



## Toxic effect of *Moringa peregrina* seeds on histological and biochemical analyses of adult male Albino rats



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### ABSTRACT

*Moringa* is multipurpose promising tree particularly for medicinal use. With its high nutritive and pharmaceutical values, every part of the tree is suitable for human consumptions. The use of vegetate parts, seeds or extracts requires toxicological evaluations to prove and verify safety uses before being added to pharmaceutical medicine, or any other products related to human diet. In this study, *Moringa peregrina* seeds, grown in high elevation mountain in Saint Catherin area, were investigated and evaluated for their toxicity with respect to its topological potential through histological and biochemical studies in Albino rats. Daily doses of 0, 500, 1000 and 2000 mg/kg body weight of dry seed of *M. peregrina* were administered orally to 4 groups of rats for 14 days. Biochemical and histopathological results were evaluated by standard methods. Measured biochemical parameters, insulin, albumin, total protein, creatinine, urea, uric acid, Follicle-stimulating hormone, Luteinizing hormone and Testosterone, revealed normal levels compared to control group. However, measured level of blood sugars, cholesterol, triglyceride and liver enzyme, displayed significant decreases. No histopathological changes were detected in the body tested organs. In consequences, intake of different doses of *M. peregrina*, even high one, exhibit no organ toxicity and are safe for human use.

### 1. Introduction

Natural products have been known as unique source for delivering many bioactive lead compounds for drug discovery. Plants, animals and microorganisms represent unlimited reservoirs for these natural products. In Particular, plants produce various metabolites that play a major role in their adaptation to certain environment. Several thousands of these compounds have been claimed to possess medicinal properties which can be used directly or as pharmaceutical agents [1]. Therefore, the plants possess specific activity; named as “herbal plants”; have been used by folk traditions for treating several diseases a century ago and represent a renewable source of natural products and chemical entities [2].

These natural products, nowadays, continue to provide a diverse and unique source of bioactive lead compounds for drug novelty especially with renewed interest for this source which considered as promising alternative medicines [3]. Additionally, there is an emerging increase in the intake of plant formulation as alternative drugs for the strong belief that these products are safe and devoid of toxic side effects [4]. Meanwhile, their acceptability, effectiveness, affordability, safety

and low cost give more attention to use them as alternative medicine [5,6]. *Moringa* tree, a member of family Moringaceae, is considered among these medicinal plants which have multiuse as folk and nutritive medicine [7]. It occurs in arid desert mountains. It is currently threatened in large part of its distribution range in the Middle East and the Arabian Peninsula due to the high rate of its consumption. It is native to the southern foothills of the Himalayas in northwestern India. Today, *Moringa* tree is widely cultivated in tropical and subtropical areas where its young seed pods and leaves are used as vegetables. There are thirteen species of *Moringa* are known worldwide, *M. oleifera* is the most distributed species beyond its origin [8]. It has already in use for several medical purposes and the evidence for its therapeutic practices have been reported by many studies [9]. The second popular *M* species is *M. peregrina* (Forssk.) Fiori., a small tree widely adapted to different harsh ecological conditions [10–12]. It geographically distributed from tropical Africa to east India. Nearly all parts of this plant species are culturally consumed by human, in its habitats, for its nutritional value and for its taste and flavor as a vegetable.

*Moringa peregrina* can also be used in herbal medicine for treatment of fever, headache, malaria, constipation, abdominal pains, muscle

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pains, hypertension, vasodilator activity, asthma, diabetes and burns in folk medicine [10]. Further pharmacological studies have shown the ability of this *M. species* to be used as antioxidant [13], analgesic, anti-inflammatory [14], antiulcer, anti-hyperglycemic [15], anti-hyperlipidemic, antimicrobial activities [14,16].

In Egypt, *Moringa peregrina* (Forssk.) Fiori. is the only native species occurs in Egyptian habitat among the thirteen species distributed all over the world. It is characterized by a very fast growth that can reach 3–5 m in height after 10 months of plantation time [12]. *M. peregrina* seeds have diminutive germination time and high seedling growth rate. Trees of *M. peregrina* bear 20–40 cm-long seed pods, each were containing 5–15 unwinged seeds. The seed kernel has high oil content in the range of 42–54% of its weight [17]. The Egyptian Bedouin used the seeds as a medicinal plant due to its composition of oil. It also used by pregnant woman for strengthen the muscles, health condition and facilitate the fetus delivery.

Many studies have been carried out to explore the medicinal properties of *Moringa* species; however, the most of the studies done were on *M. oleifera* and no studies were carried out for testing the toxicity and medical activates of naturally occurred *M. peregrina* in Saint Katherine area, South Sinai, Egypt. In the meantime, using this plant by local Bedouin often look at the medicinal benefits and neglect their toxic effects which may affect various human organs. For such purpose we collected *Moringa* seeds from its unique habitats in Saint Katherine area and used them to evaluate their toxicity for human uses.

## 2. Material and methods

### 2.1. Collection and identification of plant material

Fresh mature seed pots of *M. peregrina* trees, grown on the foot-hills and draining slopes of the higher mountains in South Sinai were collected. The plant was identified and authenticated taxonomically in Botany department, Faculty of Science, Suez Canal University. The seed pods were left to dry after then the seeds were collected from opened-dry pods. The seeds were peeled off and coat peeled-seeds were used.

### 2.2. Experimental animals

Twenty four young adult male albino rats, weighed between 120 and 150 g, were used in this study. They were fed with regular diet and tap water *ad libitum*. The rodents were housed under standard laboratory environment, and allowed to acclimatize to the laboratory environment (temperature of  $28 \pm 2^\circ\text{C}$  with relative humidity and naturally illuminated environment of 12/12 h day light/dark cycles) for two weeks before being used in the experiment. The experiment was performed in accordance with the internationally accepted standard ethical guidelines for laboratory animal use and care as described in the European Community guidelines [18].

### 2.3. Animal treatments and experimental protocol

The experimental protocol, designed to evaluate toxicity of *Moringa* seeds, was in accordance to the guideline of Diderich [19–21]. Twenty four rats were randomly divided into four groups with six rats each. Following an overnight fasting, the rats were weighted and the doses of *M. peregrina* seeds were calculated in reference to their body weight (BW). The groups were as follow: the control group, which received no treatment and feed normal diet; group one (G1), group 2 (G2) and group 3 (G3) were treated with *Moringa* seeds at doses 500, 1000 and 2000 mg/Kg BW, respectively. The seeds were crushed, after removing the outer fibrous covers, and daily administrated orally for 14 days in their diet. The rat's observation and monitoring was made for a period of 14 days for any signs of toxicity, mortality, behavior and clinical symptoms [20,21]. On the day14th of the running experiment, following an overnight fasting, all animals in designed groups were

anesthetized under chloroform vapor and blood samples were collected by cardiac puncture [22] and injected into non-heparin's bottles for biochemical investigations. Collected blood samples were allowed to clot and centrifuged according to groups; and serum was separated for the biochemical analyses. The treated groups and control group were dissected and body organs (liver, kidneys, pancreases, spleen and testes) were excised and cleaned immediately from blood by rinsing with physiological saline and then fixed in 10% buffered formalin for histopathological examination.

### 2.4. Biochemical analyses

Different biochemical tests were carried out using diagnostic kits to evaluate the toxicity of different doses of *Moringa* seeds and their effects on body function. Fasting blood glucose, expressed in mmol/L, was determined by (GOD/POD method) [23]. Serum insulin, expressed in ng/mL, was estimated by a radioimmunoassay [24]. Serum total cholesterol and triglycerides were measured colorimetric [25]. Liver enzymes, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) [26]; serum total protein concentration [27]. Serum Albumin was determined according to the method of Dumas, Watson [28]. Blood urea nitrogen, expressed in mg/dL, was assessed based on the cleavage of urea with urease according to the method of Karr [29]. Serum creatinine level (mg/dl) was determined colorimetric following the method of Heinegård and Tiderström [30]. Uric acid levels, expressed in mg/dL, was measured following the method of Kageyama [31]; Follicle-stimulating hormone (FSH), Luteinizing Hormone (LH) and testosterone were also measured according to manufacturer's structure of Midgley [32]; Niswender, Midgley Jr [33] and Vermeulen, Verdonck [34], respectively.

### 2.5. Histopathological examination

Selected organs including pancreas, spleen, liver, kidneys, and testes of treated and control rats were fixed in 10% buffered formalin in labeled bottles [35], and processed for histological examination [36]. Tissues embedded in paraffin wax were sectioned 5 mm thick, stained with Haematoxylin and Eosin, mounted on glass slides and examined under a standard light microscope. Light microscopic examinations of multiple tissue sections, from each organ in all groups, were performed and images representative of the typical histological profile were examined.

### 2.6. Statistical analysis

All data obtained, expressed in mean  $\pm$  standard error (SE), were statistically analyzed by the one-way ANOVA. The significance of the difference versus the control group was determined by Student's *t*-test. For ranking the data, Duncan Multiple Range test was applied. Values were considered statistically significant at  $p \leq 0.05$ . The Statistical Package for Social Sciences (SPSS) program, version 20, was used to run the statistical analyses.

## 3. Result

In the oral toxicity assay, all treated groups of rats at different doses of *M. peregrina* seeds (500, 1000 and 2000 mg/kg body weight) did not show any alternation in behavioral signals with no mortality during the time period of the study.

### 3.1. Biochemical studies

The biochemical results obtained from the oral *Moringa*-treated rats showed the safety use of *M. peregrina* seeds at all tested doses (500, 1000 and 2000 mg/kg body weight) when compared to control. For fasting serum glucose level, all *Moringa*-treated groups able to lower the

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