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The role of bitter melon extracts in reducing the incidence of some metabolic markers, lipid profile and morphological malformation in diabetic mother rats and their newborn

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Abstract: Diabetes mellitus (DM) is a common metabolic disease characterized by chronic hyperglycemia. Searching for convenient treatment is a potential goal that scientists seek. *Momordica charantia*, popularly stated by the term bitter melon (BM) has many benefits as it contains numerous active materials, and it has antioxidant and antihyperglycemic effects. The present study aimed to evaluate the role of BM in reducing the incidence of abnormalities in diabetic pregnant rats and their offspring. Forty pregnant albino rats were randomly divided into 4 groups: Group I, control group; Group II, BM treated group; Group III, Diabetic group; Group IV, Diabetic and BM treated group. The maternal rats with diabetes had a significant rise in the serum progesterone also estrogen hormones, while serum follicle-stimulating hormone (FSH) and Luteinizing Hormone (LH) were significantly decreased. Moreover, the total cholesterol (TC), triglyceride (TG) and low-density lipoprotein cholesterol (LDL-C) content were significantly increased in the diabetic maternal group, otherwise, high-density lipoprotein cholesterol (HDL-C) concentration was significantly decreased. There was a noticeable change in the count, weight, and length of newborn delivered to diabetic mothers. Cerebellum tissue homogenate of newborns from diabetic maternal group showed a significant increase in nitric oxide (NO) and a significant decrease in superoxide dismutase (SOD) activity. Conclusion: Treatment of maternal diabetic females with BM has improved most of the tested biochemical and morphological parameters.

keywords: Bitter melon, Diabetes mellitus, Lipid profile, Female sex hormones.

1.Introduction

Diabetes mellitus (DM) refers to a variety of metabolic diseases whose main finding is chronic hyperglycaemia. The cause is either a lack of insulin secretion or an unusual insulin effect, or mainly both **(1).** The number of people with diabetes is expected to rise to 417 million by 2030 and to 486 million by 2045 **(2)**.

Type 1 diabetes mellitus (T1DM) and type 2 diabetes mellitus (T2DM) are the two most prevalent subtypes of the different kinds of DM. T1DM accounts for about 5-10% of all patients with DM which is caused by defective β-cell function, decreased insulin release, and decreased levels of blood insulin. About 90–95% of people with diabetes have T2DM, which is the most common type of DM and is mostly caused by an insufficient response to insulin (reduced insulin sensitivity) and insulin resistance in peripheral tissues **(3)**. Another subtype of DM is gestational diabetes affects pregnant women due to hormonal changes during pregnancy **(4)**.

The usual signs of diabetes, such as polyuria, polydipsia, and polyphagia, are present in T1DM, it develops severely hyperglycemia quickly and has very high levels of hyperglycemia in people with T2DM. Severe weight loss is common only in T1DM or if T2DM remains undetected for a long period. Undiagnosed diabetes is also frequently characterized by unexplained weight loss, exhaustion, restlessness, and physical pain. Simple symptoms or those that appear gradually may also go neglected. **(5)**.

About 40% to 45% of T1DM and T2DM women experience pregnancy complications due to hypertension. **(6)**. In comparison to the general population, studies of women with preexisting diabetes showed greater incidence of perinatal death, congenital abnormalities, hypertension, preterm delivery, caesarean delivery, and other neonatal morbidities. **(7)**. Pregnancies of women with pregestational diabetes are typically affected by retinopathy, nephropathy, and neuropathy **(8).**

Outside of mainstream medicine, the usage of herbs is ancient. As clinical research and advancements in analysis and quality control demonstrate the efficacy of herbal medicine in the treatment and prevention of disease, it is becoming increasingly widely used **(9)**.

According to studies, one-third of people with DM use complementary and alternative therapies **(10)** that use dietary supplements and plant products (herbal products) in addition to or instead of conventional medical care **(11)**.

Momordica charantia, or Bitter melon (BM), is a tropical native that is part of the Cucurbitaceae family and consumed as a vegetable in nations like China, India, and others **(12)**. Tonic, stomachic, stimulant, emetic, laxative, and antihyperglycemic properties are associated with the fruit **(13)**. Its anticancer, anthelmintic, antiulcer, antifertility, antimalarial, antibacterial, antipsoriasis, and immunomodulatory properties make it a component of several polyherbal antidiabetic preparations **(14)**.

The medicinal value of BM in the treatment of diabetes has been reported internationally. In chemical terms, BM has a substance that closely resembles insulin and can often be referred to as p-insulin. Researchers have found that it can replace insulin in the body when taken frequently for a while. Additionally, it includes steroidal saponins known as charantin, peptides, and certain alkaloids that efficiently regulate blood sugar levels **(15)**.

2. Materials and methods

2.1. Materials

2.1.1. Chemicals

High purified Streptozotocin (STZ) and Nicotinamide (NA) were obtained from Sigma-Aldrich Chemical Company (St. Louis, Missouri, USA). BM fruit powder (Bixa Botanical) was ordered from NineLife in the UAE. All other chemicals and reagents used were of analytical grade.

2.1.2. Animals

Sixty albino rats weighing 150-200 g (40 adult females and 20 adult males) were used for this research. They arrived from Helwan, Egypt, *via* the Egyptian Institute for Vaccine and Serological Production. The animal house became their place of stay. Rats were kept in daily-refilled stainless-steel cages with bedding made of fine and smooth wood chips changed every other day. Before starting the experiment, all rats were let to be acclimatized for the animal house conditions for two weeks under controlled temperature (25°C ±2), cycles of 12 hours of light/12 hours of darkness and supplied with a typical commercial rat diet. During the whole experimental research time, unlimited access to water was permitted. The regional experimental animal ethics committee of the Faculty of Science has authorized the experiment to be conducted following the guidelines provided by the National Research Council about the handling, care, and treatment of laboratory animals that are employed.

2.2. Experimental design

2.2.1. Induction of diabetes

Twenty minutes prior to injecting STZ (55 mg/kg, intraperitoneal; i.p.), NA (100 mg/kg), was given i.p. to induce experimental T2DM **(16)**. NA and STZ were freshly prepared in citrate buffer (pH 4.5) **(17)**.

2.2.2. Animal groups

The adult 40 female rats were grouped into: a) Diabetic female rats (20 animals). b) Non-diabetic female rats (20 animals).

After that proper conditions for mating were prepared (each two females were incorporated with one adult male), vaginal plug or sperms in the vaginal smear was used to determine the onset of pregnancy. After that, the two groups of ten diabetic female rats were separated as follows: diabetic group only and DM treated group which received oral doses of BM (250 mg/kg) daily, and the non-diabetic female rats. Treated females were received BM daily during the whole period of pregnancy (till giving birth). After birth, the mothers and the neonatal rats from each group were used to assess the subsequent parameters. Blood samples collection and tissue preparation were proceeded.

2.2.3 Sample preparation

2.2.3.1. Blood collection and serum preparation

For serum collection, firstly blood was drawn from jugular vein of each rat at the end of experimentation period. Then, it allowed to clot at room temperature for about 20 min. Serum was separated for each blood sample by centrifugation at 1000 xg for 10 min, aliquots were made in many Eppendorf tubes and stored at - 20 °C for subsequent biochemical analyses in serum.

2.2.3.2. Isolation of cerebellum and cerebellum homogenate preparation

The cerebellum from each rat was excised immediately, washed with cold saline (0.9 %), cleaned, and dried with lint free tissue. A suitable weight (0.3 g) of left cerebellum was homogenized. The homogenate was then followed by centrifugation at 12,000 xg for 20 min at 4 °C. The supernatant for each cerebellum was collected to be used for the subsequent biochemical parameters **(18)**.

2.3.2. Determination of lipid profile

Serum triglycerides (TG) concentrations was estimated according to the method of **(19)**. Serum TC concentrations was estimated according to the technique **(20)**. Using an enzymatic colorimetric technique, the concentration of serum LDL-C was detected **(21)**. Serum HDL-C concentration was determined using enzymatic colorimetric method **(22)**.

2.3.3. Determination of hormones

The level of FSH and LH was determined according to the method of **(23)** using Rat ELISA Kit produced from MyBioSource (San Diego, California, USA). The level of progestrone was determined using Rat Progesterone ELISA Kit produced from MyBioSource (San Diego, California, USA). Catalog No.: MBS762170. The level of estrogen was determined according to the method of **(24)** using Rat FSH ELISA Kit produced from MyBioSource (San Diego, California, USA).

2.3.6. Newborn analyses

Morphological abnormalities of the newborns were detected. Activity of superoxide dismutase (SOD) in cerebellum of control and different newborns from maternal groups were assayed by the procedure of **(25)**, using kit from Biodiagnostic Co. Dokki, Giza, Egypt. Nitric oxide (NO) concentration in cerebellum tissue was determined using BioDiagnostic Kit, Egypt **(26)**.

2.4. Statistical analyses

Data from the study were conducted using GraphPad 5.0 software (GraphPad Software Inc., San Diego, California, USA). The findings are shown as mean ± the standard error of the mean (SEM) for the six subjects. One-way analysis of variance (ANOVA) was used for statistical comparisons, and the Neuman-Keuls post-hoc test was used after (**27**). Any higher significance threshold was reported, and a significant difference was assumed when the P value was < 0.05.

3. Results and Discussion

3.1. Results

Morphological abnormalities of newborn

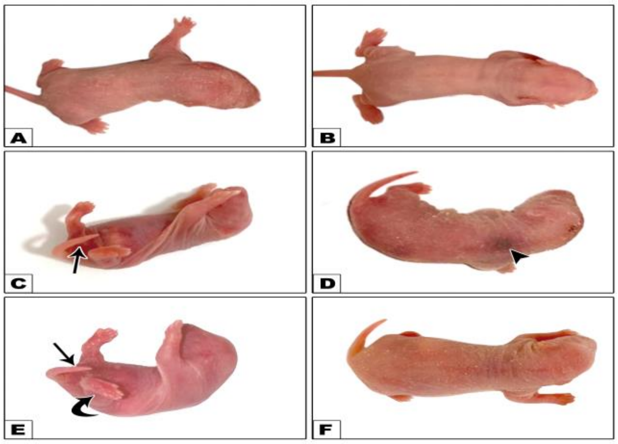

Fig. 3.1.1. Photograph of newborn rats from control mother and other studied groups on the day 21 of delivery. Control newborn (A) appeared normal morphology, BM newborn (B) as the control showing normal morphology. (C, D &E) newborn of diabetic group. (C) showed kinky tail & small size (arrow), (D) showed short neck and abnormally large size of fingers (dactylomegaly) (head of arrow), and (E) showed subcutaneous hematoma abnormal bending of the body and kinky tail with malformed limb (curved arrow). (F) Diabetic

newborn supplemented with BM with normal appearance of newborn.

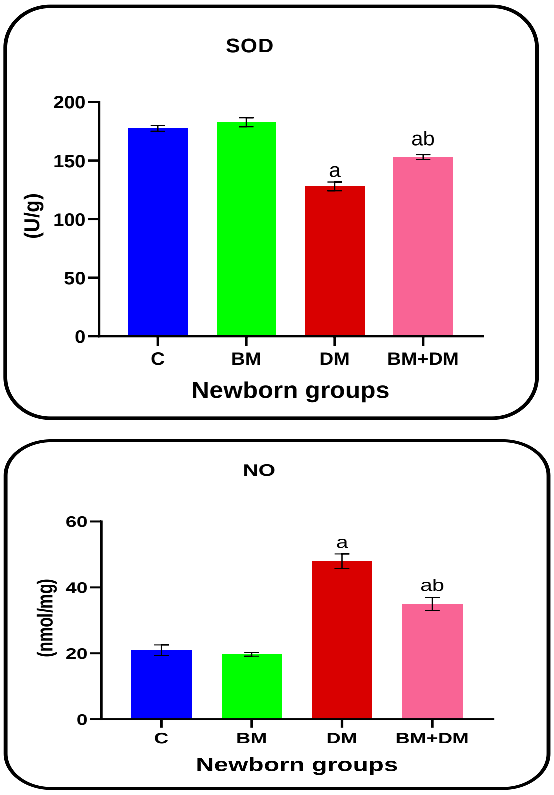

Fig.3.1.2. Superoxide dismutase (SOD) and nitric oxide (NO) levels in cerebellum of newborn in control and different newborn groups.

Table 3.1.1. Maternal reproductive performance in the control and the other experimental groups

Parameters	C	MC	DM	DM+BM
Number of pregnant rats	6	6	6	6
Number of corpuses lutea	14.5	13.6	13.3	13.6
Number of alive newborn	5.5	5.3	3	5
Weight of newborn	4.5	4.2	4	4.4
Length of newborn	2.9	2.45	1.9	2.3

Values are expressed as means.

There was a significant reduction in SOD activity in the cerebellum homogenate newborn of diabetic mother group as compared to newborn of control mothers. Newborn of diabetic mothers obtained oral treatment of BM daily till birth produced a considerable increase in SOD activity as compared to the newborns of diabetic mothers. There was a significant increase in NO content in the cerebellum homogenate of newborn of diabetic mother group as compared to newborn of control mother group. In comparison with newborn of diabetic mother group, newborn of diabetic mother group treated with BM showed a significant decrease in NO. However, NO level did not reach the levels observed in the newborn of control maternal group.

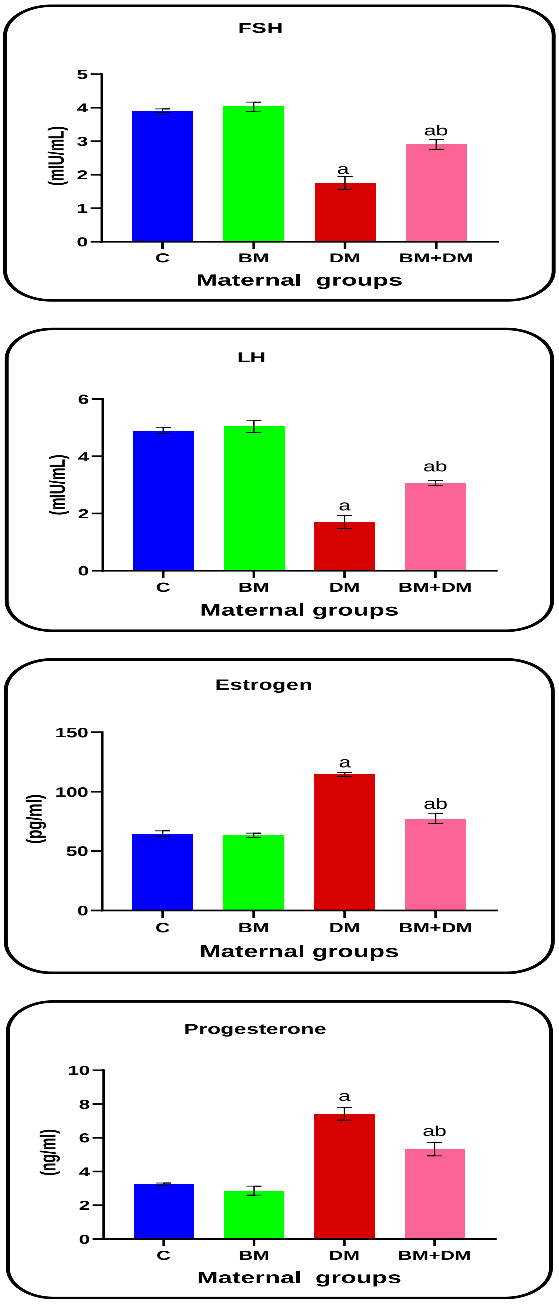

Fig.3.1.3. Serum follicle stimulating hormone (FSH), luteinizing hormone (LH), estrogen and progesterone in control and different maternal groups.

The present results showed a significant reduction in serum FSH and LH, and elevation in serum progesterone and estrogen levels when comparing maternal rats with diabetes to the control animals. In comparison with untreated rats, diabetic rats treated with BM showed a significant increase in serum FSH and LH, and a significant decrease in serum progesterone and estrogen levels.

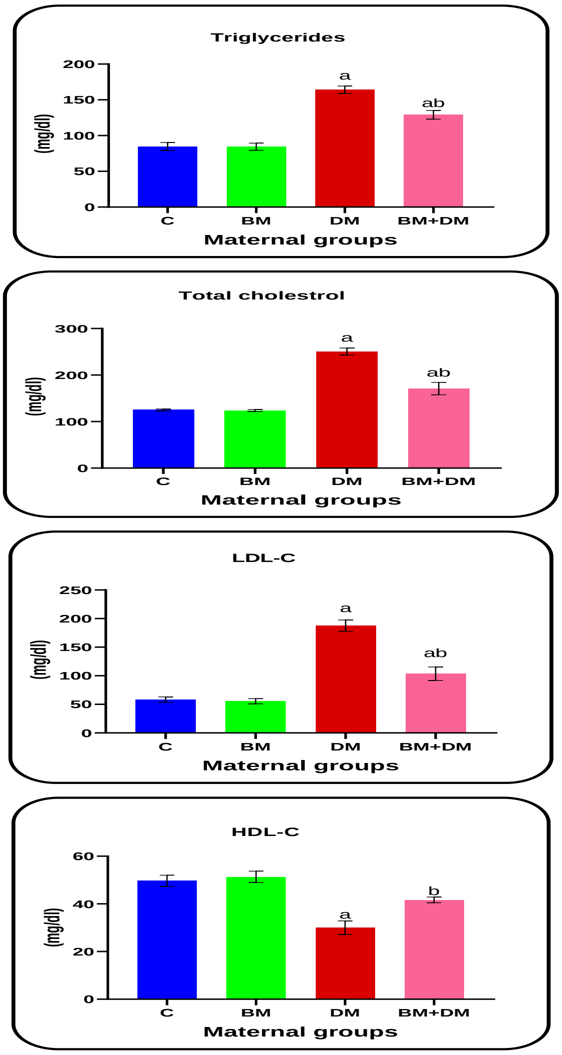

Fig.3.1.4. Serum triglycerides (TG), total cholesterol (TC), low density lipoprotein cholesterol (LDL-C) and high-density lipoprotein cholesterol (HDL-C) in control and different maternal groups.

Compared to the control mother rats, the levels of TG, TC and LDL-C were significantly higher in diabetic rats. Notably, treatment of diabetic rats with BM (250 mg/kg/day till birth) showed a reduction in serum TG, TC and LDL-C levels as compared to untreated diabetic rats, while HDL-C levels were significantly decreased in serum of diabetic rats as compared to the control rats. HDL-C in the BM supplemented groups reached normal levels when compared to the untreated diabetic rats.

3.2. Discussion

Diabetes has become an epidemic issue both in developed and developing countries in recent years **(28)**. Searching for a safe diabetes treatment is a requirement that scientists seek. Bitter melon is widely used as a vegetable in daily food in several countries in Asia **(29)**. Experimentally, the bitter melon fruit extract demonstrated potent antioxidant, hypoglycemic and potential therapeutic effects against metabolic dysfunction related to diabetes and obesity **(30)**.

In the present study, the newborn of diabetic mother group showed abnormal size, kinky tail and malformed legs compared to other groups. Diabetic embryopathy, a diabetic consequence, is more likely to result in congenital abnormalities in women with preexisting T1DM or T2DM **(31)**. Pregnancies with diabetes are up to five times more likely to result in congenital abnormalities **(32)**. Early organogenesis causes malformations during the first 10 weeks of pregnancy **(33)**. It is known that a mother's condition must be well-metabolized to reduce the danger of fetal abnormalities. Thus, in addition to the excesses of triglycerides, fatty acids, ketones, and variably glycerol, hyperglycemia is thought to be the most significant teratogen **(34)**. Reactive oxygen species (ROS) are produced during hyperglycemia throughout pregnancy, and there is no doubt that this leads to an oxidative stress state in the intrauterine environment. Damage to the embryo, placenta, fetus, and progeny may result from this disorder **(35)**.

The present results revealed that diabetic mother rats had significantly increased levels of TG, TC, and LDL-C, while HDL-C was decreased compared to the control and combined groups. These results go parallel with **(36)**. Previous studies reported similar findings **(37), (38)** for TC, **(39)** for LDL-C and TG, and **(40)** for HDL-C. Increased lipid breakdown and the release of free fatty acids from peripheral depots might be the cause of the rise. Since hormone-sensitive lipases are inhibited by insulin, they become active in the absence of insulin and promote lipolysis **(41)**. In the hepatic tissue, diabetes causes a reduction in lipogenesis and an increase in lipolysis due to the underutilization of glucose, which also stimulates the activities of gluconeogenic enzymes **(42)**. In contrast, therapy with BM in the present study substantially decreased the levels of TG, TC, and LDL-C in diabetic maternal group, whereas HDL-C levels returned to normal. These outcomes are consistent with **(43)** and **(44).** The oxidation of glucose, which reduces glutathione levels, is the cause of the difference in results between LDL-C and HDL-C. These effects are caused by elevated blood glucose levels, which raised triglyceride and LDL-C levels while lowering HDL-C levels **(45)**.

The current investigation found that the development of diabetes significantly increased serum levels of progesterone and estrogen while significantly lowering serum levels of FSH and LH in diabetic mother rats. The same results were reported by other investigations **(46)** for FSH and LH, and **(47), (48)** for estrogen and progesterone. Elevated levels of bioavailable ovarian steroid hormone are caused by hyperinsulinemia and/or insulin resistance (IR), especially in postmenopausal women with T2DM **(47)**. Diabetes affected women's hormones, causing an increase in the synthesis of androgen and estrogen **(49)**. On the other hand, when BM was administered to diabetic mother rats, serum levels of FSH and LH significantly elevated, whereas serum levels of progesterone and estrogen significantly decreased. These findings were consistent with **(50)** for FSH and LH, and **(51)** for estrogen and progesterone. FSH and LH levels of diabetic rats were elevated by the aqueous extract of BM fruits **(50)**. Because the phytochemicals in BM have antioxidant and antidiabetic potential and can counteract the effects of free radicals, it is possible that the increased serum levels of FSH and LH in the rats supplemented with BM were caused by these factors. Also, BM leaf extracts significantly decreased the levels of progesterone and estrogen **(51)**.

In the present study the neonate's cerebellar tissue homogenate from diabetic mother rats showed a significant decrease in SOD and an increase in NO content following the introduction of diabetes. equivalent outcomes have been published by **(52)** for SOD. The failure of mitochondrial electron transport and capillary degeneration caused by diabetes were avoided by overexpressing SOD **(53)**. One theory for the decline in SOD activity is the buildup of hydrogen peroxide **(54)**, other studies mimic the present results regarding NO. On the other hand, supplement of diabetic rats with BM resulted in a significant increase in SOD concentration. However, NO level did not reach the levels observed in the control maternal newborn group. BM controls the activation of apoptosis, lowers oxidative stress and inflammation, modifies mitochondrial function, and prevents lipid accumulation during the formation of liver fat **(55)**. Some of the polysaccharides found in BM have antioxidant properties in addition to the phenolic compounds **(56)**, **(57).**

Conclusion

Overall, MC is an inexpensive, readily accessible vegetable with a wide range of medicinal applications and few problems. The putative antidiabetic properties of MC align with the overall goal of maintaining a balanced diet and offer a novel, promising approach to broaden the range of diabetes treatment options.

Recommendation

We recommend elucidating the specific mechanisms of action of Momordica charantia and exploring its potential applications in human clinical settings, this includes investigating optimal dosages and administration methods.

References

1. Khan, I.A. (2021): Do second generation sequencing techniques identify documented genetic markers for neonatal diabetes mellitus? Heliyon, **7(9).**
2. Cho, N.H.; Shaw, J.E.; Karuranga, S.; Huang, Y.; da Rocha Fernandes, J.D.; Ohlrogge, A.W. and Malanda, B. (2018): IDF Diabetes Atlas: Global estimates of diabetes prevalence for 2017 and projections for 2045. Diabetes Research and Clinical Practice,**138**:271–81.
3. American Diabetes Association (2014): Diagnosis and classification of diabetes mellitus. Diabetes Care, **37(1):** S81–S90.
4. Maraschin, J.D.F. (2013): Cassification of diabetes," in Diabetes. Advances in Experimental Medicine and Biology, **771**: 12–19.
5. Ramachandran, A. (2014): Know the signs and symptoms of diabetes. *Indian Journal of Medical* Research, **140(5):** 579-581.
6. Cundy, T.; Slee, F.; Gamble, G. and Neale, L. (2002): Hypertensive disorders

of pregnancy in women with Type 1 and Type 2 diabetes. Diabetic Medicine, **19(6):** 482-489.
7. Feig, D.S.; Hwee, J.; Shah, B.R.; Booth, G.L.; Bierman, A.S. and Lipscombe, L.L. (2014): Trends in incidence of diabetes in pregnancy and serious perinatal outcomes: A large, population-based study in Ontario, Canada, 1996–2010. Diabetes Care, **37(6):** 1590-1596.
8. Schaefer-Graf, U.; Napoli, A. and Nolan, C.J. (2018): Diabetes in pregnancy: a new decade of challenges ahead. Diabetologia, **61**: 1012-1021.
9. Kumar, K.P. and Bhowmik, D. (2010): Traditional Medical uses and therapeutic benefits of Momordica charantia. *International Journal of Pharmaceutical Sciences Review and Research*, **4(3).**
10. Joseph, B. and Jini, D. (2013): Antidiabetic effects of Momordica charantia (bitter melon) and its medicinal potency. *Asian Pacific Journal of Tropical Disease*, **3(2):** 93- 102.
11. Kumari, S.; Dash, I. and Behera K.K. (2018): Therapeutic Effect of Momordica charantia on Blood Glucose, Lipid Profile and Oxidative Stress in Type 2 Diabetes Mellitus Patients: A Randomised Controlled Trial. *Journal of Clinical and Diagnostic Research*, **12(9):** BC21-BC25.
12. Cui, J.; Yang, Y.; Luo, S.; Wang, L.; Huang, R.; Wen, Q.; Han, X.; Miao, N.; Cheng, J.; Liu, Z.; Zhang, C.; Feng, C.; Zhu, H.; Su, J.; Wan, X.; Hu, F.; Niu, Y.; Zheng, X.; Yang, Y.; Shan, D.; Dong, Z.; He, W.; Dhillon, N.P.S. and Hu, K. (2020): Whole-genome sequencing provides insights into the genetic diversity and domestication of bitter gourd (Momordica spp.). Horticulture Research, **7(85).**
13. Gayathry, K.S. and John, J.A. (2022): A comprehensive review on bitter gourd (Momordica charantia L.) as a gold mine of functional bioactive components for therapeutic foods. Food Production, Processing and Nutrition, **4(1)**: 1-10.
14. Costa, J.G.M.; Nascimento, E.M.M.; Campos, A.R. and Rodrigues, F.F.G. (2011): Antibacterial activity of Momordica charantia (Cucurbitaceae) extracts and fractions. *Journal of Basic and Clinical Pharmacy*, **2(1):** 45-51.
15. Kim, S.K.; Jung, J.; Jung, J.H.; Yoon, N.A.; Kang, S.S.; Roh, G.S. and Hahm, J.R. (2020): Hypoglycemic efficacy and safety of Momordica charantia (bitter melon) in patients with type 2 diabetes mellitus. Complementary Therapies in Medicine, **52**(102524).
16. Sarkar, P.; Nath, K. and Banu, S. (2019): Modulatory effect of baicalein on gene expression and activity of antioxidant enzymes in streptozotocin-nicotinamide induced diabetic rats. *Brazilian Journal of Pharmaceutical Sciences*, **55**: e18201.
17. Furman, B.L. (2021): Streptozotocin-Induced Diabetic Modelsin Mice and Rats. Current Protocols, 1: e78.
18. Skehel, J.M. (2004): Preparation of extracts from animal tissues. Protein Purification Protocols, 15-20.
19. Fassati, P. and Prencipe, L. (1982): Trigltceride enzymatic colorimetric method. *Journal of Clinical Chemistry*, **28**: 2077.
20. Richmond, W. (1973): *Journal of Clinical Chemistry*, **19**: 1350.
21. Wieland, H. and Seidel, D. (1983): *Journal of Lipid Research*, **24**: 904.
22. Burstein, M.S.H.R.; Scholnick, H.R. and Morfin, R. (1970): *Journal of Lipid* Research, **11(6):** 583-595.
23. Fowler, P.A.; Sorsa-Leslie, T.; Harris, W. and Mason, H.D. (December 2003).
24. Ryan, K.J. (August 1982).
25. Nishikimi, M.; Roa, N.A. and Yogi, K. (1972): Biochemical Biophysical Research Communications, **46**: 849 – 854.
26. Montgomery, H. A. C. and Dymock, J. F. (1961): Analyst, **86**: 414.
27. Armitage, P. Berry, G. and Matthews, J.N.S, (2008). Statistical methods in medical research, fourth ed. Hoboken, New Jersey: John Wiley & Sons.
28. Rao, G. H. (2020): Management of diabetes epidemic: Global perspective. Current Trends in Diabetes, 25.
29. Saleem, M.; Sharif, M.K. and Saleem, R., (2022): Bitter Melon: A Comprehensive Review. Pakistan *Journal of Scientific &*

Industrial Research Series B: Biological Sciences, **65(3).**

30. Gao, Y.; Li, X.; Huang, Y.; Chen, J. and Qiu, M. (2023): Bitter melon and diabetes mellitus. Food Reviews International, **39(1)**: 618-638.

31. Loffredo, C.A; Wilson, P.D. and Ferencz, C. (2001): Maternal diabetes: an independent risk factor for major cardiovascular malformations with increased mortality of affected infants. Teratology, **64(2)**: 98–106.

32. Ludvigsson, J.F.; Neovius, M.; Soderling, J.; Gudbjornsdottir, S.; Svensson, A.M.; Franzen, S.; Stephansson, O. and Pasternak, B. (2018): Periconception glycaemic control in women with type 1 diabetes and risk of major birth defects: population-based cohort study in Sweden. BMJ, 362: k2638.

33. Evers, I.M.; de Valk, H.W. and Visser, G.H. (2004): Risk of complications of pregnancy in women with type 1 diabetes: nationwide prospective study in the Netherlands. *BMJ*, **328(7445):**915–9.

34. Han, S.; Wang, G.; Jin, Y.; Ma, Z.; Jia, W.; Wu, X.; Wang, X.; He, M.; Cheng, X.; LI, W.; Yang, X. and Liu, G. (2015): Investigating the mechanism of hyperglycemia induced fetal cardiac hypertrophy. PLOS ONE, **10(9)**: e0139141.

35. Bequer, L.; Gomez, T.; Molina, J.L.; Alvarez, A.; Chaviano, C. and Clapes, S. (2018): Experimental diabetes impairs maternal reproductive performance in pregnant Wistar rats and their offspring. Systems Biology in Reproductive Medicine, **64(1)**:60-70.

36. Samarghandian, S.; Azimi-Nezhad, M. and Farkhondeh, T. (2017): Catechin Treatment Ameliorates Diabetes and Its Complications in Streptozotocin-Induced Diabetic Rats. Dose-Response, **15(1)**.

37. Kaur, J.; Singh, P. and Sowers, J.R. (2002): Diabetes and cardiovascular diseases. *American Journal of Therapeutics*, **9(6):** 510-515.

38. Tourlouki, E.; Matalas, A.L. and Panagiotakos, D.B. (2009): Dietary habits and cardiovascular disease risk in middle-aged and elderly populations: a review of evidence. Clinical Interventions in Aging, **4**: 319-30.

39. Mealey, B.L. and Oates, T.W. (2006): American Academy of Periodontology. Diabetes mellitus and periodontal diseases. *Journal of Periodontology*, **77(8)**: 1289-1303.

40. White, J.; Swerdlow, D.I.; Preiss, D.; Fairhurst-Hunter, Z.; Keating, B.J.; Asselbergs F.W.; Sattar, N.; Humphries, S.E.; Hingorani, A.D. and Holmes, M.V. (2016): Association of lipid fractions with risks for coronary artery disease and diabetes. *JAMA Cardiol*, **1(6):** 692–699.

41. Yadav, U.C.S.; Moorthy, K. and Baquer, N.Z. (2005): Combined treatment of sodium orthovanadate and Momordica charantia fruit extract prevents alterations in lipid profile and lipogenic enzymes in alloxan diabetic rats. Molecular and Cellular Biochemistry, **268**: 111-120.

42. Gomathi, N. and Andmalarvili, T. (2009): Effect of Hibiscus rosasinensis on carbohydrate metabolizing enzymes in monosodium glutamate induced obesity in female rats. *Journal of Cell and Tissue* Research, **54(1):** 91-97.

43. Chaturvedi, P.; George, S.; Milinganyo, M. and Tripathi, Y.B. (2004): Effect of Momordica charantia on Lipid Profile and Oral Glucose Tolerance in Diabetic Rats. Phytotherapy Research, **18**: 954-956.

44. Yadav, U.C.S.; Moorthy, K. and Baquer, N.Z. (2005): Combined treatment of sodium orthovanadate and Momordica charantia fruit extract prevents alterations in lipid profile and lipogenic enzymes in alloxan diabetic rats. Molecular and Cellular Biochemistry, **268**: 111-120.

45. Chaturvedi, P. and George, S. (2010): Momordica charantia maintains normal glucose levels and lipid profiles and prevents oxidative stress in diabetic rats subjected to chronic sucrose load. *Journal of Medicinal Food*, **13(3):** 520-527.

46. Rezaei, N.; Mardanshahi, T.; Shafaroudi, M.M.; Abedian, S.; Mohammadi, H. and Zare, Z. (2018): Effects of L-Carnitine on the Follicle- Stimulating Hormone, Luteinizing Hormone, Testosterone, and Testicular Tissue Oxidative Stress Levels in Streptozotocin-Induced Diabetic Rats.

Journal of Evidence-Based Integrative Medicine, **23**: 1-10.

47. Rose, D. P.; Komninou, D. and Stephenson, G. D. (2004): Obesity, adipocytokines, and insulin resistance in breast cancer. Obesity Reviews, **5(3)**: 153–165.

48. Sun, S.; Wang, Y.; Zhou, Y.; Ma, W.; Huang, Y.; Hu, J. and Wang, Y. (2021): Serum progesterone and retinopathy in male patients with type 2 diabetes: A cross-sectional study. *Journal of Diabetes Investigation,* **12(7):** 1228- 1235.

49. Nyholm, H.; Djursing, H.; Hagen, C.; Agner, T.; Bennett, P. and Svenstrup B. (1989): Androgens and estrogens in postmenopausal insulin-treated diabetic women. The *Journal of Clinical Endocrinology and Metabolism*, **69(5):** 946– 949.

50. Soliman, G.A.; Abdel-Rahman, R.F.; Ogaly, H.A.; Althurwi, H.N.; Abd-Elsalam, R.M.; Albaqami, F.F. and Abdel-Kader, M.S. (2020): Momordica charantia Extract Protects against Diabetes-Related Spermatogenic Dysfunction in Male Rats: Molecular and Biochemical Study. Molecules, **25(22):** 5255.

51. Adewale, O.O.; Oduyemi, O.I. and Ayokunle, O. (2014): Oral administration of leaf extracts of Momordica charantia affect reproductive hormones of adult female Wistar rats. Asian Pacific *Journal of Tropical Biomedicine,* **4**(Suppl **1**): S521–S524.

52. Chen, X.; Famurewa, A.C.; Olatunde, O.O. and Olatunji, O.J. (2021): Hyperoside attenuates neuroinflammation, cognitive impairment and oxidative stress via suppressing TNF-α/NF-κB/caspase-3 signaling in type 2 diabetes rats. Nutritional Neuroscience, **25(8):** 1774-1784.

53. Kanwar, M.; Chan, P.S.; Kern, T.S. and Kowluri, R.A. (2007): Oxidative Damage in the Retinal Mitochondria of Diabetic Mice: Possible Protection by Superoxide Dismutase. Investigative Ophthalmology & Visual Science, **48(8)**: 3805–3811.

54. Udoh, A.E.; Ntui, I.; Essien, O. and Ndon, M. (2007): Red Cell Catalase Activity in Diabetics. *Pakistan Journal of Nutrition*, **6(5):** 511-515.

55. Xu, J.; Cao, K.; Li, Y.; Zou, X.; Chen, C.; Szeto, I.M.Y. and Dong, Z. (2014): Bitter Gourd Inhibits the Development of Obesity-Associated Fatty Liver in C57BL/6 Mice Fed a High-Fat Diet. The *Journal of Nutrition*, **144(4):** 475-483.

56. Liu, X.; Chen, T.; Hu, Y.; Li, K. and Yan, L. (2014): Catalytic synthesis and antioxidant activity of sulfated polysaccharide from Momordica charantia L. Biopolymers, **101(3)**: 210-215.

57. Raish, M. (2017): Momordica charantia polysaccharides ameliorate oxidative stress, hyperlipidemia, inflammation, and apoptosis during myocardial infarction by inhibiting the NF-κB signaling pathway. *International Journal of Biological Macromolecules*, **97**: 544-551.