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EVALUATING THE THERAPEUTIC EFFICACY OF MORINGA OLEIFERA EXTRACT ON ALLOXAN-INDUCED DIABETIC RATS: A COMPARATIVE STUDY

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Abstract

Introduction

The current study is aimed to determine the effects of three different dosages that is 100, 200 and 300mg/kg of moringa leaves extract on blood glucose levels of alloxan induced diabetic as a potential treatment strategy in the treatment of DM

Methodology

A comparative experimental study was conducted on n=100 young Wistar rats divided into five groups. Laboratory condition was maintained at temperature of 20 to 25^{0}C and were exposed to 12 hours of light and 12 hours of dark cycle.

Results

Between the group Analysis were performed that provided significant evidence in favor of group D in comparison to other experimental groups where the values of HbA1C were significantly lowered $p< 0.05$ than values in group D and group E

Conclusion

The study had concluded that MO leaves extract had a therapeutic effects in managing high blood glucose levels in diabetes patients.

Key Words: Diabetes Mellitus, Moringa Oleifera, Blood Glucose, Glycated Hemoglobin

Introduction

Diabetes Mellitus is a serious long term metabolic disorder that impact on individual lives, well-being, families and societies world–wide[1]. According to International Diabetes Federation (IDF) the prevalence of DM including both type 1 Diabetes (T1D) and type 2 Diabetes (T2D) has increased from 285 million of world population in 2009 to 425 million in 2017[2]. With the similar rise in trend, it is expected that by the year 2030, 578 million people will have diabetes that will increase further

to 700 million people (51% higher) in the year 2045[3]. According to the data of global diabetes prevalence the number of individuals having DM including both T1D and T2D are higher in urban population (10.8%) than rural (7.2%) population of high income countries and every one individual out of two having diabetes (50.1%) do not know that they have diabetes. This exceptionally high trend of diabetes and increase burden of disease worldwide requires serious measures that must be taken in order to curb this menace before it gets too late[4]. Multiple pharmacological treatment options are available ranging from anti-hyperglycemic medications like metformin that considered as a first line treatment approach to patients with T2D to different range of insulin like rapid acting, short acting, intermediate and long acting insulin are available that can be given to patients in TID and uncontrolled diabetes[5-6]. Studies have also provided evidences that these medication approximately cost to around $15 to $500 annually (median $177) along with other cost that includes per visit cost, laboratory cost and other thus making treatment options for DM extremely expensive thereby posing a huge financial burden on health care sectors globally[7]. Hence it becomes extremely important to find an evidence based treatment option that not only effectively treat DM but can also turn out to be cost effective as well. The plant of Moringa Oleifera (MO) is rich in various micro and macro nutrients and antioxidant agents' chiefly flavonoids, quercetin and kaempferol[8-10]. These compounds primarily works as an anti-glycemic agents by acting as a competitive inhibitor for sodium-glucose linked transporter type-1 (SGLT-1) present in the mucosa of small intestine (duodenum and jejunum), hence plummets the intestinal absorption of glucose[11-12]. The other mechanism of glucose absorption that involves GLUT-2 is also inhibited by compound called as quercetin besides inhibiting GLUT-2, quercetin present in the leaves of plant activates adenosine monophosphate-activated protein kinase (AMPK) that increases glucose uptake for the production of energy by stimulating GLUT-4[13-14]. Hence in this way not only the leaves extract of moringa oleifera plant inhibits glucose absorption via competitive inhibition method but by activating AMPK mechanism it enhances the utilization of glucose for the production of energy thereby reduces excess blood glucose levels in diabetic patients. Besides its impact on blood glucose and cholesterol levels, the extract of leaves are potential antioxidant thus it creates a major impact in increasing the immune response of body against various antigens as well[15-16]. Based on these finding the authors of current study are aimed to determine the effects of three different doses of 100, 200 and 300mg/kg of moringa leaves extract on blood glucose levels of alloxan induced diabetic as a potential treatment strategy in the treatment of DM.

Methodology

Study Setting

A comparative experimental study was conducted in the department of Biochemistry in collaboration with molecular laboratory of Isra University Hospital, Hyderabad and Suleman Roshan Medical College tandoadam

Experimental Animal

A total number of n=100 young Wistar rats (Rattus norvegicus) of same age group and body weight of 250-300g were inducted for the study. The animals were kept in polypropylene cages of 43x27x15 cm in length. Laboratory condition was maintained at temperature of 20 to 25^0C and were exposed to 12 hours of light and 12 hours of dark cycle. The bedding material was changed every second day and all animals were given Kaytee Supreme Fortified Daily Diet rat food and clean distal water at room temperature. The experiment was commenced after 10 days and the animal were kept in the lab environment for acclimatization.

Weighing Machine (Mettler Toledo PM 480 δ ranged) was used for measuring the weight of the mouse in gms at baseline and at conclusion of every week for 6 weeks. The random and fasting blood sugar levels were measured using ABBOT C- 4000 AUTOMATIC ANALYZER[17]. Enzyme linked immunoassay (ELISA) commercial kit was used to measure the levels of HbA1C in the levels of blood[18].

Grouping of Rats

A total number of n=100 rats were divided into 5 groups; n=20 in each group

Group A

The rats in group A were administered 0.9% of Normal Saline Intra muscular (IM)

Group B

The rats in group B were given alloxan at 120mg/kg of body weight intraperitonial (IP). Rats had developed diabetes within 2 days after alloxan injection.

Group C

Alloxan induced Diabetic rats were included and were given MO leaves extract at 100mg/kg 0f body weight by mixing in the food of rats for six weeks

Group D

Alloxan induced Diabetic rats Diabetic rats were inducted and were given MO leaves extract at 200mg/kg of body weight by mixing in the food of rats for six weeks

Group E

Alloxan induced Diabetic rats Diabetic rats were inducted and were given MO leaves extract at 300mg/kg of body weight by mixing in the food of rats for six weeks

Outcome Measures

The random and fasting blood sugar levels and Glycated Hemoglobin (HbA1C) levels were monitored at baseline and at the seventh day of sixth weeks using ABBOT C- 4000 AUTOMATIC ANALYZER for random and fasting blood glucose levels and Elisa commercial kit for estimating HbA1C (Crystal Chem, Chicago, IL; USA). The blood was taken by pricking the tail of rats after cleaning it with alcohol gauze swabs. Ear marking method was used for the labeling of rats for keeping the records of blood sampling

Results

A total number of n=80 Wistar rats were used in this study. The rats were weight initially before the inducting into one of the five groups and was found that the mean weight of the rats was around 283.39±2.86gm ranging from 250gm to 300gm (table 1).

Table 1 Mean and Standard deviation of weights in gram [a]

Variable	Total Numbers 'N'	Mean weight in grams ± Standard Deviation
Group A	20	285.36gm ± 3.13*
Group B	20	281.52gm ± 2.62*
Group C	20	283.84gm ± 2.91*
Group D	20	283.28gm ± 3.09*
Group E	20	282.98gm ±2.81*

*P>0.05 indicates no mean difference in weight at baseline

[a] one way analyses of variance to determine between group comparison

Estimation of Fasting and Random Blood Glucose levels

The estimation of fasting and random blood glucose levels were performed using ABBOT C- 4000 AUTOMATIC ANALYZER. The results revealed that the mean values of fasting blood glucose levels of rats at baseline was found to be 100.05mg/dl±5.62 for group A, 120 mg/dl±6.1 for group B and C both, 125.65mg/dl±5.8 for group D and 123mg/dl±5.52 for group E that remain non-significantly p=0.015 different in control group A 96±3.1, significantly increased p=0.0013 in Alloxan induced experimental group 129±5.3, decreased significantly p<0.001 in all three experimental group with an average value at week 6 were 81.25±2.7 group C, 70.65±2.2 group D and 76±2.12 in group E. Besides that the values of random blood glucose levels at within the group analysis at baseline and after six weeks were 112.61±5.4 that remains non-significantly difference 112. 05 ± 2.38 (p=0.35) at week six, in group B the values of RBS levels were 150.3 ± 2.06 that had increased to 154.5 ± 1.85 p <0.001, in group C the values were 153.05±1.43 that had reduced to

105.16±2.5 ($p<0.001$), in group D the values were 160.1±5.54 that had plummeted to 80.38±3.07 ($p<0.001$) and the values in group E were 155.05±1.83 that had reduced to 90.33±3.66 ($p<0.001$). (Table 2 and Table 3)

Table 2 Continuous Measure Annova to determine the significance of mean difference in with in group Analysis (Baseline and Week 6) for Fasting Blood Glucose levels

Variables	Baseline μ±sd	Week1 μ±sd	Week2 μ±sd	Week3 μ±sd	Week4 μ±sd	Week 5 μ±sd	Week 6 μ±sd	95% of CI[b]	p value
Group A	100±5.62	95±3.6	98±3.8	93±3.52	95±3.4	92±3.2	96±3.1	-2.2 to 5.3	0.15*
Group B	120±6.1	125±5.9	130±6.2	125±5.5	113±4.56	119±4.9	129±5.3	-5.25 to 9.65	0.0013[a]
Group C	120±6.1	90±3.2	92±3.1	88±3.01	85±2.93	80±2.95	81±2.7	26.52 to 45.15	0.00012[a]
Group D	125±5.8	88±3.2	85±3.12	80±3.3	77±2.45	75±2.5	70±2.2	33.26 to 65.12	0.000 [a]
Group E	123±5.52	85±3.3	83±3.1	82±3.2	80±2.95	79±2.33	76±2.12	32.15 to 55.12	0.0001 [a]

[b] CI is the confidence Interval measured at 95%
*P>0.05 indicates no mean difference within the group
[a] P<0.05 indicates significant mean difference with in the group

Table 3 Continuous Measure Annova to determine the significance of mean difference in with in group Analysis (Baseline and Week 6) of Random Blood Glucose levels

Variables	Baseline μ±sd	Week1 μ±sd	Week2 μ±sd	Week3 μ±sd	Week4 μ±sd	Week 5 μ±sd	Week 6 μ±sd	95% of CI[b]	p value
Group A	115	113	115	110	112	110	112	-5.3 to 4.5	0.02[a]
Group B	150	155	158	160	158	153	154	-5.2 to 6.3	0.021[a]
Group C	153	135	120	115	110	108	105	35.01 to 53.52	0.0001[a]
Group D	160	143	115	100	98	95	80	55.15 to 83.45	0.00[a]
Group E	155	138	118	110	105	100	90	55.15 to 78.52	0.00[a]

[b] CI is the confidence Interval measured at 95%
[a] P<0.05 indicates significant mean difference with in the group

Further between group Analysis were performed that revealed s significant mean difference between the group where the values in fasting blood glucose levels in group D were significantly lower $p< 0.005$ than values in group C and E (experimental group). Similarly group E values were significantly $p<0.05$ lower than group C. Likewise the values in random blood glucose test also suggested similar finding where animals in group D had shown significantly $p<0.05$ lower RBS levels than animals in other group (table 4 and table 5)

Table 4 Between Group Comparison One way Analysis of Variance Mean Difference Comparison of Fasting Blood Sugar Levels

Variables	Group A (p-value)	Group B (p-value)	Group C (p-value)	Group D (p-value)	Group E (p-value)
Group A	a	33* (0.001)[b]	15* (0.001) [b]	26* (0.001) [b]	20 (0.001) [b]
Group B	33* (0.001) [b]	a	48* (0.000) [b]	59* (0.000) [b]	53* (0.00) [b]
Group C	15* (0.001) [b]	48* (0.000) [b]	A	11* (0.01) [b]	5* (0.015) [b]

Group D	26* (0.001) [b]	59* (0.00) [b]	11* (0.01) [b]	a	6* (0.015) [b]
Group E	20* (0.001) [b]	53* (0.000) [b]	5* (0.015) [b]	6* (0.015) [b]	a
* indicates mean difference observed at week 6 in between the group a indicates no mean difference exist between group [b] p<0.05 indicates significant mean difference between the group					

Table 5 Between Group Comparison One way Analysis of Variance Mean Difference Comparison for Random Blood Sugar levels

Variables	**Group A (p-value)**	**Group B (p-value)**	**Group C (p-value)**	**Group D (p-value)**	**Group E (p-value)**
Group A	a	42* (0.00)[b]	7* (0.03) [b]	32* (0.001) [b]	22* (0.001) [b]
Group B	42* (0.00) [b]	a	25* (0.012) [b]	74* (0.00) [b]	64* (0.00) [b]
Group C	7* (0.01) [b]	25* (0.012) [b]	A	25* (0.002) [b]	15* (0.015) [b]
Group D	32* (0.001) [b]	74* (0.00) [b]	25* (0.002) [b]	a	10* (0.01) [b]
Group E	22* (0.001) [b]	64* (0.00) [b]	15* (0.015) [b]	10* (0.01) [b]	a
* indicates mean difference observed at week 6 in between the group a indicates no mean difference exist between group [b] p<0.05 indicates significant mean difference between the group					

The values of HbA1C were also determined at baseline and at week 6 and analysis of the findings had revealed that in group A the difference in the level of HbA1C was not found to be significant p=0.7, where as in group B the alloxan induced diabetes had significantly raised the difference between the baseline and at week 6 (p=0.001). In group B the administration of MO at 100mg/kg of body weight among diabetic rats significantly reduces the levels p=0.00 with an observed value of 3.5±0.81mmol/mol at week 6 compared to 6.9±1.27mmol/mol at baseline. Similarly the value in group D had also shown a significant reduction p=0.00 at baseline and at week 6 with the value measured at baseline 7.2±2.1mmol/mol that had reduced to 2.08±1.12mmol/mol at week 6. In group E the effects was also monitored and that too turned to be significant with the reduction of 7.01±1.98mmol/mol at baseline to 3.1±1.1mmol/mol at week (table 6)

Table 6 Continuous Measure Annova to determine the significance of mean difference in with in group Analysis (Baseline and Week 6) fir glycated Hemoglobin

Variables	**Baseline µ±sd**	**Week1 µ±sd**	**Week2 µ±sd**	**Week3 µ±sd**	**Week4 µ±sd**	**Week 5 µ±sd**	**Week 6 µ±sd**	**95% of CI [b]**	**p value**
Group A	3.06±1.2	3.07±1.1	3.05±1.12	3.04±1.1	3.06±1.1	3.08±1.01	3.05±1.1	-0.001 to 0.9	0.7*
Group B	6.7±2.1	7.1±2.21	8.1±2.3	8.5±2.11	8.3±1.9	8.6±2.01	8.1±2.12	-0.85 to 2.10	0.001[a]
Group C	6.9±1.27	6.3±1.4	6.2±1.12	5±1.01	4.5±1.1	4.2±1.01	3.5±0.81	-1.5 to 5.3	0.00 [a]
Group D	7.2±2.1	5.5±2.01	5±1.9	4.3±1.25	3.5±1.3	2.9±1.09	2.08±1.12	-2.8 to 5.9	0.00 [a]
Group E	7.01±1.98	6.5±2.1	6.1±1.7	4.6±1.5	4.2±1.10	3.5±1.12	3.1±1.1	-1.01 to 4.21	0.00 [a]
[a] P<0.05 indicates significant mean difference with in the group [b] CI is the confidence Interval measured at 95% *P>0.05 indicates no mean difference within the group									

Further between the groups comparison was performed using a one way Analysis of variance that revealed that effects observed in group D were significantly better than Group E and Group C (p=0.03); however no significant difference in Hb1AC levels were observed in between Group C and E (p=0.06). The detail description of between group comparisons were illustrated in table 4.8 as: (table 7)

Table 7 Between Group Comparison One way Analysis of Variance Mean Difference Comparison

Variables	Group A (p-value)	Group B (p-value)	Group C (p-value)	Group D (p-value)	Group E (p-value)
Group A	a	5.05* (0.01)[b]	0.45* (p=0.06)[c]	0.97* (0.04)[b]	0.05* (0.9)[c]
Group B	5.05* (0.01)[b]	a	4.6* (0.01)[b]	6.02* (0.001)[b]	5.0* (0.01)[b]
Group C	0.45* (p=0.06)[c]	4.6* (0.01)[b]	A	1.42* (0.03)[b]	0.4* (p=0.06)[c]
Group D	0.97* (0.04)[b]	6.02* (0.001)[b]	1.42* (0.03)[b]	a	1.02* (0.03)[b]
Group E	0.05* (0.9)[c]	5.0* (0.01)[b]	0.4* (p=0.06)[c]	1.02* (0.03)[b]	a

* indicates mean difference observed at week 6 in between the group
a indicates no mean difference exist between group
[b] $p<0.05$ indicates significant mean difference between the group
[c] $P>0.05$ indicates no significant mean difference between the group

Discussion

The analysis of the findings had revealed that Moring Oleifera (MO) leaves extract of three different doses that were 100mg/kg of body weight, 200mg/kg of body weight and 300mg/kg of body weight were found to be significantly effective in lowering the blood glucose levels in alloxan induced diabetic rats as illustrated in table 2 and table 3. The findings had further provided evidences that of all three different doses the effects of 200mg/kg of body weights were turned out to be significantly effective than 100mg/kg and 300mg/kg of MO leaves extract, hence the dose response of MO leaves extract of 200mg/kg of body weight could be used effectively for diabetes treatment (table 1). The results of this research were found to be inconsistence with the findings of another study based that was conducted on 24 male adult rats, diabetes was induced through streptozotocin and ethyl acetate extracted from Moringa Oleifera leaves was given at a dose of 200mg/kg of body weight for 30 days. The findings revealed that moringa oleifera leaves have potent therapeutic effects on diabetes mellitus where the levels glucose in rats were significantly reduced $p<0.005$ besides that levels of different pro-inflammatory markers like IL-1 β , TNF- α & IL-6 were also reduced significantly[19]. In another study efficiency of MO leaves on levels of insulin, expression of TNF-α, levels of glucose and follicle count were determined in rats' norvegicus. The study had included forty rats randomized into four set each set contain ten rats. Animals in group A were controlled, in second group poly cystic ovary syndrome (PCOs) rats were included, third group rats were PCOs that were given metformin and in last group PCOs rats were recruited that were given MO 500mg/kg of body weight. The effects were determine on TNF-α expression, insulin levels, glucose levels and follicle count. Findings revealed significant improvement in MO 500mg/kg of body weight groups in comparison to other hence suggesting MO leaves extract potency over metformin[20]. Another study that was performed to determine the anti-diabetic and anti-obesogenic effects of MO on Sprague-Dawley rats that were given high fructose diet. Study was performed on sixty rats that were divided into five groups n=12 rats in each group. Rats in group-I were provided with plain water and plain gelatin cube only, group-II rats were given 20% of fructose solution and plain gelatin cube, group III rats were provided with 20% of fructose solution with fenofibrate in gelatin cube 100mg/kg of body weight, group IV with fructose solution with low dose of MO (50mg/kg of body weight) and animals in group V with Fructose solution with high dose of MO (500mg/kg of body weight). Analysis of the findings had revealed that administration of MO both at low and high dose effectively maintained insulin concentration and HOMA-IR in alloxan induced diabetic rats[21]. Hence it was evident that MO extract produced beneficial effects on high blood glucose levels.

Conclusion

The study had concluded that MO leaves extract had a therapeutic effects in managing high blood glucose levels in diabetes patients. Three different dosages of MO leaves extract that were 100mg/kg, 200mg/kg and 300mg/kg of body weights were given to alloxan induced diabetic rats in order to determine the dose response effectiveness and the findings had suggested that dose of 200mg/kg of body weight had a better therapeutic efficacy in comparison to the dose of 100mg/kg and 300mg/kg of body weight.

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