Abstract

Cancer radiotherapy aims to effectively kill tumor cells but inexorably damages normal tissues which is undesirable. The success of radiotherapy lies in sparing normal tissues from radiation damage. Natural phytochemicals possess attribute which might protect normal cells but enhance tumor cell susceptibility followed by radiotherapy through modulating cellular molecular targets. Previous results of our laboratory and numerous recent studies on herbals have demonstrated significant cytotoxic activity to certain malignant tumors and protection of normal cell after exposure to ionizing radiation. This review describes the radio-therapeutic potential of *Moringa oleifera* (Lam.) that is easily available and common constituent of daily food. *Moringa oleifera* is an indigenous deciduous tree which possesses various therapeutic properties. The mechanisms of radioprotection of normal mammalian cells by alcoholic extracts of leaves and pods of Moringa have been discussed in terms of redox imbalance and oxidative stress. We have focused on the effect of alcoholic bioactive phytoconstituents of plant which displays synergistic cytotoxicity on tumor cells in combination with ionizing radiation. It is concluded that ethanolic extract of Moringa may be useful in efficient killing of tumor cells leading to establishment of improved protocol in cancer radiotherapy of patients.

**Key words:** *Moringa oleifera*, antioxidants, toxicity, free radical scavenging, extracts.

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is one of the prominent conventional treatment strategies where cancer cell are treated in the presence of adequate oxygen level; whereas low oxygen environment makes cancer cells more resistant to radiation and chemotherapy [3].

In chemotherapy and/or surgery, high doses of ionizing radiation are employed to treat solid malignant cells which affect normal cells and exert severe side effects including, hair loss, fibrosis, xerostomia, lung and kidney-dysfunction. Considering above points in the view, use of radioprotector is highly recommended in order to protect normal cells from harmful effects of ionizing radiation. Several synthetic radioprotectors were found to effective on normal cells and tissues, however, most of them exert severe side effects. Therefore, except amifostine and aminothiol no other radioprotector is approved for clinical application [4, 5]. Till date, safe synthetic radioprotectors are not available. Thus, there is an imperative need to identify novel, nontoxic, effective natural bioactive compounds that sensitize tumor cells and protect normal cells in radiotherapy.

Pertinent to it, the herbals mentioned in Ayurveda are used as folklore medicine for the treatment of various diseases since time immemorial. In search of potential radioprotectors to be used in cancer radiotherapy, a number of herbals and traditional compounds are under investigation. Several indigenous medicinal plants have been reported to render protection against radiation damage through improved targeting, selectively sensitizing malignant cells or protecting normal tissues by the use of natural antioxidants as a therapeutic option.

Previous research findings from our laboratory and others suggested differential role of triphala, nigella sativa, curcumin to induce apoptosis in tumor cells simultaneously protect normal cells [6, 7, 8]. The mechanism of action includes cell cycle arrest, alterations in survival signaling and up or down regulation of detoxifying enzymes. Recent investigations from our laboratory showed that combined treatment of biochanin A (a major isoflavone), was capable enough to sensitize radioresistant colon cells [9]. The mechanism was reported to involve additive increase of caspase-3 radioresistant cells alongwith substantial increase of ROS, lipid peroxidation and mitochondrial membrane potential. The present review is an attempt to discuss the radioprotection offered by alcoholic extract of *Moringa oleifera in vitro and in vivo.*

2. Brief Description of *Moringa Oleifera*

*Moringa oleifera* Lam. (MO) from the Moringaceae family is a native plant of the sub-Himalayan tracts of India, Pakistan, Bangladesh and Afghanistan. It is also known as the drumstick tree, benzolive tree, kelor, saijhan, and sajna or Ben oil tree [10]. Various parts of *M. oleifera* have long been used in habitual diets and traditional remedy in most of the tropical regions. Generation of reactive oxygen species (ROS) and other reactive radicals were found to be significantly reduced along with restored activities of antioxidant enzymes in *M. oleifera*. We have attempted to give a brief account of the progress in our understanding of the mechanisms of radioprotection of normal living cells by some major bioactive compounds of the plant such as quercetin, coumarin, and kaempherol.

MO is a fast growing soft deciduous tree about 10 to 12 m long and 30 cm in diameter. Leaves are alternate bi or tripinnate compounds, feathery, 3-5 cm
long and dark green in colour (Figure 1). White to cream colour flowers are lightly fragrant and straight stems are poorly formed. Barks are white thick corky and sticky [11]. Fruits are rounded green in colour, 15-45 cm long with 5 - 20 rounded or trilobed capsule shaped seeds [12].

3. The Phytoconstituents of *Moringa Oleifera*

The alcoholic, hydroalcoholic and aqueous extracts of various parts of MO have shown the highest antioxidant activity due to the high concentration of bioactive compounds which have defensive and curative properties. Table 1 gives a detailed account of various bioactive fractions available in different parts of the *M. oleifera*.

4. Medicinal and Nutritional Usages

Most parts of the Moringa tree e.g. leaves, fruits (pods), stem, barks, and flowers are consumed by humans in many countries. Bioactive compounds from Moringa are known to be immune boosting, hypotensive, anticancer, antibacterial alongwith antioxidant activities and involve induction of tumor suppressive effects [24, 25, 26]. Different parts of the plant have phytopharmaceutical components which have traditional, medicinal and industrial usages (Figure 1) [27]. Ethanolic extract of seeds have shown antipyretic, antiasthmatic and analgesic properties [28,29,30] hypocholesterolemic, wound healing, antithyroid., hypotensive, hepatoprotective, antitumorogenic, antiulcer, antimicrobial activities were shown by extracts of leaf, flower, stem and seed [24,31,32,17,23,19] . Extracts of stem have shown capacity to prevent cardiovascular diseases and reduce high blood pressure [33].

5. Mechanism of Action of *Moringa Oleifera*

As described earlier, radiation affects the biological systems in more than one ways. Herbals have been reported to act as a radioprotector under *in vivo* and *in vitro* conditions. The properties of bioactive compounds can facilitate therapeutic drugs to act differentially towards tumour and normal tissues, thus displaying selective metabolic effects. The alkaloids and flavonoids are among naturally occurring phytochemicals helpful in radioprotection and radiosensitization. They exhibit antimutagenic and anticarcinogenic properties, and prevent cancer [34, 35] as they are an ingredient of human diets.

5.1 Chemopreventive Potential:

Upregulation of antioxidant inhibits tumor progression and protect against damage of normal cells. Under chemo-preventive strategy, antioxidants act as bifunctional inducer that maintains balance of xenobiotic metabolism towards detoxification. Hydroalcoholic extract considerably increased antioxidant activity of pod by significant upregulation of GPx, GR, SOD, GSH, cytochrome p450 and CAT. Moreover, it slightly decreased hepatic MDA level by eliminating free radical in Skin papillomagenesis mice [23]. Sreelatha, et al (2011) reported anti-proliferation and chemoprevention in human tumor (KB) cell line. Leaf extract induced apoptosis as determined by morphological changes and DNA fragmentation. Sometimes, redox-sensitive mechanisms are known to modulate upregulation of ROS [36]. In a report, hydroalcoholic extract of *M. oleifera* leaves was found to show cytotoxic activity against HeLa cell line [37].
Figure 1: Schematic diagram showing various medicinal uses of edible parts (leaves & fruits) of *M. oleifera*

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Part Used</th>
<th>Bioactive Compounds</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Leaves</td>
<td>Gallic acid, chlorogenic acid, ellagic acid, ferulic acid, flavanoid like 5 kaempherol glycosides, quercetin glycosides, rutin, vanillin syringic acid caffeoylquinic acid glycosides, thiocarbonate, carbomate, niazirin, niaziridin, niazirinin, benzyl isothiocyanate, niaziminin A, niaziminin, ascorbic acid, carotenoids, β-sitosterol</td>
<td>[13,14,15,16,17]</td>
</tr>
<tr>
<td>2</td>
<td>Fruits/ Pods</td>
<td>Gallic acid chlorogenic acid, ellagic acid, ferulic acid kaempherol, quercetin vanillin niazirin, niaziridin, lactose, arabinise, rhamnose</td>
<td>[13,14,18]</td>
</tr>
<tr>
<td>3</td>
<td>Roots</td>
<td>Benzyl glucosionolate, niazimicin, aurentiame acetate 4, 1,3-dibenzylurea</td>
<td>[16,19,20]</td>
</tr>
<tr>
<td>4</td>
<td>Seeds</td>
<td>Benzyl isothiocyanate, moringyne, several amino acid, sterols, tocopherol, fatty acid, niazimicin, niazirin, β-sitosterol glycosides, glucomoringin</td>
<td>[21,22,23]</td>
</tr>
<tr>
<td>5</td>
<td>Bark</td>
<td>Benzyl glucosinolate, niaziridin niazirin</td>
<td>[14,16]</td>
</tr>
<tr>
<td>6</td>
<td>Flower</td>
<td>Octadecen, oleol, satol, sipo, deconoic acid, dodecanal</td>
<td>[16]</td>
</tr>
</tbody>
</table>

**Table 1: Phytoconstituents present in the parts of *M. oleifera***
5.2 Cytotoxic and Antiproliferative Mechanisms:
Charoensin et al. (2014) demonstrated that bioactive metabolite enhanced free radical scavenging activity in various cancer cell lines [38]. The free radical scavenging mechanism of ethanolic extract was found to be protective against leukemia and hepatocarcinoma cells [39]. Nair et al., (2011) have reported that aqueous extract of leaves possesses anticancerous and cytotoxic properties. Additionally, it displayed non-toxic and proliferative action on normal healthy lymphocytes. Furthermore, increase in extract concentration simultaneously decreased survival of HeLa cell line [40]. Bioactive compounds present in ethanolic seed extract are potent antioxidants that show antitumor promoting activities. Moreover, in vitro results showed that benzyl isothiocyanates, niazimicin and isolates of β-sitosterol possess ability to inhibit TPA-induced Epstein-Barr virus. Furthermore, Guevera et al. (1999) has reported that niazimicin, hindered antitumor promoting activity in vivo [22]. According to Brunelli et al. (2010), in vitro and in vivo activities of glucomoringin derived isothiocyanates (GMG-ITC) prevented damage in various cancer cell lines. The latter is reported to induce apoptosis, cell cycle perturbations and reduced the level of NF-κB activity by modulating GST/GSH pathway [41].

5.3 Radioprotective Properties:
Rao et al. (2001) demonstrated that pre administration of ethanolic leaf extract was found to be supportive in protection against γ irradiation by inhibiting free radical generation [42]. In vitro study by Berkovich et al. (2013) depicted that aqueous extract of leaf is antiproliferative and cytotoxic for pancreatic cancer cells (Panc-1, COLO 357 and p34). The mechanism was mediated by inhibition of NF-κB signaling cascade and reduces the expression of p65, p-IκBα and IκBα proteins [43]. Moreover, hydro-alcoholic extract of leaf significantly decreased radiation induced oxidative stress that causes lipid peroxidation and hinders the translocation of nuclear factor kappa B (NF-κB) in mice model. In Consequence, endogenous antioxidant such as superoxide dismutase (SOD), catalase (CAT), and glutathione (GSH), was upregulated [44, 45].

5.4 Anti-inflammatory Activities:
The boiled pod extract showed anti-inflammatory activity by inhibiting upregulation of mRNA, protein level of interleukine-6, tumor necrosis factor-alpha, inducible nitric oxide synthase, and cyclooxygenase-2 [46]. According to Sashidhara et al. (2009) demonstrated that aurantiamide acetate and 1, 3 dibenzyl urea isolated from roots produced anti-inflammatory and analgesic activities by inhibiting TNF-alpha, IL-2 cytokines [20, 47].
<table>
<thead>
<tr>
<th>Part Used</th>
<th>Properties</th>
<th>Extract</th>
<th>Cell line</th>
<th>Mechanism</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>LEAVES</td>
<td>Chemopreventive</td>
<td>Phenolics</td>
<td>Human tumor (KB) cell line</td>
<td>Induction of apoptosis</td>
<td>[36]</td>
</tr>
<tr>
<td></td>
<td>Antitumor</td>
<td>Ethanolic</td>
<td>Leukaemia and Hepatocarcinoma cell line</td>
<td>Inhibition of free radical</td>
<td>[39]</td>
</tr>
<tr>
<td></td>
<td>Cytotoxic &amp; Anticancer</td>
<td>Aqueous</td>
<td>HeLa cell line &amp; Lymphocytes</td>
<td>Inhibition of radical formation</td>
<td>[40]</td>
</tr>
<tr>
<td></td>
<td>Cytotoxic</td>
<td>Hydroalcoholic</td>
<td>HeLa Cell line</td>
<td>Induces DNA fragmentation &amp; Apoptosis</td>
<td>[40]</td>
</tr>
<tr>
<td></td>
<td>Anticancer</td>
<td>Methanolic</td>
<td>HepG2, Caco-2 and MCF-7 cancer cell line.</td>
<td>Cytotoxic towards cancer cells</td>
<td>[37]</td>
</tr>
<tr>
<td></td>
<td>Radioprotective</td>
<td>Methanolic</td>
<td>Swiss albino mice</td>
<td>Antiproliferation of cancer cell</td>
<td>[38]</td>
</tr>
<tr>
<td></td>
<td>Hepatoprotective</td>
<td>Hydroalcoholic</td>
<td>Swiss albino mice</td>
<td>Inhibition of micronucleus in erythrocytes &amp; aberrations in metaphase chromosomes</td>
<td>[42]</td>
</tr>
<tr>
<td></td>
<td>Antioxidative</td>
<td>Ethanolic</td>
<td>Swiss albino mice</td>
<td>Inhibition of NF-κB translocation and Lipid peroxidation; increases SOD, CAT, GSH</td>
<td>[44]</td>
</tr>
<tr>
<td>properties</td>
<td></td>
<td>Methanolic</td>
<td>Rat model</td>
<td>Inhibit radiation induced lipid peroxidation, increases GSH</td>
<td>[45]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ethanolic</td>
<td>Swiss albino mice</td>
<td>Increase GSH, GSH-R, GPx &amp; CAT levels in RBCs and decrease lipid peroxidation in liver</td>
<td>[50]</td>
</tr>
<tr>
<td></td>
<td>Antiproliferative</td>
<td>Aqueous</td>
<td>Pancreatic cancer cell lines (Panc-1 and COLO-357)</td>
<td>Inhibits the NF-κB signalling Pathway</td>
<td>[43]</td>
</tr>
</tbody>
</table>
### Table 2 Various bioactive fractions available in different parts of the *M. Oleifera*

<table>
<thead>
<tr>
<th>SEED</th>
<th>Antioxidative Properties</th>
<th>Antitumor Activity</th>
<th>Anticancerous Activity</th>
<th>Antitumoral Activity</th>
<th>Female rat</th>
<th>MCF cell line</th>
<th>myeloma mice model</th>
<th>Induced hepatic toxicity</th>
<th>Inhibited induction of EBV-EA</th>
<th>Cell cycle, Perturbations induces apoptosis, NF-κB inhibition</th>
<th>Cytotoxicity in cancer cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Antioxidative extract</td>
<td>Ethanolic extract</td>
<td>Ethanol extract</td>
<td>Ethanol extract</td>
<td>Female rat</td>
<td>MCF cell line</td>
<td>myeloma mice model</td>
<td>Induced hepatic toxicity</td>
<td>Inhibited induction of EBV-EA</td>
<td>Cell cycle, Perturbations induces apoptosis, NF-κB inhibition</td>
<td>Cytotoxicity in cancer cells</td>
</tr>
</tbody>
</table>

### 5.5 Antioxidative Properties

Aqueous extract of seeds are reported to efficiently inhibit arsenic induced alteration in hepatic function. Additionally, Chattopadhyay et al. (2011) demonstrated that the extract prevented DNA fragmentation, ROS initiation, oxidative stress and free radical in female albino rats [48]. Ethanol and aqueous extract of leaves and pod possesses strong reducing power, good anti-stresser and free radical scavenging activity because of its antioxidant properties. Moreover, it is shown that dose dependent concentration increased the level of endogenous antioxidant with decrease in MDA level [49]. These studies have put forward leaves and pods of *Moringa oleifera* as potent candidate to regulate redox balance.

### 5.6 Experimental Evidences of Medicinal Properties

Table 2 gives a detailed account of various bioactive fractions available in different parts of the *M. oleifera*. 

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**Table 2** Various bioactive fractions available in different parts of the *M. Oleifera*
6. Discussion
It is evident from above discussed mechanism that various parts of MO contain polyphenols, alkaloids and flavonoids that display antioxidant activity. At present numerous bioactive compounds isolated from *M. oleifera* such as quercetin, rutin, kaempherol, niazimicin, gallic acid are under preclinical or clinical trials. Based on this information as provided in Table 2, it has been presumed that among all studied parts of MO, leaves and pod displayed more potent antioxidant activity as compared to other parts. They were reported to down regulate cytokines, tumor necrosis factor-alpha, inducible nitric oxide synthase, and cyclooxygenase-2 that mediates radiation induced cancer cell death [46]. Various studies from different laboratories are in agreement that bioactive compounds present in the leaves and pod of MO protect from harmful effects of ionizing radiation. Recently, Charoensin and Wongpoomchai (2012) demonstrated free radical scavenging activity of MO leaf extract that contains polyphenols [38]. Moreover, pretreatment of leaf extract before irradiation was reported to initiate senescence and apoptosis in cancer cells by free radicals inhibition and up-regulation of endogenous antioxidants such as GSH, TRX, and CAT [39]. Berkovich et al. (2013) showed hepatoprotective effect from leaf extract through scavenging free radical and down-regulation of NF-κB and LPO [43].

7. Conclusion
Recent investigations have suggested that ethanolic extracts of *M. Oleifera* mainly containing alkaloids, flavonoids and polyphenolic compounds possess antioxidant and anti-inflammatory properties. These bioactive compounds are known to suppress cancer cell proliferation by inhibiting factors such as iNOS, COX-2, NFκB which are upregulated in majority of tumor cells. The components of extracts offer protection to normal cells against radiation by free radical scavenging and enhancing endogenous enzymes. The extract appears to have potential to modulate cell signaling pathways exhibited in therapeutic outcome. It is suggested that leaves and fruits offer greater radioprotection as compared to other parts of Moringa.

References
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