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Moringa induces its beneficial effect via hormesis

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Abstract

Moringa oleifera, a traditional Indian herb, is widely known for its capacity to induce antioxidant, anti-inflammatory and other chemoprotective effects in a broad range of biomedical models. These perspectives have led to an extensive number of studies using various moringa extracts to evaluate its capacity to protect biological systems from oxidative stress and to explore whether it could be used to slow the onset of numerous age-related conditions and diseases. Moringa extracts have also been applied to prevent damage to plants from oxidative and saline stresses, following hormetic dose–response patterns. The present paper provides the first integrated and mechanistically based assessment showing that moringa extracts commonly induce hormetic dose responses and that many, perhaps most, of the beneficial effects of moringa are due to its capacity to act as an hormetic agent.

Keywords: **Moringa: Hormesis: Dose response: Biphasic dose response: Phytomedicine: Dietary supplement**

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Introduction

The leaves of *Moringa oleifera* have become widely used for many public health goals and medically related conditions within the context of traditional cultural and medicine practices in Africa and Asia[1]. There have been many claims of human subject health benefits associated with consumption of Moringa leaf extracts (MLE) with a considerable scientific and medical literature accumulating across a broad range of biological models, cell types and endpoints of interest, including cardiovascular conditions, neurodegenerative diseases and a broad range of age-related adverse effects/deficits[2]. The MLE has been extensively analysed and is known to be a highly complex mixture of relatively high concentrations of some vitamins, carotenoids, polyphenols, phenolic acids, flavonoids, alkaloids, glucosinolates, isothiocyanates, tannins and saponins. As a result of such a diverse and high number of bioactive agents, the assessment of MLE has been one of a very challenging highly complex mixture scientific question.

The present paper addresses an important aspect of MLE biology that has yet to be integratively evaluated; that is, the nature of its dose response in the low dose zone, which may have important therapeutic applications. The particular focus of the present paper is whether and to what extent MLE induces hormetic–biphasic dose responses and their underlying mechanisms. While there is a relatively robust volume of hormetic dose response findings with respect to the effects of MLE, this paper also identifies a number of individual constituents of the MLE for their capacity to induce hormetic dose responses. Some of these agents have been extensively studied within the context of important phytochemicals, independent of their presence in MLE. For example, some of these agents include caffeic acid, chlorogenic acid, quercetin, kaempferol, rutin, gallic acid, and saponins[3–6]. These agents have been extensively studied within other biological contexts for their dose response and mechanistic aspects typically showing hormetic dose responses. Selected hormetic acting components of MLE will therefore be presented herein to complement and provide additional insight into the hormetic features of the MLE mixture. Finally, consideration is given to the issue of assessing complex mixtures in biology, toxicology and medicine and its application to MLE. Further, in the research for this paper, while many examples of hormetic dose responses were obtained for MLE, it was quite rare that these papers cited the term hormesis or recognised the hormesis concept. In fact, use of the terms hormesis or hormetic within the context of major databases such as PubMed or Web of Science would not provide access to many hormetic findings with MLE. Thus, the present paper required a more general and conceptually complex approach for conducting the literature search. As a result of this situation and the focus on hormesis and

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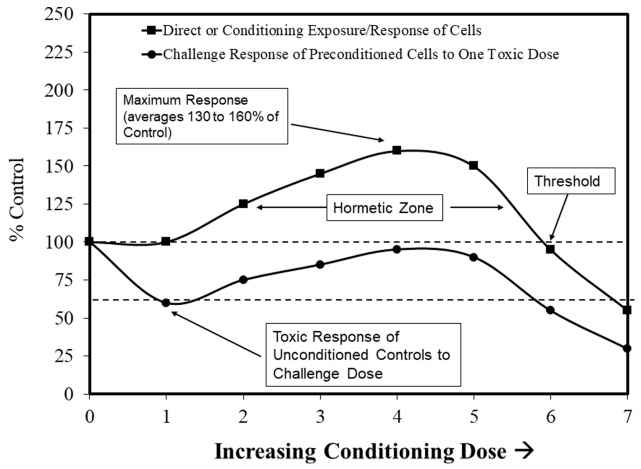


Fig. 1. The hormetic dose response concept.

MLE, this paper provides a brief overview of the hormesis concept to enhance a better appreciation of the present paper and to provide the reader with a broader perspective on the hormetic–biphasic dose response and its applications for biology, toxicology and medicine.

Hormesis overview

Hormesis is a biphasic dose–concentration response. It displays a low-dose–concentration stimulation and a high-dose–concentration inhibition[7–9]. The definition of hormesis as a biphasic dose response is decoupled from whether the response is deemed beneficial, harmful or undetermined. Judgements concerning this issue would be framed within the specific biological context of the dose–response relationship considered. It exhibits specific quantitative characteristics with a maximum stimulatory response usually about 30–60% greater than the control group (100%). The hormetic stimulatory dose–concentration range is approximately ten to twenty-fold starting from the estimated toxicological or pharmacological threshold but often shows notable variability, greater than fifty-fold and at times exceeding 1000-fold. The hormetic response results from a direct agent exposure, an hormetic preconditioning dose and a subsequent toxic dose[10,11], or a modest overcompensation stimulation after initial minor toxic responses and/or a disruption in homeostasis (Fig. 1)[9,12,13]. The hormetic dose–concentration response displays substantial generality, being independent of biological model (for example, microbes, plants, animal models, and humans), endpoint, level of biological organisation (cell, organ, organism), *in vitro* and *in vivo* evaluations, inducing agent[14–18] and mechanism[19]. Hormesis also provides an analytically illuminating framework for the study and assessment of chemical mixtures, incorporating the concept of additivity and synergism[7,11,19,20]. The most reductionist definition of hormesis then is about the beneficial or stimulatory effect caused by exposure to low doses of an agent known to be toxic at higher doses. Conceptually, this is represented by the U-shaped dose response curve for toxicity, where hormetic effects become smaller (after a maximum) with increasing dose leading to a threshold, after which toxicity increases with dose. A view that is at odds with this description holds instead that a no-threshold linear dose response curve, or a threshold dose response curve, more accurately defines biological realities[7]. Broad beneficial or adaptive effects attributed to low-dose exposure to various toxic chemical and physical agents include increased life span, improved trajectories of growth and development, decreased tumour incidence, increased resistance to infection and tolerance to radiation; the public health/environmental toxicology implications are as salient as those in the pharmacologic space.

Thus, hormetic dose responses seem likely to represent a highly conserved resource management, evolutionary-based dose response strategy that operates across all species and cells for a plethora of endpoints and defines the extent and magnitude of stimulatory responses in constitutive and adaptive processes.

Moringa mixtures

The MLE extract is comprised of a large number of metabolites, some of which have been shown to induce a broad spectrum of hormetic responses that may be central to the overall effects of the MLE-induced responses reported herein. The MLE therefore is a complex and dynamic mixture, the composition of which depends on the location or environment (for example, how stressful it is for the tree) where the moringa tree is grown, the age of the harvested samples, the extract methods used and other factors. Some of the constituents in the MLE have been extensively studied within an hormetic fashion as separate agents apart from their role within the MLE. These agents are also differentially found in other plant species that have been reported to have induced health benefits via hormetic processes. Some of these agents have been studied in sufficient breadth and depth with regard to hormetic effects that may warrant a specific detailed analysis with respect to their capacity to induce hormetic effects (for example, quercetin (Figs. S1 and S2), kaempferal (Figs. S3 and S4), caffeic acid (Figs. S5 and S6), gallic acid (Figs. S7 and S8), rutin (Figs. S9 and S10), rosmarinic acid (Figs. S11 and S12) and chlorogenic acid (Figs. S13 and S14)). In fact, the hormetic effects of ferulic acid, a significant component of MLE[21], have already been assessed in considerable depth in this regard[22]. These findings illustrate the complexity of the metabolite mixtures of MLE. The copresence of multiple bioactive hormetic agents illustrates the complex mixture status of the MLE (Table 1). While some research has been directed toward clarifying how several joint mixture constituents may affect cellular processes within an hormetic context, these findings remain of a preliminary nature.

MLE effects on sperm

Preservation of sperm under cryoprotection

Despite the widespread recognition that MLE can be highly protective for many cell types, its application to the area of sperm preservation is relatively new, with such research occurring within approximately the past 5 years with a focus on buffalo bull and ram semen in research from India and Pakistan. However,

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Table 1. List of major compounds in MLE based on Amara *et al.*[21]

Name	Relative abundance (%)
1-monolinoleoylglycerol	~1
2,4-dintrophenylhydrazine	~3·5
9-octadecynoic acid	~2
Astaxanthin	~1
β-sitosterol	~6·5
Carotene	<0·25
Decanoic acid	<0·25
Destruxin A	<0·25
Eicosapentaenoic acid	~1
Gallic acid	~42
Hexadecanoic acid	<0·25
Kaempferol	~7·75
Oleic acid	~13·5
Olyel oleate	~5
Quercetin	~5·5
Rhodotin	~3
Vitamin E	~7

the use of antioxidants to prevent damage to cryopreserved semen was supported by several prior studies, using alpha tocopherol[23], Trolox[24] and taurine[25]. These treatments were consistently successful in enhancing sperm motility and viability and also enhancing post-thawing fertility. Similar cryopreserved sperm protections were shown by Allai *et al.*[26] to be mediated by well-known antioxidant phytochemicals, such as various representative types of carotenoids, polyphenols and flavonoids. These developments are interesting since they suggest the possibility of replacing conventional antioxidants with phyto-extracts and perhaps using both within an optimised mixture.

Since the *Moringa oleifera* tree displays substantial antioxidant properties, El-Sheshtawy and El-Nattat[27] sought to clarify the potential effects of employing MLE as an additive to preserve extended semen in bovine, especially since no prior paper had addressed this question. Using standard methods for phytochemical extraction from the leaves of the *Moringa oleifera* plant and its incorporation into the standard Tris extender semen preparation, the MLE was assessed for its capacity to protect buffalo semen from oxidative damage that occurs during the cryopreservation and thawing periods (frozen sperm thawed at 37 °C for 1 min generates considerable reactive oxygen species (ROS) stress). These findings indicated that the MLE displayed an hormetic–biphasic dose response with low-dose protective and a high-dose harmful responses in the post-thawing sperm. These findings suggested that the MLE has the potential to protect the buffalo sperm and to enhance its functionality, using a 10–20% MLE mixture (Fig. 2). Of importance was that the MLE treatments did not adversely affect the quality of sperm-related parameters affecting survival, abnormal microscopic appearance and hypo-osmotic swelling. A methodologically similar follow-up study by Iqbal *et al.*[28] using water buffalo bull semen at 37 °C for 30 s for thawing also showed an hormetic-like biphasic concentration response for multiple sperm performance endpoints, including total motility, progressive motility, average path velocity, curvilinear velocity and straight-line velocity (Fig. 3). The optimal response in this experiment was shown at 15% (MLE), which was similar to the

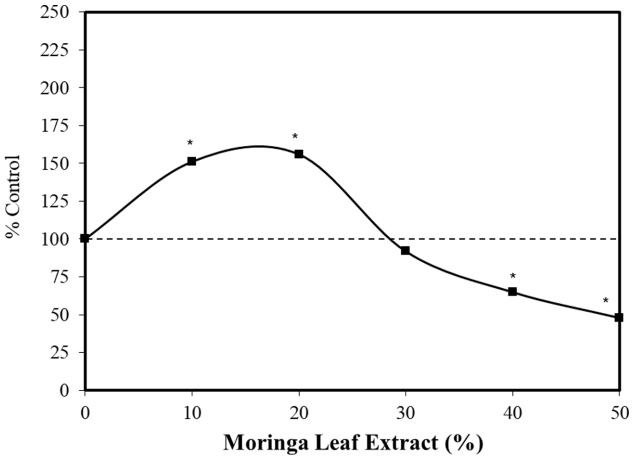


Fig. 2. Effects of a filtered moringa leaf extract (MLE) of enriched extender (MLE extract soaked in Tris-based extender) on buffalo post-thaw sperm characteristics (% is the concentration of MLE in the Tris-based extender) (modified from: El-Sheshtawy and El-Nattat[4]). $*P \leq 0{\cdot}05$.

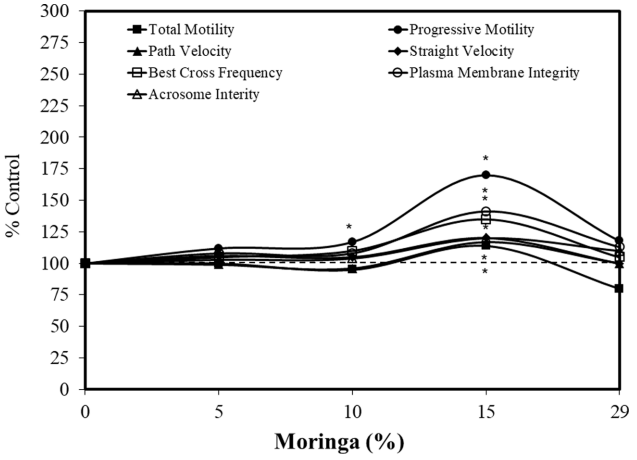


Fig. 3. Effects of a filtered moringa leaf extract (MLE) of enriched extender (MLE extract soaked in Tris-based extender) on water buffalo bull semen quality (% is the concentration of MLE in the Tris-based extender) (modified from: Iqbal *et al.*[28]). $*P \leq 0{\cdot}05$.

10– 20% range of the El-Sheshtawy and El-Nattat[27] study that enhanced motility. These authors noted that buffalo sperm have a high concentration of membrane polyunsaturated fatty acids, enhancing susceptibility to ROS damage throughout the cryopreservation process. Thus, even though an optimised concentration of ROS is necessary for sperm functionality, excessive ROS production during the cryopreservation and thawing process induces damage, overwhelming normal protective capacities of sperm, thereby showing the benefit of the MLE preparation within an optimised concentration zone. The capacity of the MLE to affect its protection was suggested due to the interactive mixture effects of a vast complex of phenolic agents, antioxidant vitamins, minerals and a mixture of tannins and saponins, flavonoids, terpenoids and glycosides.

A similar methodological approach was subsequently used to assess the effects of MLE on cryopreserved ram sperm. Two separate investigations indicate that MLE enhanced the

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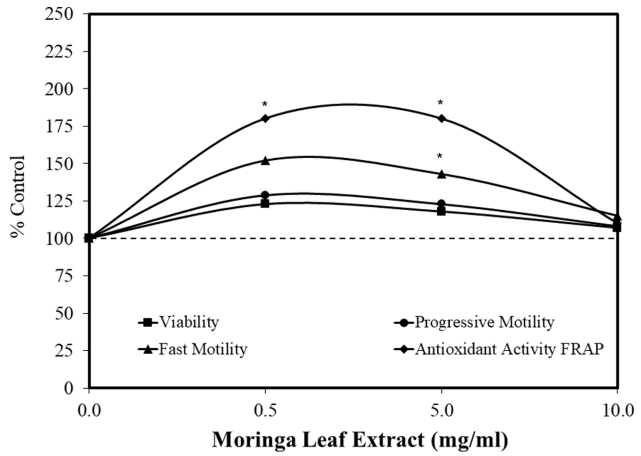


Fig. 4. Effects of a filtered moringa leaf extract (MLE) of enriched extender (MLE extract soaked in Tris-based extender) to cryopreserved ram sperm supplemented with moringa leaf extract (% is the concentration of MLE in the Tris-based extender) (modified from: Carrera-Chavez *et al.*[29]). $*P \leq 0{\cdot}05$.

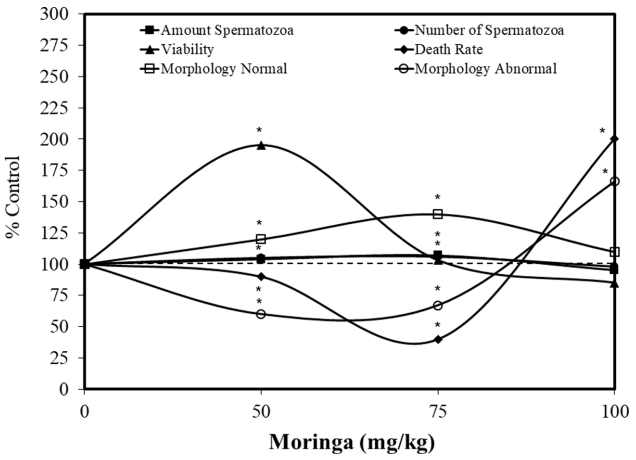


Fig. 5. Effects of a filtered moringa leaf extract (MLE) orally infused (1 ml) for 30 d on spermatozoa quality in young male white rats (modified from: Suaskara *et al.*[31]). $*P \leq 0{\cdot}05$.

functionality of cryopreserved ram sperm, showing an hormetic dose–response pattern for each endpoint measured (Fig. 4)[29,30]. The findings of MLE concerning the effects on sperm function are consistent within and between studies and across biological models. The MLE acts as an hormetic agent, protecting sperm from oxidative damage and other threats due to cryopreservation and thawing actions. What is missing in the sperm cryopreservation literature are direct test comparisons that permit practitioners to evaluate the efficacy of these protective agents as compared to each other and whether there may be optimal mixtures of these protective agents.

The use of MLE during the process of cryopreservation involves the direct application to sperm cells within an optimised chemical product mixture. This process avoids the influence of gastrointestinal tract, liver metabolism and generation of a plethora of numerous metabolites. In effect, the sperm cryopreservation system is a more simplified system as compared with an *in vivo* model. However, in the final section (Testicular function and sperm) of the sperm MLE evaluation, the MLE was administered orally, adding the far greater complexity of systems biology to the evaluation process. Given the vastly different experimental approaches it is difficult to directly and quantitatively compare the response of these different experimental approaches.

Testicular function and sperm

Suaskara *et al.*[31] explored the capacity of MLE to protect the health of young male rats, including testicular function. As was the case with the cryopreserved sperm, oral doses of MLE (50 and 75 mg/kg) enhanced their viability, morphological quality and motility (Fig. 5). These findings suggested that specific doses of MLE have the capacity to enhance sperm quality and reproductive functions. Additional investigations have indicated that MLE increased the weight of male reproductive organs, sperm functionality and levels of several hormones, such as testosterone. These findings suggested the

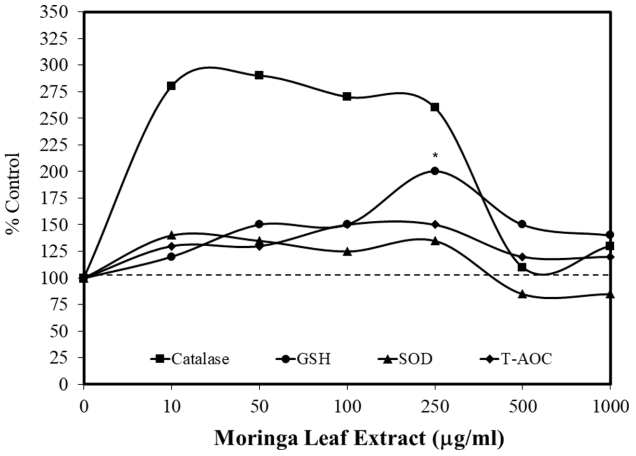


Fig. 6. Effects of aqueous filtered extract of moringa leaf extract (MLE) on antioxidant enzymes in TM3 cells, a Leydig cell line (modified from: Opuwari *et al.*[32]). $*P \leq 0{\cdot}05$.

possibility that MLE may affect the functioning of Leydig cells which comprise 2–4% of the testicular cells and provide the secretion of testosterone needed for spermatogenesis and multiple male developmental processes. Within this context Opuwari *et al.*[32] assessed the capacity of MLE to affect Leydig cell functioning including the capacity to affect the antioxidant activity using TM3 cells, a mouse Leydig cell model. While the MLE did not affect the viability in these studies, it significantly affected a coordinated set of antioxidant enzyme responses in an hormetic manner for superoxide dismutase (SOD), catalase, glutathione and total antioxidant concentration (Fig. 6). It was suggested that the MLE has the potential to prevent or reduce oxidative stress in Leydig cells affecting the health and functionality profiles of sperm. Despite the consistency between the *in vivo* rat study and the *in vitro* mouse Leydig study, the capacity to directly extrapolate or directly compare the two systems is problematic, given the likely extensive metabolism of MLE chemical product within the *in vivo* setting.

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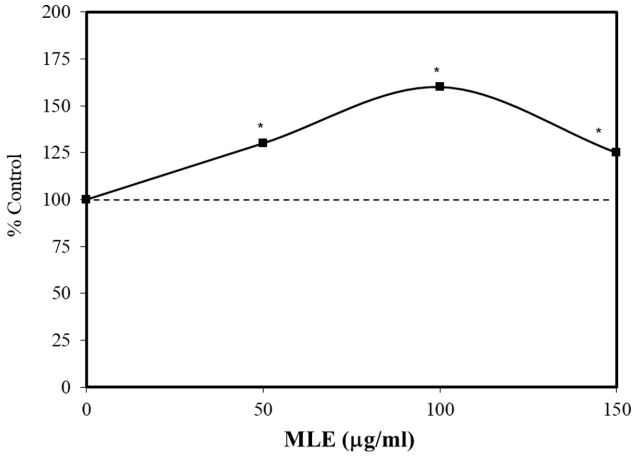


Fig. 7. Effects of moringa leaf extract (MLE) (with detailed GC/MS analysis of most prominent compounds) on SH-SY5Y cells with a preconditioning (2 h) protocol with DEHP as the stress agent (modified from: Amara *et al.*[21]). $*P \leq 0{\cdot}05$.

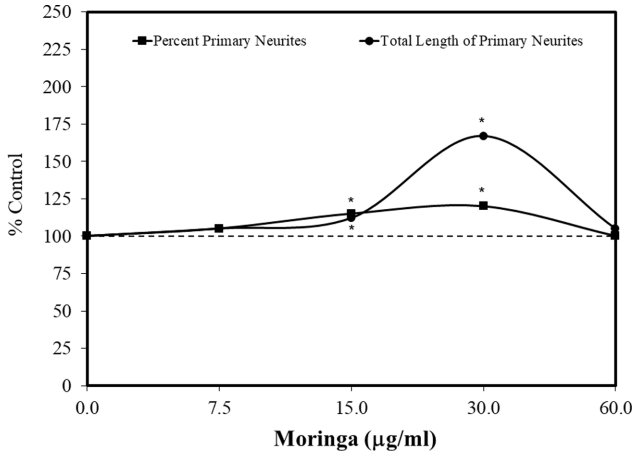


Fig. 8. Effects of ethanol extract (dissolved in DMSO) of moringa leaf on embryonic (E19) rat hippocampal neurons (modified from: Hannan *et al.*[34]). $*P \leq 0{\cdot}05$.

Neuronal survival and protection

The wealth of antioxidant constituents in MLE suggests that it may have the capacity to protect neurons. Whether the MLE may have the capacity to protect neurons from oxidative stress induced damage has become a research focus given its extensive combination of antioxidant constituents in leaves and other plant parts. The SH-SY5Y cell line has been commonly selected for use in such studies because it is a dopaminergic neuronal cell that can provide insights into a range of neurodegenerative diseases such as Alzheimer's, Parkinson's, Huntington's and amyotrophic lateral sclerosis (ALS). Since the SH-SY5Y cell line is undifferentiated, it can be differentiated to neuronal cells, altering their susceptibility to oxidative stress. In their study using differentiated SH-SY5Y cells, Jaafaru *et al.*[33] assessed the capacity of glucomoringin isothiocyanate (GMG-ITC) or moringin to directly enhance the viability of SH-SY5Y cells or to assess whether its effects within a preconditioning exposure framework (four hours) to GMG-ITC would protect against a toxic dose of hydrogen peroxide (H_2O_2). While the GMG-ITC had no stimulatory effect on the differentiated SH-SY5Y cells the preconditioning exposure protected the cells from the toxic effects of H_2O_2 at 24, 48 and 72 h showing hermetic–biphasic dose–response relationships. Complementary morphological investigations indicated that the pretreated cells displayed enhanced membrane integrity, and significantly diminished apoptotic processes. Similar findings were reported using MLE with SH-SY5Y cells that were stressed with di-(2-ethylhexyl) phthalate (DEHP) using a 24-h pretreatment exposure[21] (Fig. 7).

One of the areas targeted for research on MLE has been that of neuronal development, functioning and differentiation. Studies designed to assess the effect of MLE have involved primary mouse hippocampal neurons. These experiments assessed the effects of MLE over an eight-fold concentration range (7·5–60 µg/ml) (Fig. 8)[34]. The results show an hormetic response for the formation of primary neurites and the total length of primary neurites. The optimal concentration (30 µg/ml) was therefore selected for follow-up studies that showed an enhanced rate of neuronal differentiation, dendrite complexity, axonal development and synaptogenesis, with all responses consistent with the hormetic dose response. It is of interest to note that beta-carotene, a component of the MLE mixture, was also tested over a broad concentration range (7·5–120 µg/ml) and showed an hormetic dose response for the number of primary neurites, total length of primary neurites and the number of branching points. The findings of Hannan *et al.*[34] indicate that MLE enhances the development of primary hippocampal neurons by enhancing a broad spectrum of neural developmental processes such as its differentiation, growth and length and enhanced synaptic connectivity.

While the responses relating to neuronal survival and protection have displayed hormetic dose responses with SH-SY5Y cells and primary mouse hippocampal neurons, it is important to recognise the potential limitations and biological relevance these findings may have for *in vivo* systems. The consistency, therefore, of the hormetic findings across these two *in vitro* neuronal systems supports application of further experimental testing in the vastly more complex *in vivo* systems.

Immune responses

The biological assessment of the constituent mixture of MLE has been typically performed via the use of several types of solvent extracts as well as by the use of specific chemical constituents such as gallic acid, rutin, caffeic acid, quercetin and numerous other agents. A recent development in this regard has been the re-evaluation of novel polysaccharides. Prior research indicates that plant polysaccharides often have shown the capacity to enhance immunomodulatory activity. Experiments in this area have assessed the effects of several novel polysaccharides extracted from the leaves of the moringa tree on the function of macrophages using the cell model RAW 264·7 (Figs. 9 and 10)[35,36]. In these studies, two polysaccharides induced

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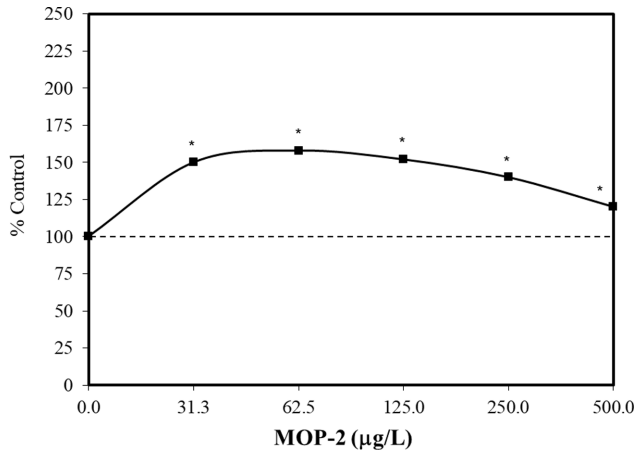


Fig. 9. Effects of a novel polysaccharide (MOP-2) from MLE on the cell viability of RAW 264·7 cells. The crude polysaccharide were separated into testable fractions (for example, MOP-2) (modified from: Dong *et al.*[35]). $*P \leq 0{\cdot}05$.

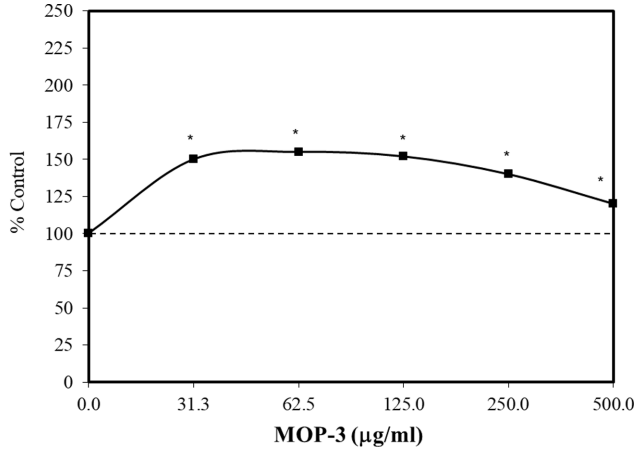


Fig. 10. Effects of different concentrations of a MOP-3, a novel polysaccharide, from MLE solutions on RAW 264·7 cell viability. The crude polysaccharide were separated into testable fractions (for example, MOP-3) (modified from: Li *et al.*[36]). $*P \leq 0{\cdot}05$.

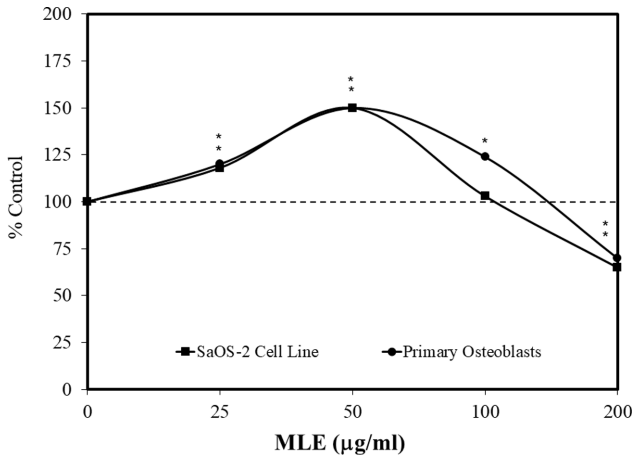


Fig. 11. Effects of moringa leaf extract in ethanol to extract soluble components on human subject osteosarcoma (Saos)-2 cell line and normal primary rat calvaria osteoblasts (modified from: Khan *et al.*[37]). $*P \leq 0{\cdot}05$.

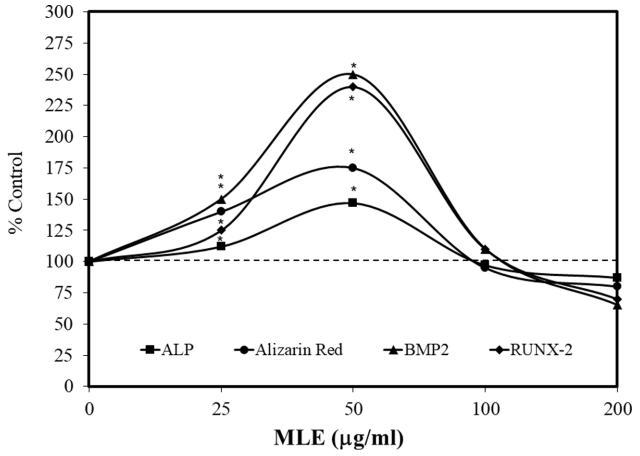


Fig. 12. Effects of moringa leaf extract in ethanol to extract soluble components on biomarkers of cellular differentiation in osteoblast-like Saos-2 cells (modified from: Khan *et al.*[37]). $*P \leq 0{\cdot}05$.

hormetic–biphasic concentration responses for cell viability, showing similar quantitative features of the dose response. These collective findings raise additional questions concerning how might these agents interact when present in mixtures as occurs when consuming the MLE. These new research initiatives on the polysaccharides suggest the possibility of identifying new biomedical and therapeutic effects of MLE.

Bone development

Despite the widespread evaluation of MLE on various biological model models and cell types, it has generated little interest in the area of bone development. However, in 2022 Khan *et al.*[37] proposed that MLE may show an hormetic effect on the growth of the human subject osteoblast-like Saos-2 cell line. In this study Khan *et al.*[37] assessed the capacity of MLE to affect cell viability, ROS, and the capacity to enhance cell differentiation (Figs. 11 and 12). Using multiple cellular assays, the MLE induced an hormetic biphasic dose response for cell proliferation and cell viability for both Saos-2 cells and primary osteoblasts. Differentiation was enhanced by MLE as measured by alkaline phosphatase (ALP) activity and Alizarin Red. Mineralised nodes, which are also phenotypic markers for osteoblasts that induce the terminal stage of differentiation, take 21 d to be induced. By the 21st day, the low-dose MLE treatment had induced the formation of the mineralised nodules; while the high doses were inhibitory. Associated with the enhanced differentiation activity was the up-regulation of bone morphogenic protein genes and for runt-related transcript factor -2 (Runx-2).

Follow-up research targeted the role of three components of the MLE mixture (β-sitosterol, quercetin and kaempferol) to enhance differentiation with each showing strong affinities for bone morphogenic protein 2 (BMP-2) and Runx-2, with β-sitosterol being most effective. The findings show that low concentrations of MLE enhanced cell proliferation, differentiation and mineralisation, as well as expression of key genes for BMP-2 and Runx-2 (Fig. 12).

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Effects on plants

Growth responses – Lepidium sativum, wheat and tomatoes

The effects of MLE to explicitly induce an hermetic–biphasic dose response on the plant species *Lepidium sativum* was addressed by Perveen *et al.*[38]. This study was undertaken within an allelopathic context for an assessment of plant secondary metabolites on the growth of adjacent plants. Multiple prior independent studies were noted that had focused on the capacity of MLE to either stimulate or inhibit the growth of various plant species. However, the interest of Perveen *et al.*[38] was to provide a more complete dose response, assessing the capacity to both stimulate growth at low concentrations and to inhibit it at higher concentrations, using an aqueous MLE mixture extract. In this study the MLE induced an hormetic dose response for shoot length and seedling dry weight with the maximum responses at 142% and 125% greater than controls, respectively (Fig. 13).

A decade earlier, a dissertation by Yasmeen[39] reported that MLE induced an hormetic effect on the germination of wheat seeds and time to germination. Likewise, the MLE also induced an hormetic increase in fresh and dry shoot weights and shoot and root lengths following an hormetic dose response pattern. Similar hormetic dose responses were reported concerning the effects of MLE on tomato plant growth in an hormetic manner via soil or foliar application.

Salt stress

Salt stress is a significant agricultural challenge in many locations. One of the approaches to decrease abiotic stresses such as excessive salinity in plants is via the application of exogenous plant extracts, including MLE[40,41]. Based on these supportive findings, Ali *et al.*[42] assessed the effects of MLE on vegetative growth within an hydroponic experimental context. Their experimental protocol involved growing maize hydroponically in the presence of elevated saline (70 mM) with the MLE treatment being administered via a foliar application using four concentration levels (5, 10, 15, 20%). The MLE foliar application was administered 3 d after exposing the plants to the elevated saline solution, thereby acting as a specific type of post-conditioning experimental protocol. The plants were then grown for 4 weeks and harvested. The saline solution by itself reduced the growth compared with the untreated control group by about 25–30% for both fresh and dry weights. However, the 3-d foliar application of MLE directly stimulated the growth of the maize in the absence of the saline stress but also with the saline stress. In both cases, there was an hormetic dose response that followed a similar pattern for all shoot and root parameters. The optimal concentration for MLE treatment (10%) was the same, whether under saline stress or not (Figs. 14 and 15). The findings of Ali *et al.*[42] showed that the foliar MLE application over only 3 d had a significant impact on multiple growth parameters. The enhanced growth was also extended to conditions when the plants were reared under a modest saline stress. Measurement of tissue levels of sodium indicated that the sodium concentrations were decreased by the MLE treatment.

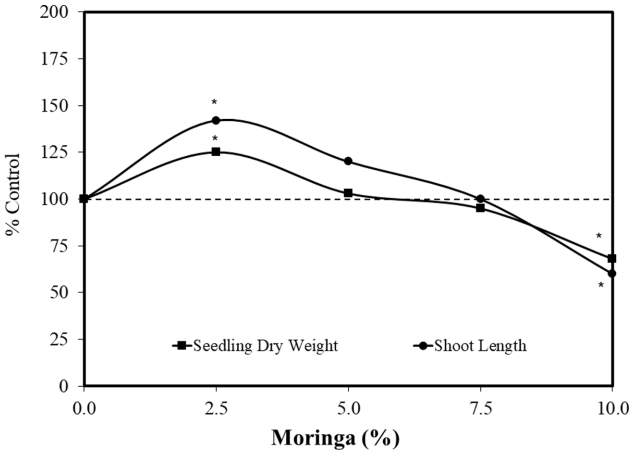


Fig. 13. Effects of moringa leaf extract on growth of *Lepidlum sativum* (modified from: Perveen *et al.*[38]). $*P \leq 0{\cdot}05$.

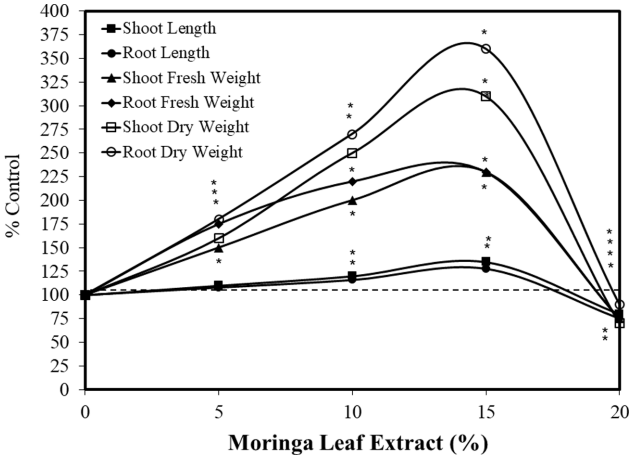


Fig. 14. Effects of moringa leaf extract on growth of maize at seedling stage (modified from: Ali *et al.*[42]). $*P \leq 0{\cdot}05$.



Fig. 15. Effects of moringa leaf extract on growth of maize at seedling stage (modified from: Ali *et al.*[42]). $*P \leq 0{\cdot}05$.

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Discussion

Hormetic dose responses by MLE have been reported herein in a broad range of experimental models and cell types. The MLE-induced hormetic responses include studies with multiple plant species (for example, tomatoes, wheat), animal reproduction and sperm preservation and functionality, several neuronal systems, bone formation and immune cell responsiveness. The MLE-induced hormetic effects have involved both the direct stimulation of the classic biphasic concentration–dose response as well as the response to a variety of external stressor agent conditions. In the case of stress-induced hormetic dose responses, the MLE has induced hormetic effects in both pre-conditioning and post-conditioning experimental protocols. The quantitative features of the dose responses were consistent also with the vast hormetic literature, as well as the information summarised in the various hormetic databases, with the maximum stimulatory response being generally in the 30–60% range with the stimulatory concentration–dose width range typically being less than fifty-fold starting with the pharmacological/toxicological threshold dose[43–46]. The mechanisms by which the MLE affects the broad range of hormetic dose responses is known only to a limited extent. The mechanism research has generally focused on the capacity of MLE to activate antioxidant systems typically via the activation of nuclear factor erythroid 2-related factor 2 (Nrf2) to prevent ROS-induced cellular damage[1,13].

An issue of importance in the present assessment is the striking diversity of MLE sample preparations. In general, only a few of the studies provided GC/MS analysis to chemically characterise the mixture constituents and their relative quantities. Thus, when the published studies are said to assess the effects of MLE on various biological systems, *in vitro* or *in vivo*, the actual MLE mixture component may be expected to vary considerably across studies. Detailed chemical mixture characterisation were provided by some of the papers assessed here[21,38].

It is notable that the experimental research showing hormesis with MLE with animals has been predominately conducted with *in vitro* systems, whereas the research with plants has involved *in vivo* plant systems. The plant studies have typically involved treatment of seeds as well as foliar applications. Thus, the emphasis of the plant research has been with a focus on practical agricultural applications. Although quite different in approach with respect to the plant research, the MLE was similarly practically applied with respect to sperm cryopreservation hormetic findings.

Of particular interest is the complex mixture of MLE constituents, many of which have been separately studied in considerable depth, independent of the role of biological actions within moringa. In fact, these agents have differential bioavailability and different tissue distribution patterns over time. The role of each agent of the MLE mixture and how they might interact in affecting hormetic responses remains with little exploration and highly uncertain. This is the case for *in vitro* studies during which a single cell type is studied and far more complex for whole organism/plant studies.

While hormesis is now a well-studied and documented scientific concept and phenomenon that is highly generalisable, being independent of biological model, cell type, endpoint, inducing agent and mechanism, research that focuses explicitly on the issue of hormesis and mixtures has also been considered in the literature[20]. However, it seems without enough recognition that mixture biology differs significantly between hormetic concepts and those of toxicological evaluation. In the toxicology domain, the goal is principally focused on documenting adverse health effects and establishing explanatory mechanisms, with some of these effects greatly exceeding additive responses, such as examples of multiplicative and synergistic effects. However, with respect to how hormesis mixtures act in the low-dose concentration zone is such that the mixture effect appears to be constrained by the limits of biological plausibility (in the 30–60% range, greater than control responses)[47]. Additive, multiplicative and synergistic effects may occur within an hormetic framework, but they are within the low-dose hormetic stimulatory 30–60% zone. These responses are therefore highly constrained in a quantitative sense as a result of the limits of biological plasticity, which importantly is not the case for toxic responses. This is the case because hormesis is concerned principally with enhancing and maximising biological performance within biological plasticity limits, which is not the case with toxicity. Therefore, both hormesis and toxicology can incorporate multiplicative and synergism concepts but how these concepts are executed is conceptually different between these two areas.

It is widely seen that complex mixtures and individual agents within such mixtures can induce hormetic responses within the same bioassay (for example, MLE and some of its individual constituents[20]). Under the current findings MLE studies provide evidence that a complex mixture acts within an hormetic fashion for multiple cell types for a range of endpoints. These findings have important implications since they can affect the nature of the study design, sample size, statistical power considerations and dose selection for therapeutic applications which is achieved by the biphasic concentration regulation with respect to MLE and other hormetic mixtures. It is important to note that the maximum stimulatory response seen with mixtures is not greater but similar to the maximum stimulatory response that is observed by individual constituents of that mixture. This is what is observed with specific constituents of MLE such as quercetin, rutin, gallic acid, caffeic acid and rosmarinic acid. All show hormetic effects and a maximum response in the 30–60% range. However, when these agents are combined within complex phytochemical mixtures the maximum stimulatory responses still remain within the 30– 60% zone[48]. Why this is the case with respect to the quantitative features of the dose response still remains to be clarified. However, these findings are consistent with the general hypothesis that the hormetic dose response, that is, its quantitative features, is constrained by the limits of biological plasticity which is a very generalised phenomenon. An important commonality of several of the principal components of the MLE mixture is their capacity to activate anti-inflammatory responses via the up-regulation of Nrf2[13]. Future research will be necessary to clarify how chemical mixtures interact with the process by which Nrf2 mediates the anti-inflammatory response. In summary, the MLE represents a complex mixture of multiple bioactive agents with many of these

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agents themselves being capable of inducing hormetic dose responses within a broad range of biological systems. This framework is important since it provides an experimental system to explore chemical interactions within an hormetic framework with potential public health and medical applications.

Supplementary material

To view supplementary material for this article, please visit https://doi.org/10.1017/S0954422423000161

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