

## Effect of propolis on sub-chronic phenol use-induced toxicity on the lymphatic system and complete blood cell count in rats

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### ABSTRACT

Phenol is naturally found in some food and animal wastes. It is used as a disinfectant, insecticide and in some pharmaceutical products. Propolis is a mixture of beeswax and resins. It has antimicrobial, anti-inflammatory, antioxidant and immunomodulatory effects. This study investigates the effect of propolis on sub-chronic phenol use-induced toxicity on the lymphatic system and complete blood cell count in rats by the assessment of serum immunoglobulin, total and differential blood cell count, and histopathological changes of thymus, spleen and lymph nodes. Eighty adult albino rats were divided into four groups; each group consists of twenty rats. The control group received water, the second group received phenol only, the third group received propolis and the fourth group received propolis with phenol for 90 days *via* gastric gavage. Phenol sub-chronic use led to toxicity manifestations such as a decrease in the serum immunoglobulin and blood cell count, disturbance in the differential white blood cell count, decrease in the body, thymus and spleen weights of rats associated with histopathological changes in thymus, spleen and lymph nodes that were improved by propolis administration. Concurrent use of propolis with phenol ameliorated its

toxicity on the lymphatic system and complete blood cell count.

**KEYWORDS:** phenol, propolis, lymphatic, blood cell

### INTRODUCTION

Phenol is a colorless or white solid aromatic organic hydrocarbon compound (monohydroxy derivative of benzene). It is known as a carboxylic acid that is moderately soluble in water and is commercially sold as a liquid. Furthermore, phenol is naturally found in some food, human and animal wastes; it is produced endogenously in the gut *via* aromatic amino acid metabolism [1]. In addition to this, it is also produced from other natural resources such as coal tar and petroleum and is used to synthesize resins and plastics. Phenol is believed to be a serious environmental and occupational hazard because of its common use as disinfectant, insecticide and in some pharmaceutical products such as antiseptic lotions, ointments, ear and nose drops; it is also used as a flavouring substance in food [2].

Phenol intoxication affects humans and animals leading to many harmful health effects such as nephrotoxicity, hematotoxicity, immunotoxicity, cytotoxicity and neurotoxicity based on the phenoxy-type radical production from phenol that has the ability to impair the epithelial cell membrane

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integrity [3]. Until now, the available data about phenol toxicity on the lymphatic, hematological and immunological systems is not adequate, and hence there is a necessity for understanding the effect of sub-chronic phenol use on these systems as an occupational and public health hazard [4].

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 and the specificity of the local flora at the site of collection. It has more than 300 of identified constituents such as plant resins, essential oils, pollen, balm, wax, minerals (zinc, copper, manganese, iron, potassium, calcium, sodium and selenium), vitamins (B<sub>1</sub>, B<sub>2</sub>, B<sub>6</sub>, A, C and E), proteins (amides, amines and amino acids) and a large number of unknown components [5]. Propolis has many beneficial biological effects such as antimicrobial, anti-inflammatory, hepatoprotective, anticancer and antioxidant. Therefore, it is used as a popular medicine in different regions of the world since ancient times till now [6].

The current study aims to investigate the effect of propolis on sub-chronic phenol-use induced toxicity on the lymphatic system and complete blood cell count in rats by the assessment of the immunoglobulin level, total and differential white blood cell count, red blood cell and platelet count, and by the evaluation of histopathological changes in spleen, thymus gland and lymph nodes.

## MATERIALS AND METHODS

Eighty healthy adult albino rats weighing 150-250 g were divided into four groups; each group comprising of twenty rats. The first group (control) received 0.5 ml of distilled water while the second group received 180 mg/kg/day of phenol (30% of oral LD50), dissolved in distilled water [7]. The third group received 90 mg/kg/day of propolis, dissolved in distilled water, while the fourth group received 180 mg/kg/day of phenol and 90 mg/kg/day of propolis, dissolved in distilled water. The daily administration of distilled water, phenol and propolis were done by gastric gavage for 90 days. Phenol (C<sub>6</sub>H<sub>5</sub>OH) was

present in the carbolic acid solution, with the molecular weight 94.11, which was obtained from Sigma-Aldrich Chemie GmbH. The oral LD50 of phenol in the rats was determined as 600 mg/kg based on the pilot study on the same species of the rats used in this experiment. Propolis was available in 400 mg capsule form that was manufactured by Sigma Pharmaceuticals Industries for International Business Establishment Co. (IBE Pharma). The propolis dose used in this study was 1% of oral LD50 in rats that was determined by Sartori *et al.*, [8] and Arvouet-Grand *et al.* [9]; it also represents 1% of oral LD50 of flavonoids in rats that are considered to be the primary constituent of propolis according to Burdock [10].

### Hematological studies

On the last day of the experiment, the rats were anaesthetized by diethyl ether. The blood samples were collected from the orbital sinus, using the heparinized capillary tubes. They were then placed in EDTA tubes for determining the red blood cell, total & differential white blood cell, and platelet counts that were performed using a quantitative, automated, differential cell counter for diagnostic use "the Beckman Coulter MAXM" [11].

### Measurement of quantitative serum immunoglobulin (Ig)

The blood samples were left at room temperature for 15-30 minutes to clot. The samples were centrifuged at 2000 rpm at 4 °C for 10 minutes to remove the clot and to separate the serum sample that was stored at -20 °C until the quantitative serum immunoglobulins assay. This assay was used for an antibody immunodeficiency evaluation by the total assessment of IgG, IgA, and IgM levels together using the rocket electrophoresis method as a quantitative electroimmunoassay [12].

### Histopathological studies

After 24 hours, following the last administration of phenol and propolis, the rats were weighed and sacrificed by cervical dislocation after being excessively anaesthetized. Chest and abdominal incisions were carried out, and thymus gland, spleen and lymph nodes were excised from the rats of the four groups for the weight measurement

and the histological studies. The tissue specimens were fixed in 10% neutral buffered formalin. The fixed specimens were trimmed, washed and dehydrated in the ascending grades of alcohol, cleared in xylene, embedded in paraffin, sectioned at 4-6  $\mu\text{m}$  thickness and stained with haematoxylin and eosin for an examination under a light microscope [13].

### Statistical analysis

Statistical analysis was performed using SPSS version 17. The data was expressed as mean  $\pm$  SD and the analysis was performed by using one-way ANOVA (Analysis of variance) and post-hoc multiple comparison tests (TUKEY) to investigate the difference between the parameters among the different groups where the *P* value of 0.05 was considered statistically significant.

### Ethical considerations

The most appropriate animal species were chosen for this research. Promotion of high standard care and animal well-being were exercised at all times. An appropriate sample size was calculated for using the fewest number of animals to obtain the valid results statistically. Painful procedures were performed under anesthesia to avoid any distress and pain that could be inflicted on the animals. Our standards of animal care and administration are consistent with the requirements and standards of international laws and regulations. The study was approved by the Institutional Research Ethics Committee.

## RESULTS

### The body, thymus, and spleen weight changes in the different rat groups

There was a statistically significant decrease in the body, thymus and spleen weights of rats in the second group (phenol) in comparison to the control group while the body, thymus and spleen weights of rats in the fourth group (phenol and propolis) had a statistically significant increase in comparison to the second group (phenol). The statistical significance was at  $p < 0.001$  (Table 1).

### The serum immunoglobulin level assay

There was a statistically significant decrease in the total serum immunoglobulin level of rats in the second group (phenol) in comparison to the control group while the total serum immunoglobulin level of rats in the fourth group (phenol and propolis) had a statistically increase in comparison to the second group (phenol). The statistical significance was at  $p < 0.001$  (Table 2).

### Hematological findings

#### A. Total blood cell count

There was a statistically significant decrease in the total count of white blood cell, red blood cell and platelet of the rats in the second group (phenol) in comparison to the control group while the total count of white blood cell, red blood cell and platelet of rats in the fourth group (phenol and propolis) had a statistically significant increase in comparison to the second group (phenol). The statistical significance was at  $p < 0.001$  (Table 3).

**Table 1.** Comparison between Mean  $\pm$  SD of the body, thymus and spleen weights in the different rat groups.

Group Parameter	First M $\pm$ S.D	Second M $\pm$ S.D	Third M $\pm$ S.D	Fourth M $\pm$ S.D	F
Body (gm)	184.45 $\pm$ 3.927	161.5 $\pm$ 3.635*	184.1 $\pm$ 4.025	172.2 $\pm$ 5.917**	120.554
Thymus (mg)	670.8 $\pm$ 6.872	614.9 $\pm$ 9.64*	670.45 $\pm$ 6.871	657.8 $\pm$ 5.406**	257.946
Spleen (gm)	2.44 $\pm$ 0.351	1.258 $\pm$ 0.1507*	2.456 $\pm$ 0.374	1.710 $\pm$ 0.119**	91.898

Number per group: 20; SD: standard deviation.

First group (control) received distilled water

Second group received 180 mg/kg/day of phenol.

Third group received 90 mg/kg/day of propolis.

Fourth group received 180 mg/kg/day of phenol and 90 mg/kg/day of propolis.

\* $P < 0.001$  (significant difference in comparison with the first group).

\*\* $P < 0.001$  (significant difference in comparison with the second group).

**Table 2.** Comparison between Mean  $\pm$  SD of the total serum immunoglobulin level in the different rat groups.

Group Parameter	First M $\pm$ S.D	Second M $\pm$ S.D	Third M $\pm$ S.D	Fourth M $\pm$ S.D	F
Serum Ig (ng/mL)	1469.93 $\pm$ 84.23755	1032.004 $\pm$ 19.69059*	1778.311 $\pm$ 85.58604	1589.619 $\pm$ 64.60446**	423.099

Number per group: 20; SD: standard deviation; Serum immunoglobulin: Ig.

First group (control) received distilled water.

Second group received 180 mg/kg/day of phenol.

Third group received 90 mg/kg/day of propolis.

Fourth group received 180 mg/kg/day of phenol and 90 mg/kg/day of propolis.

\*P < 0.001 (significant difference in comparison with the first group).

\*\*P < 0.001 (significant difference in comparison with the second group).

**Table 3.** Comparison between Mean  $\pm$  SD of the total blood cell count in the different rat groups.

Group Parameter	First M $\pm$ S.D	Second M $\pm$ S.D	Third M $\pm$ S.D	Fourth M $\pm$ S.D	F
WBCs	6.678 $\pm$ 0.6644	2.934 $\pm$ 0.1172*	12.153 $\pm$ 0.6023	8.593 $\pm$ 1.4499**	404.875
RBCs	4.636 $\pm$ 0.03880	3.082 $\pm$ 0.2148*	7.928 $\pm$ 0.4978	4.816 $\pm$ 0.3953**	548.723
PLAT	348.06 $\pm$ 17.982	67.8 $\pm$ 25.7878*	442.8 $\pm$ 11.8836	272.2 $\pm$ 45.2567**	639.414

Number per group: 20; SD: standard deviation.

WBCs: White blood cells; RBCs: Red blood cells; PLAT: Platelet.

First group (control) received distilled water.

Second group received 180 mg/kg/day of phenol.

Third group received 90 mg/kg/day of propolis.

Fourth group received 180 mg/kg/day of phenol and 90 mg/kg/day of propolis.

\*P < 0.001 (significant difference in comparison with the first group).

\*\*P < 0.001 (significant difference in comparison with the second group).

## B. The differential white blood cell count

There was a statistically significant increase in the count of lymphocytes, monocytes, eosinophils and basophils of the rats in the second group (phenol) in comparison to the control group. On the contrary, neutrophils were decreased significantly at the same time in comparison to the control group also. The count of lymphocytes, monocytes, eosinophils and basophils of the rats in the fourth group (phenol and propolis) had a statistically significant decrease in comparison to the second group (phenol) while neutrophils were increased significantly. The statistical significance was at  $p < 0.001$  (Table 4).

## Histopathological findings

### A. Histopathological findings in the thymus gland

The examination of thymus gland tissues in the control group shows normal histological structure (Figure 1A), but it shows necrosis, hemorrhage, lymphocytic depletion, fragmented nuclei and vacuolated cytoplasm in the second group (phenol) (Figure 1B). The thymus gland of the third group (propolis) displays approximately the same structure as the control group (Figure 1C) whereas the thymus gland of the fourth group (phenol and propolis) shows an injury improvement in comparison to the second group (phenol) (Figure 1D).

**Table 4.** Comparison between Mean  $\pm$  SD of the differential white blood cell count in the different rat groups.

Group Parameter	First M $\pm$ S.D	Second M $\pm$ S.D	Third M $\pm$ S.D	Fourth M $\pm$ S.D	F
Neutrophils	59.9 $\pm$ 5.6228	28.54 $\pm$ 6.2308*	52.5 $\pm$ 7.1279	66.86 $\pm$ 5.4928**	146.849
Lymphocytes	32.78 $\pm$ 5.1582	53.12 $\pm$ 5.0693*	41.022 $\pm$ 7.1366	28.14 $\pm$ 4.6101**	77.086
Monocytes	5.04 $\pm$ 0.8744	7.66 $\pm$ 2.4679*	5.41 $\pm$ 1.7919	3.54 $\pm$ 0.5092**	22.458
Eosinophils	1.64 $\pm$ 0.7611	4.432 $\pm$ 1.9131*	0.82 $\pm$ 0.3860	1.16 $\pm$ 0.6377**	45.276
Basophils	0.64 $\pm$ 0.3761	1.09 $\pm$ 0.5092*	0.248 $\pm$ 0.0617	0.5 $\pm$ 0.4401**	16.672

Number per group: 20; SD: standard deviation.

First group (control) received distilled water.

Second group received 180 mg/kg/day of phenol.

Third group received 90 mg/kg/day of propolis.

Fourth group received 180 mg/kg/day of phenol and 90 mg/kg/day of propolis.

\*P < 0.001 (significant difference in comparison with the first group).

\*\*P < 0.001 (significant difference in comparison with the second group).

### B. Histopathological findings in the spleen

The examination of spleen tissues in the control group shows normal histological structure (Figure 2A). The spleen tissues of the second group (phenol) show splenic blood sinuses congestion, fragmented nuclei and vacuolated cytoplasm within the red and white pulps, hemorrhage, lymphocytic necrosis and depletion (Figure 2B). On the other hand, the spleen tissues in the third group (propolis) show normal spleen configuration simulating the control group (Figure 2C). The spleen tissues in the fourth group (phenol and propolis) show an improvement in the spleen lesions in comparison to the second group (phenol) (Figure 2D).

### C. Histopathological findings in the lymph nodes

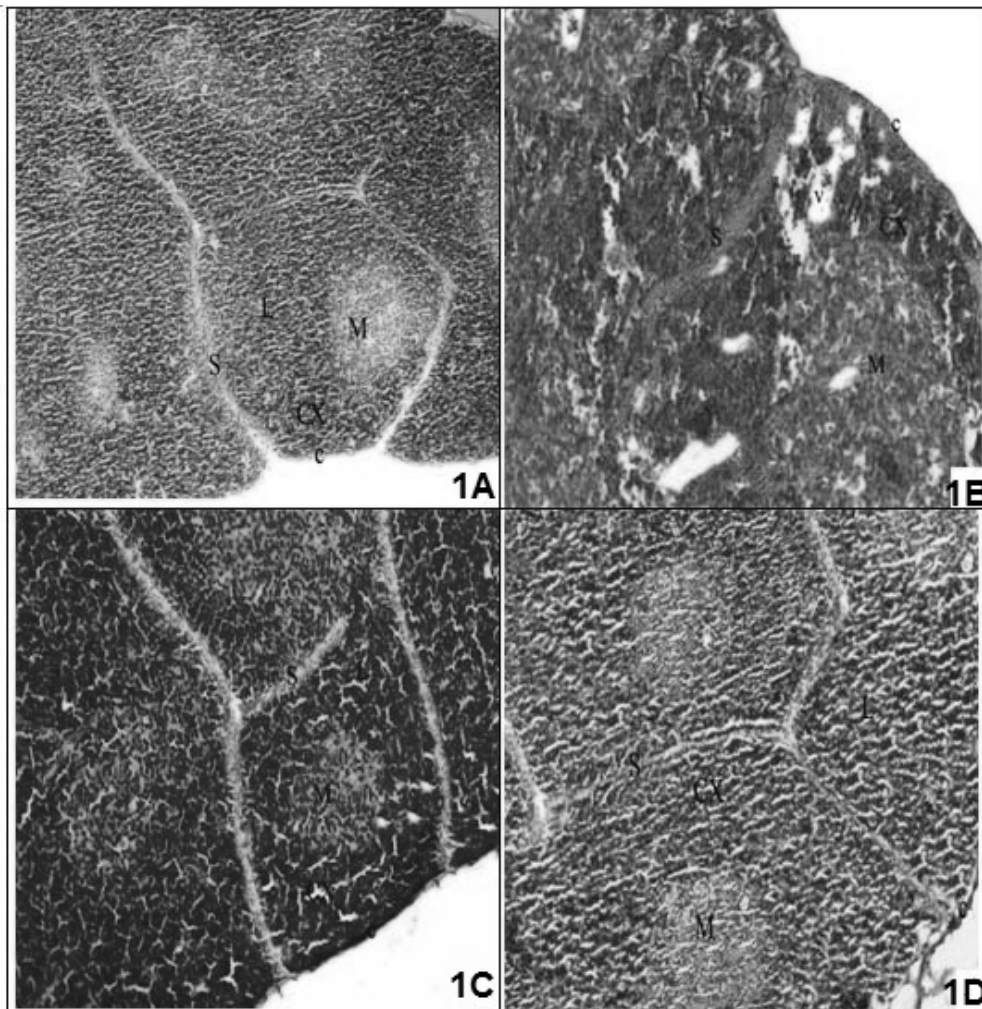
The examination of lymph nodes in the control group reveals entirely normal histological features, as illustrated in (Figure 3A), while the lymph node sections of the second group (phenol) show the distortion of lymph node architecture, dilatation and congestion of blood vessels, neutrophil and lymphocyte infiltration, macrophage proliferation and vacuolated cytoplasm within the medulla, lymphoid depletion and focal hemorrhage (Figure 3B). The lymph nodes of the third group (propolis) show near to normal appearance similar to the control group (Figure 3C). The lymph

nodes of the fourth group (phenol and propolis) show an improvement in the lesions whereas the histological structures are approximately normal in comparison to the second group (phenol) (Figure 3D).

### DISCUSSION

The risk of phenol intoxication to public health has increased in the recent years. It is considered to be one of the most common household poisoning where it is used widely as a disinfectant and insecticide [14]. Therefore, this study attempts to investigate the toxicity of sub-chronic phenol use on the lymphatic system and complete blood cell count and in addition evaluate the propolis effect on the amelioration of this toxicity.

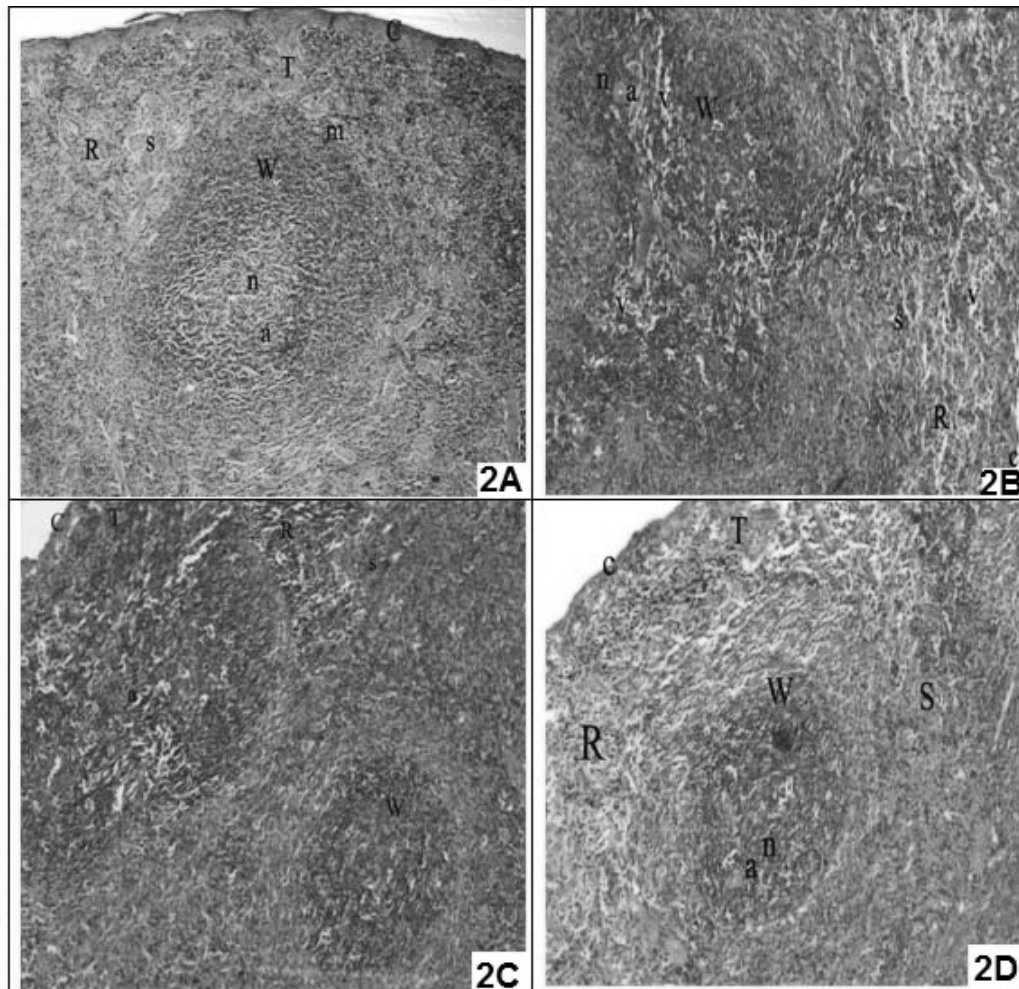
The current study showed that there is a significant decrease in the body, thymus and spleen weights of rats in the second group (phenol) in comparison to the control group which is in agreement with Monfared *et al.* [7] who observed the toxic effect of phenol on the cellularity of immune organs causing atrophy and weight reduction. On the other hand, White *et al.* [15] indicated that phenol does not have any toxic effect on the weight of spleen and thymus gland. There is a significant gain in the body, thymus and spleen weights of rats in the fourth group (propolis with phenol)



**Figure 1.** **A:** Photomicrograph of a section in the control rat thymus gland showing normal appearance of capsule (c) and septa (S) with incomplete lobules (L), dark peripheral cortex (CX) and light central medulla (M), (H&E X 80); **B:** Photomicrograph of a section in the second group rat thymus showing degeneration in the cortex and medulla, lymphocyte infiltration (L), vacuolated cytoplasm (v) in the peripheral cortex (CX) and medulla (M), irregular thickening of the capsule (c) and septa (S), (H&E X 80); **C:** Photomicrograph of a section in the third group rat thymus gland showing normal capsule (C) and septa (S) with normal lobes and incomplete lobules (L), dark peripheral cortex (CX) and light central medulla (M), (H&E X 80); **D:** Photomicrograph of a section in the fourth group rat thymus gland showing nearly normal appearance of capsule (c), septa (S), incomplete lobules (L), peripheral cortex (CX) and central medulla (M), (H&E X 80).

because the antimicrobial activity of propolis causes a better intestinal health, and then the digestion and absorption are improved which is consistent with Denli *et al.* [16]. On contrary, our results are not in agreement with Koya-Miyata *et al.* [17] who reported that propolis does not cause the weight gain although it is considered to be one of the nutritional supplements.

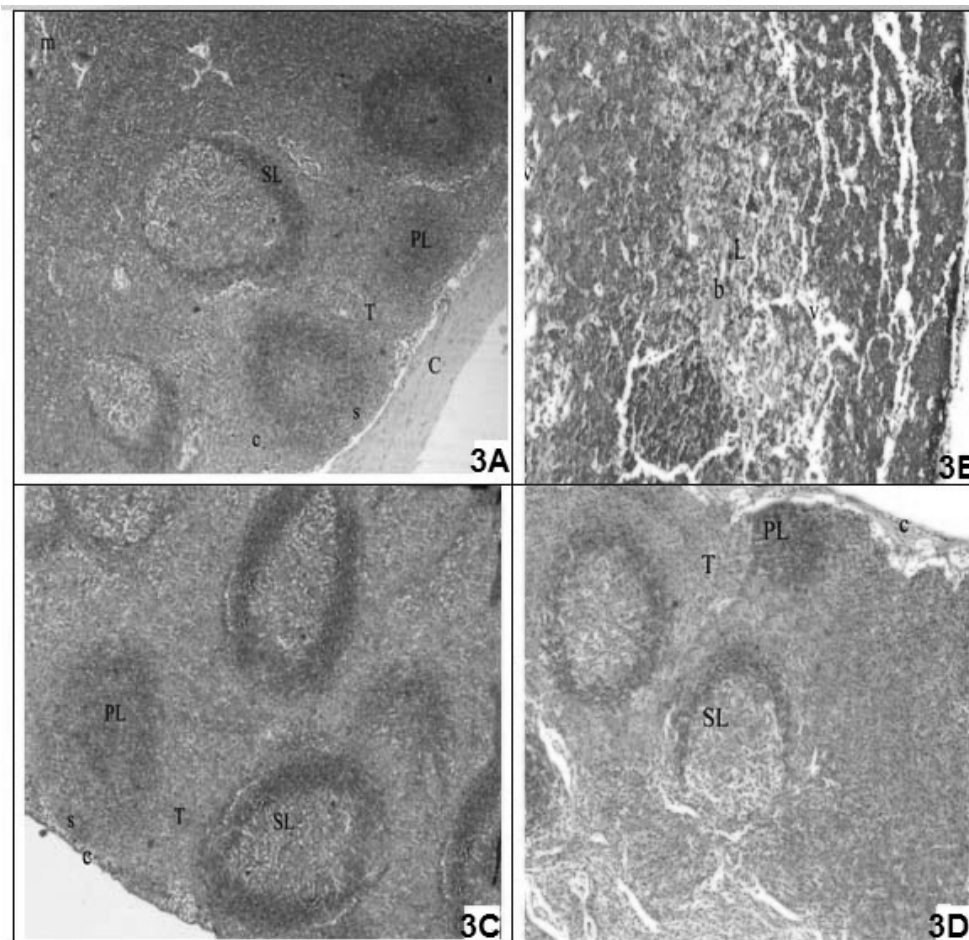
Our results showed a statistically significant decrease in the total serum immunoglobulin level and the blood cell count (platelets, white and red cells) in the second group (phenol) in comparison to the control group. In contrast with our results, Hsieh *et al.* [18] reported that phenol intoxication does not have any significant effect on the total white blood cell and platelet count but they also



**Figure 2.** **A:** Photomicrograph of a section in the control rat spleen showing normal capsule (C), trabeculae (T), red pulp (R) and white pulp (W); central artery (a) of white pulp is surrounded by lymphoid nodule (n) and the marginal zone (m), the splenic cord is separated by the splenic blood sinuses (s) in the red pulp (H&E X 80); **B:** Photomicrograph of a section in the second group rat spleen showing capsule (C), congestion of the red pulp (R) and splenic blood sinuses (s), congested thick wall of the central artery (a) in the white pulp (W), lymphoid nodule (n) with vacuolated cytoplasm (v) within the white and red pulps (H&E X 80); **C:** Photomicrograph of a section in the third group rat spleen showing normal appearance of capsule (C), red pulp (R) and white pulp (W), normal appearance of central artery (a) in the white pulp that is surrounded by lymphoid nodule (n) and the marginal zone (m), the splenic cord of the red pulp is separated by splenic blood sinuses (s) (H&E X 80); **D:** Photomicrograph of a section in the fourth group rat spleen showing nearly normal appearance of capsule (C), trabeculae (T), red pulp (R) white pulp (W), central artery (a), lymphoid nodule (n), marginal zone (m) and the splenic blood sinuses (s) in the red pulp (H&E X 80).

stated that the red blood cell count and the total serum immunoglobulin level are decreased at the same time consistent with the current study. Furthermore, White *et al.* [15] indicated that phenol toxicity does not have any significant effect on the total blood cell count and the serum

immunoglobulin level. Santa *et al.* [19] explained that the reduction in the erythrocytes count depends on the ability of phenol to destruct RBCs based on their interference with the oxidative phosphorylation, and thus the storage of the cell energy in the adenosine triphosphate molecules is



**Figure 3.** **A:** Photomicrograph of a section in the control rat lymph nodes showing normal capsule (C), trabeculae (T), subcapsular (s), cortical (c) and medullary (m) lymph sinuses, primary lymphoid nodule (PL) and the secondary lymphoid nodule (SL) in the outer cortex (H&E X 80); **B:** Photomicrograph of a section in the second group rat lymph nodes showing degeneration and absence of germinal centers in the cortex, blood vessels congestion (b), lymphocytes infiltration (L) with vacuolated cytoplasm (v) within the cortex and medulla (H&E X 80); **C:** Photomicrograph of a section in the third group rat lymph nodes showing normal appearance of capsule (c), subcapsular (s) and trabeculae (T), normal primary lymphoid nodule (PL) and the secondary lymphoid nodule (SL) with normal cortical lymph (c) (H&E X 80); **D:** Photomicrograph of a section in the fourth group rat lymph nodes showing nearly normal appearance of lymphoid cells, capsule (c) and trabeculae (T), normal primary lymphoid nodule (PL) and the secondary nodule (SL) in the outer cortex (H&E X 80).

prevented producing a disturbance in the osmotic equilibrium across the cell membranes causing a hemolysis, which is in agreement with the opinion of Michałowicz *et al.* [20]. In the contrasting context, Zaki *et al.* [21] reported that the red blood cell count is increased significantly by phenol intoxication.

The present study demonstrated that there is a statistically significant increase in all differential

white blood cell count (Lymphocytes, monocytes, eosinophils and basophils) in the second group (phenol) in comparison to the control group except for neutrophils that are decreased significantly. On the contrary, Iwata *et al.* [22] & Yao and Hou [23] reported that the phenol intoxication causes a decrease in the lymphocyte count as a result of the modulation of the gene expression of mRNA formation leading to an apoptosis associated with



the proliferation inhibition of thymocyte and lymphocyte. According to Hammam *et al.* [24], the hematological changes from phenol intoxication may be due to the reactive oxygen species that affect the substrates and lead to the deoxyribose degradation. Furthermore, the opinion of Baj *et al.* [1] is in agreement with our results for the increase in monocyte and eosinophil count but they also reported a decrease in the lymphocyte count in contrast with our results.

The current study showed many histopathological changes in the thymus gland, spleen and lymph nodes in the second group (phenol) in comparison to the control group; the thymus gland showed necrosis, hemorrhage, and lymphocytic depletion while the spleen showed blood sinuses congestion, hemorrhage, lymphocytic necrosis and depletion within the red and white pulps. The lymph nodes displayed a distortion in the architecture, neutrophil and lymphocyte infiltration with focal hemorrhage, macrophage proliferation, and lymphoid depletion. This is consistent with Monfared *et al.* [7] who indicated that phenol intoxication causes toxic histopathological changes in the thymus gland, spleen, and lymph nodes because phenol is considered to be the primary metabolite of benzene; it is hydroxylated in the liver producing the catechol that is converted into  $\alpha$  benzoquinone which is the toxic metabolite responsible for the phenol intoxication mechanism. In another context, Maleki *et al.* [25] confirmed that the toxicity of metabolites which are formed during the breakdown of phenol is lower than the toxicity of phenol itself, which is in accordance with Shadnia and Wright, [26]. It should also be noted that the free radical generation and hydrophobicity of phenol may be responsible for the phenol intoxication, which is in accordance with Abd Gami *et al.* [27]. On the contrary, Ryan *et al.* 2001 [28] stated that the phenol intoxication does not cause any alteration in the histological structure of these organs.

The concurrent use of propolis with phenol in the fourth group revealed marked amelioration in all histopathological changes in the thymus gland, spleen and lymph nodes besides an increase in the serum immunoglobulin level and the total blood cell count in comparison with the second group (phenol). There is also a statistically significant

decrease in all differential white blood cell count (Lymphocytes, monocytes, eosinophils, and basophils) in the fourth group after propolis administration with phenol except for neutrophils that increased significantly but these changes in the differential white blood cell count were within the normal limit. According to Sforcin *et al.* [29], propolis has an immunomodulatory action counteracting the toxic histopathological manifestations of phenol in the thymus gland, spleen and lymph nodes. Propolis also ameliorates other changes in the total serum immunoglobulin level and the blood cell count because it is considered as an antioxidant that can stimulate the antioxidant enzymes such as catalase, peroxidase and superoxide dismutase to reduce and prevent the oxidative damage of phenol, which is consistent with Daleprane and Abdalla [30] who confirmed that the antioxidant activity of propolis depends on the flavonoids which are capable of scavenging the free radicals and prevent the lipid peroxidation. Moreover, Sforcin *et al.* [29] reported that the flavonoids have anti-inflammatory activities, and tissue strengthening and regenerative effects to improve the lesions in the damaged organs.

## CONCLUSION

The sub-chronic use of phenol may induce toxicity on the lymphatic system and complete blood cell count that are manifested by hematological and serum immunoglobulin abnormalities associated with histopathological changes in the thymus gland, spleen and lymph nodes. Propolis may ameliorate the toxic effect of phenol on the lymphatic system and complete blood cell count based on its antioxidant properties. Further research in humans is recommended in order to verify our results using different doses of propolis for the different periods to complete this work.

## CONFLICT OF INTEREST STATEMENT

There is no potential conflict of interest with respect to the research, authorship, and the publication of this article.

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