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Haematological Parameters Alternations Resulted by Exposing Albino Mice to Shisha Smoke

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Abstract

In this study, effects of shisha smoking on some blood components were evaluated in (120) male adult mice. The animals were divided into four groups according to smoke types (cigarette, shisha, cigarette +shisha) in addition to control groups which exposed to fresh air only. A special inhalation chamber designed locally has been used to expose the animals. Exposed to cigarette smoke was done for 5 min/day while exposed to shisha smoke for 15 min /day /7days/weeks for 4,8,12 weeks. Blood samples were collected to evaluate some hematological variables. The results showed that RBC, WBC count, Hb, PCV, differential leukocyte (Lymphocyte, Monocyte, Neutrphils) were significantly high ($P \leq 0.001$) in groups which exposed to shisha smoke as compared with those exposed to cigarette smoke. These values were increased with increasing of exposure periods and become clearer in groups exposed for 12 weeks. It was concluded that shisha smoke contains high concentrations of toxicants as compared to cigarette smoke, and cause clear increase in some blood parameters that may led to cardiovascular disease.

Keywords: shisha, hematology, mice.

التغيرات الحاصله في خصائص دم الفئران البيض الناتجه من التعرض لدخان الشيشه

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الخلاصه

في هذه الدراسه، تم تقييم تاثيرات تدخين الشيشه على بعض مكونات الدم في (١٢٠) من ذكور الفئران البالغه. قسمت حيوانات التجربه الى اربعه مجاميع طبقا الى نوع الدخان الذي تتعرض له (دخان سكائر، دخان شيشه ،دخان سكائر+دخان شيشه) فضلا عن مجموعه السيطره التي كانت تتعرض للهواء النقي فقط . عرضت حيوانات التجربه الى الانواع المختلفه من الادخنه باستخذام حجره تعرض استنشاقي مصنعه محليا. كان التعرض لدخان السكائريتم خلال ٥ دقائق في/اليوم اما التعرض لدخان الشيشه فكان يستمر ل ١٥ دقيقه/اليوم ولسبعه ايام /اسبوع ولثلاث فترات تعرض ١٢،٨،٤ اسبوع.جمعت عينات الدم من الحيوانات المعرضه لتقدير بعض متغيرات الدم فيها.اظهرت النتائج ان عدد خلايا الدم الحمر وخلايا الدم البيض وتركيز الهيموغلوبين وحجم الخلايا المرصوص فضلا عن العدد التمايزي في كل من خلايا الدم البيض (الخلايا اللمفيه،الخلايا الوحيده،الخلايا العدله) اظهرت زياده معنويه عاليه ($P \leq 0.001$) في الحيوانات المعرضه لدخان الشيشه مقارنه بالحيوانات المعرضه لدخان السكائر كما سجلت هذه المتغيرات قيم عاليه بزياده فتره

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التعرض لمختلف الادخنه وظهرت الزياده بشكل واضح في المجاميع المعرضه لفتره ١٢ اسبوع. استنتج من هذه الدراسه ان دخان الشيشه يحتوي تراكيزعاليه من الملوثات السامه عند مقارنته بما يحتوي دخان السكائر من هذه الملوثات وان دخان الشيشه يسبب زياده في بعض معايير الدم التي ربما تقود الى الامراض القلبيه الوعائيه.

Introduction

Shisha (water-pipe) smoking is method of tobacco consumption in many parts of the world. It has been claimed that more than 100 million people worldwide smoke shisha daily [1]. Recently shisha cafes and waterpipe tobacco smoking have been growing in popularity in the Iraq. Shisha smoking has become more accepted and widely used among young smokers, especially university and high school students. This increasing of shisha use could be due to the misconception that shisha smoking is less harmful and less addictive than cigarettes [2-4].

Alterations in haematological parameters may be important pathophysiological determinants of the high risk of vascular disease in smokers. Cigarette smokers, in comparison with non-smokers, show increases in many haematological variables, including haemoglobin concentration (Hb), , red cell count (RBC), white cell count (WBC) and neutrophils, lymphocyte, eosinophil and monocyte counts. It has been demonstrated that subjects with 'smokers' may have a fall in Hb within a few days of cessation of smoking.

Tobacco Smoking has both acute and chronic effect on hematological parameters. During past decade, it was suggested that cigarette smoking affect the blood characteristics as well that leads to death and there is relation between smoking and white blood cell count has been well established [5].
In a number of studies, it has been found that smokers have higher white blood cell counts than nonsmokers [6]. Although in some earlier studies relationship between smoking and red blood cell was found in smokers and some scientists suggested that increase in hemoglobin level in blood of smokers could be a compensatory mechanism. However, some were of view that smoking does not increase in hemoglobin level in all smokers and this relates to tolerance potential of individual to different kind of diseases. Moreover, episodic duration of smoking and age of individual might have changed the adverse effects of smoking on blood characteristics of human being [7].Other study achieved by [8] in which Animals were exposed to cigarette, or waterpipe smoke using whole body exposure system one hour daily for 7 days as acute exposure. results refers that both cigarette and waterpipe smoke exposure caused elevation of total white blood cell count, as well as absolute count of neutrophils, and lymphocytes compared to control group.

Materials and Methods

The experiments carried out on 120 un-exposed healthy mature male Albino mice (*Mus musculus*). Their age was 7 to 8 weeks old. Mice were divided randomly into 12 groups; each group includes 10 animals for the exposed and control groups. According to inhalation exposure periods the 12 groups of animals were divided in to three groups each of them has four subgroups as it shown in table-1.

Table 1-Groups of animals in experimental design F: fresh air, S: Shisha smoke, C: cigarette smoke.

Group No.	Exposure period in weeks	Number of animals in each subgroups according to the type of exposure			
		Control (F)	Shisha smoke (S)	Cigarette smoke(C)	Shisha and cigarette smoke(S+C)
1	4	10	10	10	10
2	8	10	10	10	10
3	12	10	10	10	10

Whole body inhalation exposure was carried out using a locally made glass chamber of 80Lt. capacity. The inhalation exposure unit is a dynamic system, whole-body chamber Designed according to the WHO specifications [9, 10]**.** Animal groups were exposed to shisha smoke and cigarette smoke according to the following regime: the Subgroup (S) and subgroup (C+S) that belongs to group (1) as it mentioned in the experimental design, table -1, were exposed to shisha smoke for 15minute/ day, seven day/week.

Exposure done by burning 10 gm of Moassel using ordinary coal in shisha head. Electronic pumps switching controller was operated led to switching on the input pump, the smoke generated as puffs and transported through the parts of shisha, then fed to the inhalation chamber, where animals grouped in small cages. The one puff of smoke is almost equal to 10L in each operation cycle. After the end of exposed each of subgroups(S) and (C+S) to Shisha smoke, the subgroup(S) was removed from inhalation exposure chamber and replaced with subgroup(C) in order to started to exposed to cigarette smoke .The Cigarette smoking experiment was performed, seven days a week. The Subgroup (C) and subgroup (C+S) that belongs to group (1) were exposed to the smoke of 5 cigarettes for 5-7 minutes for each once as it recommended by the reference [11]. The main and side stream smoke was generated by burning cigarettes in a smoking box. The cigarettes were lightened and left to glow then the produced smoke was drawn into the chamber as puff where the mice placed inside it. While the subgroup (F) control group were exposed to fresh air only.The groups 2 and 3 followed the same inhalation exposure procedures of group 1, but continuously for 8 and 12 week respectively.

Blood samples for hematological analyses were collected from animals by heart puncture and placed into EDTA containing tubes, to determine the following:
Erythrocyte (RBC), leukocyte (WBC) Hemoglobin (Hb), Packed Cell Volume (PCV), leukocyte differentiation. RBC and WBC determined with standard methods in an improved Neubauer hemocytometry [12] .Microhematocrit method was used to determine the percentage (%) of PCV by using capillary tubes and microhematocrit centrifuge (12000g/min) for 5 min [13]. the hemoglobin concentrations estimated using Cyanmethemoglobin method (Drabkins method) using spectrophotometer wave length (540 nm) [14]. Blood films were made immediately after blood withdrawal. On blood films 100 leukocytes per animal were differentiated under light microscope, and concentrations of different leukocyte types per µl of blood were calculated by WBC per µl x % value of the given cell type / 100 [15].

Statistical Analysis

The obtained data of all experiments examining various variables were subjected to biometrical analysis using different tests such as Analysis of Variance (ANOVA) and Least Significant Differences (LSD).

Results and discussion

Blood test alternations

Table-2 shows mean value ± SD of RBC, WBC, Hb, PCV, neutrophils, lymphocyte and monocyte variables measured in experimental animals after being exposed to smokes released by cigarette tobacco, shisha ma'sal, cigarette tobacco & shisha ma'sal and control ample after 4, 8 and 12 weeks.

Table 2-Mean value ± SD of blood variables measured in experimental animals exposed to different types of smokes and control sample after 4, 8 and 12 weeks.

Blood variable	Mean ± SD											
	Cigarette			Shisha			Cigarette + Shisha			Control		
	4W	8W	12W	4W	8W	12W	4W	8W	12W	4W	8W	12W
RBC mm3	3407142 ±39460	3465714 ±168904	3800000 ±76594	3512857 ±102585	3715714 ±103417	3911428 ±122940	3805000 ±145979	4312857 ±263167	4318571 ±843394	3588750 ±516151	3450000 ±406890	2997143 ±244316
WBC mm3	7100 ±541	9685 ±371	8285 ±452	7757 ±844	12271 ±349	10871 ±287	7683 ±788	7942 ±725	8075 ±399	5550 ±481	5766 ±796	5471 ±430
Hb g/dl	9.19 ±1.4	10.6 ±0.91	12.3 ±1.1	12.8 ±0.7	12.2 ±2.73	15.6 ±2.72	13.6 ±2.8	14.7 ±2.02	15.5 ±4.5	11.03 ±0.3	11.1 ±0.28	10.3 ±1.23
PCV %	0.296 ±0.01	0.32 ±0.02	0.34 ±0.02	0.36 ±0.03	0.33 ±0.06	0.38 ±.0.03	0.36 ±0.013	0.41 ±0.03	0.38 ±0.06	0.32 ±0.02	0.33 ±0.02	0.33 ±0.03
Neutrophils%	0.119 ±0.01	0.126 ±0.01	0.141 ±0.04	0.143 ±0.01	0.149 ±0.01	0.154 ±0.005	0.128 ±0.015	0.163 ±0.047	0.324 ±0.05	0.13 ±0.01	0.13 ±0.01	0.133 ±0.01
Lymphocyte%	0.69 ±0.05	0.77 ±0.07	0.78 ±0.07	0.79 ±0.08	0.82 ±0.03	0.87 ±0.03	0.81 ±0.04	0.816 ±0.06	0.849 ±0.04	0.67 ±0.08	0.68 ±0.11	0.723 ±0.09
monocyte%	0.016 ±0.005	0.03 ±0.01	0.05 ±0.01	0.021 ±0.007	0.034 ±0.02	0.047 ±0.01	0.047 ±0.01	0.037 ±0.011	0.059 ±0.007	0.02 ±0.01	0.02 ±0.01	0.02 ±0.003

For RBC blood content, mean values have been found to vary from lowest value of 2997143±24431 mm^3 in control sample after 12 weeks to highest mean of 4318571±84339 mm^3 in blood sample of animal exposed for smokes induced by the combination of cigarette smoke and shisha smoke after 12 weeks, Figure-1.

In general, the control samples were recorded lowest mean values followed by those subjected to cigarette smoke and followed by sample of animals exposed to shisha smoke while highest mean values were found in sample exposed to the combination of cigarette smoke and shisha smoke.

Analysis of variance of these data shows significant effects of smoke sources and exposure periods ($P \leq 0.001$) upon mean values of RBC of examined samples. Least significant value (4239.72 mm^3) gives clear comparisons between all values. The alterations in hematological parameters serve as the earliest indicator of toxic effects on tissue [16].

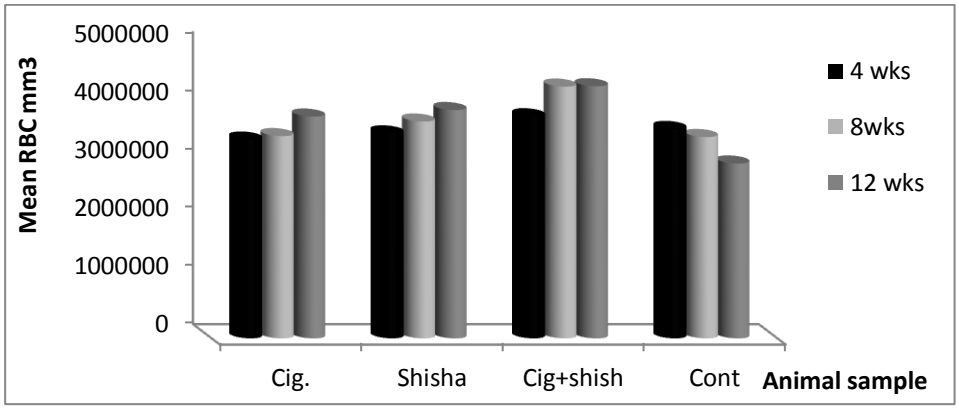

Figure 1- Mean RBC mm3in experimental animals subjected to smoke generated by cigarettes, shisha, cig & shisha and control sample after 4, 8, and 12 weeks.

Regarding WBC, the mean value was found to vary from 5471±430 mm^3 found in control sample after 12 weeks to 12271± 349 mm^3 in blood sample of animal exposed to shisha smoke after 8 weeks Figure-2.generally, control samples appears with mean value lower than those of all examined samples .Analysis of variance of these results show highly significant ($P \leq 0.001$) effects of both the source of smoke and periods of exposure upon mean value of blood WBC. LSD value (204.48 mm^3) gives obvious differences between these data.

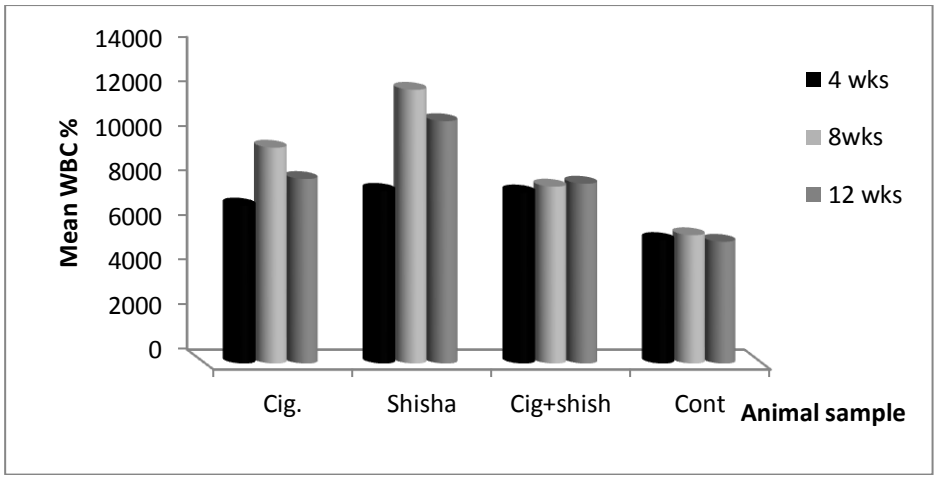

Figure 2-Mean WBC mm3in experimental animals subjected to smoke generated by cigarettes, shisha, cig & shisha and control sample after 4, 8, and 12 weeks.

In case of Hb, the mean values were found to range from 9.19±1.4 g/dl in animal blood exposed to smoke of cigarette tobacco after 4 weeks to 15.6±2.72 g/dl in those subjected to smoke of shisha moassel after 12 weeks, Figure-3. In general, control sample gave mean value lower than those of all examined sample except for that of blood sample recorded in experimental animal subjected to smoke of cigarette tobacco after 4 weeks.

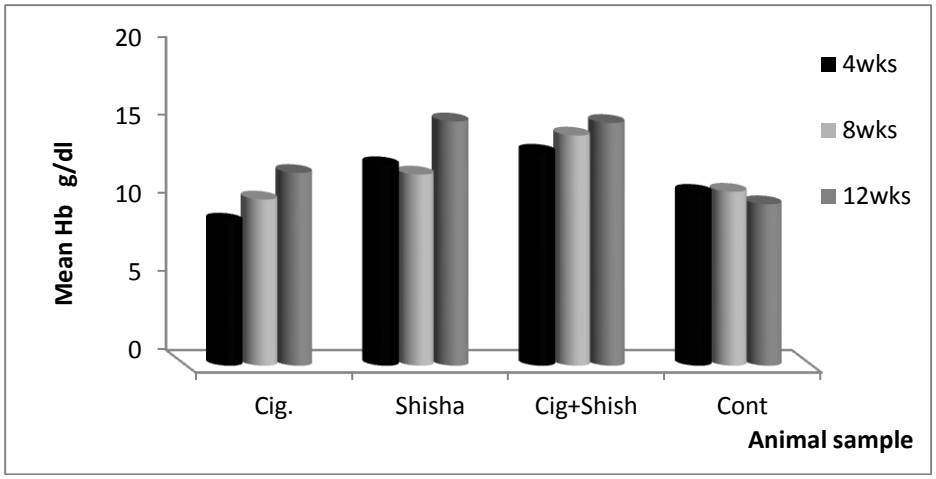

Figure 3- Mean Hb (g/dl) in experimental animals subjected to smoke generated by cigarettes, shisha, cigarette & shisha and control sample after 4, 8, and 12 weeks.

Analysis of variance of these data shows significant impact (P≤0.001) of smokes generated by cigarette & shisha smoke and also the period of exposure of experimental animals had significant influences on blood Hb. Least significant value (2.232 g/dl) gives clear comparisons between all values.

The results of present study agreed with a previous study achieved by Miri-Moghaddam et al., [17] in which they studied the effects of water pipe smoking on hemoglobin in rats. The results indicated that Hb count in chronic exposure period (12 week), were significantly higher, in rats that exposed to WP smoking (17.1g/dl) as compared with control group (15.1 g/dl).

Also Eisen and Hamond [18] observed that Hb concentrations were higher during the period of cigarette smoking than during the period of resting, and similar effects were observed in non-smokers after they had smoked six cigarettes.

Elevated levels of Hb, PCV are correlated with increased numbers of RBCs, and RBC count values were significantly high in shisha smoking [17].

In case of blood PCV, the results of present study has shown that mean value varied from 0.296±0.01% in animal blood exposed to smoke of cigarette tobacco after 4 weeks to 0.41±0.03% in blood sample subjected to smokes released in combination of cigarette tobacco and shisha ma'sal after 8 weeks ,Figure-4 ,table-2.

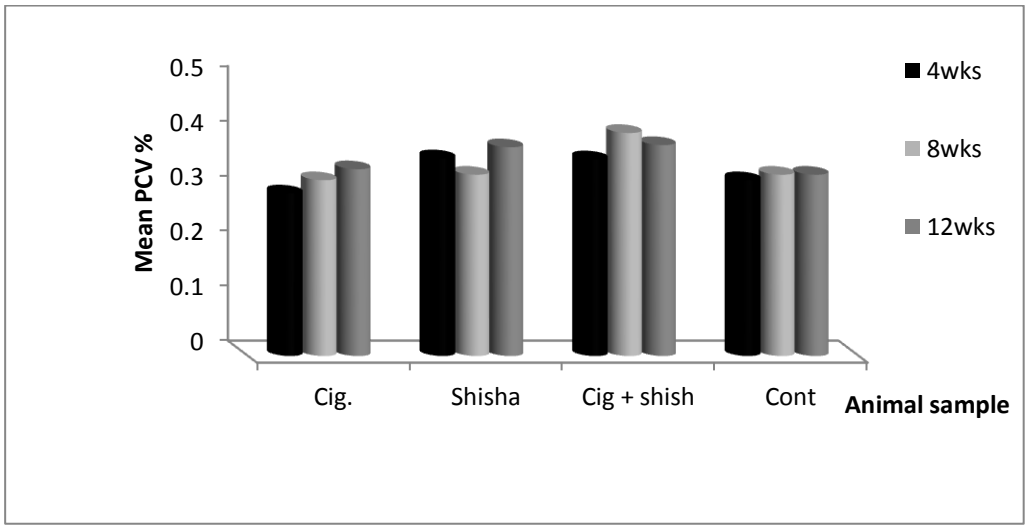

Figure 4-Mean PCV in experimental animals subjected to smoke generated by cigarettes, shisha, cig +shisha and control sample after 4, 8, and 12 weeks.

However, analysis of variance of these data shows high significant (P≤0.001) impacts of smokes generated by cigarette tobacco, shisha ma'sal and the combination of cigarette tobacco and shisha

ma'sal. Also similar significant impacts (P≤0.001) of exposure periods have been recorded on these values. However, LSD value (3.542 %) gives obvious differences between these data.

Obviously data of animal samples exposed to smoke released by the combination of cigarette smoke and shisha smoke had the highest values while the samples subjected to both cigarette smoke and shisha smoke gave mean values higher than those of control samples ,Figure-4.

The results of the present study agreed with previous study mentioned above ,the researchers observed that packed cell volume PCV, red blood count RBC concentration were higher during smoking period [18].

Higher levels of hemoglobin have been demonstrated in smokers, and these increases are likely to be compensatory for exposure to CO [7].

A person that smokes one or two pack of cigarettes per day raises a blood CO level to 20 PPM while the normal level of carbon monoxide (CO) in the blood stream is less than 8 PPM [19].

Approximately 80-90 % of the absorbed CO binds with hemoglobin to form carboxyhaemoglobin (COHb), which is specific biomarker of exposure in blood [20]. When body tissues do not receive a continuous and adequate supply of oxygen, they starve and begin to suffocate, malfunction, and finally die. Oxygen affects RBC membranes because they have more polyunsaturated fatty acids than other body tissues [21].

Nicotine leads to 13.8% increase in erythrocytes membrane oxidation at the highest concentration and its effects are dose-dependent [22], the combination of CO in tobacco with effects of nicotine disrupts oxygen delivery to tissue and stimulates the bone marrow to produce more RBCs and thereby increase Hb and PCV [23].The results in our study refers to high mean values of CO, Nicotine during exposure to shisha smoke ,these high concentrations may the essential reason for increased values of Hb , PCV blood parameters. Regarding WBC neutrophils, mean value was found to vary from 0.13±0.01% found in control sample after 4 weeks to 0.324±0.05% in blood sample of animal subjected to smoke released by the combination of cigarette tobacco and shisha ma'sal after 12 weeks ,Figure-5. It seems clearly that control sample had mean value almost constant and much lower than those of other samples under test.

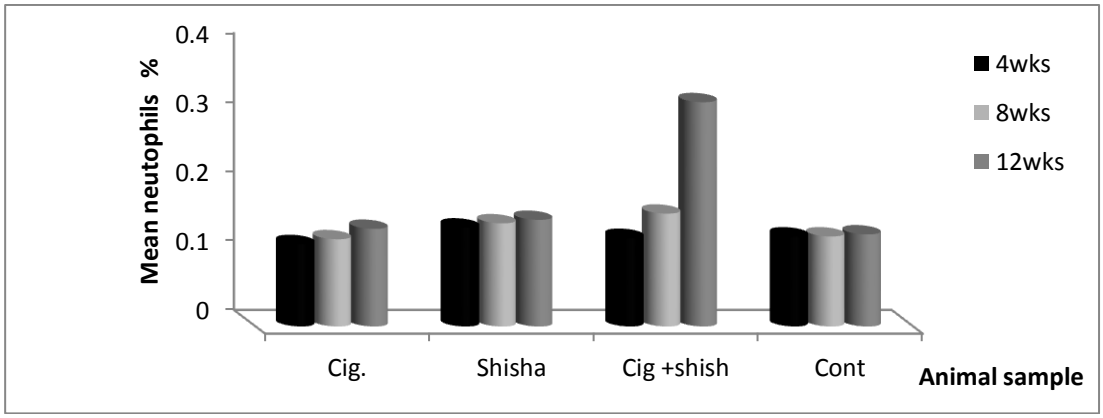

Figure 5-Mean neutrophils % in experimental animals subjected to smoke generated by cigarettes, shisha, cigarettes & shisha and control sample after 4, 8, and 12 weeks.

Analysis of variance of these results show highly significant effects (P≤0.001) of different smoke sources and various exposure periods upon the mean value of blood neutrophils. Also, the value of LSD test (2.608 %) shows clear significant differences between some of these data. For lymphocyte blood content, mean values have been found to vary from lowest value of 0.67±0.08% in control sample after 4 weeks to highest mean of 0.849±0.04 % in blood sample of animal exposed for smokes induced by the combination of cigarette tobacco and shisha ma'sal after 12 weeks, Figure-6 .In general, lowest mean values were recorded in control samples followed by those subjected to cigarette smoke while highest mean values were found in sample exposed to the combination of cigarette smoke and shisha smoke followed by those of samples of shisha smoke.

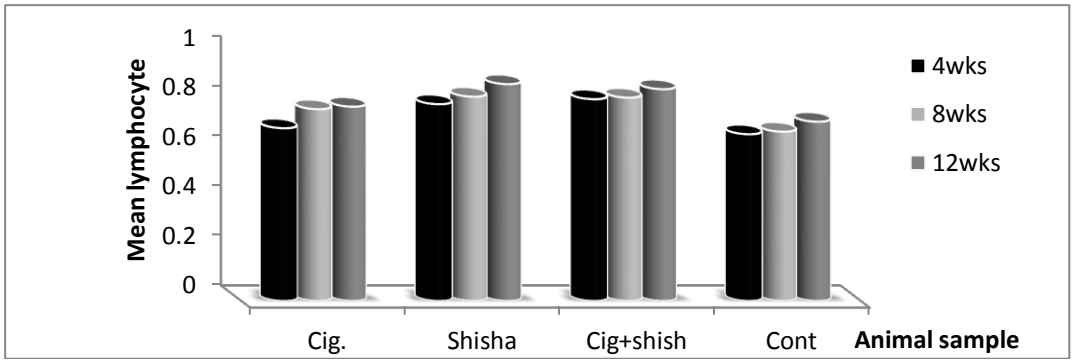

Figure 6-Mean lymphocyte in experimental animals subjected to smoke generated by cigarettes, shisha, cigarettes & shisha and control sample after 4, 8, and 12 weeks.

Analysis of variance of these data shows significant effects of smoke sources and exposure periods ($P \leq 0.001$) upon mean values of lymphocyte percentage of examined samples. Furthermore, LSD value (8.055%) gives clear differences between these data. In case of blood monocyte, the results of present study has shown that mean value varied from 0.016±0.005% in animal blood exposed to smoke of cigarette tobacco after 4 weeks to 0.059±0.007% in blood sample subjected to smokes released in combination of cigarette tobacco and shisha ma'sal after 12weeks ,Figure-7 , table-2.

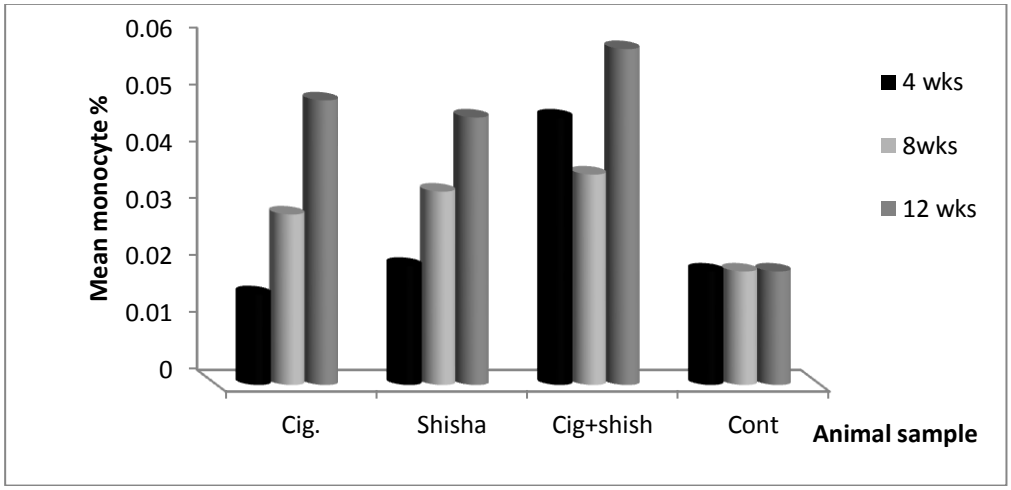

Figure 7-Mean monocyte in experimental animals subjected to smoke generated by cigarettes, shisha, cig & shisha and control sample after 4, 8, and 12 weeks.

However, analysis of variance of these data shows high significant ($P \leq 0.001$) impacts of smokes generated by cigarette tobacco, shisha ma'sal and the combination of cigarette tobacco and shisha ma'sal. Also impacts of exposure periods have similar significant ($P \leq 0.001$) in these values. However, LSD value (0.029 %) gives obvious differences between these data. Animal samples that exposed to smoke released by the combination of cigarette smoke and shisha smoke had the highest values while the samples subjected to both cigarette smoke and shisha smoke gave mean values higher than those of control samples ,Figure-7.

The results of current study agreed with study of [8] as it mentioned previously, they reached to results that, total WBC, neutrophils and lymphocytes in both cigarette and WPS were elevated as compared with control which exposed to fresh air only.

Also our results agreed with other study achieved by [24] showed that Total leukocyte count is more in smokers as compared to non-smokers and they referred that marked leukocyte has been reported even in subjects smoking less than 10 cigarettes per day, also they reached to results that the lymphocyte count increases from a mean value of 32.4% in non-smokers to 38.3% in smokers, which is found to be statistically significant, while neutrophils count showed a slight fall in smokers than non-smokers but the difference is not statistically significant.

According to some researchers effect of smoking on differential count is not uniform and is influenced by the current smoking behavior. Some studies have shown that neutrophils count rises and lymphocyte count shows a decrease, [25] as compared with this study our results have shown that both these parameters were increased.

Miri-Moghaddam [17] indicated that WP smoking led to increasing WBC count compared to control group. Friedman *et al.*, [26] observed that WBC counts in smokers were about 20-25% higher than nonsmokers and also they increased with intensity of smoking.

A group of researchers suggested that the increased leukocyte count especially lymphocyte might be due to nicotine. [7]

Increasing nicotine concentrations especially in shisha smoke samples may be responsible for increasing neutrophils, lymphocytes, monocyte in blood of animal groups whom exposed to shisha, smoke .and the increasing nicotine concentrations may be because the water bowel as part of shisha was not effective enough to filter out the toxins including Nicotine.

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