CHAPTER 7

CYTOTOXIC AND APOPTOTIC ACTIVITIES OF METHANOLIC EXTRACT OF WOODFORDIA FRUTICOSA KURZ FLOWERS AND ITS FRACTIONS ON HUMAN HEPATOMA, PLC/PRF/5 CELLS
7.1. INTRODUCTION

Liver cancer is one of the most common malignancies worldwide, especially in Asia and Africa (Qian and Ling, 2004). Hepatocellular carcinoma (HCC) is the most frequent primary hepatic tumor and the fifth most commonly diagnosed cancer, with more than one million deaths reported annually worldwide. Each year, more than 700,000 people are diagnosed with this cancer throughout the world and accounting for more than 600,000 deaths (American Cancer Society, 2012). Major risk factors for liver cancer include hepatitis viral infection, food additives, alcohol, aflatoxins, environmental and industrial toxic chemicals, air and water pollutants etc. As HCC is difficult to treat several approaches are made towards its prevention. Recently, considerable research has been carried out in the search for natural or synthetic compounds as a means of chemopreventive agents against liver cancer (Marrero, 2005; Al-Rejaie et al., 2009).

Apoptosis is a physiological process that plays an important role in the regulation of tissue development and homeostasis. Deregulation of apoptosis has been shown to contribute to the pathogenesis of a number of human diseases including cancer (Schultz and Harrington, 2003). The outstanding feature of apoptosis is its remarkable stereotyped morphology showing condensation of nuclear heterochromatin, cell shrinkage and loss of positional organization of organelles in the cytoplasm (Kerr et al., 1972). Apoptosis plays a central role in embryogenesis, morphogenesis and regulation of normal cell turnover in multicellular organisms. Morphological features of apoptosis include cell shrinkage, membrane blabbing, chromatin condensation, nuclear and cytoplasmic condensation and partition of cytoplasm and nucleus into membrane bound
vesicles (apoptotic bodies) which contain ribosomes, morphologically intact mitochondria and nuclear material (Schulte-Hermann et al., 1992). In vivo, these apoptotic bodies are rapidly recognized and phagocytosed by either macrophages or adjacent epithelial cells.

“Apoptotic induction has been a new target for innovative mechanism-based drug discovery. It is important to screen apoptotic inducers from plants, either in the form of crude extracts or as components isolated from them. Chemopreventive agents include genistein, resveratrol, S-allyl cysteine, allicin, lycopene, capsaicin, curcumin, 6-gingerol, ellagic acid, ursolic acid, silymarin, anethol, catechins and eugenol comprise a diverse group of compounds with different mechanisms of action, but their ultimate ability to induce apoptosis may represent a unifying concept for the mechanism of chemoprevention (Dorai et al., 2004; Garg et al., 2005). These agents have been shown to suppress cancer cell proliferation (Shishodia et al., 2005), inhibit growth factor signaling pathways (Siwak et al., 2005), induce apoptosis (Shi et al., 2006), inhibit NF-κB (Aggarwal et al., 2006), AP-1 (Jeong et al., 2004) and JAK-STAT activation pathways (Kim et al., 2003), inhibit angiogenesis (Aggarwal et al., 2004), suppress the expression of anti-apoptotic proteins (Aggarwal et al., 2005), and inhibit cyclooxygenase-2 (Takada et al., 2004). Thus, these chemopreventive agents have potential to be used as adjuncts to current cancer therapies. Evidence that has emerged from various studies suggests that products derived from plants are useful in the treatment as well as in the prevention of cancer. Understanding the modes of action of these compounds should provide useful information for their possible application in cancer prevention and also in cancer therapy” (Taraphdar et al., 2001).
Woodfordia fruticosa flowers have been traditionally used for the treatment of liver diseases. Besides, the flowers have been reported to possess antioxidant and antitumour properties. In addition to the reported pharmacological effects, our previous investigations demonstrated that Woodfordia fruticosa flower possess antioxidant, hepatoprotective, antifibrotic and anticancer properties particularly against N-nitrosodiethylamine (NDEA) induced hepatocellular carcinoma. Thus the present study was undertaken to investigate the cytotoxic and apoptotic activities of the methanolic extract of Woodfordia fruticosa flower (MEWF) and its sub fractions in human liver cancer cell line, PLC/PRF/5. This study was also carried out for the identification of the active apoptotic and cytotoxic fraction of the extract. Moreover, the cytotoxic and apoptotic assays of the sub fractions of MEWF, is a step towards the identification and isolation of the biologically active compounds from the extract.

7.2. MATERIALS AND METHODS

7.2.1. Chemicals

Fetal bovine serum (FBS), N-2-Hydroxyethylpiperazine-N-2-ethane-sulphonic acid (HEPES) and 4′, 6-diamidino-2-phenylindole (DAPI) were procured from Sigma Chemical Co., St. Louis, MO, USA. 5-flourouracil (5-FU) was purchased from Biochem Pharmaceutical Industries, Mumbai, India. Dulbecco’s Modified Eagle Medium (DMEM) and antibiotic-antimycotic were purchased from Gibco, Grand Island, N.Y, USA. Cell Proliferation Assay kit (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide, [MTT]) was purchased from HiMedia, India. 5,5′,6,6′ Tetrachloro-1,1′,3,3′-tetraethylbenzimidazolylcarbocyanine iodide (JC-1) was purchased from Invitrogen, Carlsbad, CA, USA. Dimethyl sulfoxide (DMSO)
was obtained from Merck, Mumbai, India. All the other chemicals used were also of high purity grade.

### 7.2.2. Cell culture

PLC/PRF/5 cell line was purchased from National Centre for Cell Science (NCCS), Pune, India and grown as a monolayer in DMEM medium containing HEPES and sodium bicarbonate supplemented with 10% FBS and 1X antibiotic-antimycotics. Cells were maintained in a tissue culture flask and kept in a humidified incubator (5% CO₂ in air at 37 °C) with a medium change in every 2-3 days. When the cells reached 70 - 80% confluence, they were harvested with trypsin - EDTA (ethylene diamine tetra acetate) and seeded into a new tissue culture flask.

### 7.2.3. Preparation of plant extracts and its sub fractions

*Woodfordia fruticosa* flowers were shade dried, powdered and soxhlet extracted with methanol (50 g in 400 ml) and were concentrated under reduced pressure using a rotary evaporator. The percentage yield of methanolic extract in our study was approximately 12.5% (w/w). The methanolic extract thus obtained was then taken in a round bottom flask of simple condenser and further fractionated using solvents in increasing polarity, viz. petroleum ether, chloroform, ethyl acetate and methanol.
**Fig. 7.1. Schematic diagram of the fractionation of W. fruticosa flower extract**

### 7.2.4. Preparation of drugs

MEWF was dissolved in DMSO at a concentration of 25mg/ml. The test solution was prepared freshly on the day of use, diluted to two different concentrations of MEWF (100µg/ml, 50µg/ml). 10 mg of petroleum ether fraction (PEF), chloroform fraction (CHF), ethyl acetate fraction (EAF) and methanolic fraction (MEF) of the methanolic extract of *W. fruticosa* flower (MEWF) were dissolved in 50 µl DMSO and made up to 1 ml with phosphate buffered saline. Subsequently, the drugs were sterilized using 0.22 µm Durapore syringe filters (Millipore, Bedford, MA, USA) and were used as stock for further experiments. On the day of experiment, test solutions were prepared by diluting the stock solutions in DMEM medium containing 10% (v/v) FBS and 1x antibiotic-antimycotics to give different concentrations (100 µg/ml and 50 µg/ml). 5-flourouracil, the standard control, was diluted to 50 µg/ml and 25 µg/ml with DMEM medium containing 10% (v/v) FBS and 1x antibiotic-antimycotics.
7.2.5. Cytotoxicity study

7.2.5.1. MTT assay of MEWF and sub fractions of MEWF

The cell viability was assessed by MTT assay (Mosmann, 1983), which determines the metabolically active mitochondria of cells.

7.2.6. Detection of apoptosis

7.2.6.1. DAPI staining assay

4’-6-Diamidino-2-phenylindole (DAPI) staining was applied for determining the apoptotic cells.

7.2.6.2. JC-1 staining

The mitochondrial membrane potential ($\Delta \psi_m$) was assayed using JC-1 (the cationic dye - 5,5’,6,6’ tetrachloro-1, 1’,3,3’-tetraethylbenzimidazolylcarbocyanine iodide) mitochondrial potential sensor (Invitrogen, USA), according to the manufacturer’s directions.

(Detailed protocols are given in chapter 2, section 2.2.11. *In vitro* cytotoxic and apoptotic assays)

7.2.7. Liquid chromatography-mass spectrometry (LC-MS) analysis of CHF

The chloroform fraction (CHF) of the methanolic extract of *W. fruticosa* flower was analyzed using LC-MS 2010A instrument (Shimadzu, Kyoto, Japan). The constituents of the CHF were identified by referring the LC-MS library, Metwin 2010 (version 2.1).

(Detailed procedure is given in chapter 2, section 2.2.12. Liquid chromatography-mass spectrometry (LC-MS) analysis.)
7.2.8. Statistical analysis

Results are expressed as mean ± standard deviation (SD). All statistical comparisons were made by means of one way ANOVA test followed by Tukey post hoc analysis and \( p \)-values less than or equal to 0.05 were considered significant.

7.3. RESULTS

7.3.1. Cytotoxicity study

7.3.1.1. MTT assay of MEWF

The dose dependent cytotoxic effects MEWF on PLC/PRF/5 cells were evaluated by MTT assay. The cells were treated with 100 and 50 \( \mu \)g/ml of MEWF. The inhibition of cell proliferation was assessed after 12 h, 24 h, 48 h and 72 h incubation. MEWF exerted cytotoxic effect on PLC/PRF/5 cells in a dose dependent manner with percentage of cell inhibition values 12.4±0.8, 23.1±0.9, 44.4±1.7 and 55.8±2.2 for 50\( \mu \)g/ml and 24.2±1.3, 33.8±1.2, 56.8±2.0 and 65.3±2.5 for 100\( \mu \)g/ml after 12 h, 24 h, 48 h and 72 h respectively. 5-flourouracil, used as positive control, showed an inhibition of 26.8±1.0, 36.2±1.5, 59.2±2.3 and 70.2±2.8 for 50\( \mu \)g/ml and 14.7±1.1, 25.2±0.8, 47.9±1.8 and 59.1±2.3 for 25\( \mu \)g/ml after 12 h, 24 h, 48 h and 72h respectively. Treatment with MEWF exhibited significant cytotoxic effect on PLC/PRF/5 cells \((p \leq 0.05)\) when compared to the cells treated with DMSO alone and were expressed in Fig. 7.2.
Fig. 7.2. Percentage inhibition of MEWF (50µg/ml and 100µg/ml) on PLC/PRF/5 cells. 

(A), PLC/PRF/5 cells treated with MEWF for 12 h; (B) PLC/PRF/5 cells treated with MEWF for 24 h (n=3).

(I) – DMSO control, (II) – MEWF 50µg/ml, (III) – MEWF 100µg/ml, (IV) – 5FU 50µg/ml, (V) – 5FU 25µg/ml. *p≤0.05 versus DMSO control.
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Fig. 7.2 (Cont.) Percentage inhibition of MEWF (50µg/ml and 100µg/ml) on PLC/PRF/5 cells.

(C). PLC/PRF/5 cells treated with MEWF for 48 h; (D) PLC/PRF/5 cells treated with MEWF for 72 h (n=3).

(I) – DMSO control, (II) – MEWF 50µg/ml, (III) – MEWF 100µg/ml, (IV) – 5FU 50µg/ml, (V) – 5FU 25µg/ml. *p≤0.05 versus DMSO control.
7.3.1.2. MTT of sub fractions of MEWF

MEWF sub fractions PEF, CHF, EAF and MEF also exerted cytotoxic effects on PLC/PRF/5 cells in a dose dependent manner. PEF exerted cytotoxic effect with percentage of cell inhibition values 10.1±1.1, 19.2±1.3, 32.4±1.8, 43.1±1.8 for 50µg/ml and 20.6±2.2, 28.5±1.6, 40.5±2.0 and 53.2±2.0 for 100µg/ml after incubation with 12 h, 24 h, 48 h and 72 h respectively. CHF showed maximum cytotoxic effect with cell inhibition values 14.5±1.5, 24.8±0.9, 44.1±2.0, 57.6±2.5 for 50µg/ml and 25.8±2.5, 35.2±1.4, 54.0±2.1 and 69.8 ±2.9 for 100µg/ml after incubation with 12 h, 24 h, 48 h and 72 h respectively. EAF has the cell inhibition values 12.6±1.0, 22.1±1.1, 38.8±1.1, 50.5±2.3 for 50µg/ml and 23.2±2.6, 31.8±1.8, 49.5±1.8 and 61.7±2.6 for 100µg/ml after incubation with 12 h, 24 h, 48 h and 72 h respectively. MEF also showed cytotoxic effect with cell inhibition values 11.6±0.9, 20.3±0.8, 36.9±1.6, 46.4±2.1 for 50µg/ml and 21.4 ±1.2, 30.1±1.8, 46.1±1.8 and 57.8±2.7 for 100µg/ml after incubation with 12 h, 24 h, 48 h and 72 h respectively. 5-flourouracil, used as positive control, showed an inhibition of 15.2±1.2, 26.8±1.6, 48.3±2.1 and 60.1±3.0 for 25µg/ml and 26.9±2.0, 37.5±1.4, 59.8±2.3 and 72.5±3.4 for 50µg/ml after 12 h, 24 h, 48 h and 72 h respectively. These values of percentage inhibition of cell proliferation of sub fractions of MEWF demonstrate the cytotoxic activity of the treated groups in the following order: 5-FU > CHF > EAF > MEF > PEF. All the treatment groups exhibited significant cytotoxic effect on PLC/PRF/5 cells ($p \leq 0.05$) when compared to the cells treated alone with DMSO. The results are graphically expressed in Fig. 7.3.
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Fig. 7.3. Percentage inhibitions sub fractions of MEWF (50µg/ml and 100µg/ml) on PLC/PRF/5 cells.

(A). PLC/PRF/5 cells treated with sub fractions of MEWF for 12 h, (B). PLC/PRF/5 cells treated with sub fractions of MEWF for 24 h (n=3).

DMSO- Dimethylsulphoxide, PEF- petroleum ether fraction, CHF- chloroform fraction, EAF- ethyl acetate fraction, MEF - methanol fraction.

(I) - DMSO control, (II) - PEF 50µg/ml, (III) - PEF 100µg/ml, (IV) - CHF 50µg/ml, (V) – CHF 100µg/ml, (VI) - EAF 50µg/ml, (VII) -EAF 100µg/ml, (VIII) - MEF 50µg/ml, (IX) - MEF 100µg/ml, (X) - 5FU 25µg/ml, (XI) - 5FU 50µg/ml. *p≤0.05 versus DMSO control.
Fig. 7.3. (Cont.) Percentage inhibitions sub fractions of MEWF (50µg/ml and 100µg/ml) on PLC/PRF/5 cells.

(C). PLC/PRF/5 cells treated with sub fractions of MEWF for 48 h, (D). PLC/PRF/5 cells treated with sub fractions of MEWF for 72 h (n=3).

DMSO- Dimethylsulphoxide, PEF- petroleum ether fraction, CHF- chloroform fraction, EAF - ethyl acetate fraction, MEF - methanol fraction.

(I) - DMSO control, (II) - PEF 50µg/ml, (III) - PEF 100µg/ml, (IV) - CHF 50µg/ml, (V) - CHF100µg/ml, (VI) - EAF 50µg/ml, (VII) -EAF 100µg/ml, (VIII) - MEF 50µg/ml, (IX) - MEF 100µg/ml, (X) - 5FU 25µg/ml, (XI) - 5FU 50µg/ml. *p≤0.05 versus DMSO control.
7.3.2. Apoptosis assays

7.3.2.1. DAPI staining

The results of DAPI staining indicated that the number of apoptotic cells were higher in drug treated cells than untreated and DMSO controls. The changes that occurred in cells as a result of PEF, CHF, EAF, MEF and 5-FU treatment are shown in Fig. 7.4. After DAPI staining, PLC/PRF/5 cells treated with the drugs showed marked nuclear fragmentation and chromatin condensation which are clear indications of apoptosis. A pronounced result of apoptotic body formation and nuclear fragmentation were observed in the cells treated with 5-FU and CHF followed by EAF, MEF and PEF.

7.3.2.2. JC-1 staining

Loss of mitochondrial membrane potential ($\Delta \psi_m$) is an early event in apoptosis. When the cells stained with JC-1, the loss of $\Delta \psi_m$ is indicated by the decrease of red fluorescence and the increase of green fluorescence. 18 h treatment of PLC/PRF/5 cells with 100 µg/ml of CHF followed by the JC-1 staining resulted in green fluorescence in majority of cells. Cells treated with 50 µg/ml of CHF and 100 µg/ml of EAF also displayed a strong green fluorescence, indicating its potent apoptotic activity. 5-fluorouracil, the positive control, showed green fluorescence in majority of cells in a dose dependent manner. MEF and PEF treated cells exhibited both red orange and green fluorescence. Whereas the untreated cells and vehicle treated control cells showed orange red fluorescence only (Fig. 7.5).
7.3.3. LC-MS of CHF

The constituent of CHF that possess potent hepatoprotective, chemopreventive and anticancer properties were identified by LC-MS analysis. The mass spectrum of CHF by LC-MS is given in Fig. 7.6 and the list of pharmacologically active phytochemicals is depicted in Table 7.1. It includes confertin, quercetin methyl ether, ellagic acid and stigmasterol. Here confertin, quercetin methyl ether and ellagic acid are pharmacologically active compounds with antitumour activity; their respective chemical structures are given in figure 7.7.
Fig. 7.4. Fluorescence images of PLC/PRF/5 cells treated with DAPI after 24 hrs incubation with the sub fractions of methanolic extract of *W. fruticosa* flowers and 5-fluorouracil. Nuclear fragmentation and chromatin condensation are indicated with red arrows.

A1 - Untreated cells; A2 - Cells treated with DMSO; B1 - 5-FU (50 µg/ml); B2 - 5-FU (25 µg/ml); C1 - PEF (100 µg/ml); C2 - PEF (50 µg/ml); D1 - CHF (100 µg/ml); D2 - CHF (50 µg/ml); E1 - EAF (100 µg/ml); E2 - EAF (50 µg/ml); F1 - MEF (100 µg/ml); F2 - MEF (50 µg/ml). Original magnification 200x.
Fig. 7.5. Fluorescence image of PLC/PRF/5 cells treated with JC-1 after 18 h incubation with the sub fractions of methanolic extract of *W. fruticosa* flowers and 5-fluorouracil. The green fluorescence indicates a decrease in mitochondrial membrane potential, an early event in apoptosis.

**A1** - Untreated cells; **A2** - Cells treated with DMSO; **B1** - 5-FU (50 µg/ml); **B2** - 5-FU (25 µg/ml); **C1** - PEF (100 µg/ml); **C2** - PEF (50 µg/ml); **D1** - CHF (100 µg/ml); **D2** - CHF (50 µg/ml); **E1** - EAF (100 µg/ml); **E2** - EAF (50 µg/ml); **F1** - MEF (100 µg/ml); **F2** - MEF (50 µg/ml). Original magnification 200 x.
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Fig. 7.6. Mass spectrum of CHF by LC-MS analysis.

(A) Mass spectrum of positive ionization, (B) Mass spectrum of negative ionization
Table 7.1. List of major antioxidant/hepatoprotective/chemopreventive/anticancer compounds present in CHF identified by LCMS analysis.

<table>
<thead>
<tr>
<th>SI No</th>
<th>Name of the compounds</th>
<th>Library sequence No.</th>
<th>Molecular mass</th>
</tr>
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<tbody>
<tr>
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<td>Confertin</td>
<td>MTW/UM/2.0.1/0014/11</td>
<td>248.33</td>
</tr>
<tr>
<td>2</td>
<td>Quercetin methyl ether</td>
<td>MTW/UM/2.0.1/2011/11</td>
<td>316.27</td>
</tr>
<tr>
<td>3</td>
<td>Ellagic acid</td>
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<td>302.19</td>
</tr>
<tr>
<td>4</td>
<td>Stigmasterol</td>
<td>MTW/UM/2.0.1/6692/11</td>
<td>412.70</td>
</tr>
</tbody>
</table>

Confertin  

Quercetin methyl ether  

Ellagic acid  

(Chemspider, 2013)

Fig. 7.7. Chemical structures of the antitumour compounds identified in CHF

7.4. DISCUSSION

Apoptosis is an important mechanism of controlled cell depletion in response to both physiological and pathological conditions (Cai et al., 2007). In cancer, there is a loss of balance between cell division and cell death and cells that should have died did not receive the signals to do so. Defects along apoptotic pathways play a crucial role in carcinogenesis and that many new treatment strategies targeting apoptosis are feasible and may be used in the treatment of various types of cancer (Wong, 2011). In the present work, we have studied the cytotoxic and apoptotic potential of MEWF and its sub fractions on human hepatoma cell line PLC/PRF/5. The cytotoxicity induced by the sub fractions of MEWF were assessed using MTT
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assay and the apoptosis inducing potential of the sub fractions of MEWF were determined by DAPI and JC-1 staining.

MTT assay is an established method of determining viable cell number in proliferation and cytotoxicity studies (Sylvester, 2011). In the present study, cytotoxic effect of MEWF and the sub fractions of MEWF on PLC/PRF/5 cells were determined based on reduction of the yellow colored water soluble tetrazolium dye 3-[4, 5- dimethylthiazol-2-yl]-2, 5-diphenyl tetrazolium bromide (MTT) to formazan crystals. Mitochondrial dehydrogenase produced by live cells reduces MTT to blue formazan product, which reflects the normal function of mitochondria and cell viability (Lau et al., 2004). A dose-dependent reduction of MTT (or color change from yellow to purple) observed in 5-FU and extracts treated cells indicate their cytotoxic potential against PLC/PRF/5 cells. Among the sub fractions of MEWF, the highest activity was observed in CHF treated cells followed by EAF, MEF and PEF.

To confirm the possible mechanism of action of MEWF and its sub fractions on the inhibition of human hepatoma PLC/PRF/5 cells, morphological analysis was used to reveal whether the cytotoxic effect was due to apoptosis or necrosis. Apoptosis and its related signaling pathways have a profound effect on the progression of cancer; therefore, the induction of apoptosis is a desirable goal for the prevention of cancer (Song et al., 2012). When hepatoma cells were treated with different concentrations of MEWF and its sub fractions for 12, 24, 48 and 72 h, at 50 µg/ml and 100 µg/ml, the cells appeared to be in a rounded-up and floating condition after 48 – 72 h of exposure. Further, the percent of apoptotic cells was seen to increase in a dose and time-dependent manner as evidenced by the results of the MTT (Denizot
et al., 1986) assay indicating that the cytotoxic response on hepatoma cells by *W. fruticosa* was due to apoptosis.

Apoptosis is initially characterized by morphological features such as cell shrinkage, membrane blabbing, chromatin condensation and nuclear fragmentation (Thompson, 1995). In the current study, the chromatin condensation and nuclear fragmentation were observed in PLC/PRF 5 cells by DAPI staining after 24 h of treatment with the sub fractions of MEWF particularly with CHF and EAF. This helped in presuming that the cell death that occurred was not due to necrosis, but due to apoptosis. The result was confirmed by JC-1 staining. Mitochondria represent key organelles for the cell survival, and their role in programmed cell death is known since several years (Lugli et al., 2005). JC-1 is a reliable probe for the analysis of mitochondrial transmembrane potential changes occurring very early in apoptosis. It is a mitochondrial lipophilic dye and becomes concentrated in mitochondria in proportion to their membrane potential ($\Delta \Psi_m$); more dye becomes accumulated in mitochondria with greater $\Delta \Psi_m$ and ATP generating capacity. Therefore fluorescence of JC-1 can be considered as an indicator of mitochondrial energy state and the dye exists as a monomer at low concentrations giving green fluorescence. At higher concentrations it forms J-aggregates giving red fluorescence. Therefore, in JC-1 staining, the apoptotic cells were identified by an increase in green fluorescence and the loss of red fluorescence (Smiley et al., 1991; Salvioli et al., 1997; Savitskiy et al., 2003). The results of JC-1 staining, observed in the present study, evidently indicate that the sub fractions of MEWF are able to decrease the mitochondrial $\Delta \Psi_m$ and thereby can induce apoptosis in PLC/PRF/5 cells. Among the sub fractions of MEWF selected for the present study, green
fluorescence was prominent in cells treated with CHF and EAF. The increase of green fluorescence and the loss of red fluorescence in cells treated with 5-FU, CHF and EAF also demonstrates the dose dependent apoptotic potential of the standard drug control and the fractions of MEWF.

Many studies revealed the anticancer properties of plants in cell culture and animal models. The phytochemical constituents present in the plants are mainly responsible for their apoptotic activity. LC-MS analysis of the most promising chloroform fraction (CHF) of MEWF revealed the presence of confertin, quercetin methyl ether, ellagic acid and stigmasterol. Here confertin, quercetin methyl ether and ellagic acid are pharmacologically active compounds with antitumour activity. Confertin showed antiproliferative effect on DLA cell lines (Thara and Zuhara, 2012). Quercetin methyl ether suppresses proliferation of mouse epidermal JB6P+ cells by targeting ERKs (Li et al., 2012). Ellagic acid is a chemopreventive and antiproliferative agent, it slows the growth of some tumors caused by certain carcinogens and it inhibits two topoisomerases (Constantinou et al., 1995).

In conclusion, this study demonstrates the antiproliferative activity of MEWF and its sub fractions and apoptotic activity of the sub fractions of MEWF in human hepatoma cell line, PLC/PRF/5. Among the sub fractions of MEWF, CHF significantly inhibited the proliferation of PLC/PRF/5 cells in a dose-dependent manner followed by EAF, MEF and PEF. CHF contain the antitumour compounds such as confertin, quercetin methylether and ellagic acid. So the components in single or in combination with other components present in the CHF might be responsible for the cytotoxic and apoptotic activity in human hepatoma PLC/PRF/5 cells. The inhibitory effect of natural bioactive substances in carcinogenesis and
tumor growth may be through two main mechanisms: modifying redox status and interference with basic cellular functions like cell cycle, apoptosis, inflammation, angiogenesis, invasion and metastasis (Kampa et al., 2007). Apoptosis has been reported to play an important role in the elimination of seriously damaged cells or tumor cells by chemopreventive or chemotherapeutic agents (Galati et al., 2000). They are quickly recognized by macrophages before cell lysis, and can then be removed without inducing inflammation. Therefore, apoptosis-inducing agents are expected to be ideal anticancer drugs. Our data support the hypothesis that *Woodfordia fruticosa* flowers may have potential in liver cancer treatment.

However, based on these works further studies are needed to isolate the active phytochemical constituents from *W. fruticosa* flower and the determination of their individual antioxidant, antifibrotic and anticancer properties. Future studies in this regard may be ear marked towards the determination of different mechanisms regulating the antiproliferative effects and apoptosis induced by the active fraction of methanolic extract of Woodfordia fruticosa.