

Full Length Research Paper

Modulatory effects of *Momordica balsamina* on Th1/Th2 cytokine profiles in immune-challenged rats

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There is an ever-growing interest to identify plants that boost the immune system functions. The objective of the present study was to evaluate the immunomodulatory effects of *Momordica balsamina* (MB) leaves extract in BCG-immunized rats. Thirty rats were randomly divided into five groups (n = 6). MB extract was suspended in 1% carboxymethyl cellulose (CMC). Firstly, animals were challenged subcutaneously with 0.05 ml BCG. The first group (vehicle control group) received 1% CMC (100 mg/kg body weight; p.o.), and the second group (positive control group) was provided with levamisole (18 mg/kg body weight; p.o.). The remaining groups (test groups) were dosed orally with different doses of MB extracts (50, 100 and 200 mg/kg body weight) for 14 consecutive days. Blood samples were collected on day 0, 7 and 14, and then plasma samples were analyzed for Th1 cytokines (TNF- α and IFN- γ) and Th2 cytokines (IL-10 and TGF- β), using cytokine specific enzyme-linked immunosorbant assay (ELISA). MB extracts significantly increased ($p \leq 0.01$) IFN- γ production in a dose- and time-dependent manner and elicited significant increase ($P \leq 0.05$) of TNF- α level at the dose 200 mg/kg after 14 days. Low doses (50 and 100 mg/kg) significantly increased ($p \leq 0.05$) TGF- β levels while a high dose (200 mg/kg) significantly ($p \leq 0.05$) reduced TGF- β levels in a time-dependent manner. No significant changes were observed on IL-10 level after plant extract treatment. MB shows immunostimulatory effects and significantly activates cell-mediated immunity.

Key words: *Momordica balsamina*, cell-mediated immunity, Th1 cytokine, Th2 cytokine.

INTRODUCTION

Interest in medicinal plants has recently burgeoned due to increased efficiency of new plant-derived drugs coupled with rising cost of conventional medicines (Karali et al., 2011; Siveen and Kuttan, 2012). In nature, various medicinal plants are believed to promote positive health

and enhance the natural resistance of the body to various human ailments. A large number of medicinal plants have been claimed to possess immunomodulatory activities (Kumar et al., 2012; Eze et al., 2017; Ahmad et al., 2018; Shruthi et al., 2018). Medicinal plants as botanical

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immunomodulators can provide alternative potential to conventional chemotherapy for a variety of immune disorders such as autoimmune diseases or conditions of impaired immune response (Mainardi et al., 2009). The therapeutic potential of plant-based immunomodulators and their curative properties have been highlighted by many researchers. In recent years, there has been growing interest to use herbal medicines as multi-component agents to modulate the immune system for prevention of infections and neuroinflammation (Jantan et al., 2015). Plant-derived natural products such as polysaccharides, flavonoids, alkaloids, sesquiterpene lactones and triterpenes have received considerable attention in recent years due to their diverse pharmacological properties. Phytochemicals such as flavonoids, polysaccharides, alkaloids, lectins, glycosides, phenolic compounds, tannins, saponins, terpenoids, sterols have been reported to modulate the immune system (Jantan et al., 2015; Venkatalakshmi et al., 2016; Oh et al., 2018).

The regulation of immune responses depends on the local production of a number of cytokines which are a diverse group of proteins that act as mediators between cells. T-cells participate in a wide range of immune responses through cytokine network. IL-2 differentiates CD4+ T-cells into two subsets of cells, T helper 1(Th1) and T helper 2 (Th2) cells. Th1 cells stimulate cell-mediated immunity through secretion of Th1 cytokines (TNF- α and IFN- γ) which activate macrophages to eradicate intracellular microbes. Th2 subset of cells stimulates antibody-mediated immunity through secretion of Th2 cytokines (IL-4, IL-5 and IL-10) (Romagnani, 2000).

Momordica balsamina, also known as Balsam Apple, belongs to the family Cucurbitaceae. It is annual or short-lived perennial herbaceous climber with yellow-orange warty fruit when ripe. The plant is widely found/cultivated throughout the drier part of tropical and subtropical Africa, Asia and Australia (Welman, 2004; Behera et al., 2011). Different parts of *M. balsamina* (leaves, fruits, seeds) contain various phytochemicals such as alkaloids, flavonoids, glycosides, steroids, terpenes, cardiac glycoside and saponins (Thakur et al., 2009; Nagarani et al., 2014). *M. balsamina* has been used as a traditional folk medicine in many countries, and it is used medicinally as anti-plasmodial, shigelloidal, anti-diarrheal, anti-septic, anti-bacterial, anti-viral, anthelmintic, anti-inflammatory, hypoglycemic, antioxidant, analgesic and hepatoprotective agent (Thakur et al., 2009; Ramallete et al., 2010; Nagarani et al., 2014).

In recent years there has been an upsurge in the clinical uses of plant-derived natural substances to strengthen natural immunity against various ailments. The aim of this study is to evaluate the immunomodulatory effect of methanolic extract of *M. balsamina* leaves in rodent model of BCG-immunized rats.

MATERIALS AND METHODS

Animals and maintenance

All animal work was carried out at the Faculty of Pharmacy, University of Khartoum (Khartoum, Sudan) in accordance with Institutional Animal Welfare Guidelines; and all experimental protocols were approved by the local Ethics Committee (Ethics Committee for Animal Experimentation, Faculty of Pharmacy - University of Khartoum, Sudan). Albino rats (average weight 180 grams) of either sex were obtained from the animal house of the Faculty of Pharmacy, University of Khartoum, Sudan. They were housed in standard plastic cages, under standard environmental conditions of temperature (18-26°C), light (12 h light/dark cycle) and controlled humidity. They had free access to standard diet and water *ad libidum* (Elahi et al., 2017).

Preparation of plant extract

M. balsamina was obtained from Northern Kordofan in Western Sudan. It was identified and authenticated by the Department of Medicinal and Aromatic Plants at the National Centre for Research, Khartoum, Sudan. Briefly, leaves of *M. balsamina* were air-dried at room temperature to avoid possible decomposition of the plant constituents and then pulverized into coarse powder. The plant's powder was macerated in 80% methanol at room temperature for 24 h with occasional shaking. The extract filtrate was concentrated under reduced pressure, using the rotary vacuum evaporator, and then dried to a solid mass under air at room temperature (Jones and Kinghorn, 2006).

Experimental protocol

Thirty rats (average body weight 180 g) were randomly divided into five groups (n = 6). *M. balsamina* extract was suspended in 1% carboxymethyl cellulose. Firstly, all animals were immunized subcutaneously with 0.05 ml BCG on day 0 to challenge the immune system. The first group (vehicle control group) received 1% carboxymethyl cellulose (100 mg/kg body weight; p.o.), and the second group (positive control group) was provided with levamisole (18 mg/kg body weight; p.o.) for 14 consecutive days (Stogaus and King, 1995). The remaining three groups (test groups) were dosed orally, for 14 consecutive days, with three doses (50, 100 and 200 mg/kg body weight) of *M. balsamina* extracts, suspended in 1% carboxymethyl cellulose.

Collection and preparation of plasma samples for assay

For quantitative measurement of cytokines in plasma, about three milliliter of blood samples were collected from the retro-orbital plexuses of individual rat under mild ether anesthesia on day 0 (just before dosing and immunization), day 7 and day 14. Plasma samples were separated after centrifugation and then stored at -20°C until further analysis (Elhag et al., 2011).

Measurement of Th1 cytokines in plasma

Levels of TNF- α and IFN- γ (Th1 cytokines) in plasma were quantified, using ELISA kits (Peprotech Company, USA), according to the manufacturer's instructions. Briefly, 96-well microtiter plates (Nunc/Thermo scientific industry, Denmark) were coated overnight at room temperature with capture antibody, washed and then

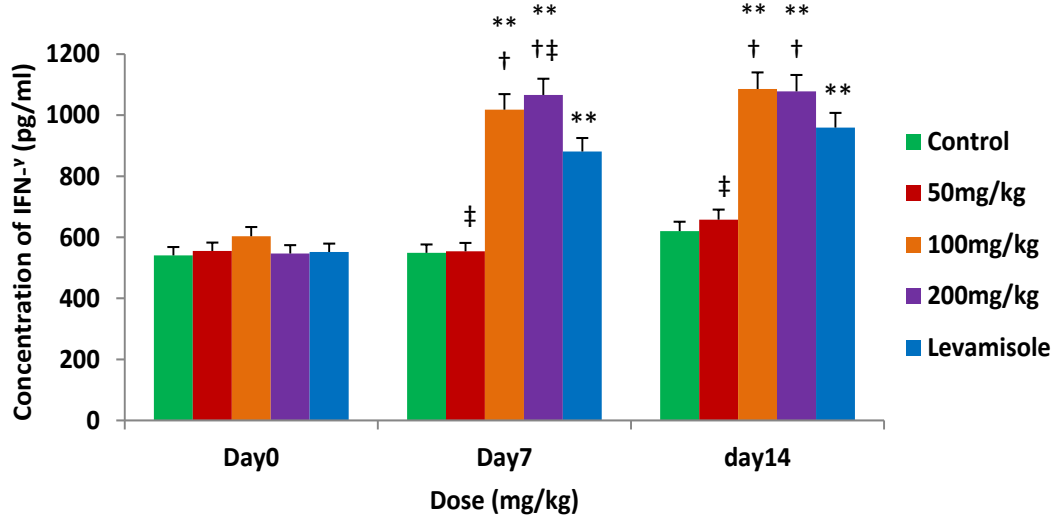


Figure 1. Dose-dependent effects of *M. balsamina* on IFN- γ levels. Rats were treated with *M. balsamina* extracts (50, 100 and 200 mg/kg), levamisole (18 mg/kg) or only vehicle. Data are presented as mean \pm S.D. **, $p \leq 0.01$ versus control. †, $p \leq 0.05$ versus 50 mg/kg. ‡, $p \leq 0.05$ versus levamisole.

blocked by addition of block buffer (1% fetal calf serum in phosphate buffer solution) for one hour at room temperature. Samples and series of diluted standards were immediately added in duplicate to the plates and incubated at room temperature for at least two hours. After washing, biotin-conjugated detection antibody was added and then incubated at room temperature for two hours. After 30 min incubation of avidin-HRP conjugate at room temperature, ABTS (2,2-Azino-bis (3-ethylbenzthiazoline-6-sulfonic acid); purchased from Sigma-aldrich, USA) liquid substrate was added and then incubated at room temperature for color development. The reactions were stopped after 15 min and the color was immediately measured at 405 nm, using automated ELISA plate reader. TNF- α and IFN- γ levels were calculated from calibration curves of diluted TNF- α and IFN- γ standard concentrations, respectively. Concentrations of the cytokines were determined in pg/ml.

Measurement of Th2 cytokines in plasma

For quantitative measurement of TGF- β 1 and IL-10 levels (Th2 cytokines), Rat TGF- β 1 and IL-10 ELISA kit (Bender Medsystem Company/eBioscience) were used, following the manufacturer's protocol. In brief, measurement of TGF- β required acidification of plasma samples for activation of inactive latent TGF- β before starting the test procedure. Diluted samples and standards were added in duplicate to precoated 96-well microtiter plates (coated with anti-rat TGF- β 1mAbs or anti-rat IL-10 mAbs) and then incubated for two hours at room temperature. After washing, biotinylated detection antibodies were added and incubated for one hour, followed by the addition of streptavidin-HRP and further incubation for one hour. Thereafter, TMB (tetramethyl-benzidine; purchased from eBioscience) substrate was added and incubated for 30 min at room temperature for color development. The reactions were stopped with 1 M phosphoric acid, and the color intensity was measured at 450 nm, using a microtiter plate reader. TGF- β and IL-10 concentrations were calculated from standard curves of TGF- β and IL-10 standard solutions, respectively. Concentrations of the cytokines were determined in pg/ml.

Statistical analysis

Data were expressed as Mean \pm S.D. Levels of difference between all groups were determined by one-way analysis of variance (ANOVA) followed by Dunnett's *t*-test. *P*-values < 0.05 were considered as statistically significant.

RESULTS

Effects of *M. balsamina* leaf extract on IFN- γ level in BCG-immunized rats

Evaluation of immunomodulatory effect of *M. balsamina* extract on Th1 cytokine revealed that the plant extract significantly increased ($p \leq 0.01$) IFN- γ levels in a dose/time-dependent manner. Daily administration of 100 and 200 mg/kg of *M. balsamina* extract exhibited a significant increase ($p \leq 0.01$) of IFN- γ level after 7 and 14 days when compared to control. Interestingly, IFN- γ levels at doses of 100 and 200 mg/kg after 7 or 14 days were greater than that observed after treatment with levamisole (positive control). *M. balsamina* leaf extract increased IFN- γ level in BCG-immunized rats as shown in Figures 1 and 2.

Effects of *M. balsamina* leaf extract on TNF- α level in BCG-immunized rats

In order to delineate the immunomodulatory effects of *M. balsamina* extract, the levels of TNF- α (Th1 cytokine) were measured after administration of three doses of the extract. Daily administration of *M. balsamina* extract

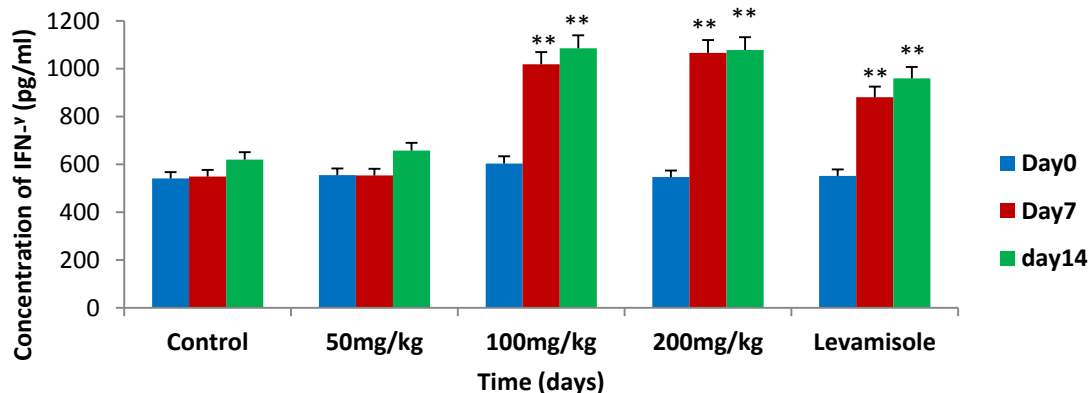


Figure 2. Time-dependent effects of *M. balsamina* on IFN- γ levels. Rats were treated with *M. balsamina* extracts (50, 100 and 200 mg/kg), levamisole (18 mg/kg) or only vehicle. Data are presented as mean \pm S.D. **, $p \leq 0.01$ versus day 0

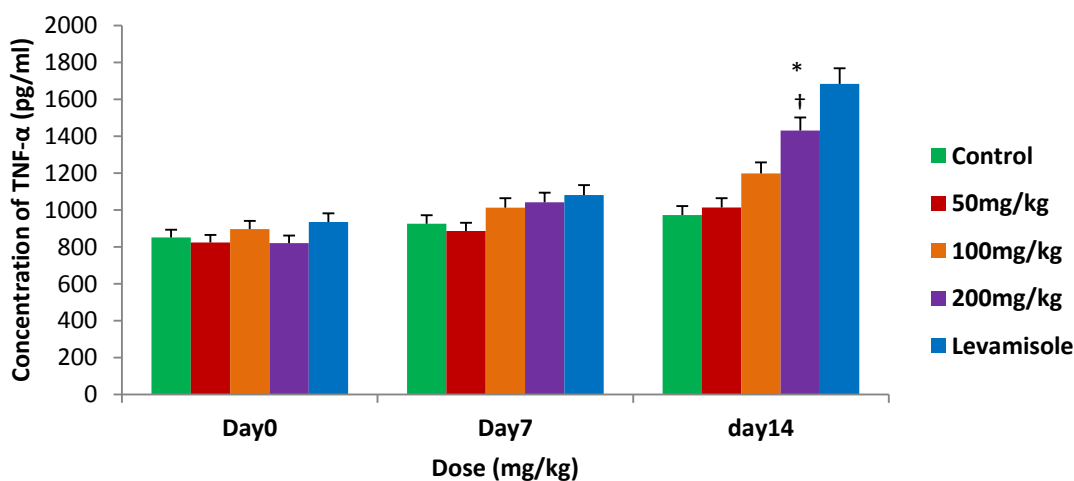


Figure 3. Dose-dependent effects of *M. balsamina* on TNF- α levels. Rats were treated with *M. balsamina* extracts (50, 100 and 200 mg/kg), levamisole (18 mg/kg) or only vehicle. Data are presented as mean \pm S.D. *, $p \leq 0.05$, versus control. †, $p \leq 0.05$ versus 50 mg/kg.

appears to exert dose-dependent increase of TNF- α levels after 14 days. Interestingly, 200 mg/kg of *M. balsamina* extract showed a significant increase ($P \leq 0.05$) of TNF- α level after 14 days when compared to control (Figure 3); and exhibited a significant increase ($P \leq 0.05$) of TNF- α level in a time-dependent manner. In administration of *M. balsamina* extract, neither 50 mg/kg nor 100 mg/kg dose affected TNF- α level with respect to time (Figure 4). *M. balsamina* leaf extract increased TNF- α level in BCG-immunized rats.

Effects of *M. balsamina* leaf extract on TGF- β levels in BCG-immunized rats

For further identification of immunomodulatory effect of *M. balsamina* extract, the levels of TGF- β were measured

as a representative of Th2 and anti-inflammatory cytokines. *M. balsamina* extract showed divergent effects on TGF- β levels depending on the dose. A significant increase ($p \leq 0.05$) of TGF- β levels was observed after 7 and 14 days of treatment with 50 and 100 mg/kg (Figure 5). In contrast, daily administration of 200 mg/kg of *M. balsamina* extract significantly ($p \leq 0.05$) reduced TGF- β levels in a time-dependent manner (Figure 6). *M. balsamina* leaf extract exhibited divergent effect on TGF- β levels.

Effects of *M. balsamina* leaf extract on IL-10 levels in BCG-immunized rats

The levels of IL-10 were measured after daily administration of three doses of *M. balsamina* extract. In

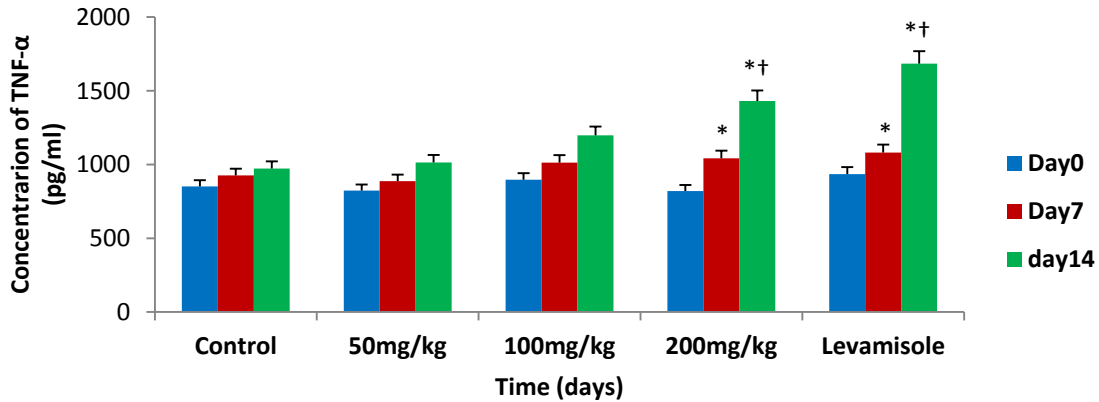


Figure 4. Time-dependent effects of *M. balsamina* on TNF- α levels. Rats were treated with *M. balsamina* extracts (50, 100 and 200 mg/kg), levamisole (18 mg/kg) or only vehicle. Data are presented as mean \pm S.D. *, $p \leq 0.05$, versus day 0. †, $p \leq 0.05$ versus day 7.

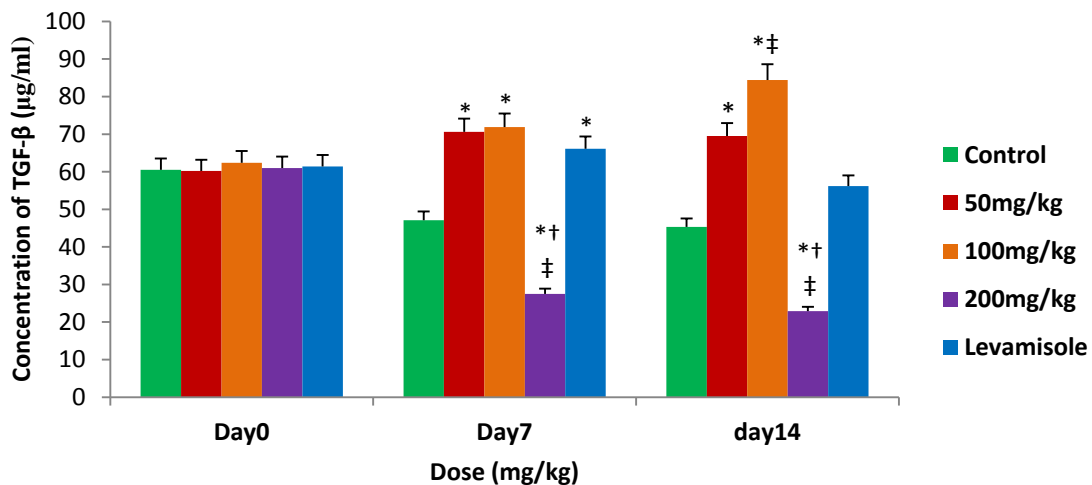


Figure 5. Dose-dependent effects of *M. balsamina* on TGF- β levels. Rats were treated with *M. balsamina* extracts (50, 100 and 200 mg/kg), levamisole (18 mg/kg) or only vehicle. Data are presented as mean \pm S.D. *, $p \leq 0.05$, versus control. †, $p \leq 0.05$ versus 50 and 100 mg/kg. ‡, $p \leq 0.05$ versus levamisole

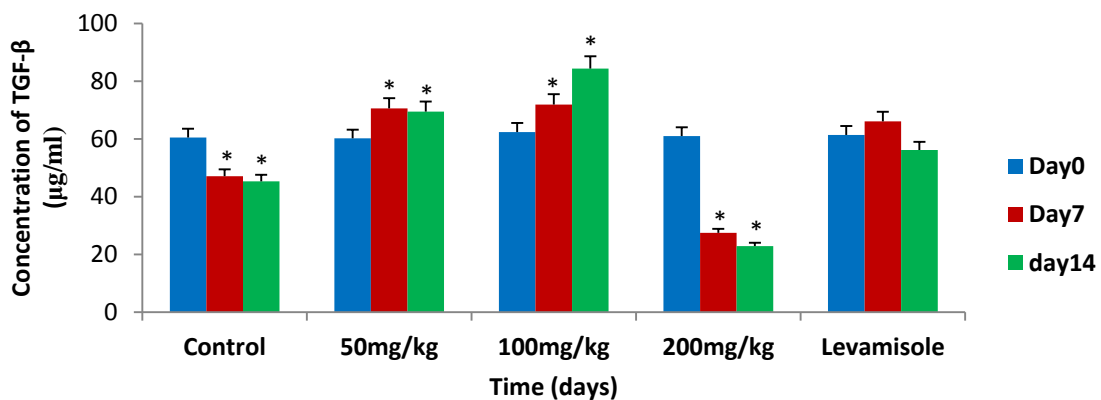


Figure 6. Time-dependent effects of *M. balsamina* on TGF- β levels. Rats were treated with *M. balsamina* extracts (50, 100 and 200 mg/kg), levamisole (18 mg/kg) or only vehicle. Data are presented as mean \pm S.D. *, $p \leq 0.05$ versus day 0.

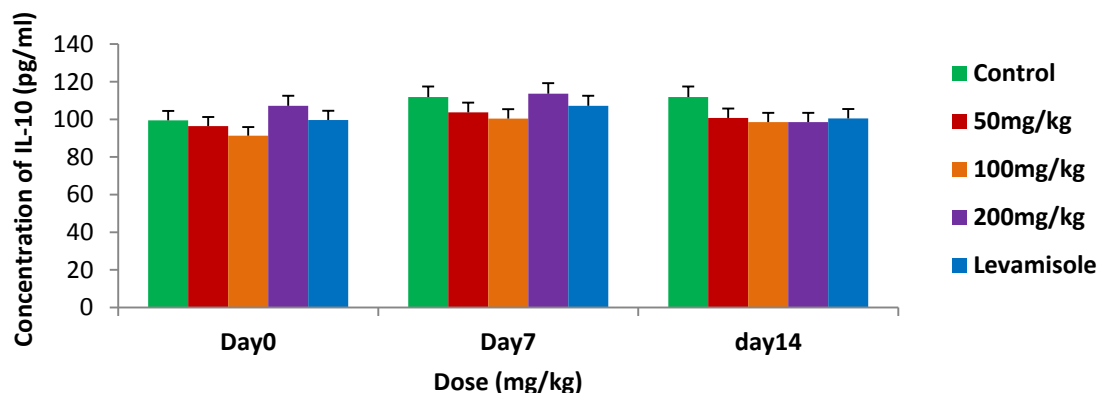


Figure 7. Dose-dependent effects of *M. balsamina* on IL-10 levels. Rats were treated with *M. balsamina* extracts (50, 100 and 200 mg/kg), levamisole (18 mg/kg) or only vehicle. Data are presented as mean \pm S.D.

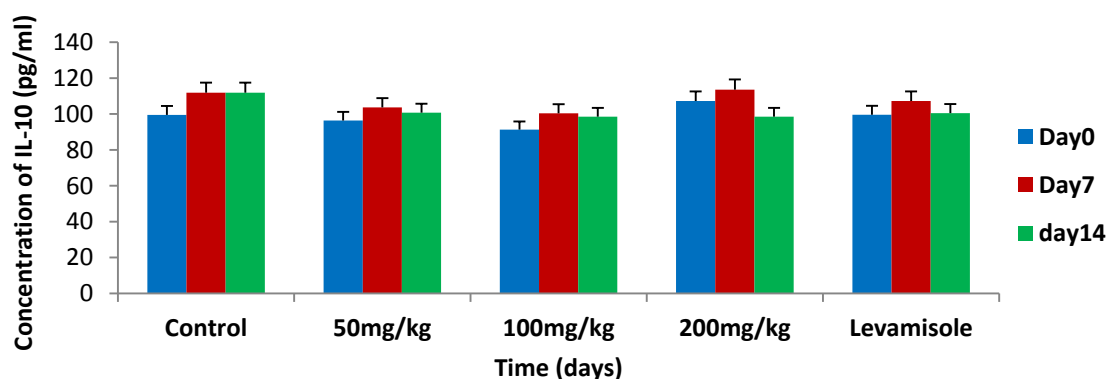


Figure 8. Time-dependent effects of *M. balsamina* on IL-10 levels. Rats were treated with *M. balsamina* extracts (50, 100 and 200 mg/kg), levamisole (18 mg/kg) or only vehicle. Data are presented as mean \pm S.D.

this respect, neither dose-dependent nor time-dependent effects of *M. balsamina* extract on IL-10 levels were observed on the three administered doses (Figures 7 and 8). Therefore, no changes were observed on IL-10 levels after treatment with *M. balsamina* extract.

DISCUSSION

Considerable attention has been paid to plant-derived products such as alkaloids, flavonoids, terpenoids, and polysaccharides due to their diverse pharmacological properties including anti-inflammatory, hepatoprotective, anti-diabetic, antimicrobial, antiviral, cytotoxic and immunomodulatory effects (Abou 2016; Kamarudin et al., 2017). Plant-based immunomodulators may act by stimulating both specific and non-specific immunity, and can provide an effective and safe alternative to conventional chemotherapy for a variety of immune disorders (Jantan et al., 2015).

This study aimed to determine the immunomodulatory

effects of *M. balsamina* that has been used traditionally to treat various infectious diseases (Madureira et al., 2012). The levels of IFN- γ , TNF- α , IL-10 and TGF- β cytokines were quantified using enzyme linked immunosorbant assay (ELISA). Selection of cytokines is based on their key role in innate and adaptive immunity which would be cell-mediated or antibody-mediated immunity. Indeed, the balance between Th1 cytokines (IFN- γ and TNF- α) and Th2 cytokines (IL-10 and TGF- β) is critical for directing the immune response toward cell-mediated or humoral-mediated responses. Thus, any factors interfere with Th1/Th2 axis might affect the outcome of the immune response (Lucey et al., 1996).

In this study, *M. balsamina* showed a significant stimulation of Th1 response indicated by the significant increase of IFN- γ and TNF- α cytokines which are known to be produced by Th1 CD⁴⁺ T cells and lesser quantity is produced by CD⁸⁺ T cells (Abbas et al., 2014). The significant elevation of both IFN- γ and TNF- α cytokines produced by higher doses indicates the immunostimulatory effect of *M. balsamina* which requires

daily administration of the plant extract for 14 days or more. Thus TNF- α and IFN- γ increment at the higher doses reflects activation of Th1 response and cell-mediated immunity that is effective in eliminating virus-infected cells and participates in defense against fungi, protozoan, cancers, and intracellular bacteria (Abbas et al., 2014).

The immunostimulatory effects of other *Momordica* species have been coincidentally described by a number of *in vitro* and *in vivo* studies. Leafy stem juices of *M. charantia* induced significant T cell proliferation and enhanced IFN- γ production (Fachinan et al., 2017). Augmented cell-mediated and antibody-mediated immunity evident by increased Delayed Type Hypersensitivity (DTH) and Haemagglutinating Antibody Titre (HAT), respectively, was observed after oral administration of *Momordica charantia* to immunized rats (Prasad et al., 2010). Moreover, the immune-promoting properties of *M. charantia* were demonstrated by enhancing natural killer cells level, IL23a and IL1 β expression in cancer patients (Bhattacharya et al., 2017; Rao, 2018). Polysaccharide of *M. charantia* significantly increased the carbolic particle clearance index, spleen index, thymus index and NK cell cytotoxicity to normal control levels in immunosuppressed mice (Deng et al., 2014). *Momordica cochinchinensis* proliferated different cells of the immune system including splenocytes, splenic lymphocytes and bone marrow cells, in a manner comparable to that of Concanavalin A (Tsoi et al., 2006). In support of the immune-enhancing properties of *M. cochinchinensis* and *M. momordica* saponins were tested as vaccine's adjuvant and reported to stimulate secretion of broad range of cytokines, suggesting that saponins may act by triggering innate immunity (Song and Hu, 2009). Additionally, saponin derived from *M. cochinchinensis* reduced the production of nitric oxide (NO), and may be considered a bioactive immunomodulator with anti-inflammatory properties (Yu et al., 2017). The immunomodulatory activity of *Momordica* species might be attributed to the presence of similar phytochemicals among these variable species. Therefore, the immunostimulatory effects of *M. balsamina*, due to enhancement of TNF- α and IFN- γ levels, may justify the usefulness of this plant as antiviral, anticancer and against numerous infectious diseases. Low doses (50 and 100 mg/kg) of *M. balsamina* extract increased TGF- β levels while the high dose (200 mg/kg) reduced the level of TGF- β . The obvious inhibitory effect of high dose (200 mg/kg) of *M. balsamina* extract on TGF- β levels might be attributed to the tolerance of T-regulatory cells (Treg cells), the main producers of TGF- β . This possibly is supported by elevated IFN- γ and TNF- α levels at dose 200 mg/kg. Therefore, it is logic to observe such an inhibitory effect on TGF- β levels upon IFN- γ induction (Goldstein et al., 2013). It was previously documented that the elevated TGF- β levels have been correlated with cancer and fibrosis severity; and

strategies to block TGF- β action have been developed to antagonize excessive TGF- β signaling activity in the aforementioned disorders (Walton et al., 2017; Xie et al., 2017). In this study, *M. balsamina* extract at high dose (200 mg/kg) increased TNF- α and IFN- γ levels and suppressed TGF- β levels. On the basis of these observations, *M. balsamina* may be a good candidate for cancer treatment at high doses (\geq 200 mg/kg). Increased TGF- β level observed at low doses (50 and 100 mg/kg) may contribute to anti-inflammatory and antioxidant effects of *Momordica* species (Nagarani et al., 2014).

The immunostimulatory activity of *M. balsamina* extract may be due to its phytoconstituents such as saponins, flavonoids, sterols and triterpenoids which were reported to modulate the immune system (Kumar et al., 2012; Venkatalakshmi et al., 2016; Nagarani et al., 2014).

Conclusion

M. balsamia poses immuno-enhancing properties by stimulating cell-mediated immune responses via elevation of IFN- γ and TNF- α levels. Moreover, low doses of the plant have anti-inflammatory activity through elevating TGF- β levels. Therefore the immunostimulatory and anti-inflammatory effects of *M. balsamina* can justify its antiviral and anticancer activities and activity against numerous infectious and inflammatory diseases. Therefore, *M. balsamia* may play a role in strengthen human immunity and improving health. Different parts of *M. balsamia* are rich sources of triterpenoids, carotenoids, saponins, flavonoids that may potentially be used as immunomodulators and antioxidants in nutraceutical industries.

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CONFLICT OF INTERESTS

No potential conflicts of interest were identified in this study.

ABBREVIATIONS

IL-10, Interleukin-10; **TGF- β** , Transforming growth factor-

beta; **TNF- α** , Tumor necrosis factor-alfa, **IFN- γ** , Interferon-gamma.

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