# Effect of Commiphora myrrha extract on some physiological parameters and histological changes in diabetic albino rats.

## Eman G.E. Helal\*, Ashraf Mahmoud \*\* Essam E. El-Badawy \*\*\* and Anwaar A. Kahwash\*

\*Zoology Department, Faculty of Science ,Al-Azhar, University. \*\*Histology Department, Faculty of Medicine, Al-Azhar, University. \*\*\*Anatomy Department, Faculty of Medicine, Tanta University.

#### Abstract

The present study aimed to clarify the antidiabetic activity of Commiphora myrrha (CM) aqueous extract on thirty adult male albino rats, which were divided into two groups; the first served as a control group, the second was injected with alloxan (120mg/Kg body weight) and divided into two subgroups the first served as diabetic group, the second treated with (CM) water extract (0.05mg/100 gm bwt). After 30 days of the treatment half of each group was sacrificed and the other half was left for other 15 days without any additional treatment (recovery period).

Our results revealed highly significant decrease (p < 0.01) in blood glucose level and highly significant increase in body weight of the diabetic rats with different histological changes in cells of islets of Langerhans. These histological and physiological changes were ameliorated in rats treated with CM.

Water extract of CM has a definite hypoglycemic, hyperinsulinimic effect, on the other hand, a significant increase in body weight,  $\beta$  cell number and liver glycogen contents were achieved.

The results of the present study clarify the role of CM as an active antidiabetic plant and suggest a relationship between drenching CM extract and insulin production. Other of investigations want be done to detect effects of different doses and time intervals of CM in diabetic animals.

#### Introduction

Diabetes is possibly the world's fastest growing metabolic disease, and as knowledge of heterogencity of this disorder increases, so does the need for more appropriate therapies (*Baily* and *Flott*, 1986).

Traditional antidiabetic plants might provide useful source of new oral hypoglycemic compounds for development countries as pharmaceutical entities, or as simple dietary adjuncts to existing therapies. A scientific investigation of traditional herbal remedies for diabetes mellitus may be valuable and leads to development of an alternative drugs and therapeutic strategies. Alternatives are clearly needed because of the inability of current therapies to control high cost and poor availability of current therapies for many rural populations, particularly in developing countries (*Marles* and *Farnsworth*, 1995).

Alloxan and streptozotocin (STZ) were found to be selectively  $\beta$ -cytotoxic agents in animals and extremely potent diabetogenic substances (*Dunn et al., 1943* and *Rakieten et al., 1963*), so alloxan and STZ have been widely used to produce diabetes in experimental animals (*Okamoto, 1984*).

Commiphora myrrha (Myrrh) Family (Burseraceae) is native to Northeastern Africa, especially Somalia. Myrrh is one of the oldest known medicines and was widely used by the ancient Egyptians. It is an excellent remedy for mouth and throat problems, with a drying, slightly bitter taste, and it also useful for skin problems, atherosclerosis, hemorrhoid, heptoses, high cholesterol, stomatosis, immunodepression and hyperglycemia. The myrrh's Gumresin-volatile oil are the main used parts, where it contains (30-60%) gum including acidic polysaccharides, resin(25-40%), volatile oil (3-8%), heerabolene, eugenol and many furansesquiterpenes (*Chevallier*, *1996* and *Duke*, *2002*).

The present study was a trial to clarify the effect of the Commiphora myrrha as hypoglycemic agent and its effect on different cells of islet of Langerhans.

# Material and Methods:

Thirty mature male albino rats (weight  $120 \pm 20$  gm) were assigned randomly into three groups. The first group (group I) served as control. The remaining twenty rats were fasted over night, then injected with a single subcutaneous dose of alloxan (120mg/kg b. wt.). After 48 hours of alloxan injection, blood glucose levels were measured by glucometer. Rats with fasting blood glucose level more than 250mg/dl considered diabetic. Then divided into two subgroups each of them has ten rats, the first (subgroup I, diabetic group), second one(subgroup II, diabetic rats treated with Commiphora myrrha (0.05g/ 100g b.wt).

After 30 days of treatment, 5 animals of each group were decapitated, while other rats were kept for 15 days more, without any additional treatment to follow up if there is any delayed effect of the treatment. Each rat was weighted at the beginning and the end of the experiment. Percentage of body weight changes were calculated. Blood sera were collected for the determination of serum glucose level (*Tietz, 1986*) & serum Insulin level (*Reeves, 1983*). Samples from liver were collected for Determination of liver glycogen content (*Joseph (1955*). The samples of pancreas were obtained and embedded in paraffin blocks then stained with Hematoxylin & Eosin (HX&E).

Sections were examined under the Microscope.Sections of the pancreas from each group were stained using 2 different techniques:

- **1-Hematoxylin and Eosin (HX & E) stain:** such stain was used for demonstrating the histological changes.
- 2-Modified aldehyde fuchsin stain (*Halami, 1952*): such stain used for detecting different cells of islets of Langerhans. And used image analysis for determination alpha, beta and delta cells number in the islet of Langerhans. The diameter of cells (alpha, beta and delta) and the nuclear diameter of them were also measured.

Data were analyzed using student (t) test, significant differences between the means of control and treated groups were considered at p<0.05 (*Sokal* and *Rohif*, 1981)

# Results

## The result of the present study showed.

- Percentage of body weight change (%):

Concerning the percentage of body weight change, highly significant decrease (p<0.01) was recorded in body weight gain in diabetic group. Otherwise, highly significant increase (p<0.01) was caused by Commiphara myrrha treated group through the treatment period then turned back to the normal value after the recovery period when compared with control rats (Fig. 1).

## **Biochemical analysis** :-

Serum glucose level:

Serum glucose level, the present data showed sever hyperglycemia (p < 0.01) in diabetic rats when compared with control group throughout the experiment. While, Commiphara myrrha treated group showed insignificant changes when compared with control rats during the experimental period (Fig 2).

## The liver glycogen content:

The present study showed high significant decrease (p < 0.01) in liver

glycogen content in diabetic group when compared with control group throughout the experimental period . Commiphara myrrha treated group showed insignificant change when compared with control group till the end of the experiment (Fig. 3)

#### The level of insulin:

In the present data sever hypoinsulinemia (p<0.01) was recorded in alloxan treated group when compared with control rats throughout the experiment. Otherwise, Commiphara myrrha extract treated group showed insignificant change when compared with control group after treated and recovery periods (Fig. 4).

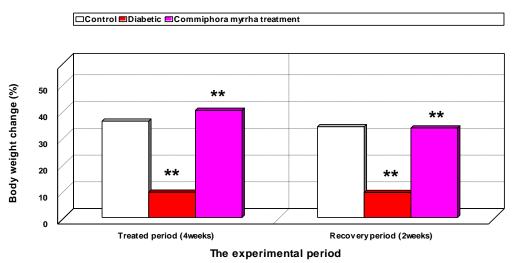
#### Histological studies:

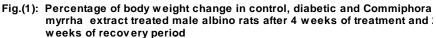
The islets of Langerhans of pancreas in normal animals were scattered throughout the pancreas as irregular, spheroidal masses with rich vascular supply and all cells are granular with central spherical nuclei. Beta-cells which are the most abundant cells and occupy the core of the islets and contain numerous granules. Alpha and delta cells form the periphery of the islets (Figs. 8, 11&12).

The current study indicated insignificant change in the number and diameter of alpha cells and their nuclear diameter in diabetic and Commiphora myrrha treated group as compared to control till the end of the experiment (Figs. 5a, 6a and 7a).

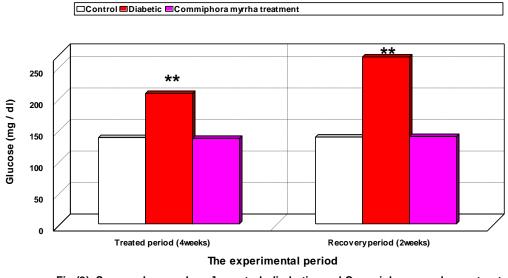
The islets of diabetic rats showed reduction in pancreatic beta cell number, severe  $\beta$ -cell necrosis, intracellular vaculation and degranulation in some surviving B-cells which recorded a significant increase in their cellular and nuclear diameter when compared with control group throughout the experimental period. While, Commiphora myrrha extract ameliorated the changes represented by increased number of islet cells. The number of B-cells appeared to be increased and the islets appeared more organized and less vacuolated. Also there is no change in cellular and nuclear diameter of B-cells when compared with normal islets during the experimental period (Figs.5b,6b,7b,9,10,12 &13).

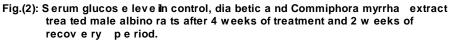
The present data showed insignificant changes in delta cells number, diameter and nuclear diameter in the diabetic group and recorded a significant increase after recovery period. Otherwise, the treated group recorded a significant decrease throughout the experiment (Figs. 5c, 6c& 7c).



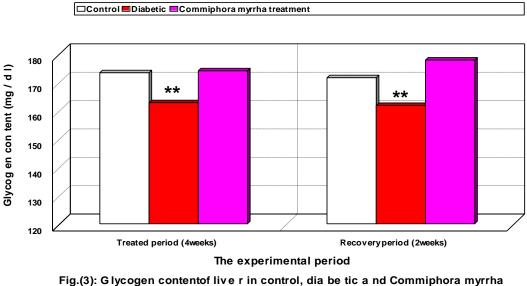


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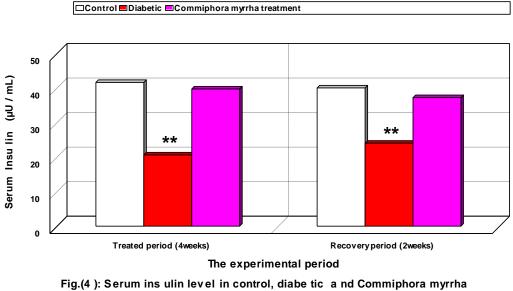


(\* = Signif icantat p< 0.05 - \*\* = Highly signif icant at p< 0.01)



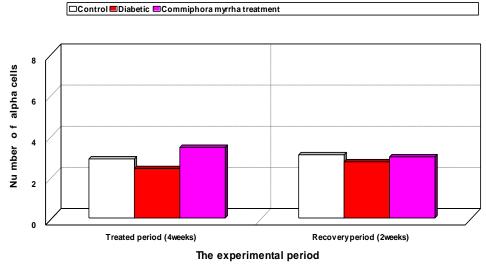
extrac ts treated ma le a lbino ra ts a fte r 4 w e eks of trea tment a nd 2 w e eks of recov e ry period.

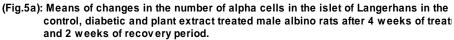
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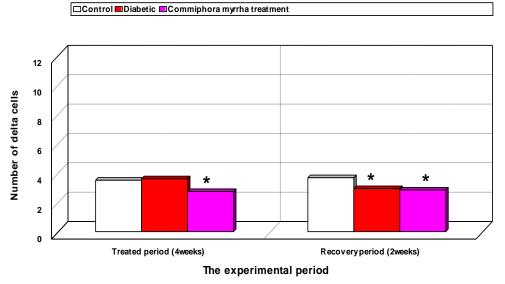
extract trea te d male albino ra ts after 4 weeks of treatment and 2 weeks of recovery period.

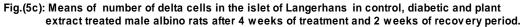
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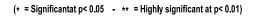




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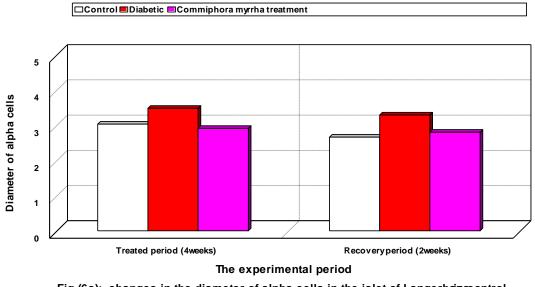
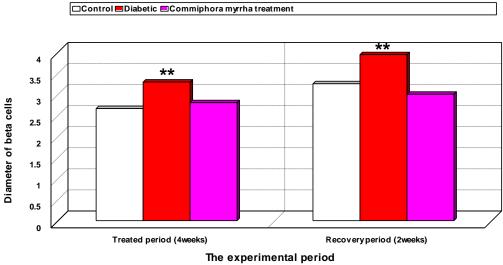
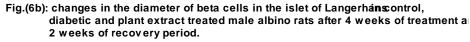
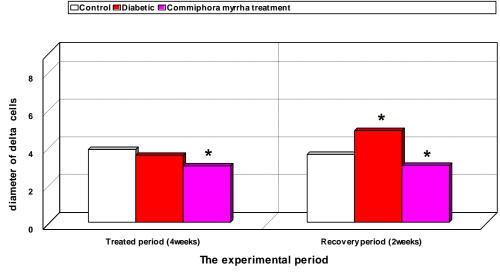


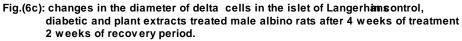
Fig.(6a): changes in the diameter of alpha cells in the islet of Langerhainscontrol, diabetic and plant extracts treated male albino rats after 4 weeks of treatmer and 2 weeks of recovery period.

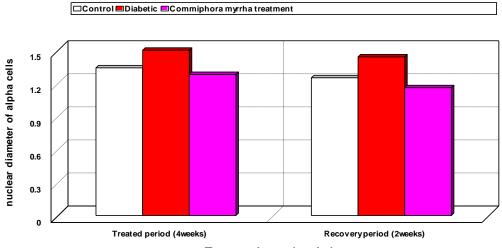




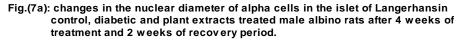
(\* = Significantat p< 0.05 - \*\* = Highly significant at p< 0.01)







The experimental period



(\* = Significantat p< 0.05 - \*\* = Highly significant at p< 0.01)

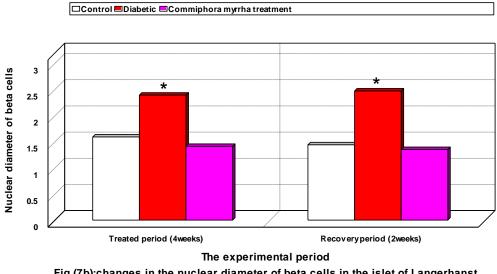
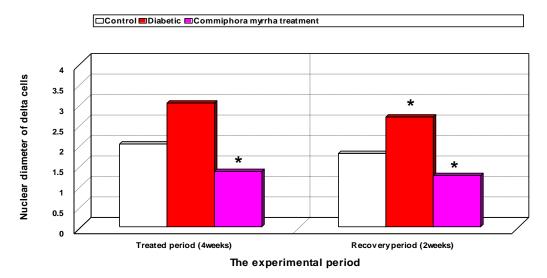
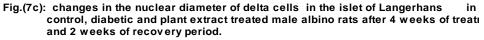
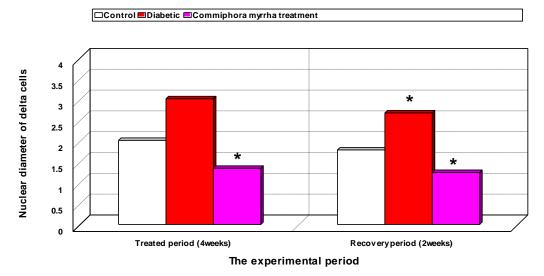


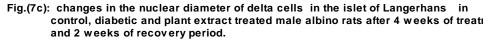
Fig.(7b):changes in the nuclear diameter of beta cells in the islet of Langerhanst in control, diabetic and plant extracts treated male albino rats after 4 weeks treatment and 2 weeks of recovery period.

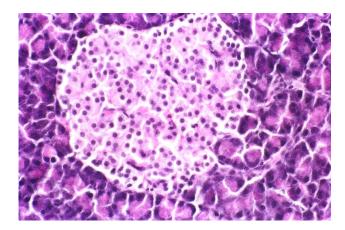




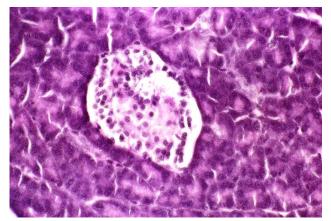
( \* = Significantat p< 0.05 - \*\* = Highly significant at p< 0.01)



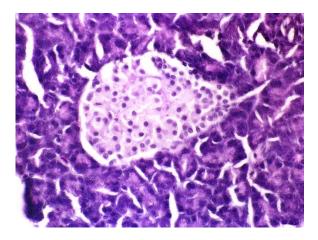




(Fig. 8): A photomicrograph of a section in the pancreas of control adult rat showing islets of langerhans, β cells and α cells. (HX&E X 400).

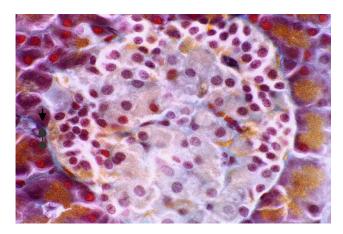


(Fig. 9): A photomicrograph of a section in the pancreas of diabetic albino rat showing pale stained cells vaculated and degenerated β cells. (HX&E X400).

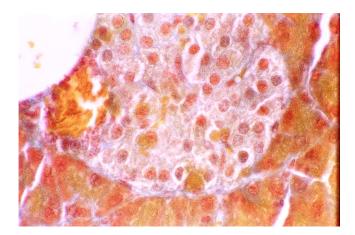


(Fig. 10): A photomicrograph of a section in the pancreas of Commiphora myrrha treated rat showing slightly vaculated  $\beta$  cells with rounded nuclei and deeply stained basophilic nuclei of  $\alpha$  cell. (HX&EX 400).

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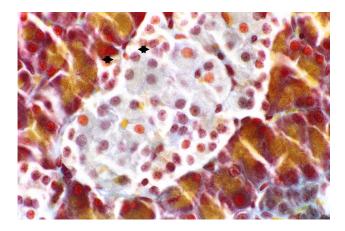


(Fig. 11): A photomicrograph of a section in the pancreas of control adult rat showing rounded or oval β cells violet, oval green δ,cells and irregular yellow α cells. (Modified aldhyde fuchsin X1000).



(Fig. 12): A photomicrograph of a section in the pancreas of diabetic albino rat showing pale disintegrated nuclei , normal structure of the exocrine glands and vaculated  $\beta$  cells , which appeared devoid of cytoplasmic organoids.

(Modified aldhyde fuchsin X1000).



(Fig. 13): A photomicrograph of a section in the pancreas of Commiphora myrrha ingested rat showing normal β cells. (Modified aldhyde fuchsin X1000).

# Discussion

Diabetes is a common disease, with major global public health Consequences (*Williams* and *Pickup*, 1999). The diabetic patients needed an alternative therapies to control all of the pathological aspects of diabetes and the high cost and poor avaliability of current therapies in many rural populations, particularly in developing countries (*Marles* and *Farnsworth*, 1995). The traditional antidiabetic plants might provide this useful source of new oral hypoglycemic compounds. So, this study is a step to evaluate and follow up the effect of Commiphara myrrha water extract as a hypoglycemic agents.

The present results revealed a significant decrease in percentage of body weight change after one moth of subcutaneous injection of alloxan in comparison with the control rats. Depression in the body weight gain may be explained by depression of synthesis of DNA and RNA in the diabetic animals and / or it is attributed to different side effects of the ability to use carbohydrates including lypolysis, glycogenolysis and acidosis *Abdel-Moneim et al. (1996), Rawi et al. (1996), Ganong (2003)* and *Helal et al.,(2003).* 

Our data also, detected an increase in the percentage of body weight change in treated group when compared with diabetic and control groups. This treatment may be stimulate most aspects of carbohydrate metabolism, including rapid uptake of glucose by the cells, enhanced glycolysis, enhanced gluconeogenesis, increased rate of absorption from the gastrointestinal tract and even increased insulin secretion with its resultant secondary effects on carbohydrate metabolism (Guyton and Hall, 2000). And may be also due to its activities in strengthening the gastrointestinal tract by increasing both the rate of secretion of the digestive juices and the motility of the gastrointestinal tract (Guyton, and Hall, 2000), so it is taken for indigestion (Chevallier, 1996 and Duke, 2002).

The present data recorded severe hyperglycemia and hypoinsulinemia in

diabetic rats. Hyperglycemia can be considered as a direct reflex to the marked hypoinsulinemia caused by the selective destructive cytotoxic effect of alloxan on the B-cells of the pancreas which lead to decrease of their number. Because it has a direct effect on membrane permeability by causing failure of ionic pumps and increased cell size. And also inhibit intracellular energy generation and insulin secretion. The decrement in B-cells number caused sudden activation of quiescent cells for a high level of protein synthesis and produced rapid and massive beta cell death (Majno and Joris, 1999).

The results of the present study also showed B-cells with vacuolated cytoplasm in diabetic group. Vacuolation of the islet is the most prominent lesion associated with functional islet abnormality and development of hyperglycemia (Bolaffi et al., 1986 and Kessler et al., 1999). Also, it may be due to the diabetogenic action of alloxan which induced highly reactive oxygen radicals, which are cytotoxic to the B-cells (Fischer and Homburger, 1980). According to Yamamoto et al. (1981) and Ronald (1988) the fragmentation of nuclear DNA of pancreatic B-cells seems to be important for the development of diabetes and supposed to be resulted from the accumulation of superoxide or hydroxyl radicals in the B-cells .

On the other hand, the treated group recorded insignificant change in beta cells number and diameter as compared to normal group. This plants may have stimulatory effect on the division of beta cells or contain nonmetabolizable 2-deoxy and 3-O-methylglucoses, which share the entry site block the diabetogenic action of alloxan and restore insulin production (*Shafrir,2003*). *Augusti* and *Sheela* (1996) mentioned that some plants exert their effect on beta cells through both protection of the already present beta cells due to their antioxidant effect and through stimulation of the beta cells to release insulin.

The hypoglycemic and hyperinsulinemic activities of Commiphora myrrha may be attributed to it's phytosterols, which have a hormonal action (*Chevallier*, 1996) or, to its polysaccharides content which have hypoglycemic activity in animals (*Evans*, 2001).In contrast to the present data *Duke et al.* (2002) reported that Commiphora myrrha had antihypo-glycemic activities.

It can be concluded from the above mentioned results that the increased rate of glycogenesis together with the decreased gluconeogenesis in diabetic rats treated with tested result in a suppression of hepatic glucose output which tends to ameliorate blood glucose level and to improve glycemic state of diabetic animals.

In the present study, the decrease in liver glycogen content of diabetic rats may be a result of increasing glucose output during insulin deficiency (Gold, 1970). And may be due to the loss of glycogen synthetase-activating system (Annamala and Augusti, 1980) and/or increased activity of glucose-6-phosphatase (Abdel-Moniem et al., 2001). It is possible that the increase in liver glycogen content after treatment with the extract of Commiphora myrrha is a result of increased insulin level which has a potent effect on glycogen synthetase activity as well as on hepatic hexokinase and glycogen-6-phosphatase activity (Sheela and Augusti, 1992).

## Conclusion

The water extract of Commiphora myrrha appeared useful agent in reducing hyperglycemia by increasing both insulin and regeneration of B-cells and increasing serum insulin level. More detailed studies on this plant must be done at different doses and different periods of observation before reaching a clear cut conclusion about the future of this plant for the treatment of diabetes mellitus.

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أجري هذا البحث لدراسة تأثير المستخلص المائي لنبات المر كمخفض لسكر الدم المستحدث في الجرذان البيضاء و الذين تم تقسيمهم الي مجموعتين . الاولي اعتبرت مجموعة ضابطة والثانية تم حقنها بعقار الالوكسان (120مج/كجم من وزن الجسم) وقسمت الي تحت مجموعتين: 1- محموعة مريضة بالسكر

2- مجموعة مريضة بالسكرو معالجة بالمستخلص المائي لنبات المر (0.01مج/كجم من وزن الجسم).

تم ذبح نصف عدد الجرذان بعد ثلاثين يوما من العلاج ثم تم ذبح النصف الأخر بعد 15 يوم بدونِ اي علاج اضافي كفترة استشفاء.

كما أظهرت النتائج زيادة ملحوظة في وزن الجسم و انخفاض كبير في سكر الدم كما أوضحت ارتفاعا كبيرا في محتوي الكبد من الجليكوجين و نسبة الانسولين في الدم و عدد خلايا بيتا في البنكرياس في المجموعة التي تم معالجتها بالمستخلص المائي لنبات المر اذا ما قورنت بالمجموعة المصابة بالسكر و التي لم يتم معالجتها. ومما سبق يتضح فاعلية نبات المر في تخفيض مستوي السكر و زيادة نسبة الانسولين في الدم.

ولكن يلزم مزيد من الدر اسات لبيان الجرعة المناسبة و كذلك الفترة المثلي للعلاج. كما يلزم تتبع آي آثار جانبية للنبات إن وجدت.