Immunostimulant effects of *Moringa Oleifera* extract on cyclophosphamide induced immunosuppression in rats

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A B S T R A C T

The present study was carried out on a total number of 90 male Albino rats. Group (1): control negative. It contained 15 rats treated with sterile saline. Group (2): (cyclophosphamide control group) treated with 50 mg / k.g B.w intramuscular. Group (3): *E.coli*-infected rats. 15 rats were injected with *E.coli* concentration intraperitoneally. Group (4): *E.coli* + cyclophosphamide group 15 rats injected with cyclophosphamide 50 mg / k.g B.w for 3 days + then experimentally infected with *E.coli* by intraperitoneal injection (one dose). Group (5): treated with M.o 500 mg / k.g B.w for 15 days then injected with cyclophosphamide 50 mg / k.g B.w for 3 days + then experimentally infected with *E.coli*. *M.o* continued with the same dose till the end of the experiment. Group (6) control *Moringa olifera*: gavaged with *M.o* 500 mg / k.g B.w all the experimental period. Results showed that, TLC in *M o* treated and *M o* control groups showed non-significant changes when compared with *E. coil* + cyclophosphamide treated and negative control groups. The level of IL-1 in the serum of *M o* treated group exhibited significant increase when compared with *E. coli* + cyclophosphamide-treated group at the 4th and 14th days after injection of *E. coli* and third dose cyclophosphamide similarly *M. o.* control group showed significant increase in levels of IL-1 when compared with control group. The levels of IL-10 in the serum of *M o* treated rats showed significant increase when compared with *E. coli* + cyclophosphamide-treated group at the 4th day after injection of *E.coli* and third dose cyclophosphamide similarly *M.O.* control group showed significant increase in levels of IL-10 when compared with control group. the levels of IgM in the scrum of *Moringa* treated rats were significantly increased when compared with *E. coil* + cyclophosphamide-treated rats at the 4th and 14th days after injection of *E. coli* and third dose cyclophosphamide, similarly *M.O.* control group showed significant increase in levels of IgM when compared with control group. Therefore, it concluded that *Moringa olifera* can be used as a protective remedy as it improves immune status in rat injected E. coil + cyclophosphamide.

Keywords: *Moringa olifera,* cyclophosphamide, *E. coil, immunostimulation*

(http://www.bvmj.bu.edu.eg) (BVMJ-32(2): 145-150, 2017)

1. INTRODUCTION

Plants play an important role in the medical practices of many peoples. There is a growing interest in medicinal botanicals as part of complementary medicine. In particular, many botanicals are sold today as dietary supplements. We focus particularly on *Moringa olifera*. There are very few data to support such use and even less information about drug toxicity or interactions. Also the immunomodulatory activities of these botanicals and the mechanisms by which these botanicals might modulate the immune system are not well known (Adedapo et al., 2009, Agrawal et al., 2008, Ajibade et al., 2012, Ali et al. 2004). The risk of accumulation of chemotherapeutic residues in meat and their potential negative impact on human health, consumer pressure in respect to food safety, and the risk of potential cross-resistance of microorganisms to the antimicrobials used to treat humans or animals gave rise to Regulation (EC) No 1831/2003 of the European Parliament and Council which provides for a ban on the prophylactic use of coccidiostats that will go into effect in 2013. In addition to the advantages previously mentioned, herbal extracts do not carry the risk of contaminating meat with harmful substances. To date, the emergence of resistance to herbal products among coccidia species has not been reported (Arczewska-Włosek and Świątkiewicz 2012). *Moringa oleifera* is the most widely cultivated species of a monogeneric family, the Moringaceae that is native to the sub-Himalayan tracts of India, Pakistan, Bangladesh and Afghanistan. This rapidly-growing tree (also known as the horseradish tree, drumstick tree, benzolive tree,

kelor, marango, mlonge, moonga, mulangay, nébéday, saijhan, sajna or Ben oil tree), was utilized by the ancient Romans, Greeks and Egyptians; it is now widely cultivated and has become naturalized in many locations in the tropics. It is a perennial softwood tree with timber of low quality, but which for centuries has been advocated for traditional medicinal and industrial uses. It is already an important crop in India, Ethiopia, the Philippines and the Sudan, and is being grown in West, East and South Africa, tropical Asia, Latin America, the Caribbean, Florida and the Pacific Islands. All parts of the Moringa tree are edible and have long been consumed by humans. This tree has in recent times been advocated as an outstanding indigenous source of highly digestible protein, Ca, Fe, Vitamin C, and carotenoids suitable for utilization in many of the so-called "developing" regions of the world where undernourishment is a major concern (Fuglie 1999, Anwar et al. 2007, Auwal et al. 2013, Awodele et al., 2012). Moringa leaves contained more Vitamin A than carrots, more calcium than milk, more iron than spinach, more Vitamin C than oranges, and more potassium than bananas," and that the protein quality of Moringa leaves rivals that of milk and eggs (Fuglie 2000, Bennett et al., 2003, Bharali et al., 2003). Leaves from four different Moringa species (*Moringaoleifera*, *Moringa peregrina*, *Moringa stenopetala* and *Moringa drouhardii*) all contained high levels of nutrients and antioxidants. Vitamin A was found to be at its peak during the hot-wet season, whereas iron and vitamin C was highest during the cool-dry season. This little-plant had the potential to improve nutrition, boost food security and support sustainable land use practices (Price 2007).

Therefore, this study will aim to evaluate the prophylactic, hematological, immunostimulatory and anti -inflammatory effects of *Moringa oleifera* on experimentally immunosuppressed and *E.coli*-infected rats

2. MATERIALS AND METHODS

2.1. Expermintal animals:

The present study was carried out on a total number of 90 male Albino rats (180-200 gm body weight). They were obtained from laboratory animal center, faculty of veterinary medicine, Benha University. They were housed for two weeks in the same environmental and nutritional conditions similar to those under which the experiment would be performed for accommodation. Rats were randomly allocated into 6 groups (15 rats/group) and housed in separate cages. Each group of rats was provided by suitable feeder and water.

2.2. Moringa olifera.

M. olifera was obtained from Desert Research Institute Elshekh Zoed - Sinai – Egypt.

2.3. Bacteria

Esherichia coli bacteria obtained from the Department of Microbiology and Immunity, Faculty of Veterinary Medicine, Benha University, Egypt. Type of *E. coli* bacteria was O125 (pathogenic) and isolated from hens. *E. coli* broth saline was prepared by suspension of *E.coli* culture in broth at concentration of 1×10^9 CFU, by using turbidimetric method according to Lee, (2009).

2.4. Cyclophosphamide

Cyclophosphamide (Endoxan) was obtained from Baxter Oncology GmbH, kantstrasse 2, D-33790 Halle, Germany.

2.5. Materials used for clinicopathological examinations:

Dipotassium salt of ethylene diamine tetra-acetic acid (EDTA): The anticoagulant was obtained from Sigma Chemical Company, (USA) and used according to Feldman et al., (2000). Automatic cell counter (H.A vet Clindiag, Belgium) was used for complete blood count. Commercial diagnostic kits used for estimation of concentration of different biochemical parameters were obtained from Qumica Clinica Alpicada (QCA) (Spain). The diagnostic kits of interleukin (IL)-1β, interleukin (IL)-10, IgG and IgM were obtained from MBS Company (USA). The diagnostic kits of IL-1β, IL-10, IgG and IgM were measured by using Spectra 2000 (USA).

2.6. Experimental design:

Group (1): control negative. It contained 15 rats treated with sterile saline (1 ml / 200 gm body weight). Group (2): (cyclophosphamide control group) treated with 50 mg / k.g B.w intramuscular (Wojcik, 2014). Group (3): *E.coli*-infected rats. 15 rats were injected with *E.coli* concentration ($(1x10^9)$ intraperitoneally at 18^{th} day. Group (4): *E.coli* + cyclophosphamide group 15 rats injected with cyclophosphamide 50 mg / k.g B.w for 3 days (16th , 17th and 18th days) + then experimentally infected with *E.coli* (1×10^9 CFU) by intraperitoneal injection (one dose) at 18^{th} day (Wojcik, 2014). Group (5): treated with M.o 500 mg / k.g B.w for 15 days then injected with cyclophosphamide 50 mg / k.g B.w for 3 days (16th , 17th and 18th days) + then experimentally infected with *E.coli* (1×10^9 CFU) by

intraperitoneal injection (one dose) at 18th day. *M.o* continued with the same dose till the end of the experiment (Gupta et al., 2010 and Wojcik, 2014). Group (6) control *Moringa olifera*: gavaged with *M.o* 500 mg / k.g B.w all the experimental period.

2.7. Blood Samples:

Blood samples were collected by the retro orbital bleeding method from 5 rats from each group at 24 hrs. 96 hrs. and 14 days of experiment and blood samples were divided into 2 parts, first part (0.5-1 ml) collected on dipotassium salt of EDTA-tubes for complete blood count and second part (3 ml) collected on plain tubes for serum separation.

2.8. Statistical Analysis:

Statistical analysis was performed using the statistical software package SPSS for Windows (Version 16.0; SPSS Inc., Chicago, Ill.). Student's *t*-test was used to determine significant differences between two experimental groups. The significance of differences between more than two groups was evaluated by one-way analysis of variance (ANOVA). If one-way ANOVA indicated a significant difference, then differences between individual groups were estimated using Fishe's least significant difference (LSD) test. Results are expressed as the mean ± standard error of mean (SEM). A *P*-value of less than 0.05 was considered significant (Kinner and Gray, 2008).

3. RESULTS

3.1. Changes in leukogram among the experimental rat groups:

WBCs, Granulocytes, Lymphocytes and monocytes counts in the blood of control, *E. coli*-infected, cyclophosphamide-treated rats in the absence and presence of *Moringa olifera* treatment were examined after 24 hours, 4 and 14 days of injection of *E.coli* and third dose cyclophosphamide (table 1).

3.2. Changes in serum cytokines (IL-1β and IL-10) and immunoglobulins (IgG and IgM) among the experimental rat groups:

IL-1β, IL-10, IgG and IgM levels in the serum of control, *E.coli*-infected, cyclophosphamide-treated rats in the absence and presence of Morinaga-treatment were examined after 4 and 14 days of injection of *E.coli* and third dose cyclophosphamide (table 2).

Table (1): Changes in the mean values ± SE of leukogram in different experimental groups:

Parameters	Groups Collection	Control	*E.coli*	Cyc.	*E.coli* + cyc	Treated M.	C. *Moringa*
Leucocytes	First	7.16 ±0.55^{b}	10.62 ±1.69^{a}	1.44 ±0.22^{c}	1.52 ±0.45^{c}	0.94 ±0.22^{c}	–
	Second	6.7 ±0.35^{b}	10.08 ±0.43^{a}	0.85 ±0.11^{c}	0.94 ±0.24^{c}	0.92 ±0.16^{c}	–
	Third	9.84 ±0.69^{b,c}	13.88 ±1.91^{a,b}	7.45 ±0.85^{c}	15.8 ±2.45^{b,c}	12.53 ±1.6^{b,c}	9.02 ±0.65^{b,c}
Lymphocyte	First	5.70± 0.70^{a}	6.59± 1.00^{a}	1.01± 0.17^{b}	1.26± 0.41^{b}	0.70± 0.14^{b}	–
	Second	4.47± 0.54^{a}	5.25± 0.72^{a}	0.50± 0.11^{b}	0.41± 0.06^{b}	0.61± 0.16^{b}	–
	Third	6.38± 0.44^{a}	6.79± 1.22^{a}	12.18± 0.22^{a}	8.28± 1.25^{a}	6.65± 1.86^{a}	5.98± 1.05^{a}
Monocyte	First	0.94± 0.16^{a}	0.90± 0.12^{a}	0.11± 0.02^{b}	0.11± 0.03^{b}	0.06± 0.01^{b}	–
	Second	0.84± 0.27^{a}	0.72± 0.10^{a}	0.05± 0.01^{b}	0.06± 0.01^{b}	0.06± 0.00^{b}	–
	Third	0.90± 0.08^{a}	1.25± 0.17^{a}	2.06± 0.51^{a}	1.41± 0.24^{a}	1.11± 0.25^{a}	0.9± 0.14^{a}
Granulocyte	First	2.96± 0.65^{a}	3.16± 0.59^{a}	0.36± 0.04^{b}	0.21± 0.03^{b}	0.23± 0.06^{b}	–
	Second	3.04± 0.56^{a}	3.14± 0.44^{a}	0.25± 0.07^{b}	0.52± 0.20^{b}	0.29± 0.03^{b}	–
	Third	4.01± 0.37^{a,b}	5.88± 0.57^{a,b}	12.26± 0.44^{a,b}	6.14± 0.98^{b}	3.13± 0.87^{b}	3.56± 0.70^{b}

Table (2): Changes in the mean values ± SE of IL-1β, IL-10, IgG, and IgM in different experimental groups:

Parameters	Groups Collection	Control	*E.coli*	Cyc.	*E.coli* + cyc	Treated M.	C. *Moringa*
IL-1β	First	–	–	–	–	–	–
	Second	40.93±0.84^{c}	72.16±3.03^{b}	39.1 ±1.03^{c}	59.20±4.11^{b}	113.76±8.39^{a}	–
	Third	45.05 ±2.65^{f}	64.35 ±1.67^{e}	68.8 ±2.08^{d}	66.45 ±2.65^{e,d}	90.15±1.91^{b,c}	94.95±0.61^{a}
IL-10	First	–	–	–	–	–	–
	Second	18.13 ±0.46^{b}	27 ±2.95^{a}	28.9 ±2.63^{a}	17.63 ±0.65^{b}	24.03 ±1.69^{a}	–
	Third	34.75 ±2.00^{d}	32.05 ±2.32^{d,e}	29.5 ±1.55^{e}	41.8 ±0.97^{b}	38.55 ±0.61^{c}	56.6 ±0.97^{a}
IgG	First	–	–	–	–	–	–
	Second	571.50 ±71.43^{a}	529.53 ±19.59^{a}	663.63 ±18.35^{a}	625.03 ±78.44^{a}	631.90 ±36.82^{a}	–
	Third	738.25 ±20.37^{d}	822 ±5.96^{b,c}	759.65 ±9.92^{c,d}	985.4 ±5.79^{a}	767.2 ±13.06^{c,d}	781.2 ±14.20^{d}
IgM	First	–	–	–	–	–	–
	Second	19.7 ±1.12^{c}	37.33 ±2.25^{b}	21.9 ±1.4^{b}	26.53 ±1.43^{b}	43.6 ±2.53^{b}	–
	Third	25.45 ±1.51^{d}	30.25 ±0.77^{c}	34.4 ±0.98^{b}	34.7 ±0.65^{b}	35.55 ±0.93^{b}	37.45 ±0.61^{b}

4. DISCUSSION

TLC in *M.O* treated and *M.O.* control groups showed non-significant changes when compared with *E. coil* + cyclophosphamide treated and negative control groups respectively, our finding came in harmony with Mahajan *et aL., (2011).* On studying Dose-dependent effect of ethanolic and aqueous extract of *Moringa olifera* fruit and leaf on WBC count of mice blood there were insignificant changes. Our results disagreed with *Gupta et al., (2010)* they found that *M.O.* leaves extract showed significant dose dependent increase in WBC and percent neutrophils in mice. Our results disagreed with *Lee et al., (2003)* because of different plant organ as their experiment was carried out on *Moringa olifera* seed extract. Discrepancies among scientific reports may be attributable to many factors. Studies differ in the plant species used, type of extract made including commercially prepared vs. laboratory prepared), and precise method of extraction changes. Our results disagreed with *Gupta et al., (2010)* they found that *M.O.* leaves extract showed significant dose dependent increase in WBC and percent neutrophils in mice. Our results disagreed with *Lee et al., (2003)* because of different plant organ as their experiment was carried out on *Moringa olifera* seed extract. Discrepancies among scientific reports may be attributable to many factors. Studies differ in the plant species used, type of extract made including commercially prepared vs. laboratory prepared), and precise method of extraction.

In assessing the role of *Moringa olifera* on cytokines, the level of IL-1β in the serum of *M.O.* treated group exhibited significant increase when compared with *E. coli* + cyclophosphamide-treated group at the 4th and 14th days after injection of *E. coli* and third dose cyclophosphamide similarly *M.O.* control group showed significant increase in levels of IL-1β when compared with control group, our results not consistent with Rastogi *et al., (2009)* as they reported that the report reveals the significant increase in serum TNF-a and IL-1β in atherogenic diet fed rats. Treatment with *M olifera* (100 and 200 mg/kg/b.wt) significantly decreased these parameters which is taken as the anti-inflammatory activity of the *M.O.* Meanwhile, *Sudha et al., (2010),* Sutar et al (2008) mentioned that a methanol extract of *M olifera* leaves given orally at doses of 250 and 750 mg/kg stimulated both cellular and humoral immune responses. These discrepancies in results were attributed to the differ in experimental procedures.

The levels of IL-10 in the serum of *M.O.* treated rats showed significant increase when compared with *E. coli* + cyclophosphamide-treated group at the 4th day after injection of *E.coli* and third dose cyclophosphamide similarly *M.O.* control group showed significant increase in levels of IL-10 when compared with control group, our results coincident with *Hukkeri et al.,*

(2006); *Muangnoi et al., (2012)* they found that Amelioration of inflammation associated chronic diseases could be possible with the potent anti-inflammatory activity of *M.O.,* bioactive compounds N-butanol extract of the seeds of *M.O.* showed anti-inflammatory activity against ovalbumin-induced airway inflammation in guinea pig. (IL-10) was associated with anti-inflammatory activities *(Durbin et al., 2000)* The levels of IL-10 in the serum of *M.O.* treated rats showed significant increase when compared with *E. coli* + cyclophosphamide-treated group at the 4th day after injection of *E.coli* and third dose cyclophosphamide similarly *M.O.* control group showed significant increase in levels of IL-10 when compared with control group, our results coincident with *Hukkeri et al., (2006)*; *Muangnoi et al., (2012)* they found that Amelioration of inflammation associated chronic diseases could be possible with the potent anti-inflammatory activity of *M.O.,* bioactive compounds N-butanol extract of the seeds of *M.O* showed anti-inflammatory activity against ovalbumin-induced airway inflammation in guinea pig. (IL-10) was associated with anti-inflammatory activities *(Durbin et al., 2000)*

the levels of IgM in the scrum of *Moringa* treated rats were significantly increased when compared with *E. coil* + cyclophosphamide-treated rats at the 4th and 14th days after injection of *E. coli* and third dose cyclophosphamide, similarly *M.O.* control group showed significant increase in levels of IgM when compared with control group, our results consistent with *Gupta et al., (2010)*; *Sudha et al., (2010)* their results indicated that *M.O.* leaves significantly reduced cyclophosphamide induced immunosuppression by stimulating both cellular and humoral immunity.

The data of IgG levels obtained in our study revealed significant decrease in the levels of IgG in *E. p* treated group and *M.O.* treated group when compared with *E. coli* + cyclophoaphamide group at 14th day after injection of *E.coli* and third dose cyclophosphamide this result agreed with that obtained by *Mishima et al., (2004).*

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