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EFFECTS OF IMIDACLOPRID-CONTAMINATED FEED EXPOSURE ON HEMATOLOGICAL PARAMETERS IN ADULT RABBITS (*Oryctolagus cuniculus*)

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ABSTRACT

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Blood parameters are an important index to assess the body homeostasis in mammals. Here, the effect of pesticide (imidacloprid) contaminated feed exposure on the haematological parameters of adult rabbits (n=15) was studied. Pesticides (Imidacloprid, Bildor® 0.5ml (100mg)/L water spray on vegetables and green grass) exposed feed fed to rabbits with wash (washed feed rabbit) or without a wash (not washed feed rabbit) in the once-daily morning for every alternative day up to 15 days. Control rabbits have received a standard diet (fresh vegetables and green grass). The blood cell analysis showed that the total erythrocyte count, packed cell volume, and haemoglobin values were not changed significantly in pesticide exposed rabbit. For differential leukocyte count, the percentage of neutrophil and eosinophil was significantly decreased in the pesticide-exposed rabbit as compared to the control rabbit. The lymphocyte percentage was increased significantly in pesticide-exposed rabbits compared to control rabbits. Interestingly, the number of lymphocytes was significantly increased in not washed feed rabbits compared to wash feed rabbits. The results of the present study suggest that residue of agriculturally used pesticides may affect the body homeostasis of animals.

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INTRODUCTION

Blood is both a tissue and a fluid. It is composed of cells suspended in a fluid matrix (plasma) with the major function of maintaining homeostasis (Isaac et al., 2013). Blood parameters are good indicators of the physiological status of animals (Khan and Zafar, 2005). Blood parameters provide an opportunity to determine the presence of toxic metabolites and their constituents in the body, which plays a vital role in determining the body status of mammals (Etim et al., 2014). Pesticides or toxic chemicals have become a part of our food chain, and it is tough to escape its exposure, especially in occupational life exposure. There is widespread evidence that most of the crop, vegetables, and fruit farmers in Bangladesh use pesticides (Miah et al., 2014; Kobir et al., 2020). The unregulated use and its aerial application over large agricultural and urban areas have caused severe threats to public health and animal health (Al-Saleh, 1994).

To enhance more food production, farmers often use pesticides unrestrictedly. Additionally, the misusing of agricultural pesticides in Bangladesh is common (Kobir et al., 2020). According to the Department of Agricultural Extension, approximately 5,661 products of 359 pesticides are permitted to be used in Bangladesh (Hossain, 2018). Our previous survey report showed that imidacloprid was the top used insecticide in agro-based areas of Bangladesh (Kobir et al., 2020). Imidacloprid is the representative of neonicotinoid systemic insecticides marketed as an alternative of organophosphorus insecticides, firstly in 1991 by Bayer AG (a German multinational pharmaceutical and life sciences). The chemical name of imidacloprid is 1[(6-chloro-3-pyridinyl) methyl]-N-nitro-2-imidazolidinimine and the most widely used insecticide against sucking and chewing pests (Malev, 2012 and Jeschke et al., 2010). This insecticide, once it gets inside the body via oral, dermal, or inhalation, affects the organs of different systems of the body (Komal, 2018). At the end of the metabolization, metabolites of imidacloprid come into the blood circulation. Therefore, blood parameters may be a useful tool for the determination of the presence of metabolites and their constituents in the body, which plays a vital role in determining the body condition of mammals. It is reported that blood parameters are an important index to assess the body homeostasis in mammals. Blood acts as a reflector of the status of the body of animals, whether it is exposed to any toxicant or other adverse condition (Olafedehan et al., 2010). There is a very scanty database regarding pesticide toxicity in animals and mammals in Bangladesh. Recently, the effects of the pesticides on blood parameters of fish were reported (Mostakim et al., 2015 and Khan, 1994), but no evidence on the animal model to investigate the residual effects on the blood. The objective of this study was to investigate the effects of ingested pesticide (imidacloprid) on haematological parameters in rabbits under experimental conditions. Rabbits were chosen as model animals for this study because domestic animals could be exposed to the pesticide through farmlands, crops, water, and natural pastures.

MATERIALS AND METHOD

Experimental animal

A total of 15 numbers of adult rabbits of both sexes were collected from the local market. The rabbits were then acclimatized in the animal shade of the Department of Anatomy and Histology, Bangladesh Agricultural University, for 7 days having sufficient light and airflow. The rabbits were supplied with fresh vegetables (cabbage, bean, and tomato) and green grass with water *ad libitum*. The rabbits were categorized into control and pesticide exposed rabbits. Pesticide-exposed rabbits were again divided into two groups designated as not washed feed rabbit (pesticide sprayed vegetables and green grass were fed without wash) and washed feed rabbit (pesticide sprayed vegetables and green grass were fed after washing). The control rabbits received normal fresh vegetables and green grass that are free from pesticides.

Chemical preparation and exposure to animals

Imidacloprid (Bildor[®], marketed by Corbel International Limited) was collected from the local market and handled carefully with appropriate precautions. The insecticide imidacloprid 0.5ml (100mg) /liter was properly mixed with fresh tap water and sprayed in vegetables (cabbage, bean, tomato) and field green grass mixing with fresh water in the evening. The previously sprayed vegetables and green grass were collected the next morning and supplied as such as collected to rabbits (designated as not washed feed rabbits) and after washing to rabbits (designated as washed feed rabbits) for every alternative day up to 15 days.

Collection of blood and analysis of blood

At the end of the pesticide exposure (days 16), blood was collected from pesticide-exposed and control rabbits using light chloroform anaesthesia. About 5ml of blood was collected from the marginal ear vein with a sterile disposable syringe and needle. The blood was immediately transferred into sterile ethylene diamino-tetra-acetic acid (EDTA) embedded vials, properly mixed, and kept in the refrigerator for haematological analysis. The total erythrocyte count (TEC), erythrocyte sedimentation rate (ESR), total leukocyte count (TLC), and differential leukocyte count (DLC) were estimated by the haematocytometer method. Haemoglobin (Hb) was measured by Sahli Hemoglobinometer. Packed cell volume (PCV) was determined by the use of haematocrit method.

Statistical analysis

All the values of the routine blood test were input and analyzed using Microsoft Excel (version -2013) software. The values were expressed as mean \pm standard deviation (SD). Student t-test was performed for all parameters to observe the significant changes among the pesticide exposed and control rabbit.

RESULTS

The physical appearance and body weight of pesticide exposed rabbits and control rabbits were normal (Figure 1). The total erythrocyte count (TEC), haemoglobin (Hb) concentration, packed cell volume (PCV), and erythrocyte sedimentation rate (ESR) in the control animals were 3.47 ± 0.66 , 12 ± 1.14 , 40.5 ± 1.2 and 1.2 ± 0.45 , respectively (Table 1). The value of TEC, ESR, and Hb concentration was decreased (at non-significant level) in pesticide exposed feed rabbits in comparison with control rabbits (Figure 2A-C). The erythrocyte indices mean corpuscular haemoglobin (MCH), mean corpuscular volume (MCV), and mean corpuscular haemoglobin concentration (MCHC) were decreasing tendency with exposure to pesticide (Table 1).

Total leukocyte count (TLC) and the differential leukocyte count are presented in Table 2. The TLC was $7.36 \pm 4.13 \times 10^3/\mu\text{l}$ in normal control rabbits. The TLC was not changed significantly in pesticide exposed not washed feed, and washed feed rabbit compared to control rabbit. In the case of the DLC, the percentage of neutrophil and eosinophil significantly decreased in pesticide-exposed rabbits compared to control rabbit (Table 2). The lymphocyte percentage was increased significantly ($p < 0.005$) in both pesticide exposed rabbit compared to control rabbit. Interestingly, the lymphocyte percentage significantly higher in pesticide exposed, not washed feed rabbits compared to that of pesticide exposed, washed rabbits (Figure 3). The monocyte percentage showed a decreased tendency in both pesticides exposed to rabbits compared to control rabbit (Figure 2).

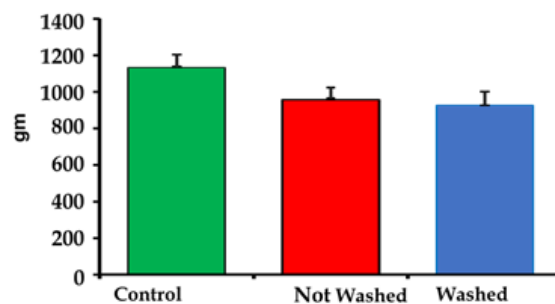
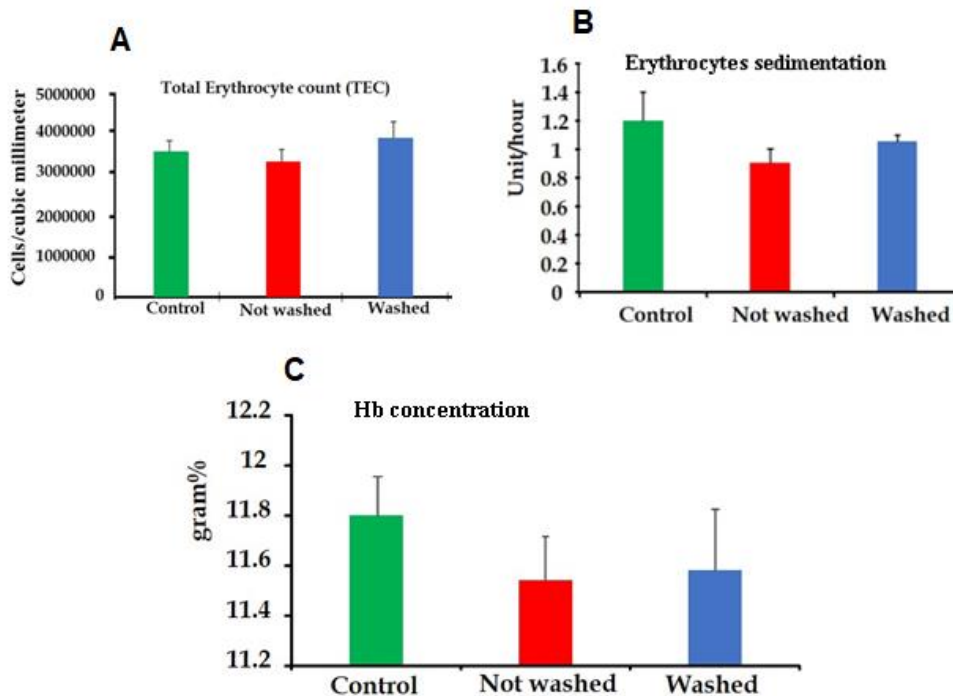
Table 1. The total erythrocyte count, haemoglobin concentration, and packed cell volume (mean \pm SD) of imidacloprid treated rabbits

Parameters	Control	Imidacloprid exposed rabbit (mg/kg bw)	
		Not washed fed rabbit	Washed fed rabbit
Total erythrocyte count (TEC $\times 10^6/\mu\text{L}$)	3.47 ± 0.66	3.31 ± 0.78	3.82 ± 1.03
Hemoglobin concentration(g/dl)	12 ± 1.41	11.42 ± 0.53	11.65 ± 0.60
Packed cell volume (%)	40.5 ± 1.29	37 ± 1.83	38.12 ± 3.09
MCH	36.13 ± 11.32	35.45 ± 5.77	32.26 ± 9.44
MCV	120.46 ± 26.73	110.91 ± 24.16	104.43 ± 24.11
MCHC	3.39 ± 0.27	3.16 ± 0.13	3.27 ± 0.25

Table 2. Total leukocyte and differential leukocyte count of imidacloprid treated rabbits

Parameters	Control	Imidacloprid exposed rabbit (mg/kg bw)	
		Not washed fed rabbit	Washed fed rabbit
Total leukocyte count(TLC $\times 10^3/\mu\text{L}$)	7.36 \pm 4.13	6.45 \pm 5.51	7.91 \pm 3.36
Neutrophil (%)	25.6 \pm 2.70	11.8 \pm 3.70 ^a	12.2 \pm 4.26 ^a
Eosinophil (%)	14.6 \pm 2.70	5.8 \pm 0.84 ^a	13.4 \pm 6.11 ^{ab}
Lymphocyte (%)	48 \pm 1.58	73 \pm 2.24 ^a	64.6 \pm 9.09 ^{ab}
Monocytes (%)	9.8 \pm 2.77	10.4 \pm 3.44	8 \pm 4.18

Student t-test, a and b are indicating significant (P value <0.05) difference between control vs not washed or wash feed rabbit and the difference between not washed feed rabbit vs washed feed rabbit, respectively.

**Figure 1.** Graphical illustration of body weight showing no significant difference between pesticide exposed rabbits and control rabbits**Figure 2A-C.** Effects of pesticide on total erythrocyte count (A), erythrocyte sedimentation rate (B), haemoglobin concentration (C) showing the values of TEC, ESR and Hb concentration decreased in not washed feed rabbit compared to the control rabbit. Student t-test showed a statistically non-significant

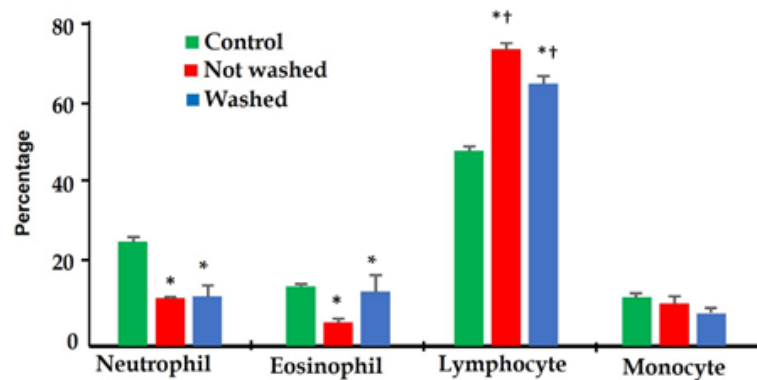


Figure 3. Effects of pesticide on differential leukocytes count showing the percentage of neutrophil; eosinophil was significantly decreased in both pesticide exposed rabbits compared to control rabbit whereas the lymphocyte percentage was increased significantly in both pesticide exposed not washed and washed rabbit compared to control rabbit. The monocyte percentage was reduced in both pesticide exposed rabbit. Student t-test, * and † indicating significant (P value <0.05) difference between control vs not washed or washed feed rabbit and the difference between not washed feed rabbit vs washed feed rabbit, respectively. Values were given as mean \pm SE

Discussion

Toxicity due to unsafe use of pesticides in the field is the most prevalent and serious occupational hazards of agricultural workers as well as pasture animals in developing countries. The use of pesticides not only affects our environment but also affects the health of animals and humans (Aktar et al., 2009). In this work, the result showed no significant differences between TEC, ESR, Hb, and PCV values in pesticide exposed rabbits and control rabbits. The value of erythrocyte indices was also not changed. Similar results were reported in the pesticides exposed rabbits (Basir et al., 2010), chickens (Tras et al., 2000), and agriculture workers (Aktar et al., 2009).

The TLC was not changed significantly in pesticide exposed, not washed feed and washed feed rabbit compared to control rabbit. In the differential leukocyte count, the percent of neutrophil and eosinophil in pesticide exposed both not washed and washed feed rabbit was significantly reduced whereas lymphocyte percentage increased significantly in pesticide exposed both not washed and washed feed rabbit compared to control rabbit. These results are similar to the findings of Suseela et al. (2015) in lambda-cyhalothrin exposed albino mice. So it is evident that pesticides have a direct association with the production of DLC. Besides, in the pesticide exposed groups, the lymphocyte percentage significantly higher in pesticide exposed washed feed rabbit compared to that of pesticide exposed, not washed rabbits. It may be suggested that very low residue level of pesticide may act as an effective stimulator for the production of leukocytes. The monocyte percentage was reduced in pesticide-exposed rabbits compared to control rabbits. The result of monocyte percentage in this study is similar in case pesticide exposed rabbit with the report of Basir et al. (2011) in female rabbit using lambda cyhalothrin intoxication.

CONCLUSION

The present study shows that pesticide exposed rabbits are more likely affected as compared to control rabbits in association with a decrease in neutrophil and eosinophil and an increase in lymphocytes. The total erythrocyte count, packed cell volume, and haemoglobin values were not changed significantly in pesticide exposed not washed and washed feed rabbit as compared to control rabbit. For differential leukocyte count, the percentage of neutrophil and eosinophil in pesticide-exposed rabbit were significantly decreased. The lymphocyte percentage was increased significantly in pesticide-exposed rabbits compared to control rabbits. Interestingly, the number of lymphocytes was significantly increased in not washed feed rabbits compared to wash feed rabbits. These results indicate that pesticide exposure at low level alter inflammatory cells mainly lymphocytes and affect body homeostasis, which acts as a co-factor for other diseases or infection.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this paper.

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