The Effect of Some Natural Herbs on ... Suffering from Diabetes

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Abstract

The present study was carried out to evaluate the effect of different ratios of *Moringa oleifera* (MO), *Gymnema sylvestre* (GS) leaves, and their combination on diabetic rats. Male albino rats (40 rats) were used in this study. The rats were divided into two main groups. The first main group (5 rats) was fed on a basal diet as a control negative group (c-). While the second main group was diabetic induced by a single intraperitoneal injection of alloxan (120 mg/kg b.wt) then divided into seven equal subgroups. One of them fed on a basal diet as a control positive group (c+), and the other groups fed on a basal diet containing 2.5% & 5% from MO leaves, 2.5% & 5% from GS leaves, and their equal combination by the same ratio respectively. Fino bread was prepared with the same levels from MO and GS, and their combination. The results showed that injected rats of alloxan-induced defectiveness in all parameters. Feeding rats that suffered from diabetes with diets containing the two levels from MO and GS leaves and their combination led to a decrease in serum glucose, triglycerides, total cholesterol, low and very low density lipoprotein cholesterol, kidney functions (uric acid, urea nitrogen, and creatinine), and liver enzymes activity (AST, ALT and ALP).While HDL-c increased, as compared to the positive control group. The results of the sensory evaluation showed an acceptable fino bread prepared in most proportions of the study. Therefore, the study recommends the use of *Moringa oleifera* leaves, *Gymnema sylvestre*, and their combination in preparing fino bread suitable for a diabetic.

***Key words*: *Moringa oleifera*, *Gymnema sylvestre*, serum glucose, lipid profile, liver enzymes, kidney functions.

INTRODUCTION:

Diabetes mellitus (DM) is a lifelong continue worldwide pestilence that is spreading worldwide resulted in a substantial socioeconomic encumbrance and impacts the person family and society, raising a substantial morbidity and death rate **(Nabolsi, 2020).**

Diabetes is among the upper ten reasons for mortality in adult people all over the world where the last estimates for 2019 are that it has caused 4.2 million deaths in the world. Diabetes spread is ventured to increase from 463 million in 2019, to 700 million in 2045 by 51% from 2019 to 2045 worldwide **(Smokovski, 2020).**

Diabetes mellitus is one of the popular non-communicable diseases (NCDs) globally. Led persistent high blood glucose which in the end boosts abdominal fat, lipogenesis and increases total and low-density lipoprotein (LDL), triglyceride, and cholesterol level alongside for changed platelet job and raised glycoprotein metabolism **(Oza & Kulkarni, 2016)**. It is a chronic disease. Affects many organs of the body leads to many health complications such as microangiopathic, retinopathy, neuropathy, arterial hypertension, and atherothrombosis. The action of chronic hyperglycemia leads to other complications which may be tall term or short-term
(Das *et al*., 2020 and Scheen, 2020).

The inception and development of long-term diabetic diabetes mellitus problems appear to be connected with hyperglycemia and metabolism. While numerous conventional medicines are available to treat hyperglycemia, demand for the usage of anti-diabetic plant items is growing. Some of the considerations that contribute to a significant preference for hypoglycemic medicines of plant origins that are thought to be suited for chronic therapy include. Many medicinal plants are beneficial in the management of diabetes **(Adejoh *et al*., 2016).**

Interestingly, several native plants that are found to possess antidiabetic and antioxidant characteristics include flavonoids, glycosides, alkaloids, terpenoids and carotene. *Moringa oleifera* showed notable various pharmacological activities like antidiabetic, immunomodulator, anti-inflammatory, antiulcer, cardioprotective, antihypertensive, hepatoprotective, anti-nephrotoxicity and anti-microbial activities to arouse **(Prabu *et al*., 2019).**

Moringa oleifera (Moringaceae, M. oleifera) is an extremely nutrient-rich, therapeutic plant utilized in many health issues, and it has remarkable medicinal qualities. It offers a rich combination of minerals, amino acids, antioxidants, anti-aging, and anti-inflammatory substances, and it is used for a wide range of medications, especially in South Asia and India. In many tropical and subtropical nations of Asia and Africa, *Moringa oleifera* is grown in the Nile valley. The leaves are used for many months without losing in nutritive content as a leafy vegetable with fresh, cooked and kept as dry powder. The combined actions of various bioactive found in the plant potassium, calcium, phosphorus, zinc, manganese and iron, vitamins A, D, E and C, alkaloids found in Moringa, carotenoids as well as essential amino acids are attributed to the therapeutic effects of *Moringa oleifera* leaves, in addition, it includes three phytochemical structure groups with various therapeutic effects. They are glucosinolates, such as glucomoringin, quercetin, and kaempferol flavonoids, and phenol acids, chlorogenic acids. These phytochemicals have been reported to possess antioxidant, hypoglycemic **(Yassa & Tohamy, 2014; Konmy *et al*., 2016 and Magaji *et al*., 2020)**.

Gymnema is an herb used in Indian traditional medicine. It is also called gurmar because of its characteristic that suppresses sweet taste. The earliest scientific validation that *Gymnema sylvestre* leaves have been used in human diabetics more than 90 years ago, it helps to that urine glucose in diabetics has been decreased and reduces fat buildup **(Gunasekaran *et al*., 2019)**. *Gymnema sylvestre* (Asclepiadaceae) has been recognized as a possible herbal medicine that regenerates both the ß-cell and stimulates the production of insulin. It also has anti-obesity, immunomodulatory and anti-wound cure activity, anti-hyperlipidemic, anti-inflammatory, and anti-cancer antioxidants **(Yadav *et al*., 2019).**

The *Gymnema sylvestre* leaves include triterpenoid saponins. In *Gymnema sylvestre*, there are 20 different saponins and glycosides. There are also gymnemic acids wherein Several studies show that anti-diabetic by encouraging islet cells to regenerate, enhance insulin production and limit intestinal glucose absorption **(Krishnamurthy *et al*., 2016 and Laha & Paul, 2019).**

Therefore, the present study aimed to investigate the effect of different ratios of *Moringa oleifera*, *Gymnema sylvestre* leaves and their combination on biological and biochemical parameters in diabetic rats. In addition to studying the possibility of including different ratios of *Moringa oleifera*, *Gymnema sylvestre* leaves and their combination in making fino bread.

MATERIAL AND METHODS:

Materials:

1- Dried *Moringa oleifera* Leaves (MO) and *Gymnema sylvestre* Leaves (GS) were obtained from the local market, Cairo, Egypt.
2- Ingredients of food product (wheat flour, dry yeast, salt, sugar, skim milk powder, corn oil) obtained from the local market, Damietta Governorate, Egypt.
3- Casein, all vitamins, minerals, choline chloride, cellulose and alloxan were obtained from El-Gomhoriya Company for Trading Drugs, Chemicals and Medical instruments, Cairo, Egypt.
4- Corn oil and corn starch were obtained from the local market, Damietta Governorate, Egypt.
5- Forty normal male albino rats (Sprague Dawley strain) weighing (155 ±5g) were obtained from the Nile Center for Experimental Researches, Mansoura City.

6-Kits used to determine serum glucose, triglycerides (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-c), low-density lipoprotein cholesterol (LDLc), very low-density lipoprotein-cholesterol (VLDL-c) ,aspartate amino transferase (AST), alanine amino transferase (ALT), alkaline phosphatase (ALP), uric acid, urea nitrogen and creatinine were obtained from El-Gomhoriya Company for Trading Drugs, Chemicals and Medical instruments, Cairo, Egypt.

Methods:

Preparation of Plant Samples:

The dried *Moringa oleifera* and *Gymnema sylvestre* leaves were milled separately into powder and it stored in containers.

Biological experiments:

Male albino rats Sprague Dawley strain (40 rats) weighting (155±5g.) Rats were inhabited under record conditions (12 h. light–dark cycles, 5 rats per 1500 cm^2 cage in 22±3^o C) for one week to adaptive before the experimental study. During this period, rats were nurtured on the normative basal diet with freedom access to food and water. The basal diet consists of 14% Casein (Protein > 80%), corn oil 4%, cellulose5%, vitamin mixture 1%, salt mixture 3.5%, choline chloride 0.25% and the remainder was corn starch **(Reeves *et al*.,1993)**. The experiment on rats was carried out according to the National regulations on animal welfare and Institutional Animal Ethical Committee. The biological experiments performed complied with the rulings of the Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council **(NRC, 2011).**

Experimental Design

After the period of adaptation on a basal diet (one week), the rats were divided into two main groups as follows

-The first main group (5 rats) was fed on a basal diet as a control negative group (C-).

-The second main group (35 rats) were injected subcutaneously by alloxan solution at a rate of (120 mg/kg b.wt) to induce hyperglycemia according to the method described by **Kumar *et al*. (2010)**. Then the rats were fed on the basal diet for 48h during which hyperglycemia was developed. Blood samples were withdrawn after alloxan injection to ensure the occurrence of diabetes in rats. The rats in the second main group were divided into seven subgroups (n=5):

- Group 2 (C+): fed on a basal diet as a positive control group
- Group 3(MO_1): fed on a basal diet containing 2.5% *Moringa oleifera*
- Group 4(MO_2): fed on a basal diet containing 5% *Moringa oleifera*
- Group 5(GS_1): fed on a basal diet containing 2.5% *Gymnema sylvestre*
- Group 6(GS_2): fed on a basal diet containing 5% *Gymnema sylvestre*
- Group7 (MO_1GS_1): fed on a basal diet containing (1.25% *Moringa oleifera* + 1.25% *Gymnema sylvestre*).
- Group 8 (MO_2GS_2): fed on a basal diet containing (2.5% *Moringa oleifera* + 2.5% *Gymnema sylvestre*).

Biological determination:

During the experiment period (28 days), the quantities of diet, which were consumed and/or wasted, were recorded every day. In addition, the rat's weight was recorded weekly, to determine body weight gain%, food intake and food efficient ratio according to the method of (**Chapman *et al*., 1959).**

Body weight gain% was determined using the following equation:

$$\text{Body Weight Gain} = \frac{\text{Final weight (g) - Intial weight (g)}}{\text{Initial weight (g)}} \times 100$$

Food Efficiency Ratio (FER) = Body weight gain (g) / Food intake (g)

Blood Sampling

At the end of the experimental period, the rats were fasted overnight before being sacrificed, and blood samples were collected from the aorta. The blood samples were centrifuged for 20 min at 3000 rpm to separate the serum. The serum was carefully separated into dry clean Wassermann tubes by using a Pasteur pipette and kept frozen until analysis at (-20°C).

Biochemical analysis of serum

Serum glucose was determined in the serum according to the method described by **Trinder, (1959).** Serum total cholesterol (TC), triglycerides (TG) and high density lipoprotein–cholesterol (HDL-C) were determined according to the method described by **Allain *et al*., 1974; Trinder & Ann, 1969** and **Lopes-Virella *et al*., 1977)**, respectively. Serum low density lipoprotein–cholesterol (LDL-c) and very low-density lipoprotein-cholesterol (VLDL-c) were determined according to the method described by **FriedWald *et al*. (1972)**. Serum uric acid, urea nitrogen and creatinine were determined by **Fossati *et al*., 1980**; **Patton & Crouch, 1977 and Bohmer, 1971)**, respectively. Aspartate amino transaminase (AST), Alanine amino transaminase (ALT) and alkaline phosphatase (ALP) activities were measured according to the method described by **Reitman & Frankel, 1957 and Belfield & Goldberg, 1971)**, respectively.

<u>Histopathological Examination:</u>

After sacrificing animals were taken pancreas laundered with a saline solution to get rid of blood put into 10% formalin solution. Tissues from the pancreas of the sacrificed rats were examined as described by **Bancroft & Gamble (2008).**

Preparing of fino bread:

Fino bread is prepared by the straight dough method as described in **A.A.C.C. (2002)**. **The different batches of fino bread were classified as follows:**

1- Control: control fino bread was made from 100% wheat flour (72% extraction),
2- Different formulas:
a- Treatment was made from replaced wheat flour with MO at ratios of 2.5 and 5%, respectively.
b- Treatment was made from replaced wheat flour with GS at ratios of 2.5 and 5%, respectively.
c- Treatment was made from replaced wheat flour with a mixture of MO and GS at ratios of 1.25 +1.25 % and 2.5+ 2.5%, respectively.

Chemical analysis

The proximate chemical composition of fino bread samples was determined; moisture, protein, crude fat, crude fiber and ash by the **A.O.A.C. (2005)**, while total carbohydrates were calculated by the differences. Carbohydrates (%) = [100 – (moisture + fat +protein +crude fiber +ash)].

Sensory evaluation:

Sensory evaluation was participated by invited ten staff panelists from the Home Economics Department, Faculty of Specific Education, Damietta University, Damietta, Egypt. Each panelist was asked to evaluate seven samples from Fino bread according to color, odor, texture, taste, and general acceptability.

The evaluation was carried out according to the method of **(Abd El – latif, 1990)**.

Statistical analysis:

The data obtained were statistically analyzed by using computer using, the results were expressed as mean ± standard deviation **"SD"** and tested for significance using one-way analysis of variance" **ANOVA"** test, according to Duncan's multiple range test at ($P \leq 0.05$) probability According to the method described by **Armitage & Berry (1987).**

RESULT AND DISCUSSION

Body weight gain%, feed intake and food efficiency Ratio:

Data in table (1) showed that body weight gain % (BWG%) of the positive control group decreased significantly ($P \leq 0.05$), as compared to the healthy rats in the negative control group. Treated rats with *Moringa oleifera* leaves (MO) 2.5 & 5% and *Gymnema sylvestre* leaves (GS) 2.5% showed a non-significant increase ($P \leq 0.05$) in body weight gain% as compared to the control positive group. On the other hand, treated groups with 5% MO and the combination by 1.25%MO + 1.25GS and 2.5%MO + 2.5%GS showed a significant decrease ($P \leq 0.05$) in BWG% as compared to the control positive group.

Injected rats with alloxan increased the mean value of feed intake by about 14.55% than that of the control negative group. Treating diabetic rats with 2.5 & 5% MO, 2.5% & 5%GS and their combination (1.25%MO+1.25%GS and2.5%MO+2.5%GS) led to a slight decrease in the mean value of feed intake, as compared to the (C+) group Date also showed that treated rats with 2.5 & 5%MO, 2.5%GS increased the mean value of FER by comparing with the control positive control. Nevertheless, treated rats with 5% GS and the combination (1.25%MO+1.25%GS and 2.5%MO+2.5% GS) revealed a significant decrease ($P \leq 0.05$) of FER by comparing with the positive control group.

In this respect, **Abo Baker& Moawad (2020)** showed that diabetic animals had a significant decrease in their body weight. Whereas, in animals who received *Moringa oleifera*, recovery of decreased weight was observed. Diabetes mellitus causes metabolic disorders in distinct organs, including body weight decrease and tissue destruction.

These findings are supported by showing that, **Bamagous *et al*. (2018)** showed that, the bodyweight of diabetic control group animals showed significantly reduced weight gain as compared to normal rats ($P<0.05$). Diabetic rats treated with *Moringa oleifera* showed significantly better weight gain ($P<0.05$) in contrast to diabetic control rats. Food intake of diabetic control group animals was higher ($P<0.05$) as compared to normal group animals. The food intake levels were reduced in diabetic rats treated with *Moringa oleifera* ($P<0.05$). An average food efficiency ratio of diabetes control rats was significantly lower than normal rats ($P<0.05$) whereas rats in diabetic rats treated with *Moringa oleifera* showed a higher food efficiency ratio as compared to diabetic group rats ($P<0.05$).

Also, **Gopalakrishnan *et al*. (2020)** showed that the body weight was significantly decreased, whereas food intake was significantly increased in diabetic rats when compared with normal control rats. On oral administration of *Gymnema sylvestre* (250 mg/kg b.wt) for 45 days the body weight and food intake significantly decreased when compared with untreated diabetic control rats.

Table (1): Effect of MO, GS leaves and their combination on BWG%, FI and FER of rats suffering from diabetes.

Parameters / Groups	B W G (%)	FI (g/day/rat)	FER
C-	18.09 ± 2.21^{a}	14.03	1.28 ± 0.15^{a}
C+	12.91 ± 1.32^{b}	16.42	0.78 ± 0.07^{bc}
MO_1	15.51 ± 2.37^{ab}	16.28	0.95 ± 0.16^{b}
MO_2	14.20 ± 1.47^{b}	14.75	0.95 ± 0.09^{b}
GS_1	12.95 ± 2.76^{b}	16.07	0.80 ± 0.17^{bc}
GS_2	9.70 ± 2.37^{c}	14.35	0.67 ± 0.16^{cd}
MO_1GS_1	8.41 ± 2.20^{cd}	16.00	0.52 ± 0.13^{de}
MO_2GS_2	5.81 ± 1.95^{d}	14.25	0.40 ± 0.13^{e}

MO: *Moringa oleifera*, **GS**: *Gymnema sylvestre*
Values which have different letters in each column differ significantly at ($p \leq 0.05$).

Serum Glucose:

Data in the table (2) showed the effect of *Moringa oleifera*, *Gymnema sylvestre* leaves and their combination on serum glucose of diabetic rats. The mean value of serum glucose of positive control groups increased significantly ($p \leq 0.05$), as compared to the negative control group Serum glucose increased by about 95.55% in the positive control group. The increase in serum glucose may suggest disrupted carbohydrate metabolism due to the enhanced breakdown of liver glycogen **(Abd el Halim, 2020)**.

Also, from the same table, it could be noticed that significant decreases ($p \leq 0.05$) were recorded in glucose levels between diabetic rats fed on MO leaves (2.5%, 5 %); GS leaves (2.5%, 5%) and their combination (1.25%MO+ 1.25%GS), (2.5%MO+2.5%GS) as compared to the positive control group. On the other hand, the highest decrease in serum glucose was recorded for the group fed on the combination

(2.5%MO+2.5%GS) and (1.25%MO+ 1.25%GS). The mean value of serum glucose of treated groups fed on (1.25%MO+ 1.25%GS), and (2.5%MO+2.5%GS) showed non-significant difference ($p \leq 0.05$), as compared to the negative control group.

These results are in agreement with those found by **Ekeh *et al.* (2019)** who mentioned that the groups that received 500 mg/kg *Moringa oleifera* showed a 65.47 % decrease in blood glucose levels. When compared to the diabetic control group, the treated groups had a substantial ($p<0.05$) drop in blood glucose levels. Also, **Youl *et al.* (2020)** reported that adding at 100 mg/kg body weight, an aqueous ethanol extract of *Gymnema sylvestre* leaves led to significantly reduced
($p \leq 0.05$) blood glucose.

Studies have shown that in rats with type 2 diabetes, leaves of *Moringa oleifera* significantly reduce glucose concentration. Leaves are indeed a powerful source of polyphenols, responsible for hypoglycemia. *Moringa oleifera* also has an enhancing effect on glucose intolerance that can be mediated by quercetin-3-glucoside and fibers contained in leaf powder **(Jacques *et al.*, 2020)**.

Gymnema sylvestrein traditional medicine for the treatment of diabetes. It has been reported to have potent primary metabolites like proteins, amino acids, and secondary metabolites consisting of alkaloids, flavonoids, glycosides, Saponins, Tannins and anthraquinones and phenolic compounds (**Rahangdale, 2019)**. *Gymnema sylvestrein* having antidiabetic and antioxidant activity due to its bioactive compounds like oleanines (gymnemic acid, gymnema saponins), anthraquinones, flavones, hentriacontane, pentatriacontane, phytin, resin, glycosides and anthraquinones, alkaloid like gymnamine, flavonoids, cinnamic acid, folic acid and ascorbic acid **(Laha & Paul, 2019)**.

Table (2): Effect MO, GS leaves and their combination on serum glucose of rats suffering from diabetes.

Parameters / Groups	Serum Glucose Mg/dl
C-	72.00± 10.07 [c]
C+	140.80±4.86[a]
MO_1	106.60±18.83[b]
MO_2	102.20±12.39[b]
GS_1	108.80±5.01[b]
GS_2	104.40±13.52[b]
MO_1GS_1	83.20±10.35[c]
MO_2GS_2	80.20±6.49[c]

MO: *Moringa oleifera*, **GS**: *Gymnema sylvestre*
Values which have different letters in each column differ significantly at ($p \leq 0.05$)

Lipid Profile:

Effect of *Moringa oleifera*, *Gymnema sylvestre* leaves and their combination on blood lipid profile is presented in table (3). The results showed significant increases ($p \leq 0.05$) in total cholesterol, triglyceride, LDL-c, and VLDL-c of the positive control group compared with the negative control group. While all treated groups showed a significantly decreased ($p \leq 0.05$) as compared to the positive control group. The treated group which fed on 5% GS and mix (2.5%MO+2.5%GS) showed a non-significant change in serum cholesterol and serum LDL-c, as compared to the negative control group.

On the other hand, Serum triglycerides in all treated groups with *Moringa oleifera*, *Gymnema sylvestre* leaves and their combination decreased significantly ($p \leq 0.05$), as compared to the positive control group. Serum triglyceride decreased gradually with increasing the levels of MO, GS, and their combination. While, all treated groups showed a significant decreased ($p \leq 0.05$) in serum VLDL-c, as compared to the positive control group. On the other hand, the mean value of total serum HDL-c decreased significantly ($p \leq 0.05$) in the positive control group, as compared to the negative control group. Where data showed significant increases ($p \leq 0.05$) in serum HDL-c for all diabetic rats group fed on *Moringa oleifera*, *Gymnema sylvestre* leaves and their combination compared with the control positive.

These results were in agreement with the data of **Sun *et al*. (2019)** who found that the diabetic control group mice showed higher TG, TC, and LDL, but lower HDL. Compared with the negative control group, the *Moringa oleifera* leaf extract administration group (120 mg/kg) showed lower TG ($P \leq 0.01$) and LDL ($P \leq 0.01$), but higher HDL ($P \leq 0.01$).

Also, **Shah *et al*. (2019)** showed that, after 8 weeks, there was a significant increment in TGL, total cholesterol and LDL while a decrease in HDL was observed in diabetic control rats compared with normal rats. In treated rats with *Gymnema sylvestre* significantly decreased total cholesterol, triglycerides (TGL) and low-density lipoprotein (LDL) level while increased the high-density lipoprotein (HDL).

**Table (3): Effect of MO, GS leaves and their combination on serum lipids profile
of rats suffering from diabetes.**

Parameters / Group	Cholesterol	TG	HDL-c	LDL-c	VLDL-c
	Mg/dl				
C-	75.00 $\pm$ 8.48^{e}	50.40 $\pm$11.80^{f}	57.60 $\pm$ 4.33^{a}	7.32 $\pm$ 4.24^{f}	10.08 $\pm$ 2.36^{f}
C+	134.80 $\pm$ 7.19^{a}	84.20 $\pm$ 3.56^{a}	39.40 $\pm$ 0.8^{b}	78.56 $\pm$ 7.38^{a}	16.84 $\pm$0 .71^{a}
MO_1	99.60 $\pm$ 6.42^{b}	66.60 $\pm$ 3.36^{b}	53.60 $\pm$ 2.50^{a}	32.74 $\pm$ 7.04^{b}	13.32 $\pm$ 0.67^{b}
MO_2	94.40 $\pm$ 9.88^{bc}	64.20 $\pm$ 8.49^{bc}	54.20 $\pm$ 2.77^{a}	27.76 $\pm$ 12.19^{bc}	12.84 $\pm$ 1.69^{bc}
GS_1	89.40 $\pm$ 2.07^{bcd}	62.00 $\pm$ 2.91^{bcd}	55.40 $\pm$ 10.26^{a}	21.60 $\pm$ 10.53^{cd}	12.40 $\pm$0 .58^{bcd}
GS_2	83.40 $\pm$ 12.7^{de}	58.20 $\pm$ 2.94^{cde}	56.20 $\pm$ 5.11^{a}	15.56 $\pm$ 8.51^{def}	11.64 $\pm$ 0.58^{cde}
MO_1GS_1	87.60 $\pm$ 8.93^{cd}	56.20 $\pm$ 4.14^{def}	58.40 $\pm$ 3.36^{a}	17.96 $\pm$ 6.95^{cde}	11.24 $\pm$0 .82^{def}
MO_2GS_2	82.40 $\pm$ 3.78^{de}	54.40 $\pm$ 1.34^{ef}	62.00 $\pm$ 20.91^{a}	9.52 $\pm$ 2.84^{ef}	10.88 $\pm$ 0.26^{ef}

MO: *Moringa oleifera*, **GS**: *Gymnema sylvestre*
Values which have different letters in each column differ significantly at ($p \leq 0.05$)

Liver Enzymes:

From the data presented in table (4), it could be observed that, the mean value ± SD of serum (ALT, AST and ALP) in the positive control group increased significantly ($p<0.05$), as compared to the negative control group. Injected rats with alloxan to induce hyperglycemia led to increased (ALT, AST and ALP) Enzymes by about 54.43%, 22.34% and 46.41% in the positive control group than that of the negative control group. A significant decrease ($p \leq 0.05$) in the mean values of ALT enzyme was observed between the groups treated with MO (5%), GS (5%) and their combination (1.25%MO +1.25%gs and 2.5%MO +2.5%GS) and the positive control.

Data in this table showed a significant decrease ($p \leq 0.05$) in the mean values of AST enzyme was observed between the groups which fed on a diet containing MO leaves (5%) , GS leaves (5%), their combination (1.25%MO +1.25%GS and 2.5%MO +2.5%GS) and the positive control group. On the other hand, a significant decrease ($p \leq 0.05$) in the mean values of ALP enzyme was observed between the groups which treated with different levels from MO leaves (5%), GS leaves (5%) and their combination (1.25% MO +1.25% GS), (2.5% MO +2.5% GS) and positive control group.

The study was in agreement with **Bamagous *et al.* (2018)** who reported that, in the group of rats with diabetes-induced diabetes the mean serum activity of AST, ALT and ALP has been increased ($P \leq 0.05$). The level of diabetes-related liver damage indicated the high amounts of certain enzymes. *Moringa oleifera* ethyl extract the blood level ALT, AST and ALP was considerably lowered ($P \leq 0.05$) compared to the serum rat's diabetes Group. Almost comparable results have been found in serum AST, ALT, and ALP levels in the group of normal rats.

Also, **Hamzah (2018)** indicated that rats were given 100, 300, 600 mg/kg of extract weight daily for 21 days in their respective groups. All the *Gymnema sylvestre* extracts were able to the activity of serum liver enzymes (AST, ALT and ALP) significantly decreased ($p \leq 0.05$) compared with the diabetic untreated group.

Table (4): Effect of MO, GS leaves and their combination on liver enzymes of rats suffering from diabetes.

Parameters / Group	ALT	AST	ALP
	(U/dl)		
C-	20.50 ± 2.12^{c}	96.50 ± 9.19^{bc}	299.50 ± 2.12^{b}
C+	31.66 ± 1.52^{a}	118.06 ± 7.78^{a}	438.50 ± 31.81^{a}
MO_1	28.00 ± 2.64^{ab}	107.00 ± 5.19^{ab}	340.40 ± 14.63^{ab}

MO_2	26.66 ± 4.04^{b}	102.00 ± 7.93^{bc}	300.00 ± 81.30^{b}
GS_1	28.33 ± 3.18^{ab}	106.00 ± 2.82^{ab}	331.75 ± 49.60^{ab}
GS_2	26.25 ± 3.77^{b}	100.00 ± 5.65^{bc}	302.66 ± 8.14^{b}
MO_1GS_1	25.33 ± 2.51^{bc}	94.33 ± 11.01^{bc}	298.75 ± 106.98^{b}
MO_2GS_2	24.00 ± 1.73^{bc}	91.33 ± 2.30^{c}	290.00 ± 14.14^{b}

MO: *Moringa oleifera*, **GS**: *Gymnema sylvestre*
Values which have different letters in each column differ significantly at ($p \le 0.05$).

Kidney Functions:

Statistical analysis in table (5) diabetic rats induced an increase, in the mean values of serum (urea nitrogen, uric acid, and creatinine) levels, as compared to the negative control group. The mean value of serum urea nitrogen of treated groups 5%MO and mix (2.5% MO + 2.5% GS) decreased significantly ($p \le 0.05$), as compared to the positive control group. Additionally, non-significant differences in the mean value of serum uric acid were observed between the groups treated with *Moringa oleifera*, *Gymnema sylvestre* leaves and their combination and the negative control group (normal rats). All treated groups revealed a significant decrease ($p \le 0.05$) in creatinine, as compared to the positive control group (untreated group).

These findings are in agreement with those of **Tuorkey (2016)** who found that, in diabetic untreated mice, plasma creatinine levels were considerably elevated compared to the control group. The mice receiving *Moringa oleifera* showed an insignificant change from the negative control group. The levels of creatinine have considerably decreased due to the treatment of diabetic mice with *Moringa oleifera*. On the other hand, urea levels in the diabetes untreated groups were considerably increased. The urea level has decreased considerably in the mice that took *Moringa oleifera* compared to the untreated diabetes group.

While, **Morolahun *et al.* (2019)** cleared that Results that in rats that were diabetic compared to control rats, the uric acid level increased significantly. However, administration with *Moringa* *oleifera* aqueous extract for 4 weeks resulted in a substantial reduction in uric acid level ($P \leq 0.05$).

Khan *et al*. (2019) cleared that in alloxan-induced diabetic rats were utilized, the levels of urea, uric acid, and creatinine in blood were considerably ($p \leq 0.05$) raised, while *Gymnema sylvestre* leaf extract led to significantly decreased ($p \leq 0.05$) in the high levels of urea, uric acid, and creatinine in diabetic rats.

Table (5): Effect of MO, GS leaves and their combination on kidney Functions of rats suffering from diabetes.

Parameters / Group	Urea	Uric acid	Creatinine
	Mg/dl		
C-	31.00 ±4.00[c]	1.90±0.38[b]	0.94 ±0.05[ab]
C+	49.60±8.53[a]	3.22±1.07[a]	1.00± 0.00[a]
MO_1	42.20±7.98[ab]	2.40±0.12[b]	0.84 ± 0.11[bc]
MO_2	38.00±4.41[bc]	2.30±0.38[b]	0.80 ± 0.14[cd]
GS_1	43.20±7.39[ab]	2.10±0.49[b]	0.78 ± 0.08[cd]
GS_2	43.00±6.44[ab]	2.00±0.51[b]	0.76 ± 0.05[cd]
MO_1GS_1	43.40±3.13[ab]	2.32±0.43[b]	0.76 ± 0.05[cd]
MO_2GS_2	35.60±9.31[bc]	2.12±0.62[b]	0.72 ± 0.04[d]

MO: *Moringa oleifera*, **GS**: *Gymnema sylvestre*
Values which have different letters in each column differ significantly at ($p \leq 0.05$)

Histopathological examination of pancreas:

The pancreas of rats from group 1 revealed normal pancreatic acini and normal islets of Langerhan's **photo (1)**. On the other hand, the pancreas of rats from group 2 showed vacuolations of cells of islets of Langerhan's and cystic dilatation of pancreatic duct **photo (2)**. Meanwhile, the pancreas of rats from group 3 revealed vacuolations of some cells of islets of Langerhan's **photo (3)**. However, the pancreas of rats from group

4 and some sections from group 5 showed no histopathological changes and normal pancreatic tissue **photo (4) & photo (5a)**. Whereas, other sections from group 5 revealed congestion of pancreatic blood vessel **photo (5b)**. Moreover, the pancreas of rats from group 6 showed no histopathological changes and normal pancreatic tissue **photo (6)**. Also, examined sections from groups 7 & 8 showed revealed no histopathological changes and normal pancreatic tissue **photo (7) & photo (8).**

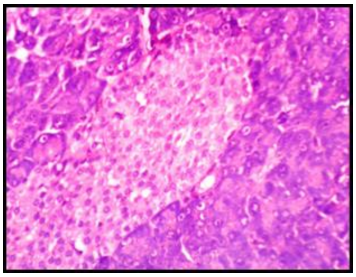

photo (1): Pancreas of rat fromgroup 1 showing normal pancreatic acini and normal islets of Langerhan's (H & E X 400).

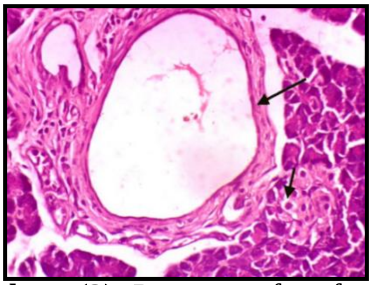

photo (2): Pancreas of rat from group2 showing vacuolations of cells of islets of Langerhan's and cystic dilatation of pancreatic duct (H & E X 400).

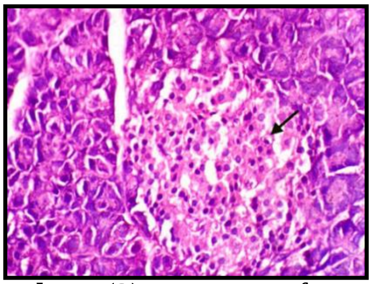

photo (3): Pancreas of rat from group 3 showing vacuolations of some cells of islets of Langerhan's (H & E X 400).

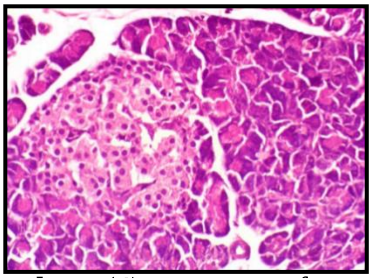

photo (4): Pancreas of rat from group 4 showing no histopathological changes and normal pancreatic Tissue (H & E X 400).

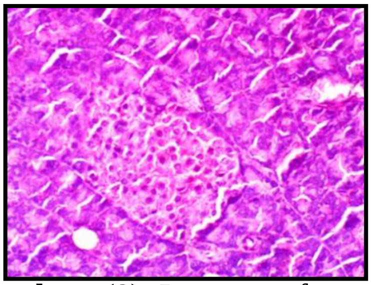

photo (8): Pancreas of rat from group 8 showing no histopathological changes and normal pancreatic tissue (H & E X 400).

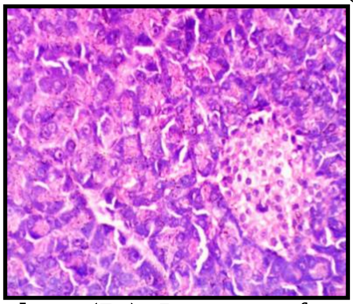

photo (5a): Pancreas of rat from group 5 showing no histopathological changes and normal pancreatic tissue (H & E X 400).

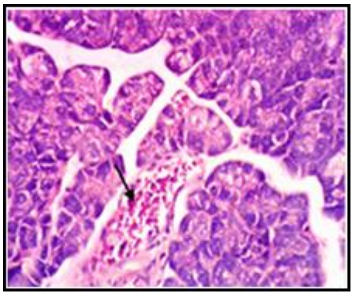

photo (5b): Pancreas of rat from group 5 showing congestion of pancreatic blood vessel (H & E X 400).

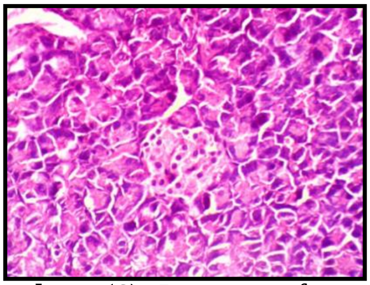

photo (6): Pancreas of rat from group 6 showing no histopathological changes and normal pancreatic tissue (H & E X 400).

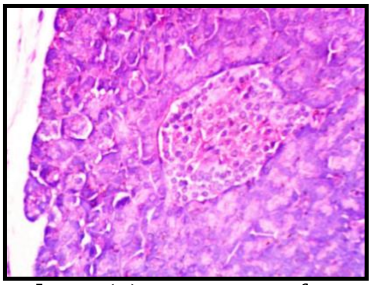

photo (7): Pancreas of rat fromgroup 7 showing no histopathological changes and normal pancreatic tissue (H & E X 400).

Chemical composition of fino bread supplemented with different levels of MO, GS leaves and their combination:

Data in table (6) shows the chemical composition of fino bread. The total carbohydrates represented the major component in fino bread, it contains a high proportion of total carbohydrates, the mean values of carbohydrates decreased gradually with increasing the level of *Moringa oleifera*, *Gymnema sylvestre* leaves and their combination. The moisture content decreased non-significantly ($p \le 0.05$) as compared with the control unsupplemented fino bread). Protein in supplemented fino bread with different levels of (2.5% & 5%) *Moringa oleifera*, (5%) *Gymnema sylvestre* and mix (2.5%MO+2.5%GS) increased significantly ($p \le 0.05$), as compared with the control (unsupplemented fino bread). Data showed that also, a significant increase ($p \le 0.05$) in fat of fino bread supplemented with 5% *Moringa oleifera* as compared with control (unsupplemented fino bread). This data also cleared revealed that the mean values of crude fiber increased gradually with increasing the level of *Moringa oleifera*, *Gymnema sylvestre* leaves and their combination. The highest score was recorded for 5%GS, 5%MO and mix (2.5% MO + 2.5% GS). The mean values of ash increased gradually with increasing the level of *Moringa oleifera*, *Gymnema sylvestre* leaves and their combination. Significant increase ($p \le 0.05$) in ash between fino bread supplemented with levels of 5% *Moringa oleifera*, 5%*Gymnema sylvestre* leaves and their combination (2.5% MO + 2.5% GS), as compared with control unsupplemented fino bread).

Table (6): Chemical composition of fino bread

Nutrient / Treatment	Moisture (%)	Protein (%)	Fat (%)	Crude fiber (%)	Ash (%)	Carbohydrates (%)
Control	13.19 ± 0.30[a]	10.13 ± 0.20[e]	6.23 ± 0.10[b]	0.47 ± 0.10[c]	0.61 ± 0.20[d]	69.37 ± 0.90[a]
2.5%MO	13.01 ± 0.30[a]	10.52 ± 0.20[bc]	6.38 ± 0.10[ab]	0.93 ± 0.20[b]	0.70 ± 0.10[bcd]	68.46 ± 0.30[abc]
5% MO	12.84 ± 0.20[a]	10.91 ± 0.30[a]	6.54 ± 0.10[a]	1.25 ± 0.20[a]	0.94 ± 0.10[a]	67.52 ± 0.90[c]
2.5%GS	13.05 ± 0.30[a]	10.31 ± 0.10[cde]	6.32 ± 0.20[ab]	0.93 ± 0.10[b]	0.67 ± 0.20[cd]	68.72 ± 0.90[abc]
5%GS	12.93 ± 0.10[a]	10.48 ± 0.20[bcd]	6.43 ± 0.10[ab]	1.26 ± 0.02[a]	0.88 ± 0.10[abc]	68.02 ± 0.52[bc]
1.25%MO +1.25%GS	13.03 ± 0.20[a]	10.14 ± 0.10[de]	6.34 ± 0.20[ab]	0.93 ± 0.10[b]	0.68 ± 0.10[bcd]	68.88 ± 0.70[ab]
2.5% MO + 2.5% GS	12.88 ± 0.10[a]	10.70 ± 0.20[ab]	6.48 ± 0.20[ab]	1.25 ± 0.20[a]	0.91 ± 0.10[ab]	67.78 ± 0.80[bc]

MO: *Moringa oleifera*, **GS**: *Gymnema sylvestre*
Values which have different letters in each column differ significantly at ($p \leq 0.05$).

Sensory evaluation of fino bread supplemented with different levels of MO, GS leaves and their combination.

The average scores obtained by the fino bread product in the sensory evaluation are presented in Table (7). Revealed that, color in all supplemented fino bread with different levels of *Moringa oleifera*, *Gymnema sylvestre* leaves and their combination decreased significantly ($p \leq 0.05$) in scores of color, as compared with the control unsupplemented fino bread) because the fino bread samples became darker. Also data showed a significant decrease ($p \leq 0.05$) in the odor of the fino supplemented with different levels of *Moringa oleifera*, *Gymnema sylvestre* leaves and their combination as compared with control. The mean values of the texture of supplemented fino bread showed, non - significant differences ($p \leq 0.05$), between supplemented fino bread with 2.5%GS and 5%GS as compared with control. While the general acceptance of fino bread supplemented with 2.5% GS was the most liked among all the judges. The lowest score of general acceptance was recorded for the fino bread supplemented with 5%MO. On the other hand, data showed that the mean values of total scores decreased gradually with increasing the level of *Moringa oleifera*, *Gymnema sylvestre* leaves, and their combination. The results of the sensory evaluation showed an acceptable fino bread prepared in most proportions of the study.

Table (7): Sensory evaluation score of the fino bread Treatments with different levels of MO, GS leaves and their combination.

Treatment	Color (20)	Odor (20)	Texture (20)	Taste (20)	General acceptable (20)	Total Score (100)
Control	19.30 $\pm$ 0.67 ^{a}	19.60 $\pm$ 0.51 ^{a}	19.30 $\pm$0.94^{a}	19.60 $\pm$0.51^{a}	19.30 $\pm$ 0.67^{a}	97.10 $\pm$ 2.37^{a}
2.5%MO	16.20 $\pm$ 2.78 ^{bc}	16.60 $\pm$ 2.22 ^{b}	16.42 $\pm$3.11^{bcd}	16.65 $\pm$2.80^{b}	16.85 $\pm$ 2.35^{bc}	82.72 $\pm$ 11.32^{b}
5% MO	14.20 $\pm$ 3.35 ^{c}	14.20 $\pm$ 3.70 ^{b}	13.80 $\pm$4.02^{d}	13.95 $\pm$3.87^{bc}	14.10 $\pm$ 3.57^{d}	70.05 $\pm$ 17.70^{c}
2.5%GS	16.75 $\pm$ 2.07 ^{b}	16.35 $\pm$ 3.44 ^{b}	17.05 $\pm$2.98^{ab}	15.75 $\pm$3.45^{bc}	17.38 $\pm$ 1.80^{ab}	83.28 $\pm$ 12.06^{b}
5%GS	14.90 $\pm$ 3.03 ^{bc}	15.20 $\pm$ 3.45 ^{b}	16.70 $\pm$2.86^{abc}	14.40 $\pm$3.68^{bc}	16.60 $\pm$ 2.50^{bc}	77.80 $\pm$12.85^{bc}
1.25% MO + 1.25% GS	16.05 $\pm$ 2.60 ^{bc}	16.20 $\pm$ 2.44 ^{b}	15.94 $\pm$2.89^{bcd}	15.10 $\pm$3.03^{bc}	17.23 $\pm$ 2.32^{ab}	80.52 $\pm$10.51^{bc}
2.5% MO + 2.5% GS	14.00 $\pm$ 3.55 ^{c}	14.60 $\pm$ 3.23 ^{b}	14.20 $\pm$3.48^{cd}	13.40 $\pm$3.65^{c}	14.90 $\pm$ 3.90^{cd}	71.00 $\pm$ 15.69^{c}

MO: *Moringa oleifera*, **GS**: *Gymnema sylvestre*

Values which have different letters in each column differ significantly at ($p \leq 0.05$)

Conclusion:

From the thorough study and investigation of the available literature of *Moringa oleifera* and *Gymnema sylvestre* Leaves, they are clearly shown that the plant serves as an important source of many therapeutically efficient chemicals. From the previous results, this research can conclude that the *Moringa oleifera*, *Gymnema sylvestre* Leaves, and their combination improved serum glucose levels, liver functions, serum lipid profile, and kidney functions in diabetic rats. Such improvements were increased with the increase of the tested plant concentration. This confirms the nutrition and health benefits of the *Moringa oleifera* and *Gymnema sylvestre* Leaves which might be due to flavonoids, tannins, fibers, phenolic acids, alkaloids and gymnemic acids. Found in the herbs. Therefore, we recommended the use of *Moringa oleifera*, *Gymnema sylvestre* leaves and their combination as additives in the area of pharmaceutical industries and different food applications.

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" تأثير بعض الأعشاب الطبيعية علي الفئران المصابة بالسكري "

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ملخص البحث

أجريت هذه الدراسة لتقييم تأثير النسب المختلفة من مطحون أوراق المورينجا ، الجيمنيما سيلفستر ومخلوطهما علي الفئران المصابة بداء السكري، واستخدم في هذه الدراسة 40 فأراً من فئران الألبينو وتم تقسيمهم إلى مجموعتين رئيسيتين،المجموعة الرئيسية الأولى (5 فئران) تتغذى على النظام الغذائي الأساسي كمجموعة ضابطة سالبة ، بينما المجموعة الرئيسية الثانية تم إصابتها بالسكري عن طريق الحقن بمادة الألوكسان

(120 ملجم / كجم من وزن الجسم) ثم قسمت الى سبع مجموعات فرعية ، إحدى هذه المجموعات تم تغذيتها على الغذاء الأساسي فقط كمجموعة ضابطة موجبة ، أما باقي المجموعات فقد تم تغذيتهم على غذاء أساسي يحتوى على مجفف أوراق (المورينجا اوليفيرا(2.5% و 5%) ، الجيمنيما سيلفستر(2.5% و 5%) ومخلوطهما بالتساوي وبنفس النسب السابقة علي التوالي. كما تم إعداد خبز فينو باضافة مجفف أوراق المورينجا ، الجيمنيما سيلفستر ومخلوطهما بالنسب السابق ذكرها. أظهرت النتائج أن حقن الفئران بمادة الالوكسان تسبب في تغير جميع المؤشرات الحيوية، كما أدت تغذية الفئران على الوجبات الغذائية التي تحتوي علي مجفف أوراق (المورينجا ، الجيمنيما ومخلوطهما) إلى انخفاض في مستوى سكر الدم، الكوليسترول الكلي، الدهون الثلاثية، البروتين الدهني منخفض الكثافة، البروتين الدهني منخفض الكثافة جدا، حمض اليوريك، نتروجين اليوريا، الكرياتينين، ونشاط انزيمات الكبد، بينما لوحظ زيادة البروتين الدهني عالي الكثافة مقارنة بالمجموعة الضابطة الموجبة. وأظهرت نتائج الاختبارات الحسية وجود درجة تقبل مناسبة لخبز الفينو المعد بمعظم نسب الدراسة ، لذلك توصي الدراسة بالاستفادة من أوراق المورينجا ، الجيمنيما سيلفستر ومخلوطهما في إعداد خبز فينو يصلح لتغذية مرضي السكر.

الكلمات المفتاحية : المورينجا اوليفيرا ، الجيمنيما سيلفستر ، جلوكوز الدم ، وظائف الكبد ، دهون الدم ، وظائف الكلى.