

Some Hematological-Studice ON Diabetic RATS treated with certain hypoglycemic plants

Eman G.E. Helal*, Abeer S.M. Gawish**,A. Kahwash*

*Zoology Department. Faculty of science ,Al -Azhar, University.

**Oral Medicine, Periodontology ,Oral Diagnosis and Radiology department, Faculty of Dental
Medicine (girls section) Al- Azhar, University.

Abstract

The present study was designed to evaluate the effect of some antidiabetic plants on the hematological parameters in aloxan induced diabetes in albino rats.

Eighty adult albino rats (120±20 gm b.wt) were randomly divided into eight groups ten on each group, the first group conserved as control group. The remaining rats were injected with aloxan to induce diabetes. The second group conserved as diabetic group. The third group was diabetic rats treated with a mixture consists of *Nigella Sativa*, *Aloe Vera*, *Ferula assa-foetida* , *Boswelli Carteri*, *Commiphora Myrrha*. (0.1ml/100gm b.wt),

Fourth group treated with 0.1ml/100gm b.w of *Nigella Sativa*, Fifth group treated with 0.05ml/100gm b.w of *Aloe Vera*,Sixth group treated with 0.1ml/100gm b.w of *Ferula assa-foetida*, Seventh group treated with 0.1ml/100gm b.w of *Boswelli Carteri*, Eighth group treated with 0.1ml/100gm b.w of *Commiphora Myrrha*. Haematological parameters includes: red blood cell count (RBCs), hemoglobin concentration (Hb), hematocrit value (Hct%), total and differential white blood cell cont (WBCs) were done after 30 days of treatment and after 15 days of recovery period.

The result of RBCs count, Hb concentration and Hct value were significantly decreased in diabetic, *Nigella Sativa* and *Aloe Vera* treated groups, throughout the experiment, except *Aloe Vera* treated group showed insignificant change after the recovery period.while insignificant changes was recorded in the remnant groups during the experimental period.

WBCs count, lymphocyte and neutrophil percentage were highly significant increased in diabetic and *Ferula assa-foetida* treated rats after treatment and recovery periods. While insignificant differences were observed in the other treated groups. Moreover, no significant changes were recorded in eosinophils and monocytes in all groups throughout the study period.

Diabetes mellitus in experimental animal can be treated with some plants which have antidiabetic effect, the present investigation was designed to illustrate any hazard effect of these plants on some hematological parameter. Further investigations on each plant and their mixture are needed to role out its effect on both diabetes and the vital parameters.

Introduction

For long time ago, many plants and plant extracts which posses marked hypoglycemic activity have been tried in the treatment of diabetes mellitus in folk medicine (Asmal&Marble, 1984). More than 400 traditional plant treatment s have been recorded, but only a small part of these plants have been investigated scientifically and medically evaluate their efficacy (Lot-likar &Rajaram,1966).

A scientific investigation of herbal remedies for diabetes mellitus may be helpful in the development of alternative therapeutic strategies (Marls& Farnsworth, 1995).

A number of plants have been shown to exhibit hypoglycemic activity with animal models.

Nigella Sativa has been used as a natural medicine for treatment of diabetes,

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hypertension as well as some dermatologic conditions (Meral et al,2001).

Aloe Vera has been used all over the world for their various medicinal properties. However, there have been controversial reports about its hypoglycemic properties, which may be due to the differences in the parts of the plant used or to the model of diabetic chosen (Okyar et al, 2001).

Ferula assa-foetida, the asafoetida's Olea-gum-resin are the main parts used, the volatile oil contains disulphides. The sulphur compounds in the oil may protect against fat induced hyperlipdemia (Duke, 2002).

Commiphora Myrrha, the myrrh's gum-resin- volatile oil are the main used parts. It is an excellent remedy for hyperglycemia (Chevallier, 1996 & Duke, 2002).

A water extract of a mixture of the above mention five plants were studied for identification of its active components (Al Awadi & Shoukry 1988). Only the extracts of myrrh and Olea gums effectively increased glucose tolerance in both normal and diabetic rats. While the remaining components showed no effect.

Alloxan was found to be selectively β -cytotoxic agents in animals, extremely potent diabetogenic substance and may induce hemolysis of red blood cell in experimental animals (Okamoto, 1984).

The present investigation was carried out to illustrate if any one of these plant extracts has any hazard effect on hematological picture.

Materials and Methods

The present study was carried out on eighty male albino rats weights 120 \pm 20 grams of local strain from NAMRU medical center. They were divided into eight groups (10/ group).

Preparation of the mixture:

Plants were grinded and 10 gm of each plant were mixed and boiled in 100 ml water for a period of 10 min then cooled at room temperature and filtered. The extract was given orally with a dose of 1 ml/100

gm b.w (by using the incubation tube, every day till 30 days). This dose equal to the therapeutic dose related to human dose (Paget and Barnes, 1964).

Group I serve as control

The remaining 70 fasting rats over night were injected with subcutaneous dose of alloxan (120/kg b.wt) to induce diabetes. After 48 hours blood glucose level were measured by glucometer, diabetes was considered as blood glucose \geq 250mg/dl

Group II served as diabetic.

Group III diabetic rats treated with mixture of treatment plants (0.1ml/100gm b.w)

Group IV diabetic rats treated with 0.1ml/100gm b.w of *Nigella Sativa*

Group V diabetic rats treated with 0.05ml/100gm b.w of *Aloe Vera*

Group VI diabetic rats treated with 0.1ml/100gm b.w of *Ferula assa-foetida*

Group VII diabetic rats treated with 0.1ml/100gm b.w of *Boswellia Carteri*

Group VIII diabetic rats treated with 0.1ml/100gm b.w of *Commiphora Myrrha*

After 30 days of treatment (treatment period), 5 animals of each group were sacrificed, while the other half of each group was kept for 15 days more without any treatment to follow up if there is any delayed effect (recovery period).

Hematological assessment

Two blood samples were taken from each rat: after both the treated period and the recovery period

The following analyses were done

- 1- Estimation of hemoglobin concentration (Drabkin & Austin, 1932)
- 2- Measurement of hematocrit value (Hct) (Rodak, 1995) by using heparinized capillary tube
- 3- Red blood corpuscles count (RBCs) visually counted according to Dacie & Lewis (1991) method
- 4- White blood cell count (WBCs) visually counted according to Mitruka *et al.*, 1977)

5- Calculation of differential leucocytic count, were done according to Dacie & Lewis (1991)

All the data were statistically analyzed using student t test.

Results

The present study showed highly significant decrease ($p < 0.01$) in RBCs count, Hb concentration and Hct value in diabetic rats compared with control group during the experimental period (table 1)

Moreover, the groups treated with mixture, *Ferula assa-foetida* *Boswellia Carterii* *Birdw* & *Comiphora Myrrha* showed insignificant changes in RBCs count, Hb concentration and Hct values as compared with control group, while there was a highly significant increase ($p < 0.01$)

when compared with diabetic group throughout the experiment.

Nigella Sativa treated group showed highly significant decrease ($p < 0.01$) in RBCs count, Hb concentration and Hct value when compared with controls group, and no significant changes was recorded in these parameters when compared with diabetic group.

On the other hand, the group treated with *Aloe vera* showed significant decrease ($p < 0.05$) in RBCs count, Hb concentration and Hct value when compared with controls group after treated period, and no significant changes was recorded after recovery period. However, there was a significant increase ($p < 0.05$) in these parameters after recovery period when compared with diabetic group. (Table 1,2)

Table (1) : Red blood cell count (RBCs), Hemoglobin conc.(Hb) And Hematocit value (Hct) in control, diabetic and plant extract treated groups after 4 weeks (treated period)

Parameter \ Group		Treated period (4weeks)							
		control	diabetic	mixture	<i>Nigella stavia</i>	<i>Aloe vera</i>	<i>Ferula Assa-foetida</i>	<i>Boswellia carterii Birdw</i>	<i>Commiphora myrrh</i>
6 RBCs X10	mean	7.58	5.65	7.56	5.74	6.52	6.75	6.95	7.04
	±SD	0.15	0.21	0.14	0.16	0.26	1.22	1.07	1.08
	A		**		**	*			
	B			**		*	**	**	**
Hb(gm%)	mean	14.8	12.08	14.66	11.64	13.86	14.58	14.76	14.58
	±SD	0.25	0.31	0.02	0.43	0.25	0.22	0.27	0.19
	A		**		**	*			
	B			**		*	**	**	**
Hct%	mean	48.40	40.24	47.98	38.92	44.60	48.96	48.32	49.12
	±SD	0.75	1.01	0.07	1.30	1.45	0.54	0.64	0.33
	A		**		**	*			
	B			**		*	**	**	**

A- in comparison with control group

B –in comparison with diabetic group

* Significant ($p < 0.050$)

** Highly significant ($p < 0.01$)

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Table(2) : Red blood cell count (RBCs) ,Hemoglobin conc. (Hb) And Hematocit value (Hct) in control ,diabetic and plant extract treated groups after recovery period (2 weeks)

Group Parameter		Recovery period (2weeks)							
		<i>control</i>	<i>diabetic</i>	<i>mixture</i>	<i>Nigella stavia</i>	<i>Aloe vera</i>	<i>Ferula Assa-foetida</i>	<i>Boswellia carterii Birdw</i>	<i>Commiphora myrrh</i>
RBCsX 10 ⁶	mean	7.16	5.97	7.12	5.92	6.93	7.15	7.27	7.16
	±SD	0.09	0.07	0.10	0.06	0.16	0.04	0.07	0.03
	A		**		**	**			
	B			**		**	**	**	**
Hb(gm %0)	mean	15.38	11.36	14.84	11.85	14.34	14.82	14.72	14.78
	±SD	0.31	0.04	0.31	0.09	0.39	0.12	0.29	0.31
	A		**		**				
	B			**		**	**	**	**
Hct%	mean	.1450	43.08	49.32	44.86	47.56	47.56	48.90	45.25
	±SD	0.85	1.05	0.47	0.91	0.82	0.37	1.01	0.48
	A		**		**				
	B			**		**	**	**	**

A- in comparison with control group

B –in comparison with diabetic group

* Significant(p<0.05)

** Highly significant(p<0.01)

Total and differential white blood cells count:

Concerning white blood cell count the present data showed a significant increase (p<0.05) in diabetic group which was highly significant (p<0.01) after recovery period when compared with control group (table 3&4)

Diabetic group showed insignificant changes in the percentage of eosinophils, monocyte and staff neutrophils, however, there was highly significant increase (p<0.01) in segmented neutrophils . Lymphocytes showed highly significant decrease(p<0.01) when compared with control group throughout the experimental period.

The groups treated with mixture, *Nigella Sativa*, *Aloe Vera*, *Boswellia Carterii Birdw* & *Comiphora Myrrha* showed insignificant change in their count when compared with control group during the experiment, while a significant decrease

(p<0.05) was recorded after the treatment period and showed highly significant decrease (p<0.01) after recovery period when compared with diabetic group

Concerning the percentage of eosinophils, neutrophils (Staff and Segmented) ,monocyte and lymphocytes insignificant change had been recorded in treated groups when compared with control group during the experimental period. However, when compared with diabetic group a high significant decrease was detected in segmented neutrophils (p<0.01)and high significant increase (p<0.01) in lymphocytes , while the percentage of eosinophils, staff neutrophils and monocytes showed insignificant changes.

On the other hand, the group treated with , *Ferula assa-foetida* showed significant increase in its WBCs count when compared to control group , while insignificant changes was clear when

compared to diabetic group during the study period.

Concerning the percentage of eosinophils, staff neutrophils and monocyte showed insignificant change Ferula assa-foetida treated group when compared with control group during the experimental period. However, Segmented Neutrophils showed high significant increase ($p<0.01$) as compared

with control group and insignificant change when compared with diabetic group.

Lymphocytes showed high significant decrease ($p<0.01$) as compared with control group and insignificant change when compared with diabetic group all over the study period (Table 3&4).

Table (3) : White blood cells count and differential leucocytic count (Eosinophils, Segmented neutrophils ,Staff neutrophils ,Monocyte and Lymphocyte)in control ,diabetic and plant extract treated groups after 4 weeks (treated period)

Group Parameter		Treated period (4weeks)							
		<i>control</i>	<i>diabetic</i>	<i>Mixture</i>	<i>Nigella stavia</i>	<i>Aloe vera</i>	<i>Ferula Assa-foetida</i>	<i>Boswellia carterii Birdw</i>	<i>ommiphora myrrh</i>
WBCsX10	mean	7.53	9.33	7.64	7.41	7.85	9.27	8.01	8.09
	±SD	0.95	0.33	0.45	0.70	0.47	0.26	0.38	0.36
	A		*				*		
	B			*	*	*		*	*
Eosinophil s%	mean	0.83	.460	0.73	0.53	0.67	0.80	0.50	0.57
	±SD	0.31	0.21	0.31	0.21	0.29	0.28	0.22	0.24
	A		-	-	-	-	-	-	-
	B			-	-	-	-	-	-
Segmented neutrophils	mean	.3330	.1457	31.33	25.33	29.50	55.50	27.67	28.50
	±SD	2.23	2.29	2.42	3.96	2.96	3.96	1.96	2.22
	A		**				**		
	B			**	**	**		**	**
Staff neutrophils	Mean	1.17	1.32	0.77	0.85	0.83	1.53	0.88	0.78
	±SD	0.33	0.42	0.31	0.31	0.31	0.18	0.42	0.45
	A		-	-	-	-	-	-	-
	B			-	-	-	-	-	-
Monocyte	mean	2.69	1.67	2.29	2.66	2.00	1.96	2.02	2.92
	±SD	0.43	0.56	0.73	0.23	0.73	0.43	0.33	0.62
	A		-	-	-	-	-	-	-
	B			-	-	-	-	-	-
Lymphocyte	mean	64.98	39.01	64.87	71.02	67.00	40.21	68.93	67.23
	±SD	1.92	2.96	1.75	3.71	2.13	2.98	1.72	1.53
	A		**				**		
	B			**	**	**		**	**

A- in comparison with control group

B –in comparison with diabetic group

* Significant ($p<0.05$)

** Highly significant ($p<0.01$)

- Insignificant

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Table (4) : White blood cells count and differential leucocytic count (Eosinophils, Segmented neutrophils ,Staff neutrophils ,Monocyte and Lymphocyte)in control ,diabetic and plant extract treated groups after recovery period(2 weeks)

group parameter		Recovery period (2 weeks)							
		control	diabetic	mixture	Nigella stavia	Aloe vera	Ferula Assa-foetida	Boswellia carterii Birdw	Commiphora myrrh
WBCs	mean	7.86	10.83	7.38	7.52	7.77	9.34	7.88	8.06
	±SD	0.47	0.13	0.32	0.26	0.38	0.25	0.34	0.36
	A						*		
	B			**	**	**		**	**
Eosinophils%	mean	0.88	.430	0.83	0.60	0.60	0.67	0.72	0.67
	±SD	0.45	0.33	0.65	0.34	0.34	0.42	0.52	0.42
	A		—	—	—	—	—	—	—
	B								
Segmented neutrophils	mean	.6230	60.63	31.17	27.78	30.31	50.57	29.33	32.83
	±SD	0.96	3.96	1.05	1.86	2.22	3.21	1.75	2.83
	A		**				**		
	B			**	**	**		**	**
Staff neutrophils	mean	1.50	1.60	1.36	0.83	1.00	1.47	1.17	1.50
	±SD	0.34	0.45	0.40	0.40	0.68	0.54	0.45	0.02
	A		—	—	—	—	—	—	—
	B			—	—	—	—	—	—
Monocyte	mean	2.55	1.67	2.17	7.73	2.16	1.57	2.12	.093
	±SD	0.62	0.65	0.33	1.50	0.62	0.33	0.42	0.62
	A		—	—	—	—	—	—	—
	B			—	—	—	—	—	—
Lymphocyte	mean	64.45	35.68	64.47	68.26	66.13	45.74	66.65	61.91
	±SD	1.52	1.65	2.54	2.39	1.84	1.34	3.33	2.07
	A		**				**		
	B			**	**	**	**	**	**

A- in comparison with control group

B –in comparison with diabetic group

* Significant $p < 0.05$

** Highly significant $p < 0.01$

Discussion

The traditional antidiabetic plants might provide useful source of new oral hypoglycemic compounds. The antidiabetic action of plant extract may be mediated through decreased hepatic gluconeogenesis (Al Awadi *et al.*, 1991).

The present study is a step to evaluate and follow up the effect of these plant extracts on some hematological parameter.

The present results showed that diabetic rats recorded a decrease in RBCs

count , Hb content and Hct value .These rats suffered from anemia which may be results from the toxic effect of alloxan used to induce diabetes in these rats . This anemia could be attributed to destruction of RBCs and reduced rate of its release from the bone marrow to blood. Several studies attributed this anemia to increase in lipid peroxidation of the erythrocyte cell membrane (Kang-Xin *et al.*,1990 and Helal, 2000).

The results of the present investigation showed significant decrease in RBCs , Hb and Hct value in *Nigella Stavia* (N.S)treated group. The lower serum iron might be due to the effect of N.S which deprives iron from body fluid to be conjugated with proteins. This was contradictory with Hedaya (1995) who found that low dose of N.S extract caused significant increase in Hb, PCV and RBCs

Tizard (1995) mentioned that iron is essential for bacterial invasion to the body in which , iron is associated with iron binding protein transferring, lactoferrin, heptaglobin and ferritin. The lower serum iron in the present investigation might be due the effect of N.S. which eliminate iron from the body fluid to be conjugated with proteins. This may be due to the antibacterial effect of N.S as reported by El-Fatary (1975), Hanafy & Hatem (1991) and Kandil *et al* (1994)

Otherwise, a general decrease in all hematological parameters in the diabetic state was ameliorated by the treatment with mixture, *Aloe vera*, *Bosweelia Carterri Bidw* and *Commiphora Myrrha*. This indicates that these plants could reduce the destructive effect of alloxan .

The total WBCs count in diabetic group showed a significant increase. This increase in total WBCs count may be due to the increased hemopoitic activity as a result of the hemolysis of RBCs in diabetic rats.In the diabetic group , lymphocytes were decreased in number. This may be as a response to stressful condition after antigen (alloxan) injection which was in agreement with Sovneyl *et al.*, 1990, or it may be due to the production of specific or non specific antibodies against different antigens, since lymphocytes are responsible for achieving the defense mechanism in the body (El Feki *et al.*,1997).

The increasing of neutrophil number may be due the engagement of these cells in the phagocytic process against different antigens. The increase in neutrophils also may be due to decrease its phagocytic activity in hyperglycemia and due to increase in hemopoitic activity after releasing the granules of neutrophil by exocytosis to lyses the antigens extracellularly (Ganong,2003).

Ferula ass.foetida showed an improvement in percentage of lymphocytes when compared to diabetic group. This may be attributed to ass-foetida's volatile oil which contain sodium ferulate which prevent lymphocytes apoptosis(Lu *et al*,1998), and activate the lymphoid tissues of the body to form lymphocytes. This is in accordance with Chevallier (1996) who mentioned that *ass-foetida's* volatile oil, has antibiotic action and acts as immunoenhancer.

In the present result, mixture, *Nigella Stavia*, *Aloe vera*, *Bosweelia Carterri Bidw* and *Commiphora Myrrha* treated groups showed an increase (turned back to normal value) in WBCs count, neutrophils percentage and a decrease in lymphocytes, this may be due to the changes in the immunological parameters achieved by plant extracts during diabetes and after treatment period. This indicates that the treatment with plants affect the defense mechanism and immune response to inhibits the inflammation resulting from alloxan treatment .This is in accordance with Kollar &Roan, 1980 and Shakoori *et al.*, 1992.

From the present study, the use of, *Aloe vera*, *Bosweelia Carterri Bidw* and *Commiphora Myrrha* could ameliorate the damage effect of aloxan.Using *Nigella Stavia* increase, the hazards state which turn back to normal value after stopping the usage of the plant extract. So, it is well recommended not to use *Nigella Stavia* in anemic patients.

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بعض قياسات الدم في الفئران البيضاء المصابة بالسكر والمعالجة ببعض النباتات المخفضة للسكر

* ايمان جمال الدين هلال ، ** عيبر سعد محمد جاويش ، * أنوار الكامل كحوش
* قسم الحيوان – كلية العلوم – جامعة الأزهر
** قسم طب الفم و أمراض اللثة و التشخيص والأشعة – كلية طب الأسنان – جامعة
الأزهر

أجري هذا البحث لدراسة مدي فعالية بعض النباتات التي تستخدم كمخفضات لسكر الدم و تأثيرها علي صورة الدم. ولقد تم اجراء هذه الدراسة علي ثمانين من ذكور الجرزان البيضاء البالغة يزن الواحد منهم حوالي (20±120 جم) و لقد تم توزيعها بصورة عشوائية الي ثمان مجاميع (كل مجموعة تحتوي علي 10 جرذان كالاتي:
المجموعة الاولى : اعتبرت مجموعة ضابطة
المجموعة الثانية : اعتبرت مجموعة مصابة بالسكر.
المجموعة الثالثة : جرذان مصابة بمرض السكر تم معالجتها بالمستخلص المائي للخليط (1مل/ 100 جم من وزن الجسم)
المجموعة الرابعة : جرذان مصابة بمرض السكر تم معالجتها بالمستخلص المائي لنبات حبة البركة (1مل/ 100 جم من وزن الجسم)
المجموعة الخامسة : جرذان مصابة بمرض السكر تم معالجتها بالمستخلص المائي لنبات الصبر (0.05مل/ 100 جم من وزن الجسم)
المجموعة السادسة : جرذان مصابة بمرض السكر تم معالجتها بالمستخلص المائي لنبات الحلثيت (1مل/ 100 جم من وزن الجسم)
المجموعة السابعة : جرذان مصابة بمرض السكر تم معالجتها بالمستخلص المائي لنبات اللبان (1مل/ 100 جم من وزن الجسم)
المجموعة الثامنة : جرذان مصابة بمرض السكر تم معالجتها بالمستخلص المائي لنبات المر (1مل/ 100 جم من وزن الجسم)
تم تقييم للتغيرات لصورة الدم بعد العلاج لمدة ثلاثون يوما و كذلك بعد فترة الاستشفاء (بعد 15 يوم)
و قد أظهرت النتائج التي تم الحصول عليها من الدراسة حدوث انخفاض ذو دلالة احصائية في كرات الدم الحمراء و تركيز الهيموجلوبين و الهيماتوكريت في كل من الجرذان المصابة بالسكري و الاخري المصابة و التي معالجتها بالمستخلص المائي لكل من حبة البركة و الصبر طوال فترة التجربة . كما أظهرت تحسنا ملحوظا في باقي المجموعات عند مقارنتها بالمجموعة الصابة بالسكر و التي لم يتم معالجتها خلال فترتي المعاحة و الاستشفاء.
كما أظهرت النتائج زيادة احصائية في عدد كرات الدم البيضاء و نسبة كل من الخلايا الليمفاوية و المتعادلة في العدد النوعي لكرات الدم البيضاء في كل من المجموعة بالسكر و

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التي لم يتم معالجتها و الاخري المصابة والتي تم معالجتها بالمستخلص المائي لنبات الحلتيت .

و لم تظهر باقي المجموعات المعالجة اي تغيير ذو دلالة احصائية. كما اتضح أنه ليس هناك تغير تغيير ذو دلالة احصائية في نسبة كل من خلايا كرات الدم البيضاء الحامضية و الاحادية في كل من المجموعات المصابة بالسكر و التي لم يتم معالجتها و الاخري التي تم معالجتها بأي من هذه النباتات أو الخليط منهم.