JCBPS; Section B; November 2018 – January 2019, Vol. 9, No. 1; 010-023, E- ISSN: 2249 –1929
[DOI: 10.24214/jcbps.B.9.1.01023.]

Journal of Chemical, Biological and Physical Sciences

An International Peer Review E-3 Journal of Sciences

*Available online at*www.jcbsc.org

Section B: Biological Sciences

CODEN (USA): JCBPAT Research Article

Effect of integrated diets comprising of *Sesamum indicum*, *Emblica officinalis* and *Foeniculum vulgare on* Blood Cells during Gestation and Lactation in Swiss mice

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Received: 20 October 2018**; Revised:** 11 November 2018; **Accepted:** 30 November 2018

Abstract: The dietary components have measureable effect on blood constituents. Haematological values are extensively used to determine systemic relationship and pathological evaluation of general health conditions and in diagnosis of various types of animal diseases. In the present study, effect of different combinations of diet on morphological changes in blood cells of pups and numerical changes in blood cells of their mothers were studied. Thirty six Swiss albino mice divided into 6- groups of 6 – mice in each, served as subjects for this study. Animals in these six groups were fed with different diets according to the experimental protocol. **Group-1** Control diet (control), **group- 2** High fat (sesame seed), **group- 3** *Emblica officinalis*, **group- 4** High Fat + *Emblica officinalis*, **group- 5** *Foeniculum vulgare* and **group- 6** High Fat + *Foeniculum vulgare*. After parturition the blood sample of mothers were taken from all experimental groups and RBC and WBCs count were assessed. Blood smear of pups were prepared on post-natal day (PND) 1, 21 and 49. In all studied groups statistically highly significant ($P<0.01$) changes were observed in RBC and WBC count of mother at all PND when compared to control. On the other hand no major morphological and structural changes were observed in blood cells of the pups on different post natal days. It can be concluded that various combinations of diet given during gestation and lactation have substantial effects on blood cells of mother and their developing foetus, and this dietary impact on pre and post-natal development remains till adulthood.

Keywords: diet, development, foetus, post-natal, RBC, WBC

1. INTRODUCTION

Blood is very important component of human body; the cells present in the blood are RBC, WBC and platelets. Leucocytes are either granulocytes, cells with granule which includes neutrophils, basophils and eosinophils or agranulocytes, cells without granule which includes lymphocytes and monocytes. The percentage of different leucocytes in human blood ranges as neutrophils 50-70%, eosinophils 1-5%, basophils 0-1, monocytes 2-10%, and lymphocytes 20.45% [1.].Blood cells are continuously formed in the body through a process called haemopoiesis and erythropoiesis. Certain dietary components increase production of blood cells and others may interfere in this processes. Erythropoiesis is an enormously active process that needs several metabolites for synthesis of haemoglobin, which contains globin as protein part and haem as prosthetic group. Previously several natural ingredients with high antioxidant value were reported from herbal plants. For the present study Emblica officianlis (amla) and Foeiniculum vulgare (fennel) were selected, having anti-inflammatory, antioxidant, anticancer, hepatoprotective, immunostimulant, gastroprotective, nephroprotective and dermoprotective properties.

Flavonoids present in *Sesamum indicum's* were found very effective in raising the haemoglobin content in rats [2]. Sesame oil proved beneficial in improving the blood glucose, glycosylated haemoglobin, lipid-peroxidation, and antioxidant levels in diabetic induced female rats [3-4]. Amla maintains the function of the liver, increases haemoglobin and red blood cell count. It regulates blood sugar, lipids in cell membranes and control blood pressure. It promotes the absorption of iron and naturally increases Hb% in blood. During pregnancy iron plays important role in producing new blood cells to carry oxygen and nutrients to the foetus [5]. Fennel shows distinctive beneficial effect on blood, it prevents platelet aggregation and clot retraction[6].

Many studies were already performed in past to study the effects of amla and fennel seeds on blood parameters of animal models[7] ,but the morphological and numerical variation in RBC and WBC in relation to different combinations of diets are not widely studied. To fulfil this lacuna the present study was conducted on Swiss albino mice with the aim to assess the blood cells after exposing pregnant and lactating females to various combinations of selected antioxidant with high fat diet

2. MATERIALS AND METHODS

The proposed experiments were conducted in the Environmental and Developmental Toxicology Research Laboratory, Department of Zoology, University College of Science, Mohanlal Sukhadia University, Udaipur, Rajasthan, India to observe the role of dietary components during pregnancy and lactation in Swiss mice.

2.1 Animals: Healthy adult female Swiss mice 8-10 weeks old and weighing 25-30gm were used for this study. Animals were obtained from the animal house of our department. Mice were kept in the ratio of 1:4 (male and females) for breeding, and females showing vaginal plugs were isolated and their gestation periods were recorded. Confirmed pregnant females were housed in polyvinyl chloride cages (270×220×140mm) wrapped with rice husk bedding, and maintained under standard laboratory conditions. The laboratory animals were kept in well ventilated animal room with relative humidity of 70-80%. The room lighting consisted of alternate 12 hours light and dark periods. During the entire experimental period, the animals were fed on a standard diet and water ad libitum. The maintenance and handling of the animals were done as per the guidelines of Purpose of Control and Supervision of

Experimental Animals, Ministry of Environment and Forests, Government of India. All the experimental work was approved by the institutional animal ethics committee (No.CS/Res/07/759).

2.2 Experimental design: The selected pregnant females were separated in the following groups (six animals in each group and sub-group), to study the variation in blood cell count and morphology (RBC and WBC) on 1st, 21st and 49th days. The combination of diets was introduced to the animals during the complete experimental duration i.e. from 1st day of gestation up to the end of lactation. After the termination of the experiment all the animals were fed with control diet. In all the following groups each animal was given 10 gm diets in different combination in different experimental groups.

- Group 1- Control diet (Each mice receiving (3.85gm Wheat + 3.85gm Maize + 1.55gm Gram + 0.75gm Groundnut = 10gram).
- Group 2- **High fat** (Sesame seeds) (2.31gm Wheat + 2.31gm Maize + 0.93gm Gram + 0.45grm Groundnut + **4gm Sesame seeds**)
- Group 3- ***Emblica officinalis*** (fruit powder) (3.66gm Wheat + 3.66gm Maize + 1.47gm Gram + 0.71grm Groundnut + **0.50gm *Emblica officinalis***)
- Group 4- **High fat + *Emblica officinalis*** (2.12gm Wheat + 2.12gm Maize + 0.85gm Gram + 0.41grm Groundnut + **4gm Sesame seeds + 0.50gm *Emblica officinalis***)
- Group 5- ***Foeniculum vulgare* (seeds)** (3.66gm Wheat+3.66gm Maize + 1.47gm Gram + 0.71grm Groundnut+**0.50gm *Foeniculum vulgare***)
- Group 6- **High fat + *Foeniculum vulgare*** (2.12gm Wheat+2.12gm Maize +0.85gm Gram + 0.41grm Groundnut+**4gm Sesame seeds+0.50gm *Foeniculum vulgar e***)

Blood samples for blood cell count were obtained from the tail of each mouse. The tip of the tail was cleaned with spirit before being cut with a sharp blade and was not squeezed to avoid dilution of blood by tissue fluid. The first drop of blood was discarded and the blood was diluted with Thomas baker RBC and WBC diluting fluid. The blood cell count was performed with the help of haemocytometer (Neubauer improved chamber made in Germany) method described by Dacie and Lewis (1975). Data obtained s analysed statistically using one way ANOVA and the values were expressed as mean ± Standard deviation.

Morphological changes in RBC and WBC were observed by making blood films. These blood films were fixed in absolute methanol for 15 minutes and stained with freshly made Giemsa stain then the washed slides were allowed to dry and the haematological observations were taken.

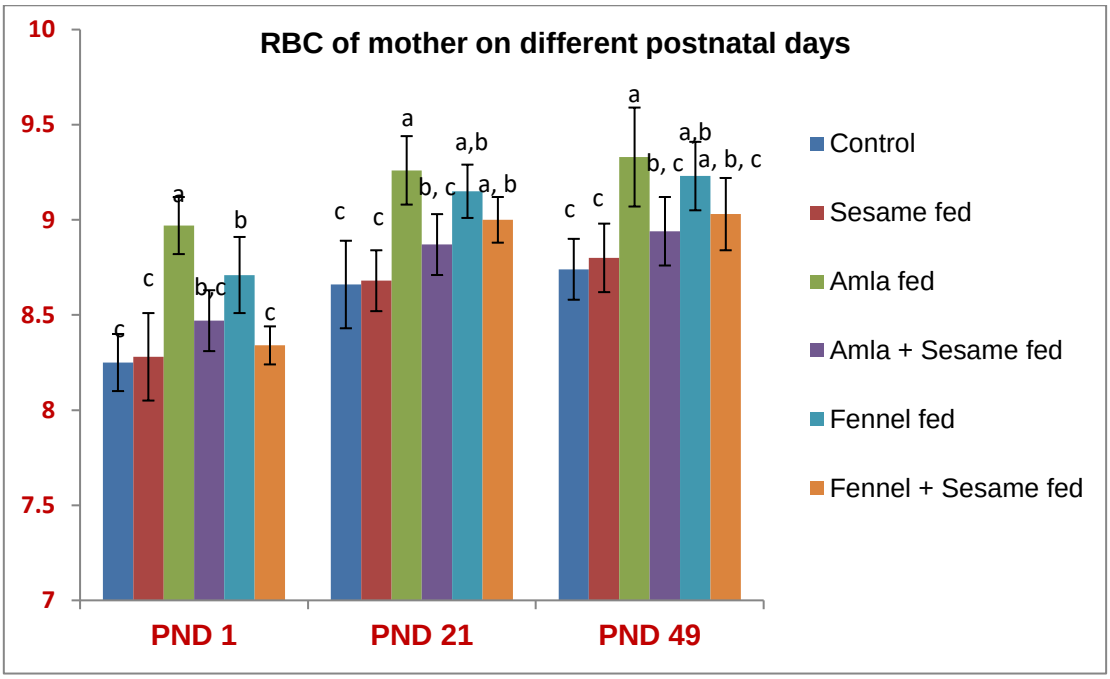
3. Results

3.1 Effect of different combination of diets on RBC count ($\times 10^6$ cells/mm^3) of mother on various PND 1, 21 and 49: Details of RBCs count of various experimental groups in comparison to control are shown in **table 1 and graph 1.** Statistically highly significant ($P<0.01$) changes were observed on PND 1 at the time of birth, PND 21 at the end of lactation and on PND 49 after puberty. At the time of birth it was observed that the RBCs count increased in following order Amla, Fennel, HF + Amla, HF + Fennel and HF group in comparison to control group. At the end of lactation and after puberty highest increase in RBC counts were observed in amla and fennel fed group. While other group showed decrease in RBC counts compared to above two groups.

Table 1: Variations in RBC counts 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.

Treatments	RBC of mother on following experimental days		
	1st day	21st day	49th day
Control	8.25 ± 0.15^{c}	8.66 ± 0.23^{c}	8.74 ± 0.16^{c}
High fat	8.28 ± 0.23^{c}	8.68 ± 0.16^{c}	8.80 ± 0.18^{c}
Amla	8.97 ± 0.15^{a}	9.26 ± 0.18^{a}	9.33 ± 0.26^{a}
HF + Amla	8.47 ± 0.16^{b,c}	8.87 ± 0.16^{b,c}	8.94 ± 0.18^{b,c}
Fennel	8.71 ± 0.20^{b}	9.15 ± 0.14^{a,b}	9.23 ± 0.18^{a,sb}
HF + Fennel	8.34 ± 0.10^{c}	9.00 ± 0.12^{a,b}	9.03 ± 0.19^{a,b,c}
F-values	10.88**	6.57**	5.58**

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 rang test.



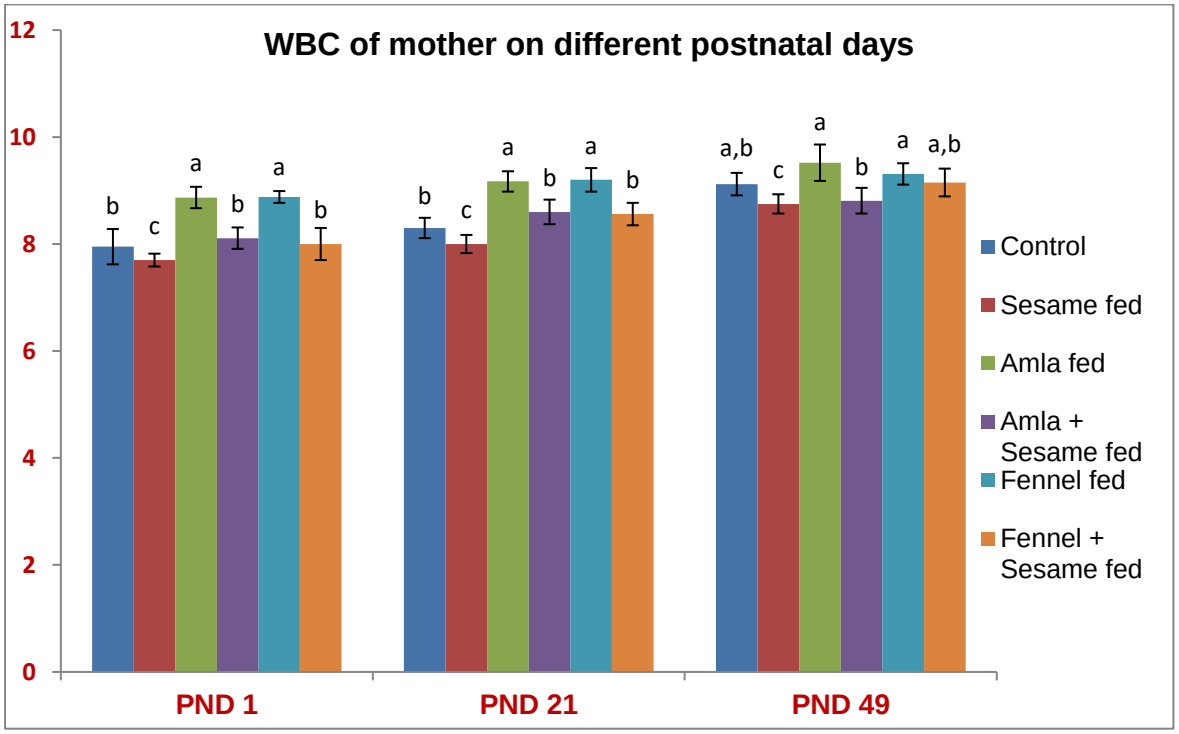
Graph 1: Variations in RBC counts (x10^{6}/ mm^{3}) of mother in different experimental groups on PND 1, 21, and 49

3.2 Effect of different combination of diets on WBC count of mother on PND 1, 21 and 49: Details of WBCs count of various experimental groups in comparison to control are shown in **table 2 and graph 2**. Statistically highly significant (P<0.01) changes were observed on PND 1st, 21st and 49th. At the time of birth the highest increase in WBCs count was observed in Amla, Fennel, HF + Amla and HF + Fennel compared to control. At the end of lactation and after puberty highest increase in WBCs count was observed in amla and fennel group but remaining groups showed decrease in WBCs count. HF group showed decrease in WBCs count compared to control group on PND 1st, 21st and 49th.

Table 2: Variations in WBC counts (x10^{3}/mm^{3}) 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.

Treatments	WBC counts of mother on following experimental days		
	1st day	21st day	49th day
Control	$7.95 \pm 0.33^{b,c}$	$8.3 \pm 0.19^{b,c}$	9.12 ± 0.21^{ab}
High fat	7.70 ± 0.12^{c}	8 ± 0.17^{c}	8.75 ± 0.18^{b}
Amla	8.87 ± 0.20^{a}	9.17 ± 0.19^{a}	9.52 ± 0.34^{a}
HF + Amla	8.11 ± 0.20^{b}	8.60 ± 0.23^{b}	8.81 ± 0.24^{b}
Fennel	8.88 ± 0.11^{a}	9.20 ± 0.22^{a}	9.31 ± 0.20^{a}
HF + Fennel	8.00 ± 0.30^{b}	8.56 ± 0.21^{b}	9.15 ± 0.26^{ab}
F-value	19.34^{**}	21.46^{**}	5.55^{**}

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 rang test.



Graph 2: Variations in WBC counts ($x10^3/mm^3$) of mother in different experimental groups on PND 1, 21, and 49

3.3 Morphological and structural evaluation of RBC and WBC on PND 1, 21 and 49: The present study was accomplished to evaluate the role of high fat (sesame seeds) and antioxidant containing amla and fennel during pregnancy and lactation on morphology of RBC and WBC of pups of Swiss albino mice.

In present investigation different type of WBCs were observed, which are listed in **table 3.**

- ✓ **Group I (Control) –** Control neutrophil, lymphocyte, band shaped neutrophil, monocyte

- ✓ **Group II (High Fat) –** Degenerated neutrophil, segmented neutrophil, ring neutrophil, large lymphocyte, Bi-nucleated lymphocyte, oval lymphocyte
- ✓ **Group III (Amla) –** Control neutrophil, band shaped neutrophil, lymphocyte
- ✓ **Group IV (HF + amla)** –Developing neutrophil, segmented neutrophil
- ✓ **Group V (Fennel)** – Band shaped neutrophil, lymphocyte, monocyte
- ✓ **Group VI (HF + Fennel)** – Segmented neutrophil, ring neutrophil, monocyte

In present investigation different type of RBCs were observed which are listed in table 4.

Group I (Control) - Normal biconcave RBCs without nucleus.

Group II (High Fat) - Elliptocyte, echinocyte, roulex, stomocyte and macrocytosis.

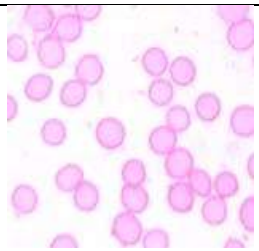
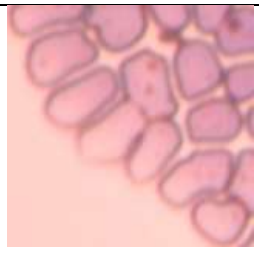
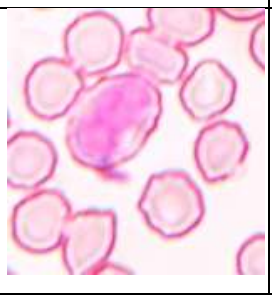
Group III (Amla) - Normal and elliptocytes

Group IV (HF + Amla) - Echinocyte, roulex and dacrocyte.

Group (Fennel) - Normal and microcytes.

Group (HF+ Fennel) - Microcytes, roulex, dacrocytes and Echinoytes.

Table 3: Morphological and structural evaluation of Red blood cells in control and all experimental groups on PND 21:

S. No	Structural appearance	Type of cells	Description of cells	Presence in group
1.		Normal red blood cells	Pink, biconcave, disc-shaped cell with a flattened centre on both sides without nucleus	Control group, amla and fennel fed group
2.		Elliptocytes (Ovalocyte)	Red blood cells that are elongated with blunt ends and parallel side.	High fat group and high fat + amla group, high fat+ fennel
3.		Echinocytes (Burr cells/Crenated cells)	Red blood cell with, multiple, small, delicate, regular shaped spines distributed evenly around the membrane	High fat group

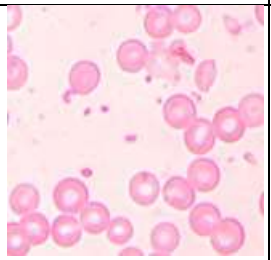
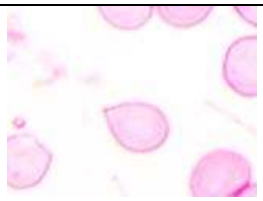
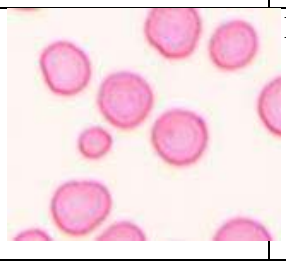
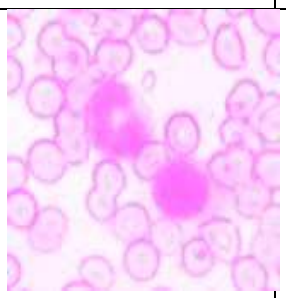
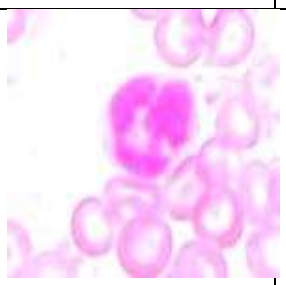
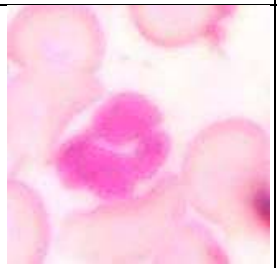
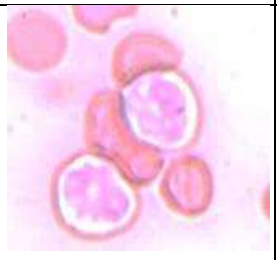
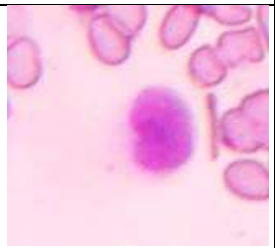
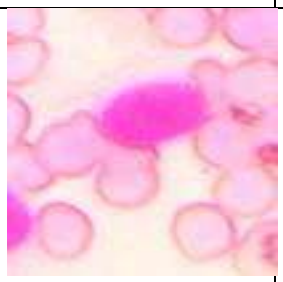
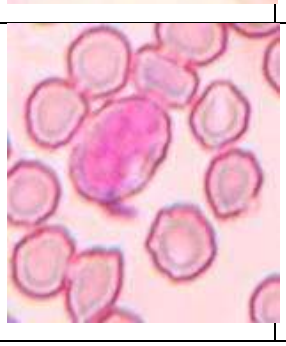
4.		Rouleaux pattern	In this type of blood cell pattern four or more red cells organized in a linear arrangement like a stack of coins	High fat group
5.		Stomocytes	Oval or rectangular area of central pallor and opening appearing like mouth	High fat and High fat + fennel group
6.		Dacrocytes (tear drop cells)	Red cells shaped like a tear drop or pear	High fat and High fat + Fennel group and high fat + amla fed group
7.		Macrocytosis	Presence of abnormally large red cells.	High fat group
8.		Microcytosis	Decrease in the size of red blood cell	High fat group

Table 4: Morphological and structural evaluation of white blood cells in control and all experimental groups on PND 21:

S. No	Structural appearance	Type of cells	Description of cells	Presence in group

1.		Control neutrophil	A very characteristic nucleus divided into 3-7 lobes connected by thin strands of chromatin	Control group, amla and fennel
2.		Band shaped neutrophil and lymphocyte	A type of WBC with a nuclear membrane that has parallel sites, although slight indentation may be present. the band is round to oval Lymphocyte is usually round or slightly oval. The nucleus appear dense chromatin material	High fat and high fat + amla
3.		Developing neutrophil	3-5 lobed, condensed chromatin material	Amla group, control
4.		Segmented neutrophil	It is the prominent white blood cell in the peripheral blood. The nucleus is lobulated and the lobes are connected by a thin filament.	Amla and fennel group
5.		Degenerated neutrophil	An average number of lobes increased. Lobes are fused. cell become roundish and compacting chromatin material	High fat group

6.		Ring neutrophil	A type of WBC with nucleus ring or doughnut shaped	High fat group and amla+ fat group
7.		Monocyte	Band shaped or kidney shaped nucleus, have fine chromatin pattern	Fennel group, control
8.		Developing monocyte	2 types of cells associated with one another, and clear cytoplasm is seen.	Fennel group
9.		Oval lymphocyte	Nucleus turns round to oval in shape and cytoplasm is hardly seen	Control group, amla andfennel
10		Large lymphocyte	Large granular lymphocytes are commonly found with viral infections.	High fat group,

(Identification of blood cells is based on: Veterinary Hematology sixth edition, Atlas and Veterinary hematology, Evaluation of cell morphology and introduction to platelet and white blood cells morphology).

4. DISCUSSION

In the control group the RBCs were pinkish, biconcave discs and very numerous. They usually have diameter measure 6-7µm with flattened center on both sides without nucleus. Early postnatal RBCs were large at birth and the cell size decreased gradually during different developing stages.

The primary function of RBCs is to transport oxygen from the lungs to all tissues, RBCs are also important regulatory components of hemorheology i.e. the dynamics of blood flow [8-9]. It is postulated

that the alterations in hematological changes serve as the earliest indicator of toxic effect on tissue[10]. Togun *et al*.[11] postulated that hematological studies are useful in the diagnosis of many diseases as well as investigation of the intensity of damage to blood. Hematological parameters are good indicators of the physiological status of animals[12]. It is also observed that changes in hematological parameters are often used to determine various health conditions and also to determine strains due to environmental, nutritional and pathological factors[13].

In present study high fat group showed non-significant changes in RBC counts of mother on all postnatal days (PND 1st, 21st, 49th) while significant decrease in WBC counts at all PND was observed. Blood smear of pups fed with HF diet showed different type of neutrophil and lymphocyte (degenerated neutrophil, segmented neutrophil, ring neutrophil, large lymphocyte, Bi-nucleated lymphocyte and oval lymphocyte). Hall *et al*.[14] also observed that diet with omega-6 and omega-3 Fatty acid in ratio of 1.4:1 had significant effects on T- lymphocytes. High-fat diet promotes red blood cell dysfunction and pro-inflammatory monocyte activation, which contribute to atherosclerosis in obesity [15]. Maternal HFD increased AHR (airway hyper-reactivity) in the offspring compared to control and significant increase in neutrophil counts [16].

Njidda and Isidahomen[17] reported that nutritional status of animals were directly related with hematological parameters. Minerals play vital role in bone mineralization, enzyme synthesis, red blood cell production, hormone production and regulation of cardiac and skeletal muscles activities. Essien *et al*.[18] and Abdul-Rahman *et al*.[19] observed that after eating sesame seeds in diet there was increase in the RBCs and WBCs count in rats but in the present investigation it was observed that in sesame seeds fed group, there was non-significant change in RBCs count but WBCs count decreased in comparison to all other groups. The findings of the present study are in accordance to the results of Okon and Umoh[20], who observed that Sesame extracts significantly increased RBCs count but significant decreased WBCs count and this significant increases or decrease in the hematological parameters were dose-dependent. Sesame seeds enhance erythropoiesis and Sesame lignin have many antioxidant and health promoting activities [21]. Flavonoids present in Sesamum indicum's were effective in raising the haemoglobin level in rats [2].

The results of present study are also in favour of many previous findings but contradiction with other investigators finding may be due to the level of doses in various combinations given at different physiological state of animals and due to different types of fatty acids used in the diet. Previous work was mostly performed on males and at specific condition (like diabetes, ulcer, ketonaemia etc.), while the present study was conducted during gestation and lactation to establish the truth that dietary status during these periods not only affects the health of mothers but also their offspring. Due to the presence of strong placental bond, some negative impact of high fat diet comprised of sesame seeds during these specific periods were observed both in mothers as well as their pups.

Amla has been used as household remedy against various human aliments from ancient time. In present study amla group showed significant increase in RBCs and WBCs count with in normal range compared to control and all other groups. Amla is an immune boosting agent so elevation in RBCs and WBCs may be due to this property. In the blood smear of pups some WBC, like normal and band shaped neutrophils and few lymphocytes were observed.

According to Paul and Khanna [22] amla is a potent immune-stimulant and known as the best rejuvenating herb in Ayurveda. Amla was combined in aquarium water to Clarias batrachus and the immune-stimulant potential was assessed by analysing the haematological parameter like Hb%, blood cell counts etc. The authors concluded that after administration of aqueous extract of amla in fishes, increase in lymphocyte and granulocyte was observed. It was also observed that RBC counts were increased in

fishes kept in 300ml extract of amla. Thus the work of Paul and Khanna supports the fact that amla powder when added to diet of fishes has definite immune-modulatory effect. The findings of present study are also in favour of the results of Paul and Khanna. On the other hand Nahak and sahu [23] observed the immune-modulatory activity of aqueous leaf extract of Ocimum basilicum (Tulsi) in fishes, they noticed there was no significant differences in lymphocyte, eosinophil and monocyte count, between the experimental and control groups, but the values were gradually increased with duration of time and doses.

Amla is rich source of vitamin C, iron, amino acids and flavonoids which naturally increases Hb% in blood. During pregnancy iron plays important role in making new blood cells to carry oxygen and nutrients to the foetus [5]. It was observed that after feeding animals with Phyllanthus emblica there was increase in haematological parameters because it contains vitamin C and flavonoids. Our findings are also in agreement with the above study, results of present study showed highly significant increase in RBCs and WBCs count in amla group, compared to control and other groups. Increase in the blood cells count was in normal range and not cause any type of abnormalities in experimental animals. Gopinathan and Rameela [24] postulated that treatment with combined juice of Aloe-Vera and Amla fruit enhanced the haemoglobin content, RBC and WBCs count was also brought to normal range.

In present study it was observed that after addition of fennel powder in experimental diet during gestation there was significant improvement in the blood cells count (RBC and WBC) within normal range but it was not an indication of any disease. Elevation in blood cells count may be due to immune-boosting effect of fennel seeds. It is also a good source of flavonoids, phenolic components and galactagogues which all positively affect haematological parameters. In the blood smear of pups some morphological and structural variations in cells were also observed. Some band shaped neutrophils, lymphocytes and monocytes were observed. It was reported that when fennel seeds added to experimental diets there was significant ($p<0.05$) increase in number of red blood cells, and non-significant increase in WBC compared with the control group. In general these differences among treatment groups may be due to active ingredients in fennel such as trans-anethole and estragole [25]. The elevation in blood cells and hemoglobin content may be caused by the improvement of metabolism and increase the absorption of nutrients [26].

Tognolini *et al*. [6] suggested that fennel seeds showed characteristic effect on blood parameters when added as 1, 2 and 3g/kg in the diet of chicks and had significantly increased red blood cell counts, haemoglobin and PCV (packed cell volume) compared with the control group. It was suggested that fennel seeds increase the RBC and WBC values, especially at dose 250 mg/mL and 500mg/mL compared to the control group. Fennel increased the red and white blood cells probably due to the presence of polyphenols and antioxidants, and it also reduced the negative effects of free radicals on blood cells. Mansouri et al. already reported that antioxidant activity and phenolic components maintains the RBC membrane against oxidizing factors and increases the process of erythropoietin in rats [27].

The highest level of WBC counts was reported in diet containing 400 mg/kg fennel essential oil and there was non-significant increase in RBC counts [28]. Present findings are parallel to the interpretation resolved by Tognolini *et al*. [6] and Mansouri *et al*. [27]because it was also observed that animals fed with fennel seeds powder, showed elevation in number of RBCs and WBCs compared to control.

So at the end of this segment it can be resolved that amla and fennel improved all the selected hematological parameters because they boost the immune system owing to the presence of antioxidants. There was elevation in the RBC and WBC count in mothers because the selected plants are enriched with vitamin C and iron (amla), and anethole, phenolic component and galactagogues (fennel), which

reduced the negative impact of free radicals and increased cell counts during and after lactation on PND 1st 21st and 49th. Due to the overall positive impact on hematological parameters of mother, their infants were also benefited because there is a strong bond between mother and developing foetus through placental connection.

5. CONCLUSION

The results of present study demonstrates that gestation and lactation periods are multifaceted, so during these periods pregnant mothers are directly affected by the diet and this also indirectly onset its impact on developing fetus. The study clearly establishes the fact that pregnant mother's diet including high fat induced alterations in RBC and WBC counts and also in their morphology. Antioxidants are molecules that inhibit oxidation of other molecules and are man-made or natural substances that may prevent cell damage. Plants with antioxidant properties like amla and fennel when consumed during pregnancy, cure many health related problems. Vitamin C, vitamin B complex, iron, flavonoids and phenolic components present in amla and fennel improved the blood constraints in mice due to their free radicals scavenging properties. Developing stages are very sensitive to environmental fluctuations and synthetic formulations may have their negative influence on mother and fetus so it is considerably safe to include plant base antioxidants in diet during gestation and lactation.

ACKNOWLEDGEMENT

The Authors are obliged to concerned department for providing all the basic necessary facilities required in this work. Neelam Jain is thankful to DST, New Delhi for providing the financial support (Inspire fellowship). Help provided by Dr. Harish, Assistant professor, Department of Botany, M. L. S. University, Udaipur, for statistical analysis has also acknowledged.

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Online publication Date: 30.11.2018