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التأثيرات العلاجية لأوراق المورينجا وبذور الشيا على الفئران البيضاء المصابة بالسكرى

Remedial Effects of Moringa Leaves and Chia Seeds on Diabetic White Rats

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المستخلص:

يهدف هذا البحث إلى تقييم تأثير أوراق المورينجا وبذور الشيا والخليط منهما علي ذكور الفئران المصابة بالسكرى. تم تقسيم ثلاثون فأر من الذكور البالغين سبراغ داولي إلى خمس مجموعات. مجموعة (١): وهي المجموعة الضابطة السالبة (–) تغذت على الوجبة الأساسية ، المجموعة (٢): وهي المجموعة الضابطة الموجبة (+) وهي الفئران المصابة بالسكرى وتغذت على الوجبة الأساسية. المجموعة (٣): الفئران المصابة بالسكرى التي تغذت علي اوراق المورينجا بنسبة ٥%. المجموعة (٤): الفئران المصابة بالسكرى التي تغذت على بذور الشيا بنسبة ٥ %. المجموعة (٥): الفئران المصابة بالسكرى التي تغذت على الاثنين معا (أوراق المورينجا وبذور الشيا) بتركيز ٥%.. في نهاية التجربة، بعد ٢٨ يومًا من التغذية، تم تقدير الاختبارات البيوكيميائية للدم. الحقن بالألوكسان سبب ارتفاع الجلوكوز واليوريا والكرياتينين وحامض اليوريك ALP, AST, ALT, ومستوى الكوليسترول الكلي والجلسريدات ثلاثية والليبوبروتين منخفض الكثافة والليبوبروتين منخفض الكثافة جدا وانخفاض الليبوبروتين مرتفع الكثافة ووزن الجسم والمأخوذ من الغذاء ومعدل الاستفادة من الغذاء في الفئران المصابة بالسكر وتحسنت النتائج باستخدام الأغذية المعالجة. وقد كان أفضل غذاء هو المجهز من اوراق المورينجا و بذور الشيا معا – مما يشير الى تأثير معزز– يضاف إلى ذلك أن غذاء بذور الشيا كانت نتائجه أفضل من غذاء أوراق المورينجا.

الكلمات المفتاحية:

مرض السكرى – اوراق المورينجا – بذور الشيا – الليبوبروتين.

Remedial Effects of Moringa Leaves and Chia Seeds on Diabetic White Rats

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Abstract:

This investigation aimed to evaluate the effect of moringa leaves, chia seeds and mixture of both on male diabetic rats. Thirty (30) adult male Sprague Dawley rats were divided into five groups. Group (1): Normal rats fed on basal diet as control negative (C-), Group (2): Control positive (C+) (untreated group). Group (3): Diabetic rats fed on basal diet and moringa leaves (5%). Group (4): Diabetic rats fed on basal diet and chia seeds (5%). Group (5): Diabetic rats fed on basal diet and mixture of both plants (5%). At the end of experiment, after 28 days of feeding, all serum samples were analyzed for biochemical parameters. Injection with alloxan caused a significant decrease in the level of HDL, BWG, FI & FER while a significant increase was recorded in TC, TG, VLDL, LDL, U.A, Creatinine, Urea, GOT, GPT, ALP & Glucose. For diabetic rats treated with various experimental diets, the results showed the improvement in all previous parameters. The best diet was that of the mix, indicating synergistic action, moreover chia seeds diet gave better results than moringa leaves diet. This indicated better remedial effect in the first case

Key words:

Diabetes – Moringa leaves – Chia seeds – basal diet – Injection.

Introduction:

Moringa oleifera **Lam. is a tree that grows widely in many tropical and subtropical countries. It is grown commercially in India, Africa, South, Central America, Mexico, Hawaii, and throughout Asia & Southeast Asia. It is known as the drumstick tree based on the appearance of its immature seed pods, the horseradish tree based on the taste of ground root preparations, and the ben oil tree from seed-derived oils. In some areas, immature seed pods are eaten, while the leaves are widely used as a basic food because of their high nutrition content (Mbikay, 2012), (Abdull Razis *et al*., 2014). Seeds, leaves, oil, sap, bark, roots, and flowers are widely used in traditional medicine. Moringa leaves have been characterized to contain a desirable nutritional balance, containing vitamins, minerals, amino acids, and fatty acids Additionally, the leaves are reported to contain various types of antioxidant compounds such as ascorbic acid, flavonoids, phenolics, and carotenoids (Alhakmani *et al.*, 2013), (Vongsak *et al.*, 2014).**

According to several commentaries (Mbikay, 2012), (Abdull Razis *et al.*, 2014) various preparations of *M. oleifera* are used for their antiinflammatory, antihypertensive, diuretic, antimicrobial, antioxidant, antidiabetic, antihyperlipidemic, antineoplastic, antipyretic, antiulcer, cardioprotectant, and hepatoprotectant activities. The therapeutic potential of *M. oleifera* leaves in treating hyperglycemia and dyslipidemia was reviewed by (Mbikay, 2012).

Chia (*Salvia hispanica* L.) is an annual herbaceous plant that belongs to the Lamiaceae family native from southern Mexico and northern Guatemala. The chia seed has been described as an important source of oil, protein, dietary fiber, minerals, and polyphenolic compounds (da Silva Marineli *et al.*, 2014). Chia oil is unique because it contains the highest proportion of □-3 linolenic acid (C18:3) of any known natural source. Many studies have provided evidence that regular consumption or dietary supplementation with long chain □-3 polyunsaturated fatty acids result in numerous health benefits (da Silva Marineli *et al.*, 2015).

Some studies report that consumption of chia seed promotes health benefits (Vuksan *et al.*, 2010), improving biological markers related to dyslipidemia, inflammation, cardiovascular disease, glucose homeostasis, and insulin resistance, without promoting adverse effects. In sucrose-fed rats, dietary chia seed improved adiposity and normalized hypertriacylglycerolemia and insulin resistance without affecting glucose homeostasis in dyslipidemic rats (da Silva Marineli *et al.*, 2015).

Materials and Methods:

Materials:

(*Moringa Oleifera*) Moringa leaves and chia (*Salvia hispanica*) seeds were obtained dry from herb shop in Cairo, Egypt.

Chemicals:

Alloxan obtained from El-Gomhoria Company, Cairo. Egypt.

Animals:

Thirty (30) adult male Sprague Dawley rats, average body weight (150±10 g) were used in this study. Rats were obtained from Research Institute of Ophthalmology, Medical Analysis Department, Giza, Egypt.

Methods:

Basal diet composition of tested rats:

The basal diet in the experiment consisted of casein (12%), corn oil (10%), mineral mixture (4%), vitamin mixture (1%), cellulose (5%), chorine chloride (0.2%), methionine (0.3%) and the remained is corn starch (67.5%) according to AIN (1993).

Preparation of materials:

All materials were milled to soft powder by using electric grinder and kept in dusky stoppered glass bottles in a cool and dry location till use according to Russo (2001).

Inducing diabetes in rats:

Rats were injected by Alloxan at 150 mg /kg body weight to induce diabetes in rats. Injection repeated for 3 consecutive days then fed on basal diet for 7 days before determination of serum glucose. Rats with serum glucose near: 200 mg/dl considered diabetic.

Experimental design and animal groups:

Rats were housed in wire cages under the normal laboratory condition, and were fed on basal diet for a week as an adaptation period. The rats were divided into 5 groups each of 6 rats. All groups of rats were housed in wire cages at room temperature 25 C^0, and kept under normal healthy condition. Rats were divided into the following groups:

Group (1): Control negative group (-), in which normal rats were fed on basal diet.

Group (2): Control positive group (+), in which diabetic rats were fed on basal diet.

Group (3): Diabetic rats fed on moringa leaves diet 5%.

Group (4): Diabetic rats fed on chia seeds diet 5%.

Group (5): Diabetic rats fed on mixture of both diet 5%.

Determination of Biochemical Blood Parameters:

Blood samples were collected after 12 hours fasting at the end of experiment using the abdominal aorta. The rats were scarified under ether anaesthesia. Blood samples were received in clean dry centrifuge tubes, in which blood was left to clot at room temperature, and then centrifuged for 10 minutes at 3000 r.p.m to separate the serum. Serum was carefully aspirated and transferred into clean cuvette tubes and stored frozen at-20^{o}C for biochemical analysis as described by Schermer, (1967). All serum samples were analyzed for the determination of the following parameters:

Urea was determined according to the enzymatic method of Patton and Crouch (1977), creatinine was determined according to kinetic method of Henry (1974) and uric acid was according to the enzymatic colorimetric test of Fossati and Prencipe (1980). Aspartate amino transaminase (AST) and alanine amino transferase (ALT) were carried out according to the method of Yound (1975) and Tietz (1976). Alkaline phsphatase (ALP) was determined according to Belfield and Goldberg (1971). Total cholesterol (TC) was determined according to Allen (1974), and high density lipoprotein cholesterol (HDL-c) according to Lopez (1997). The calculation of low density lipoprotein cholesterol (LDL-c) was carried out according to the method of Lee and Nieman (1996), atherogenic index (AI) was calculated according to Kikuchi *et al*., (1998) and triglyceride determination carried out as Fossati and Prencipe (1982). Serum glucose determined according to Kaplan (1984). Biological parameter (BWG, FI & FER) were also calculated.

Statistical Analysis:

The data were statistically analyzed using a computerized Costat Program by one way ANOVA using a Completely Randomized Factorial Design (SAS, 1988), when a significant mean effect was detected, the means were separated with the Duncan's Multiple Range Test. Differences between treatments at $P \leq 0.05$ were considered significant. The results are presented as mean ± SD.

Results and Discussion:

Data presented in table (1) illustrate the effect of moringa leaves, chia seeds and mixture of both on BWG, FI and FER of diabetic rats.

It could be observed that the mean value of (BWG) of control (-) group was higher than control (+) group, being 1.14±0.025 and 0.71± 0.009 g respectively. The best (BWG) level showed for groups 5 (rats fed on basal diet containing 5% mixture of both plants) when compared to control (+) group.

It could be noticed that the mean value of FI of control (-) group was higher than control (+) group, being 22.46±0.003 and 18.16± 0.005 g

respectively. The best (FI) level showed for group 5 (rats fed on basal diet + 5% mixture of both) when compared to control (+) group.

Also, data of table (1) showed that the mean value of (FER) of control (-) group was higher than control (+) group, being 0.051±0.0009 and 0.039±0.0001 respectively. The best FER was shown for group 5 (rats fed on basal diet + 5% mixture of both) when compared to control (+) group.

Table (1): Effect of moringa leaves, chia seeds and mixture of both on body weight gain (BWG), feed intake (FI) and feed efficiency ratio (FER) of diabetic rats

Parameters / Groups	BWG (g) Mean ± SD	FI (g) Mean ± SD	FER (%) Mean ± SD
G1: Control –ve	1.14^{a}±0.025	22.46^{a}±0.003	0.051^{a}±0.0009
G2: Control +ve	0.71^{e}± 0.009	18.16^{e}± 0.005	0.039^{d}± 0.0001
G3: Moringa leaves (5%)	0.80^{d}± 0.007	18.62^{d}± 0.026	0.043^{c}± 0.0007
G4: Chia seeds (5%)	0.85^{c}± 0.005	18.70^{c}± 0.009	0.046^{b}± 0.0004
G5: mixture of both (5%)	0.95^{b}± 0.034	19.00^{b}± 0.008	0.050^{a}± 0.0005
LSD	0.036	0.024	0.001

Values of same letters in the same column indicate nonsignificant difference at ($p \leq 0.5$).

Data presented in table (2) illustrate the effect of moringa leaves, chia seeds and mixture of both on total cholesterol and triglycerides of diabetic rats. It could be observed that the mean value of total cholesterol (TC) of control (+) group was higher than control (-) group, being 256± 2.39 and 105± 2.12 mg/dl respectively. The best serum (TC) level was showed for groups 5 (rats fed on basal diet containing 5% mixture of both) when compared to control (+) group.

It could be noticed that the mean value of triglycerides TG of control (+) group was higher than control (-) group, being 248± 2.07 and 123±1.89 mg/dl respectively. The best serum (TG) level showed for group 5 (rats fed on basal diet + 5%mixture of both) when compared to control (+) group.

Aborhyem *et al*., (2016) reported that *Moringa Oleifera* showed a significant decrease in total cholesterol and triglycerides in hyperlipidemic rats.

Alamri (2019) indicted that chia seeds (white & black) decreased total cholesterol and triglycerides in treated groups compared to the control groups in diabetic rats.

Table (2): Effect of moringa leaves, chia seeds and mixture of both on total cholesterol (TC) and triglycerides (TG) level of diabetic rats

Parameters / Groups	TC (mg/dl) Mean ± SD	TG (mg/dl) Mean ± SD
G1: Control –ve	105^{d}± 2.12	123^{b}±1.89
G2: Control +ve	256^{a}± 2.39	248^{a}± 2.07
G3: Moringa leaves (5%)	197^{b}± 2.61	125^{b}± 1.16
G4: Chia seeds (5%)	195^{b}± 1.55	122^{b}± 1.63
G5: mixture of both (5%)	170^{c}± 2.28	111^{c}± 2.44
LSD	4.04	3.43

Values of same letters in the same column indicate nonsignificant difference at ($p \leq 0.5$).

Data presented in table (3) show the effect of moringa leaves, chia seeds and mixture of both on HDLc, LDLc, VLDLc & AI of diabetic rats.

It could be observed that the mean value of ($VLDL_C$) of control (+) group was higher than control (-) group, being 49.6±1.24 and 24.6±1.11 mg/dl respectively. The best serum VLDLc was shown for group 5 (rats fed on basal diet + 5% mixture of both) when compared to control (+) group.

It could be showed that the mean value of (HDLc) of control (-) group was higher than control (+) group, being 61± 1.85 and 38±1.93 mg/dl respectively. The best serum HDLc was shown for group 5 (rats fed on basal diet containing 5% mixture of both) when compared to control (+) group.

The same table indicated that the mean value of (LDLc) of control (+) group was higher than control (-) group, being 168.4± 2.28 and 19.4±1.25 mg/dl respectively. The best serum LDLc was shown for group 5 (rats fed on basal diet +5% mixture of both) when compared to control (+) group.

Also, data of table (3) observed that the mean value of (AI) of control (+) group was higher than control (-) group, being 5.74±0.024 and 0.72±0.009 respectively. The best AI was shown for group 5 (rats fed on basal diet + 5% mixture of both) when compared to control (+) group.

Aborhyem *et al*., (2016) indicted that *Moringa Oleifera* decreased low-density lipoprotein (LDL) & very low-density lipoprotein (VLDL) and increased high-density lipoprotein (HDL) in hyperlipidemic rats.

da Silva *et al*., (2019) found that chia reduced low-density lipoprotein and increased HDL in adult female rats.

Table (3): Effect of moringa leaves, chia seeds and mixture of both on (VLDLc), (HDLc), (LDLc) (mg/dl) and atherogenic index (AI) of diabetic rats

Parameters / Groups	VLDL (mg/dl) Mean ± SD	HDL (mg/dl) Mean ± SD	LDL (mg/dl) Mean ± SD	AI Mean ± SD
G1: Control –ve	**24.6^{b}±1.11**	**61^{a}± 1.85**	**19.4^{d}±1.25**	**0.72^{e}±0.009**
G2: Control +ve	**49.6a± 1.24**	**38d± 1.93**	**168.4a± 2.28**	**5.74a±0.024**
G3: Moringa leaves (5%)	**25.0b± 0.42**	**55c± 1.26**	**117b± 1.68**	**2.58b±0.003**
G4: chia seeds (5%)	**24.4b± 1.17**	**56bc± 1.71**	**114.6b± 1.58**	**2.48c±0.039**
G5: mixture of both (5%)	**22.2^{c}± 0.49**	**59^{ab}± 1.41**	**88.8^{c}± 2.69**	**1.88^{d}±0.005**
LSD	**1.74**	**3.005**	**3.58**	**0.038**

Values of same letters in the same column indicate nonsignificant difference at ($p \leq 0.5$).

Results of table (4) show the mean value of serum creatinine, urea and uric acid (mg/dl) on diabetic rats fed on various diets.

It could be observed that the mean value of uric acid of control (+) group was higher than control (-) group, being 6.86±0.005 and 2.91±0.008 mg/dl respectively. Group 5 (basal diet containing 5% mixture of both) recorded the best result as compared to control (+) group.

The same table (4) results illustrate that mean value of creatinine of control (+) group was higher than control (-) group, being 1.54±0.003 and 0.70± 0.001 mg/dl respectively. In concern to creatinine the best treatment was recorded for the group 5 (rats fed on basal diet +5% mixture of both) when compared to control (+) group.

It could be noticed that the mean value of urea of control (+) group was higher than control (-) group, being 82±1.39 and 17±1.15 mg/dl respectively. Group 5 (rats fed on basal diet +5% mixture of both) recorded the best result as compared to control (+) group.

El-Hak *et al*., (2018) found that *Moringa peregrina* seeds decreased uric acid, urea and creatinine levels in serum compared to control group in adult male albino rats.

Table (4): Effect of moringa leaves, chia seeds and mixture of both on uric acid (U.A), creatinine and urea (mg/dl) levels of diabetic rats

Parameters / Groups	U.A (mg/dl) Mean ± SD	Creatinine (mg/dl) Mean ± SD	Urea (mg/dl) Mean ± SD
G1: Control –ve	**2.91^{e}±0.008**	**0.70^{b}± 0.001**	**17^{d}±1.15**
G2: Control +ve	**6.86^{a}± 0.005**	**1.54^{a}± 0.003**	**82^{a}± 1.39**
G3: Moringa leaves (5%)	**3.50^{b}± 0.009**	**0.59^{c}± 0.008**	**38^{b}± 1.85**
G4: chia seeds (5%)	**3.36^{c}± 0.006**	**0.56^{d}± 0.005**	**36^{b}± 1.92**
G5: mixture of both (5%)	**3.11^{d}± 0.002**	**0.54^{e}± 0.007**	**21^{c}± 1.77**
LSD	**0.012**	**0.009**	**2.89**

Values of same letters in the same column indicate nonsignificant difference at ($p \leq 0.5$).

Data of table (5) illustrate the effect of moringa leaves, chia seeds and mixture of both on serum levels of AST, ALT, ALP enzymes & (AST/ALT) ratio of diabetic rats.

It could be observed that the mean value of AST enzyme of control (+) group was higher than control (-) group, being 162±2.81 and 128±2.75 (U/L) respectively. The best treatment was observed for group 5 (basal diet containing 5% mixture of both) when compared to control (+) group.

It could be noticed that the mean value of ALT enzyme of control (+) group was higher than control (-) group, being 128±2.82 and 38±1.01 (U/L) respectively. The best treatment was observed for group 5 (basal diet containing 5% mixture of both) when compared to control (+) group.

It could be noticed that the mean value of (AST/ALT) of control (-) group was higher than control (+) group, being 3.37±0.37 and 1.27± 0.009 respectively. The best treatment was observed for group 5 (basal diet containing 5% mixture of both) when compared to control (+) group.

Data of the same table (5) show the mean value of ALP enzyme of control (+) group was higher than control (-) group, being 123±2.71 and 59±2.89 (U/L) respectively. Group 5 showed the lowest mean value of ALP enzyme level as compared to control (+) group which and recorded the best result**.**

Toppo *et al*., (2015) reported that treatment with *Moringa oleifera* 500 mg/kg significantly ($p<0.01$) decreased the elevated ALP, AST & ALT in rats with cadmium toxicity.

Alamri, (2019) indicted that both black and white chia seeds is effective in reducing the levels of liver enzymes (AST and ALT) in rats.

Table (5): Effect of moringa leaves, chia seeds and mixture of both on AST, ALT, AST/ALT and ALP (U/L) of diabetic rats

Parameters / Groups	AST (U/L) Mean ± SD	ALT (U/L) Mean ± SD	AST/ALT Mean ± SD	ALP (U/L) Mean ± SD
G1: Control –ve	**128^{c}±2.75**	**38^{c}±1.01**	**3.37^{a}±0.37**	**59^{d}±2.89**
G2: Control +ve	**162^{a}± 2.81**	**128^{a}± 2.82**	**1.27^{d}± 0.009**	**123^{a}±2.71**
G3: Moringa leaves (5%)	**133^{b}± 2.51**	**52^{b}± 1.66**	**2.56^{bc}± 0.001**	**72^{b}±2.26**
G4: Chia seeds (5%)	**111^{d}± 2.47**	**49^{b}± 1.74**	**2.27^{c}± 0.007**	**69^{bc}±2.45**
G5: mixture of both (5%)	**105^{e}± 2.19**	**40^{c}± 1.28**	**2.63^{b}± 0.005**	**65^{c}±2.58**
LSD	**4.65**	**3.3**	**0.30**	**4.71**

Values of same letters in the same column indicate nonsignificant difference at ($p \leq 0.5$).

Data presented in table (6) show the effect of moringa leaves, chia seeds and mixture of both on serum glucose of diabetic rats. It could be noticed that the mean value of glucose of control (+) group was higher than control (-) group, being 380± 2.16 and 95± 2.89 (mg/dl) respectively. The best serum glucose was observed for group 5 (basal diet containing 5% mixture of both) when compared to control (+) group.

El-Hak *et al.*, (2018) found that *Moringa peregrina* seeds induced significant decreases in fasting serum glucose level when compared to control group male albino rats.

Vuksan *et al.*, (2017) reported that chia seeds reduced blood glucose under the curve over 120 min by 82.5±19.7 mmol/l (P<0.001) in healthy individuals.
da Silva *et al.*, (2019) found that chia reduced glucose in adult female rats.

Table (6): Effect of moringa leaves, chia seeds and mixture of both on serum glucose (mg/dl) level of diabetic rats

Parameters / Groups	Glucose (mg/dl) Mean ± SD
G1: Control –ve	95^{e}± 2.89
G2: Control +ve	380^{a}± 2.16
G3: Moringa leaves (5%)	122^{b}± 2.62
G4: Chia seeds (5%)	110^{c}± 2.28
G5: mixture of both (5%)	105^{d}± 2.76
LSD	4.56

Values of same letters in the same column indicate nonsignificant difference at ($p \leq 0.5$).

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