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**Life Sciences** **Research Article……!!!**

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# *IN VITRO* CYTOTOXICITY OF *GMELINA ARBOREA*

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**Keywords:**
*Gmelina arborea* , *in vitro* cytotoxicity.

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## ABSTRACT

*Gmelina arborea* (GA) belongs to the family *Verbenaceae* widely distributed in tropical regions and extensively used in South India for various diseases. The present investigation evaluates the in vitro anticancer activity of the methanol extract of *Gmelina arborea* against two types of cell lines viz., Hep-2 and vero. From data obtained, it was evident that the maginitude of the cytotoxicity was predominant on 1.25 mg/ml concentration of the methanol extract against the death rate of both the cell lines. Present study thus confirms the cytotoxic property of *Gmelina arborea* and also demonstrated the role of its in traditional medicine.

## INTRODUCTION

In the United States in 1999, it is estimated that over 1.2 million persons will be diagnosed with invasive forms of cancer, and over 1,500 people will die as a result of cancer each day [1]. Medicinal plants play a key role in human healthcare. About 80% of the world population relies on the use of traditional medicine, which is predominantly based on plant materials [2]. Scientific studies indicate that the promising phytochemicals can be developed from the medicinal plants for many health problems [3]. Natural products from plants have been valuable sources for anticancer drug discovery [4]. Often the different components in a herb have synergistic activities or buffer toxic effects. Mixtures of herbs are even more complex and so might have more therapeutic or preventive activity than single products alone. In fact, several studies have demonstrated that extracts from several herbal medicines or mixtures had an anticancer potential in vitro or in vivo [5, 6, 7, 8]. Plant secondary metabolites also show promise for the cancer chemoprevention, which has been defined as the use of non-cytotoxic nutrients or pharmacological agents to enhance physiological mechanisms that protect the organism against mutant clones of malignant cells [9]. Phenolic and flavonoids contents provide antioxidant activities that may underlie the anticancer potential [10] Cancer is an ailment that affects more or less 200 types of cells. The major characteristic is the lack of control of the cell proliferation, differentiation and health, invading organs and tissues. There are many difficulties in the treatment but the more frequently are the drug resistance, toxicity and low specificity. Cancer is the second leading cause of death in the world. The prognosis for a patient with metastatic carcinoma of the lung, colon, hepatic or prostate remains a concern and accounts for more than half of all cancer deaths. Since almost all artificial agents currently being used in cancer therapy are known to be toxic and produce severe damage to normal cells. Therefore, chemoprevention or chemotherapy via nontoxic agents could be approach for decreasing the incidence of these cancers. Naturally occurring dietary antioxidants found in medicinal plants could in theory serve as alternatives to chemically designed anticancer agents [11, 12]. In the regulation of development and homeostasis in multicellular organism, cell death is as important as cell proliferation. Although, physiological cell death is proliferated and inhibited apoptosis are major characteristics of cancer cells. Apoptosis in a gene-regulated phenomenon that is included by many chemotherapeutic agents in cancer treatment. The induction of apoptosis in tumor cells is considered to be useful not only for the management and treatment and treatment of cancer, but also for its prevention [13, 14, 15]. Several plants have reputed applications and are deliberately used in treatment of cancer and inflammatory diseases [16]. Plant derived natural products such as flavonoids, terpenes, alkaloids [17, 18, 19] and so on have received considerable attention in recent years due to their diverse pharmacological properties including cytotoxic and cancer chemopreventive effects [20]. The rich and diverse plant sources of India are likely to provide effective anticancer agents. One of the best

approaches in search for anticancer agents from plant resources is the selection of plant based on ethano medical leads and testing the selected plant's efficacy and safety in light of modern science.

**MATERIALS AND METHODS**:

For the assessment of the anticancer activity of the studied plant, the following were used: Vero and Hep-2. The cell lines were purchased from Amla Research Institute,Trichur.MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5- diphenyl teterazolium bromide) assay [21]. Cells were grown in RPMI-1640 medium at 37ºC under incubated for 6-7hrs. 5% CO2 in a humified incubator. Cells were harvested, counted (3×104cells/ml), and transferred into a 24 well plate, and incubated for 24hrs. Prior to the addition of test compound. Serial dilutions of test samples were prepared by dissolving compounds in DMSO followed by dilution with RPMI-1640 medium to give final concentration at 10, 50, 2.5, 1.25, 0.625, 0.3125 and 0.156mg/ml. Stock solutions of samples were prepared. Sample at 10µl and cell lines at 90µl were incubated for 72hrs. MTT solution at 5mg/ml was dissolved in 1ml of Phosphate Buffer Solution (PBS), and 10µl of it was added to each of the 24wells. The wells were wrapped with aluminium foil and incubated at 37ºC for 4hrs. The solution in each well containing media, unband MTT and dead cells were removed by suction and 150µl of DMSO was added to each well. Then the plants were shaken and optical density was recorded using a microplate reader (spectrophotometer) at 595nm. DMSO as a blank. Controls and samples were assayed and replicated for each concentration and replicated three times for each cell line. After 24h incubation of the mononuclear cells with plant extracts, the cytotoxicity on the cancer cell lines was evaluated using MTT assay. The cytotoxicity was obtained by comparing the absorbance between the samples and control. The values were then used to iteratively calculate the concentration of plant extracts required to cause a 50% reduction (IC50) a growth (cell number) for each cell lines.

$$\text{Cell viability (\%)} = \text{Mean OD/Control OD} \times 100$$

**RESULTS:**

The standardization of methanolic extract [Table 1 and 2, Fig 1 and 2] of plant material has been carried out as per the standard guidelines and the anticancer activity of extract was conducted against two different cell lines of which methanolic extract have shown the anticancer activity against larynch cell lines [Refer Table 1 and 2, Fig 1 and 2].

**Table 1: GA Against Hep 2 Cell line**

| S.no | Concentration (mg/ml) | Dilutions | Absorbance | Cell viability |
|---|---|---|---|---|
| 1 | 5 | Neat | 0.03 | 5.88 |
| 2 | 2.5 | 1:1 | 0.16 | 31.37 |
| 3 | 1.25 | 1:2 | 0.22 | 43.13 |
| 4 | 0.625 | 1:4 | 0.29 | 56.86 |
| 5 | 0.3125 | 1:8 | 0.35 | 68.62 |
| 6 | 0.156 | 1:16 | 0.39 | 76.47 |
| 7 | 0.078 | 1:32 | 0.47 | 92.15 |
| 8 | Cell control | - | 0.51 | 100 |

**MTT ASSAY**

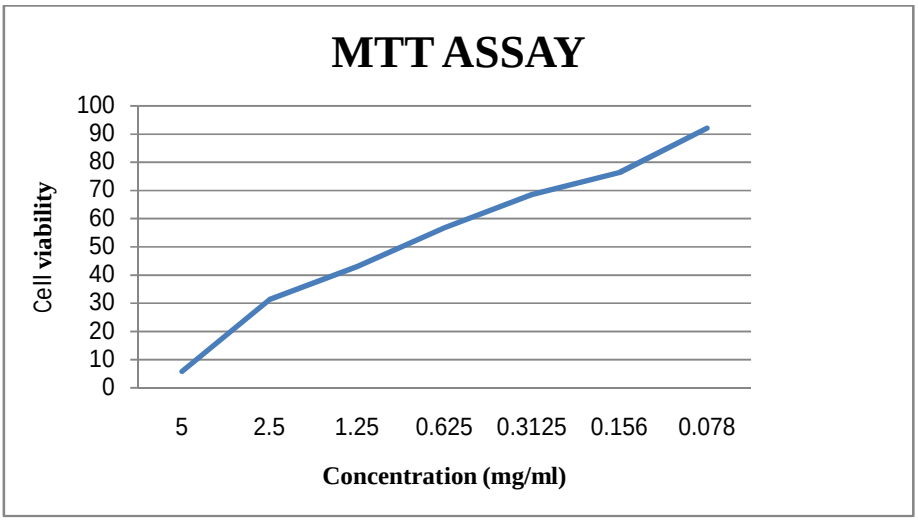
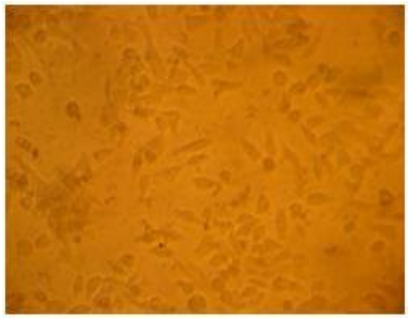



**Fig1:GA Concentration (mg/ml) against Hep 2 cell line**

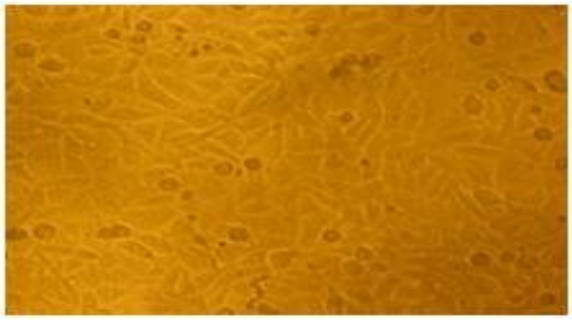
**1+ Toxicity**

**2+Toxicity**

**3+ Toxicity**



**4+Toxicity**



**Normal Hep 2 cell line**

**Table 2: GA Against Vero cell line**

| S.no | Concentration (mg/ml) | Dilutions | Absorbance | Cell viability |
|---|---|---|---|---|
| 1 | 5 | Neat | 0.07 | 13.72 |
| 2 | 2.5 | 1:1 | 0.11 | 21.56 |
| 3 | 1.25 | 1:2 | 0.19 | 37.25 |
| 4 | 0.625 | 1:4 | 0.23 | 45.09 |
| 5 | 0.3125 | 1:8 | 0.32 | 62.74 |
| 6 | 0.156 | 1:16 | 0.46 | 90.19 |
| 7 | 0.078 | 1:32 | 0.50 | 98.03 |
| 8 | Cell control | - | 0.51 | 100 |

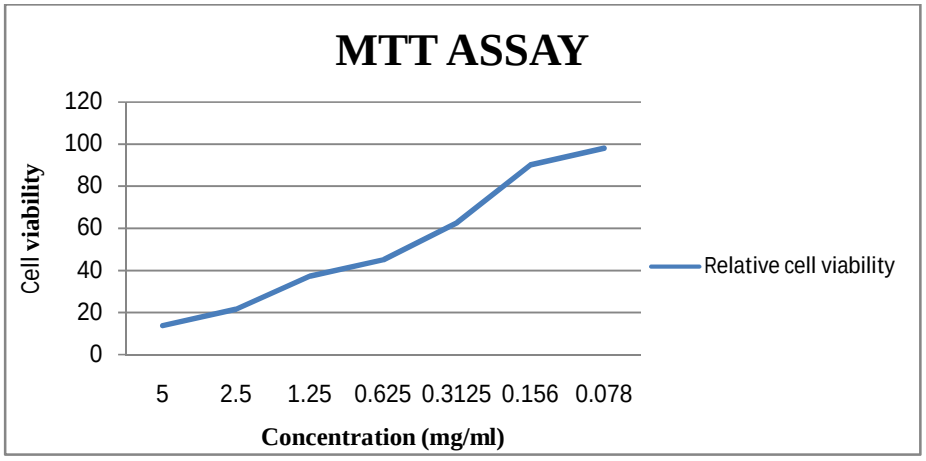
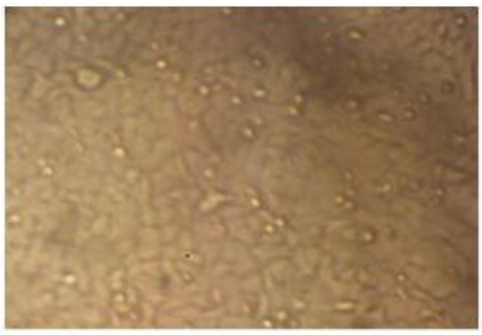
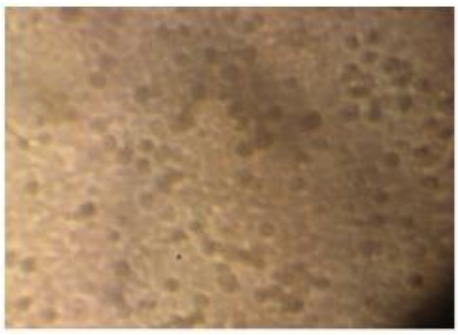


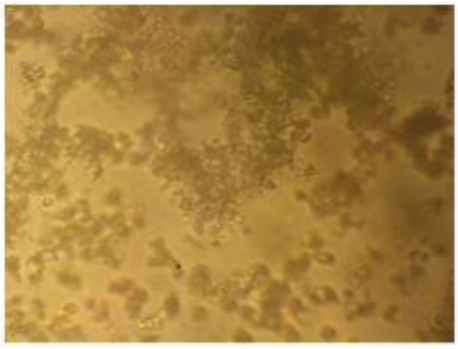

**FIG 2: GA Concentration (mg/ml) Against vero cell line**



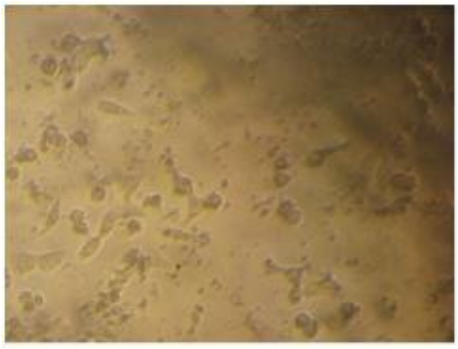
**1+cytotoxicity**



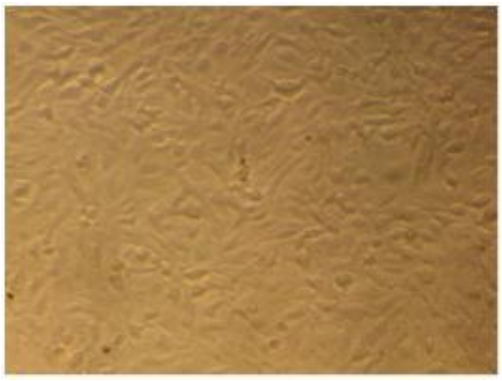
**2+cytotoxicity**



**3+cytotoxicity**



**4+cytotoxicity**



**Normal Vero cell line**

**1+ For 25% dead cells**

**2+ For 50% dead cells**

**3+ For 75% dead cells**

**4+For100% deadcells**

**DISCUSSION:**

Plant substances continue to serve as viable source of drugs for the world population and several plant-based drugs are in extensive clinical use [22]. Agents capable of inhibiting cell proliferation, inducing apoptosis or modulating signal transduction are currently used for the treatment of cancer [23]. The use of multiple chemopreventive agents or agents with multiple targets on cancer cells are considered to be more effective in cancer

treatment [24]. Medicinal plants are playing an important role in the health care immemorial. Activities of medicinal plants were due to the safe, compared with costly synthetic drugs that have adverse effects. Flavonoids also are potent water soluble antioxidants and free radical scavengers, which prevent oxidative cell damage, have strong anticancer activity [25, 26, 27 ]. Further more, flavonoids have a chemopreventive role in cancer through their effects on signal 1+ For 25% dead cells 2+ For 50% dead cells 3+ For 75% dead cells 4+ For 100% dead cells transduction in cell proliferation [28] and angiogenesis [29]. The cytotoxicity and anticancer properties are due to the presence of flavonoids. Phenolic compounds, including flavonoids are especially promising candidates for cancer prevention [30]. Much information is available on the reported inhibitory effects of specific plant phenolic compounds and extracts on mutagenesis and carcinogenesis [10]. The potential ability of polyphenol combinations to prevent cancer progression has not been adequately studied. Scientistis have suggested that it appears extremely unlikely that any one substance is responsible for all of the associations seen between plant foods and cancer prevention because of the great variety of dietary phenolics, including flavonoids and the many types of potential mechanisms reported [31, 8 ]. Plant extracts containing catechin, epicatechin, quercetin, kaempferol, rutin etc, have shown to decrease proliferation of breast, pancreatic, prostate and other cancer cell lines [32]. Multi-component prescription is a common feature in cancer treatment. Our observations are in agreement with that made by [33]. Usually in cancer chemotherapy the major problems that are being encountered are of myelosuppression and anemia [34, 35].

**CONCLUSION**:

It has become clear that in *Gmelina arborea* plant might provide effective anticancer, therapeutics. In developing countries for prevention and treatment of dangerous diseases like cancer. The extract should be considered as good sources for drug discovery. Further studies are in progress in our laboratory in synthesis of novel derivatives and investigation of molecular mechanisms responsible for the cytotoxic activity of this plant. This study may contribute to the improvement of scientific understanding of chemical constituents and functionally of the tested traditional medicinal plants.

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