**In-vitro Anti-Cancer and Anti-Inflammatory Screening of Dodonaea viscosa**

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**Authors’ contributions**

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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**ABSTRACT**

**Background:** The current investigation was done to assess the in vitro anticancer property of *Dodonaea viscosa* (*D. viscosa*) in three malignant growth cell lines and mitigating impact in RAW 264.7 macrophages.

**Methods:** The hydroalcoholic remove *D. viscosa* was ready and tried against HCT-116 colon malignancy, MCF-7 bosom disease HeLa cervical disease cell lines. The cytotoxicity of concentrate was affirmed by MTT cheeky. The calming movement of concentrate was assessed utilizing LPS invigorated RAW 264.7 macrophages and the degree of incendiary middle people was estimated.

**Results:** The anticancer impact of *D. viscosa* onHCT-116, MCF-7 and HeLa cell line with the IC50 worth of 60.43 ± 0.76 μg/ml, 75.26 ± 0.45 μg/ml and 72.12 ± 0.87μg/ml individually. Further, in LPS stimulatedRAW264.7 macrophage cells, treatment with *D. viscosa* extract altogether decreased the raised level of NO, TNF-α and PGE2.

**Conclusion:** This examination gave the proof to *D. viscosa* an anticancer and mitigating specialist. Further bioactive confinement and atomic examinations are needed to prove the impact of plant remove.
Keywords: D. viscosa; cancer cell lines; cytotoxicity; RAW264.7 macrophage; inflammatory mediators.

1. INTRODUCTION

Cancer is a staggering sickness all around the world which forces huge dismalness and mortality. Because of need early discovery of the illness and the greater part of the patients are identified at the last stage, the administration becomes troublesome [1,2]. But there is an uncontrolled progression in the field of medication for the administration of different infection, yet the board of malignant growth stays as one of the immense difficulties to specialists [3]. Among the different illnesses, death rate in disease is high because of its metastatic potential influencing other essential organs [4]. Mounting epidemiological and pharmacological investigations demonstrate that every day admission of specific phytochemicals can minify the pervasiveness of wide scope of malignancy [5,6]. Normal items have been generally utilized in the administration of different sorts of malignancy because of its apoptosis and hostile to proliferative impacts [7].

Greater part of the counter malignancy drugs, around 60% supported for the administration of disease are plant-based medications or normal items detailing [8]. Likewise, aggravation and oxidative pressure arrange a vital part in the advancement of different diseases [9]. At present, nonsteroidal calming drugs (NSAIDs) are the backbone in the administration of incendiary illnesses, notwithstanding it evokes harmful unfriendly impacts, for example, gastro-poisonousness and furthermore causes colossal monetary weight [10].

Dodonaea viscosa Linn. (Sapindaceae) ordinarily known as 'virali' is an evergreen enduring bush broadly circulated in Western Ghats and Tamilnadu. The old stories guarantee uncovers that the leaves have been utilized for the treatment of cerebral pains and spinal pains by the Muthuvan clans of the Kerala area. High temp water decoction of leaves is utilized to diminish swellings, spinal pains and steam inward breath is utilized to lighten cold. Further, in conventional clinical practice D. viscosa is utilized to ease stomach torment, heaps and ulcer. Past investigations have revealed the mitigating, antimicrobial, neighbourhood sedative and hostile to disease action of D. viscosa [11,12]. Hence the current examination was meant to assess the counter malignancy and calming of hydro alcoholic concentrate of D. viscosa on different invtro models.

2. MATERIALS AND METHODS

2.1 Cell Lines

HCT 116 (Colon malignancy), MCF-7 (Breast disease), HeLa (cervical carcinoma) and RAW264.7 murine macrophage were bought from Sigma Aldrich, USA.

2.1.1 Plant material

The leaves of D. viscosa were gathered in the month August 2018, from Trichy, Tamilnadu, India. The plant material was distinguished and verified by the botanist. The plant materials were dried under conceal, cut into little pieces, pounded utilizing a mechanical processor and went through 40 lattice strainer and put away in a water/air proof compartment for additional utilization.

2.1.2 Extraction of plant material

The powdered leaves of D. viscosa were removed with hydro liquor at room temperature. After thorough extraction, the dissolvable was gathered and separated. The dissolvable was focused under lessen pressure at 50-55°C. The concentrated hydro liquor extricates were kept in desiccators for additional utilization.

2.1.3 Qualitative phytochemical analysis

The rough hydro alcoholic concentrate of D. viscosa leaves were investigated for the presence of different phytoconstituents by keeping standard phytochemical conventions. The presence of alkaloid (Dragendorff reagent, Mayer's reagent, Hagers reagent and Wagner's reagent), flavonoids (Shinoda-Paw test), steroids (Liberman Burchard test and Salkowski's response), terpenes (Vanillin sulfuric corrosive reagent) and carbs (Fehlings test and Molisch test) were dissected.

2.2 In vitro anticancer activity

The in vitro anticancer movement of D. viscosa extract was assessed utilizing HCT 116, MCF-7 and HeLa cell lines. HCT116 colon disease cell line was filled in RPMI 1640 medium enhanced with 10% fetal ox-like serum (FBS). MCF-7 bosom malignancy and HeLa cervical carcinoma
cell lines were filled in Eagle's base fundamental medium enhanced with 10% FBS and developed at 37°C in a humidified 5% CO₂ hatchery.

For cytotoxicity test, the cells were cultivated at 2 × 10³ cells/well into 96-well cell culture plates. Then, at that point the plant removes at different focuses (2.5, 5, 10, 20, 40, 80 and 120 μg/mL) were added to the 96 well plate for 72 h. MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) colorimetric test was done to quantify the development of cell [13]. The absorbance of each all-around was estimated at the frequency of 540 nm utilizing a microplate per user.

2.3 In vitro ANTI-INFLAMMATORY ACTIVITY

2.3.1 Cell culture and cytotoxicity assay

The in vitro calming movement of D. viscosa was assessed in RAW264.7 murine macrophage cells. Momentarily, the cells were filled in DMEM medium enhanced with 10% FBS and developed at 37°C in a humidified 5% CO₂ hatchery. Then, at that point the cells were cultivated at 2 × 10³ cells/well into 96-well cell culture plates with different convergences of the plant removes (2.5, 5, 10, 20, 40, 80 and 120 μg/mL) and brooded for 24 h. MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) colorimetric examine was done to quantify the development of cell. The absorbance of each very much was estimated at the frequency of 540 nm utilizing a microplate per user.

2.3.2 Measurement of no production

The NO creation was estimated utilizing the strategy for Griess et al. [14]. Then the RAW264.7 cells were cultivated at 5 × 10⁵ cells / well into 96-well cell culture plates with different groupings of the plant extricates (2.5, 5, 10, 20 μg/mL) and hatched for 1 hour, trailed by expansion of 1 μg/mL LPS and afterward brooded for 24 hours. After the hatching time frame, the supernatant was gathered and utilized for the assessment of PGE2, TNF-α in RAW264.7 cells utilizing ELISA packs as per the assembling’s data (Sigma Aldrich, USA).

3. STATISTICAL TOOLS

3.1 Results

3.1.1 Cytotoxic effect of D. viscosa in various cell lines

In this investigation, D. viscosa extract displayed a checked cytotoxicity towards HCT-116, MCF-7 and HeLa cells in portion subordinate way. The IC₅₀ for HCT-116, MCF-7 cells and HeLa cells was found to 60.43 ± 0.76 μg/ml, 75.26 ± 0.45 μg/ml and 72.12 ± 0.87 μg/ml separately. The outcomes were displayed in Fig. 1.

3.1.2 Effect of D. viscosa on no release in LPS stimulated RAW264.7 macrophages

Our outcomes uncovered that the concentrate of D. viscosa displayed critical restraint of LPS-actuated NO creation in portion dependant way when contrasted with LPS-treated cells without plant extricates. The outcomes were displayed in Fig. 2.

3.1.3 Anti-inflammatory activity of D. viscosa in LPS stimulated RAW264.7 macrophages

In this examination, LPS invigorated RAW264.7 macrophages showed huge height of PGE2 and TNF-α in the medium. In the meantime, treatment with D. viscosa at different focuses, for example, 5, 10, 20 μg/mL showed critical hindrance of PGE2 and TNF-α levels in a portion subordinate way when contrasted with LPS alone invigorated cells. The outcomes were displayed in Fig. 3 and Fig. 4.
Fig. 1. Cytotoxic effect of *D. viscosa* on various cell lines; The percentage cell viability of HCT-116, MCF-7 and HeLA cancer cell lines were markedly decreased when the drug concentration were increased.

Fig. 2. Effect of *D. viscosa* on NO inhibition in LPS stimulated RAW264.7 macrophages. Treatment with LPS significantly increased the NO level and treatment with *D. viscosa* at the concentration of 5,10,20 µg/ml significantly reduced NO level.

Fig. 3. Effect of *D. viscosa* on PGE2 inhibition in LPS stimulated RAW264.7 macrophages. Treatment with LPS significantly increased the PGE2 level and treatment with *D. viscosa* at the concentration of 5,10,20 µg/ml significantly reduced PGE2 level.
study uncovers the anticancer capability of exercises including hostile to malignancy effects phenolics evokes wide scope of natural metabolites like alkaloids, terpenoids and with great adequacy and less incidental effects.

Chemotherapy is one of the principal lines of treatment for malignancy and its associated ailments. Albeit, allopathic medicines are easy available and elicits good efficacy, herbal plants are gaining lot of attention due to their less adverse effects [15]. In the present study, we assessed the invitro anticancer and antiinflammatory activity of hydro alcoholic extract of D. viscosa in various cancer cell lines and LPS induced inflammation in RAW264.7 macrophages. The unrefined hydro alcoholic concentrate of D. viscosa uncovered the presence of different phytoconstituents like flavonoids, phenolic compounds, terpenoids, and alkaloids. Reports show that flavonoids and phenolic compounds are important for the numerous drug details and have cancer prevention agent, anticancer and mitigating properties [16].

Chemotherapy is one of the principal lines therapies for the therapy of malignancy and its persistent openness can prompt extreme antagonistic intricacies. Subsequently, the specialists are focussing on elective medications with great adequacy and less incidental effects. Report shows that plant inferred optional metabolites like alkaloids, terpenoids and phenolics evokes wide scope of natural exercises including hostile to malignancy effects [17]. However, their clinical utility is restricted because of their ill-advised bioavailability and along these lines primary change is needed to work on their anticancer effects [18,19]. Our study uncovers the anticancer capability of D. viscosa in HCT-116 colon malignant cell line, MCF-7 bosom disease cell line and HeLa cervical malignancy cell line in a portion subordinate way. Past examinations show that Kaempferol, a generally circulated flavonoid present in D.viscosa displayed apotosis intervened malignant growth cell passing was explored for the blockage of apoptotic cell death [20]. In expansion, gold nanoparticles arranged from D. viscosa plant separate showed huge cytotoxic action against non-little cell cellular breakdown in the lung’s cells [21]. In another examination, ethanolic concentrate of D. viscosa showed stamped cytoxicity against HT-29 human colon malignancy cell line [22].

Irritation emerges because of physiological reaction to cell injury intervened by synthetic compounds or drug, physical or by irresistible specialists. The fiery responses happen in two stage, intense and constant. Nitric oxide (NO) is one of the significant synthetic middle people associated with the fiery interaction and estimation of serum NO levels is a dependable marker to survey the inflammation [23]. Thus, focusing on the pathways to decrease the NO level is one of the compelling procedures to relieve provocative illnesses. NF-κB is the sign transduction factor which directs the quality articulation of different proinflammatory arbiters in case of incendiary cycle. The initiation of NF-κB pathway is set off by the proinflammatory upgrades delivered by IL-1 and TNF-α. The intense provocative cycle is interceded by histamine, serotonin, and COX-2, in the interim the ongoing responses are intervened by PGE2, NO, and lipoxygenases [24]. Mounting reports shows that constant aggravation is one of the...
significant offenders in the advancement of different sicknesses like fiery entrail infection, rheumatoid joint inflammation, and cancer [25].

In our examination, LPS invigorated RAW macrophages showed checked expansion in the level of NO, PGE2 and TNF-α when contrasted with control. Treatment with D. viscosa significantly diminished the level of NO, PGE2 and TNF-α when contrasted with LPS alone treated cells. Past report shows that, hurricanic corrosive disconnected from D. viscosa displayed powerful calming action in kaolin/carrageenan actuated monoarthritic by lessening the degree of proinflammatory cytokines [26].

5. CONCLUSION

The current examination uncovers the anticancer and calming capability of D. viscosa. In any case future atomic investigations and detachment of dynamic phytochemicals are justified to explain the component of the concentrate.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES


