*International Research Journal of Environment Sciences*_____ ISSN 2319–1414
Vol. **3(5),** 86-91, May **(2014)** *Int. Res. J. Environment Sci.*

Toxic Impacts of Sub-Chronic Inhalation of Mosquito Coil Smoke in Rabbits

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Available online at: www.isca.in, www.isca.me
Received 24th April 2014, revised 1st May 2014, accepted 19th May 2014

Abstract

Various Asian countries including china, Thailand, India, Malaysia Korea and Japan commonly used mosquito coils as mosquito repellent. Mosquito coils are slow-burning product which release smoke containing one or more insecticides; it burns for several hours in confine places in order to provide protection to humans against malaria, which spread due to mosquitoes. Present study was intended to examine the toxic impacts of inhaling mosquito coil smoke on male rabbits. Mosquito coils are widely used to kill mosquitoes. A total of forty rabbits were used in this study. These rabbits were divided into two major groups i.e., Control Group and Experimental Group. Experimental group was further divided into 3 sub-groups i.e., E1, E2 and E3.Each group contain 10 rabbits as shown in figure 2.1. Control Group was not exposed to mosquito coil smoke while Experimental groups were exposed to mosquito coil smoke for 3 hours/day. Exposure time remain the same but exposure days vary with each Experimental group i.e., E1 was exposed for 5 days, E2 was exposed for 10 days and E3 was exposed for 15 days. After the completion of exposure day's blood was collected from the ear pinna of rabbits for the analysis of Red Blood Cells, White Blood Cells and Platelets. Blood samples were analyzed by using Hematology Analyzer. Effect of mosquito coil smoke on weight of rabbits was also observed and morphological changes due to mosquito coil smoke was also evaluated. Results were analyzed by comparing the data of Experimental groups with Control group. According to results a significant decrease was observed in weight of Group E3 rabbits. Results also demonstrated that with the increase of exposure day's number of White Blood Cells and Platelets were also increased while the numbers of Red Blood Cells become decreased.

Keywords: Red blood cell, white blood cell, platelets, complete blood count.

Introduction

The urban population is expected to increase more than 70% in developed countries[1] resulting in the increased usage of urban pests. Recently, several new mosquito control devices are available in markets in order to reduce or eliminate the number of mosquitoes by effectively trapping or preventing them from residential properties[2]. Mosquito coils are slow-burning product which release smoke containing one or more insecticides; it burns for several hours in confine places in order to provide protection to humans against malaria, which spread due to mosquitoes[3]. Mosquito coils are widely used as a mosquito repellent in various Asian countries including china, Thailand, India, Malaysia Korea and Japan. Burning one mosquito coil would release same amount of $PM_{2.5}$ mass which is equivalent to burning 75-137 cigarettes[4]. A World health organization (WHO) report estimated the world wide annual consumption of mosquito coils to be approximately 29 billion pieces[5].

Most of mosquito coils consist of an active ingredient known as pyrethroids, consist of about 0.3-0.4% of the coil mass[6]. Other ingredients of mosquito coil includes various binders, fillers, dyes, d-allethrin along with wood powder, coconut shell powder starch and additives capable of smoldering. Many mosquito coils also contain a chemical term S2 (octa-chlorodipropyl ether) during burning of coil S2 degraded into another chemical known as bis-chloromethyl ether (BCME), it is a potential cancer causing agent[7].

Epidemiological studies have shown that mosquito coil smoke can stimulate asthma and persistence wheeze in children when they are exposed to it for a long period of time[8]. There are also some irritants which are released in the coil smoke cause sneezing in rats such as aldehydes and sulphates[9]. Animal studies with long term exposure to mosquito coil smoke showed abnormal growth of skin cells, per weight loss and lung damage[10].

Hematological studies on rats exposed to mosquito repellents carried out by Ayorinde et al. (2012), in this study rats were divided into two groups of 6 males and 6 females and they were exposed to mosquito repellents per day for four weeks. The results showed increase in white blood cell (WBC) counts in the females and males exposed for two weeks. Platelets (PLT) counts in female rats (after two weeks) also increased when compared with the control group[11].

Material and Methods

Experimental Rabbits: The present study was undertaken to determine the toxic effects of mosquito coil smoke on

mammals, for this purpose male rabbits were used. Total of 40 adult male rabbits free from any apparent clinical ailment were purchased from the local market. Rabbits were kept in wire cages in Botanical garden of Lahore College for Women University and they were divided into two main groups: 1) Control group (C), and 2) Experimental group. Fresh grass and green fodder and drinking water were provided, around the clock.

Mosquito Coil: The commonly used brand of mosquito coil (Mortein Coil) was purchased from local Market. Mosquito coil used measured 12 cm diameter and 15.0 g weight.

Experimental Design: Control group comprised of 10 rabbits and they were exposed to normal room ($125m^3$) fresh air. While Experimental group comprised of total 30 rabbits and they were further divided into three groups of E1, E2 and E3. Each group consisted of 10 rabbits E1, E2, and E3. E1 group was exposed to mosquito coil smoke for 5 days/3 hrs, E2 group were exposed for 10 days/3 hrs and E3 group were exposed to coil for 15 days/3 hrs respectively. They were marked with different colors permanent markers (blue, red and green) for individual identity.1ml blood was collected from the ear pinna of rabbits before and after the exposure.

Parameters: The parameters to be studied include: i. Morphological and behavioral changes, ii. Body weight, iii. Complete blood count (CBC).

Instruments: i. Hematology Analyzer (Model:Sysmex KX-21), ii. Weighing balance

Apparatus: For Tagging: i. Permanent markers. For Exposure: i. Mortein mosquito coil, ii. Match box. For Blood Collection: i. Syringes, ii. Cotton, iii. EDTA blood collection glass tubes, iv. 90% Ethanol.

Body Weight Analysis: Electrical weighing balance of Model ALPHALA, range: 35 kg was used for calculation of subsequent changes in weight due to mosquito coil smoke. Initial, continuous and final body weights of male rabbits after exposure period of mosquito coil smoke were documented according to their group. Each value was counted twice and their mean value was taken for the comparison of weight change between control group and experimental groups.

Exposure to Mosquito Coil Smoke: Mosquito Coil smoke was got inhaled by rabbits according to their groups. Coil was placed 6 inches away from rabbits and cages were covered from sides so that proper circulation of smoke occur. About 3 hours exposure had been given to rabbits daily according to their exposure days as shown in figure 1. Control group remained in fresh air.

Blood Sampling: Blood was collected from both experimental rabbits as well as control ones. Rabbit's ear pinna veins (intravenous blood collection) were chosen for blood collection. The auricular vein is a common venipuncture site in rabbits. It can easily be seen on the dorsal surface of ear in the center between the two marginal ear veins.

Before blood collection withdrawal site was cleaned with 90% Ethanol as disinfectant. The ear had to warm or tapped with fingers in order to dilate the vessels. Sterile 23G (gauge) needle with BD 5 ml syringes was used for blood collection and also to limit tissue trauma and discomfort.1 ml blood was drowned at each collection from every rabbit. The marginal ear veins were used to collect up to 1 millimeter of blood samples from the tip of the ear, away from the base of the ear. Applying finger pressure on cotton placed at the blood sampling site for approximately 2 min in order to stop the blood flow before rabbits returned to its cages.

According to rabbit exposure and defined days blood samples were collected as taken after 5 days from E1 group rabbits, after 10 days from E2 group rabbits and after 15 days from E3 group rabbits correspondingly. Collected Blood was poured in EDTA blood collection glass tube and slightly shakes to prevent clotting formation.

Hematological Analysis: The blood samples of experimental rabbits and control group rabbits were analyzed to test the effect of mosquito coil smoke on complete blood counts (CBC) of rabbits. For this purpose hematology analyzer (Model: Sysmex KX-21) as shown in figure; was used to analyze the following: White blood cell (WBC), Red blood cell (RBC), Hemoglobin (HGB), Platelets (PLT), Lymphocytes(LYM), Neutrophils (NEUT), Red cell distribution width (RDW), Platelet distribution width (PDW), Mean platelet volume (MPV), and Ratio of large platelets (P-LCR).

Statistical Analysis: Data of study was investigated through comparing all the experimental data with control. Data were expressed as mean ± standard error of the mean. Statistical significant was performed using the analysis of variance (ANOVA).

Results and Discussion

Observed morphological toxicity symptoms in present study were include Wheezing, Sleepiness, Nausea, Slight salivation, Slight eye irritation, Lacrimation, Heavy fits, Productive cough and Scratching of nose. While weakness, laziness and loss of appetite were observed in each rabbis of all Experimental groups.

Pyrethroid is considered as an important ingredient of mosquito coil. Exposure to very large amounts of pyrethroids leads to dizziness, headache, and nausea that might last for several hours. Larger amounts could cause muscle twitching, reduced energy, and changes in awareness. Even larger amounts could

cause convulsions and loss of consciousness that could last for several days[12] .

According to the research conducted by Baron ,this study revealed that Cyanide, which is released through coil smoke, reduces the oxygen carrying capacity of erythrocytes, which leads to reduce metabolism and energy output which indicates the weakness of body[13].

An infection can stimulate the body to produce higher number of bacterial infection. An increased number of lymphocytes may be produced with the viral infection. In certain states of diseases such as leukemia, abnormal white blood cells multiply rapidly ultimately leading to increase the number of WBCs count**.** Present study also investigated the increase in white blood cells count in all experimental groups after mosquito coil smoke inhalation.

Red Blood Cells are also known as Erythrocytes. They are produced in bone marrow and released into the blood stream as they become mature. If insufficient amount of RBCs are present then in that condition person suffer from anemia, fatigue and weakness[14]. In present study the amount of RBCs count was decreased. Control group did not show any change in RBCs count while experimental groups showed slight decrease in RBCs count.

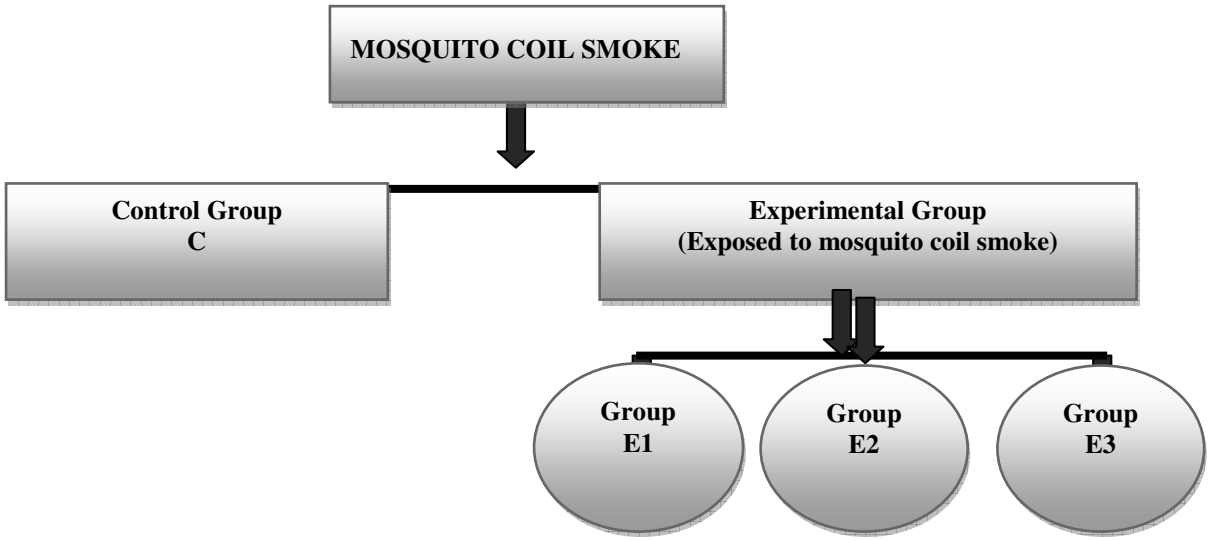

Figure-1
Flow sheet Diagram showing Mosquito Coil Smoke Exposure Pattern and Group Division

Table-1
Effect of Mosquito Coil Smoke on Weight (kg)

Experimental Groups		Control Group(C)	ANOVA(Group wise Comparison)
E1 Group (5 days exposure)	1.1530±0.0471	1.4370±0.0710	**
E2 (10 days exposure)	1.0630±0.0641		***
E3 (15 days exposure)	1.0470±0.0714		***
ANOVA (Intergroup Comparison)	***		

Values given are Mean ± SE of results obtain from forty rabbits. **X=**Insignificant difference of mean value, ***=**Significant difference (P<0.05), ****=**More Significant difference (P<0.01), *****=**Highly Significant difference (P<0.001)[11].

Table-2
Effect of Mosquito Coil Smoke on White Blood Cells (10^3/µL)

Experimental Groups		Control Group(C)	ANOVA(Group wise Comparison)
E1 Group (5 days exposure)	4.760±0.428	5.170±0.705	X
E2 (10 days exposure)	6.240±0.554		X
E3 (15 days exposure)	8.000±0.905		*
ANOVA (Intergroup Comparison)	**		

Values given are Mean ± SE of results obtain from forty rabbits. **X=**Insignificant difference of mean value, ***=**Significant difference (P<0.05), ****=**More Significant difference (P<0.01), *****=**Highly Significant difference (P<0.001)[11]

If the numbers of platelets count become greater than 600×10^3/µL then myeloproliferative disorders of the stem cells occurred in the bone marrow[15]. In present study significant increase was observed in platelets count of experimental groups as compared with control group.

Conclusion

This study was conducted in order to determine the toxic impacts of mosquito coil smoke on blood count parameters (WBCs, RBCs and PLTs) in male rabbits. Results of study showed that mosquito coil smoke induced morphological symptoms in experimental groups which include laziness, Wheezing, Sneezing, Slight eye irritation, lacrimation, heavy salivation, heavy fits, convulsions, loss of appetite, Scratching of nose and weakness. Mosquito coil smoke also decreased the weights of Experimental Group rabbits. This study also showed that as the number of exposure days increased but the exposure time remain the same then the amount of WBCs (10^3/µL) and PLTs (10^3/µL) count were increased while amount of RBCs (10^6/µL) count become decreased as compared with Control Group which exposed to fresh air.

Table-3
Effect of Mosquito Coil Smoke on Red Blood Cells (10^6/µL)

Experimental Groups		Control Group(C)	ANOVA(Group wise Comparison)
E1 Group (5 days exposure)	4.9950±0.0819	5.668±0.157	**
E2 (10 days exposure)	5.250±0.101		X
E3 (15 days exposure)	4.757±0.175		**
ANOVA (Intergroup Comparison)	***		

Values given are Mean ± SE of results obtain from forty rabbits. X=Insignificant difference of mean value, *=Significant difference (P<0.05), **=More Significant difference (P<0.01), ***=Highly Significant difference (P<0.001)[11].

Table-4
Effect of Mosquito Coil Smoke on Platelets (10^3/µL)

Experimental Groups		Control Group(C)	ANOVA(Group wise Comparison)
E1 Group (5 days exposure)	659.5±40.1	521.3±60.4	X
E2 (10 days exposure)	769.2±53.5		**
E3 (15 days exposure)	917.0±86.5		**
ANOVA (Intergroup Comparison)	***		

Values given are Mean ± SE of results obtain from forty rabbits, X=Insignificant difference of mean value, *=Significant difference (P<0.05), **=More Significant difference (P<0.01), ***=Highly Significant difference (P<0.001)[11]

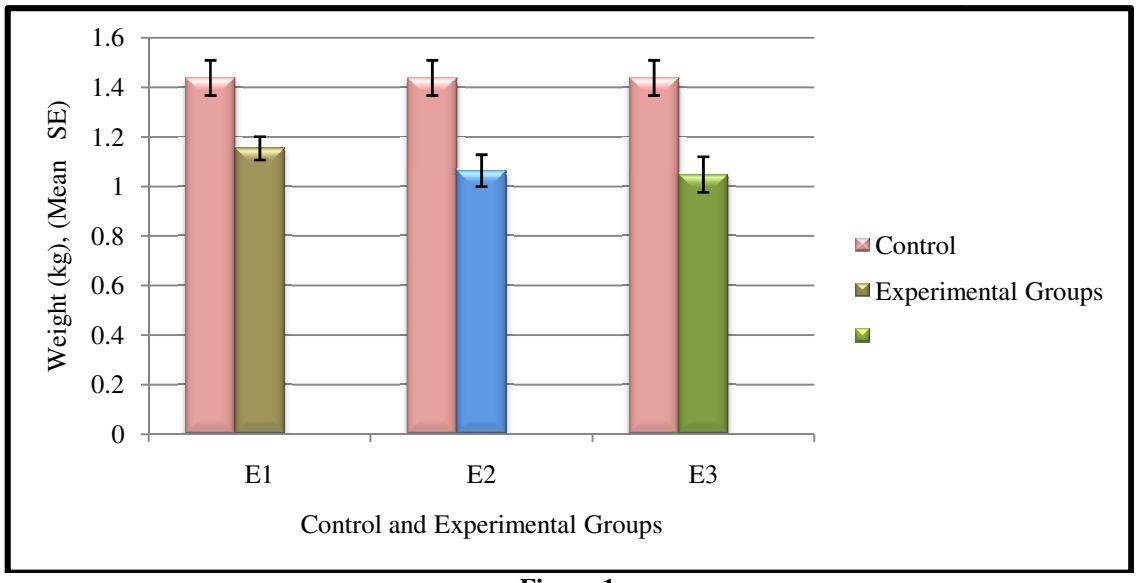

Figure 1
Histogram showing the comparison of (Mean± SE) values of Weight(kg) of Control and Experimental Groups

After Mosquito coil smoke exposure significant decrease was observed in body weight of Experimental Groups as compare to Control group. Inter group comparison showed highly significant difference while in group wise comparison Group E2 and E3 showed highly significant differences in weights as compare to Control Group (figure-1).

Mosquito coil smoke exposure induced alteration in WBCs count of Experimental groups as compare to Control. Decrease was observed in Group E1 while Group E2 and E3 showed increase in WBCs count. Inter group comparison showed more significant differences in WBCs count .On other hand in group wise comparison Group E3 exhibited the significant difference as compare to Control while Group E1 and Group E2 did not show statistically significant differences in WBCs count (figure-2).

Mosquito coil smoke exposure caused reduction in RBCs count of Experimental groups as compare to Control. Highest decrease was observed in Group E3 which was exposed for 15 days. Inter group comparison showed highly significant differences in RBCs count .While in group wise comparison Group E1 and E3 exhibited the more significant difference as compare to Control but Group E2 did not show statistically significant differences in RBCs count compared with Control Group (figure-3).

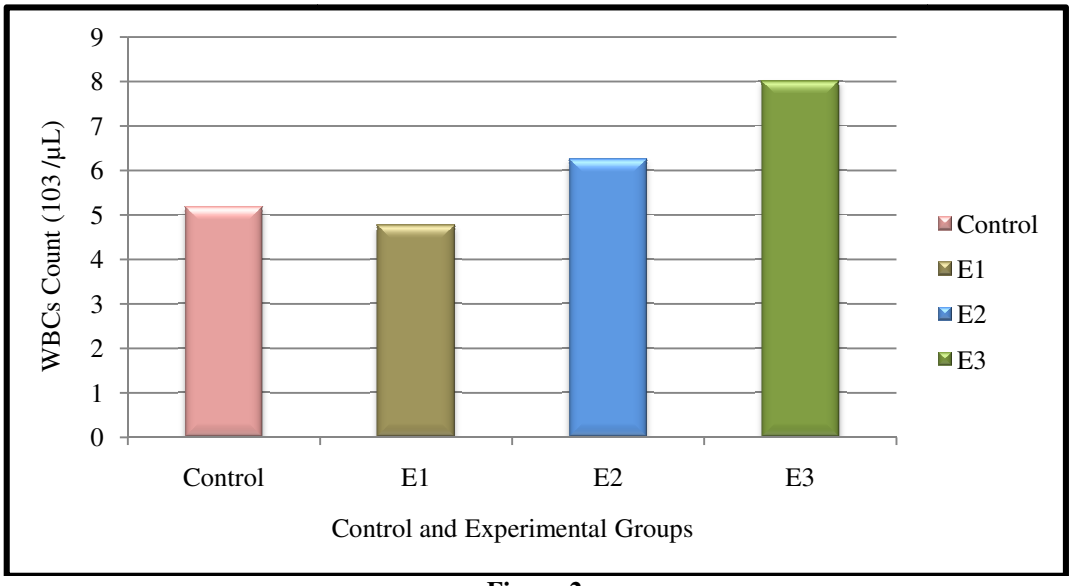

Figure 2
Bar graph showing the comparison of White Blood Cell Counts of Control and Experimental Groups

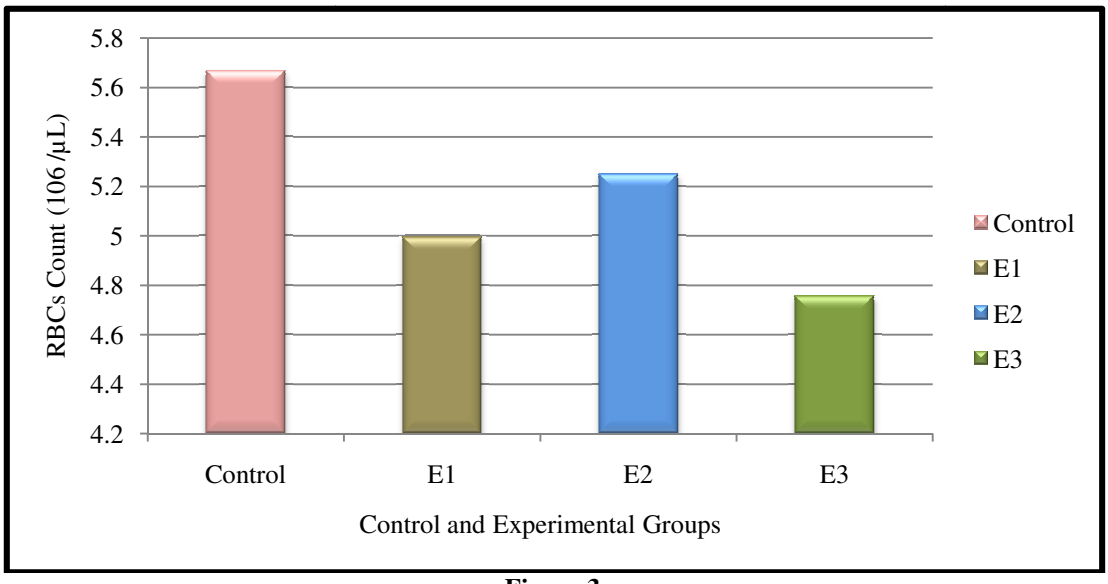

Figure 3
Bar graph showing the comparison of Red Blood Cell Counts of Control and Experimental Groups

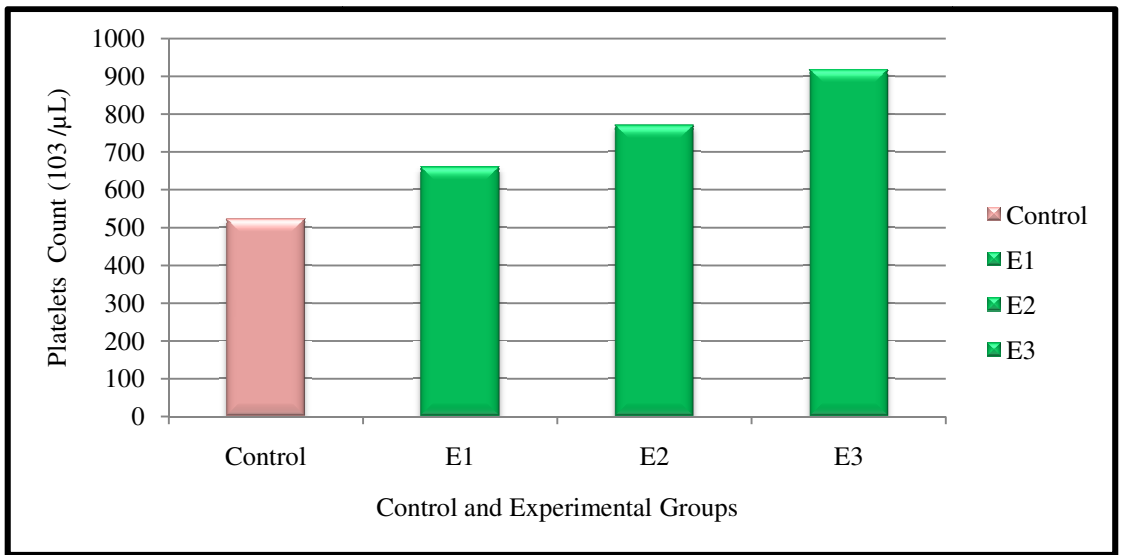

Figure 4
Bar graph showing the comparison of Platelet Counts of Control and Experimental Groups

Explanation: Mosquito coil smoke exposure increased the Platelets count of Experimental groups as compare to Control. Highest increase was observed in Group E3 which was exposed for 15 days to mosquito coil smoke. Inter group comparison showed highly significant differences in Platelets count .While in group wise comparison Group E2 and E3 exhibited the more significant difference as compare to Control but Group E1 did not show statistically significant differences in Platelets count compared with Control Group (figure-4).

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