Investigation of the anti-dengue and platelet-enhancing potential of Cedrus deodara heart wood extract: In vitro and in vivo evaluation in a Hydroxyurea-induced thrombocytopenia model

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Abstract

The purpose of the study was to assess the ethanol extract of *Cedrus deodara* bark's anti-dengue properties. Authenticated heart wood of *Cedrus deodara* was shade-dried, defatted with petroleum ether and extracted with ethanol using a Soxhlet apparatus. An *in vivo* hydroxyurea-induced thrombocytopenia model in albino rats was used to assess the ability of CDEE to increase platelet count. Hematological parameters including platelet count, clotting time, bleeding time, Hemoglobin, RBC count, MCHC, PCV, MCV, and MCH were measured. At study completion, Rats were put to death while sedated with thiopental sodium, and bone marrow samples were subjected to histopathological analysis. In vitro, the anti-dengue activity of CDEE was tested by inoculating normal Vero cells with DENV-2 at various concentrations, and MTT assay was used to evaluate cell viability. The IC50 value of the extract was also determined. The CDEE significantly increased RBC, WBC, platelet count, and hemoglobin levels, and normalized PCV, MCV, MCH, and MCHC in treatment groups, with effects comparable to standard. Platelet recovery also corrected bleeding and clotting times. In the cell viability assay, CDEE protected vero cells from DENV-2, enhancing normal cell viability and indicating inhibitory activity against the virus. The ethanol extract of *Cedrus deodara* demonstrated significant in vivo and in vitro anti-dengue activity. To clarify its mode of action, more research is required.

1.0 Introduction

In recent decades, dengue hemorrhagic fever, an infection spread by mosquitoes, has become a significant global public health concern. It is prevalent in tropical and subtropical regions, especially in urban and semi-urban areas of India, Sri Lanka, and other countries. Other names for the illness include "breakbone fever" and "dandy fever," and it is spread by *Aedes mosquitoes*. A persistent high fever lasting two to seven days, hemorrhagic tendency (positive tourniquet test, petechiae, or epistaxis), thrombocytopenia (platelet count < 100 × 10^9/L), and plasma leakage exhibited by hemoconcentration (hematocrit ↑20% above normal), pleural effusion, or ascites are among its primary clinical features. Despite advancements in medicine, there is still no authorized vaccine or specialized medication [1, 2].

Acute viral infections like dengue can have deadly consequences. In a Chinese medical encyclopedia, it was initially identified as "water poison" connected to flying insects [3, 4]. The first recognized epidemics occurred in the 1780s in Africa, North America, and Asia. In 1789, Benjamin Rush described the Philadelphia epidemic of 1780 and coined the term “breakbone fever” due to severe myalgia and arthralgia. Annually, about 50 million infections occur, with 500,000 hospitalizations for dengue hemorrhagic fever, mostly in Southeast Asia, the Pacific, and the Americas. A global strategy has been advocated, emphasizing surveillance, outbreak response, behavior change, integrated vector management, and accurate early diagnosis. Antivirals and vaccines under development may further aid control [5, 6]. Symptoms include fever, headache, severe muscular and joint pain, and rashes. Despite advances in healthcare, no effective medicine exists. However, Ayurveda describes several herbal remedies, including papaya leaf juice. Dengue, known in Ayurveda as *Dandak jwara*, was treated with extracts such as *Amrita* (Guduchi), *Tulasi* (Holy basil), *Shunthi* (dried ginger), *Erand-karkati* (papaya), and *Brassica*. Extracts of *Carica papaya* have shown benefits, though scientific validation of *Cedrus deodara* remains lacking [7, 8]. Hence, this study was designed to evaluate the effects of *Brassica campestris* extracts on dengue complications. Similarly, *Cedrus deodara* (Himalayan cedar) has been traditionally used against dengue, but no scientific evidence supports its efficacy [9]. Therefore, the present study was undertaken to evaluate the *in vivo* thrombolytic and *in vitro* anti-dengue potential of ethanol extract of *Cedrus deodara*.

2.0 Materials and Methods

2.1 Phytochemical investigation

2.1.1 Collection and authentication of plant material

Dr. V Rama Rao of the Regional Research Institute for Metabolic Disorder in Bangalore verified the authenticity of the *Cedrus deodara* heartwood, which was bought from the local market. The Institute Herbarium Library is where the specimen herbarium was kept (Ref. No. RRCI-mus124).

2.1.2 Preparation of ethanol extract

Cedrus deodara wood bark was promptly dried in the shade, ground, and the coarse powder was gathered. The defatted product was obtained after 250g of dried coarse powder of *Emblica officinalis* was first defatted using petroleum ether (40°–70°C) by hot continuous percolation using a Soxhlet device. The leftover marc was then stored in hot air at 90°C. For 72 hours, the defatted coarse powder was once more extracted using methanol [10].

2.1.3 Preliminary phytochemical investigation

Following the steps outlined by Khandelwal, the methanol extract of *Cedrus deodara* (CDEE) was assessed for the initial phytochemical constituents. [11].

2.1.4 HPLC Analysis of ethanol extract of Cedrus deodara

Phenolic acids in WF were quantified using an Agilent 1290 Series HPLC system (Agilent Technologies, USA). The HPLC pumps, autosampler, and column oven were controlled through Agilent MassHunter Workstation Data Acquisition software. Chromatographic separation was achieved on a Waters ACQUITY HPLC BEH HILIC column (1.7 µm, 2.1 × 100 mm). The autosampler was maintained at 4 °C, and the column oven temperature was set to 35 °C. The mobile phase consisted of solvent A (0.1% formic acid in acetonitrile) and solvent B (0.1% formic acid in water). The gradient program was as follows: 0–8 min, 5–13% A; 8–12 min, 13–23% A; 12–21 min, 23–35% A; and 21–26 min, 35–100% A. The flow rate was set at 0.2 mL/min, and the injection volume was 2 µL.

2.2 In vivo anti-dengue potentials against Hydroxyurea induced thrombocytopenia

Studies were conducted both in vitro and in vivo to assess the anti-dengue properties of *Cedrus deodara* ethanol extract.

2.2.1 Animals

Albino wistar rats of either sex (150-200g) will acclimatized for seven days under conventional husbandry settings, i.e.; room temperature of 25 ± 10 C; relative humidity 45-55% and a 12:12h light/ dark cycle. The rats had to free access to normal rat pellet, with water supplied ad libitum under strict hygienic circumstances. For 48 hours before the experimental protocol, the animals were acclimated to the laboratory environment in order to reduce any non-specific stress. The Institutional Animal Ethics Committee of East West College of Pharmacy in Bangalore approved the protocol (Ref No: EWCP/CPCSEA/IAEC/II/2019/05), and all studies and protocols were carried out strictly in accordance with the ethical norms of CPCSEA, New Delhi.

2.2.2 Hydroxyurea induced thrombocytopenia in wistar rats

The *in vivo* Hydroxyurea induced thrombocytopenia in albino rats model was carried out to test the ability of the ethanol extract to increase platelet count in dengue fever [12,13]

2.2.3 Induction of thrombocytopenia

Freshly produced Hydroxyurea (Cytodrx, Cipla Ltd., India) mixed in distilled water was administered orally to induce thrombocytopenia [12,13,14].

2.2.4 Group design

The present investigation consists of six group wistar rats consisting 6 animals in each. On the first day, a single dosage of hydroxyurea (15 mg/kg, p.o.) was given to every animal except the normal group. The detailed treatment protocol was given below as follows.

- Group 1-Normal: Given with 2 % tween 20 for 21 days
- Group 2-Toxic control: Given with single dosage of hydroxyurea (15 mg/kg, p.o.) on 1st day and 2 % tween 20 for 21 days
- Group 3-Standard: Given with single dosage of hydroxyurea (15 mg/kg, p.o.) on 1st day and caripil syrup (275mg/kg, p.o.) for 21 days
- Group 4-CDEE 100 mg: Given with single dosage of hydroxyurea (15 mg/kg, p.o.) on 1st day and low dose of ethanol extract of *Cedrus deodara* (100mg/kg, p.o.) for 21 days.
- Group 5-CDEE 200 mg: Given with single dosage of hydroxyurea (15 mg/kg, p.o.) on 1st day and medium dose of ethanol extract of *Cedrus deodara* (200mg/kg, p.o.) for 21 days
- Group 6- CDEE 200 mg: Given with single dosage of hydroxyurea (15 mg/kg, p.o.) on 1st day and medium dose of ethanol extract of *Cedrus deodara* (200mg/kg, p.o.) for 21 days

2.2.5 Parameters

The blood sample from all the experimental rats were collected and biochemical parameters such as were Thrombocyte count, Hemoglobin, clotting time, bleeding time, white blood cell count, and red blood cell count determined on day first and end of the study for the evaluation of benefits of *Cedrus deodara* against Hydroxyurea induced thrombocytopenia in albino wistar rats.

2.2.6 Histopathological examination of bone marrow

At the end of treatment, femurs were excised and fixed in 10% neutral-buffered formalin, followed by decalcification using 10% EDTA solution. Tissues were processed, paraffin-embedded, and sectioned at 5 µm thickness. Sections were stained with Hematoxylin and Eosin (H&E) for histopathological evaluation, while reticulin staining was used to assess stromal network when required. Slides were examined under a light microscope and photographed. Parameters evaluated included bone-marrow cellularity, fat-to-cell ratio, myeloid:erythroid (M:E) ratio, trabecular osteocytes lined by osteoblasts, and presence or absence of megakaryocytes in multiple high-power fields. Group-wise comparisons of hypocellularity, normalcy or hypercellularity, along with megakaryocyte integrity, provided evidence for the protective effects of CDEE against hydroxyurea-induced thrombocytopenia.

2.3 Evaluation of in vitro anti-dengue properties

2.3.1 Cell Lines and Virus Culture

Vero cells (ATCC® CCL-81™) and human endothelial EA.hy926 cells (ATCC® CRL-2922™) were used for dengue virus propagation and infection studies. Cells were cultured and maintained in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% heat-inactivated (decomplemented) fetal bovine serum (FBS) and 0.5% penicillin–streptomycin solution. Cultures were incubated at 37 °C in a humidified atmosphere containing 5% CO_2. Dengue virus serotype-2 (DENV-2) used in this study was obtained from the laboratory repository of the Department of Molecular Biology and Biotechnology, GM University, Davangere.

2.3.2 Propagation of DENV-2 in Vero Cells

DENV-2 was propagated in Vero cells following standard procedures. Briefly, Vero cells were seeded into either 96-well plates (3.0×10^4 cells/well) or T-25 culture flasks (2.0×10^6 cells/flask) and incubated for 24 h at 37 °C with 5% CO_2 until approximately 80% confluency was achieved. Spent culture medium was aspirated, and fresh growth medium (50 µL per well or 4 mL per flask) was added. Subsequently, DENV-2 inoculum from frozen viral stocks (50 µL per well or 1 mL per flask) was added, and cultures were incubated for 6 days under standard culture conditions. Following incubation, cell culture contents were collected into 15 mL centrifuge tubes and centrifuged at 150 × g for 7 min at 4 °C. The clarified supernatant containing viral particles was collected, aliquoted into cryovials, and stored at −80 °C until further use [16,17].

2.3.3 Optimization of Adherent Cell Seeding Density for MTT and SRB Assays

EA.hy926 cells were trypsinized, centrifuged, and resuspended in complete culture medium. Two-fold serial dilutions ranging from 2.0×10^5 to 1.56×10^3 cells per well were prepared to optimize cell density for both the MTT and sulforhodamine B (SRB) assays. Cells were seeded in triplicate into 96-well plates at each concentration. Blank wells containing medium alone were included for background correction. Plates were incubated overnight at 37 °C in a humidified 5% CO_2 atmosphere prior to assay performance.

2.3.4 MTT Assay

Cellular metabolic activity was assessed using the MTT assay as described by van Tonder *et al.* with minor modifications. After overnight incubation, spent culture medium was aspirated, and cells were washed once with pre-warmed 1× phosphate-buffered saline (PBS). Subsequently, 200 µL of MTT solution (0.5 mg/mL) was added to each well, and plates were incubated for 4 h at 37 °C in 5% CO_2, protected from light. Following incubation, the MTT solution was removed, and isopropanol was added to solubilize the formazan crystals. Plates were shaken at room temperature for 30 min to ensure complete dissolution, and absorbance was measured at 570 nm using a microplate reader [16,17].

2.3.5 Sulforhodamine B (SRB) Assay

The SRB assay was performed according to the method described by Vichai and Kirtikara, with minor modifications. Briefly, spent medium was aspirated and cells were washed twice with pre-warmed 1× PBS. Cells

were then fixed by adding DMEM without FBS followed by ice-cold trichloroacetic acid to a final concentration of 10% and incubated at 4 °C for 1 h. Fixed monolayers were washed five times with tap water and air-dried. Subsequently, 100 µL of SRB solution (0.2 mg/mL) was added to each well and incubated at room temperature for 30 min. Excess dye was removed by washing five times with 1% acetic acid. Bound dye was solubilized with 100 µL of Tris base, and plates were shaken for 1 h at room temperature before measuring absorbance at 540 nm [16,17].

2.3.6 Determination of Non-Toxic Concentrations of CDEE in EA.hy926 Cells

2.3.6.1 SRB-Based Cytotoxicity Assessment

EA.hy926 cells were seeded into 96-well plates at a density of 1.0×10^5 cells/well, as determined during assay optimization, and incubated overnight. Spent medium was aspirated, and cells were washed with pre-warmed PBS. Cells were then treated with varying concentrations of CDEE (7.8–1000 µg/mL) prepared in serum-free DMEM, in triplicate, and incubated for either 30 min or 24 h at 37 °C with 5% CO_2. Untreated cells served as controls. Following treatment, cells were washed twice with PBS, and the SRB assay was performed as described above. Cell viability was calculated using the following equation:

$$\text{Cell viability (\%)} = \left(\frac{\text{Absorbance of treated cells}}{\text{Absorbance of untreated cells}} \right) \times 100$$
cells

2.3.6.2 MTT-Based Cytotoxicity Assessment

EA.hy926 cells were seeded at a density of 5.0×10^4 cells/well in 96-well plates and incubated overnight. Cells were washed once with PBS and treated with different concentrations of CDEE (7.8–1000 µg/mL) for 30 min, 2 h, or 4 h at 37 °C in 5% CO_2. Following treatment, cells were washed with PBS, and the MTT assay was performed as described earlier [16,17].

2.3.6.3 Optimization of DENV-2 Infection in Endothelial Cells

To determine the optimal viral inoculum for endothelial cell infection, EA.hy926 cells were infected with DENV-2 diluted at a factor of 10. Following inoculation, cells were incubated for either 2 h or 4 h at 37 °C in a humidified 5% CO_2 atmosphere. After incubation, cells were washed to remove unbound virus, and cellular metabolic activity was assessed using the MTT assay [16,17].

2.3.7 Assessment of Endothelial Cell Function Following Interaction with DENV-2 in the 2.3.7.1 Presence of CDEE

Endothelial cell metabolic activity following viral exposure and extract treatment was evaluated using the MTT assay under three experimental conditions:

a. pre-treatment of DENV-2 with CDEE,
b. sequential treatment of DENV-2 followed by CDEE, and
c. sequential treatment of CDEE followed by DENV-2.

2.3.7.2 Pre-Treatment of DENV-2 with CDEE

DENV-2 was incubated with different concentrations of CDEE (7.8–500 µg/mL) for 1 h at 37 °C in a humidified 5% CO_2 atmosphere. Subsequently, 200 µL of the virus–extract mixture was added to EA.hy926 cell monolayers and incubated for 4 h under standard culture conditions.

2.3.7.3 Sequential Treatment of DENV-2 Followed by CDEE

EA.hy926 cell monolayers were inoculated with DENV-2 and incubated for 4 h at 37 °C. The viral inoculum was then removed, and cells were washed to eliminate non-adsorbed virus. Cells were subsequently treated with varying concentrations of CDEE (7.8–500 µg/mL) for 1 h.

2.3.7.4 Sequential Treatment of CDEE Followed by DENV-2

Washed EA.hy926 cell monolayers were treated with different concentrations of CDEE (7.8–500 µg/mL) and incubated for 1 h at 37 °C in 5% CO_2. Excess extract was removed, and cells were washed with PBS. DENV-2 was then inoculated, and cells were incubated for an additional 4 h.

2.3.7.5 Evaluation of Endothelial Cell Viability

Following all treatment protocols, cells were washed with 1× PBS, and the MTT assay was performed to assess endothelial cell viability. Protective effects of CDEE against DENV-2-induced cytotoxicity were quantified based on metabolic activity relative to untreated control cells.

2.5 Statistical analysis

Statistical analysis was carried out using GraphPad Prism version 5.0. Data were expressed as mean ± standard error of the mean (SEM). One-way analysis of variance (ANOVA) and the Student-t test was performed for in vivo study and Two-way ANOVA was employed to evaluate the effects of the CDEE on DENV-3 infection by comparing treated endothelial cells with untreated controls. A p value < 0.05 was considered statistically significant.

3.0 Results & Discussion

3.1 Preparation of extract

Cedrus deodara's ethanol extract had a light brown color and a 9.45% yield percentage. and has revealed the existence of several phyoconstituents, including carbohydrates, polyphenols, alkaloids, flavonoids, glycosides, and tannins [Table 1].

Table 1: Results of preliminary phytochemical investigation on CDEE

Sl. No	Phyto-constituent	Presence
1.	PPL	+++
2.	FLV	+++
3.	GLS	+++
4.	ALK	+
5.	CH	++
6.	Proteins	++

3.2 HPLC analysis of CDEE

About Nineteen pharmacologically active monomeric components were collected through experimental study and analysis. Further purification has been revealed the presence of seventeen monomeric components is shown in picture are as follows (Figure 1).

1) Gallic acid, 2) p-Hydroxybenzyl alcohol, 3) Protocatechuic acid, 4) p-Hydroxybenzoic acid, 5) p-Coumaric acid-β-D-glucoside, 6) Chlorogenic acid, 7) Vanillic acid, 8) Caffeic acid, 9) Syringic acid, 10) p-Coumaric acid, 11) Vanillic acid-β-D-glucoside, 12) Sinapic acid, 13) Benzoic acid 14) Phenylacetic acid, 15) Salicylic acid, 16) Cinnamic acid, 17) Ferulic acid.

3.3 Assessment of in vivo anti-dengue potentials

The current study evaluated *Cedrus deodara* extract's *in vitro* anti-dengue properties in vivo and vitro approaches.

3.3.1 Effect of CD on blood cell count

There was significant (P<0.01) reduction in RBC, WBC and platelet cell count was observed in animals of disease control group treated with hydroxyurea alone compare to normal animals. The animals pretreated with standard drug and CDEE (200mg/kg and200mg/kg) have significant (P<0.01) increase in total count of RBC, WBC and Thrombocytes [Table 2 and 3].

3.3.2 Effect of CDEE on hemoglobin

The amount of hemoglobin was significantly (P<0.01) reduced in toxic control animals treated with hydroxyurea alone comparing to normal animals. The animals pretreated with standard drug and CDEE (200mg/kg and200mg/kg) could significantly (P<0.01) stimulate hemoglobin concentration in therapeutic group [Table 2 and 3].

3.3.3 Effect of CDEE on bleeding and clotting time

When compared to the normal group of animals, the toxic group of animals treated with hydroxyurea alone had significantly longer bleeding and clotting periods (P<0.01). However, animals pretreated with normal medication plus CDEE (200 mg/kg and 200 mg/kg) showed a significant reduction in both bleeding and clotting times [Table 2 and 3].

Table 2: Effect of CDEE on total blood cell count against hydroxyurea induced thrombocytopenia in wistar rats on day 1

Group	Blood parameters					
	Thrombocytes	Leukocytes	Erythrocytes	Hemoglobin (g%)	Bleeding time (secs)	Clotting time (secs)
Normal	5.31±0.45	7.32±0.37	5.78±0.75	13.9±0.94	140.71±9.55	152.2±9.61
Toxic	5.18 ^{ns} ± 0.08	7.35 ^{ns} ± 0.19	5.64 ^{ns} ±0.27	14.05 ^{ns} ±0.14	139.22 ^{ns} ± 10.84	149.66 ^{ns} ± 12.35
Standard	5.22±0.32	7.11±0.32	5.54±0.18	14.32±1.27	142.17±10.56	139.21±4.9
CDEE-100mg/kg	5.39 ^{ns} ±0.2	7.37 ^{ns} ± 0.32	5.28 ^{ns} ±0.35	13.98 ^{ns} ±0.78	151.27 ^{ns} ±9.56	152.3 ^{ns} ±9.65
CDEE-200mg/kg	5.11^{ns}± 0.28	7.2 ^{ns} ±0.3	5.75 ^{ns} ±0.18	14.3 ^{ns} ±1.32	145.05 ^{ns} ±10.74	143.7 ^{ns} ±12.54
CDEE-400mg/kg	5.27^{ns}± 0.27	7.3 ^{ns} ±0.27	5.49 ^{ns} ±0.25	13.87 ^{ns} ±1.15	148.35 ^{ns} ±7.60	139.7 ^{ns} ±4.34

Statistical significance is indicated by n=6 symbols, and the values are mean ± S.E.M. Toxic versus Normal control, Standard versus Toxic group, and CDEE treated versus Toxic (ns p>0.05)

Table 2: Effect of CDEE on total blood cell count against hydroxyurea induced thrombocytopenia in wistar rats on day 21

Group	Blood parameters					
	Thrombocytes	Leukocytes	Erythrocytes	Hemoglobin (G%)	Bleeding time (secs)	Clotting time (secs)
Normal	5.2±0.38	7.4±0.65	5.95±0.33	14.4±1.32	138.62±12.76	191.2±7.087
Toxic control	0. 81$^{\$}$±0.1	2.2$^{\$}$±0.2	2.32$^{\$}$±0.1	4.9$^{\$}$±0.65	258.35$^{\$}$±19.55	506.5$^{\$}$±15.73
Standard	5.13*±0.42	7.1*±0.32	5.78*±0.23	13.9*±1.55	163.28*±14.21	191.3*±5.737
CDEE-100mg/kg	2.22^{ns}±0.2	2.8 ^{ns}±0.32	3.29 ^{ns} ±0.21	4.7 ^{ns} ±0.67	255.41^{ns}±12.64	477. ^{ns} ±10.54
CDEE-200mg/kg	4.36*±0.31	4.2*±0.2	3.98*±0.2	8.9*±0.95	195.14*±12.11	352.3*±17.11
CDEE-400mg/kg	5.1*±0.35	6.9*±0.32	5.62*±0.38	14.2*±1,21	144.29*±9.54	229.3*±5.578

Statistical significance is indicated by n=6 symbols, and the values are mean ± S.E.M.

$^{\$}$p<0.05-Toxic vs. Normal control * p<0.05- Standard vs. Toxic group and also CDEE treated vs. toxic,

3.3.4 Hematological analysis of bone marrow

Normal: Bone marrow sections showed well-defined trabeculae with normally cellular hematopoietic elements. The fat-to-cell ratio was maintained at 1:4 (20% fat, 80% cellular), and the M:E ratio was 2.5:1, indicating balanced hematopoiesis. Megakaryocytes were present and morphologically normal. Trabeculae contained osteocytes within lacunae and were lined by active osteoblasts (Figure 2).

Toxic Control: Marked marrow hypocellularity was observed with an increased fat-to-cell ratio of 3:1 (75% fat, 25% cellular). The M:E ratio was mildly reduced to 2:1. Megakaryocytes were absent, suggesting impaired megakaryopoiesis. Trabecular structure and osteoblastic rimming were preserved (Figure 2).

Standard: Near-normal marrow cellularity was restored. The fat-to-cell ratio was 1:2.3 (30% fat, 70% cellular), and the M:E ratio normalized to 3:1. Megakaryocytes were present and intact, with preserved trabecular architecture (Figure 2).

CDEE 100 mg: Pronounced marrow hypocellularity was noted with a fat-to-cell ratio of 3:1 and reduced M:E ratio of 2:1. Megakaryocytes were absent, indicating suppressed hematopoiesis (Figure 2).

CDEE 200 mg: Bone marrow architecture appeared near normal with restored cellularity. Fat-to-cell ratio (1:2.3) and M:E ratio (3:1) were comparable to the standard group. Megakaryocytes were intact (Figure 2).

CDEE 400 mg: Hypercellular marrow was observed, indicating enhanced hematopoietic activity. The fat-to-cell ratio decreased to 1:9 (10% fat, 90% cellular), with a stable M:E ratio of 3:1. Megakaryocytes were normal and abundant (Figure 2).

3.4 In Vitro evaluation of CDEE against DENV-2

3.4.1 Propagation of DENV-2 in Vero cells

In this study, DENV-2–induced cytopathic effects in Vero cells were qualitatively assessed to evaluate virus-mediated cellular damage. Normal Vero cells exhibited typical fibroblast-like, elongated morphology and grew as a uniform monolayer. In contrast, DENV-2–infected cells progressively lost these characteristics, showing cell rounding, spindle-shaped morphology, and detachment from the culture surface, which became prominent from day 5 post-infection. Similar morphological alterations after 10 days of incubation following DENV-2 inoculation have been previously reported [20,21].

3.4.2 Determination of optimum EC plating concentration for MTT and SRB assays

Optimal cell densities for MTT and SRB assays were determined in a single optimization experiment. Based on linearity and assay performance, 5.0×10^4 cells/well for the MTT assay and 1.0×10^5 cells/well for the SRB assay were selected for subsequent experiments (Fig. 3). Two-fold serial dilutions of ECs ($0.15–20 \times 10^4$ cells/well) were analyzed in triplicate using MTT (3A) or SRB (3B). Error bars represent ± SEM, and R^2 values indicate goodness of fit to the linear regression.

3.4.3 Determination of the optimum interaction time of DENV-2 with EA.hy926 cells

The direct cytopathic effect (CPE) of DENV-2 on EA.hy926 cells was evaluated following virus–cell interaction for 2 and 4 h. Treatment with the DENV-2 stock (1×) for 4 h induced significantly greater cellular damage, resulting in 49.8% cell viability ($p < 0.001$), compared with 58.7% viability after 2 h exposure ($p < 0.01$) (Fig. 4). These findings indicate a more pronounced cytopathic effect with prolonged interaction. Consequently, a 4 h interaction with the DENV-2 stock was selected for subsequent evaluation of the effect of the CDEE on DENV-2–EA.hy926 interactions.

$$Optical\ density\% = \frac{Uninfected\ cell\ absorbance - Infected\ cell\ absorbance}{Uninfected\ cell\ absorbance}\ x\ 100$$

3.4.4 Determination of DENV-2 titer

The viral titer was estimated using the MTT_{50} assay as an alternative approach to determine infectious viral load, defined as the virus concentration at which the mean optical density (OD) of infected cells was reduced to 50% of that of uninfected cells . The OD values of individual wells were plotted against the –log (dilution factor) (Fig. 5A and 5B). The results indicated viral titers of 4.1 $\log_{10}$ (MTT_{50}/mL) and 3.4 $\log_{10}$ (MTT_{50}/mL) following 2 h and 4 h interactions with DENV-2, respectively.

3.4.5 Nontoxic concentration of CDEE on EA.hy926 cells

To evaluate the effect of CDEE on DENV-2 in relation to endothelial cell (EC) functionality, the nontoxic concentration range of the plant extract (7.8–500 µg/mL) was first determined using total cellular protein estimation, with cell viability maintained above 72% following 30 min and 24 h exposures (Fig. 6). These concentrations were further validated by assessing the metabolic activity of ECs at intermediate exposure durations (2 and 4 h), in addition to the short-term exposure (30 min), to identify a time window during which cellular metabolic stability was preserved across different concentrations of the CDEE. A modest increase in cellular metabolic activity was observed at nearly all concentrations after 30 min and 2 h of incubation; however, a reduction was noted at the lowest concentration (7.8 µg/mL) following 4 h exposure (Fig. 6B).

3.4.6 Effect of CDEE on DENV-2 Interaction with EA.hy926 Cells

The direct effect of CDEE on DENV-2 during its interaction with endothelial cells (ECs) was investigated. A significant reduction in cell viability was observed in the presence of DENV-2 compared with non-infected, CDEE-treated cells. However, two concentrations of CDEE (15.6 and 31.3 µg/mL) used for pre-treatment of DENV-2 showed no significant difference in cell viability when compared to treated cells without viral infection (Fig. 7).

As shown in Fig. 8, significant protection of ECs was observed, with enhanced protection when DENV-2 was pre-treated with the extract prior to interaction with ECs (lowest $p = 0.0003$). The highest level of cellular protection was detected at CDEE concentrations of 15.6 and 31.3 µg/mL ($p < 0.0001$). Although pre-treatment of the virus with the extract is not a clinically practical strategy, it provides valuable mechanistic insight into how the extract may protect ECs or prevent virus-induced cellular damage. The observed increase in protection suggests that the plant extract may exert its effects through direct inactivation of the virus.

Furthermore, the lack of significant protection when ECs were treated with CDEE prior to viral inoculation indicates that the observed protective effects are primarily due to direct interactions between the extract and DENV-2 particles rather than modulation of host cell susceptibility. This finding supports the conclusion that CDEE is capable of inactivating DENV-2 particles before their entry into ECs.

4.0 Discussion

Hydroxyurea-induced thrombocytopenia is a well-established experimental model that mimics bone marrow suppression and platelet depletion observed in dengue-associated hematological complications [18, 19]. In the present study, administration of hydroxyurea resulted in a significant reduction in platelet count, erythrocytes, leukocytes, and hemoglobin levels, along with prolonged bleeding and clotting times, confirming successful induction of thrombocytopenia. These alterations are attributable to suppression of megakaryopoiesis and generalized hematopoietic toxicity.

Treatment with *Cedrus deodara* ethanol extract demonstrated a dose-dependent restoration of hematological parameters. Notably, medium and high doses significantly improved platelet counts, hemoglobin concentration, and total blood cell counts when compared to the toxic control group. The reduction in bleeding and clotting times further indicates functional recovery of platelet activity and hemostasis. These findings suggest that CDEE exerts a protective and regenerative effect on hematopoietic tissue.

Bone marrow histopathological findings strongly support the hematological results. Hydroxyurea caused marked marrow hypocellularity with complete absence of megakaryocytes, whereas CDEE treatment, particularly at 200 and 400 mg/kg, restored marrow cellularity and megakaryocyte population. The hypercellular marrow observed at the highest dose suggests stimulation of hematopoietic progenitor cells, which is crucial for platelet regeneration.

The platelet-enhancing effect of CDEE may be attributed to its rich polyphenolic and flavonoid content identified by phytochemical and HPLC analyses. Compounds such as gallic acid, ferulic acid, caffeic acid, and chlorogenic acid are reported to possess antioxidant, antiviral, and hematopoietic-stimulating properties. These bioactive constituents may protect bone marrow cells from oxidative stress and enhance megakaryocyte differentiation.

The present in vitro investigations were designed to elucidate the anti-dengue potential of CDEE and its protective effects on endothelial cells during DENV-2 infection. Dengue virus endothelial interactions play a pivotal role in disease pathogenesis, particularly in vascular leakage, thrombocytopenia, and hemorrhagic manifestations.

Therefore, assessing viral cytopathic effects and endothelial cell protection provides valuable insight into the therapeutic relevance of plant-derived antiviral agents [20, 21].

Propagation of DENV-2 in Vero cells resulted in pronounced cytopathic effects, including cell rounding, spindle-shaped morphology, and detachment from the monolayer, confirming successful viral replication. These morphological alterations are characteristic of dengue virus–induced cellular injury and validate the suitability of Vero cells as a permissive host for DENV-2. The observed cytopathic changes are consistent with earlier reports demonstrating progressive cell damage following dengue virus infection, thereby establishing a reliable viral stock for subsequent assays.

Optimization of cell density for MTT and SRB assays was essential to ensure assay sensitivity, linearity, and reproducibility. The identification of distinct optimal seeding densities for the two assays reflects their different biochemical endpoints—mitochondrial metabolic activity for MTT and total cellular protein content for SRB. Such optimization minimizes experimental variability and strengthens the validity of cytotoxicity and antiviral assessments conducted using these assays.

Evaluation of the interaction time between DENV-2 and EA.hy926 endothelial cells revealed a time-dependent increase in cytopathic effects, with a 4-hour interaction producing significantly greater loss of cell viability compared to 2 hours. This finding underscores the rapid susceptibility of endothelial cells to dengue virus infection and supports previous observations that dengue virus can initiate cellular injury soon after adsorption. The use of EA.hy926 cells, a well-established endothelial cell model, further enhances the physiological relevance of the study, given the central role of endothelial dysfunction in dengue-associated vascular complications.

The estimation of viral titer using the MTT_{50} assay demonstrated measurable infective potential of DENV-2 following both 2- and 4-hour interactions. Although conventional methods such as $TCID_{50}$ or plaque assays are commonly employed, the MTT_{50} approach offers a practical alternative by quantifying virus-induced metabolic impairment in host cells. The observed titers indicate effective viral infectivity and support the use of metabolic assays as surrogate markers for viral cytopathogenicity in antiviral screening studies.

Assessment of CDEE cytotoxicity revealed that the extract was well tolerated by EA.hy926 cells across a wide concentration range, maintaining acceptable cell viability even after prolonged exposure. The modest enhancement of metabolic activity at certain concentrations suggests a possible stimulatory or protective effect on endothelial cell function. Importantly, the absence of significant cytotoxicity establishes a therapeutic window within which antiviral activity can be evaluated without confounding host cell damage.

The most significant finding of the in vitro study was the protective effect of CDEE against DENV-2–induced endothelial cell damage. Pre-treatment of the virus with CDEE resulted in marked preservation of endothelial cell viability, particularly at concentrations of 15.6 and 31.3 µg/mL. This observation strongly suggests that the extract exerts a direct virucidal or virus-neutralizing effect, likely through interaction with viral envelope proteins or disruption of viral integrity. In contrast, pre-treatment of endothelial cells with CDEE did not confer comparable protection, indicating that the extract does not primarily act by enhancing host cell resistance but rather by directly targeting the virus.

Such direct antiviral mechanisms have been reported for several plant-derived polyphenols, terpenoids, and flavonoids, which are known constituents of *Cedrus deodara*. These bioactive compounds may interfere with viral attachment, entry, or membrane fusion, thereby preventing successful infection [22, 23, 24, 25]. The concentration-

dependent protection observed further supports a specific antiviral action rather than a nonspecific cytoprotective effect.The findings indicate that *Cedrus deodara* heartwood extract possesses significant platelet-enhancing and hematopoietic restorative potential, supporting its possible therapeutic role in managing dengue-associated thrombocytopenia.

5.0 Conclusion

The present in vivo suggesting that the ethanol extract *Cedrus deodara* possesses to protect platelets against hydroxyurea induced destruction while in vitro study revealed the significant antiviral property. Further study is require to conduct *in vivo* antiviral study for exact benefits of *Cedrus deodara* against dengue infection. And also research is essential to specific constituent causing anti-dengue potentials and also to determine their mechanism.

Declarations

Competing interest

We now declare that no conflicts of interest exist.

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The authors declare that no funding was received for the present research work. All requirements related to the study were provided by the affiliated institutions of authors.

Author Contribution

ToThe Editor-in-ChiefJournal of Pharmaceutical InnovationSpringer NatureSubject: Author Contribution and Declaration for Manuscript SubmissionDear Editor-in-Chief,We hereby submit our manuscript entitled “Investigation of the anti-dengue and platelet-enhancing potential of Cedrus deodara heart wood extract: In vitro and in vivo evaluation in a Hydroxyurea-induced thrombocytopenia model” for consideration for publication in the Journal of Pharmaceutical Innovation.We confirm that all authors have made substantial intellectual and practical contributions to this work. The specific contributions of each author are detailed below:Sl.No Name of Author Details of contribution1. Keshava Kyatanahalli Shekharappaa Conceptualization, study design, execution of in vitro experiments, data analysis, and preparation of the original manuscript draft.2. Ramesh C Assistance in experimental work, data collection, interpretation of results, and manuscript drafting.3. Parikshit Roychowdhury: In vivo experimentation, animal handling, and biochemical and hematological assessments.4. Girish Bolakattic Supervision of the study, Statistical analysis, data validation, and interpretation of experimental findings.5. Gowthamarajan Kuppusamy Supervision of the study, critical revision of the manuscript, project administration, and overall responsibility for the integrity of the work.6. Amith Kumar B Methodology development, laboratory support, and experimental troubleshooting.7. Rakesh S. A Data curation, literature review, and manuscript editing.8. Pratiksha CC Visualization of results, preparation of figures and tables, and manuscript review.9. Sahed Alikhan Technical support, quality control of experimental procedures, and final manuscript proofreading.All authors have read and approved the final manuscript and agree to be accountable for all aspects of the work.We further declare

that the manuscript is original, has not been published previously, and is not under consideration for publication elsewhere. All animal experiments were conducted in compliance with institutional and national ethical guidelines, with appropriate approvals obtained. The authors declare that there are no conflicts of interest associated with this manuscript.We believe that the findings presented in this study will be of significant interest to researchers and professionals working in the areas of pharmaceutical innovation, phytopharmacology, and antiviral therapeutics.Thank you for your time and consideration.Yours sincerely,Dr. Gowthamarajan KuppusamyCorresponding Author(On behalf of all authors)

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References

1. Gubler DJ. Dengue and dengue hemorrhagic fever. Clin Microbiol Rev 1998;11(3):480–496.
2. Aswinikumar CR,Vinay P. Clinical manifestations and trend of dengue cases admitted in a tertiary care hospital, Udipi district, Karnataka. Ind J Commu Med 2010;35(3):386–90.
3. Normile D. Tropical medicine. Surprising new dengue virus throws a spanner indisease control efforts. Science 2013;342(6157):417.
4. Chen LH, Wilson ME. Dengue and chikungunya infections in travel ers. Curr Opin Infect Dis 2010;23(5):438–44.
5. Tayal A, Kabra SK, Lodha R. Management of Dengue: An Updated Review. Indian J Pediatr. 2023;90(2):168–177.
6. Idrees S, Ashfaq UA. A brief review on dengue molecular virology, diagnosis, treatment and prevalence in Pakistan. Genet Vaccines Ther. 2012;10(1):6.
7. Sreedharan A, R VL, B V. Ayurvedic management of dengue haemorrhagic fever with menorrhagia: A case report. J Ayurveda Integr Med. 2024 May-Jun;15(3):100923.
8. Abd Kadir SL, Yaakob H, Mohamed Zulkifli R. Potential anti-dengue medicinal plants: a review. J Nat Med. 2013;67(4):677–89.
9. Bisht A, Jain S, Misra A, Dwivedi J, Paliwal S, Sharma S. Cedrus deodara (Roxb. ex D.Don) G.Don: A review of traditional use, phytochemical composition and pharmacology. J Ethnopharmacol. 2021;279:114361.
10. Kokate CK. Practical Pharmacognosy. New Delhi; Vallabh Prakashan: 1994;4:100–114.
11. KR Khandelwal, Practical Pharmacognosy-Techniques and Experiments. Pune; Nirali Prakashan; 2000.
12. Geetha M. Evaluation of efficacy of *Carica papaya* leaf extracts to increase platelet count in hydroxyurea induced thrombocytopenia in Albino rats. International Journal of Basic & Clinical Pharmacology. 2018;7(1):173.
13. Khamis AM, Oda SS, Khafaga AF, Hashem MA. Hematocytological evaluation of hydroxyurea-induced toxicity in male Rats. Alexandria J Vet Sci 2017;55(2).
14. Wiggins RW, Woo J, Cauba JN, Mito S. Evaluating the Potential of Herbal Extracts as Treatment in Immune Thrombocytopenia: A Review of Evidence and Limitations. App Biosci 2025; 4(1):1.

15. Munezero PC, Handunnetti S, Fernando N, Ranaweera L, Hapuarachchi SD, Nyamweya BM, Ndacyayisenga J. Preliminary study on the effect of *Munronia pinnata* (Wall) Theob.(Meliaceae) aqueous extract on functional change in endothelial cells infected by dengue virus. Scientific African. 2023;21:e01861.
16. Flores-Ocelotl MR, Rosas-Murrieta NH, Moreno DA, Vallejo-Ruiz V, Reyes-Leyva J, Domínguez F, Santos-López G. Taraxacum officinale and Urtica dioica extracts inhibit dengue virus serotype 2 replication in vitro. BMC complementary and alternative medicine. 2018;18(1):95.
17. Tauscher J, Siegel F, Petrides PE. Hydroxyurea induced oscillations in twelve patients with polycythemia vera. Haematologica. 2010;95(7):1227–9.
18. Al-ameedi AI, Al-Ani MM, Sahib BS. Acute toxicity of hydroxyurea in male albino mice. Int J Pharm Sci & Res 2018;9(7):2960–4.
19. Gammulle A, Ratnasooriya WD, Jayakody JR, Fernando C, Kanatiwela C, Udagama PV. Thrombocytosis and anti-inflammatory properties and toxicological evaluation of Carica papaya mature leaf concentrate in a murine model. International Journal of Medicinal Plants Research. 2012;1(2):21–30.
20. MithuN M, Rajashekaraiah V. Antioxidants As supplements during drug-induced thrombocytopeniA: A compArAtive AnAlysis of vAnillic Acid, l-cArnitine And cAripil. The Ukr Biochem J. 2024;96(1):49–59.
21. Joseph B, Sankarganesh P, Ichiyama K, Yamamoto N. In vitro study on cytotoxic effect and anti-DENV2 activity of Carica papaya L. leaf. Frontiers in Life Science. 2015;8(1):18–22.
22. Jain J, Kumar A, Narayanan V, Ramaswamy RS, Sathiyarajeswaran P, Devi MS, Kannan M, Sunil S. Antiviral activity of ethanolic extract of *Nilavembu kudineer* against dengue and chikungunya virus through in vitro evaluation. J Ayur and Int Med. 2020;11(3):329–35.
23. Munezero PC, Handunnetti S, Fernando N, Ranaweera L, Hapuarachchi SD, Nyamweya BM, Ndacyayisenga J. Preliminary study on the effect of *Munronia pinnata* (Wall) Theob.(Meliaceae) aqueous extract on functional change in endothelial cells infected by dengue virus. Scientific African. 2023;21:e01861.
24. Mohd Abd Razak MR, Norahmad NA, Md Jelas NH, Jusoh B, Muhammad A, Mohmad Misnan N, Zainol M, Thayan R, Syed Mohamed AF. Preliminary study on the expression of endothelial cell biology related genes in the liver of dengue virus infected mice treated with Carica papaya leaf juice. BMC research notes. 2019;12(1):206.
25. Inyoo S, Suttitheptumrong A, Pattanakitsakul SN. Synergistic effect of TNF-α and dengue virus infection on adhesion molecule reorganization in human endothelial cells. Japanese journal of infectious diseases. 2017;70(2):186–91.

Figures

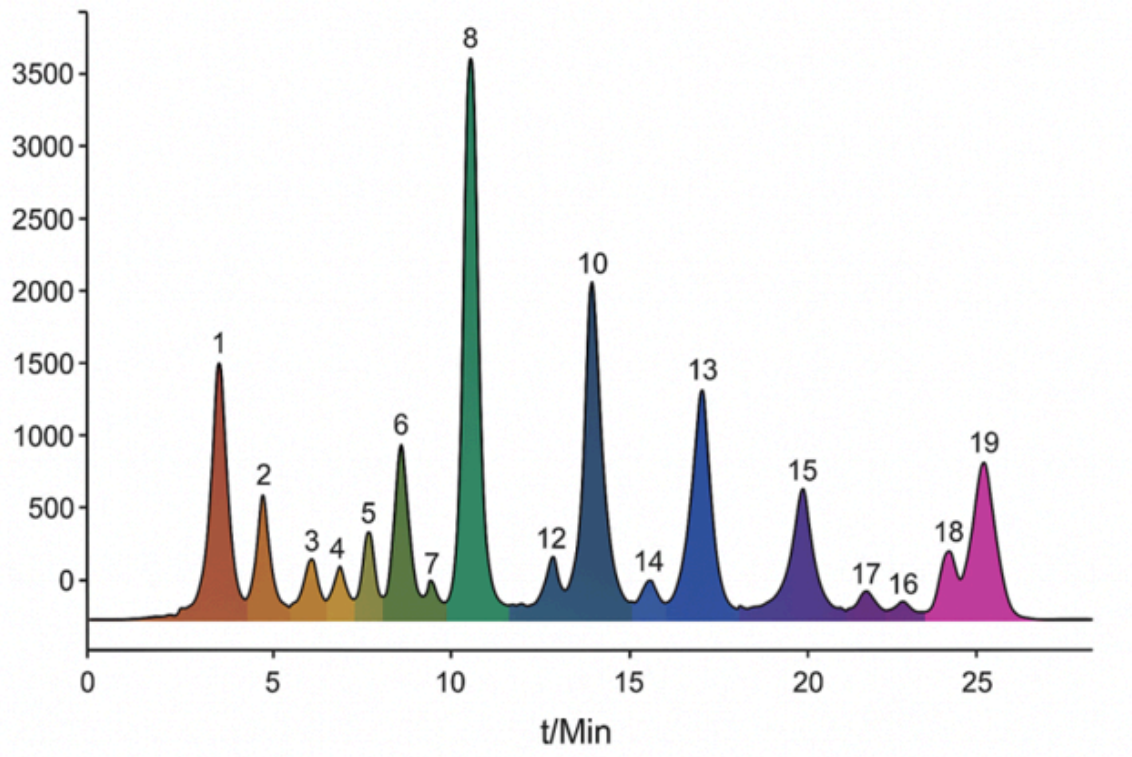

Figure 1

HPLC chromatogram of CDEE medicinally potential componds

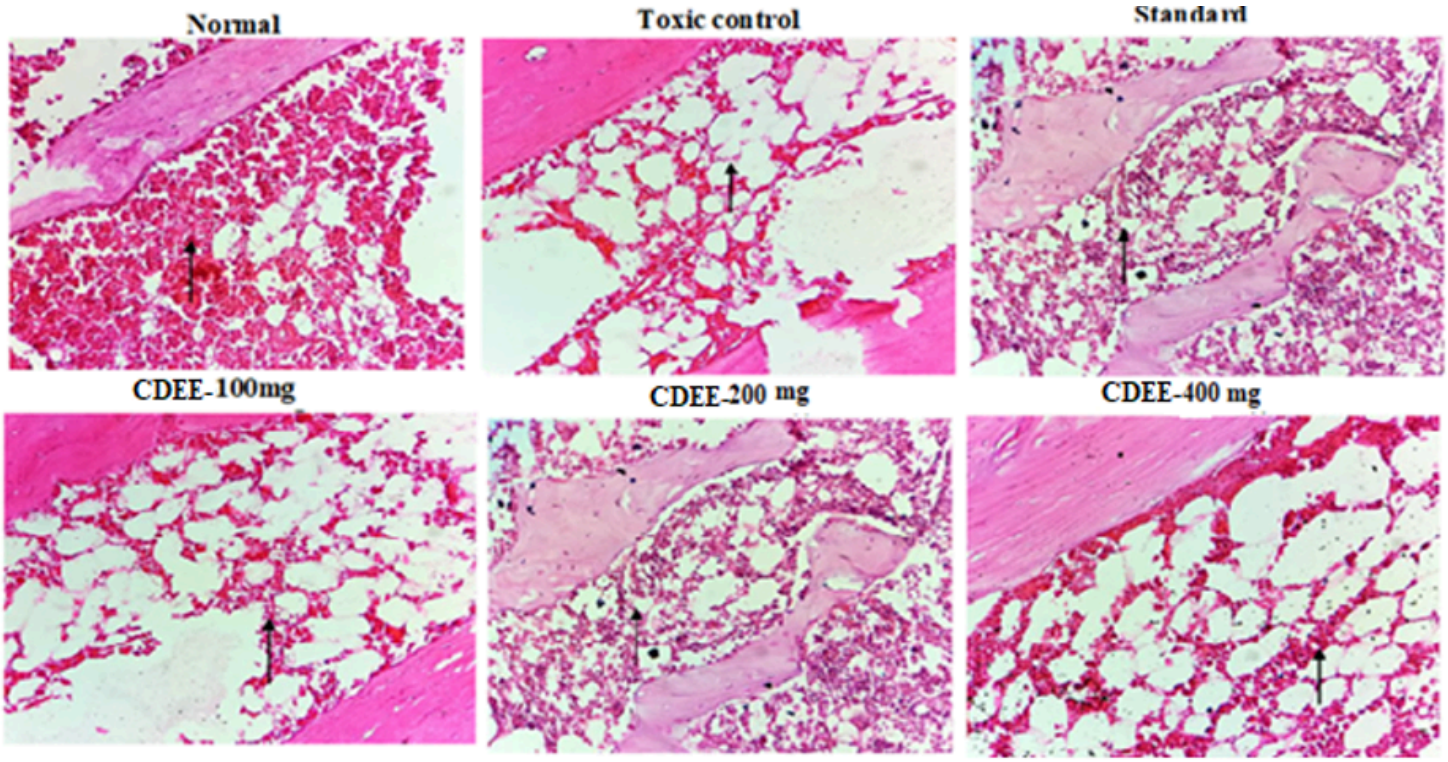

Figure 2

Effect of CDEE on histopathology of bone marrow against hydroxyurea induced thrombocytopenia

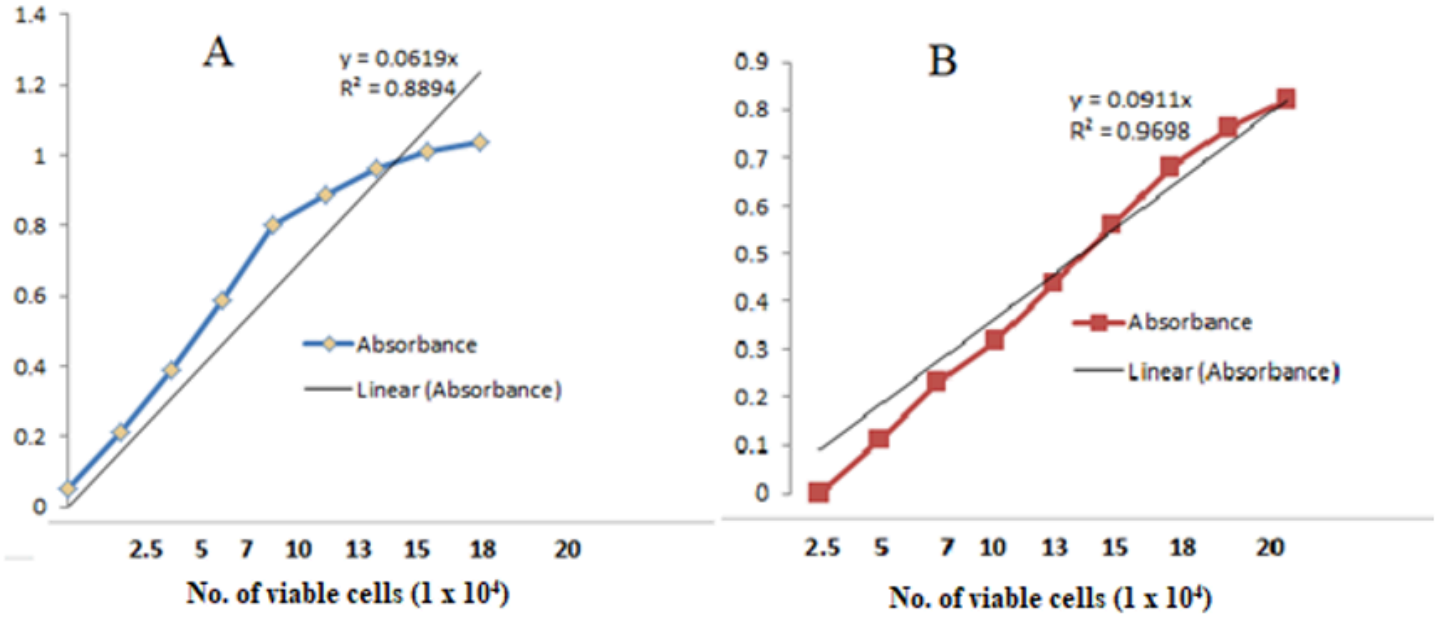

Figure 3

The MTT and SRB assay optimization EC curve

Error bars represent ± SEM and R2 (R-squared value) represent the fitness of data points to the linear trend.

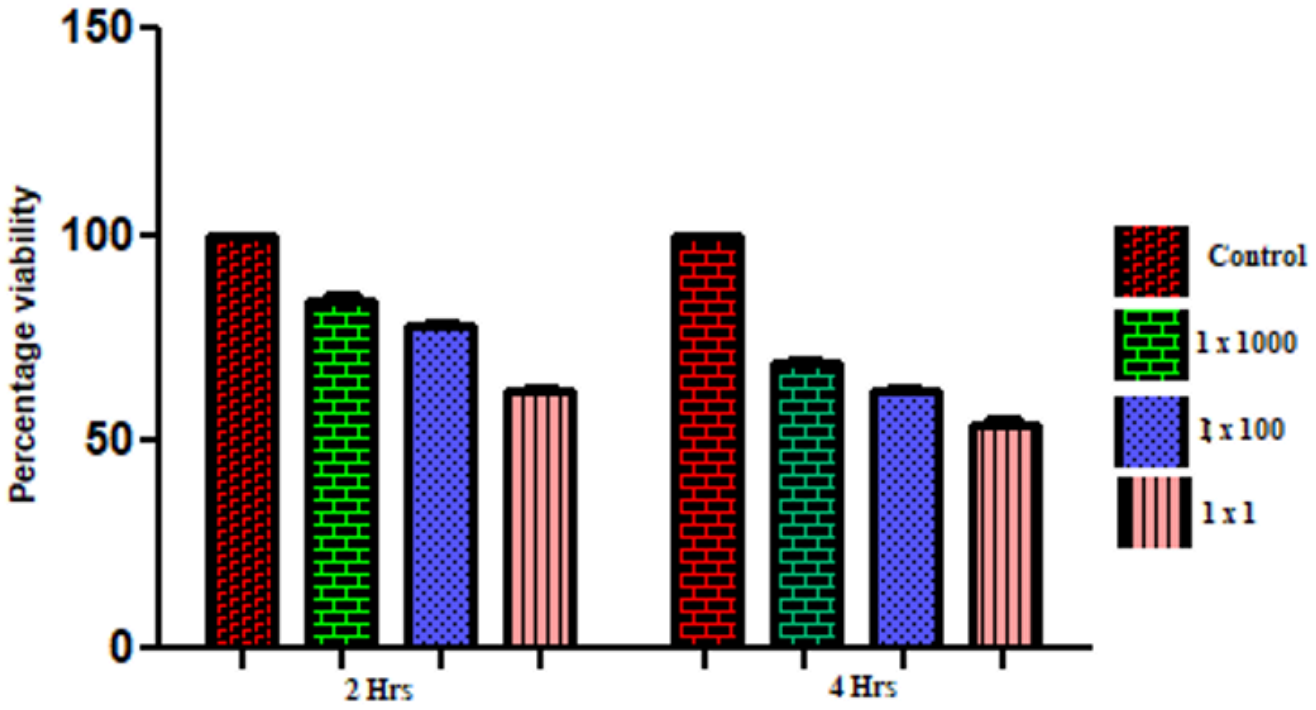

Figure 4

Effect of CDEE on cell viability of EA.hy926 against DENV-2

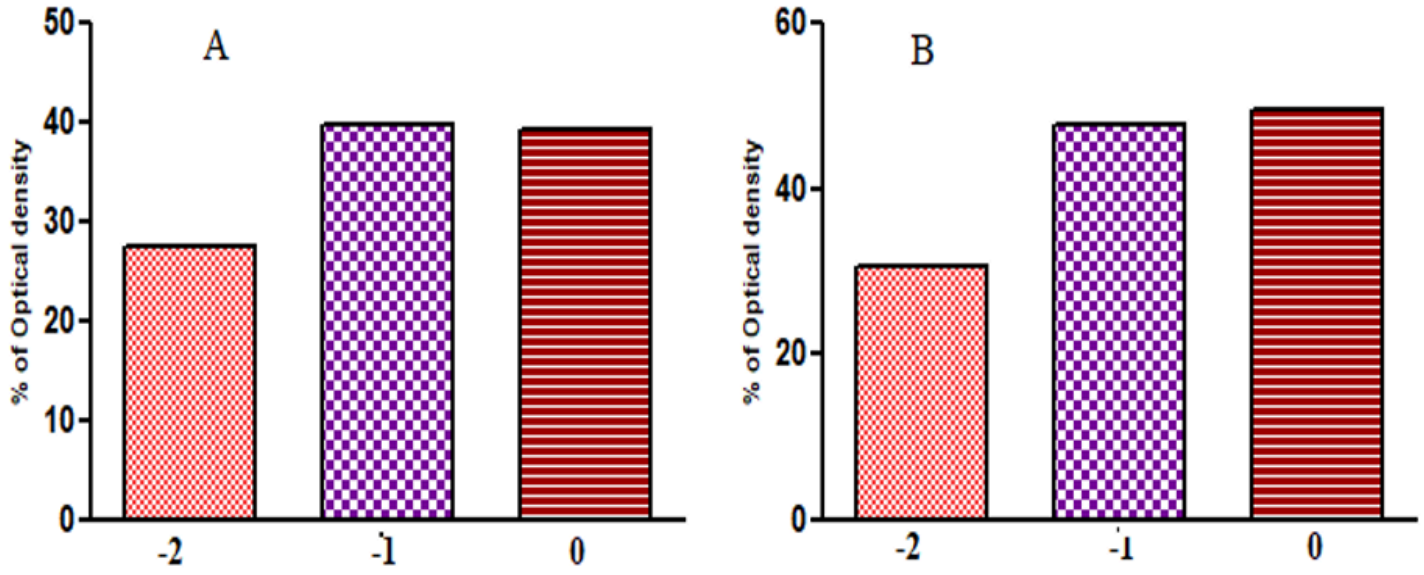

Figure 5

Impact of CDEE at varying dilutions on the MTT titer of DENV-2 stock.

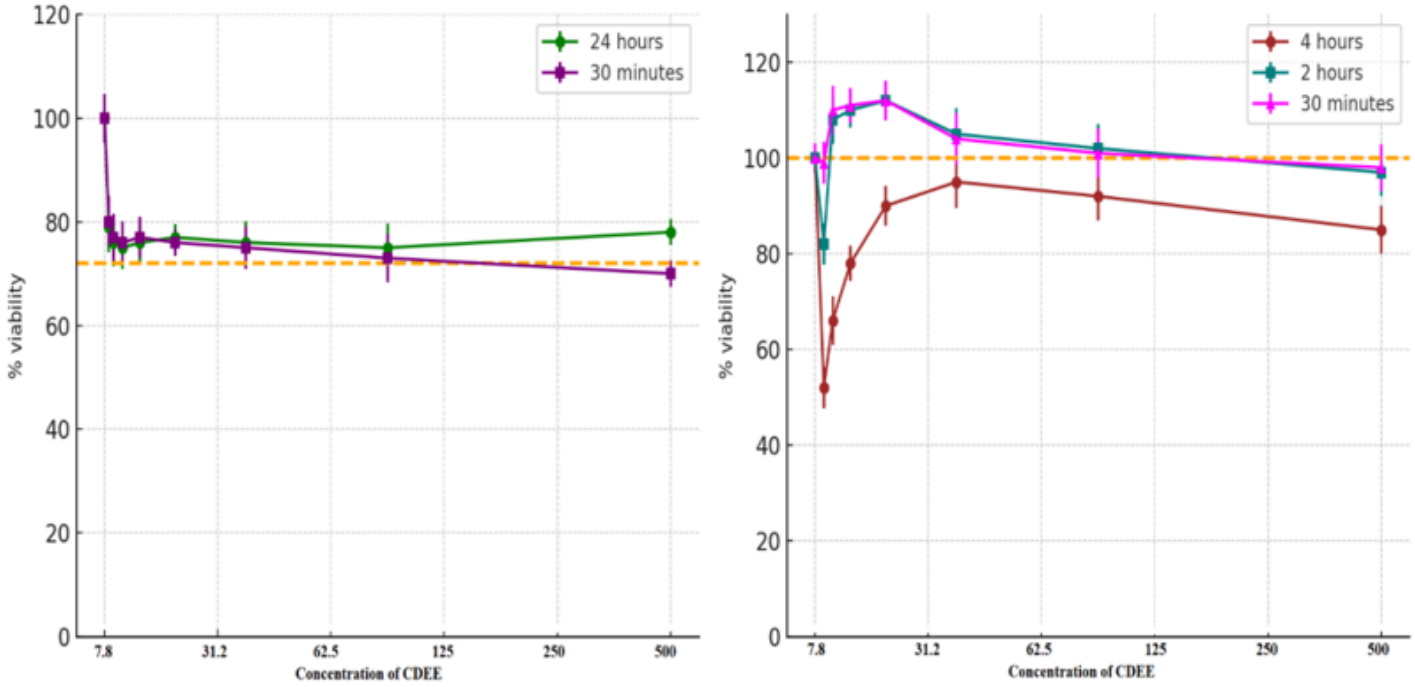

Figure 6

***Cedrus deodara*ethanol extract's impact on ECs' cellular protein levels.**

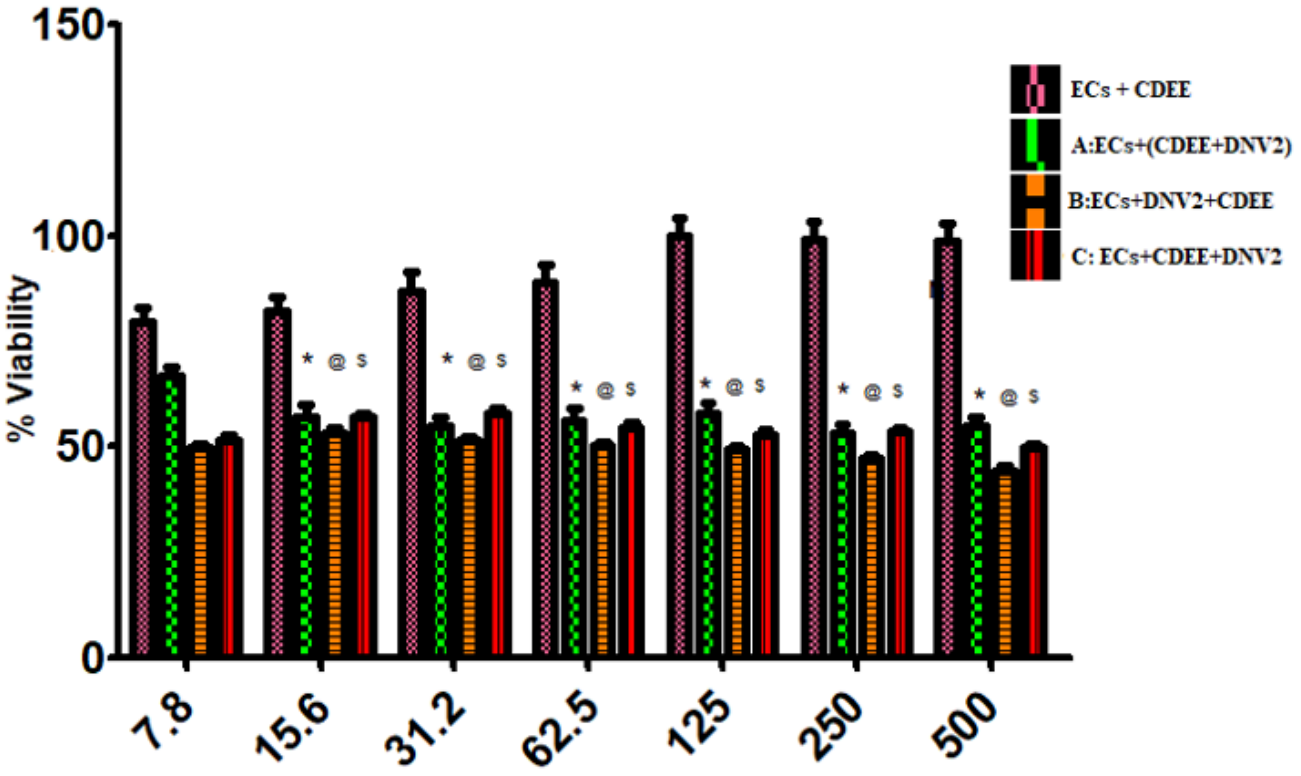

Figure 7

Effect of CDEE against growth of DENV-2 interacted with EA.hy926

Data were analyzed by two-way ANOVA with Dunnett's post-test; *, @, and $ indicate $p < 0.01$. Error bars represent ± SEM.

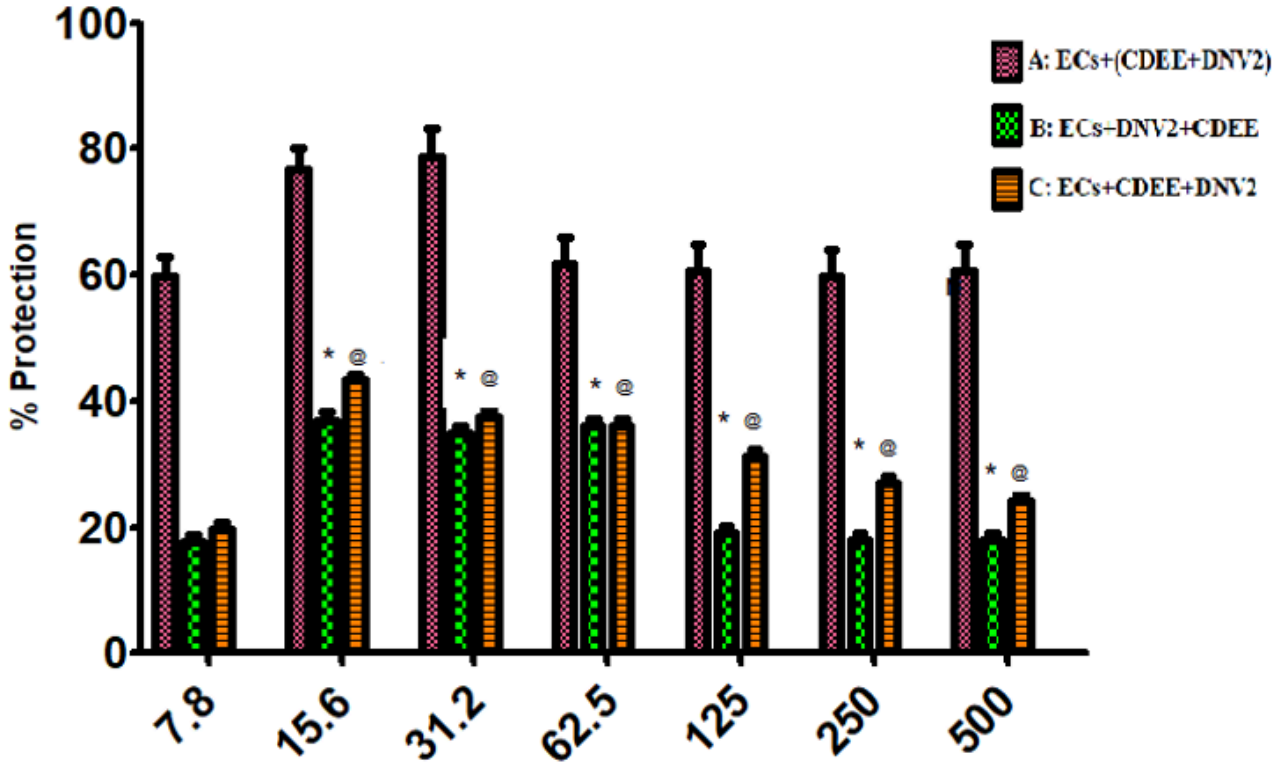

Figure 8

Effect of CDEE against protection of DENV-2 interacted with EA.hy926

The percentage cell death of EA.hy926 after interaction with pre-treated DENV-5 with CDEE at concentration from 7.8 µg/mL to 500 µg/mL in triplicate were compared to the cell death of infected cells in the absence of CDEE to obtain the resulting protection of ECs in contact with DENV-3 and CDEE (error bars represent ± SEM). The correlation between protection and CDEE concentration was calculated after excluding 7.8 µg/mL.