injury represents a major global health concern, contributing to acute liver failure and chronic hepatic disorders. Hepatotoxins originate from environmental contaminants, industrial chemicals, pharmaceutical drugs (e.g., acetaminophen), alcohol, and naturally occurring toxins [3]. The mechanisms involve metabolic activation of inert compounds into reactive metabolites by cytochrome P450 enzymes, particularly CYP2E1, triggering excessive oxidative stress, lipid peroxidation, mitochondrial dysfunction, and inflammatory activation, ultimately causing hepatocellular damage [4, 5].

Oxidative stress, defined as an imbalance between pro-oxidant production and antioxidant defenses, is a central molecular driver in liver disease pathogenesis, including viral hepatitis, fibrosis, cirrhosis, and hepatocellular carcinoma [6]. The liver possesses an intricate antioxidant defense system comprising enzymatic antioxidants (SOD, CAT, GPx) and non-enzymatic molecules (GSH, vitamins E and C,

flavonoids) [7]. Under sustained toxic insult, these defenses become overwhelmed, leading to biomolecular damage [8].

Given limitations of conventional synthetic hepatoprotective drugs—including side effects and limited efficacy—interest in natural alternatives, particularly medicinal herbs, has grown substantially [9]. Herbal remedies exhibit potent antioxidant, anti-inflammatory, and membrane-stabilizing properties through phytochemicals such as flavonoids, phenolic acids, tannins, and terpenoids, which neutralize free radicals and enhance endogenous defenses [10].

Decalepis hamiltonii, commonly known as swallow root, is a climbing shrub native to Peninsular India, traditionally valued for aromatic tuberous roots used in Ayurvedic formulations [11]. While root hepatoprotective and antioxidant effects are documented [12], seed-based scientific validation remains limited [13]. This investigation assessed the methanolic seed extract of *D. hamiltonii* against CCl₄, paracetamol, and ethanol-induced liver injury in rats, examining biochemical markers, antioxidant enzyme systems, and histopathological outcomes to address this research gap.

2. Materials and Methods

2.1. Plant Material and Extraction

Seeds of *D. hamiltonii* were procured and authenticated. They were cleaned, shade-dried at ambient temperature for five to seven days, and ground into powder. The powder was sieved (mesh 40) for uniform consistency and stored in an airtight amber container [14].

The seed powder was defatted using petroleum ether (40–60°C) in a Soxhlet apparatus for 6–8 hours. The defatted material was air-dried, and methanolic extraction was performed via maceration for seven days with intermittent agitation and daily solvent replacement. The mixture was filtered (Whatman No. 1), and filtrates were concentrated in a water bath at 45°C to syrup-like consistency. The final concentrate was stored at 4°C [15].

2.2. Phytochemical Screening

Preliminary qualitative screening of the methanolic extract followed standard procedures to identify bioactive compound classes [16, 17]. Tests included: flavonoids (Shinoda test, alkaline reagent), tannins (ferric chloride, gelatin test), saponins (froth test), alkaloids (Dragendorff's, Wagner's, Mayer's reagents), terpenoids (Liebermann–Burchard test), glycosides (Keller–Killiani test), carbohydrates (Molisch's, Fehling's tests), proteins/amino acids (Millon's, Biuret, Ninhydrin tests), and inorganic elemental analysis [18].

2.3. Acute Toxicity Test

Acute oral toxicity followed OECD Guideline 423 [19]. Nine mice were divided into three groups (n = 3) and administered extract at 10, 100, and 1000 mg/kg orally. Toxicity signs and mortality were monitored

over 24 hours. A second phase administered 1600, 2900, and 5000 mg/kg to another nine mice, with 14-day observation [20].

2.4. Animals

Adult Wistar rats (180–250 g, either sex) were housed under controlled conditions (12-hour light/dark cycle) with unrestricted water access. Procedures adhered to institutional animal care guidelines with ethical clearance [21].

2.5. Antioxidant Activity Assessment

Seven in vitro assays evaluated antioxidant potential:

DPPH Scavenging

Measures hydrogen/electron donation to DPPH radical. Absorbance at 517 nm; percentage inhibition calculated [22].

ABTS Scavenging

Evaluates ABTS radical cation quenching. Absorbance at 734 nm; results expressed as Trolox Equivalent Antioxidant Capacity (TEAC) [23].

FRAP

Assesses electron-donating capacity by reducing Fe^{3+} -TPTZ to Fe^{2+} -TPTZ. Absorbance at 593 nm; results as $\mu\text{mol TEAC/mg}$ [24].

Phosphomolybdenum Assay

Measures total antioxidant capacity based on Mo(VI) reduction to Mo(V). Absorbance at 695 nm [25].

Metal Chelating Activity

Evaluates Fe^{2+} binding, inhibiting ferrozine- Fe^{2+} complex formation. Absorbance at 562 nm [24].

Superoxide Radical Scavenging

Neutralizes superoxide anions from PMS-NADH system. Absorbance at 560 nm [24].

H_2O_2 Scavenging

Assesses hydrogen peroxide neutralization. Absorbance at 230 nm [25].

2.6. CCl_4 -Induced Hepatotoxicity

Rats were fasted 24 hours and allocated to five groups (n = 6). Group I (Normal Control) received normal saline (10 mL/kg, p.o.). Group II (Toxic Control) received CCl₄ (1 mL/kg, i.p., 1:1 in liquid paraffin). Groups III–V received extract at 50, 100, and 200 mg/kg orally for seven days. On day 7, one hour after final treatment, Groups II–V received CCl₄. After 24 hours, blood was collected for biochemical markers (ALT, AST, ALP, total bilirubin, total protein). Liver tissues were preserved in 10% formalin for histopathology [26].

2.7. Paracetamol-Induced Hepatotoxicity

Animals were fasted 24 hours and assigned to five groups (n = 6). Group I received normal saline. Group II received paracetamol (2 g/kg orally). Groups III–V received extract at 50, 100, and 200 mg/kg p.o. for seven days. On day 7, paracetamol was administered one hour post-extract to all except normal control. After 24 hours, blood and liver tissues were collected for biochemical and histopathological analysis [27].

2.8. Alcohol-Induced Liver Injury

Rats were divided into groups and pre-treated with extract or silymarin before ethanol exposure (6 mL/kg). Biochemical markers (ALT, AST, ALP, total bilirubin) and hepatic antioxidants (SOD, CAT, GSH) were evaluated, with histopathological assessment [28].

3. Results

3.1. Phytochemical Screening

The methanolic extract tested positive for alkaloids, steroids, carbohydrates, flavonoids, saponins, glycosides, and tannins. Fixed oils, fats, proteins, amino acids, and terpenoids were absent in the aqueous fraction but detected in other fractions. The presence of flavonoids and tannins is notable for established antioxidant and anti-inflammatory properties; saponins suggest anti-ulcer and mucosal protection potential (Table 1) [29].

Table 1
Phytochemical Screening of *D. hamiltonii* Seed Extracts

Constituents	Methanolic	Aqueous	Pet. Ether	Chloroform
Terpenoids	+	–	–	–
Proteins/Amino Acids	–	–	+	–
Tannins	+	+	+	+
Fixed oils, fats	–	–	–	–
Saponins	+	+	+	+
Flavonoids	+	+	+	+
Steroids	+	+	+	+
Glycosides	+	+	+	+
Carbohydrates	+	+	+	+
Alkaloids	+	+	+	+

3.2. Acute Toxicity Study

Oral doses up to 5,000 mg/kg showed no toxicity signs, behavioral alterations, or mortality over 14 days. The oral LD₅₀ exceeds 5,000 mg/kg, classifying the extract as practically non-toxic per GHS criteria. The effective dose (ED₅₀) for biological actions was approximately 200 mg/kg, indicating a wide therapeutic window (Table 2) [30].

Table 2
LD₅₀ and ED₅₀ of *D. hamiltonii* Seed Extract

Plant	ED ₅₀ (mg/kg)	LD ₅₀ (mg/kg)
<i>D. hamiltonii</i>	200	> 5,000

3.3. Antioxidant Activity

The extract demonstrated strong, dose-dependent antioxidant potential across all assays.

DPPH Scavenging

Inhibition increased from 58.16% (50 mg/kg) to 81.53% (200 mg/kg); IC₅₀ decreased from 198.7 to 126.6 µg/mL [31].

ABTS Scavenging

TEAC values rose from 931.5 (50 mg/kg) to 1432.1 µM/mg DW (200 mg/kg) [32].

FRAP

Values increased from 38.62 (50 mg/kg) to 62.50 μ M TEAC/mg (200 mg/kg) [33].

Superoxide Scavenging

Inhibition ranged from 43.18% (50 mg/kg) to 67.82% (200 mg/kg); IC_{50} = 139.6 μ g/mL [34].

H₂O₂ Scavenging

Inhibition increased from 41.23% to 58.24%; IC_{50} = 172.2 μ g/mL [35].

Phosphomolybdenum

Total antioxidant capacity rose from 186.48 (50 mg/kg) to 280.43 μ g AAE/mg (200 mg/kg) [36].

Metal Chelating

Activity increased from 42.38% (50 mg/kg) to 62.71% (200 mg/kg); IC_{50} = 192.4 μ g/mL [37].

3.4. CCl₄-Induced Hepatotoxicity

Pre-treatment significantly attenuated CCl₄-induced damage dose-dependently. ALT in the toxic control (172.4 IU/L) was reduced to 48.6 IU/L at 200 mg/kg (71.85% protection), compared to silymarin's 83.61% protection (28.3 IU/L). AST decreased from 191.7 to 59.0 IU/L (69.22% protection). ALP and bilirubin showed similar dose-dependent reductions, with 200 mg/kg achieving 75.18% and 74.18% protection, respectively [38, 39].

Antioxidant enzyme depletion by CCl₄ was reversed: SOD restored from 2.34 to 5.88 U/mg protein; CAT from 18.2 to 42.6 U/mg protein; GSH from 2.11 to 5.36 μ mol/g tissue at 200 mg/kg, approaching silymarin efficacy [40, 41, 42].

3.5. Paracetamol-Induced Hepatotoxicity

Paracetamol (2 g/kg) elevated ALT to 176.2 IU/L and AST to 184.9 IU/L. Extract pre-treatment at 200 mg/kg reduced ALT to 56.8 IU/L (67.76% protection) and AST to 59.1 IU/L (68.02% protection). Silymarin provided ~ 80% protection [43, 44].

Hepatic antioxidants were restored dose-dependently: SOD to 5.73, CAT to 41.2, and GSH to 5.12 units at 200 mg/kg, comparable to silymarin [45, 46].

3.6. Alcohol-Induced Liver Injury

Ethanol exposure significantly elevated all liver enzymes. At 200 mg/kg, extract reduced ALT to 52.6 IU/L (68.74% protection), AST to 58.4 IU/L (67.48%), ALP to 111.3 IU/L (73.96%), and bilirubin to 0.91 mg/dL. Antioxidant markers showed parallel restoration: SOD to 5.61, CAT to 40.4, and GSH to 4.98 units [47, 48, 49].

3.7. Histopathological Analysis

Normal controls displayed intact hepatic architecture. Toxic controls showed widespread necrosis, ballooning degeneration, fatty infiltration, sinusoidal congestion, and inflammatory infiltrates. Silymarin restored normal morphology. Extract-treated animals showed graded improvement, with 200 mg/kg producing near-complete restoration comparable to silymarin [50, 51].

4. Discussion

This investigation provides compelling evidence for the hepatoprotective potential of *D. hamiltonii* seed extract against three distinct hepatotoxic agents. The consistent, dose-dependent protection across all models—supported by biochemical, antioxidant, and histopathological data—underscores therapeutic promise [52].

In all models, hepatotoxic agents sharply elevated serum ALT, AST, ALP, and bilirubin, markers of hepatocellular damage and compromised membrane integrity [53]. The extract produced progressive, dose-dependent improvement. In the CCl₄ model, 200 mg/kg reduced ALT from 172.4 to 48.6 IU/L (~72% protection), approaching silymarin's ~84% efficacy. Similar trends for AST, ALP, and bilirubin across all models demonstrate robust hepatic functional protection [54, 55].

The biochemical recovery was substantiated by profound enhancement of intrinsic antioxidant defenses. Hepatotoxins induce severe oxidative stress by generating excessive ROS and depleting endogenous antioxidants [56]. CCl₄ exposure markedly depleted SOD, CAT, and GSH. Pre-treatment at 200 mg/kg substantially revived these markers, restoring levels toward physiological ranges and matching silymarin improvements [57]. This suggests effective ROS neutralization, membrane stabilization, and free radical damage mitigation [58].

Histopathology provided crucial morphological corroboration. Toxic controls displayed severe alterations including necrosis, ballooning degeneration, steatosis, and inflammatory infiltrates [59]. Extract-treated rats, particularly at 200 mg/kg, exhibited marked preservation of hepatic cords, minimal degenerative changes, reduced inflammation, and near-normal morphology—comparable to silymarin [60]. This reflects membrane stabilization, lipid peroxidation inhibition, and inflammatory pathway suppression [61].

The activity is attributed to rich phytochemical composition—flavonoids, phenolic acids, saponins, and steroids—known for synergistic hepatoprotection through antioxidant, anti-inflammatory, and anti-apoptotic mechanisms [62, 63]. In vitro assays confirmed impressive DPPH scavenging (81.53%), ABTS quenching (1432.06 µM TEAC/mg), and broad-spectrum antioxidant capacity via FRAP, metal chelation, and superoxide scavenging, indicating interference with oxidative chain reactions through multiple mechanisms [64, 65]. The dose-dependent normalization of liver enzymes, restoration of antioxidant defenses, and histopathological recovery collectively indicate comprehensive hepatoprotection extending beyond simple antioxidation to modulation of inflammatory and degenerative pathways [66].

5. Conclusion

This study establishes *D. hamiltonii* seed extract as a potent hepatoprotective agent with significant antioxidant efficacy. Across CCl₄, paracetamol, and ethanol models, pre-treatment consistently attenuated liver injury, normalizing ALT, AST, ALP, and bilirubin while robustly restoring SOD, CAT, and GSH levels. Histopathological examinations confirmed pronounced improvements in hepatic architecture and reduced necrosis. The multifaceted antioxidant profile—demonstrated through DPPH, ABTS, FRAP, phosphomolybdenum, superoxide, H₂O₂, and metal chelation assays—suggests phytochemical synergy. These findings validate traditional uses and highlight promising potential for standardized phytopharmaceutical development. Future studies should focus on bioactive compound isolation, mechanistic elucidation, pharmacokinetic profiling, and clinical assessment [67].

Declarations

Author Contributions

GT: Conceptualization, methodology, investigation, data curation, writing—original draft preparation, visualization.

GSR: Conceptualization, formal analysis, supervision, writing—review and editing, project administration.

PN: Conceptualization, Statistical Analysis and Final manuscript Writing.

All three authors have read and agreed to the published version of the manuscript.

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. All authors certify that they have no affiliations with or involvement in any organization or entity with any financial interest in the subject matter or materials discussed in this manuscript. The study was conducted in accordance with institutional ethical guidelines, and ethical clearance was obtained from the Animal Ethics Committee of the affiliated institution. The authors confirm that the data supporting the findings of this study are available within the article. All authors have approved the final manuscript and agree to be accountable for all aspects of the work.

Funding

The authors declare that no funding was received for this research.

Acknowledgments

The authors acknowledge the support of management and the laboratory staff for their assistance during the research work.

Conflict of Interest

The authors declare no conflict of interest.

References

1. Trefts E, Gannon M, Wasserman DH (2017) The liver. *Curr Biol* 27(21):R1147–R1151
2. Michalopoulos GK, Bhushan B (2021) Liver regeneration: biological and pathological mechanisms and implications. *Nat Rev Gastroenterol Hepatol* 18(1):40–55
3. Jaeschke H, McGill MR, Ramachandran A (2012) Oxidant stress, mitochondria, and cell death mechanisms in drug-induced liver injury: lessons learned from acetaminophen hepatotoxicity. *Drug Metab Rev* 44(1):88–106
4. Guengerich FP (2006) Cytochrome P450s and other enzymes in drug metabolism and toxicity. *AAPS J* 8(1):E101–E111
5. Cederbaum AI (2015) Molecular mechanisms of the microsomal mixed function oxidases and biological and pathological implications. *Redox Biol* 4:60–73
6. Cichoz-Lach H, Michalak A (2014) Oxidative stress as a crucial factor in liver diseases. *World J Gastroenterol* 20(25):8082–8091
7. Matés JM, Pérez-Gómez C, de Castro IN (1999) Antioxidant enzymes and human diseases. *Clin Biochem* 32(8):595–603
8. Poli G (2000) Pathogenesis of liver fibrosis: role of oxidative stress. *Mol Aspects Med* 21(3):49–98
9. Saleem U, Ahmad B, Rehman K et al (2017) Hepatoprotective effect of a plant extract on paracetamol-induced hepatotoxicity in mice. *Bangladesh J Pharmacol* 12(4):392–396
10. Kumar S, Pandey AK (2013) Chemistry and biological activities of flavonoids: an overview. *Sci World J* 2013:162750
11. Reddy MC, Murthy KSR (2013) A review on *Decalepis hamiltonii* Wight & Arn. *J Med Plants Res* 7(46):3395–3402
12. Srivastava A, Shivanandappa T (2010) Hepatoprotective effect of the root extract of *Decalepis hamiltonii* against carbon tetrachloride-induced oxidative stress in rats. *Food Chem* 118(2):411–417
13. Navya K et al (2021) Evaluation of *Decalepis hamiltonii* seed extract for seed priming and enhanced germination. *Int J Seed Sci* 15(2):112–118
14. Harborne JB (1998) *Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis*, 3rd edn. Chapman & Hall, London
15. *Hand book of Ayurvedic Medicinal Plants* (2017) Boca Raton: CRC
16. Trease GE, Evans WC (2002) *Pharmacognosy*, 15th edn. Bailliere Tindall, London
17. Sofowora A (2008) *Medicinal Plants and Traditional Medicine in Africa*, 3rd edn. Spectrum Books, Nigeria
18. Kokate CK, Purohit AP, Gokhale SB, *Pharmacognosy* (2021) 52nd ed. Pune: Nirali Prakashan

19. OECD (2002) Test No. 423: Acute Oral Toxicity—Acute Toxic Class Method. OECD Guidelines for Testing of Chemicals, Section 4. OECD Publishing, Paris
20. Lorke D (1983) A new approach to practical acute toxicity testing. Arch Toxicol 54(4):275–287
21. CPCSEA (2003) Guidelines for Laboratory Animal Facility. Indian J Pharmacol 35(4):257–274
22. Brand-Williams W, Cuvelier ME, Berset C (1995) Use of a free radical method to evaluate antioxidant activity. LWT-Food Sci Technol 28(1):25–30
23. Re R, Pellegrini N, Proteggente A et al (1999) Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radic Biol Med 26(9–10):1231–1237
24. Benzie IF, Strain JJ (1996) The ferric reducing ability of plasma (FRAP) as a measure of antioxidant power: the FRAP assay. Anal Biochem 239(1):70–76
25. Prieto P, Pineda M, Aguilar M (1999) Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. Anal Biochem 269(2):337–341
26. Srivastava A, Shivanandappa T (2010) Hepatoprotective effect of the root extract of *Decalepis hamiltonii* against carbon tetrachloride-induced oxidative stress in rats. Food Chem 118(2):411–417
27. Tatiya AU, Surana SJ, Sutar MP, Gamit NH (2011) Hepatoprotective effect of poly herbal formulation against paracetamol induced hepatotoxicity in rats. J Pharmacogn Phytochem 1(1):1–6
28. Meera R, Devi P, Kameswari B et al (2009) Antioxidant and hepatoprotective activities of ethanolic extracts from *Ocimum basilicum* and *Trigonella foenum-graecum* against H₂O₂ and CCl₄-induced oxidative stress. J Pharm Res 2(8):1363–1367
29. Reddy MC, Murthy KSR (2013) A review on *Decalepis hamiltonii* Wight & Arn. J Med Plants Res 7(46):3395–3402
30. Lorke D (1983) A new approach to practical acute toxicity testing. Arch Toxicol 54(4):275–287
31. Brand-Williams W, Cuvelier ME, Berset C (1995) Use of a free radical method to evaluate antioxidant activity. LWT-Food Sci Technol 28(1):25–30
32. Re R, Pellegrini N, Proteggente A et al (1999) Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radic Biol Med 26(9–10):1231–1237
33. Benzie IF, Strain JJ (1996) The ferric reducing ability of plasma (FRAP) as a measure of antioxidant power: the FRAP assay. Anal Biochem 239(1):70–76
34. Nishikimi M, Appaji Rao N, Yagi K (1972) The occurrence of superoxide anion in the reaction of reduced phenazine methosulfate and molecular oxygen. Biochem Biophys Res Commun 46(2):849–854
35. Ruch RJ, Cheng SJ, Klaunig JE (1989) Prevention of cytotoxicity and inhibition of intercellular communication by antioxidant catechins isolated from Chinese green tea. Carcinogenesis 10(6):1003–1008
36. Prieto P, Pineda M, Aguilar M (1999) Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex. Anal Biochem 269(2):337–341

37. Dinis TCP, Madeira VMC, Almeida LM (1994) Action of phenolic derivatives (acetaminophen, salicylate, and 5-aminosalicylate) as inhibitors of membrane lipid peroxidation and as peroxyl radical scavengers. Arch Biochem Biophys 315(1):161–169
38. Srivastava A, Shivanandappa T (2010) Hepatoprotective effect of the root extract of *Decalepis hamiltonii* against carbon tetrachloride-induced oxidative stress in rats. Food Chem 118(2):411–417
39. Tatiya AU, Surana SJ, Sutar MP, Gamit NH (2011) Hepatoprotective effect of poly herbal formulation against paracetamol induced hepatotoxicity in rats. J Pharmacogn Phytochem 1(1):1–6
40. Matés JM, Pérez-Gómez C, de Castro IN (1999) Antioxidant enzymes and human diseases. Clin Biochem 32(8):595–603
41. Cichoz-Lach H, Michalak A (2014) Oxidative stress as a crucial factor in liver diseases. World J Gastroenterol 20(25):8082–8091
42. Poli G (2000) Pathogenesis of liver fibrosis: role of oxidative stress. Mol Aspects Med 21(3):49–98
43. Tatiya AU, Surana SJ, Sutar MP, Gamit NH (2011) Hepatoprotective effect of poly herbal formulation against paracetamol induced hepatotoxicity in rats. J Pharmacogn Phytochem 1(1):1–6
44. Meera R, Devi P, Kameswari B et al (2009) Antioxidant and hepatoprotective activities of ethanolic extracts from *Ocimum basilicum* and *Trigonella foenum-graecum* against H₂O₂ and CCl₄-induced oxidative stress. J Pharm Res 2(8):1363–1367
45. Matés JM, Pérez-Gómez C, de Castro IN (1999) Antioxidant enzymes and human diseases. Clin Biochem 32(8):595–603
46. Cichoz-Lach H, Michalak A (2014) Oxidative stress as a crucial factor in liver diseases. World J Gastroenterol 20(25):8082–8091
47. Meera R, Devi P, Kameswari B et al (2009) Antioxidant and hepatoprotective activities of ethanolic extracts from *Ocimum basilicum* and *Trigonella foenum-graecum* against H₂O₂ and CCl₄-induced oxidative stress. J Pharm Res 2(8):1363–1367
48. Cichoz-Lach H, Michalak A (2014) Oxidative stress as a crucial factor in liver diseases. World J Gastroenterol 20(25):8082–8091
49. Poli G (2000) Pathogenesis of liver fibrosis: role of oxidative stress. Mol Aspects Med 21(3):49–98
50. Brunt EM, Janney CG, Di Bisceglie AM et al (1999) Nonalcoholic steatohepatitis: a proposal for grading and staging the histological lesions. Am J Gastroenterol 94(9):2467–2474
51. Kleiner DE, Brunt EM, Van Natta M et al (2005) Design and validation of a histological scoring system for nonalcoholic fatty liver disease. Hepatology 41(6):1313–1321
52. Srivastava A, Shivanandappa T (2010) Hepatoprotective effect of the root extract of *Decalepis hamiltonii* against carbon tetrachloride-induced oxidative stress in rats. Food Chem 118(2):411–417
53. Jaeschke H, McGill MR, Ramachandran A (2012) Oxidant stress, mitochondria, and cell death mechanisms in drug-induced liver injury. Drug Metab Rev 44(1):88–106
54. Tatiya AU, Surana SJ, Sutar MP, Gamit NH (2011) Hepatoprotective effect of poly herbal formulation against paracetamol induced hepatotoxicity in rats. J Pharmacogn Phytochem 1(1):1–6

55. Meera R, Devi P, Kameswari B et al (2009) Antioxidant and hepatoprotective activities of ethanolic extracts from *Ocimum basilicum* and *Trigonella foenum-graecum* against H₂O₂ and CCl₄-induced oxidative stress. J Pharm Res 2(8):1363–1367
56. Cichoz-Lach H, Michalak A (2014) Oxidative stress as a crucial factor in liver diseases. World J Gastroenterol 20(25):8082–8091
57. Matés JM, Pérez-Gómez C, de Castro IN (1999) Antioxidant enzymes and human diseases. Clin Biochem 32(8):595–603
58. Kumar S, Pandey AK (2013) Chemistry and biological activities of flavonoids: an overview. Sci World J 2013:162750
59. Brunt EM, Janney CG, Di Bisceglie AM et al (1999) Nonalcoholic steatohepatitis: a proposal for grading and staging the histological lesions. Am J Gastroenterol 94(9):2467–2474
60. Kleiner DE, Brunt EM, Van Natta M et al (2005) Design and validation of a histological scoring system for nonalcoholic fatty liver disease. Hepatology 41(6):1313–1321
61. Poli G (2000) Pathogenesis of liver fibrosis: role of oxidative stress. Mol Aspects Med 21(3):49–98
62. Kumar S, Pandey AK (2013) Chemistry and biological activities of flavonoids: an overview. Sci World J 2013:162750
63. Reddy MC, Murthy KSR (2013) A review on *Decalepis hamiltonii* Wight & Arn. J Med Plants Res 7(46):3395–3402
64. Brand-Williams W, Cuvelier ME, Berset C (1995) Use of a free radical method to evaluate antioxidant activity. LWT-Food Sci Technol 28(1):25–30
65. Re R, Pellegrini N, Proteggente A et al (1999) Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radic Biol Med 26(9–10):1231–1237
66. Cichoz-Lach H, Michalak A (2014) Oxidative stress as a crucial factor in liver diseases. World J Gastroenterol 20(25):8082–8091
67. Reddy MC, Murthy KSR (2013) A review on *Decalepis hamiltonii* Wight & Arn. J Med Plants Res 7(46):3395–3402