

TOXIC EFFECTS OF THE MORTEIN, KINGTOX, AND FINIS MOSQUITO COIL'S SMOKE ON BLOOD PROFILE OF ALBINO RATS (*RATTUS ALBUS*)

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خلاصہ

موجودہ تحقیق کا مقصد مچھر کنڈلی (M.C) کا چوہوں کے دمویاتی سرگرمیوں (Haematological parameters) پر اثرات کا جائزہ لینا تھا۔ خون کے خاکے (blood parameters) کا تجزیہ بہت سی بیماریوں مثلاً اینیمیا (خون کی کمی)، متعدی امراض اور سوجن، جلن کا پتہ چلانے میں مدد دیتا ہے۔ آٹھ گھنٹے روزانہ چھ ماہ کے لئے چوہوں کو تین مختلف مچھر کنڈلیوں (مورٹن، کنگ ٹوکس، فنس) کے دھوئیں میں رکھا گیا، اور ان کے خون کے نمونوں کو جمع کیا گیا۔ اڑتالیس چوہوں کو یکساں طور پر چار گروہوں (A, B, C, D) میں تقسیم کیا گیا، انکے خون کے نمونوں کا چھ ماہ تک مشاہدہ کیا گیا اور ہر ماہ کے آخر میں خون کے نمونوں کی جانچ کی گئی۔ کل سرخ جیسے (RBC)، ہیموگلوبن، پیک سیل والیم (PCV)، اور سفید خونی خلیات (WBC) کو تمام مچھر کنڈلیوں کے دھوئیں میں چھوڑے گئے چوہوں میں شمار کیا گیا اور جائزہ لیا گیا۔ نتائج سے یہ بات ظاہر ہوئی کہ مچھر کنڈلیوں کا دھواں خون میں موجود ہیموگلوبن، سرخ جیسے (RBC)، ہیماٹوکریٹ (Hct)، ایم سی وی (MCV)، ایم سی ایچ (MCH)، ایم سی ایچ سی (MCHC)، سفید خونی خلیات (WBC) اور دموی لوہجین (PLT) کے اعداد پر برے اثرات مرتب کرتا ہے۔ موجودہ تحقیق کے دوران ان عوامل خاص کی تعداد میں کمی پیشی مشاہدہ کی گئی اور حاصل ہونے والی ہیموگلوبن (Hb)، سرخ جیسے (RBC)، ہیماٹوکریٹ (Hct)، سفید خلیات (WBC) اور دموی لوہجین (PLT) کی اعداد میں تمام چوہوں کے گروہوں میں اضافہ دیکھا گیا کنٹرول گروہ کے مقابلے میں، جبکہ ایم سی وی (MCV)، ایم سی ایچ (MCH) اور ایم سی ایچ سی (MCHC) چھ ماہ کی تحقیق کے دوران کنٹرول کے مقابلے میں کم مشاہدہ کی گئی۔

Abstract

The purpose of this research was to assess the impact of mosquito coil's smoke on the hematological activities in albino rat (*Rattus albus*). Blood profile analysis help to detect diseases such as anemia, infections and inflammation etc. Blood is collected from albino rats treated with Mortein, Kingtox and Finis mosquito coils smoke 8 hours daily for 6 months. Forty-eight rats were equally distributed into four groups (A, B, C, and D). The blood was collected after one-month interval from each group for six months. Total erythrocytes, hemoglobin (Hb), packed cell volume (PCV) and leukocyte counts were determined in all the treated groups. Results disclosed that mosquito coils smoke of Mortein, Kingtox and Finis coils causes effect on the number of haemoglobin (Hb), red blood cells (RBCs), haematocrit (Hct), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), white blood cells (WBCs), and platelets (PLT) count as these parameters either increased or decreased during the experiment. The detected values of Hb, RBCs, Hct, WBCs and PLT during six months in all the treated groups of albino rats shows elevation than control group values and likewise the detected values for MCV, MCH and MCHC during six months showed decrease in group A, B and C than the untreated rats group.

Key words: Pyrethroids, Mosquito coils, Toxicity, hematological activities

Introduction

Mosquitoes are trajectories of various illnesses most specially dengue, malaria, filarial elephantiasis, yellow fever, West Nile fever and Japanese encephalitis. To inhibit the impact of insect bites mainly mosquitoes actions should be taken to defend the diseased person and to stop the blowout of illness (Katz *et al.*, 2008). For this purpose, different types of pyrethroid based mosquito repellents are used worldwide to combat mosquito troubles and diseases. In the Domestic context different pyrethroid's products are used to impede the mosquito nibble. The occurring of mosquito borne diseases on large scale are common in Pakistan and appearance of dengue is a recent example. The first outburst of dengue fever in Pakistan was in 1994 and after 2010 Pakistan faced dengue pandemic that has triggered 16,580 confirmed dengue patients and 257 deaths in the Lahore city and approximately 5000 patients and 60 deaths recorded from the rest of the country (WHO., 2018). Furthermore, the fright of blowout of chikungunya is also a serious worry in this country. Similarly, the feast of malaria disease remained a major issue in Pakistan. With such a history in Pakistan the usage of pyrethroid based mosquito repellents has increased in the domestic and agriculture circles therefore it is a wide zone of

research. Extra preventive measures should be taken to impede the mosquito bites of pregnant women and their new-born babies. Revelation to mosquito repellents throughout the pregnancy period possibly can cause health impact to mother, fetus and newborn baby.

In underdeveloped countries specially Pakistan where public mostly have no idea about protective measures, the extra attention should be taken to avert the mosquito nibble to newborn babies and children. This protective measure comprises mosquito coils, mosquito repellent lotion and liquid vaporizers in which the main active ingredient is allethrin. Among these repellents, mosquito coil is extensively used in the Pakistan domestic context due to low price and efficacy but 30 % of the pesticides sold in underdeveloped countries do not fulfil the internationally accepted quality standard (FAO., 2011). The mosquito coils sold in the Pakistan contains d-Allethrin and d-trans Allethrin. Burning of mosquito coils release 2.5 micrometer particulate matter, carbon monoxide (CO), and methane gas (Lee & Wang., 2006). So, new generation are impinged to allethrin noxiousness from the first day when they are created in mother's uterus or after the birth.

The medical consequences and health damage triggered by inspiration of allethrin-based mosquito coil's smoke are revealed by using rodent model systems. Subjection of rats to mosquito coil's smoke significantly modify haematological and biochemical values in different tissues, organs and sperm (Idowu *et al.*, 2013). Although the pyrethroid bombard the central nervous system of target and non target species, the continuous use of pyrethroid insecticides in the domestic context may outcome into toxicity in various organs and so has the negative impact on the health status (Liu *et al* 2003; John & John., 2015). In treated animals, RBCs are increased while Hb decrease in female rat (Fetoui *et al.*, 2008). Red cells count and packed cell volume (PCV) remained augmented in all laid open rats to coil's smoke (Garba *et al.*, 2007). The number of RBCs was found decreased in Nadia and Anum (2014) conclusions. Significant increase in red blood cells (RBC) count was demonstrated by (Parker *et al.*, 1984; EPA, 1991a; Shakoori *et al.*, 1992). In a study, haemoglobin (Hb) displayed no significant shift ($p>0.05$) when matched with control groups, while increased in treated groups of rats (Garba *et al.*, 2007). Haemoglobin (Hb) content showed significant increase in a study of (Parker *et al.*, 1984; EPA., 1991a; Shakoori *et al.*, 1992). Allethrin motivated erythropoiesis and haemoglobin (Hb) production (Haratym-Maj., 2002). Rats on exposure to pyrethroids based mosquito coils smoke for 90 days reported anemia in all the groups (Schoenig., 1995). In the mosquito coils treated rats white cell counts (WBCs) remained considerably ($p>0.01, 0.05$) elevated in all the groups (Garba *et al.*, 2007). White blood cells (WBCs) increased after the treatment with synthetic pyrethroids insecticides (Shakoori *et al.*, 1992; Fetoui *et al.*, 2008). Leucocytosis is observed in mammals after the treatment with pyrethroid based mosquito coil (Shakoori *et al.*, 1992). Leukopenia is recorded in rats after the treatment with pyrethroid based insecticides (Dameshek, W., 1944; Institoris *et al.*, 1999; Matsushima *et al.*, 2003; Khan *et al.*, 2012). White blood cells (WBCs) and platelets escalate in all experimental groups after the inhalation of coils smoke (Nadia and Anum., 2014). Haematocrit (Hct) decrease in male rats on the exposure to coils smoke (Anonymous., 1989). While haematocrit (Hct) showed significant increase in studies reported by (Parker *et al*, 1984; EPA, 1991a; Shakoori *et al.*, 1992). In one study mean corpuscular volume (MCV) increased (Sayim *et al.*, 2005; Shah *et al.*, 2007). Sayim *et al.*, (2005) reported Mean Corpuscular haemoglobin was either increased or decreased but was found rather unaffected according to shah *et al.*, (2007) observations. Mastushima *et al.*, (2003) and Basir *et al.*, (2011) recorded decrease in Mean Corpuscular Haemoglobin Concentration (MCHC) which remained unaffected in Sayim *et al.*, (2005) and Shah *et al.*, (2005) analysis. The current investigation was planned to facsimile the residential and daily exploitation of three allethrin based mosquito coils Mortein, King tox and Finis impact in the Karachi city by implementing rats as a model to prove the possible health insinuation and risk of mosquito coil smoke exposure.

Materials and Methods

A total of forty-eight (48) albino rats (*Rattus albus*), weighing in between male 200- 240 gm and female 130-160 gm are used in this research, acquired from the Pakistan Council of Scientific and Industrial Research (PCSIR), Karachi. The rats were held in the concrete cages at ordinary temperature of 32 ± 6 C°. They had excess to tap water and diet such as bread, cucumber, grams, and sunflower seeds etc. Experimental rats were divided into four groups namely group A, group B, group C and control group. Group A was exposed to Mortein coil's smoke which contained 1.0 g/kg d-trans Allethrin (Esbiothrin 75:25) as active ingredient. One Mortein coil was burnt 7 to 8 hours daily throughout the night from 10 pm to 6 am for 6 months. Group B was exposed to Kingtox coil's smoke which contain 0.25 % d-Allethrin as an active ingredient. One Kingtox coil was burnt 7-8 hours daily throughout the night from 10 pm to 6 am for 6 months. Group C was exposed to Finis coil's smoke which contain 0.15 % d-Allethrin as an active ingredient. One Finis coil was burnt 7 to 8 hours daily throughout night from 10 pm to 6 am for 6 months while the fourth one group was control group which was not exposed to any kind of coil's smoke. Three categories of Mosquito coils Mortein, Kingtox, and Finis were commercially purchased for experiment from the various outlets located in Karachi city, which contained d-trans Allethrin 1.0 g/kg (Esbiothrin) 75:25, d-Allethrin 0.25 %, and d-Allethrin 0.15 % as active ingredients respectively mention

on coil's box. Blood samples were taken from direct heart puncture (Hoof *et al*, 2000; Beeton *et al*, 2007) and collected in purple cap test tube containing anticoagulant EDTA for blood profile and checked by 5-part differential automated CBC analyzer in PCSIR lab which uses volume conductivity scatter and cytometry to determine diameter, granularity and inner complexity of blood cells.

Results and Discussion

Mosquito coils are extensively used in Asian countries because they are cheap and easily available. Smoke emitted from the test coils causes significant alteration in haematological parameters in treated albino rats. Under the different environmental conditions and for the examination and analysis of drugs or chemically induced haemolysis, haematological parameters detections are crucial for the many systematic functions (Atamanalp and Yanik., 2003). Though, data concerning haematological modifications after exposure to pyrethroid insecticides is diverse. Inhalation of allethrin based mosquito coils (Mortein, kingtox and Finis) smoke in *Rattus albus* (albino rat) revealed toxic effects on haematological parameters reported in the current investigation demonstrated in table 1 which showed the significant difference in treated groups A, B and C as compared to untreated group. Table 1 demonstrate variations in the red cell count and its indices in the experimental animals exposed to allethrin based mosquito coils for six months. Haemoglobin (Hb), Red blood cells (RBCs), hematocrit (Hct), white blood cells (WBCs) and Platelets were higher in the experimental groups when matched with control group while mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) shows decrease in test class when matched with untreated class and these results remained statistically significant ($p < 0.005$) in all treated groups exposed to coils smoke.

The detected significant values of haemoglobin (Hb) were 17.3 ± 1.44 in group A, 16.51 ± 1.50 in group B and 15.98 ± 1.52 in group C which displayed increase than control group values 14.28 ± 1.36 , similarly the detected significant values of red blood cells (RBC) were 13.96 ± 1.24 in group A, 12.91 ± 1.14 in group B and 11.83 ± 0.88 in group C which was found increased than control group values 7.48 ± 0.80 , and the observed significant values of haematocrit (Hct) were 53.5 ± 4.32 in group A, 50.83 ± 4.44 in group B and 49 ± 4.47 in group C which showed elevation than the control group values 43 ± 3.84 , and the noted significant values of mean corpuscular volume (MCV) were 38.34 ± 1.22 in group A, 39.36 ± 0.51 in group B and 41.36 ± 1.17 in group C which expressed decrease than control group values 57.97 ± 8.03 . The noted values of mean corpuscular haemoglobin (MCH) were 12.39 ± 0.40 within group A, 12.78 ± 0.17 within group B and 13.52 ± 0.38 within group C considerably decreased when matched with control class values 19.25 ± 2.69 . The noted values of white blood cells (WBC) were 23.93 ± 1.02 in group A, 21.35 ± 0.39 in group B and 19.4 ± 0.59 in group C which were remained significantly high in all the classes as compared to untreated class values; 8.38 ± 1.77 . The spotted values of platelets count were 1841.6 ± 75.84 in group A, 1703.8 ± 32.63 in group B and 1607.5 ± 7.81 in group C which appeared high as compared to control group values; 515.5 ± 39.40 .

Table.1: Effects of mosquito coil smoke inhalation on hematological indices of experimental animals (*albino rattus*) exposed to mosquito coils (Mortein, Kingtox and Finis) for six months

Blood Parameters	Control	Group A (Mortein)	Group B (kingtox)	Group C (Finis)
Hb (g/dl)	14.28±1.36	17.3±1.44	16.51±1.50	15.98±1.52
RBC ($10^6/\mu\text{l}$)	7.48±0.80	13.96±1.24	12.91±1.14	11.83±0.88
PCV/HCT (%)	43±3.84	53.5±4.32	50.83±4.44	49±4.47
MCV (fl)	57.97±8.03	38.34±1.22	39.36±0.51	41.36±1.17
MCH (pg)	19.25±2.69	12.39±0.40	12.78±0.17	13.52±0.38
MCHC (g/dl)	33.20±0.26	32.32±0.27	32.48±0.36	32.70±0.48
WBC ($10^9/L$)	8.38±1.77	23.93±1.02	21.35±0.39	19.4±0.59
PLT ($10^9/L$)	515.5±39.40	1841.6±75.84	1703.8±32.63	1607.5±7.81

Hb=haemoglobin, RBC=Red blood cell, Hct=Haematocrit, MCV=Mean corpuscular volume, MCH=Mean corpuscular haemoglobin, MCHC=Mean corpuscular haemoglobin concentration, WBC=white blood cell, PLT=Platelets.

*significant difference ($p < 0.005$)

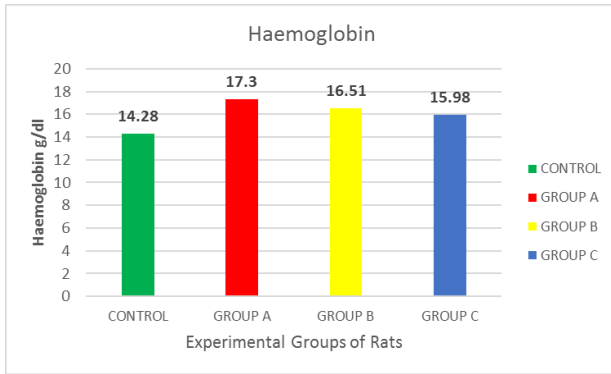


Fig.1. Haemoglobin concentration of albino rats exposed to Mortein, Kingtox and Finis coils

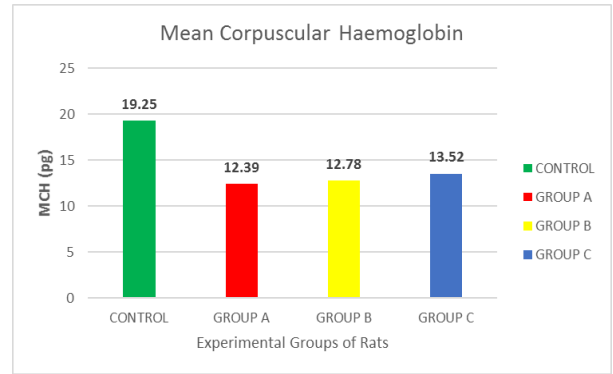


Fig.5. MCH concentration of albino rats exposed to Mortein, Kingtox and Finis coils

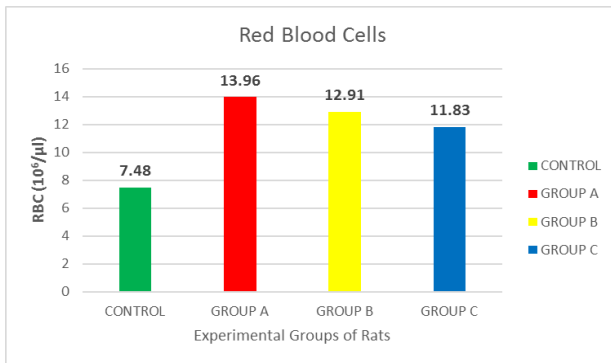


Fig.2. RBCs of albino rats exposed to Mortein, Kingtox and Finis coils

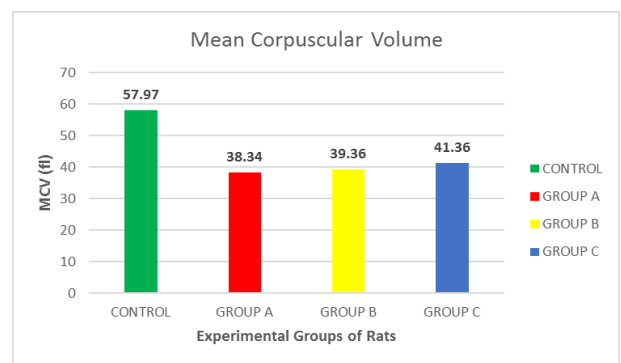


Fig.4. MCV of albino rats exposed to Mortein, Kingtox and Finis coils

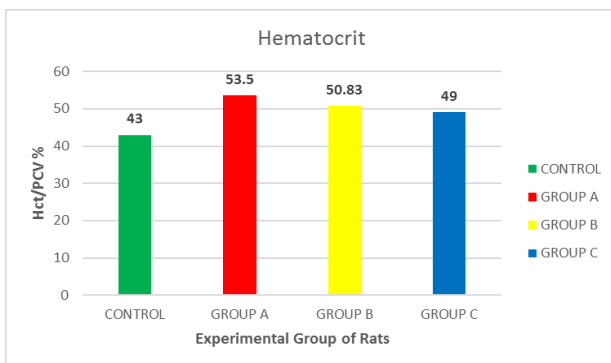


Fig.3. Hematocrit percentage of albino rats exposed to Mortein, Kingtox and Finis coils

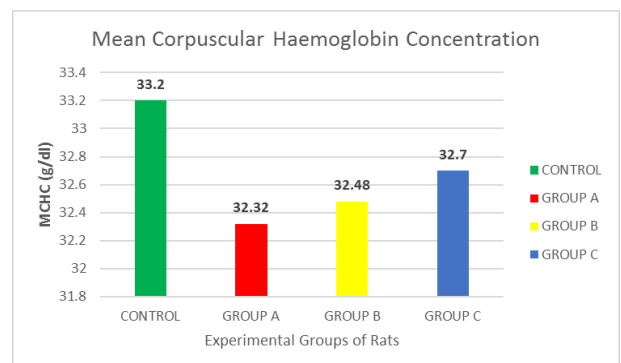


Fig.6. MCHC concentration of albino rats exposed to Mortein, Kingtox and Finis coils

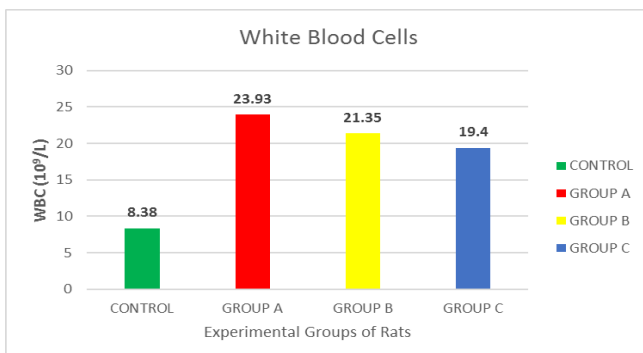


Fig.7. WBC values of albino rats exposed to Mortein, Kingtox and Finis coils

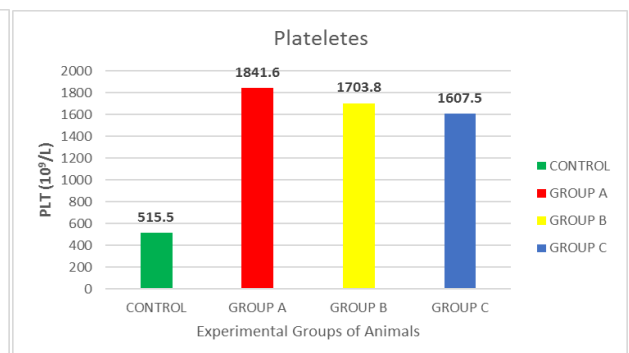


Fig.8. Platelets counts of albino rats exposed to Mortein, Kingtox and Finis coils

The derive results for each of the hematological parameters were evaluated with standard reference values generated by Charles River Laboratories in 2008 for specific age sets and sexes of the rats (Giknis and Clifford, 2008). Estimates from our study exhibited that there were significant differences in the normal values of some of the hematological parameters between the rats of treated groups. In current investigation hemoglobin (Hb), Red blood cells (RBC), Packed cell volume (PCV) or hematocrit, white blood cells (WBC) and platelets were found significantly ($p < 0.05$) augmented in all the experimental groups treated with mosquito coils smoke as compared to control group and standard reference values of Charles River Laboratories while mean corpuscular volume (MCV), Mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were found decreased in all exposed groups of rats. Haemoglobin (Hb) increase in exposed rats contradict to Fetoui *et al.*, (2008) findings and was analogous with Parker *et al.*, (1984); EPA, (1991a); Shakoori *et al.*, (1992); Garba *et al.*, (2007) analysis.

In this study it is found that cyanide which is released through mosquito coils smoke reduces the O_2 carrying capacity of RBCs and causes decrease in metabolism and energy production, consequently weakens the body and augmented hemoglobin in exposed albino rats that was found consistent with Karim *et al.*, (2020) and Baron *et al.*, (1992) findings.

The emission of particulate matters by burning of mosquito coils lead to extrinsic allergic alveolitis (hypersensitivity pneumonitis) which is an inflammation of lungs and correlate with the hardening of lungs, difficulty in breathing, dry cough and result in significant reduction of O_2 supply to tissues. Low oxygen (O_2) in the blood circulation could lead to hypoxic condition and tissues do not get enough oxygen, which is identified by the kidneys and ultimately can cause excitement of erythropoietin synthesizing cells in the kidney to formulate more erythropoietin hormones and as a result, stimulate the red blood cells (RBC) construction which was found steady with Parker *et al.*, (1984a); EPA., (1991a); Shakoori *et al.*, (1992a); Garba *et al.*, (2007) study but it doesn't agree with Nadia and Anum., (2014) analysis. Clonal stem-cell proliferation of red blood cells (RBCs) increased haematocrit (HCT) or packed cell volume (PCV) indicating polycythaemia condition (Spivak J.L., 2002). In the present research WBCs count increased which is comparable with Nadia and Anum., (2014) experiment in which WBCs escalated in all the rabbits exposed to mosquito coils smoke, which is due to inflammation or leukemia infection in which WBCs divide rapidly and consequently increase WBCs counts, RBCs count contradicted with Nadia and Anum., (2014) analysis where RBCs decreased in rabbits due to RBCs breakdown. Furthermore, escalation in platelets count was noticed in the current investigation. The hiked in the platelets count above $600 \times 10^3/\mu\text{L}$ in current investigation is suggestive of myeloproliferative disorder of stem cells in the bone marrow. Increase in platelets counts was found similar with Goodnough *et al.*, (1999) findings. In present study, White cell count (WBCs) significantly ($p > 0.05$) escalated in all the classes treated with mosquito coils smoke which affirms the Garba *et al.*, (2007) findings. In the current investigation decline in MCV, MCH, and MCHC was noticed which was found consistent with Sayim *et al.*, (2005); Shakoori *et al.*, 1992 and contradict with Pauluhn and Mohr (2006); Ratnasooriya (2002); Shakoori *et al.*, (1988) analysis.

Conclusion

Based on our results, it is concluded that the inhaled smoke of the Mortein, Kingtox and Finis mosquito coils in albino rats has adverse effects on the haematological parameters. It was noted that among these test coils Mortein mosquito coil (d-trans Allethrin 1.0 g/kg; Esbiothrin 75:25) is highly toxic than Kingtox and Finis coil, Kingtox (d-Allethrin 0.25 %) is less toxic than Mortein but highly toxic than Finis (d-Allethrin 0.15 %) which was found less toxic than Mortein and Kingtox.

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