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Effects of some medicinal plants on bone formation and bone resorption in normal and diabetic rats

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Abstract

This experiment was conducted to study the effects of some medicinal plants on bone formation and bone resorption in normal and diabetic rats. The studies included two experiments; the first was in summer while the second was in winter season. Each experiment included 120 adult male albino rats averaged 100±10 gm live body weight. Normal rats were distributed into 4 groups (15 rats each). Group1 fed control diet, Group2 fed garlic 5%, Group3 fed *Nigella sativa* 5%, Group4 fed garlic 2.5% + *Nigella sativa* 2.5 %, while four groups included diabetic rats, Group5 (Control), Group6 fed garlic 5 %, Group7 fed *Nigella sativa* 5% and Group8 fed garlic 2.5% + *Nigella sativa* 2.5%. Blood samples were collected after 4 and 8 weeks from the start of the experiment and centrifuged at 3000 rpm for 15 minutes to obtain serum. Results show that serum lactic dehydrogenase, alkaline phosphatases, glucose and parathyroid hormone were significantly increased in diabetic rats than normal rats while treatment of diabetic rats with medicinal plants efficiently modify this effect. Serum osteocalcin was significantly decreased in diabetic rats than normal rats. Conclusion, treatment with medicinal plant has a beneficial effect on regulating bone formation and resorption either in normal or diabetic rats.

Keywords: parathyroid hormone, lactate dehydrogenase, alkaline phosphatase, body weight, rectal temperature.

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1. Introduction

Plants consider the nature's green pharmacy, which provides drugs to maintain the good health and to restore failed health of humans. The medical arts had its origin when mankind early began to use remedial measures to get rid of pains, sufferings and other illnesses (Badr *et al.*, 2012). Al–Yahya *et al.* (1987) and Haney (1978) stated that many herbs and other plants were very early recognized by their medicinal properties. Drugs have been extensively developed from these herbs during the last two centuries. Diabetes Mellitus affects skeletal system and bone metabolism through multiple pathways and it recognized as a major risk factor for osteoporosis (Leidig-Bruckner and Zeigler, 2001). Process of bone formation (osteogenesis) involves three main steps: 1- production of the extracellular organic matrix (osteoid); 2- mineralization of the matrix to form bone; 3-bone remodeling by resorption and reformation (Khalil, 2012). The cellular activities of osteoblasts, osteocytes, and osteoclasts are essential for the process. Osteoblasts synthesize the collagenous precursors of bone matrix. Also, regulate its mineralization. As the process of bone formation progresses, the osteoblasts come to lie in tiny spaces (lacunae) within the surrounding mineralized matrix which later called osteocytes. The cell processing of osteocytes occupy minute canals (canaliculi) which permit the circulation of tissue fluids. To meet the requirements of skeletal growth and mechanical function, bone undergoes dynamic remodeling by a coupling process of bone resorption through osteoclasts and reformation by osteoblasts. Diabetes mellitus consider the commonest endocrine disease resulting from deficiency of the secretion or action of the pancreatic hormone, insulin, which in turn produce profound abnormalities of metabolism (Celik *et al*., 2002) and alteration of neuronal and/or vascular functions (Hilton *et al*., 1983; Shalaby *et al*., 1989 and Wick *et al*., 2005). The main objective of this work was to determine the effect of garlic or *Nigella Sativa* on bone formation and bone resorption in normal and diabetic rats during summer and winter seasons.

2. Materials and methods

This study was carried out in Animal House Laboratory, Department of Animal Production, Faculty of Agriculture, Al-Azhar University, Cairo, Egypt which provided the standard laboratory chemicals and equipment for this study. Glucose commercial determination kits glutamic oxaloacetic transaminase, alkaline phosphatase and lactic dehydrogenase, were performed using diamond-Egypt kits. Alloxan was obtained from B.D.H. Chemical LTD, England. Parathyroid and osteocalcin hormones were analysis at Faculty of Pharmacy, Al-Azhar University, Cairo, Egypt.

2.1 Experimental animals

The albino rats used in this study were originally bought from El Osman Farm, Cairo, Egypt. Animals were housed in cages under ambient temperature (ranged from 30 to 43 °C) in summer and between 6 and 18 °C in winter. Light-dark cycle was maintained at 12-hour. The laboratory standard for chow and tap

water were provided ad libitum. All animals were healthy and clinically free from diseases.

2.2 Induction of diabetes mellitus in rats

Diabetes mellitus was induced by interperitoneally injection of alloxan solution (0.1 ml/100 gm body weight). Alloxan solution consisted of 0.12 gm (120 mg/kg) alloxan hydrasin per 1 ml buffer solution. Alloxan buffer is prepared by the addition of 7.5 ml of 5.7% glacial acetic acid to 29.5 ml of 8.2% sodium acetate solution. Injected animals were fasted 18 and 2 hrours before and after injection, respectively according to Malaisse (1982) and Mohammed (2005). Ten days later, animals injected with alloxan considered diabetic where they showed some symptoms as increased urine volume and body weight loss. Some rats were resistant to alloxan so they were excluded. Blood sugar was measured after one week from injection and the blood sugar ranged.

2.3 Experimental outline

One experiment was carried out during summer (First of July to 30th of August, 2017). One cold stress experiment was carried out during winter (15th of December 2017 until 15th of February 2018). The experiments started after one week of alloxan injection in summer and winter. In all experiments, the micrometeorological data were recorded at intervals simultaneous with measurements of physiological parameters.

2.4 Summer and winter experiments

In summer and winter, total of 120 animals were assigned into eight groups each contain 15 rats for each season. G1: Normal rats fed control diet, G2: Normal rat fed 5% garlic, G3: Normal rat fed 5% *Nigella sativa*, G4: Normal rat fed 2.5% garlic + 2.5% *Nigella sativa*, G5: Diabetic rats fed control diet, G6: Diabetic rat fed 5% garlic, G7: Diabetic rat fed 5% *Nigella sativa* and G8: Diabetic rat fed 2.5% garlic + 2.5% *Nigella sativa*. In summer season, the mean ambient temperature (AT) and relative humidity (RH) for each group at ambient temperature were 30.20 ± 10 °C and 70.8 ± 10.22 %. In winter season the mean ambient temperature and relative humidity at ambient temperature were 12 ± 5.1 °C and 61.1 ± 8.75%.

2.5 Serum collection

Blood samples were obtained from rats by withdrawing blood from the orbital venous plexuses using a capillary tube. Samples were collected at weeks 4 and 8from the start of the experiment. Blood samples were collected and centrifuged at 3000 rpm for 20 min to obtain serum. Serum was transferred to Eppendorf tube and stored at –20^{o} C until subsequent analyses.

2.6 Serum parameters

Glucose assay: The glucose concentration determined by glucose oxidase method (Trinder, 1969) using commercial kit (bio-Meieux, Lyon, France). Alkaline phosphatase (ALP) measured by a

colorimetric method as described by Marsh *et al*. (1959) using diamond-Egypt kits. Lactic dehydrogenase (LDH) was measured by LDH stabino Kit. LDH specifically catalyzes the oxidation of lactate to pyruvate with the subsequent reduction of NAD to NADH2. The rate at which NADH forms is proportional to LDH activity. The method described determines the increase in NADH absorbance per minute (Henry *et al*., 1974). Osteocalcin hormone in serum was measured by radio-immunoassay (RIA) (Garnero, 1996). In all these methods the second antibody separation technique was used. PTH hormone in serum was measured by radio-immunoassay (Silverman and Yalow, 1973). In all these methods the second antibody separation technique was applied.

2.7 Statistical analyses

Data was subjected to analysis of variance using the General Linear Models procedure of SPSS software program package (SPSS, 2001, version 11.0). All percentages were first transformed to arcsine then analysed to approximate normal distribution before ANOVA. In addition, significant differences among means were determined by Duncan's multiple range test (Duncan, 1955) at 5% level of significance. Data were analysed by one-way analysis method.

3. Results and Discussion

3.1 Rectal temperature

Table (1) shows that during summer season after 1, 4 and 8 weeks from the start of the experiment all medicinal plant did not show any significant effect on rectal temperature (RT) on health and diabetic rats. The results also show that during summer diabetes did not show any significant changes on rectal temperature. Meanwhile, in winter (Table 1) after 4 weeks from the start of the experiment, the healthy rats fed medicinal plants did not show any significant effect on rectal temperature as compared with the control health rats. The results also show that after 4 weeks with treatment in winter, rectal temperature significantly decreased in diabetic rats compared with control group. Treatment of diabetic rats with medicinal plants for 4 weeks did not show any significant effect on rectal temperature as compared with control diabetic rat. Meanwhile at one- or 8-weeks during winter all medicinal plants did not show any significant effect on rectal temperature in both healthy and diabetic rats as compared with the control health group. The results also show that during winter, rectal temperature was decreased in diabetic rats (36.156 – 36.567 °C) than normal rats (37– 37.556 °C). During winter season's diabetic rats fail to maintain the balance between heat production and heat loss which may be due to that the heat production was decreases in diabetic rats than normal rats.

Table (1): Means of rectal temperature affected by medicinal plants in healthy and diabetic rats during summer and winter seasons.

Groups		Weeks one		4 weeks		8 weeks	
		Mean ± SE*	Dt**	Mean ± SE*	Dt**	Mean ± SE*	Dt**
		Summer					
G1	Normal rats fed control diet	37.745±.110	a	37.750±.6455	a	37.745±.06455	a
G2	Normal rat fed 5% garlic	37.832±.345	a	37.5360±345	a	37.865±.534	a
G3	Normal rat fed 5% *Nigella sativa*	37.645±.547	a	37.265±.543	a	37.345±.677	a
G4	Normal rat fed 2.5% garlic + 2.5%	37.454±.978	a	37.675±.643	a	37.757±.567	a
G5	Diabetic rats fed control diet	37.451±.225	a	37.346±.542	a	37.976±.776	a
G6	Diabetic rat fed 5% garlic	37.656±.193	a	37.265±.945	a	37.564±.466	a
G7	Diabetic rat fed 5% *Nigella sativa*	37.758±.243	a	37.456±.654	a	37.456±.876	a
G8	Diabetic rat fed 2.5% garlic + 2.5%	37.575±.154	a	37.978±.234	a	37.865±.936	a
		Winter					
G1	Normal rats fed control diet	37.122±.324	a	37.556±.325	a	37.0500±.274	a
G2	Normal rat fed 5% garlic	37.232±.235	a	37.434±.656	ab	37.455±.565	a
G3	Normal rat fed 5% *Nigella sativa*	37.123±.577	a	37.334±.534	ab	37.455±.566	a
G4	Normal rat fed 2.5% garlic + 2.5%	37.323±.436	a	37.322±.346	ab	37.234±.943	a
G5	Diabetic rats fed control diet	36.355±.346	a	36.156±.476	b	36.567±.564	a
G6	Diabetic rat fed 5% garlic	36.353±.345	a	36.166±.214	b	36.255±.833	a
G7	Diabetic rat fed 5% *Nigella sativa*	36.536±.446	a	36.245±.547	b	36.677±.542	a
G8	Diabetic rat fed 2.5% garlic + 2.5%	36.653±.467	a	36.135±.345	b	36.455±.234	a

*S.E = standard error, **Dt. = Duncan's Multiple Range test between group, Mean within each row with similar letters are not significant different at p ≥0.05.

3.2 Body weight

Table (2) shows that during summer or winter seasons after 4 or 8 weeks from the start of the experiment on healthy rats, all medicinal treatments did not show any significant effect on body weight (BW) as compared with the control group. The results also show that during summer or winter seasons body weight was significantly decreased in diabetic rats compared with their control group. Treatment of diabetic rats with medicinal plants did not show any significant effect on body weight as compared with control diabetic rats. These results agree with Johan *et al.* (1990) who observed that after 12 weeks of diabetes, the adult diabetic body weight of rats had lost considerable weight; their final weight was only 60% of that of control rats. Bernard *et al*. (1970) observed that animals respond to the severe alloxan-induced diabetes. Approximately half of all the examined animals either became "obese" or strikingly emaciated and moribund.

3.3 Serum parathyroid hormone concentration

Table (3) shows that during summer season, after 8 weeks from the start of the experiment, treatment of healthy rats with 5% garlic or 2.5% garlic +2.5% *Nigella sativa* significantly decreased serum parathyroid hormone (PTH) compared with the control of normal group. Meanwhile, treatment of healthy rats with 5% *Nigella sativa* for 8 weeks

during summer season showed increase in PTH but insignificant compared with the control group. After 8 weeks from the start of the experiment, during summer season, diabetes significantly increased serum PTH compared with the normal groups. Treatment of diabetic rats with 5% *Nigella sativa* did not show significant effect on serum PTH as compared with diabetic rats without medicinal plants. The results also show that diabetic rats treated during summer season with 5% garlic or 2.5% garlic +2.5% *Nigella sativa* significantly decreased serum PTH as compared with diabetic control rats. This effect was more pronounced after treatment with 2.5% garlic +2.5% *Nigella sativa*.

Table (2): Means of body weights (g) affected by medicinal plants in healthy and diabetic rats during summer and winter seasons.

Groups		4 weeks		8 weeks	
		Mean ± SE*	Dt**	Mean ± SE*	Dt**
Summer					
G1	Normal rats fed control diet	172.566±3.22749	a	249.435±1.145	a
G2	Normal rat fed 5% garlic	171.766±4.22749	a	241.775±5.100	a
G3	Normal rat fed 5% *Nigella sativa*	171.657±3.52749	a	240.234±6.987	a
G4	Normal rat fed 2.5% garlic + 2.5% *Nigella sativa*	170.788±4.60749	a	239.566±8.675	a
G5	Diabetic rats fed control diet	105.345±1.44338	b	121.234±5.0234	b
G6	Diabetic rat fed 5% garlic	109.897±2.40338	b	125.243±1.0224	b
G7	Diabetic rat fed 5% *Nigella sativa*	111.346±5.47338	b	125.223±1.567	b
G8	Diabetic rat fed 2.5% garlic + 2.5% *Nigella sativa*	115.768±8.42338	b	119.244±6.567	b
Winter					
G1	Normal rats fed control diet	160.404±7.256	a	230.740±9.393	a
G2	Normal rat fed 5% garlic	165.560±4.39	a	233.340±6.345	a
G3	Normal rat fed 5% *Nigella sativa*	170.780±1.244	a	240.560±2.213	a
G4	Normal rat fed 2.5% garlic + 2.5% *Nigella sativa*	169.290±9.948	a	238.890±2.343	a
G5	Diabetic rats fed control diet	110.500±7.333	b	115.000±6.8867	b
G6	Diabetic rat fed 5% garlic	112.450±5.987	b	120.000±1.456	b
G7	Diabetic rat fed 5% *Nigella sativa*	118.780±1.443	b	122.000±1.232	b
G8	Diabetic rat fed 2.5% garlic + 2.5% *Nigella sativa*	116.290±2.25	b	120.000±2.456	b

*S.E = standard error, **Dt. = Duncan's Multiple Range test between group, Mean within each row with similar letters are not significant different at $p \geq 0.05$.

Treatment of diabetic rats with 2.5% garlic + 2.5% *Nigella sativa* succeeded to decrease serum PTH in diabetic rats to reach the normal range of the control group. The results in Table (3) also did not show significant effect on serum PTH as compared with the control group except treatment with 2.5% garlic +2.5% *Nigella sativa* that decreased serum PTH compared with the control group. Diabetes significantly increased serum PTH as compared with the normal groups. Treatment of diabetic rats with 5% *Nigella sativa* did not show significant effect on serum PTH compared with diabetic rats without medicinal plants. While 5% garlic or 2.5% garlic + 2.5% *Nigella sativa* significantly decreased serum PTH compared with diabetic control rats. This

effect was more pronounced after treatment with 2.5% garlic + 2.5% *Nigella sativa*. Treatment of diabetic rats with 2.5% garlic + 2.5% *Nigella sativa* succeeded to decreased serum PTH in diabetic rats to reach to the range in normal control group. The significant increase in serum PTH in diabetic rats during summer may be due to the effect of diabetes on decreasing serum T3 and T4 or may be due to the effect of diabetes on calcium metabolism. Para thyroid hormone responds to the moment by moment fluctuation of Ca^{++} in the blood and other extracellular fluids at the following way; when concentration of Ca^{++} in the blood perfuse the parathyroid gland so that it drops below its set point (2.2 – 2.5 mmol/L) and the gland releases parathyroid hormone (PTH) into the circulation. PTH, in turn, affects the osteoclasts and the osteocytes by stimulating them to affect bone resorption, thus releasing Ca^{++} and phosphatase from the bone into the circulation (Khalil, 2013). During summer, significant decrease noticed on serum T3 and T4. Thyroid hormones play an important role on mineral metabolism (Allain, 1993; Mosekilde, 1990). Thus, the significant decrease in T3 andT4 affect mineral metabolism. Segal *et al*. (1989) showed that T3 produced a rapid increase in concentration of Ca^{++}-ATPase activity. Kumar and Prasad (2003) demonstrated that Ca^{++} uptake into intestinal Brush Border Membrane Vesicles (BBMV) increased in response to thyroid hormone and showed that hypothyroid rats showed a significant decrease in initial uptake of Ca^{++}. It could recognize that the decrease in T3 and T4 during summer will lead to a decrease in Ca^{++}-ATPase activity. This decrease in Ca^{++}-ATPase activity will lead to decrease the absorption of calcium from the intestine which leads to increase PTH secretion. During summer, in diabetic rats, we showed more increase in serum PTH than in normal rats. This result may refer to the effect of diabetes on calcium metabolism. Sultan Entessar *et al.* (2008) showed that serum calcium levels of diabetic group were significantly lower compared with the control group, (9.96±1.9 mg/dl, $p<0.05$). They also showed that diabetic group showed significant increase in levels of urinary calcium (270.66±41.7 and 300.56±55.67 mg) than the control group (244.23±51.5 mg), especially the uncontrolled one ($p<0.05$ and $p<0.001$) respectively. The significant increase in urinary calcium and significant decrease in serum calcium lead to significant increase in serum PTH. The results indicate that summer plus diabetes have a double effect on increasing bone resorption. The results indicate that during winter season diabetes significantly increased serum PTH which consequently increased bone resorption. These results agree with those found by Caroline *et al*. (2014) who observed increase of PTH levels with diabetes. Sultan Entessar *et al.* (2008) observed that PTH level was higher in diabetic group compared with control group

(overweight, due to type 2 diabetes). The results also indicated that medicinal plants succeeded to decrease serum PTH that increased after induction of diabetes. These results mean that medicinal plants can decrease the bone resorption which increased in diabetic rats. Treatment with mix of garlic and *Nigella sativa* had more effect on decreasing bone resorption than other treatments.

Table (3): Least square means and standard errors of serum parathyroid hormone levels (pg/ml) affected by medicinal plants for healthy and diabetic rats during summer and winter.

Groups		Summer 8 weeks		Winter 8 weeks	
		Mean ± SE*	Dt**	Mean ± SE*	Dt**
G1	Normal rats fed control diet	10.0375±0.78702	cd	10.5000±0.23805	cd
G2	Normal rat fed 5% garlic	9.1750±0.41508	e	10.1000±0.40825	cd
G3	Normal rat fed 5% *Nigella sativa*	11.3000±0.45092	c	11.8000±0.27386	c
G4	Normal rat fed 2.5% garlic + 2.5% *Nigella sativa*	6.0000±0.45644	f	9.1000±0.27988	e
G5	Diabetic rats fed control diet	21.1100±0.82731	a	20.8775±1.71780	a
G6	Diabetic rat fed 5% garlic	16.9500±0.73655	b	16.7000±0.89536	b
G7	Diabetic rat fed 5% *Nigella sativa*	21.5000±0.70828	a	21.0250±0.67992	a
G8	Diabetic rat fed 2.5% garlic + 2.5% *Nigella sativa*	9.9750±0.33510	cD	10.4500±0.53774	cd

*SE = standard error, **Dt = Duncan's Multiple Range test between group, Mean within each row with similar letters are not significant different at p ≥0.05.

3.4 Serum osteocalcin concentration

Table (4) show that during summer or winter season after 8 weeks from the start of the experiment treatment of healthy rats with 2.5% garlic + 2.5% *Nigella sativa* significantly increased serum osteocalcin, while treatment of healthy rats with 5% *Nigella sativa* significantly decreased serum osteocalcin as compared with the normal control group. Meanwhile, treatment of healthy rats with 5% garlic for 8 weeks did not show significant effect on serum osteocalcin as compared with its control group. The results also showed that diabetes significantly decreased serum osteocalcin level as compared with the comparable healthy rats. Treatment of diabetic rats with 5% garlic or 5% *N. sativa* during summer or winter season significantly increased serum osteocalcin as compared with control diabetic rats but did not reach to the normal range in the normal control group. Treatment of diabetic rats with 2.5% garlic + 2.5% *N. sativa* succeeded to increase serum osteocalcin as compared with the other groups of diabetic rats to reach the normal range of the healthy control group. The results indicated that diabetes significantly decreased serum osteocalcin. These results indicated that diabetes decreased bone formation. This result is in accordance with those obtained by Abd El-Ghfar (2016) and Hasani-Ranjbar *et al.* (2012) who observed lower level of osteocalcin at diabetic groups compared with control group. Sultan *et al.* (2008) observed lower level of osteocalcin in both diabetic groups (patients who were overweight, with type 2 diabetes) compared with control group. The results also indicated that medicinal plants succeeded to increase serum osteocalcin that will decrease after induction of

diabetes. These results mean that medicinal plants can increase the bone formation that decrease in diabetic rats. Treatment with mix of garlic and *Nigella sativa* had more effect on increasing bone formation than other treatments.

Table (4): Least square means and standard errors of serum osteocalcin levels (ng/ml) affected by medicinal plants for healthy and diabetic rats during summer and winter.

Groups		Summer 8 weeks		Winter 8 weeks	
		Mean ± SE*	Dt**	Mean ± SE*	Dt**
G1	Normal rats fed control diet	2.0000±.09129	b	1.6500±.018484	bc
G2	Normal rat fed 5% garlic	1.8650±.02533	b	1.8675±.03425	b
G3	Normal rat fed 5% *Nigella sativa*	1.3500±.10157	c	1.3725±.04715	c
G4	Normal rat fed 2.5% garlic + 2.5% *Nigella sativa*	2.3500±.18484	a	2.4500±0.15546	a
G5	Diabetic rats fed control diet	.5725٠±.08320	e	.5225±0.10957	e
G6	Diabetic rat fed 5% garlic	.9075±.01436	d	.7525±.010610	d
G7	Diabetic rat fed 5% *Nigella sativa*	.8875±.08440	d	.9200±0.21672	d
G8	Diabetic rat fed 2.5% garlic + 2.5% *Nigella sativa*	1.8500±.11902	b	1.9950±0.04113	b

*SE = standard error, **Dt = Duncan's Multiple Range test between group, Mean within each row with similar letters are not significant different at p ≥0.05.

3.5 Serum lactate dehydrogenase concentration

Table (5) shows that during summer season after 4 or 8 weeks from the start of the experiment treating healthy rats with 5 % *Nigella sativa* or 5 % garlic did not show any significant effect on serum lactate dehydrogenase (LDH) as compared with their control group, while treatment of healthy rats with 2.5% *Nigella sativa* + 2.5% garlic significantly decreased serum LDH as compared with the healthy control group. The results in Table (5) also show that diabetes significantly increased serum LDH level as compared with the normal control rats. Treatment of diabetic rats with different medicinal plants for 4 or 8 weeks during summer season significantly decreased serum LDH as compared with the diabetic control group but did not reach to the normal rang in the normal control group. The best treatment in decreasing serum LDH, that increase by diabetes, was the mix of 2.5% *Nigella sativa* + 2.5% garlic. Table (5) shows that during winter season after 4 weeks from the start of the experiment treating healthy rats with 5 % *Nigella sativa* or 5 % garlic did not show significant change on serum LDH compared with the normal control group, while treatment of healthy rats with 2.5% *Nigella sativa* + 2.5% garlic significantly decreased serum LDH compared with the control group. While after 8 weeks from the start of the experiment, during winter season, all medicinal plants significantly decreased serum LDH in healthy rats as compared with their control group. The results in Table (5) also show that diabetes significantly increased serum LDH level compared with the healthy control rats. Treatment of diabetic rats with medicinal plants for 4 or 8 weeks during winter season significantly decrease serum LDH compared with diabetic control rats but did not reach the rang of the normal control group. The best treatment in decreasing serum LDH

that increased by diabetes during winter season was the mix of 2.5% *Nigella sativa* + 2.5% garlic. Lactate dehydrogenase is an intracellular enzyme that widely distributed in the tissues of body, particularly the kidney, heart, skeletal muscle, brine, liver, and lungs. Increases in the reported value usually indicate cellular death and leakage of the enzyme of cell (Frances *et al*., 2003). The results indicated that diabetes significantly increased serum LDH, these results agree with Rajeswarareddy (2012) who observed that the activity of LDH was significantly ($p < 0.01$) increased in the kidney in diabetic rats. However, El-Demerdash *et al*. (2005) found in alloxan-diabetic rats that activities of plasma LDH, was significantly ($p < 0.05$) increased by 37%, relative to their normal levels. Bernard *et al.* (1970) showed that there was an abrupt and marked increase in LDH levels in the fourth day coincident with the hepatic lipidosis and the other stressful conditions accompanied the acute onset of severe diabetes.

Table (5): Least square means and standard errors of serum lactate dehydrogenase (u/l) affected by medicinal plants for healthy and diabetic rats during summer and winter seasons.

Groups		4 weeks		8 weeks	
		Mean ± SE*	Dt**	Mean ± SE*	Dt**
Summer					
G1	Normal rats fed control diet	106.2500±2.454	e	153.9500±2.92874	e
G2	Normal rat fed 5% garlic	102.1000±3.452	ef	150.7250±2.90556	e
G3	Normal rat fed 5% *Nigella sativa*	106.5750±2.98	e	147.3500±3.49869	e
G4	Normal rat fed 2.5% garlic + 2.5% *Nigella*	91.6250±1.432	f	135.7000±1.36443	f
G5	Diabetic rats fed control diet	398.0000±2.863	a	283.1500±3.74889	a
G6	Diabetic rat fed 5% garlic	221.7000±5.764	c	216.6000±2.47487	c
G7	Diabetic rat fed 5% *Nigella sativa*	307.7250±3.875	b	249.1750±6.45657	b
G8	Diabetic rat fed 2.5% garlic + 2.5% *Nigella*	152.8750±5.865	d	186.3900±5.54486	d
Winter					
G1	Normal rats fed control diet	151.1000±3.70473	e	149.1750±1.31236	e
G2	Normal rat fed 5% garlic	144.9750±2.79713	e	133.7250±2.00265	f
G3	Normal rat fed 5% *Nigella sativa*	140.8250±3.80950	ef	136.1000±1.85068	f
G4	Normal rat fed 2.5% garlic + 2.5% *Nigella*	131.2750±0.43851	f	126.5250±1.32059	g
G5	Diabetic rats fed control diet	291.5250±4.11225	a	289.2250±3.00122	a
G6	Diabetic rat fed 5% garlic	212.8750±3.10520	c	216.4750±2.28851	c
G7	Diabetic rat fed 5% *Nigella sativa*	248.4000±8.32046	b	243.2750±2.94742	b
G8	Diabetic rat fed 2.5% garlic + 2.5% *Nigella*	171.9000±3.00333	d	178.2000±3.03507	d

*SE = standard error, **Dt = Duncan's Multiple Range test between group, Mean within each row with similar letters are not significant different at $p \geq 0.05$.

Again, the arteriosclerotic female breeders displayed the greatest increase in serum LDH levels, *i.e.*, 204 % increase, in the fourth day following the injection of alloxan. The results also indicated that treatment of diabetic rats succeeded to decrease serum LDH that increased by diabetes. Medicinal plant prevents the lessons effect of diabetes on most cells.

3.6 Serum alkaline phosphatase activities

Table (6) shows that during summer season after 4 or 8 weeks from the start of the experiment treatment of healthy rats with medicinal plants significantly

decreased serum alkaline phosphatase (ALP) activity as compared with their control group. The results in Table (6) also show that diabetes significantly increase serum ALP level as compared with the normal control rats. Treatment of diabetic rats with mixed medicinal plants for 4 or 8 weeks during summer season significantly decreased serum ALP activities compared with control diabetic rats and to a level near the range of normal control group.

Table (6): Least square means and standard errors of serum alkaline phosphatase (u/l) affected by medicinal plants for healthy and diabetic rats during summer and winter seasons.

Groups		4 weeks		8 weeks	
		Mean ± SE*	Dt**	Mean ± SE*	Dt**
	Summer				
G1	Normal rats fed control diet	198.4550±7.17799	d	214.7950±6.74880	d
G2	Normal rat fed 5% garlic	176.9850±4.21924	ef	171.7700±6.48269	ef
G3	Normal rat fed 5% *Nigella sativa*	178.5800±3.01434	ef	183.0400±2.85476	e
G4	Normal rat fed 2.5% garlic + 2.5% *Nigella*	169.4950±4.15108	f	160.8375±3.29719	f
G5	Diabetic rats fed control diet	304.5550±4.30906	a	321.0250±6.45476	a
G6	Diabetic rat fed 5% garlic	270.3000±3.30883	c	242.8000±6.85796	c
G7	Diabetic rat fed 5% *Nigella sativa*	291.8625±2.29066	b	281.9225±2.36831	b
G8	Diabetic rat fed 2.5% garlic + 2.5% *Nigella*	189.2150±4.19040	de	203.9175±3.70547	d
	Winter				
G1	Normal rats fed control diet	138.8375±3.93845	c	115.1650±3.56257	e
G2	Normal rat fed 5% garlic	122.6925±1.78493	cd	104.7875±1.46918	e
G3	Normal rat fed 5% *Nigella sativa*	131.2375±1.53449	c	104.3375±1.36186	e
G4	Normal rat fed 2.5% garlic + 2.5% *Nigella*	117.4950±3.20075	e	91.7825±.63925	f
G5	Diabetic rats fed control diet	275.6450±5.42851	a	257.6500±3.21494	a
G6	Diabetic rat fed 5% garlic	242.1750±3.56333	b	226.5750±3.00953	c
G7	Diabetic rat fed 5% *Nigella sativa*	267.5750±8.31760	a	237.4900±2.14416	b
G8	Diabetic rat fed 2.5% garlic + 2.5% *Nigella*	142.4875±3.11596	c	141.3700±7.85170	d

*SE = standard error, **Dt = Duncan's Multiple Range test between group, Mean within each row with similar letters are not significant different at $p \geq 0.05$.

Table (6) shows that during winter season after 4 or 8 weeks from the start of the experiment treating healthy rats with 5 % *Nigella sativa* or 5 % garlic did not show significant effect on serum ALP activities compared with their control group. While treatment of healthy rats with 2.5% *Nigella sativa* + 2.5% garlic significantly decreased serum ALP activities than control group. The results in Table (6) also show that diabetes significantly increased serum ALP activities compared with the normal control group. Treatment of diabetic rats with mixed medicinal plants for 4 weeks during winter season significantly decreased serum ALP activity to be near the range in the normal control group. While treating for 8 weeks was able to decrease ALP level, but still significantly higher than normal control group. The results indicated that diabetes significantly increased serum ALP activity. These results agree with Hasani-Ranjbar *et al*. (2012) who observed that bone serum alkaline phosphatase activities significantly higher with diabetic groups (diabetic patients with chronic disease such as chronic renal failure and diabetic patients) compared with control group. Sultan Entessar *et al.*

(2008) showed that PTH level in all diabetic patients was correlated positively with ALP level (r= 0.54, P<0.01) and negatively with serum calcium (r =-0.65 and P<0.01). Radhia *et al*. (2012) observed a significant increase in the level of ALP with diabetic groups compared with control group. Sultan Entessar *et al*. (2008) reported that the detected elevation in ALP level could be explained by the prolonged exposure to PTH which eventually increases osteoblastic activity. The results also indicated that medicinal plants significantly decreased serum ALP activity and this effect was more pronounced after treatment with mix of 2.5% *Nigella sativa* + 2.5% garlic. These results agree with those found by Mahmoud *et al*. (2002) because of using *Nigella sativa* oil (2.5 ml/kg), where ALP decreased in liver compeer with the control, El-Shenawy Siham and Hassan Nabila (2008) showed that rats given garlic alone showed a significant decrease in ALP (–7.16%) compared to the control group. Faoziyat *et al.* (2014) showed that Serum ALP was significantly reduced with *Allium sativum*. However, El-Demerdash *et al*. (2005) showed increase in the activities of plasma alkaline phosphatase, in the diabetic rats but using the garlic extracts decreased the level of ALP.

4. Conclusions

It's concluded that the use of medicinal plants especially the mixture between garlic and *Nigella sativa* could improve the condition of bones either in normal or diabetic animals.

References

Abd El-Ghfar, S. S. (2016), *Effects of diabetes on bone formation and bone resorption*, M.Sc. Thesis, Faculty of Agriculture, Al-Azhar University, Cairo, Egypt.

Allain, T. J., Chambers, T. J., Flanagan, A. M. and McGregor, A. M. (1993), "Triiodothyronine stimulates rat osteoclastic bone resorption by an indirect effect", *Journal of Endocrinology*, Vol. 133, pp. 327–331.

Al-Yahya, M. A., Mossa, J. S. and Al-Meshal, I. A. (1987), *Medicinal Plants of Saudi Arabia*, King Saud University libraries, Saudi Arabia.

Badr, M. I., Khalifa, H. H., Abd El-Hamid, A. A., Khalil, M. H. and Kotb, E. (2012), "Protective effect of some medicinal plants on blood parameters kidney and liver function and histopathological features of kidney in rats", *Egyptian Journal of Animal Production*, Vol. 49 No. 2, pp. 233–240.

Bernard, C. W., Jack, S. and Joseph, J. (1970), "Variations in response to severe alloxan diabetes in arteriosclerotic and non-arteriosclerotic rats", *Journal of Diabetes Research*, Vol. 19 No. 5, pp. 311–323.

Caroline, K. K., Balakumar, S., Anthony, J., Hanley, P. W., Connelly, M. S., Bernard, Z. and Ravi, R. (2014),

"Vitamina D and parathyroid hormone status in pregnancy: Effection insulin sensitivity, β-cell function, and gestational dDiabetes mellitus", *The Journal of Clinical Endocrinology and Metabolism*, Vol. 99 No. 12, pp. 4506–4513.

Celik, I., Yegin, E. and Odabasoglu, F. (2002), "Effects of experimental diabetes mellitus on plasma lactae dehydrogenase and glutamic oxaloacetic transaminase levels in rabbits", *Turkish Journal of Biology*, Vol. 26, pp. 151–154.

Duncan, D. B. (1955), "Multiple range and multiple F test", *Biometrics*, Vol. 11, pp.1–42.

El-Demerdash, F. M., Yousef, M. I. and Abou El-Naga, N. I. (2005), "Biochemical study on the hypoglycemic effects of onion and garlic in alloxan-induced diabetic rats", *Food and Chemical Toxicology*" Vol. 43, pp. 57–63.

El-Shenawy Siham M. and Hassan Nabila S. (2008), "Comparative evaluation of the protective effect of selenium and garlic against liver and kidney damage induced by mercury chloride in the rats", *Pharmacological Reports*, Vol. 60, pp. 199–208.

Faoziyat, A. S., Muinat, O. K., Ahmed, M. W. O., Temowoa, I. O. A., Faridat I. Z., Temitope, A. A., Azido, N. and Oluyomi, S. A. (2014), "Antimicrobial and toxic potential of aqueous extracts of *Allium sativum, Hibiscus sabdariffa* and *Zingiber officinale* in Wistar rats", *Journal of Taibah University for Science*, Vol. 8 No. 4, pp. 315–322.

Frances, T., Lippincott, W. and Wilkins, P. (2003), A manual of Laboratory and diagnostic tests, 7th edition, Lippincott Williams & Wilkins, Philadelphia, Pennsylvania, USA.

Garnero, P. and Delmas, P. D. (1996), "New Developments in Biological Markers for Osteoporosis", *Calcif Tissue International*, Vol. 59, No, 1, pp. 2–9.

Haney, A. (1978), *Plant life*, Macmillan publishing Co., London, United Kingdom.

Hasani-Ranjbar, S., Jouyandeh, Z., Qorbani, M., Hemmatabadi, M. and Larijani, B. (2012), "The effect of semelil (angipars®) on bone resorption and bone formation markers in type 2 diabetic patients", *DARU Journal of Pharmaceutical Sciences*, Vol. 20, 84.

Henry, R. J., Cannon, D. C. and Winkelman, J. W. (1974), *Clinical Chemistry: Principles and Techniques*, Harper and Row, New York, USA, pp. 407–421.

Hilton, J. W., Hodson, P. V. (1983), "Effect of increased dietary carbohydrate on selenium metabolism and toxicity in rainbow

trout (*Salmo gairdneri*) ", *The Journal of Nutrition*, Vol. 113, pp. 1241–1248**.**

Johan, V., Erik, V. H., Walter, J. V., Aastrid, M. H., Suiker, M., Thomasset, T. A., Einhorn, E. F. and Roger, B. (1990), "Bone and mineral metabolism in BB rats with long-term diabetes", *Diabetes*, Vol. 39, pp. 477–482.

Khalil, M. H. (2012), *Human Physiology Man*, 3rd edition, University Book House, Al-Ain, United Arab Emirates.

Khalil, M. H. (2013), *Endocrinology*, 3rd edition, Al-Ain Book Shop, United Arab Emirates, pp. 152.

Kumar, V. and Prasad, R. (2003), "Thyroid hormone stimulate calcium transport systems in rat intestine", *Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease*, Vol. 1639, pp. 185–194.

Leidig-Bruckner, G. and Zeigler, R. (2001), "Diabetes mellitus and risk factor for osteoporosis?", *Experimental and Clinical Endocrinology & Diabetes*, Vol. 109, pp. 493–514.

Malaisse, W. J. (1982), "Alloxan toxicity to the pancreatic B-cell. A new hypothesis", *Biochemical Pharmacology*, Vol. 31, pp. 3527.

Marsh, W. H., Finger hut B. and Kirsch, E. (1959), "Adaptation of an alkaline phosphatase method for automatic colorimetric analysis", *Advances in Clinical Chemistry*, Vol. 5, pp. 119–125.

Mosekilde, L., Eriksen, E. F. and Charles, P. (1990), "Effect of thyroid hormones on bone and mineral metabolism", *Endocrinology & Metabolism Clinics of North America*, Vol. 19, pp. 35–64.

Rajeswarareddy, S., Lavany, T., Narasimhulu, G. and Sathyavelureddy, K. (2012), "Effect of pimpinellatirupatiensison oxidative enzymes in STZ-induced diabetic rat kidney", *Iranian Journal of Pharmaceutical Research*, Vol. 11 No. 1, pp. 277–286.

Segal, J., Johnm, H. and Sidney, I. H. (1989), " Stimulation of calcium-ATPase activity by 3,5,3'-tri-iodothyronine in rat thymocyte plasma membranes. A possible role in the modulation of cellular calcium concentration", *Biochemical Journal*, Vol. 261, pp. 749–754.

Shalaby, T. H., Yousef, M. K. and Dupre, R. K. (1989), "Thermoregulatory responses of diabetic rats", *Comparative Biochemistry and Physiology Part A Comparative Physiology*, Vol. 94 No. 1, pp. 153–157.

Silverman, R. and Yalow, R. S. (1973), "Heterogeneity of parathyroid hormone. Clinic and physiologic implications", *Journal of Clinical*

Investigation, Vol. 52, pp. 1958–1971.

Sultan Entessar, M. D., Inas Taha S. B. and Saber Lobna M. (2008), "Altered bone metabolic markers in type 2 diabetes mellitus: Impact of glycemic control", *Journal of Taibah University Medical Sciences*, Vol. 3 No. 2, pp. 104–116.

Trinder, P. (1969), "Determination of glucose in blood using glucose oxidase with an alternative oxygen acceptor", *Annual Clinical Biochemistry*, Vol. 6 No. 1, pp. 24–27.

Wick, D. E., Robrts, S. K., Basu, A. P. Sandroni, Fealey,R. D., Slatten, D. and Charkoudian, N. (2005), "Delayed threshold for active cutaneous vasodilation in patients with type 2 diabetes mellitus", *Journal Applied physiology*, Vol. 100 No. 2, pp. 637–664.