Apoptotic and antioxidant activities of methanol extract of *Mussaenda roxburghii* leaves

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Abstract: Present research work was designed to study the anticancer and antioxidant activities of methanol extract of *Mussaenda roxburghii*. Anticancer activity of MMR has been carried out on Ehrlich ascites carcinoma (EAC) cells with three different doses (20, 40 and 60 mg/kg/day) by observing different parameters such as tumor weight, survival time of EAC-bearing mice, growth inhibition of EAC cells, morphological changes and nuclear damage of EAC cells etc. whereas antioxidant activity was determined by measuring total antioxidant, DPPH free radical scavenging, ferrous reducing capacity assay. The extract showed highest anticancer activity at 60 mg/kg day^{-1}(i.p.). It caused 81.4% ($P<0.01$) cells growth inhibition and reduced tumor burden significantly (78.5%; $P<0.001$) in comparison to control. It also increased life span of EAC-bearing mice significantly (73.5%; $P<0.01$). MMR treated EAC cells showed membrane blebbing, chromatin condensation, nuclear fragmentation (apoptotic feature) in Hoechst 33342 staining under fluorescence microscope. DNA fragmentation assay in agarose gel (1.5%) electrophoresis also rectified that it causes EAC cells death by apoptosis. MMR also exhibited moderate antioxidant properties in dose dependent manner. Thus, this plant can therefore be considering a resource for natural chemo-preventive drugs as well as a possible pharmaceutical supplement.

Keywords: Apoptosis, Intrinsic pathway, anticancer agents, antioxidant, *Mussaenda roxburghii*, EAC cells, Chemo protective drugs etc.

INTRODUCTION

Plants have been serving an important role for mankind's medicine from the very beginning of human history and there is an incredible usage of plant preparations in medicine (Suffiness and Douros, 1982). Scientific research endeavor on plants used in ethno-medicine led to the researcher to develop many valuable drugs such as bleomycin, taxol etc (Kinghorn and Balandrin, 1993). Active phytochemical principle such as flavonoids, polyphenols, proanthocyanidin etc. act as strong free radical scavenger. Free radicals including super oxide radicals, hydroxyl radicals, singlet oxygen and hydrogen peroxide (reactive oxygen species; ROS) are produced as by-product of biological processes, or from external sources. These ROS can induce lipid per-oxidation of cell membrane, decrease membrane permeability and caused cancer (Cerutti, 1991). Cancer is a non-communicable disease and due to lack of effective drugs/treatment modalities, it is rating the top second cause of death (Rasida *et al.*, 2012). Effective ROS scavenger may prevent free radicals-mediated diseases such as cancer (Ames *et al.*, 1995). Anti-oxidants, the radical remover can guard human body against ROS. Drugs or modalities based on anti-oxidant for cancer engaged many researchers worldwide to explore the chemo-therapeutic prospective of plants of nature.

Mussaenda species member of the Rubiaceae (coffee family) are indigenous to West Africa, Indian sub-continent, South East Asia and Southern China. These plants used in Chinese, Fijian and Indian folkloric preparations for various ailments such as diuretic, antiphlogistic, antipyretic etc (Vidyalakshmi *et al.*, 2008). Active ingredients from these family plants also have potential cytotoxic, antibacterial, antiviral, anti-RSV activity (Jayasinghe *et al.,* 2002; Sunit *et al.,* 2003; Yaolan *et al.,* 2004). Exploration of an indigenous medicinal plant, *Mussaenda roxburghii* (family: Rubiaceae) commonly known as Himalayan Mussaenda which is a shrub and distributed in Bangladesh, Myanmar and Bhutan, is under investigation. Root of this plant is used in the tongue for the treatment of boils (Narayan and Sanjay, 2002) and a recent study showed that it inhibits the growth of both *Staphylococcus aureus and Escherichia coli* effectively (Chandra *et al.,* 2012). As there is no further pharmacological report of this plant elsewhere, we first here present the anti-neoplastic properties on EAC cells and antioxidant activity to find out effective chemo preventive drugs.

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MATERIALS AND METHODS

Test animals

Male adult Swiss albino mice, five to six weeks old (25±3 gm body weight) were purchased from animal resource branch of the International Centre for Diarrheal Disease Research, Bangladesh (ICDDR'B) and used in this studies. The experimental animals were kept in poly-propylene cages containing sterile paddy husk as bedding material. They were maintained under standard conditions (12:12 h light-dark), temperature (25±5°C). The mice were fed with standard mice food-pellets (obtained from ICDDR'B) and water was given in *ad libitum*.

Cell lines

The cancer cells (EAC) were collected from Indian Institute of Chemical Biology (IICB), Kolkata, India and were maintained as Ascites tumour in mice by intra-peritoneal inoculation of 2×10^6 cells/mouse.

Ethical clearance

The protocol used in this work, in mice model for anti-cancer drug development was approved by the Institutional Animal, Medical Ethics, Biosafety and Bio security Committee (IAMEBBC) for Experiment on Animal, Human, Microbes and Living Natural Sources (225/320-IAMEBBC/IBSc), Institute of Biological Sciences (IBSc), Rajshahi University, Bangladesh.

Plant materials

The collected leaves of the plant were sun dried for several days and made powder. At room temperature, the powder was extracted with methanol (yield 8.5%). The resulted extract was filtered three times by cloth and then Whatman No.1 filter papers. Rotary evaporator (at 45°C and reduced pressure) was used to concentrate the extract. The crude extract designated (MMR) then dried and preserved in an airtight container. MMR was dissolved in 2% (V/V) dimethylsulfoxide (DMSO) for the experiments.

Chemical screening of MMR

The chemical constituents of the plant were tested using standard protocol (Harborne, 1998). The compounds analyzed for were saponin, saponin-glycosides, steroids, glycoside, proanthocyanidins, anthraquinone, tannin, flavonoid, alkaloids, volatile oil, phenol and balsams (gum).

Determination of median lethal dose (LD_{50})

Methanol extract of *M. roxburghii* (MMR) was dissolved in 2% (v/v) DMSO and were given intra-peritoneally (i.p.) to seven groups of mice (n=4) at different doses [200, 400, 500, 550, 600, 650, 700 and 750mg.kg^{-1}]. LD_{50} value was then calculated by the procedure described in the literature (Litchfield and Wilcoxon, 1949).

Determination of cell growth inhibition

Cells growth reduction with the extract was performed by standard method (Sur and Ganguli, 1994). For this experiment, five groups of Swiss albino mice (n=6) weighing 25±3 gm were used. For therapeutic evaluation 2×10^6 cancer cells were inoculated per mouse of every group on day "0". MMR and *bleomycin* (standard clinically used anticancer drug) treatment were commenced after 24 hours of tumor implantation. The treatment was continued for five consecutive days of tumor inoculation. Here group I-III were treated with MMR at the doses of 20, 40, and 60mg.kg^{-1} per day respectively and group four treated with *bleomycin* at 0.3 mg.kg^{-1} whereas the last group (control group) receiving solvent only. Animal from every group were sacrificed on 6th day and total EAC cells were collected using saline water. At first, viable tumor cells were marked with trypen blue dye and then counted with haemo-cytometer under inverted microscope (XDS-1R, Optika, Italy). Numbers of cancer cells of MMR supplemented groups were compared to control (EAC-bearing) mice.

Bioassay of EAC cells

Effect of MMR on bioassay of EAC was studied by the method described in the literature (Abbott, 1976). For this experiment, 2 groups of mice (n=6) were inoculated with 17×10^5 EAC cells on first day. Group 1 received MMR at 60 mg.kg^{-1}/days for five days and group 2 used as control. On the 7th day, EAC from MMR receiving mice (first group) were collected in cold saline water, pooled, centrifuged and re-inoculated into two new mice groups (n=6). Further treatment with MMR was not given on these animals. On 5th day of tumor re-inoculation, they were sacrificed and EAC/mouse was estimated.

Effect of MMR on survival time and cancer burden

A brief description of the method used by the literature (Sur and Ganguli, 1994), is given bellow. For this experiment, 5 groups of adult male mice (n=6) were used. On 1st day, 2×10^6 cancer cells/mouse was given. Treatments with MMR as well as bleomycin for 10 consecutive days were started from 2nd day of EAC implantation. Cancer burdens were observed by recording regular weight of animal. Survival time of EAC hosts were recorded and designated as MST (mean survival time) in days. Increase of life span (ILS) EAC-bearing animal was calculated by the following formula:

$$\text{MST} = \frac{\text{Survival time of each mouse in a group}}{\text{Total number of mice (n)}}$$

$$\%\ \text{ILS} = \frac{\text{MST of MMR treated group-1}}{\text{MST of control group}} \times 100$$

MMR effects on macrophages

Number of macrophages and peritoneal cells in normal mice treated with MMR were measured by standard protocol (Meyer *et al.,* 1982). Normal mice (n=6) were treated with MMR at the dose of 20, 40 and 60mg per

kg/day for 3 days. On day 4, the mice were sacrificed and the number of intra-peritoneal exuded-cells and macrophages were counted in presence of 1% neutral red under microscope.

Morphological changes and nuclear damage of EAC cells

Cellular apoptosis induced by the extract (MMR) was studied by the method described earlier with little modification (Rahman *et al.,* 2012). Morphological observation of cells, in absence and presence of MMR (60 mg/day) were studied using a fluorescence microscope (Olympus IX71, Korea). At first EAC cells were collected from culture plates receiving MMR and saline (none treated control plates) and then stained with 0.1 µg/ml of Hoechst 33342 at 37^{o}C for 20 min. After that EAC cells were washed with PBS (phosphate buffer saline) and re-suspended in PBS for observation of morphological changes under fluorescence microscopy. In addition, to determine the necrotic or late apoptotic cell death, EAC cells were further washed by 0.01% Sodiun azide containing 0.9% NaCl and then stained with Propidium Iodide (PI).

Effect of caspase inhibitors

In order to study the role of caspases in MMR-mediated tumor cell killing, EAC were cultured in CO_2 incubator in presence of Z-DEVD-FMK (caspase-3 blocker, 2µmol/ml) and Z-IETD-FMK (caspase-8 blocker, 2 µmol/ml) for 1 hour. Then the tumor cells were treated with MMR and kept for another 24 hour (Yinyuan *et al.,* 2011).

DNA fragmentation assay

DNA fragmentation assay in agarose gel electrophoresis was determined by the method described previously (Chun-Ping *et al.,* 2012). EAC cells obtained from mice treated with and without extract (1×10^6/ml) at the dose 60 mg/kg/day for five consecutive days. After two times washing with PBS genomic DNA was extracted with DNA isolation kit (Promega, USA). Purified DNA was analyzed in electrophoresis (1.5% agarose gel in presence of 0.1µg/ml ethidium bromide).

Antioxidant properties of MMR

Antioxidant activity of MMR was evaluated by examining the parameters like total antioxidant content (TAC), determining DPPH radical scavenging and ferrous reducing potency assays.

Total antioxidant capacity assay

Antioxidant potential of MMR and catechin (CA) was measured by the standard method with some modifications (Prieto *et al.,* 1999). For this experiment, different concentrations of MMR/CA (0.5ml) were added to three ml reaction mixture (0.6 M sulphuric acid, 28 mM sodium phosphate and 1% ammonium molybdate) into each test tube. To complete the reaction all tubes were incubated at 95^{o}C for 10 minutes and then absorbance of samples were taken at 695 nm with a spectrophotometer against blank at room temperature.

Assay of ferrous reducing capacity

Ferrous reducing capacity of MMR/Ascorbic acid (AA) was measured by the method of Oyaizu (Oyaizu, 1986). For this purpose, different concentration of MMR/AA solution (0.25 ml) were added to reaction mixture (0.625 ml of potassium buffer and 0.625 ml of 1% potassium ferricyanide [K_3Fe $(CN)_6$] in all test tubes. They were incubated for twenty minutes at 50^0 C and after that 0.625 ml TCA (Trichloro acetic acid, 10%) was added to each test tube. Content of each tube was centrifuged at 3000 rpm for 10 minutes. Then, supernatant (1.8 ml) was withdrawn from each tube and mixed with water (1.8 ml). Finally, $FeCl_3$ solution (0.36 ml, 0.1%) was added to each tube. Absorbance of all samples was taken at 700 nm against blank.

Assay of DPPH radical scavenging capacity

DPPH radical scavenging assay (DRSA) of MMR was studied following standard protocol (Choi *et al.,* 2000). For this experiment, methanol solution of DPPH (0.1mM) was prepared. 2.4ml of the prepared solution was added to MMR solution (1.6ml) of various concentrations. All samples were vortexed thoroughly and kept in dark for 30 minutes. Finally, absorbance was taken at 517 nm. In this experiment, AA was used as reference standard and % DRSA was determined by following formula: (% DRSA) = $\{(Bo - B1)/Bo\} \times 100$

Where, A_0 is the absorbance of blank, and B1 is the absorbance of MMR/AA. Then % of inhibition was plotted against concentration, and from the graph IC_{50} was determined.

STATISTICAL ANALYSIS

The experimental results are presented as the Mean ± SD (Standard Deviation). Data have been analyzed by one way ANOVA followed by Dunnett 't' test using statistical package for social science (SPSS) software of 10 version.

RESULTS

Chemical screening of MMR

Chemical screening of crude extracts of *M. roxburghii* indicated that the plant had polyphenols, flavonoids, steroids, glycoside and also alkaloids. The compounds like anthraquinones, hydrolysable tannin, saponin, glycoside etc were not found in MMR (table 1).

Determination of median lethal dose (LD_{50})

LD_{50} of MMR was 600mg/kg (i.p.), in adult male albino mice. The experimental animals showed toxicity at this dose regarding body weight, food intake as well as general appearance.

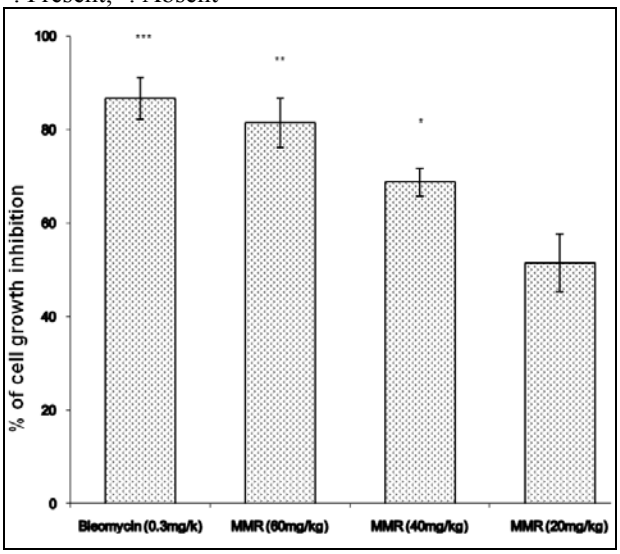
Determination of cell growth inhibition

Effects of MMR on tumor growth on 6th day of EAC inoculation is presented in fig. 1. Maximum EAC growth retardation was observed at 60 and 40mg/kg as evident from 81.4% and 68.7% inhibition respectively, whereas *bleomycin* showed 86.62% inhibition (0.3 mg/kg/day).

Table 1: Phytochemical components of crude methanol extract of *M. roxburghii*

Phytochemical components	*E. camadulensis*
Alkaloids	+
Saponins	-
Saponin glycosides	-
Tannins	-
Hydrolysable tannins	-
Phlobatannins	-
Anthraquinones	-
Glycosides	+
Cardiac Glycosides	-
Flavonoids	+
Steroid	+
Volatile oils	-
PolyPhenols	+

+: Present; -: Absent



Results are shown as mean ±SD (Standard Deviation), where significant values are *P<0.05, **P<0.01 and ***P<0.001 when (EAC+ MMR) treated mice compared with EAC bearing control mice (EAC bearing only).

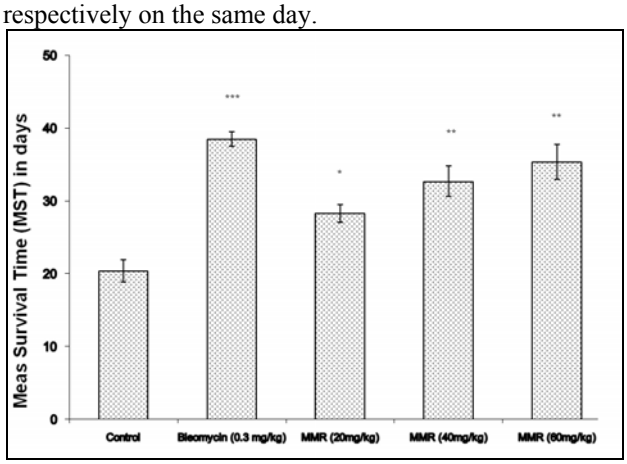
Fig. 1: Effects of MMR on EAC cells growth inhibition

Bioassay of EAC cells

Transplantability of EAC cell receiving MMR decreased remarkably as 51.4% reduction of EAC cell growth was observed when EAC cells from MMR treated mice (at the dose 60mg/kg i.p.) were re-inoculated into fresh mice and sacrificed and compared with control on day 5.

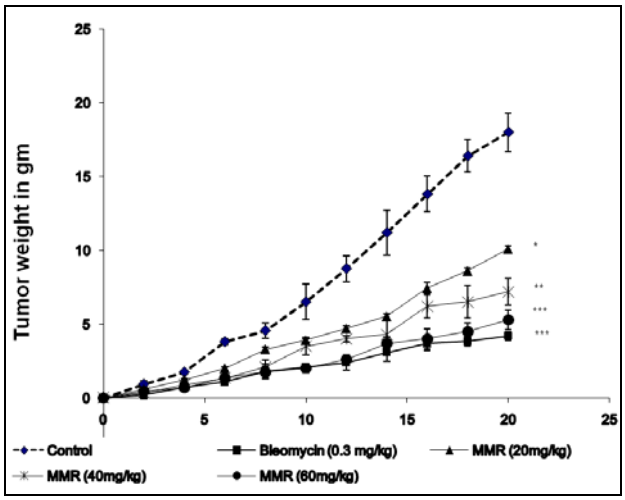
Effect of MMR on survival time and tumor burden

All effective chemo-preventive agents exhibit strong impact on survival time of tumor-bearing animals. Effects of MMR and bleomycin on survival time are shown in fig. 2. It was observed that EAC-bearing mice receiving MMR at doses 20, 40 and 60mg/kg shown a significant increased of life spans, which were 41, 60.3, 73.5% respectively compared to that of control mice. On the other hand, *bleomycin* increased life span by 88.7% when compared to EAC-bearing mice. Results of MMR at 20, 40, 60mg/kg (i.p.) and antitumor drug *bleomycin* (0.3mg/kg) on average tumor burden is given in fig. 3. Supplementation of MMR to EAC-bearing mice caused significant reduction of cancer burden. In control EAC-bearing mice, tumor burden raised by 68.5% on 20th day in comparison to normal animal. Experimental animal receiving MMR at 20, 40 and 60 mg/kg/day (i.p.), the tumor burden increased by 37.6, 28.52 and 21.5 % respectively on the same day.



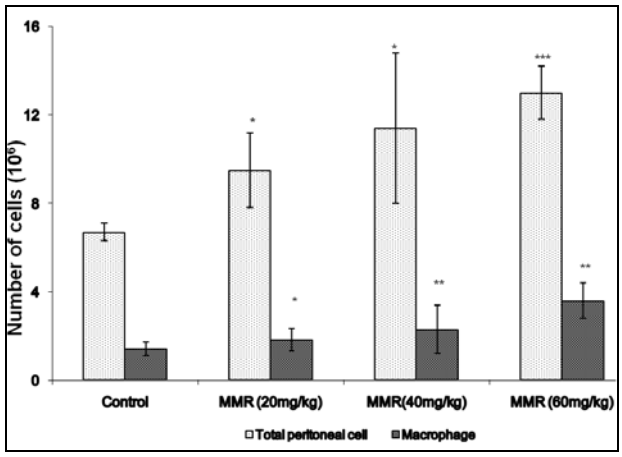
Results are shown as mean ± SD (Standard Deviation), where significant values are *P<0.05, **P<0.01 and ***P<0.001 when (EAC+ MMR) treated mice compared with EAC bearing control mice (EAC bearing only).

Fig. 2: Effects of MMR on survival time of tumor bearing mice



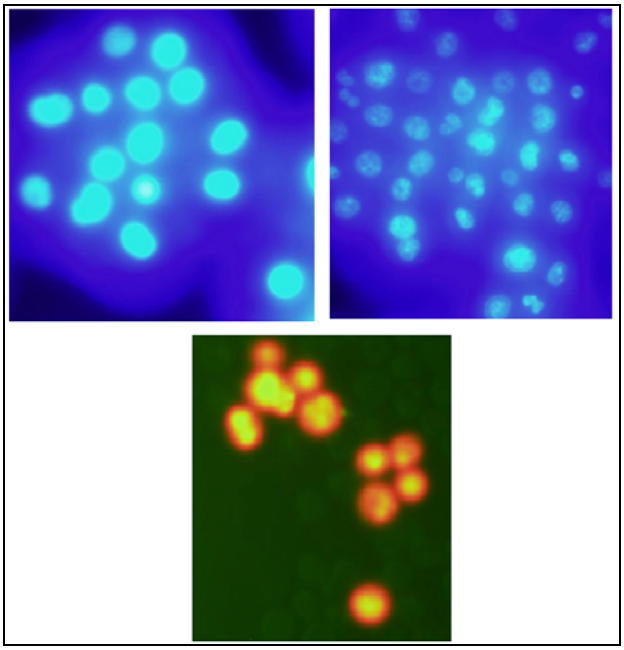
Results are shown as mean SD (Standard Deviation), where significant values are *P<0.05, **P<0.01 and ***P<0.001 when (EAC+ MMR) treated mice compared with EAC bearing control mice (EAC bearing only).

Fig. 3: Tumor weight of EAC bearing mice treated with MMR and *bleomycin*



Results are shown as mean ± SD (Standard Deviation), where significant values are *P<0.05, **P<0.01 and ***P<0.001 when (Normal+ MMR) treated mice compared with control mice.

Fig. 4: Effects of MMR on the enhancement of macrophages and peritoneal cells

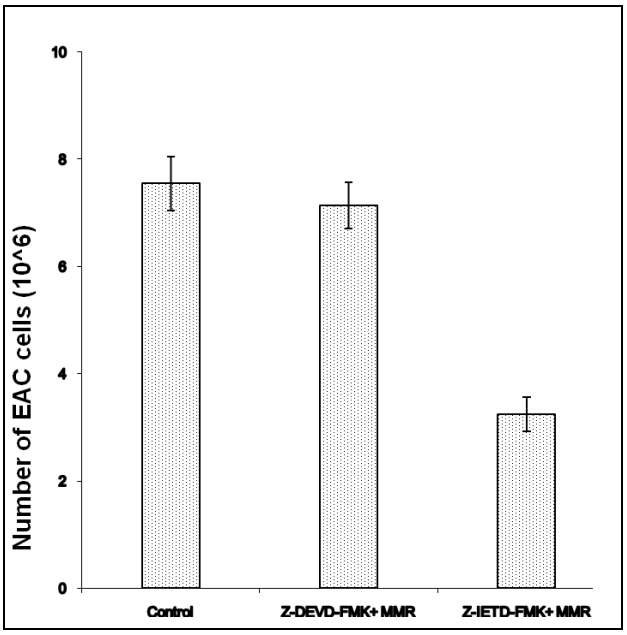


A) EAC of normal mice shown no apoptotic feature. B) EAC cells treated with extract shown nuclear condensation, fragmentation (black arrow), cell membrane blebbing (red arrow), and apoptotic bodies (white arrow) etc. C) Cells undergone late apoptosis (black arrow) and normal dead (white arrow) shown in PI staining.

Fig. 5: Fluorescence microscopic view of control and treated EAC cells

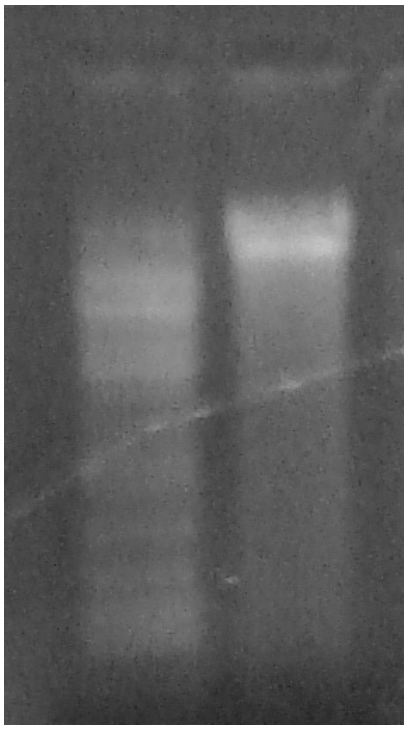
MMR effects on macrophages

Normal mice treated with MMR have had increased number of macrophages and other peritoneal exudates cells. It is observed that mice receiving MMR having (13±1.15) $\times 10^6$ peritoneal cells whereas the macrophage count of this animal was (3.6±0.32) $\times 10^6$ at 60 mg/kg/day respectively. Supplementation of MMR at 60mg/kg/day for 3 days remarkably increased macrophages (fig. 4).



Results are shown as mean ± SD (Standard Deviation).

Fig. 6: Effect caspases inhibitors on EAC cells



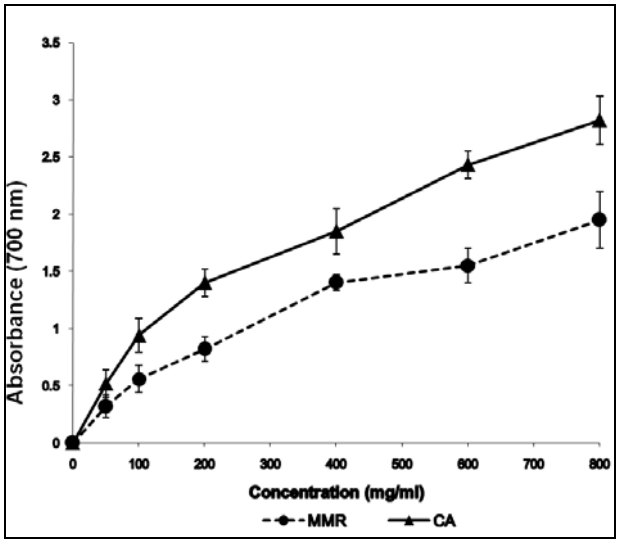
DNA run and detected on 1.5% agarose gel electrophoresis. A) DNA from control EAC cells, B) DNA from MMR treated EAC cells (DNA fragmentation detected from treated EAC cells).

Fig. 7: *In vivo* effects of extract on DNA fragmentation of EAC cells

Morphological changes and nuclear damage of EAC cells

Morphological changes of EAC cells were examined by Hoechst 33342 staining after culturing the cells with MMR and without extract (60µg/ml) for 24 hrs. EAC

nuclei were round, regular and homogeneously stained with Hoechst 33342 in control group as shown in fig. 5A. Whereas MMR treated EAC cells exhibit DNA fragmentation in nuclei (fig. 5B). Fluorescence microscopic observations of MMR treated EAC cells showed membrane blebbing, condensation of nuclear materials, apoptotic bodies etc. Results of this experiment indicated that MMR induced apoptosis of tumor cells. Necrotic or late apoptotic cell death caused by extract was also observed by staining with PI as shown in fig. 5C. Here the numbers of necrotic cells were found to be very low.



Results are shown as mean ± SD (Standard Deviation).

Fig. 8: Determination of total antioxidant capacity of *M. roxburghii*

Effect of caspase inhibitors

Caspase blockers/inhibitors Z-DEVD-FMK (caspase-3 blocker) and Z-IETD-FMK (caspase-8 blocker) were used to understand which pathway was switched on with the MMR treatment. The cytotoxicity of extract towards Z-IETD-FMK-pretreated EAC cells was significantly reduced to 56.95%, whereas Z-DEVD-FMK-pretreated cells did not any reduction of cytotoxicity in comparison to control (fig. 6).

DNA fragmentation assay

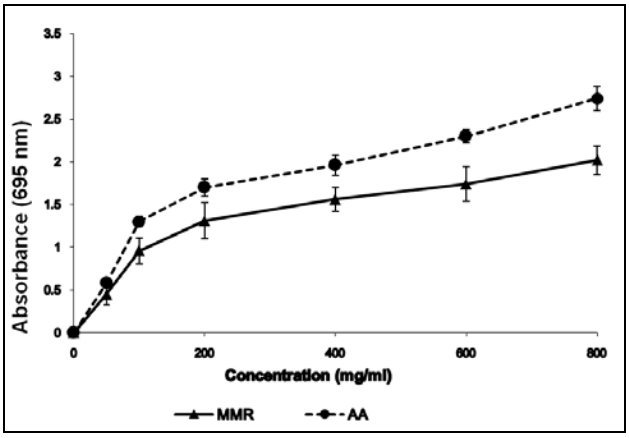
Activation of Ca^{2+}/Mg^{2+} dependent endonuclease caused cleavage of inter-nucleosomes and produced nucleotide fragments (180-200 base-pair). DNA from MMR treated EAC cells produced ladder like bands in electrophoresis and which is the characteristic feature of apoptosis induction, whereas DNA from untreated mice generated smear-like DNA bands, which was shown in fig. 7.

Total antioxidant capacity (TAC)

TAC of MMR was shown in fig. 8. The plant *M. roxburghii* showed potent antioxidant activity like reference standard catechin at the dose used in this experiment. Spectrophometric absorbance of MMR and standard CA were 1.95 and 2.82 respectively at 800 mg/ml.

Ferrous reducing antioxidant capacity assay

Fig. 9 showed the ferrous reducing potentials of MMR, which was quite comparable to Ascorbic acid. Reductive capacity of MMR was moderate at the dose used here and further increment of dose will showed promising activity.

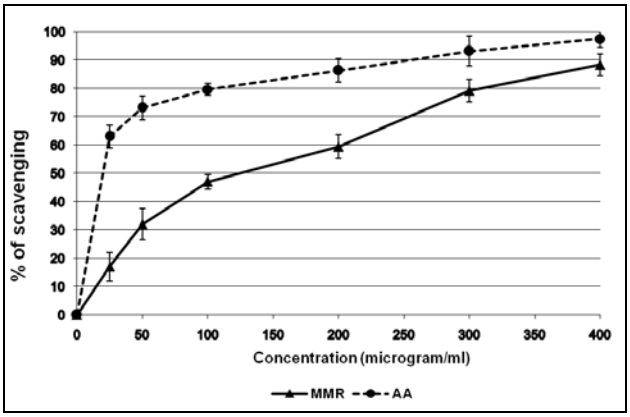


Results are shown as mean ± SD (Standard Deviation).

Fig. 9: Determination of ferrous reducing antioxidant capacity of *M. roxburghii*

Assay of DPPH radical scavenging

DPPH radical scavenging activity of MMR is presented in fig. 10. With increasing concentration of MMR this activity was found to be increased. Here, IC_{50} value of MMR was 120.4µg/ml and IC_{50} value of AA was 18.52µg/ml.



Results are shown as mean ± SD (Standard Deviation).

Fig. 10: Determination of DPPH radical scavenging activity of *M. roxburghii*

DISCUSSION

Reduction of tumor burden, enhancement of life span of cancer bearing mice, tumor cell growth inhibition is the important contributing factors of a potential anticancer agent. The efficiency of MMR as anticancer agents were compared clinically used anti-tumor chemo-agent

bleomycin and also with those found in similar type of extracts available in the literature (Muhammad *et al.,* 2011). The average tumor weight/burden reducing capacity of this extract has been examined. For EAC-bearing mice, body weight was raised gradually with time period. Treatment of such mice with the extract reduced the grown up remarkably. The extract, MMR also inhibited the cell growth rate effectively; as more than 71 % inhibition was found at the dose of 60 mg per kg body weight, this results is fairly as good as *bleomycin* (76.6%; 0.3 mg/kg).

Supplementation of MMR enhanced life span of EAC-bearing animals very effectively and its potency was found to increase with the enhancement of dose. In the present experimental design, a dose up to 60mg kg^{-1} was used. It is speculated that further enhancement of the life span will therefore be expected using higher doses. It is noted that the enhancement of life span has been assigned as a very important parameter for judging the suitability and efficacy of a compound as anticancer agent (Price and Greenfield, 1958). The high LD_{50} value (600 mg/kg) of this crude extract indicated little toxicity to the host.

It has been found that the extract of *M. roxburghii* has significant impact on the increment of macrophages and peritoneal cells in normal animals. Treatment with MMR at 60 mg kg^{-1} enhanced macrophages to a good number. This is being considered to be a very important parameter for acquiring self-destroying ability of the animals or living beings towards cancer cells (Fernandes and Klubes, 1979).

EAC cells treated with extract (MMR) shown nuclear condensation, fragmentation, cell membrane blebbing, apoptotic bodies etc. under the fluorescence microscope which implies that the extract induce EAC cells apoptosis. PI staining of EAC cells also indicates the late phase apoptosis induction after treatment with the extract. The integrity of the DNA was also assessed by agarose gel electrophoresis. DNA isolated from cells showed a ‘‘ladder’’ pattern in apoptosis. Genomic DNA isolated from treated and untreated cells shown apoptotic pattern in agarose (1.5%) electrophoresis. This characteristics ladder like DNA band in the gel, further conform the induction of apoptosis in EAC cells to the treatment of MMR.

There are two main signaling pathways of apoptosis induction (extrinsic and intrinsic). The first one is mediated by death receptor whereas intrinsic pathway is executed by caspases (Fulda *et al.,* 2010). To understand the involvement of specific signaling pathway trigger by MMR in EAC cells, the specific inhibitors of candidate molecules such as caspase-8 and caspase-3 were used. Data obtained from our experiment shows that EAC cells pretreated with caspase-8 inhibitor (caspase-8 blocked/inactivated and caspase-3 remain active) showed 56.95% growth inhibition in comparison to control whereas, cells pretreated with caspase-3(caspase-3 inactive and caspase-8 active) shown very little or no cells growth inhibition. This result leads one to conclude that caspase-3 mediated signaling pathway is involved in EAC cells apoptosis induced by the treatment of *M. roxburghii*.

Results obtained from antioxidant study revealed that MMR had modest antioxidant activity. It is reported that combination chemotherapy with antioxidants caused cancer regression and/or enhanced longevity cancer patients (Chinery *et al.,* 1997). Resistance against chemo-agents of cancer is believed owing to lower accumulation of agents into cancer cells (Robert, 1999). Recently it is demonstrated that antioxidants compounds like flavonoid increased the volume of chemo-agents in cancer cells and the glycosides changed the tumor cells' signaling by binding to Na^+/K^+-ATPase complex (Newman *et al.,* 2008). This altered signaling events "switch on" Src, phosphositide-3 kinase and phospholipase-C which in turn caused cancer cells death by apoptosis or autophagy related mechanism (Xie, 2001). Glycosides for instance oleandrin induced apoptosis of different cell lines by increasing the expression of Fas and Tumor Necrosis Fasctor receptor 1 (TNF1) in cancer cells (Sreenivasan *et al.,* 2006). Thus this compounds deserve promising potential in cancer (Winnicka *et al.,* 2006). Plant alkaloids in contrast caused cell cycle arrest by altering microtubules structure/dynamics, which in turn leads the cancer cells death through apoptosis.

It is thought that glycosides, flavonoids and alkaloids presence in MMR could be attributed to the anti-tumor activity of *M. roxburghii* through apoptosis but further research to identification specific active compound involved is to be carried out on different tumor cell lines and also in higher animals.

From above observations, it is said that MMR showing potential anticancer activity through intrinsic mitochondrial pathway and might be considered as one of the promising resources in cancer chemotherapy. It also showed moderate antioxidant activity, which might be providing save guard against damaging oxidants as well as boost up chemo-preventive potential.

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