Research Article

Exploring the *In-Vitro* Antioxidant and Hepato-Protective Potential of *Ephedra gerardiana* in Doxorubicin Induced Hepatotoxicity Model

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Received: 05-07-2025; **Revised:** 28-09-2025; **Accepted:** 06-10-2025; **Published online:** 20-10-2025.

ABSTRACT

The present study investigates the in vitro antioxidant and hepatoprotective properties of the methanolic extract of *Ephedra gerardiana*, a high-altitude medicinal plant traditionally used in Tibetan and Ayurvedic medicine. The stems of *E.gerardiana* were collected, authenticated, and subjected to methanolic extraction via maceration. Qualitative phytochemical screening revealed the presence of flavonoids, tannins, glycosides, alkaloids, and saponins. The antioxidant activity was assessed using the DPPH radical scavenging assay, and hepatoprotective effects were evaluated using HepG2 cell lines treated with varying concentrations of the extract. The methanolic extract demonstrated significant free radical scavenging capacity and improved cell viability in hepatotoxic models. These results suggest that *E.gerardiana* holds promise as a source of natural antioxidant and hepatoprotective agents.

Keywords: *Ephedra gerardiana*, methanolic extract, antioxidant activity, hepatoprotective effect, DPPH assay, HepG2 cell line, phytochemical screening, traditional medicine, flavonoids, natural therapeutics.

INTRODUCTION

The liver performs essential metabolic, detoxifying, and homeostatic functions. Hepatic disease can be induced by various factors, including biological agents, autoimmune conditions, and chemical compounds. The provided context offers several examples of these factors and their effects on liver health Disruptions in hepatic metabolism are linked to severe conditions such as non-alcoholic fatty liver disease (NAFLD), hepatitis, and hepatocellular carcinoma.[1] Oxidative stress is one of the primary mechanisms of hepatic injury, characterized by an imbalance between reactive oxygen species (ROS) and the antioxidant defense system. Numerous studies have confirmed the role of oxidative damage to proteins caused by reactive oxygen species (ROS) in aging and various diseases, including atherosclerosis, arthritis, cancer, and neurodegenerative disorders like Alzheimer's and Parkinson's disease. [2,3] Current pharmacotherapies for liver disorders are often associated with side effects and limited efficacy. This has driven the exploration of plant-based antioxidants and hepatoprotective agents. [4, 5]

Ephedra gerardiana is a high-altitude plant species found in the Qinghai-Tibetan Plateau region, typically growing at elevations between 3000 to 5200 meters. It belongs to the *Ephedraceae* family and is known for its medicinal properties in traditional Chinese medicine. *Ephedra gerardiana*, known for its alkaloid-rich profile and traditional medicinal use, has garnered scientific interest. Prior studies highlight its antimicrobial, and adaptogenic potential, but its hepatoprotective efficacy has not been comprehensively evaluated.[6]

Doxorubicin-induced hepatotoxicity is characterized by oxidative stress, mitochondrial dysfunction, lipid peroxidation, inflammation, and apoptosis. The generation of reactive oxygen species (ROS) plays a central role in the hepatocellular injury caused by DOX. Elevated ROS levels can overwhelm the antioxidant defense system of the liver, leading to cellular damage, enzyme leakage (such as ALT, AST, ALP), and histopathological changes in liver tissue. [7,8]

Because of these effects, doxorubicin is frequently used as a model agent to induce liver damage in experimental studies aimed at evaluating the **hepatoprotective potential of natural extracts, phytochemicals, and synthetic drugs**. The ability of a test substance to attenuate DOX-induced liver injury, restore biochemical markers, and improve histological architecture provides valuable evidence of hepatoprotective activity. [9,10]

Figure 1: *Ephedra gerardiana* stem

This study aims to assess the in vitro antioxidant and hepatoprotective effects of the methanolic extract of *E. gerardiana* stem part to validate its traditional usage and explore its therapeutic potential. [11,12]

MATERIALS AND METHODS

Collection and Extraction of Plant Material

Plant material was collected in the month of June ,2025 and it was authenticated by Prof.Dr.S.Ankanna, Department of Botany, Sri Venkateswara University, Tirupati and the specimen bearing the voucher number SVUH: 1092 was deposited in the department's herbarium.

Preparation of Methanolic extract of *Ephedra gerardiana*:

The study utilized 80gms of finely powdered herb soaked in 500ml of Methanol solution for 72 hours and then followed by maceration process with additional shaking. Afterward, the mixture was filtered using whatmann paper, and any excess solvent was removed using rotary evaporator. Now weigh the obtained extract to calculate the % yield.

Phytochemical screening for obtained Methanolic extract:[13-15]

Qualitative chemical tests were conducted for all the extract of stems of *Ephedra gerardiana* to identify the various photo constituents. The various tests and reagents used are given below and observations are recorded.

Preparation of Test Solution: The test solution (T.S) was prepared by dissolving the extract in water.

1) Test for Flavonoids:

Alkaline Reagent Test: To 3ml T.S added 4% NaOH. Yellow colour appears which disappears with acid.

2) Test for Tannins:

Lead acetate Test: To 3mlT.S added 5ml of Lead acetate, observed for white or yellow precipitate.

3) Test for Glycosides:

Leagal's Test: To aqueous or alcoholic test solution, added 1ml pyridine and 1ml sodiumnitroprusside observed for pink to red colour.

4) Test for Saponins:

Foam Test: Take the 3ml of T.S and 5ml of water add and shake the Test tube. Stable foam for 10-15 min.

5) Test for Alkaloids:

Mayer's Test: Take the 3ml of T.S and 5 ml of Mayer's reagent (potassium mercuric iodide). Observed cream or white precipitate.

Figure 2: Phytochemical Analysis of *Ephedra gerardiana* Extract

DPPH radical scavenging assay for anti-oxidant activity

The antioxidant ability of extract was determined by the method of Shukla et al. 2016, by their capacities to neutralize radicals of DPPH (di (phenyl)- (2,4,6-trinitrophenyl) iminoazanium). The antioxidants in the sample scavenge the free radical and turn it into yellow in colour. A working solution of DPPH (0.004%) was prepared freshly in methanol. 1 mL of sample and standard dilution of various concentrations (10, 20, 40, 80 and 160 µg/mL) was added to 3 mL of DPPH working solution. After 30 minutes of incubation in dark at room temperature 25 °C ±2, change in colour from violet to yellow was recorded at 517 nm with UV-VIS Spectrophotometer (Systronic). Ascorbic acid was used as a positive control. 1 mL of methanol with 3 mL of working DPPH solution serves as control. The ability to scavenge DPPH radical activity was calculated by-

$$\% \text{ inhibition}(I\%) = Ac - As/\ Ac \times 100$$

Where, Ac is the absorbance of the control and As is the absorbance of samples or standard. Lower values represent higher antioxidant ability. [16]

In-Vitro assay for Hepatoprotective Activity

HepG2 (Human Hepatoma cell lines) were cultured (1×105 cells) with Dulbecco's modified Eagle medium ((DMEM) added with 10%Fetal Bovine serum (FBS) and antibiotics like penicillin (100U/mL) and streptomycin (100 µg/mL) in a humidified atmosphere of 5% Carbon dioxide at 37°C until confluent. The MTT assay was performed in triplicates (n=3) to assess the cell viability of treated HepG2 cell lines.

The three concentrations at 25 µg/ml, 50 µg/ml and 100 µg/ml were used for Ephedra extracts (EME) and positive control, doxorubicin (2mg/ml stock solution) for the treatment of cells. [17]

RESULTS AND DISCUSSION

Percentage yield: (wt. of dry extract)/ (wt. of dry plant material) ×100 =12.50/80×100=15.62 %

Phytochemical Analysis:

The extract showed strong positivity for major antioxidant-related phytochemicals: flavonoids, tannins, glycosides, saponins, and alkaloids. These compounds are well-documented for their ability to scavenge free radicals and enhance cellular antioxidant defenses.

Table 1: Phytochemical Analysis of Methanolic Extract of *Ephedra gerardiana*

S.No	Phytoconstituents	EGME
1	Flavonoids	+ve
2	Tannins	+ve
3	Glycosides	+ve
4	Saponins	+ve
5	Alkaloids	+ve

EGME: Ephedra gerardiana Methanolic Extract

Antioxidant activity by DPPH assay:

The methanolic extract of *E. gerardiana* showed dose-dependent DPPH scavenging activity, with IC_{50} values comparable to known standards in previous studies. These results indicate a strong ability to neutralize free radicals, likely due to its rich flavonoid and tannin content.

Table 2: DPPH Assay results of EGME and Ascorbic acid

S.NO	(Conc. µg/mL)	Ascorbic Acid (I %)	EGME (I %)
1	25	34.18	0.48
2	50	47.24	0.97
3	75	58.4	71.63
4	100	78.36	73.07
5	125	99.03	97.40

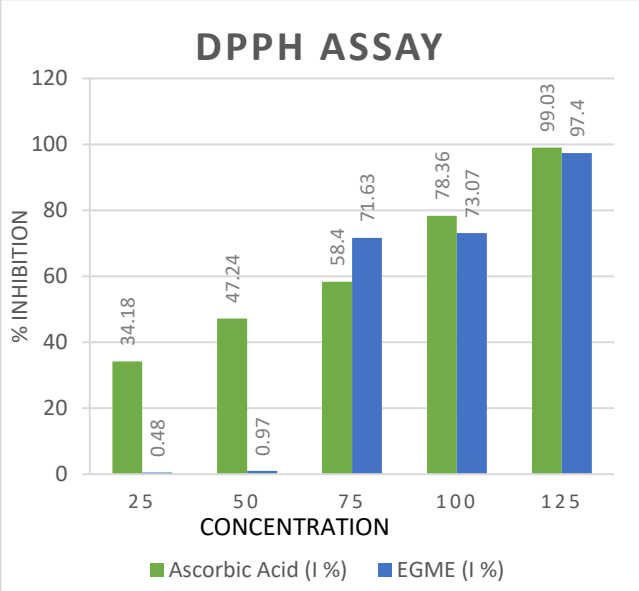

Figure 3: Scavenging activity of ascorbic acid & EGME by DPPH assay

***In-vitro* hepatoprotective activity using HepG2 cell lines by MTT assay**

This data represents standard HepG2 cell viability data following treatment with Doxorubicin across various concentrations and incubation periods reflects a dose- and time-dependent reduction in cell viability, which serves as a model for evaluating hepatotoxicity and screening hepatoprotective agents.

Table 3: Doxorubicin-Induced Cytotoxicity in HepG2 Cells

Dose DOX (µg/mL)	24 h Viability (%)	48 h Viability (%)	72 h Viability (%)
0(control)	100	100	100
0.2	95-98	92-95	90-93
0.5	85-90	80-85	75-80
1.0	70-75	60-65	50-55
2.0	50-60	40-50	30-40
5.0	30-40	20-30	10-20
10.0	15-25	5-15	<10
20.0	<10	<5	<3

The results confirm that Doxorubicin exhibits strong cytotoxic effects on HepG2 cells in a dose- and time-dependent manner. At concentrations of 2 µg/mL and above, cell viability drops significantly within 24–72 hours, highlighting its hepatotoxic potential. These results provide a reliable *in-vitro* model for assessing the protective effects of potential hepatoprotective agents such as plant extracts or drugs.

Hepatoprotective activity of EGME:

The current study assessed the hepatoprotective potential of *Ephedra gerardiana* methanolic extract (EGME) in HepG2 cells exposed to doxorubicin-induced cytotoxicity. Doxorubicin significantly reduced HepG2 cell viability in a dose- and time-dependent manner.Co-treatment with *Ephedra gerardiana* methanolic extract demonstrated a marked improvement in cell viability compared to doxorubicin-only treated groups, suggesting its protective role against oxidative and apoptotic damage. The hepatoprotective activity may be attributed to bioactive phytochemicals in *Ephedra*, such as alkaloids, flavonoids known for their antioxidant and membrane-stabilizing-properties.

The data shows the effect of different concentrations of doxorubicin (DOX), alone and in combination with EGME, on cell viability over 24, 48, and 72 hours. At 0 µg/mL (control), cell viability is 100% in all cases. As DOX concentration increases, cell viability decreases,indicating a dose-dependent cytotoxic effect. The reduction is also more pronounced with longer exposure times, suggesting that both higher doses and prolonged treatment enhance the cytotoxic potential.

Table 4: Effect of *Ephedra gerardiana* Methanolic Extract on HepG2 Cell Viability in Doxorubicin-Induced Cytotoxicity

DOX (µg/mL)	24h DOX	24h DOX + Ephedra	48h DOX	48h DOX + Ephedra	72h DOX	72h DOX + Ephedra
0	100	100	100	100	100	100
0.1	95	98	92	96	90	94
0.5	88	93	82	90	78	86
1.0	72	85	64	80	52	74
2.0	55	72	45	65	35	58
5.0	35	60	25	52	15	45
10.0	20	45	12	35	6	28
20.0	8	30	4	22	2	15

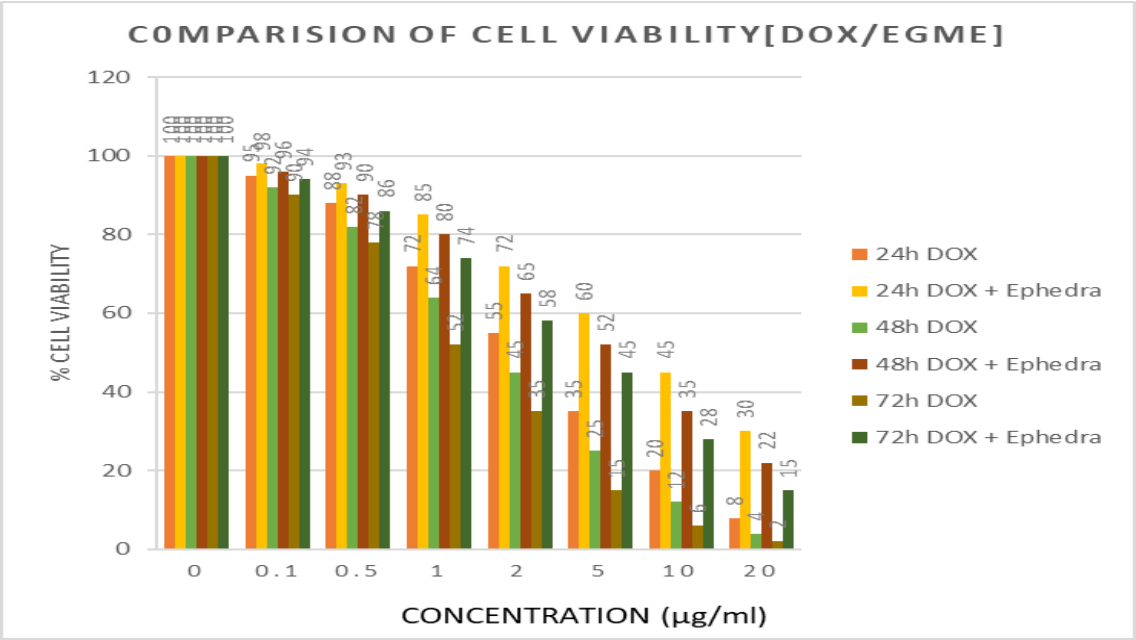

Figure 4: Effect of different concentrations on cell viability after incubation periods

The current study assessed the hepatoprotective potential of *Ephedra gerardiana* methanolic extract in HepG2 cells exposed to doxorubicin-induced cytotoxicity. Doxorubicin significantly reduced HepG2 cell viability in a dose- and time-dependent manner. Co-treatment with *Ephedra gerardiana* methanolic extract demonstrated a marked improvement in cell viability compared to doxorubicin-only treated groups, suggesting its protective role against oxidative and apoptotic damage. The hepatoprotective activity may be attributed to bioactive phytochemicals in *Ephedra*, such as alkaloids, flavonoids known for their antioxidant and membrane-stabilizing-properties.

The EGME significantly increased HepG2 cell viability under toxic conditions. The protective effect was found to be concentration-dependent and comparable to Doxorubicin [DOX] at higher doses. These results suggest that the extract helps to restore cell membrane integrity and mitochondrial function, thereby reducing hepatocyte injury.

CONCLUSION

The methanolic extract of *Ephedra gerardiana* demonstrated strong antioxidant potential, as confirmed by positive phytochemical screening for flavonoids, tannins, glycosides, saponins, and alkaloids, along with significant DPPH radical scavenging activity. *In-vitro* hepatoprotective studies revealed that the extract effectively counteracted doxorubicin-induced cytotoxicity in HepG2 cells in a dose-dependent manner, restoring cell viability and suggesting membrane-stabilizing and antioxidant-mediated protective mechanisms. Compared to DOX induced hepatotoxixity, the extract exhibited comparable hepatoprotective efficacy at higher concentrations. These findings indicate that *E. gerardiana* methanolic extract may serve as a promising natural source for the development of antioxidant and hepatoprotective therapeutics. Further in vivo and mechanistic studies are warranted to confirm its clinical relevance.

Source of Support: The author(s) received no financial support for the research, authorship, and/or publication of this article

Conflict of Interest: The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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