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Palliative effects of *Moringa olifera* ethanolic extract on hemato-immunologic impacts of melamine in rats

Yasmina M. Abd-Elhakim[a,*], Khlood M. El Bohi[a], Sahar Khalifa Hassan[b], Shorouk El Sayed[c], Sabry M. Abd-Elmotal[b]

[a] Department of Forensic Medicine and Toxicology Zagazig University, Zagazig, Egypt
[b] Department of Pharmacology, Zagazig University, Zagazig, Egypt
[c] Department of Microbiology, Zagazig University, Zagazig, Egypt

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ABSTRACT

Melamine (MEL) is a widespread food contaminant and adulterant. *Moringa olifera* is a widely known medicinal plant with various pharmacological properties. Herein, this study aimed to investigate, for the first time, the probable protective or therapeutic role of *M. olifera* ethanolic extract (MOE) against MEL induced hemato-immune toxic hazards. Fifty Sprague Dawely male rats were orally treated with distilled water, MOE (800 mg/kg bw), MEL (700 mg/kg bw), MOE/MEl or MOE + MEl. Erythrogram and leukogram profiling were evaluated to assess hematological status. Innate immune functions were evaluated via measuring lysozyme levels, nitric oxide concentration, and bactericidal activity of phagocytes. Serum immunoglobulin levels were estimated as indicators of humoral immunity. Histologic and immunohistochemical evaluations of splenic tissues were also performed. The results indicated that MEL caused a significant decline in RBC, Hb, PCV, total WBC, neutrophil, lymphocyte, phagocytes bactericidal activity, lysozyme activity, nitric oxide, total IgM and IgG levels. Also, MEL induced various pathologic lesions in the spleen with strong expression of CD4 and CD8 positive cells. MOE significantly counteracted the former anaemic, leucopenic, innate and humoral depressant effects of MEL particularly at co-exposure. In conclusion, these findings revealed that MOE could be candidate therapy against MEL hemato-immunotoxic impacts.

1. Introduction

Melamine (MEL) (2, 4, 6-triamino-1, 3, 5-triazine) is an industrial nitrogen heterocyclic triazine compound containing high nitrogen level, 66.7% by mass (Chen et al., 2017; Guo et al., 2014). MEL has become more commonly used in food contact items like cups, plates, bowls, and utensils as they are dishwasher safe, inexpensive, and durable (Bolden et al., 2017). The passage of MEL monomers from plastic tableware to foods has been recently reported (Mannoni et al., 2017). Also, it has been used as a fertilizer, a nonprotein nitrogenous source for feeding cattle and lately as an adulterant in pet, livestock, and fish foods (Hau et al., 2009). In addition, MEL is detected as a metabolite and a degradation product of the pesticide and veterinary drug cyromazine as well as a trace contaminant in nitrogen-containing feed supplements like urea (EFSA, 2010).

MEL has been intentionally added to milk or other food products to boost the plain protein content (Chen et al., 2017). In 2008, WHO arose a serious food safety alert due to the elevated occurrence of kidney stones and renal failure in infants due to ingestion of MEL-adulterated infant formula in many countries. Also, a huge number of accidental pet deaths following ingestion of MEL-tainted foods have been lately reported (Dorne et al., 2013; Rumbeiha et al., 2010). Additionally, various hazards have been accompanied with MEL consumption including bladder cancer (Ogasawara et al., 1995), kidney injury (Dobson et al., 2008), nerve dysfunction (Wang et al., 2011), and reproductive toxicity (Khalil et al., 2017; Yin et al., 2013).

In particular, immunocompromise signs were observed in children consuming MEL-contaminated powdered formula (Zhou et al., 2010). Additionally, in an earlier hematologic and immunologic study, it was reported that the prolonged (60 days) oral exposure of rats to MEL could alter both host hematology and immunology significantly (Abd-Elhakim et al., 2016).

Various allopathic drugs are used to amend the immune system. However, these drugs are costly, not easily accessible, and in most cases are associated with adverse drug reactions. As a result, the majority of people especially in the rural areas of the developing world turn to the

* Corresponding author. Department of Forensic Medicine and Toxicology, Faculty of Veterinary Medicine, Zagazig University, Zagazig, Egypt.
E-mail address: yasmina_forensic@hotmail.com (Y.M. Abd-Elhakim).

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use of medicinal plants that are widely accepted, accessible, cheaper, and have fewer side effects (Chan et al., 2008).

Moringa oleifera Lam. (Moringaceae) is a medicinal plant distributed all over the world and has been frequently known as Saguna, Sainjna, and Drumstick tree (Lockett et al., 2000). Many parts of this plant, particularly leaves, have dietary and medicinal importance owing to high contents of ascorbic acid, carotene, vitamin E, calcium, essential amino acids and iron (Makkar and Becker, 1996; Sivasankari et al., 2014). Several phytochemicals have been isolated from *M. oleifera* known for their useful pharmacological properties including antifungal, antihypertensive, anticancer, antispasmodic, antitumor, antiulcer, cholesterol lowering, diuretic, hepatoprotective, and hypoglycemic capabilities (Anwar et al., 2007; Fahey, 2005; Fakurazi et al., 2008; Hamza, 2010; Mbikay, 2012; Mishra et al., 2011; Pinto et al., 2015; Sreelatha et al., 2011).

Previous reports demonstrated that *M. oleifera* leaf extract can protect against the chromium-induced testicular (Sadek, 2014) and cyclophosphamide-induced urinary bladder toxicity (Taha et al., 2015). Leaf extracts have been adopted as a therapy against hyperthyroidism and an anti-Herpes Simplex Virus Type-1 (Lipipun et al., 2003; Tahiliani and Kar, 2000). The *M. oleifera* leaf extract has been reported to have high antioxidant content, stimulate lactation and improve the immune system (Anwar et al., 2007; Leone et al., 2015; Siddhuraju and Becker, 2003; Singh et al., 2009; Verma et al., 2009).

M. oleifera leaf powder supplementation stimulated the immune response in HIV–positive people (Burger et al., 2002). Various studies have reported the immunomodulatory activity of *M. oleifera* leaf extracts in cyclophosphamide immunosuppressed Wistar albino rats (Gaikwad et al., 2011; Gupta et al., 2010). Also (Nfambi et al., 2015), reported that methanolic leaf extract of *M. oleifera* at doses of 250, 500, and 1000 mg/kg bwt caused a remarkable immunostimulatory effect on both the cell-mediated and humoral immune systems in the Wistar albino rats.

Given the previous layout, the current study was contrived to address the following issue: could M. *oleifera* safeguard against MEL-induced hemato immune toxic effects.

2. Material and methods

2.1. Tested compounds, reagents and chemicals

MEL (99.5% purity) was purchased from Alpha Chemica (Mumbai, India). It was diluted using pre-warmed distilled water to working stock concentrations for use in the experiments. Commercial enzyme-linked immunosorbent assays (ELISA) kits (Kamiya Biomedical, Tukwila, WA) were used to evaluate immunoglobulin G (IgG) and IgM, as well as of lysozyme activity (MyBioSource, San Diego, CA). All other reagents/chemicals used purchased from (Sigma, St. Louis, MO) and were of analytical grade.

2.2. Preparation of M. oleifera ethanol extracts

The leaves of M. *oleifera* were obtained as a precious gift and authenticated by Prof. Dr. Aboul Fetoh M. Abd-Allah, head of the Egyptian Scientific Society of Moringa, National Research Center, Egypt. Following the protocol of (Okechukwu et al., 2013), the leaves were thoroughly washed with distilled water and dried under room temperature at (29–35 °C) for three weeks. Using acrestor high speed milling machine, the leaves were crushed to coarse form. Then, the later form (1000 g) was macerated in absolute ethanol for 48 h and subsequently filtered. The resultant ethanol extract was concentrated and evaporated to dryness using a rotary evaporator at (40–45 °C). The concentrated extract was diluted using a distilled water with1% carboxy methyl cellulose to 1000 ml and stored in the refrigerator.

2.3. Gas chromatography/mass spectrometry analysis (GC–MS) of M. oleifera ethanol extracts

The *M. oleifera* ethanol extracts (MOE) samples were subjected to GC–MS analysis using a 1310 TRACE GC Ultra Gas Chromatographs (Thermo Fisher Scientific Inc., Waltham, MA, USA), coupled with a THERMO mass spectrometer detector (ISQ Single Quadrupole Mass Spectrometer) at the Regional Center for Mycology and Biotechnology, Al-Azhar University Campus, Nasr city, Cairo, Egypt. The isolated components of the extract were identified by matching their mass spectra with the NIST published data.

2.4. Animal grouping and experimental design

Fifty Sprague Dawely male rats (8-weeks-of-age), with average weight from (140 gm to 160 gm) were purchased from the Laboratory Animal Farm at Zagazig University. All rats were housed in stainless steel cages in a pathogen-free environment maintained at a controlled temperature (21–24 °C) with a relative humidity of (50–60%) and a 12-h light–dark cycle. Along the experimental period, feed and water were offered *ad-libtum*. The Ethics of the Animal Use in the Research Committee (EAURC) of Zagazig University approved the present protocols. All experimental procedures were applied following the NIH general guidelines for the Use and Care of the Laboratory Animals in scientific investigations.

Experimental rats were allocated into five equal groups, each of 10 rats. The first group received distilled water with1% carboxy methyl cellulose and kept as control. The Second group (MOE): had orally received a dose of 800 mg MOE/kg bw once a day for two weeks (Aja et al., 2015). The third group (MEL): orally received 700 mg MEL/kg bw once a day for two weeks (Early et al., 2013). The Fourth group of (MOE/MEL): received MOE as 800 mg/kg bw once a day for two weeks then received MEL as the previously mentioned durations and doses. The fifth group (MOE + MEL) co-treated with MOE and MEL at the doses given in groups 2 and 3. These regimens had administered by feeding needle of (14 gauge). The rats had been carefully monitored during the experiment for the detection of the signs of toxicity, mortality, and morbidity.

2.5. Sampling

By the end of the dosing, three separate blood samples were collected from the retro-orbital plexus from each rat:

1. The first blood sample was collected into K_2EDTA plastic tube for use in hematological evaluations.
2. The second blood sample was collected into a glass serum tube and left for 20 min at room temperature to coagulate; after centrifugation at 3000 rpm for 10 min, the resulted serum was isolated and placed at −20 °C until used (within 2 weeks) in the biochemical assays outlined below.
3. The third blood sample was collected into lithium heparin blood collection tube to separate phagocytes to assess bactericidal activity.

All rats of each group were then weighed and euthanized by cervical dislocation. Small pieces of the spleen were collected and fixed in 10% buffered neutral formalin solution for histopathological and immunohistochemical examinations.

2.6. Hematological evaluation

Using a Hema Screen 18 automated hematology analyzer (Hospitex Diagnostics, Sesto Fiorentino, Italy), total red blood cells (RBC), hemoglobin (Hb), packed cell volume (PCV), mean cell volume (MCV), mean corpuscular hemoglobin concentration (MCHC), total leukocytes, granulocytes, lymphocytes, and monocytes were determined (Weiss