How to Cite:
Al-Musawi, J. F. H. (2022). The efficacy of Momordica charantia L. (herbal agent) in treatment of type 1 diabetes mellitus in rats in comparison to insulin (animal model). *International Journal of Health Sciences*, *6*(S3), 6061–6070.
https://doi.org/10.53730/ijhs.v6nS3.7339

The efficacy of Momordica charantia L. (herbal agent) in treatment of type 1 diabetes mellitus in rats in comparison to insulin (animal model)

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Abstract---This study was done for a total (24) Black Rats, over a time of 28 days (divided into 1st, 7th. 14th and 28th day) in which (6) Rats in each group, which reflects that ALLOXAN can be caused hyperglycemia with highly significant results (P<0.0001). This increased in blood glucose level can be corrected by HERBAL DRUGS (Momordica Charantia L), with high significant effects (P<0.0001), when compared this with insulin which had high significant results in control hyperglycemia of Alloxan causes (P<0.0001).

Keywords---Momordica charantia L, alloxan, insulin, hyperglycemia, hypoglycemia.

Introduction

The Momordica charantia L is; bit gourd; Bitter squash; balsam-pear (1). Is a sub-tropical and tropical vine coming under the family Cucurbitaceous, is abundantly cultivated in major regions of Asia, Africa, and Caribbean for the usefulness of edible fruit. The existence of different varieties of this edible fruit is diversified across its shape and bitterness. During the 14th century, the bitter melon originally originated in India was successfully introduced in the regions of China. (2). The edible fruit has wide applications and hence it is widely used in Asian and their neighboring regions of South Asia and East Asia (3). The fruit turns orange and gets mushy when it is fully ripe. Further it divides into parts that curve back in the approach of revealing seeds encased in bright red pulp. (4). *Momordica charantia* is considered as a useful fruit which can be used for treating many clinical diseases like preventing cancer, diabetes treatment, fever, HIV and AIDS (5).

Persistent Diarrhea, pain in the abdominal region, high fever, hypoglycemic condition, problem in urine flow and abnormal pain in chest are several side

effects which are reported based on the application of *Momordical charantia*. The clinical symptoms are mild which does not demand treatment measures and can be recovered by taking rest. (6). Bitter melon is not recommended during the time of pregnancy because it might cause bleeding, contractions, and miscarriage (7). M. charantia L. has been studied for its anti-diabetic efficacy in a variety of animal models of type 2 diabetes mellitus. (8, 9).

When rodents and a variety of other animal species are supplied with alloxan, a poisonous glucose analogue that has the potential to selectively damage insulin-producing cells (beta cells) in the pancreas. This causes these animals to develop an insulin-dependent diabetes mellitus (called "Alloxan diabetes"), which has many similarities to type 1 diabetes in people. Because of its preferential absorption by the GLUT2 glucose transporter, Alloxan is highly harmful to insulin-producing beta cells in the pancreas. In the presence of intracellular Thiols, Alloxan produces reactive oxygen species (ROS) in a cyclic process with its reduction product, dialuric acid. The free radicals created during such a redox reaction start the beta cell toxicity of Alloxan. According to studies, Alloxan does not induce diabetes in humans (10). Others reported a significant variation in plasma Alloxan levels between children with type 1 diabetes and those without. (11).

Because it destroys the insulin-producing beta cells in the pancreas, Alloxan is used to induce diabetes in experimental animals (12, 13). This is most likely due to the compound's preferential absorption because to its structural similarity to glucose and the effective uptake mechanism of the beta-extremely cell. (GLUT2). Additionally, Alloxan has a high affinity for SH-containing biological molecules, lowering glutathione levels. Additionally, Alloxan molecule is inhibiting the action of glucokinase, an SH-containing enzyme required for glucose-induced insulin release (14). The majority of investigations have demonstrated that Alloxan is not harmful to human beta cells, even at extremely high doses, most likely due to the fact that humans and rats have distinct glucose uptake systems (15, 16).
Doses differ between researches; nevertheless, it is believed that single doses of 50–150 mg/kg cause chronic hyperglycemia in rodents, depending on the mode of administration and strain (17, 18).

(Alloxan has been demonstrated to begin acting within two minutes (as plasma insulin levels rise, resulting in a brief phase of hypoglycemia preceding long-term hyperglycemia). (19). Another substance used to cause D.M. Streptozotocin, also known as streptozocin (INN, USP) (STZ), is an alkylating anticancer drug that can cause the hyperglycemic condition called the diabetes mellitus. In mammals, this chemical compound is particularly harmful to the pancreas' insulin-producing beta cells. It is used in medical applications to treat malignancies and tumours in the islets of Langerhans, as well as in research to establish an animal model for hyperglycemia, Alzheimer's disease, and Type 2/ Type 1 diabetic symptoms at various dose levels. . (20).

Materials and Methods

A-Establishment of mice model

We were able to visualize a model in "Black rats", for induction of hyper glycaemia by intraperitoneal injection of ALLOXAN. The administration of ALLOXAN which are used is with 150 mg/kg mg/kg/body weight from 1st day to 30th day (21). The number of each group was 6 rats. These dose which was selected can produce hyperglycemia within 1-2 hours (22). Momordica charantia L was injected also by the same intra-peritoneal pathway as a solution for 30 days. The best results were obtained with a dose of 300 mg/kg body weight. Furthermore, MCP exhibited no harmful symptoms throughout a restricted toxicity study in rats and mice (5). Insulin subcutaneously administered in a dosage (4IU per gram body weight). The Injection group got daily subcutaneous (SC) injections of 4 IU/200 g of body weight of long-acting insulin molecule (100 UI/mL dosage Insulin Lantus; France) (23)

Procedure of animal experiments for in vivo assays includes the following steps

The research trials were performed on “24 Black Rats". With body weights around “75-90 g -, in which these rats maintained on special diet (24). These animals were grouped into "four groups" of "six animals" each:

1. First group was the healthy group, which is the CONTROL group, only given Tap water.
2. The Second group was treated with Momordica charantia L, in a dose of 250-300mg/kg body weight (5), which is not exposed to (not administered) Alloxan.
3. We followed each animal by measuring of blood sugar after administration of each agent.
4. The other third and fourth groups were treated with150mg/kg body weight/24hrs of ALLOXAN, as following: - from the 1st day, the 7th day, the 21st day and the 28th day (25). Also we followed the blood sugar of the animal after administration of the Alloxan. The third group were treated again with with250-300mg/kg body weight /24hrs of Momordica charantia L, also from the 1st day the 7th day, the 21st day and the 28th day (5, 26).
5. The fourth group treated again by subcutaneous INSULIN in a dose of 2 IU/kg body weight (26, 27).

After administration of each agent we measure the level of blood sugar frequent time, (fasting and postprandial). Animal studies were in accordance with the guidelines for the care and use of laboratory animals and approved by the corresponding animal care and use committees (Data Sciences International, Eli Lilly). The POCG tested included one veterinary device (POCG A) and 4 human-use instruments (POCG B through E) (A*&B*). POCG: point-of-care glucometer (28, 29).

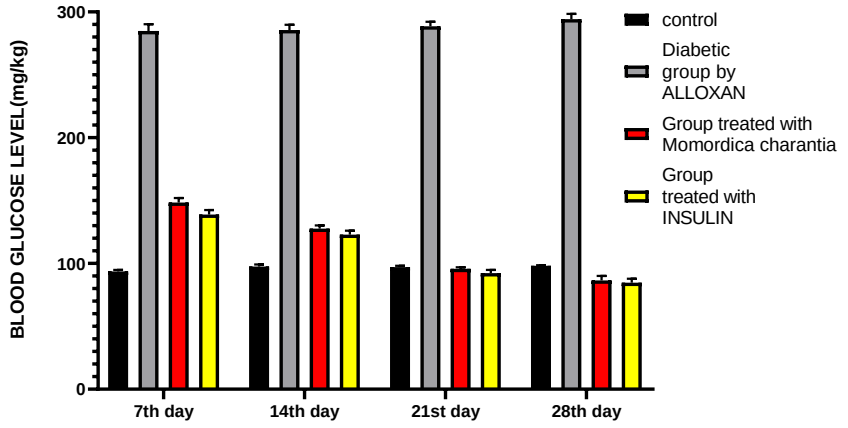
Results

The effect of *Momordica charantia* L. in treatment of Type 1 Diabetes Mellitus in Comparison to Insulin in Rats in this study as follow :- The mean ± SD of the hypoglycemic effect of Momordica charantia L in black rats were illustrated in table (1) and statistical figure (1), in the following ; 7th, 14th, 21st and 28th days respectively which were (148.42 +/-3.65), (127.66 +/-2.520), (95.78 +/-1.18), (86.54 +/-3.55), with a number of 6 animals (Black rats) for each group.

Table 1
The efficacy of Momordica charantia L. in treatment of Type 1 Diabetes Mellitus in Comparison to Insulin in Rats

BLOOD GLUCOSE LEVEL(mg/kg)					
Treatment	Dose (mg/kg)	7th day	14th day	21st day	28th day
Control group	----	93.59 +/- 1.32	97.53 +/- 1.58	97.11 +/- 1.04	98.10 +/- 0.52
Diabetic group by ALLOXAN	150mg/kg Intra-personally	284.83 +/- 5.37	285.58 +/- 4.22	288.64 +/- 3.56	294.32 +/- 4.16
Group treated with Momordica charantia	0.5 g/kg* Intra-personally	148.42 +/- 3.65	127.66 +/- 2.52	95.78 +/- 1.18	86.54 +/- 3.55
Group treated with INSULIN	2U/Kg Subcutaneo-usly	138.91 +/- 3.61	122.88 +/- 3.18	92.25 +/- 2.60	84.75 +/- 3.09
	X±SD	166.43+/- 3.48	158.41 +/- 2.87	143.44 +/- 2.10	140.92 +/- 2.83

figure No1:-The efficacy of Momordica charantia L. in treatment of Type 1 Diabetes Mellitus in Comparison to Insulin in Rats



Source of Variation	% of total variation	P value	P value summary	Significant?
Interaction	0.3631	0.0218	*	Yes
Row Factor	97.22	<0.0001	****	Yes
Column Factor	1.064	<0.0001	****	Yes

This mean that there is very clear effectiveness of Momordica charantia L on reduction of hyperglycemia caused by Alloxan. In which this hypoglycemic effect of *Momordica charantia* L, look like Insulin which is highly significant, (P<0.0001) for Momordica charantia L), and also (P<0.0001 for Insulin).

The significant effect of Momordica charantia L in reduction of high Blood: - Glucose Sugar in these animals were illustrated also by Table (No2) and Figure No.2 in this study. That the mean and SD of hypoglycemic effect of both groups which were treated with Momordica charantia L (P<0.0001) and those who were treated with Insulin (P<0.0001), in comparison to the group which were treated previously by ALLOXAN (causing severe hyperglycemia (P<0.0001), which caused highly significant result in increasing blood glucose level). This mean that there is highly significant results of hypoglycemic effect of Momordica charantia L on black rats treated with Alloxan (which caused highly significant effect to induce hyperglycemia) Table No.2 & Figure No2.

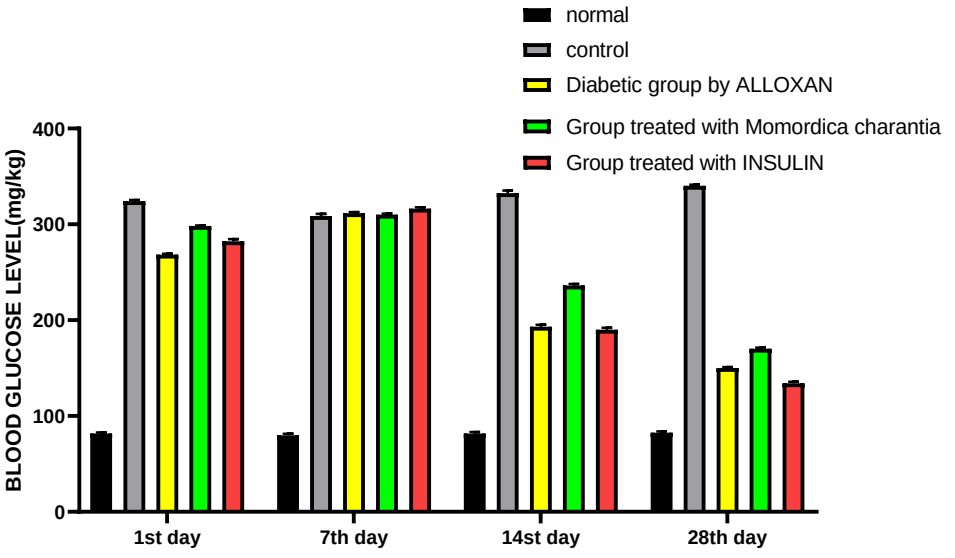
Table 2
The significant effects of Momordica charantia L. in treatment of Type 1 Diabetes Mellitus in Comparison to Insulin in Rats

GROUP	1st day	7th day	14th day	28th day
Normal Group	81.64 +/- 1.06	79.94 +/- 1.50	81.75 +/- 1.52	82.63 +/- 1.32
Control group	324.32 +/- 1.12	308.70 +/2.14	332.5 +/- 2.71*	340.2 +/- 2.07*
Standard group (on	268.40 +/- 0.96	311.70 +/- 1.03	193.12** +/1.99	150.05 +/- 1.06***

ALLOXAN)				
Group treated with MCL	298.22 +/- 0.52	310.02 +/- 1.07	236.1 +/- 1.62*	170.2 +/- 1.09**
Group treated with Insulin	282.4 +/- 2.16	316.30 +/- 1.28	189.9 +/- 2.12**	134.01 +/- 1.64***
X±SD	250.98 +/- 1.164	265.32 +/- 1.40	206.67+/- 1.99	175.42+/- 1.43

M N1: Normal control group
N2; standard DM
N3&N4 treated by Momordica charantia in a dose o.5mgkg, and 2U Insulin respectively
No. of each group was 6 RATS

figure No2: The significant effects of Momordica charantia L. in treatment of Type 1 Diabetes Mellitus in Comparison to Insulin in Rats



ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Interaction	4863	3	1621	F (3, 40) = 692.9	P<0.0001
Row Factor	195836	3	65279	F (3, 40) = 27903	P<0.0001
Column Factor	6338	1	6338	F (1, 40) = 2709	P<0.0001
Residual	93.58	40	2.339		

The animal studies were in accordance with the guidelines for the care and use of laboratory animals and approved by the corresponding animal care and use committees (Data Sciences International, Eli Lilly). The POCG tested included one veterinary device (POCG A) and 4 human-use instruments (POCG B through E) (A*&B*). POCG: point-of-care glucometer (28, 29).

Discussion

Momordica charantia (bitter melon) is a plant that has been widely used as a traditional medicine for treating the hyperglycemic condition called the diabetes mellitus (DM). The research studies have proved that Momordica charantia (Mc) has an increase of blood sugar level on humans and animals, but we are not aware whether this sudden impact is retaining in the chronic state or if extract obtained from the stems and leaves of the plant is influencing the effect of anti-hyperglycemic condition. (30). Alloxan, or Alloxan hydrate, is the chemical compound having the chemical formula as OC (N (H) CO) 2C (OH) and categorized as a pyrimidine derivative. Because it kills the insulin-producing beta cells in the pancreas, Alloxan is used to induce diabetes in experimental animals. (31, 32).

Lantus® (insulin glargine injection) is a molecule resembling the category of insulin which is utilized for the application of treating patients with type 2 diabetes, as well as adults and children aged 6 and above who have type 1 diabetes. In this study we use this type of insulin that is why we used this type of insulin in this study (33).

In this study we found that there is a significant results of Alloxan on increased the blood glucose level of rats, which had high significant effect ($P<0.0001$) which caused severe hyperglycemia in rats, and this result in agreement with Danilova I.G., Sarapultsev P.A., Medvedeva S.U et al, and also accepted by Mrozikiewicz, A.; Kielstrokczewska-Mrozikiewicz et al and also in agreement with Szkudelski T, who illustrated; that they written as following:- Alloxan is used to induce diabetes in animals because it kills beta cells in the pancreas, which produce insulin. Because of its structural similarity to glucose and the beta-efficient cell's absorption mechanism, this is most likely owing to the compound's selective uptake (GLUT2). Glutathione levels are further reduced by Alloxan's high affinity for SH-containing biological molecules. It also inhibits the action of glucokinase, and thiol (SH)-containing enzyme essential for the release of insulin from glucose. (11, 12, and 18).

The other 12 hyperglycemic rats due to Alloxan were treated later by either Momordica Charantia L, or Insulin, The 6 animals treated by Momordica charantia and this reflected good response with high significant result by improve hyperglycemia induced by Alloxan ($P<0.0001$), this this is discussed by Wang, Limei; Waltenberger et al that, theMomordica charantia (bitter melon) is a plant that has been widely used in traditional medicine to treat diabetes mellitus (DM). Momordica charantia (Mc) has been proven to an increase of blood sugar level on humans and animals, but we are not aware whether this sudden impact is retaining in the chronic state or if extract obtained from the stems and leaves of the plant is influencing the effect of anti-hyperglycemic condition. (34). In this study, we found the results reflect that; the hyperglycemic condition of Momordica Charantia L caused by Alloxan in Black Rats was the same effect of Insulin, this is in agreement with Parker Nelson M, Edmond K. Farhad.et al that they found the effect of the insulin on beta-cells of the pancreas was by stimulation of these cells and control hyperglycemic effects caused by Alloxan or Streptozotocin (35). (Table No1 and No2 & Figure No1&2) illustrate these result statistically.

Conclusion

We conclude in this study that there is a semi effectiveness of *Momordica charantia L* on diabetes mellitus type 1 in rats (Animal model), that both Momordica *charantia L* and Insulin reduce blood glucose by the same measure.

Conflict of interest: None.

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Statistical Analyses

Table No1:-The efficacy of Momordica charantia L. in treatment of Type 1 Diabetes Mellitus in comparison to Insulin in Rats

BLOOD GLUCOSE LEVEL(mg/kg) Treatment Dose (mg/kg)	7th day	14th day	21st day	28th day
Control group	----	93.59 +/- 1.32	97.53 +/- 1.58	97.11 +/- 1.04
	98.10 +/-0.52			
Diabetic group by ALLOXAN 150mg/kg Intra-peritoneal				
y	284.83 +/- 5.37	285.58 +/- 4.22	288.64 +/- 3.56	294.32 +/- 4.16
Group treated with Momordica charantia 0.5 g/kg* Intra-peritoneal				
y	148.42 +/- 3.65	127.66 +/- 2.52	95.78 +/- 1.18	86.54 +/-3.55
Group treated with INSULIN 2U/Kg Subcutaneous-ugly	138.91 +/- 3.61	122.88 +/- 3.18	92.25 +/- 2.60	84.75 +/-3.09
X±SD	166.43+/-3.48	158.41 +/- 2.87	143.44 +/- 2.10	140.92 +/-2.83