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PHYSIOLOGICAL STUDIES OF HONEYBEE PRODUCT (PROPOLIS) ON IMMUNITY AND SOME HAEMATOLOGICAL PARAMETERS IN MALE RATS

By

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ABSTRACT

Propolis or bee glue is one of the most powerful hive products of honeybees. It is considered an important natural dietary supplement. The present study aimed to clarify the physiological effect of propolis on immunity and some haematological parameters in male rats. This study was carried on 45 Wister male rats, 21 days old. The experiment lasted for 2 months; rats were equally allocated into three groups, the control group, fed on a basal diet, low dose propolis group supplemented daily with 750mg propolis/kg diet, high dose propolis group supplemented daily with 1.5 g propolis/kg diet. Whole blood and serum samples were obtained for measuring; some immunological parameters [total leucocytic count (TLC), lymphocyte and neutrophil %, lymphocyte transformation test (LTT), nitroblue tertrazolium (NBT) and total immunoglobulin G (T.IgG)], some hematological parameters [red blood cells count (RBCs),haemoglobin (Hb) and packed cell volume (PCV)].The obtained result revealed that, propolis is a powerful immunomodulatory natural product via increasing lymphocyte % and consequently increasing antibody production, also propolis improved all haematological parameters studied. Conclusively, the dietary supplementation of high dose propolis for 2 months for improvement of immunological and haematological parameters is recommended.

Key words:

Propolis, immunity, haematological parameters, male rats.

INTRODUCTION

Propolis (bee glue) is a resinous and sticky bee product, which is used as a building and sheltering material.The term propolis comes from two Greek words, means pro (in defense of) and polis (the city); thus, propolis means in defense of the city or beehive **(Ghisalberti, 1979)**. Propolis is a mixture of beeswax and resins that is collected from different parts of the plant

(flowers and leaf buds) by honeybees. The chemical composition of propolis depends on the type of plant that is accessible to honeybees, the specificity of the local flora at the site of collection and the season **(Markham *et al.*, 1996; Bankova *et al.*, 1998).** More than 500 compounds have been identified in propolis including phenolic components, terpenes, lipid-wax substances, beeswax and other substances such as vitamins such as B_1, B_2, B_6, A, C and E, proteins, amino acids, minerals such as zinc, copper, manganese, iron, potassium, calcium, sodium and selenium and sugars **(Kurek-Gorecka *et al.*, 2014)**. Among them, plant phenolics constitute the most abundant group of chemical components, including flavonoids, phenolic acids and aldehydes,simple phenols and their esters,coumarins,stilbenes and lignans **(Bankova, 2005)**.

Propolis has been reported recently as one of the most important natural dietary supplements. It has immunomodulatory, antioxidative, cytostatic and antimutagenic properties.

These properties of propolis are due to its higher content of phenolic compounds. Flavonoids are thought to be responsible for many of its biological and pharmacological activities including anticancer, anti-inflammatory, antimicrobial and antioxidant effects **(Seven *et al.*, 2010).**

MATERIAL AND METHODS

The present study was done to investigate the physiological effect of propolis on immunity and some haematological parameters.

Propolis:

Chinese propolis was obtained from the Faculty of Agriculture-Cairo University in the form of dark brown powder. The two selected doses of propolis are 750 mg /kg diet (low dose) and 1.5 g/kg diet (high dose) **(Chopra *et al.*, 1995).** Daily ground propolis was weighed and mixed well with diet.

Animals:

Forty-five Male Wister rats (21 days old) were obtained from faculty of veterinary medicine, animal management department and housed in faculty of veterinary medicine, physiology department, Cairo University. Animals were allocated in plastic cages with wood shaving bedding in a well-ventilated room. Temperature was controlled at 18-20^{o}C with constant 12 /12 h light dark cycles. Rats were maintained on standard rat laboratory chew and

ad-libitum water supply throughout the experimental period (2 months).Their weights were 30 - 40 gm. Rats were randomly allocated into 3 groups:

Group I:

Fifteen rats served as control, maintained on standard rat laboratory chew and ad-libitum water supply throughout the experimental time.

Group II:

Fifteen rats served as low dose propolis group and supplemented daily with 750 mg propolis /kg diet for 2 months **(Chopra *et al*., 1995).**

Group III:

Fifteen rats served as high dose propolis group and supplemented daily with 1.5 gm propolis /kg diet for 2 months **(Chopra *et al*., 1995).**

Blood sampling:

Blood samples were collected under light ether anesthesia monthly along the experimental period (2 months) via heparinized capillary tube from orbital sinus into tubes containing EDTA or sodium heparin or gel for obtaining whole blood and serum samples. Blood samples were collected in gel tubes then immediately centrifuged at 4000 rpm for 15 minutes,

Measurements:

1- Anticoagulated blood sample (sodium heparin):

Whole anticoagulated blood samples were used for assaying some immunological parameters. Lymphocyte transformation test (LTT) was measured according to method of **Baehar *et al.* (2012).** Nitroblue tetrazolium (NBT) was measured according to the method of **Yonar *et al.* (2014).**

2-Serum samples:

Serum samples were used for measuring total immunoglobulin G.

Total IgG was measured according to the method of **Karamese *et al.* (2016)** using rat immunoglobulin G ELISA Kit purchased from ELAB company-Egypt.

Principle:

The microtiter plate provided in this kit has been pre-coated with an antibody specific to target antigen. Standards or samples are then added to the appropriate microtiter plate wells with a biotin-conjugated antibody preparation specific for target antigen and then avidin conjugated to Horseradish Peroxidase (HRP) is added to each microplate well and incubated.

Then a Tetramethylbenzidine (TMB) substrate solution is added to each well. Only those wells that contain target antigen, biotin-conjugated antibody and enzyme-conjugated Avidin will exhibit a change in colour. The enzyme-substrate reaction is terminated by the addition of a sulphuric acid solution and the colour change is measured spectrophotometrically at a wavelength of 450 nm±2 nm.The concentration of target antigen in the samples is determined by comparing the optical density (O.D) of the samples to the standard curve.

3-Anticoagulated blood sample (EDTA):

A-Red blood cells count (RBCs), total leucocytic count (TLC) and differential leucocytic count (lymphocyte and neutrophil %) were determined by coulter counter **(Marshall, 2003).**

B- Packed cell volume (PCV %):

Packed cell volume was measured by microhematocrite method. Heparininzed capillary tube centrifuged at 15000 rpm for 3-5 minutes, blood was separated into 3 layers (PCV layer, buffy coat and plasma) and PCV layer were read by using microhematocrite special reader **(Bulls *et al*., 2000).**

C**-Haemoglobin (Hb)**

Haemoglobin was measured according to the methodof **Burtis*et al*.(1999)** using a commercial kit purchased from diamond company - Egypt.

Principle:

Hemoglobin is oxidized by potassium ferricyanide into methaemoglobin, which is converted into cyanomethaemoglobin, by potassium cyanide.

The intensity of the colour formed is proportional to the hemoglobin concentration in the samples.

Statistical analysis:

Data are expressed as means± standard error of five replicate determinations.

Statistical analysis was performed using two-way analysis of variance (ANOVA) followed by least significant difference test (LSD) to assess significant differences among the different means. The results were considered to be significant at $p<0.05$. All statistical analyses were performed using SPSS software program version 16 **(Nie *et al*., 1970).**

RESULT

Table (1): Effect of low and high dose of propolis on total, differential leucocytic count (lymphocyte and neutrophil %) and immunological parameters in male rats after first and second month.

Groups / Parameter	Time (month)	Control group	Low dose group	High dose group
Total leucocytic count/ µL	1m	$5660^{b}\pm218.2$	$7132^{b}\pm303$	$7220^{b}\pm120$
	2m	$10280^{a,b}\pm372.02$	$16560^{a,b,c}\pm994.79$	$19280^{a,b,c}\pm1660.84$
Lymphocyte %	1m	$65\%^{a,b}\pm1.55$	$74.4\%^{a,b,c}\pm1.69$	$82.6\%^{a,c}\pm2.36$
	2m	$74.8\%^{a,b}\pm0.92$	$84.8\%^{a,b}\pm1.59$	$83.4\%^{a}\pm1.029$
Neutrophil %	1m	$29.4\%^{a,b}\pm1.47$	$23\%^{a,b,c}\pm1.45$	$14.4\%^{a,c}\pm2.06$
	2m	$21.2\%^{a,b}\pm0.37$	$13.4\%^{a,b}\pm1.83$	$12.2\%^{a}\pm1.43$
Lymphocyte transformation test	1m	$2.0394^{a,b}\pm0.06$	$2.328^{a,b}\pm0.09$	$2.318^{a,b}\pm0.07$
	2m	$0.4696^{a,b}\pm0.02$	$0.6108^{b}\pm0.04$	$0.6994^{a,b}\pm0.04$
Total IgG (mg/dl)	1m	$427.85^{a}\pm 25.6$	$588.16^{a,b}\pm23.69$	$563.13^{a,b}\pm41.77$
	2m	368.38 ± 24.32	$403.8^{b}\pm 5.59$	$382.66^{b}\pm 16.89$
Nitro blue tetrazolium	1m	$1.027^{a}\pm0.05$	$0.758^{a,c}\pm0.01$	$0.603^{a,b,c}\pm0.02$
	2m	$1.0298^{a}\pm0.08$	$0.681^{a,c}\pm0.02$	$0.406^{a,b,c}\pm0.02$

Means ± SE.

Means have the same superscript (a, c) within the same raw are significantly different at P≤0.05 according to two way ANOVA followed by LSD test.

Means have the same superscript (b) within the same column are significantly different at P≤0.05 according to two way ANOVA followed by LSD test.

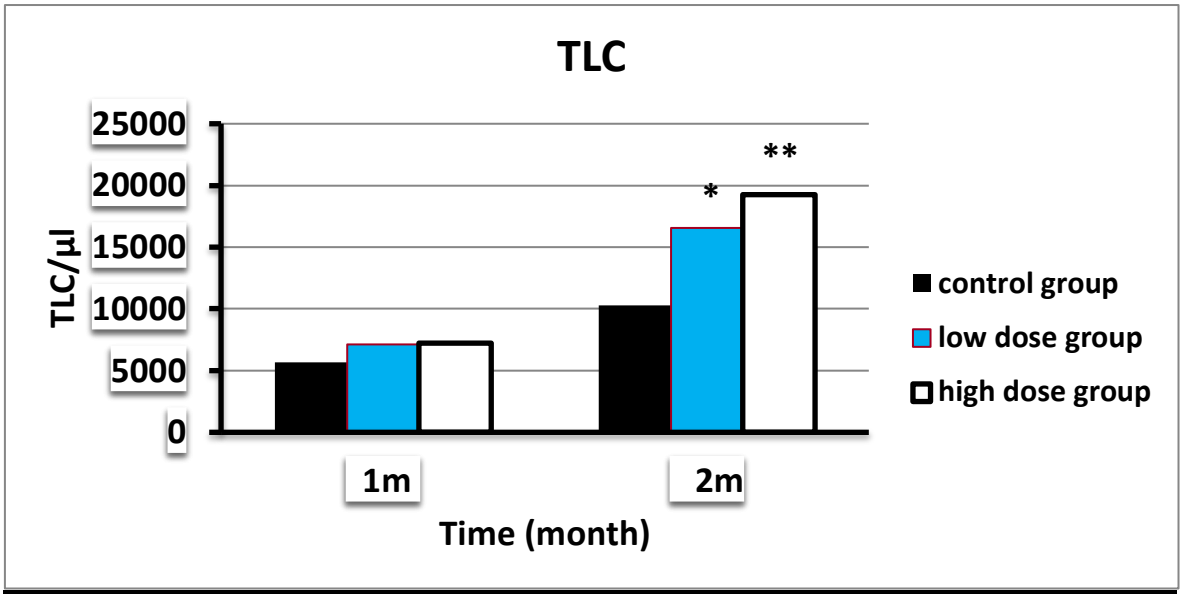

Fig. (1): Effect of low and high dose of propolis on total leucocytic count in male rats after first and second month.

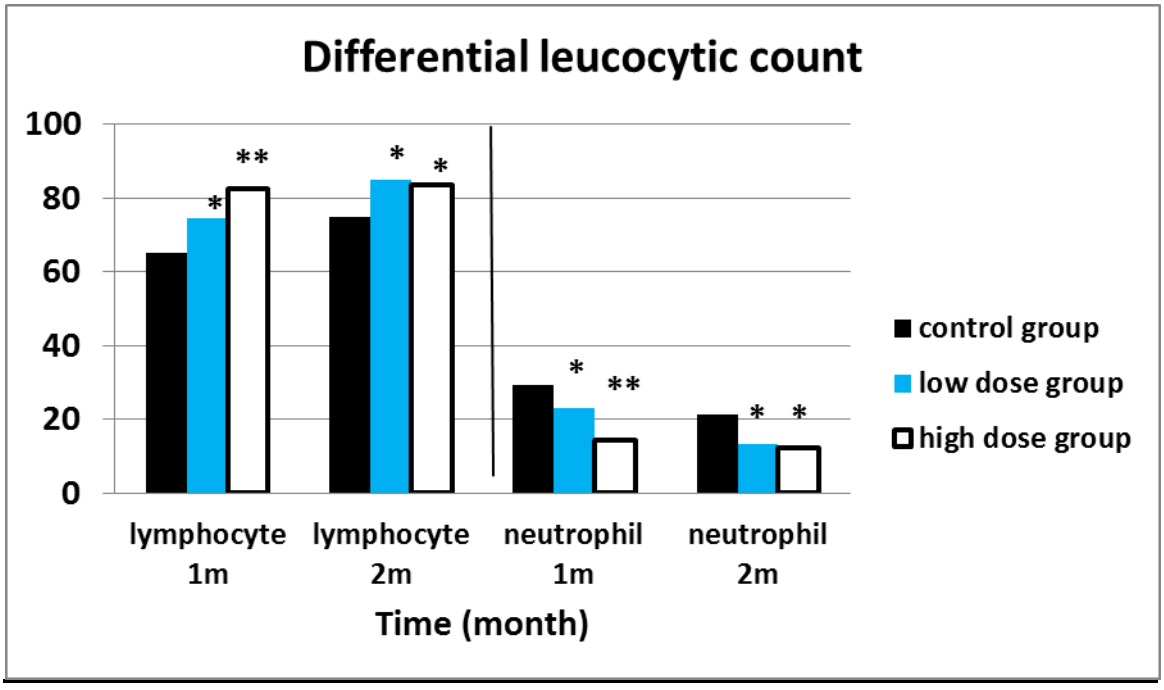

Fig. (2): Effect of low and high dose of propolis on lymphocytic and neutrophil % in male rats after first and second month.

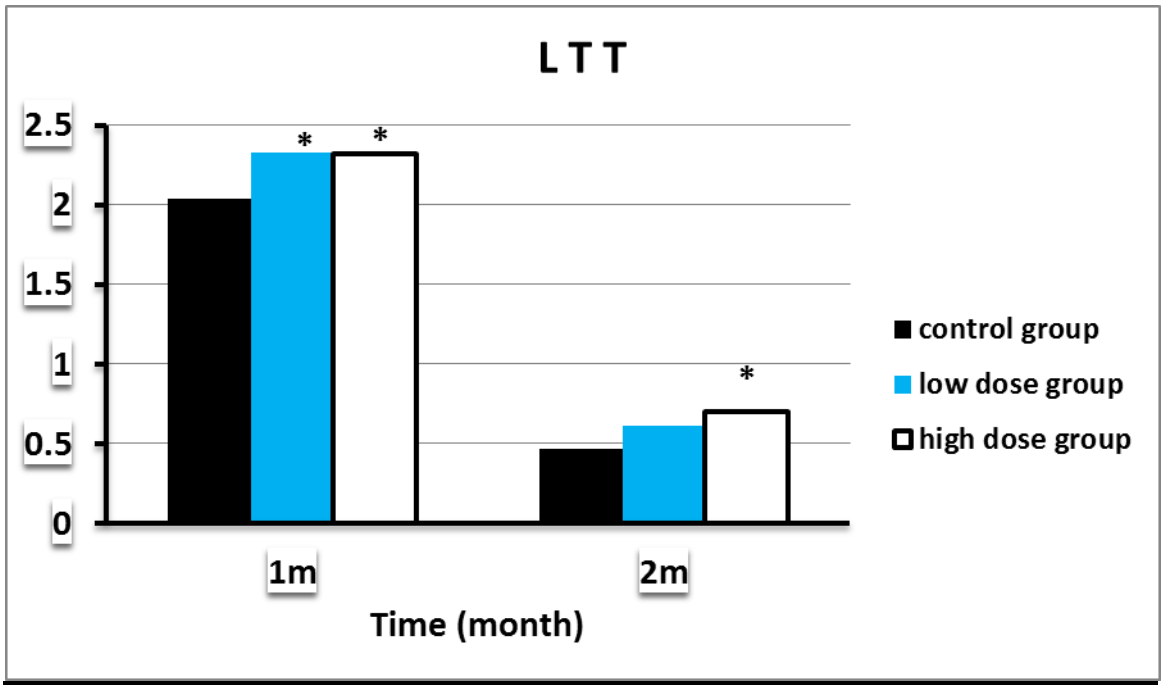

Fig. (3): Effect of low and high dose of propolis on lymphocyte transformation test in male rats after first and second month.

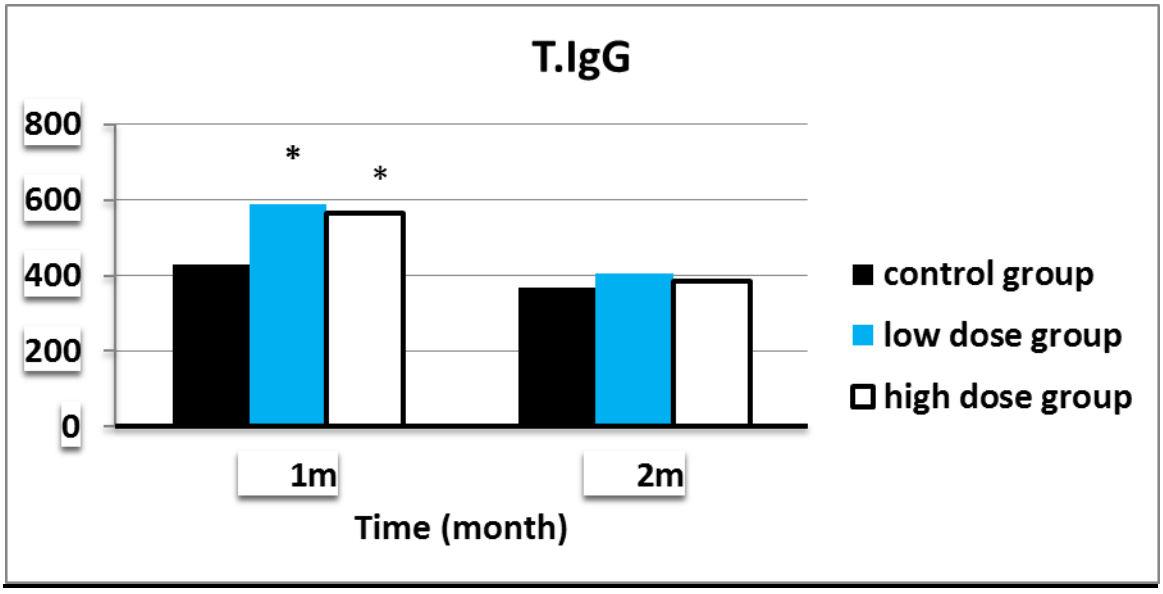

Fig. (4): Effect of low and high dose of propolis on total IgG in male rats after first and second month.

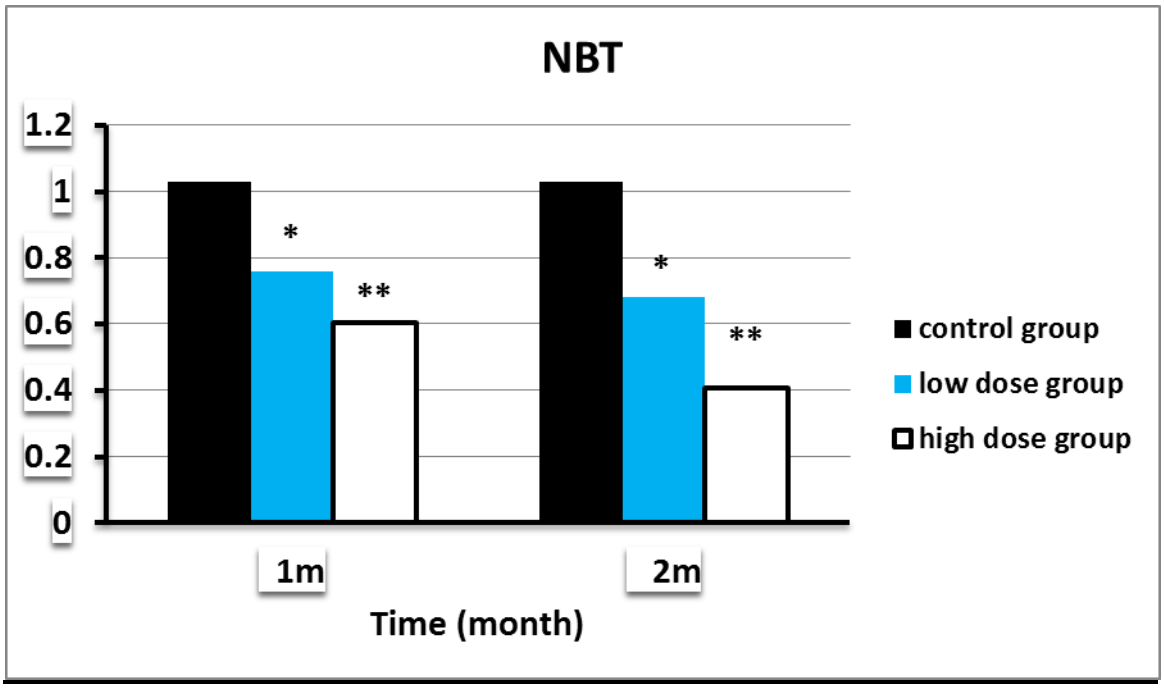

Fig. (5): Effect of low and high dose of propolis on nitroblue tetrazolium in male rats after first and second month.

Effect of low and high dose of propolis on total, differential leucocytic count (lymphocytic and neutrophil %) and some immunological parameters in male rats after first and second month:

Data presented in (Table 1) and illustrated in Fig. (1, 2, 3, 4 and 5) revealed that, total leucocytic count were not altered by either low or high dose of propolis supplementation when compared to the control group during the 1st month. However, during 2nd month of the experiment propolis supplementation in low and high dose resulted in significant increase in TLC when compared to control. Moreover, high dose resulted in significant increase in TLC compared to the low dose. Additionally, TLC of either low or high dose of propolis in the 2nd month were higher than those of the same groups during the 1st month.

Lymphocytic % was significantly increased by either low or high dose of propolis supplementation when compared to the control group during the 1st and 2nd month. Moreover, high dose resulted in significant increase in lymphocyte % compared to the low dose at the 1st month. Additionally, lymphocyte % of the low dose of propolis in the 2nd month was higher than those of the same group during the 1st month.

Neutrophil % was significantly decreased by either low or high of propolis supplementation when compared to the control group during the 1st and 2nd month. Moreover, high dose resulted in significant decrease in neutrophil % compared to the low dose at the 1st month. Additionally, neutrophil % of the low dose of propolis in the 2nd month was higher than those of the same group during the 1st month.

Lymphocyte transformation test (LTT) was significantly increased by either low or high dose of propolis supplementation when compared to the control group during the 1st month. Moreover, during the 2nd month propolis supplementation in high dose resulted in significant increase in LTT when compared to the control group but the low dose was not altered.

Total Immunoglobulin G was significantly increased by either low or high dose of propolis supplementation when compared to the control group during the 1st month.

Nitroblue tetrazolium was significantly decreased by either low or high dose of propolis supplementation when compared to the control group during 1st and 2nd month. Moreover, high dose of propolis resulted in significant decrease in nitroblue tetrazolium compared to the low dose at the 1st and 2nd month.

Table (2): Effect of low and high dose of propolis on some haematological parameters RBCs, Hb and PCV in male rats after first and second month.

Parameter \ Groups	Time (month)	Control group	Low dose group	High dose group
RBCs ($\times 10^6$ /µL)	1m	4.46 ^{a,b}±0.12	5.63 ^{a,b}±0.10	5.33^{a,b}±0.12
	2m	5.48 ^{a,b}±0.29	6.28 ^{a,b}±0.14	7.2^{a} ^{,b}±0.45
Hb (g/dl)	1m	12.58^{a,b}±0.58	13.78 ^{a,b}±0.55	14.55^{a,b}±0.41
	2m	14.36^{a,b}±0.29	15.72^{b}±0.32	16.21^{a,b}±0.20
PCV (%)	1m	44.8 ^{b}±2.67	46 ^{b}±1.05	45.6 ^{b}±0.24
	2m	50.2^{a,b}±1.49	52.2^{b}±1.02	55 ^{a,b}±1.22

Means ± SE.

Means have the same superscript (a) within the same raw are significantly different at P≤0.05 according to two way ANOVA followed by LSD test.

Means have the same superscript (b) within the same column are significantly different at P≤0.05 according to two way ANOVA followed by LSD test.

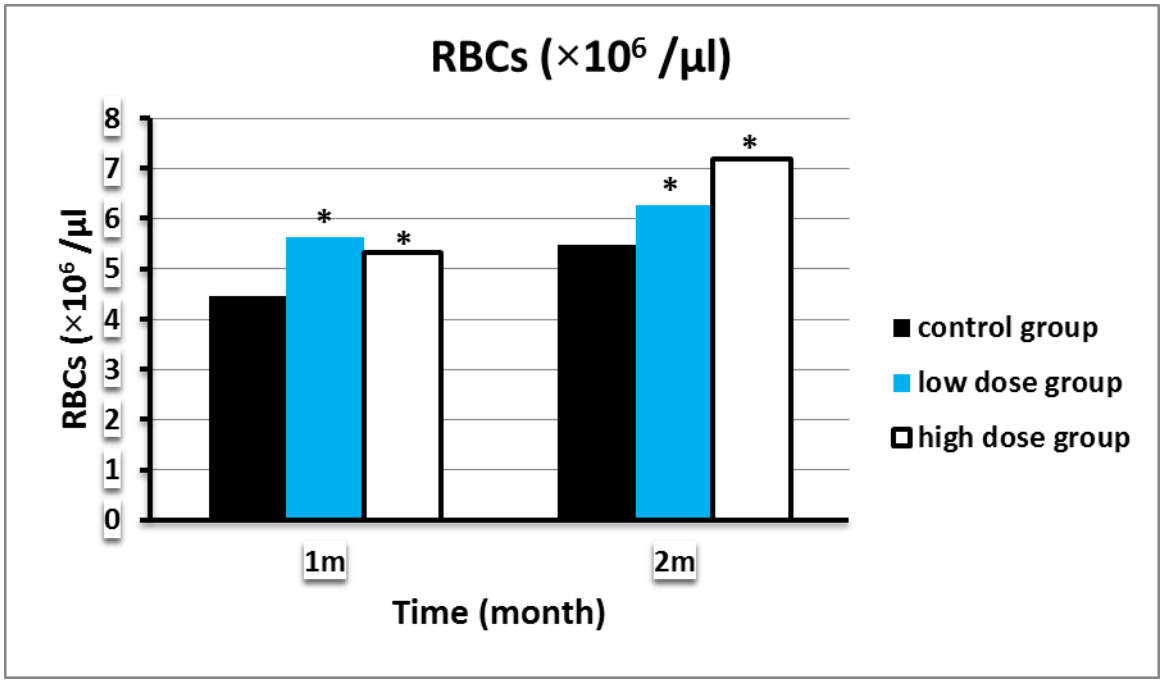

Fig. (6): Effect of low and high dose of propolis on red blood cells count in male rats after first and second month.

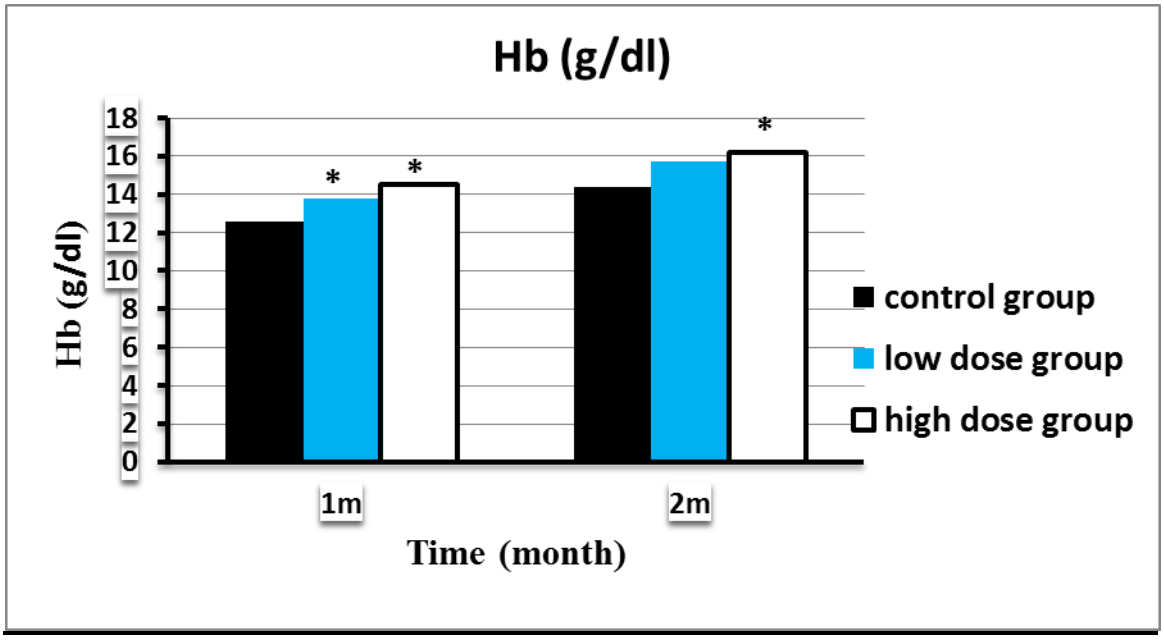

Fig. (7): Effect of low and high dose of propolis on haemoglobin (g/dl) in male rats after first and second month.

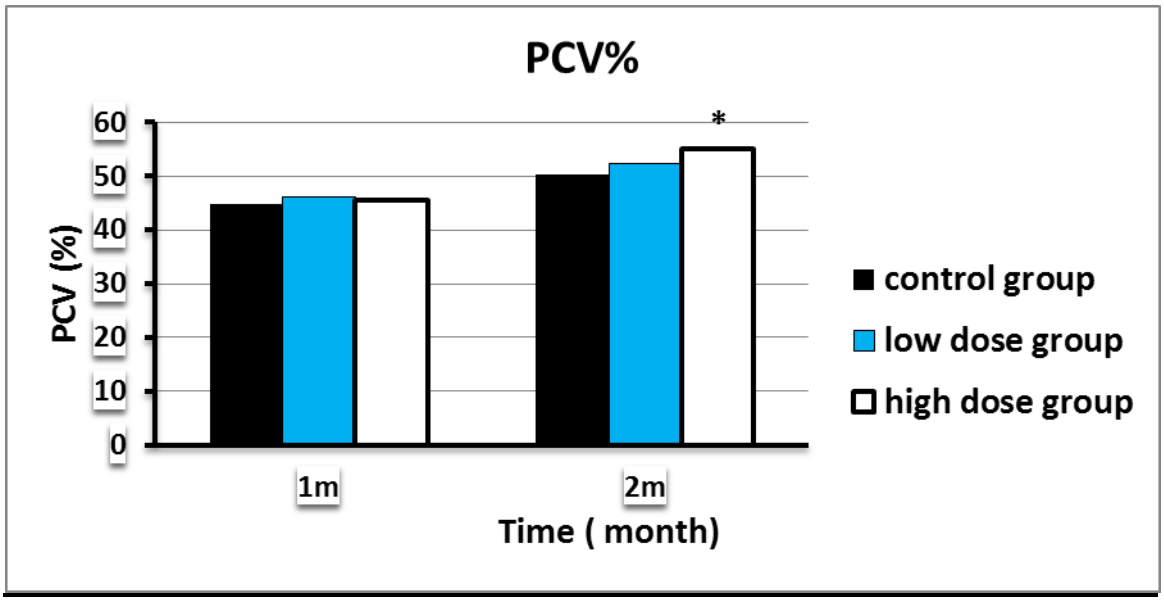

Fig. (8): Effect of low and high dose of propolis on packed cell volume in male rats after first and second month.

Effect of low and high dose of propolis on some haematological parameters RBCs, Hb and PCV in male rats after first and second month:

Data presented in (Table 2) and illustrated in Fig. (6, 7, 8) revealed that, red blood cells count was significantly increased by either low or high dose of propolis supplementation when compared to the control group during 1st and 2nd month.

Haemoglobin was significantly increased by either low or high dose of propolis supplementation when compared to the control group during 1st month. Moreover, during the 2nd month propolis supplementation in high dose resulted in significant increase in haemoglobin content when compared to the control group. Additionally, haemoglobin of low and high dose of propolis in the 2nd month was higher than those of the same groups at the 1st month.

Packed cell volume was significantly increased by high dose of propolis supplementation when compared to the control group at the 2nd month.

DISCUSSION

The present study was planned to investigate the role of propolis, one of the natural honeybee's products on immunity and some haematological parameters in male rats. The two selected doses of propolis were 750mg propolis/kg diet (low dose) and 1.5 g propolis/kg diet (high dose) supplemented for 2 months.

Effect of low and high dose of propolis on total, differential leucocytic count (lymphocytic and neutrophil %) and some immunological parameters in male rats after first and second month:

The obtained results revealed that, propolis increased total leucocytic count significantly when compared with control group. In addition, propolis increased both lymphocytic % and lymphocytic activity which was confirmed by the results of lymphocyte transformation test. These results concering percentage of lymphocyte and their functional activity coincided with previous results of **Orˇsoli´c *et al.*(2005);Orˇsoli´c *et al.*(2007); Sforcin (2007)** who reported that phenolic compounds of propolis may be associated with activation of macrophages that secreted cytokines regulating activities and functions of B, T and NK cells. These results were further documented by the increase in total IgG concentration in propolis groups.

These results coincided with **Orsi *et al.*, (2000); Murad *et al.*, (2002); Sforcin *et al.*(2002); Sforcin (2007)** who stated that propolis has a potent effect on different cells of the immune response. Also, the data obtained in the present study coincided with that obtained by **Sforcin *et al.* (2005)** who showed that propolis has a transit short immunostimulatory effect; however, the immunostimulatory effect reported in the present study lasted for 1 month.

The difference between the results of **Sforcin *et al.* (2005)** and those of the present study may be attributed to the difference in the route of propolis administration. On the other hand, the data obtained in the present study disagree with **You *et al.* (1998)** who stated that flavonoids have an immunosuppressive effect on the lymphoproliferative response.

The immunosuppressive effect reported in the former study may be due to usage of a single propolis component (flavonoids), while in the present investigation; whole propolis was used as supplement. Thus, the immunostimulatory effect recorded may be due to the other propolis component. The obtained results in present study revealed that, propolis led to decease in % of neutrophil; this was confirmed by the results of nitroblue tetrazolium test which is responsible for measuring oxidative radical production of neutrophil. These results coincided with previous results of **Sadik and Luster (2012); Moelants *et al.* (2013); Bueno-Silva *et al.***

(2016) who reported that propolis reduce TNF-α secretion which is considered an important cytokine related to neutrophil functions where it stimulated neutrophil migration, adhesion, rolling and transmigration. Propolis decreased the functional activity of neutrophil including respiratory burst activity. These results coincided with that obtained by **Cristina *et al.* (2007)** who stated that propolis decreased neutrophil/lymphocyte ratio.

Effect of low and high dose of propolis on some haematological parameters RBCs, Hb and PCV in male rats after first and second month:

Concerning haematological parameters, dietary supplementation of high dose of propolis for 2 months improved all parameters studied. This ameliorative effect of propolis on haematological parameters may be attributed to bioflavonoid and vitamin C content of propolis that enhanced iron absorption **(Haro *et al.*, 2000).** Dietary propolis supplementation improved digestive utilization of iron and Hb regeneration efficiency and consequently stimulated erythropoiesis**.** Moreover, **Suwalsky *et al.* (2008); Morireia *et al.* (2010)** stated that propolis administration decreased the osmotic fragility of erythrocytes this may be to phenolic compounds and flavonoids of propolis which stabilize the erythrocyte membrane by interaction with membrane phospholipids resulting in decreasing erythrocyte hemolysis in spleen. All of these factors were reflected on increasing red blood cells count.

The present results go hand by hand with those of **Cristina *et al.* (2007)** who showed that propolis supplementation increased Hb concentration which correlated positively with PCV. On the other hand, the present study contradicted with those of **Jasprica *et al.* (2007)** who showed that propolis supplementation did not affect either RBCs count or Hb content.

The difference between the results of **Jasprica *et al.* (2007)** and those of the present study may be attributed to usage of propolis extract in the above-mentioned study while in the present study raw propolis was used.

CONCLUSION

It is concluded that high dose of propolis (1.5 g/kg diet) for 2 months has an immunostimulatory effect via increasing lymphocyte % and consequently increasing antibody production and alleviate all haematological parameters studied (RBCs, Hb and PCV).

Thus, we recommend dietary supplementation of high dose propolis for 2 months.

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