VOLUME 10 ISSUE 2 2024

ISSN 2454 – 3055

INTERNATIONAL JOURNAL OF ZOOLOGICAL INVESTIGATIONS

Forum for Biological and Environmental Sciences

Published by Saran Publications, India

International Journal of Zoological Investigations Vol. 10, No. 2, 582-601 (2024)

International Journal of Zoological Investigations

Contents available at Journals Home Page: www.ijzi.net

Editor-in-Chief: Prof. Ajai Kumar Srivastav

Published by: Saran Publications, Gorakhpur, India

ISSN: 2454-3055

Effects of Dietary Potato, Cinnamon, and Cumin on Blood Composition in Gestating and Lactating Swiss Albino Mice

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Received: 11th February, 2024; **Accepted:** 13th June, 2024; **Published online:** 4th September, 2024

https://doi.org/10.33745/ijzi.2024.v10i02.056

Abstract: Nutrition taken during gestation impose its direct effect on the growth and development of the offsprings. In the present investigation effects of potato, cinnamon and cumin were observed on the blood composition during the period of gestation and lactation in Swiss albino mice. Animals divided in 6 different groups were given different diets during the period of gestation and lactation; i.e. group I Control (CN), group II high carbohydrate (HC), group III cinnamon (Ci), group IV high carbohydrate + cinnamon (HC+Ci), group V cumin (Cu) and group VI high carbohydrate + cumin (HC+Cu). After parturition, on PND1, PND 21 and PND49, blood cell count (WBC and RBC) was calculated in mothers and the morphology of blood cells was examined on blood smears of pups. It was found that in mothers, RBC count remained same in group I and II; whereas it increased in group V and decreased in group III. Further, WBC count was found to be higher in group III, and remained unchanged in group IV (HC+Ci). In blood smears of pups, it was observed that the morphology of the blood cells RBC and WBCs in group II was same as that in group I; whereas group IV showed different type of RBCs and group III showed large neutrophils in aggregation. Hence, it is concluded that Cinnamon and Cumin can replace synthetic drugs for boosting immunity, treating initial stages of anaemia and as a natural source of anti-oxidants.

Keywords: Gestation, RBC, WBC, Cinnamon, Potato, Cumin, Lactation

Citation: Rani Deepika and Sharma Ragini: Effects of dietary potato, cinnamon, and cumin on blood composition in gestating and lactating Swiss albino mice. Intern. J. Zool. Invest. 10(2): 582-601, 2024.

https://doi.org/10.33745/ijzi.2024.v10i02.056

Introduction

The natural environment is necessary for the existence of present and future generation and has provided all the basic needs of humans and animal societies throughout history. Along with the environmental factors proper nutrition also widely affects the development of an organism. Carbohydrates, proteins, lipids, minerals, vitamins, and water are the essential nutrients and when they are present in correct amount which is required by the body is called optimum nutrition, adequate nutrition, or balanced diet. Plants organic molecules imparts crucial and beneficial impacts on human physiology for example various antioxidants, which inhibit oxidation processes.

Basically, the requirement of such antioxidants is closely tied to the existence of reactive oxygen species (ROS) within cells, which can rapidly engage in redox reactions, leading to significant changes and harm to other metabolites, DNA, proteins, or fatty acids. (Auten and Davis, 2009; Saed-Moucheshi *et al*., 2014). As compared to meat diet, plant-based diet is widely recognized as healthier and more sustainable (Sabate and Soret, 2014; Fresan and Sabate, 2019; Wiseman 2019; Spence, 2019).

Pregnant and nursing mothers, infants and children are the most sensitive segments of a population from the nutritional standpoint. Pregnancy is the stage when food requirement of the body increases for the proper development of baby and for mother's health. Maternal energy requirement increases during pregnancy (Miese-looy *et al*., 2008). Every year around more than 20 million infants are born with low birth weight (Akin *et al*., 2010). More than one-third of child deaths are due to maternal and child malnutrition (Black *et al*., 2008). On the other hand, a low-fat diet during pregnancy and lactation helps to prevent metabolic disturbances in the offspring of obese mice with type 2 diabetes mellitus (Gallou-Kabani *et al*., 2007).

Blood is said to be the largest connective tissue of the body which helps in communicating every part of the body from each other directly or indirectly. Thus, any change in the morphology or count of different blood components can be an important indicator of normal and abnormal functioning of particular organs or the body. Blood also contributes in immunity. WBCs help in phagocytosis and killing of invading disease-causing microorganisms and form body's primary line of defence against infections (Van Fur, 1982).

Along with the primary function of RBCs to transport oxygen from the lungs to the tissues, RBCs are also important regulatory component of hemorheology, the dynamics of blood flow (Baskurt and Meiselman, 2003; Nash *et al*., 2008). High vitamin C intake leads to the higher percentage of lymphocytes which is the protective function of vitamins (Gravina *et al*., 2012). Vitamin C stimulates body to produce more white blood cells, which in turn helps the body to fight infections (Braun, 2013). Vitamin C can also stimulate the activity of neutrophils, monocytes, and lymphocytes (Hespel *et al*., 2006) and enhance immunoregulation (Bhaskaram, 2002).

Any change in blood component is an initial indicator of improper functioning of body (Paprika and Sharma, 2003). Even in the diagnosis of many diseases hematological studies are proven to be very important (Togun *et al*., 2007). Omitoyin (2006) reported that hematological parameters are crucial for analysing the type of response of an animal exposed to any toxicant. Physiological status of animals can be easily overseen by observing hematological parameters (Khan and Zafar, 2005; Olafedehan *et al*., 2010). Stressed condition of the body generated by environmental, nutritional and/or pathological factors can also be determined by hematological parameters according to Afolabi *et al.* (2010).

The proposed experiments were conducted at the Environmental and Developmental Toxicology Research Laboratory, Department of Zoology, University College of Science, Mohanlal Sukhadia University, Udaipur, Rajasthan, India; to study the antioxidant properties of some plants during development of Swiss albino mice fed with high carbohydrate diet. In the present investigation effects of potato, cinnamon and cumin were observed on the blood composition during the period of gestation and lactation in Swiss albino mice.

Materials and Methods

Experimental Animal:

This study was conducted on mice (*Mus musculus*). The selection of mice as a model organism in the present research was due to their small size, short generation time, relatively accelerated life span (i.e. one mouse's one year = 300 human years) (Nichols and Simmons, 1970), manipulatable genome, 3 billion base pair DNA as humans, easy handling and most importantly 99% genome similarity with humans.

Animals were provided by the animal house of the aforementioned institute. The laboratory in which animals were kept was well-ventilated with relative humidity of 70-80%, with alternate 12 h of light and dark periods. Animals were kept in a rectangular poly vinyl cage covered with steel grill. Animals were maintained on standard diet and provided with 24 h of drinking water. The maintenance and handling of the animals were done as per guidelines of Committee for the Purpose of Control and Supervision of Experimental Animals, Ministry of Environment and Forest, Government of India. The experimental protocol is approved by the Institutional Animal Ethical Committee of the University (No. CS/Res/07/759).

In the present study healthy adult female Swiss albino mice (*Mus musculus)* 8-10 weeks of age and average body weights (BW) of 30 g were selected to perform experiments. After reaching an average weight of 30 g, four females were kept with one male in the ratio (4:1) for the act of copulation to take place. Then female mice were examined every day in the morning and if they showed vaginal plug, they were isolated and that day was counted as first day of gestation period and were proceed with experimental diet.

Diet Selection:

The diet selection for experiment was based on its known nutritional value, medicinal value, economical availability, and its commonly domestic use. *Solanum tuberosum* (potato) was taken as high carbohydrate diet, *Cinnamomum zeylanicum* (Cinnamon - Dalchini) taken as antioxidant and *Cuminum cyminum* (cumin) is taken as antioxidant and lactating agent. Diet was calculated according to the recommended dietary allowance for humans.

Experimental Design:

Selected females with vaginal plug were randomly separated into six experimental groups, each experimental group consist of 6 females.

Group I: Control diet (each mouse was given 10 g (3.85 g Wheat + 3.85 g Maize + 1.55 g Gram + 0.75 g Groundnut).

Group II: High Carbohydrate (*Solanum tuberosum*) (2.31 g Wheat + 2.31 g Maize + 0.93 g Gram + 0.45 g Groundnut + 4 g *Solanum tuberosum*)

Group III (a): *Cinnamomum zeylanicum* (bark) (3.66 g Wheat + 3.66 g Maize + 1.47 g Gram + 0.71 g Groundnut + 0.5 g *Cinnamomum zeylanicum*)

Group III (b): *Cinnamomum zeylanicum* (bark) (3.74 g Wheat + 3.74 g Maize + 1.52 g Gram + 0.75 g Groundnut + 0.25 g *Cinnamomum zeylanicum*)

Group IV: High Carbohydrate diet + *Cinnamomum zeylanicum* (2.12 g Wheat + 2.12 g Maize + 0.85 g Gram + 0.41 g Groundnut+ 4 g *Solanum tuberosum* +0.5 g *Cinnamomum zeylanicum*)

Group V: *Cuminum cyminum* (seeds) (3.66 g Wheat + 3.66 g Maize + 1.47 g Gram + 0.71 g Groundnut + 0.5 g *Cuminum cyminum*)

Group VI: High Carbohydrate diet + *Cuminum cyminum* (2.12 g Wheat + 2.12 g Maize + 0.85 g Gram + 0.41 g Groundnut + 4 g *Solanum tuberosum* + 0.5 g *Cuminum cyminum*)

Hematological Parameters:

Structural changes in red and white blood cells were observed for hematological investigation. For determination of haematological parameter, blood samples were collected at postnatal day (PND) 1, 21 and 49, from tail of the Control and Experimental female mice for RBC and WBC count post parturition and blood smears of pups was prepared on same days to evaluate the morphology of RBC and WBC.

Immediately after collection of blood, blood smear was prepared as soon as it dries, slides were dipped in methanol for 15 min for blood cell fixation and then stained by Giemsa stain so that blood cells in smear were easy to spot and evaluate the morphological changes if any.

Results

EFFECT OF HIGH CARBOHYDRATE DIET (POTATO) AND ANTIOXIDANTS (CINNAMON AND CUMIN) ON

RBCs count of mother on PND 1, 21 and 49 (Table 1):

As compared to CN (I) highly significant changes were observed in RBC count of PND 1, PND 21 and PND 49.

At PND 1 (time of birth), PND 21 (end of lactation) and PND 49 (after puberty): **I**ncrease in RBCs count was observed in Cu group (V) and decrease in RBCs was observed in HC group (II).

(1) Effect of High Carbohydrate diet (potato) on RBCs count of mother on PND 1, 21 and 49 (group II- HC)

- On PND 1 [At the time of birth], PND 21 [at the end of lactation] and PND 49 [After puberty], HC group (II) showed statistically significant decrease in RBCs count when compared to CN (I) and all the other experimental groups.

(2) Effect of Antioxidant I (Cinnamon) on RBCs count of mother on PND 1, 21 and 49 (group III b- Ci)

- On PND 1 [At the time of birth], Ci group (III b) showed almost equal number of RBC like CN (I), statistically significant increase in RBCs count than HC group (II) and decrease from rest of the experimental groups.
- PND 21 [at the end of lactation], Ci group (III b) showed statistically significant decrease in RBCs count than CN group (I), Cu (V) and HC + Cu group (VI) and almost equal to HC + Ci group (IV) and increase when compared to HC group (II).
- PND 49 [After puberty], Ci group (III b) showed statistically significant decrease in RBCs count than CN group (I) and all the other experimental groups, except HC group (II).

(3) Effect of High carb + cinnamon diet on RBCs count of mother on PND 1, 21 and 49 (group IV- HC+ Ci)

- On PND 1 [At the time of birth], HC+ Ci group (IV) showed statistically significant increase in RBC count than CN group (I), Ci (III b) and HC group (II) and decrease than groups HC + Ci (IV), Cu (V) and HC+ Cu group (VI).
- On PND 21 [at the end of lactation], HC+ Ci group (III b) showed statistically significant decrease in RBCs count than CN group (I) and all the other experimental groups, except HC group (II).
- On PND 49 [After puberty], HC+ Ci group (IV) showed statistically significant decrease in RBC count than CN group (I) and rest; almost equal number of RBC count to Ci (III b) and increase number when compared to HC group (II).

(4) Effect of Antioxidant II (Cumin) on RBCs count of mother on PND 1, 21 and 49 (group V- Cu)

- On PND 1 [At the time of birth], PND 21 [at the end of lactation] and PND 49 [After puberty], Cu group (V) showed statistically significant increase in RBC count than CN group (I) and all the other groups.

(5) Effect of High carb + cumin diet on RBCs count of mother on PND 1, 21 and 49 (group VI- HC+ Cu)

- On PND 1 [At the time of birth], PND 21 [at the end of lactation] and PND 49 [After puberty], HC + Cu group (VI) showed statistically significant increase in RBC count than CN group (I) and all the other groups, except Cu group (V).

EFFECT OF HIGH CARBOHYDRATE DIET (POTATO) AND ANTIOXIDANTS (CINNAMON AND CUMIN) ON WBCS COUNT OF MOTHER ON PND 1, 21 AND 49 (Table 2):

WBCs are cells of immune system that response and trigger immune responses. They protect the body from foreign antigens (pathogen and allergens). As compared to control CN (I) statistically highly significant changes were observed in WBC count on PND 1, PND 21 and PND 49.

At PND 1 (time of birth), PND 21 (end of lactation) and PND 49 (after puberty): Increase in WBC count was observed in Ci group (III b) and decrease in WBC was observed in HC group (II).

Table 1: Variations in RBCs count (×106 cells/mm3) of mother in different experimental groups on different postnatal days. Values are expressed as mean ± S.D. for six females per group

Groups	RBCs of Mother On Following Postnatal Days (PND)		
	PND 1	PND21	PND49
Control	8.24 ± 0.23^{a}	8.87 ± 0.16^{a}	8.94 ± 0.18^{a}
HC	$7.95 \pm 0.27^{a, b}$	$8.14 \pm 0.34^{a, b}$	$8.39 \pm 0.28^{a, b}$
Cinnamon	$8.25 \pm 0.15^{b, c}$	$8.68 \pm 0.24^{b, c}$	$8.74 \pm 0.17^{b, c}$
HC+ Cinn	8.48 ± 0.16^{c}	8.66 ± 0.20^{c}	8.80 ± 0.18^{c}
Cumin	8.98 ± 0.16^{c}	9.27 ± 0.18^{c}	9.34 ± 0.27^{c}
HC+ cumin	8.72 ± 0.20^{d}	9.15 ± 0.14^{d}	9.23 ± 0.19^{d}
F value	13.240	13.365	10.38

P value >0.05 = non-significant (ns), <0.05 = significant (*) and <0.01 = highly significant (**). Mean followed by the same alphabet within columns are not significantly different (P < 0.05) using Duncan's multiple range test.

Table 2: Variations in WBCs count (×103 cells/mm3) of mother in different experimental groups on PND 1, PND 21 and PND 49 days. Values are expressed as mean ± S.D. for six females per group

Groups	WBCs of Mother on Following Postnatal Days (PND)		
	PND 1	PND 21	PND49
Control	7.95 ± 0.33^{a}	8.53 ± 0.19^{a}	9.12 ± 0.22^{a}
HC	7.35 ± 0.18^{a}	7.8 ± 0.21^{a}	8.11 ± 0.21^{a}
Cinnamon	8.88 ± 0.21^{b}	9.18 ± 0.19^{b}	$9.53 \pm 0.34^{a, b}$
HC+ Cinn	8.84 ± 0.17^{b}	9.2 ± 0.23^{b}	$9.31 \pm 0.21^{a, b}$
Cumin	8.11 ± 0.21^{b}	8.3 ± 0.19^{b}	8.81 ± 0.25^{b}
HC+ cumin	8 ± 0.30^{c}	8.5 ± 0.30^{c}	9.15 ± 0.27^{c}
F value	23.428	23.353	15.443

P value >0.05 = non-significant (ns), <0.05 = significant (*) and <0.01 = highly significant (**). Mean followed by the same alphabet within columns are not significantly different (p < 0.05) using Duncan's multiple range test.

(1) Effect of High Carbohydrate diet (potato) on WBCs count of mother on PND 1, 21 and 49 (group II- HC)

- On PND 1 [At the time of birth], PND 21 [at the end of lactation] and PND 49 [After puberty], HC diet group (II) showed statistically significant decrease in WBC count when compared to CN (I) and all the other experimental groups.

(2) Effect of Antioxidant I (Cinnamon) on WBCs count of mother on PND 1, 21 and 49 (group (III b- Ci)

- On PND 1 [At the time of birth], PND 21 [at the end of lactation] and PND 49 [After puberty], Ci diet group (III b) showed statistically significant increase in WBC count than CN group (I) and all other experimental groups; almost equal count like HC + Ci group (IV).

(3) Effect of High carb + cinnamon diet on WBCs count of mother on PND 1, 21 and 49 (group IV- HC+ Ci)

- On PND 1 [At the time of birth], HC+ Ci group (IV) showed statistically significant increase in WBC count than CN group (I) and all the other groups; almost equal to Ci diet group (III b).
- PND 21 [at the end of lactation] and PND 49 [After puberty], HC+ Ci group (IV) showed statistically significant increase in WBC count than CN group (I) and all the other groups; decrease than Ci diet group (III b).

(4) Effect of Antioxidant II (Cumin) on WBC count of mother on PND 1, 21 and 49 (group V- Cu)

- On PND 1 [At the time of birth], Cu group (V) showed statistically significant increase in WBC count than CN group (I) and all the other groups, except Ci (III b) and HC+ Ci group (III b).
- PND 21 [at the end of lactation], Cu group (V) showed statistically significant decrease in WBC count than CN (I) and all the groups, increase than HC group (II); almost equal count like HC+ Cu group (VI) .
- PND 49 [After puberty], Cu group (V) showed statistically significant decrease in WBC count than CN (I) and all the groups, increase than HC group .

(5) Effect of HC + Cu diet on WBC count of mother on PND 1, 21 and 49 (group VI- HC+ Cu)

- On PND 1 [At the time of birth], PND 21 [at the end of lactation] and PND 49 [After puberty], HC + Cu group (VI) showed almost equal WBC count when compared to CN (I), statistically significant increase in WBC count than HC (II) and decrease in rest of the groups.

MORPHOLOGICAL AND STRUCTURAL EVALUATION OF RED BLOOD CELLS OF PUPS FROM BIRTH TO MATURITY:

Figure 1 shows the blood smear of pups depicting different type of RBCs found in different experimental groups.

Group I (CN):

Morphologically RBC_s were normal in shape and size. They were round, spherical and without nucleus. Rarely morphology of some cells was distorted from normal shape to tear drop (dacrocyte), elliptocyte. Figure 1(1): Size between normal and macrocyte (2); Dacrocyte (tear drop cell)(3), rouleax (10).

Group II (HC):

RBCs were normal in shape and size as that of control. Other than normal cells, some other cells like acanthocytes and stomocytes were also noticed in the blood smear of pups. Figure 1(2): Dacrocyte (tear drop cell) (3), Acanthocytes (Spur cells) (4); Echinocyte (Creanated and burr cells) (5); Blister cells (7); Codocytes (Target cells) (6).

Group III b (Ci 0.25):

Shape and size of RBCs was distorted from the normal cells. Size of cells increases (macrocytes). Margins of the cells became irregular which results in elliptocytic RBCs. Nucleated RBCs were also noticed. Figure 1(3) **:** ElliptocAyte (Cigar cell, Ovalocyte) (8), nucleated RBC (9).

Group IV (HC+ Ci):

Shape and Size of RBCs was distorted from the normal RBCs. Cells increases in size but size remains between normal cells and macrocytes. Cell margins were irregular, but irregularity noticed was less than that of Cinnamon treated (group III b). Figure 1 (4)**:** Dacrocyte (tear drop cell) (3), Elliptocyte (Cigar cell, Ovalocyte) (8), rouleax (10),

Group V (Cu group):

Cell size very between normal and macrocytes. Irregularity in RBCs was less then Group Ci (III b), from round shape, cells become oval and some were elliptocytes. Figure 1(5): Elliptocyte (Cigar cell, Ovalocyte) (8), nucleated RBC (9).

Group VI (HC+ Cu):

Mostly cells were normal in shape and size. Cell size varies from normal to microcytes to increase in size but less than that of macrocytes.

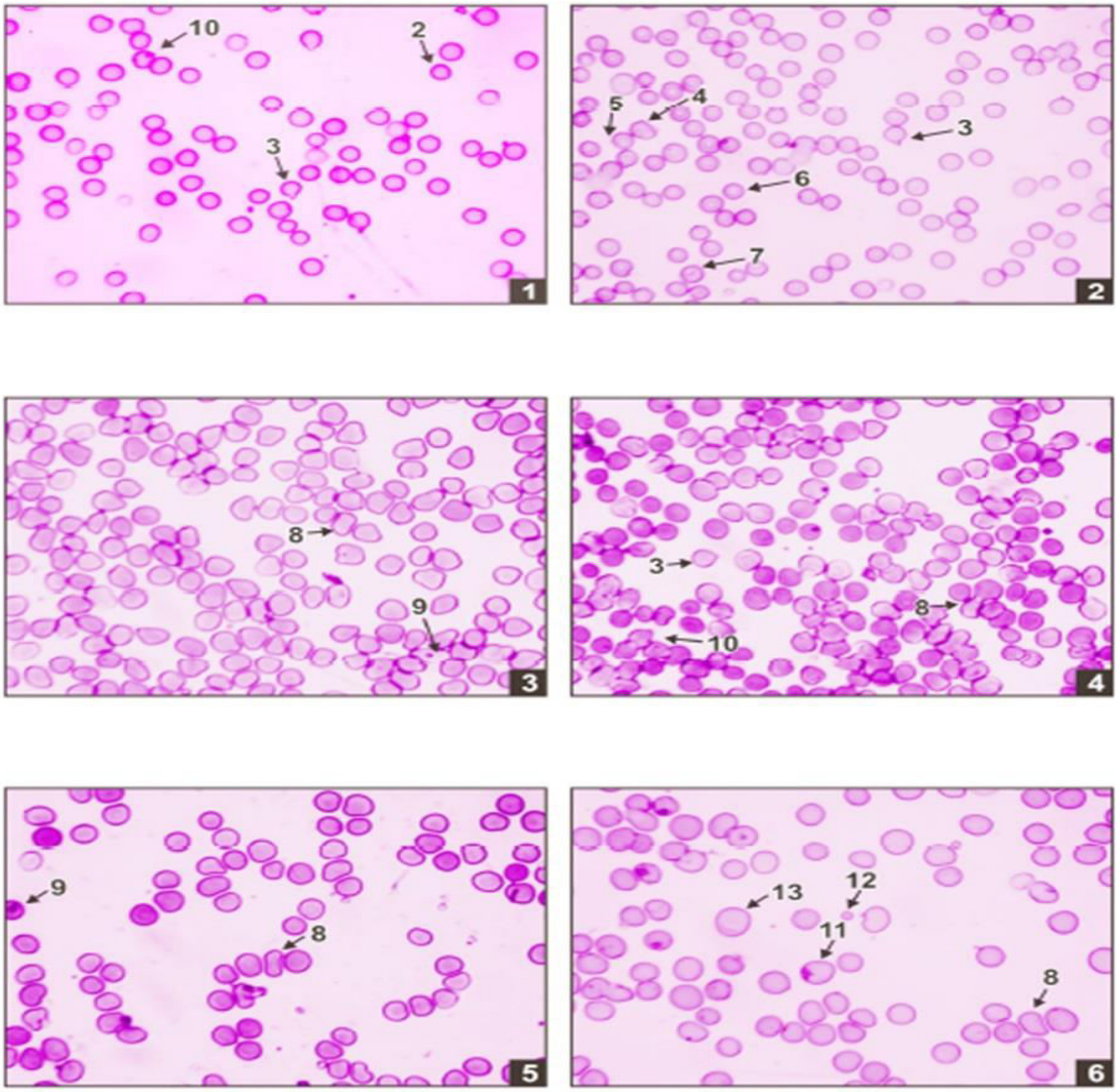

Fig. 1: Distribution of red blood cells in different experimental groups (PND21) 450X, Giemsa Stain
(1) CN;(2) HC; 3 (Ci b); 4 (HC+ Ci); 5 (Cu); 6 (HC+ Cu).

Irregularities in the margins of cells were noticed but these cells were very few in number. Figure 1(6): Macrocyte (13); Microcyte (12); Elliptocyte (Cigar cell, Ovalocyte) (8); Howell jolly body (11). Figures 2 and 3 show the detail morphological RBCs structure found in different experimental groups

MORPHOLOGICAL AND STRUCTURAL EVALUATION OF WHITE BLOOD CELLS OF PUPS FROM BIRTH TO MATURITY:

Morphology in the structure of WBCs was also studied in the present study in the influence of high carbohydrate diet with antioxidants (cinnamon and cumin) during gestation and lactation. Different types of WBCs were observed in the smear of pups in different experimental groups. WBCs were normal in control, neutrophil with characteristics nucleus with 3-5 lobes which increase to 5 to 6 till puberty. This increase was due to cell age normally up to 7 lobes were observed in very old neutrophils in adults. Lymphocytes were round, ovoid; later becomes notched and slightly indented. Normally nucleoli not visible. Monocytes with smooth margins, oval

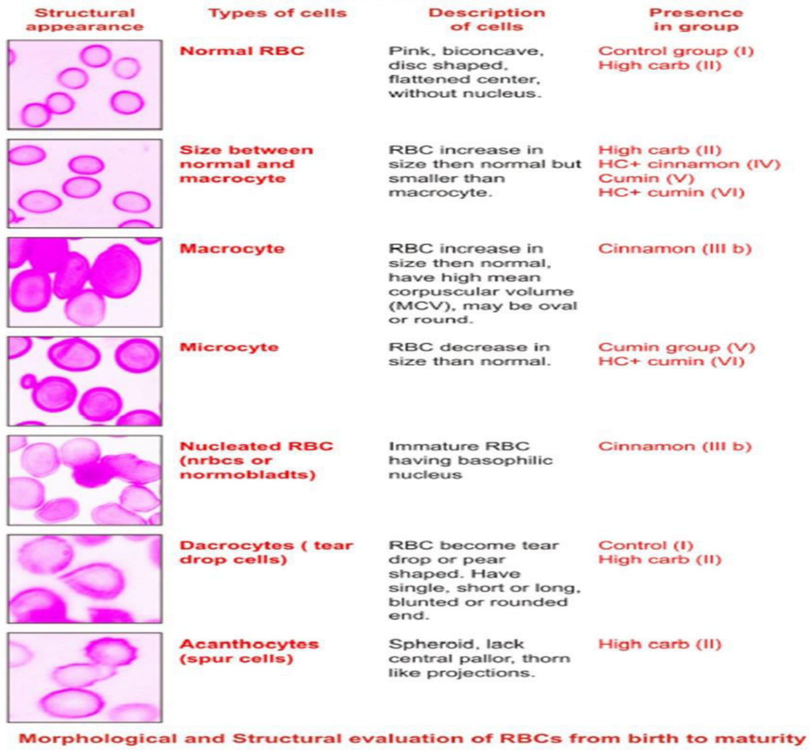

Fig. 2: Different types of RBCs in different experimental groups.

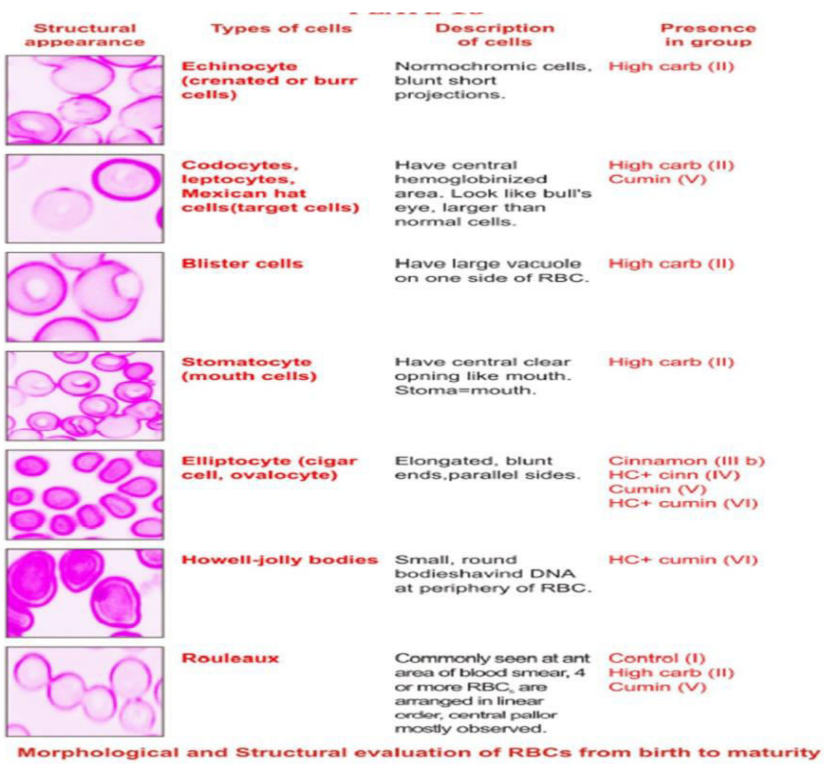

Fig. 3: Different types of RBCs in different experimental groups.

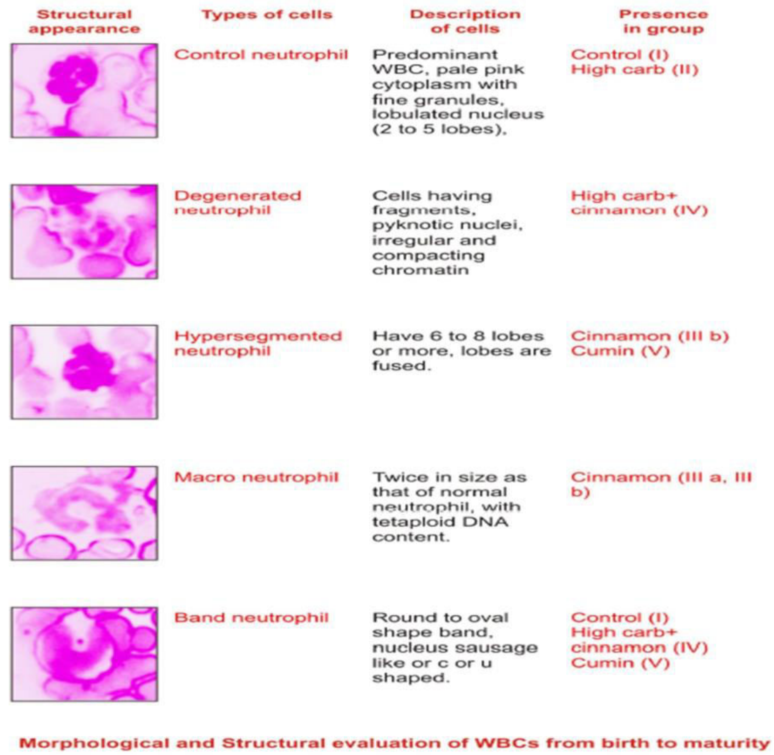

Fig. 4: Different types of WBCs found in different experimental groups.

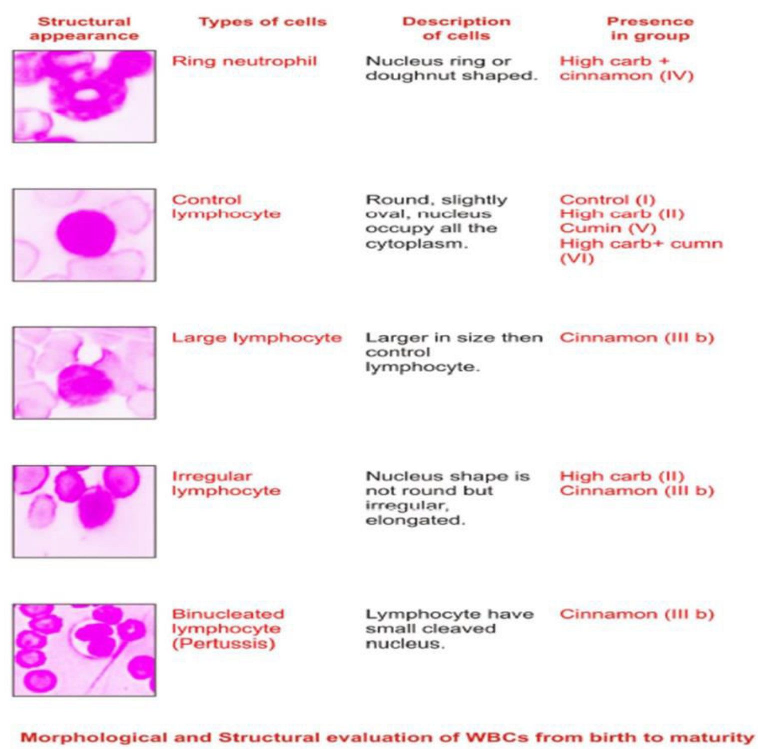

Fig. 5: Different types of WBCs found in different experimental groups.

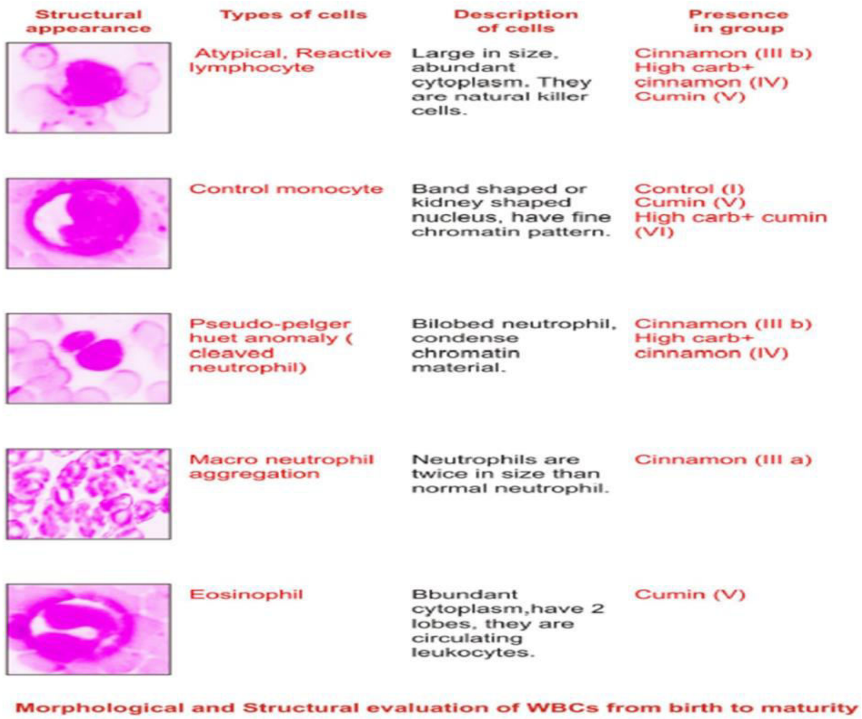

Fig. 6: Different types of WBCs found in different experimental groups.

nucleus little bit folded. Clumped chromatin material but less then neutrophil and lymphocyte were observed.

In treated groups neutrophils are distorted from normal shape and size like:

- Degenerated neutrophils were observed which have less condense chromatin material, have unequal fused lobes which were difficult to identify and have reduced cytoplasm.
- Hyper segmented neutrophil was also observed which have 5 to 7 lobes interconnected, no visible cytoplasm and chromatin material noticed, even have broken strands.
- Ring neutrophils were also noticed which were doughnut shape, differentiated chromatin material with no clear cytoplasm and they showed vacuolization.
- Distorted lymphocytes found in the treated group were: (i) Reactive lymphocyte which has very large irregular flattened nucleus and blue stained cytoplasm; (ii) large lymphocytes with clumsy chromatin material, large nucleus which cover all cytoplasm; and (iii) along with them oval, irregular, bi-nucleated and cleaved lymphocytes were also noticed in the treated groups.

Type of WBCs observed in different experimental groups (Figs. 4, 5, 6):

Group I (Control) – control lymphocyte, band neutrophil, 2 to 4 lobed neutrophil, control monocyte.

Group II (High carbohydrate) – control lymphocyte, irregular lymphocyte, 2 to 4 lobed neutrophils.

Group III b (cinnamon 0.25) – reactive, irregular, cleaved lymphocyte, mega and hyper segmented neutrophil.

Group IV (High carb + cinnamon 0.5) – Degenerated, ring and band neutrophil, reactive lymphocyte.

Group V (Cumin) – band, multi-lobed neutrophil, control and reactive lymphocyte, monocyte.

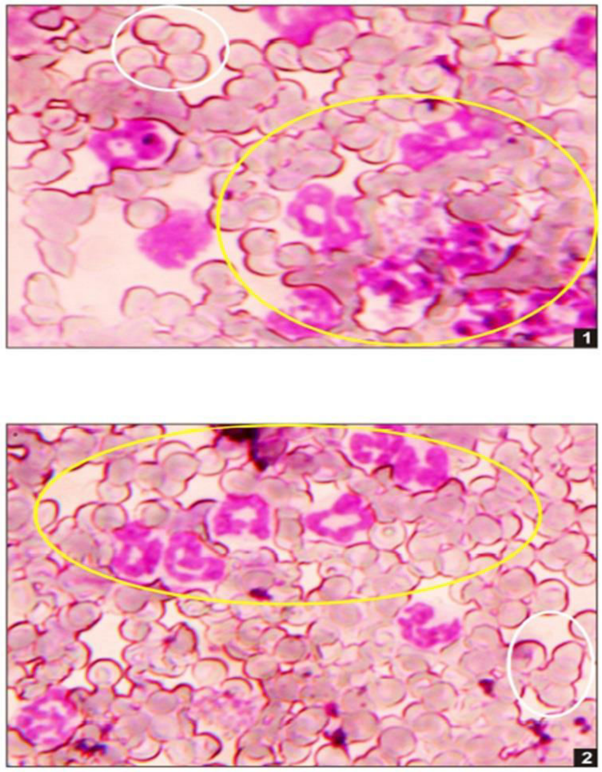

Fig. 7: RBC and WBS distribution in group III Ci (a) higher dose - 1- RBCs; 2- WBCs.

Group VI (High carb + cumin) – control lymphocyte, monocyte, neutrophil (3 to 5 lobed).

CINNAMON (PND 1) (Ci III a) (Ci- 0.50 g/10 g):

One of the important aspects of this study was to evaluate the safe dose of selected ingredients, hence, the amount of Ci was reduced to half for the same purpose. So group Ci III was sub divided into Ci (III a 0.5 mg/10 g)- higher dose and Ci (III b 0.25 mg/10 g)- lower dose. At low dose it was observed that pregnant female was normal during gestation period. Observation of blood cells of group III (b) was shown above with other experimental groups. Blood smear of higher dose of cinnamon group III (a) showed :

In group Ci (III a) blood smear of pups shows rouleaux pattern of RBC (In White circle; Fig. 7 -1).

In group Ci (III a) Neutrophil aggregation with hyper-lobed macro neutrophils is visible (In Yellow circle; Fig, 7 -2).

Discussion

Experimental Diet and Blood Development Outcomes (*Blood Cell Count and High Carbohydrate Diet*):

Anaemia is strongly related to under-nutrition in both animals and humans. It has been reported that starvation suppressed or decreased erythropoiesis (Levy *et al*., 1996). In the present study no such effect of HC diet was observed on morphology and blood cell count of animals. Carbohydrate does not directly affect the components of blood. Weber (1982) studied total eosinophil counts of dogs during starvation and refeeding at early fasting and observed that eosinophil counts fluctuated and then gradually diminished. Refeeding the fasting dogs with high carbohydrate diet however, did not return the eosinophil count to normal, but HCD remind their low count during fasting by activating pituitary-adrenal cortical activity which generally becomes active when eosinophil count decreases. They observed that this activation was continued by consumption of a diet high in carbohydrates.

Few studies showed that type of carbohydrate effects glycosylated haemoglobin and plasma glucose levels of mother as well as foetus which in turn related to fatal growth and infant birth weight but did not directly act on blood components (Scholl *et al*., 2004; Moses *et al*., 2006). Food and Agriculture Organization (FAO) of the United Nations identified Potato (*Solanum tuberosum*) as a staple and sustainable food for the growing world population, which provides a high-calorie-based, nutrient-rich diet and it is more conservative in land and water usage compared to other major crops, such as wheat or rice (Mekonnen and Hoekstra, 2011; Devaux *et al*., 2014). Study done by Beaudin and Stover (2007) states that during the stages of rapid growth like pregnancy and foetal development, folates are very important and its deficiency of which results in increased risk of cardiovascular diseases, anaemia, and cancers, also mild cognitive impairment, and depression (Bailey *et al*., 2003; Araujo *et al*., 2015). According to RDA values for adults of age 19-51 years 1.3 mg of vit B6 is required and for males and females above 51 years, recommended RDA is slightly higher i.e. 1.7 mg and 1.5 mg, respectively (Otten *et al*., 1988). 100 g of white fleshed raw potato provides approximately 12% of the RDA value or 0.239 mg (USDA National Nutrient Database for Standard Reference, Release 17), hence, Potato is a very good source for the vitamin. Different potato varieties like white versus red fleshed, the vit B6 content can vary significantly; vit B6 content also varies with age of the tubers (Mooney *et al*., 2013; Goyer *et al*., 2019). Recent study done by Magana *et al*. (2020) showed that folate-dependent metabolism is activated by vitamin C. For humans, plants folate biosynthesis is the main food source, because humans cannot synthesize the vitamin de novo (Hanjo Hellmann *et al*., 2021). For adults 400 g/day and for pregnant women 600 g/day is the required folate RDA. Medium size potato contains 6% of the recommended daily intake. But between potato varieties folate content varies (Goyer and Sweek, 2011; Robinson *et al*., 2015, 2019). Also, higher folate concentrations are found in younger tubers (Goyer and Navarre, 2009). After 8 months of storage at 7.8 °C, there is up to 141% increase in the Folate concentrations (Goyer *et al*., 2019). Potatoes are also a very good source for vit C. Raw potatoes (143 g) contain 27 mg of vit C, which is ~30–40% of the RDA values for adult woman and men (75 and 90 mg), as per Food and Drug Administration (FDA) (Beals, 2019). According to the study of Kallner *et al.* (1982) estimated turn-over rate of vit C is around 1 mg kg^{-1} body per day, so it requires continuous supplement by food, with an RDA of 65 and 75 mg for adult (>19 years) women and men, respectively (Monsen, 2000). Vitamin C or L-ascorbic acid is one of the most potent antioxidants; it is said to be a central antioxidant in living cells because it neutralized broader range of ROS such as hydrogen peroxide, peroxy radicals superoxide anion radicals, hydroxy radicals, and singlet oxygen (Kazmierczak-Baranska *et al*., 2020).

Potato also contain Vitamin B1 (thiamine) which is important for growth and development, proper functioning of the heart, muscles, and nervous system (Harper, 2006; Whitfield *et al*., 2018). Vitamin B6 (pyridoxine) is important for anaemia, neurological disorders, premenstrual syndrome, cardiovascular diseases, and cancer. Vitamin B9 (folate) is must for production of red blood cells, critical during periods of rapid growth (e.g., pregnancy, foetal development) (Beaudin and Stover, 2007; Araujo *et al*., 2015). Glycoalkaloids (SGA) boost immune system (Itkin *et al*., 2013; Nahar *et al*., 2017; Lange *et al*., 2020; Yu *et al*., 2020; Kiyama *et al*., 2021).

In the present investigation, no major changes were observed in the count of RBC and WBC. Both blood cell counts were low when compared to control and even to all the experimental groups, but in a healthy range. Production of RBCs is normal, this might be because of the Vit B9 which is present in potato. As examined, the RBC and WBC count on PND 1, 21 and 49 in HC group was steady and there was no change in the count even on different PND's. Hence, the observation of present investigation is also parallel to the previously done studies that HC directly not affects the blood components.

Blood Cell Count and Cinnamon:

Abd Elmawla *et al*. (2001) reported that Cinnamon contains manganese, dietary fibre, iron, calcium and cinnamaldehyde which are converted into cinnamic acid by oxidation. Cinnamic acid in liver is converted into benzoate by βoxidation (Abd Elmawla *et al*., 2001), which is a sodium salt (NaB). NaB is approved as a drug by FDA for the treatment of hepatic metabolic defects related with hyperammonaemia like urea cycle disorder in children (Leonard and Morris, 2002; Scaglia *et al*., 2004) and used in food and cosmetic preservative (Nair, 2001). Cinnamon's health benefit includes blood sugar control, antimicrobial and anticoagulant properties, enhancing brain function and calcium and fibre protection against heart disease (Mateljan, 2005). 41 mg of iron are present per 5 g of cinnamon. Many seasonings, herbs, and spices have a good iron content, but its portion is needed to be taken care off (Hassan, 2017). It is experimentally proven that cinnamon may reduce inflammation, have antioxidant effects, and fight bacteria (Hassan, 2017). During the period of the menstrual cycle cinnamon increase the levels of iron which results in increase of haemoglobin (Hassan, 2017).

NaB also alter the structure of T cells and protect allergic encephalomyelitis in animal model (Brahmachari and Pahan, 2007). Herbal extract increased the lymphocyte count in growing pigs which revealed that ET (eugenol) and CT (cinnamaldehyde) treated pigs has greater lymphocyte count compared with control treatment (Yan and Kim, 2012). *In vitro* studies on cinnamon revealed that it enhanced human lymphocytes to proliferate (Shan *et al*., 2005). In rat nephrotoxic serum (NTS) nephritis, *Cinnamon* inhibited the excretion of protein into the urine and increases the peripheral leukocyte counts (Nagai *et al*., 1982).

Present investigation is in line with the above findings; there was highest increase in WBC count in the pregnant Swiss mice when fed with cinnamon during gestation and lactation. Pregnant female mice of this group showed statistically significant increase in WBC count not only in comparison to control group but also to the other experimental groups on all the selected PND's.

Cinnamon bark decreased the platelet counts to comparable levels with the control group and a slight increase in haemoglobin content (Onderoglu *et al*., 1999). In the present investigation there were no major changes observed in the RBC count of Ci group, on PND 1 RBC count was equal to CN. Also, there was no change in morphology of the RBC membrane by different doses of aqueous cinnamon extract.

Cinnamon when consumed for a longer period significantly decreased the platelet counts in normal rats (Onderoglu *et al*., 1999). Shah *et al*. (1998) noticed a fall in haemoglobin level by cinnamon. In this study on PND's 21 and 49 there was decrease in the RBC count when compared to CN which is in conformity with studies of Shah *et al.* (1998).

Blood Cell Count and Cumin:

Cumin being an antioxidant and a good lactating agent, also widely affects the RBC and WBC count. Change in the blood cell count by cumin in dose dependent manner was proved by many previously studies.

Al-Hader *et al*. (1993) observed that cumin oil decreased serum lipids and glucose levels, and leukocytes and platelets count in rats, whereas it increased haematocrit and haemoglobin concentrations. In the present investigation, cumin treatment to pregnant mice during gestation and lactation also showed increase in RBC count and almost same count of WBC was observed when compared to CN on PND 1. Similar findings were also noticed by Srivastava (1989). Srivastava and Mustafa (1994) reported that acute and sub chronic treatment of cumin oil leads to the decrease in WBC count and increase of the haemoglobin concentration, haematocrit, and platelet counts. Active compound present in cumin countered on depleted T-lymphocytes, decreased the elevated corticosterone levels and size of adrenal glands, and increased the weight of thymus and spleen in restraint stress induced immune-suppressed animals (Chauhan *et al*., 2010). Acute and sub chronic administration of cumin oil increased the haemoglobin concentration, haematocrit, and platelet counts (Deepak, 2013). When haemoglobin level is less than 12 g/dl it is said to be Anaemia in women (age ≥15 years) (WHO, 2011). Anaemia decreases performance, work productivity, threatens the safety of mothers and babies during pregnancy and childbirth (Fadlun, 2014; Kementerian Kesehatan Republik Indonesia, 2016). 100 g of cumin contains 66.36 mg of iron and 10 μg of folic acid (Singh *et al*., 2017). This increased haemoglobin levels have been proved in experimental animals such as rats, ducks, sheep, chickens, and rabbits (Adel *et al*., 2016; Berrama *et al*., 2017; Chougule *et al*., 2017). Synthesis of haemoglobin required iron with vit C, which helps in absorption of nonheme iron by up to 4 times (Almatsier, 2009; Ciesla, 2012). Folic acid plays important role in the maturation of red blood cells, so that they have a normal life span (Hall John, 2016). Protein is also a component for the synthesis of red blood cells (Ciesla, 2012) and for the synthesis of globin which is a part of cellular protein and haemoglobin hence, protein deficiency can affect haemoglobin synthesis (Aris, 2009). It reduces the strength, elasticity, and flexibility of the membrane (McCance and Huether, 2014). Putri *et al*. (2021) suggested that cumin helps in the absorption and formation of haemoglobin in the blood.

In this study, on PND's 21 and 49, there was slight increase in the RBC count and drastic decrease in the WBC count on same PND's in comparison to CN group.

Result of present investigation does not align with few previous studies. Al-Kassi (2010) showed dose dependent reduction in RBC in broiler chicks. According to him increase in Cumin Crop Residue (CCR) in a diet resulted in the reduction of RBC number. Hence, the animals that consume 21% CCR, had the least RBC value. According to Ibrahim *et al*. (2007) decrease in RBC was due to materials found in cumin which cause derangement of haemopoietic process of the body. Cumin does not directly affect the blood cells as Kitajima *et al*. (2003) observed that cumin improved anaemia which occurs due to increase in non-enzymatic glycosylation in membrane proteins of RBC in diabetics and via reduction of lipid peroxidation in RBC haemolysis and thus does not affect RBC level directly.

In contrast to the present investigation, Houghton *et al*. (1995); Nair *et al*. (1991); Daly (1998) and Haq *et al.* (1995) reported that cumin significantly protects mice and rats from cisplatin (reduction done by cancer) induced deduction in leukocytes counts and affects leukocytes activities. Meral *et al.* (2004) reported that cumin reduced the number of HGB, WBC and RBC in carbon tetrachloride treated rabbits. In the present investigation it is concluded that there is also some protective role played by cumin in maintaining the blood cell because irrespective of whether Cu was increasing or decreasing the blood cell count, such increase or decrease was in safe range and never too steep.

Unlike that of the present investigation Jami *et al.* (2015) does not notice any change in the number of WBC from cumin and study of Al-Kassi (2010) does not supports the result of present investigation, he observed that higher dosages of cumin in broiler chicks resulted in the increase in WBC count.

After observing the individual effect of HC, Ci and Cu diet on the RBC and WBC count of pregnant Swiss mice during gestation and lactation, this effects in combination, over the same period were also reported in the present investigation. From the result it was concluded that Ci when given in combination with HC diet (HC) Ci) during the same period there was slight increase in the RBC count on PND 1 when compared to only Ci diet, but this increase was very high in comparison to HC diet even on PNDs 21; on PND 21 RBC count was noticed to be the same. When Cu is given in the combination with HC diet then there was decrease in the RBC count when compared to the individual given Cu and HC diet on the PNDs 1 and 21.

There was no major changes in WBC count when Ci was given in combination with HC diet, WBC number was almost same in Ci and HC+ Ci group on PND 1 and 21. When Cu was given in combination with HC during gestation and lactation, WBC count was noticed to be almost same on PND 1 but it increased on PND 21.

In the present investigation it was concluded that Cu increase the RBC count hence, can be used in anaemic condition whereas Ci can be used when the WBC count fall below to the normal number. Also, Cu can be used in the conditions where WBC increases above the normal number.

Alteration in the Shape of Blood Cells:

Diet also influence the morphology and in turn function of blood cells. In the patients of G6PD deficiency high reticulocyte count along with some abnormal RBC in peripheral blood was observed in comparison to healthy person (Champe *et al*., 2005; Smith *et al*., 2005). Similar result was obtained by other researchers also (Zinkham and Lenhard, 1956; Hafez *et al*., 1986). In the present study also, varied changes in the morphology of blood cells in the pups of different experimental groups was observed.

When the size of RBC increased from its normal size it is called as macrocytosis. Soivio *et al*. (1974) opined that size of red blood cell increased due to increase in mean corpuscular volume (MCV) mainly due to the increase in their activity. Increase in the size of RBC was due to many factors but it was mainly seen in the condition of stress (Weber *et al*., 1992). In the present investigation, macrocytes were noticed in Ci, HC+ Ci and in Cu treated groups, whereas microcytes were seen in HC+ Cu treated groups, this may be due to the increase in oxidative stress instead of anti-oxidative property and thus it was concluded that the period of pregnancy may need more antioxidants to rectify oxidative stress.

Echinocytes (crenated cells) increased viscosity of blood hence affects the blood flow and hinders microcirculation (Reinhart and Chien, 1987). According to Zeni *et al.* (2002), this change in RBC was due to cellular adaptation of physiological parameters which leads to shape maintenance.

Apart from macro and microcyte RBC many other morphological changes in the RBC were observed in this study because of selected diet plan, morphologically RBC_s are normal in shape and size in CN and HC group whereas shape was distorted in Ci and HC+ Ci group. Rarely tear drop (dacrocyte), elliptocyte were seen in CN but acanthocytes and stomocytes and RBCs with irregular cell margins were noticed in other groups. Nucleated RBCs were also noticed in Ci group.

Fait *et al*. (2005) compared the early pregnancy with non-pregnant stage and noticed that in an uncomplicated pregnancy peripheral polymorphonuclear leukocytes (PMNL) and neutrophil count were elevated due to increase in ROS and OS which occur during pregnancy. Result of present research goes parallel with the above study; increased neutrophil and multilobed neutrophil were seen in Ci and HC+ Ci group along with the aggregation of neutrophils.

Some abnormal monocytes were also noticed, reactive monocytes were seen with intensely basophilic and vacuolated cytoplasm in Cu and HC+ Cu group. Finding of DeNicola *et al.* (1991) supports the present study with the evidence of reactive monocytes which are mainly due to chronic inflammation in cat.

Having black cumin seeds, thyme, cinnamon bark, or their mixture as biological feed additives leads to the significant increase in RBC count (Demir *et al*., 2008). Acute and sub chronic administration of cumin oil leads to the decrease in WBC count and increased the haemoglobin concentration, haematocrit, and platelet counts and LDL/HDL ratio was reduced to half (Johri, 2011).

Conclusion

In the present investigation different types of RBC and WBC was observed in different experimental groups. Apart from normal shaped RBC; acanthocytes, stomocytes, macrocytes, elliptocytic, Nucleated $RBC_{s,}$ microcytes were also noticed in different diet groups. Morphologically control, irregular reactive, lymphocyte, band, 2-4 lobed, mega, and hyper segmented, ring neutrophils, monocyte were seen along with normal WBC. Hence, it was concluded that different diets, along with RBC and WBC count also alter the morphology of blood cells.

Acknowledgements

Authors are thankful to the authorities at Department of Zoology, College of Science, M.L.S. University for providing necessary facilities for the present research work.

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