



Sudan University Science and Technology College of veterinary medicine



Effect of feeding different levels of *prosopis juliflora* (mesquite) leaves on some haematological parameters of albino rat

A dissertation submitted for partial fulfillment of the Requirements of B.V.sc Honc.s Degree in Veterinary Medicine.

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الآية

تعالى: {وَمَا يَعْلَمُ تَأْوِيلَهُ إِلَّا اللَّهُ وَ الرَّاسِخُونَ فِي الْعِلْمِ يَقُولُونَ آمَنَّا بِهِ كُلُّ مِنْ عِنْدِ رَبِّنَا}

آل عمران (7)

Dedication

To our Parents

To our Families

To our teachers

To our Friends

Acknowledgements

Praise is in the first place being to Almightily Allah, who gave us health and strength to complete this work.

We are deeply indebted to our supervisor Dr. Randa Amin Basheer, for her guidance, advice, encouragement and considerable help throughout the execution and writing of this study.

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Abstract

This study was conducted at the College of Veterinary Medicine- Sudan University of Science and Technology Hillat Kuku. The aim of the study was to investigate the effects of feeding mesquite leaves on some blood parameters and body weight of rat. Mesquit leaves were collected from Hillat Kuku and were air dried in a room. four rat diets were formulated containing the dried leaves at four levels (0%, 5%, 10% and 15%).

Twenty male rats, with an average weight of 55-60 gm were used .The rat of this study divided randomly into four groups and were allotted randomly to one of the four diets. Blood samples were collected from the retrorbital plexus in EDTA containing tubes and analyzed by Sysmex autoanalyzer (KX-2n/Spain) for determination of erythrocytes count, hemoglobin concentration, packed cells volume, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration and total leucocytes count .

The animals which were fed mesquite leaves at the highest level (15%) showed bilateral alopecia, all the other animals did not show any signs of discomfort or illness. Adding mesquite leaves to the diet of albino rat did not have any significant effect on the studied hematological parameters. All the obtained values are within the normal range reported in the literature for albino rat

It is concluded that the mesquite leaves can be added to young male mice feed u to 10% without causing any hematological or clinical alterations.

مستخلص

هذه الدراسة اجريت في كلية الطب البيطري جامعة السودان للعلوم والتكنولوجيا حلة كوكو. وكان الهدف من هذه الدراسة التعرف في آثار تغذية أوراق المسكيت على بعض مكونات الدم و وزن الجسم من الفئران. تم جمع اوراق المسكيت من حلة كوكو وجففت بالهواء في غرفة وطحنت ناعما واعدت أربع وجبات للجرذان تحتوي على الأوراق المجففة بأربعة مستويات (0%، 5%، 10% و 15%). عشرون ذكر جرذ استخدمت للدرسة ، حيث يبلغ متوسط وزن 55-60 جم والجرذان قسمت عشوائيا إلى عشرون ذكر جرذ استخدمت للدرسة ، حيث يبلغ متوسط وزن 55-60 جم والجرذان قسمت عشوائيا اللي المحلل التلقائي (KX) -2n / اسبانيا) لتحديد عدد كريات الدم على أنابيب وتحليلها بواسطة SYSMEX المحلل التلقائي (KX) -2n / اسبانيا) لتحديد عدد كريات الدم الحسواء , وتركيز الهيموجلوبين ،حجم الخلايا المتراصة ، متوسط حجم الكرية الحمراء ، هيموغلوبين الكرية الحسواء و متوسط تركيز الهيموغلوبين والعدد الكلي لكريات الدم البيضاء الحيوانات التي غذيت على أعلى مستوى من أوراق المسكيت (15٪) أظهرت الثعلبة الثنائية، اما بقية الحيوانات الأخرى فلم تظهر عليها علامات للانزعاج أو المرض. إضافة أوراق المسكيت إلى النظام الغذائي الجرذان البيضاء لم يكن لديه أي تأثير كبير على قياسات الدم التي شملتها الدراسة كل القيم التي تم الحصول عليها هي ضمن المعدل الطبيعي التي ذكرت في الدراسات السابقة للجرذان البيضاء عليها هي ضمن المعدل الطبيعي التي ذكرت في الدراسات السابقة للجرذان البيضاء ويستنتج من ذلك أنه يمكن ان يضاف اوراق المسكيت إلى الفئران الذكور ف الطعام حتي 10٪ دون أن تسبب أي أمراض الدم أو تغيرات.

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CHAPTER ONE

Introdaction

Mesquite is considered an important type of plant to study; because of its variable uses as forage, wood ,some of medicinal preparation and environmental management.

Mesquite (*Prosopic* . *Juliflora*) was introduced into Sudan in 1917 planted in Khartoum. The success attained in establishment of the tree and its ability to tolerate drought and fix sand dunes encouraged the introduction of the tree into various agro ecologies on dry areas , planted in shelterbelts around farms, irrigated schemes and along the Nile. Now mesquite has become a noxious weed threatening bio-diversity, invading natural range lands, water courses, flood plains and irrigated schemes .Eradication of mesquite has been proven to be impossible . Utilization of mesquite different parts is one of the proposed strategies of controlling mesquite; so this study was carried out to investigate the potentiality of mesquite leaves as albino rat feed.

General objective:

To study the effect of feeding mesquite leaves on albino rats some haematological parameters .

Specific objectives:

To measure the effect of feeding albino rat mesquite leaves on erythrocytes count Haemoglobin concentration, Packed cell volume, Mean corpuscular volume, Mean corpuscular haemoglobin, Mean corpuscular hemoglobin concentration. To measure the effect of feeding albino rat mesquite leaves on total of white blood cell count.

1.1.Albino rat:

Albino rat is the laboratory animal which we use in the experiments; it is an iconic model organism for scientific research in variety of fields with their white hair coat and pinky eyes.

The first time one of these albino mutants was brought into laboratory for study was in 1828, in an experiment on fasting. Over the next 30years rats were used for several more experiments and eventually the laboratory rat become the first animal domesticated for purely Scientific reasons. They are small and relatively easy to care after, plentiful and reproduce quickly. (Kuramato.*et al* 2012)

1.1.1. Origin of albino laboratory rats:

It is one of the most frequently used laboratory animals across the world however the origin of albino laboratory rats remains unclear . DNA samples for 117 albino rat strains that have been used worldwide were studied and it was found that all the strains share the same albino mutation.

This finding suggested that albino mutation had very likely occurred originally in the hooded rat. It can be presumed that hooded rats were used as laboratory animals_and that an albino rat emerged as a product of the breeding of hood rats. (Kuramato.et al 2012).

1.1.2. Causes of choosing albino rats use in research:

The use of rats in laboratories has deep roots in history, stretching back several hundred years.

Rat is natural choice for laboratory testing for several reasons:

- a. They are highly intelligent and when tamed are friendly and docile.
- b. They are small and relatively easily to care.
- c. They are plentiful and reproduce quickly so it is easy to get subjects
- d. They have a short life spans which make them ideal for studding some medical topics.
- e.Rats have also evolved with humans for millions of year.

f. They eat most of food as we do and live in all of the same places.

g. They can get many of the same disease we do. (Kuramato.et al 2011).

1.2.Blood:

Blood is a.....

Functions of blood are included in the following list

Distribution of nutrients absorbed from the digestive tract

- Transport of oxygen from the lungs to cells throughout the body
- Transport of carbon dioxide from metabolizing cells to the lungs
- Transport of waste products from metabolizing cells to the kidneys for excretion
- Transport of hormones from endocrine glands to target cells

- Assistance in body temperature control by transporting heat from deeper structures to the surface of the body
- Assistance in maintaining a constant pH of body fluids by providing chemical buffers
- Assistance with the prevention of excessive loss of blood from injuries by providing
- proteins and other factors necessary for blood coagulation.
- Assistance in the defense of the body against disease by providing antibodies, cells, and other factors of body defensa. (Brown, 1984)

1.2.1. Blood cells:

Three types of blood cells (corpuscles) are recognized: erythrocyte (red cells), leukocyte (white cell) and thrombocyte (platelets). (Brown, 1984)

1.2.1.1. Erythrocytes:

Erythrocytes in circulatory blood of mamals are non nucleated, non motile cell They usually appear as biconcave circular disks with central pale spot . The biconcavity increases the surface area, thus facilitating the exchange of oxygen and carbon dioxide carried by them.

Hemoglobin, the pigment of erythrocytes is a complex, iron – containing, conjugated protein composed of a pigment(heme) and a simple protein that is globin. The red color of hemoglobin is due to the heme molecule. (Reece, 1991)

1.2.1.2. Leukocytes:

Leukocytes, the white blood cells, are much less numerous than erythrocyte in the circulatory blood.

Leukocytes normally found in the blood are classified as granulocytes

(Neutrophils, basophiles and esinophils) and agranulocytes (lymphocyte and

Monocytes). (Altura, 1980)

1.2.1.3Platelets:

Platelets (thrombocytes) are small, colorless, round or rod -shape bodies in the

circulatory blood of the mammals. The average of cell diameter is $(3\mu\text{m}\)$ but in

some cases they are considerably larger. (brown ,1984)

1.3. prosopis . juliflora:

1.3.1. Scientific Classification:

Kingdom: plantae

Order: fabales

Family: fabaceae

Subfamily: mimosoideae

Tribe: mimoseae

Genus: prosopis

Species: prosopis. juliflora

1.3.2.Description:

prosopis. Juliflora is a shrub or a small tree in the family fabaceae a kind of mesquite.

It is native of Mexico, South America and the Caribbean. It has become established as an invasive weed in Africa, Asia and Australia.(Golubov, *et al* 1999).

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The mesquite tree grows to a height of up to 12 meters (39 ft) and has a trunk with a diameter of up to 1.2 meters (3.9 ft)

1.3.3.Tree size and form:

Tree size and form vary considerably between species, population and individuals both due to genetic and environment influences. The trunk is short and soften or twisted. The bark is grey-brown, rough and fibrous. Tree form varies from erect tree to flat topped. Also tree with decumbent branches touch the ground this branches are upright at first but take horizontal form before becoming pendulous at distal ends. Smaller branches are green or greenish-brown and take on a zigzag appearance. (Ferreyra, 1979).

1.3.4. Leaves and flowers:

Leaves are deciduous, bi-pinnate, light green, compounded with 12 to 20 leaflet and petiole plus rachis 5-20 cm. Immature leaves and dry, fallen foliage both have higher palatability than mature, fresh foliage on the tree. Leaves of palatable species can be directly picked off the plant or from lopped branches. Leaflets vary greatly in size(5-23 m) long and (1-7) m wide, (Burkart, 1976).

Prosopis leaves have many adaptations to drought, such as the bipinnate, compound leaves typical of the Mimosoideae. Possession of larger numbers of smaller leaflets is considered to be a response to high temperatures, being a means of dissipating increased heat loads. In the *Prosopis. juliflora - P. pallid* complex, the action of the pulvinus can cause the leaflets to fold, protecting stomata on the upper leaf surfaces from water loss during periods of high evapo-transpiration. Leaflets of *Prosopis* species possess specialised adaptations promoting efficient utilisation and retention of water such as sunken stomata, more stomata on adaxial than on abaxial surfaces, thick and waxy cuticles and the presence of mucilaginous cells

leaves are composed principal of lignin and cellulose in the wall to give rigidity to the leaf structure. comparison of the proximate analysis of various authors show high levels of crude protein (14-26%) and crude fiber (21-25%), with ether extract (fat) of 3-9%, carbohydrate at 30-46% and highly variable levels of ash (1-16%). Elemental mineral content (Nitrogen, Potassium, Calcium, Magnesium and Phosphorus). Flowers appear shortly after leaf development, the flowers are in 5-10 cm long green- yellow cylindrical spikes, its root are able to grow to a great depth in search of water: in 1960, they were discovered at a depth of 53 meters.

1.3.5. Chromosome Numbers:

In Sudan ,the chromosome numbers of most recognized species of *prosopis* have been determined, where all taxa are having diploid with a haploid number of n=14 (2n=28) (Burkart 1976)

1.3.6. Chemistry of prosopis.juliflora:

prosopis commonly called mesquite is a prominent member of the flora

prosopis.juliflora complex been investigated taxonomically but it has also been the subject of ecological studies. The flavonoids, one of the most bioactive compounds naturally existing in the plant kingdom. A total of 21 flavonoids were found in a population representing the five species of *prosopis*, two varieties and a putative hybrid. 12 of the flavonoide have been identified: 6 flavones-- apigenin, luteolin, apigenin 6, 8-C-glycosides, chrysoeriol 7-O-glucoside, luteolin 7-O-glucoside, and 6 flavonols--kaempferol 3-O-methyl ether, quercetin 3-O-methyl ether, isorhamnetin 3-o-glucoside, isorhamnetin 3-o-rutinoside, quercetin 3-o-rutinoside, and quercetin 3-o-diglycoside (glucose, arabinose). (Johnston, 1962).

1.4.Mesquite In Sudan:

It was introduced into sudan in 1917 from South Africa and Egypt an planted in Khartoum(1929). The success attained in establishment of the tree and its abilities to tolerate drought and fix sand dunes provided the impetus for introduction of the tree in to various agro ecologies with emphasis on dry areas. (ELFADL, 1997).

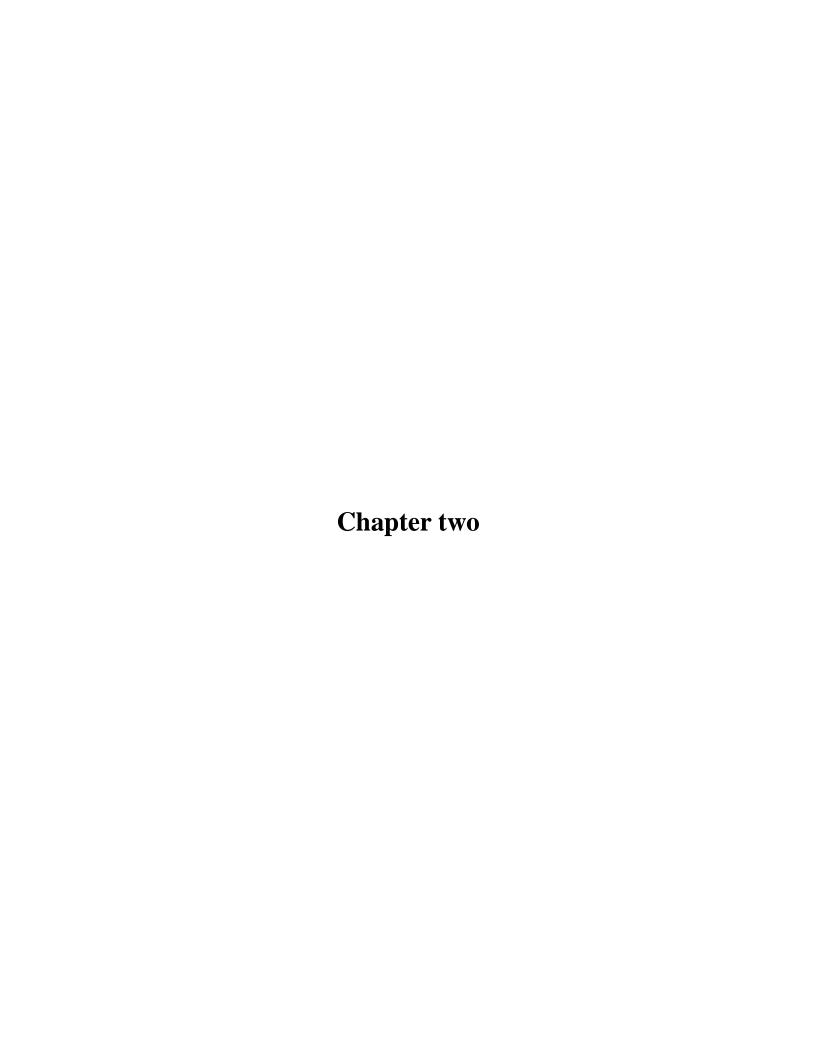
1.5. Environmental effects:-

Effect on soil fertility; over time, *prosopis.juliflora* exhibit the ability to improve soils via biological nitrogen fixation, leafe litter addition and incorporation, nutrient pumping, changes in soil structure, soil fauna and microbial populations, there is an increase in organic matter, soil macronutrient and many micronutrient. (Sharma, 1968).

An increase in silt and clay particles was also noted under canopies of *prosopis.juliflora* trees in Sudan by (EL FADAL, 1997), with a corresponding decrease in sand particles. This was thought to be due to a reduction in wind and water erosion and entrapment of windblown dust resulting in surface sealing and an increase in surface run off and soil cracks.

1.6.. Medicinal use of prosopis. juliflora:-

Prosopis.juliflora is used in the preparation of medicinal products to treat human ailments, such as eye infections, stomach disorders, skin ailments and superficial wounds. (Aqeel *,et al* 1991).



Materials and methods

This is a case control study carried out in East Nile in Sudan University of

Science and Technology (SUST) in January to mid February 2016.

2.1. Plant material:

The plant was approved at Medical and Aromatic Plants and Traditional Medicine

Research Institute (MAPTMRI) as prsosopis. juliflora (botanical name).

2.2. Experimental Animals:

Twenty male Wibster Albino rats with an average age 3-4 weeks and average body

weight 55-60g, were brought from Medical and Aromatic Plants and Traditional

Medicine Research (MAPTMRI). Experimental animals were divided randomly

into four groups. They had been held for one week as adaptation period before the

start of the experiment.

2.3. Feed:

Four rations were formulated containing conventional ingredients (flour ,salt ,meat

milk, oil) and mesquite leaves were added at four different levels.

The rats' four groups were allotted randomly to one of the following diets:

Control group: the diet contain 0% of mesquite leaves

5% group: the diet contain 5% mesquite leaves

10% group: the diet contain 10% mesquite leaves

15% group: the diet contain 15% mesquite leaves

The experimental animal were kept in separate standard cages (five rats each).

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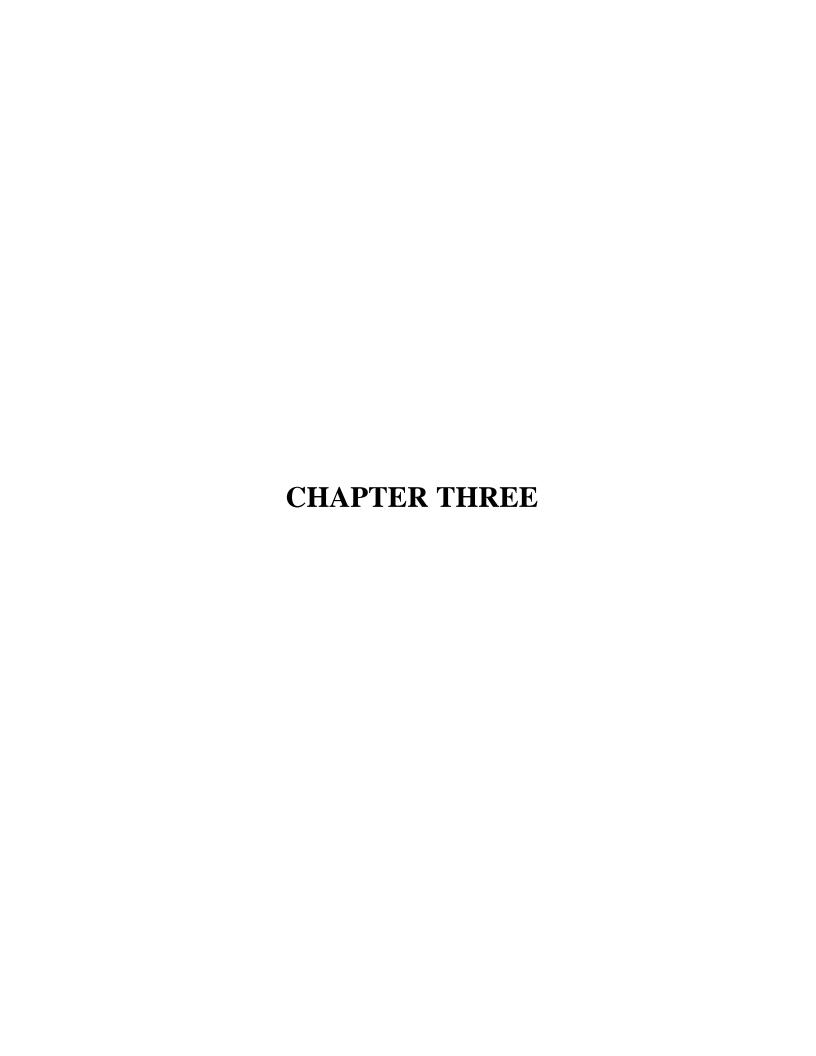
2.5. Blood collection and analysis:

Blood samples were taken from each rat three times during the study. Blood was collected in EDETA tubes using capillary tube by puncturing retrorbital plexus , blood was analyzes using sysmex auto analyzer ($kx_2n/spain$) for determination of red blood cells count , hemoglobin concentration ,

mean corpuscular hemoglobin concentration, mean corpuscular hemoglobin, packed cell volume, white blood cells count and Lymphocyte.

2.6.Data analysis:

All the data was presented as mean \pm SD. Data was analyzed using statistical package for social science (SPSS version 16) by ANOVA. (GOMEZ 1984).



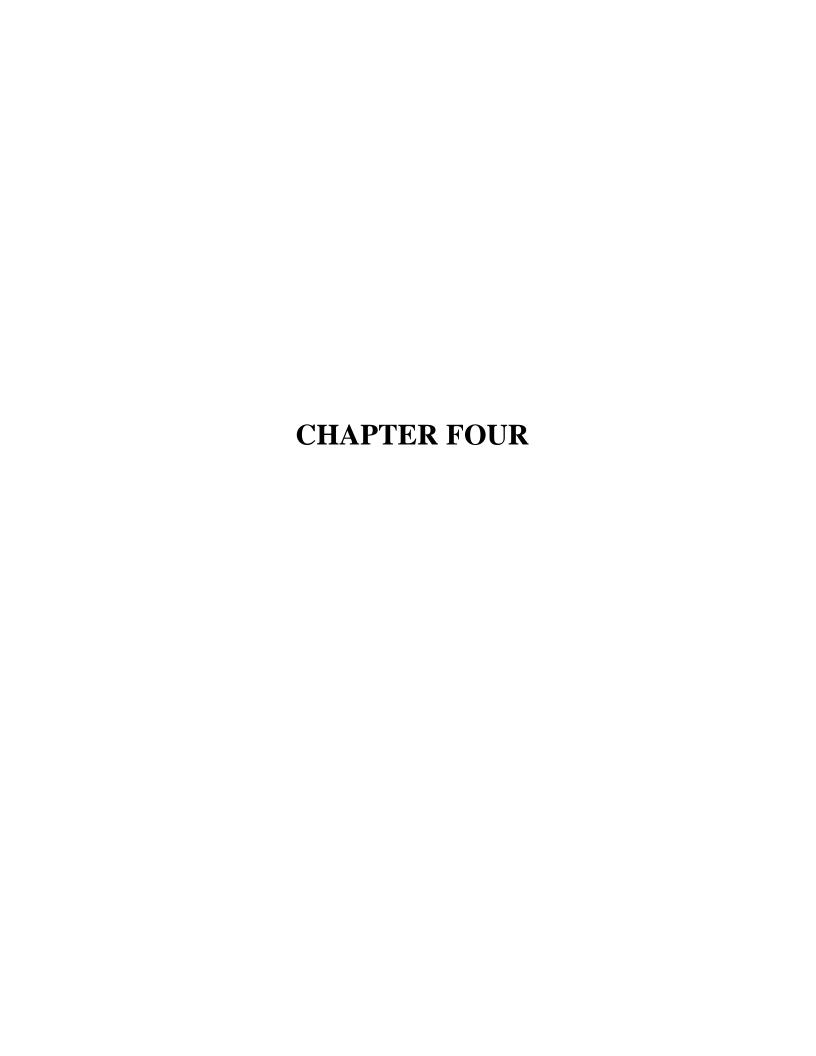
Results

All the animals did not show any signs of distress or illness except of group four which showed a bilateral alopecia

3.1. The effect of feeding different levels of *prosopis.juliflora* on albino rats some hematological parameters

The highest RBCs count was found in the control group and the lowest count was found in 15% group. The lowest count of HGB was found in 5% and highest at control group and there is no significant variation among groups. The highest HCT count was found in15% group and the lowest count was found in 5%. Also The lowest MCV count was found in control group while the 5% group has the highest count. The highest MCH count was found in 5% group and lowest was found in control group. Also the highest MCHC count was found in 5% group and lowest at control group.

The WBCs count has the lowest count at control group and the highest count at 10% and 15% groups.



discussion

There are many causes for alopecia in rats like, bacterial, parasitic allergy or nutritional. The rats in this study did not suffer from any bacterial or parasitic infestations, so most probably the alopecia seen in the rats fed mesquite leaves at 15% level is due to nutritional factors.

Although vitamin and mineral have been posited as causes hair loss the nutritional regulate hair production parameters that might have been evaluated comprehensively. Much of the focus has been on zinc, vitamin D, protein. Studies of the role of zinc in hair loss demonstrated the principle that both too much substance can have deleterious effect Rushton (2002). Also vit A deficiency was found to be one of the causes of hair loss, but in this work vitamin A deficiency can not be responsible for the hair loss, because (Mejia et al 1979) found that vit A deficiency caused

Hair loss in rat with constellation of clinical signs that included aneima and alopecia which were not observed in the current study.

Osno *et al* (2016) administered female winstar albino rats orally with ethanolic leaf extracts of *prosopis.juliflora* the dosage level of 100 mg|kg body weight. Aftar28 day they found insignificant increase in corpuscular hemoglobin compared to the control which is on line with the current work. Alternatively, in the current work there was an insigninficant decrease in the RBCs and an insigninficant increas in the WBCs, mean corpuscular hemoglobin cocentration compared to the control group which is condradicting the finding of the previous autlhors—at the same dosage. All the studied parameter are with in the normal range of the rat and this

may be due to the several alkaloids have been isolated from leaf extracts having pharmacological properties (Aqeel *et al, 1989*).

Rat fed mesquite leaves at the highest level (15%) had insignificant lower body weight than the control and the other mesqite fed groups . This may be attributed to unpalatability of the mesquite leaves at this level leading to inappetance and consequently weight loss.

Recommendations

- ❖ Increase the palatability of mesquite leaves by adding feed additive as flavors.
- ❖ More studies should be performed using more animals for long periods.
- ❖ The ratio of mesquite leaves in albino rats diet must not exceed 15%.
- ❖ Investigate the causers of the symmetrical truncal alopecia

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Appendix

Appendex (1). Normal parameter of albino rats:

Parameters	Values
Normal blood pressure	60 - 90/75_120
Blood volume	5 - 7.ml/100g
Clotting time	2.5/miniute

Appendix (2)

Rat hematologic reference ranges:

Parameters	Values
Red blood cells	6.76 - 9.75×10/mm ³
White blood cells	6.6 - 12.6×10 ³ /mm ³
Hemoglobin	11.5 - 16.1g/dl
Neutrophils	1.77 - 3.38×10 ^{3/} mm ³
Lymphocyte	4.78 - 9.12×10 ³ /mm ³
Esinophilis	0.03 - 0.08×10 ³ /mm ³
Monosytes	$0.01 - 0.04 \times 10^3 / \text{mm}^3$
Basophiles	$0.00 - 0.3 \times 10^3 / \text{mm}^3$
Platelets	150 - 400×10 ³ /mm ³
Packed cell volume	37.6 - 50.6%

(lea and Febbiger, 1993

Table (3). Proximate analysis of prosopis.juliflora leaves.

Location	Dry matter	Crude protein		Ether extract	Ash	Nitrogen Source free extract%
	%	%	%	%	%	

PJ Brazil	-	18.5	21.2	5.6	-	-
PJ India		26.3	24.8	8.5	1.4	31.8
Gohl 1981		20.3	24.0	0.5	1.4	31.0
PJ S. Africa Touzeau	-	19.0	21.6	2.9	8.5	29.7
1973						
PJ Niger Anttilet al	38.1	13.7	22.9	4.8	15.9	42.7
1993						
PJ Kenya	-	21.7	21.0	4.8	-	45.8
Anttila et al 19	993					

Table (4). Mineral content of prosopis.juliflora leaves.

Location	N	P	K	Ca	Mg	Na	Source
	%	%	%	%	%	%	

PJ Brazil	3.24	0.10	1.16	1.72	0.75	-	Lima 1994
PP Brazil	3.13	0.10	1.35	1.49	0.75	-	Lima 1994
PJ India	4.10	0.25	2.10	1.61	0.78	0.67	Singh et al 1988b
PJ India	3.55	0.19	1.00	1.0	1 0.70	0.73	Singh et al 1990
PJ India	3.31	0.30	2.11	2.25	0.38	4.08	Sharma 1968
PJ India	5.57	0.90	3.11	1.01	-	-	Patel 1986
PJ Niger	-	0.14	0.83	4.20	0.56	-	Touzeau 1973
PJ Honduras	4.39	0.27	1.92	2.19	0.32	-	Hunter and Stewart
							1993
PJ Kenya	PJ Kenya 2.76 0.19 2.42 1.32 0.34 - Maghembe <i>et al</i> 1983					nghembe et al 1983	

Table(5) Effect of feeding different levels of Mesquite leaves on some hematological parameters of albino rats

Groups/Paramet	0%	5%	10%	15%	P value
ers					
RBCs×10 ⁶ /ml	6.33±1.61	6.08±0.91	5.99±1.15	6.27±1.23	0.70
HGB g/dl	13.34±1.51	13.49±1.5 9	12.99±1.5 9	12.69±1.3 2	0.27
НСТ%	37.31±3.67	36.59±4.1	36.95±5.2 2	37.82±4.8 8	0.39
MCV fl	55.72±2.29	56.66±1.1	55.96±1.1	56.40±1.2	0.72
MCH pg	18.60±0.69	19.37±0.6 9	18.72±0.9 6	19.07±1.0 8	0.81
MCHCg/dl	33.15±0.74	34.03±0.8 2	33.45±1.6	33.78±1.4	0.35
WBCs×10 ³ /ml	8.25+2.41	8.51+2.12	9.97+3.29	9.64+3.69	0.12

Significance level at $P \le 0.05$.