Print ISSN: 2319-2003 | Online ISSN: 2279-0780

IJBCP International Journal of Basic & Clinical Pharmacology

DOI: http://dx.doi.org/10.18203/2319-2003.ijbcp20190145

Original Research Article

Effect of turmeric on alloxan induced diabetes mellitus in albino rats

Mugdha Rajeeva Padhye, Sangita Devrao Jogdand*

Department of Pharmacology, Jawaharlal Nehru Medical College, Sawangi (Meghe), Wardha, Maharashtra, India

Received: 27 November 2018
Accepted: 28 December 2018

***Correspondence to:**
Dr. Sangita Devrao Jogdand,
Email: drsangitaraj@gmail.com

ABSTRACT

Background: Diabetes mellitus is a chronic metabolic disorder of glucose metabolism characterised by hyperglycaemia. Long standing diabetes mellitus leads to various complications affecting multiple organ systems. Management of diabetes mellitus includes lifestyle modification and pharmacotherapy. Pharmacotherapy of diabetes mellitus includes a wide variety of drugs that help in achieving adequate glycaemic control. Anti-diabetic medications are however associated with several adverse effects. Phytochemicals are being used extensively for the treatment of various diseases. Use of phytochemicals would minimize adverse effects due to various anti-diabetic drugs and improve patient compliance. In the present study, authors studied the effect of turmeric on alloxan induced diabetes mellitus in albino rats.
Methods: Albino Wistar rats of either sex weighing 180 - 250grams were utilized for the present study. Diabetes mellitus was induced by intraperitoneal administration of alloxan. Ethanolic extract of turmeric was administered to diabetic rats daily orally for duration of 28 days. Blood glucose levels were monitored using glucometer before and after intervention with turmeric.
Results: Statistically significant reduction in mean blood glucose levels (p value <0.05) was seen after intervention with turmeric in diabetic rats. There was a significant reduction in mean blood glucose levels.
Conclusions: Ethanolic extract of turmeric showed antihyperglycemic effect in diabetic rats.

Keywords: Alloxan, Diabetes mellitus, Phytochemicals

INTRODUCTION

A chronic disorder of impaired glucose metabolism, Diabetes mellitus (DM) is characterized by underutilization and overproduction of glucose leading to hyperglycaemia.[1] Based on the latest available data, global prevalence of diabetes mellitus as per the International Diabetes Federation (IDF) is estimated to be 151 million in 2000, 194 million in 2003, 246 million in 2006, 285 million in 2009, 366 million in 2011, 382 million in 2013 and 415 million in 2015.[2]

The major modality in the treatment of DM is lifestyle modification which includes diet and physical exercise. Majority of the patients with DM however require pharmacotherapy to achieve target glucose concentration.[3] Pharmacotherapy aims at maintaining glycaemia levels and preventing long term complications of DM. The only available therapy for Type 1 DM is insulin therapy.[4] Various classes of drugs for the management of Type 2 DM are biguanides, thiazolidinediones, meglitinide analogues, sulfonylureas, alpha glucosidase inhibitors, Dipeptidyl peptidase IV (DPP-4) inhibitors, selective sodium-glucose transporter-2 (SGLT-2) inhibitors, Glucagon like peptide-1 (GLP-1) agonists and Insulin. All classes of anti-diabetic medications are associated with various adverse effects.[1]

Adverse effects due to use of synthetic anti-diabetic agents have led to an increased demand for natural ingredients as

hypoglycaemic agents. About 1200 plants have been screened as hypoglycaemic agents. Curcuma longa (Turmeric) belonging to the family Zingiberaceae, known as "Haldi" in Hindi is an extensively used dietary spice. It is extensively used for various diseases in various branches of medicine such as Ayurveda, Siddha and Unani.[5]

The current research work was conducted to study the effect of turmeric on blood sugar level after chemical induction of DM in experimental animals.

The aim of the present study was to study the effect of turmeric on alloxan induced diabetes mellitus in albino rats.

Objectives

- To induce diabetes mellitus in albino rats using alloxan.
- To evaluate the effect of alloxan on fasting blood glucose levels.
- To evaluate the effect of turmeric on fasting blood glucose levels.

METHODS

Duration of study was 2 months. We utilized 36 Albino Wistar Rats for the study. It was an experimental study. The study was performed in the Central Animal House of Department of Pharmacology, Jawaharlal Nehru Medical College, Sawangi (Meghe).

Table 1: Experimental animals were grouped.

Group	Intervention	Duration
Group I	Normal saline 10ml/kg/day IP (intraperitoneally).	60 days
Group II	Single dose of alloxan 125mg /kg dissolved in 0.9% normal saline IP (intraperitoneally).	Single dose
Group III	Single dose of alloxan 125mg /kg dissolved in 0.9% normal saline IP (intraperitoneally) along with alcoholic extract of turmeric orally.	Alcoholic extract of turmeric will be given daily for 28 days.

The study included 36 Albino Wistar rats of either sex weighing 180- 250gm. Study was initiated after approval from the IAEC (Reference No. DMIMSDU/IAEC/2016-17/12.

- All the animals were handled as per guidelines given by CPCSEA. Before starting the study, all animals were acclimatized for 15days. During the period of study food and water was given ad libitum.
- Animals with body weight 180 - 250gm were selected for the study.
- They were fasted for 16hrs.
- On the 16th day before any intervention baseline fasting blood sugar level of all animals was estimated.
- Blood samples were obtained by sequential snipping of rat tail. Blood glucose levels were recorded using glucometer
- Body weight of all animals was taken on a weekly basis throughout the experiment.
- Animals in group I were given normal saline 10ml/kg/day IP.
- Animals in group II were given single dose of Alloxan 125mg /kg dissolved in 0.9% normal saline IP.
- Animals in group III were given single dose of Alloxan 125mg /kg dissolved in 0.9% normal saline IP.
- For prevention of fatal hypoglycaemia, a result of massive pancreatic insulin release, alloxan administered rats were provided with 10% glucose solution after 6 hours for next 24 hours.
- After this fasting blood sugar levels were estimated in each group on days 1, 3, 7, 14, 21 and 28. Animals from group II and III with a blood sugar level more than 200mg/dl were considered as diabetic.
- On the 29th day after giving Alloxan, turmeric was given to diabetic rats in group III in a dose of 300mg/kg/day orally once daily for next 28 days. Blood sugar level was estimated in all group III on days 1, 3, 7, 14, 21 and 28 after administration of turmeric.

Preparation of ethanolic extract of turmeric

Dry rhizomes of Turmeric in powdered form were purchased from local market. It was packed into thimble of filter paper and put in Soxhletapparatus in 5 batches of 200gm each.

It was subjected to continuous extraction with 99.99% ethanol for about 48 hours at 60^{0}C till solvent in the siphon tube became colourless. Thus, process required around 8-10 cycles/200gram turmeric powder.

To avoid bumping of the solvent small porcelain pieces were added to the flask. The solvent thus obtained was distilled and was heated using a water bath to get concentrated thick extract. The extract was then diluted in Tween80 and administered to the rat by oral route once daily.

Method of blood collection

Blood was collected by sequential snipping of tip of rat tail.

Estimation of blood glucose

Blood glucose was estimated using Accu-Chek glucometer active. It is quick, reliable and easy to use method of blood glucose estimation. It makes use of glucose oxidase specific strips and works on principle of reflectance photometry.

The test strip is inserted into the glucometer and the drop of blood sample is placed directly on the strip. The value of blood glucose appears on the screen within a few seconds and is expressed in mg/dl.

Statistical analysis

Statistical analysis was done by using descriptive and inferential statistics using student's paired t test, one way ANOVA and Multiple comparison, Tukey Test and software used in the analysis were SPSS 22.0 version and $p < 0.05$ is considered as level of significance

RESULTS

As shown in Table 2, the mean value of blood glucose level in Group I was 94.00±7.76, group II was 94.08±7.37, group III was 89.83±7.70. By applying One-way ANOVA no statistically significant variation was found in mean blood glucose levels among experimental rats of group I, group II and group III (F = 1.22, p value = 0.308, NS).

Table 2: Comparison of mean fasting blood glucose level (mg/dl) in three groups.

Group	N	Mean	Std. deviation	Std. error	95% Confidence Interval for mean		Minimum	Maximum
					Lower bound	upper bound		
Group I	12	94.00	7.76	2.24	89.06	98.93	83.00	106.00
Group II	12	94.08	7.37	2.13	89.39	98.77	84.00	104.00
Group III	12	89.83	7.70	2.22	84.93	94.73	75.00	103.00

Table 3: Comparison of mean blood glucose level (mg/dl) in three groups after 28 days of giving alloxan in group II and group III.

	N	Mean	Std. deviation	Std. error	95% Confidence Interval for mean		Minimum	Maximum
					Lower bound	Upper bound		
Group I	12	92.00	1.62	0.46	90.97	93.02	89.83	94.17
Group II	12	238.08	5.88	1.69	234.34	241.82	233.00	249.83
Group III	12	234.11	3.51	1.01	231.88	236.34	229.00	241.17

As shown in Table 3, mean value of blood glucose level in Group I was 92.00±1.62, Group II was 238.08±5.88, Group III was 234.11±3.51. By applying One way ANOVA statistically significant variation was found in mean blood glucose levels among experimental rats of group I, group II and group III (F = 5024.35, p value = 0.0001, S).

As shown in Table 4, one-way ANOVA showed a statistically significant variation in mean blood glucose levels in all the 3 groups (p value = 0.0001, S) on days 1, 3, 7, 14, 21 and 28 after oral administration of ethanolic extract of Turmeric.

By using multiple comparison Tukey test statistically significant difference was seen in mean blood glucose levels between group I and group II (p value = 0.0001, S), group I and GROUP III (p value = 0.0001, S) on days 1, 3, 7, 14, 21 and 28 respectively.

Statistically significant difference was also seen in the mean blood glucose levels between Group II on Group III (p value = 0.0001, S) on days 7, 14, 21 and 28 respectively.

However, no statistically significant difference in the mean blood glucose level between group II and group III on day 1 (p value = 0.568, NS) and day 3 (p value = 0.781, NS).

Comparison of body weight at day 1 to day 56

- Group I: t=0.25, p-value=0.80, not significant
- Group II: t=49.17, p-value=0.0001, highly significant
- Group III: t=49.38, p-value=0.0001, highly significant
- Mean bodyweight of rats in Group I on day 1 was 216.73±6.96 and on day 56 was 216.93±5.09. The difference in mean bodyweight was not significant in this group (p value = 0.80, NS). The mean value of bodyweight of rats in group II on day 1 was

212.31±4.37 and on day 56 was 136.75±3.69. and was found to be highly significant (p value = 0.0001, S). Experimental rats in group III on day 1 weighed 215.29±3.73 and on day 56 weighed 166.27±1.35. The difference in mean bodyweight was highly significant (p value = 0.0001, S).

Table 4: Comparison of mean blood glucose level in three group between day 1 and day 28 after giving intervention in group III with turmeric.

		N	Mean	Std. deviation	Std. error	95% Confidence Interval for mean		Minimum	Maximum
						Lower bound	Upper bound		
Day 1	Group I	12	94.00	7.76940	2.24	89.06	98.93	83.00	106.00
	Group II	12	235.83	7.49343	2.16	231.07	240.59	226.00	249.00
	Group III	12	238.83	6.17669	1.78	234.90	242.75	230.00	250.00
Day 3	Group I	12	91.58	8.27327	2.38	86.32	96.83	75.00	103.00
	Group II	12	229.50	8.64975	2.49	224.00	234.99	218.00	245.00
	Group III	12	232.00	10.26025	2.96	225.48	238.51	222.00	260.00
Day 7	Group I	12	91.08	8.19599	2.36	85.87	96.29	75.00	107.00
	Group II	12	235.83	6.78010	1.95	231.52	240.14	229.00	248.00
	Group III	12	218.00	11.07003	3.19	210.96	225.03	209.00	248.00
Day 14	Group I	12	92.66	9.46124	2.73	86.65	98.67	79.00	106.00
	Group II	12	246.16	3.78594	1.09	243.76	248.57	242.00	252.00
	Group III	12	188.00	3.54196	1.02	185.74	190.25	184.00	197.00
Day 21	Group I	12	91.58	8.27327	2.38	86.32	96.83	75.00	103.00
	Group II	12	236.16	6.42203	1.85	232.08	240.24	229.00	248.00
	Group III	12	164.83	3.61395	1.04	162.53	167.12	160.00	172.00
Day 28	Group I	12	91.08	8.19599	2.36	85.87	96.29	75.00	107.00
	Group II	12	245.00	6.38179	1.84	240.94	249.05	239.00	257.00
	Group III	12	144.75	3.25087	0.93	142.68	146.81	140.00	149.00

Table 5: Comparison of body weight (gm) changes of experimental animals in three groups.

Group	Day 1	Day 7	Day 14	Day 21	Day 28	Day 35	Day 42	Day 49	Day 56
Group I	216.73 ±6.96	217.30 ±6.24	216.02 ±5.98	216.95 ±5.19	216.71 ±4.84	217.30 ±6.24	216.02 ±5.98	216.95 ±5.19	216.93 ±5.09
Group II	212.31 ±4.37	192.71 ±2.90	183.50 ±3.39	169.20 ±2.74	162.55 ±1.97	157.97 ±1.57	151.99 ±1.57	144.64 ±3.58	136.75 ±3.69
Group III	215.29 ±3.73	201.08 ±1.90	190.71 ±3.09	181.34 ±2.78	168.62 ±2.26	167.55 ±1.80	166.91 ±1.65	166.6 ±1.02	166.27 ±1.35

DISCUSSION

In present study to evaluate the effect of Turmeric on Alloxan induced diabetes mellitus, 36 albino wistar rats of either sex were included in the study. They were divided into 3 groups i:e group I, group II and group III with 12 rats in each group.

Group I was control group, administered normal saline 0.9% intraperitoneally (I.P.) at a dose of 10ml/kg, group II and group III were the diabetic rat group. In group II and group III rats diabetes was induced by giving single dose of Inj. alloxan 125mg/kg IP. These rats were then monitored for blood glucose levels for another 28 days. On the 29th day we started administering ethanolic extract of Turmeric in Group III diabetic rats.

Mean blood glucose level before giving any intervention, in group I was 94.00±7.76, Group II was 94.08±7.37 and group III was 89.83±7.70. There was no statistically significant difference in the mean blood glucose value among the 3 groups at the start of study (p value = 0.308, NS).

The mean value of blood glucose from day 1 to day 28 after giving single dose Inj. alloxan 125mg/kg in group II and group III was compared with that of group I. Mean blood glucose level in group I was 92.00±1.62, group II was 238.08±5.88 and group III was 234.11±3.51. There was a

statistically significant difference in the mean blood glucose level between groups I and II (p value = 0.0001, S) and groups I and III (p value = 0.0001, S) respectively. However, there was no statistically significant difference in the mean blood glucose levels between group II and group III (p value = 0.057, NS).

Alloxan undergoes a series of cyclic reactions leading to generation of superoxide and hydroxyl free radicals. Hydroxyl radical is one of the most toxic radicals in the cell and plays major role in beta cell death leading to alloxan induced diabetes. Ighodaro et al, reported that there is a rise in blood glucose levels approximately 1 hour after administration of alloxan. This is coupled with a reduction in plasma insulin concentration.[6]

Mean value of blood glucose after giving alloxan in group II on day 1 was 235.83±7.49, day 3 was 229.50±8.65, day 7 was 235.83±6.70, day 14 was 246.16±3.74, day 21 was 236.16±6.43 and day 28 was 245.00±6.39. Although there was a rise in mean blood glucose level after giving Alloxan, there was a fluctuating response in mean blood glucose levels from day 1 to day 28 i: e mean blood glucose levels decreased to 229.50±8.65 in comparison with day 1where the blood glucose level was 235.83±7.49. Mean blood glucose increased to 246.16±3.74 on day 14 and again decreased to 236.16±6.43 on day 21, there was again a rise in blood glucose to 245.00 ± 6.39 on day 28. Glucose and alloxan share a similar chemical structure and are up taken by a common GLUT2 (Glucose Transporter 2) present of pancreatic beta cells. Increased levels of blood glucose protect the pancreatic islet beta cells against the toxicity and diabetogenicity of alloxan by lowering binding of alloxan to GLUT2 receptor. Moreover, GLUT2 has a greater affinity for glucose than Alloxan. A similar study carried out by Ighodaro et al, stated that Alloxan shows a multiphasic response characterized by inconsistent increase or decrease in blood glucose levels.[6]

Ethanolic extract of turmeric in a dose of 300mg/kg/day P.O. was administered to rats in group III on 29th day of the study which was considered as day 1 of intervention with Turmeric in group III. The mean blood glucose levels were monitored in group III on days 1, 3, 7, 14, 21 and 28 after daily administration of Turmeric and were compared to mean blood glucose levels in group I and group II. There was a statistically significant difference in mean value of blood glucose between groups I and II (p value = 0.0001, S) and Groups I and III (p value = 0.0001, S) on days 1, 3, 7, 14, 21 and 28 respectively. However, there was no statistically significant difference in mean blood glucose levels between group II and group III on day 1 (p value = 0.568, NS) and day 3 (p value = 0.781., NS) respectively. On day 7 mean blood glucose in Group II was 235.83±6.70 and group III was 218±11.03. On day 14 mean blood glucose in group II was 246.16±3.74 and group III was 188.00±3.56. On day 21 mean blood glucose in group II was 236.16±6.43 and group III was 164.83±3.65. On day 28 mean blood glucose in group II was 245.00±6.39 and group III was 144.75 3.27. The above results showed that there was statistically significant difference in mean blood glucose level between group II and group III (p value =0.0001, S) from day 7, 14, 21 till day 28.

Zhang et al, reported that oral administration of various doses of curcumin in alloxan induced diabetic rats i:e 80mg/kg body weight (BW) for 21 days and 45 days, 60mg/kg BW for 14 days, 90mg/kg BW for 15days, 150mg/kg BW for49 days, 300mg/kg BW for 56 days 100mg/kg BW for 4 weeks, 7 weeks and 8 weeks were able to lower blood glucose levels and glycosylated haemoglobin (HbA1C) and improve sensitivity to insulin.[7] Ghorbani et al, reported that oral administration of Curcumin in an experimental model of streptozotocin (STZ)-induced diabetes, oral administration of curcumin at a dose of 100 mg/kg/day for 8 weeks, significantly reduced blood glucose levels and improved renal function.[8] Several studies have reported that curcumin increased insulin sensitivity by activation of PPARγ. It also decreased TNF-α, IL-6, IL-8 secretion in isolated human erythrocytes and high glucose-treated cultured monocytes. It protects the islets of Langerhans from cytokine induced cell death by scavenging ROS, decreases cytokine induced translocation of NF-kB. Thus, reduction in oxidative stress is a plausible mechanism for the hypoglycaemic effect of curcumin.[9] Maithili Karpaga Selvi et al, stated that Curcumin is a potent scavenger of a variety of reactive oxygen species including superoxide anion radicals, hydroxyl radicals, and nitrogen dioxide radicals and these protective effects are attributed to its antioxidant property. Studies have also shown that curcumin exhibits strong antioxidant activity and plays a vital role against oxidative stress-mediated diseases like diabetes, obesity, cardiovascular disease, etc.[10]

Mean bodyweight of rats in group I before giving any intervention was 216.73±6.96 and on the last day of the study was 216.93±5.09. There was no statistically significant difference in the mean bodyweight of animals in group I (p value = 0.80, NS).

Mean bodyweight of rats in Group II before giving any intervention was 212.31±4.37 and on the last day of study was 136.75±3.69. The difference in mean bodyweight of animals in Group II was highly significant (p value =0.0001, S). Mean bodyweight declined in this group during the study. Mean bodyweight of rats in group III before giving any intervention was 215.29±3.73 and on the last day of study was 166.27±1.35. The difference in mean bodyweight of animals in group III was highly significant (p value = 0.0001, S). This group also experienced significant weight loss. However, in comparison with group II where mean bodyweight before giving any intervention was 212.31±4.37 and on the last day of study was 136.75±3.69, the weight loss was comparatively less. Similar study conducted by Zhang et al, stated that Curcumin when administered orally in a dose 0f 300mg/kg bodyweight for 4 weeks was able to prevent loss of bodyweight.[7]

CONCLUSION

Authors concluded that alloxan was able to induce Diabetes mellitus in experimental animals. Blood glucose levels were significantly high after administration of alloxan. Oral administration of ethanolic extract of Turmeric led to a significant reduction in blood glucose levels.

Funding: No funding sources
Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

REFERENCES

1. Madhu S, Srivastava S. Diabetes Mellitus: Diagnosis and Management Guidelines. 2015;28(1):4.
2. Guariguata L, Whiting DR, Hambleton I, Beagley J, Linnenkamp U, Shaw JE. Global estimates of diabetes prevalence for 2013 and projections for 2035. Diabetes Res Clin Pract. 2014 Feb;103(2):137-49.
3. Asmat U, Abad K, Ismail K. Diabetes mellitus and oxidative stress-a concise review. Saudi Pharmaceut J. 2016 Sep;24(5):547-53.
4. Ahangarpour A, Oroojan AA, Khorsandi L, Kouchak M, Badavi M. Solid lipid nanoparticles of myricitrin have antioxidant and antidiabetic effects on streptozotocin-nicotinamide-induced diabetic model and myotube cell of male mouse. Oxidative Med Cellular Longevity. 2018;2018.
5. Silva TBC, Almeida PHRF, Araújo VE, Acurcio F de A, Guerra Júnior AA, Godman B, et al. Effectiveness and safety of insulin glargine versus detemir analysis in patients with type 1 diabetes: systematic review and meta-analysis. Ther Adv Endocrinol Metab. 2018 Jun 22;9(8):241-54.
6. Ighodaro OM, Adeosun AM, Akinloye OA. Alloxan-induced diabetes, a common model for evaluating the glycemic-control potential of therapeutic compounds and plants extracts in experimental studies. Medicina. 2017 Jan 1;53(6):365-74.
7. Zhang DW, Fu M, Gao SH, Liu JL. Curcumin and diabetes: a systematic review. Evidence-Based Complementary Alternative Med. 2013;2013.
8. Ghorbani Z, Hekmatdoost A, Mirmiran P. Anti-hyperglycemic and insulin sensitizer effects of turmeric and its principle constituent curcumin. Int J Endocrinol Metabol. 2014 Oct;12(4).
9. Mohammed A, Islam MS. Spice-derived bioactive ingredients: potential agents or food adjuvant in the management of diabetes mellitus. Frontiers Pharmacol. 2018;9:893.
10. Maithili Karpaga Selvi N, Sridhar MG, Swaminathan RP, Sripradha R. Curcumin attenuates oxidative stress and activation of redox-sensitive kinases in high fructose-and high-fat-fed male Wistar rats. Sci Pharmaceut. 2014 Nov 4;83(1):159-75.

Cite this article as: Padhye MR, Jogdand SD. Effect of turmeric on alloxan induced diabetes mellitus in albino rats. Int J Basic Clin Pharmacol 2019;8:264-9.