INTRODUCTION

The harmful tumors are one of the major and driving reasons for grimmness and mortality in people for around the world, with roughly 14 million new cases in 2012 and have high effect in industrialized nations [1-3]. These days, disease is the second driving reason for death all around and was in charge of 8.8 million passings in 2015. Internationally, about 1 in 6 passings is because of malignancy [4]. This growing trend indicates the deficiency in the current cancer therapies, which include surgery, radiotherapy, and chemotherapy [5,6]. There is a basic requirement for hostile to tumor operators with higher viability and less symptoms that can be obtained at a reasonable cost [3,7,8]. In such manner, the hunt and advancement of new viabilities and markers is expected to confirm the cytotoxicity.

RESULTS

Ethyl acetate fraction from the flower extract of A. mexicana L. was tested for its anticancer activity against HepG2 cells (liver cancer) at various concentrations by MTT assay. It was confirmed that the IC50 value of this sample was 72±1.7 μg/ml against liver cancer HepG2 cell line.

CONCLUSIONS

A. mexicana L. is a potential plant with anticancer activity. The isolation of the pure compounds and determination of the structure of individual compounds will be further performed.

Keywords: Argemone mexicana L, Anticancer activity, MTT assay, HepG2, Cytotoxicity.
tetrabromide (MTT), and DMSO were purchased from Sisco research laboratory chemicals, Mumbai. The various chemicals and reagents were obtained from Sigma-Aldrich, Mumbai.

**Principle of MTT assay**

The MTT assay (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) decrease test was the main homogeneous cell viability examine created for a 96-well organization that was reasonable for high throughput screening. The MTT tetrazolium test innovation has been broadly received and stays well known in scholastic labs as prove by a huge number of distributed articles. The MTT substrate is set up in a physiologically adjusted arrangement, added to cells in culture, more often than not at a last grouping of 0.2-0.5 mg/mL and hatched for 1-4 hrs. The amount of formazan (directly correspondent to the quantity of suitable cells) is measured by recording changes in absorbance at 570 nm utilizing a plate perusing spectrophotometer. Viable cells with dynamic digestion change over MTT into a purple shaded formazan item with an absorbance most extreme almost 570 nm [20, 21]. At the point when cells kick the bucket, they lose the capacity to change over MTT into formazan; accordingly shading development fills in as a valuable and advantageous marker of the viable cells. The correct cellular instrument of MTT lessening into formazan is not surely known, but rather likely includes response with NADH (the redox responses of nicotinamide adenine dinucleotide) or comparative decrease atom that exchange electrons to MTT. Theory in the early writing including particular mitochondrial proteins has prompted the supposition specified in various productions that MTT is measuring mitochondrial movement. The formazan result of the MTT tetrazolium gathers as an insoluble encourage inside the cells and additionally being stored close to the cell surface and in the way of life medium. The formazan must be solubilized preceding recording absorbance readings. An assortment of techniques have been utilized to solubilize the formazan item, balance out the shading, stay away from dissipation, and lessen obstruction by phenol red, and other culture medium parts. Different solubilization techniques incorporate utilizing: Fermented isopropanol, DMSO, dimethylformamide, sodium dodecyl sulfate (SDS), and mixes of cleanser and natural dissolvable. Fermentation of the solubilizing arrangement has the advantage of changing the shade of phenol red to yellow shading that may have less obstruction with absorbance readings. The pH of the solubilization arrangement can be changed in accordance with give most extreme absorbance if affectability is an issue; be that as it may, other test advances offer substantially more noteworthy affectability than MTT.

**In vitro assay for cytotoxicity activity (MTT assay)**

The cytotoxicity of the sample (*A. mexicana* L) on HepG2 (liver) cell line was determined by the MTT assay. Cells (1×10⁵/well) were plated in 1ml of medium/well in 24-well plates (Costar Corning, Rochester, NY). Following 24 hrs incubation, the cell achieves the conversion. Then, cells were incubated in the presence of various concentrations of the samples in 0.1% DMSO for 24 hrs at 37°C. After removal of the sample solution and washing with phosphate-buffered saline (pH 7.4), 200 µl/well (5 mg/ml of 0.5% 3-(4,5-dimethyl-2-thiazoyl)-2,5-diphenyl-tetrazolium bromide (MTT) phosphate-buffered saline solution was added to cells. After 4 hrs incubation, 100 µl solubilization solution (pH 4.7 for 40% (v/v) dimethyl ether in 2% (v/v) glacial acetic acid and 16% (w/v) SDS) were added. Viable cells were controlled by the absorbance at 570 nm. Estimations were performed, and the fixation required for a half inhibition of viability (IC₅₀) was resolved graphically. The absorbance at 570 nm was measured with an ultraviolet (UV)-spectrophotometer utilizing wells without test containing cells as spaces. The effect of the samples on the proliferation of HepG2 was expressed as the % cell viability, using the following formula:

\[
\% \text{Cell viability} = \left( \frac{A_{570} \text{of treated cells}}{A_{570} \text{of control cells}} \right) \times 100
\]

**Statistical analysis**

All the data were reported as the mean ± standard deviation. All statistical analysis was performed by means of one-way analysis of variance (ANOVA) and Student’s t-test using GraphPad Prism statistical software package version 7.02. The IC₅₀ concentration was ascertained from nonlinear relapse examination utilizing the GraphPad Prism programming with the condition: \( Y = 100 \left[ 1 + 10^{d \cdot \log_{10} C} \right] \). Only a value of \( p<0.05 \) and 0.01 was considered statistically significant.

**RESULTS**

**In vitro assays (cytotoxic studies)**

The anticancer activity of the *A. mexicana* L. flower was confirmed by MTT assay (Table 1; Figs. 1 and 2). MTT is considered to be a reliable assay to determine the extent of cell viability. The control HepG2 cells showed high proliferation that has been taken as 100%. Test samples showed effective IC₅₀ value against HepG2 (liver) cell line was found to be 72±1.7 µg/ml. It induced cytotoxicity in a significant manner which implicit the damage to the membrane integrity of the cell when compared with control. The cytotoxicity was increased with increase in the concentration of the sample and near normal level was attained at various concentrations (10, 25, 50, 100, and 250 µg/ml) and the

| Concentration (µg/ml) | Cell viability (%) | IC₅₀ (µg/ml) |
|-----------------------|--------------------|-------------|
| Control               | 100                | 72±1.7      |
| 10                    | 84.06±0.67**       |             |
| 25                    | 71.17±1.00**       |             |
| 50                    | 57.33±0.88**       |             |
| 100                   | 41.25±0.58**       |             |
| 250                   | 28.11±1.00**       |             |

Values are shown as for means±SD of triplicate. **p<0.05 and 0.01 compared with control (one-way ANOVA and t-test)

**Fig. 1:** Graphical representation of the cell viability (%) values of the solid obtained from the ethyl acetate fraction of *Argemone mexicana* (flowers) against HepG2 cell line

**Fig. 2:** (a-f) Effect of the solid obtained from the ethyl acetate fraction of *Argemone mexicana* flowers against human liver cancer HepG2 cell line in different concentration
maximum effect was found when treated at 250 µg/ml, which showed cell viability of 28.11±1.00%. From the above results, it was confirmed that the solid obtained from ethyl acetate fraction of A. mexicana L. extract at 250 µg/ml seems to offer significant protection and maintains the structural integrity of the hepatic membrane.

**DISCUSSION**

Horrible free radicals are created in the body amid ordinary digestion and furthermore on presentation to ecological toxins, for example, irresistible operator, UV light, and radiation et cetera. Destructive free radicals are not killed by the body’s essential and optional resistance component on overabundance of omen free radicals [21]. Existing clinical reviews have additionally demonstrated that supplemental levels of hostile to oxidant vitamins (E, C, and B complex) decrease, the individual hazard for certain disease [21-24]. Huge numbers of the restorative plant have been observed to be compelling in trial and clinical instances of growth. Therapeutic plants overwhelm immunomodulatory and hostile to oxidant properties, prompting against growth action [21,25-27]. This plant has been accounted for free radical rummaging impacts and antioxidant property [28,29]. In this study, the cytotoxic impact was expanded with the concentrate of the test sample (Fig. 2). From the results, the fraction had an IC₅₀ value of 72±1.7 µg/ml which assumed cell viability 50%. The United States National Cancer Institute (US-NCI) estimates that an extract or fraction that shows an IC₅₀ value of <100 µg/ml is considered active [5,30,31]. Hence, it is clear that the solid obtained from the ethyl acetate fraction of A. Mexicana L. flowers has cytototoxic effect against HepG2 cell line.

**CONCLUSION**

The outcomes acquired from the in vitro thinks about performed utilizing the HepG2 cell lines uncovers that the strong gotten from the ethyl acetate fraction of the EtOH concentrate of A. mexicana L. flowers has a direct anticancer action despite the fact that cell development restraint was expanded when grouping of the test was expanded. These fixations could prompt apoptosis on human malignancy cell lines, and its anticancer action was observed to be exact. In addition, work is required keeping in mind the end goal to build up the character of the substance constituent in charge of anticancer movement. Studies are in advance in our research facility to illustrate the subatomic structure of the compound. This contributes toward the advancement of intense anticancer medication.

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