Benefits of using plant extracts in fertility preservation with special reference to *Moringa oleifera*

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Summary

Due to the increase in childhood cancer survivors, fertility preservation has become an important issue in the field of cancer management and infertility treatment. However, the limitations of current methods such as semen cryopreservation, testicular tissue cryopreservation, and *in vitro* spermatogenesis, urges the researchers to explore alternative and practically feasible approaches to protect the gonadal tissue from the cytotoxic chemotherapeutic agents. This review highlights the possible application of natural products, especially the *Moringa oleifera* leaf extracts in protecting the gonadal tissue from cytotoxic agents, their possible mechanism of action, advantages, current situation and scope for future research.

KEY WORDS: male fertility preservation, gonadal damage, infertility, natural products, plant extract, *Moringa oleifera*.

Introduction

In the last few decades a tremendous development in the field of cancer therapy has been noticed due to which the survival rate in cancer victims has increased. Especially in pre-pubertal age group, the survival rate has increased up to 80% (1). Even though there are concerns of long term effects of cancer treatments on general health of the individual, majority of these children reaches the reproductive age and think of having their own family. Since treatment modalities such as radiotherapy and chemotherapy, either alone or in combination with other treatment modalities are cytotoxic in nature, the gonadal tissue is most commonly affected (2, 3). The adverse effects of these treatments may have reversible or irreversible effects on the gonadal function and hence the concern about the future fertility potential of the cancer survivor is always uncertain.

In recent days, fertility preservation has become an important issue in planning the cancer treatment for an individual. Especially, fertility preservation in prepubertal age group is a challenging area of research since in this group of patients gametogenesis is not completely attained. The present review is focused on the current approaches of male fertility preservation with emphasis on the research advances in fertility preservation using plant products, their advantages and future research scope.

Fertility preservation approaches

Various approaches have been followed in the past to ensure that the fertility potential of the cancer victim is retained after completion of the treatment. In males, if the individual is adult and has adequate number of spermatozoa, well estab-
lished method like semen cryopreservation is useful (4). However, in pre-pubertal boys testicular tissue cryopreservation is most suitable due to the absence of mature spermatozoa which has to be followed by transplantation of the testicular tissue or spermatogenesis in vitro. Even though these techniques have shown some promising results in animal studies (5) their applicability in clinical service is yet to be established. Therefore, alternative approaches are explored rigorously.

Chemoprotection of gonadal tissue

Chemoprotection can be defined as protecting the normal tissue from the hazardous effects of cytotoxic treatments like radiotherapy and/or chemotherapy. This concept gained lot of attention during the World War II in view of nuclear and chemical warfare (6). An ideal chemoprotective agent should be able to protect the normal tissues without interfering with cancer cure. Studies have demonstrated that few chemoprotective agents not only prevented normal tissue toxicity, but also increased cytotoxic activity of chemotherapeutic agents on cancer cells (7, 8). On the contrary, D’Andrea (9) demonstrated that these agents can also reduce the efficiency of the cancer therapy. However, due to the technical difficulties with other approaches explained earlier, chemoprotection of the gonadal tissue seems to be a practically feasible, easiest and physiological option at present.

a. Gonadotrophin releasing hormone analogues (GnRHa): this approach is based on the concept that gonadotropin-releasing hormone analogs suppress the pituitary gonadotropins and follicle-stimulating hormone (FSH)-dependent germ cell proliferation. Since the testicular tissue enters a quiescent state following continuous administration of GnRHa, cytotoxicity of the chemo and/or radiotherapy is expected to be lowest. Earlier studies have demonstrated that this approach is beneficial in male fertility preservation (10, 11). However, few other studies have failed to observe such gonadoprotective role in experimental models and humans (11-13).

b. Hormone administration: this approach is opposite of the previous approach which is based on stimulating the spermatogenesis by administration of FSH. Van Alphen et al. (14) observed that administration of FSH can protect the testis from radiation induced testicular damage in Rhesus monkey which is probably related to the proliferative effect of FSH on spermatogonial cells in normal monkeys (15) and increased DNA repair activity (16). Pretreatment with testosterone, testosterone and 17β estradiol results in enhanced spermatogenesis after irradiation or procarbazine treatment (17, 18) hypothesizing that the hormonal pretreatment will help in protecting the spermatogonial stem cells. In addition, Meistrich et al. (19) demonstrated that the spermatogenesis can be initiated by administration of GnRH agonist following cytotoxic therapy. However, these approaches did not show impressive clinical results in oncological patients.

c. Synthetic compounds: the most commonly explored synthetic compound for mitigating the normal tissue toxicity during cancer treatment is amifostine (WR-2721). It is an organic phosphorylated thiol, used extensively as a radio and/or chemoprotective agent in the 80’s and 90’s. Majority of the studies has focused on exploring the protective effect on hemopoeitic and sensory system (20-23). However, reports on its gonadoprotective function do not appear to be convincing (24, 25). Even though AS101, an immunomodulator (26) and pyrrolidine dithiocarbamate (PDTC), a nuclear factor-kB inhibitor (NF-kB) (27) have shown promising results, there are no supporting studies to prove their clinical utility.

d. Natural products: from time immemorial, natural products have played an important role in disease management and drug development. Several chemicals have been isolated from natural compounds such as plants, microbial organisms, marine flora and organisms and animals with potent pharmacologic action. Among these, plant products occupy a special position since they are consumed regularly in the form of diet, or traditional Ayurvedic and Chinese medicinal systems. In the last four decades plant extracts have been tried extensively as radioprotective and chemoprotective agents (28-32). They can be used in the form of crude extract, multiherbal formulations or
isolated compounds. Crude plant extract usually contains concentrated portion of medicinally active components extracted with the help of a suitable solvent. On the other hand, multiherbal formulations are the medicinally concentrated portions of various plants that are mixed in a suitable proportion to get maximum pharmacological effect. Similarly, the plant derived compounds are the pure phytochemicals with medicinal property that are isolated, purified and characterized using sophisticated separation techniques.

Fertility preservation using plant extracts

a. Crude plant extract. A large number of plant extracts have been screened for their beneficial effect on protecting the normal tissues from radiotherapy and chemotherapeutic drugs (33). However, studies focusing mainly on the gonadal tissue protection are limited (Table 1). Extracts of plants Phodophyllum hexandrum (28), Hippophae rhamnoides (34), Spinacia oleracea (35), Hibiscus sabdariffa (36), Mentha piperita (29), Gingko biloba (31), Carya illinoensis (37) have shown promising protective role in experimental animals. A recent study by Mohammadi et al. (38) has demonstrated that oral administration of Gingeber officinale extract from rhizome protects the rat testis from cyclophosphamide induced adverse effects. Our laboratory has been exploring the beneficial properties of Moringa oleifera plant in male fertility preservation. It is a plant with high nutritional and medicinal significance and, cultivated widely in India, other Southeast Asian countries, Central America, Caribbean islands and Africa. The plant belongs to Moringaceae family with common names- drumstick or horseradish tree (Figure 1). Leaves, bark, pods, roots and flowers of this plant have been used in preparation of various dishes as

<table>
<thead>
<tr>
<th>Plant extract/isolated</th>
<th>Plant part used</th>
<th>Type of isolated compound</th>
<th>Gonadotoxic agent</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phodophyllum hexandrum</td>
<td>Rhizome extract</td>
<td>-</td>
<td>Radiation</td>
<td>Samanta and Goel, 2002 (28)</td>
</tr>
<tr>
<td>Mentha piperita</td>
<td>Leaf extract</td>
<td>-</td>
<td>Radiation</td>
<td>Samarth and Samarth, 2009 (29)</td>
</tr>
<tr>
<td>Moringa oleifera</td>
<td>Leaf extract</td>
<td>-</td>
<td>Cyclophosphamide</td>
<td>Nayak et al., 2015 (32)</td>
</tr>
<tr>
<td>Ficus racemosa</td>
<td>Stem bark extract</td>
<td>-</td>
<td>Doxorubicin</td>
<td>Ahmed et al., 2013 (44)</td>
</tr>
<tr>
<td>Carya illinoensis</td>
<td>Nut shell extract</td>
<td>-</td>
<td>Cyclophosphamide</td>
<td>Benvegnu et al., 2013 (37)</td>
</tr>
<tr>
<td>Gingko biloba</td>
<td>Leaf extract</td>
<td>-</td>
<td>Cisplatin</td>
<td>Amin et al., 2012 (31)</td>
</tr>
<tr>
<td>Gingko biloba</td>
<td>Leaf extract</td>
<td>-</td>
<td>Doxorubicin</td>
<td>Yeh et al., 2009 (30)</td>
</tr>
<tr>
<td>Astragalus membranaceus</td>
<td>Root extract</td>
<td>-</td>
<td>Cyclophosphamide</td>
<td>Kim et al., 2012 (45)</td>
</tr>
<tr>
<td>Crataegus monogyna</td>
<td>Fruit extract</td>
<td>-</td>
<td>Doxorubicin</td>
<td>Jalali AS and Hasanzadeh, 2013 (46)</td>
</tr>
<tr>
<td>Camellia sinensis</td>
<td>Leaf extract</td>
<td>-</td>
<td>Cyclophosphamide</td>
<td>Sato et al., 2010 (47)</td>
</tr>
<tr>
<td>Spinacia oleracea</td>
<td>Leaf extract</td>
<td>-</td>
<td>Radiation</td>
<td>Sisodia et al., 2008 (35)</td>
</tr>
<tr>
<td>Resvaratrol</td>
<td>NA</td>
<td>Stelbinoid</td>
<td>Methotrexate</td>
<td>El-Sheikh et al., 2014 (48)</td>
</tr>
<tr>
<td>Astaxanthin</td>
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<td>Carotenoid</td>
<td>Cyclophosphamide</td>
<td>Tripathi and Jena, 2008 (49)</td>
</tr>
<tr>
<td>Kolaviron</td>
<td>NA</td>
<td>Flavonoid</td>
<td>Radiation</td>
<td>Adaramoye et al., 2012 (50)</td>
</tr>
<tr>
<td>Curcumin</td>
<td>NA</td>
<td>Polyphenol</td>
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<td>Ilbey et al., 2009b (51)</td>
</tr>
<tr>
<td>β-carotene</td>
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<td>Carotenoid</td>
<td>Methotrexate</td>
<td>Vardi et al., 2009 (52)</td>
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<tr>
<td>Ellagic acid</td>
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<td>Cisplatin</td>
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<td>Vitamin C</td>
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<tr>
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<td>NA</td>
<td>Organo-sulfar</td>
<td>Cyclophosphamide</td>
<td>Kim et al., 2013 (55)</td>
</tr>
</tbody>
</table>
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In a recent study conducted by our group, we have demonstrated that administration of ethanolic extract of Moringa oleifera leaves (MOE) significantly protects the prepubertal testicular tissue from cyclophosphamide induced toxic effects (32). Administration of MOE (25 mg/kg, intra peritoneally) was able to overcome the reduction in sperm count and motility, defective head morphology and poor DNA integrity of the spermatozoa induced by cyclophosphamide (50 mg/kg body weight). Similar observations were made with another chemotherapeutic agent busulfan (BS). MOE administration prior to BS treatment (30 mg/kg, intra peritoneally), significantly improved the sperm count, motility, sperm head morphology and DNA integrity further confirming its chemoprotective property (unpublished data). The mechanism of action of MOE in protecting testicular tissue from the chemotherapy induced damage appears to be complex. Significant reduction in lipid peroxidation and increase in the activities of antioxidant enzymes such as superoxide dismutase (SOD) and Catalase indicates that the protective effect is mediated through efficient scavenging of free radicals generated following chemotherapy (32). In addition, the upregulation of mRNA expression of pluripotency gene Oct-4 and stem cell factor C-kit helps in replenishing the germ cell depleted after cyclophosphamide or busulfan treatment (unpublished data). These results indicate that Moringa oleifera leaf extract has promising future in male fertility preservation.

a. Mechanism of action of plant extracts in fertility preservation. Owing to their complexity with respect to the phytochemical composition, it is difficult to elucidate the exact mechanism by which plant extracts brings about chemoprotection/fertility preservation. However, the most probable mechanism appears to be mediated through its scavenging action on free radicals generated by chemotherapeutic drugs or radiation (29, 30, 56). Majority of the plant extracts are rich in antioxidants such as flavonoids, polyphenols, vitamins, etc. (57, 58). These agents may act directly on the free radicals and neutralize their effect. In addition, they elevate the activity of battery of antioxidant enzymes such as catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx), or increase the level of cellular antioxidant reduced glutathione (GSH) (59, 60). Earlier studies have shown that the plant extracts decrease the DNA damage and increase the DNA repair ability (61) thereby reducing the loss of proliferating cells. Altogether, these complex mechanisms together may help in improving the gonadal function after chemotherapy.

b. Isolated compounds from plants. The nutritional and medicinal properties of plants extracts are due to the presence of wide variety of active phytochemicals. According to WHO
report (62) more than 121 drugs prescribed in USA today have been isolated from the natural sources out of which more than 90 are from plant source (63). Moreover, studies carried out in animal models have shown that various isolated compounds like curcumin, β-carotene, ascorbic acid, ellagic acid, L-cysteine, vitamin E, resveratrol, etc., ameliorated cytotoxic and genotoxic effect induced by chemotherapeutic agents on gonadal cell and increased reproductive potential of the animals (48, 49, 51-53, 55, 64). Most of these compounds acts as natural antioxidants and helps in scavenging the reactive oxygen species generated by toxic compounds and maintains tissue oxidative balance which probably helps in reestablishment of testicular homeostasis.

Conclusion

Natural compounds are a part of traditional medicinal system even before the civilization. Their beneficial role in enhancing the gonadal function has been observed after traditional Ayurvedic and Chinese medicinal system. Thus, the rich ethnomedical knowledge justifies the use of natural compounds as a potent and safe strategy to mitigate the gonadotoxic effects. Since we consume these plant products regularly in various forms of diet, they may not exert any toxicity on their own. Their rich content in potent antioxidant phytochemicals might also help in improving the immune status of the individual following cancer treatment and thereby improving the general health status (57). Earlier studies have shown that leaf extract of *Moringa oleifera* has significant stimulatory effect on cellular and humoral immunity (65-67). However, the seed extracts possess immunosuppressant function (68, 69). The toxicity data available in the literature suggests that the leaf extract is well tolerated by the animals without any systemic toxicity and organ dysfunction (70, 71). As we have enormous heritage of medicinal plants, it is worth exploring the efficient, economically viable and clinically acceptable plant products or their formulations to identify a best plant based compound/extract for fertility preservation. However, using the plant extracts for therapeutic purposes are not without any concerns. The presence of multiple phytochemicals makes it difficult to understand the mechanism of action by which these crude extracts bring about the protective effect and also their pharmacokinetics which can help in planning an ideal treatment schedule. To overcome this problem, even though the active principle present in the extract can be identified and separated, the magnitude of the efficiency of the isolated compounds may not be similar compared to crude extract (72, 73). Other problems such as batch to batch variation and seasonal variation of the extract with respect to the concentration of phytochemicals, avoiding microbial organisms/ pathogens during collection, preparation and storage of the crude extract are quite common. In conclusion, using the plant extracts for fertility preservation seems to be a safe approach. However, in depth studies are required in future to ascertain that they do not interfere with the cancer cure. Our laboratory is working on in this direction to further explore the possibility of using the plant extracts for fertility preservation in cancer patients.

Acknowledgements

Authors acknowledge the partial financial assistance from Indian Council of Medical Research (ICMR) (Grant. No.59/51/2010/BMS/TRM).

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