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"Antidiabetic Effects Of Magnetized Water in normal and Alloxan-Diabetic Rats"

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Abstract : Diabetes is one of the most prevalent abnormalities in all over the world. Nowadays, there are lots of attempts to reduce its disturbances and defects by changing life style to access some moderate effect with minimum side effects. The aim of this paper ,were to evaluate the hypoglycemic , hypolipidemic and improvement the antioxidant status of Magnetized Water (2mT) on normal and diabetic rats. Twenty four albino rats were divided into two main groups, the first is normal group (n=12), which were divided into two subgroups (6 rats each) as follow (subgroup 1 served as negative control group which drank tab water; subgroup2 normal rats drank magnetized water, The second is the diabetic rats (n=12) which were divided into two subgroups (6 rats each) as follow: subgroup 3: served as positive control group, subgroup 4: drank magnetized water. After completing the treatment period (six weeks), blood samples were collected to used for the biochemical analysis as blood glucose, insulin, Hemoglobin A1c (HbA1c), Insulin sensitivity index (HOMA-IR), lipid profile and antioxidant status . The results indicated that drinking diabetic rats to magnetized water resulted in a significant decrease in the elevated level of glucose , Insulin, HbA1c, antioxidant status and lipid profiles . In conclusion, magnetizing water with 20 Gauss improve blood glucose level, Insulin, HbA1c, HOMA, lipid profile and antioxidant status of normal and diabetic rats.

Key Words: Magnetized water, diabetic rats, blood parameters, serum parameter

1. introduction: Diabetes mellitus (DM) is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction, and failure of different organs, especially the eyes, kidneys, nerves, heart, and blood vessels (**Gaba *et al.*, (2015) and A.D.A, (2014)**). Although the leading mechanism of diabetic complications remains unclear, much attention has been paid to the role of oxidative stress. It has been suggested that oxidative stress may contribute to the pathogenesis of different diabetic complications (**Ceriello, 2000**). Furthermore, with diabetes, several features appear including an increase in lipid peroxidation, alteration of the glutathione redox state, a decrease in the content of individual natural antioxidants, and finally a reduction in the antioxidant enzyme activities (**Gumieniczek, 2005**). Diabetes prevention and treatment are high priorities in medical research. It has been shown recently that magnetized water has an efficient antioxidant effect and has a high ability to diffuse rapidly into tissues (**Lee and Kang,(2013); Nakashima-Kamimura *et al.*,(2009); Ohsawa *et al.*,(2007); Ohno *et al.*,(2005)**). Water molecule consists of hydrogen and oxygen atoms are partly positive and partly negative that forming by weak attraction allowing to the formation of hydrogen bond. The magnetic and electric fields extremely affected in liquid water through hydrogen bond that changes some physical and chemical properties of magnetized water (**Samir, 2008**). Magnetized water means passing water through magnetic tubes, or by putting a magnet in water so properties of water turned into very fertile and active causing high oxygen ratio (**Batmanghelidj, 2005**). It has also been shown that magnetized water administration can reduce blood glucose level and improve the antioxidant status and lipid profiles in the heart, spleen, and lung of diabetic rats (**Lee and Kang, 2013**). Even more, drinking magnetic water may have a useful role in inhibiting blood parameter disorders of type 2 diabetes mellitus (**Kajiyama *et al.*,2008**). Moreover, magnetized water is believed to have an antioxidant effect which could be a result of the increase in glutathione peroxidase concentration in serum (**Ali, 2012**).

Lack of information about disorders accompanying induced type 2 diabetes and how to rescue these disorders prompted us to use a diabetic rat model. Therefore, the objective of the current study was to investigate the potential effects of magnetized water to improve blood glucose level, Insulin, HbA1c, HOMA, lipid profile and antioxidant status of normal and diabetic rats.

2. Materials And Methods

Materials:

Static Magnetic Field Device :

Water magnetization performed by putting tap water in contact with static magnetic field device 2mT (UO 50 mg. OS inch, output 4- 6 m2/hr productions by magnetic technologies L.C obtained from Faculty of Science, Zagazig University, Egypt , according to **Orlando *et al.*,(2016)**



Fig.(1): Static magnetic field device

The magnetic field intensity measured to be 20 G using SE- 8606 a digital Gauss- Tesla meter. Glass flask had been washed carefully with distilled water several times to remove any impurities then it had been filled with a water sample and the magnetized device put in the water flask at room temperature for one hour, while The magnetized water was changed every day, as the shelf life of the magnetized water was 1 day.

Chemicals :Casein, cellulose, choline chloride, di-methionine, vitamins mixture minerals mixture were obtained from El-Gomhoria pharmaceutical Company , Cairo , Egypt. Alloxan obtained from Sigma , USA and used for inducing hyperglycemic in rats .Kits for estimating biochemical analysis were purchased from Alkan Medical Company, El-Doky, Giza, Egypt. Malondialdehyde (MDA), of glutathione-S-

transferase (GST) and catalase activity (CAT) kits were obtained from Biodiagnostic Co. Dokki, Giza, Egypt .

Rats: Twenty – four adult male albino rats, Sprague Drawley stain, weighing 200 ± 5 g were purchased from Medical Insects Research Institute, Doki, Cairo, Egypt .

Methods :

Inducation of experimental diabetes mellitus : Diabetes was induced in normal healthy male albino rats by intra – peritoneal injection of alloxan (150 mg/kg body weight) , according to the method described by (**Desai and Bhide , 1985**).

Biological investigation :The experimental study was done in the Faculty of Home Economics, Minufia University, Shebin El-kom. The rats were housed in Plastic cage under controlled condition. The diet was introduced to rats in special food container to a void scattering of food and contamination. Tap water and magnetized water were provided to rats by mean of glass tubes projecting through Plastic cages from inverted bottles supported to one side of the cage rats. Rats were fed standard diet for 7 days for adaptation according to AIN - 93 guidelines (**Reeves *et al.*, 1993**). The rats were randomly divided into two main groups (normal group and diabetic group), the first is normal group (n=12), which were divided into two subgroups (6 rats each) as follow :subgroup 1 served as negative control group which drank tap water, subgroup 2 normal rats drank magnetized water. The second is the diabetic rats (n=12) which were fasted overnight prior to injection of alloxan dissolved in normal saline at a dose of 150 mg/kg body weight given intraperitoneally (**Aruna *et al.*,1999**). Diabetes was identified by visual observations and measuring blood glucose concentration 72h after injection of alloxan (**N.D.D.G,1994**). Rats with a fasting blood glucose level above 200 mg/dl were considered diabetic. After divided into two subgroups (6 rats each) as follow: subgroup 3: served as positive control group, subgroup 4: drank magnetized water. After completing the treatment period (6 weeks) , animals were sacrificed under diethyl ether anesthesia. Blood samples were collected from the hepatic portal vein, for used to the biochemical assays .

Biochemical assays: Glucose was measured in blood according to **Hugget and Nixon (1957)**. The concentration of serum insulin was determined with a rat insulin ELISA kit Insulin sensitivity from the final fasting insulin and glucose values was estimated by the Homeostasis model assessment of insulin resistance (HOMA-IR) according to the

following formula: [fasting glucose (mM)× fasting insulin (mUI/L)] / 22.5 (Cordero-Herrera *et al.*, 2015). Triglycerides (TG) ,Total cholesterol and high density lipoprotein (HDL) were determined according to Fassati and Precipel (1982), Allain (1974) and Fnedewaid (1972) respectively. Very low density lipoproteins (VLDL-c) and low density lipoproteins (LDL-c) were calculated to Lee and Nieman (1996) as the following equations :VLDL-c (mg / dl) = triglycerdes / 5, LDL-c (mg / dl) = total cholesterol – (HDL-c + VLDL-c). Malonaldehyde (MDA), glutathione reduced (GSH.Rd) and catalase (CAT) were assayed according to the methods described by Ohkawa *et al.*, (1979).

Statistical analysis : Results were expressed as the mean ± SD. Data for multiple variable comparisons were analyzed by one-way analysis of variance (ANOVA). For the comparison of significance between groups, Duncan's test was used as a post hoc test according to the statistical package program (Artimage and Berry, 1987).

3.Results and discussion:

Data presents in Table (1) revealed the effect of drinking magnetized water on glucose in blood in normal and diabetic rats . The results indicated that the level of blood glucose was significantly ($p \leq 0.05$) increased in diabetic rats compared to negative control group. Our results corroborate the findings of Vinuthan *et al.*, (2004) observed that alloxan has been shown to destruct beta cells of pancreas producing hyperglycemia, the diabetes was characterized by hyperglycemia. Also Bouwens, (2004) showed that the alloxan dose used induced an irreversible hyperglycaemic state in all mice.

The data indicated that diabetic rats had significantly ($p \leq 0.05$) high blood glucose level as compared with normal rats. After 21th days of experimental period, level still high in the untreated diabetic rats (positive control groupe) which was (583.83 mg/dl) but in diabetic rats treated with magnetized water blood glucose level were decrease which were (191.33 mg/dl) , compared to the initial day. At the 33th day of the experimental period, blood glucose level still high in the untreated diabetic rats which was (584.67 mg/dl) but in diabetic rats treated with magnetized water blood glucose level were decrease which were (106.83 mg/dl) , compared to the initial day and After two weeks (11th days) . The highest reduction in blood glucose level was noted in diabetic rats treated with magnetized water in (33th day) groups .

The obtained results are in accordance with data of **Alhammer *et al.*, (2013)** who indicated that magnetically treated water significantly decreased the glucose level. This may be attributed to magnetically treated water has increased the water conductivity, this may increase the blood circulation and by which increases the glucose uptake by the cells **Alhammer *et al.*, (2013); High *et al.*,(2000) and Bonhomme-Faive *et al.*,(1998)** . **On the other hand, the study of Yacout *et al.*, (2015)** reported that groups drank magnetic water showed a significant increase in glucose level compared with drank unmagnetic water.

The data indicated that Table (2) revealed the effect of drinking magnetized water on insulin, HbA1c and (HOMA-IR) in normal and diabetic rats. Untreated diabetic rats had significantly ($P \leq 0.05$) low insulin ,HbA1c and HOMA-IR level as compared with normal rats and the other diabetes groupe .Our results corroborate the findings of **Morgan and Lazarow (1965)** who suggested that alloxan damaged beta cells did not release their insulin in response to hyperglycemia but that the insulin was destroyed within the beta cell. Also **Vinuthan *et al.*,(2004)** observed that alloxan has been shown to destruct beta cells of pancreas producing hyperglycemia, the diabetes was characterized by hyperglycemia.

There are significant ($P \leq 0.05$) differences between subgroup 1 and subgroup 2 in Insulin and HbA1c but no significant ($P > 0.05$) difference in HOMA-IR between them . The results revealed that diabetic rats which drank tap water (subgroup 3) had significantly higher ($P \leq 0.05$) in Insulin, HOMA-IR and HbA1c levels compared with normal groups and treated groups. Diabetic rats which drank magnetized water has recorded more effective to improvement Insulin, HOMA-IR and HbA1c compared with other groups.

Data in Table (3) showed the effect of drinking magnetized water on serum lipid profile in normal and diabetic rats . Cholesterol is a vital lipid component important for the proper body function and normal cellular metabolism. However, high blood fatty contents (cholesterol and triglyceride) are a risk factor that increases the chance of getting diseases like diabetes, heart disease, and atherosclerosis. To investigate whether induced type 2 diabetes affects the level of blood fatty profiles, total blood cholesterol and triglyceride levels were measured . We also observed high fatty substances (hypercholesterolemia and high triglyceride) in diabetic rats compared with other groups . Remarkably, in subgroup 4 has recorded more effective to increase HDL.c and decrease T.C, T.G, LDL.c and VLDL.c level compared with other groups . Our results corroborate the finding of **Yadav *et al.*, (2004)** who

observed that alloxan diabetic rats showed the level of triglycerides and total cholesterol in blood serum increased significantly during diabetes. Similar results were obtained by **Yadav et al., (2005)** who reported that the results show that there was a significant ($p < 0.01$) increase in serum triglycerides and total cholesterol levels after 21 days of alloxan diabetes.

We found that HDL was decreased in subgroup 3 compared with other groups . To investigate the rescue ability of MW on the high blood fatty contents in type 2 diabetic rats, we treated these diabetic rats with MW, then we measured both cholesterol forms (T.G , T.C, HDL.c ,LDL.c and VLDL.c) .We noticed an HDL increase and decrease T.C, T.G,LDL.c and VLDL.c after MW treatment compared to non treated diabetic rats. These findings are supported by **Khudiar and Ali , (2012)**. which they found that groups treated daily for 60 days with magnetic water showed a significant increase in serum concentration of high density lipoprotein-cholesterol, and a significant decrease in serums total cholesterol, triacylglycerol, low density lipoprotein - cholesterol, and very low density lipoprotein cholesterol concentrations .

Data presented in Table (4) show the effect of drinking magnetized water on enzymatic antioxidants in serum in normal and diabetic rats . There are significant ($P \leq 0.05$) differences between subgroup 1 and subgroup 2 in SOD and GSH. Furthermore diabetic rats which drank tap water (subgroup 3) had significantly lower ($P \leq 0.05$) in SOD and GSH levels in liver tissues compared with normal groups and treated groups . These results of this study came in accordance with that reported by **Jemai et al., (2009)** who reported that alloxan - diabetic rats showed depletion in the antioxidant enzymes activities. **Ravikumar and Anuradha , (1999)** observed that Increased in circulating antioxidants in alloxan - diabetic rats .

Diabetic rats which drank magnetized water has recorded more effective to increase SOD and GSH levels compared with other groups .These findings are supported by **Hafizi et al., (2014); Shah and Nagarajan, (2013) ; Wagh and Lippes, (1993); Wang et al.,(2002) and Raymond-Whish et al.,(2007)** which they found that magnetized water could influence effectively on the oxidant-antioxidant balance, for instance, it could decrease the amounts of malondialdehyde (MDA), increase the superoxide dismutase (SOD) activity in the heart, kidney and liver and also decrease the amounts of nitric oxide which all result in decreasing oxidative stress .

Data in Table (5) showed the effect of drinking magnetized water on non enzymatic antioxidants in serum in normal and diabetic rats. The results indicated that the activity of CAT in diabetic rats was significantly lower ($p \leq 0.05$) compared with negative control group while MDA had an opposite trend. These results of this study came in accordance with that reported by **Punitha et al., (2006)** who confirmed that several studies have shown a decrease in nonenzymatic antioxidants in the plasma of alloxan-induced diabetic rats. There are significant ($P \leq 0.05$) differences between subgroup 1 and subgroup 2 in MDA but no significant ($P > 0.05$) difference in CAT between them. Furthermore diabetic rats which drank tap water (subgroup 3) had significantly lower ($P \leq 0.05$) in CAT levels in liver tissues compared with normal groups and treated groups, while MDA had opposite trend. Diabetic rats which drank magnetized water has recorded more effective to increase CAT levels and decrease MDA level compared with other groups. **Yacout et al.,(2015)** indicated that Magnetic water showed a higher glutathione peroxidase (GSH-Px), catalase (CAT) and superoxide dismutase (SOD) compared to groups that drank unmagnetized water. **Khudiar and Ali, (2012)** found that magnetic water showed a significant increase in serum glutathione concentration.

Table (1): Effect of drinking magnetized water on glucose in blood in normal and diabetic rats

Parameters		Normal Group		Diabetic Group	
		subgroup 1	subgroup 2	subgroup 3	subgroup 4
Glucose in blood (mg/dl)	1 st day	132.00 ^{Ab} ± 4.05	130.50 ^{Ab} ± 2.88	583.33 ^{Aa} ± 3.27	583.17 ^{Aa} ± 2.14
	11 th days	132.00 ^{Ac} ± 4.05	125.83 ^{Bd} ± 3.19	584.17 ^{Aa} ± 2.86	221.33 ^{Bb} ± 2.94
	21 th days	127.67 ^{Ac} ± 1.86	120.83 ^{Cd} ± 2.32	583.83 ^{Aa} ± 2.86	191.33 ^{Cb} ± 2.73
	33 th days	132.00 ^{Ab} ± 4.86	106.83 ^{Dc} ± 3.66	584.67 ^{Aa} ± 2.16	106.83 ^{Dc} ± 3.66

Each value in the table is the means ± SD. Small letters (a,b,c,d,.....) in the same row significantly different ($p \leq 0.05$) among experimental periods. Capital letter (A,B,C,D,.....) in the same column significantly different ($p \leq 0.05$) among groups.

Table (2) : Effect of drinking magnetized water on insulin, HbA1c and (HOMA-IR) in normal and diabetic rats

Parameters	Normal Group		Diabetic Group	
	subgroup 1	subgroup 2	subgroup 3	subgroup 4
Insulin (mIu/ml)	0.20 ^c ± 0.00	0.17 ^d ± 0.01	0.50 ^a ± 0.03	0.36 ^b ± 0.02
HbA1c (ng/ml)	0.93 ^b ± 0.09	0.53 ^c ± 0.03	2.45 ^a ± 0.46	0.59 ^c ± 0.10
Insulin sensitivity index (HOMA-IR)	2.73 ^c ± 0.10	2.21 ^c ± 0.08	20.07 ^a ± 1.23	3.94 ^b ± 0.22

Data are expressed as mean \pm SD. Values within a row having different superscripts are significantly different ($p \leq 0.05$); where the small letters indicate significant among dietary treatment groups as indicated by one-way ANOVA followed by Duncan's multiple range test ($a > b > c > d > e$). (HOMA-IR) : [fasting glucose (mM) \times fasting insulin (mUI/L)] / 22.5 .

Table (3) : Effect of drinking magnetized water on serum lipid profile in normal and diabetic rats

Parameters	Normal Group		Diabetic Group	
	subgroup 1	subgroup 2	subgroup 3	subgroup 4
T.C (mg/dl)	96.71 ^c \pm 3.16	98.12 ^c \pm 1.88	175.04 ^a \pm 2.25	102.89 ^b \pm 1.50
T.G (mg/dl)	204.91 ^b \pm 1.50	200.51 ^c \pm 3.62	423.89 ^a \pm 1.88	155.13 ^d \pm 3.65
HDL.c (mg/dl)	32.43 ^b \pm 2.07	42.58 ^a \pm 1.07	27.60 ^c \pm 1.13	41.66 ^a \pm 2.14
LDL.c (mg/dl)	23.30 ^c \pm 1.87	15.43 ^d \pm 2.53	62.66 ^a \pm 3.76	30.21 ^b \pm 4.00
VLDL.c (mg/dl)	40.98 ^b \pm 0.30	40.10 ^c \pm 0.72	84.78 ^a \pm 0.38	31.03 ^d \pm 0.73

Data are expressed as mean \pm SD. Values within a row having different superscripts are significantly different ($p \leq 0.05$); where the small letters indicate significant among dietary .

Table (4) : Effect of drinking magnetized water on enzymatic antioxidants in serum in normal and diabetic rats

Parameters	Normal Group		Diabetic Group	
	subgroup 1	subgroup 2	subgroup 3	subgroup 4
SOD (U/L)	0.25 ^a \pm 0.01	0.17 ^b \pm 0.07	0.09 ^c \pm 0.03	0.15 ^b \pm 0.02
GSH (ng/ml)	0.23 ^b \pm 0.02	0.27 ^a \pm 0.01	0.13 ^d \pm 0.03	0.18 ^c \pm 0.03

Data are expressed as mean \pm SD. Values within a row having different superscripts are significantly different ($p \leq 0.05$); where the small letters indicate significant among dietary treatment groups as indicated by one-way ANOVA followed by Duncan's multiple range test ($a > b > c > d > e$) .

Table (5): Effect of drinking magnetized water on non enzymatic antioxidants in serum in normal and diabetic rats

Parameters	Normal Group		Diabetic Group	
	subgroup 1	subgroup 2	subgroup 3	subgroup 4
CAT(ng/ml)	0.21 ^{ab} \pm 0.03	0.26 ^a \pm 0.06	0.07 ^c \pm 0.01	0.17 ^b \pm 0.05
MDA(nmol/ml)	0.09 ^d \pm 0.02	0.13 ^c \pm 0.02	0.32 ^a \pm 0.02	0.25 ^b \pm 0.01

Data are expressed as mean \pm SD. Values within a row having different superscripts are significantly different ($p \leq 0.05$); where the small letters indicate significant among dietary treatment groups as indicated by one-way ANOVA followed by Duncan's multiple range test ($a > b > c > d > e$).

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التأثيرات المضادة للسكري للماء الممغنط على الفئران الطبيعية والفئران المصابة بالسكر

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الملخص العربي:

يعد مرض السكري أحد أكثر الأمراض انتشارًا في جميع أنحاء العالم فهناك الكثير من المحاولات للحد من الاضطرابات والعيوب من خلال تغيير نمط الحياة للوصول إلى الحد الأدنى من الآثار الجانبية ويهدف هذا البحث إلى تقييم التأثير الخافض للسكر والخافض لدهون الدم وتحسين الحالة التأكسدية للماء الممغنط بشدة ٢٠ جاوس علي الفئران الطبيعية والفئران المصابة بالسكر. تم استخدام أربعة وعشرين فأر مختبرياً من النوع Albino في هذه التجربة قسمت إلى مجموعتين رئيسيتين ، المجموعة الرئيسية الأولى هي المجموعة السليمة (عدها ١٢ فأر) وتم تقسيمها إلى مجموعتين فرعيتين (٦ فئران لكل منهما) على النحو التالي المجموعة الفرعية ١ (المجموعة الكنترول السالبة) و قد شربت ماء الطبيعي ؛ المجموعة الفرعية ٢ شربت الفئران الماء الممغنط ، و المجموعة الرئيسية الثانية تم إصابتها بمرض السكري (عدها ١٢ فأر) وتم تقسيمها إلى مجموعتين فرعيتين (٦ فئران لكل منهما) على النحو التالي: المجموعة الفرعية ٣ (المجموعة الكنترول الموجبة) ، المجموعة الفرعية ٤: شربت الماء الممغنط. و بعد انتهاء التجربه (سنة أسابيع) تم جمع عينات الدم لاستخدامها في التحليل الكيميائي الحيوي مثل الجلوكوز في الدم ، الأنسولين ، السكر التراكمي (HbA1c)، مؤشر حساسية الأنسولين (HOMA-IR) ، صورة الدهون في الدم وحالة مضادات الأكسدة. وقد اظهرت النتائج أن الماء الممغنط أدى إلى انخفاض كبير في المستوى المرتفع من الجلوكوز ، الأنسولين ، السكر التراكمي HbA1c، حالة مضادات الأكسدة صورة الدهون. **الخلاصة:** يستنتج من هذا البحث أن الماء الممغنط بشدة ٢٠ جاوس يحسن الحالة الصحية من الجلوكوز ، الأنسولين ، السكر التراكمي (HbA1c)، حالة مضادات الأكسدة وملاحح الدهون للفئران العادية والمصابة بإرتفاع سكر الدم .