Review Article

Moringa Oleifera- A Wonder Plant for Male Fertility Preservation

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Abstract

Since time immemorial, *Moringa oleifera* Lam. (MO) is a highly valued plant with potential application in the area of nutrition and medicine. All parts of the plant have been used in preparing Ayurvedic medicines while the leaves and pods have been used in preparing traditional dishes. The leaves of this plant are most commonly used as they are rich in amino acids, vitamins, poly-phenols, flavonoids, alkaloids and phytosterols, which attribute to the nutritive and medicinal properties. The tissue and cyto-protective action of MO against various toxic agents is gaining tremendous importance in the field of toxicology. However, very few reports have highlighted the protective effect of MO and its active components against gonadotoxic agents. The present review mainly focuses on the medicinal properties of MO with special reference to its role in male fertility preservation.

Key words: Moringaoleifera, Chemotherapy, Testicular damage, Chemoprotection, Antioxidant

Introduction

Moringa oleifera (MO) is a fast growing tree belonging to monogeneric family Moringaceae, a native of India. The genus Moringa has 13 other species native to the African continent[1] and are distributed in tropical and subtropical regions of the world (Fig 1). The plant has been used from ancient times to prepare various dishes and herbal medicines owing to its rich nutrient contents and therapeutic values. Hence, it is popularly known as 'Miracle tree' or 'Nature gift' or 'Mothers best friend'. In India, MO is commonly referred to as 'drumstick tree', due to the shape of its fruit which resembles a drum stick. It is also called as 'horse radish tree' due to the pungent smell of its roots. The plant has a diverse application such as source of food, animal fodder, natural coagulants, forestry products, fertilizer, alley cropping and fueling.[2]

Fig 1: Global distribution of Moringa species (shown in green color)

Taxonomy of *Moringa oleifera* Lam.

Kingdom: Plantae
Phylum: Tracheophyta
Class: Magnoliopsida
Order: Brassicales
Family: Moringaceae
Genus: *Moringa*
Species: *oleifera*

Moringa oleifera plant as a nutritive source

Moringa oleifera is a highly nutritive plant. MO seeds are rich in monounsaturated fatty acids and proteins with sulfur containing amino acids.[3] Interestingly, Moringa species grow in zones where malnutrition is more evident such as drought affected areas. The leaves, pods and seeds (Fig 2) of this plant are nutritious and most commonly used for preparing traditional dishes. In India, M. oleifera is commercially cultivated mainly for its pods as a vegetable[4]; the pods are rich in proteins, carbohydrates and dietary fibers.[5] The leaves are rich in proteins, essential amino acids, vitamins, minerals and antioxidants which serve as natural remedy in preventing malnutrition in children of under-developed countries.[6-8] A randomized blind placebo control study showed that MO leaf powder can improve nutritional intake and nutritional status in HIV infected patients undergoing anti-retroviral therapy thereby ensuring good immuno-metabolic response.[9]

Fig 2: Different parts of *Moringa oleifera* plant

Use of *Moringa oleifera* in Ayurvedic medicine

Moringa oleifera is the plant of choice for the preparation of various traditional medicines since ancient times. All parts of the plant have shown immense health benefits, particularly in improving function of reproductive, circulatory and immune system. Various studies have shown that MO and its active ingredients, either alone or as a multi-herbal preparation play a promising role in combating a wide range of pathologies including cancer, diabetes, ulcer, oxidative stress, infection and infertility (Table 1). Among different parts of the plant, leaves have been widely studied for its various therapeutic properties as detailed below.

a. Anticancer effect: Aqueous extract of MO leaves hinder the proliferation of alveolar cancer cells by inducing oxidative stress, DNA fragmentation and apoptosis. Moreover, the anti-proliferative effect of the extract was greater in cancer cells than normal cells[10]; thus it can be speculated that an active principle of the extract specifically targets the cancer cells, thereby it can potentially decrease the side effects on normal tissues unlike many of the chemotherapeutic drugs. Aqueous extract of MO leaves increased the cytotoxic effect of chemotherapeutic agents on pancreatic cancer cells[11] by down-regulating nuclear factor-kappa beta (NF-kB) indicating chemo-sensitization of the extract. Ethanolic extract of leaves exhibited anticancer activity in breast and colorectal cancer cell lines.[12] The crude extract and certain isolates of the plant such as glucomoringin isothiocyanate, quercetin and benzyl isothiocyanate have been shown to increase the apoptosis in cancer cell lines.[13-16]

Parts	Composition	Medicinal property
Roots	Benzyl isothiocyanate Phenethyl isothiocyanate	Anticancer Neuro-protective Anti-fertility
Stem (Bark)		Antibacterial action Anticancer effect Insulin sensitization
Leaves	Glucomoringin isothiocyanate, Quercetin, Benzyl isothiocyanate, Vicenin-2, Chlorogenic acid, Gallic acid, Kaempferol, Rosmarinic acid and Rutin	Anticancer Chemo-sensitization
		Anti-diabetic
		Anti-bacterial Anti-viral
		Antianalgesic, anti-inflammatory and antioxidant
		Wound healing
Pods and seeds	Isothiocyanates	Anti-hypertension Antioxidant and antibacterial action Anti-inflammatory action Antiulcer
Flowers	Trypsin inhibitor	Larvicidal action on *Aedes aegypti* (dengue fever)

Table 1: Medicinal properties of different parts of *Moringa oleifera* plant

b. Antidiabetic effects: Methanolic extract of MO leaves prevented diabetes induced nephrotoxicity through its hyperglycemic, antioxidant and anti-inflammatory action.[17] Aqueous extract of leaf showed potent anti-diabetic effect against streptozotocin-induced diabetes in rats.[18] The study on type 2 diabetes-induced mice and rats revealed that the extract of MO exhibited hypoglycemic effect, ameliorated oxidative stress, renal and hepatic dysfunction and improved glycogen synthesis and modulated lipid metabolism.[19-22] It has been demonstrated that MO leaf extract can mitigate alloxan-induced diabetes by regenerating β-cells and modulate the expression of pyruvate carboxylase.[23] Furthermore, the extract from bark showed improvement in insulin resistance induced by dexamethasone.[24]

c. Antimicrobial action: The water-soluble lectin extracted from MO exhibited antibacterial action against *Serrata marcescens* and Bacillus species by inducing cell wall damage.[25] The bark showed significant beneficial effect in the management of urinary tract infections.[26] The aqueous extract from MO leaves showed protection against HBV infection by reducing fibrosis markers, IL-6 and HBsAg secretion.[27] The flower extract inhibited the larval growth of *Aedes aegypti,* a vector of dengue fever, by targeting trypsin and acetyl choline esterase activity.[28]

d Other properties: Aqueous extract of MO leaves contain phytochemicals like vicenin-2, chlorogenic acid, gallic acid, quercetin, kaempferol, rosmarinic acid and rutin which exhibited potential wound healing property.[29,30] The methanolic extract showed analgesic, anti-inflammatory and antioxidant properties in animal models.[31-33] The active compounds in pods and seeds exhibited hypotensive and anti-ulcer properties respectively.[34,35] The hydro-ethanolic extract of MO leaves showed cerebro-protective effect against ischemic stroke by decreasing oxidative stress[36] and penicillin induced convulsion, locomotor behavior by modulating the secretion of neurotransmitters.[37]

Role of *Moringa oleifera* in tissue protection

The tissue protective action of MO is summarized in Table 2. The studies have shown that MO and its active components impart protective action against certain toxic chemicals and radiation. Aqueous extract of MO leaves showed amelioration of radiation-induced oxidative stress in mouse hepatocytes by inhibiting translocation of Nf-kb, decreasing lipid peroxidation and increasing the activity of antioxidant enzymes.[38] The toxic effect of radiation on bone marrow cell was mitigated by the extract by decreasing induction of chromosomal aberrations and micronucleus formation.[39] A recent study revealed that butyl p-hydroxyphenyl-acetate (MIMO2), a novel compound isolated from MO leaves prevented oxidative stress and DNA damage induced by a metal vanadium, a potent neuro-toxicant.[40] Further, MO leaf extract showed nephro-protective effect against acetaminophen, a common analgesic and antipyretic by modulating activity of antioxidant enzymes and anti-inflammatory molecules.[41] Similarly, another study exhibited the mitigating effect of MO leaves on acetaminophen induced hepatotoxicity by restoring glutathione level.[42] The ethanolic extract of MO leaves showed ameliorating effect against anti-tuberculosis drugs (isoniazid, rifampicin, and pyrazinamide) - induced hepatotoxicity.[43] The oil extracted from MO seeds showed protective effect against carbon tetrachloride (CCl_4)-induced hepatitis by decreasing lipid peroxidation and antioxidant status in rat hepatocytes .[44]

MO Extract / Phytochemicals	Toxic agents	Tissue/ cell
Leaf extract	Radiation	Liver
		Bone marrow
Leaf extract	Acetaminophen	Kidney
		Liver
Leaf extract	Antituberculosis drugs- isoniazid, rifampicin, and pyrazinamide	Liver
Leaf extract	Chromium	Testis
Leaf extract	Cyclophosphamide	
Butyl p-hydroxy phenyl-acetate	Vanadium	Neurons

Table 2: Tissue protective action of *Moringa oleifera* leaf extract and its phytochemicals.

Protective role of *Moringa oleifera* in male gonadal toxicity

It is known that certain chemicals and radiation are highly toxic to testes as they affect structure and/or functioning of testes. In literature, there are very few reports on protective action of MO against testicular toxicity. The ethanolic extract of leaves showed the mitigating effect against chromium-induced testicular toxicity and improvement in sperm functions by preventing oxidative stress and increasing testosterone in rats.[45] The oil produced from MO, mitigated mercury induced testicular toxicity by improving steroidogenesis and antioxidant status in rats.[46] However, there are no studies on the protective role of MO against chemotherapy and radiotherapy; which are known to cause severe damaging effects on testes imparting infertility. Therefore, we have conducted experiments to assess the testicular protective effect of ethanolic extract of MO leaves against cyclophosphamide, a broad spectrum anticancer drug.

Role of *Moringa oleifera* in Fertility Preservation

In recent years, the number of cancer survivors have been increasing due to the tremendous advancement in diagnostic and therapeutic strategies in cancer treatment. However, due to the non-specific action of radiation and anticancer drugs, these agents can cause toxicity in normal cells leading to various long-term health issues. Testis is highly vulnerable to these toxic agents due to the presence of rapidly proliferating spermatogonial stem cells (SSC). Depletion of SSC leads to temporary or permanent arrest in spermatogenesis. Therefore, cancer survivors have compromised fertility potential which in turn affects the quality of life post chemotherapy and radiotherapy.

Fertility preservation is a promising option to preserve reproductive capacity of cancer patients undergoing chemo- and radio- therapies. The currently available methods of fertility preservation are depicted in Fig 3.

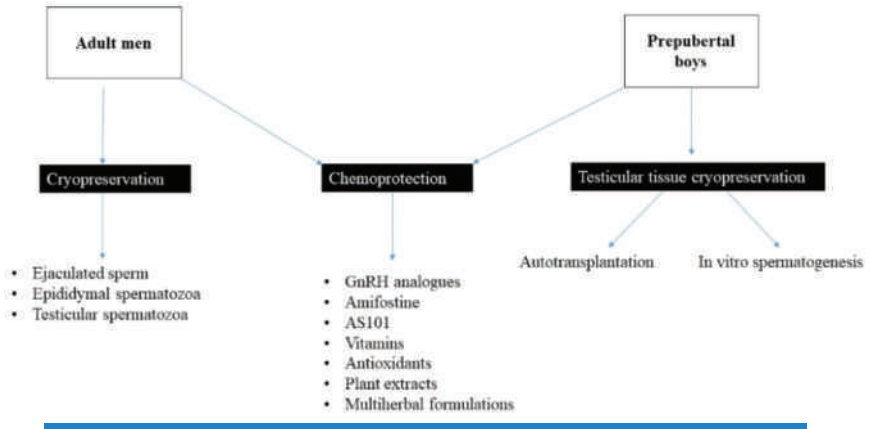

Fig 3: Schematic representation to show fertility preservation options for males

Semen cryopreservation is a well-established method for fertility preservation in adult males. However, it cannot be applicable for pre-pubertal individuals as they do not produce spermatozoa; testicular cryopreservation or SSC cryopreservation followed by derivation of spermatozoa by auto-transplantation or in vitro culture are the only available options. But these protocols are still in the experimental stages and have technical limitations. Since the survival rate in childhood cancer has increased more than 80%[47], there is a need for developing a strategy for fertility preservation, which is practically feasible and economically affordable.

Chemoprotection is considered to be an ideal, alternative option for fertility preservation using cyto-protective agents. These include Gonadotropin releasing hormone (GnRH) analogues[48,49], synthetic compounds like amifostine (WR-2721), AS101 (immuno-modulator)[50,51] and natural products like antioxidants, vitamins and plant extracts.[52-63] Among these, natural products have gained popularity because they are part of our diet and well tolerated by the body. Moreover, the clinical trials with GnRH analogs and other synthetic agents did not show any promising role in preventing chemotherapy-induced male gonadal toxicity.[64,65] The chemoprotective effect of various plant extracts and natural compounds against chemo and radiotherapy induced testicular toxicity are mentioned in Table 3.

Extract/active compounds	Chemotherapeutic agent	Beneficial effect
Zingiber officinale	Busulfan	Increased sperm count and testosterone
Ginkgo biloba	Doxorubicin	Improved sperm functions, decreased oxidative stress and apoptosis
Allium sativum		
Amaranthus viridis	Cyclophosphamide	Improved sperm functions, endocrine functions, antioxidant status and decreased apoptosis
Rosmarinus officinalis	Etoposide	
Podophyllum hexandrum	Radiation	Improved spermatogenesis and antioxidant status
Mentha Piperita		
β-carotene	Methotrexate	Decreased oxidative damage and apoptosis
Curcumin	Cisplatin	
Vitamin C	Cisplatin	
Ellagic acid	Cisplatin Cyclophosphamide	Increased sperm quality, improved spermatogenesis and antioxidant status
Lycopene	Cyclophosphamide	

Table 3: Chemo-protective role of herbal extracts and its active principles in chemo and radiotherapy-induced testicular toxicity

Our earlier study demonstrated that administration of ethanolic extract of MO leaves (MOE) to pre-pubertal mice mitigated the cyclophosphamide (CP) induced testicular toxicity and improved the sperm functional characteristics when mice attained puberty.[66]
The improvements in the testicular functions were associated with decreased lipid peroxidation and increased activity of antioxidant enzymes like superoxide dismutase (SOD) and catalase suggesting that MOE prevents testicular tissue by preventing oxidative stress induced by CP. Further, administration of MOE prior to CP was able to decrease the DNA damage and apoptosis in spermatogonial cells of pre-pubertal mice which are highly sensitive to CP. The protective effect of MOE is further supported by gene expression analysis by quantitative reverse transcriptase PCR (qRT-PCR), where it has been shown to modulate the expression of genes related to DNA damage response, pluripotency and stem cell survival.[67]

Mechanism of protective action

High Performance Thin Layer Chromatography (HPTLC) analysis showed that MOE is rich in quercetin and chlorogenic acid that are known to possess antioxidant and anti-apoptotic properties. CP is an alkylating agent which mainly targets rapidly proliferating spermatogonial cells. In our study, it was demonstrated that MOE increased the activity of antioxidant enzymes like superoxide dismutase (SOD), catalase, glutathione peroxidase (GPX4), glutathione S transferase (GST) and glutathione reductase (GSR). These enzymes can help in CP-induced oxidative stress and prevent oxidative damage of DNA, RNA and proteins. Single cell gel electrophoresis (Comet assay) and Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay showed that DNA damage and apoptosis induced by CP was significantly decreased by MOE administration in spermatogonial cells thereby ensuring the normal spermatogenesis and improvement in quality of spermatozoa. Further, in support of the above findings, flow cytometric analysis showed that MOE attenuated CP-induced depletion of differentiating and haploid population. Gene expression analysis showed that MOE modulated the expression of genes related to apoptosis (P53, Bax, Bcl2 and CytC), pluripotency (Oct4) and stem cell survival (c-Kit) suggesting the molecular mechanism underlying protective effect. The study on kinetics of DNA damage and repair following CP treatment in spermatogonial cells revealed that MOE administration modulated the expression of γ-H2AX (double strand break sensor) as well as RAD51 and KU80 (repair proteins). These observations further confirm that MOE can alter the CP mediated DNA damage response, thereby rescuing the spermatogonial cells leading to normal spermatogenesis (unpublished findings).

Conclusion and future prospects

Administration of MOE seems to be a promising strategy to prevent chemotherapy-induced testicular toxicity. This can serve as an ideal option for pre-pubertal boys undergoing chemotherapy. The use of MO during cancer treatment is expected to have complementary effects due to the anticancer properties of the extract in addition to its normal tissue protective effect. The anti-proliferative activity of the extract helps in reducing the dose of anticancer drugs and in turn can further reduce the testicular toxicity. However, further studies are required to establish the dual role of MO as an anticancer and protective agent.

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