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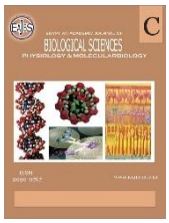

In Vitro Anti-inflammatory Activity of Bee Venom Melittin and Phospholipase A2 on Murine Splenocytes Stimulated with *Schistosoma mansoni* Antigens

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ABSTRACT

Schistosomiasis is a fatal parasitic disease caused by trematode worms of the genus *Schistosoma*. Bee venom (BV) was used as a potential drug for many diseases due to its pharmacologically active molecules. The major biopeptides of *Apis mellifera* V are melittin (MEL) and phospholipase A2 (PLA2) which have a wide range of biological activities. The present *in vitro* study aimed to elucidate the anti-inflammatory effects of MEL and PLA2 on murine splenocytes activated with *Schistosoma mansoni* egg and worm antigens (SEA and SWA). Splenocytes were collected from the spleen of Balb/c mice, and then cultured in the presence of MEL or PLA2. Proliferation was estimated by MTT assay. Levels of tumor necrosis factor-α (TNF-α), Interleukin-10 (IL-10) and nuclear factor-κB (NF-κB) were estimated by enzyme-linked immunosorbent assay (ELISA). MEL and PLA2 induced proliferation of murine primary splenocytes in a concentration and time-dependent pattern, where the proliferation of 24 h incubated splenocytes was significantly ($p < 0.05$) increased at 0.5 µg/ml and 0.05 µg/ml concentrations of MEL and PLA2, respectively compared to non-stimulated cells. The results indicated that MEL and PLA2 modulate an inflammatory reaction by decreasing the levels of both TNF-α and NF-κB and significantly elevating IL-10 levels. These results may suggest their potent anti-inflammatory effects that can be applied in the treatment of many inflammatory diseases including *S. mansoni* infection. Further, *in vivo* studies are required to confirm these results.

INTRODUCTION

Schistosomiasis is ranked as the second-most neglected parasitic disease in terms of the number of deaths it causes (Alemu *et al.,* 2018; Mohamed *et al.,* 2018; Díaz *et al.,* 2023). According to the World Health Organization (WHO, 2022), it was reported that Schistosomiasis causes 11,792 deaths annually. The Middle Eastern and North African regions are endemic areas for schistosomiasis (El-Kassas *et al.,* 2024). Current schistosomiasis treatment is based on the administration of praziquantel (PZQ), which is most effective against all Schistosomial species infecting humans (Niu *et al.,* 2022; Barry *et al.,* 2023). Repeated treatment is sometimes necessary to get rid of all parasites (Hoekstra *et al.,* 2020). Searching for novel, safe and effective anti-schistosomal drugs is necessitated due to resistance problems of *Schistosoma* to PZQ and side effects (Mnkugwe *et al.,* 2020; Akrasi *et al.,* 2022; Alwan *et al.,* 2023).

Venom derived from *Apis mellifera* has various biological effects as anti-inflammatory, anti-bacterial, anti-viral, anti-cancer, and hepato-protective in addition to immune modulation activities (Hossen *et al.*, 2017; Zhang *et al.*, 2018; Kaplan and Orhan, 2022). Bee venom (BV) contains several biologically active peptides, including melittin (MEL) and enzymes such as phospholipase A2 (PLA2) (Jang and Kim, 2020; Yaacoub *et al.*, 2021). MEL is the major component of BV (Pandey *et al.*, 2023), where it possesses anti-inflammatory, anti-protozoan, antibacterial, antiviral, anti-cancer and radio-protective properties as reported in several studies (Lee and Bae, 2016; Komi *et al.*, 2017; Memariani *et al.*, 2019; Memariani and Memariani, 2021). Additionally, it was used as an alternative treatment for drug-resistant infections (Karyne *et al.*, 2020; Askari *et al.*, 2021). MEL inhibited the expression of pro-inflammatory cytokines, such as Tumor necrosis factor-α (TNF-α), Interleukin-1β, IL-6 and Interferon gamma through the blockade of the nuclear factor-κB (NF-κB) signaling pathway (An *et al.*, 2018; Kim *et al.*, 2021; Jung *et al.*, 2022). Moreover, MEL increases the release of anti-inflammatory cytokines such as IL-10 and IL-12 (Kim *et al.*, 2020).

PLA2 is a major enzymatic protein derived from BV, where it is the second-most prominent biomolecule of the BV (Wehbe *et al.*, 2019; Yaacoub *et al.*, 2023). Several studies have confirmed the anti-parasitic effects of PLA2 against many protozoans (Soltan-Alinejad *et al.*, 2022). The therapeutic efficacy of MEL and PLA2 have been reported for various diseases including some protozoal infections but their anti-schistosomal effects haven`t been studied (Rehman *et al.*, 2017). Therefore, the search for potential anti-schistosomal drugs based on natural sources is necessitated (Toth *et al.*, 2023). The present study aims to investigate the *in vitro* anti-inflammatory effects of MEL and PLA2 on murine primary splenocytes and identify their related immune modulation pathways.

MATERIALS AND METHODS

1. Preparation of Murine Splenocytes Single Cell Suspension:

Male BALB/c mice (20-25g) aged 6 weeks, was obtained from Theodor Bilharz Research Institute (TBRI, Giza, Egypt) and maintained in the Zoology and Entomology department, Faculty of Science, Helwan University, Egypt. Mice were maintained in a 12/12 h light/dark cycles. Ethical approval was granted for this study from the Animal Ethics Committee of the Zoology and Entomology Department, Faculty of Science, Helwan University (no. HU-IACUC/Z/OR1006-47). After the mice were acclimatized for 1 week, they were sacrificed then splenocytes were isolated from mice under aseptic conditions according to the procedure described by Vardeu *et al.*, (2022); and Amon *et al.*, (2023).

Splenocyte suspensions were prepared from aseptically isolated spleens by gently pressing in RPMI-1640 medium (Lonza BioWhittaker., Verviers, Belgium) supplemented with 10% heat-inactivated fetal bovine serum (FBS, HyClone, UK), 1% penicillin-streptomycin and 1% L-glutamine. Cell suspensions were collected and spun at 500 ×*g* for 10 min then erythrocytes were lysed by incubation with Ammonium–Chloride–Potassium (ACK) lysis buffer (Sigma, St. Louis, MO) (150 mM NH_4Cl, 10 mM $KHCO_3$ and 0.1 mM Na_2EDTA). Splenocytes were washed and re-suspended in RPMI 1640-FBS medium. The viability was determined by the trypan blue exclusion test (Biochrom AG, Leonorenstr. Berlin) according to Lebeau *et al.* (2019). Cell viability was greater than 95%. Splenocyte suspensions were prepared as 2×10^6 cells/ml.

2. MTT Assay:

The cell viability assay was performed as described previously (Mosmann, 1983). Murine primary splenocytes were isolated from Balb/c mice spleens and seeded in 96-well plates in complete RBMI. After stimulation with different concentrations of MEL or PLA2 (0-500 μg/ml, SIGMA-ALDRICH Co., St.

Louis, MO, USA.) for 24, 72 and 120 h. Lipopolysaccharides (LPS, 5 μg/ml) from *E. coli* (L- 2654, Sigma-Aldrich Co.) were used as a control. 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl-tetrazolium bromide assay (MTT, SERVA Electrophoresis GmbH, Heidelberg, Germany) was performed. The amount of viable cells was assessed based on the formation of formazan dye by mitochondrial dehydrogenases. Formazan product was estimated using a microplate reader at 570 nm absorbance (BioTek, USA).

3. Optimization of Soluble Egg Antigens (SEA) and Soluble Worm Antigens (SWA) of *Schistosoma mansoni*:

SEA and SWA were obtained from Theodor Bilharz Research Institute (TBRI, Giza, Egypt) as a lyophilized, endotoxin-free product. Isolated splenocytes were cultured with a series of SEA and SWA (0-100 μg/ml) for 24 h at 37 °C in a 5% CO_2 incubator. Each experiment runs in triplicates. The supernatant and pellets were collected and kept at -80 °C for measuring tumor necrosis factor-alpha (TNF-α), interleukin 10 (IL-10) and nuclear factor-κB (NF-κB) levels. Optimum concentrations for SEA and SWA were found 6.25 μg/ml and 50 μg/ml, respectively which are suitable for subsequent treatment experiments.

4. *In vitro* Treatment with MEL or PLA2:

In a 96-well microculture plate, 100 μl/ well of splenocyte suspension was added. Splenocytes activated with 6.25 μg/ml SEA and 50 μg/ml SWA were treated with 0.5 μg/ml MEL and 0.05 μg/ml PLA2. Experiments were carried out in triplicates. Cultured splenocytes were incubated at 37 °C in 5% CO_2 for 24 h. Both culture supernatants and pellets were collected and stored at -80 °C to determine TNF-α, IL-10 and NF-κB levels.

5. Measurement of Cytokines and NF-κB by ELISA:

Cytokines and NF-κB levels were measured using the Sandwich ELISA technique according to the instructions of the manufacturer (Murine ELISA kit, Sunlong Biotech, China). TNF-α and IL-10 (Cat. No. SL0547Mo and SL0310Mo, respectively) were measured in the supernatant of cultured splenocytes while NF-κB was detected in splenocytes homogenate (Cat. No. SL0723Mo).

6. Statistical Analysis:

Analyses were performed using Graphpad Prism software version 8.4.3. Data represented as mean ± SD or percentage change. Two-way analysis of variance (ANOVA) was used to compare splenocyte proliferation at different concentrations and incubation times, confirmed by Tukey's multiple comparisons test. One-way ANOVA was used to compare between different treatments. Statistical change is significant at $p < 0.05$. Percentage change representing the percent of variation with respect to control and calculated as:

$$\% \text{ Change} = \frac{\text{Mean of the experiment} - \text{Mean of control}}{\text{Mean of control}} \times 100$$

RESULTS

1. Proliferative Effect of MEL and PLA2 on Murine Primary Splenocytes:

The proliferative effect of both MEL and PLA2 at different doses and incubation times on healthy splenocytes was investigated using an MTT assay. MEL and PLA2 tend to inhibit the proliferation of splenocytes in a dose and time-dependent pattern where splenocyte proliferation decreased significantly ($p < 0.05$) with increasing concentrations of MEL and PLA2 in the range of (0.05 μg/ml – 500 μg/ml) and incubation time from 24 h to 120 h compared to non-treated splenocytes. MEL and PLA2 treatment at 0.5 μg/ml and 0.05 μg/ml, respectively act as proper concentrations at which proliferation of splenocytes increased significantly ($p < 0.05$) at 24 h as shown in Figures 1 and 2. Also, these concentrations were suitable for subsequent splenocyte experiments and confirmed that MEL has lower cytotoxic and higher cell proliferative effects than PLA2.

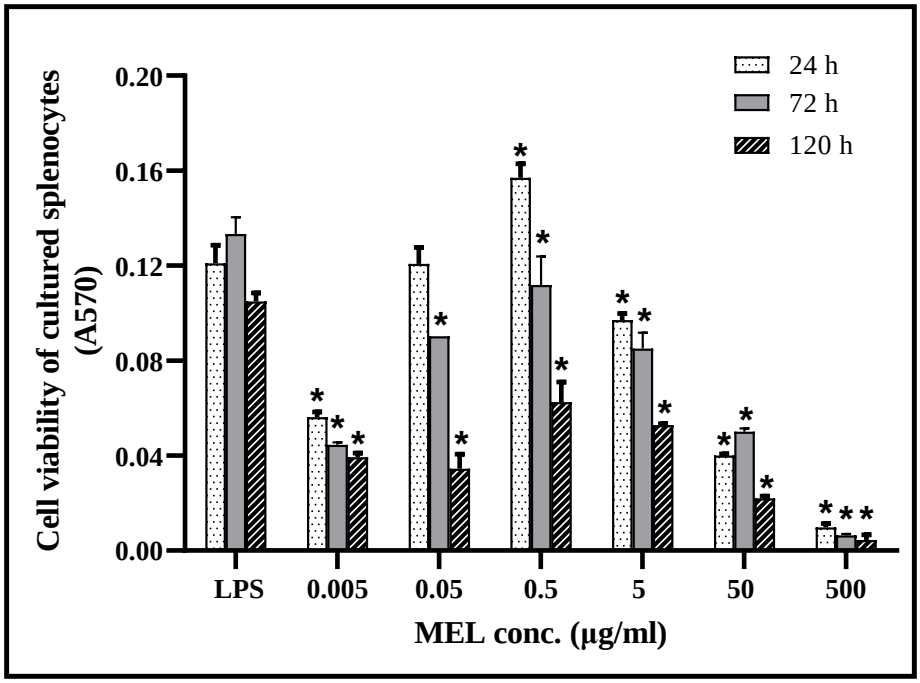

Fig. 1. Effect of MEL on the viability of splenocytes.
Data displayed in the form of mean ± SD, Number = 3, Two-way ANOVA was used for comparing cell proliferation at different concentrations and incubation times, confirmed by Tukey's multiple comparisons test. *Significant at $p < 0.05$ with respect to control. The original cell density was 2×10^6 cells/ml. LPS: Lipopolysaccharides. MEL: Melittin.

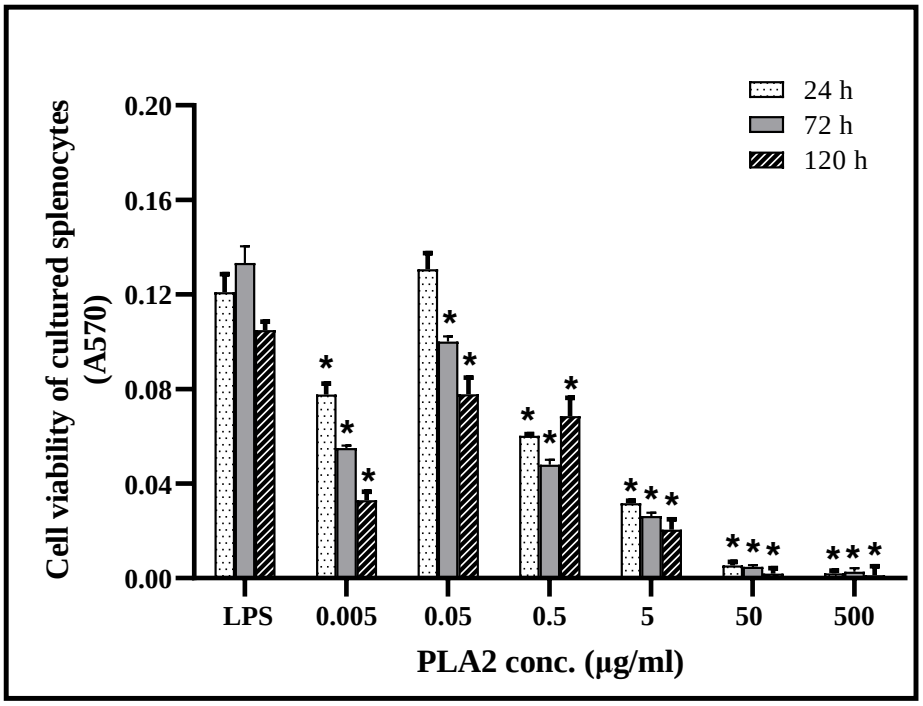

Fig. 2. Effect of PLA2 on the viability of splenocytes.
Data shown mean ± SD, Number = 3, Two-way ANOVA compared cell proliferation at different concentrations and incubation times, confirmed by Tukey's multiple comparisons test. *Significant at $p < 0.05$ with respect to control. The original cell density was 2×10^6 cells/ml. LPS: Lipopolysaccharides. PLA2: Phospholipase A2.

2. Effect of SEA on Expressions of Cytokines and NF-κB:

The levels of TNF-α, IL-10 and NF-κB produced by SEA-stimulated splenocytes (0 - 100 µg/ml) are shown in Figure 3. We found that SEA from 6.25 to 50 µg/ml was capable of significantly ($p < 0.05$) increasing TNF-α, NF-κB and IL-10 levels in stimulated splenocytes compared to non-stimulated ones. SEA at 6.25 µg/ml was recommended to induce inflammation in splenocytes as evidenced by the maximum levels of pro-inflammatory TNF-α and NF-κB along with the minimum expression level of anti-inflammatory IL-10 at this concentration.

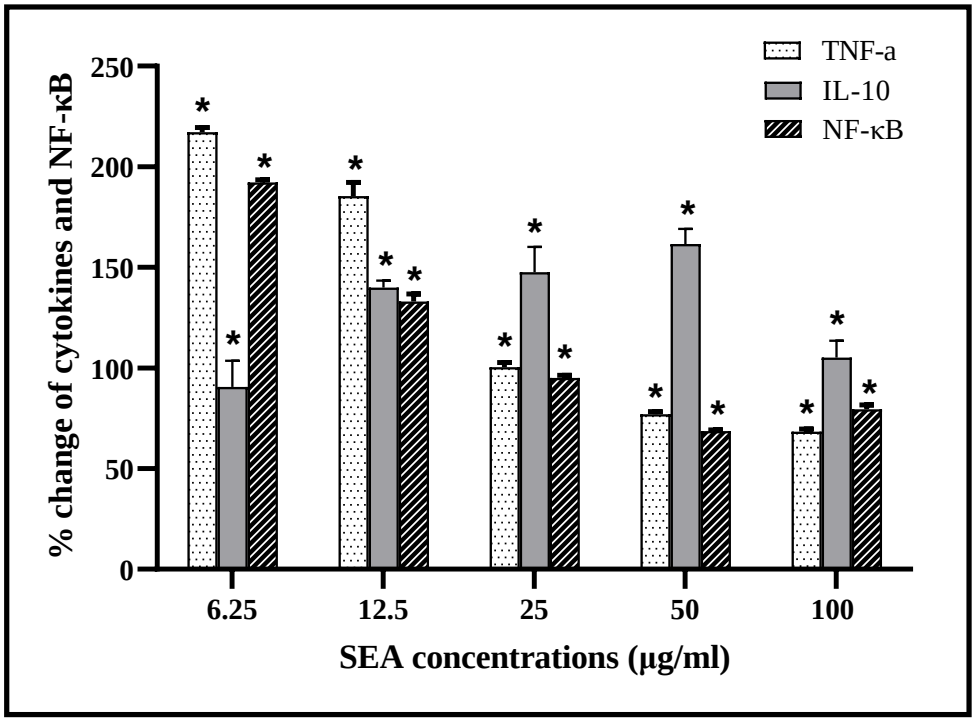

Fig. 3. *In vitro* effect of concentrations of SEA on cytokines and NF-κB levels of activated splenocytes.

Data represented by percent change corresponding to their value of non-stimulated splenocytes. Number = 3, One-way ANOVA was performed to compare between different groups. *Significant at $p < 0.05$ with respect to control. SEA: *Schistosoma mansoni* soluble egg antigen.

3. Effect of SWA on Expressions of Cytokines and NF-κB:

Primary splenocytes were stimulated with different concentrations of SWA at 24 h to detect their inflammatory effect and select the suitable concentration for subsequent splenocyte treatment experiments. Splenocytes stimulated with a concentration range of SWA (6.25 µg/ml - 100 µg/ml) displayed a significant ($p < 0.05$) increase in TNF-α and NF-κB levels compared to control as shown in Figure 4. While IL-10 levels were significantly ($p < 0.05$) increased at 50 and 100 µg/ml of SWA. The maximum levels of TNF-α and NF-κB were estimated at 50 µg/ml of SWA which was selected as the proper dose for inducing inflammatory reaction and following treatment experiments.

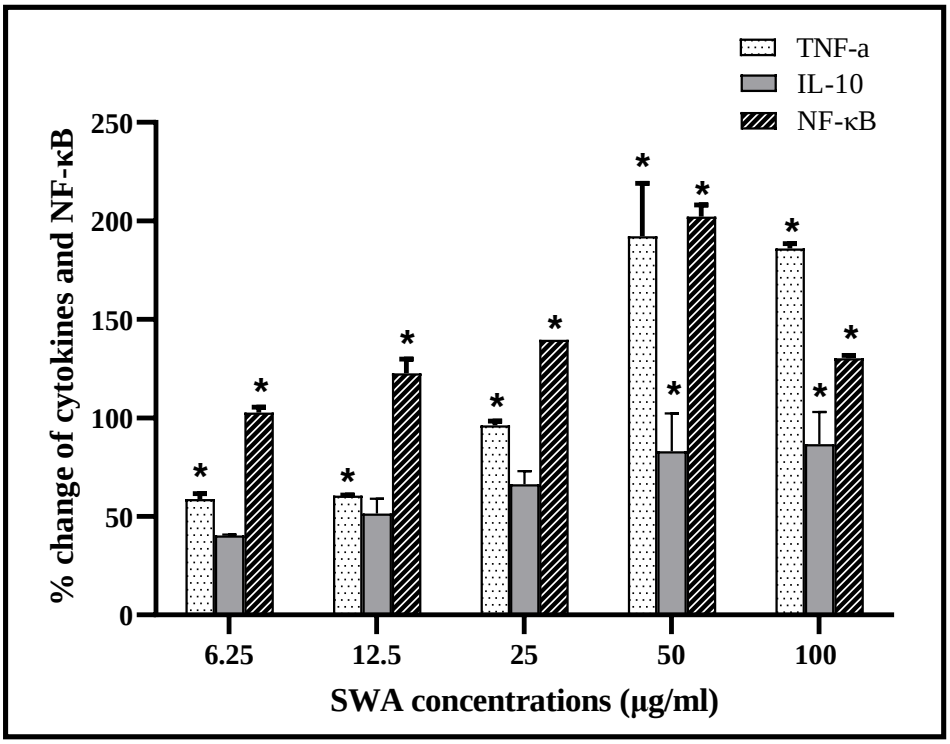

Fig. 4. *In vitro* effect of concentrations of SWA on cytokines and NF-κB levels of activated splenocytes *in vitro*.

Data represented by percent change corresponding to their value of non-stimulated splenocytes. Number = 3, One-way ANOVA was performed to compare between different groups. *Significant at $p < 0.05$ with respect to control. SWA: *Schistosoma mansoni* soluble worm antigen.

4. Effects of MEL and PLA2 on the Production of Cytokines and NF-κB by SEA-Activated Splenocytes.

MEL and PLA2 at the pre-determined non-cytotoxic doses were added to SEA and SWA-activated splenocytes for 24 h to investigate their effect on the expression of inflammatory parameters (Fig. 5). Compared to non-treated splenocytes (-SEA), a significant ($p < 0.05$) increase in TNF-α, IL-10 and NF-κB levels was recorded in +SEA, SEA+MEL and SEA+ PLA2 treated splenocytes. MEL and PLA2 treatment resulted in a significant ($p < 0.05$) decrease in TNF-α and NF-κB compared to infected splenocytes. However, IL-10 levels were significantly ($p < 0.05$) increased in SEA+MEL and non- significantly increased in SEA+PLA2.

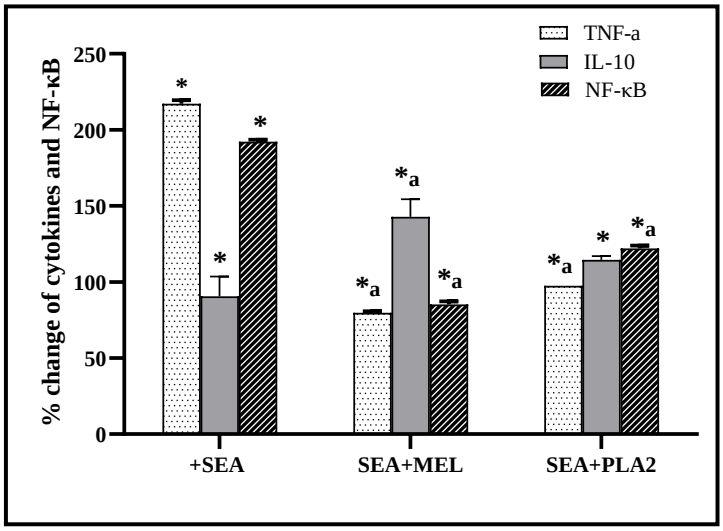

Fig. 5. *In vitro* effects of MEL and PLA2 on the levels of cytokines and NF-κB of SEA-activated splenocytes *in vitro*.

Data represented by percent change corresponding to their value of non-stimulated splenocytes. Number = 3, One-way ANOVA was performed to compare between different groups. *Significant at $p < 0.05$ with respect to control. a Significant change at $p < 0.05$ with respect to SEA-activated splenocytes (Positive control). +SEA: splenocytes activated with *Schistosoma mansoni* soluble egg antigen. MEL: Melittin. PLA2: phospholipase A2.

5. Effects of MEL and PLA2 on the Production of Cytokines and NF-κB by SWA-Activated Splenocytes.

The detected levels of TNF-α, IL-10 and NF-κB showed a significant ($p < 0.05$) increase in all MEL and PLA2-treated splenocytes compared to non-infected ones. A significant ($p < 0.05$) decrease in TNF-α and NF-κB levels was observed after MEL and PLA2 treatment compared to infected cells. However, MEL and PLA2 treatment was not capable of increasing IL-10 levels significantly compared to non-treated splenocytes (Fig. 6).

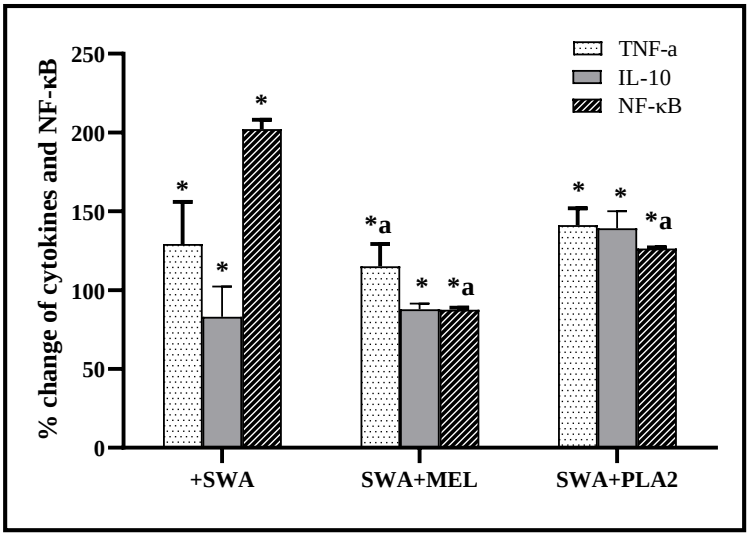

Fig. 6. *In vitro* effects of MEL and PLA2 on the levels of cytokines and NF-κB of SWA-activated splenocytes *in vitro*.

Data represented by percent change corresponding to their value of non-stimulated splenocytes. Number = 3, One-way ANOVA was performed to compare between different groups. *Significant at $p < 0.05$ with respect to control. a Significant change at $p < 0.05$ with respect to SWA-activated splenocytes (Positive control). +SWA: splenocytes activated with *Schistosoma mansoni* soluble worm antigen. MEL: Melittin. PLA2: phospholipase A2.

DISCUSSION

In traditional medicine, BV has long been used against various diseases including skin diseases, rheumatoid arthritis, Alzheimer's disease, inflammation, and cancer (Zhang *et al.,* 2018; Badawi *et al.,* 2020). The effect of BV on *S. mansoni*-infected mice showed a reduction in the worm load and the number of eggs in the liver tissues (Mohamed *et al.,* 2016). BV consists of many different components which are mixtures of peptides, lipids, carbohydrates, amino acids and enzymes like MEL and PLA2, which are the most common derivatives of BV. They exhibited anti-parasitic, antimicrobial, anti-viral, anti-inflammatory, antifungal, anti-protozoan and antischistosomal properties (Mohamed *et al.,* 2016; Kurek-Górecka *et al.,* 2020; Saleh *et al.,* 2020; Memariani and Memariani, 2021; Akhzari *et al.,* 2022).

In vitro cytotoxicity assessment is essential to evaluate natural products' safety profile before being applied in the biomedical field. Thus, the present study aimed to investigate the proliferative effects of several concentrations of MEL and PLA2 and their related inflammatory effects on murine primary splenocytes. Remarkably, MEL treatment significantly increased splenocyte proliferation and viability at 24 h and a concentration of 0.5 µg/ml. Several studies ensured that MEL effects on the viability of many protozoans and different cancer cell lines are dose and time-dependent (Abhari *et al.,* 2019; Soliman *et al.,* 2019; Moghaddam *et al.,* 2021; El-Dirany *et al.,* 2021; Akhzari *et al.,* 2023).

Yu *et al.* (2020) reported that the major *in vitro* stimulated cell types of murine splenocytes are B cells and T cells in addition to other cell types. Thus, MEL may be responsible for enhancing the proliferation of B and T cells of splenocytes. The decreased proliferation of stimulated splenocytes observed at 0.005, 50 and 500 µg/ml concentrations of MEL might be due to cellular apoptosis and autophagy as was recorded by Moghaddam *et al.* (2021) who reported that cytotoxic doses of MEL-induced cell lysis by disruption of phospholipid bilayer integrity via forming pores in the membrane surface leading to leakage of ions and increased permeability. In addition, MEL induced apoptosis in ovarian cancer cells by targeting mitochondrial-related pathways by stimulating death receptors and inhibiting the JAK2L/STAT3 pathway (Tu *et al.,* 2008; Kim *et al.,* 2011; Jo *et al.,* 2012). Similarly, El-Dirany *et al.* (2021) also reported that MEL affected the viability of different developmental forms of *Trypanosoma cruzi* by inducing morphological changes in nuclei, mitochondria, and membrane extensions of the parasite.

The increased splenocyte proliferation after PLA2 treatment may be due to its capability of stimulating B cells and/or T cell proliferation (Yu *et al.,* 2020). Conversely, the decreased splenocyte proliferation by a high cytotoxic dose of PLA2 can be explained by its direct lipolytic action on the membrane phospholipids producing lyso-phospholipids which are responsible for cellular death (Yaacoub *et al.,* 2021). Consequently, the alteration in membrane organization impairs the proper function and expression of macromolecules and membrane receptors disrupting cell survival and calcium-mediated signal transduction pathways (Boutrin *et al.,* 2008; Hossen *et al.,* 2017).

Soltan-Alinejad *et al.* (2022) reported that PLA2 has anti-parasitic activity against *Trypanosoma brucei* and inhibits intra- erythrocytic development of *Plasmodium* in concentration gradient. It was also indicated that PLA2 isolated from BV and *Daboia russelii* venom (Eastern India Russell's viper) prevented the proliferation of tumor cells in dose and time-dependent patterns (Khunsap *et al.,* 2011; Hossen *et al.,* 2017). Conversely, PLA2 extracted from BV showed no significant cytotoxic effects on the HCT116 cell line as demonstrated by Yaacoub *et al.* (2021).

In the present study, the *in vitro* inflammatory effects of MEL and PLA2 on

splenocytes were investigated. The current expression of pro-inflammatory TNF-α was increased due to an inflammatory reaction stimulated with *Schistosoma* SEA or SWA. Splenocyte activation by *Schistosoma* antigens observed in the present study may be due to up-regulation of CD80, CD86 markers, and MHC-II that increase the production of TNF-α and IL-10 by dendritic cells activated with *S. mansoni* warm glycolipids as was previously explained by Van Stijn *et al.* (2010). A correlation was reported between elevated TNF-α levels and increased risk of *S. mansoni* pathological changes including periportal fibrosis, granuloma formation and hepatocyte apoptosis (Lee *et al.*, 2014; Kamdem *et al.*, 2018; Mutengo *et al.*, 2018). A current significant reduction in the level of TNF-α observed in both MEL and PLA2-treated splenocytes was in agreement with previous *in vitro* studies by Pereira *et al.* (2016) and Mirzaei *et al.* (2020) who reported that MEL-treated macrophages showed effective anti-leishmanial activity that contributed to the decreased levels of TNF-α. The current significant downregulation in NF-κB expression and TNF-α detected in MEL-treated splenocytes was also reported by Park *et al.* (2007) who attributed the inhibited expression of TNF-α and NF-κB to the anti-inflammatory effects of MEL. In the same context, Wullaert *et al.* (2006) and Lee *et al.* (2014) indicated that MEL inhibits NF-κB nuclear translocation and signaling that in turn reduces TNF-α level.

The current study revealed that PLA2 downregulated the expression of pro-inflammatory TNF-α, NF-κB and upraised IL-10 levels and this can be explained by splenocyte cellular changes induced by PLA2. Baek *et al.* (2020) reported that PLA2 binds to mannose receptor (CD206) on dendritic cells and upregulates COX-2 expression and prostaglandin E2 (PGE2) secretion by these cells. PGE2 then binds to EP2 inducing regulatory T-cell differentiation which contributes to immune suppression in various inflammatory diseases by several mechanisms including IL-10 production. In contrast to the present results, previous studies indicated that MEL-treated macrophages showed a notable drop in IL-10 levels that may reduce the disease progression through macrophage deactivation and parasite persistence (Pereira *et al.*, 2016; Mirzaei *et al.*, 2020). Although MEL and PLA2 reduced *schistosoma* antigens-induced inflammation, additional *in vitro* studies are needed to support the therapeutic advantage of MEL and PLA2 versus traditional treatment with PZQ. Moreover, it is necessary to focus on the *in vitro* molecular mechanistic action of MEL and PLA2 linking to proliferation and apoptosis signaling pathways.

CONCLUSIONS

The current findings concluded that MEL and PLA2 isolated from *A. mellifera* venom might have *in vitro* cell- proliferative and anti-inflammatory effects where MEL was found to be more effective in reducing inflammation induced by *Schistosoma* antigens than PLA2. MEL and PLA2 may represent potential therapeutic agents that can be used in medical applications for the treatment of inflammatory diseases including Schistosomiasis.

Declarations:

Ethical Approval: The study was approved by The Animal Ethics Committee of the Zoology and Entomology Department, Faculty of Science, Helwan University (no. HU-IACUC/Z/OR1006-47).

Conflict of interests: The authors declare that the research proceeded in the absence of any financial or commercial relationships and they are responsible for the content of the paper.

Authors Contributions: All authors contributed equally, and have read and agreed to the published version of the manuscript.

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Availability of Data and Materials: The data underpinning the findings of this study are accessible upon request from the corresponding author.

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REFERENCES

Abhari, F.M., Pirestani, M., Dalimi, A. (2019) Anti-amoebic activity of a cecropin-

melittin hybrid peptide (CM11) against trophozoites of *Entamoeba histolytica*. *Wien Klin Wochenschr,* 131(17-18), 427-434. doi: 10.1007/s00508-019-01540-9.

Akhzari, S., Nabian, S., Taheri, M., Salimabadi, Y. (2023) Therapeutic evaluation of activatable melittin for selective treatment of leishmaniasis *in vitro* and *in vivo*. *Bulgarian Journal of Veterinary Medicine*, 27(1), 57-67. doi:10.15547/bjvm.2022-0019.

Akhzari, S., Nabian, S., Shayan, P., Taheri, M. (2022) Evaluation of the effect of liposome carriers and albumin nanoparticles containing activated melittin on inhibiting the growth of *Leishmania* major amastigote *in vivo. Journal of Ilam University of Medical Sciences*, 29(6), 36-47. doi:10.52547/sjimu.29.6.36.

Akrasi, W., Brah, A.S., Essuman, M.A., Osei, V., Boye, A. (2022) Adverse drug effects among students following mass de-worming exercise involving administration of Praziquantel and Albendazole in KEEA Municipality, Ghana. *PLoS Neglected Tropical Dis*eases, 16(9), e0010680. doi: 10.1371/journal.pntd.0010680.

Alemu, M., Zigta, E., Derbie, A. (2018) Under diagnosis of intestinal schistosomiasis in a referral hospital, North Ethiopia. *BMC Research Notes*, 11(1), 245-249. doi: 10.1186/s13104-018-3355-0.

Alwan S.N., Taylor, A.B., Rhodes J., Tidwell, M., McHardy, S.F., LoVerde, P.T. (2023) Oxamniquine derivatives overcome Praziquantel treatment limitations for schistosomiasis. *PLOS Pathogens,* 19(7), e1011018. doi: 10.1371/journal.ppat. 1011018.

Amon, L., Dudziak, D., Backer, R.A., Clausen, B.E., Gmeiner, C., Heger, L., Jacobi, L., Lehmann, C.H.K., Probst, H.C., Seichter, A., Tchitashvili, G., Tochoedo, N.R., Trapaidze, L., Vurnek, D. (2023) Guidelines for DC preparation and flowcytometry analysis of mouse lymphohematopoietic tissues. *European Journal of Immunology,* 53(12), e2249893. doi: 10.1002/eji.202249893.

An, H.J., Kim, J.Y., Kim, W.H., Gwon, M.G., Gu, H.M., Jeon, M.J., Han, S.M., Pak, S.C., Lee, C.K., Park, I.S., Park, K.K. (2018) Therapeutic effects of bee venom and its major component, melittin, on atopic dermatitis *in vivo* and *in vitro*. *British Journal of Pharmacology,* 175(23), 4310-4324. doi: 10.1111/bph.14487.

Askari, P., Namaei, M.H., Ghazvini, K., Hosseini, M. (2021) *In vitro* and *in vivo* toxicity and antibacterial efficacy of melittin against clinical extensively drug-resistant bacteria. *BMC Pharmacology and Toxicology,* 22(1), 42. doi: 10.1186/s40360-021-00503-z.

Badawi, H.M., Abdelsalam, R.M., Abdel-Salam, O.M., Youness, E.R., Shaffie, N.M., Eldenshary, E.D.S. (2020) Bee venom attenuates neurodegeneration and motor impairment and modulates the response to L-dopa or rasagiline in a mice model of Parkinson's disease. *Iranian Journal of Basic Medical Sciences,* 23(12), 1628-1638. doi: 10.22038/ijbms.2020.46469.10731.

Baek, H., Park, S.Y., Ku, S.J., Ryu, K., Kim, Y., Bae, H., Lee, Y.S. (2020) Bee Venom Phospholipase A2 Induces Regulatory T Cell Populations by Suppressing Apoptotic Signaling Pathway. *Toxins (Basel)*, 12(3), 198. doi: 10.3390/toxins12030198.

Barry, A., Kabatende, J., Telele, N.F., Mnkugwe, R.H., Mugisha, M., Ntirenganya, L., Bienvenu, E., Aklillu, E. (2023) Effect of pharmacogenetic variations on praziquantel plasma concentration and safety outcomes among school children in Rwanda. *Scientific Reports,* 13, 1446. https://doi.org/

10.1038/s41598-023-28641-w.

Boutrin, M.C., Foster, H.A., Pentreath, V.W. (2008) The effects of bee (*Apis mellifera*) venom phospholipase a2 on *trypanosoma brucei brucei* and enterobacteria.*Experimental Parasit ology*, 119(2), 246-251. doi: 10. 1016/j.exppara.2008.02.002.

Díaz, A.V., Walker, M., Webster, J.P. (2023) Reaching the World Health Organization elimination targets for schistosomiasis: the importance of a One Health perspective. *Philosophical Transactions of the Royal Society B: Biological Sciences,* 378(1887), 20220274. doi: 10.1098/rstb.2022.0274.

El-Dirany, R., Shahrour, H., Dirany, Z., Abdel-Sater, F., Gonzalez-Gaitano, G., Brandenburg, K., Martínez de Tejada, G., Nguewa, P.A. (2021) Activity of Anti-Microbial Peptides (AMPs) against *Leishmania* and Other Parasites: An Overview. *Biomolecules*, *11*(7), 984. doi: 10. 3390/biom11070984.

El-Kassas, M., Sheemy, R.E., Elbadry, M. (2024) Strategies and achievements in controlling and eliminating schistosomiasis from Egypt. *Egyptian Liver Journal*, 14(1),31. https://doi.org/10.1186/s43066-024-00339-2.

Hoekstra, P.T., Casacuberta-Partal, M., van Lieshout, L., Corstjens, P., Tsonaka, R., Assaré, R.K., Silué, K.D., Meité, A., N'Goran, E.K., N'Gbesso, Y.K., Amoah, A.S., Roestenberg, M., Knopp, S., Utzinger, J., Coulibaly, J.T., van Dam, G.J. (2020) Efficacy of single versus four repeated doses of praziquantel against *Schistosoma mansoni* infection in school-aged children from Côte d'Ivoire based on Kato-Katz and POC-CCA: an open-label, randomised controlled trial (RePST). *PLOS Neglected Tropical Diseases*, 14(3):e0008189. doi: 10. 1371/journal.pntd.0008189.

Hossen, M.S., Shapla, U.M., Gan, S.H., Khalil, M.I. (2017) Impact of Bee Venom Enzymes on Diseases and Immune Responses. *Molecules*, 22(1), 25. doi: 10.3390/molecules22010025.

Jang, S., Kim, K.H. (2020) Clinical Effectiveness and Adverse Events of Bee Venom Therapy: A Systematic Review of Randomized Controlled Trials. *Toxins (Basel),* 12(9), 558. doi: 10.3390/toxins12090558.

Jo, M., Park, M.H., Kollipara, P.S., An, B.J., Song, H.S., Han, S.B., Kim, J.H., Song, M.J., Hong, J.T. (2012) Anti-cancer effect of bee venom toxin and melittin in ovarian cancer cells through induction of death receptors and inhibition of JAK2/STAT3 pathway. *Toxicology and Applied Pharmacology*, 258(1), 72-81. doi: 10.1016/j.taap.2011.10.009.

Jung, H., Kim, Y.S., Jung, D.M, Lee K.S., Lee, J.M., Kim, K.K. (2022) Melittin-derived peptides exhibit variations in cytotoxicity and antioxidant, anti-inflammatory and allergenic activities. *Animal Cells and Systems,* 26(4), 158-165. doi: 10.1080/ 19768354.2022.2099971.

Kamdem, S.D., Moyou-Somo, R., Brombacher, F., Nono, J.K. (2018) Host Regulators of Liver Fibrosis During Human Schistosomiasis. *Frontiers in Immunology,* 9, 2781. doi: 10.3389/fimmu.2018.02781.

Kaplan, N.A., Orhan, I.E. (2022) Bee Venom and its Biological Effects. *Current Perspectives on Medicinal and Aromatic Plants*, 5(1), 86-105. doi: 10.38093/cupmap.1127949.

Karyne, R., Lechuga, G.C., Souza, A.L.A., Carvalho, J.P.R.S., Villas-Bôas, M.H.S., De Simone, S.G. (2020) Pan-Drug Resistant Acinetobacter baumannii, but Not Other Strains, Are Resistant to the Bee Venom Peptide Mellitin. *Antibiotics (Basel)*, 9(4),178. doi: 10.3390/antibiotics 9040178.

Khunsap, S., Pakmanee, N., Khow, o., Chanhome, L., Sitprija, V., Suntravat,

M., Lucena, S.E., Perez, J.C., Sánchez, E.E. (2011) Purification of a phospholipase A2 from *Daboia russelii siamensis* venom with anticancer effects. *Journal of Venom Research,* 2, 42-51.

Kim, S.K., Park, K.Y., Yoon, W.C., Park, S.H., Park, K.K., Yoo, D.H., Choe, J.Y. (2011) Melittin enhances apoptosis through suppression of IL-6/sIL-6R complex-induced NF-κB and STAT3 activation and Bcl-2 expression for human fibroblast-like synoviocytes in rheumatoid arthritis. *Joint Bone Spine*, 78:471-477. 78(5) 471-477. doi: 10.1016/j.jbspin.2011.01.004.

Kim, J.Y., Leem, J., Hong, H.L. (2021) Melittin Ameliorates Endotoxin-Induced Acute Kidney Injury by Inhibiting Inflammation, Oxidative Stress, and Cell Death in Mice. *Oxidative Medicine and Cellular Longevity*, 2021, 8843051. doi: 10.1155/2021/8843051.

Kim, H., Hong, J.Y., Jeon, W.J., Baek, S.H., Ha, I.H. (2020) Bee Venom Melittin Protects against Cisplatin-Induced Acute Kidney Injury in Mice via the Regulation of M2 Macrophage Activation. *Toxins (Basel)*, 12(9), 574. doi: 10.3390/toxins12090574.

Komi, D.E.A., Shafaghat, F., Zwiener, R.D. (2017) Immunology of bee venom. *Clinical Reviews in Allergy & Immunology*, 54(3), 386-396. doi: 10.1007/s12016-017-8597-4.

Kurek-Górecka, A., Górecki, M., Rzepecka-Stojko, A., Balwierz, R., Stojko, J. (2020) Bee products in dermatology and skin care, *Molecules*, 25(3), 556. doi: 10.3390/molecules25030556.

Lebeau, P.F., Chen, J., Byun, J.H., Platko, K., Austin R.C. (2019) The trypan blue cellular debris assay: a novel low-cost method for the rapid quantification of cell death. *MethodsX*, 6, 1174-1180. doi: 10.1016/j.mex.2019.05.010.

Lee, W.R., Kim, K.H., An, H.J., Kim, J.Y., Chang, Y.C., Chung, H., Park, Y.Y., Lee, M.L., Park, K.K. (2014) The Protective Effects of Melittin on Propionibacterium acnes–Induced Inflammatory Responses *In Vitro* and *In Vivo*. *Journal of Investigative Dermatology*, 134(7), 1922-1930. doi: 10.1038/jid.2014.75.

Lee, G., Bae, H. (2016) Bee venom phospholipase A2: yesterday's enemy becomes today's friend. *Toxins (Basel),* 8(2), 48. doi: 10.3390/toxins8020048.

Memariani, H., Memariani, M. (2021) Melittin as a promising anti-protozoan peptide: current knowledge and future prospects. *AMB Express*, 11(1), 69. doi: 10.1186/s13568-021-01229-1.

Memariani, H., Memariani, M., Shahidi-Dadras, M., Nasiri, S., Akhavan, M.M., Moravvej, H. (2019) Melittin: from honeybees to superbugs. *Applied microbiology and biotechnology,* 103(8), 3265-3276. doi: 10.1007/s00253-019-09698-y.

Mirzaei, A., Maleki, M., Masoumi, E., Maspi, N. (2020) A historical review of the role of cytokines involved in leishmaniasis. *Cytokine*, 145, 155297. doi: 10.1016/j.cyto.2020.155297.

Mnkugwe, R.H., Minzi, O.S., Kinung'hi, S.M., Kamuhabwa, A.A., Aklillu, E. (2020) Efficacy and safety of praziquantel for treatment of *Schistosoma Mansoni* infection among school children in Tanzania. *Pathogens,* 9(1), 28. doi: 10.3390/pathogens9010028.

Moghaddam, F.D., Akbarzadeh, I., Marzbankia, E., Farid, M., khaledi, L., Reihani, A.H., Javidfar, M., Mortazavi, P. (2021) Delivery of melittin-loaded niosomes for breast cancer treatment: an *in vitro* and *in vivo* evaluation of anti-cancer effect. C*ancer Nanotechnology,* 12(1), 14. https://doi.org/10.1186/s12645-021-00085-9.

Mohamed, A.H., Hassab El-Nabi, S.E., Bayomi, A.E., Abdelaal, A.A. (2016)

Effect of bee venom or proplis on molecular and parasitological aspects of *Schistosoma mansoni* infected mice. *Journal of parasitic diseases,* 40(2), 390-400. doi: 10.1007/s12639-014-0516-5.

Mohamed, I., Kinung'hi, S., Mwinzi, P.N.M., Onkanga, I.O., Andioge, K., Muchiri, G., Odiere, M.R., Vennervald, B.J., Olsen, A. (2018) Diet and hygiene practices influence morbidity in schoolchildren living in Schistosomiasis endemic areas along Lake Victoria in Kenya and Tanzania-A cross- sectional study. *PLoS neglected tropical diseases*, 12(3), e0006373. doi: 10.1371/journal. pntd.0006373.

Mosmann, T. (1983) Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *Journal of Immunological Methods,* 65(1-2), 55-63. doi: 10.1016/0022-1759(83) 90303-4.

Mutengo, M.M., Mduluza, T., Kelly, P., Mwansa, J.C.L., Kwenda, G., Musonda, P., Chipeta, J. (2018) Low IL-6, IL-10, and TNF-[alpha] and High IL-13 Cytokine Levels Are Associated with Severe Hepatic Fibrosis in *Schistosoma mansoni* Chronically Exposed Individuals. *Journal of Parasitology Research,* 2018(2), 1-8. doi: 10.1155/2018/ 9754060.

Niu, X., Hu, T., Hong, Y., Li, X., Shen, Y. (2022) The Role of Praziquantel in the Prevention and Treatment of Fibrosis Associated with Schistosomiasis: A Review. *Journal of Tropical Medicine*, 2022(4), 1-8. doi:10.1155/2022/ 1413711.

Pandey, P., Khan, F., Khan, M.A., Kumar, R., Upadhyay, T.K. (2023) An Updated Review Summarizing the Anticancer Efficacy of Melittin from Bee Venom in Several Models of Human Cancers. *Nutrients*, 15(14), 3111. doi: 10. 3390/nu15143111.

Park, H.J., Son, D.J., Lee, C.W., Choi, M.S., Lee, U.S., Song, H.S., Lee, J.M., Hong, J.T. (2007) Melittin inhibits inflammatory target gene expression and mediator generation via interaction with IkappaB kinase. *Biochemical Pharmacology*, 73(2), 237-247. doi: 10.1016/j.bcp.2006. 09.023.

Pereira, A.V., de Barros, G., Pinto, E.G., Tempone, A.G., Orsi, R.de O., Dos Santos, L.D., Calvi, S., Ferreira, R.S., Pimenta, D.C., Barraviera, B. (2016) Melittin induces *in vitro* death of *Leishmania* (*Leishmania*) *infantum* by triggering the cellular innate immune response. *Journal of Venomous Animals and Toxins including Tropical Diseases*, 22, 1. doi: 10.1186/s40409-016-0055-x.

Rehman, A., Ullah, R., Jaiswal, N., Khan, M.A.H., Rehman, L., Beg, M.A., Malhotra, S.K., Abidia, S.M.A. (2017) Low virulence potential and *in vivo* transformation ability in the honey bee venom treated *Clinostomum complanatum*. *Experimental Parasitology,* 183, 33-40. doi: 10.1016/j.exppara. 2017. 10.007.

Saleh, A.H., Badr, A.M., Mahmoud, S.S., Mahana, N.A., Abo Dena, A.S. (2020) *In-vitro* investigation of the effect of bee venom on *Schistosoma mansoni* eggs. *International Journal of Veterinary Science,* 9(3), 473-475. doi:10.37422/IJVS/036.

Soliman, C., Eastwood, S., Truong, V.K., Ramsland, P.A., Elbourne, A. (2019) The membrane effects of melittin on gastric and colorectal cancer. *PLoS ONE*, 14(10), e0224028. doi: 10. 1371/journal.pone.0224028.

Soltan-Alinejad, P., Alipour, H., Meharabani, D., Azizi, K. (2022) Therapeutic Potential of Bee and Scorpion Venom Phospholipase A2 (PLA2): A Narrative Review. *Iranian journal of medical sciences,* 47(4), 300-313. doi:

10.30476/IJMS.2021.88511.1927.

Toth, K., Alwan, S., Khan, S., McHardy, S.F., LoVerde, P.T., Cameron, M.D. (2023) Addressing the oxamniquine *in vitro-in vivo* paradox to facilitate a new generation of anti-schistosome treatments. *International Journal for Parasitology: Drugs and Drug Resistance,* 21, 65-73. doi: 10.1016/j.ijpddr.2023.01.003.

Tu, W.C., Wu, C.C., Hsieh, H.L., Chen, C.Y., Hsu, S.L. (2008) Honeybee venom induces calcium-dependent but caspase-independent apoptotic cell death in human melanoma A2058 cells. *Toxicon*, 52(2), 318-29. doi: 10.1016/j.toxicon.2008.06.007.

Van Stijn, C.M., Meyer, S., van den Broek, M., Bruijns, S.C., van Kooyk, Y., Geyer, R., van Die, I. (2010) *Schistosoma mansoni* worm glycolipids induce an inflammatory phenotype in human dendritic cells by cooperation of TLR4 and DC-SIGN. *Molecular Immunology,* 47(7-8), 15441552. doi: 10.1016/j.molimm. 2010.01.014.

Vardeu, A., Davis, C., McDonald, I., Stahlberg, G., Thapa, B., Piotrowska, K., Marshall, M.A., Evans, T., Wheeler, V., Sebastian, S., Anderson, K. (2022) Intravenous administration of viral vectors expressing prostate cancer antigens enhances the magnitude and functionality of CD8+ T cell responses. *Journal for ImmunoTherapy of Cancer*, 10(11), e005398. doi: 10.1136/jitc-2022-005398.

Wehbe, R., Frangieh, J., Rima, M., El Obeid, D., Sabatier, J.M., Fajloun, Z. (2019) Bee Venom: Overview of Main Compounds and Bioactivities for Therapeutic Interests. *Molecules* , 24(16):2997. doi: 10. 3390/molecules24162997.

World Health Organization. (2022, February 14) Guideline on control and elimination of human schistosomiasis. Available at: https://www.who.int/publications/i/item/9789240041608.

Wullaert, A., Heyninck, K., Beyaert, R. (2006) Mechanisms of crosstalk between TNF-induced NF-kappaB and JNK activation in hepatocytes. *Biochemical Pharmacology*, 72(9), 1090-1101. doi: 10.1016/j.bcp.2006.07.003.

Yaacoub, C., Rifi, M., El-Obeid, D., Mawlawi, H., Sabatier, J.M., Coutard, B., Fajloun, Z. (2021) The Cytotoxic Effect of *Apis mellifera* Venom with a Synergistic Potential of Its Two Main Components—Melittin and PLA2—On Colon Cancer HCT116 Cell Lines. *Molecules*, 26(8), 2264. doi: 10.3390/molecules26082264.

Yaacoub, C., Wehbe, R., Roufayel, R., Fajloun, Z., Coutard, B. (2023) Bee Venom and Its Two Main Components Melittin and Phospholipase A2 As Promising Antiviral Drug Candidates. *Pathogens,* 12(11), 1354. doi: 10.3390/pathogens12111354.

Yu, Y., Song, Q., Huang, L., Shen, M., Yu, Q., Chen, Y., Xie, J. (2020) Immunomodulatory activities of sulfated *Cyclocarya paliurus* polysaccharides with different degrees of substitution on mouse spleen lymphocytes. *Journal of Functional Foods,* 64, 103706. doi: 10.1016/j.jff.2019.103706.

Zhang, S., Liu, Y., Ye, Y., Wang, X., Lin, L.T., Xiao, L.Y., Zhou, P., Shi, GX., Liu, C.Z. (2018) Bee venom therapy: Potential mechanisms and therapeutic applications. *Toxicon*, 148, 64-73. doi: 10.1016/j.toxicon.2018.04.012.