EFFECT OF *ZEA MAYS* HAIRS ON HEMATOLOGICAL PARAMETERS

by

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CERTIFICATE

This is to certify that the dissertation entitled "**EFFECT OF *ZEA MAYS* HAIRS ON HEMATOLOGICAL PARAMETERS**" is the bonafide record of research work done by **MR. CHEE CHEW SIM** during the period from June 2004 to March 2005 under our supervision.

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ABSTRAK

Ekstrak bulu jagung *Zea mays* diberikan kepada tikus albino *Sprague Dawley* dengan dua dos berlainan – 50mg/kg berat badan dan 100mg/kg berat badan. Tikus-tikus dibahagikan kepada empat kumpulan – kontrol, rawatan 7 hari, rawatan 14 hari dan rawatan 28 hari. Seterusnya, tikus-tikus dikorbankan dan darah tikus dikumpul untuk ujian analisis hematologi. Parameter-parameter yang digunakan termasuk pembilangan sel darah merah, kontent hemoglobin, tahap hematokrit, pembilangan sel darah putih, pembilangan beza sel darah putih dan pembilangan platelet. Pada keseluruhannya, tahap hematokrit didapati meningkat dengan signifikannya mungkin disebabkan kesan diuretik bulu jagung *Zea mays*. Pembilangan beza sel darah putih juga menunjukkan sedikit sebanyak perubahan yang signifikan: peratus neutrofil meningkat pada permulaannya manakala peratus kedua-dua limfosit dan eosinofil menurun dengan signifikan.

ABSTRACT

The extract of *Zea mays* hairs was administered to *Sprague Dawley* albino rats with two dosages – 50mg/kg body weight and 100mg/kg body weight. The rats were divided into four groups – control, 7 days treatment, 14 days treatment and 28 days treatment. After that the rats were sacrificed and the collected blood was tested for hematological analysis. The parameters used were RBC count, hemoglobin content, hematocrit level, total WBC count, WBC differential count and platelet count. Overall, the hematocrit level was significantly increased probably due to the diuretic effect of *Zea mays* hairs. WBC differential count also showed some degree of significance: neutrophil percentage was increased initially whereas both lymphocyte and eosinophil percentage were reduced significantly.

INTRODUCTION

Zea mays is a tall annual cereal grass bearing kernels on large ears. It is a very popular vegetable and one of the major and most common staple foods grown all over the world. *Zea mays* grows fast up to 2m annually. It flowers from July to October while from September to October the seeds ripen. The flowers are monoecious (individual flowers are either male or female, but both sexes can be found on the same plant). They are pollinated by wind. It is warm-loving with few soil requirements.

The plant grows well in light (sandy), medium (loamy) and heavy (clay) soils and needs well-drained, moist soil. Acid and neutral soils are preferred. It is not able to thrive in the shade. Below is the scientific classification of this well-known plant:

Scientific name	:	*Zea mays*
Kingdom	:	Plantae
Division	:	Magnoliophyta
Class	:	Liliopsida
Order	:	Poales
Family	:	Poaceae
Genus	:	*Zea*
Species	:	*Zea diploperennis*
		Zea luxurians
		Zea mays ssp. huehuetenangensis
		Zea mays ssp. mays
		Zea mays ssp. mexicana
		Zea mays ssp. parviglumis
		Zea nicaraguensis
		Zea perennis

There are a number of varieties of corns, some of which are as follow:

- *Zea mays amylacea* - corn having kernels almost entirely of soft starch
- *Zea mays everta* - corn having small ears and kernels that burst when exposed to dry heat
- *Zea mays indentata* - corn whose kernels contain both hard and soft starch and become indented at maturity
- *Zea mays indurata* - corn having kernels with a hard outer layer enclosing the soft endosperm
- *Zea mays rugosa/ Zea saccharata* - corn whose young ears are sweet and suitable for eating as a vegetable

(www.webster-dictionary.org/definition/Zea%20mays)

As food, maize is used in various forms. The most important agricultural groups are:

Ornamental Corn *(Z. indurata)* - This comes in a wide range of shapes, colors and sizes. The dried kernels can be ground for corn meal and young ears can be harvested at milk stage and boiled or barbecued. This kind of corn, however, is mainly grown for ornament. The ears are shucked back to expose the beautiful, colorful kernels and the husks are tied into bunches.

Popcorn *(Z. praecox)* - Popcorn is a type of corn which puffs up when it is heated in oil or by dry heat. It is a popular snack. Each kernel of popcorn contains a small drop of water stored inside a circle of soft starch. The soft starch is surrounded by the kernel's hard outer surface. As the kernel heats up, the water begins to expand,

and pressure builds against the hard starch. Eventually, this hard surface gives way, causing the popcorn to explode. As it explodes, the soft starch inside the popcorn becomes inflated and bursts, turning the kernel inside out. The steam inside the kernel is released, and the popcorn is popped.

Sweet Corn *(Z. rugosa)* - Sweetcorn, also known as sugar corn, is a hybridized variety of maize (*Zea mays*), specifically bred to increase the sugar content. The fruit of the sweetcorn plant is the corn kernel, a type of fruit called a grain in which the pericarp of the fruit is fused with the seed coat and a type typical of the grasses. The cob is a collection of grains. It is close to a multiple fruit in structure, except that the individual fruits (the kernels) never actually fuse into a single mass. Sweetcorn is commonly eaten as a vegetable, rather than a grain *(www.botany.com/zea.html)*.

For our study, sweet corn is used. This type of corn can be found abundantly in this country and thus they are suitable to be used as the source of substance for our research.

The corn has many other common names according to regions such as Ble De Turquie, Corn, Dari, Ganma Sham, Indian Corn, Jagong, Jagung, Mais, Mais Vert, Maiz, Maize, Misir, Oswego Arrowroot, Thurah Safrah, Yu Kao Liang, and Yu Shu Shu.

The original habitat of corn is unclear, probably S. America or Mexico. From middle America, cultivation has today spread predominantly to North and South America,

Europe, and Asia including China, Dominican Republic, Haiti, India, Iraq, Japan, Java, Kurdistan, Malaya, Peru, Spain, Trinidad, Turkey, Venezuela etc.

Almost all parts of the plant are used for cooking purposes or for medicinal value. In cooking, various usage such as flour, starch, syrup, oil etc is in practice. In addition, *Zea mays* is also a reputed folk remedy for many ailments such as strangury, dysuria, tumors, nephritis, urinogenital disorders etc.

Zea mays hairs are believed to have the properties of cholagogue, demulcent, diuretic, and vasodilator. Summarized below are the edible uses and medicinal uses of the plant:

Edible Uses:

Seed :
- Eaten raw or cooked
- Ground into flour as thickening agent in foods
- Starch extracted used in confectionery, noodles etc
- Dried seeds to make popcorn
- Edible oil
- Roasted seeds to make coffee substitute

Pollen :
- Soup ingredient

Stem :
- Chewed like sugar cane
- Can make syrup

Medicinal Uses:

Leaves/ Roots	:	- Used in strangury, dysuria and gravel
Seed	:	- As diuretic and a mild stimulant - Emollient poultice for ulcers, swellings and rheumatic pains - Used in the treatment of cancer, tumors and warts
Corn silks	:	- Are cholagogue, demulcent, diuretic, lithontripic, mildly stimulant and vasodilator and hypertensive agent - Reduce blood sugar levels of diabetes mellitus - Also used in cystitis, gonorrhoea, gout etc

(http://www.ibiblio/pfaf/cgi-bin/arr_html?Zea+mays)

Morphology:

Plate 1: Picture of *Zea mays*

Zea mays is a monoecious plant. It can grow up to 60-80 cm high. It has broad leaf-blades and separate staminate (male) and pistillate (female) inflorescences. The spikes or ears proceed from the stalls at various distances from the ground, and are closely enveloped in several thin leaves, forming a sheath called the husk; the ears consist of a cylindrical substance, a pith called the cob; on this the seeds are ranged in eight rows, each row having thirty or more seeds. From the eyes or germs of the seeds proceed individual filaments of a silky appearance and bright green colour; these hang from the point of the husk and are called 'the silk'. The silks are developed from the ovary which extend from the cob and receives the pollen from the tassel *(www.fao.org/ag/AGP/doc/GBASE/data/PF000342.HTM)*.

REVIEW OF LITERATURE

Corn is one of the vegetables that we eat. It is a good source of protein and simple and complex carbohydrates which provide both short and long term energy. An ear of white corn contains four grams of soluble fiber whereas the yellow corn contains half of it. Both types provide plentiful thiamin, the B-vitamin that is required for converting food to energy *(Smart Guide to Healing Foods, Katharine Colton, 1999).*

In the article entitled *"Seeds That Heal"* by Charles H. Coleman, research scientists Bianco Magno and Paolo Rovesti have found that different vitamins, oils, and other substances are contained in corn juice and from the juices of other seeds as well. They took a pile of shelled corn to which they added water. The grains began to sprout and after they had germinated, the scientists made their extractions. Dermatological tests have indicated that these juices stimulate and nourish the skin. It is pointed out in the article also that not all varieties of corn contain the same beneficial agents. Kernels of maize, a sugary corn, contain more niacin than do kernels of starchier varieties *(Nature's Medicines, Richard Lucas, 1979).*

Scientific research shows that the seeds of the corn contains sugar, zeaxanthin, protein, hexaphosphoric acid, maizenic acid, kalium, calsium and vitamin B. On the other hand, corn silks contain galaktan, xylan, dextrose, kalium nitrate, vitamin K and yushushu acid. The oil has linoleic acid, oleic acid, palmatic acid and stearic acid *(Perubatan Herba: Konsep dan Pendekatan, Prof. Madya Dr. Wan Omar Abdullah, 1998).*

In a similar study, the lipid composition of a plasma membrane isolated from corn roots was examined. The fatty acid composition of the membrane was predominantly linoleic (60%) and palmitic (30%) *(Phytochemistry, John W. Gronewalda et al, Volume 21, Issue 4 , 1982, Pages 859-862).*

There are many types of maize and the grain may be yellow, white or red. Yellow maize contains a pigment, cryptoxanthin, which is a precursor of vitamin A. The maize kernel contains Zein and lesser amounts of Glutelin in the endosperm and in the germ it has Glutelin. Maize has useful amounts of biotins and carotenoids. The constituents generally include starch, sugar, fat, salts, water, yellow oil, maizenic acid, azotized matter, gluten, dextrine, glucose, cellulose, silica, phosphates of lime and magnesia, soluble salts of potassa, soda and etc. *(www.fao.org/ag/AGA/AGAP/FRG/AFRIS/DATA/549.htm)*

One of the possible therapeutic benefits of corn is its ability to lower risk of certain cancers *(The Food Pharmacy, Jean Carper, 1994).* There is little evidence on the pharmacological actions of corn. However, its seeds are rich in protease inhibitors, which are known to prevent cancer in laboratory studies *(Smart Guide to Healing Foods, Katharine Colton, 1999; The Food Pharmacy, Jean Carper, 1994).*

Another study reported in 1981 by Pelayo Correa of the Louisiano State University Medical Center found a strong correlation between corn consumption and low death rates from colon, breast, prostate cancer and heart disease. *(The Food Pharmacy, Jean Carper, 1994).*

Corn silk was believed to be a diuretic and mild stimulant *(The Food Pharmacy, Jean Carper, 1994; Herbal Medicine for Everyone, Michael McIntyre, 1988; Herbal Medicine In Primary Care, Sue Eldin et al, 1999)*. Corn silks besides being a diuretic and having other beneficial effects on the urinary system are also anti-inflammatory and demulcent in action. A major advantage of using corn silks as a diuretic agent is its high potassium ion content *(Herbal Medicine In Primary Care, Sue Eldin et al, 1999)*.

Corn silks can help strengthen the kidney. The diuretic effect might be owing to its saponins. The corn silks can also be used to encourage cleansing process via the kidneys. Toxic elements like tissue wastes or fatty deposits accumulated over many years might be gently released *(Herbal Medicine for Everyone, Michael McIntyre, 1988)*.

Furthermore, corn silk has stone-reducing properties. Also, it has been used for cystitis, urethritis, nocturnal enuresis, prostates, and specifically for acute or chronic inflammation of the urinary system *(Pharmacodynamic Basis of Herbal Medicine, Manuchair Ebadi, 2002)*.

In addition, corn silk is rich in magnesium. Dried corn silk makes a wonderful kidney tea, particularly for one who has a tendency to have gravel in the kidneys and bladder. It helps to dissolve the gravel and break it down *(Nature Has a Remedy, Dr. Bernard Jensen, 2001)*.

In fact, *Zea mays* is used alone or in combination by Iraqi herbalists to propel urinary stones. The aqueous extract of the hair of *Z. mays* was studied to

determine its diuretic activity. *Z. mays* hair aqueous extract failed to show significant diuresis when given alone (22% increase) when compared with control, furosemide (179% increase). However, *Z. mays* caused significant increase in the amount of urinary sodium, potassium and chloride when administered alone *(Journal of Ethnopharmacology, Muneer Al-Ali et al, Volume 85, Issues 2-3 , April 2003, Pages 257-260).*

Corn oil is rich in polyunsaturated fat and has long been known to lower total cholesterol better than other vegetable oils *(Smart Guide to Healing Foods, Katharine Colton, 1999; The Food Pharmacy, Jean Carper, 1994).*

However, corn oil also lowers the HDL-type cholesterol which is the good type. So, it is not considered as a good heart protective choice *(Smart Guide to Healing Foods, Katharine Colton, 1999).*

On the contrary, in one of the studies done by Kenneth Carroll at the University of Western Ontario, excess corn oil had been shown to produce cancer in laboratory animals. This has led to some authorities to recommend restriction of polyunsaturated oils, such as corn oil not to exceed ten percent of total fat intake.

In more recent studies, corn oil also lowers immunity in mice, making them more susceptible to infections and cancer *(The Food Pharmacy, Jean Carper, 1994).*

Moreover, corn was shown to have the ability to lower risk of heart disease *(The Food Pharmacy, Jean Carper, 1994).*

The cob, root and hairs are used for hypertension *(Perubatan Herba: Konsep dan Pendekatan, Prof. Madya Dr. Wan Omar Abdullah, 1998).*

Also, corn was reported to decrease risk of cavities *(The Food Pharmacy, Jean Carper, 1994).* Another survey of forty seven countries found that in areas where people consume starch in corn rather than wheat and rice, there are lower rates of dental cavities *(The Food Pharmacy, Jean Carper, 1994).*

In parts of Mexico corn is used to treat dysentery. In various countries the seed has credibility in treating diabetes *(The Food Pharmacy, Jean Carper, 1994).*

The cob is also used for menorrhagia and nose bleed. The root is for blenorea (urinary tract infection) whereas the decoction of the cob can be used for gaster disorders *(Perubatan Herba: Konsep dan Pendekatan, Prof. Madya Dr. Wan Omar Abdullah, 1998).*

Besides, corn has anti-viral and estrogen boosting capabilities. However, it is also an allergen and may contribute to migraine-induced epilepsy, rheumatoid arthritis and irritable bowel syndrome *(Nutrition Almanac, Fourth Edition, Gayla J. Kirschmann et al, 1996).*

Another point needs to be cautioned is that the corn tends to accumulate nitrates, which may result in poisoning of cattle. Under particular conditions, a cyanogenic glycoside may also be produced *(Medical Botany, Walter H. Lewis et al, 1977).*

LACUNAE IN LITERATURE

As discarded substance, *Zea mays* hairs are eaten by the cattle. Since many parts of the plant are useful and used in herbal medicine, these corn silks besides being used mainly as diuretics, may have other beneficial usage. So far, there is no documentation or published work available in the literature regarding the effect of *Zea mays* hairs on the hematological parameters.

OBJECTIVE OF THE STUDY

To study the effects of *Zea mays* hairs on some hematological parameters viz.,

- RBC total count
- WBC total count/ WBC differential count
- Platelet count
- Hemoglobin content
- Hematocrit level

MATERIALS AND METHODS

Ethical Clearance:

The protocol of the study is approved by the Animal Ethical Committee of the University.

Extraction of Plant Material:

Fresh *Zea mays* hairs were obtained from a local plant supplier in Kelantan. The aqueous extract of the plant were used for the study. The corn silks were dried overnight in the oven. After that, the dried corn silks were ground into powder using a blender. Aqueous extract of this powder was obtained by using soshlet apparatus based on the traditional practice. The extraction procedures were continued for three days. Then the extract was transferred into a clean container until it was cool. Following this, the decoction was rotavaporised until the volume reduced and concentrated. The extract was then frozen dried and kept in the fridge.

It was reconstructed with distilled water for use. Two different doses of the extract - 50mg and 100mg per kg body weight were administered orally to the rats by gavage.

Experimental Animals:

Forty two Albino rats of the Sprague Dawley strain were used for this study. All the animals were of the same age group and body weight ranging between 150 to 200g. Only male rats were used in the study because hormonal changes in the

female rats undergoing cyclic changes might interfere with the end results. All animals were maintained under standard laboratory conditions where food and water were freely available to all the animals.

Animal Groups:

The rats were randomly divided into seven equal groups, each group consisting of six rats.

Group 1: Control Group

The rats were sacrificed without the administration of the extract.

Group 2: 50mg for 7 days

The rats of this group were administered daily with 50 mg extract of *Zea mays* hairs orally for seven days. On the eight day, these rats were sacrificed.

Group 3: 50mg for 14 days

The rats of this group were administered daily with 50 mg extract of *Zea mays* hairs orally for fourteen days. On the fifteen day, these rats were sacrificed.

Group 4: 50mg for 28 days

The rats of this group were administered daily with 50 mg extract of *Zea mays* hairs orally for twenty eight days. On the twenty ninth day, these rats were sacrificed.

Group 5: 100mg for 7 days

The rats of this group were administered daily with 100 mg extract of *Zea mays* hairs orally for seven days. On the eight day, these rats were sacrificed.

Group 6: 100mg for 14 days

The rats of this group were administered daily with 100 mg extract of *Zea mays* hairs orally for fourteen days. On the fifteen day, these rats were sacrificed.

Group 7: 100mg for 28 days

The rats of this group were administered daily with 100 mg extract of *Zea mays* hairs orally for twenty eight days. On the twenty ninth day, these rats were sacrificed.

All the animals were given mild ether anesthesia before sacrificing. The blood sample was collected directly from the heart, mixed with EDTA and used for hematological studies.

Parameters:

1. RBC total count
2. WBC total count/ WBC differential count
3. Platelet count
4. Hemoglobin content
5. Hematocrit level

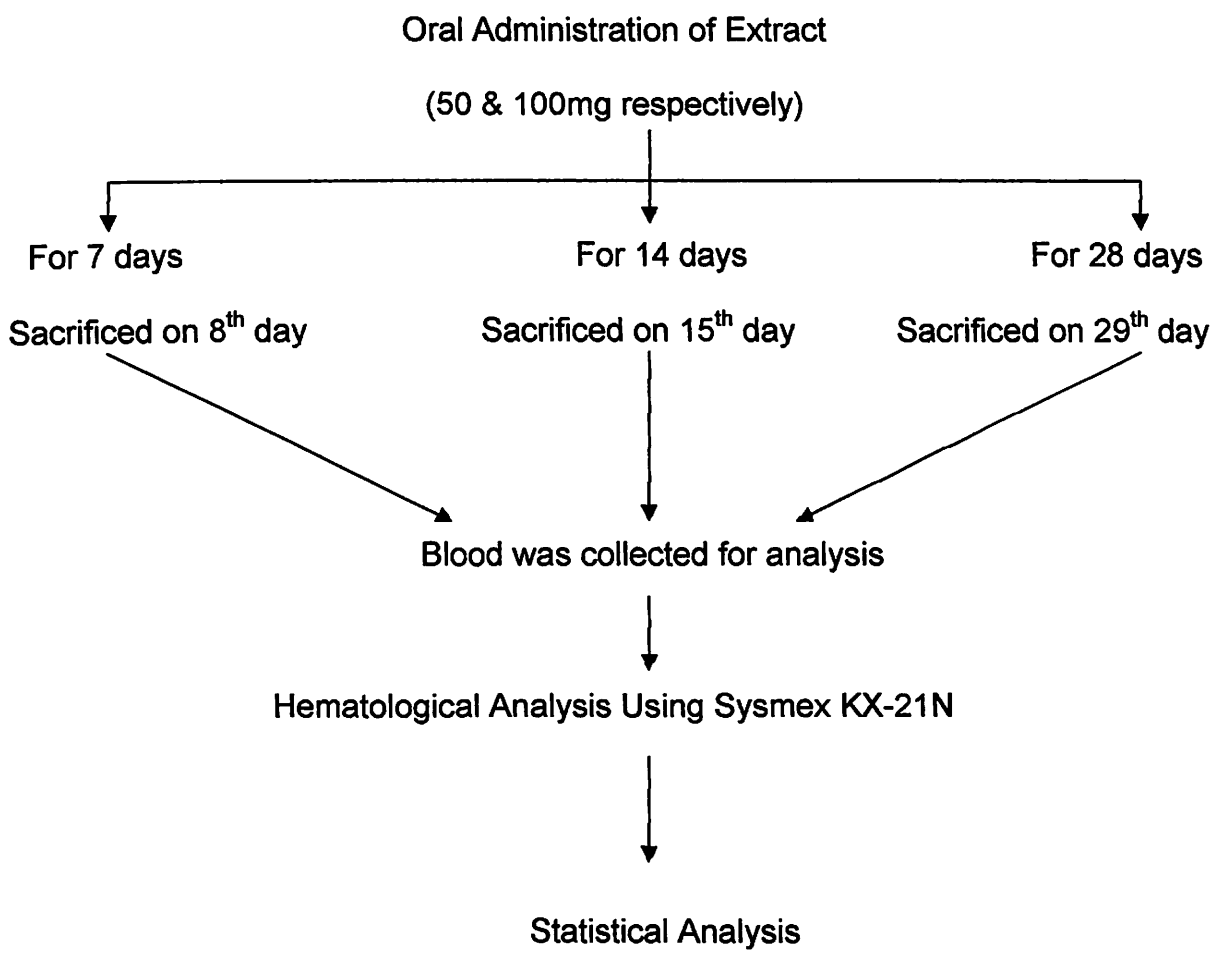
All the experimental procedures were conducted in the forenoon between 8am and 10am to avoid the influence of the circadian rhythm.

Blood cell counts were carried out using the blood cell counter (Sysmex KX-21N). The reagents used were manufactured from the company as kits which are CELLPACK as diluent and STROMATOLYSER-WH as WBC/HGB lyse reagent. The principle of analysis used in the blood cell counter for hemoglobin is the non-cyanide hemoglobin analysis method. Hematocrit values were determined by RBC pulse height detection method. RBC, WBC and platelet counts were done using the DC detection method in the machine. WBC differential count is done manually using Wright's Stain method.

Statistical Analysis:

The data of results were analyzed using the computerized statistical software SPSS program. The results of each parameter were analyzed by applying the mean values. This was followed by Paired-samples t- test, to determine the significant difference of means of the groups for each parameter. The significance level was fixed at $p<0.05$.

Experimental Protocol



RESULTS

The results of RBC count, hemoglobin content, hematocrit level, total WBC count, WBC differential count – percentage of neutrophil, lymphocyte, eosinophil and monocyte as well as platelet count are given in separate tables and figures. The table shows Mean ± Standard Error of Mean (SEM), t-values and its significance. The data of result of each parameter is presented in a bar diagram. The bar diagram is used to differentiate result of each group for each parameter.

RBC Count:

50mg/ kg treatment:

There was no significant change in the RBC count for 7, 14 or 28 days treatment. (Table 1; Figure 1)

100mg/ kg treatment:

Here also no significant change was noticed after 7, 14 or 28 days treatment. (Table 10; Figure 10)

Hemoglobin Content:

50mg/ kg treatment:

There was no significant change observed in 7, 14 or 28 days treatment. (Table 2; Figure 2)

100mg/ kg treatment:

No significant change was seen in all the three groups. (Table 11; Figure 11)

Hematocrit Level:

50mg/ kg treatment:

There was a significant increase in 28 days treatment but no significant change was seen in 7 and 14 days treatment. (Table 3; Figure 3)

100mg/ kg treatment:

Here a significant increase was noticed in 7 and 28 days treatment but not in 14 days treatment. (Table 12; Figure 12)

Total WBC Count:

50mg/ kg treatment:

No significant change was noticed after 7, 14 or 28 days treatment. (Table 4; Figure 4)

100mg/ kg treatment:

Here also no significant change was observed in all three groups. (Table 13; Figure 13)

Neutrophil Percentage:

50mg/ kg treatment:

Significant increase was seen in 14 days treatment. However, 7 and 28 days treatment did not show significant change. (Table 5; Figure 5)

100mg/ kg treatment:

There was no significant change in 7, 14 and 28 days treatment. (Table 14; Figure 14)

Lymphocyte Percentage:

50mg/ kg treatment:

There lymphocyte percentage decreased after 14 days treatment and the decrease was statistically significant. But no significant change was seen after 7 and 28 days treatment. (Table 6; Figure 6)

100mg/ kg treatment:

No significant change was noticed in all three groups. (Table 15; Figure 15)

Eosinophil Percentage:

50mg/ kg treatment:

A significant decrease was seen after 7 days treatment but there was no significant change in 14 and 28 days treatment. (Table 7; Figure 7)

100mg/ kg treatment:

There was a significant decrease for 7 and 14 days treatment. However, 28 days treatment did not show any significant change. (Table 16; Figure 16)

Monocyte Percentage:

50mg/ kg treatment:

There was no significant change for 7, 14 or 28 days treatment. (Table 8; Figure 8)

100mg/ kg treatment:

No significant change was noticed for all three groups. (Table 17; Figure 17)

Platelet Count:

50mg/ kg treatment:

Platelet count did not show any significant change in 7, 14 and 28 days treatment. (Table 9; Figure 9)

100mg/ kg treatment:

Here also no significant change was noticed in all three groups. (Table 18; Figure 18)

Dose: 50mg/kg body weight

Table 1: Effect of *Zea mays* Hairs Extract on Red Blood Cell Count ($X\ 10^{12}/L$)

Group	Mean±SEM	t values	Significance
Control	6.30±0.271	-	-
7 Days Treatment	5.84±0.615	0.658	0.539
14 Days Treatment	6.83±0.160	-1.793	0.133
28 Days Treatment	6.28±0.177	0.054	0.959

Figure 1: Effect of *Zea mays* Hairs Extract on Red Blood Cell Count ($X\ 10^{12}/L$)

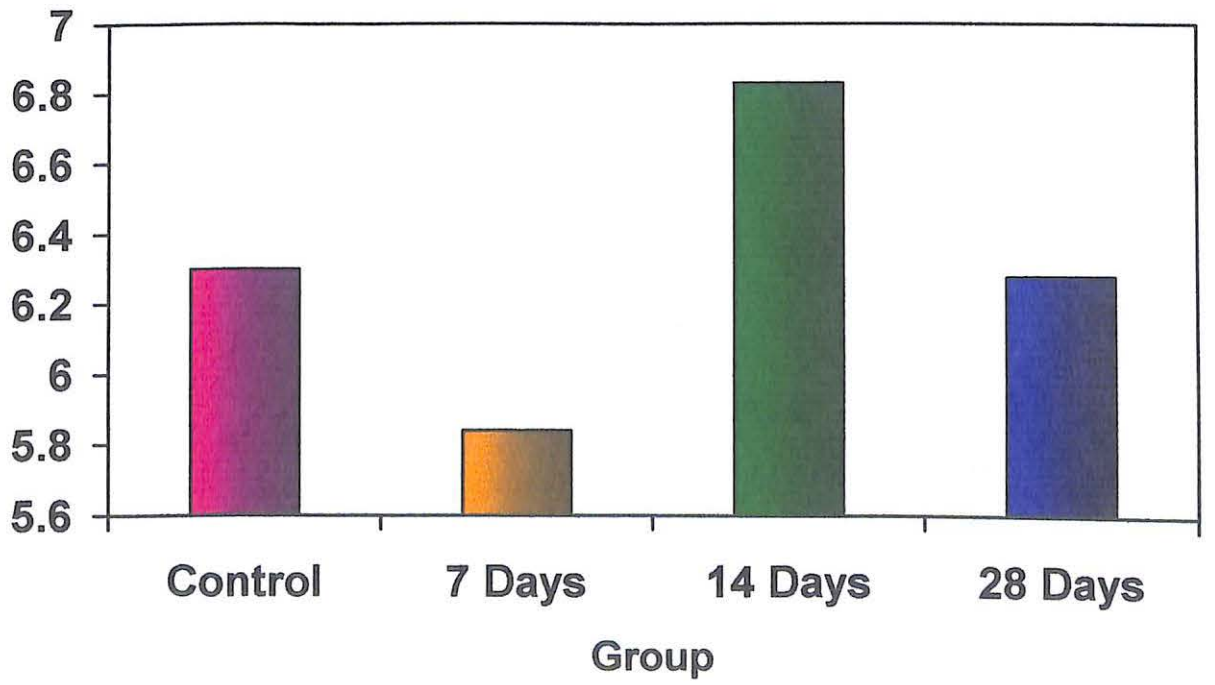


Table 2: Effect of *Zea mays* Hairs Extract on Hemoglobin Content (g/dL)

Group	Mean±SEM	t values	Significance
Control	12.93±0.590	-	-
7 Days Treatment	12.68±1.520	0.138	0.895
14 Days Treatment	13.42±0.244	-0.871	0.423
28 Days Treatment	13.43±0.280	-0.625	0.559

Figure 2: Effect of *Zea mays* Hairs Extract on Hemoglobin Content (g/dL)