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Antifungal and immunomodulatory potentials of propolis nanoparticles against *Fusarium solani* in Swiss Albino mice

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Abstract: The nanotechnology has an important role in improving the efficacy and safety in therapy. The present study explains the antifungal and the immunomodulatory potency of propolis nanoparticles (PNPs) against *Fusarium solani* in Albino Mice. Disc diffusion method was used for antifungal efficacy of PNPs. Moreover, PNPs exhibited a progressive decrease in the % of viable Vero cells and suppressed the virulence factors of *Fusarium saloni* including adhesion and biofilm formation. In animal experiments, lymphoid cell, peritoneal phagocytes, red blood cell RBCs and white blood cell WBCs counts and the activities of T and B- lymphocytes were determined. In addition, T-cell mitogenesis cells, serum level of interleukin 2 (IL-2) and haemoglobin Hb concentration were measured. PNPs exhibited high inhibition zone with most of *Fusarium solani* isolates. The results indicated that treatment of mice by PNPs showed a marked rise in the number of cells from thymus, spleen, mesenteric lymph nodes and bone marrow. Furthermore, PNPs caused statistically significant raised in Hb concentration and WBCs count. These results confirmed that many biological activities attributed to PNPs and is as an adjuvant for immune enhancement.

Keywords: Propolis nanoparticles, *Fusarium solani*, cytotoxicity, macrophages and lymphocytes.

INTRODUCTION

The use of natural products in the treatment of various diseases has been of great interest by researchers due to their low side effects and inexpensive therapeutic approach (Dutta *et al*., 2019). Propolis or bee glue is one of these products that have proven to be effective for the overall health of the body and in the treatment of many diseases. It is substances collected by honey bees from buds and other parts of certain plants to protect and reconstruction the beehive (Salatino & Salatino, 2017). It is a resinous mixture with bee wax and pollens as well as salivary gland secretions of honey bees (Silva-Carvalho *et al*., 2015).

Propolis has several biological and pharmacological properties such as anticancer, anti-inflammation, immunomodulatory, antioxidant, antifungal and antibacterial activities (Xavier *et al*., 2017). The pharmacological activities of propolis are due to the presence of flavonoids and other component which can bind to biological polymers and scavenge free radicals as well as catalyst electron transport (Coneac *et al*., 2008).

The nanotechnology has an important role in improving the efficacy and safety in therapy (Boisseaua and Loubatonb, 2011; Kazemi *et al*., 2019). Therefore, PNPs have been used in our research because we are expected that PNPs will be more effective than bulk propolis in preventing the structural changes in cell molecules. On the other hand, studies on finding a natural product against fungi, specially Fusarium spp., are urgently required. The aim of the present study was the evaluation of antifungal and immunomodulatory potency of PNPs in fusarium toxin-fed mice.

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MATERIALS AND METHODS

Chemicals

Propolis was purchased from CC Pollen High Desert, USA. Serum IL- 2 Duoset ELISA Development kit was purchased by R&D Systems Inc, MN, USA. Other chemicals were supplied by Sigma for Chemicals, Tanta, Egypt.

Preparation of PNPs

Raw propolis was divided into small parts. 20g of propolis was dissolved in 80mL of ethanol (70%). The solution was incubated at 37°C on a shaker at a speed of 150 rpm for 48h. The impurities were removed from the extracted propolis by a filter paper. The filtered solution was kept at 4°C for 24h and then filtered again.

The preparation of PNPs was done by shaking the ethanolic extract at 250 rpm in a 37°C incubator for 24h. Then the solution was lyophilized, and the dried powder was dissolved in 70% alcohol (Kazemi *et al*., 2019).

In vitro studies

Detection of susceptibility to antifungal agents

Minimal inhibitory concentration of antifungal drugs as nystatin, amphotercin, fluconazole and ketoconazole were determined. A serial half dilution of each antifungal drug

was prepared in test tubes (10000, 5000, 2500, 1250, 625 and 312.5 µg/ml). Half ml of each drug concentration was added to 19.5 ml of YNBA plates to obtain concentrations of 250, 125, 62.5, 31.65, 15.62 and 7.8 µg/ml). Tested Fungi isolate inoculum was prepared by suspending the colonies in YNB (yeast nitrogen base) medium to obtain a turbidity equal to 1 McFarland tube. Inoculation was done using automatic pipettes to inoculate equal drops of each one isolates into the 6 plates of the drug concentrations used. The tip was changed and another one was used to inoculate next strain and so on. Inoculated plates could dry, then incubated at 30°C for 24-48h. MIC (Minimal Inhibitory Concentration) for each strain is the lowest concentration of the drug not yielding growth, as one discrete colony (Lefler and Stevens, 1984).

Effect of PNPs on the growth of Fusarium solani strains

A sterile filter paper disc was soaked with the extract and dried aseptically, then loaded on the surface of *Fusarium solani* inoculated plates (Balouiri *et al*., 2016).

Cytotoxicity Assay of PNPs

For the cytotoxicity assay, 1 × 10^4 Vero cells were seeded in a 96-well plate and allowed to attach overnight. On the following day, three different concentrations of PNPs (5, 20 and 40µg/mL) were added. The percentage of cell viability was quantified (Iadnut *et al*., 2019).

Adhesion ability of Fusarium solani

Adhesion ability of *Fusarium solani* was determined using fluorescence assay. the percentage of adhesion was quantified (Iadnut *et al*., 2019).

Biofilm formation

Fusarium biofilm mass measured by the XTT dye [2,3-bis (2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide sodium salt] (Invitrogen, NY). the colorimetric change of XTT reduction was measured by a microplate reader at 492 nm (Haque *et al*., 2016).

***In vivo* studies**

Animals

Male Swiss Albino mice (25 ± 5 g) were kindly supplied by animal unit from Helwan Research Animal Center, Cairo, Egypt. The animals were housed (8 per cage) and kept at an ambient temperature. All the animal experiments were conducted in accordance with international guidelines.

Experimental groups

Forty mice were used in this research and randomly divided into 5 groups (n=8). They were orally treated by gavage as follows:

Group I (Control): include healthy mature mice that were treated with ethanol (70%).

Group II: includes Fusarium toxin-fed mice that were treated with ethanol (70%).

Group III, IV and V: includes Fusarium toxin-fed mice that were treated with PNPs (50, 200 and 400 µg/mL) for 14 days.

Determination of lymphoid cell counts in primary and secondary lymphoid organs

24 hours after the last injection, control, Fusarium toxin-fed mice and Fusarium toxin-fed mice treated with PNPs (50, 200 and 400 µg/mL) for 14 days were anesthetized by ether. Thymuses, spleens, peripheral lymph nodes and mesenteric lymph nodes were excised, cleaned, defatted and weighed. Single Cell suspensions were prepared by squeezing the respective organs between two slides in PBS, followed by filtration through a nylon sieve. Bone marrow was obtained from femurs and tibiae, suspended in PBS and filtered. The respective cell suspensions were washed three times, suspended in PBS, and the cells were counted with a hemocytometer and calculated per gram of tissues (Othman *et al*., 2018).

Peritoneal phagocytes count

To obtain inflammatory peritoneal phagocytes, control, Fusarium toxin-fed mice and Fusarium toxin-fed mice treated with PNPs (50, 200 and 400µg/mL) for 14 days were intraperitoneally injected with 2.0 ml of starch suspension. Three days later, mice were anesthetized with ether and peritoneal macrophages were obtained by peritoneal lavage with 5.0 ml of phosphate buffered saline PBS. Cells were resuspended in PBS. Peritoneal phagocytes counts were determined using hemo-cytometer, where phagocytes stained with neutral red (El-Shaikh *et al*., 2006).

Activity of T- lymphocytes

The determination of T- cell function by intraperitoneal injection of $1x10^8$ SRBC in 0.2ml saline. After 7 days, spleens from control, Fusarium toxin-fed mice and Fusarium toxin-fed mice treated with PNPs (50, 200 and 400µg/mL) for 14 days were excised and cleaned. Single cell suspensions were prepared and mixed with an equal volume of 0.5% SRBC in test tube and incubated for 2- 4 hours at 37°C. The rosettes were counted with a hemocytometer and calculated per million mononuclear cells (Hsu *et al.,* 1975).

Activity of B- lymphocytes

B-cell function was determined by intraperitoneal injection of $1x10^8$ SRBC in 0.2 ml saline. After 5 days, spleens from control, Fusarium toxin-fed mice and Fusarium toxin-fed mice treated with PNPs (50, 200 and 400µg/mL) for 14 days were excised and cleaned. The assay mixture was prepared by adding 50µl of 25% SRBC and 50µl of guinea pig complement to 100µl of spleen cell suspension and plated to a slide chamber and incubated for 30- 45 minutes at 37°C. The plaques were counted microscopically and calculated per million mononuclear cells (Brousseau *et al.*, 1999).

Spleen lymphocytes proliferation assay
The determination T-cell mitogenesis was detected by preparation single spleen cell suspension after teasing through a sterilized autoclaved mesh. The adjusted cells were placed in a 96-well microtiter plate and added with 200 mL aliquots per well. Con A (5mg/mL) and RPMI - 1640 medium were set as a positive and negative control, respectively in the presence of PNPs (5, 20 and 40 µg/mL). After 68 hours, cells were counted by MTT assay (Lao *et al*., 2013).

Serum IL-2 level
The release of serum IL-2 was measured to evaluate the immune response using the Duoset ELISA Development kit (R&D Systems Inc, MN, USA).

Colorimetric determination of Hb concentration
Drabkin's Reagent was used for the quantitative determination of Hb concentration in whole blood at 540 nm (Lerberghe *et al*., 1983) from control, Fusarium toxin-fed mice and Fusarium toxin-fed mice treated with PNPs (50, 200 and 400 µg/mL) for 14 days.

RBCs count
Blood was carefully drawn to the 0.5 mark of the RBC pipette. Control saline was drawn to the 101 mark to dilute the blood. The tip of the pipette is touched to the side of the hemocytometer chamber and a drop of a fluid was run under the cover glass. Total number of cells in 5 squares in the center of counting chamber was determined (Rahman., 2016).

WBCs count
Blood was carefully drawn to the 0.5 mark of the WBC pipette. WBC reagent (Glacial acetic acid 3%) was drawn to the 11 mark to dilute the blood. The tip of the pipette was touched to the side of the hemocytometer counting chamber and a drop of a fluid was run under the cover glass. Total number of cells in 4 squares at the corner of counting chamber was determined (Rahman 2016).

STATISTICAL ANALYSIS

All *in vivo* results were expressed as the mean + SD of groups consisting of 8 mice. The *in vitro* data was also expressed as the mean + SD of groups consisting of four wells. By using Student's t-test, all data were analyzed for significance using SPSS Statistical program version 22. Data was designated at the level of $P<0.05$.

RESULTS

Effect of different antifungal drugs against Fusarium solani strains
The results recorded in table 1 illustrated that Fluconazole was the most effective against the growth of strains than the others which recorded the clear inhibition zones ranged from 5-34 mm followed by ketoconazole which recorded the clear inhibition zone ranged from 2-23 followed by Amphotericin which recorded the clear inhibition zone ranged from 2-19 followed by Nystatin which recorded the clear inhibition zone ranged from 1-16.

Effect of PNPs on Fusarium solani strains
Through the table 2, the antifungal effect of PNPs was clear recorded at most of the strains, especially number 1, 2, 4, 7, 8 which recorded the clear inhibition zones ranged from 11-38 mm, and the maximum inhibition zone was recorded 38 mm at strain 7 followed by 25 mm at strain number 4 and 24 mm at strain number 2.

Cytotoxicity Assay of PNPs
As shown in table 3, *in vitro* incubation of PNPs (5, 20 and 40µg/mL) with 1 × 10^4 Vero cells elicited a progressive decrease in the % of viable Vero cells as compared with those of vehicle control. This decrease was significantly with a dose 40µg/mL ($P<0.05$).

Adhesion ability of Fusarium solani
Table 4 described that the percentage of adhesion ability of Fusarium solani was gradually decreased in the presence of PNPs (5, 20 and 40µg/mL) as compared with those of vehicle control. This decrease was significantly with doses 20 and 40 µg/mL ($P<0.05$).

Fusarium biofilm formation
As explained in table 5, the percentage of Fusarium biofilm mass was decreased in presence of PNPs (5, 20 and 40µg/mL) as compared with those of vehicle control. This decrease was significantly with a dose 20µg/mL ($P< 0.01$)

Effect on lymphoid cell counts in primary and secondary lymphoid organs
As shown in table 6, the total number of cells from thymus, spleen, peripheral lymph node, mesenteric lymph nodes and bone marrow of Fusarium toxin-fed mice were reduced as compared to those of control mice. Treatment of Fusarium toxin-fed mice with PNPs (50, 200 and 400 µg/mL) for 14 days caused a statically significant rise in the number of cells from thymus, spleen, mesenteric lymph nodes and bone marrow compared with Fusarium toxin-fed mice ($p<0.05$ and $p<0.01$), although fusarium toxin-fed mice treated with *PNPs* did not cause any significant effect on the number of cells from the PLN.

Effect on the phagocytic cell count
As shown in table 7, the macrophage number of Fusarium toxin-fed mice were reduced as compared to control group. Treatment of Fusarium toxin-fed mice with PNPs (50, 200 and 400 µg/mL) for 14 days repaired this effect and elicited a statistically significant increase in the number of phagocyte cells ($p<0.05$ and $p<0.01$) at doses (50, 200 and 400 µg/mL) as compared with the control group.

Table 1: Effect of different antifungal drugs against *Fusarium solani* strains

Inhibition zone of antifungal drugs on the growth of *Fusarium solani* (mm)				
Nystatein	Fluconazole	Amphotericin	Ketoconazole	*Fusarium solani* strains
16±0.58**	28±0.45**	18±0.59**	21±0.37**	1
14±0.58**	29±0.45**	17±0.59**	23±0.37**	2
9±0.58**	18±0.45**	9±0.59**	12±0.37**	3
13±0.58**	26±0.45**	15±0.59**	22±0.37**	4
4±0.58**	11±0.45**	6±0.59**	8±0.37**	5
2±0.58**	5±0.45**	3±0.59**	3±0.37**	6
14±0.58**	34±0.45**	19±0.59**	22±0.37**	7
14±0.58**	15±0.45**	11±0.59**	13±0.37**	8
1±0.58**	4±0.45**	2±0.59**	2±0.37**	9
11±0.58**	8±0.45**	11±0.59**	9±0.37**	10

** Significantly different from Fusarium- fed group at P < 0.01.

Influence on the activity of T and B lymphocytes

Table 8 showed that the number of activated T-lymphocytes and activated B- lymphocytes of Fusarium toxin-fed mice were reduced as compared to control group. Treatment of Fusarium toxin-fed mice with PNPs (50, 200 and 400µg/mL) for 14 days repaired this effect and caused a gradual increase in the number of activated T- lymphocytes and activated B- lymphocytes as compared to the control group. This increase in the number of activated T and B-lymphocytes were statistically significant ($p<0.05$) with a dose (200 µg/mL) as compared with the control group.

Table 2: Effect of PNPs on *Fusarium solani* strain

Fusarium solani strains	Diameter of inhibition zone (mm)
1	19±0.63**
2	24±0.63**
3	8±0.63**
4	25±0.63**
5	5±0.63**
6	3±0.63**
7	38±0.63**
8	19±0.63**
9	3±0.63**
10	13±0.63**

**Significantly different from Fusarium- fed group at P < 0.01

Effect on the mitogenic response of T- Lymphocytes

As described in table 9, in the absence of Con A mitogen, PNPs by itself had no mitogenic effect under the cultured conditions explained. However, in the presence of Con A (5µg/ ml), PNPs significantly motivate the proliferation of cultured splenocyte ($p<0.05$) at doses (20 and 40 µg/mL).

Effect of PNPs on serum IL-2 level

As explained in table 10, the serum IL-2 levels of Fusarium toxin-fed mice were clearly reduced as compared to control mice. Treatment of Fusarium toxin-fed mice with PNPs (50, 200 and 400µg/mL) for 14 days improved this effect and caused statistically significant raised in serum levels of IL-2 ($p<0.05$) with a dose (200 µg/mL) as compared to the control group.

Table 3: The percentage of cell viability of 1 × 10^4 Vero cells was quantified in the absence or presence of PNPs (5, 20 and 40µg/mL).

% of viable Vero cells	Treatment
94 ± 3.61	Vehicle
86 ± 3.61	PNPs (5µg/mL)
63.33 ± 6.11	PNPs (20 µg/mL)
47.33 ± 3.06*	PNPs (40 µg/mL)

Table 4: The percentage of adhesion ability of *Fusarium solani* was quantified in the absence or presence of PNPs (5, 20 and 40µg/mL)

% of adhesion ability of *Fusarium solani*	Treatment
86 ± 4	Vehicle
66.67 ± 2.52	PNPs (5µg/mL)
57.33 ± 5.51*	PNPs (20 µg/mL)
52.33.33 ± 4.51*	PNPs (40 µg/mL)

Table 5: the percentage of *Fusarium* biofilm mass was quantified in the absence or presence of PNPs (5, 20 and 40µg/mL).

% of *Fusarium* biofilm mass	Treatment
84.33 ± 4.04	Vehicle
57 ± 3.61	PNPs (5µg/mL)
49.33 ± 4.04**	PNPs (20 µg/mL)
42 ± 6.56	PNPs (40 µg/mL)

**Significantly different from control at P < 0.01

Effect of PNPs on Hb concentration and RBCs and WBCs count

From table 11, Hb concentration, RBCs and WBCs counts of Fusarium toxin-fed mice were clearly reduced as

Table 6: Total number of leukocytes from thymus (Thy), spleen (Spl), peripheral lymph nodes (PLN), mesenteric lymph nodes (MLN) and bone marrow (BM) in control and in Fusarium toxin-fed mice treated with PNPs (50, 200 and 400 µg/mL) for 14 days

Total cell count/ g of tissue (Mean± SD)					Treatment
BM $x10^6$	MLN $x10^9$	PLN $x\ 10^9$	Spl $x10^9$	Thy $x10^9$	
2.13±0.16	1.08±0.13	1.40±0.15	2.32±0.19	1.92±0.12	Control
1.14±0.08	0.81±0.09	0.98±0.14	1.69±0.04	0.86±0.07	Fusarium toxin-fed + Vehicle
1.27±0.06**	0.83±0.04	1.05±0.24	1.82±0.06**	0.91±0.08	Fusarium toxin-fed + PNPs (50 µg/mL)
1.81±0.14	1.11±0.16*	0.96±0.06	1.83±0.10*	1.00±0.17*	Fusarium toxin-fed + PNPs (200 µg/mL)
1.72±0.11*	0.95±0.09	1.13±0.10	2.12±0.17	1.26±0.20*	Fusarium toxin-fed + PNPs (400 µg/mL)

Table 7: Number of macrophages in control and in Fusarium toxin-fed mice treated with PNPs (50, 200 and 400 µg/mL) for 14 days

Treatment	No. of macrophages (Mean ± SD x10^6)
Control	4.10 ± 0.56
Fusarium toxin-fed + Vehicle	1.49 ± 0.17
Fusarium toxin-fed + PNPs (50 µg/mL)	2.38 ± 0.23*
Fusarium toxin-fed + PNPs (200 µg/mL)	3.12 ± 0.25**
Fusarium toxin-fed + PNPs (400 µg/mL)	2.85 ± 0.07*

*Significantly different from Fusarium- fed group at P<0.05.
**Significantly different from Fusarium- fed group at P<0.01

Table 8: Number of activated T and B- lymphocytes / 10^6 nucleated spleen cells in control and in Fusarium toxin-fed mice treated with PNPs (50, 200 and 400µg/mL) for 14 days.

Treatment	No. of activated T- Lymphocytes / million nucleated spleen cells (Mean ± $SDx10^3$)	No. of activated B- Lymphocytes / million nucleated spleen cells (Mean ± $SDx10^3$)
Control	2.17 ± 0.12	1.42 ± 0.04
Fusarium toxin-fed + Vehicle	1.04 ± 0.11	0.78 ± 0.16
Fusarium toxin-fed + PNPs (50 µg/mL)	1.26 ± 0.07	1.01± 0.09
Fusarium toxin-fed + PNPs (200 µg/mL)	1.81 ± 0.14*	1.37 ± 0.13*
Fusarium toxin-fed + PNPs (400 µg/mL)	1.97 ± 0.18	1.20 ± 0.13

* Significantly different from Fusarium- fed group at P < 0.05.

compared to control mice. This situation has improved after treatment of Fusarium toxin-fed mice with PNPs (50, 200 and 400µg/mL) for 14 days and caused statistically significant raised in Hb concentration and WBCs count (p<0.05 and p<0.01) as compared to the control group, although Fusarium toxin-fed mice treated with PNPs did not cause any significant effect on the number of cells from RBCs.

DISCUSSION

The current study showed that the antifungal effects of PNPs were clear recorded at most of the strains, especially number 1, 2, 4, 7, 8 which recorded the clear inhibition zones ranged from 11-38 mm, and the maximum inhibition zone was recorded 38 mm at strain 7 followed by 25 mm at strain number 4 and 24 mm at strain number 2. This study agreed with the data of Veiga *et al*., (2018) that made it clear that the main components of propolis extract are cinnamic acids, terpenoid compounds, artepillin C, and phenolic substances. The active substances in propolis extract are the phenolic substances, which are responsible for the anti-inflammatory, antimicrobial, and antifungal activity of propolis.

The present study showed that the percentage of adhesion ability of *Fusarium solani* was gradually decreased in the presence of PNPs (5, 20 and 40 µg/mL). This study is consistent with the study of Feldman *et al*., 2012 reported that EEP-NP 2 possessed unique physicochemical properties due to their biocompatible properties. The possible mode of action of phenolic compound was an anti-adhesion ability of *C. albicans*. The hydrophobic interaction generally plays an important role for the adherence of *C. albicans* to host cells or abiotic surface; it is usually considered as a good predictor for adhesion ability.

Our study showed that the percentage of Fusarium biofilm mass was decreased in presence of PNPs (5, 20 and 40 µg/mL). Shahzad *et al*. reported two polyphenol

Table 9: T cell mitogenic response *in vitro*. Cultured splenocytes (2 x10^6 cells/ ml) were exposed to Culture medium (Control), Con A (5µg/ml) in the absence or presence of PNPs (5, 20 and 40µg/mL) for 72 hours.

Optical density (570 nm)		Treatment
Con A (5µg/ml)	Control (Culture medium)	
0.38 ± 0.04	0.25 ± 0.04	Vehicle
0.61 ± 0.05	0.31 ± 0.03	PNPs (5µg/mL)
0.79± 0.13*	0.32 ± 0.05	PNPs (20 µg/mL)
0.78 ± 0.07*	0.34 ± 0.03	PNPs (40 µg/mL)

* Significantly different from Fusarium- fed group at P<0.05.

Table 10: Serum levels of IL-2 in control and Fusarium toxin-fed mice treated with PNPs (50, 200 and 400µg/mL) for 14 days.

Treatment	IL-2 (pg/mL)
Control	28.18±0.83
Fusarium toxin-fed + Vehicle	19.00 ± 1.17
Fusarium toxin-fed + PNPs (50 µg/mL)	22.61 ± 0.59
Fusarium toxin-fed + PNPs (200 µg/mL)	25.44 ± 1.86*
Fusarium toxin-fed + PNPs (400 µg/mL)	23.62 ± 2.10

* Significantly different from Fusarium- fed group at P<0.05.

Table 11: Hb concentration, RBCs and WBCs counts of blood in control and in Fusarium toxin-fed mice treated with PNPs (50, 200 and 400µg/mL) for 14 days.

Treatment	Hb concentration (gm/ 100 ml)	RBCs count (Mean ± SDx10^6)	WBCs count (Mean ± SDx10^3)
Control	12.12±0.47	4.85±0.31	5.33±0.57
Fusarium toxin-fed + Vehicle	8.82±0.39	2.98±0.37	2.75±0.46
Fusarium toxin-fed + PNPs (50 µg/mL)	9.27±0.29	3.68±0.10	3.43±0.25**
Fusarium toxin-fed + PNPs (200 µg/mL)	11.16±0.42*	4.04±0.26	4.21±0.34**
Fusarium toxin-fed + PNPs (400 µg/mL)	10.62±0.42**	3.82±0.36	4.13±0.24*

*Significantly different from Fusarium- fed group at P<0.05.
**Significantly different from Fusarium- fed group at P<0.01.

compounds, curcumin and pyrocatechol, which down regulated *HWP1* and *ALS3* expressions, leading to the decrease of the biofilm formation of *C. albicans*. The inhibitory effect and the invasive ability of *C. albicans* are reduced by the release of LDH from Vero cells, which is an indicator for the cell damage.

Also, the data of the current work revealed that Treatment of Fusarium toxin-fed mice with PNPs (50, 200 and 400 µg/mL) for 14 days caused a statically significant rise in the number of cells from thymus, spleen, mesenteric lymph nodes and bone marrow compared with Fusarium toxin-fed mice although Fusarium toxin-fed mice treated with PNPs did not cause any significant effect on the number of cells from the PLN. The data of this study are agreed with the data of Gao *et al*., (2014) that indicated that propolis improved immune function in aged mice and had a direct regulatory effect on basic functional properties of immune cells as a result of its analytical components of polyphenols, flavonoids, cinnamic acid and artepillin-C. Kalsum *et al*., (2017) proved that terpenoid is one of the essential ingredients in bee glue increase bone marrow cellularity.

The present study also showed that treatment of Fusarium toxin-fed mice with PNPs elicited a statistically significant rise in the number of phagocytic cells at doses (50, 200 and 400µg/mL) as compared with the control group in agreement with the study of Bueno-Silva *et al*., (2017) who confirmed that peritoneal macrophages were activated by Brazilian green propolis administration through reducing NO levels and diminished release and expression of pro-inflammatory cytokine and genes. do Nascimento *et al*., (2016) proved that nanoparticles of Brazilian red propolis extract will act as agents that inhibit oxidative processes and act as antioxidants in biological systems. In addition, Kalsum *et al.*, (2017) was able to prove propolis can enhance the activity of the lymphocyte activating factor that can directly activate macrophages because propolis has an activity such as

IFN-γ cytokines which function can activate macrophages followed by differentiation and proliferation. These differentiation and proliferation processes led to an increased number of macrophages circulating through the body.

Our study also confirmed that treatment of Fusarium toxin-fed mice with PNPs caused a marked increase in thenumber of activated T and B- lymphocytes and serum IL-2 levels with a dose (200µg/mL) as compared to the control group. These results documented what Sforcin., (2007) said propolis used as a potential immune regulator. The study conducted by Kalsum *et al*., (2017) showed Propolis contains alkaloids, flavonoids, triterpenoids, and saponins. propolis extracts increases the potential for the humoral immune response when compared to animals that received antigen without propolis (negative control). Terpenoids increased the total antibody production, antibody producing cells in the spleen. On the other hand, Tao *et al*., (2014) found that propolis flavonoids liposome not only could promote the phagocytic capability and the cytokine production of murine peritoneal macrophages *in vitro*, but also could enhance the humoral immune response and cellular immune response. This is in addition to Al Ghamdi *et al*., (2014) who explained that treating diabetic mice with propolis significantly restored both the levels of IL-2 and IL-7 and the proliferation of B and T lymphocytes.

The current study demonstrated that treatment of fusarium toxin-fed mice with PNPs (50, 200 and 400µg/mL) for 14 days caused a clear raised in Hb concentration and WBCs count. These results were agreed with Hegazi *et al*., (2014) that found flavonoids in propolis may be responsible for the improvement in the anemic status of mice and protect red blood cells from deformation. The current study also agreed with El-Aidy *et al*., (2015) that explained sensitized aqueous propolis extract and sensitized ethanolic propolis extract treated groups exhibited significant increases in the percentage of peripheral blood neutrophils, lymphocytes and monocytes.

CONCLUSION

Our results concluded that the PNPs have effective antifungal and immunomodulatory activities. These finding push the studies toward the therapeutic effect of nanotechnology as an effective treatment against many diseases.

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