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Determination of antioxidant and anti-inflammatory activity of ethanolic extract of Ailanthus excelsa on SHSY-5Y cells

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Abstract

Ailanthus excelsa plant belonging to the Simaroubaceae family has been used for various biological activities due to the presence of various sterols, phenols, saponins, flavonoids, glycosides and proteins which were also confirmed by performing preliminary studies. It is hepatoprotective, hypoglycemic, anti-fungal, anti-bacterial, anti-oxidant and anti-inflammatory in nature. Using SHSY-FY human neuroblastoma cell lines, the antioxidant, cytotoxic, and anti- inflammatory properties of A. excelsa ethanolic extract (AEEE) were investigated in this present work. Firstly, stem bark of this plant were collected and progressively extracted using ethanol as a solvent with the help of cold maceration method. The percentage yield was then computed. The concentration of phenols and flavonoids was evaluated to assess the anti-oxidant potential which was calculated as 39.51±0.167 mg GAE/gm and 24.92±0.261 mg QE/gm respectively. DPPH (2,2-diphenyl-1-picrylhydrazyl) method was also used to evaluate the antioxidant profile in which percentage inhibition was observed as 78.751±0.005 % which was compared with the standard i.e. ascorbic acid (89.165±0.233 %). Moreover, MTT assay was also performed which has showed a significant decline in the cell viability at a concentration of 200-1200 µg/ml. Along with this, it also consists anti-inflammatory activity and antioxidant activity. The cells were exposed with the concentrations of 100 and 200 µg/ml of ethanolic extract of A. excelsa in which it decreased the level of IL-6, MDA and increased the level of GSH (glutathione). Therefore, all the results suggested that A. excelsa could be a potential therapeutic approach for the treatment of neurodegenerative diseases which are related with oxidation and inflammation.

Keywords: Ailanthus excelsa, DPPH, Antioxidant, IL-6, and ethanolic extract, SHSY-5Y cell line.

Introduction

Many disorders can now be treated with therapeutic medicines derived from natural resources.

Ailanthus plants belonging to the Simaroubaceae family are of extremely valuable because of their numerous therapeutic benefits [1]. In traditional medicine, several portions of this plant are used to treat a wide range of diseases. It has been observed that the root bark has cytotoxic and anticancer properties in both animals and cell cultures [2]. Extracts from stem bark shown strong antifungal and antibacterial properties [3, 4]. The alcohol extract derived from the bark of leaves and stems demonstrates exceptionally strong anti-implantation and early abortifacient properties [5]. A. excelsa is filled with diverse chemical compounds with a wide range of possible biological uses such as sterols, alkaloids, phenols, terpenoids, flavonoids, and quassinoides [6]. Since free radicals can trigger inflammatory reactions, antioxidants play a crucial role in reducing inflammation. Inflammatory diseases like arthritis, heart problems, and neurological disorders have been related to chronic oxidative stress. By lowering oxidative damage, plant species containing antioxidant property help to promote cellular health and modulate inflammatory pathways [7]. In this study, SHSY-5Y cell lines were used which are neuroblastoma cells to check the anti-oxidant and anti-inflammatory potential due to which this plant extract can be used as a therapeutic approach for the treatment of neurodegenerative diseases.

Material and methods

Preparation of Extract

Plant material (stem bark) was purchased from Khari Baoli, Delhi, India, and authenticated at CCS University, Meerut, Uttar Pradesh, India. Firstly, the stem bark was properly washed, shade dried, grinded in powdered form, and finally extracted by cold maceration method using ethanol as a solvent. The obtained extract was reduced with a rotary evaporator, weighed, collected in a air tight container, and preserved in a refrigerator for further work.

Evaluation of total phenol and flavonoid content

In this plant extract, the concentration of phenol was demonstrated by the Folin Ciocalteu method used by Singleton et al., 1999 and total flavonoid by AlCl3 method described by Ebrahimzadeh et al., 2008 [8, 9].

Culture of cells

The SHSY-5Y cells were cultured at 37°C in a humidified atmosphere with 5% CO2 in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS), growth factors, and antibiotics [10].

DPPH assay

The free radical scavenging activity of AEEE was evaluated by DPPH method used by Munda et. al., 2019 [11].

Cell viability Assay

SH-SY5Y cells were cultured in high-glucose DMEM with 10% fetal bovine serum. The cytotoxicity of extract doses was evaluated using the MTT test. After 24 hours, cells were treated with various extract doses. After a 24-hour break, MTT was applied to each well, and the iMarkTM Microplate Absorbance Reader determined the absorbance [12].

Determination of IL-6

The concentration of interleukin-6 was determined by the method described by Dietrich et. al., 2020 [13].

Determination of GSH

The study used SH-SY5Y cells to detect GSH levels, which were then stimulated with LPS for

23 hours. The DetectX Glutathione Fluorescence Detection Kit and iMARkTM microplate absorbance reader were used to detect total and free GSH from cell lysate, with excitation and emission wavelengths set to 370-410 nm and 450 nm, respectively [14].

Determination of MDA

Enzyme-Linked Immunosorbent Assay was used to quantify the cell-permeable concentrations of MDA. A twelve-well plate with 125 SH-SY5Y cells was pre-treated with NAC and LPS for 23 hours. Cell pelletization was done, and liquid was collected. The iMARkTM microplate absorbance reader was used to read the samples at 450 nm [15]

Statistical analysis

The study used Graph Pad Prismsoftware, Version 5.01, to interpret results using T-test, One- way ANOVA, and Tukey post hoc test, with $p<0.05$ probability value.

Results

Total phenol and flavonoid concentration

The anti-oxidant potential which was observed as 39.51±0.167 mg GAE/gm and 24.92±0.261 mg QE/gm respectively

Inhibitory activity of AEEE on DPPH

Significant percentage inhibition was observed by AEEE (78.751±0.005 %) which was compared with ascorbic acid ((89.165±0.233 %). (Figure-1)

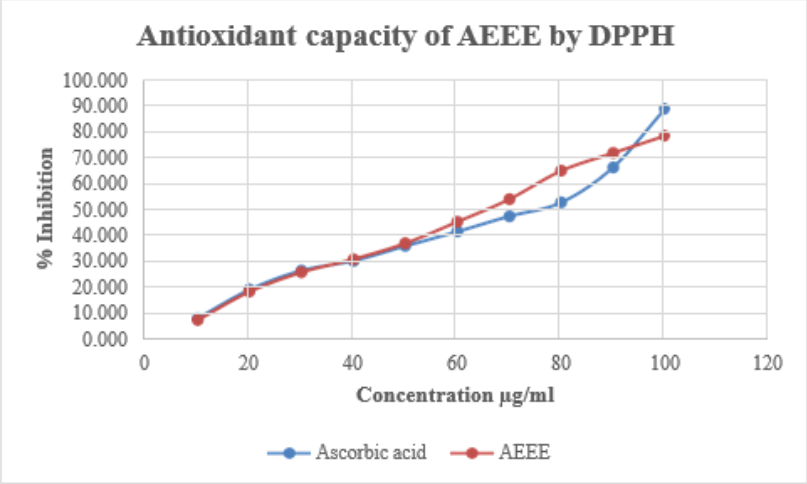

Figure:1 Antioxidant potential of AEEE.

The % inhibition of the test sample was compared with Ascorbic acid at different concentrations.

Inhibitory activity of AEEE on cell viability

The plant extract AEEE decreased the concentration the percentage of cell viability when treated with different doses (200-1200 µg/ml) ($p<0.0001$).(Figure-2)

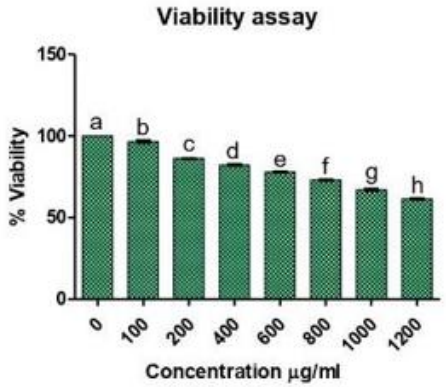

Figure:2 Cytotoxic effect of AEEE.

After treatment of the cells, cell viability was measured according to the concentration of formazan produced by mitochondrial dehydrogenase enzymes in the cells and compared to the untreated cells. Data were measured by One-way ANOVA followed by Tukey post hoc test and represented as mean±SEM. $p<0.05$.b, c, d, e, f, g, h versus a.

Inhibitory activity of AEEE on IL-6

The plant extract AEEE at a dose 200 µg/ml decreased the concentration of IL-6 more significantly than the dose 100 µg/ml as compared to LPS treated cells ($p<0.001$).(Figure-3).

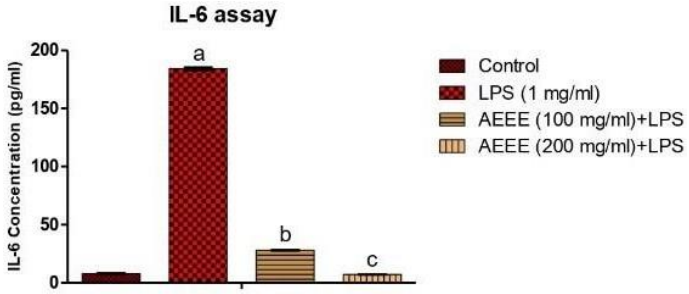

Figure:3 Inhibitory activity of AEEE on IL-6 concentration.

Group-1 normal control, and the negative control group (Group-2) was given LPS (1 µg/ml), and both test groups (Group-3 & 4) were treated with AEEE (100 and 200 µg/ml). Data were measured by One-way ANOVA followed by Tukey post hoc test and represented as mean±SEM. a versus Normal control; b versus LPS (1 µg/ml); c versus AEEE (100 µg/ml).

Inhibitory activity of AEEE on GSH

In comparison to the normal control group, the LPS-treated cells showed a significant decline in the level of GSH ($p<0.0001$). Whereas, AEEE at a dose 100µg/ml treated cells significantly increased the level of GSH as compared to the LPS-treated group. Moreover, the AEEE at a dose 200µg/ml group demonstrated a significant elevation in the level of GSH than AEEE (100µg/ml) ($p<0.001$).(Figure-4).

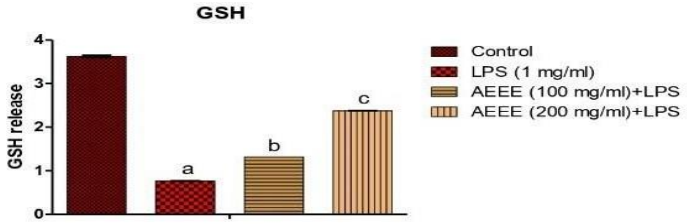

Figure:4 Protective action of AEEE on GSH.

Group-1 was normal control, and the negative control group (Group-2) was given LPS (1 µg/ml), and both test groups (Group-3 & 4) were treated with AEEE (100 and 200 µg/ml). Data were measured by One-way ANOVA followed by Tukey post hoc test and represented as mean±SEM. a versus Normal control; b versus LPS (1 µg/ml); c versus AEEE (100 µg/ml).

Inhibitory activity of AEEE on MDA

In comparison to the normal control group, the LPS-treated cells showed a significant increase in the level of MDA ($p<0.0001$). Whereas, AEEE (100µg/ml) treated cells significantly decreased the level of MDA as compared to the LPS-treated group. Moreover, the AEEE (200µg/ml) group demonstrated an effective decline in the level of MDA than AEEE (100µg/ml) ($p<0.0001$).(Figure-5).

Figure:5 Inhibitory activity of AEEE on MDA.

Group-1 was normal control, and the negative control group (Group-2) was given LPS (1 µg/ml), and both test groups (Group-3 & 4) were treated with AEEE (100 and 200 µg/ml). Data were measured by One-way ANOVA followed by Tukey post hoc test and represented as mean±SEM. a versus Normal control; b versus LPS (1 µg/ml); c versus AEEE (100 µg/ml).

Conclusion

The ethanolic extract of A. excelsa has demonstrated antioxidant and anti-inflammatory properties due to its high concentration of phenols and flavonoids, demonstrating notable radical scavenging activity in the phytochemical study. The stem ark of this plant has also showed anti- inflammatory activity, reducing IL-6 concentration, MDA and increasing GSH. Further studies are needed to identify compounds responsible for these effects. The anti-oxidant and anti-inflammatory activity of this plant suggested that it could be a therapeutic potential for the treatment of various neurological diseases.

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Conflict of Interest

The authors declare no conflict of interest.

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