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Research Paper

COMPARATIVE IMMUNE STIMULANT AND QUALITY CONTROL PARAMETER OF BUFFALO MILK AND GOAT MILK

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Abstract

Milk is used by humans from childhood as a nutrient and growth promoter but the other therapeutic value of milk is it also promotes immunity. As we know immunity plays an important role in human health. In this we worked to know about the immunity from different milk samples. Two different milk samples were tested for immunostimulant activity in that one is buffalo milk and 2nd sample is goat milk and these milk samples were given to mice y and test group is compared with control group . The immunity parameters of the 2 groups were checked by Hematology instrument . From the results of the experimental work of buffalo milk and goat milk 2nd sample goat milk has shown better immune stimulant action.

Key words: Buffalo milk, Goat milk, immunity Haematological parameters.

INTRODUCTION

Milk, as the firstly fodder for mammals, materials every part of the energy and nutrients required for the polite evolution and advance of the neonate.Moreover, it is a supply of countless bioactive components, such as high-quality proteins, lipids, carbohydrates, lactose, vitamins, minerals, enzymes, hormones, immune globulins, and expansion factors. These machinery not single avoids appointment person dietary requirements, but as well performance an applicable responsibility in preventing a choice of disorders such as hypertension[1]and cardiovascular diseases[2], obesity[3], osteoporosis[4], dental caries[5], impoverished gastrointestinal health [6], colorectal cancer[7], ageing[8], and others[9]. Early immune system discoveries were largely fuelled by a desire to prevent the spread of disease and develop better treatments forthe sick[10]. The innate immune system is so called because it provides protection from pathogens without theneed for preconditioning from the environment. In different words, when the innate system encounters an infective agent, it will

react straight off to kill or to get rid of it from the host[11]. Milk proteins too fat as carriers of encrypted functional sequences that, while unconfined as peptides, use biological functions, counting antimicrobial and immunomodulatory activity, which may perhaps be part of the cause to the infant's competitive success. Our fresh examination of milk samples demonstrates that these milk proteases persist to digest milk proteins contained by the infant's stomach, perhaps similar to a bigger limit than the infant's acknowledge proteases. As the neonate has reasonably trough digestive capacity, the interest of milk proteases in the infant may offer of great consequence assistance in digesting milk proteins.

Composition of milk[12]

The biological process price of milk is especially high because of the balance of the nutrients that comprises. The composition varies among animal species and breeds at intervals constant species, and additionally from one form to the opposite, counting on the amount of lactation and diet. For instance, Milk contains several groups of nutrients.Organic substances are present in about equal quantity and are divided into elemental builders, proteins, and energy components, carbohydrates and lipids. It also comprises functional elements, such as traces of vitamins, enzymes and dissolved gases, and contains dissolved salts, especially in the form of phosphates, nitrates and chlorides of calcium, magnesium, potassium and sodium.It also contains dissolved gases (5% by volume), mainly carbondioxide (CO_2), nitrogen (N) and oxygen (O_2) Water: 87.3% (85.5 - 88.7%), Milk fat: 3.9 % (2.4 - 5.5%),Proteins: 3.25% (2.3-4.4%),Casein: 2.6% (1.7-3.5%),Serum proteins, Minor proteins, Carbohydrates (Lactose): 4.6% (3.8-5.3%),Minerals: 0.65% (0.53-0.80%), Cationic: K, Ca, Mg, K, Anionic chloride, phosphate, citrate, carbonate, Organic acids: 0.18% (0.13-0.22%) ,Citric, lactic, formic, acetic, oxalic,Enzymes - peroxidase, catalase, phosphatase, lipase,vitamins - A, C, D, thiamine, riboflavin, Gases – CO_2 , N_2 , O_2 (CO_2 lost after drawing)This research work will be helpful for finding out best nutrient milk samples for promoting immunity in the body.

MATERIALS AND METHODS:

Determined quality control parameters for milk samples are: starch in milk,added urea in milk,ammonium compounds in milk,nitrates in milk,total ash on dry basis in dried milk.

Immunity determination through animals (mice)by oral feeding method.

Experimental design for Quality Control Methods:

A)Detection of Starch in Milk:

Take concerning five metric capacity unit of milk during a tubing. Bring to boiling condition and permit the tubing to chill to temperature.Add 1-2 drops of iodine answer to the tubing.Development of blue colour indicate presence of starch,which disappears when sample is boiled and reappears on cooling. [13]

B)Detection of Added Urea in Milk:

Mix one milliliter of milk with one milli liter of 1.6% DMAB chemical agent. Distinct yellow color is determined in milk containing else organic compound. The control (normal milk) shows a slight yellow colour due to the presence of natural urea. The limit of detection of method is 0.2%.[14]

C)Detection of Ammonium Compounds in Milk

Take 1.0 millilitre of milk add 0.5 millilitre of 2% caustic soda, 0.5 millilitre of 2% sodium hypochlorite and 0.5millilitre of 5% phenol solutionHeat for twenty seconds in boiling water bathtub, light-blue color turns deep blue in the presence of ammonium ion salt. The development of pink colour shows that the sample is free from Ammonium sulphate.[15]

D)Detection of Nitrates (Pond Water) in Milk:

Take two ml of milk during a tubing.Rinse the tube with the milk and drain the milk from the tubing. Add two-three drops of the chemical agent on the aspect of the tubing. Note the developed colour. Deep blue colour will be formed in the presence of nitrate in the milk sample.Pure milk sample will not develop any colour.[16]

E)Determination of Total Ash on Dry Basis in Dried Milk:

Weigh accurately about 3 g of the dried milk sample in the crucible, previously dried in a hot air oven and weighed. Heat the crucible gently on a burner or hot plate at 20°C till grey ash is obtained. Cool±first and then strongly in a muffle furnace at 550 20°C for 30±the crucible in a desiccator and weigh it. Heat the crucible again at 550 min. Cool the crucible in a desiccator and weigh. Repeat this process of heating for 30 min, cooling and weighing until the difference between two successive weighing is less than 1 mg. Record the lowest mass.[17]

F)Test for Presence of Skimmed milk Powder in Natural milk:

Take fifty cubic centimeter of milk in an exceedingly sixty cubic centimeter centrifuge tube.

Place the tube within the centrifuge and balance it properly. Centrifuge at 5000 rpm for 15 minutes. Decant the supernatant creamy layer carefully. Add 0.5 metric capacity unit of four-dimensional ethanoic acid to milk portion for natural action of macromolecule. Centrifuge the tubes at 5000 rpm for 5 min. Decant the supernatant and wash the precipitate with distilled water twice. Discard the washings. Then, add two milliliter of I Chronicles phosphomolybdic acid to the washed precipitates.Mix the contents totally and warmthem in an exceedingly water bathtub at boiling temperature for quarter-hour then cool.The curd obtained from pure milk shall be greenish in colour,whereas the curd of sample containing skimmed milk powder

shall be bluish in colour. The intensity of bluish colour depends on the amount of the skim milk powder present in the sample.[18]

MATERIALS:

Milk of Buffalo and Goat purchased from local vendor,cellHematology instrument was used.

Preparation of animals:

The animals were randomly selected, marked to permit individual identification, and kept in their cages for at least 5 days prior to dosing to allow for acclimatization to the laboratory conditions

Mice of weight around 35-45gms were taken and divided into 3 groups.Each group contains 5 animals. The animals were acclimatized in an air conditioned animal house at a temperature of 22±2ºC,Relative humidity of 57±2º % and photo cycle of 12:12hours light and dark. The animals were provided with standardized pellet food and drinking water *adlibitum*. All the experimental procedures were carried out in accordance with the guidelines of the institutional animal ethical committee.

Drug profiling:

- Group I served as control and were given water and pellets
- Group II served as Test sample-1 and were given Buffalo milk daily (1ml/100g b.w) twice per day.
- Group III served as Test sample-2 and were given Goat milk daily (1ml/100g b.w) twice per day.

Experimental design for immunity determination:

The animals were given milk orally for a period of 45days. The animals were kept under standard conditions(day/night rhythm 8:00 A.M to 8:00 P.M.,22ºC room temperature, standard diet.)

Measurement of parameters for immunity determination:

White blood cells[WBC], Lymphocytes[LYM], Monocytes[MON], Granulocytes [GRA], Lymphocytes% [LYM%], Monocytes% [MON%,],Granulocytes%[GRA%],Red Blood Cell [RBC], Hemoglobin[HB], Hematocrit[HCT], Mean Corpscular Volume[MCV], Mean Cell Hemoglobin[MCH], Mean Cell Hemoglobin Concentration [MCHC], Red blood cell

distribution width [RDWC], primeld Lymphocyte Test[PLT],Platelets, Procalcitonin[PCT], Mean Platelet Volume[MPV], Platelet Distribution width[PDWC] Were measured by placing blood sample in Cell Hematology instrument.[19]

Table 1: Results of Quality control parameters for two milk samples

Quality Control Test	Test sample-1	Test sample-2
Starch in Milk	+	+
Urea in Milk	+	+
Ammonia Compound in Milk	+	+
Nitrates in Milk	-	+
Skimmed Milk Powder	-	+
Alkaline Phosphate Test	+	+

+ indicates Positive, - indicates negative

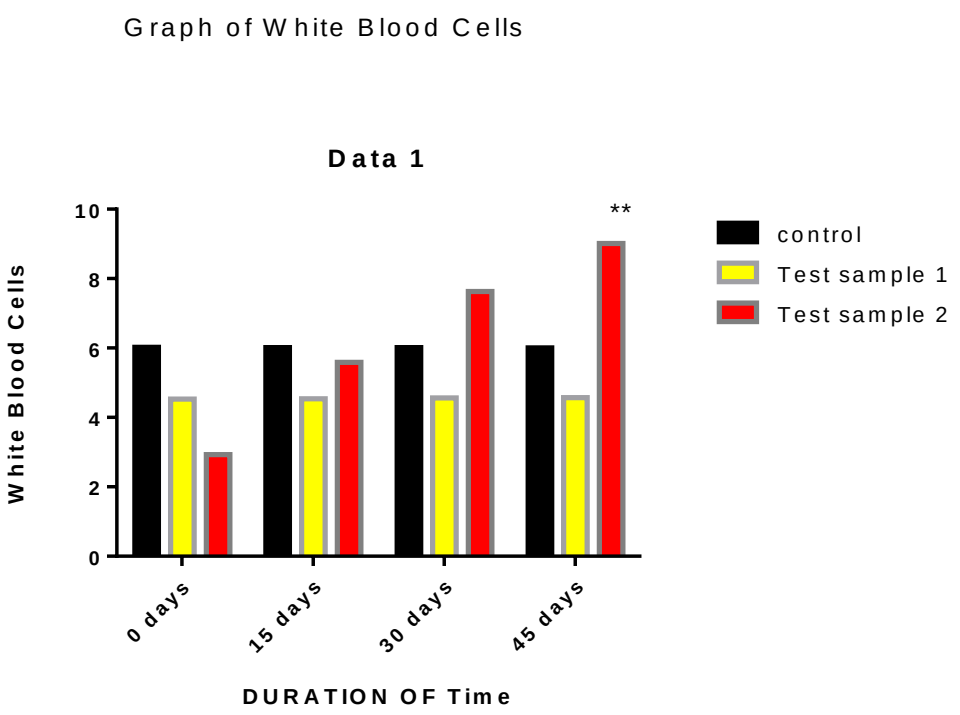
Table 2: Results of heamatological parameters measured at regular intervals

Parameter	0 days			15 days			30 days			45 days		
	I	II	III	I	II	III	I	II	III	I	II	III
WBC $10^3/\mu l$	6.02	5.17	4.36	6.01	5.20	5.69	6.01	5.29	7.42	6.00	5.31	7.63**
LYM10^3 /µl	5.74	3.75	2.70	5.73	3.82	3.63	5.73	4.05	4.87	5.72	4.15	5.09**
MON10^3 /µl	0.70	0.48	0.625	0.70	0.62	0.789	0.71	0.71	1.35	0.71	0.89*	1.84**
GRA10^3 /µl	3.04	0.91	0.15	3.05	0.93	1.20	3.04	0.96	1.23	3.05	0.98	1.39**
LYM%	55.95	71.1	65.3	55.96	72.0	72.9	55.95	73.5	83.5	55.97	74.1	85.2**
MON%	9.25	10.2	14.14	9.24	10.9	14.3	9.26	11.2	20.1	9.25	11.5*	21.3**
GRA%	34.8	18.65	3.35	34.9	18.80	20.3	35.0	19.01	21	34.9	19.20*	23**
RBC10^6/µl	5.285	5.12	3.145	5.284	5.19	3.59	5.285	5.21	5.56	5.285	5.25*	6.14**
HB g/dl	7.3	7.9	4.25	7.4	8.1	5.15	7.5	8.3	7.9	7.4	8.4*	8.5**

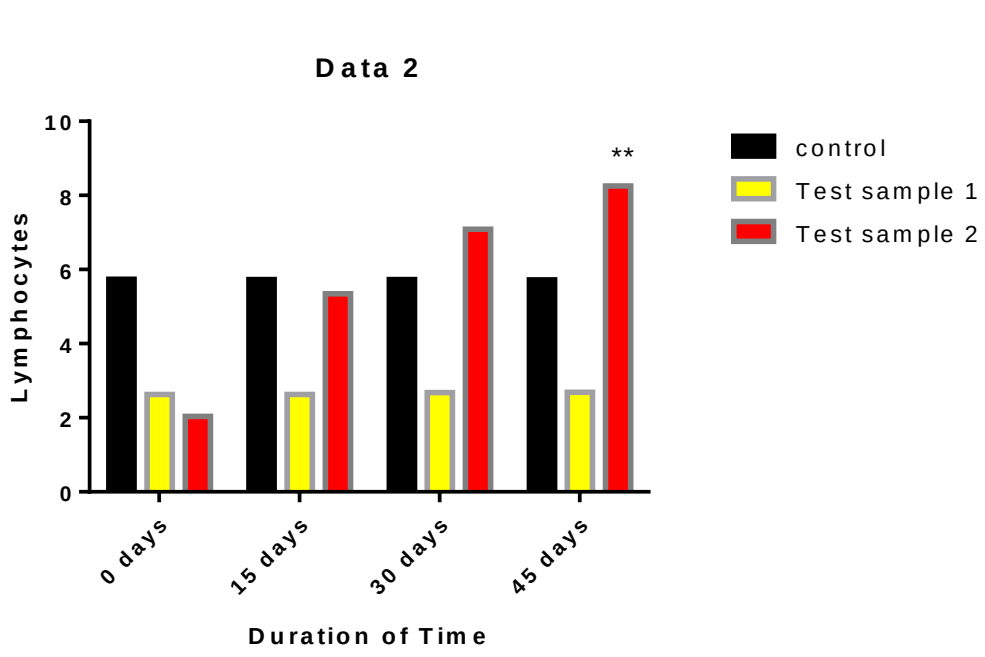
HCT %	29.05	30.89	15.9	29.04	30.91	20.55	29.05	30.93	28.8	29.03	30.96*	29.7**
MCVfl	54.5	59.5	51.5	54.5	60.1	52	54.4	60.3	57.05	54.5	60.5*	59.04**
MCHpg	13.75	15.45	13.75	13.76	15.50	14.23	13.75	15.53	14.54	13.76	15.55*	15.65**
MCHC g/dl	25.0	26.15	24.7	25.1	26.21	26.6	25.0	26.25	27.2	25.2	26.28*	28.3**
RDWC %	19.75	17.85	13.15	19.75	17.91	20.6	19.76	17.95	21.05	19.75	17.99*	22.7**
PLT10^3/ µl	547	416	432.5	548	420	472	548	429	473.5	549	431	484.7**
PCT %	0.155	0.145	0.10	0.156	0.149	0.134	0.155	0.152	0.287	0.157	0.155	0.39**
MPVfl	2.65	3.6	2.9	2.64	3.9	4.65	2.64	4.1	6.05	2.65	4.4*	6.94**
PDWC %	30.45	32.1	12.2	30.44	33.3	30.7	30.45	34.9	35.3	30.45	35.1**	36.4*

P<0.001 is considered as significant
Values are mean of 5 animals
I=Control
II=Buffalo[Test sample-1]
III=Goat [Test sample-2]

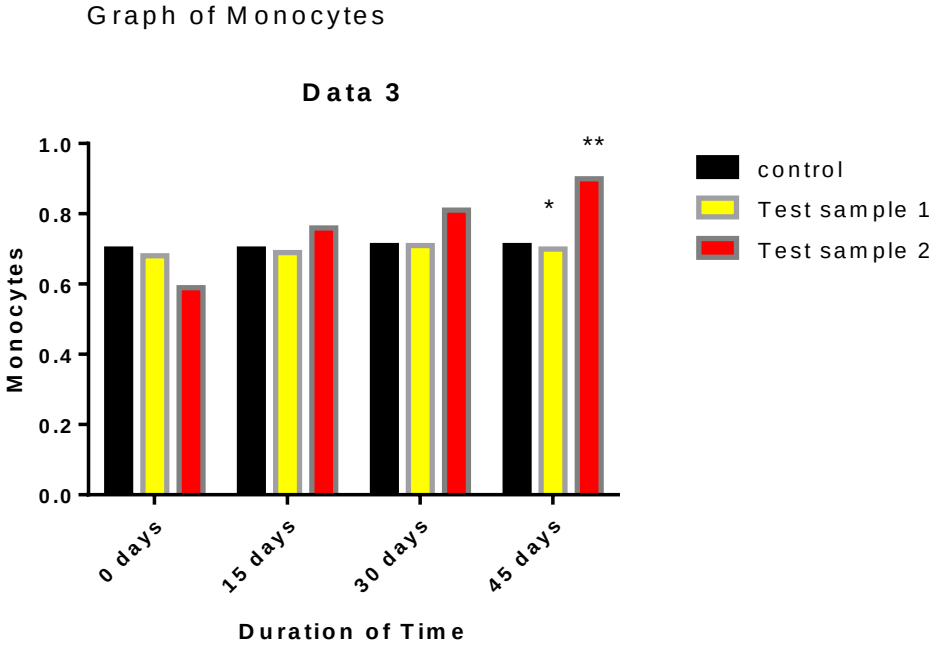
Graphical representation of white blood cells



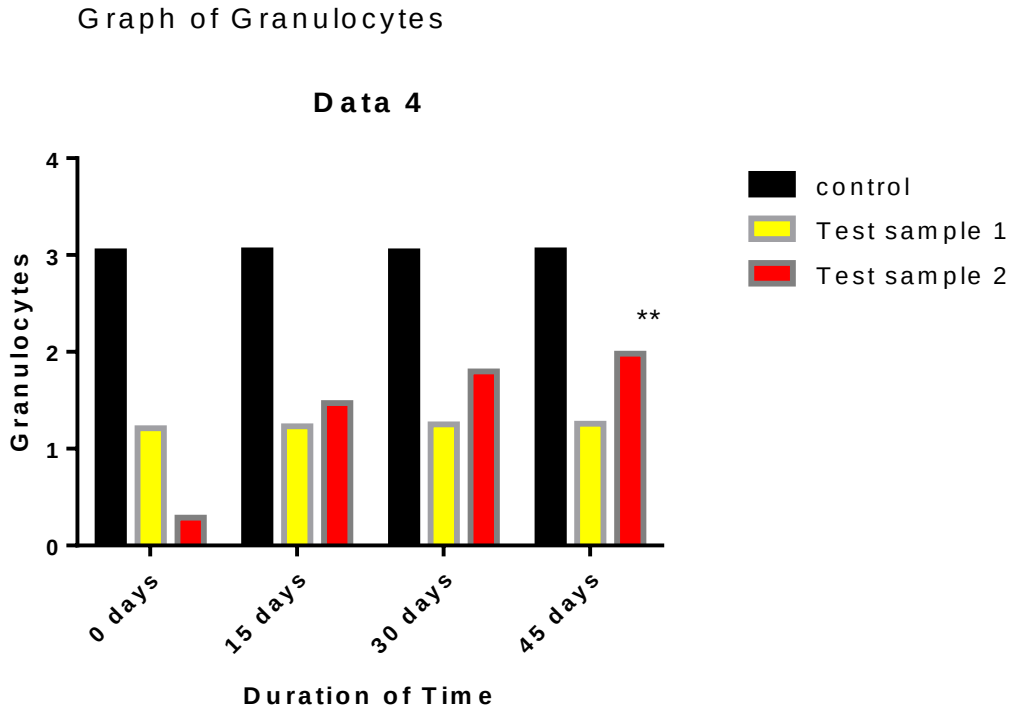
Graphical representation of Lymphocytes



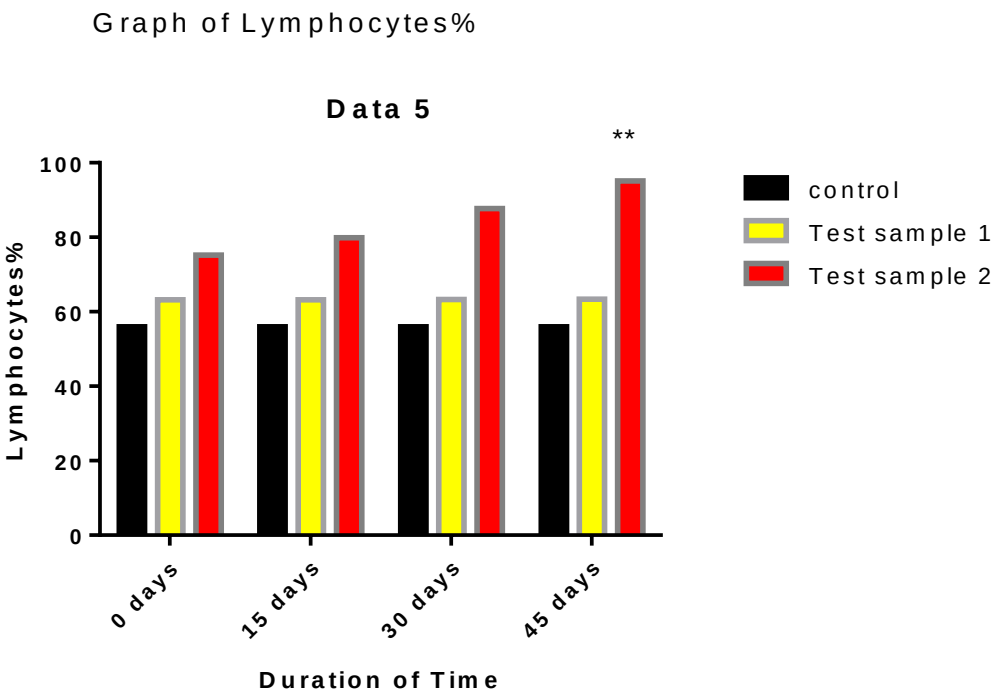
Graphical representation of Monocytes



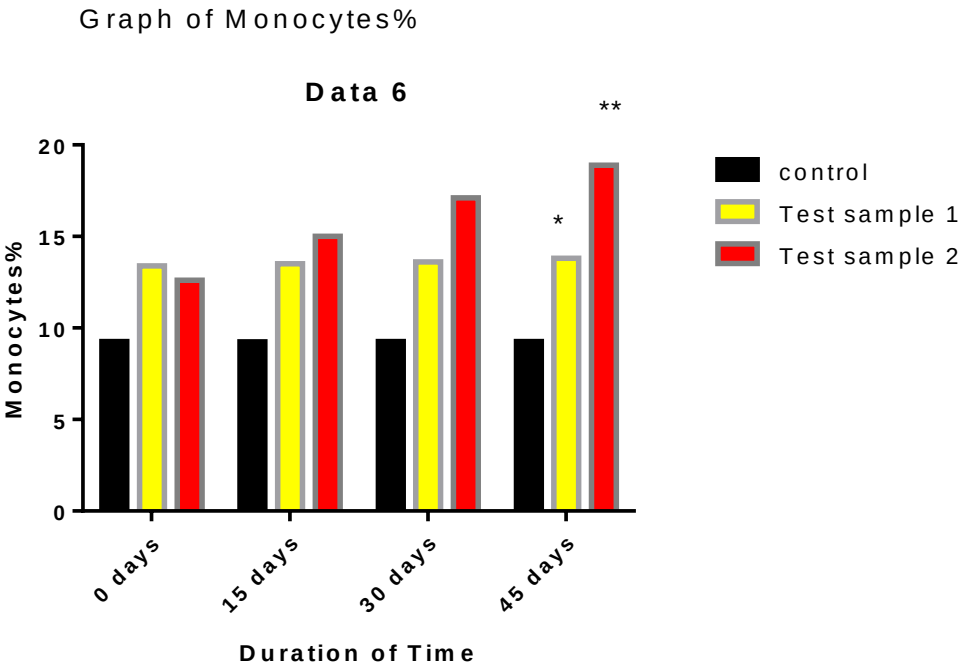
Graphical representation of Granulocytes



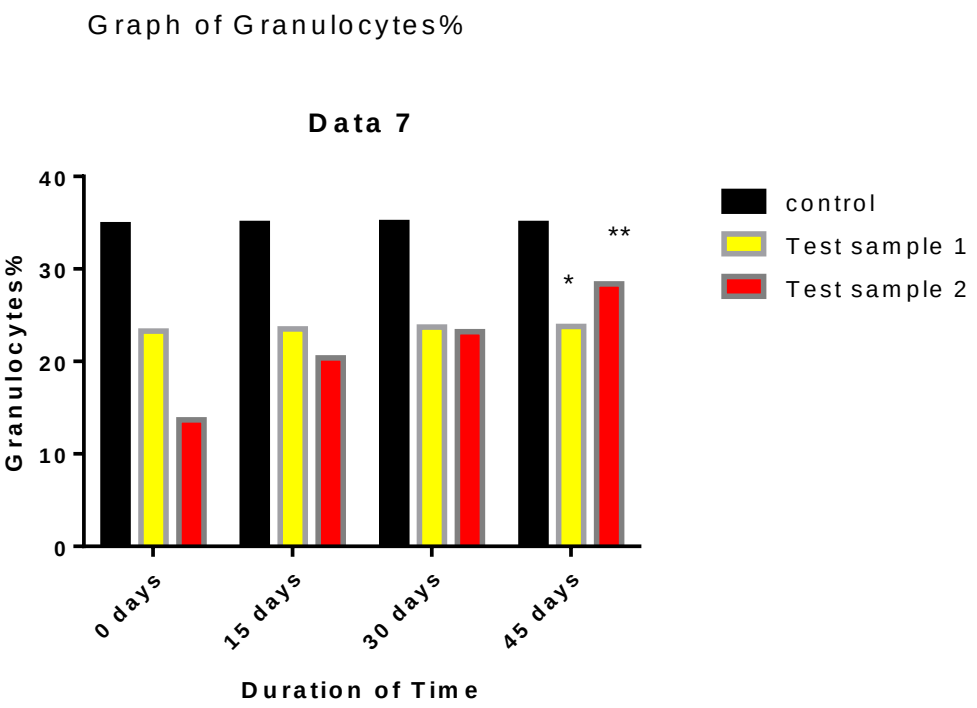
Graphical representation of Lymphocytes%



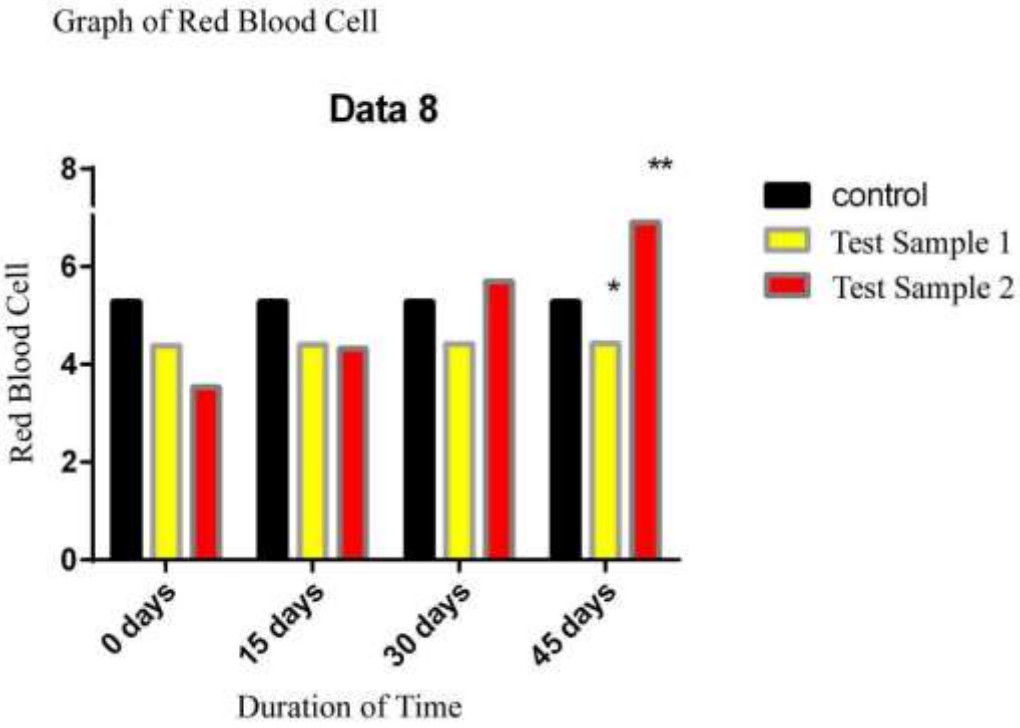
Graphical representation of Monocytes%



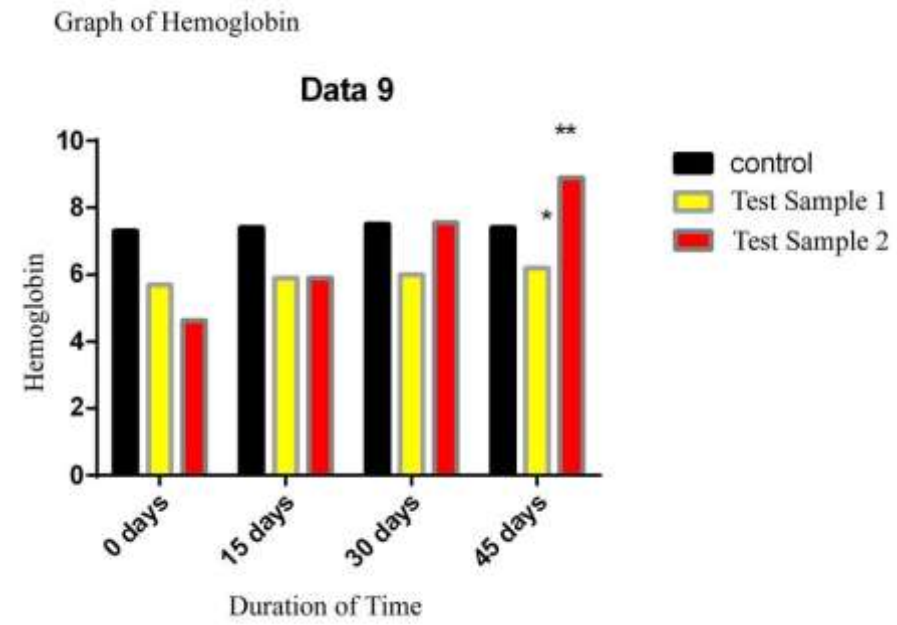
Graphical representation of Granulocyte%



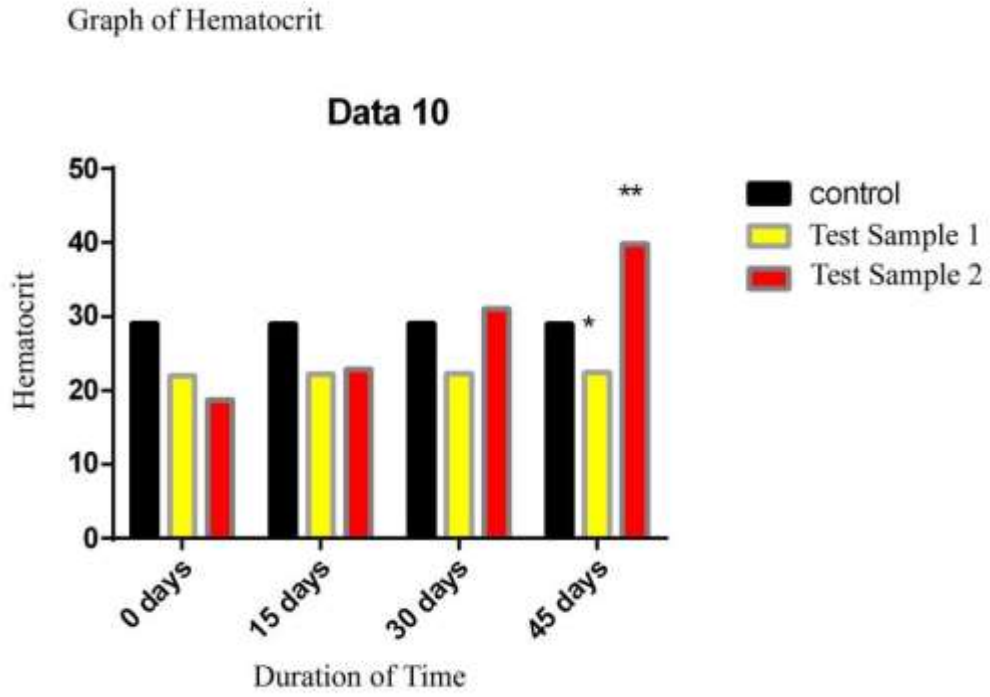
Graphical representation of Red blood cell



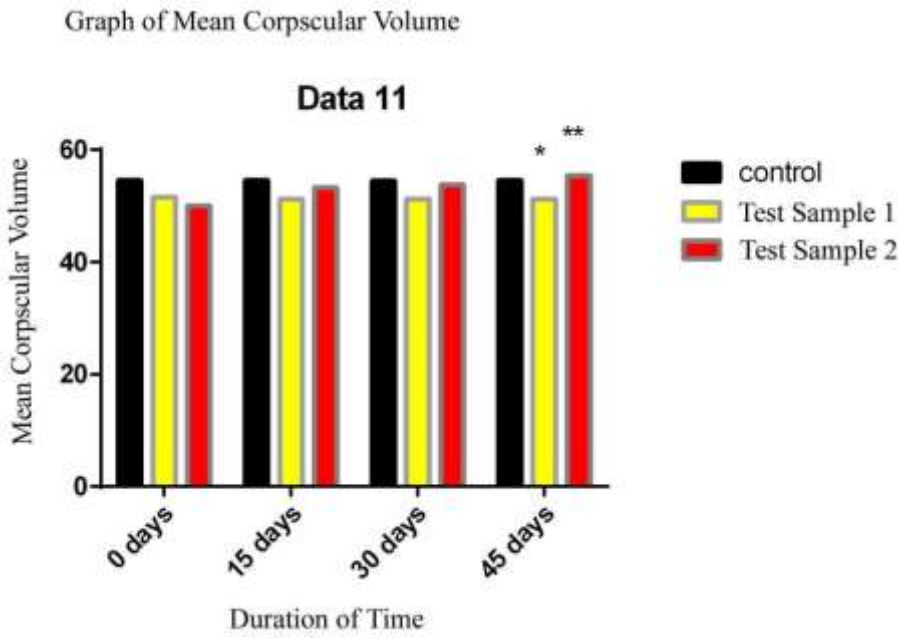
Graphical representation of Hemoglobin



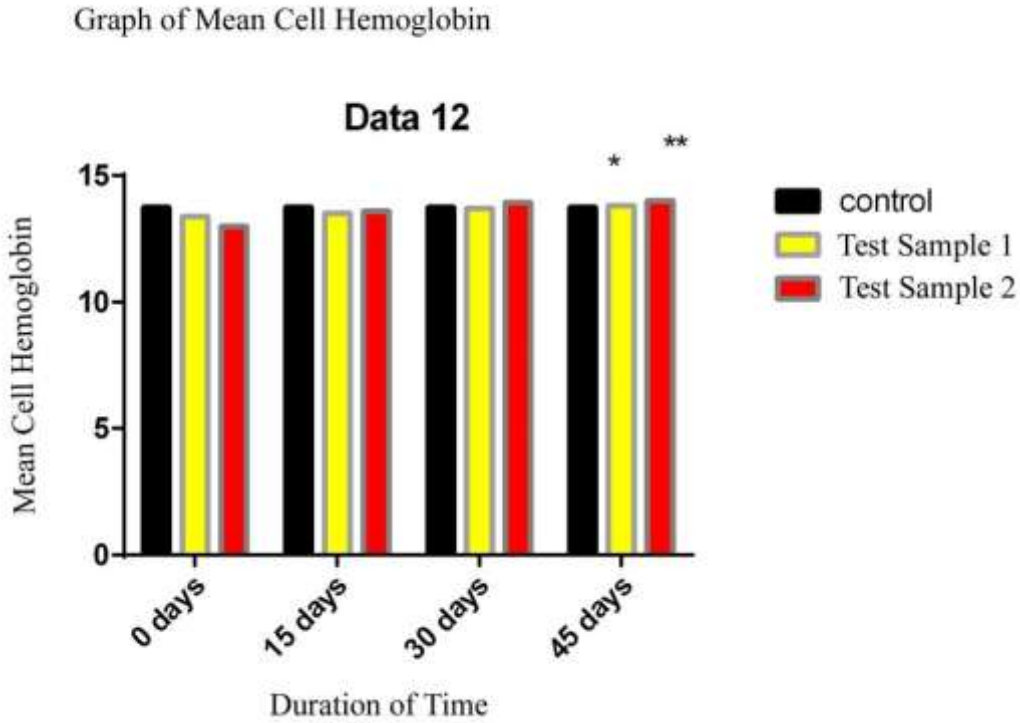
Graphical representation of Hematocrit



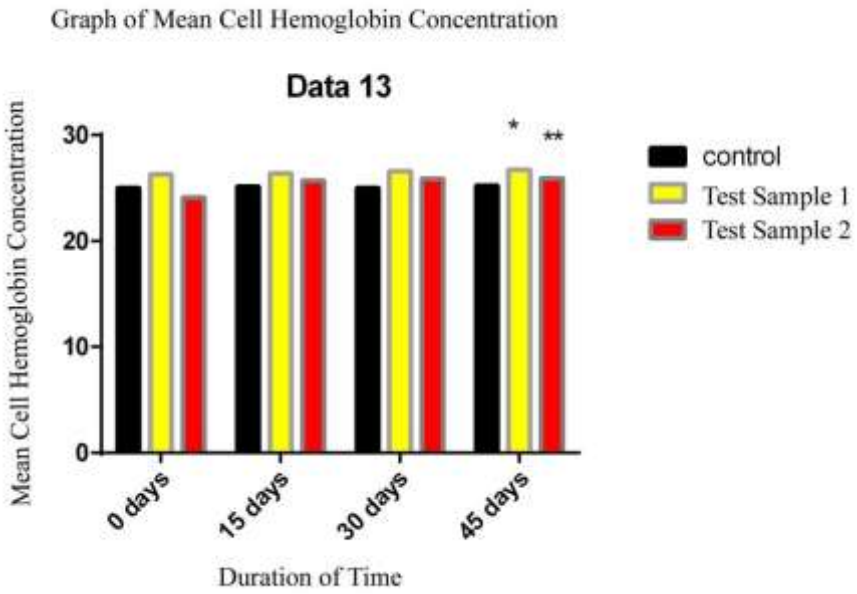
Graphical representation of Mean corpscular volume



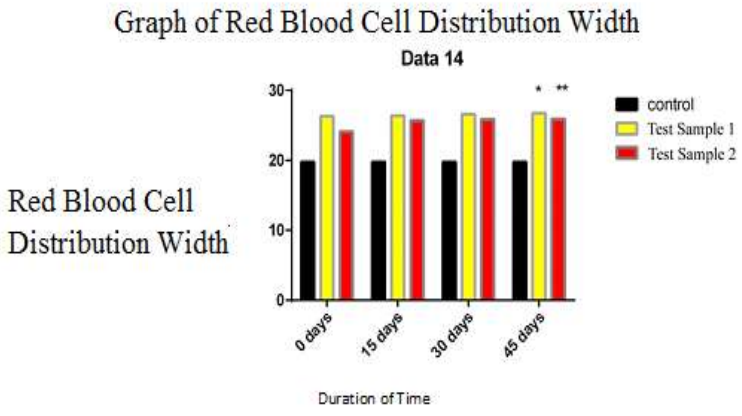
Graphical representation of Mean cell Hemoglobin



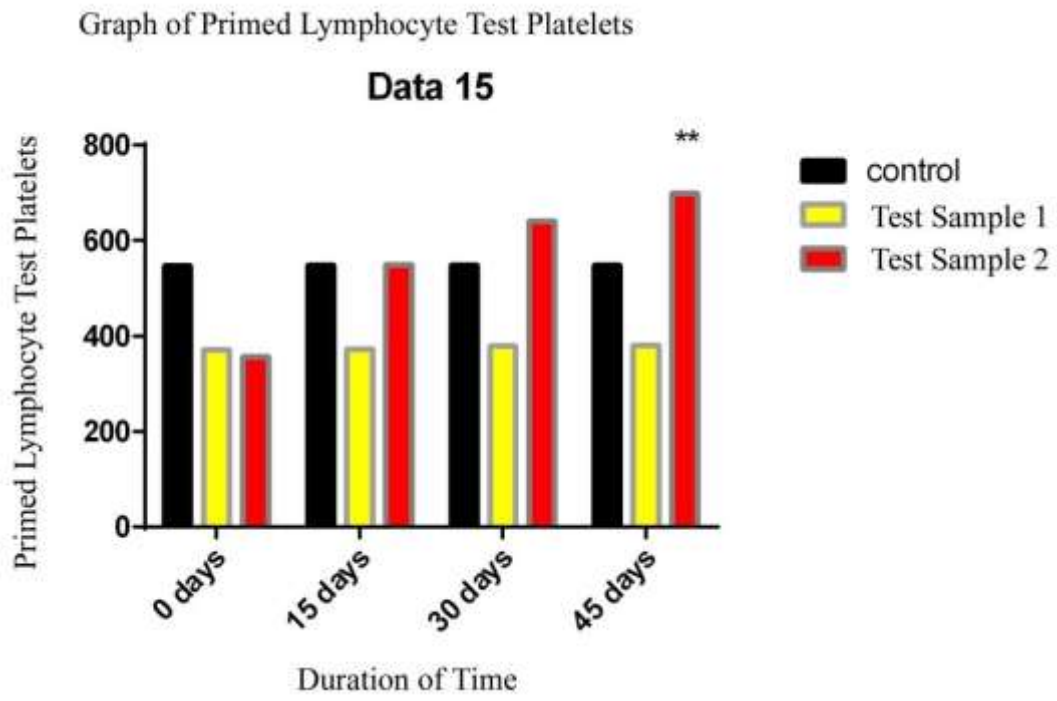
Graphical representation of Mean cell Hemoglobin concentration



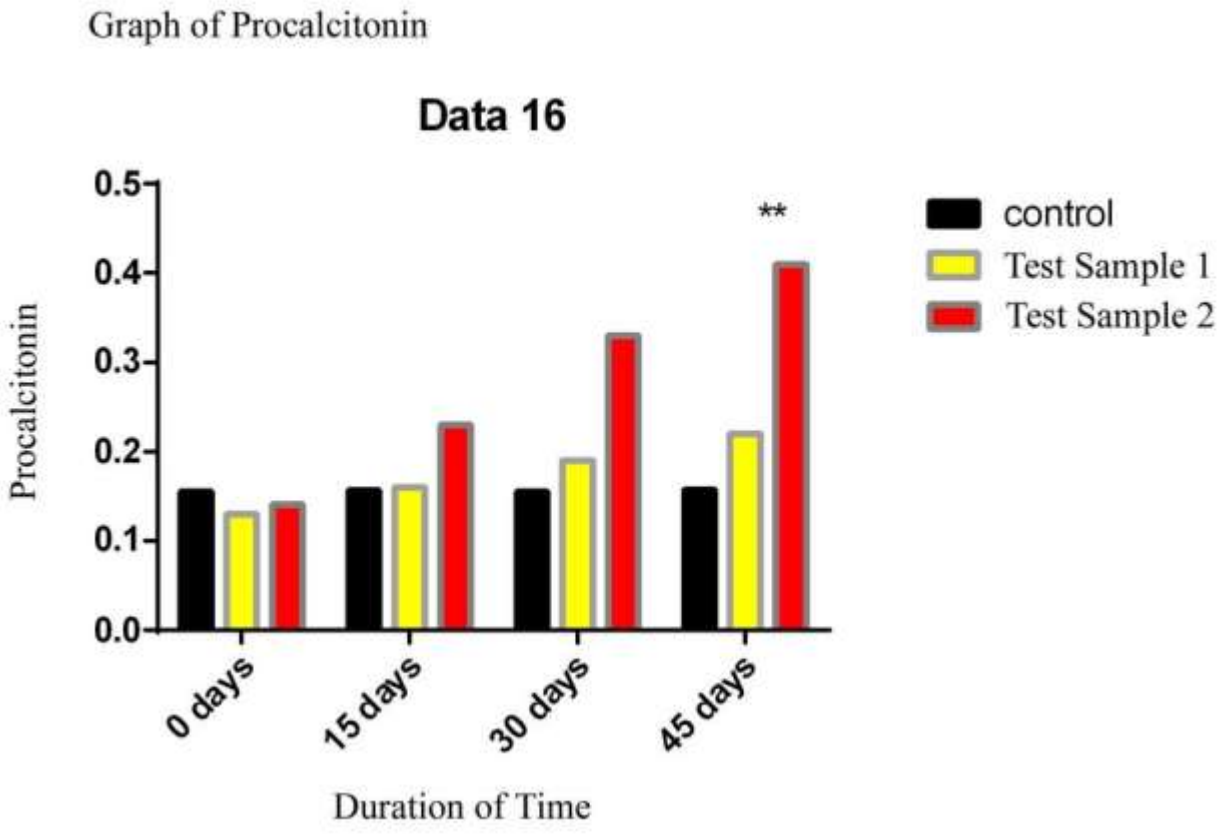
Graphical representation of Red blood cell distribution width



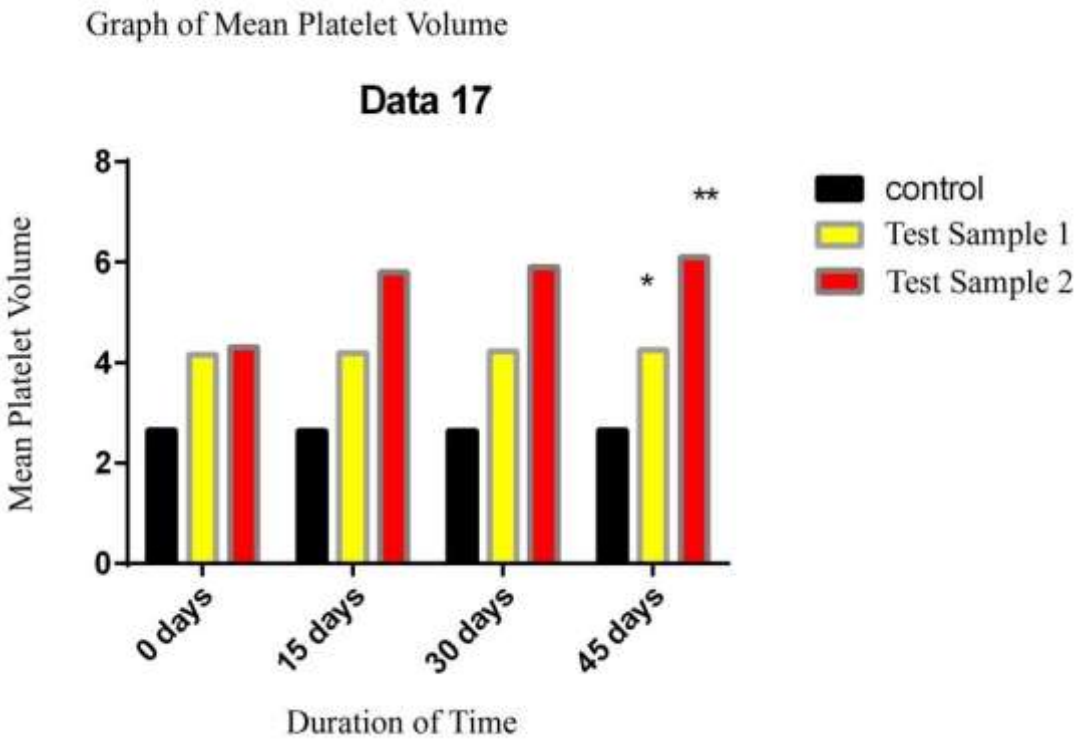
Graphical representation of primed lymphocyte test platelets



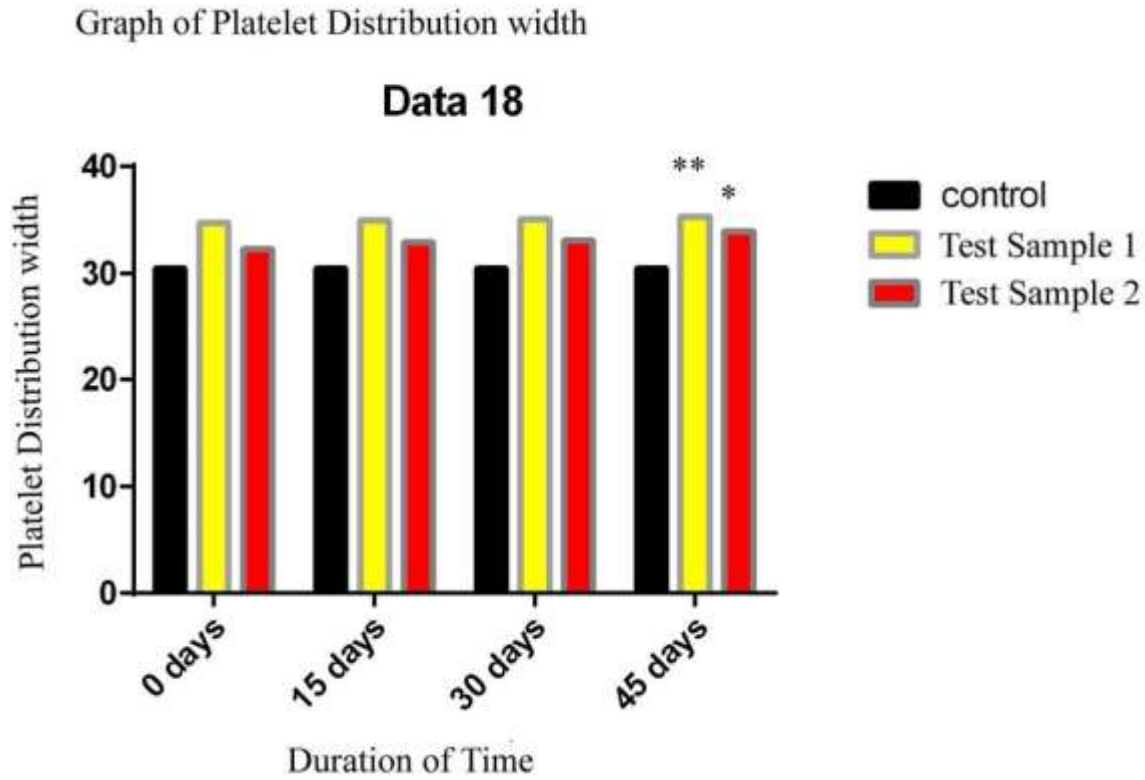
Graphical representation of procalcitonin



Graphical representation of Mean platelet volume



Graphical representation of platelet distribution width



DISCUSSION

WBC : white blood cell

They play a most important role in phagocytosis and immunity and therefore in defense against infection[20].WBC count of Buffalomilk in 0days is 5.17 and after treatment of 45days it shown the 5.31.wbc count of Goat milk in 0days 4.36and after 45 days it shown the 7.63.when comparing the Goat milk with Buffalo milk,goatmik has shown the increased level of wbc count as shown in graph1.

LYMPHOCYTES :

The T and B lymphocytes (T and B Cells) are involved in the acquired or antigen-specific immune response given that they are the only cells in the organism able to recognize and respond specifically to each antigenic epitope. The B Cells have the ability to transform into plasmocytes and are responsible for producing antibodies (Abs). Thus, humoral immunity depends on the B Cells while cell immunity depends on the T Cells.[21] Lymphocyte count of Buffalo milk in 0days is 3.75 and after treatment of 45days it shown the 4.15. lymphocyte count of Goat milk in 0days 2.70 and after 45 days it shown the 5.09.when comparing the Goat milk with Buffalo milk, goat milkhas shown the increasedlevel of lymphocyte count as shown in graph2.

Monocytes :

Role of Monocytes in Homeostasis, Bacterial Infection, Barotrauma, and Ischemia-Reperfusion[22] Monocyte count of Buffalo milk in 0days is 0.48 and after treatment of 45days it shown the 0.89 .monocyte count of Goat milk in 0days 0.625 and after 45 days it shown the 1.84.when comparing the Goat milk with Buffalo milk,goat milk has shown the increasedlevel of monocyte count as shown in graph3.

Granulocytes :

Granulocytes are made within the bone marrow and discharged into the blood and tissues, wherever they act because the 1stline of defense in host resistance and wound healing[23] Granulocytes count of Buffalo milk in 0days is 0.91 and after treatment of 45days it shown the 0.98. granulocytes count of Goat milk in 0days 0.15 and after 45 days it shown the 1.39.when comparing the Goat milk with Buffalo milk,goat milk has shown the increased level of granulocytes count as shown in Graph4.

Lymphocyte % :

Lymphocyte % of Buffalo milk in 0days is 71.1 and after treatment of 45days it shown the 74.1.lymphocyte % of Goat milk in 0days 65.3 and after 45 days it shown the 85.2.when comparing the Goat milk with Buffalo milk, goat milkhas shown the increased levels of lymphocyte % as shown in Graph5.

MONOCYTES % :

Monocyte %of Buffalo milk in 0days is 10.2 and after treatment of 45days it shown the 11.5.monocyte % of Goat milk in 0days 14.14 and after 45 days it shown the 21.3.when comparing the Goat milk with Buffalo milk , goat milk has shown the increased monocyte % as shown in graph6.

GRANULOCYTES % :

Granulocyte% of Buffalo milk in 0days is 18.65 and after treatment of 45days it shown the 19.20 Granulocyte % of Goat milk in 0days 9.35 and after 45 days it shown the 23.when comparing the Goat milkwith Buffalo milk,goat milk has shown the increased level of granulocyte % as shown in graph7.

RBC (Red blood cell) :

Red blood cells are ideal vehicles for delivering oxygen to tissues[24] RBC count of Buffalo milk in 0days is 5.12 and after treatment of 45days it shown the 5.25.RBC count of Goat milk in 0days 3.145 and after 45 days it shown the 6.14.when comparing the Goat milk with Buffalo milk, goat milk has shown the increased level of RBC count as shown in Graph-8.

HEMOGLOBIN :

Hemoglobin is the predominant protein in the red blood cell and is responsible for transporting oxygen, carbon dioxide, and protons between the lungs and tissue[25] Hemoglobin count of Buffalo milk in 0days is 7.9 and after treatment of 45days it shown the 8.4.hemoglobin count of Goat milk in 0days 4.25 and after 45 days it shown the 8.5.when comparing the Goat milk with Buffalo milk,goat milk has shown the increased level of hemoglobin count as shown in graph9.

HCT (HEMATOCRIT) :

The hematocrit is the percentage of the total blood volume occupied by red blood cells[26]HCT count of Buffalo milk in 0days is 14.80 and after treatment of 45days it shown the 29.21HCT count of Goat milk in 0days 15.9 and after 45 days it shown the 30.93.when comparing the Goat milk with Buffalo milk,goat milk has shown the increased level of HCT count as shown in graph 10.

MCV (Mean cell volume) :

The MCV is that the average size of red blood cells.When the RBC is decided by a hematology instrument, the MCV is measured.When cell counts ar determined by a haemocytometer, MCV is calculated.MCV count of Buffalo milk in 0days is 59.5 and after treatment of 45days it shown the 60.5MCV count of Goat milk in 0days 61.5 and after 45 days it shown the 69.04.when comparing the Goat milk with Buffalo milk,goat milk has shown the increased level of MCV count as shown in graph 11.

MCH (Mean cell hemoglobin) :

MCH count of Buffalo milk in 0days is 15.45 and after treatment of 45days it shown the 15.55 MCH count of Goat milk in 0days 15.75 and after 45 days it shown the 16.65when comparing the Goat milk with Buffalo milk,goat milk has shown the increased level of MCH count as shown in graph 12.

MCHC (Mean cell hemoglobin concentration) :

MCHC count of Buffalo milk in 0days is 26.15 and after treatment of 45days it shown the 26.28 MCHC count of Goat milk in 0days 26.7 and after 45 days it shown the 30.3when comparing the buffalo milk with Goat milk,buffalo milk has shown the increased level of MCHC count as shown in graph 13.

RDWC (Red Blood Cell Distribution Width):

RDWC count of Buffalo milk in 0days is 17.85 and after treatment of 45days it shown the 17.99 RDWC count of Goat milk in 0days 19.15 and after 45 days it shown the 22.7when comparing the buffalo milk with Goat milk,buffalo milk has shown the increasedlevel of RDWC count as shown in graph 14.

PLT (PLATELETS) :

Platelets play an important role in the vessel. Following their formation from megakaryocytes, platelets exist in circulation for 5–7 days and primarily perform as regulators of hemostasia and occlusion.[27]Platelets count of Buffalo milk in 0days isis547 and after treatment of 45days it shown the 549 platelets count of Goat milk in 0days 532.5 and after 45 days it shown the 584.7 when comparing the Goat milk with Buffalo milk,goat milk has shown the increased level of platelets count as shown in graph 15.

PCT (Procalcitonin test) :

Procalcitonin may be a substance made by many varieties of cells withinthe body, typically in response to micro organism infections however conjointly in response to tissue injury.The level of procalcitonin with in the blood will increase considerably in general micro organism infections and infection. This test measures the level of procalcitonin in the blood.[28] PCT count of Buffalo milk in 0days is 0.145 and after treatment of 45days it shown the 0.155 PCT count of Goat milk in 0days 0.10 and after 45 days it shown the 0.39 when comparing the Goat milk with Buffalo milk,goat milk has shown the increased level of PCT count as shown in graph 16.

MPV(Mean platelet volume) :

MPV count of Buffalo milk in 0days is 3.6 and after treatment of 45days it shown the 4.4 MPV count of Goat milk in 0days 2.9 and after 45 days it shown the 6.94when comparing the Goat milk with Buffalo milk,goat milk has shown the increased level of MPV count as shown in graph 17.

PDW (PLATELET DISTRIBUTION WIDTH) :

PDW count of Buffalo milk in 0days is 32.1 and after treatment of 45days it shown the 35.1 PDW count of Goat milk in 0days 26.2 and after 45 days it shown the 36.4 when comparing the buffalo milk with Goat milk,buffalo milk has shown the increased level of PDW count as shown in graph 18.

Conclusion:

From the Quality control studies and immune modulatory studies,out of 2 samples buffalo milk and goat milk.Goat milk has shown the best immunomodulatory activity as it increases theheamoglobinconcentration,WBC, Lymphocytes, Monocytes, Granulocytes, Lymphocytes%, Monocytes%, Granulocytes%, Red Blood Cell, Hemoglobin, Hematocrit, Mean corpscular volume, Mean Cell Hemoglobin, Mean Cell Hemoglobin Concentration,Red blood cell distribution width ,primeldLymphocyte Test,Platelets,Procalcitonin, Mean Platelet Volume,Platelet Distribution width.which are required for immunity to fight against disease conditioning of body.

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