ABSTRACT
The aim of the present work is to evaluate the anti-tumour potential of Madhuca indica flowers through preliminary cytotoxic analysis. Cytotoxic potential of methanol extract of Madhuca indica flowers was evaluated on chicken liver cell line through cell viability assay. Antioxidant study was carried out to evaluate the free radicals scavenging effect of flower extract. The positive response for cytotoxic and antioxidant activities of Madhuca indica flower extract may be due to its major nutritional components.

Keywords: Madhuca indica, cytotoxicity, antioxidant.

INTRODUCTION
Cancer is one of the most dreaded diseases of the 20th century and spreading further with continuance and increasing incidence in 21st century. Thus, advances in the clinical research for anticancer drugs have been increased over the years (Merina et al., 2012). According to World Health Organization, 80% of the people depend on medicinal herbs as primary healthcare system since they are commonly available, safe and comparatively economical. Some herbs protect the body from cancer by enhancing detoxification functions of the body. Compounds derived from medicinal plants are known to inhibit growth of cancer by modulating the activity of specific hormones and enzymes, by reducing the toxic side effects of chemotherapy and radiotherapy. Scientists all over the world are concentrating on the herbal medicines to boost immune cells of the body against cancer and designing herbal formulations to attack the cancerous cells without harming normal cells of the body (Sakarkar and Deshmukh, 2011).
"Madhuca indica" is a large, shady deciduous tree both commonly known as Mahua, found in different parts of India (Kirtikar and Basu, 1987). The medicinal properties attributed to this plant are stimulant, demulcent, emollient, heating and astringent (Awashthi and Mitra, 1967). Large numbers of Mahua trees are found in India and estimated production of its flowers is more than one million tonne in the country. The extract of Mahua flowers is used in food industries for making jams, jellies, biscuits and other food products due to its nutritional components like vitamins, sugars, amino acids, organic acids, enzymes and other compounds (Betaine, tannins and crude pigments) and antioxidant activity (Patel and Naik, 2010). Thus, in the present study cytotoxic and antioxidant potential of methanol extract of "Madhuca indica" flowers was studied.

MATERIAL AND METHODS

Preparation of Plant Material Extract

Flowers of Madhuca indica were collected from the forest area of Jhabua District of Madhya Pradesh. Methanol extract (10 g) of Mahua fresh flowers was prepared using cold extraction method by continuous shaking in orbital shaker at 100 rpm (25°C). After 24 hours, the extract was filtered and residue of the sample was re-extracted by the same procedure using methanol as the solvent for the period of one week (Umachigi et al., 2007). After one week, the collected filtrates were concentrated by evaporating the methanol at 50°C. Dried residue of the sample was re-dissolved in methanol and used for the further analysis.

Cytotoxic study

Freshly harvested liver from chicken was used for preparation of liver cell lines. For this the liver was initially cleaned with sterile saline and the four lobes were separated using sterile scissors aseptically. One of the lobe was transferred to Petri dish containing sterile Hanks Balanced Salt Solution (HBSS) and washed properly with the salt solution. This was chopped into small pieces and again washed with HBSS. The slices were then treated with 0.25% trypsin-EDTA solution and shaken to loosen the cells. The cell suspension was then filtered through cheese cloth to remove debris. The suspension was centrifuged at 5000 rpm for 3 min. The supernatant was discarded and the cells were dispensed in growth medium [DMEM enriched with 10% FBS, Penicillin (100 units/ml), Streptomycin (100 μg/ml) and amphotericin B (2.5 μg/ml)]. The cells were dispensed into T-25 cm² tissue culture flasks and incubated at 37°C. The cells were then trypsinised and used for viability assay (Senan et al., 2013).
Viability is a measure of the metabolic state of a cell population, which is indicative of the potential of the cells for growth. The prepared liver cell line (2000 cells/tube) were treated with different concentrations of methanol extract of Mahua flowers (2, 4, 6, 8 and 10 mg/ml) dissolved in DMSO and incubated at 37°C for 24 hrs. Each treatment was carried out in triplicate. DMSO was used as solvent blank. The cells were then trypsinised using 100 μl of 0.25% Trypsin EDTA solution for 1 minute and neutralized using 100 μl of FBS. The viability was determined by Trypan blue dye exclusion method (Talwar, 1994). The percentage of cells that were not stained with Trypan blue (viable cells) is the measure of the viability.

The percentage viability of cells over the control was calculated using the formula,

\[ \text{Percentage Viability} = \left( \frac{\text{Number of viable cells in drug treated}}{\text{Mean number of viable cells in control}} \right) \times 100 \]

Percent Cytotoxicity = 100 - Percent Viability.

**Antioxidant study**

**Total antioxidant activity**

Total antioxidant activity of methanol extract of Mahua flowers was determined according to the standard method. An aliquot of flower extract of 1.0 ml (1.0 mg/ml) was combined with 1.0 ml of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes were incubated in a boiling water bath at 95 °C for 90 min. Then, the samples were cooled to room temperature and the absorbance was measured at 695 nm against blank prepared in the same conditions by replacing sample with 1.0 ml of distilled water. All the analyses were performed in triplicate and the results were averaged. Antioxidant capacity was expressed as Ascorbic acid equivalents (mmol/mg) (Prieto et al., 1999).

**Ferric reducing antioxidant power**

The reducing power of methanol extract of Mahua flowers and Ascorbic acid was determined according to the method of Oyaizu (1986). 2.0, 4.0, 6.0, 8.0 and 10.0 mg/ml of the extracts and 0.2, 0.4, 0.6, 0.8 and 1.0 mg/ml of standard (ascorbic acid) was mixed with phosphate buffer (2.5 mL, 0.2 M, pH 6.6) and potassium ferricyanide [K₃Fe(CN)₆] (2.5 mL, 1%). The mixture was incubated at 50°C for 20 minutes. 2.5 ml of trichloroacetic acid (10%) was added to the 2.5 ml of the reaction mixture, which was then centrifuged at 3000 g for 10 minutes. The upper layer of the solution (2.5 mL) was mixed with distilled water (2.5 mL)
and FeCl₃ (0.5 mL, 0.1%) and the absorbance was measured at 700 nm against blank prepared in the same conditions by replacing sample with 1.0 ml of distilled water. All the analyses were performed in triplicate and the results were averaged. Increased absorbance of the reaction mixture indicated increasing reducing power.

RESULT AND DISCUSSION

Cytotoxic study

Figure 1, showed the cytotoxic effects of methanol extract of Mahua flowers on liver cells and the results were found to be significant. Cell viability was found to decrease as the concentration of extract increases and cytotoxic effect was found to increase. At 10 mg/ml of extract concentration the effect was found to be better. In cell viability assay, it was found that dead cells were stained blue while live cells were colourless (Figure 4). Cytotoxic effect of plant extract determines its anti-tumour effect. Cytotoxicity testing is important for the sole purpose of determining the potential toxicity of the compounds being studied. Cytotoxic agents unselectively kill and damage cancerous cells by interfering with either, the cellular process or mechanical process. Cytotoxicity studies with normal culture systems (tissue culture) of local plant extracts or folk medicinal plant extracts has not been studied extensively and this is vital for the safety evaluation or any herbal preparations. Thus the significant results for cytotoxic effect of Madhuca indica flowers, can reveals its further use as a safe herbal anti-cancer agent (Hanisa et al., 2014).

Antioxidant Study

Total antioxidant activity

Total antioxidant activity is a quantitative assay, since the antioxidant activity is expressed as the number of equivalents of Ascorbic acid. The assay is based on the reduction of Mo (VI) to Mo (V) by the sample analyte and the subsequent formation of a green phosphate/Mo (V) complex at acidic pH with the maximal absorption at 695nm (Prieto et al., 1999). The linear equation of ascorbic acid for total antioxidant activity was found to be $y=2.676x$ with $r^2=0.9979$ (Fig. 2). The antioxidant activity of flower extract was found to be $0.29±0.02$ mM Ascorbic acid/mg extract of Mahua flowers.

Ferric reducing antioxidant power

The reducing ability of a compound generally depends on the presence of reductants which have been exhibited antioxidative potential by breaking the free radical chain, donating a hydrogen atom (Saha et al., 2008). The presence of reductants (i.e. antioxidants) in plant
extract causes the reduction of the Fe3+/ferricyanide complex to the ferrous form. Therefore, the Fe2+ can be monitored by measuring the formation of Perl’s Prussian blue colour at 700 nm. Increase in absorbance at 700 nm reflects an increase in reductive ability (Duh et al., 1999). As the concentration of flower extract and Ascorbic acid increases the ferric reducing antioxidant power increases (Fig. 3). The reducing power of extract of was very potent and it increases as the quantity of sample increases.

Figure 1: Cytotoxic potential of methanol extract of Madhuca indica flowers.

Figure 2: Standard calibration curve of Ascorbic acid for total antioxidant activity analysis of Madhuca indica flower extract.

Figure 3: Ferric reducing antioxidant power of methanol extract of Madhuca indica flowers and Ascorbic acid.
Figure 4: Cytotoxic effect of Methanolic extract of Madhuca indica flowers, a= Live cells (without extract treatment), b= Dead cells (extract treatment).

CONCLUSION
The methanol extract of Mahua flowers showed potent cytotoxic effect on liver cells, along with antioxidant activity, may be due to the presence of different nutrient components as mentioned in the literature. Thus, the Madhuca indica flower extract can be introduced as safe source of herbal anti-tumour agent for cancer therapy. Further studies can be done on its cytotoxic effect on cancerous line along isolation of bioactive compound.

ACKNOWLEDGMENT
Authors are thankful to the management of Birla College to provide all the facilities for the completion of research work.

REFERENCES


